STANDARD LABORATORY ORGANISMS FOR WATER QUALITY STUDIES PROGRAMME

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THE WATER RESEARCH COMMISSION

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EXECUTIVE SUMMARY

The two digit numbers in the summary corresponds to those of the chapters in the main report.

1. INTRODUCTION, BACKGROUND AND MOTIVATION

Aquatic toxicologists in South Africa use standard laboratory organisms, such as *Daphnia pulex*, most of which are from lentic or standing water environments for the determination of pollutant tolerances. The aim of this project is the selection, maintenance and captive breeding of suitable test species from lotic or flowing water habitats for the use in an artificial stream system which is being developed at Rhodes University (Palmer *et al* 1995). At the artificial stream system in the Institute for Water Research, water quality guidelines for the natural environment will be determined using these indigenous taxa. A range of representative taxa should be selected to be tested because responses to the same chemical differ from species to species. Standard test species enable different researchers to produce similar results from a species reared under known circumstances. It has been established that the full consequences of pollution for the aquatic ecosystem can not be determined unless the responses of member species of these communities are assessed. Niederlehner & Cairns (1990) and Stokes (1986) found much diversity in the responses of species to pollutants, which resulted ultimately in the decrease of species richness and composition through reduction in egg production and growth reduction, while primary productivity, decomposition and nutrient processing remained essentially the same.

1.1 AIMS OF THE PROJECT

- To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.
- To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.
- To attempt to establish methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.

1.2 APPROACH IN PLANNING THE PROJECT

The establishment of a laboratory culture method for riverine invertebrates requires the selection of suitable candidate species and the investigation of the responses of the selected species to the variety of abiotic conditions such as temperature and photoperiod which will prevail in a laboratory. The project plan was formulated with the recognition that information about the habitat requirements would dictate the type of container which would offer the best option for laboratory maintenance. Information about the life cycle would dictate the periods when mature animals would be available for laboratory breeding, and the functional feeding group to which the species belongs would determine what type of food should be offered. The effect of other biotic variables such as density and fecundity on the production potential of the programme also has to be investigated. The design of the experimental programme took into consideration which combination of abiotic and biotic factors would have the greatest impact on the growth and survival of the test candidates.

The research programme had a dual nature which overlapped in all areas. The selection of suitable taxa from the lotic habitat and the investigation of the biological and environmental requirements of these species for laboratory maintenance and breeding was closely interwoven with the development of the equipment and methods to maintain macroinvertebrates in aquaculture.

2. SUMMARY OF PROGRESS - SELECTION AND SCREENING OF CANDIDATE SPECIES

2.1 SELECTION CRITERIA

Certain criteria to be considered in the selection of organisms, suitable as subjects for ecotoxicological studies, have been developed by researchers in the field over the years. These can be grouped into several categories.

Sensitivity

♦ *High susceptibility to pollutant stress.*

Different taxonomic groups respond more, or less, sensitively to different pollutants. Therefore we have focused on abundant, widely distributed taxa, that are not present under grossly impaired conditions.

Ecology & Life History Information

♦ *Importance in terms of abundance or productivity, physical community structure or regulatory properties in the system* (ie the role of the chosen species in the ecosystem should be relatively important).

Multi-species test systems should include representatives of producer, regenerator and circulating phases but these taxa do need to be present in sufficient numbers for reliable collection to be suitable for laboratory breeding.

♦ *Wide distribution*

Test results which are obtained from species with a wide distribution are theoretically applicable to a wide range of ecosystems. However, the wide distribution may be an indication of phenotypic plasticity.

Provenance

♦ *Species identification.*

In cases where problems are experienced with identity it is necessary to collect parent stock from a limited range and keep could record of the identifying features.

♦ *The ecology and physiology of the species.*

Information on temporal and spatial variation in life-history features of aquatic invertebrates is essential in planning laboratory schedules for field collections.

♦ *Low genetic and biological variability.*

The importance of low variability in achieving reproducible test results is obvious, but when these results are extrapolated to the responses of wild populations which have naturally more variability the guidelines set may appear conservative.

Maintenance And Breeding

♦ *Easily held or cultured in laboratory for experimental ecotoxicological procedures.*

Herbivores or detritivores such as shredders, grazers or filter feeders are generally easier to maintain than predators or parasites with host requirements.

♦ *Easily sampled* i.e large and robust.

Hierarchy of Considerations

After three years of experience in the selection and maintenance of taxa we believe these should be ranked as follows;

1. Availability in the field and size of the organism.
2. Suitable life history and biology for maintenance in laboratories.
3. Accurate identification.
4. Position and function in ecosystem.
5. Sensitivity to range of test chemicals.

Ideal and achievable aims are seldom the same and compromises in the selection process had to be made in this complex and unpredictable procedure. Unless a field population of the candidate species is easily accessible, replenishing stocks for the early laboratory investigation can become prohibitively expensive. Problems associated with life history styles such as aerial phases can be circumvented in many but not all cases, but if a species has very narrow and specific habitat requirements this may make their culture uneconomic. We have come to the conclusion that a problematic taxonomy is not insurmountable if the identifying features are carefully described. However species complexes from the same habitat must be regarded with caution.

2.2 SCREENING OF ORGANISMS

After several screening trials and from observations during experiments both in this programme and during toxicological investigations conducted by Palmer *et al* (1995) as well as in field collections the following taxa were selected for further investigation. Those taxa which have been subject to detailed investigation are listed below.

Ephemeroptera Leptophlebiidae.

- *Choroterpes elegans:* A good candidate, reasonably accessible (Buffalo river, eastern Cape) with wide distribution, of good size, easy to feed and fairly robust, but fairly tolerant as it occurs in lower reaches of rivers. Its temperature range is possibly lower than taxa from upland rivers. Taxonomy well established.

- *Adenophlebia auriculata:* This is an excellent candidate, which is easily available, comparatively large and easy to feed. It is found in numerous upland rivers with good water quality so is quite sensitive and widely distributed. Robust with careful handling. Taxonomy is reliable and there are two species in the genus. However the breeding

biology is obscure.

Mollusca Ancylidae

♦ *Burnupia stenochorias:* An excellent candidate and easy to feed, robust with specialist handling and breeds easily in the laboratory. A better candidate than other gastropods as it is more susceptible to external conditions due to the anatomy. All life stages are aquatic.

Three other candidates which have good potential as laboratory candidates are *Thricorythis* spp. (Ephemeroptera); *Cheumatopsyche* spp. (Trichoptera) & Planaria.

3 SUMMARY OF PROGRESS - DEVELOPMENT OF METHODS

3.1 FIELD COLLECTION AND TRANSPORT

Animals should be handled as little as possible during collection and transported in cool well aerated water with pliable solid substrate. If possible, animals should be collected and transported on the same day. It was proved experimentally that touching limpets increased mortality rates and it was decided to use an anaesthetic during field collection and to line all containers in which the limpets are grown with plastic bags so that they could be moved without being handled.

3.2 MAINTENANCE EQUIPMENT

3.2.1 Artificial Streams - Recirculating Channels

Two sizes of recirculating channels of the same basic design were constructed (Fig 3.3.2.1). A plastic or PVC channel containing substrate, through which water is pumped from a sump by submersible aquarium pumps via a horizontal spreader. There are some problems associated with the use of these systems

- difficulty in the maintenance while in use.
- no easy method to allow trapping of adult insects on emergence.
- small juveniles escape easily from the outflow.

3.2.2 Static Water - Bubblepots

Bubblepots are cylindrical opaque or transparent plastic containers of a variety of sizes, equipped with

aerators. These are the most suitable containers for rearing small juveniles. They are easily maintained and transported. It was shown experimentally that one airstone produces the same level of dissolved that oxygen to all volumes of water and temperature and dissolved oxygen are negatively correlated.

3.2.3 Substrate

Ovoid kaolin stoneware substrate (6cm x 4cm x 4cm) have been manufactured to standardize the available algal food growing surface and to provide refugia (Fig 3.3.2.1 c). In addition, periphyton is cultivated on 8cm x 8cm unglazed tiles which provide a more accurately measurable surface of available food. A variety of materials were tested as substrate for mayflies. Observations from all experiments confirmed that solid substrate are essential for the optimal survival of stream macro-invertebrates. For the transport of invertebrates softer substrate such as fine textured plastic foam rubber, plastic sheeting or leaves from the collection site should be offered.

3.2.4 Food provision

The limpet is a grazer and the mayflies are brusher collectors so that both animals collect their food by scraping the surface from stones. Periphyton grown in the laboratory has proved to be an adequate basic food to sustain growth in both species. Degraded leaves, river detritus and TETRAMIN have all proved capable of sustaining mayflies.

3.3 CONCLUSIONS ABOUT METHODS OF LABORATORY MAINTENANCE

In conclusion, the species investigated so far can be adequately housed and fed prior to being made available for experimental purposes. The most important conditions for the successful maintenance and breeding of invertebrates are:

- Correct light conditions for algal growth to ensure good food supply.
- Temperature regulation.
- A good water supply, preferably from a non municipal source, with the ability to regulate its quality and condition.

4. SUMMARY OF RESULTS - INVESTIGATION OF *BURNUPIA STENOCHORIAS*

4.2 EXPERIMENTAL INVESTIGATIONS

4.2.1 OPTIMAL REARING CONDITIONS

4.2.1.1 A Pilot study on suitable laboratory conditions

A pilot study was conducted during which limpets were kept in 500ml bubblepots and were offered three different feed types. It was established that i) the limpets between 4-6mm shell length were sexually mature and laid eggs within 4 weeks while ii) limpets which were between 2-4mm in shell length were not sexually mature but did mature and then laid eggs with in 8 week of the start of the experiment. iii) Spat from both sizes of limpets grew between 0.019and 0.32mm/day on the diet provided. iv) In some containers a second generation of spat was produced. This pilot study showed that it would be possible to cultivate the limpet in the laboratory.

4.2.1.2 Handling requirements

Touching the limpets, either in the laboratory or transporting from the field, has a very negative effect on both their growth and survival. To overcome this not only was the use of anaesthesia investigated as stated above, but a measuring template was devised to enable measurement without removal from the container. We believe that what is lost in accuracy of measurement is gained in the increase in survival. The survivorship of the limpet in the laboratory has not been very high (20%). Initial losses after hatching are high and this is not unexpected as similar mortalities have been reported (Russell-Hunter, 1953).

4.2.1.3 Dietary requirements

Although the specific food requirements of *Burnupia* have not yet been investigated the species which most closely resembles it, *Ancylus fluviatilis* is a microherbivore of epilithic algae, particularly diatoms. *Burnupia* feeds by rasping the stone surface with the radula which is similar to the behaviour reported for *Ancylus*. Artificial food (pellets of *Spirulina*, fishmeal, starch and essential amino acids), natural periphyton and periphyton from an enriched environment all gave the same growth rate for smaller limpets (2-4mm) but these differed in the larger size group (4-6mm) where the artificial food proved to be the better food source and also gave the highest yield of eggs.

The debate as to whether test animals should be fed during ecotoxicological testing to minimise stress was addressed experimentally and it was found that 96 hours (acute test period) with no food did not affect the survival of either adults or sub-adults.

4.2.1.4 Hydraulic requirements

Although the limpets are naturally found in lotic habitats, it is presumed that this is in response to their need for high levels of oxygen within the water body. In the laboratory it has been found that a greater number of eggs are laid in aerated standing than in flowing water (section 4.2.1.5). The suitability of the design of channel used was clearly demonstrated in sections 4.2.1.4 to 4.2.1.7 where small channels (50cm long) consistently gave poor survival, and this was ascribed to the large angle of slope, and the lack of a good foothold on the smooth surface of these channels for the limpets. Large channels (1.5 M Long) gave a higher survival rate than standing, aerated water, although the difference in the survival of hatchlings between the channels and the standing water needs to be investigated further.

4.2.1.5 Temperature effects

At 15°C, 20°C and 25°C steady temperature, the higher temperature regime resulted in a significantly increased growth rate, at densities of up to 30 limpets per 500ml volume of water ($p<0,001$).
Work reported on other snails suggests that a circadian temperature fluctuation, rather than a constant temperature favours all physiological activities. This is a factor which will be taken into consideration when a dedicated laboratory is designed.

4.2.1.5 Density effects

Under high densities intensified grazing pressure and increased output of metabolic wastes may cause a shift in the species composition and succession of the periphyton assemblage (Bronmark 1989). Densities of 10 limpets per 500ml volume of water (230cm^2 available surface area) attained higher growth rates than densities of 20 and 30. The effect of density on fecundity has not yet been investigated but may prove significant. Chernin & Michelson (1957a & b), Wright (1960) and Eisenberg (1966) all showed a negative effect on the mean clutch size with increasing density in other snail species.

4.2.2 REPRODUCTION AND FECUNDITY

4.2.2.1 Breeding biology

As hermaphrodites (Brown, 1980) these limpets are able to produce young without copulating, but copulation has been observed, usually a smaller limpet acting as the male (Bantustan, 1950; pers. obs.). In hermaphrodite animals it is always difficult to determine if self or cross-fertilisation has taken place without genetic investigation. Further it is also often impossible to determine which partner act as female without direct observation. Because of our lack of understanding of the complexities involved in the reproductive style in the early period of the project, the results from the fecundity experiments conducted to date are not clear cut. However some indication of expected reproductive capacity and possible yield of juveniles can be made. Other researchers have also found a large degree of variation, and the difficulty we have experienced in determining individual fecundity is also universal.

The size of limpet at sexual maturity was always greater than 3,4mm shell length

4.2.2.3 Fecundity

The number of capsules and eggs produced by an individual is widely variable. The number of eggs/capsule ranges from 1 to 13. The average number produced by a group of limpets seemed to vary slightly depending on the history of the parents. Mature wild reared limpets laid capsules with an average of 5.75 eggs per capsule when brought into the laboratory. Juvenile limpets (approx. 2,5mm,) reared in the laboratory either as pairs or singly produced on average 3,7 egg/capsule. From August to October 1995 a field sampling programme has been underway and the average number of eggs per capsule has been determined as 5,4.

The numbers of capsules recorded from one pair of limpets in the laboratory also ranged widely. From pairs of field reared adults brought into the laboratory 14-44 (avg 24.7) capsules were recorded and from laboratory reared pairs 1-16 (avg 8.1) per pair was recorded.

More capsules were laid in the aerated, standing water, than in the flowing water channels in the laboratory.

4.2.2.4 Embryology

The embryological development of the eggs has been described and appears to follow a generalised

molluscan pattern in the initial stages but like other Ancylidae does not undergo torsion in the last stages before hatching. *Burnupia* displays direct development, with the young emerging as crawling snails. Hatching success in the laboratory is 91%. The embryonic period is 14 to 17 days, at an average temperature of 19°C. Cooler temperatures extend the time of hatching up to 21 days at 13°C (pers. obs.).

4.3 FIELD INVESTIGATIONS

After 8 months sampling of wild populations we have been able to determine that a small number of eggs are laid throughout the winter, but that there is a build up to peak egg-laying in spring. Growth rates and cohort responses to temperature variations in the field can only be analyzed after several seasonal cycles have been recorded.

4.4 CONCLUSIONS

The greatest advantage that the limpet has for laboratory rearing is, perhaps, the readiness with which it lays eggs in captivity. The most serious bottleneck in the production potential of the limpet is low survivorship of hatchlings but it is believed that enough knowledge has been accumulated to enable a pilot maintenance project to be launched. It appears from preliminary investigations that the limpet may have a similar life history to that of the mayfly.

5. SUMMARY OF RESULTS - INVESTIGATION OF *ADENOPHLEBIA AURICULATA* AND *CHOROTERPES ELEGANS*

5.1.1 INTRODUCTION

Two candidate species from the mayfly family Leptophlebiidae, *Adenophlebia auriculata* and *Choroterpes elegans*, were investigated, primarily in the field with fewer laboratory experiments being conducted than with the limpets. The emphasis on field work was because the life cycle, breeding strategy and mating behaviour of these animals are largely unknown. It is unlikely that laboratory maintenance will involve natural mating and egg laying as is the case with the limpets. However the knowledge of life history patterns gathered will enable optimal use of field collections for sub-adults. Investigation of artificial fertilization will be continued.

Growth rates, moulting frequency and suitable feeds have been investigated in laboratory experiments with

A. auriculata and *C. elegans.*

5.2.1 LABORATORY MAINTENANCE AND REARING CONDITIONS

5.2.1.1 Hydraulic requirements

Habitat requirements such the hydraulic conditions and substrate type was investigated. In this instance it was discovered that solid substrate are an essential prerequisite and that although the species is found most abundantly in riffles and runs it survives the best in static bubbled water in the laboratory.

5.2.1.2 Dietary requirements

It was found that if the natural diet of decaying leaves, detritus and periphyton was supplemented with TETRAMIN, a commercial fish food, optimal conditions for growth were provided if water quality was good.

5.2.1.3 Temperature effects

Rising temperatures depressed levels of dissolved oxygen and mortalities of *Choroterpes* sp. occurred when the DO reached 30 % at 25°C, except in the largest volumes of water where onset of mortality was delayed.

The average instar period for nymphs of *A. auriculata* is inversely correlated to temperature. (Table 6.5.1)

Table 6.5.1. Average instar length of *A. auriculata* at three temperatures in two experiments and overall instar period calculated by ANOVA.

Treatment	25°C	20°C	15°C
Petri-dish	7.7(2.66)	8.8(3.12)	13.5(6.20)
Bubblepot	7.4(3.43)	10.4(4.47)	14.9(6.23)
Overall avg.	7.52	9.24	10.12

Summary of growth rates in response to diet and temperature variation.

Table 6.5.2 Absolute growth rates (mm/day) calculated from all experiments in channels and bubblepots in the laboratory

Experiment no.	Growth rate mm/day	Temperature	Hydraulic/diet conditions
5.2.3	0.0191- 0.0220	19-22°C	Flowing/periphyton
5.2.4	0.0142-0.0277	17-22 °C	Static/ leaves & tetramin
5.2.5	0.0047/0.0085/0.015	15, 20, 25 °C	Static/ leaves
RANGE	0.005-0.03	15-25°C	

5.3 FIELD INVESTIGATIONS

Field populations have been studied over a three year period. *A. auriculata* in the Palmiet river in riffles and runs have a similar size distribution but higher densities were found in runs. Hatchlings have not yet been captured in the field. In 1993 there appeared to be a late autumn/early winter and a late winter/early spring emergence of subimagoes. This was confirmed when a weekly sampling regime was instituted in 1994. In addition a large summer emergence was found which encompassed several weeks in February and March.

Aspects of the life history of the mayfly that still need investigation include; a) accurate measurements of the effect of temperature on growth rate; b) factors which influence fecundity; c) metamorphoses; d) the habitat of hatchling nymphs (< 0.4mm HW.) which may be gravel and sand (de Moor pers comm.) or leaf packs; e) cues which triggers emergence; f) determination of growth rates in the field to verify those obtained in laboratory culture.

5.4 REPRODUCTION AND FECUNDITY

5.4.1 ARTIFICIAL FERTILIZATION

The successful captive mating of mayflies have never been accomplished. It was decided to resort to the well tried piscicultural practice of artificial fertilisation. It was demonstrated that the eggs of *A. auriculata* could be artificially fertilised and that viable offspring resulted. The most successful method involved dissecting the seminal vesicles out of a male imago in a freshly prepared solution of insect Ringers. The

female was then induced to release her eggs into the solution after decapitation by dipping the tip of her abdomen onto the surface of the mixture. This mixture of eggs and sperm was then left for at least 15 minutes before the eggs were transferred to a suitable substrate in river water where they rapidly adhered to the surface. Egg development was fastest at 25 °C. and took 16 -22 days to hatch. However, further work is necessary to perfect the techniques and to increase the hatch rate.

Sufficient numbers of new hatchlings have never been available for experimental investigation of rearing but in the petri-dishes where they hatched they reached the second instar. The smallest sizes (0.2 -.4 mm head width) of field caught nymphs have all survived well in the laboratory (Ch.5.2.).

5.5 CONCLUSIONS

Sufficient supply from laboratory stocks is still not possible as several problems associated with reproduction in captivity and survival of a large proportion of both selected species have not been solved.

6 ACHIEVEMENT OF AIMS

i. *To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.*

♦ Screening was accomplished in the eastern areas of the country, and in addition invertebrate workers country wide were consulted by questionnaire and nine suitable candidates were identified. Of these three species have been investigated to varying degrees.

ii. *To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.*

♦ A pilot programme has been established and methods to feed and house invertebrates are in place. However the pilot project is not yet operational because of difficulties with the long term survival of the limpet and artificial fertilisation and rearing of the hatchlings of the mayfly.

iii. *To attempt to establish methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.*

♦ Until the above problems are solved, a sufficient supply of experimental organisms will not be possible.

However during the course of the project toxicological experiments were supplied with limpets on two occasions and many of the problems associated with handling, transporting, and maintaining the test species were addressed and solved.

Although not stated explicitly in the aims of the project, implicit in the work programme and the development of the methods, has been the investigation of the biology and life history of the two species selected. Knowledge gained about the previously unknown responses of these two species to environmental variables has been significant and will contribute to the understanding of ecosystem function of subtropical rivers.

7 RECOMMENDATIONS FOR FUTURE WORK

Requirements of the cultivation, maintenance and supply of sufficient experimental animals for ecotoxicological experiments in artificial stream laboratory (which is now fully operational):

- Further knowledge of the breeding requirements, and better survival of juvenile *Burnupia*.
- Development of techniques for artificial insemination and maintenance of early instars of *A. auriculata*.
- The investigation of optimal growing conditions for the cultivation of periphyton.
- Increasing the scale of laboratory maintenance facilities to provide more capacity for the maintenance of larger laboratory populations.
- The design and construction of a dedicated laboratory for production and maintenance of experimental populations of invertebrates. (see section 7.1).

7.1 DEVELOPMENT OF A DEDICATED REARING AND MAINTENANCE LABORATORY

Present facilities in the Institute for Water Research are inadequate for the envisaged production of experimental animals. The requirements for a purpose-built facility are as follows:

- Floor space of at least 100 m^2.

- Adequate temperature control, by air-conditioning.
- Adequate natural light, e.g. through sky-lights.
- Periphyton cultivation facilities which are separate from the insect rearing facilities.
- Water purification facilities, e.g. by deionisation.
- Rearing and breeding channels, as well as extensive population holding facilities, with temperature and light control.

Two initiatives are proposed for the second phase of this project, which begins in January 1996:

- A visit to Stroud Water Research Centre, Pennsylvania, where a large amount of mayfly research is conducted and Virginia Polytechnic in Blackenberg where ecotoxicological testing has been ongoing for 20 years, to learn about the conditions described above.
- The engagement of a fund-raiser, to obtain funding for the proposed laboratory.

7.1.1 DEVELOPMENT OF THE REARING FACILITY

The development of this facility should take into consideration the needs which are peculiar to the culture of lotic invertebrates. However, they rely on general principles of aquacultural design which have been developed throughout the world for many years. Huguenin & Colt (1989) produced a guidebook in which much of the available literature on general principles of aquacultural design needs for both sea and freshwater have been summarised (Appendix 6). The requirements which are particular to this project revolve around the provision of the correct feed by the cultivation of periphyton, and the addition of decaying leaves supplemented by TETRAMIN as the basic diet for the mayflies. A subsidiary consideration is the maintenance of large numbers of smaller-sized specimens (those not fully grown) in a holding facility in readiness for use as toxicology test subjects.

Laboratories where invertebrates are cultured on a routine basis should be investigated. Information will be obtained from two sources: Stroud Water Research Centre, Pennsylvania where mayfly culture has been ongoing for at least 20 years; and the Virginia Polytechnic where ecotoxicology work is also of long standing.

Once these investigations are complete plans can then be drawn up for the construction of a dedicated facility in Grahamstown. A fund-raiser will be engaged to obtain funding for the project, and after the plans have been approved, and funding is obtained, the building will proceed. Prof.T.Hecht and Mr.P.Britz at the Department if Ichthyology and Fisheries and Science at Rhodes both have extensive experience in the construction of aquaculture facilities and have agreed to oversee the planning.

The expertise being developed in this and during the earlier project can be applied to other areas of environmental management such as the rehabilitation of damaged rivers and streams. The restocking of rivers with macroinvertebrates will speed their recovery. This was a contributing factor towards the interest in collaboration shown by the Dept. of Nature and Environmental Conservation.

ACKNOWLEDGEMENTS

In a project with a diverse nature such as this one has proved to be, the people who are called on for advice and information come from many professions. We would like to acknowledge the assistance of aquatic biologists country wide, the irrigation engineers and aquaculturists we have consulted. We have been fortunate that in the eastern Cape and particularly at Rhodes University many experts were easily accessible.

To Dr. Jay O'Keeffe and Dr. Tally Palmer, goes our gratitude for creating the opportunity together with the Water Research Commission, to embark on what has been a fascinating and at times frustrating project. Jay and Tally thank you, for firm guidance and loyal support. To our colleagues in the Institute we would like to give thanks for help, support and lots of fun. In particular, I would like to thank Margi Rogers our ever cheerful and unflappable secretary who, together with Ann McKane, helped to keep the finances of the project ordered. To Patsy Goetsch, thank you for encouragement and editorial help and Gaye Youthed for technical support. As project researcher, I would like to thank Heather for bringing better order and structure the project, and Nkosnathi for making and maintaining the equipment as well as for valuable assistance in the field.

Colleagues at Rhodes University who have been ready to give sound advice include Prof. Tom Hecht, Peter Britz, Sarah Radloff and Martin Villet. Horst Kaiser must be singled out, for although he acted as official consultant, for statistical analysis, his input has been enthusiastic and unstinting. He tackled some nasty analytical problems and came up innovative solutions.

One of the other advantages of working at a university is the availability of students eager to find suitable projects every year. I have been fortunate to have had three sterling young people working with me. Thank you; Lisa Horne, Bruce Davison and Simon Burton for your input.

The WATER RESEARCH COMMISSION fulfils an important role in the scientific community of the country by supporting many scientists and giving them the scope to pursue work which is not only of their own interest but of great national importance. We in this project has been extremely fortunate in having Dr. Steve Mitchell as Project Manager. He has been encouraging, supportive and always at hand with guidance. More than anything Steve I want to thank you for having confidence in us, for that has allowed our confidence to grow. Although Dr Peter Reid is no longer with the Commission, we would nevertheless like to acknowledge his contribution, which was often one of grasping the nub of a problem, in wide ranging discussions.

Finally the members of the steering committee of the project must be thanked for their input and time. The members not mentioned already were; Professors MN Bruton, JA Day, IG Gaiger, Dr. KCD Hamman. Mr D Roux and Prof. JHJ van Vuren.

Guide to this report.

Each chapter in this report stands on its own with its own reference list. The appendices are numbered according to the relevant chapter except Appendix 3 which refers to both Chapter 3 and 5. Table 5.5.1 appear in both the Executive Summary and in Chapter 5 (summary).

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OBJECTIVES AND BACKGROUND

Introduction

Aquatic toxicologists in South Africa use standard laboratory organisms for the determination of pollutant tolerances. Most of the invertebrate test organisms used are lentic inhabitants. The aim of this project is the selection, maintenance and captive breeding of suitable test species from lotic habitats for the use in an artificial stream system which is being developed at Rhodes University (Palmer *et al* 1995). Laboratory toxicological bioassays assess the possible effects of proposed new chemical products or the reasons how and why chemicals have affected the target organisms. Ecotoxicology involves the investigation of the fate and effect of toxic agents, as well as the tolerances of test species to these substances, in the ecosystem (De Kruijf 1988). The artificial stream system in the Water Research Institute at Rhodes will be employed in identifying these responses and in doing so, determine water quality guidelines for the natural environment. To test the tolerances of flowing water taxa to the water quality variables this facility needs a reliable and uninterrupted supply of indigenous test organisms from the lotic environment.

1.1 PROJECT AIMS

- To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

- To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.

- To attempt to establish methodologies for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.

1.2.1 TARGETS

GANNT CHART - APPROXIMATE SCHEDULING OF PROJECT TASKS.

YEAR	1993				1994				1995			
QUARTER	1	2	3	4	1	2	3	4	1	2	3	4
1. Literature review	X	X	X	X	X	X	X	X	X	X	X	
2. Life history data		X	X	X	X	X	X	X	X	X	X	X
3. Habitat		X	X	X	X	X	X	X	X	X		
4. Interim report						X						
5. Lab maintenance				X		X	X	X	X	X	X	X
8. Pilot scale rearing						X	X	X	X	X	X	
9. Transport methods				X	X	X	X	X				
10. Final report										X	X	X

1.3 BACKGROUND TO PROJECT

The establishment of biological tolerance criteria for the aquatic environment has become critical in this country with its limited water resources, as ever more pressure is being exerted on this resource through increased utilisation. The full consequences of pollution on the aquatic ecosystem can not be determined unless the responses of member species of these communities are assessed. Examples of the variety of responses from resident species to pollutants are cited here to highlight the need for a representative selection of species to be investigated. Niederlehner & Cairns (1990) found that members of periphyton communities showed a variety of significant responses by slight changes in pH, which often resulted in the biomass remaining the same whilst the community structure and perhaps function changed. This was consistent with previous findings of Stokes (1986) that species richness decreased and composition changed while primary productivity, decomposition and nutrient processing remained essentially the same to a pH of 5.6. From studies on other commonly tested multicellular organisms these authors extrapolated that a pH decline from 6.0 will begin to have adverse effects on insects (chironomids), fish, crustacea (Cladocera) and some amphibians. Six fish species, cladocerans, copepods, rotifers and protozoans were tested at various levels of ammonia exposure by Hermanutz *et al (1987) and revealed widely differing responses to concentration as well as type i.e. reduction in egg production, growth reduction or histological lesions in some organs.*

Petersen et al (1992) state that some ecological concepts are often not considered in ecotoxicology, and discusses their importance in assessing the effects of hazardous substances on aquatic systems at the population, community and ecosystem levels. He suggests that the study of a small group of ecologically related organisms, a guild, can provide more information on the effect of toxic substances than the study of the whole community. A key to this analysis is the recognition of the difference in species sensitivity and it is suggested that the specialist-generalist concept can be used to predict which species will be most affected by toxic substances.

1.3.2 APPROACH IN PLANNING THE PROJECT.

The establishment of a laboratory culture method for riverine invertebrates requires firstly the selection of suitable candidate species and then investigation of the responses of the selected species to the variety of conditions which will prevail in a laboratory. Naturally, considerable variation occurs in temporal and spatial scales of life-history features such as emergence, feeding and growth, and movements and migrations of aquatic insects (Rosenberg & Resh, 1993). Information on the degree of variation which occurs is needed to plan laboratory investigations, and field sampling programmes, for toxicological

experiments. Since experimental toxicology was started concurrently with the breeding programme; and the breeding programme is dependent on "seeding" populations in the field; this project aimed both to provide information on field populations, and on the breeding potential of selected stream organisms. If sampling is done at times when the largest number and/or the most vulnerable stages are readily available, progress of the work will be optimised.
The project plan was formulated with the recognition that information about the habitat requirements would dictate the type of container which would offer the best option for laboratory maintenance. The life cycle would dictate the periods when mature animals would be available for laboratory breeding, and the functional feeding group to which the species belongs would determine what type of food should be offered. The design of the experimental programme took into consideration which of the laboratory variables would have the greatest impact on the growth and survival of the test candidates.

Single species tests are important for establishing the effect of chemicals on growth, reproductive success, behaviour and other biologically important information, and these have been refined over the last 40 years. Although Cairns (1992) has made some progress in the use of multi-species test systems these involve lower orders. We have used the single species approach, but have selected invertebrate taxa from different orders.

1.4 SUMMARY OF PROGRESS

1.4.1 SCREENING OF ORGANISMS

Aim

To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

Results

A protocol for selecting appropriate experimental organisms was developed through reviewing the literature pertaining to the use of artificial streams and associated types of research. Rivers in the Mpumalanga, eastern Cape and western Cape were sampled and faunal complexes returned to the laboratory where the survival of the various species was recorded. From these experiments a short list of suitable species was compiled. At the same time the laboratory culture methodologies were investigated. A questionnaire was circulated to invertebrate biologists at various universities and their responses are recorded in Chapter 2.

1.4.2 MAINTENANCE OF REPRODUCING POPULATIONS

Aim

To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.

Results

In attempting to satisfy this aim a two-pronged approach was used. To be able to design optimal holding and breeding conditions for any species some understanding of the life history, the habitat requirements and the breeding biology is needed. Consequently, the responses of the selected species to laboratory conditions were recorded experimentally, while the ecology and life history were recorded by regular field investigations.

Ancylidae

The limpet *Burnupia stenochorias* was found to reproduce readily in the laboratory. Consequently a number of experiments were conducted to ascertain the responses of this animal. The following areas have been and continue to be investigated.

1) Growth rates
    a) suitability of various hydraulic water conditions.
    b) influence of temperature (15°C, 18-20°C and 25°C)
    c) effects of disturbance
2) Food requirements
3) Reproductive biology
    a) Fecundity and hermaphroditism.
    b) Developmental period and basic embryology.
4) Field studies, initiated in 1995.

It is believed that enough knowledge has been accumulated to enable a pilot maintenance project to be launched.

Leptophlebiidae

Two candidate species from the mayfly family Leptophlebiidae, *Adenophlebia auriculata* and *Euthraulus (Choroterpes) elegans*, were investigated, primarily in the field with fewer laboratory experiments being conducted than with the limpets. The emphasis on field work was because the breeding strategy and mating behaviour of these animals are largely unknown. To date the life history of *A. auriculata* has been monitored, although a direct correlation of environmental cues and breeding has not yet been established. Laboratory maintenance experiments using *A. auriculata* and *Choroterpes*

spp. as subjects, have investigated growth rates, moulting frequency and suitable feeds. It is unlikely that laboratory maintenance will involve mating and egg laying, as the cues for these are unknown. However, a knowledge of life history patterns in the field will enable optimal use of field collections. Investigation of artificial fertilisation will be outlined further.

1.4.3 METHODS

Aim

To develop methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.

Results

One of the essential components of successful toxicity testing is reliable survival of control populations. To this end it was important to establish optimal conditions in the experimental systems. In addition, conditions under which maintenance and breeding will be optimal needed investigation. At this stage we have identified the following key parameters: hydraulic conditions, light, temperature and food requirements. To date, control survival is reliably 80 - 90% over 96 hours, however, the sufficient supply from laboratory stocks is still far from possible as several problems associated with reproduction in captivity and life long survival of both selected species have not been solved.

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CHAPTER 2
SELECTION AND SCREENING OF LOTIC INVERTEBRATES SUITABLE FOR LABORATORY REARING AS EXPERIMENTAL ANIMALS

2.1 A REVIEW OF THE SELECTION CRITERIA TO BE APPLIED TO CANDIDATES FOR CAPTIVE REARING FOR ECOTOXICOLOGICAL STUDIES

The choice of a standard laboratory organism for rearing in large numbers should obviously be governed by carefully selected criteria. In many ways these criteria are similar to those that apply to the choice of bio-monitoring indicator species which are utilised to monitor the effects of xenobiotics on the natural community. Ideally, indicator organisms are those species that have narrow and specific environmental tolerances. Conversely, organisms that have wide tolerances for different environmental conditions, and whose patterns of distribution or abundance are only slightly affected by substantial variations in environmental quality, are poor indicators.

The principal underlying assumption in using indicator organisms for water quality assessment is that the abundant presence of the indicator signifies that its physical, chemical, and nutritional requirements are being met. Table 2.1.1 lists the characteristics of "ideal" indicator organisms as proposed by Rosenberg and Wiens (1976) and Hellawell (1986) and the desirable properties for "ideal" test species

suggested by De Kock, de Kruijf & van de Guchte (1988) and the high degree of similarity in the requirements for both biomonitoring and laboratory test procedures is evident. The implications of these criteria highlights the need to have an understanding of the biology of those species which are to be used in toxicology tests.

Table 2.1.1 Desirable selection criteria for indicator species compared to those for laboratory test species. Several criteria can be grouped and are discussed under the same heading.

IDEAL INDICATOR SPECIES (Rosenberg and Wiens (1976) & Hellawell (1986))	IDEAL TEST SPECIES (De Kock, de Kruif & van de Guchte, (1988))
Taxonomic soundness and easy recognition by the nonspecialist. Taxonomic uncertainties will complicate long-term monitoring and between-site interpretation.	Species identification should be reliable.
Cosmopolitan distribution (or distribution involving an ecological analog), which would allow for comparative studies on local, national, and international scales.	Species should be widely distributed.
	Species should have high susceptibility to pollutant stress.
Numerical abundance allows for ease of sampling and for conclusions about quantitative distribution patterns.	Species should be abundant or dominant components of the natural system.
Low genetic and ecological variability (indicators should have relatively narrow ecological demands).	Species should have low genetic and biological variability.
Ecological characteristics should be well known. Background physiological and autecological information should be available widely.	Information from previous ecological or physiological studies should be available as well as abundant ecological data.
Suitable for use in laboratory studies to allow for determination of causality	Species should be easily held or cultured in the laboratory for experimental procedures.
Large body size which would facilitate sampling and sorting.	Species should be easily sampled.
Limited mobility and relatively long life history to allow for ease of integration on spatial and temporal scales.	

2.1.1 SENSITIVITY

♦ *High susceptibility to pollutant stress.*

Over a period of twenty years, investigations at the Virginia University Institute for Environmental and Hazardous Materials Studies have established that naturally derived, unstressed communities of aquatic microbes and macro-invertebrates are composed of species showing a normal distribution of stress tolerance from sensitive to resistant. Cairns (1986) addresses the concept of the "most sensitive species " and the erroneous perception that responses from sensitive species can be extrapolated to a wider array of species at different levels of biological organisation. Firstly, the sensitive species may not be resident in the ecosystem for which management decisions are to be taken and incorrect criteria could be set, and secondly, responses to chemicals are known to vary from species to species. In addition, different taxonomic groups respond more, or less, sensitively to different pollutants. Therefore we have focused on abundant, widely distributed taxa, that are not present under grossly impaired conditions.

2.1.2 ECOLOGY & LIFE HISTORY INFORMATION

♦ *Importance in terms of abundance or productivity, physical community structure or regulatory properties in the system* (ie the role of the chosen species in the ecosystem should be relatively important).

An understanding of ecosystem function and structure is essential in the selection of species to breed for laboratory testing. Therefore representatives from different trophic levels should be selected. Cairns (1975) also suggests that not all species are equally important in the functioning of a system and coined the term "critical species" for those which have the most telling effect. It has been demonstrated several times that the removal of top predators can result in population explosions while the overproduction of lower level producers has resulted in severe population imbalances and the development of pest species.

The distribution of individuals within species follows a recognizable pattern: a few species are represented by many individuals, many species are represented by few individuals, and some species are intermediate (Williams, 1953). Pearson *et al* (1983) proposed using species of intermediate abundance as pollution indicators. He argued that very abundant taxa may be rejected as indicators because they may have opportunistic characteristics such as high reproductive capacity and good dispersal mechanisms, rather than being tolerant to the pollutant which may have made them less suitable. Species need to be present in sufficient numbers for reliable collection.

For ecotoxicological testing, those species which play a key role in the functioning of ecosystems need to be identified. In water bodies these are likely to be the predators, algal grazers and the litter processors as well as decomposers (bacteria and fungi). Most of these are benthic inhabitants.

♦ *Wide distribution*

Test results which are obtained from species with a wide distribution are theoretically applicable to a wide range of ecosystems. However, the wide distribution may be an indication of phenotypic plasticity. Thus, species with restricted distribution abilities could be adversely affected by incorrect tolerance criteria set for their habitats if derived from non-residents.

2.1.3 PROVENANCE

The provenance of parent populations of test animals should be of prime concern and:

♦ *Species identification should be reliable*

In southern Africa this could prove to be a stumbling block as taxonomists are rare and the identification of many taxa is difficult. However, the importance of accurate identification must be of prime concern. In cases where problems are being experienced the necessity of collection parent stock from a limited range is important and the need to establish the identifying features accurately is of prime importance. This information will be of value when the taxonomy is established formally.

♦ *The ecology and physiology of the species should be known.*

Information on temporal and spatial variations in life-history features of aquatic invertebrates is essential in planning laboratory schedules for field collections, for the replenishment of laboratory stocks. The local knowledge base of the variations is rather shallow and narrow; and most indigenous aquatic invertebrates have not been investigated. Guidelines of expected responses may be ascertained from literature if the life histories of related organisms, but this information should always be regarded as indicative of expected emergence patterns and reproductive periods. Once a test organism has been selected, it is important to investigate these aspects of its biology.

Invertebrates may acclimate physiologically or behaviorally to a pollutant but this resistance would not be inherited (Johnson *et al, 1993). Alternatively, they may develop increased resistance by natural selection (genetic adaptation), and Klerks and Weis (1987) found that most populations in polluted areas have some form of increased resistance. Investigations of the same species from related but pristine and altered systems may assist in the identification of vulnerable indicator species.*

♦ *The species should have low genetic and biological variability.* Populations from pristine conditions are ideal stock material as behavioral and biochemical adjustments to foreign influences have not taken place. While the importance of low variability in achieving reproducible test results can be understood, it is when results are extrapolated to the responses of wild populations in field conditions the may be found to be

conservative. Conservative results are appropriate for setting environmentally protective guidelines.

2.1.4 MAINTENANCE AND BREEDING

♦ *Easily held or cultured in laboratory for experimental ecotoxicological procedures.*
Ideally good laboratory culture subjects should reproduce in one medium and not have an aerial phase but this would exclude many aquatic insects, limiting the range of species from the aquatic system to be tested. A parthenogenetic or self fertilising life style would facilitate breeding as complex environmental and behavioural mating cues are then excluded.

Predacious feeding habits are complex to maintain in laboratory situations but omnivores, herbivores or detritivores such as shredders, grazers or filter feeders are generally easier to maintain. Information on feeding biology from the literature can be of assistance in selecting likely candidates.

♦ *Easily sampled.*
If animals are too small or fragile they may disintegrate before their mortalities can be recorded during LC_{50} tests. Sublethal tests on the other hand investigate such processes as osmoregulation, cellular respiration and ventilation, gas transport and haemopeitic systems, and the test species should offer the researchers the option of analysing the affected organs or observing various behavioral responses with reasonable ease. If the species is too small these observation are more difficult to record.

2.1.5 HIERARCHY OF CONSIDERATIONS

What is the hierarchy of considerations in the selection process?
After three years of experience we would rank them as follows;

1. Availability in the field and transportability.
2. Suitable life history and biology for maintenance in laboratories.
3. Classification and relationships.
4. Position and function in ecosystem.
5. Sensitivity to range of test chemicals.

Compromise is inevitable in such a complex and unpredictable procedure. Unless a field population of the candidate species is easily accessible, replenishing stocks for the early laboratory investigation can become prohibitively expensive. Problems associated with life history styles such as aerial phases can be circumvented in many but not all cases, but if a species with very narrow habitat requirements requires highly specific keeping conditions this may make their culture uneconomic. We have come to the conclusion that uncertain taxonomy may not be an insurmountable problem if the identifying features are carefully described. However species complexes from the same habitat must be regarded with caution. The

most important criterion for the first species to be tested is laboratory survival.

2.2 SITES SURVEYED AND SPECIES SCREENED

Introduction

To date, the aquatic species employed in laboratory testing are used mainly due to their ease of culture and well researched life histories. Species used for aquatic toxicology work can be found most commonly among the Pisces and Crustacea, with a few members of the Insecta represented from the order Diptera (Chironomidae). To widen the range of possible test species, communities from riffles and runs of rivers were collected using suitable methods (Chapter 3) and screened for survival under a variety of laboratory conditions. Once this simple screening had been accomplished and some background knowledge had been acquired on the survivorship and responses of the taxa collected, more rigorous and replicated experiments were conducted.

Aim

To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

2.2.1 SITES SURVEYED

A number of relatively pristine areas were visited to bring back live material from suitable locations for screening; it was presumed that the resident populations would not have become habituated to foreign substances. The following rivers were visited: Buffalo, Nahoon and Kologa Rivers: East London and Stutterheim; Kowie, Berg and Palmiet Rivers: Grahamstown district; Sabie River: Kruger National Park; Kirstenbosch Stream: Cape Town.

2.2.2 AVAILABILITY AND ABUNDANCE OF TAXA

The availability of suitable species from the sites visited is reported in Table 1, Appendix 2. Invertebrate experts country-wide were consulted to gather information and solicit opinions on the suitability and availability of species for use as regional standard taxa. Before compiling a questionnaire several invertebrate experts were interviewed: Prof. Schoonbee (RAU), Mr. Bickerton (CSIR, Stellenbosch) and Dr. Hart (UCN) (culture methods): Dr. King & Prof. Davies and Day (UCT; insect keeping): Prof C. Appleton (UN, Pmb; molluscan culture). The anecdotal information gathered was considered when selecting suitable taxa, and when suitable equipment was designed. The questionnaire was then compiled and circulated to invertebrate workers throughout the country (Results; Table 2.2.2). The questionnaire is appended. (Appendix 2.2).

Table 2.2.2 List of possible suitable candidate species.

GROUP	*SPECIES*	AREA	RESPONDENT
CRUSTACEA	*Paramelita nigricolus* *P. capensis*	W. Cape	Day; Griffiths
	Caridina spp.	Natal	Rayner; Alletson; Hart
	Palaemon capensis	W Cape	Day
	Macrobrachium sp.	E Tvl.	Van Vuren
	Streptochephalus sp.	E Cape	Day
MOLLUSCA	*Bulinus tropicus*	Natal	Schiff; Rayner
	Ferissia sp.	Natal	Schiff; Rayner
	Burnupia sp.	Wide	O'Keeffe
	Lymnaea natalensis	E. Cape	
	Physopsis globosus		
PLANARIA	*Dugesia sp.*		Day
INSECTA Ephemeroptera	*Trichorythus spp.* *Afronurus harrisoni* *Adenoplebia auriculata* *Choroterpes elegans*	Wide	 Alletson; Palmer King
Trichoptera	*Cheumatopsyche afra &* *C. thomasetti*	Wide	Palmer

2.2.3 SCREENING OF SUITABLE TAXA

Introduction

A broad screening programme involves testing suitable maintenance equipment and feed as well as testing the responses of the animals to the provided laboratory conditions, the variation of which must be monitored and recorded. Most riffle dwelling invertebrates are thigmotactic. The substrate are usually the food source as well. As the screening progressed we tested the suitability of various substrates and food supplements as well as the two hydraulic conditions of flowing versus turbulent water (channels or raceways against bubblepots (Appendix 3).

Aims

i. To investigate the survivorship of riverine macro-invertebrates in a range of laboratory conditions in order to ascertain optimal holding conditions.

ii. To ascertain if selected riffle dwellers are obligately rheophylous (require flowing water) and their response to changing levels of temperature and dissolved oxygen (see Chapter 3 appendix 3).

iii. To screen various types of substrates for suitability (see Chapter 3 Appendix 3)

iv. To test the suitability of commercial food as diet of invertebrates as opposed to naturally occurring food from a relatively pristine river.

General methods

Collections were made according to methods described in Chapter 3. During the trials the oval raceways, and a range of bubblepots including 500ml jars were used as experimental containers while plastic netting and foam rubber pads were tested as suitable substrates. Both ground TETRAMIN, and detritus and decayed leaves from the home stream were offered as food. The collected animals were placed in raceways with stones and detritus from the collection site. In bubblepots, stones or plastic mosquito netting or rigid foam-pads were provided as substrate. In Grahamstown the laboratory was air conditioned to a steady 19°C but in the Kruger Park such facilities did not exist.

Faunal complexes from the following rivers were screened:
Buffalo, Kologa, Palmiet (E. Cape) and Sabie (Mpumalanga) rivers.
Detailed experimental reports are in Appendices 2 & 3.

Results

The results in Table 2.2.3 are summarized from six trials and numerous laboratory observations while conducting experiments with other aims. The best survivors among the insecta were Trichoptera (Hydropsychidae, *Cheumatopsyche* sp.) & Ephemeroptera (Trichorythidae, *Thrichorythus* spp. and Leptophlebiidae *Choroterpes & Adenophlebia* spp). Good survival rates were also shown by larval Sphaenidae and Planaria. The cumulative mortalities recorded in the controls of the salinity tolerance experiments conducted in the Kruger Park (Palmer *et al* 1995) support the fact that *Trichorythus* spp and *Choroterpes* spp are both good candidate species for laboratory culture as average mortality seldom

exceeded 10% in raceways. In contrast to the excellent survivorship in raceways, mortalities in excess of 80% were recorded in 500 ml bubblepots with inadequate substrate and temperatures rising (16-28°C) over four hours. Heptageniidae were the worst candidates as they are physically fragile. Freshwater limpets of the genus *Burnupia* showed the best survival rate.

Trials indicated that both raceways and bubblepots provided adequate holding/maintenance containers provided that sufficient oxygen, food and correct substrate is provided. Temperatures should not rise rapidly to above 25°C.

Table 2.2.3 Results of all screening trials summarized.

Poor = <20%; Moderate 20-40%; Good > 40% ; Excellent = 60% survival over 45 days. Container code: BP = bubblepot; RW = raceway; C = channel. Feed code:D = detritus; A = algae; T = tetramin; L = leaves.

ORDER FAMILY	SPECIES	SURVIVAL	TEMP. °C	CONTAINER	FEED
TRICHOPTERA Hydropsychidae	*Cheumatopsyche thomasetti* & *C. afra*	good	19 - 23	RW & BP	D & T
Philopotamidae	*Chimara* sp.	poor	19 - 24	RW & BP	D & T
Leptoceridae	*Leptoceris* sp. & *Oecetis* sp		19 - 24	RW & BP	D & T
EPHEMEROPTERA Leptophlebiidae	*Choroterpes* spp *Adenophlebia auriculata*	good excellent	19 - 24 15 - 25	RW & BP BP+ RW & C	T, D & A T, D, L, A
Baetidae	*Centroptilum*,sp *Afroptilum*,sp *Pseudocloeon*,sp *Beatis* spp.	good (not separately screened)	14 - 23	BP & RW	D & T
Heptageniidae	*Afronurus* , *sp* *Compsonuriella* spp.	poor	14 - 25	BP	D & T
Trichorythidae	*Trichorythus sp* *Neuroceanis* spp.	excellent (not screened separately)	14 - 24	RW & BP	
Caenidae	*Austroceanis* sp.	moderate	14 - 24	BP	D & T
COLEOPTERA Gyrinidae		poor			
Elmidae		poor			
Dryopidae		poor			
Psephenidae		good	19	RW	A
MOLLUSCA Ancylidae	*Burnupia stenochorias*	excellent	14-25	RW & BP	A
PLANARIA		good	15-23	BP	D & T

Species selected for further investigation.

Once these trials had been concluded the genera that emerged as candidates for further investigation were:

- Trichorythidae and Leptophlebiidae.
  - *Trichorythus.* Although a strong candidate, accessibility is a problem as there are no local stocks. It would be better to perfect the technique of invertebrate aquaculture before investigating this genus. The taxonomy is also unclear.
  - *Choroterpes* A good candidate, reasonably accessible (Buffalo river, eastern Cape) with wide distribution, of good size, easy to feed and fairly robust, but fairly tolerant as it occurs in lower reaches of rivers. Its temperature range possibly higher than taxa from upland rivers. Taxonomy would be easy to establish.
  - *Adenophlebia* This is an excellent candidate, which is easily available, of good size and easy to feed. It is found in numerous upland rivers with good water quality so is quite sensitive and widely distributed. Robust with careful handling. Taxonomy is reliable and there are two species in the genus. However the breeding biology is obscure.
- Baetidae. Suitable for field collection and use in experiments but the identification is difficult and the nymphs are too small.
- Psephenidae. Good candidate but sparsely distributed and nothing is known about the biology other than that adults do not fly to mate.
- Trichoptera have been kept in laboratory by other researchers and the taxonomy good.
  - *Cheumatopsyche sp.* A good candidate on survival ratings but there may be problems with feeding, keeping pupae and the breeding biology is obscure. In this case we also feel that it would be worth investigating once the techniques are established.
- Ancylidae.
  - *Burnupia* An excellent candidate and easy to feed, robust if carefully handled and breeds easily in the laboratory. A better candidate than other gastropoda as it is more susceptible to external conditions due to the exposed foot. It has no aerial stage.
- Planaria. A good candidates and easy to keep as it is robust if rather small. It is widely distributed and always found in riffle collections. The taxonomy may be obscure. There has not been enough time to investigate experimentally. It may be difficult to observe in large experimental streams (Palmer *et al* 1995).

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CHAPTER 3

DEVELOPMENT OF METHODS

Aims:

(i) To investigate and develop methods for collecting live invertebrates from rivers and transporting them from the field to the laboratory and from the breeding/maintenance facility to the testing laboratory of the client.

(iii) To develop practical, versatile receptacles such as pots and channels in which to maintain, breed and move invertebrates at an acceptable cost.

3.1 COLLECTION OF INVERTEBRATES FROM NATURAL WATERS

3.1.1. HANDLING IN THE FIELD

Animals should not be handled during collection. If rocks are rinsed into the containers the least damage is inflicted. Where this is not possible the occupants of movable rocks must be washed into a fine-meshed (10μm) net held immediately downstream from a rock. The rock is lifted rapidly and placed either in the mouth of the net or washed into the collecting bucket. Water and substrate under the rock is then fanned through the net which is emptied into the bucket with extreme care. These nets should be fairly shallow, with a wide mouth. On the bank of the river the collection is then placed into a cooler box with chilled aerated water. Collections can be stored overnight in aerated cooler boxes with foam rubber pads, detritus and leaves from the collection site. The sorting of the undamaged specimens and measuring can then take place the following day. A variable acclimation period (2-5 days) may be necessary before the experimental conditions are instituted. The experimental containers should have a number of specimens excess to that needed to accommodate mortalities. Nevertheless reserves from the remainder of the collection should be kept in similar conditions to those of the experimental containers. The containers should be supplied with detritus and leaves to enable the animals to survive an acclimation period. As soon as the mortality rate stabilizes the animals can be measured and the numbers controlled for the start of the experiment. Animals which are more difficult to remove from rocks can be moved by a small firm paintbrush or the edge of an exposed, washed film. If possible, animals should be collected and transported on the same day.

Sessile limpets are easily damaged during manual removal from rocks. We have found that the technique of anaesthetizing animals in the field by using 2% solution of magnesium sulphate to be effective:

Method

1) Mix 25 litre 2% $MgSO_4$ in a large sturdy plastic bucket using river water.
2) Suspend a slightly smaller net bag in the bucket with a tie around the mouth of the bucket. Quite large rocks can then be completely submerged in the solution.
3) Submerge the sample rock in this solution for a period of 2-5 min. after which the limpets will be comatose.
4) Move the rock and the net bag to a container with river water and wash the animals off the rocks into the bottom of the net.
4) Place the collection in plastic bags filled with well oxygenated river water in insulated containers. The limpets usually recover in 5-10 minutes.

This method was developed by Mrs HI White in the Department of Ichthyology and Fisheries Science (Rhodes) for use in abalone farming. Preliminary investigations have indicated 95% survivorship for specimens collected in this manner. However, long term effects must be investigated experimentally before this could be adopted as a method of choice. A similar effect can be achieved with CO_2 bubbled through

water around the rock, but the logistics of transporting gas cylinders or dry ice have not been investigated.

Plastic bags and sheets can be used to line all the containers in which animals are reared or maintained and will facilitate moving collections without having to handle the specimens too frequently. This procedure has only been used in the last few months and has not been tested rigorously

3.2 TRANSPORT

Introduction

The handling and transport of small aquatic animals in large numbers without damage presents problems which centre around handling, buffeting by the water movement, temperature and oxygen supply. Ideally, cultured animals should be kept in the containers in which they are maintained until they reach the experimental facility, whereupon they have to be transferred with extreme care. Successful transport methods must conform to the following criteria.

Temperature

The temperature should be kept between 8°C and 15°C at all times if possible. A second insulated box with ice taken on collecting trips assists in keeping the collection cool. Animals should be placed in insulated containers immediately after being washed off rocks in the field.

Substrate

Benthic animals require perches but hard objects damage the animals during transport. Finely textured plastic foam appears suitable. Leaf detritus from the collection site also offers suitable perches.

Oxygenation

Oxygen levels should be maintained between 50 and 85% using portable air pumps.

Methods

1) Equip a cooler box with a battery driven aerator pump connected to an air-stone suspended from tubing running through a hole in the lid. Take a portable pump which can run off the cigarette lighter socket in a motor vehicle as backup.
2) For field collecting, commercially available insulated boxes have been modified in the following way. Line the collecting box with a plastic bag of appropriate size and place a thin, fairly rigid layer of foam rubber on the bottom. A deeper layer of foam slightly larger than the internal area of the box is placed on the surface. The bottom layer serves as perch for the animals and the top layer prevents excessive wave action of the water during travel.
3) Small cooler boxes, similarly modified will be useful for the transport of groups of like animals from the culture facility to the testing laboratory.

4) On arrival at the collection site, put river water in the cooler box and add about 250 ml of ice to lower the temperature. The cooler box acts as a store for the animals which are collected into a bucket in the river, and must remain closed. The aerator pump can be started as soon as the animals are added to the cooler box.

5) a) When limpets and snails are collected, a generous layer of leaf detritus from the site, added to the containers, will act as perches as well as facilitating removal from the container, and counting and measuring in the laboratory, without handling the animals.

b) An alternate perch was made for mayflies and stoneflies, consisting of a plastic frame, of the same interior dimension of the container, covered with net and suspended in the container. If water from the collecting buckets is carefully poured through the screen, mayflies find an immediate perch. This method also facilitates sorting.

3.3 MAINTENANCE

Introduction

Frutiger (1984) states that stream-dwelling organisms are physiologically dependent on current and that their behaviour may be seriously affected by being kept in lentic conditions. Consequently, laboratory investigations with running water animals should, in principle, only be carried out under conditions of flow.

Pontash & Cairns (1989) tested the responses of a community of riffle dwellers to four hydraulic conditions. Static and flow through systems with current maintained the largest diversity and numbers of specimens but certain species did well in all conditions. They showed that a microcosm of riffle insect communities can be maintained for up to 30 days with moderate current and minimal flow through with temperature and photoperiod nearly identical to ambient conditions. Adequate quality and quantity of available food proved a limiting factor, and a supplemental source of nutrition may be required during long-term experiments with large numbers. Algae, detritus and decaying organic matter form part of the diet of the macro-benthos. Therefore conditions suitable for the growth and reproduction of algae which form part of the diet of the species to be investigated must be provided (See section 3.3.4.2). Conditions suitable for algal cultivation is discussed in section 3.3.5.2 (Laboratory conditions).

3.3.1 DESCRIPTION OF HOLDING EQUIPMENT

3.3.1.1 Artificial Streams - Raceways

Although all the early trials were conducted in the 'PERSPEX' raceways designed by Ciborowski (1979) (Ch. 2 & 5) these proved unsuitable for long term, uninterrupted use. The channels leaked and the motors failed,

while mobile substrates smaller than 2cm jam the paddle wheel.

3.3.1.2 Artificial Streams - Planted Stream

A small stream in which stream macro- and microphytes were grown on sand, gravel and rocks was constructed in May 1993. This system was modified several times during its existence and tested the feasibility of constructing and maintaining a miniature stream ecosystem indoors. The maintenance of this stream for nearly 2 years demonstrated the importance of the correct light and temperature to the healthy growth of stream plants.

This system consisted of four flow-through pools and a return flow pool. Water was recirculated by submersible aquarium pumps with variable output. The stream substrate consisted of gravel overlaid with sand from the parent stream into which sedges and reeds from the parent stream was planted. These were: Cyperaceae; *Fuirena hirsuta* and *Scirpus proliber* and Juncaceae; *Juncus dregeanus*. Rocks with periphyton and algae from the parent stream were introduced.
Initially the stream was kept in a constant environment room (CER) with a photoperiod of 14 hrs, with an initial temperature regime of 15 °C night and 19 °C day. After a 12 week period during which only artificial light supplied by fluorescent tubes and incandescent globes was available, the plants were etiolated with brown marks on their leaves. Eight Lindner "Linodym" grow lights with additional red spectrum output were installed in the C.E. room which improved the plant condition. This brought the available light in the room up from 12 to 15 micromoles/m^2/sec quanta and from 20.4 to 25.6 PAR (photosynthetic assimilation rate). Pyronometer readings went up from 10.7 to 21.2 micromoles/m^2/sec quanta. The light intensity in the room varied from 13 to 69.0 micromoles/m^2/sec quanta, depending on where the SKYE ELE SKR 100 meter SKR 10 sensor was positioned. According to Mr. B. Sonnenberg of the Botany Department at Rhodes University (pers. comm.) a riverine thicket at midday usually has between 50 to 70 micromoles/m^2/sec quanta. Periphyton and algae cultivated on rocks and artificial substrate thrive under these conditions.

This stream was moved to a west facing laboratory with natural light where it functioned very well.
but was dismantled due to space constraints in March 1995.

3.3.1.3 Artificial Streams - Recirculating Channels

Recirculating systems (Fig 3.3.2.1) consist of a plastic or PVC channel through which water is pumped from a sump by submersible aquarium pumps via a horizontal spreader. Ovoid kaolin stone and small square tiles are placed in the channels for substrates. The sumps are plastic bins varying in size from 5 to 85 litre (Figure 3.3.2.1 a & b). Two scales of channels were constructed. The larger channels consist of 250 mm heavy duty PVC piping which are halved lengthwise and stoppered at both ends. These rest on metal adjustable stands. The smaller channels are 0.5 m lengths of square profile white guttering (Marley) stoppered at both ends.

A variety of makes of submersible aquarium pumps have been tested (Natura, Idra, Aquaclear and Reno). The head required and the pump setting will vary the output, but their range is between 500 and 1000 litres/hour^2. Flow rate can be varied by changing the slope of each channel, by altering the delivery rate of the pumps or by a control valve set into the pipe from the sump.

The small channels with individual 5 litre buckets as sumps are used as replicated experimental systems. The large channels are holding and growing systems for experimental animals and the cultivation of algae and periphyton. These systems are housed in a laboratory with natural light conditions as well as daylight tubes. During the course of the project the experimental systems have been housed in three different localities of which a north west facing laboratory with large windows provided the best growing conditions for algae.

Flow through pots

Individual mayflies sometimes had to be isolated in flowing water for various tests and containers which allowed water to flow through them but kept the mayfly isolated were constructed. The flow-through pots were made from transparent plastic 500ml bottles (similar to those used for the small bubblepots) with mesh windows inserted on opposing sides. Three mesh sizes (2.0mm, 1.5mm & 1.0mm) were chosen to confine animals of different dimensions. The mesh size must be considered when the animals to be placed the pot is selected other wise they escape. In situations where the water has a high suspension load the mesh can clog up very quickly.

Problems encountered with these systems to date include;

a) difficulty in the maintenance while in use.

b) no easy method to allow trapping of adult insects on emergence.

3.3.1.4 Static Water - Bubblepots

Bubblepots are cylindrical opaque plastic buckets with straight or sloping sides and lids, equipped with aerators. A variety of sizes of containers were tested but the most often 500ml bottles, 5 litre buckets and 50cm diameter plastic basins were used. These containers are used in a variety of experiments and the larger containers proved ideal for raising limpet hatchlings and maintaining leptophlebiid mayflies (Fig 3.3.1.1 a).

A small number of cylindrical containers were equipped with a central spiral baffle which created a directed current. These SPIRAL POTS were labour intensive to make and materials to manufacture the baffles were difficult to obtain locally. They were also difficult to clean and maintain while only a small number of animals could be kept in them. They were discarded as an impractical option.

3.3.2 SUBSTRATE PROVISION

Clifford *et al* (1989) tested the substrate preference of benthic macro-invertebrates in natural streams, between rough and smooth tiles as a place on which to perch. It was found that rough tiles were generally preferred to smooth tiles particularly by chironomids. However, Heptageniidae (mayfly) nymphs were found in consistently higher numbers on smooth tiles. This highlights the fact that not only are substrates essential but that they should be of the right quality.

Ovoid kaolin stones (6cm x 4cm x 4cm) have been manufactured to standardize the available food surface and to provide refugia (Fig 3.3.1.1 c). In addition, periphyton is cultivated on 8cm x 8cm unglazed tiles. These provide a more accurately measurable surface of available food, can be used in shallow water, and are practical for monitoring the growth of small invertebrates as they afford a uniformly flat surface for microscopic observation and measurement. Both these types of substrate are pale in colour to facilitate observation of the organisms.

3.3.3 FOOD PROVISION

3.3.3.1 Introduction

The provision of the correct feed is of paramount importance in establishing successful culture systems. Particle size, composition and presentation are all important factors to be considered. Benthic macro-invertebrates have been classified into several functional feeding groups, according to the methods in which food is assimilated (Cummins & Klug 1979). Each of these groups consume a specific type of food in a specific way and the presentation of the food can be as important as the content.

It has been amply illustrated that food quality can contribute significantly to growth rate and therefore to duration of life-cycle, size at maturity and fecundity (Anderson & Cummins 1979; Sweeney *et al* 1986). In the laboratory optimal growth in the shortest period for rearing and reproductive purposes is desirable, while growth retardation, once the correct size for testing purposes has been reached, to maintain adequate stock, is essential. Therefore feeding on optimal diet and the development of this diet often forms the largest portion of aquaculture research project. However the scope of this investigation does not allow for such elaborate investigation and it was therefore decided to test commercially formulated fish food for suitability. Tetramin is such a food and readily available and has been used by other workers to supplement the natural diet of insects. On the other hand it was felt that the cultivation of periphyton as a suitable general feed should not present too many difficulties, so we embarked on a trial and error periphyton growing project.

Food Preferences

Food generally is accepted as a major component of a population's environment and when a population reaches epidemic proportions, food shortages are likely to occur, particularly within our laboratory streams where there may be maximum densities and the problem may be one of quality and not quantity.

Limpets

Little is known about the ability of freshwater pulmonates to select from within the spectrum of algal foods available to them. Work by Calow (1973 b) shows very clearly that *Ancylus fluviatilis*, a close relative of *Burnupia*, which has the same method of rasping action in order to acquire its food, actively seeks out algae in preference to, although also consuming, lichen, detritus and fungi. Calow maintains that in the absence of epilithic algae, lichen may be used but it results in less rapid growth and must be considered as a less rich energy source. In 1973(a) he showed that the preferred algae were the diatoms. *Burnupia* is very likely a micro-herbivore grazer with the same preferences. Small sand grains (mineral particles) are potentially important as they allow greater trituration of food (Hunter 1980), of particular importance in the digestion of diatoms which Calow (1970) suggests are less efficiently assimilated than green filamentous algae due to their high ash content and protective exoskeleton. Eisenberg (1970) showed very clearly with *Lymnaea elodes* that the inverse relationships between snail density and mean size, mean number of eggs per egg mass, as well as total eggs were found to disappear with food additives. Food additives appear to also have improved the survivorship of the snails, and he presents evidence which indicates that the food limitation in this snail is one which probably involved accessory growth factors. In the case of *Burnupia* we have to ask the question - is the algal assemblage provided in the streams sufficient for maximum growth and fecundity?

Mayflies

Cummins & Klug (1979) found that animals that belong to a certain functional feeding group may be unable to digest food which does not form part of their preferred diet. For example, shredders lack an enzyme such as cellulase to digest complete polymers such as cellulose but the semi-digested or hydrolysed polymer may well be digestible. So conditioned or semi-composed leaves with a high microbial fauna are consumed faster than leaves less colonised and faster growth may result. Rosillon (1988) showed experimentally that growth responses of *Ephemerella ignita* to water temperature variations differed according to the content of the food available for that species. Work on the growth rates of the mayfly *Leptophlebia intermedia* on leaves from different trees has shown that the diet can influence adult size and fecundity but not growth rate (Sweeney *et al* 1986). It is therefore important that the diet offered should contain the right proportions of protein, carbohydrate and lipids in a digestible form but also of the correct physical formulation.

3.3.3.2 Periphyton

The multi-specific complex of diatoms, filamentous algae and bacteria which develops on the surface of stones probably forms the most generally acceptable food source for a range of functional feeding groups. It was decided to cultivate periphyton on artificial stone substrates in the channels as a basic food source to be supplemented for the specific needs of macroinvertebrates.

Three aspects concerning the growth of algae (and hence the basic source of food) in the laboratory streams should be considered:

(1) What species of algae do the limpets or mayflies prefer and which will increase growth to a maximum rate.

(2) What species of algae grow in the laboratory streams; how the chemical conditions of the water, the physical form of the substrates, and the light conditions all affect the growth.

(3) What is the interaction between the limpets and the algae, particularly the effect that the grazing action has on algal species composition and water conditions.

Laboratory Conditions

Periphyton distribution in streams is often a mosaic of assemblages on different successional trajectories with the degree of patchiness related to spatial and temporal variation in the environment (Fisher, 1983). Succession within a patch is bounded by the composition of the species pool and the physiological characteristics of the species, and it is influenced by the interaction of factors such as invasion rate, nutrients, light, temperature, current, substrate and herbivory (DeNicola and McIntire 1990). A review of the literature highlighted the complexity of the interaction between environmental variables such as current speed, light intensity, substrate quality and algal species in the colonisation of substrates.

(a) Lamberti *et al* (1989) studied the interactions between algal assemblages and herbivorous snails (*Juga silicula*) and found that a light intensity of 100μmol/m^2/s is ideal for laboratory stream algal growth and 250 snails/m biomass equivalent. Diatoms remain dominant in the system and grazing is sufficient to "control" filamentous and blue-green algae. In their experiment, this light intensity is not high enough (compared to 400μmol/m^2/s) to produce rapid algal growth, causing diatoms to decrease in number. They found that at intensities of less than 100μmol/m^2/s the grazing impact was too great for algal growth to be sustained. Lamberti *et al* (1989) also found that high light intensity gave high algal growth and consequent sloughing of algae to form detritus. This would contribute to changes in the chemical nature of the water as these algae break down, possibly to the detriment of the stream system. Water should then be changed regularly for this reason alone. It appears then that the light intensities recorded in the CER and the laboratory which ranged between 13 and 69μmol/m^2/s are inadequate for good growth.

In a Leptophlebiidae experiment conducted in our laboratory, it was found insufficient change of water caused the rapid build-up of sewage fungus, an indicator of high organic nutrients, which in turn rapidly increased in volume and presumably consumed large amounts of the dissolved oxygen.

(b) DeNicola and McIntire (1990) examined the effect of water flow on the algal assemblage in re-circulating laboratory streams, comparing the colonization of algae on both stones and unglazed clay tiles. At flow rates of 2 litres/min periphyton accumulation was greater on recessed regions behind substrates than on the tops. The higher water velocities above the substrates increased the rate at which algal cells flowed over them, but the relatively unidirectional flow and higher shear velocities

may have inhibited cell attachment. In contrast, the zone behind the substrates had multidirectional flow with lower colonization of algal cells (Munteanu and Maly 1981, Stevenson 1983). McIntire (1966), and Steinman and McIntire (1986) both observed a higher accumulation of biomass in faster currents (38cm/s) than in slower (9cm/s) currents. Studies which show that current flow enhances nutrient uptake, respiration, photosynthesis and growth in algae (Whitford, 1960) support the hypothesis that biomass accumulation should be higher in faster current regimes after initial establishment. Results by DeNicola and McIntire (1990) also showed that assemblages in the different current regimes became more similar over time. The major difference in the species was in the greater abundance of *Stigeoclonium tenue*, a member of the Chlorophyceae (green filamentous) family, on recessed substrates towards the end of the experiment. This genus appears to be dominant in our own laboratory streams (Ms. Josca, Botany Dept, University of Cape Town, 1994).

McIntire (1966) found green filamentous algae increased biomass much faster in slow currents (2 l/min). Faster currents showed a more felt-like dense growth on the substrate, dark green or brownish in colour, with a predominance of diatoms. Whitford and Schumaker (1964) (in McIntire 1966) demonstrated that lotic and lentic species of *Oedogonium* and *Spirogyra* have higher rates of respiration and phosphorus uptake when subjected to a current velocity of 15cm/s than in still water, which led to a greater growth rate.

Steinman and McIntire (1986), in comparing both light (450 and 50$\mu E/m^2/s$ and current velocity (5cm/s and 15cm/s) in laboratory streams, found the seven most commonly found diatoms exhibited different patterns of response to the experimental conditions: *Synedra ulna* and *Fragilaria vaucheriae* had relatively high mean biomasses at the higher light level and lower current velocity; *S. rumpens* var. *familiaris* and *Nitzschia oregona* had greater mean biomasses at both the hight light level and higher current velocity; the mean biomasses of *Nitzschia dissipata* had a negative relationship with light energy and a positive relationship with current velocity; *Nitzschia linearis* had greater biomasses at the lower current velocity while *Achanthes lanceolata* had lower mean biomasses at the lower current velocity. At the end of the experiment (32 days), *Stigeoclonium tenue* formed an extensive reticulation on tiles from the higher photon flux density. In contrast, algal assemblages from the lower photon flux density retained a dense understorey of *Achanthes lanceolata* and an upper layer of larger diatoms. However several species of *Nitzchia* were indifferent to treatment effects, suggesting other factors were important in influencing structure.

Decisions as to how best to provide food for the test species on the basis of light incidence and current velocity, are therefore almost impossible until further studies on both the exact food requirements and the responses of diatoms which can be identified from the species habitat have been completed.

(c) The following literature was helpful in making the decision as to whether clays tiles or artificial stones would be more suitable for the growth of *Burnupia:* Lamberti and Resh (1985) compared introduced tiles and natural substrates for sampling algae and found least variability between tiles.

DeNicola and McIntire (1990) showed the differences between tiles and stones which affected in their colonization by, and the subsequent growth of algae to be the degree of roughness. Colonization began with small diatoms, then the large diatoms, blue-greens, ciliates, and finally green filamentous, with the slower currents encouraging green filamentous on the stones in particular. This latter work clearly indicates that tiles are a more suitable substrate for algal growth for the limpets than artificial stones.

Tuchman and Stevenson (1980), when comparing diatoms communities on clay tile, sterilized rock and natural substrate in a small stream, found clay tiles yielded the least variability between replicate samples. For trial purposes with *Burnupia* this is the ideal means of providing food.

The application of the above mentioned background information, (sections (b) and (c)) to the maintenance and breeding of suitable test species must be investigated experimentally.

In the initial phase of the project the algal colonisation within the recirculating systems was initiated by stones and water brought in from the field collecting sites. A few days elapsed before significant colonisation of the clean artificial stones occurred. The substrates are maintained in conditioned tap water in the recirculating systems.

Initially nutrient enrichment in the form of the mixture given in **section 3.3.6** was used to encourage algal growth to ensure the algae would not be a limiting factor in any of the experiments. However, we later found investigations by Marks and Lowe (1989) confirmed our observations that nutrient enrichment, particularly nitrogen and phosphorus, causes a shift in the algal composition from diatoms to less useable filamentous green algae over a 28 day period. Without any herbivorous activity in the periphyton cultivation streams, experience has shown it necessary to continually scrub the substrates to decrease the amount of filamentous algae.

When used as a food source for *Adenophlebia* and in the initial growth of *Burnupia* the periphyton stones are placed in bubblepots. Aeration provides a high percentage of dissolved oxygen, required by these species and presumably sufficient for the algae. In later stages of growth we envisage the limpets will be transferred to the laboratory streams where they will complete their development, and from where many will be transferred to the ecotoxicological work. Future work on the algal assemblages will be necessary, monitoring the species present through each of these above stages.

Effects of Grazing on the Algae and Water Conditions

DeNicola *et al* (1990) compared substrates grazed by the snail *Juga silicula* with ungrazed substrates ($500/m^2$) in laboratory streams, found in ungrazed streams after, 40 days, the algal assemblage consisted of a thick mat of diatoms and the chlorophyte *Scenedesmus obliquus*, with an overstorey of filaments of the chlorophyte *Stigeoclonium tenue*. In general, introductions of grazers at any stage altered this pattern by

removing biomass, accelerating the replacement of *S. obliquus* by diatoms, and suppressing the growth of filaments. Grazing also reduced the relative abundance of the larger diatom *Nitzschia oregona* but increased the relative abundance of the smaller adnate diatoms *Nitzschia frustulum* var. *perpusilla* and *Navicula minimum*. These results give and indication of the feeding preferences of the snail.

Hunter (1980) evaluated the effects of *Lymnaea, Mysa*, and *Helisoma* on periphyton on tiles in a pond, and found these species were essentially non-selective within their "normal" range of food dimensions (medium to large size diatoms) with massive reductions in living cells, detritus and particulate inorganic matter alike. This had the effect of reducing the quantity of periphyton by reduced dry weight, carbon and chlorophyll *a* per dm^3. In contrast, grazing increased aufwuchs quality as indicated by increased chlorophyll *a* and nitrogen per mg dry weight and by decreased C:N ratio. Grazing at this density (216 snails/m^2) reduced both the abundance and diversity of the attached community from 80 889 individuals/cm^2 and 24 taxa on the controls to 501 individuals/cm^2 and 8 taxa on grazed substrates.

Marks and Lowe (1989), in their work on the interactive effects of snail grazing and nutrient enrichment on periphyton communities, found that nutrient enrichment had a much greater effect on altering community structures than did grazing. Although the snail ratio per unit area was smaller than we would expect to see with *Burnupia*, grazers had little effect on diversity and relative abundance of major classes in low-nutrient communities, although they did alter the species composition. In nutrient-rich communities grazing decreased diversity and caused a shift in relative abundance of the major classes, and encourages the presence of *Stigeoclonium tenue* a filamentous algae and therefor not a food item of choice for grazing snails.

The chemical conditioning of the water by the limpets may affect the growth of the periphyton: they remove ions, amino acids, and oxygen and add ammonia, carbon dioxide, ions, mucopolysaccharides and organic acids. Their grazing action is particularly important in causing plant factors to be released into the medium, organic acids in particular which will acidify the water medium. Thomas, Goldsworthy and Benjamin (1974) showed this very clearly with *Biomphalaria glabrata.*

Ms.P. Josca (University of Cape Town, 1994) confirmed the extreme diversity of the periphyton which had been cultivated in our laboratory streams, including numerous species of pennate diatoms, desmids, cyanophyte and unicellular and filamentous Chlorophytes.

3.3.3.3. <u>Feed Other Than Periphyton</u>.

Choroterpes elegans and *Adenophlebia auriculata* are both classed as brusher collectors (Palmer *et al*, 1993) and therefore have fairly omnivorous feeding requirements. In the field these nymphs are most frequently found under rocks where fine sediment collects and in large leafpacks. It was therefore decided to test ground Tetramin as a suitable food source and offer this in addition to periphyton as an alternative to detritus. When nymphs are kept in large bubblepots, leaf packs are always brought back from the field as a supplement to periphyton. Containers of autumn leaves matured in water for 2-3 weeks have been

documented as suitable feed for a number of mayfly species (Sweeney *et al* 1986). See (Ch.5.2.4) for results.

3.3.4 WATER COMPOSITION AND HYGIENE

Although water from the river of origin of the animals to be investigated is the logical medium for use in transferring the populations from the field to the laboratory and for maintaining populations and conducting experiments, transporting large volumes of water on a regular basis presents problems. When considering a laboratory source of water, we have found tap water allowed better algal growth than distilled water. Total use of dechlorinated tap water, while readily available, may not be equally suitable for non-filamentous algal growth which is the food source in the majority of experiments. In other centres, micro-elements have been added to water to obtain better results in the cultivation of plants and animals. The addition of nitrogen and phosphorus to agar plates dispersed in New Zealand streams substantially increased the algal growth taking place on them (Winterbourn, 1990).

The two sets of chemical additives listed below are considered good nutrient for algal cultivation (pers. com. M. Logie; Microbiology Dept., Rhodes University). Those chemicals listed on the right are made up into a one litre stock solution:

NaHCO 4,20 g/litre	FeCl 0,2mg/l
KNO 0,51 g/l	EDTA 1,8mg/l
MgSO 1,23 g/l	H BO 14,0mg/l
CaCl 0,044 g/l	MgCl 0,8mg/l
KH PO 0,027 g/l	ZnCl 0.1mg/l
	CoCl 0.002mg/l
	CaCl 0.02mg/l

The stock solution was used in our laboratory streams at a rate of 1ml per litre of water, and all other chemicals were used at a dilution of 15% of the volume of tap water. However, both the expense of the nutrients, and the density and type of algae (filamentous Chlorophytes) which grew the most rapidly necessitated discontinuing their use.

Stein (1973) suggests water should be changed every two to three weeks when used to culture both algae and limpets because:

(a) CO_2 is depleted by the algae.

(b) the submersible pumps produce ozone, lethal to the algae.

She reported that the use of black, white, and clear polyethylene material is safe for the culturing of freshwater algae, with no significant leaching of elements into the water.

Water was monitored daily or weekly in the laboratory streams by electronic CIBA CORNING probes which measure temperature, Ph, conductivity and dissolved oxygen (DO). Nutrient analysis has to date not been done on a regular basis as the expertise to do the analyses has not been developed, although the facilities are available.

The necessary chemical composition and balance of water for the growth of algae and invertebrates need to be investigated further by consultation with Prof. Braam Pieterse (Potchefstroom Univeristeit) and some experimentation.

3.3.5 DENSITY

Chernin and Michelson (1957 a & b) studied the effects of population density on *Australorbis (Planorbis) glabratus*, and found that snails maintained under crowded conditions grew more slowly and were less fecund than those in less densely populated aquaria. Wright (1960) showed that there is no single factor which can be held responsible for diminished performance under high densities and suggested three factors may be involved: (1) food, (2) collisions, and (3) chemical pollution. DeNicola et al (1990) demonstrated that grazing by stream invertebrates has an impact on both periphytic species composition and abundance, especially in laboratory streams.

In developing management strategies the effect of density in aqualcultural situations is of great economic interest. Density effects on limpets were investigated and the results are reported in Chapter 4.2.2.2

3.3.6 REQUIREMENTS FOR BREEDING

The art of breeding animals in captivity rests on providing the correct cues in terms of photoperiod and temperature as well as understanding the mating behaviour. Unless the laboratory animals are reared with optimal nutrition, fecundity will be lowered. Care must also be taken to maintain a broadly based gene pool with the regular importation of wild parent stock (De Kock and van Eeden 1981). To date the breeding behaviour of the limpet has been observed (Chapter 4.2.3). The effects of programmed circadian temperature fluctuations on the population dynamics of Burnupia will have to be considered (Appleton *(et al)* De Kock and van Eeden (1986) found that egg production, hatching rate, survival and growth rates were all positively influenced by daily temperature fluctuations. Photoperiod will also have to be considered in its influence on the limpets. The causes and methods to prevent the high mortalities must be investigated. Periphyton seems to provide adequate nutrition but species composition will be investigated to a greater degree.

The successful captive breeding of mayflies has eluded all workers. It was decided to resort to the time honoured piscicultural practice of artificial fertilisation with which we have had limited success. The technique has not been perfected and will need further investigation. Several authors have reported similar experiences (Chapter 5.4). With regard to mayfly hatchlings, sufficient numbers of new hatchlings have never

been available for experimental investigation but the smallest (0.2 -.4 mm head-width) field caught nymphs have all survived well on a diet of decayed leaves and ground Tetramin added to periphyton (Ch.5.2.).

3.3.7 EXPERIMENTAL INVESTIGATION OF EQUIPMENT

(see Appendix 3 for experimental detail)

Five experiments were conducted to determine conditions within the containers and to explore optimal maintenance conditions. The experimental results are reported here under the numbers assigned in Appendix 3.

a) EXPERIMENT 3.1.1.1: One air-stone produces the same level of dissolved oxygen to all volumes of water while temperature and dissolved oxygen are negatively correlated.

b) EXPERIMENT 3.1.1.2: When testing the effect of rising temperature on the survival rate of Choroterpes sp. (Leptophlebiidae) in different volumes of water, it was found that rising temperature depressed levels of dissolved oxygen and mortalities occurred when the DO reached 30 %, after the temperature had peaked at 25°C from 11 hours. In 500ml of water, mortality onset was delayed until 32 hours at which stage in all the other conditions 80 % mortality had occurred.

c) SUBSTRATES; EXPERIMENTS 3.1.2.1 & 2: A variety of materials were tested for suitability as substrates for mayflies. Foamrubber pads, kaolin artificial stones and plastic mosquito netting were tested on *Choroterpes spp.* The survival rate with foam-pads and stones was consistently higher than that with netting, which was as poor as that in the controls where no substrate was supplied. This was true for both running and standing water. Each time a container was inspected all the live specimens were found under the substrate, suggesting that a refuge from light and turbulence is needed. Observations from other experiments, (not conducted to test substrates) confirmed this finding (Ch. 5.2.3).

The method in which the animals were transported from the Kruger National Park ensured survival in excess of a month in the experimental conditions tested. Expected residence time of nymphs in the laboratory can be extrapolated from these results.

d) HYDRAULIC CONDITIONS Although the species selected for experimental investigation are all found in riffles and runs in rivers, they may not be obligate rheophyllic. Conclusions from five of the experiments which were conducted to determine the responses of the two selected species to variation in hydraulic conditions are reported here.

EXPERIMENT 3.1.3.1. The growth rates of *B. stenochorias* in flowing and bubbled water was compared using dechlorinated tap water. In the experimental channels some unknown factor caused early mortality of the limpets and no results for comparison with growth in the pots were available.

EXPERIMENT 3.1.3.2, A & B: Two similar experiment was conducted on *Adenophlebia auriculata* nymphs. The condition in the pots and the channels are not only hydraulic different, resulting in diffrences in the shear forces exerted on the nymphs (excacerbated by insufficient refugia) but the temperature regime in the pots is 2-3 °C lower than in the channels due to evaporative cooling.

3.1.3.2.A: In the first experiment three kaolin stones were provided in both the channels and the bubblepots. The follwing differences were observed;

a) adult emerged sooner earlier in the relatively warmer channels.

b) a rapid decline in numbers in the channels between days 15 and 26 due to both mortalities and emergences. There was no significant difference between the replicates in either the channels or the pots (ANOVA F ratio 0.097, $p>0.05$ for channels and F ratio 1,29 $p=0.2884$) for pots) although the variation in the survival rates in the pots is greater. It is quite clear from Fig 3.1.3.2 that the variation in the sample number in bubblepots is much larger that from those in the channels which could be ascribed to high survival rate (58% and 35%) in two pots.

c) there was a significant difference in the survival rates between channels and bubblepots (ANOVA f ratio 6.7 and $p < 0.05$.) and a multiple range test confirmed the difference.

3.1.3.2.B: In the second experiment the channels contained 6 substrates were tested against 5 litre bubblepots with the same number of substrates. The survival period recorded in this experiment was much higher than in A. Firstly the nymphs survived for at least 7 week in both conditions but longer in bubblepots. Of the 68 nymphs which were placed in each set of replicates at the start of the experiment, 54% died in bubblepots and 69% in channels. The early decline of numbers in the channels was due to both the emergence of adults and mortalities. In Fig. 3.1.1.5 (Appendix 3) altogether 20% of the nymphs emerged from the channels and 18% from the pots. The difference in the survival rates between channels and bubblepots was significant (f ratio 6.7 and $p < 0.05$.).

A. auriculata did not survive well in channels with three stones. A mean of 33% and 32% of the original sample survived three weeks in the CER and laboratory respectively. The low survival rate of nymphs in channels in the laboratory could be due to two reasons. The flow rate in the system may be too rapid or there may have been too few stones in the channels. In the Palmiet River the nymphs favour the rocky edges of the stream out of direct current where coarse grained sediment collects under rocks as substratum i.e. that they inhabit low flow areas out of direct current while few smaller nymphs are found in moderate currents. The smooth plastic of the channels and angle caused a fairly strong current to flowed over a few smooth rocks which most probably did not offer sufficient refuge or foothold for the nymphs. This may have caused large energy expenditure to keep positions.

It can be concluded from the above two trials (APPENDIX 3: 3.1.1.4 A & B) that bubblepots are the optimal rearing container for *A. auriculata* and that they are not obligately rheophilous.

e) DIET EXPERIMENT 3.1.1.5: The suitability of commercial fish food as diet for *A. auriculata* was tested by monitoring survival over a 12 week period. Suspensions of ground TETRAMIN powder and river detritus was offered as food. The survival rate of *A. auriculata* was the best on Tetramin when 50% survived for five weeks, 30% survived for six weeks and 10% of the population survived for eight weeks. Detritus on the other hand sustained 50% of the population for only 2-3 weeks with a more rapid decline in numbers thereafter and all perished after 8-9 weeks. However again it must be pointed out that some losses was due to emergences.

3.3.8 SUMMARY

In conclusion, the three species investigated so far can be adequately housed and fed prior to being made available for experimental purposes. The fact that neither species was obligately rheophilic makes the prospect of rearing small animals to a size suitable for experimental purposes much less intimidating. Bubblepots are easy and cheap to set up, and more easily managed in terms of water quality and food provision. If the pot is lined with a plastic bag before limpet eggs are introduced, it will be easier to move these to channels once they have reached a size larger than 1.5 mm.

Although several groups of limpets and mayflies has been fed on the periphyton cultivated in the laboratory, it is not yet certain that we have achieved optimal growth under experimental conditions. Therefor indications drawn from a literature review that:

a) Light intensity of 100μmol/m^2/s is ideal for laboratory stream algal growth, and at less than 100μmol/m^2/s the grazing impact was too great for algal growth be sustained and
b) Current speed can significantly influence both the species and the rate at which diatoms colonise substrates, will have to be investigated once the dietary preference of *Burnupia* is determined.

Chemical conditioning of the water by the limpets may affect the growth of the periphyton: they remove ions, amino acids, and oxygen and add ammonia, carbon dioxide, ions, mucopolysaccharides and organic acids. Their grazing action is particularly important in causing plant factors to be released into the medium, organic acids in particular which will acidify the water medium. Thomas, Goldsworthy and Benjamin (1974) showed this very clearly with *Biomphalaria glabrata.*

The importance of the correct conditions in which to house a facility in which natural food can be grown was demonstrated to us by the variety of laboratories in which conditions varied considerably which we have had to occupy during the project period. That in itself proved an test of certain type. In our experience to date

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the most important conditions for the successful maintenance and breeding of invertebrates are:

1. Correct light conditions for algal growth.
2. A method to exercise thermal regulation.
3. A supply of good quality water, preferably from a non municipal source with the ability to regulate its quality and condition.
4. A manager who is meticulous, aware of the necessity of hygiene yet sensitive to the needs of invertebrates.

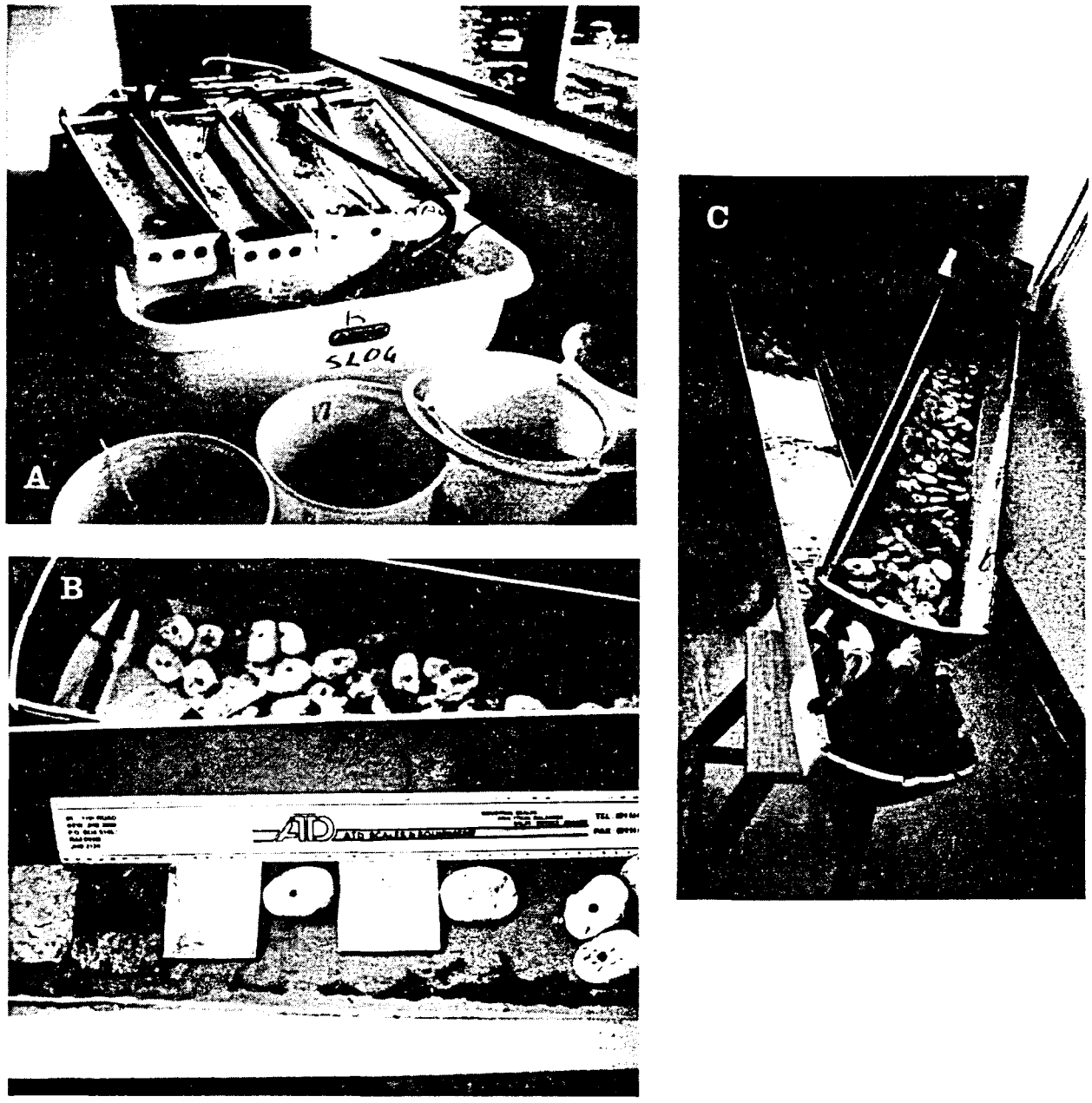

Fig. 3.3.1.1. a) Bubblepots and small recirculating streams. b) Artificial kaolin stones and unglazed tiles for perches and the cultivation of periphyton. c) Large recirculating stream with artificial stones.

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CHAPTER 4

INVESTIGATIONS OF THE LIMPET *BURNUPIA STENOCHORIAS* (MELVILL & PONSONBY).

CONTENTS

4.1 BACKGROUND TO *BURNUPIA STENOCHORIAS* (Pulmonata; Basommatophora)

The freshwater limpet *Burnupia stenochorias* proved to be an excellent survivor in the initial trials to screen riffle and run inhabitants for laboratory culture. It was therefore decided the life history of this species should be investigated and in doing so, attempt to establish optimal conditions for its laboratory culture.

The fresh water pulmonates make up a large proportion of the benthic animal biomass of the margins of larger lakes and rivers, but are particularly well adapted and successful in smaller, more variable aquatic habitats such as ponds, marshes, streams and ditches (Russell-Hunter, 1978). The Ancylidae are worldwide in distribution, comprising 7 genera (Hubendick, 1964), three of which occur in Africa; *Ferrissia* which is cosmopolitan, *Ancylus* with a mainly palaearctic range and *Burnupia* which is restricted to Africa (Brown, 1980). The genus *Burnupia* is found mainly in rivers but also on vegetation in pools and lakes. There are nine nominal species in southern Africa, and Brown (1967,1980) gives some indication of the distribution patterns, and the general systematic state of the genus. A revision of the genus (states Brown 1967) would reveal cases of synonymy among the large number of nominal forms recognised by Walker (1923) and Connolly (1939), and consequently one has to proceed with caution in establishing the identity of any population being investigated.

These limpets seem to have an especially high requirement for oxygen, as they are almost entirely confined to small, stony streams and the shores of lakes which are exposed to considerable wave-action (Brown 1980). Although easily overlooked, members of the Ancylidae are present in most freshwater habitats, those with clean, well oxygenated water being favourable for species of *Burnupia*, while the members of *Ferrissia* are characteristic of stagnant waters (Brown, 1980).

The ancylids have shells analogous to those found in the Capulidae, in that the shell is not conical but is slightly coiled, and there is no metamorphosis of the shell in growth (Russell-Hunter, 1953). The shell of *Burnupia* can reach lengths of up to 10mm, with the apex usually prominent, turned to the right and with rows of radial pits (Brown, 1980). The edge of the shell consists of relatively flexible uncalcified material which enables it to conform to the irregularities of the surfaces to which these limpets attach themselves. Unlike *Patella* and other marine littoral species, there is no "homing" in freshwater limpets and consequently, instead of the rock surface at one particular place becoming shaped to fit the limpet's shell, the shell must be flexible enough to become shaped to the rock surface of many different resting-places (Russell-Hunter, 1953).

The ancylid fresh water limpets represent the most advanced fresh water basommatophorans. They have lost nearly all traces of the pulmonary cavity and, to an even greater degree than the closely related

Planorbidae, have developed well vascularized, extensive neomorphic gills from evaginations of rectal mantle lobes between the foot and the roof of the mantle on the left side of the body. These gills provide an extensive surface area for aquatic gas exchange, allowing the respiration of fresh water limpets to be entirely cutaneous and therefore totally aquatic (Basch, 1963; Hubendick, 1964, 1970; Purchon, 1977; Russell-Hunter, 1978).

In the southern African sub-continent no investigations into the biology of the Ancylidae have been conducted, with one exception: Oberholzer (1963) reported the morphology and histology of *Burnupia mooiensis*. The closest relatives of the *Burnupia* spp which have been extensively investigated are within the genus *Ancylus*, in particular *Ancylus fluviatilis* which occurs widely in Europe and North Africa in lotic freshwater environments. It appears to have many similarities of life history style to *B. stenochorias*. Eggs are laid in capsules of a similar design and in similar numbers (1-13). Roughly 10 to 20 egg capsules are deposited by each individual over the entire life span. The shell sizes attained by populations fall in similar ranges to those measured to date for *B. stenochorias*. Hatched size is between 0.80-1.00 mm shell length with average adult size not exceeding 6.00-8.00 mm. Eggs are laid at night and under stones as has been observed in *B. stenochorias* (Hubendick, 1970; Russell Hunter, 1953; 1961a & 1961b; Geldiay, 1956; Lamberet, 1966). *Ancylus fluviatilis* feeds on periphyton, mostly diatoms and green algae, on smooth stone and rock surfaces. Locomotion takes place on top of the stones and resting periods underneath (Schwenk & Schwoerbel, 1973; Streit, 1981).

Ecology

Although *B. stenochorias* is an inhabitant of streams and is found largely in riffles and runs it has been collected in some pool habitats where very slow flow has been recorded. Appleton (1978) discussed the range of abiotic factors which may limit the distribution of Planorbid snails and states that while qualitatively certain abiotic factors may not be limiting to distribution, quantitatively they may well have some limiting influence. Of these, temperature and current velocity seem to have the largest influence while ionic composition does not seem to have a limiting effect on distribution but rather on levels of abundance.

Current velocity has a marked effect on both *Bulinus* sp. and *Biomphalaria pfeifferei* in that they seldom occur in velocities exceeding 0.3 m/sec. Temperature is the most important limiting factor in standing waters and current velocity in flowing systems where it is allied to rock hardness or erodability. In British streams, calcium ion concentrations of below 3 and 5 mg/l proved to be limiting to the distribution of *Lymnaea pereger* and *Planorbis albus*. High magnesium content on the other hand severely limited egg production of *Biomphalaria* under laboratory conditions. Many such magnesium rich waters occur in S.A. especially in Swaziland and Eastern Transvaal areas. Snails appear to be most abundant in harder waters above approximately 30mg/l of calcium ions. The conditions in local rivers should be correlated to the distribution of *Burnupia spp.*

Population Control and Breeding Strategy

Aldridge (1982) reports on extensive research on density dependant control mechanisms in freshwater snails. Many different mechanisms have been implicated including trophic limitations (van der Steen, 1967; Eisenberg, 1970; Mooij-Vogelaar et al, 1973), pheromone-like substances (Berrie & Visser, 1963; Berrie 1968) metabolite accumulation in the environment (Hiruta & Kawabata 1954) and the frequency of tactile contact between snails (Chernin & Michealson, 1957; Berrie, 1968; Eversole, 1974). In *Heliosoma* increasing adult density had a more marked effect on fecundity than egg density (Eversole, 1974). In *Leptoxis* density of eggs, not adults, affects fecundity and there appears to be a lag time between early egg laying when egg density has no effect and later when *Leptoxis* females respond negatively to high egg density ± 1 week prior to the hatching of the earliest eggs. This suggests the possibility of a mechanism that allows females to track and compensate for variation in egg mortality.
Russell-Hunter (1953) observed in his study on *A. fluviatilis*, that from mid-April to mid-August 10% of adults survived and less than 1% survived to mid-September.

It has been reported by several authors that freshwater pulmonates show remarkable phenotypic plasticity and variation in life cycle patterns. *L. palustris* can have a simple one generation per year pattern with incomplete replacement to a two generation overwintering pattern and a pattern intermediate between these. The degree of plasticity can be correlated to the degree of specialisation with specialised species like *Ferrisa rivularis* showing inter-populational variation on the same scale as *L. palustris* shows on the intra-populational level.

Bondesen (1950) showed that *A. fluviatilis* in Europe produces very large eggs in comparison to other fresh water gastropods of similar size, and he suggested that this contributes to the survival of young under unfavourable conditions. With regard to growth as observed in the field and laboratory it is noteworthy that different embryos within a single capsule may show marked differences in growth rate and these differences may continue long after hatching (Prinsloo & Van Eeden, 1973). This fact can be explored further.

Temperature

Three North American planorbid families have an optimal temperature range of between 24°C-26°C. At higher temperatures growth is faster but egg production is limited. *Lymnaea stagnalis* shows an optimal range around 22°C. Temperature appears to affect the gonad development through impaired tissue development. It could be advantageous to support early growth in elevated thermal conditions and to move the populations to lower temperatures at late juvenile stages. Daily warming and cooling rates have been held to provide stimuli responsible for the observed nocturnal and activity patterns of several pulmonate snails of both aquatic and terrestrial habit.

Streit (1985) reported that assimilation and rates of feed, grazing rate and radular movement are directly

linked to temperature in *Ancylus fluviatilis*. Optimal temperatures for this species appear to lie between 13°C and 19°C. Reduction of the metabolic rate to a winter resting state in this species may occur anywhere between 5°C and 10°C depending on the ecological history of the population, those from colder areas having a lower switch trigger and undergoing complete rest. It is most likely to be in the area of temperature responses that *Burnupia* may show its biggest differences from *A. fluviatilis* because of the relatively elevated temperature regimes in which it has evolved in Africa.

Feeding and Growth

In the various habitats Ancylidae are found, they feed by rasping at rock surfaces with their radulae (Moss, 1980). *Lymnaea palustris* and *L. pereger* feed on aufwuchs with little ability to select diatoms in preference to filamentous algae. with food taking an average of 90 minutes to travel the length of the gut. It was found that radula stroke rate diminished with increasing food richness or density. When feeding on thin coatings of food the snails move forward faster than when feeding on thicker coatings of food. Under the latter circumstances the sideways sweeps of the head increase (Hunter, 1975). Streit (1985) found that low levels of periphyton on substrates were ignored by foraging adults; that feeding rates varied with temperature, with radular rate and area grazed increasing with temperature. However, he found that assimilation rate decreases at high temperatures due to increased metabolic demands. Juveniles have relatively larger teeth than adults which allows them to graze similar grazing areas and feed to adults.

Turner (1926) found that the standard length of *L. pereger* doubled every four weeks over the period of exponential growth which was confirmed by Calow (1981) who found that, in common with other freshwater snails, growth under laboratory conditions is sigmoid with an initial linear phase followed by an exponential phase, after which size increase decelerates to a steady state. As regards the influence of temperature, the optimal temperature for growth and fecundity was found to be between 16°C and 22°C with both growth and egg production rates reducing sharply as temperature increased.

Calow (1973) suggests that variations in diet may affect growth and presents evidence to suggest that a lichenophilous habitat, as opposed to one with a pre-dominance of algae particularly diatoms, may result in reduced growth of *A. fluviatilis*.

Respiration

The respiration of freshwater snails displays an increase in oxygen consumption with increase in temperature but the rate and level of consumption differs with species. However the consumption rates fall in a fairly narrow range and pulmonates and prosobranchs seem to have similar rates of respiration. The incipient limiting or critical level of oxygen supply for freshwater gastropods was investigated by Berg & Ockelmann (1960) and they reported a species specific variety of responses to declining oxygen concentrations.

For example:

Lymnaea auriculata is able to maintain its oxygen consumption in relation to decreasing concentration to about 11% DO after which uptake decreases.
L. pereger uptake decreases with decreasing oxygen concentration particularly below 8% DO.
L. palustris has decreasing uptake with decreasing concentration but there seems to be an increase as the DO level approaches 12-13%, after which uptake decreases again.
Myxas glutinosa maintained uptake to 12 % DO after which it decreases, particularly below 6% DO.
Bythia tentaculata oxygen consumption decreases with oxygen concentration.
Physa fontinalis is able to maintain uptake with decrease in concentration after the initial small decline. Uptake begins to decline at 13% DO with a rapid drop at concentrations below 6% DO.
Valvata piscinalis is the same and rapid decline in uptake start at concentration of 9-10%.
Bithnia leachi consumption is maintained or may increase with decreasing concentration till 13-14% DO whereupon uptake decreases but the decline is not as rapid as in other species i.o.w consumption at all oxygen conditions is relatively close to normal.

Berg et al (1958) demonstrated that the oxygen consumption of *Ancylus fluviatilis* increases 1.3 to 2 times in spring and summer over the winter values and he regards this increase due to sexual activity. Similar results were reported for *Lymnaea pereger* and *L. palustris*.

Reproduction and Fecundity

Like most pulmonates, Basommatophorans are hermaphrodite, apparently produce eggs and sperm simultaneously and are capable of self-fertilisation, but the copulatory organs seem to be designed to promote cross-fertilisation (Brown, 1980). Observations of mating among *B. stenochorias* have been infrequent, although observations have not been made during the nights. Protandrous hermaphroditism can not be deduced from the observations carried out thus far in this study.

In the laboratory Durrant (1976) concluded that specimens of *A. fluviatilis* as small as 4.00 mm in length had active ovotestes and laid egg capsules producing viable spat. It was seen that no spat were produced by individuals smaller than 4.00 mm. Bondeson (1950) and Geldiay (1956) reported that *A. fluviatilis* lays between 1-11 eggs per capsule. Russel-Hunter (1953) showed that the average number of eggs laid by an individual *A. fluviatilis* is 46 contained in 12 capsules while Streit 1976 reported a much higher value of between 66-113.

Russell-Hunter (1953) made the observation that the majority of the adult population die soon after spawning. This observation may hold true for populations of *B. stenochorias*. This semelparous reproductive cycle compared to the iteroparous condition when parents survive reproduction and live to reproduce again (Calow, 1978) may be an adaptation to variable habitats such as found in streams.

Jarne & Delay (1990) examined the effects of self fertilisation and cross fertilisation in *Lymnaea pereger*.

Cross fertillsation resulted in a larger number of eggs laid, and young hatching and reaching sexual maturity. Jarne *et al* (1991) provided similar evidence with *Bulinus globosus* where selfing snails and snails produced by selfing were less fit in terms of fecundity and the hatchability of their eggs.

Data on other Basommatophora have also provided evidence for an inbreeding depression. Boycott et al (1930) showed that allosperm (*ie* that from the second individual) can be stored and remain viable after copulation, but as it becomes exhausted or dies, the self-fertilisation rate gradually increases (Duncan 1975). Vianey-Liaud (1976) observed a decrease of 40% in the number of eggs laid in *Biomphalaria glabrata* when comparing snails during a grouping period with the same snails one month after isolation. It was suggested that snails had exhausted their allosperm and had progressively switched to self fertilisation. In this case, previous copulations did not prevent an inbreeding depression (estimated by the number of eggs laid).

The possibility of a similar drop in reproductive fitness in *Burnupia* has not yet been examined. However literature indicates that for a general breeding programme, there is a need for continual re-introduction of individuals from an external source to maintain a high reproductive fitness.

Russell-Hunter (1953) observed that in a population of *A. fluviatilis* eggs that hatched at the start of the period of intense spawning had a better chance of surviving the early stages of development than those which hatched from egg masses laid later. This illustrates the phenomenon of competition between individuals where a high mortality rate will result from a dense population.

Toxicity Tests

The need to obtain life history information and apply this information to macroinvertebrate toxicity tests is emphasised by Buikema and Benfield (1979). However, very little work has been accomplished in understanding the life history variables that may affect toxicity test results within snails, and limpets in particular (Wurtz and Bridges 1961; Cairns and Messenger 1974). Examples where life history patterns have been used in determining toxicities are Weir and Walter (1976) and Harman (1974), where immature snails are documented as showing greater sensitivity to toxicants than adults; Flannagan (1974) showed that the influence of a toxicant impairs reproduction through four generations of *Heliosoma trivolvis*. Cocks (1973) and Spronk *et al* (1971) examined the effect of toxicants on the water balance within *Biomphalaria* and *Lymnaea* and Ishak and Mohamed (1975) showed the significant physiological disorders resulting form high doses of copper sulphate in *Biomphalaria alexandrina*. Nduku and Harrison, (1976) demonstrated the detrimental effects on egg production, hatching and shell development due to lowered calcium levels in water. There is a large body of work reporting on the acute and subacute effects of various toxic substances, including halogens, molluscicides, insecticides, fertilizers, copper salts and other compounds on snails, but it is not the intention of this chapter to deal exhaustively with these. No work of an ecotoxicological nature seems to have been completed on the Ancylidae.

4.2 EXPERIMENTAL INVESTIGATION OF *B. STENOCHORIAS*

The investigation to establish optimal conditions for the rearing, handling and breeding of *Burnupia* has been conducted along the following lines, but not necessarily in this sequence:

(4.2.1) Growth and survival;

Pilot study to investigate methods an explore the responses of *Burnupia*
influence of handling
influence of hydraulic conditions (standing with aeration or flowing)
influence of temperature
influence of density

(4.2.2) Reproductive biology; fecundity and hermaphroditism
developmental period and basic embryology

4.2.1. ESTABLISHMENT OF OPTIMAL REARING CONDITIONS - GROWTH AND SURVIVAL.

4.2.1.1 Pilot study on the feeding, growth, survival and reproduction of *B. stenochorias*, conducted by Miss L Horne as a student project.

The project write-up has been shortened.

Aims

To test the effect of three different types of food on the growth and reproduction of *Burnupia stenochorias* under ambient conditions of light and temperature and to assess the survival of the captive population under given laboratory conditions in an effort to establish the suitability of this animal for laboratory culture.

Method

1) Limpets collected from the Blaauwkrantz River were carefully removed from the rocks (to minimize damage to the shells), measured and divided into two size groups with a shell length ranging between 4.0mm to 6.0mm and 2.5mm to 3.5mm.
2) Stones, some of which had been allowed to grow periphyton, were placed into four replicates of two 500ml bubble pots. The stones in the controls (NF) and the artificial food (BR) replicates were scrubbed of all periphyton and sterilized in alcohol. The artificial food was specially formulated by Mr P. Britz from the Department of Ichthyology and Fisheries Science at Rhodes University, for the commercial culture of Abalone. The pellet consists of Spirulina, fish meal, starch and essential amino acids. Periphyton stones were left in ponds at the fish farm for 2-3 weeks to allow the periphyton to grow (FP) or brought back from the river (RF). The 15 limpets in each container were allowed to settle onto the rocks before the air stones were introduced.

Table 4.2.1.1.1. Experimental Design

POPULATION (lg.)			POPULATION (ss.)			FOOD TYPE
Label	Stone volume	Limpet size (mm)	Label	Stone volume	Limpet size (mm)	
NF1	120	4-6	NF2	50	2-4	none
FP1	60	4-6	FP2	75	2-4	Periphyton from fish farm
RF1	50	4-6	RF2	50	2-4	Periphyton from river
BR1	120	4-6	BR2	20	2-4	Artificial food

3) Shell length of specimens were measured monthly using a micrometer eyepiece at a magnification of 120. The spat were counted and a sub-sample of 15 were measured.

4) Containers were kept at ambient temperature throughout the duration of the experiment which lasted from 16 March to 31 August. Water was changed once a week and topped up to the 500ml mark when the level dropped.

Results & Discussion

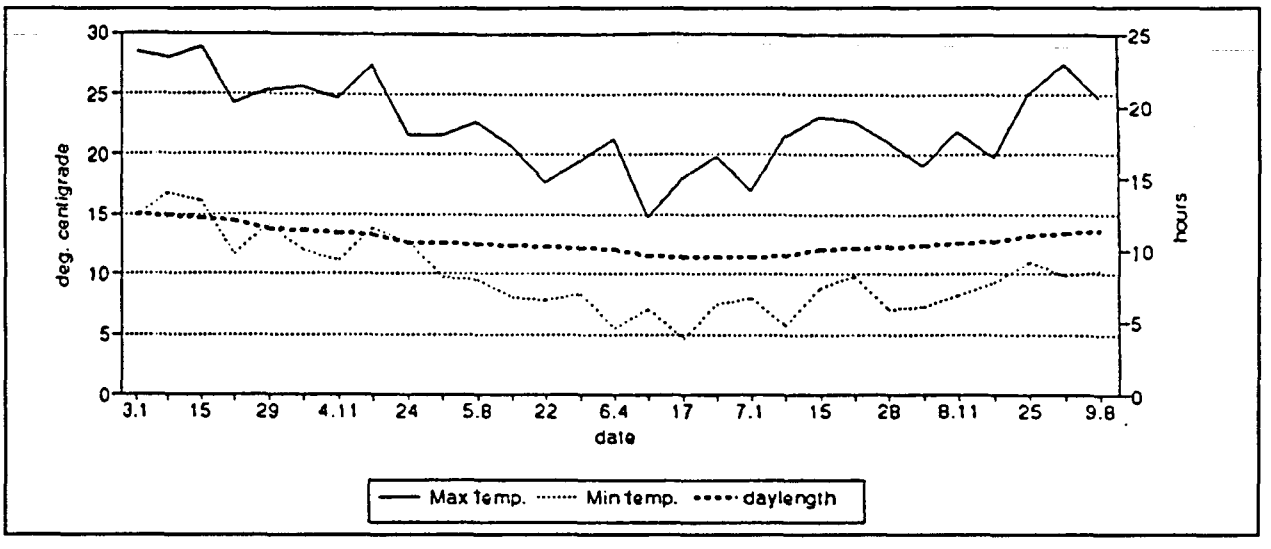

Fig. 4.2.1.1.1 Ambient temperatures and daylength for the experimental period.

In those containers which began with sterile rocks, periphyton grew which provided a food source and enabled the spat to survive and grow. Where artificial food was provided, the periphyton grew more densely than in the others, possibly due to the enrichment of the water by the artificial food. In those containers with natural periphyton the growth became dense and had to be regularly scraped away.

Growth of adults

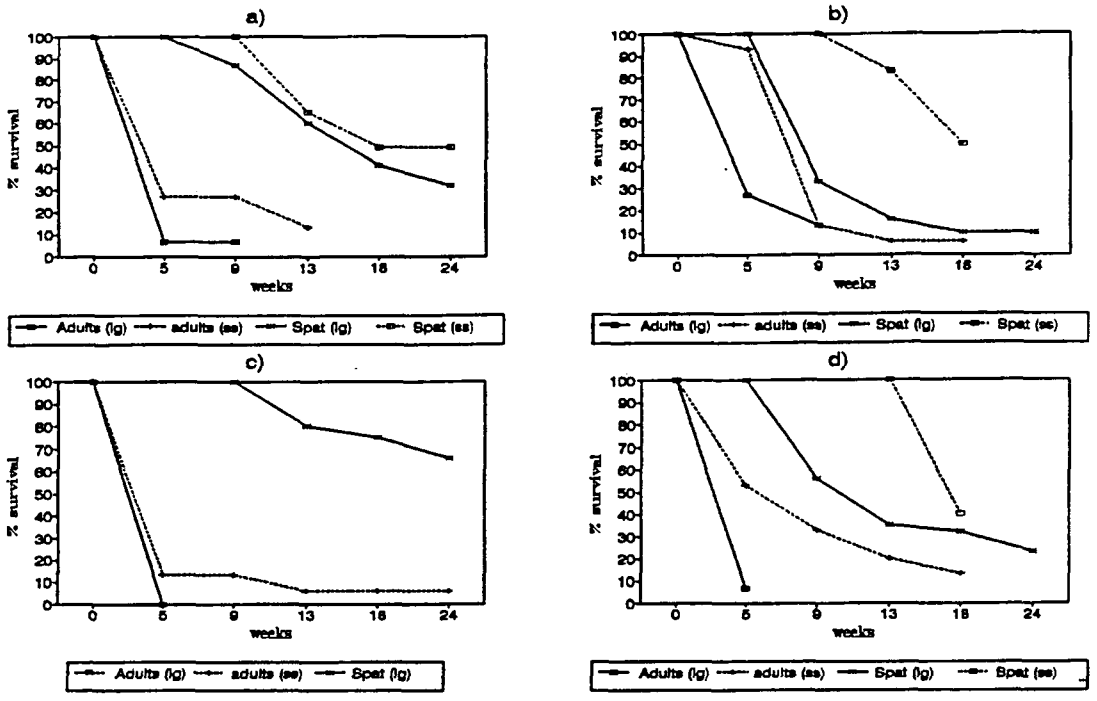

Fig 4.2.1.1.2 a) to d) Graphs to show the percentage survival of limpets under four feeding regimes.

Table 4.2.1.1.2 Average growth rates per day for the adult populations (mm). BR = artificial food; FP = fish farm periphyton; RF = river periphyton; NF = no food 1 = population size range (4-6mm); 2 = population size range (2-4mm).

Feed	Time (Days)	Ave size (mm) at start	Ave size (mm) at end	Growth (mm)	Ave growth rate/day (mm)
BR1	63	3.54	5.0	1.46	0.023
BR2	92	2.24	5.0	2.76	0.030
FP1	63	3.50	3.70	0.20	0.003
FP2	127	2.26	5.0	2.74	0.022
RF1	38	3.68	4.40	0.72	0.018
RF2	127	2.12	4.60	2.48	0.019
NF1	38	3.73	0.00	0.00	0.000
NF2	168	2.32	6.0	3.68	0.022

The low average growth rate of 0.003mm/day which is seen in the FP1 replicate is due to the largest specimens dying in the first month, affecting the average size considerably. The best growth was achieved in containers BR2 and BR1 which were fed on artificial food. The two samples fed on river periphyton showed similar growth rates (0.018-0.019mm/day) while the sample of small animals (FP2) fed on fish farm periphyton, showed slightly better growth approximating that of the larger animals fed on artificial food(0.22 versus 0.23 mm/day). The sample of small animals with no food continued to grow to a maximum length of 6mm after 24 weeks at the rate of 0.022mm/day.

There were not enough available data points for the adult populations and so a linear regression analysis could not be conducted.

Growth of spat

Table 4.2.1.1.3 Average growth rates per day for spat populations (mm). BR = artificial food; FP = fish farm periphyton; RF = river periphyton; NF = no food 1 = large population (4-6mm); 2 = small population (2-4mm).

Feed	Time (days)	Ave size at start (mm)	Ave size at end (mm)	Growth (mm)	Ave growth rate/day (mm)
BR1	125	1.41	4.29	2.88	0.023
BR2	105	1.04	4.44	3.40	0.032
P1	125	1.14	3.94	2.80	0.022
FP2	64	1.20	2.74	1.54	0.024
RF1	125	1.56	3.94	2.38	0.019
RF2	35	1.20	1.85	0.65	0.019
NF1	105	1.40	5.0	3.60	0.034

NOTE: No spat were produced in NF2.

The spat produced by all the adults showed positive growth in all conditions. Best growth rates were obtained on artificial food supplement. Spat in the container which originally had no food had the highest growth rate.

A simple regression analysis showed a fit of 96%, 90%, 97% of the sample to the regression equation (line). Although there appears to be a difference in growth rates, a Kruskal-Wallis analysis of final sizes reached by spat populations in the three food types showed no significant differences.

Reproduction

In Table 4.2.1.1.4 the reproductive gain which was realised in each treatment is laid out. As can be seen the reproductive gain varied considerably between replicates. The adults which were between 4 and 5mm shell length at the start of the experiment died off sooner than smaller limpets and on average produced more young (60/12; 62/10; 91/65) except in the container where no periphyton was supplied where the reverse was true (5/75). The animals in the RF2 containers were smaller than those in the NF2, FP2 and BR2 containers which could be the reason for the two periods of egg laying observed. On the whole the reproductive gain from the N1 generation which were reared under laboratory conditions was lower than that from the wild reared parents.

Table 4.2.1.1.4 Reproductive gain in each treatment. Column 2 = the average size with standard deviations of the 15 limpets at the start of the experiment : STD = standard deviation: Column 3 = number of survivors which were present when the first spat were observed; Column 4 = average size with standard deviations: Date = date on which spat were observed; the last three columns refer to second (N2) generation spat which appeared in the containers from the N1 generation as well as the average size and the number of the parents present in the container and the date on which observed. 3^1 = first measurement and spat count. 2^2 = second measurement and spat count.

Treatment	Ave size(STD) at start	Survi-vors	Size	N1 Spat	Date	N2 Spat	Date	NI Parent Ave (STD)	No.
NF2	2.9(0.24)	1	7.5	75	31/8				
NF1	4.65(0.62)	0	0	5	18/5				
FP2	2.833(0.25)	2	4.63(0.38)	12	18/5	55	31/8	3.42(0.12)	6
FP1	4.38(0.39)	4	4 .38(0.13)	60	28/4	44	31/8	4.92(0.19)	6
RF2	2.65(0.44)	3^1	5.67(0.42)	10	16/6				
		2^2	5.75(0.25)	200	31/8				
RF1	4.6(0.42)	1	5.5	62	28/4	10	31/8	4.99(0.11)	14
BR2	2.8(0.26)	4	3.25(0.25)	65	16/5	15	18/7	4.29(0.34)	32
BR1	4.433(0.39)	1	6.0	91	28/4	48	31/8	5.36(0.56)	29

The interval between the start of the experiment and the first observation of spat was shorter for the larger adults (5-9 weeks) than for the smaller adults (9-13 weeks). We can deduce that the smaller animals had not reached sexual maturity when the experiment started. We observed that eggs were not laid by individuals under 4mm in length and can therefore suggest 3.5-4.0mm to be the minimum size required for B.stenochorias to reach sexual maturity.

Survival

It is clear from Figures 4.2.1.1 a)-d) that the larger animals died sooner than the smaller adults. However they produced a larger number of offspring except in the no-food situation where one of the smaller adults not only survived for the longest period but also produced the largest number of spat. If the slopes of the lines are compared a rapid mortality in the early period of the experiment can be seen followed by a decrease in the mortality rate. This is unlikely to be a reflection of the density of the spat (presuming the greater the density, the greater the mortality) because in BR1, 91 spat showed a slow decline while 60 spat in FP1 showed a much more rapid decline.

The spat survived for longer than the adults. This is depicted by more gradual slopes in the spat than in the adult

limpets. The spat were hatched under laboratory conditions and thus did not experience any physical disturbance. A possible explanation for the higher mortalities in adults could be due to their reaching the end of their natural life span.

Two Chi-square tests were performed to test if the survival of the populations of spat from the two sizes of parents were different. These tests proved that there was a highly significant relationship between the survivorship of the spat and the three food types in conditions of turbulent water ($X=197.0$; $D.F=9$; $p<0.000010$). The test did not however tell us which food type gave us the greatest survival. However, it can be observed in Fig 4.2.1.2.1 that the spat from the population of larger adults in the container with artificial food had a higher survival rate of 45% compared to 30% and 10% on other food types 13 weeks after hatching. The survival rate of the spat from the larger adults with no food proved to be the highest with a 70% survival.

The average shell length of the spat when first observed was 1.12mm. As egg laying or hatching was not observed it is not possible to determine the early growth rate or the size at hatching. This size is comparable with that reported by Russel-Hunter (1953) for *Ancylus fluviatilis*. In the laboratory, Durrant (1976) concluded that specimens of *A.fluviatilis* had active ovotestes and laid egg capsules producing viable spat at 4.0mm in length. This result correlates favourably with the results obtained on the minimum size required to enable reproduction of *B.stenochorias*.

It has been suggested by various authors that Basommatophorans are hermaphroditic. Städler et al. (1993) observed that *A.fluviatilis* has the ability to self-fertilise but appears to switch to self-fertilisation only when mates are unavailable. This phenomenon of switching from cross-fertilisation to self-fertilisation may explain why in the container with no food there was only one adult individual left in the container after week 13 and after 24 weeks a population of spat was observed. If *B.stenochorias* is able to self-fertilise we can deduce that this population of spat arose from the single adult survivor as the result of self-fertilisation. In the light of later experiment (B:4.2.10) this deduction appears to be correct.

It is evident that the greatest numbers of spat hatched in the container with artificial food. It was also observed (Table 4.1.2.3) that this population had the greatest survival rate and thus it can be postulated that the artificial food provides a better source of nutrition as it is able to sustain a population containing more individuals without a high mortality rate.

Bondesen (1950) shows that *A. fluviatilis* in Europe produces very large eggs in comparison with other fresh water gastropods of similar size, and he suggests that this contributes to the survival of young under unfavourable conditions. With regard to growth as observed in the field and laboratory it is noteworthy that different embryos within a single capsule may show marked differences in growth rate and these differences may continue long after hatching (Prinsloo & Van Eeden, 1973). Russell-Hunter (1953) also observed that in his study on *A.fluviatilis*, from mid April to mid August 10% of adults survived and less than 1% survived to mid September. These results compare favourably to the results obtained in this study (Figs. 4.2.2 a)-d) where *B.stenochorias* fed on artificial food, fish farm periphyton and river periphyton displayed a survival rate of 10% over approximately

the same time period. It must be noted however, that this survival rate was observed from the population of initially smaller sizes (2-4mm) and not of the larger size range (4-6mm). This may be due to the semelparous life history and proves that the larger adults were close to the end of their natural lives.

A species of oligochaete (unknown) was often found between the mantle and the disc of the foot of *B.stenochorias*, probably as a commensal rather than as a parasite. The oligochaete *Chaetogaster limnei* (Baer) was found on the species *Ancylus fluviatilis* (Geldiay, 1956).

As the experiment progressed the shells of *B.stenochorias* became covered with various algal species and limpets were often seen browsing on each other with small ones frequently found on the shells of older limpets.

Conclusion

This investigation proved to be valuable as a pilot study indicating the potential as a species for laboratory culture. Areas which require further investigation became apparent. These include density effects on reproduction and survivorship and responses to food types and other abiotic variables such as the hydraulic regime and temperature. The experimental design will have to be improved to allow for adequate statistical analysis.

4.2.1.2 Growth and Survival in Disturbed and Undisturbed Conditions

Aim

To establish growth rates at a stable temperature and to establish if the measurements taken every two weeks, which disturbed the limpets, had any effect on the survival and growth rates.

Method

1) 300 adult limpets were collected from the Blaauwkrantz River on 13 October, 1993 and held 14h00 photoperiod and 16-23±2°C which were ambient conditions. These limpets were kept in a shallow bubblepot with ample substrate covered with periphyton on which egg cases were deposited.
2) 14 x 5 litre bubblepots were each half filled with water from the river of origin, covering a tile with periphyton growth. Six of the pots were labelled numerically and eight alphabetically.
3) Stones with eggs cases were placed in these pots 12 days after the adult were collected and placed in a CER at 18 °C and 14hOO photoperiod. The population in the pots labelled alphabetically were not measured until they were 94 days old but were counted 2 weekly intervals which meant that they were undisturbed. In order to determine the developmental period the egg cases on the stones were examined every two days in the numbered containers until the hatching was completed. Thereafter a sub-sample of the limpets were removed to be measured and counted every 14 days, until age 139 days.

Results

See Table 4.2.1.2 and Figures 4.2.1.2.1; .2; .3 and .4.

The Analysis of Variance conducted on the average sizes reached by populations in all containers at 94 days showed an F ratio of 22.870, df = 13,339, and $p < 0.0001$ which indicates a significant difference between treatments. A multiple range test conducted on the population size between all containers showed that significant differences appeared between some containers (f=12.123; df 7,216; p<.00001) : grouping containers that showed the smallest differences together resulted in groups ADEJLN; 123; 45; and CD (Fig 4.2.1.2.2). When these groups were tested against each other in the sequences given below, all the comparisons yielded significant differences:

123-ABEJLN; 45-ABEJLN; ABEJLN-C; ABEJLN-D; 123-45; 123-C; 123-D; 45-C.

The sizes achieved showed that those animals that had been disturbed for measuring were smaller (1,4 - 1,8 mm) on average than those which had not been disturbed (2.0 - 2.6 mm).

Mortalities

Regression analyses were conducted between the percentage survival, and age of the population in each container in both treatments using BMDP.

(a) Undisturbed population:

At the 5% level of probability, there was a significant difference between containers when all containers were tested together. F = 3.045; df = 16,27; p value = 0.00523.

Graphs in Fig. 4.2.1.2.4 (a) and (b) show that the slopes of containers A and K are different from the others. The regression slopes of A and K are respectively -9.05 and -25.6 while that of all the other populations are between -14.7 and -17.8. Therefore excluding A and K, F = 1.617; df = 12,21; p = 0.16154. When tested this proved that there was no significant difference between the regression slopes in containers BCEFJLN.

(b) Disturbed population:

The regression slopes ranged between -27 and -19 in all the containers and there was no significant difference between the regressions of the population in these containers (F ratio = 1.308.517 df 10,18 and p = < 0.029762). However when the populations in the two treatments were compared to one another there was a significant difference between the rate of decline (with or without A & K). The mortality rate in disturbed populations had a regression line of -21 and that of the undisturbed populations was -16 (F ratio = 95.517 df=1,28 p value= <0.00001).

Discussion and Conclusion

Physical handling of the limpets has a detrimental effect on both the growth and survival rate of juvenile limpets, between the sizes of 0,6mm and 2,4mm shell length. Minimal handling is therefore necessary when collecting from the field and when measuring the limpets during experiments. Fig. 4.2.1.2.3 shows the difference in the % of the sample surviving after 30 days in undisturbed (a,b) and disturbed (c) pots. The vulnerable nature of the juveniles is demonstrated by the fact that between 70 and 90% of the animals survived in 7 out of 9 containers

in undisturbed while less than 70 % of the animals survived the first 30 days if disturbed. However after the initial period, survival rate stabilized until day 66 and then declined more sharply except in container 1. In the undisturbed containers the high level of survival was sustained until day 45 with the exception of K and F but after that the mortality rate increased. In two containers a total mortality was recorded which could perhaps be ascribed to a pathogen. However if the undisturbed containers are compared to those which were regularly disturbed it is clear that survival was on the average 10% higher when undisturbed.

As mentioned in Chapter 3.1, field handling has been reduced since this experiment by using an anaesthetic, and a template made of transparent photographic film with measured punctured holes ensuring minimal handling when measuring the limpets.

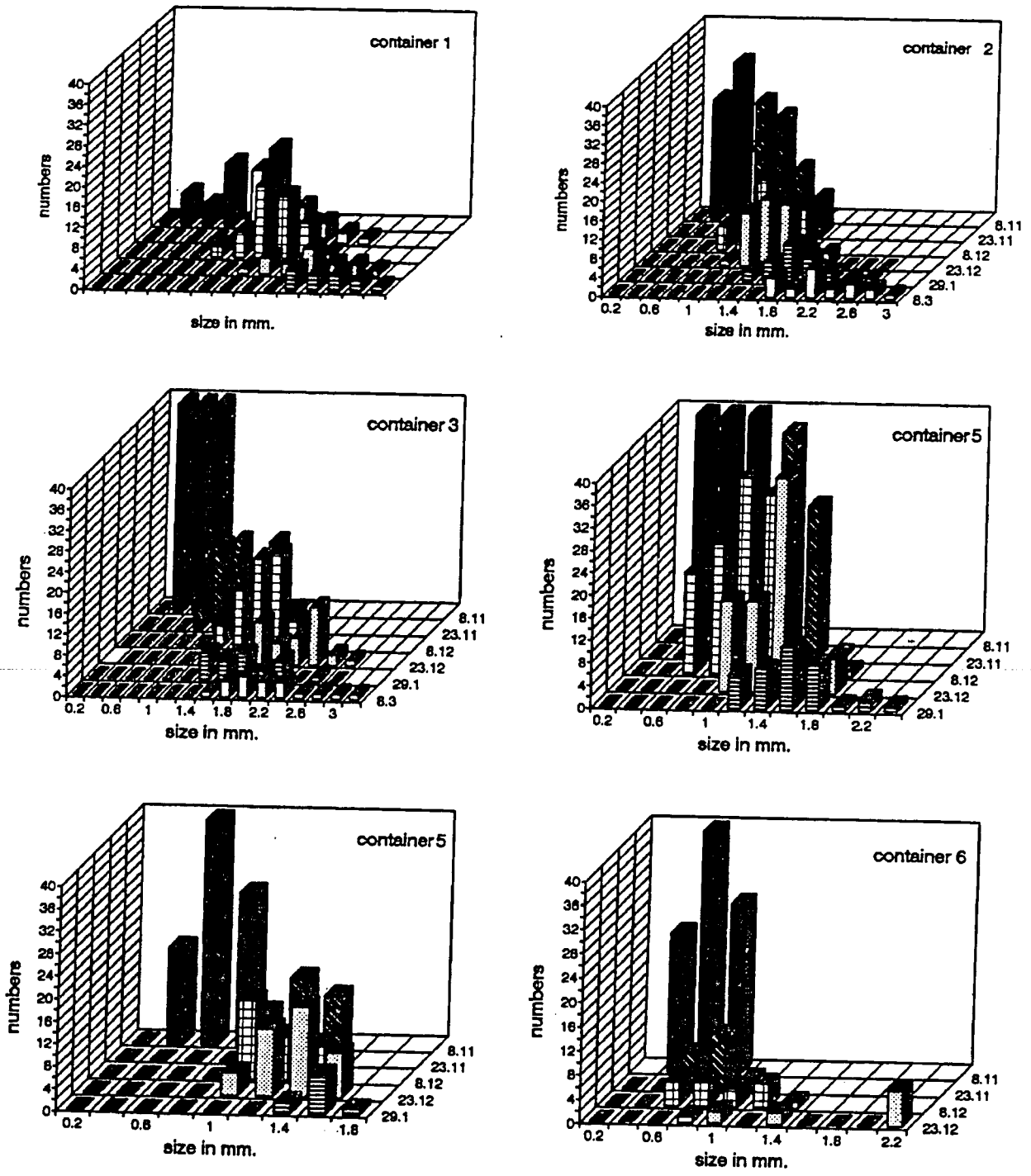

Fig. 4.2.1.2.1 Frequency distribution of the population size at a number of dates during the experimental period. The numbers 3.11 - 8.11 are the dates during which 50% of the eggs had hatched.

Table 4.2.1.2.1. Table showing the numbers in each container at the start of the experiment, after 94 days; the average shell length with standard deviation, achieved in each container and daily growth rate in mm. Disturbed containers are labelled 1-6; undisturbed containers are A-N.

POT	INITIAL NUMBERS	FINAL NUMBERS	AVERAGE SIZE (mm)	STANDARD DEVIATION	GROWTH RATE
1	295	17	1.79	.057	0.018
2	165	23	1.84	.066	0.017
3	247	31	1.75	.056	0.014
4	371	41	1.38	.043	0.0088
5	94	14	1.46	.040	0.009
6	101	11	1.90	.152	0.014
A	85	60	1.96	.040	0.018
B	67	23	2.17	.062	0.019
C	104	23	2.39	.080	0.0237
E	96	21	2.57	.104	0.024
F	94	29	2.00	.071	0.016
J	64	16	2.08	.113	0.021
L	79	30	1.88	.053	0.017
N	98	22	2.05	.042	0.020

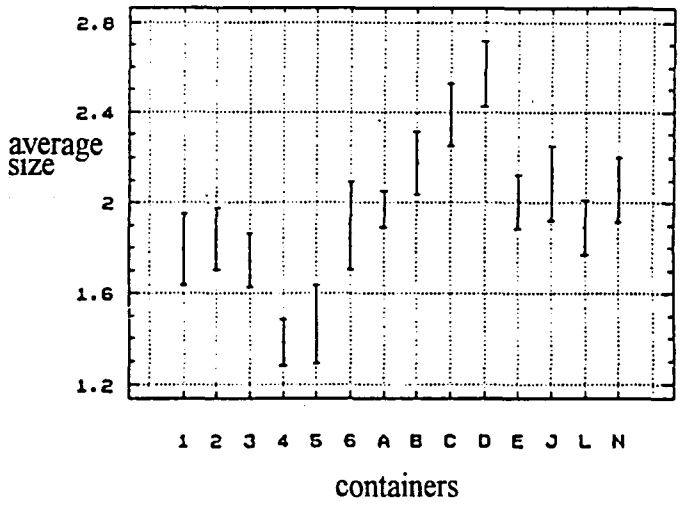

Fig. 4.2.1.2.2 Average size the sample achieved after a period of 94 days in both disturbed (1-6) and undisturbed (A-N) containers.

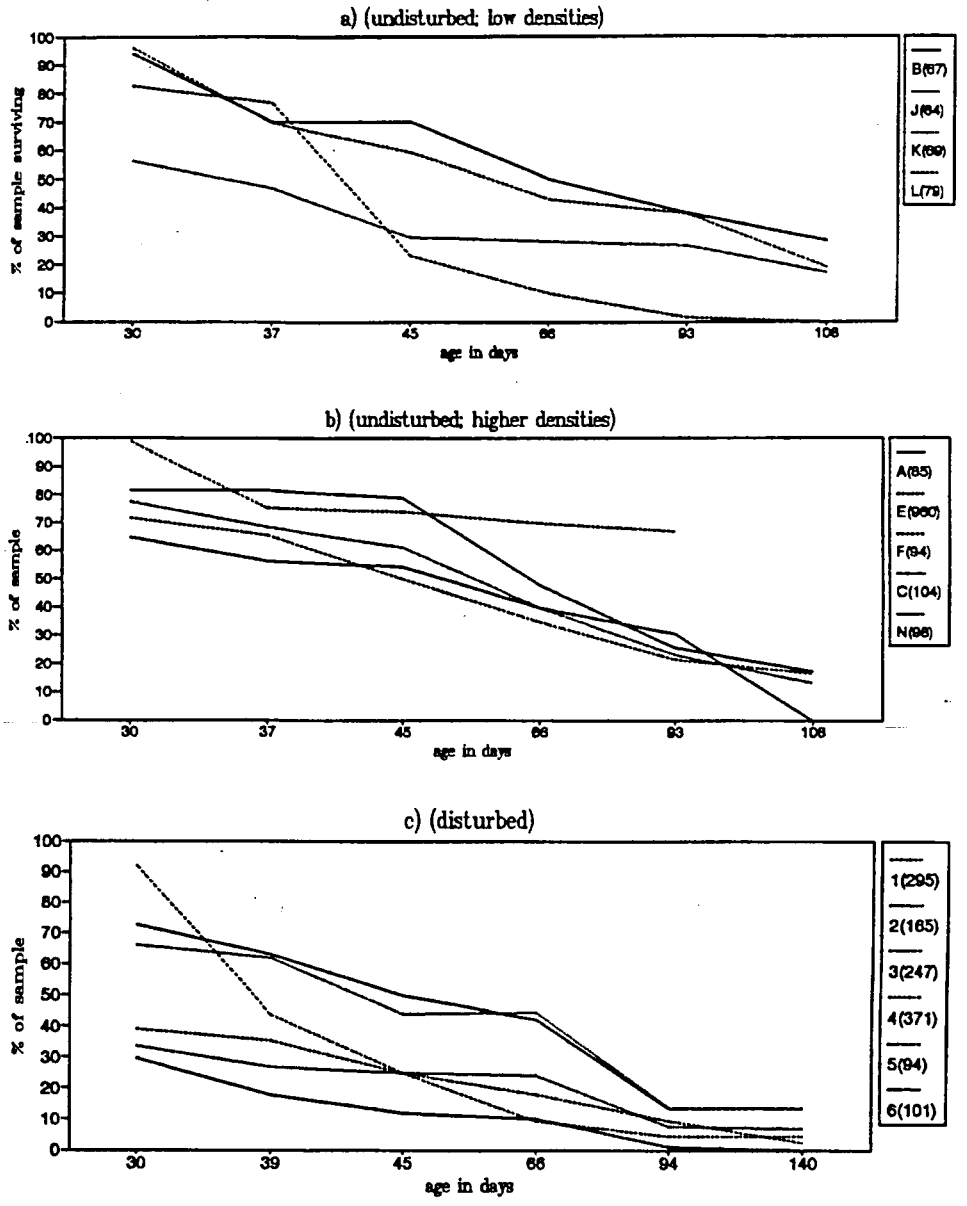

Fig. 4.2.1.2.3 Percentage of sample surviving during the experimental period. The legends indicate the initial size of the sample in each container. (a) and (b) undisturbed containers (c) disturbed samples (removed for measuring)

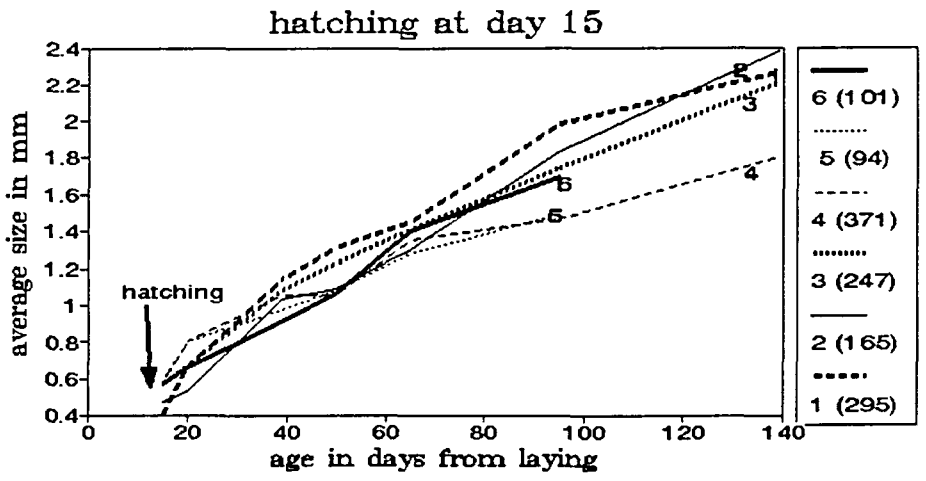

Fig. 4.2.1.2.4 Average growth rate of *B. stenochorias* juveniles over 139 days from deposition Sample size legend

As can be seen all the animals in container 6 had died, and in container 5 three remained.

4.2.1.3 A Comparison of Survival Rates Between Fed and Unfed Populations

Aim

To assess the length of time a given number of adults and sub-adults can survive without food.

The debate as to whether test animals should be fed during ecotoxicological testing is unresolved. The question about the stress caused by lack of food and the attendant change in response to toxins needs to be resolved. This experiment is the first step in gaining insight into this problem. The projected experimental period for toxicology testing is four days.

Method

1) 8 channels, each 50cm in length with approximately equal amounts of water circulating through at a known rate and depth within each channel from a common water sump were set up. The water source was Grahamstown tap water, allowed to floe through the system for 2 days to allow for de-chlorination.
2) 4 channels had 3 ceramic stones of similar size, covered in periphyton, placed in each channel. The source of the stones was from the ongoing laboratory culture of algal growth where stones are held in recirculating water mixed from various sources (riverine and predominately tap water). As these channels are in the same laboratory as this experiment, temperatures did not alter and therefore there was no change in the growth of the periphyton. The stones were visually

assessed to ensure approximately the same amount of periphyton was placed in each replicate.

3) 4 channels had 3 ceramic stones of similar surface area to those of the above, placed in each channel. These stones were scrubbed and dried to ensure there was no periphyton. All channels were treated similarly, to ensure there was no food available.

4) On each stone in all of the 8 channels 2 adult (approx. 2,5mm in length) field-caught limpets of known length and height were placed, particular care being taken not to damage the animals in any way when moving them. The limpets were acclimatised for one week to the laboratory conditions.

5) Twice daily temperatures (at 08h00 and 15h30) and daily levels of TDS, pH and % oxygen were measured.

Results

Water conditions did not differ between all the replicates over the fifteen days. One way analyses were conducted on the two treatment populations at 4,8,12 and 16 days and at no stage were any significant differences detected.

Table 4.2.1.3. Statistical data of ANOVA. Day = number of days after treatment. df = degrees of freedom.

Day	F ratio	df	p value
4	2,6667	2,7	0,1835
8	0,667	2,7	0,6151
12	0,267	2,7	0,8469
15	1,250	2,7	0,3629

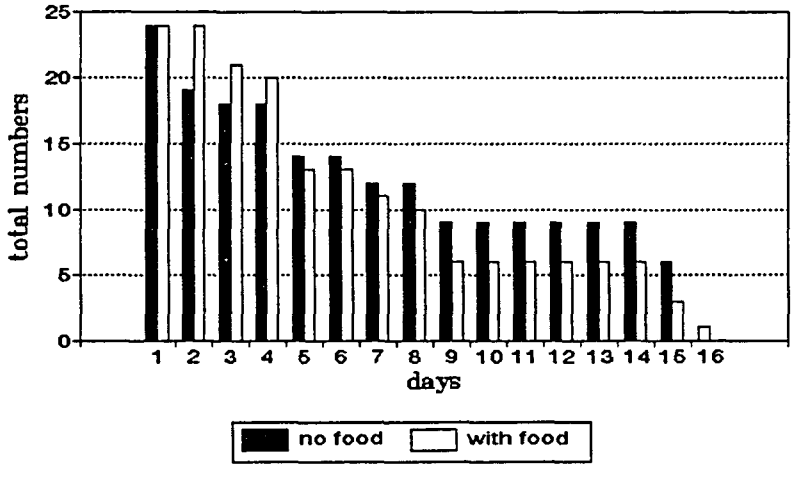

Fig. 4.2.1.3 Total number of limpets remaining in each treatment.

Conclusion

A period of 4 days (96 hours), that period under which ecotoxicological testing is completed, without food does

not appear to affect the survival of adults and sub-adults.

4.2.1.4 Hydraulic conditions

Several trials were conducted to test the responses of the limpets to channels (flowing water) and bubblepots (standing aerated water). Full procedures are reported in Appendix 3, section 3.1.3. dealing with the development of equipment.

4.2.1.4.1 Aim: To compare the growth rate of *B. stenochorias* in flowing water with that of aerated, standing water.

Method

Four 50 cm channels, each with approximately equal volumes of Grahamstown de-chlorinated tap water and four bubblepots of known and equal size, each with approximately equal volumes of water and air flow were housed in the CER at 19°C (day), 14°C (night) and a photoperiod of 14hrs. Within each pot or channel were placed 3 ceramic stones with periphyton as food source and 12 limpets of equal size.

Results and Discussion

Despite the fine net covering the holes through which the water flowed the limpets escaped and landed in the water sump. This made it difficult to asses growth rates of limpets in the channels. Survival in bubblepots (60% at 35 days and 40% at 79 days) was significantly better than in channels (225 at 35 days and 5% at 79 days). In the channels many of the limpets lost their shells or had extremely soft shells, which indicate unsuitable water chemistry despite pH levels which were neutral or slightly alkaline. The experiment was terminated after 3 months because of the above difficulties.

In the bubblepots the daily growth rates achieved in the first 35 days ranged between 0.01mm and 0.03mm and during the subsequent 39 days 0.01mm to 0.02mm at an average temperature of 19°C.

Conclusion

Narrow channels with three stones are not suitable for the rearing of *Burnupia* in the laboratory. More attention will be paid to water composition in future experiments (see section **3.3.6 Water Composition and Hygiene**).

4.2.1.4.2 Aim: To test the effects of density and flow on the survival and reproductive capacity of limpets.

Method

Two sets of four channels, each set with a common sump and 8 bubblepots were housed in the laboratory at an average temperature of 17.2 °C in channels and 15.2°C in pots. Three periphyton stones, and a known number of measured limpets ±100 and ±10, were placed in each channel and bubblepot. Dead limpets were replaced with an individual of similar size from a field-caught sample until the 48th day, to maintain the original densities. Dissolved oxygen, Ph, and TDS were monitored weekly and temperature daily. The trial ran for 162 days over the winter period when day-length declined from 11 to 9 hours and then increased again to 10 hours.

Results

The limpets moved from the channels, into the sumps from where they had to be moved back to the channels daily to maintain the density. This continual disturbance has previously been shown (see section 4.2.2) to have a detrimental effect on the growth of the limpets. The eggs deposited during the experimental period were not removed so that some indication of the reproductive gain in the various conditions was given. The highest gain of 6 hatchlings per adult was achieved in a bubblepot with low density of limpets. In the rest of the containers a negative reproductive gain was recorded.

The mortality in two bubblepots was high (77% & 63.6%). In two channels mortalities in excess of 20% were recorded. In the rest of the channels and bubblepots mortalities ranged between 4 and 19% (ave. 13%)

Conclusion

More egg capsules were laid in the bubble pots than in the flowing channels, especially at low density of limpets. The survival rate did not appear to differ markedly between the flowing and standing waters with the exceptions stated above. However the highest mortalities were recorded in the pots which could indicate a pitfall of pots as rearing equipment, that if a pathogen or any other cause of mortality enters such a confined space the entire sample in that pot is at risk.

From this exercise we therefor conclude that small numbers of adult limpets in a bubblepot will give the greatest numbers of offspring at an average survival rate. If some way could be found to preventing large numbers of limpets from escaping the channels, these would provide adequate facility in which to rear limpets.

4.2.1.4.3 Aim: To compare the survival of limpets in flowing versus standing, aerated water.

Method

About 700 limpets were collected in the field, using anaesthetics and maintained in the laboratory streams with de-chlorinated tap water for a few days to allow for any that died to be removed. Equal numbers of 2mm to 4mm

limpets were then placed on tiles which had accumulated a layer of periphyton, in three replicates of the large recirculating streams and three 10 litre basins, set up in the laboratory under ambient conditions of temperature (22°C-17°C, ave 19°C) and light. Dead limpets were counted and removed, and egg cases were counted daily. TDS, temperature, % oxygen and pH were monitored with each change of water. The experiment was allowed to run for 9 weeks.

Results

A greater number of egg capsules were laid in the pots, (64; 74; 115; ave. 84.3%) than in channels (47; 90; 103; ave. 80) The mortality rate varied considerably, between and within all the replicates. The average mortality for the channels was 64,5%, (71%; 62%; 58%;) for the pots, 75,6% (67% 72%; 88%).

Conclusion

In this experiment, the channels gave a better average survival rate than the pots where more egg capsules were laid.

4.2.1.4.4. Aim: To compare the growth rate of *B. stenochorias* in flowing water and turbulent standing water at 15°C, 20°C and 25°C.

Method. This experiment was conducted in a CER at 15°C. Four water baths were equipped with an aquatic thermoregulator each. Two were maintained at 20 and two at 25 °C. In two baths four five litre sumps were placed from which four channels was supplied. In the other two baths five litre bubble pots were placed. Two further baths were maintained at the ambient temperature of 15°C, one equipped with bubblepots and one with channels and sumps. Twenty limpets of known size were placed in each of the bubblepots and channels and fed on periphyton.

Conclusion

The aquatic thermoregulators used to maintain the water temperature in the sumps of the channels were unreliable and consequently unavoidable temperature fluctuations occurred. The results could not be used. It was therefore decided to conduct an experiment in which only bubblepots were used in an attempt to ascertain the growth rate of the limpets at three temperatures.

4.2.1.5. The Effect of Temperature and Density on Growth.

Aim

To determine the effect of temperature and density on the growth and survival of *B. stenochorias* with a view to determining optimal stocking density and ambient water temperature in a laboratory culture.

Introduction

Temperature and food are the most frequently reported factors which affect life history traits in aquatic invertebrates. Temperature directly affects growth by influencing metabolic rates and feeding rates (Giberson and Rosenberg 1992). It also affects food quality and quantity by influencing algal production and growth rates on detritus (Ward and Stanford 1982). When evaluating growth and development for the purposes of aquaculture, not only should temperature and food be considered but also density. At high densities competitive interaction may affect feeding rates and growth.

Method

1) The Controlled Environment Room was set at a stable 15°C.
2) Egg capsules laid on ceramic stones in the laboratory streams by field-caught *Burnupia* were placed in 500ml transparent plastic jars (called bubble pots) which were filled with 400ml of water, aerated, and allowed to acclimatise to the CT room temperature. A few days after hatching (which was completed by 29 September 1994) many of the spat moved to the sides of the containers. These pots were then manipulated so that there were 12 pots each of 10, 20 or 30 *Burnupia* per pot. The ceramic stones were removed, as were any excess numbers of spat which were kept separately and used to replace any limpets that died in the first two weeks of the experiment. This method ensured no handling of the spats occurred. First measurements were taken on the 13 October 1994 @ 14 days old.
3) Four water baths were used, each a rectangular white plastic container 50cm x 25cm x 25cm deep. In each water bath twelve of these bubble pots were placed, with 4 representatives from each of the densities. Each pot was given a clear soft-plastic lid held in place with an elastic band. Through this lid an aerator was suspended and into each bubble pot was placed one ceramic stone previously allowed to grow periphyton. The water in the bath was then filled and maintained at the same level as that in the bubble pots.
4) Each water bath was maintained at a different temperature, namely ambient 15°C, 20°C and 25°C.
5) The water within each pot was maintained at 400ml with distilled water and replaced every week with aerated dechlorinated calcified tap water (this water was kept aerated in the C.E Room, with crushed shells suspended, acting as the source of calcium). The pH, TDS and were monitored weekly.
6) The *Burnupia* were measured weekly for the first 4 weeks and thereafter fortnightly.

Measurements were taken with a prepared template through the sides of the pots. Any deaths within the first 2 weeks were replaced with individuals from the initial population of hatchlings.

7) The light intensity in the C.E. room was maintained at 15microm/m^2/sec quanta with daylight bulbs to increase the available range wavelength. The photoperiod was 14 hours.

Results

See Table 1, Appendix 4; Table 4.2.1.5. and Fig. 4.2.1.5.

Table 4.2.1.5. BMDP analyses of temperature versus density results. D** refers to density of 10, 20 or 30 limpets per container while T** refers to the temperature at which the experiment was conducted.

ANALYSIS				F RATIO	PROBAB.
at all temps	D10 slope 0,01994	D20 slope 0,01896	D30 slope 0,01820	25,189	<0,001
at temp 15°C	D10 slope 0,0231	D20 slope 0,02236	D30 slope 0,02153	11,417	<0,001
at temp 20°C	D10 slope 0,01822	D20 slope 0,0290	D30 slope 0,01795	30,138	<0,001
at temp 25°C	D10 slope 0,0231	D20 slope 0,02236	D30 slope 0,02153	11,417	<0,0011
T15 vs T20 at all densities	T15 slope 0,01682	T20 slope 0,01926		84,186	<0,001
at all densities	T15 slope 0,01682	T20 slope 0,01926	T25 slope 0,02242	122,121	<0,001
at density 30	T15 slope 0,01663	T20 slope 0,01795	T25 slope 0,02153	44,283	<0,001
D10 vs D20 at all temperatures	D10 slope 0,01994	D20 slope 0,01896		5,165	<0,005

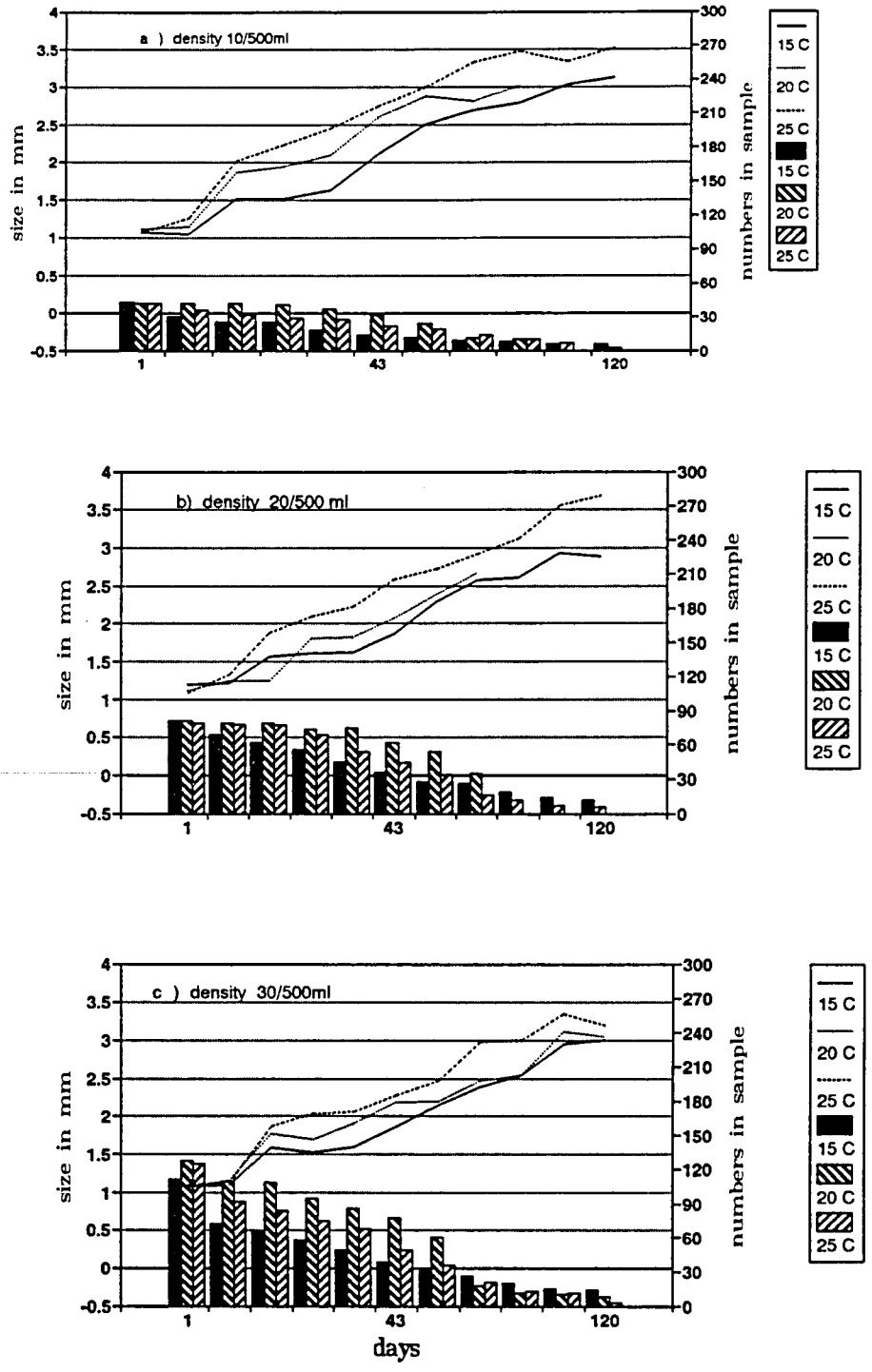

Fig. 4.2.1.5. The average size of *B. stenochorias* in all the replicates in each treatment (lines) and the total number of limpets in each treatment (bars).

Discussion

Temperature

It is generally agreed by ecologists that temperature is one of the most important physical influences of any biotope, in particular of the freshwater biotope (Shiff 1964). The apparent ability of snails to withstand wide variations in temperature has been noted by many workers. However Shiff (1964) points out that many of these inadequately measured the overall conditions of the habitat, and states that over very hot periods water at a depth of 25cm may be 5°C cooler than that at the surface. Mozley (1959) also stressed the importance of microhabitats in which animals may seek refuge from overall conditions. It is thus probable that the apparently wide thermal tolerance shown by aquatic snails may be due to their ability to select the more suitable microhabitats. This opportunity was not afforded by this *Burnupia* experiment.

Population patterns are strongly influenced by temperature, as demonstrated in the genus *Physa* (Duncan 1959, Russel-Hunter 1961(b), Girod 1969, DeWitt 1955, Eckblad 1973 and others). These authors found that differences in the number of generations through different geographical regions is linked to differences not only in food source quality, but also in higher mean growth rates and shorter generation times, all as a result of the influence of their ambient temperature. Similarly, temperature has been directly correlated with growth rate in many freshwater pulmonate snails (McMahon 1975(a). In general growth rates tend to increase with increasing temperature up to a critical temperature (usually near the upper limit) after which growth rate declines. Lambert (1989) showed the rate of individual growth and demographic parameters (survivorship, fecundity and net reproductive rate) of *Lymnaea peregra* are strongly influenced by temperature. The freshwater snail *Indoplanorbis exustus* showed very clearly (Raut *et al* 1992) an optimal temperature (30°C) for growth and number of capsules and eggs produced per week.

Table 4.2.1.5.1 reveals highly significant results ($p<0,001$):

(a) when all densities were combined, increasing temperature increased growth rate.

(b) at a density of 30, increasing temperature increased growth rate.

(c) when comparing temperatures of 15°C and 20°C a higher growth rate was seen at 20°C.

For the analysis of accumulated mortalities as opposed to growth, to see if either temperature or density affected mortality, it was necessary to find the time at which 50% mortality occurred within each replicate, using a detoured probit analysis (STATGRAPHICS). A two way ANOVA using these values at 50% mortalities, revealed that neither temperature nor density was found to have any effect on the time taken to reach the 50% mortality.

In order to calculate maximum ingestion rates for different sizes and temperatures, Streit (1985) showed the speed of rasping is a strong function of temperature, with the least time needed being at 22°C. Similarly the speed of

the radular movements, and hence the amount ingested per unit time, is a function of size, food concentration and temperature, with maximum ingestion at 25°C for the large limpets. When comparing production efficiencies of juveniles and larger limpets, he found the juveniles are better converters with a production efficiency of 83.9% at 10°C, but only 8% at 19°C for the large limpets.

These findings indicate difficulty in assessing the results obtained when comparing the growth rate of *Burnupia* at three temperatures. Duncan (1959) found that temperature was of fundamental importance in affecting the rate of development, and in influencing the sexual maturity and oviposition in *Physa fontinalis*; Streit (1975) showed for *Ancylus maximum* ingestion and assimilation rates were at 25°C, maximum growth and egg laying were at 19°C, maximum value of production/ingestion was at 13°C, and the lower limit of egg laying was between 7°C and 10°C. These results may also suggest that for any large scale production different temperatures may be necessary for the optimisation of the growth of different size groups.

Density

If we were to consider a natural population, and to decide what ultimate mechanism(s) determine the numbers in a population, one view is that they are regulated in a density-dependent fashion, and that this regulation is mediated through intra- and interspecific relationships which result directly from density feedback (Eisenberg 1966). Within this experiment, presuming the only variable within one temperature is density, a density-independent model must predict that convergence would not occur and that high and low density replicates would remain disparate in time. As various density-independent processes acted on the sub-populations, they would fluctuate, but a plot of the log densities with time would give a series of quasi-parallel lines. On the other hand, density-dependent models would predict that high and low density replicates would converge in time, although the level at which they converged would almost certainly be influenced by density-independent processes (Eisenberg 1966).

Chernin and Michelson (1957a & b) studied the effects of population density of *Australorbis glabratus*, and found that snails maintained under crowded conditions grew more slowly and were less fecund than those in less densely populated aquaria. They also found that, whereas a doubling of the number of snails in a given volume of water produced a marked reducing effect on the growth and reproductive potential of the individuals, a similar effect was not obtained by keeping the numbers constant and halving the water volume. In re-assessing these results, Wright (1960) showed that there is no single factor which can be held responsible for poor growth and lowered fecundity under high densities. He suggested three factors involved: (a) food, (b) collisions, and (c) chemical pollution.

(a) FOOD: Density can affect growth rate by limiting available food especially among grazers such as *Burnupia*. The effect of snail grazing on periphyton is manifested in a shift in the species composition and succession of periphyton assemblages (reviewed in Bronmark 1989). Succession of a periphyton assemblage generally proceeds from a monolayer assemblage dominated by small, adnate diatoms, to a more structurally complex community

with stalked diatoms and small or large filamentous algae (Steinman et al, 1987). An increase in grazing pressure will halt succession at an intermediate stage or earlier, relative to the pressure (Cattaneo 1983). Although the food requirements of *Burnupia* have not yet been investigated, Calow (1973, 1975), Schwenk and Schwoerbel (1973) & Streit (1975) found that *Ancylus fluviatilis* is a microherbivore, the preferred food being epilithic algae, particularly diatoms. *Burnupia* exhibits feeding behaviour similar to that reported for *Ancylus*, which is random rasping of the stone surface with the radula. Thus it seems likely that it's food requirements will be similar to *Ancylus*. During this experiment there was no consistent analysis of the periphyton assemblage. Visually there appeared to be very little differences in the replicates, with the presence of filamentous green algae in each replicate. Periodic scrapings of the surfaces of both the ceramic stones and the sides of each container revealed an abundance of both single and filamentous diatom species throughout the replicates, although their relative abundance was not calculated. Visually it appeared that grazing pressure was having very little effect on the periphyton assemblage at these densities. Thomas *et al* (1974), Muller (1983) and Wetzel (1983) remind us that nutrient concentrations contribute to the control of periphyton assemblages. Snails will alter the chemical composition of the environment by removing ions, amino acids, and oxygen, and by adding substances including ammonia, carbon dioxide, ions, mucopolysaccharides and organic acids. They claim that mollusc excrement or defecation increase nutrient availability, which in turn enhance algal productivity. In this way it overrides the effects of high density (and therefore grazing) on periphyton growth.

We conclude, with regard to food within this trial:

(i) initially food was not a limiting factor and is unlikely to have contributed to any differences between the three densities of limpet tested here.

(ii) After approximately 43 days the numbers of limpets in the high relative to the low density replicates had dropped to similar levels (see Fig 4.2.1.5) and thus the feeding pressure was likely to have been similar between all replicates. At this stage the periphyton consisted of a large proportion of filamentous algae. Streit (1985), in his work on *Ancylus fluviatilis*, showed there was an upper limit to the thickness of periphyton layer that this limpet could cope with because of the limitations resulting from the size of the radula teeth, of similar size to *Burnupia* (Oberholzer 1963). The thickness of periphyton is very likely to have been at a level where the limpets were limited in their food uptake, contributing to growth impediment as the trial progressed.

(b) COLLISIONS: Density dependent stimulation of tactile and visual receptors is a second possibility for density effects, suggested by Wright (1960). Close proximity of individuals of the same species with a resulting antagonistic behaviour is seen in a number of the Physidae, for example in *Physella virgata* (Brown, Carman, and Inchausty, 1994) but this appears not to have been recorded in the Ancylidae (Kawata 1993). Under natural conditions *Burnupia* are regularly seen in close proximity to each other on the same rock. Observations have shown that all sizes of individuals move very small distances, seldom even moving from rocks of approximately 500ml in volume, in natural streams (pers. obs.). The confines of the 500ml jars used in this experiment would therefore be an unlikely source of irritation to the *Burnupia* under any of the tested densities.

(C) CHEMICAL POLLUTION: Wright (1960) showed that chemical pollution by the snail *Bulinus forskalii* played a major part in affecting growth and fecundity. In a confined, static environment any chemical inhibitor that is emitted will have a more pronounced effect. Whether this inhibition of growth and survival with *Bulinus* is brought about by a chemical in their waste products or by some specific substance of a pheromonal nature has not been ascertained. Since Chernin and Michelson (1957a and b) were unable to demonstrate by chemical means any important differences in the composition of the water samples taken from the different densities they studied with *A glabratus*, it seems likely the active substance is at a very low concentration (and therefore possibly a pheromone).

This phenomenon of growth inhibition has been demonstrated in various other plant and animal groups. Overcrowding and consequent growth inhibition was reported by Rose (1960) in tadpoles. Growing tadpoles of *Rana pipiens* release more of this substance and become less sensitive to a given concentration of it. Thus large tadpoles will suppress the growth of smaller ones kept in the same container. The substance produced was species specific. Berrie and Visser (1963) showed very clearly with *Biomphalaria sudanica* (Martens) that a chemical is produced by the individuals that at high densities produces a growth inhibition. When artificially increased to just beyond the level that was found in natural pools, this chemical became lethal. They found that the greater the number of individuals, and the larger they grew, the greater the inhibition of growth. These conclusions were somewhat contradicted by the work of Thomas *et al* (1975) who showed that with *Biomphalaria glabrata* (Say) that an increase in growth of juveniles and natality rates was achieved with crowding *ie* by increasing snail numbers or by decreasing the volume available to critical thresholds. Further increase in snail numbers or a decrease in volume available to each snail beyond the optimum levels showed a decrease in growth and natality rates of the snails. In closed systems with equivalent densities of 20 *Burnupia* per 500ml water, they proved conclusively with this species that no inhibitory pheromone was produced by the snails which was toxic to growth or reproduction rates under this density, which appeared to be the optimum condition.

Unlike *Biomphalaria*, however, *Burnupia* is not naturally found in pools and stagnant water (Brown 1980) but under such experimental conditions, it would not be surprising to find that pheromones did in fact have a greater part to play on the growth of individuals than under natural flowing systems. On the basis of this, our results would be difficult to interpret. Initial growth of small individuals would show some variation. Would those larger than the average, within each container, then have a growth inhibiting influence on those smaller, by the production of one or more chemical factors? Examination of each single growth regression curve for high density replicates (graphs not provided) reveals a large disparity in the sizes of limpets surviving in each replicate. Survivorship within the high density replicates was much poorer than in the lower densities (see Fig 4.2.1.5), possibly also partially explained by these growth factors.

Table 4.2.1.5 shows highly significant results ($p<0,001$) when considering the density results:

(a) when combining all temperatures, increasing the density decreased the growth rate.

(b) at 15°C, D10 had a greater growth rate than D20 or D30.

(c) at 20°C a density of 20 had the greatest growth rate.

(d) at 25°C D10 had the greatest growth rate.

(e) when comparing the growth rate at all temperatures of D10 and D20, D10 was higher, but at a less significant value ($p<0,005$).

Conclusion to Section 4.2.1.5.

This experiment suggests that a temperature of 25°C and a density of 10 per 500ml water would attain the greatest growth rate for *Burnupia*. Although this trial was investigating the effects of temperature and density on the growth and survival of limpets, an important factor, when considering the propagation of these animals, is the effect that temperature and density will have on their fecundity. The mean number of eggs per egg case and the viability of those eggs should now be considered, under specified conditions of temperature and density. Chernin and Michelson (1957a and b), Wright (1960) and Eisenberg (1966) all showed in their laboratory results with Basommatophorans that there was a negative effect on the mean clutch size with increasing density. Streit (1985) suggests carbon partitioning between growth and egg production is also a function of temperature, remaining constant for *Ancylus* from 13°C to the lethal temperature. The situation within *Burnupia* needs to be investigated (see section **4.2.2.**).

4.2.2 REPRODUCTION AND FECUNDITY

The fecundity and breeding biology of an animal under given conditions is essential information in the establishment of an aquacultural programme. The family Ancylidae is recognised as hermaphroditic but there is no information in the literature on the reproductive biology of the genus *Burnupia*.

Questions that need addressing include:

a) How many egg-cases and eggs can be expected from a single or pair of adults and what is the range and average number of eggs produced during the period of egg deposition under given conditions? Is the sequence in egg-case deposition similar to that reported for *Ancylus fluviatilis*?

b) What is the duration of the egg-laying period and which cues initiate reproductive activity?

c) Is cross fertilisation essential to reproductive activity and viable eggs?

d) Is reproductive maturity of both male and female gonads sequential or simultaneous and at what age or size does maturity occur?

The following experiments aimed at answering these questions.

4.2.2.1 Egg Development

Aim

To ascertain from each egg capsule laid, the number of eggs within each capsule, the number of spat that emerge, the number of days to emergence and to ascertain embryological development on a daily basis.

Method

1) Adults were collected from the Blaauwkrantz River and conditions at the time of collection were recorded from midstream, the area from where the majority of adults were collected. Riverine water was used as the holding water for the adults, in an aerated basin at 19°C in the CER.

2) Numbered glass microscope slides 2,5cm x 7,6cm were previously prepared by placing in a recirculating stream where they developed a thin layer of green algae.

3) A series of 9 bubble pots (labelled 1-9) were set up. Numbers 4 and 5 were white 5 litre buckets, and the remainder were 500 ml transparent plastic jars. Each bubble pot was kept half-filled with water. A sling of white netting supported a prepared slide on which the adults were placed, in the water. The lids of the small pots were placed loosely on the top in order to reduce the evaporation rate of the water. The water used was from the Belmont River collected at the same time as the adults, and distilled water was used for any replenishment in order to maintain the levels of total dissolved salts. TDS, temperature and pH were monitored twice weekly. After 9 days the water was replaced with Palmiet River water and TDS, temperature and pH were

again noted.

4) One adult between 4,5mm and 6mm in length was placed on each slide. When an adult died it was replaced with either one or a pair of adults of approximately the same size.

5) Daily checks at approximately 08h00 were carried out to monitor newly laid egg capsules. When new capsules were noted the slide was moved to a labelled 500 ml bubble pot equipped as described above, in order to follow the individual development of each egg capsule. The adult in the original bubble pot was placed on a new, prepared slide .

6) The daily development of each egg capsule was recorded on a separate data sheet. Black and white photographs and coloured slides were made throughout the entire development of the capsules. Photographs were taken with a WILD Heerbrugg M400 microscope and magnification was usually 10 x 32 unless stated otherwise in the captions to the photographs.

7) The experiment was considered complete when no further egg laying occurred, and all the young had emerged. Adults were placed in the system on 1 February, the experiment proceeding for three weeks.

Results

See Table 4.2.2.1.

Summary of table

During the experimental period 26 egg capsules were laid of which 4 (15,38%) were accidentally damaged. These will be excluded from the remainder of the summary. From the remaining 22 egg capsules laid, 134 eggs were counted (average = 6,09/ capsule). These eggs yielded 122 spat which is a hatching rate of 91,04%.
The average period from lay date to hatching was 14,9 days (n = 21).

Water conditions

Table 4.2.2.1 Water quality parameters recorded during the experiment.

Parameter	River water	Laboratory water
TDS	506 mg/l	148-591 mg/l
pH	6.8	6.22-7.62
Temperature	23.1°C	19.0-23.4°C

Temperatures varied between 19°C and 23,4°C averaging 21°C in all the containers. There was a small variation between the pots at any one time despite identical ambient conditions. This may indicate that differences in the aeration rates between the pots affect evaporation rates and hence temperature.
Similarly, the turbulence created by the aeration is substantially less in pots 4 and 5 with larger volumes of water than the other smaller pots. Note that a greater number of eggs were laid in these two pots.

Oviposition and embryonic development

Most pulmonates exhibit direct development and emerge as crawling snails. In *B. stenochorias* as for other Ancylidae, the oviduct opens to the left, as seen in Fig.4.2.2.1.3 a) which depicts a copulating pair.

The Basommatophora show extreme variability in the processes preceding oviposition and these appear partly dependant on external factors. Observations have shown that Ancylidae, although hermaphrodite, do copulate, with usually the smaller (younger) individual acting as the male (Bondesen 1950). This has been observed in the artificial streams with *B. stenochorias* and it has been established that some individuals will lay eggs without the benefit of copulation (4.2.2.4). Chain copulation as was seen in Planorbidae and Lymnaeidae species (Duncan 1975) has not been observed with *B. stenochorias*. It has been observed that sexual development can vary from one hermaphroditic individual to another, but usually within the Ancylidae the male organs develop first (Duncan 1975). The sexual development of *B. stenochorias* will be elucidated when stained sections of individuals of known ages are studied in October 1995 (see section **4.2.3**).

Oviposition

Oviposition in freshwater snails is primarily dependant on sexual maturity and water temperature, and since growth and maturation are in turn dependant on temperature, this factor has a major role in controlling reproductive cycles (Duncan 1975). Brown (1967) shows that the distribution of the genus *B. stenochorias* is linked to the warmer regions of South Africa. Temperatures within this experiment varied from 19°C to 23,4°C.

In this experiment eggs were laid between 11h00 and 05h00. The majority of Gastropoda appear to oviposit during this time period although *Ancylus fluviatilis* (Muller) has been known to lay until midday (Bondesen 1950). Oviposition takes place underneath any substrate provided, but egg capsules are also deposited on the surface of the containers.

Table 4.2.2.2 The number of egg capsules deposited during the experimental period. The figures in brackets in the table represent the following: (one capsule)(no. eggs)(no. spat hatched)(no. days to hatching).

Date	Pot 1	Pot 2	Pot 4	Pot 5	Pot 7	Pot 9
2/2		1(1)(1)(14½)	1(8)(8)(13½) 1(8)(8)(13½)			
3/2	1(7)(7)(13½)		1(8)(killed)	1(9)(9)(15½)		
4/2	1(6)(6)(14½)			1(8)(8)(14½) 1(9)(9)(13½)	new adult	new adult
5/2	1(1)(0)					
6/2			new adult			
7/2		new adult		1(8)(8)(13½)		
8/2			1(5)(5)(15½)			
9/2		new pair	mating			new pair
10/2			1(8)(killed) 1(6)(6)(15½)			
11/2			1(5)(5)(17½)		1(5)(5)(14½)	1(4)(2)(15½) 1(3)(3)(14½)
12/2		1(7)(6)(13½)		1(6)(6)(17½)		
13/2				1(1)(1)(16½)		
14/2		dead adult				
15/2		1(5)(killed) 1(2)(killed)		1(6)(6)(15½)		
21/2			1(8)(8)(15½)	1(5)(5)(15½)		

Generally the size of eggs produced relative to the amount of albumen and yolk varies in pulmonate gastropods (Bondesen 1950). In *B. stenochorias* the area of the egg case is slightly smaller than the area of the shell of the fully developed egg. The freshwater pulmonates possess an external membrane, the continuation of which is referred to as the 'egg string', an important phylogenetic character. *B. stenochorias* does not have this feature which confirms it as a member of the family Ancylidae. The terminology of the egg capsules is shown in Fig 4.2.2.1.1.

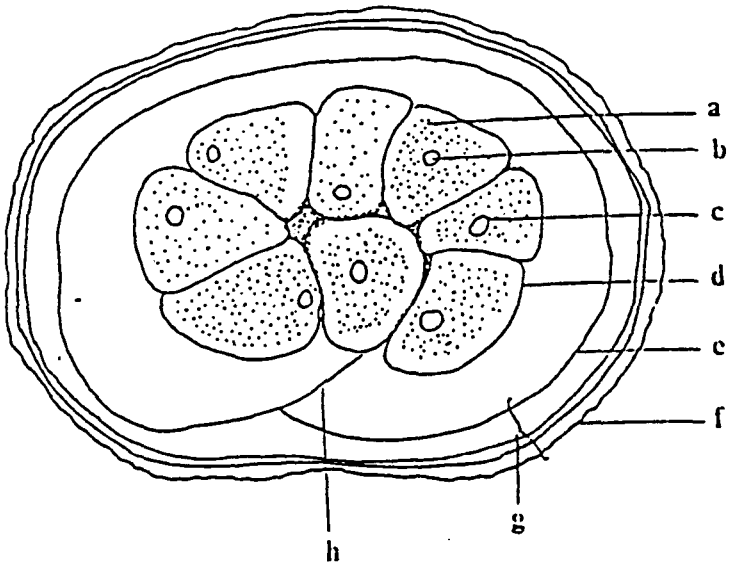

Fig. 4.2.2.1.1. Terminology of the egg capsule structure of *Burnupia stenochorias* (a = albumen, b = yolk (a & b = egg or ovum), c = yolk membrane (primary membrane), d = internal membrane (egg case or secondary envelope), e = internal capsule, f = quaternary envelope, g = external capsule, h = terminal tail).

The capsule of *B. stenochorias* is gelatinous, disc-shaped and slightly convex, 1,3 - 1,8 mm wide and 1,4 - 1,9 mm long. The quaternary envelope (as seen in Fig. 4.2.2.1.1) is unique to the Ancylidae and is an irregular membrane secreted by the foot, covering the capsule itself. This affords protection against parasites and foreign bodies. The resilient nature of the capsule perhaps makes it more suitable for laboratory handling than other non-Ancylidae species. The operculate suture which acts as a preformed line of rupture through which the young will emerge, appears to follow the edge of the capsule but can not be differentiated in our photographs. The capsule appears transparent immediately after oviposition, changing to pale yellow as it matures. The eggs are initially transparent and gradually become darker yellow as embryos develop. Because of the transparency of the structure it is extremely difficult to see with the naked eye on opaque surfaces. Since studying the egg capsules so intensively we have succeeded in observing eggs in the field.

The terminal tail of the internal capsule is closed and turned more or less over the initial point and covered by the external capsule, which indicates that *B. stenochorias* does not turn as it oviposits, a behaviour which is characteristic of most freshwater pulmonates. The eggs are not in a spiral formation as in the Lymnaeidae but are in one layer. The position of each egg relative to the others indicates the order of succession of lay, and when viewed from the upper side, the first egg is at the top, the second egg has its front edge under the rear edge of the first egg, etc. Each egg is of a polygonal shape and varies in number from 1 - 13 per capsule, although in this experiment, the maximum number recorded was nine.

Embryogenesis

The daily recording of the embryonic development revealed variations in the period of time taken for organogenesis and hatching from the capsule. No attempt was made to determine the cause or to quantify the extent of this variation. Development is reported here in 24 hour periods although observations were made more frequently.

The embryonic period consists of two phases, namely cleavage and organogenesis.

Cleavage phase

Hours 0-24; DAY 0-1

Cleavage of the cells take place. Blastulation occurs and micromeres and macromeres are formed. The embryo is uniformly pale yellow and appears to remain the same size.

Hours 24-48; DAY 1-2,

During the gastrulation phase the cells which are formed during the blastulation are differentiated into the three primary germ layers. The divisions of the macromeres and micromeres are not synchronous, and consequently the embryo rotates in the albumen.

Organogenesis phase

DAY 2.5-4.5, Fig 4.2.2.1.2a

There is an increase in the rate of rotation of the embryo. The colour becomes deeper and the shape elongates from its initial circular form. The albumen is slowly absorbed by the embryo so that it is approximately ½ to ⅓ the size of the egg case, and appears as a concentrated grey mass: the albumen is stored in albumen cell complexes which deepen the colour of the embryo's developing digestive organs to a golden yellow. The remainder of the embryo is grey. Differentiation between the digestive area and the podocephalic area is visible. The embryo increases in size in relationship to the egg, which indicates considerable mitotic activity.

DAY 4.5-5.5, Fig. 4.2.2.1.2b

There is a dramatic increase in the size of the embryo in relationship to the egg and a clear difference in colour and form between the digestive region and the podocephallic area. The globular nature of the embryo is changing with the podocephalic area distinct from the remainder of the body.

DAY 6.5, Fig. 4.2.2.1.2c

The embryo tumbles very slowly within the egg case relative to previous days. The tentacular /optic lobes begin differentiating. The albumen continues to decrease in volume.

DAY 7.5, Fig. 4.2.2.1.2d

Tentacular lobes develop and there are visible signs of the eyes. A faint heart pulse is observed in a few individuals.

DAY 8.5 The eyes are red and clearly visible. Heart beat is obvious in the most developmentally advanced specimens with vortices of fluid movement around and within the embryo. Relative to other gastropods, the pulmonates are characterised by a relatively early organogenesis of the radula (Moor, 1983) and in *B. stenochorias* the position of the radula can now be seen in some individuals. Shell development can now be seen clearly, just covering the organs posteriorly and appearing to grow towards the anterior end

to eventually cover the head region. From the ventral side the foot can be seen developing. The albumen has been almost totally absorbed. There is considerable individual variation in developmental rate.

DAY 9.5 The internal membrane begins to collapse. Heart beat and the vortex movement of fluid are both easily observed. Further development of the shell shows the initial laying down of the circular patterns seen in the adult.

DAY 10.5 Fig 4.2.2.1.2e

The shell appears almost complete in the development of its circumference, deepening in colour to a golden brown as the striations develop. The inner egg membrane continues to collapse.

DAY 11.5

The embryos are all actively moving, bumping into each other. The shell colour deepens, and the rim now completely covers the entire animal. There is a strong heart beat and the radula is moving, while vortices of fluid movement are seen within the body. Remnants of albumen are still visible.

DAY 12.5-13.5

Movement of radula and heart is stronger and the larvae are active, occasionally turning over in their position. Internal membranes can still be seen. During this period the first hatchings occur from the egg cases.

DAY 14.5 The shell becomes more domed in shape. This is the average hatching date for this population. All internal membranes have collapsed.

DAY 15.5 Individuals are immobile prior to hatching from the capsule. Emergence from the capsule occurred in this experiment in the early hours of the morning until approximately 08h15, emerging as crawling snails, as seen with most pulmonates (Fretter and Peake 1975). However, unlike the *Ancylus* species, there is no lid opening; instead the larvae crawl under the external capsule, apparently by nudging head and shell against the edge until it lifts. As each individual emerges (with approximately 10 minutes between each one) it appears easier to move under the capsule and out. There appear to be no egg remains in the capsule.

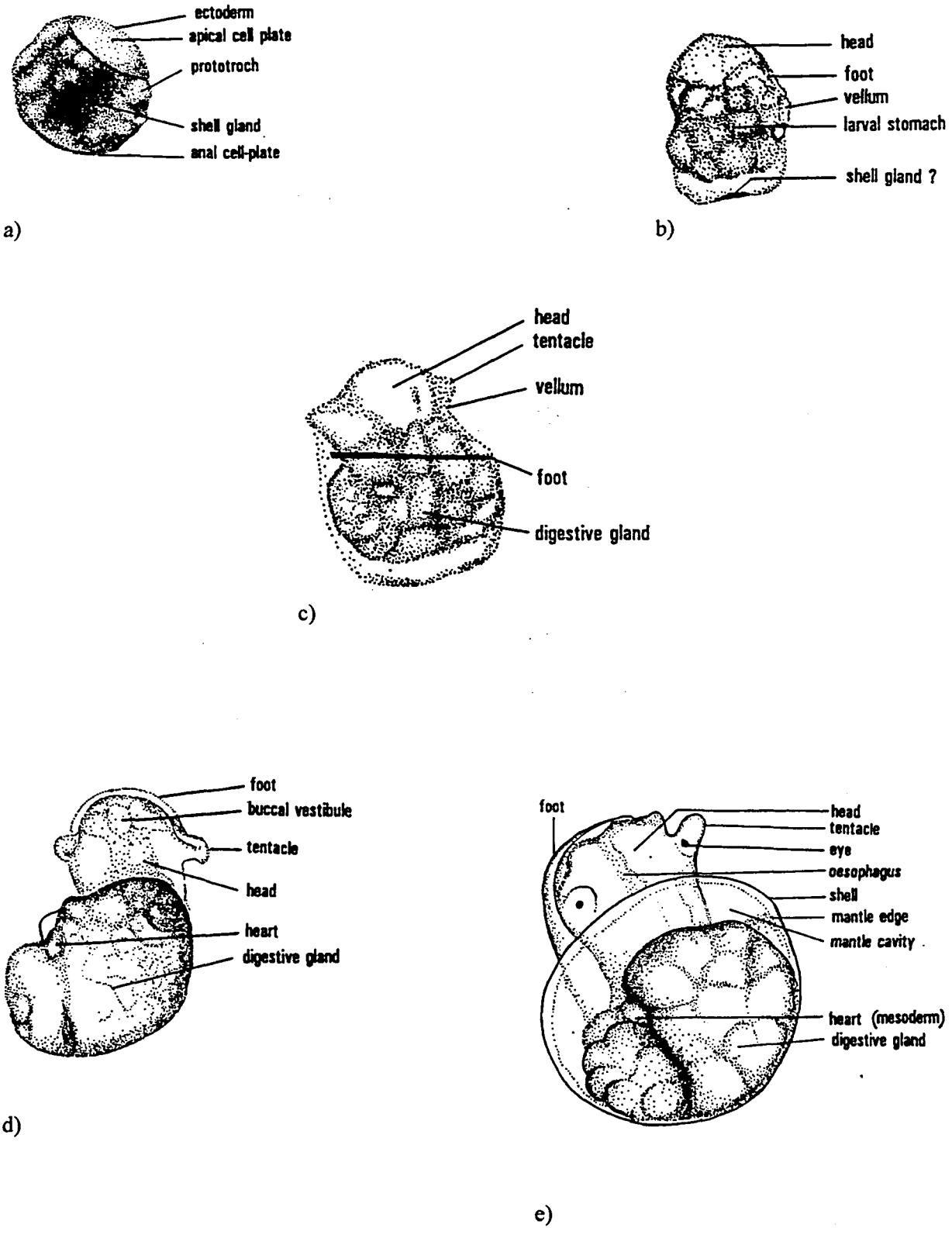

Fig 4.2.2.1.2 a) Embryo at about 96 hours old. Early organogenesis. b) Embryo at 5.5 days, body is elongating. c) In embryos at 6.5 days and d) at 7.5 days the adult morphology can be

discerned.

Discussion

This general overview of the major developmental events which take place in gastropods has been compiled from Raven (1975) and Moor (1983). Observations made during this work is related to their information. The embryos are very small and under available magnification details of the development, especially in the early stages of blastulation, were difficult to distinguish.

At the third division of the cells, spiral cleavage results in the formation of animal micromeres and vegetative macromeres. It has been elucidated in earlier studies that determination of the fate of micromeres and macromeres takes place at the very early stages of blastulation (after the third division). Cleavage gives rise to a coeloblastula with a wide cleavage cavity. Gastrulation takes place by invagination of the archenteron at the vegetative pole. The embryos at 2 days old depict this stage. The eggs of pulmonates in the cleavage stage begin to ingest albumen in all the cells. This was seen during day 2. The albumen is laid down in the ectoplasmic part of the cells in special albumen vacuoles, thought to be by a process of pinocytosis. After gastrulation, the uptake of albumen is more and more restricted to the endoderm. Part of the endoderm develops into the albumen sac, or larval digestive gland, which plays an important part in the uptake and digestion of albumen.

Organogenesis takes place in the following sequence:

The trochophore stage is where the dorsally situated head vesicle, the median apical plate and the ventrolateral cephalic plates are differentiated. During days 3-5 this differentiation became visible in the embryos. From the median apical plate the cerebral ganglia, tentacles and eyes develop. As in most other animals, the nervous system is mainly of ectodermal origin.

Before gastrulation is complete, cells in the dorsal (post trochal) area of the embryo enlarge to form a prospective shell field (Fig.4.2.2.1.2a). This area invaginates to form the shell gland, a narrow pit with a circular opening. The cells which secrete the periostrachum (early shell) are brought close together at the rim of the shell gland and thus the shell is formed with no hole in it. Subsequently the shell gland evaginates again and the shell spreads. The shell producing cells divide mitotically and the shell grows. In gastropods the shell field is saucer-shaped and this is visible in Fig.4.2.2.1.2b. The shell gland gradually changes to the mantle, from the edge of which the principal growth of the shell takes place (Fig.4.2.2.1.2e). The mantle edge therefore also contributes to the development of the mantle cavity.

The lung primordium arises posteriorly as an ectodermal invagination and the mantle cavity later surrounds this invagination and gradually the pneumostome forms. The walls of the pulmonary cavity fold to enclose adjacent blood vessels forming the vascular reticulum of the lung. This development was not observable under binocular microscope.

The foot originates as an ectodermal thickening on the ventral side behind the mouth at an early embryonic stage and only later an outgrowth covered with cilia appears, clearly seen during day 8.

The invagination of the stomadeum (mouth) starts on the ventral side and from there the remainder of the digestive system is formed. Basommatophora have a larval digestive gland, as described above, that disappears at the end of the embryonic period. The development of the radula starts early in embryonic life and by the time hatching takes place several radular teeth have already been lost. The earliest visible signs of the radula were during day 8.
The archenteron is the primordial lumen which later develops into the mid- and hindgut, and in the Ancylidae is lined with giant cells which contain the ingested albumen. The pronephridia consist of 2 - 4 cells with the efferent duct consisting of three cells.

The environmental factors which may influence egg laying include the level of turbulence and the TDS of the water. It was observed but not quantified that in the pots where the bubbling was the most severe, no eggs were laid. Two further interesting points emerged with regard to the TDS levels of water:

(a) pots 10 (8 eggs), 11 (1 egg), 12 (7 eggs), 18 (1 egg) had a TDS of 500. Only pot 18's single egg did not hatch.

(b) No egg capsules were laid where TDS remained below 95.

Future work should investigate the effect of turbulence and TDS levels on egg laying and hatching rate.

4.2.2.2 Capsule Size Distribution (in relation to number of eggs per capsule)

Aims

To establish the range and average size of egg capsules (number of eggs within each) laid by adults collected from the field, and to establish the developmental time from laying to hatching of the same capsules at 17°C.

Method

1) Approximately 300 limpets were collected in the field from the Blaauwkrantz River on 10 October 1993. The population was placed in a 44cm diameter plastic basin with natural stones, as well as artificial substrates which were covered with laboratory grown periphyton. The basin was maintained at ambient light and temperature conditions (16±2°C - 23±2°C, 14h00 photoperiod) with water from the river which was aerated.
2) After 12 days, 18 stones and tiles which had periphyton cover were removed from the basin and placed in six 5 litre bubble pots in the C.E. room at 18 °C and 14h00 photoperiod. The bubble pots, labelled 1 to 6, contained a mix of river water and conditioned tap water. The water was kept at the same level by the addition of both mature tap water and distilled water to maintain TDS and pH at a similar level.
3) Every second day the substrates were examined and the development of the capsule and

eggs noted. Before and after hatching the population in each container was counted and a sub-sample measured under a Nikon stereo-microscope equipped with an eyepiece micrometer. The measurements continued at two week intervals for four months, the last being taken at 139 days. The early counts of the population once the juveniles had started moving around were inaccurate as the animals were small (0.8 - 1.0 mm shell length) and pale in colour which made them difficult to see with the naked eye, particularly once they moved onto the container. Early numbers are therefore extrapolations from later, more accurate, numbers of survivors.

Results

Table 4.2.2.2.1 Water conditions prevailing in disturbed pots.

CONTAINER	1	2	3	4	5	6
Ave TDS	422.3	374.4	332.5	335.1	336.6	381.1
STD	115.7	93.1	87.2	93.8	102.8	100.1
MAX	679	650	650	565	606	602
MIN	248	271	227	220	230	253
Ave pH	7.54	7.45	7.56	7.6	7.53	7.47
STD	0.53	0.50	0.58	0.64	0.60	0.57
MAX	8.23	8.16	8.38	8.48	8.31	8.21
MIN	6.69	6.59	6.62	6.65	6.75	6.76

Table 4.2.2.2.2 Water conditions in undisturbed pots.

CONTAINER	H	I	J	K	L	M	N	A	B	C	E	F
Ave TDS	234.6	255	185.7	209	222.6	314.9	263.35	402.7	396.3	331.5	237.5	182.5
STD	18.62	13.41	195.92	23.52	20.68	97.62	36.36	56.90	62.27	42.11	49.03	195.26
Max.	255	274	739	249	275	453	314	490	555	381	373	700
Min.	210	245	67	171	187	0	201	312	281	242	141	75
Ave pH	7.57	7.32	7.6	7.4	7.4	7.3	7.3	7.36	7.53	7.15	7.66	7.68
STD	0.43	0.40	0.30	0.32	0.32	0.33	0.37	0.38	0.44	0.25	0.64	0.24
Max.	8	7.9	7.9	8	8	8	8	8	8.1	7.5	8.9	8.1
Min.	7.13	7	7.01	7.06	7.07	7	7	7	7	6.9	7.1	7.4

Table 4.2.2.2.3 Number of egg capsules, average number of eggs per capsule and total number of eggs examined in each container.

Container	Egg Capsules	Eggs	Average (std) Eggs/Capsule
1	60	285	4.8 (2.45)
4	51	276	5.4 (2.77)
2	35	184	5.3 (2.27)
3	41	229	5.6 (2.91)
5	13	67	5.2 (2.41)
6	9	55	6.1 (2.92)
TOTALS	209	1096	5.3

Average number of eggs in total number capsule = 5,4 (1-12).

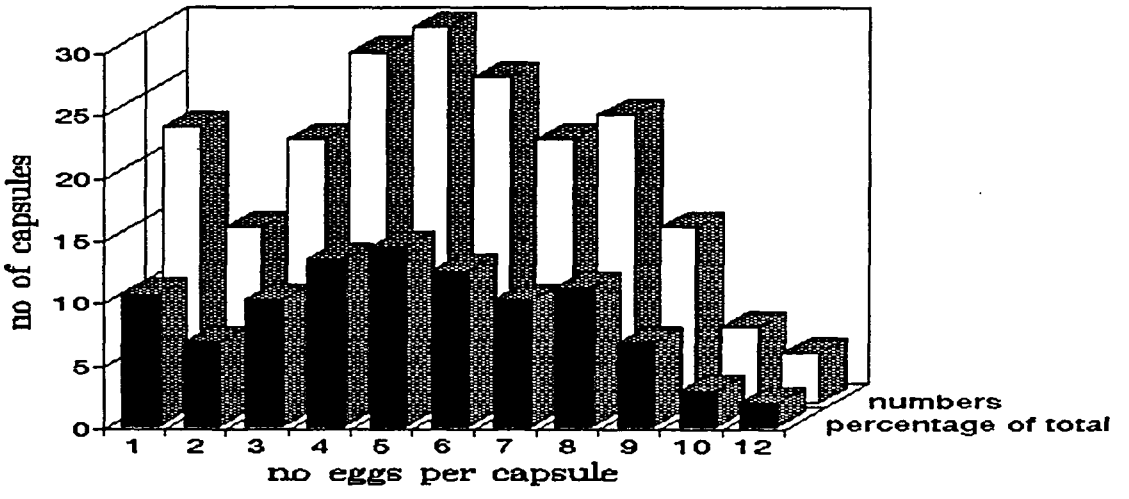

Fig 4.2.2.2. Frequency distribution graph of the range and frequency of size of capsules deposited in late spring to early summer (October 1993).

Initially the capsules are totally transparent, but after an average of six days the eggs within the capsule become visible and can be counted. See Fig 4.2.2.2.2 and Tables 4.2.2.2.3 and 4.2.2.2.4. Note the low number of capsules containing 2 eggs and the relatively larger number containing only one egg (Fig. 4.2.2.3). Although not reflected in the bar graph the largest number of eggs per capsule found was 13.

Developmental period

If one assumes that egg-laying commenced on 11 or 12 October, by 22 October the oldest embryos were visible and hatching commenced between 1 and 3 November, 21-24 days after capture. The results obtained in the section on development showed that the incubation period is 14-17 days but the longest period recorded to date is 19-20 days. This would put the earliest lay-date in this copulation at 15 October. The last possible lay-date, therefore, is 21 October which gives a range in age of 5 days. Most hatching was complete by 8 November. If these eggs were deposited between 19 and 21 October the developmental time was 18 days.

Table 4.2.2.2.4 Dates of developmental events noted in six sample populations of egg capsules. E L-D = earliest lay date; L L-D = latest lay date; E Hatch = date of earliest hatch; E Days = number of days to hatch; 50% H-D = date when 50% hatched; 50% Days = number of days to 50% hatched.

Rep	E L-D	L L-D	E Hatch	E Days	50% H-D	50% Days
1	16.10	21.10	2.11	17	8.11	19
2	15.10	21.10	1.11	17	7.11	19
3	15.10	21.10	1.11	17	8.11	20
4	14.10	18.10	31.10	17	3.11	17
5	16.10	21.10	2.11	13	7.11	18
6	16.10	21.10	1.11	17	8.11	18

Size and age

Because the late embryos are easily observed through the eggcase prior to hatching accurate measurements were obtained for the pre- and post hatching period. When eyes can be seen the embryo is usually between 0.3 - 0.5mm long. Sizes of 14 to 17 day old embryos immediately prior to hatching ranged from 0.32 - 0.72mm. At this stage the shell is golden brown in colour. At hatching sizes range from 0.65 - 0.8mm. At a shell length of 1.2mm, the shell is a golden brown colour. At about this size the shell darkens and the first transparent rim of the newly deposited shell can be observed by 1.3mm.

Conclusion

The number of eggs in each capsule varied from 1 to 12 (occasionally some capsules containing 13 have been noted), with an average of 5,4 per capsule. Developmental time under these conditions of temperature, pH and TDS was on average 18 days.

4.2.2.3 Fecundity in the Laboratory Under Different Temperatures

Aims

To establish the fecundity of *B. stenochorias* under different temperature regimes by determining the number of egg-capsules and eggs a mature adult limpet produces: and to determine if fecundity is affected by short-term temperature changes.

Method

1) 6 x 5litre bubblepots each had a sling of white netting suspended from the rim onto which was suspended a ceramic tile 6cm x 6cm. All tiles had a covering of periphytic growth. Each bubblepot was half filled with water from the river of origin, covering the tile, and was stabilised for two days before the adults were introduced.
2) Experiment (A) had two adults of similar size which had been taken from the natural habitat placed on the tile after their length had been measured. Experiment (B) had four adults of similar size placed on each tile. In both cases adults that died were replaced by similar sized animals in the initial stage of the experiment. When no more adults were available or more than 50% of the adults had died the experiment was terminated.
3) Observations were made every second day. The egg capsules deposited on both the ceramic tiles and the sides of the pots were counted and the number of eggs noted.
4) Both experiments were conducted under three temperature regimes: (A) 19°C, 25°C and 14h00 photoperiod. (B) 15°C, 20°C and 14h00 photoperiod.

Results

Table 4.2.2.3 Summary of results obtained from two experiments to determine the reproductive capacity of *B. stenochorias* in the laboratory.

Treatment	Total adults	Total		Ave eggs /adult	Ave eggs/ treatment	STD.
		Capsules	Eggs			
22°C (amb.)B	23	134	395	17	43	38
15°C B	16	49	184	12	26	11.5
20°C B	17	81	384	23	64	113
20°C(amb.) A	15	34	178	12	25	n/c
19°C A	19	61	335	17	48	n/c
25°C A	9	13	72	8	10	n/c

Despite the use of the sling the limpets occasionally moved onto the sides of the pots where a large number of the eggs were laid. This caused difficulty in determining the number of eggs in each of these capsules. Table 4.2.2.3 shows the number of adults used in each of the three temperature regimes for the

entire duration of the experiment, the total number of eggs produced by these adults in each of the treatments, the average number of eggs per adult per treatment and the standard deviation in each of the treatments.

The possible number of eggs produced by an adult ranged from 4 to 49. As four eggs per adult was clearly an unlikely result in view of the largest possible number these were not included in the frequency distribution but were included in the assessment of the standard deviations.

Discussion

Both fecundity experiments produced inconclusive and widely variable results. The number of cases per adult limpet ranged from one to five but no conclusions could be drawn from this experiment with regard to the fecundity of this species. Further experiments were required before any substantial information and conclusions could be drawn. The cause of the high adult mortality was not determined, but is likely to have been the chemistry of the water. Such premature death of an adult does not give a true reflection of its reproductive capacity.

The findings of Appleton and Eriksson (1984) and de Kock and van Eeden (1986) indicate that *Biomphalaria pfeifferi* would benefit under laboratory conditions from a temperature regime with a daily fluctuation of more than 10°C. Constant temperatures in previous experiments (de Kock and van Eeden, 1981) revealed that the highest values for different population parameters such as natality, survival and growth rates were generally attained at different constant temperatures. With *B. pfeifferi* the lower temperatures, as a rule, favoured longevity while the higher temperatures favoured growth rates and natality. This suggests that a circadian temperature fluctuation within a breeding system for *Burnupia* may favour all physiological activities. The importance of combining the temperatures experienced with day-length hours to give Degree Days, a known physiological activator, is emphasised here. The results of the analyses of the field data of ambient conditions, combined with the data pertaining to size, relative numbers and egg-laying presently being accrued (section **4.2.3**) should be linked to the fecundity experiments.

4.2.2.4 Eggs from Single Limpets

Aim

To determine whether unmated individuals will lay eggs, how many they will lay and the frequency of laying.

Method

1) On 30 September 1994 single limpets taken from the Blaauwkrantz River, from 2,5mm in length, were placed in 500ml bubblepots together with two ceramic stones which had previously been allowed to grow periphyton. The pots were kept at ambient temperature

and light in the laboratory.

2) By 28 November 1994 all limpets had a shell length of 3mm or longer, and the first eggs were laid (not necessarily in all the pots).

Results

See Table 4.2.2.4.

Average capsules per individual (#10) = 6,7. Average eggs per individual (#10)= 20,7

Average laying frequency (# 7) = 5,8 days. Average size at initial laying (length) = 4,4

Average water pH = 6,64 (6,51-7,21) and TDS = 183 (144-228)

Table 4.2.2.4 Summary of the results of the egg-laying capacity of single limpets.

Size = length of each individual from 28 November 1994 until 19 February 1995 at the end of the experiment, or at the time of death; Size laying = size (mm length) at which laying began. Caps = no. of capsules layed. Frequency = average no. of days between each capsule laid, from first day of lay. * = smallest size at which laying occurred. Hatchlings = total number of young which grew to 1mm in length.

Pot no	size mm	Size laying	Date died	Caps	Eggs in capsules	Total no eggs laid	Frequency days	Hatch lings
1	3-4	-	14.2.95	0	-	0	-	-
2	3,6-4	3,6 *	20.1.95	17	1-5 ave.3,6	62	3,6	26
3	3,7-4,3	4	11.1.95	6	1-8 ave.3,8	23	8,3	10
4	3,5	-	12.12.95	0	-	0	-	-
5	3,7-6	5	19.2.95	7	1-6 ave.3,2	22	7,5	22
6	4,4-5	-	14.2.95	0	-	0	-	-
7	4,4-5	4,7	28.1.95	11	1-4 ave.2,3	25	3,2	24
8	3,5-4,6	4,5	19.2.95	4	1-3 ave.1,5	6	6,2	1
9	3,5-4,5	4,5	-	5	1-3 ave.1,75	9	7,2	1
10	4,2-5	4,5	14.2.95	17	1-6 ave.3,5	60	3,6	27

Discussion

Observations have shown *Burnupia* to be greater than 3mm in length when mating, therefore it is unlikely that these limpets had mated before being brought from the field and separated in the laboratory. Water conditions were acidic with low TDS levels when compared to the natural conditions of the Blaauwkrantz River (see Section **4.2.3**): this may have been the cause of premature death for the limpets, thus potentially decreasing the total number of capsules laid per individual within this experiment. Approximately 70%

of the limpets laid, at this time of the year, without mating. Of those that laid, all were 3,6mm or greater in length. There was a great variability in the number of capsules and eggs laid, which has been a consistent observation through all the *Burnupia* experiments.

4.2.2.5 Eggs from Mating Pairs, July 1994

Aim

To determine the number of eggs laid by mating pairs.

Method

1) Large numbers of limpets 3mm in length were collected from the Blaauwkrantz River and maintained in the laboratory streams.
2) Those pairs that were then found mating (11) were carefully removed from the streams, measured for length, and placed, still as mating pairs, in 500ml bubblepots. They were kept in tap water under laboratory conditions of ambient temperature and light.
3) The number of capsules and eggs laid was monitored.

Results

Table 4.2.2.5 Number of eggs and capsules laid by mated pairs. Male = length (mm) of "male" in mating pair, at the time of mating. Female = length (mm) of "female" in mating pair, at the time of mating.

Pair no	No of capsules	No of eggs	Male mm	Female mm
1	44	160	5,15	4
2	24	68	4,3	4,15
3	20	85	4,6	5,3
4	24	130	5	5,7
5	21	92	5,15	5,4
6	30	134	4,15	4,9
7	19	91	4,7	4,6
8	21	84	4,4	4
9	5	16	3,4	4
10	28	98	5	4
11	14	44	3	4
Average	22,7	91,1	4,2	4,55

Average laying frequency = 2,2 days

Water Averages; pH = 6,9 (6,2-7,4); TDS = 135 (119-188); temperature = 17,6°C (14°-23°C)

Discussion

It has often been observed that when moving Ancylidae from natural conditions into the laboratory, Ancylidae will immediately mate and invariably lay eggs (Calow 1978). This was the case here. As can be seen from Table 4.2.2.5 the sizes of the limpets within each mating pair differed considerably. With the exception of one, all the limpets were 3,4mm in length or greater, and as personal observations have shown limpets greater than 3,4 are capable of laying eggs, it seems very likely that many of these limpets had already laid eggs in the field before they were collected, an important factor when considering the total potential offspring of each pair, or each individual. Similarly, many of these limpets could have already mated in the field, before again mating in the laboratory. The effect this has on the fecundity of each limpet is unknown, particularly as the Ancylidae possess the ability to store all allosperm (from a second limpet) in their spermatheca . This will be discussed further when the field and sexual development trial, section **4.2.3**, is completed in 1996.

Nevertheless, the results of this experiment can be compared to that in single limpets (section **4.2.2.4**) and pairs of limpets (section **4.2.2.6**).

4.2.2.6 Eggs from Paired Limpets, March 1995

Aim

To determine the number of eggs laid by a pair of limpets, not necessarily having mated.

Method

1) 25 pairs of small (1,8-2mm length) limpets were collected from Blaauwkrantz River and placed in 500ml bubblepots, together with 2 ceramic stones previously allowed to grow periphyton, and tap water.
2) When a limpet died it was replaced with the same size individual which had been taken from the Blaauwkrantz River at the same time. Water was replaced every week.
3) This is an on-going experiment. The number of capsules and eggs laid are monitored, as well as the occurrence of any observed mating.

Results

For the first 30 days the mortality was 60%. Although each dead limpet was replaced, this did not include sizes greater than 3,5mm, the sizes at which mating occurs (pers. obs.). Any eggs which were produced were either a result of the pair mating, or a result of self-fertilisation. Only 2 of the 25 pairs were observed to have mated (however, observations were only made during office hours). Capsules were first laid on 4 May 1995, 55 days after collection from the field.

Water Averages; pH = 6,7 (6,0-7,47); TDS = 146 (116-201); temperature = 17,4°C (15,1°-19,5°C)

To date, 50% of the limpet pairs have not laid capsules (the data shows the 12 that have laid). The length of time an individual lived after the final replacement at 30 days varied considerably. At 91 days (9 June) 25% of the limpets had died, and this figure was 42% after 102 days (20 June) leaving 21 of the original 25 pairs as single limpets. Although on-going, no eggs have been laid since 20 June 1995.

Table 4.2.2.6 Summary of the results of the egg-laying capacity of pairs of limpets. Size laying = size (mm length) of the smaller of the pair at which laying began. Capsules = no of capsules laid. Frequency days = average no of days between each capsule laid, from first day of lay. * = smallest size at which laying began.

Pair no	Size laying	Capsules	Eggs in capsules	Total no eggs	Frequency days
1	3,7	16	1-5 ave.3,5	53	3,6
2	3,5	9	2-4 ave.3,1	28	3,3
3	3,5	10	2-5 ave.3,8	38	2,1
4	4,2	12	2-6 ave.3,5	41	4,3
5	3,5	1	3	3	-
6	3,5	9	1-4 ave.2,3	21	2,3
7	3,7	6	1-3 ave.3	17	5.2
8	3,6	14	1-5 ave.3	42	1,6
9	3,7	10	1-5 ave.3,1	31	5,4
10	3,4 *	4	3 ave.3	12	3,3
11	4	2	2-3 ave.2,5	5	6,5
12	3,8	4	2-4 ave.3	12	4,5
Total average	3,8	8,1	2,88	25,3	3,8

Average capsules per individual = 8,1. Average eggs per individual = 25,3
Average laying frequency = 3,8 days; Average length at initial laying = 3,8 mm

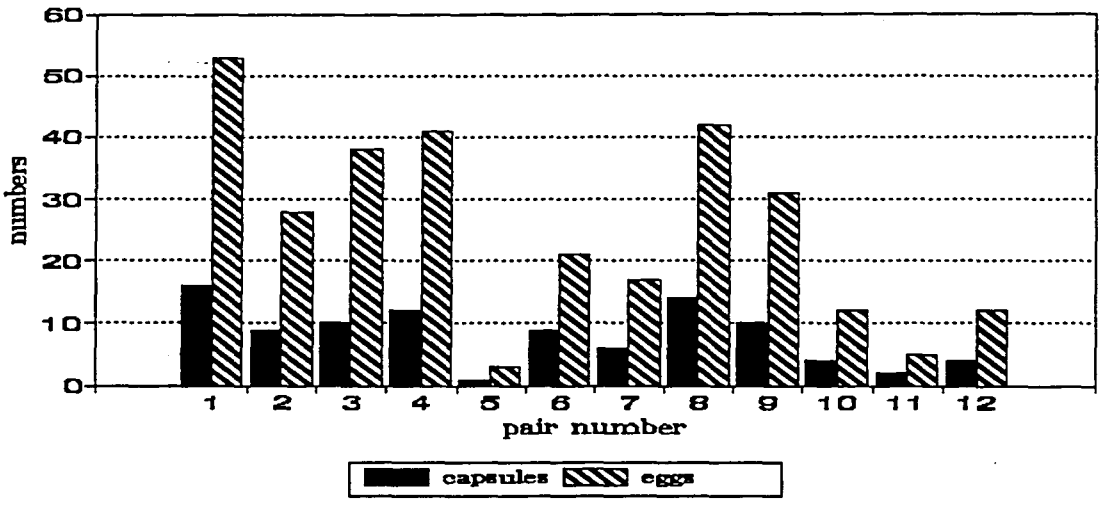

Fig. 4.2.2.6 Graph showing the number of capsules and eggs laid by the mating pairs, March 1995.

Discussion

The pH and TDS values are low when compared to the Blaauwkrantz River values. To date, none of the limpets have grown larger than 4mm, the majority being 3,5mm or less, and it is suggested that these water conditions are having a significantly detrimental effect on both the growth and fecundity (Macan 1961, Dussart 1979). Although the water conditions of the two pair experiments (this and section 4.2.2.5) were very similar, the number of capsules and the number of eggs per capsule are significantly different, that of the mating pairs showing the greater fecundity. This difference may be due to the size of each limpet, the mating pairs being larger; or as a result of decreased fecundity from being reared in the laboratory. The number of eggs per mass in some pulmonates is also related to the quality and quantity of food available to the snails (McMahon *et al* 1974, Hunter 1975, McMahon 1975a): this may also account for differences between the two pairs experiments, where the mating pairs had a natural, more ideal food supply before and during maturation of their reproductive parts, compared to the pairs which had been grown in the laboratory. When they are completed, the results of the field study should therefore be compared to these.

4.3 NATURAL POPULATIONS

Introduction

It is necessary to investigate the natural reproductive cycle of any animal before it can be bred in the laboratory. The patterns of reproduction which emerge from field studies may not necessarily reflect a typical cycle, but may answer many questions, such as: what time of the year, and how often does the species reproduce. These are two vital points when considering rearing in the laboratory. It is appreciated that snails in particular show interpopulation variations in individual growth (Russel-Hunter 1961, Hunter 1975), age and size at reproduction (Brown 1985), fecundity (Browne 1978, Aldridge 1982) and other life history traits (Russel-Hunter 1978, McMahon 1983). There are two possible, non-mutually exclusive explanations, according to Lam and Calow (1989): (i) the differences could be a result of microevolutiuonary adjustments in isolated populations to local selection pressures, in ways that can be predicted by life history theory; (ii) interpopulation divergences might entirely be an expression of developmental plasticity in response to proximate environmental differences.

Very few species within the Ancylidae have been studied in southern Africa and life history variations have not been studied within the genus *Burnupia*. The majority of the *Burnupia* species are found in Africa, south of the Equator, with a small pocket to the north of the Equator (Brown 1980). However, much of the available literature dealing with Ancylidae and other freshwater pulmonates refers to northern hemisphere molluscs living under temperate conditions. Those species closely related to *Burnupia* will provide basic guidelines to various aspects of any investigation into *Burnupia*, but will not provide life history patterns.

Method and Sites

(1) This field study began in February 1995 and is on-going.

(2) Two sites on the Blaauwkrantz River system were chosen because of their easy accessibility. The first, in the Belmont Valley 9 kms from the centre of Grahamstown (referred to as BV), is partially shaded by trees and a steep bank on the north-eastern edge. This site has a bed of small rocks and large boulders (ranging in longest length from 6cm to 41cm) and current speeds ranging to date from 0,32 to 1m/sec . The second site at Manley Flats (called MF) is approximately 4km downstream with grassed banks and considerable light incidence throughout the day. The bed is of fine silt with boulders ranging from 17-38cm longest length, and current speeds ranging to date from 0,06 to 0,42m/sec.

(3) Individuals are measured biweekly to the nearest 0,5mm, and 300 recordings are taken, which includes the number of eggs. At MF these are found on 3 or 4 rocks: at BV many rocks are sampled in order to reach this number. Each rock is lifted out of the water and all individuals are measured *in situ* using a template of washed photographic film into which measured holes have been punched. The rock is then replaced. Biweekly measurements of TDS, $\%O_2$, pH, temperature and current speed are also taken.

(4) 6-10 limpets ranging in size from 2,5mm to the 6mm are removed from outside the measuring sites, and their shells are measured for length, width and height before being preserved using Bouin's fluid, for later mounting in wax, and sectioning in order to monitor the sexual development of cohorts throughout the year. The sectioning of limpets will begin in October 1995, when the development of the penis, the maturation of the ovotestis and the bursa copulatrix will be analyzed.

(5) From August 1995, samples of egg capsules from each site, away from the area of rocks which are measured, are brought back to the laboratory, still attached to the rock, where they remain in aerated water until the number of eggs within each capsule can be counted. These rocks are then returned to the stream.

(6) Three further sites have been chosen which will be monitored in the same way, excluding measurements of sexual development, every 3 months in order to encompass a wider variety of field site conditions.

(7) From October 1995, biomass of periphyton at the MF and BV sites will be estimated using a method similar to that of Lam and Calow (1989), in order to monitor seasonal and site variations. Similarly gut contents will be monitored, to a limited degree because of the lack of specimens which can be removed and dissected from the BV site.

Results

Daily measurements taken during late February at both sites determined that *Burnupia* moved very little from one rock to another, suggesting biweekly measurements would very likely be measuring the same individuals each time, with any recruitment from other rocks upstream or nearby being minimal.

No results have been included from the three sites which will be monitored every three months, as there is insufficient data to date. Neither is there any data showing daylight hours, as this will be considered when all field work has been completed.

Table 4.3.1 Water conditions at the two sites, from February to August 1995.

Site: Blaauwkrantz R.	Factor	Maximum	Minimum	Average
Manley Flats	Temp °C	21,8	9,5	14,1
	TDS	842	284	632
	pH	8,4	5,7	7
Belmont Valley	Temp °C	22,8	9,5	14,1
	TDS	843	401	643
	pH	8,3	5,7	7

Fig 4.3.1 and Fig 4.3.2 show the changes in current flow and water temperatures (monitored at the time

of sampling) at Manley Flats and and Belmont Valley.

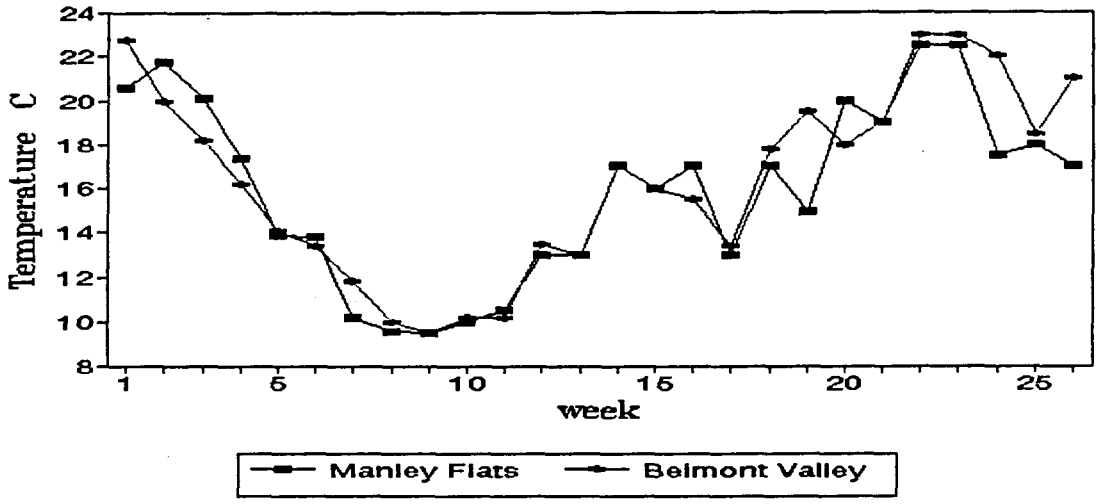

Fig 4.3.1 Changes in temperature (°C) at the Manley Flats and Belmont Valley sites.

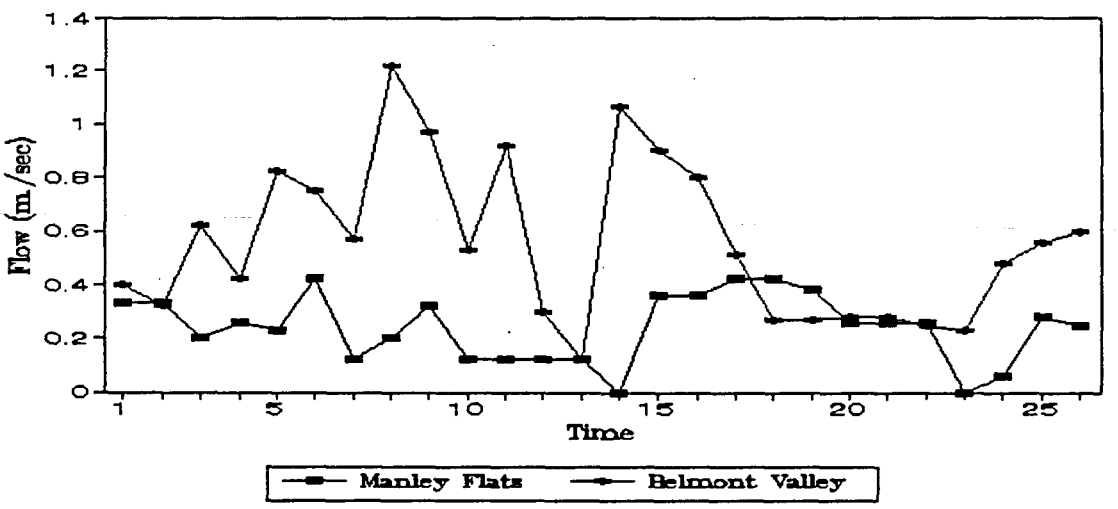

Fig 4.3.2 Changes in flow rate of water at the Manley Flats and Belmont Valley sites.

The results given in Fig 4.3.3 and Fig 4.3.4 show the numbers of limpets found at each site, with each graph depicting the month's samples. Each bar represent the number of limpets measured during the biweekly sampling. The size 0mm indicates the number of egg capsules. Because the samples were taken biweekly, there are three sample dates within the month of March. Only one sample was taken in July, with previous and post sample dates being 23/06/95 and 3/08/95 respectively and this sample is included in the June graph.

No attempt has yet been made to date to identify periphyton species. However, visual observations at the two sites (MF and BV) show a great difference in the algal growth. The MF site has no filamentous growth greater than 2mm on any of the rocks. The epilithic growth appears minimal and is very difficult to detect on any rock in comparison to the BV site, only appearing (dark green) when the river flow dropped considerably after 10 July (see fig 4.3.1). The BV always has growth on every rock, most

having filamentous growth (with filaments up to 60cm in length) and black epilithic, sometimes slimy growth where the limpets are to be found.

The monitoring of eggs within each capsule has proved to be very difficult, mainly because of the inability to magnify the capsules sufficiently in order to differentiate them in the capsule: microscopes cannot be used as the rocks do not fit under the eyepiece. Only scattered results have therefore been accumulated which will be presented in the final write-up of this field trial.

Discussion

When considering the algal growth of the two sites, there is likely to be a difference in the suitability of the food present, with the BV providing less suitable species for the limpets to consume: as previously discussed, the first storey, high diatom content, algal layer is preferred by the Ancylidae (Calow 1973). Filamentous algae are difficult to consume (Calow 1975). When comparing the maximum sizes attained by the limpets at the two sites, it can be seen in the graphs that those at MF are larger than at BV, to date, perhaps due to this difference in food. This will be analyzed in greater detail from October 1995, and should lead to a key factor when considering propagation of limpets in the laboratory.

This preliminary analysis of the sizes attained in the field (plotting frequency bar graphs, or modes and means of successive samples) is of limited biological significance because populations are composed of individuals that belong to different cohorts. Determining the peak times of egg laying, or the number of generations per year using the graphs depicted in fig 4.3.3 is extremely difficult. It can be seen, however, that there are eggs present throughout the sampling period, at both sites, despite the cold temperatures of June and July (interval 8,9 and 10 on graph fig 4.3.2). Observations of copulation, which may give an indication of peak egg-laying periods, are infrequent: personal observations have shown copulation in *Burnupia* occurs during the night and in the early hours of the morning until as late as 09H00, seldom after this time which is when the field samples are taken. The possibility of using polymodal analysis using frequency versus shell height at each collection time will be considered when the field data is complete for at least a 12 month period (Harding 1949, McMahon 1975, Brackenbury 1989). The results, presented as growth curves, should reveal how many cohorts are present through the year. The life span of each cohort in the field will then be seen, as well as the times of egg-laying.

The purpose of monitoring the sexual development of the limpets is to determine any correlation between sexual development and size. The number of eggs per mass has been shown to be directly correlated with size in a number of freshwater pulmonates (Hunter 1972, McMahon 1972, De Witt 1954a and b). Data on the bursa copulatrix and penis will determine at what size an individual can act as a functional female or male respectively. These results can then be linked to those in Section 4.2.2 where egg-laying has been monitored.

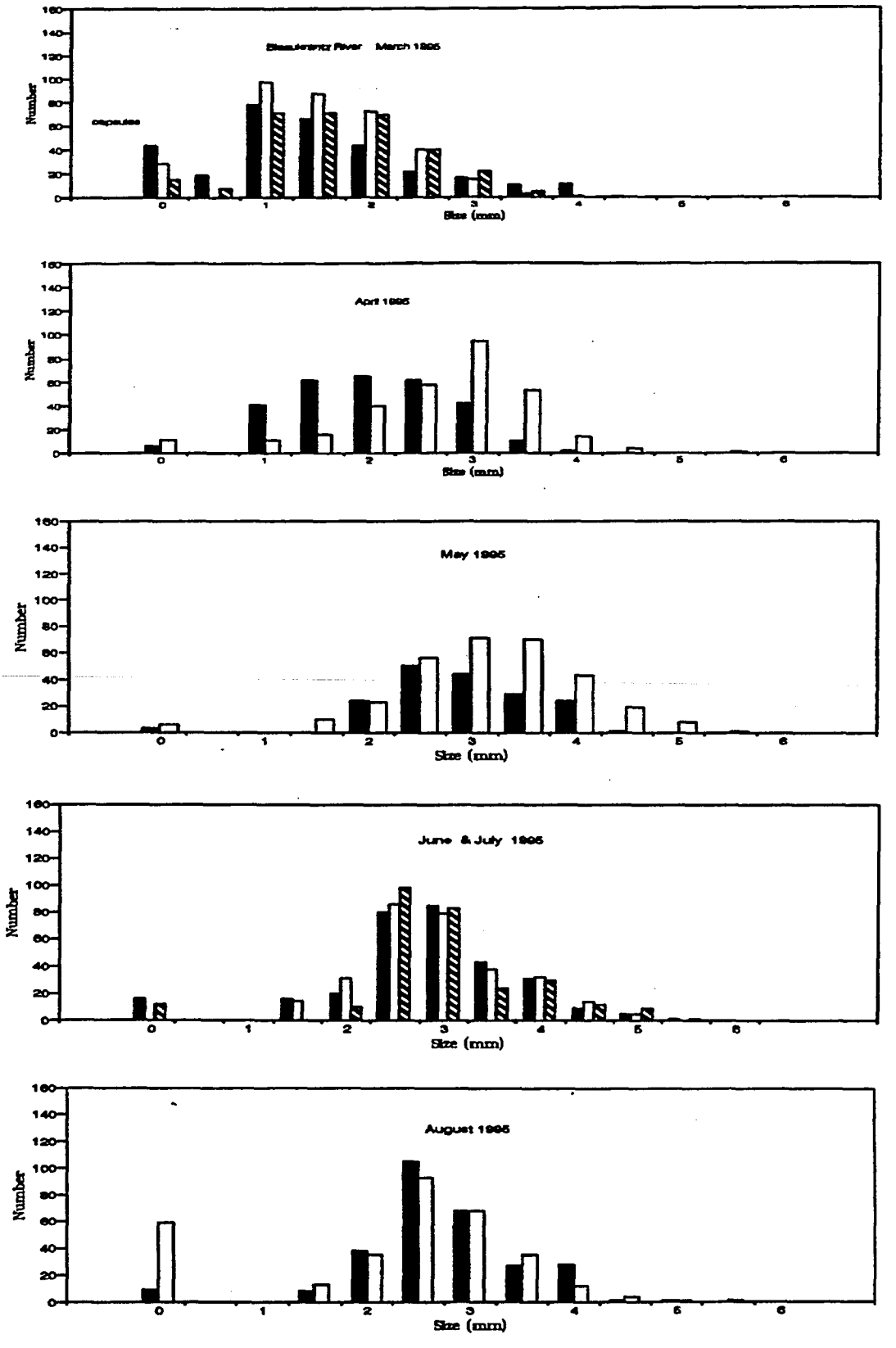

Fig.4.3.3 Sizes and proportional numbers of limpets sampled monthly from March to August 1995 from the Belmont site on the Blaauwkrantz River.

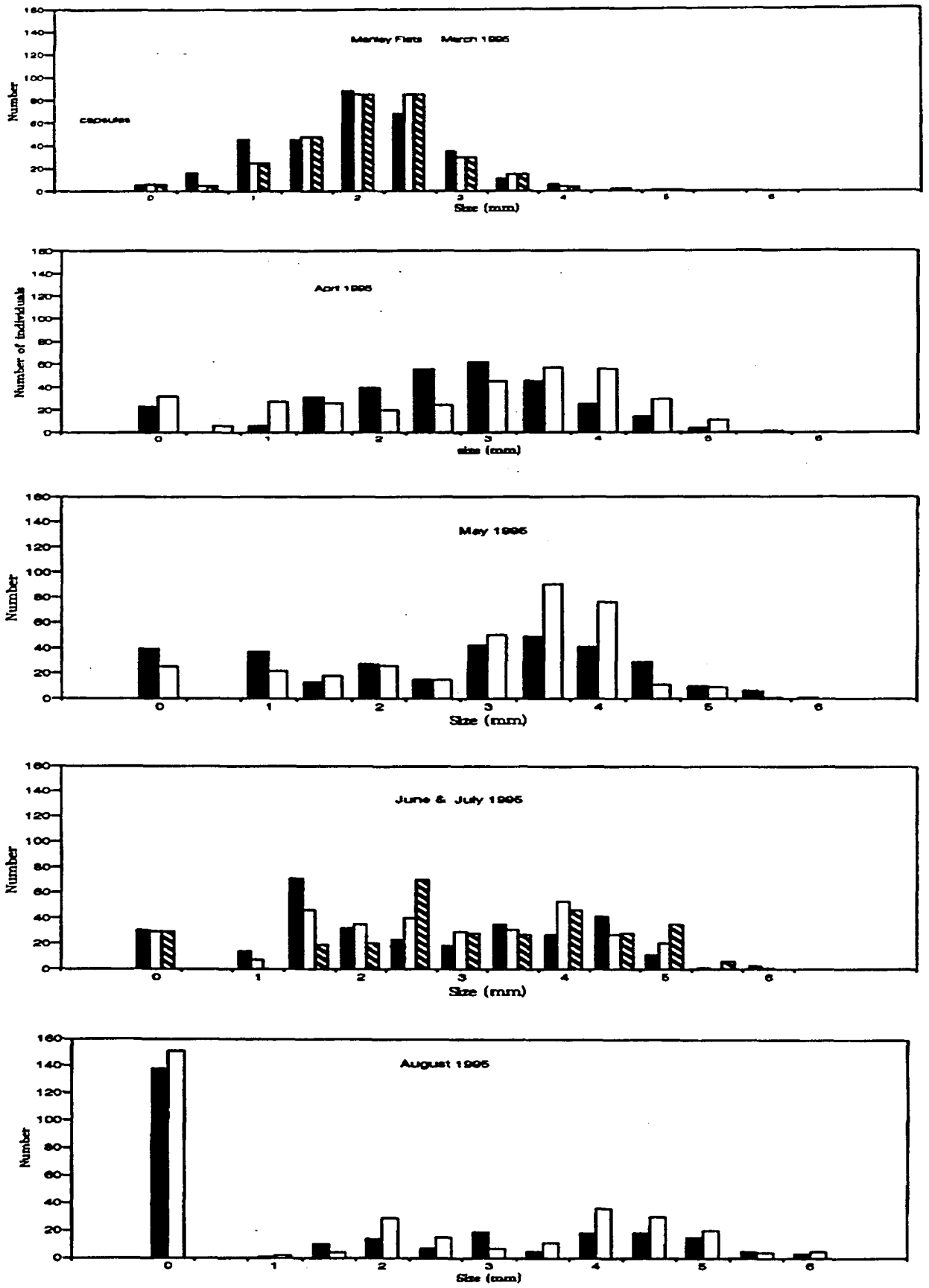

Fig. 4.3.4 Sizes and proportional numbers of limpets sampled monthly from March to August 1995 from the Manley Flats site on the Blaauwkrantz River.

4.4 CONCLUSIONS

With *Burnupia* surviving well in the initial trials it was decided to investigate the life history of this limpet and establish optimal conditions for its laboratory culture. There have been no previous investigations into the biology of any of the African Ancylidae, with the exception of Oberholzer's work (1963) on the morphology and histology of *Burnupia mooiensis*. This lack of any background information on life histories necessitated literature searches further afield, where this information was gained from predominantly the Ancylid *Ancylus fluviatilis*, which is a European and north African species found in lotic freshwater habitats and which appears to have many similarities of life history style to *Burnupia* (Russel Hunter, 1953; 1961; Calow, 1973; 1981; and others). Many of the papers on the bilharzia snails *Biomphalaria* and *Bulinus*, on *Physa*, and on Lymnaeid species which were consulted (although acknowledged as different families with different habitat requirements), revealed valuable information on ecology; population control and breeding strategies; and the effects of temperature, density and food on growth and reproduction.

The attempts to establish optimal rearing conditions revealed the following points:

1) Touching the limpets, either in the laboratory or transporting from the field, has a very negative effect on both the growth and survival.

2) Dietary requirements.
Although the food requirements of *Burnupia* have not yet been investigated, Calow (1973, 1975), Schwenk and Schwoerbel (1973), and Streit (1975) found that *Ancylus* is a microherbivore which prefers epilithic algae, particularly diatoms. *Burnupia* exhibits feeding behaviour similar to that reported for *Ancylus*, which is a rasping action of the stone surface with the radula. Thus, it seems likely that they will have the same food requirements. Optimal feed provision for a laboratory population is always a major part of the development of an aquaculture project. However the cultivation of the most suitable periphyton, under optimal growth conditions of water nutrients and light spectra and intensity, has not been investigated. In an experiment (section 4.2.1.2) with various food sources, artificial food (pellets of *Spirulina*, fish meal, starch and essential amino acids), river periphyton, and fish farm periphyton all proved to be suitable for the survival of the smaller limpets (<4mm), but the artificial food gave better survival rates for those greater than 4mm.
When limpets of all sizes were left for four days without food, that time during which ecotoxicological testing is completed, the survival of either adults or sub-adults was not affected.

3) Hydraulic conditions.
In the laboratory it has been found that a greater number of eggs are laid in aerated standing than in flowing water (section 4.2.1.5), although the possible negative effect of high aeration and consequent turbulence should be considered (section 4.2.2.1). Limpets held in channels made of heavy duty PVC piping proved to have a greater survival rate than standing, aerated water.

4) Water conditions.

As indicated in section 3.3.6, the correct water composition suitable for rearing and holding the limpets has proved to be very difficult to determine. In all experiments the water was considered to be a limiting factor in achieving the optimal survival, growth and reproductive rates and will need careful consideration in the next phase of this project.

5) Temperature.

It is generally agreed by ecologists that temperature plays one of the most important roles in influencing population patterns (Shiff 1964). The strong influence of temperature on the life cycle of *Physa* has been demonstrated by a number of authors (Duncan 1959, Russel-Hunter 1961, Girod 1969, DeWitt 1955, Eckblad 1973 and others), strongly linked to food source. Many freshwater pulmonate snails have their growth rate directly correlated with temperature, increasing up to an optimal or critical temperature, after which growth declines (McMahon 1975). The freshwater snail *Indoplanorbis exustus* showed very clearly (Raut *et al* 1992) an optimal temperature for growth and number of capsules and eggs produced per week. Where our experiments where water baths could be maintained at 15°C, 20°C and 25°C (section 4.2.1.8), increasing temperature increased growth rate, at densities of up to 30 limpets per 500ml volume of water ($p<0,001$).

6) Density.

When considering the breeding of limpets in the laboratory, the effect of density on the growth and fecundity is important. Under high densities, the availability of food may be limiting, or may cause a shift in the species composition and succession of the periphyton assemblage (Bronmark 1989). In the experiment where the effect of different combinations of density and temperature were analyzed (section 4.2.1.8), the density of 10 limpets per 500ml volume of water attained the greatest growth rate for *Burnupia* at temperatures of 15°C and 25°C, being marginally less significant than a density of 20 limpets at 20°C. Although not considered here the effect of density on fecundity may be significant. Chernin & Michelson (1957a & b), Wright (1960) and Eisenberg (1966) all showed a negative effect on the mean clutch size with increasing density.

Various experiments were conducted to further understand the fecundity and breeding biology of *Burnupia*, under given conditions, essential information for any aquaculture programme. The following information has been accumulated (section 4.2.2, Reproduction and Fecundity, and 4.2.3, Natural Populations):

1) *Burnupia* is hermaphrodite. Copulation commences at size greater than 3,4mm in shell length, although eggs have been deposited by limpets reared in isolation. The sectioning of limpets (described in section 4.3) will accurately correlate size of limpet to sexual development.

2) The total development of the eggs has been described (section 4.2.2.1).

3) The number of capsules and eggs produced by an individual is widely variable (section 4.2.2.2 and 4.2.2.3). The number of eggs per capsule varies from 1 to 13; they take 14 to 15 days to hatch from date of lay, under water conditions of average pH > 7,3 (6,6-8,4), average TDS = 280 (185-420), and average temperature 19°C. Cooler temperatures extend the time of hatching up to 21 days at 13°C (pers. obs.). Those limpets that were collected from the streams laid capsules with an average of 5,75 eggs per capsule in the laboratory. Those that were collected at a very small size (approx. 2,5mm, before expected sexual maturity) and reared in the laboratory either as pairs (4.2.2.6) or as singles (4.2.2.4) produced on average fewer eggs per capsule (3,7) than those limpets collected at a larger size from the streams, and allowed to lay eggs in the laboratory (6,1). Generally, water conditions in the laboratory had a pH of <7,0 and an average TDS = 136. It has been found (section 4.3) that the average number of eggs per capsule laid in natural conditions is 5,4 (August to October). The river conditions have an average pH of >7,0 and average TDS of >300. If the water conditions in the laboratory had adversely affected their development and fecundity, this supports the views of Macan (1961) and Dussart (1979) who found that water conditions in the laboratory, using tap water as a source, has a significant effect on both growth and fecundity.

4) The number of capsules laid per limpet pair is estimated to range from 1 to 21.

5) Hatching in the laboratory is at a rate of 91%. They display direct development, with the young emerging as crawling snails.

6) From the field observations and analyses (section 4.3) a clear indication of the cohorts within each year should be obtained. From this, egg-laying times through the seasons will become clear, and when linked to the data relating to Degree-days, it is hoped that this information can be used to obtain optimal rearing in the laboratory.

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CHAPTER 5.
INVESTIGATIONS OF THE LEPTOPHLEBIID MAYFLIES *ADENOPHLEBIA AURICULATA* (EATON), 1871 AND *CHOROTERPES ELEGANS* (BARNARD, 1932).

5.1 BACKGROUND

5.1.1 INTRODUCTION

A survey by Mayer & Ellersieck (1986) indicated a global need to develop acute toxicity methods for baetid and burrowing mayflies, caddisflies and stoneflies. We have selected genera from the family Leptophlebiidae as useful mayflies to breed in the laboratory as future toxicity test organisms. The members of this family is widespread and fairly large in size, robust and easy to identify. They therefore fulfil several of the selection criteria (Chap. 2). According to Johnson *et al* (1993) the life history indicators of environmental stress are survival, growth and reproduction. This, to us, emphasises that information about these life history parameters, under natural unstressed conditions, must be available as a baseline against which to judge results from toxicological experiments. Even results from the experimental controls should be judged against this information. The experiments which will determine optimal conditions for laboratory maintenance and reproduction will give much information in the areas mentioned above, but observations of the responses of natural population to field conditions are essential to verify laboratory results which in turn shed light on areas such as growth rates, which are difficult to determine in the field.

5.1.2 EPHEMEROPTERA AND TOXICOLOGY

Mayflies are an important component of stream ecosystems and are recognised indicator species in biomonitoring. Pontasch & Cairns (1991) suggest that mayflies are the group most sensitive to the deleterious effects of effluent (effect of concentration being dependant on species) while chironomid densities increased after exposure to effluent. There is a growing literature of laboratory and field studies in toxicology and ecotoxicology in which a variety of mayfly species have been the subject of investigation. The effects of a wide range of chemicals have been reported but those receiving the most attention seem to be heavy metals.

The effect of copper and zinc from both food (diatoms) and water on the growth and emergence of *Epeorus latifolium* was evaluated in an indoor model stream by Hatakeyama (1989). It was found that the moult interval was nearly double that of the control at the concentrations tested. Frick & Herrmann (1990) experimentally studied the occurrence of aluminum accumulation in nymphs of *Heptagenia sulphurea* at low pH (4.5). Jop (1991) investigated the bioconcentration of cadmium, copper, lead and zinc in various stages of the field collected *Ephemera danica* Muller, *Ephemera vulgata* (L.), *Leptophlebia vespertina* (L.) and *Baetis vernus* Curt from South Poland, while Gerhardt (1992) investigated acute toxicity of cadmium to *Baetis rhodani* & *Leptophlebia marginata* at pH 5 and 7 simultaneously in static (ST) and flow through (FT) systems. He found *L. marginata* to be more tolerant than *B. rhodani* and that both species tolerated Cd better in the ST system than in the FT system, especially at pH 5. Saouter *et al* (1991) tested the bio-accumulation of inorganic mercury in *Hexagenia rigida*, analysing various anatomical regions on a structural and ultrastructural level and found that gills and gut could be transfer routes for mercury absorption, but also target organs for metal accumulation. Cadmium and mercury content in emergent *Hexagenia bilineata* from the Upper Mississippi River was analyzed by Dukerschein *et al* (1992).

The responses which are useful guides to the effects of pollutants have also been investigated. For instance Diamond, *et al* (1992) used *Stenonema modestum* (eastern United States & Canada) in subacute tests and showed that moult production was a more sensitive indicator of pollutant effects than length of organism or width of head capsule measurements. Tests using sodium chloride or silver nitrate and coal mine effluent demonstrated that these methods and end points provide repeatable and relatively sensitive data. Comparisons of scale were reported by Van Wijngaarden (1993) when he evaluated the field results against those from laboratory ecotoxicological research when the response of *Cloeon dipterum* to chlorpyrifos (Dursban) was evaluated in a laboratory toxicity test, in indoor microcosms, outdoor artificial ponds and experimental ditches. It was concluded that the laboratory toxicity tests gave good prediction of acute direct effects. *Cloeon triangulifer* was evaluated as a bioassay organism by assessing life history response and body burden to the exposure of chlordane by Sweeney *et al* (1993) and was judged to be well suited to laboratory testing because it has a relatively short egg and larval stage and can be readily

cultured.

We are in agreement with Buikema & Voshell (1993) that life history information would not only be useful for culturing laboratory test species but also for identifying sensitive life stages and pertinent ecological variables which could modify organism sensitivities. In this manner defensible chronic test methods can be developed. Thus, in planning the investigation of the mayflies, it was decided to concentrate on gathering information on the life history in the field and to amplify that with laboratory investigation.

5.2 LABORATORY MAINTENANCE EXPERIMENTS

5.2.1 DEVELOPMENT OF HOLDING CONDITIONS

Four experiments which are reported in Appendix 3.3.3 were conducted to determine optimal holding conditions for leptophlebiid mayflies. The findings are reported here.

5.2.1.2. Substrate selection

The necessity of and most suitable type of substrate was examined in two experiment with *Choroterpes* as subject. Substrate that were offered included stone, plastic foam, and rigid plastic mesh.
In bubblepots (App.3; Fig.3.3.8.2) the survival on netting was similar to the control where no substrate was offered. if substrate was offered 90% of the samples were still alive after four days. All three the substrates were tested in both flowing and bubbled water for a month and it wad found that 70% of the sample survived for 10 days and 20% for 30 days on foam-pads and stones in contrast to mesh and the controls where survival rate dropped from 70% survival at 5 days to 25% survival by day 10 (App.3; Fig. 3.3.8.2.3)

5.2.1.3 Hydraulic conditions

The suitability of the small channels and bubble pots were tested in two experiments. In the first channels were placed in the CER and the laboratory and in the second channels and bubblepots were placed in the laboratory.
After three weeks in the first experiment 32 and 33% of the sample had died and at 4 weeks 100% whereas 30% of the sample survived for 7 weeks in the channels in the second experiment. However the survival rates in the bubblepots were significantly higher than those in the channels in the second experiment. (ANOVA result f ratio 6.7 $p<0.05$) In two of the replicates 40% of the nymphs were still123

alive after 9 weeks.

Growth rates for individual nymphs were calculated in the first experiment and are summarised in Table 5.2.1.1. There was no significant difference between the growth rates of nymphs in the CER and in the laboratory ($P > 0.05$) as the average temperatures were similar

Table 5.2.1.1. Growth rates calculated from survivors in each channels in the laboratory and CER results (Growth rates in mm/day). Average ambient temperature 19°C.

	CER	LAB
Channel 1	0.015	0.017
Channel 2	0.043	0.017
Channel 3	0.022	0.028
Channel 4	0.014	0.009
Mean	0.023	0.019

Conclusions

An absolute growth rate of between 0.019-0.022mm/day means that a nymph could grow from hatching to a maximum size in four months. Temperatures in the field stream fluctuate around 15-24 °C and growth rate has been found to be linked to temperature in invertebrates.

It can be concluded from the above trials that bubblepots are the optimal rearing container for *A. auriculata* and that they are not obligately rheophilous.

5.2.2 FEEDING TRIALS.

5.2.2.1 Suitability of commercial fish food for survival

In the first six months of the project period, a trial to test the suitability of TETRAMIN as feed for stream invertebrates was conducted. TETRAMIN is a registered fish food consisting of carbohydrate, protein and lipid, with a total energy value of ** kilojoules/ gm. 70% of the nymphs of *A. auriculata* survived for 7 weeks while in the controls which were fed on detritus 25% of the sample survived for 7 weeks (Appendix 3.3.8.5).

5.2.2.2 Growth rates on two feeds

Aim: To assess the growth of the nymphs in the laboratory at ambient conditions, and to determine if TETRAMIN as an alternate to decaying leaves gives a better growth rate.

Introduction

It has been amply illustrated that food quality can contribute significantly to growth rate and therefore to the generation period, size at maturity and fecundity (Anderson & Cummins 1979; Sweeney *et al* 1986). In the laboratory optimal growth in the shortest period for rearing and reproductive purposes is desirable. If, however, the aim is to have large number of animals of a certain size in stock for extended periods, the fact that growth is retarded at low temperatures becomes important. This option can then be exercised once the correct size for testing purposes has been reached, to move the animals into a cooler situation with reduced feeding and in a sense put them in suspension. Feeding on optimal diet to suit each purpose and the development of this diet often forms the largest portion of aquaculture research project. However the scope of this investigation does not allow for an in-depth investigation into diet and it was therefore decided to see if the addition of TETRAMIN as a supplement to periphyton, instead of the natural diet of decaying plant matter (Leaves) would provide an adequate diet.

Materials and Method

1) Eight x 5 litre replicate of bubblepots were furnished with 10 substrates with periphyton grown in the laboratory placed in the bottom. In 4 replicates the nymphs were fed *ad libitum* on decaying leaves from the study site in addition to the periphyton while the other 4 replicates received 0.5ml ground TETRAMIN weekly in addition to the available periphyton. A leaf shape of inert black plastic was placed in these pots to resemble the real leaf.
2) Nymphs of *A. auriculata* were collected in the field and after being sorted, were allowed to acclimate for a week before being measured. The dead animals were replaced from the remainder which had been kept in a large bubblepot with stream detritus and leaves adjacent to the experimental pots. The experiment started two weeks after the collection date. In some containers up to 80% of the

nymphs died and were replaced. Twenty one nymphs between the sizes of 0.4 and 0.8 mm headwidth were placed in each bubblepot. All the nymphs were measured weekly, the water and food were replaced and the number of shucks were recorded.

3) The average head width of each sample was calculated weekly. The absolute growth rate was calculated weekly, and over a period of 47 days which was the period prior to a large number of emergences. Statistical analysis was performed on STATGRAPHICS 7 software package.

Results Fig. 5.2.2.2.1, and 2a) & 2b). The results of this experiment were ambiguous and difficult to interpret. The average rate of growth was difficult to calculate as, beside the mortalities which occurred, a number of adults emerged. Whenever an adult emerges the average size of the remainder of the sample becomes smaller and the growth rate then appears negative or retarded. Survivals had to be calculated taking both the mortalities and the emergences into consideration. Although the experiment lasted for 12 weeks, the average size increases up to day 50 only is depicted (Fig 5.2.2.2.1). To record accurate growth rates, nymphs would have to be reared in singly.

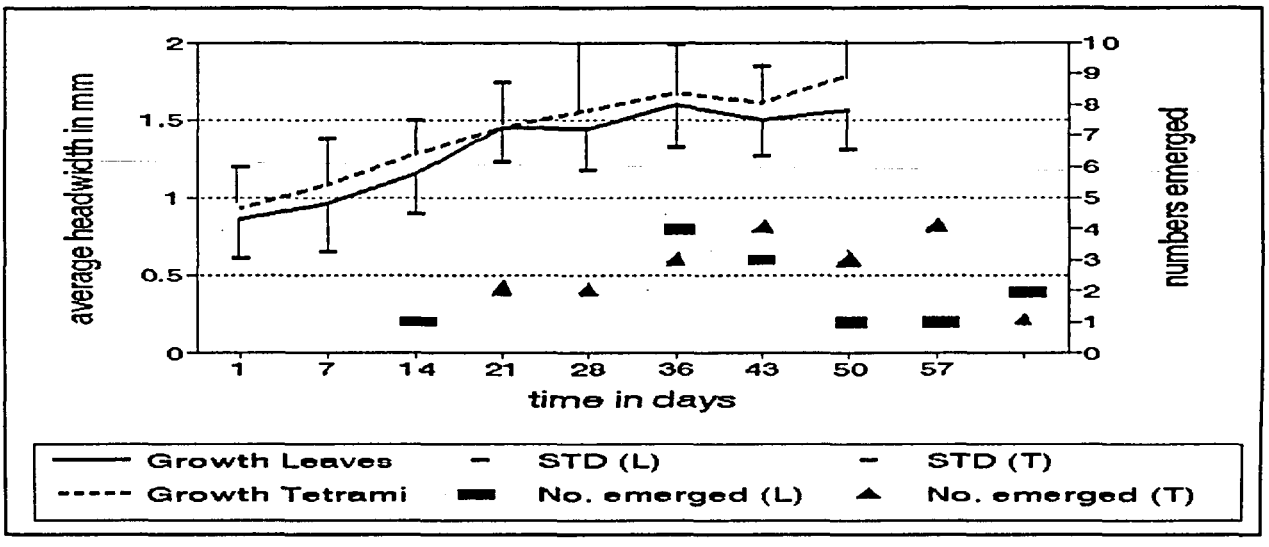

Fig. 5.2.2.2.1 Growth of nymphs of *A. auriculata* in bubblepots fed on decaying leaves or TETRAMIN in conjunction with periphyton. The average headwidth of all the specimens in all the replicates is given with and error bar indicating standard deviation from the mean. The emergences of adults are included in this graph indicated by symbols. The influence of the emerged adults which are no longer a factor in the calculation is reflected by the negative slope of the line between weeks 36 and 43.

A simple regression performed on the two treatments yielded a slope 0.013 for growth on leaves and 0.016 for growth on TETRAMIN. In Fig. 5.2.2.2.1 this is borne out by the difference in the slopes yielded from the line graph constructed by calculating the average size of sample each week.

Table 5.2.2.2.1 Average absolute growth rates mm/day calculated for each week from average headwidth of the total number of nymphs in all replicates. In the last row the growth rates have been calculated for the total number of days given in the left column.

Time, weeks	Growth on leaves, mm/day	Growth on TETRAMIN, mm/day
1	0.0146	0.02
2	0.0267	0.029
3	0.0419	0.024
4	0.004	0.016
5	0.0219	0.0008
7	-0.014	0.024
Average growth over 14 days 21 days 57 days	0.0206 0.0277 0.0142	0.0243 0.0241 0.0157

In Table 5.2.2.2.1 the daily growth rate is calculated on a weekly basis. In those weeks when large animals died or emerged the growth appeared to be negative which is an artifact. Growth rates calculated over a longer period as in the last row of the table during which 7 emergences had taken place in both treatments. The mean growth rate is nearly halved. However it was clear during the course of the experiment that some animals did exhibit no growth and this could be morbidity due to pathogens. There was evidence of fungal growth in pots 8 and 6 both of which were fed on TETRAMIN.

Table 5.2.2.2.2 an ANOVA performed to test differences between all replicates in which the growth achieved by *A.auriculata nymphs* on leaves(L) were tested against that achieved on TETRAMIN(T). The results of multiple range analysis is given below. Each replicate has the treatment type next to the number (7.Leaves).

Method 95% LSD			
Level	Count	LS Mean	Homogeneous groups
7.L	78	1.1864234	X
5.L	132	1.1911134	X
2.T	132	1.2067042	X
4.L	107	1.2979697	-X
6.T	92	1.3334692	-XX
10.L	141	1.3467826	-XX
8.T	117	1.3916301	--XX
9.T	80	1.4675727	---X

An ANOVA comparing the two treatments over time produced an F ratio 24.08 at P= 0.0000 which shows a significant difference between the treatments, despite the fact that there is such a degree of overlap between the replicates.

During the experimental period a total of 63 specimens died and 21 emerged in those containers where leaves formed the diet and 60 died and 24 emerged when TETRAMIN was the diet.

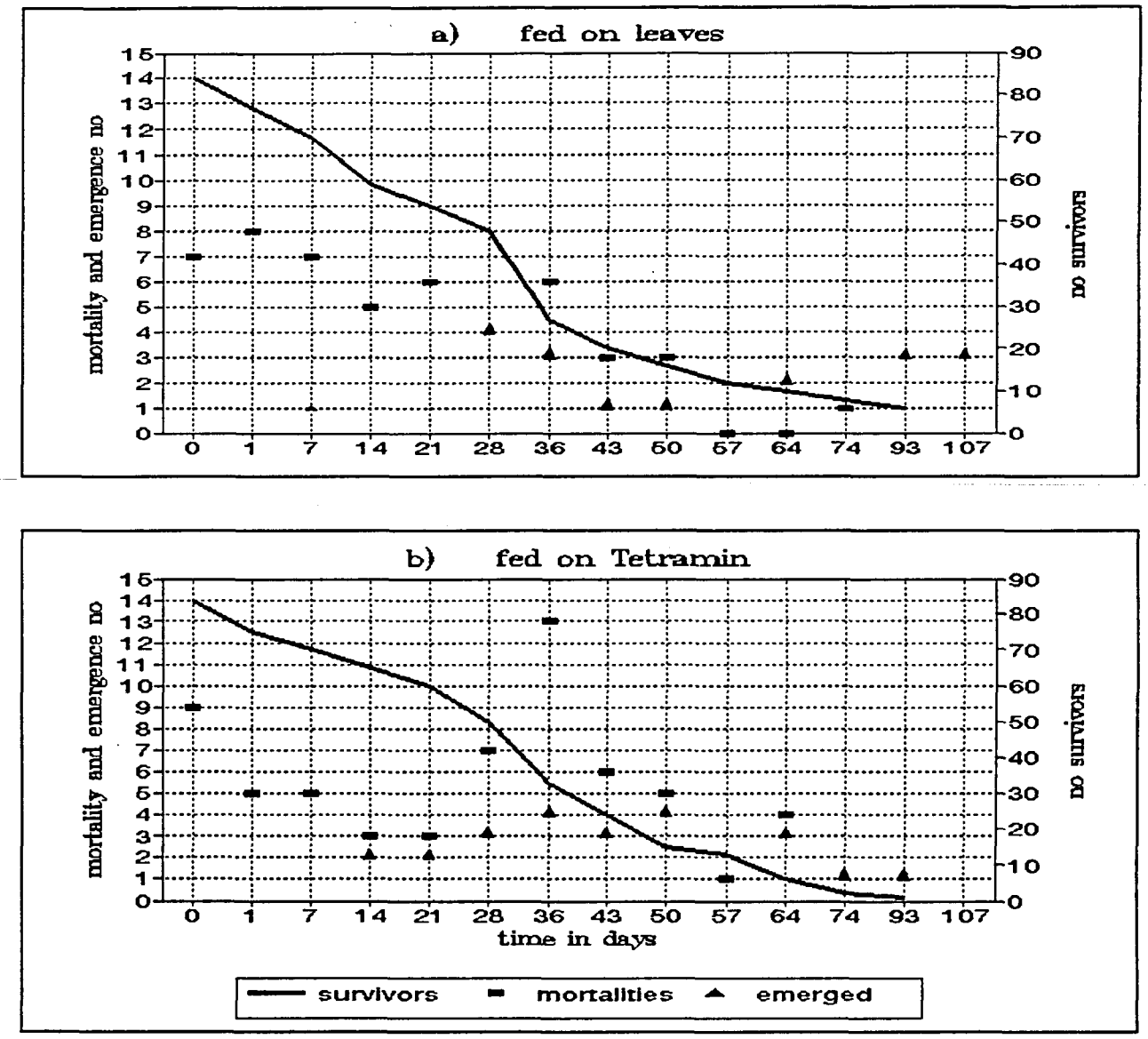

Fig. 5.2.2.2.2. a) & b) Survival, mortality and emergence (total numbers in all replicates) of nymphs of *A. auriculata* in bubblepots when fed on a) leaves and periphyton and b) TETRAMIN and periphyton.

The largest nymph at the start of the experiment (1.2 mm HW) emerged after 2 weeks. Those which were between 1.1 and 1.0 mm HW emerged after 5-6 weeks. The smallest nymphs in this trial was between 0.4 and 0.6 mm HW and the last emergences took place after 93-107 days which was 13-15 weeks on natural food. This gives some indication of the summer growth period which may be found of nymphs in the field. It is notable that the growth period is shorter and that emergences took place earlier in the TETRAMIN treatment.

Conclusion

A supplement of TETRAMIN to the diet of *A. auriculata* nymphs will increase the growth rate. Care has to be taken to maintain water quality as the largest numbers of early deaths were recorded in those pots where TETRAMIN had been added. The results from the experiment using only leaves as feed give a growth rate of 0.013 from the regression and 0.02 if the growth rate over the first 21 days is calculated. This indicates that it may take 10 - 14 weeks for a specimens of HW 0.6mm to reach sub-adult stage in summer conditions. and 14 - 23 weeks to grow from hatching to sexual maturity.

5.2.3 TEMPERATURE EFFECTS

5.2.3.1 Temperature effects on moulting rates.

Aim

To determine instar period for *A. auriculata* at three temperatures.

Mayflies have a number of post-embryonic moults, with estimates of between 10 and 50; however, most are in the range 15-25 (Brittain 1982). The number of instars is also influenced by temperature (Needham & Traver 1972) and has been cited as a useful indicator of pollution stress (Diamond *et al*1992).

Materials and Methods.

Two separate experiments were conducted to determine instar period and the effect of temperature on the moulting frequency.

1. a) Forty five 9 cm petri-dishes each containing a decaying leaf, from leafpacks in which the nymphs were found, were used as containers. To simulate the subdued light under rocks these were stored in brown cardboard boxes.

   b) Fifteen 5 litre bubblepots with approximately 500ml of water were furnished with periphyton stones in the second experiment.

2) All containers were equally divided into CERs at 15°C and 25°C and the laboratory at ambient (17-22°C). Photoperiod was 14h in the CER and 14-13h (ambient for summer) in the laboratory.

3) (a) One animal was placed in each container and if it died it was replaced. Sizes ranged between 0.6 and 1.2.mm HW.

   (b) Three animals of small, medium and large size (range 1.2 - 1.6mm HW) were placed in each container so that shuck size could be correlated to the moulted animal.

4) Containers were observed daily and in (a) the specimens were measured whenever a shuck was observed. Experimental period was 18 week for (a) and 9 weeks for (b).

4) The results are portrayed as a frequency range of instar period.

Results. Fig. 5.2.3.1. a) & b); Fig. 5.2.3.2 and Table 5.2.3.1.

Results.

Instar period.

Table 5.2.3.1. Average instar period at three temperatures for nymphs in the laboratory. The figures in brackets are standard deviations for the average numbers of days between moults.

PETRI-DISHES	**25°C**	**17-22°C (ambient)**	**15°C**
Number of observations	353	373	959
Average no of days (STD.)	7.67(2.66)	8.88(3.12)	13.5(6.20)
BUBBLEPOTS	25°C	20°C	15°C
Observations	266	355	239
Average instar length	7.4(3.43)	10.44(4.47)	14.94(6.23)
Ave(SE) calculated by ANOVA	7.52(0.54)	9.24(0.53)	10.12(0.31)

The average instar period for nymphs of *A. auriculata* at the three temperatures in the two separate experiments and fed on two different but natural diets are within one day of each other except for those experiments which were held at room temperature.

An ANOVA and multiple range analysis for all treatments indicated significant differences between all temperatures for both treatments (f ratio 35.9 $p<0.0000$) but no significant difference between the results of the two experiments (F ratio 3.3 $p=0.0683$).

In Fig. 5.2.3.1(a) instars of three days can be observed for nymphs kept at both 25 and 15 °C. While it may be feasible at 25 °C it is highly unlikely that a three day intermoult would take place at 15 °C due to the reduction in metabolic rate. It is therefore suggested that this brief period can be ascribed to observer error as different observers were used during the long run of the experiment. However stress of capture may cause frequent moulting and can not be altogether discounted. If the outliers of 3 and 4 days are removed a more realistic image emerges.

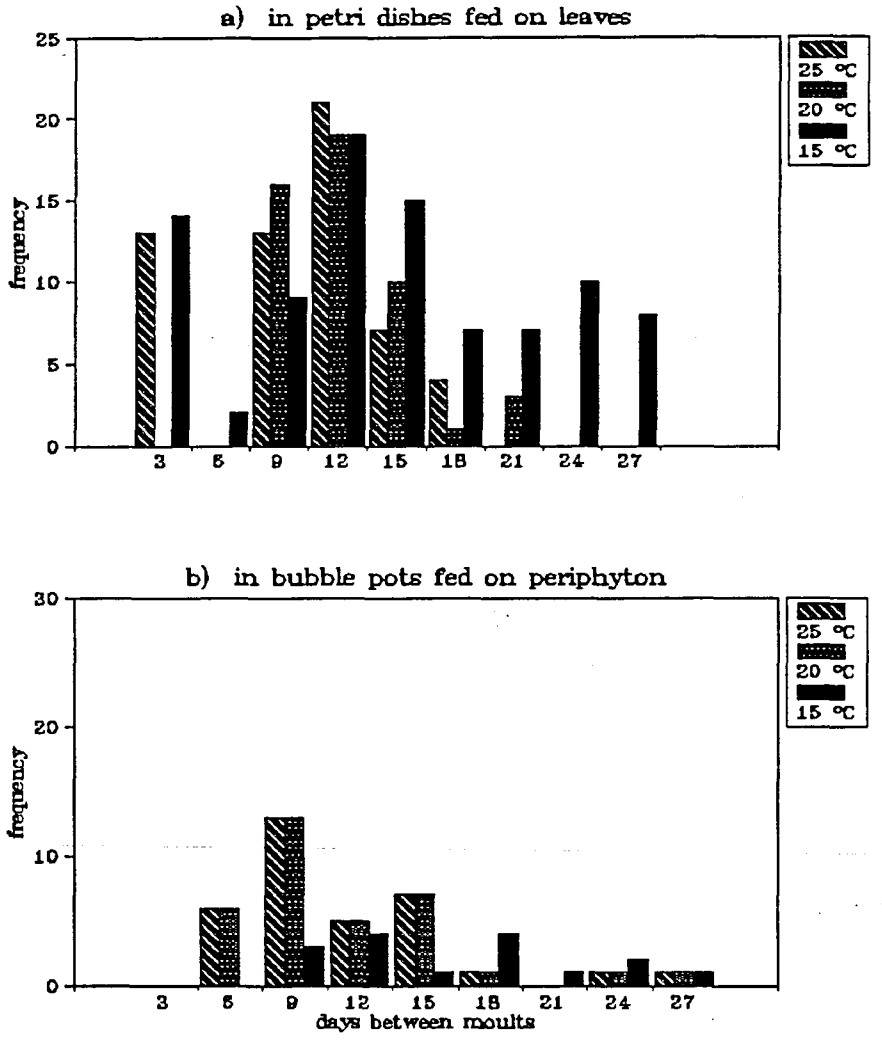

Fig.5.2.3.1 Frequency distribution of instar period for nymphs *A. auriculata* at three temperatures.

a) single nymphs were placed in petri dishes and fed on decaying leaves (b) two or three nymphs placed in 5 litre bubblepots and fed on periphyton.

The range in the number of days between moults is wide but there is a clear trend towards the lengthening of the instar period with lowering of temperature as can be expected. In (a) observations were carried out for a longer period (18) weeks) than for (b) (9 weeks). This was due to the fact that smaller animals were used in experiment (a).

There is a degree of overlap in instar period between all temperatures (6 and 12 days). The present data set is not large enough for this to show clear trends. If the instar length can be correlated with the age and size of nymphs as well as the stage in the experimental period when it occurred more clarity may be obtained for the reason for this overlap.

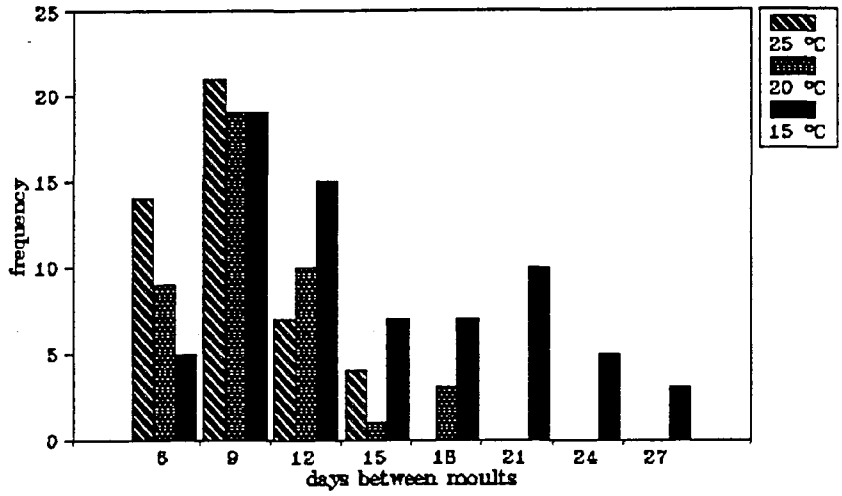

Fig. 5.2.3.2 Frequency distribution of instar periods, with outliers removed, of nymphs of *A.auriculata,* fed on leaves, reared singly in petri dishes.

b) Survival and growth rate in experiment (a)

The survivorship is very different at the three temperatures. At 15°C 3 animals died, at ambient (20°C) temperature 8, while at 25°C, 22 died. The largest number of mortalities occurred 2 weeks after the start of the experiment at 25°C when 12 nymphs died within 7 days.

The daily growth rates of the longest lived animals at each temperature regime were calculated. At 15 °C 8 nymphs survived for between 70 and 126 days at 20°C 5 animals between 40 and 111 while at 25°C 4 animals between 40 and 93 days. The growth rates recorded for these long lived nymphs were

Table 5.2.3.2. Survival period and daily growth rate of longest lived nymphs, at three temperatures in petri-dishes with leaves as food.

TEMP.	RANGE DAYS SURVIVED	AVE. no. DAYS	GROWTH RATES mm/day	AVE. GROWTH RATE
15°C	(n=8) 128-71	117	0.003-0.008	0.00478
20°C	(n=5) 40-111	74.5	0.008-0.010	0.0085
25°C	(n=4) 40-93	70	0.009-0.022	0.01500

Although there are difference between the growth rates the ranges recorded are large and there was a degree of overlap between the temperatures. Nevertheless, the animals at 15°C grew much more slowly

than at the other two temperatures.

Discussion.

Several authors have found a variation in instar period and Newbold *et al* (1994) has discussed the possibility of a pre-emergence diapause period during which a population of nymphs may be able to synchronise emergence in order to facilitate mating possibilities.

It seems that leptophlebiid nymphs are vulnerable at higher temperatures as high mortality was observed. This could be as a result of oxygen deprivation as the petri-dishes were not aerated.

5.3. FIELD INVESTIGATIONS.

THE LIFE HISTORY OF THE MAYFLY *ADENOPHLEBIA AURICULATA* (EATON) (EPHEMEROPTERA, LEPTOPHLEBIIDAE) IN A SUBTROPICAL FOURTH ORDER STREAM IN SOUTHERN AFRICA.

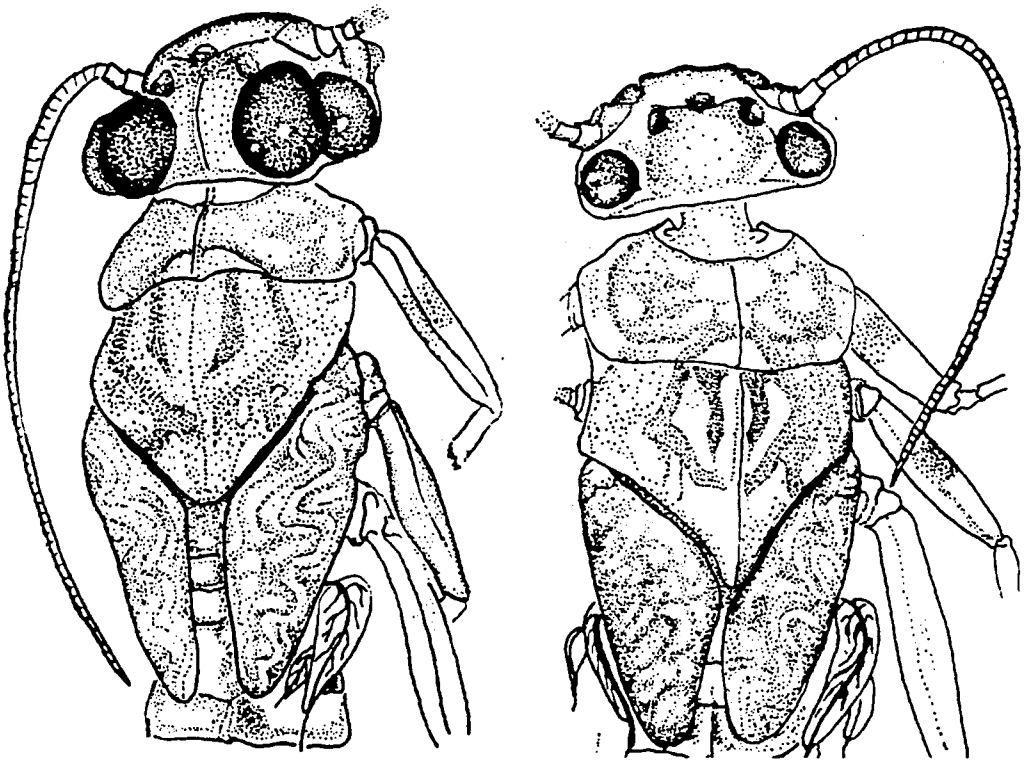

Fig. 5.3.1. a) Male of late instar nymph of *A. auriculata* showing the large eyes and b) female nymph with long wingbuds indicating that they are undergoing metamorphoses.

Introduction

The order Ephemeroptera dates from Carboniferous and Permian times and today their highest diversity is in lotic habitats where they are an important component of the ecosystem. They consume organic matter such as leaves and fine particulate organic matter as well as algae, and this energy in then made available to predators in the system (King *et.al* 1987 a & b). These processes are essential in the upper reaches of rivers where the majority of energy in the system is imported from outside in the form of leaves and detritus.

Mayflies are unique among the insects in that they have two winged adult stages, the subimago and the imago. There is much debate as to whether this is a primitive or derived state (Edmunds & McCafferty 1988).

The presence of long dark wingbuds usually indicates imminent emergence. During this preadult phase males with large double eyes can be distinguished from females which tend to be larger (Fig. 5.3.1) In leptophlebiids the pharate nymph crawls a few centimetres out of the water usually onto a rock or up vegetation and moults into the subimago (Needham & Traver 1972; Edmunds & McCafferty 1988). The subimago may then fly a short distance to cover before resting. Subimagoes are generally not strong fliers and seldom fly far (Edmunds & McCafferty 1988). After 24-48 hours the subimago moults into an imago (Needham & Traver 1972) which differs from the subimago in that the dark, opaque wings with a fringe of fine cilia become clear and reflective and the cerci and forelegs are longer (Needham & Traver 1972; Brittain 1982; Edmunds & McCafferty 1988). The only two functions of adult mayflies are mating and oviposition and the adults have no functional digestive system (Needham & Traver 1972; Brittain 1982; Edmunds & McCafferty, 1988). Hence, adult mayflies seldom survive for more than one week and usually die due to desiccation (Edmunds 1972). The general breeding behaviour of mayflies involves the males forming a swarm, any female flying through the swarm is usually copulated with immediately (Needham & Traver 1972; Brittain 1982; Edmunds & McCafferty 1988). This breeding behaviour therforee requires fairly closely synchronised life cycles and it is uncertain how the loosely synchronised emergence pattern observed in *A. auriculata* allows for successful mating if this was their mating strategy. However we have never observed swarms of adults in the river.

In the Palmiet River in the eastern Cape, the leptophlebiid *Adenophlebia auriculata* is an abundant component of the aquatic macroinvertebrate fauna, and is an important pollution indicator species as it is fairly widespread and inhabits upper reaches of rivers and streams. Detailed information on its response, to laboratory conditions as well as knowledge of its population distribution, seasonality and habitat preference is needed to determine whether *A. auriculata* will be a suitable subject for laboratory culture for ecotoxicological testing.

This population of mayflies has now been investigated since March of 1993. Our sampling programme has been modified as more information on the life cycle has become available.

Aims

1) To determine the seasonal population densities, habitat preferences, life history style (uni/bi/multivoltine) and the peak emergence periods of *A. auriculata* in the Palmiet river by analysing the proportions of the nymphal population in three size classes.
2) To attempt to correlate the rate of growth determined experimentally for *A. auriculata* nymphs to population dynamics in the field and, in doing this, estimate the life cycle.

Environmental influences

Investigations into the environmental factors which govern the life cycle of stream macroinvertebrates are numerous and mayflies, particularly, have received much attention in recent years. The total development time is governed by such external factors as temperature, available food and photoperiod which all influence the expression of the genotype of which temperature may be the most important (Sweeney 1978 & 1984). Investigations have revealed similar traits in large numbers species from similar biomes (Wiggins, 1977; Clifford, 1982; Newbold *et.al,* 1994; Jackson & Sweeney, 1995) while differences in life history is also found between occupants of the same biome (Sweeney & Vannotte, 1981; Jacobi & Benke, 1991; Sweeney, *et al* 1995). This leads one to the conclusion that the genotype will determine the life history style: how the species apportions the available energy between growth, maintenance activities and reproduction, and how the energy made available for reproduction is utilised. The various life history styles which have evolved in similar biomes give insight into the variety of responses which have evolved in response to the same environmental variables.

Sweeney (1978), investigating the metabolism of Isonychia bicolor showed that there is little metabolic compensation or acclimation to thermal fluctuations within this species and that summer and winter generations will exhibit the same metabolic rate and response to thermal changes. It was found that respiration rates of both winter and summer nymphs were the same when measured at 15 °C. Similar responses were recorded from gastropods by Berg *et al* (1958) & Calow (1975). It is therforee not surprising that several studies (Wise, 1980; Giberson & Rosenberg, 1994; Sweeney, *et al*, 1995; Pritchard & Zloty 1994) have revealed that intra-specific voltinism may be affected by the thermal regime of the environment. This results in a uni or semi-voltine life cycle gradually becoming bi-voltine as the thermal regime of the environment increases. Furthermore it has become clear that as the ambient temperature of an aquatic system increases with a concomitant decrease in seasonal fluctuation, the life history styles of the assemblages of invertebrate inhabitants tend toward multivoltinism (Jackson & Sweeney 1995; Jacobi & Benke 1991). Under these circumstances the development speeds up with increase in temperature.

However, adult size and fecundity, which are positively correlated (Anderson & Cummins 1979), and developmental period can be adversely affected by temperatures outside the optimal range for a given species. Sweeney & Vanotte (1978) hypothesised that this is due to a disequilibrium between larval growth rate and the timing of metamorphoses caused by a shift in the energy partitioning.

Materials and Methods

Study site and field data.

1) The study site is a stretch about 0.8km upstream of the confluence of the Berg and Palmiet rivers at the national road and comprises a series of riffles, runs and pools (Wadeson 1993) (Fig 5.3.2). During 1993 a stretch of about 0.5 Km consisting of 2 riffles, 2 runs and a pool is sampled at monthly intervals from February to September. In 1994-95 the stretch which is sampled weekly, is the run where the highest densities of *A. auriculata* were recorded throughout the year in 1993. During the weekly field samples the specimens which were collected were measured as described below and immediately returned to the upstream area of the study site in order to minimise the impact of the investigation. Animals which were captured and returned to the laboratory were collected downstream from the study site.

Water temperature, conductivity and pH were taken weekly and averaged for each quarter (table 5.3.1.) while the level of the stream is also noted. Daily maximum and minimum air temperature as well as rainfall was acquired from the local weather station. Cumulative weekly aerial and aquatic thermal inputs were summed from degree days using the formula (DD=T0-Tmax*dayL/24)*7. Where To = 10°C. 10 °C is chosen from literature as the temperature below which no development may take place but will have to be verified experimentally.

Table 5.3.1. Average water quality information from the Palmiet River 1994-1994.

YEAR	SUMMER			AUTUMN			WINTER			SPRING		
Condition	pH	TDS	°C	H	TDS	°C	pH	TDS	°C	pH	TDS	°C
1994	5.81	68.4	24.8	7.2	84	21	6.0	86	17.6	7.0	71	17.2
1995	6.0	73.5	20.1	7.0	71.3	19.4	8.0	86	12.1	7.0	96	20

Sampling method and measuring techniques.

2) The collecting method followed the method described in Chapter 3. The collection methods for the derivation of quantitative data was for the same collector to collect for a standard time period. These samples were preserved and returned to the laboratory. Rocks for sampling were selected randomly over the entire sample area regardless of size or position unless habitat-specific samples were being taken. Habitat specific samples were taken from rocks in and out of direct current. The sizes rocks sampled were noted. The subimaginal shucks on rocks at the edges of the stream is

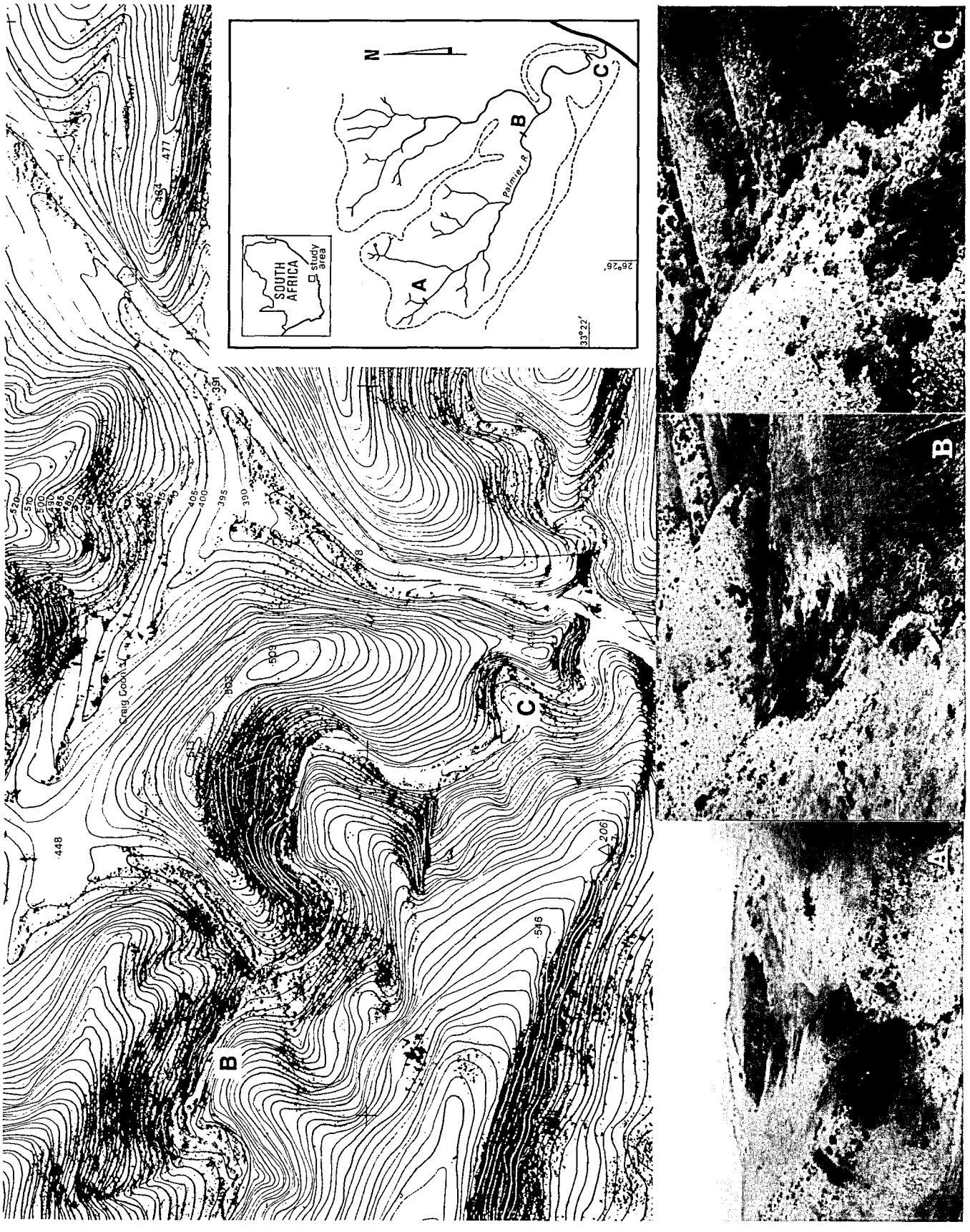

Fig. 5.3.2 Contour map of the Palmiet river Grahamstown, eastern Cape, RSA with aerial views of selected sites; a) headwaters b) second order reach c) 4 or 5th order reach at the study site.

counted each week as were adults, either resting on the edges of the stream, drowned, or caught in spider webs.

3) The dorsal head-widths (HW) across the widest points at the eyes were measured in the laboratory using a calibrated microscope eyepiece at 250 times magnification or on a calibrated 'V' on graph paper. The head widths show a closer correlation to the instar than does body length (Palmer pers.comm.). During the weekly sampling of 1994-95 the head-widths of fifty randomly selected insects were measured in the field. Once measured the insects were placed in a holding bucket to prevent re-sampling and were released into the same site once the sample was complete. Sizes below 1mm HW were not randomly selected but carefully sought as these animals are more difficult to capture. The smallest individuals (<0.04mm HW) were never captured, which leads to an underestimation of the proportion of hatchling nymphs in the population as well as difficulty in estimating hatching period and the duration of the earliest part of the post embryonic period. In this study there is a need to distinguish late instar nymphs from earlier stages. These nymphs are termed subadult nymphs and should not be mistaken or confused with the subimagoes which refer to the winged pre-adult stage.
4) Analysis. The collected measurements were grouped in three size classes. Those below 0.9mm HW were grouped and represent the recruits from the hatchlings of the previous laying. The sizes between 1.0 and 1.6 mm HW were considered the standing stock of the population. The sizes above 1.7mm represent the sub-adult, maturation phase with the development of wing-buds and gonads. The presence of long dark wing-buds usually indicate imminent emergence.

Results

Temporal variation in population.

Change in densities over time.

The changes in density and size frequency distribution of the nymphs in the five habitats in this reach of over a seven month period gives an indication of possible seasonal effects. As stated in the methods section, it must be kept in mind that newly hatched and very small nymphs are not readily captured so that there is always an under estimation of numbers and it is difficult to determine hatch dates.

At monthly sampling intervals in 1993 it is possible to monitor the changes in population structure and to predict possible emergence periods. The most striking aspect of these samples is the presence of all size classes throughout the year (Fig. 5.3.3).

The March sample was small with the largest number of nymphs found in riffle 1. However this was the first sample collected and an element of inexperience may account for the low numbers. In April there was an increase in the numbers of recruits in all sites but most marked in riffle 3. By May the recruits

appeared in larger numbers in run 2, and the standing stock size class numbers had increased markedly at all sites. The first sub-adults appeared at runs 2 & 4. The population profile looks very similar in July except that some larger nymphs were captured in the pool. In August there is a dramatic increase in numbers at site 2, in both the standing stock and the sub-adult size classes. This trend carries through to September, with a decline in numbers of sub-adults.

The overall trend seen in the reach over the 7 month sampling period in 1993 showed a overall increase in the total number of nymphs caught from March to September (late summer to spring) as well as an increase in the density of the nymphs with a drop in density in July (Fig. 5.3.3)

The presence of a strong cohort of fairly large nymphs in September indicated a spring emergence. A fairly synchronous spring or early summer emergence is implied by the sudden appearance of late instars in August, and the appearance of large numbers of shucks in the stream.

At this stage of the investigation we made the following deductions:

1. There may be an extended non synchronous mating/laying period indicated by the presence of small nymphs throughout the year.
2. The nymphs of this species are to be found in runs where the current speed is less than in riffles.

However the information clearly had large areas of uncertainty such as what happens in summer period. We therfore needed more frequent sampling over periods when emergence was to be expected. We hoped to be able to deduce the cues for emergence and flight, and to estimate the duration of the life cycle from this sampling regime.

Consequently, a new sampling regime was devised for 1994, aimed at discovering breeding periodicity and life cycle. At first we sampled weekly at those times when the results from 1993 had led us to expect an adult emergence but by the spring of 1994 it was decided to sample weekly for a period of at least eighteen months and after that to review the data.

Habitat preferences.

There appeared to be a seasonal change in the density distribution pattern of nymphs in the different habitats. In the colder late summer and winter months the nymphs appear to aggregate in the runs, with higher densities in the run with little canopy cover from July. The decrease in densities in July from May in all the habitats indicates a winter emergence of adults (Fig.5.3.4). The appearance of larger numbers of nymphs in the samples in May and September could be an indication of the increase in the nymphal size of the population which makes for easier collection for experimental purposes.

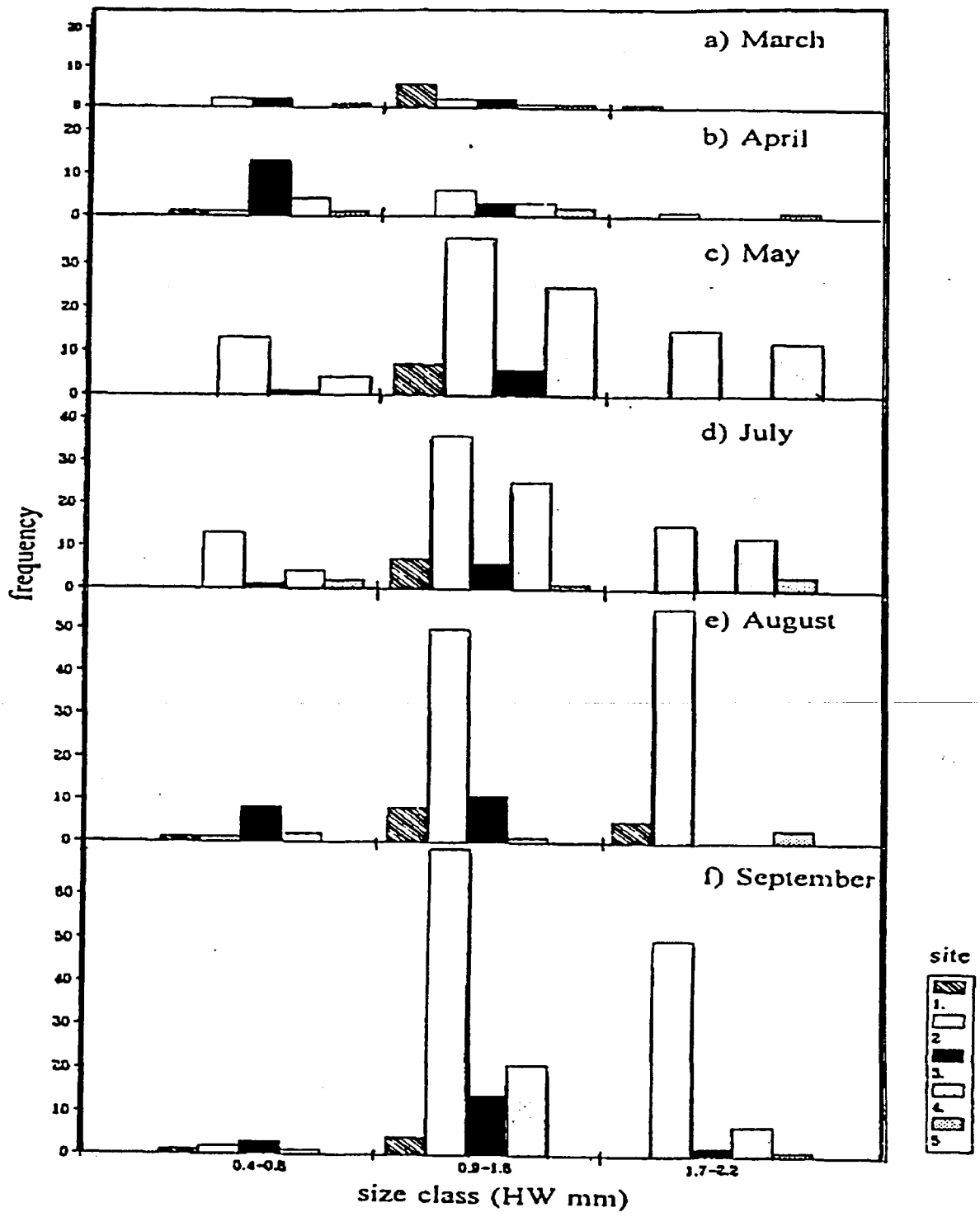

Fig. 5.3.3 Size frequency distribution of nymphs of *A.auriculata* sampled during six months (1993) at five sites in the Palmiet river. The samples have been dived into three size classes and the number of each size class is shown for each site which is coded with a different pattern, i.e. Site 1= riffle; Site 2 = run; site 3 = riffle; Site 4 = run; Site 5 = pool.1. There may be 2 generations per year with eggs being laid in May and September.

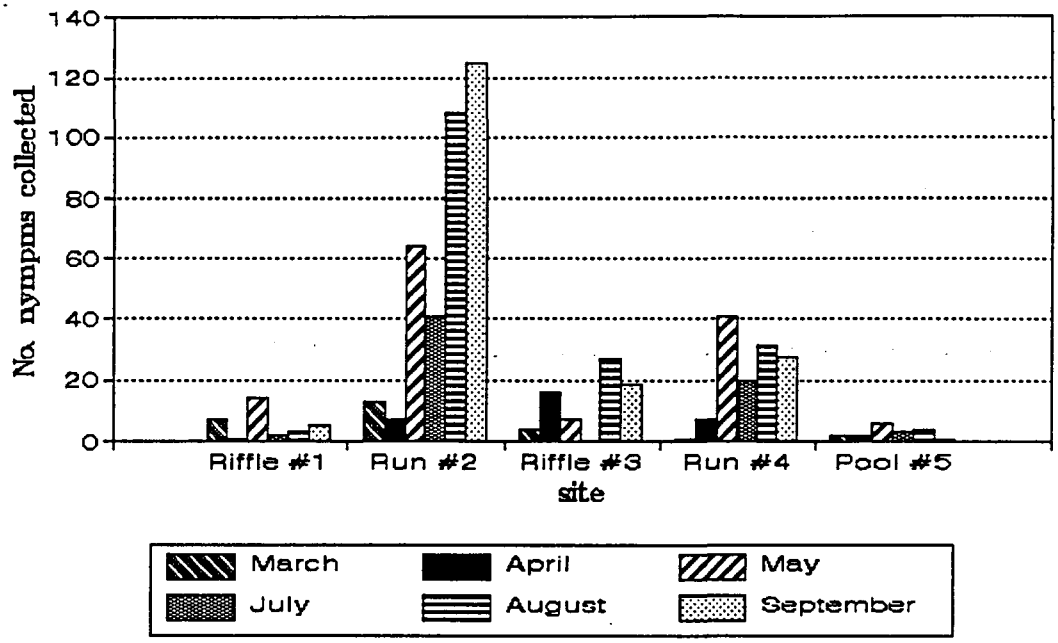

Fig. 5.3.4. Temporal spatial fluctuation of the population *A. auriculata* nymphs in the Palmiet River during 1993, showing numbers of nymphs captured each month at each site. Population densities were also calculated for the area sampled and showed a similar pattern of rise in autumn, followed by a decline in winter with numbers rising again in spring. It can be seen that site 2 and 4 consistently yielded higher numbers of nypmhs.

Temporal population dynamics

We deduce from Fig.5.3.5 that there are several major emergence periods during the year but that a low level of emergences take place at frequent intervals. However, the most notable feature of the population profile is the standing stock of nymphs (1 - 1.59 mm HW) comprising an average of 50% of the samples except for brief periods when the percentage of the standing stock in the sample was below 42% such as in week 45 and 59, 69, 88. Although the numbers of recruits in the samples fluctuate considerably but it is clear from the figure that recruitment continues throughout the year. Similarly ther are always sub-adults nymphs present in all but a few weeks during the year. However this does not indicate that emergences take place throughout the year, as the large nymphs are not necessarily pre-emrgence. In the period before emerging the sub-adult metamorphoses developing wingbuds, testes and ovaries (Fig.5.3.1). We therfore conclude that *Adenophlebia auriculata* is a multivoltine species.

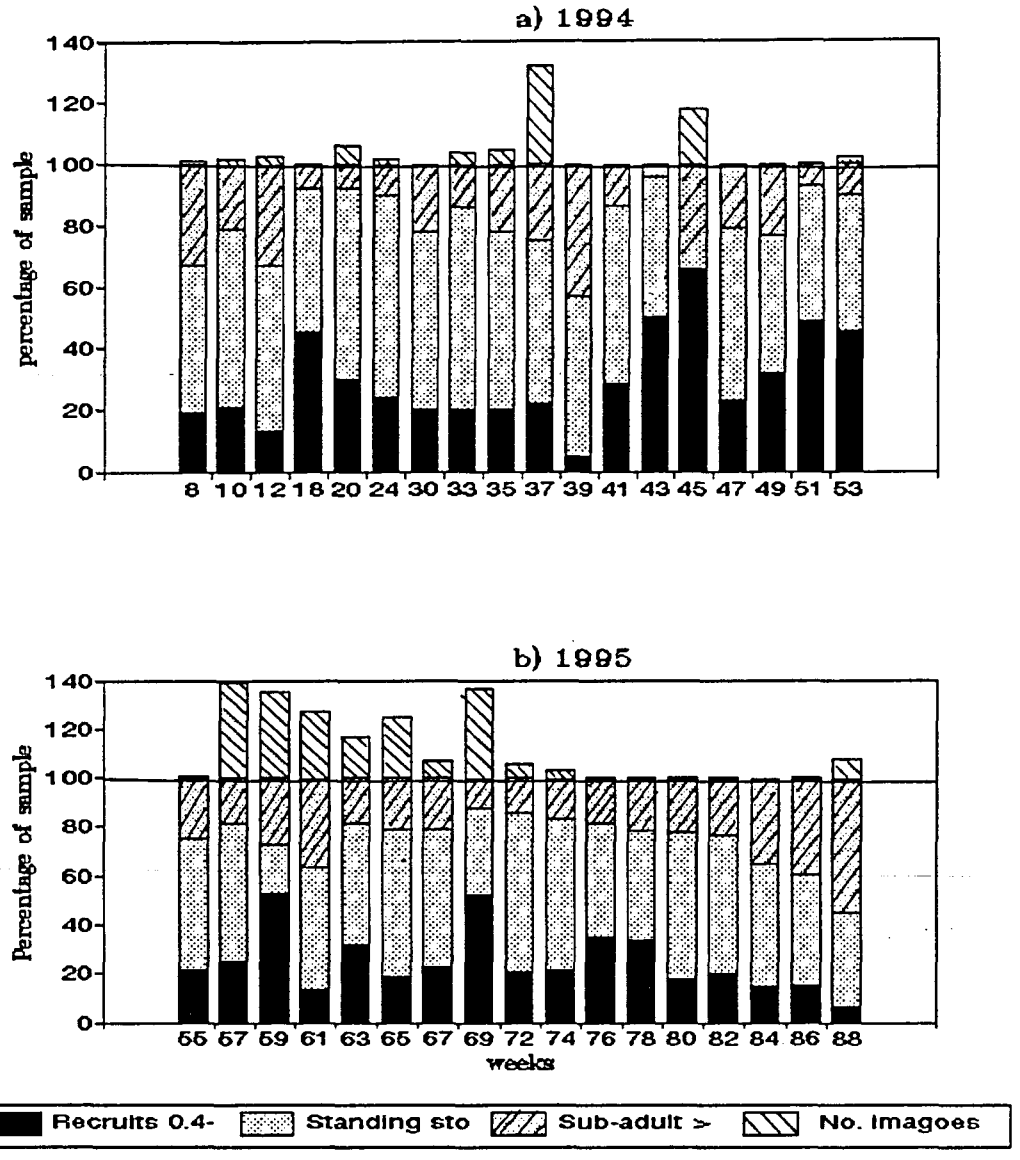

Figure 5.3.5 a & b). Stacked bar graphs depicting the population structure at one site in the Palmiet river over a two year period. The weekly samples are pooled at fortnightly intervals which would encompass an intermoult period of a large number of the population (see Exp. 5.2.3.1. instars between 8 and 15 days). a) Samples for 1994; b) samples in 1995. The X-axis displays weeks from February 1994 to 1995.

It must be noted that January, February, and June 1994 and part of April were not sampled. From August 1994 sampling was as described. The size classes are represented as percentage of the samples but the emerged adults and shucks have been added as numbers in the top line.

The question about the life cycle and the breeding periodicity remains unresolved, for although it is now known when the mating periods are, unless we can determine the period between hatching and sexual maturity we will be unable to deduce if the species is in fact multivoltine, or perhaps bivoltine. The factors which complicate interpretation of the available information are largely due to the scarcity of visible adults and the difficulty in finding the eggs, which makes the distinguishing of cohorts difficult. Further information which has not become apparent are the cues for emergence and the degree of synchronicity.

Firstly, consider the major emergence events. Emergences occurred in late April (weeks 16-18), when 7 adults were caught in the emergence trap and adults were observed at the site and 19 final instar shucks were found on rocks at the site. Further evidence of an emergence in April was the high proportion of final instar nymphs in late March, the very low proportion of recruits. It is possible that emergences were occurring throughout March and April. The results of samples from weeks 63 to 65 indicate a similar pattern for April 1995.

The next major emergence which was tracked occurred in weeks 35-37, (spring) when 5 adults were caught in the emergence trap and 22 final instar shucks were found at the site. The relatively short emergence period is most likely an artifact (Fig.5.3.6) as there was a rainstorm the week following, which could have washed the shucks off the rocks. This deduction is supported by the fact that the number of sub-adult were still high in the nymphal population despite the large emergences the preceding weeks.

Six weeks later a less numerous emergence occurred followed by the major summer emergence which was tracked from weeks 57 through 64. In May, (wk 70), another smallish emergence was recorded. During the winter spring which followed (weeks 72-86) no shucks were recorded and the sub-adults were present, but no maturation was noted. The expected early spring emergence in week 84 did not happen and the first shucks were counted in early October.

To enable interpretation of these data and to determine which emergences are related, mayflies have been reared in the laboratory at ambient temperatures in summer in order to get estimate lifespan. In Experiment 5.2.2 (Fig. 5.2.2.1) it became evident that it takes between 10 and 14 weeks for an animal of 0.6mm HW to reach maturity at 20 °C. From other observation (Exp. 5.4.1.) it has been established that the eggs hatch in 17 to 20 days (2-3 weeks). It has been estimated that a hatchling can reach 0.6 mm HW in 4-6 weeks. This estimate was obtained indirectly when a leaf pack with no nymphs in it was kept in a bubblepot and six weeks later small nymphs were found in the container, suggesting that leaf-packs may be the area to search for eggs.

Given all this information it can tentatively be inferred that the lifecycle of *A. auriculata* during summer is between 18 and 24 weeks. The spring emergence seems to give rise to the adults which emerge in

summer and the autumn emergence gives rise to those adults of the October emergence (Fig 5.3.5 (a & b)). Estimated growth rates were used to calculate possible generation periods of 14 to 23 weeks.

To display environmental variation and the changes in the sub-adult portion of the samples Fig. 5.3.6 was developed by the addition of recorded water temperature and average degree-days for the relevant interval after which the rainfall was overlaid on the numbers of sub-adults and imagoes. The most obvious fact that emerges from this is the difference in period between the autumn/late summer emergences and the spring/early summer flights (22 weeks versus 28 weeks). This may be related to the decline in the amount of heat energy available. The number of sub-adults trapped appears also to be correlated with the amount of rain.

To test the hypothesis that it is the total amount of available energy which governs the generation period, the degree-days were summed over the two apparent generation times (Fig 5.3.7), and these figures overlaid on the preadult nymphal and adult numbers. The similarity in the amount of summed energy which brought a generation to maturity becomes apparent.

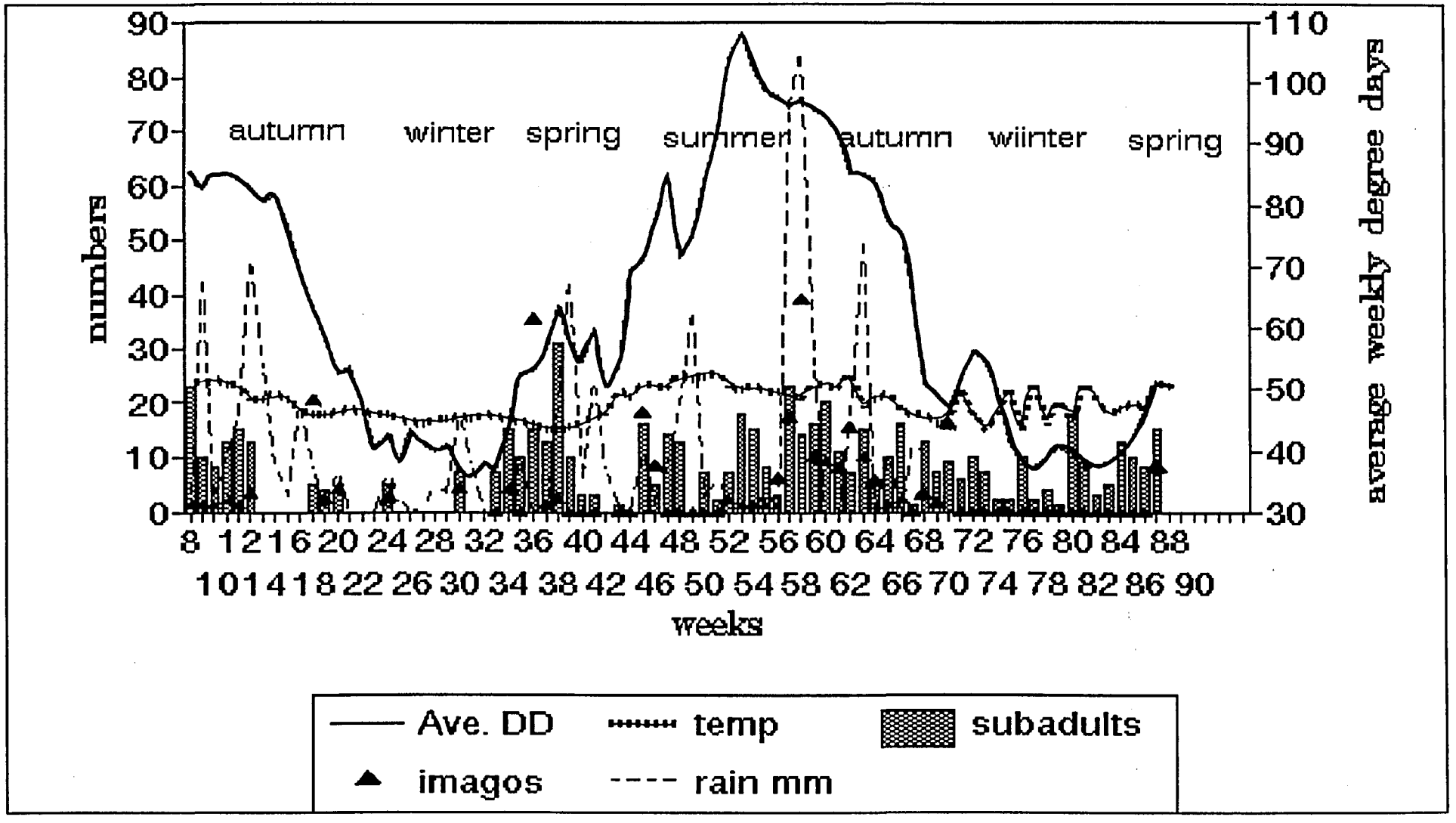

Fig. 5.3.6 The ambient environmental parameters (1994-95) in the Grahamstown district and average weekly temperatures in the Palmiet river overlaid on the sub-adult nymphal groups.

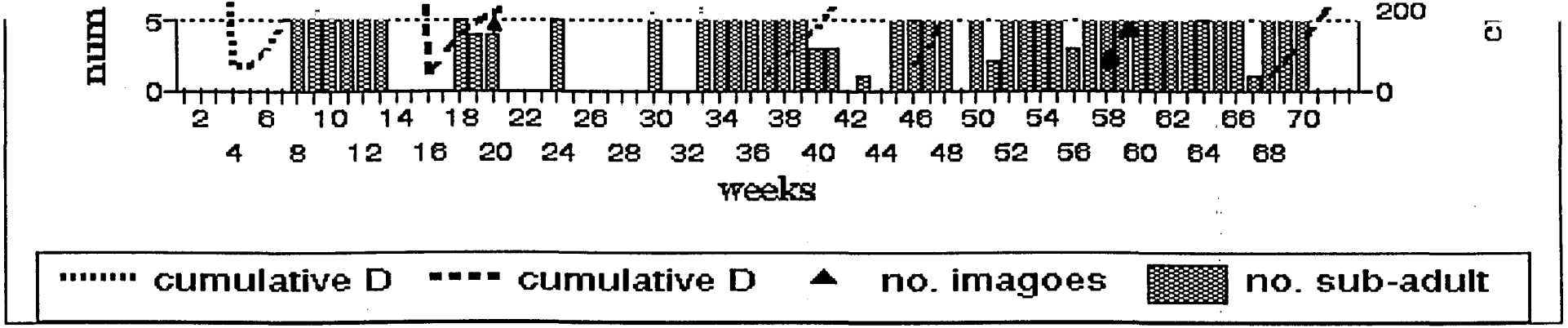

Fig. 5.3.7. Degree-days (DD) summed over the proposed lifespan of nymphs of *A. auriculata* in the Palmiet River, superimposed over bars representing subadults and triangles representing imagoes.

Discussion

Samplin

In designing a field sampling protocol the dilemma
the most comprehensive and representative insight in
which we encountered were an adult phase which i
poorly documented behaviour and an apparent over
when he questions the validity of using the captured
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Discussion

Sampling method.

In designing a field sampling protocol the dilemma is to choose a sampling procedure which will yield the most comprehensive and representative insight into the dynamics of the life history. The problem areas which we encountered were an adult phase which is not only short lived but sparsely distributed, with poorly documented behaviour and an apparent overlap in size classes. We concur with Malicky (1989) when he questions the validity of using the captured aerial phases of aquatic insect to deduce quantitative measures such as secondary production. To counter this problem, the presence or absence of the adult and subimaginal shucks in the surrounds of the stream was used as an indication of emergence. However, an absence of the shucks could not be taken as an indication that no emergence activity was taking place as rain and rising river levels could wash the shucks off the rocks. We have concluded that the condition of the pre-adult nymphs will have to be more carefully documented and that perhaps a third size class will be created to separate nymphs with wingbuds from those of the same size but not in metamorphoses.

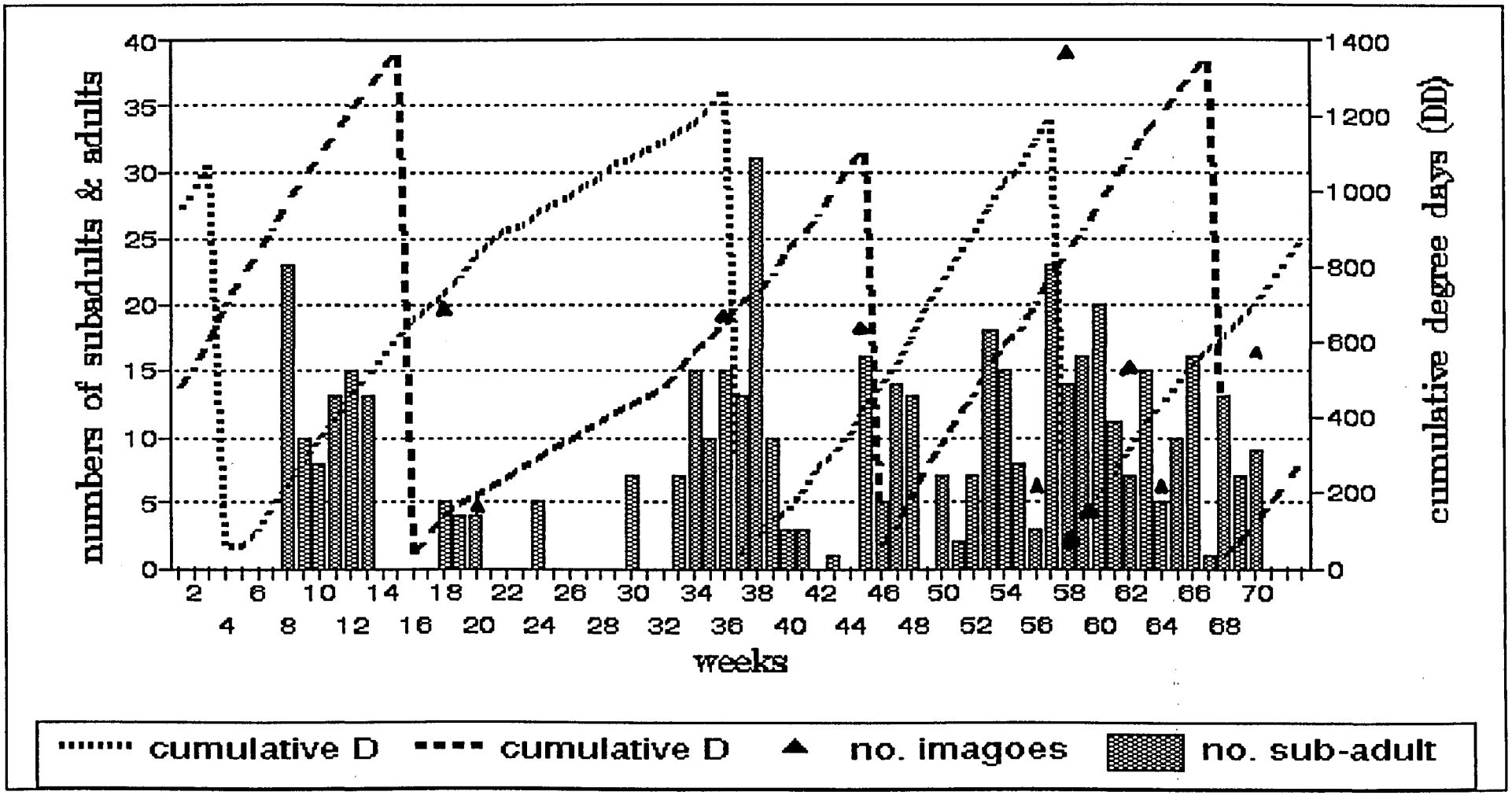
Cues for population changes

Studies have shown that flooding in unstable rivers, changes in water temperature and pH, pollution and food availability are amongst the factors that affect survival and distribution of other leptophlebiid mayflies (Collier & Winterbourn, 1990; Dudgeon, 1989; 1990; Graesser, 1988; Hall *et al*, 1988; Jowett *et al*, 1991; King *et al*, 1988; Scrigmeour *et al*, 1988; Scrigmeour and Winterbourn, 1989; Scrimgeour, 1991). During the period of investigation several flooding periods occurred when the river level rose dramatically and sampling was somewhat difficult. It was found that the numbers which were required for the sample could be collected in the detritus which collects at the edges of high water.

Temporal population dynamics and emergence periodicity

Brittain (1976) has shown that another leptophlebiid, *Leptophlebia vespertina,* in the northern hemisphere, has an adult emergences in late autumn and early winter. However it has become increasingly clear that the voltinism of aquatic insects in temperate areas are quite different from those in sub-tropical areas. This is largely due to the fact that temperature seems to be the major controlling factor influencing emergence periodicity as previous studies have shown (Brittain, 1976,1982, Sweeney *et al*;1978, Newbold *et al* 1994, Giberson & Rosenberg, 1994).

At monthly sampling intervals during 1993, it was possible to monitor the changes in population structure and to predict possible adult emergence periods for *A. auriculata* in spring and autumn. This complemented the findings of Sweeney (1978) who showed that *Isonychia bicolor* winter generations underwent a fairly synchronous emergence in the first ten days of spring in response to higher water temperatures in Pennsylvania. That the emergence in spring is fairly synchronous, is implied by the



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General conclusions

The introductory study of *A. auriculata,* in the Palmiet River suggested that the population distribution of nymphs in similar habitats (riffles and runs) are similar in size distribution but that higher densities were found in runs and that density of the size class 0.6-1.6 mm HW also fluctuated during the year. It has been impossible to estimate the size of hatchling as they are difficult to capture. This sampling monthly sampling regime over a seven month period indicated a possible late autumn/early winter and a late winter/early spring emergence of subimagoes. This was confirmed when the sampling regime was more frequent and extended over the summer period when a large summer emergence was found. The fact that emergences occur between these major events makes the analysis of the captured samples to determine the life cycle extremely complex. Laboratory growth trials which have been conducted give some indication of the growth rate but this cannot be taken as absolute until confirmed by field growth

trials. Temperature and food availability experiments will be conducted to examine the responses of this species to these variables.

Questions remaining include; a) what triggers emergence; b) what is the exact influence of temperature on growth rate; c) factors which influence fecundity and metamorphoses; d) do the hatchling nymphs (< 0.4mm HW.) inhabit the gravel and sand (de Moor pers comm.) or leaf packs as we suspect.

The correlation of environmental cues {photic = (day length and moon phases), thermal = (water temperature and degree-day) and partial pressure} with emergence periodicity requires a data set which covers more than one year to ensure replication of seasonal variations. Only if similar responses occur over a number of seasons, can conclusions be drawn about the cause and effect of climatic control on the life history of this mayfly.

5.4 ARTIFICIAL FERTILISATION

Aim

To attempt to artificially fertilize eggs in the laboratory and study the embryological development.

5.4.1 BACKGROUND

In the family Leptophlebiidae, female imagos lay eggs in suitable habitats by descending to the water and releasing a few eggs at a time when the tip of their abdomen is dipped into the water (Needham & Traver 1972; Brittain 1982). The eggs are covered with fine spring-like hairs which uncoil on contact with water and become adhesive (Needham & Traver 1972). The eggs presumably adhere to the first solid object that they come in contact with. The time taken for the eggs to hatch is strongly influenced by temperature. Most mayfly eggs may hatch in two to three weeks but those laid in cold streams (<10 °C) may diapause over winter (Needham & Traver 1972; Wise, 1980 and Brittain, 1982). The effect of temperature on hatching also appears to be linked to the natural environmental regime of the species.

Suter & Bishop (1989) incubated eggs of *Atalophlebia australis, Nousia inconspicua, N. fuscula & Baetis soror* from South Australia under constant temperature conditions in the laboratory (Range 4-24 °C). Embryonic development occurred at all temperatures, but hatching did not occur below 12 °C for *A. australis,* below 15 °C for *N. inconspicua* or below 5 °C for *N. fuscula & B. soror*. Photoperiod length had no effect on the incubation period. The hatch rate of eggs of *Potamanthus formosus* which was 34-53% over a temperature range of 15-30 °C, dropped abruptly below 15 °C to almost zero at 13°C (Watanabe,1992). Brittain & Campbell(1991), incubated the eggs of *Coloburiscoides* sp. in the laboratory at constant temperatures at 5°C intervals between 5 °C and 30 °C. Hatching success was high (> 80%) at temperatures between 10 °C and 25 °C. No eggs hatched either at 5 °C or 30 °C. Artificially fertilized eggs had low (< 10%) hatching success. Beside temperature, the pH of the water in which the species evolved must be considered when developing methods of artificial incubation. Rowe *et al* (1988) reared *Leptophlebia cupida, Habrophlebia vibrans, Stenonema femoratum & Baetis flavistriga* at pH levels (4.0, 4.5, 5.0, and 6.5) in the laboratory and the proportion of eggs undergoing eclosion did not vary with pH. Hatching rate was affected at the three lower pH values for *H. vibrans, & B. flavistriga* but was unaffected in the other three species which occur in low pH waters. Punzo & Thompson (1990) investigated the combined effects of pH and temperature on hatching and hatchling survival of *Caenis diminuta & C. hilaris* and found high mortality at pH 3.5 over a temperature range of 10-30 °C with best hatching and survival success 20 °C over a pH range of 4.0-7.2.

5.4.2 AN EXPLORATION OF METHODS TO ACHIEVE ARTIFICIAL FERTILIZATION FOR *A. AURICULATA.* S Burton (investigator) & E H Haigh (supervisor)

Aim

To achieve artificial fertilisation of the eggs of a leptophlebiid mayfly.

Introduction

At present most animals used in laboratory experiments are collected from the field. This is not only time consuming but may lead to over-sampling in the field. Hence, an artificial breeding program in the laboratory would be invaluable with regard to saving time and maintaining numbers in the field.

Artificial fertilization of egg of Ephemeroptera has been reported by several authors as part of a programme of investigating growth rates and life histories. Sweeney (1978) reports combining the sperm and eggs (*Isonychia bicolor*) directly on a glass slide without any other solution, leaving the mixture together for 5 minutes and the washing the eggs into a container of filtered stream water. Giberson & Rosenberg (1992a) on the other hand strip the egg from female sub imagoes into Yeagar's solution and then macerate the terminal segments of the males and place these with the eggs for 10 minutes. They also report that the fertilized eggs of *Hexagenia limbata* could be stored at 8 °C for extended periods and will nevertheless develop normally when returned to higher temperatures.

Material and Methods

Late or final instar nymphs were collected from a site removed from the field study site and returned to the laboratory and placed in bubble pots in a CER. Large rocks were placed in the bubble pots to provide crawl out area for the emergence of the sub imagoes. The time taken for the final instar nymphs to emerge could be reduced by placing them in a warm CER (20-23 °C). Subimagoes were removed from the bubble pots and placed in ventilated jars until they moulted into imagoes.

Fertilization

A number of methods were attempted to effect artificial fertilization. These included:

a) pinning a male and removing the head and then attempting artificial copulation with a female by holding their genitalia together. This method has proved successful with mosquitoes (WHO Report 1975).

b) dissecting the eggs out of a female in insect Ringers, Yaeger's solution and a mixture of insect Ringers and Yaeger's solution immediately dissecting out the seminal vesicles of the male and mixing the sperm and eggs. Both sperm and eggs were examined microscopically to check viability.

c) dissecting a male in insect Ringers and then stimulating a female to release its eggs onto the sperm by removing its head and dipping the tip of its abdomen in the Ringers above the sperm.

d) fertilization was also attempted between;

i. a male imago and a female subimago,

ii. between a male subimago and a female imago and

iii. between male and female subimagoes to determine whether the subimagoes contained viable eggs or sperm.

Eggs were then left in the sperm/Ringers mix for 5, 10, 15, 20 and 30min before being transferred onto ground glass slides in stream water to determine the minimum time that the eggs and sperm should be mixed to yield a sufficiently high fertilization rate.

Incubation

In the first series of experiments the newly fertilized eggs were placed in 500ml bottles of stream water with no lid containing an air stone with a nozzle to create a current and left at ambient temperature. In the second series of experiments the newly fertilized eggs were placed on ground glass slides in petri dishes of stream water and incubated at either 17 °C or 25 °C in CERs. Giberson (pers. comm.), found this method of artificial fertilization succeeded. Small samples of roughly 15-20 eggs were removed from the egg/sperm mix and transferred to small bottles. Eggs were also removed immediately after being placed in the petri dishes of water, every 24 hours for a week and thereafter once a week. All eggs were preserved in formaldehyde for microscopic examination. Drawings were made of the egg development.

Results

Microscopic examination (1000X) showed that the sperm were motile and were still viable after being dissected out of the seminal vesicles, handled with a pipette and transferred to a Ringers solution. The eggs were ellipsoid and approximately 200 μm by 90 μm in size. Fig 5.4.1.(a) shows the surface, shape and size of the eggs prior to fertilization. Hence, examination under 100X magnification was sufficient for observation of embryological development. Due to the three dimensional nature of the eggs examination was difficult at any higher magnification. The eggs are translucent white in colour and are covered with many small boss-like protuberances. After submergence in water a coat of fine 'hairs' is evident so that the eggs look similar to ciliated protozoans. These 'hairs' enable the eggs to adhere to surfaces as smooth as glass.

The first series of experiments (a) accomplished fertilization but development did not take place. Within 24h of fertilization a perivitelline space was evident in the majority of eggs (Fig. 5.4.1 b)) but no further development occurred. This perivitelline space did not form in eggs that were not fertilized in a control experiment and these eggs broke down after a period of approximately 2 weeks.

Fertilization was also accomplished in the second series of experiments (b & c) and development followed.

The length of time that the sperm and eggs were in contact influenced the proportion of fertilized eggs with approximately 50% fertilized after 5min, 70% fertilized after 10min, 80% fertilized after 15min and 85% fertilized after 20 and 30min. Hence, in all of the following experiments in the second series the sperm and eggs were left together for 15min as a fertilization rate of 80% was thought to be sufficient. It was also found that subimagoes did contain viable sperm or eggs and fertilization rates were no different when male and female imagoes, a male imago and a female subimago, a male subimago and a female imago and male and female subimagoes were used for the fertilization experiment. Nymphs that managed to break totally free from the chorion were regarded as having hatched.

As was found by Giberson (pers. comm.) the hatch rate of artificially fertilised eggs was less than 15%. Further, eggs hatched in only three of the experiments. All three successful experiments involved a male imago and a female imago. The eggs in the first two were fertilized by dissection of both imagoes and yielded very low hatch rates. Only 5% and 6% hatched in the first and second successful experiments respectively. In the third successful experiment (c) the female imago was not dissected but induced to release her eggs as described above. In this experiment a hatch rate of approximately 15% occurred. Those eggs that did hatch hatched between 16 and 22 days at 25 °C. No eggs incubated at 19 °C have hatched but many appeared to still be developing at the termination of this trial.

Fig 5.4.1. (b-g) shows the sequence of development over time. Once again the perivitelline space formed in approximately 24 hours. After five days a small embryo was visible as a dark area in the egg that had displaced some of the yolk. This increased in size and length and a head area, a thoracic area and an abdominal area were evident on the tenth day. After 14 days the embryo's began to resemble mayfly nymphs with a head, three thoracic segments and a number of abdominal segments. An eye patch, antennae and legs were also visible. Fig. 5.4.1.(f) shows a hatched egg with the hole in the chorion clearly visible. In all hatched eggs the hole was a longitudinal slit orientated near one pole of the egg. The first instar nymphs were approximately 350 μm long and resembled the later instars except for their pentagonal shaped heads lack of filamentous gills; simple tarsi and three equally sized ocelli (Fig.5.4.2 a) These nymphs were able to swim and crawl and fed by brushing the bottom of the petri dishes. Gills began to develop in the later instars and these instars were more active (Fig.5.4.2 b). Only two replicates of each experiment were performed due to a lack of available adults.

Discussion

Artificial Fertilization

The reasons for the failure of the first series of artificial fertilization experiments are not clear. Perhaps the containers in which the eggs were placed were not suitable (too deep, not sufficient oxygen). It was observed that algae soon covered the eggs on the slides which would not occur in the high current areas

in the stream where the eggs would settle.

The second series of experiments during which the eggs were incubated in shallow petri dishes was more successful. Algal growth was suppressed by covering the petri dishes with a semi-transparent lid, thus reducing the light intensity. In her successful experiments with a different species of mayfly, Giberson (pers.comm.) used Yeager's solution. Yeager's solution, however, triggered the adhesive 'hairs' prematurely and resulted in the eggs not adhering when placed in water. Thereafter, a fresh insect Ringers solution and a combination of the two were used and both proved successful. The reasons for the high fertilisation rate and low hatch rates are not clear and can only be determined with further experimentation. Many eggs could have succumbed to bacteria or other microorganisms so it may be necessary to use filtered stream water. Further, 25 °C may have been too high and the eggs may have been oxygen deprived. A possible method for this would be to fertilise a large number of eggs as described above and 'seed' them onto a fine mesh screen in still water. Once the eggs adhere which takes a few minutes, a current can be directed over the screen to ventilate the eggs. A detailed study of the development has not been accomplished at this stage as insufficient numbers of eggs and hatchlings were available.

Conclusion

It has been demonstrated that the eggs of *A. auriculata* can be artificially fertilised in the laboratory and viable offspring can be obtained. The most successful method involved dissecting the seminal vesicles out of a male imago in a fresh solution of freshly prepared insect Ringers. The female can then be induced to release her eggs into the solution by decapitation and dipping the tip of her abdomen onto the surface of the mixture. This mixture of eggs and sperm is then left for at least 15 minutes before the eggs are transferred to a suitable substrate in river water. Egg development is fastest at 25 °C. However, further work is necessary in this field to increase the hatch rate.

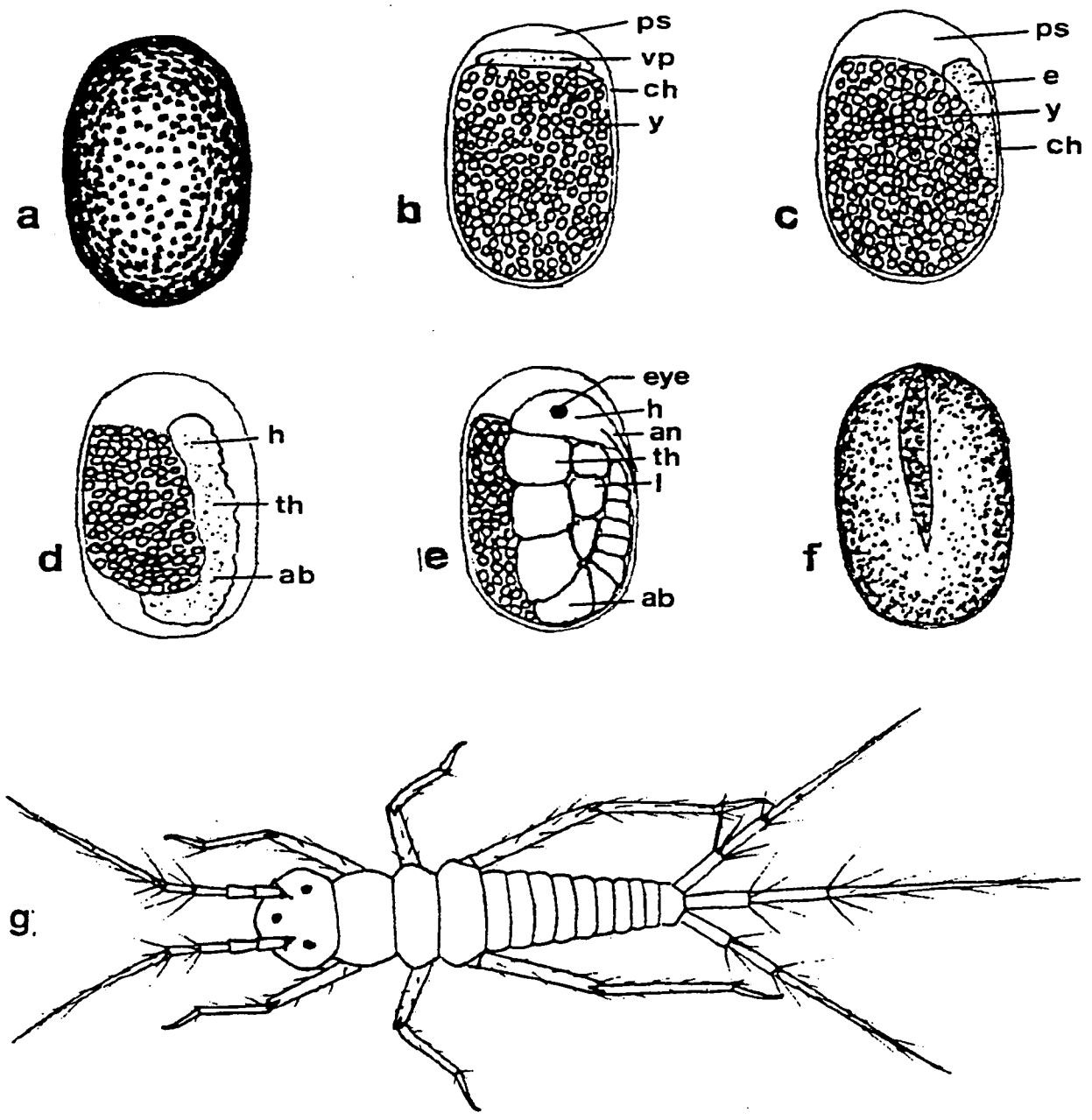

Fig. 5.4.1. Embryonic development of *A. auriculata* after artificial fertilization. (a) Surface of egg prior to fertilization; (b) fertilized egg with perivitelline space, 24 hours; (c) early embryological development, five days; (d) embryo with head, thoracic and abdominal regions, 10 days; (e) late embryological development, 14 days; (f) chorion of hatched egg, 16 days; (g) Newly hatched first instar nymph. ch- chorion; vp- ventral plate; ps- perivitelline space; y- yolk; e- embryo; h- head; th- thorax; ab- abdomen; an- antennae; l- leg. (Interpretation based on an embryological study of a heptageniid mayfly by Needham & Traver (1972))

ich were hatched from artificially fertilized
ith two gills. (Mag.x 3200) c) Field caught
tions.

5.5 SUMMARY.

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b) Diet and temperature variables. In this
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growth rate in response to diet and temp
The average instar period for nymphs o
the range was wide. The average instar

5.5 SUMMARY.

When a new field of research is broached the planning of the protocols is similar to a journey into unknown territory. Maps are available in the form of guidelines from previous work in related areas and established methods which can be adapted, but the topography of the landscape is unknown and the pitfalls are only discovered through experience.

In the case of an invertebrate aquaculture project the methods employed in pisciculture have offered useful guidelines as far as methods are concerned, and the literature of basic research on aquatic invertebrate biology has offered ample information to indicate the life history style which may be encountered in the selected candidate. Principles which were to be heeded in the execution of the project were found in the review of literature on the use of stream macroinvertebrates in ecotoxicology.

According to Johnson *et al* (1993), the life history indicators of environmental stress are survival, growth and reproduction. This was an indication that information about these life history parameters, under natural unstressed conditions, must be compiled and utilized as a baseline against which to judge results from toxicological experiments. Buikema & Benfield (1979) reviewed a hundred publications on toxicology and found that 50% used no life history information in the analysis of the results. A survey by Mayer & Ellersieck (1986) indicated a global need to develop acute toxicity methods for baetid and burrowing mayflies, caddisflies and stoneflies because these orders form an important component of stream ecosystems and are recognised indicator species in biomonitoring. Pontasch & Cairns (1991) suggest that mayflies are the group most sensitive to the deleterious effects of pollutants.

The experimental work on *A.auriculata* was planned in such a manner as to give information on their responses to:

a) Habitat variables such as hydraulic conditions and substrate type. It was discovered that solid substrate was an essential pre-requisite and, that although the species is found most abundantly in riffles and runs, it survives better in static aerated water than in experimental channels. This concurs with observations in the field that *A.auriculata* is seldom captured in the full current but tends to be found under stones on the edges of runs where detritus collects.

b) Diet and temperature variables. In this investigation it was found that the natural diet of decaying leaves and periphyton could be successfully supplemented by the addition of TETRAMIN, a commercial fish food, and provide optimal conditions for growth provided that good water quality was maintained. Daily growth rate in response to diet and temperature variation was calculated and is given in table 5.5.1 below. The average instar period for nymphs of *A. auriculata* was negatively correlated with temperature and the range was wide. The average instar period calculated was 7.5 days at 25°C; 10.44 days at 20°C and

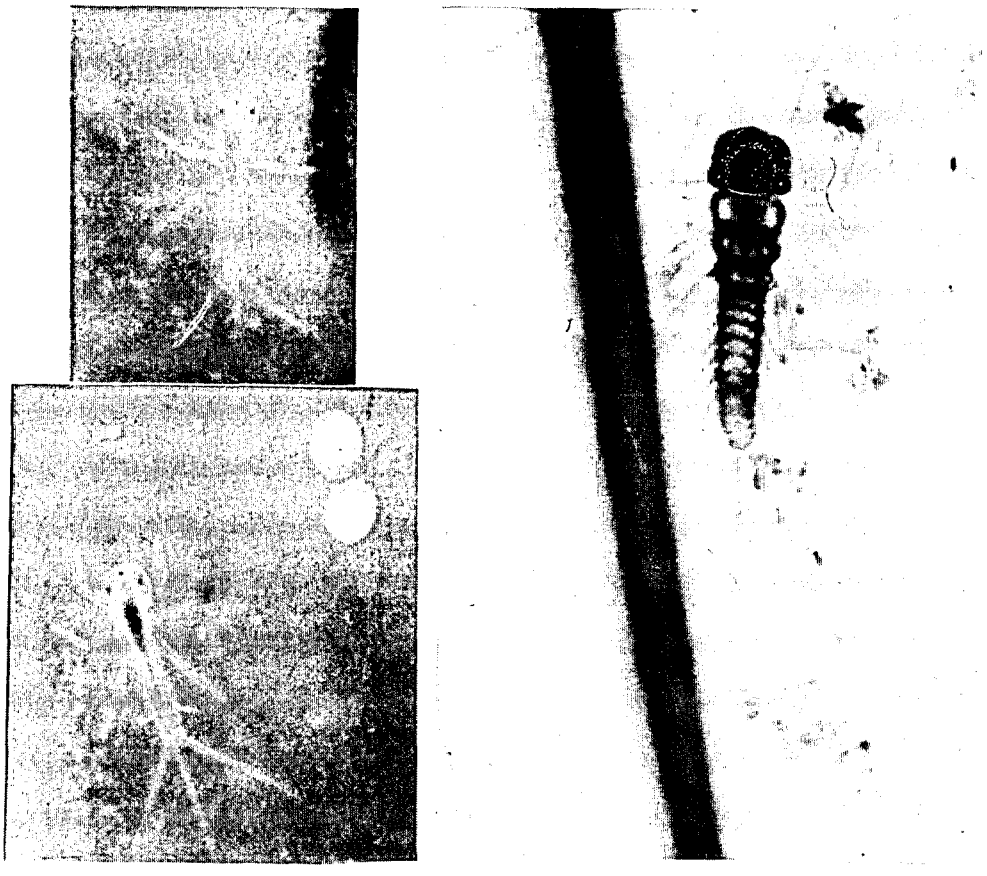

Fig. 5.4.2. Photomicrograph of nymphs of *A. auriculata* which were hatched from artificially fertilized egg. a) first instar nymph b) second instar nymph with two gills. (Mag.x 3200) c) Field caught nymph of the smallest size which is found in collections.

15 days at 15°C. The shortest instar in all cases was 4 days and the longest 27 days.

Table 5.5.1 Growth rates (mm/day) pre sub-adult nymphs calculated from all experiments in channels and bubblepots in the laboratory

EXPERIMENT NO.	AVE. GROWTH RATE MM/DAY	TEMPERATURE CONDITIONS	HYDRAULIC COND. & DIET
5.2.1	0.019-0.023	19-22°C	Flowing; periphyton
5.2.2	0.014-0.042	17-22 °C	Static; leaves TETRAMIN
5.2.5	0.005 0.009 0.015	15 °C 20 °C 25 °C	Static, leaves
RANGE	0.005-0.04	15-25°C	

Buikema and Benfield (1978) discussed the importance of using both life cycle and life history information in designing and assessing toxicity tests. For many reasons, different life stages, such as immatures and gravid females, have been found to be more sensitive to toxicants. Reproductive impairment and moulting frequency are good measures of stress. Therforee, if the selected test species is to become a successful laboratory animal, its responses to test conditions should be accurately interpretable against adequate natural life history information. To fulfil some of these requirements, the field population was studied over a three year period. We determined that *A. auriculata* probably has a multivoltine life cycle. There was a major emergence period from February throughout March and April at ever decreasing numbers. Then in May, after a hiatus, another brief emergence was recorded followed by a relatively short emergence period in spring. During winter and summer no emergences were recorded and the sub-adults present in the samples showed no visible signs of metamorphoses.

Using the estimated experimental growth rates to calculate possible generation periods, a figure of 14 to 23 weeks is reached for *A. auriculata.* The spring emergence could give rise to the adults which emerge in late summer and the autumn emergence may give rise to late spring adults.

A captive breeding programme is essential to have sufficient numbers of test subjects of a guaranteed quality and genetic. Given the current level of knowledge about the breeding behaviour and cues for *A. auriculata*, natural reproduction in the laboratory is not yet feasible, so methods for artificial fertilisation were investigated. The most successful method involved dissecting the seminal vesicles out of a male

imago in freshly prepared insect Ringers solution. The female was induced to release her eggs into the solution by decapitation and dipping the tip of her abdomen onto the surface of the mixture. This mixture of eggs and sperm is left for at least 15 minutes before the eggs are transferred to a suitable substrate in river water. Egg development is fastest at 25 °C and viable offspring of *A. auriculata* resulted.

Further investigations of this species should include:

a) A detailed experimental investigation of artificial fertilisation techniques and methods to increase hatch rate.
b) Rearing of hatchlings.
c) Determination of growth rates and generation periods in the field.
d) Field investigations of mating behaviour.

The completion of this work should enable us to rear several other mayfly species from similar habitats and with similar food requirements in the laboratory. These may include *Choroterpes elegans* from the Buffalo River and other *Choroterpes* species. This genus has already passed the first test of survival as has *Trichorythus* spp. from the Sabie River (Kruger Park).

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CHAPTER 6
SUMMARY OF RESULTS

Introduction

The sustained cultivation of an organism in an artificial environment is dependant on several related factors, primarily the provision of a suitable environment. The design of the environment should be as closely modelled as possible to the natural environment in which the organism occurs; using those environmental influences considered to be the most important, including adequate uninterrupted food supply; the correct ambient temperature and photoperiod to optimise growth and fecundity; and in the case of aquatic organisms, water with the correct nutrient balance. Literature surveys may obviate the need for extensive and time consuming experimental investigation and results from short term research projects provide salient parameters. Once the abiotic factors and habitat are selected, the impact of other biotic factors such as density effects can be determined.

In this project the habitat requirements, some of the dietary requirements, the responses to temperature variation and a description of the natural environment of both species have been addressed.

6.1. SUMMARY OF RESULTS

The selection process for species to be maintained in laboratory systems was determined by the needs of the artificial stream project, in their investigation of tolerances of lotic macro-invertebrates to water quality variables. This lead to the investigations into the biological and environmental requirements of those species selected, and the necessary equipment and methods necessary for maintaining them.

6.1.1. SCREENING OF ORGANISMS.

There are certain criteria to be considered in the selection of organisms in determining their suitability as subjects for ecotoxicological studies. For the purposes of both the Artificial Streams Project and the Standard laboratory Organisms Project, the hierarchy of considerations in the selection process were ranked as follows;

1. Availability in the field and size of the organism. A field population of the candidate

species must be easily accessible, so that stocks for early laboratory experiments can be replenished.

2. Suitable life history and biology for maintenance in laboratories. Problems associated with aerial phases can be overcome. Narrow habitat requirements would necessitate specific, possibly expensive, holding conditions and would, therefore, be undesirable. So too would very specific dietary requirements.
3. Accurate identification.
4. Sensitivity to a range of test chemicals.
5. Position and function in the ecosystem.

Several screening trials, observations during toxicological investigations conducted by Palmer *et al* (1995), and field collections contributed to the identification of 18 possible taxa, found among the Crustacea, Mollusca, Insecta and Platyhelminthes. Those taxa which have been subject to detailed investigation as a result of this selection process are:

- Ephemeroptera; Leptophlebiidae.
  Euthraulus (Choroterpes) elegans and *Adenophlebia auriculata:*
- Mollusca; Ancylidae
  Burnupia stenochorias:

Further genera which have potential as laboratory candidates are *Tricorythis* sp. (Ephemeroptera); *Cheumatopsyche* spp. (Trichoptera) & Planaria. Among the Crustacea, an amphipod, *Paramelita* sp., is already used as a test subject at the University of Cape Town and *Caridina* sp. has been successfully bred at the University of Natal.

6.1.2 METHOD DEVELOPMENT

The conditions which were a prerequisite for maintenance and breeding of macroinvertebrates from rivers were investigated, and the following key parameters were identified: hydraulic conditions and water quality, substrate, diet and temperature.

With regard to collection and transport, animals should be handled as little as possible during collection and transported in cool, well aerated water with substrates. Two forms of maintenance equipment were tested, namely recirculating channels of two sizes, and cylindrical plastic bubblepots of various sizes. These proved to be the most suitable containers for rearing small juveniles of both the limpets and the mayflies. Observations from all experiments confirmed that solid substrates are essential for the optimal survival of the selected stream macro-invertebrates. However, for their transport, softer substrates such as fine textured plastic foam rubber, plastic sheeting or leaves from the collection site should be offered.

With regard to dietary requirements, the limpet is a grazer and the mayflies are brusher collectors: both animals collect their food by scraping the surface of stones. Periphyton grown in the laboratory has proved to an adequate basic food to sustain growth in both species, with degraded leaves, river detritus and TETRAMIN also suitable for mayflies.

In conclusion, the species investigated to date can be adequately housed and fed prior to being used for experimental purposes in the Artificial Streams Project. The most important conditions for the successful maintenance, and particularly breeding, of invertebrates are:

1. Correct light conditions for algal growth to ensure good food supply.
2. Adequate thermal regulation.
3. A good water supply, preferably from a non municipal source with the facility to regulate its quality and condition.

6.1.3 INVESTIGATION OF SELECTED SPECIES

In attempting to satisfy the second aim of the project (to develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions) a two-pronged approach was used. To be able to design optimal holding and breeding conditions for any species some understanding of the life history, the habitat requirements and the breeding biology is needed. Consequently, the responses of the selected species to laboratory conditions were recorded experimentally, while the ecology and life history were recorded by regular field investigations.

6.1.3.1 Laboratory Maintenance and Rearing Conditions

Hydraulic requirements

It would appear that neither species is obligately rheophillic as both species showed good survival in bubblepots. It was clear that the provision of substrates is essential in both hydraulic conditions. Hatchlings of limpets survive better in bubblepots, mainly because they are easily swept from channels. The limpets laid a greater number of eggs in aerated, standing rather than in aerated, flowing water.

Dietary requirements

Burnupia is a microherbivore which feeds by rasping the stone surface with the radula. It has been adequately maintained on laboratory cultured periphyton (see Chapter 4). It does not require feeding during short experiments (96 hours, acute test period: see Section 4.2.1.3). The mayflies are collector gatherers and it was found that the natural feed of decaying leaves, detritus and periphyton could be supplemented by TETRAMIN, which provided optimal conditions for growth.

Temperature

Temperature effects

Both species responded in a predictable manner to various, constant temperatures (15°C, 20°C and 25°C) with increased growth rates, and the mortalities of the limpets did not appear to be significantly affected. Mayflies responded with significantly shortened instar periods but with increased mortalities at 25°C.

Density effects

The best survival was attained at a density of 20 limpets at 20°C. This can then be regarded as the optimal stocking density. However, the effect of this density and temperature on the fecundity is unknown, and it has been stated by many authors that temperature which rises above certain optimal limits has a detrimental effect on the fecundity (section 4.2.2)

Handling requirements

Handling adversely affects the growth and survival of limpets. Anaesthetics must be used when collecting from the field, and a measuring template during laboratory experiments. Mayflies are more robust and can be easily handled with a small camel hair paintbrush.

6.1.3.2 Biology of selected species

Breeding biology of *Burnupia stenochorias* .

As hermaphrodites (Brown, 1980) these limpets are able to produce young without copulating, but copulation has been observed, with the smaller limpet acting as the male. Copulation takes place at night and early in the morning, with eggs being laid at night under stones. The size of limpet at time of egg-laying was always observed to be at greater than 3,4mm shell length. Field observations for eight months have determined that a small number of eggs are laid throughout the winter, but that a substantial increase in egg-laying occurs in the months of September and October.

Embryology

Burnupia displays direct development, with the young emerging as crawling snails. Hatching success in the laboratory is 91%. The embryonic period is 14 to 17 days at a temperature of 19°C. Cooler temperatures extend the time of hatching up to 21 days at 13°C (pers.obs.).

Fecundity

The number of eggs per capsule ranges from 1 to 13. Those limpets reared in the wild laid between 14-44 (ave 24.7) capsules per pair with an average of 5,75 eggs per capsule when brought into the laboratory. Juvenile limpets (approx. 2,5mm) reared in the laboratory either as pairs or singly produced on average 3,7 egg/capsule and 1-16 (ave 8.1) capsules per pair.

Breeding biology of *A. auriculata*

The breeding biology of *A. auriculata* is unknown and literature searches indicate no captive mating has ever been described. However it was demonstrated that the eggs could be artificially fertilised, producing viable hatchlings.

Field investigations

The field population has been studied over a three year period in the Palmiet River where the largest numbers of *A. auriculata* are found in riffles and runs. The size distribution of nymphs is similar in these two habitats but higher densities occur in runs. Hatchlings have not yet been captured in the field. There are major emergences in late autumn/early winter, late winter/early spring and a large summer emergence over several weeks in February and March. Size distributions of nymphs over a twelve month period indicates overlapping cohorts. Laboratory growth trials have given an indication of the growth rate and therefore possible generation period in laboratory conditions.

6.2 COMPLETION OF AIMS

i. *To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.*

♦ Screening was accomplished in the eastern areas of the country. Invertebrate researchers countrywide were consulted by questionnaire and eighteen candidates suitable for laboratory cultivation were identified. Of these, three species were investigated further.

ii. *To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.*

♦ A pilot programme has been established and methods to feed and house these chosen invertebrates are in place. Problems do, however, exist concerning long term survival of the limpet (through more than two generations) and the artificial fertilisation and rearing of hatchlings of the mayfly.

iii. *To attempt to establish methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and test for macroinvertebrate to various conditions in the experimental stream project.*

♦ Until the problems referred to in (ii) are solved, the sufficient supply is not yet feasible. However during the course of the project toxicological experiments were supplied with limpets on two occasions and several problems associated with handling and transporting the test species were addressed and solved.

6.3 CONCLUSION AND DISCUSSION

We believe that the new knowledge gained about responses of these two species to environmental variables has been significant, contributing to the understanding of ecosystem function of subtropical rivers.

It is believed that enough knowledge has been accumulated to enable a sustainable pilot maintenance project to be launched. From the figures obtained about growth rate, expected survival and density effects recorded, the productive potential of each of the large channels or bubblepots can be calculated, despite the areas of uncertainty which remain.

Aspects of the life history of the mayfly that still need investigation include;
a) the exact influence of temperature on growth rate and field growth rates;
b) fecundity and factors which influence it;
c) metamorphoses;
d) the abode of the nymphs less than 0.4mm HW.;
e) cues which trigger emergence.
f) techniques for artificial fertilization and hatchling rearing, the most important aspect to be investigated.

With regard to the limpet, the high mortality experienced in the laboratory must be overcome. It is suspected that both the water supplied to the limpets in the laboratory, to date, and the inadequate supply of the correct diatom combination, could be the causes. Detailed investigation of the diet should be completed.

CHAPTER 7
RECOMMENDATIONS FOR FUTURE WORK

Given the problems highlighted in Chapter 6, the sufficient supply of test species to the stream laboratory on an onoging basis is not yet possible. The bottlenecks we have identified are reproduction in captivity of the mayfly and life long survival of a large proportion of both selected species.
We estimate that the further requirements for the cultivation, maintenance and supply of sufficient experimental animals for ecotoxicological experiments to artificial stream laboratory can be listed as follows:

- Further knowledge of the cues for breeding, and better survival of juvenile *Burnupia.*
- Development of techniques for artificial insemination and maintenance of early instars of *A. auriculata.*
- The investigation of optimal growing conditions for the cultivation of periphyton.
- Increasing the scale of laboratory maintenance facilities to provide more capacity for the maintenance of larger laboratory populations.
- The design and construction of a dedicated laboratory for production and maintenance of experimental populations of invertebrates.

7.1 DEVELOPMENT OF A DEDICATED REARING AND MAINTENANCE LABORATORY

Present facilities in the Institute for Water Research are inadequate for the envisaged production of experimental animals. The requirements for a purpose-built facility are as follows:

- Floor space of at least 100 m^2.
- Adequate temperature control, by air-conditioning.
- Adequate natural light.
- Periphyton cultivation facilities which are separate from the insect rearing facilities.

- Water purification facilities, e.g. by ultraviolet irradiation and filtration.

- Rearing and breeding channels, as well as extensive population holding facilities, with temperature and light control.

Two initiatives are proposed for the second phase of this project, which begins in January 1996:

- A visit to Stroud Water Research Centre, Pensylvania, where a large amount of mayfly research is conducted and Virginia Polytechnic in Blackenberg where ecotoxicological testing has been ongoing for 20 years, to learn about the conditions described above.

- The engagement of a fund-raiser, to obtain funding for the building of a proposed laboratory.

7.2 OBJECTIVES FOR THE SECOND PHASE OF THE PROJECT.

The objectives of the proposed project can be divided into three main aims:

1) To complete the experimental and field investigations on the selected test species:
Burnupia stenochorias. Field studies of natural reproduction and laboratory studies on specific feeding requirements and handling techniques wil be executed.
Adenophlebia auriculata. The laboratory studies on artificial fertilization, and the rearing of hatchlings will be continued.

2) To scale up the experimental techniques developed to a larger production level. To test the suitability of various biological filtration systems at this larger scale to ensure optimal water quality. To optimise the culture of selected diatom species present in periphyton.

3) The investigation of design features and costing for a laboratory dedicated to the large-scale production of the selected species.

7.3 PROPOSED WORK PROGRAMME.

7.3.1 *BURNUPIA STENOCHORIAS.*

a) The field investigation currently being conducted into the reproductive biology of the limpet, should be completed by August 1996. Fortnightly observations which involve the measurement of 300 individuals and the recording of environmental variables, are made at two sites on the Blaauwkrantz River.

b) To identify the onset of sexual maturity by sectioning individuals of known age and size collected through the year will be completed by September 1996. This will link individual size to sexual maturity.

c) Investigation of the dietary requirements of the limpet will be initiated in January 1996, followed by the investigation of fecundity in response to diet.

d) Analysis of the results will be conducted during the experimental period but final interpretation will be possible at the completion of the experimental work.

Mrs Heather Davies-Coleman will be responsible for this work with support from and supervision by Mrs. Haigh and Prof. O'Keeffe.

e) Experiments to test handling methods using two anaesthetics will be conducted by Mrs Haigh in collaboration with Mrs HI White of the Department of Fisheries Science and Ichthyology, Rhodes University.

f) The identity of the various members of the genus *Burnupia* is not clear because shell shape which is the major identifying feature varies not only between species, but also between sites, depending on current speed and type of substratum (Brown, 1980). To identify species with certainty it is necessary to investigate the genetic basis of variations among populations and taxa. This would form the basis for postgraduate thesis. The uncertainty in this area is a problem with the development of the limpet as a country wide test species

7.3.2 *ADENOPHLEBIA AURICULATA.*

a) The current field investigation of this species in the Palmiet river will be completed by May 1996.

b) Experimental work on artificial fertilization and rearing of the hatchlings will be ongoing in 1996 and early 1997.

c) The influence of temperature on growth and fecundity will be investigated as part of the rearing programme.

Mrs Lil Haigh will be responsible for the investigations of the mayfly.

7.3.3 PERIPHYTON.

The investigation of the identity of the component periphyton species and optimal water quality conditions for the growth of periphyton will be conducted in collaboration with Prof. Braam Pieterse at Potchefstroom University and possibly with Prof. Grobbelaar from University of the Free State during 1997.

7.3.4 DEVELOPMENT OF THE REARING FACILITY

A preliminary agreement has been reached with Dr Anton Bok of the East Cape Department of Nature and Environmental Conservation to conduct a trial at Amalinda fish hatchery in East London, where an existing indoor pond which appears to be suitable for the maintenance of invertebrates on a large scale is situated. Water of excellent quality. This trial will enable us to monitor the interior environmental conditions in a structure of this type for an extended period and establish the suitability of this design. It is foreseen that the commissioning of the trial will be undertaken by Mrs Haigh but the monitoring will be carried out by staff at the hatchery under her supervision.

Once these investigations have been completed plans can then be drawn up for the construction of a dedicated facility in Grahamstown. A fund-raiser will be engaged to obtain funding for the project. Prof. T. Hecht and Mr. P. Britz at the Department of Ichthyology and Fisheries and Science at Rhodes both have extensive experience in the construction of aquaculture facilities and have agreed to oversee the planning phase.

7.4 REFERENCES

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APPENDIX 2

2.1 INVESTIGATIONS INTO THE IDENTITY AND LOCATION OF SUITABLE TAXA.

Table 2.1.1. Collecting trips undertaken.

DATE	RIVER AND LOCATION
January, September & October 1993	Buffalo River; East London -Maden Dam
February 1993 to date	Berg & Palmiet Rivers; Grahamstown district
March 1993 to date	Belmont Valley stream; Grahamstown
July - August 1993	Sabie River; Kruger National Park
August 1993	Nahoon river; East London
March 1994	Kologa & Buffalo River; Stutterheim

Table 2.1.2. Availability of suitable species from the sites visited over the period of the project to date. Abundance has not been quantified. XX indicates that sufficient numbers (at least 180) of animals of similar size in good condition were collected during a two to three hour period for a replicated experiment (s/s = small sizes).

LOCALITY	SPECIES	TIME /DATE	ABUNDANCE		
			Good	Fair	Poor
PALMIET (Grahamstown)	Leptophlebiidae, Baetidae Trichoptera	DECEMBER -MARCH			X
		MARCH - MAY	XX		
		MAY - JULY		X	
		AUGUST - DECEMBER	XX		
BLAAUWKRANZ stream Grahamstown	Ancylidae	AUGUST - NOVEMBER	XX	X	
		JANUARY - APRIL			X s/s
	Baetidae	AT ALL TIMES	XX	X	
BUFFALO upper reaches	Leptophlebiidae Baetidae, Trichoptera Ancylidae	JANUARY		X	
		MARCH - APRIL	XX		
BUFFALO lower reaches (East London)	Leptophlebiidae	SEPTEMBER	X		
KOLOGA river	Ephemeroptera many spp. Ancylidae Trichoptera	APRIL		X	
SUNDAYS river, Carlisle bridge	Baetidae Trichoptera			X	

2.1.1 CONSULTATION WITH OTHER RESEARCHERS

Invertebrate experts country-wide have been consulted to gather information and solicit opinions on the suitability and availability of species for use as regional standard taxa. A questionnaire was compiled and circulated to invertebrate workers throughout the country. Those which were returned, have been analyzed and the table below is the result.

Several people were interviewed during the past year. These include; At RAU Prof. Schoonbee and Dr. Bickerton at the CSIR (Stellenbosch) on the culture of Crustacea. Drs Day and King and their staff and Prof. Davies and his co workers at UCT. Consultation was also held with Prof C. Appleton from UCN on the subject of molluscan culture. The anecdotal information thus gathered, was used to help during the designing of the equipment and in selecting suitable taxa. Questionnaire appended (APPENDIX 2a)

Table 2.1.3. List of possible suitable candidate taxa.

GROUP	*SPECIES*	AREA	RESPONDENT
CRUSTACEA	*Paramelita nigricolus* *P. capensis*	W. Cape	Day; Griffiths
	Caridina spp.	Natal	Rayner; Alletson
	Palaemon capensis	W Cape	Day
	Macrobrachium sp.	E Tvl.	Van Vuren
	Streptochephalus sp.	E Cape	Day
MOLLUSCA	*Bullinus tropicus*	Natal	Rayner
	Ferissia sp.	Natal	Rayner
	Burnupia sp.	Wide	O'Keeffe
	Lymnaea natalensis	E. Cape	
	Physopsis globosus		
Planaria	*Dugesia sp.*		Day
INSECTA Ephemeroptra	*Trichorythus spp.* *Afronurus harrisoni* *Adenoplebia auriculata* *Choroterpes elegans & other spp.*	Wide	Alletson; Palmer
Trichoptera	*Cheumatopsyche afra &* *C. thomasetti*		

2.2 SCREENING TRIALS FOR THE SELECTION OF TAXA SUITABLE FOR LABORATORY CULTIVATION.

Screening of faunal complexes involved placing collections in a variety of laboratory conditions and noting their responses and survival under laboratory conditions. Screening was carried out in the Eastern Cape and in the Kruger Park (Mpumalango) where fauna from the Sabie River was screened. Samples were placed in raceways, bubble pots and flow-through channels and observed.

2.2.1 BUFFALO RIVER FAUNA IN RACEWAY

In January 1993 the first field collection was made from the Buffalo river. The opportunity was also used to investigate collection and transport methods on an informal basis. The collected samples were transported in cooler boxes with leaves and detritus. The collected animals were placed in a raceway after being sorted in the laboratory. Stones and detritus from the collection site was placed in the raceway. The laboratory was air conditioned to a steady 19°C. The survival of the captive population was monitored every third day until the air conditioning failed. Rising temperature rendered conditions unsuitable for continued work and resulted in all the animals dying after three weeks.

Results

The following deductions were made from the observations of the species in the raceway.

a) Ephemeroptera. Several families of mayflies were represented in the collection but only Leptophlebiidae (*Adenophlebia & Choroterpes)* were present in significant numbers. Members of these families were regularly found on or under the rocks positioned closest to the paddle wheel where the water had the greatest amount of turbulence.

b) Coleoptera. A small number of Psephenidae larvae survived and these grazed on the surface of the greenest rocks in the straight of the raceway. However these were the first to die when the temperature rose.

c) Mollusca. Freshwater limpets of the family Ancylidae (*Burnupia*) showed the best survival rate and were found in the straight and on the sides of the raceway.

2.2.2 PALMIET RIVER FAUNA IN RACEWAY and SMALL BUBBLEPOTS

Aim

To discover if directional current is essential for the survival of animals found in riffles and runs in rivers. Artificial channels require expensive construction while containers such as basins and buckets are readily available at any supermarket shelf, can easily be equipped to provide static aerated water with readily available aquarium equipment.

Materials and methods

A collection was made in the Palmiet/Berg River east of Grahamstown and kept under similar conditions

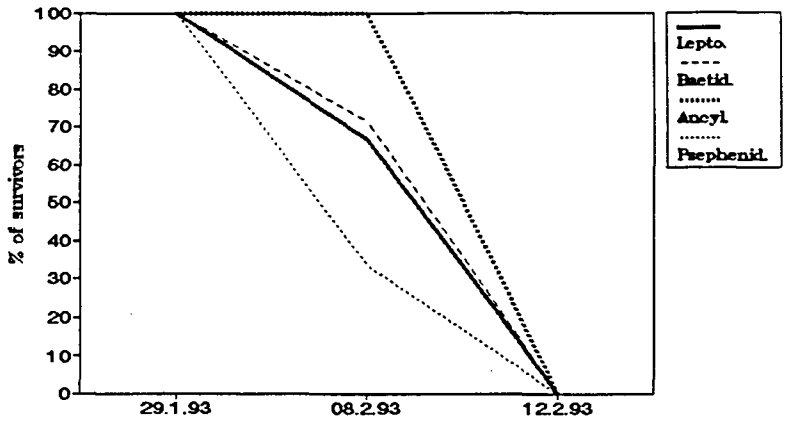

Fig. 2.2.1 % survivors in raceway during 1993.

to those described in 2.2.1 above. An emergence cage was constructed for the raceway to trap emerged adults.

Fifty-four Leptophlebiidae, 31 Baetidae, 9 Trichoptera, 7 Platyhelminthes and 3 Chironomidae were brought back from the field and placed in the raceway.

Three 500ml plastic bottles were equipped with air-stones and pebbles. Two to three *Adenophlebia auriculata* mayfly nymphs were placed in each of these bottles.

Results

Breakdowns of equipment such as failure of the air-conditioning and the burnout of the paddle well motor contributed to the death of the experimental animals.

As can be seen from Fig. 2.3.2.1, 57% of the Baetidae and 60% of the Leptophlebiidae survived for three weeks. The reduction in numbers of the mayflies can largely be ascribed to adult emergences. Altogether 14 (42%) Baetidae and 4 (7.5%) Leptophlebiidae emerged. Of the mayflies placed in the bubblepots 80% survived for four weeks and two of these emerged.

This simple holding exercise gave an indication that both raceways and bubblepots could be good holding facilities provided that sufficient food is available. The survival rate obtained in small bubblepots prompted an investigation into the suitability of this method for long term maintenance and feeding. Several trials were conducted.

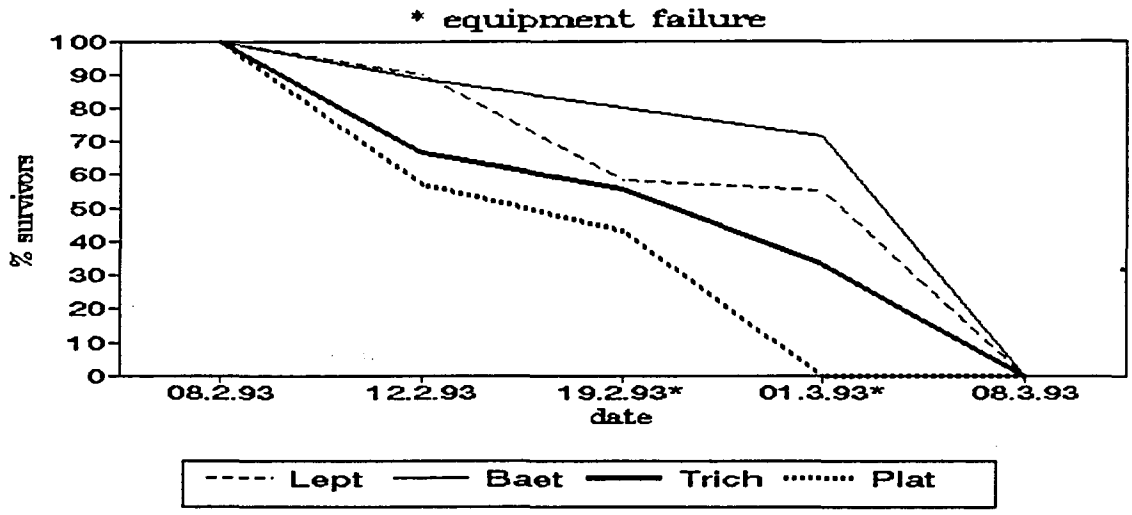

Fig. 2.2.2 % survivors of fauna from the Palmiet river Grahamstown in raceway.

2.2.3 SCREENING OF INVERTEBRATE FAUNA FROM THE SABIE RIVER (MAPUMALANGA)

Aim

To screen the most abundant taxa of riffles in the Sabie River in the Kruger National Park for suitability to captive maintenance under laboratory conditions. The survival response to different feeds and a variety of substrates was assessed.

The following trials were conducted.

1. To test the suitability of commercial food for feeding of invertebrates from a relatively pristine river.
2. Response of fauna to DO levels (see Ch. 2 & App. 2).
3. Screening of various substrates for suitability (see Ch 2 & App. 2).

Materials and methods

This was the general experimental protocol which was followed in the Kruger National Park

Sets of three or four replicates of 500ml bubblepots were used. River water was used in all cases. The pots were aerated by electrical aquarium air-pumps. No temperature or daylight control was attempted. The controls contained no food or substrate.

Families and genera used in the experiment:

ORDER	FAMILY	GENERA & SPECIES
Trichoptera	Hydropsychidae	*Cheumatopsyche thomasetti* & other spp.
	Philopotamidae	*Chimara* sp.
ORDER	FAMILY	GENERA & SPECIES
Trichoptera	Leptoceridae	*Leptoceris* sp., *Oecetis* sp.
Ephemeroptera	Leptophlebiidae	*Choroterpes* spp.
	Baetidae	*Centroptilum* sp., *Afroptilum* sp., *Pseudocloeon* sp., *Baetis* spp.
	Heptageniidae	*Afronurus* spp., *Compsonuriella* sp.
	Tricorythidae	*Thricorythus* sp., *Neuroceanis* sp.
	Caenidae	*Austrocaenis* sp.
Coleoptera	Gyrinidae	
	Elmidae	
	Dryopidae	

2.2.4 SURVIVAL TEST OF TAXA KEPT IN BUBBLEPOTS, FED ON TETRAMIN AND DETRITUS SEPARATELY AND MIXED

Aim

To test the suitability of commercial food for feeding of invertebrates from a relatively pristine river.

Method

1) Two screening exercise was attempted but were attempted to screen fauna in the Sabie river. Replicates of bubblepots were supplied with a variety of feed as given below.
2) Tetramin : 1 mg in 50ml of water; 5ml of suspension in each 500ml bottle.
   Detritus : suspension from collection site, 5ml in each 500ml bottle.
   A mixture of detritus and Tetramin suspension as above (D & T): 5ml in each 500ml bottle.
   No food was (no fd) added to the control.
3) Air supply to the pots was evenly regulated. The containers were monitored daily. The ambient photoperiod was 11H00.

Results

Ambient and water temperature ranged between 14-25°C.
Mortalities were extremely high in the first two trials. Only after foam rubber pads were tested as an alternative substrate to netting did survival rates improve.

The results which are of primary interest are the survival rates of the various taxa. However, as far the diet offered is concerned, the smallest percentage of all taxa survived in Tetramin and the largest percentage in the control with no food (Fig.2.3.3.1). Baetidae did best on detritus and Leptophlebiidae on

mixed food while the Tricorythidae fared best under all circumstances.

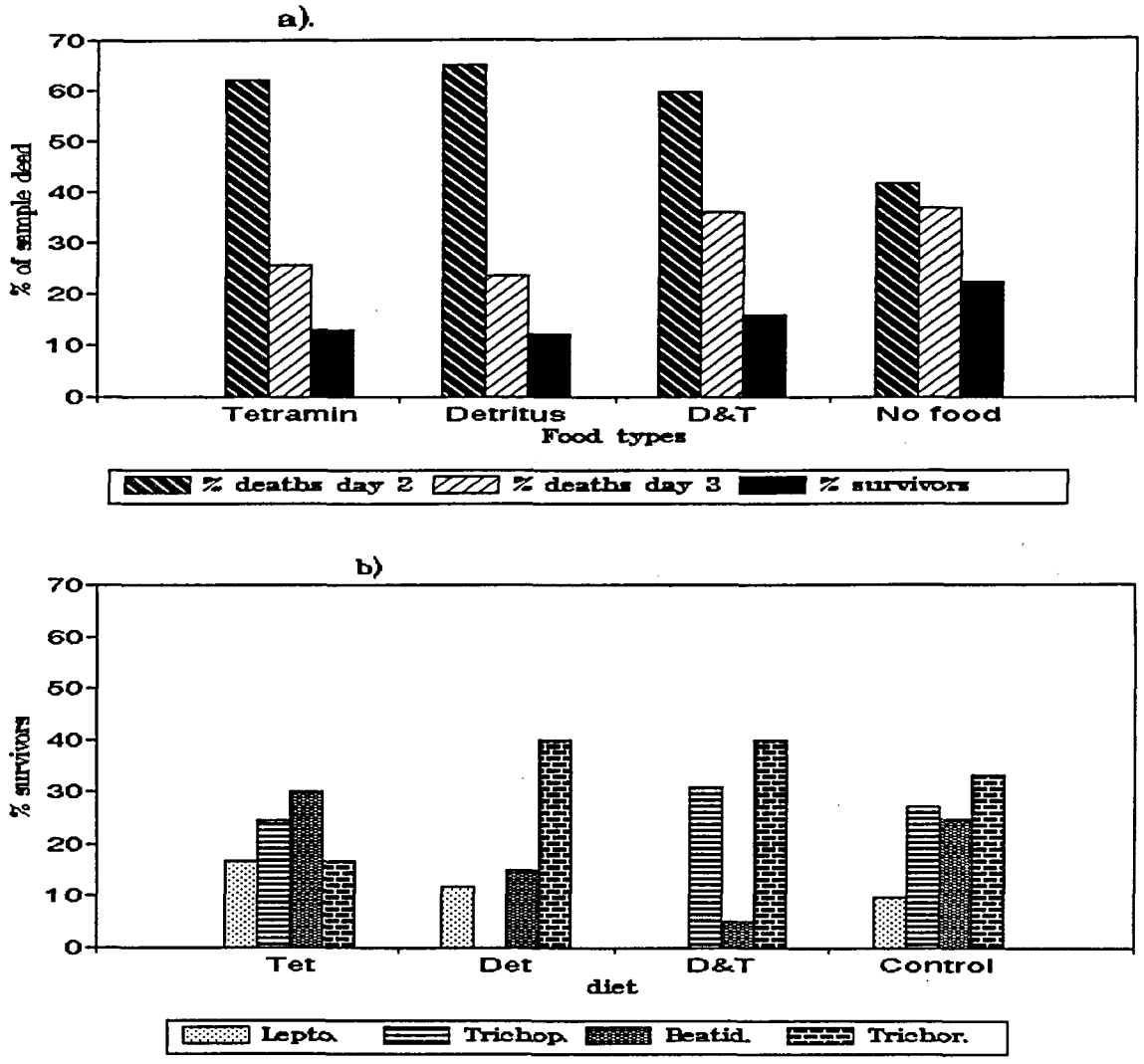

Fig.

Fig. 2.2.4 Bar graphs showing (a) the percentage of the population that died and survived during this period (b) Percentage survivals of each population in all the replicates in each food treatment.

Conclusions

1) The best survivors of all the taxa which were screened in all the experiments conducted in the Kruger National Park were TRICHOPTERA (Hydropsychidae, *Cheumatopsyche* sp.); EPHEMEROPTERA, (Trichorythidae, *Thrichorythus* spp., and Leptophlebiidae, *Choroterpes* spp.).

2) No conclusions could be drawn about the suitability of the food as these experiments as survival was not long enough to make such deductions. The only deduction which can be made from these observations is that invertebrates should not be offered any food when they are returned from the field but allowed to acclimate in water from the environment only.

3) The 500ml bubblepot with netting as substrate is not a suitable container. Netting provided inadequate refuge from the turbulence in the pots, which could have damaged the specimens. Test with foam pads substantiated this conclusion.

4) High water temperatures could have affected the metabolic rate and the available oxygen.

APPENDIX 3

Appendix 3 contains trials conducted to ascertain the effects of various types of equipment and the conditions created in these. Abbreviated result are reported in Chapters 3, 4 and 5.

3.1 DEVELOPMENT OF EQUIPMENT AND METHODS. (Ch. 3.3.8)

Several tests were conducted to establish the conditions in and the suitability of equipment for experimental and holding conditions. A brief report on each experiment is given here.

3.1.1 DISSOLVED OXYGEN

3.1.1.1 TEST OF THE LEVELS OF DISSOLVED OXYGEN DELIVERED BY AIRSTONES IN VARYING VOLUMES OF WATER AND AT FLUCTUATING TEMPERATURES

Aim. To ascertain if airstones will delivers the same amount of oxygen despite a variation in water volume.

Method.
Two replicates each of 1, 2,3 and 5 litres of water were supplied with the same size and make of airstone from a central supply. The temperature and dissolved oxygen levels were measured regular intervals. The containers were moved between two CER one set to fluctuate between 15 - 20 °C and one at 25 °C.

Results
The air-stones appear to aerate the water to the same level despite the different volumes. Temperature appears to have a marked negative correlation with the amount of dissolved oxygen in the water, with corresponding peaks and valleys between rising temperature and falling DO levels.

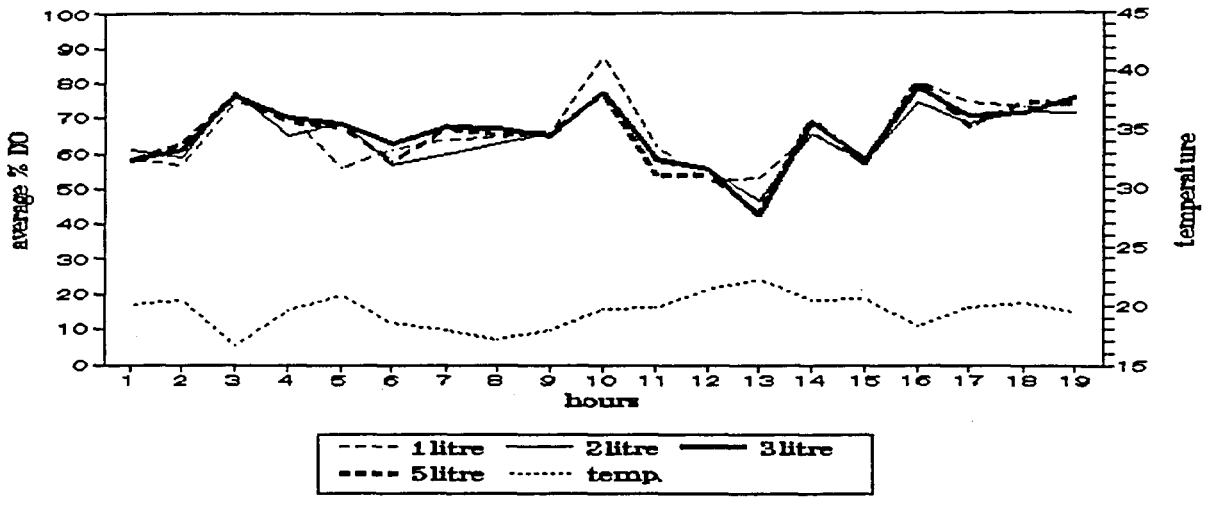

Fig 3.1.1.2.1 Average temperatures in four volumes of water at two temperature regimes.

3.1.1.2 INTERACTION BETWEEN WATER VOLUME, DISSOLVED OXYGEN LEVEL TEMPERATURE AND THE RESPONSE OF *CHOROTERPES* SPP. TO THESE VARIABLES.

As it has been established that temperature and levels of dissolved oxygen are related the next test was to establish the effects of these variable on the survival of mayflies. The levels of dissolved oxygen in riffles and runs of rivers are generally quite high and therefor could be a limiting factor in the survival of rheophyllic animals in static water.

Method

1) Eight 500 ml bubblepot in replicates of two containing with 500 ml, 350 ml and 250 ml of aerated river water and controls with 250 ml non-aerated water were prepared and furnished with plastic netting as substrate.
2) Ten *Choroterpes* nymphs were placed in each bubblepot.
3) Pots were checked hourly during the day but not at night for 36 hours.

Results

Mortalities started occurring as the temperature rose and after 32 hours, 80% mortality was reached.

The most obvious difference in amount of DO is between the 250ml of aerated and static water as can be expected. In the 250ml non-aerated water, the oxygen levels started dropping as soon as the ambient temperature rose and as time progressed overnight oxygen levels dropped down to 30%. This did not occur in any of the other replicates. In 250ml non aerated pots mortalities occurred in the middle of the second day when DO levels had been below 20% for four hours. This would indicate that *Choroterpes* sp. has the ability to respire at relatively low DO levels for short periods. In all other volumes of water DO remained at similar levels but mortality was different. The smallest volume, 250ml aerated water,

showed the most rapid early mortality which may be ascribed to the higher turbulence in the container due to a smaller volume of water. The next most rapid mortality was at 350ml. In 500ml volume of water, which had both the highest levels of DO and the least turbulence, the best survival was evident. Unfortunately only the ambient temperature was recorded so direct temperature effects can not be examined. However the water temperatures were generally 1°C lower than ambient.

This test reveals:

a) The resilience to low DO levels for short periods by *Choroterpes*

b) The possible effect of turbulence on survival of mayflies

c) The essentiality of aeration for the maintenance of constant levels of dissolved oxygen in standing water for the long-term survival of mayflies.

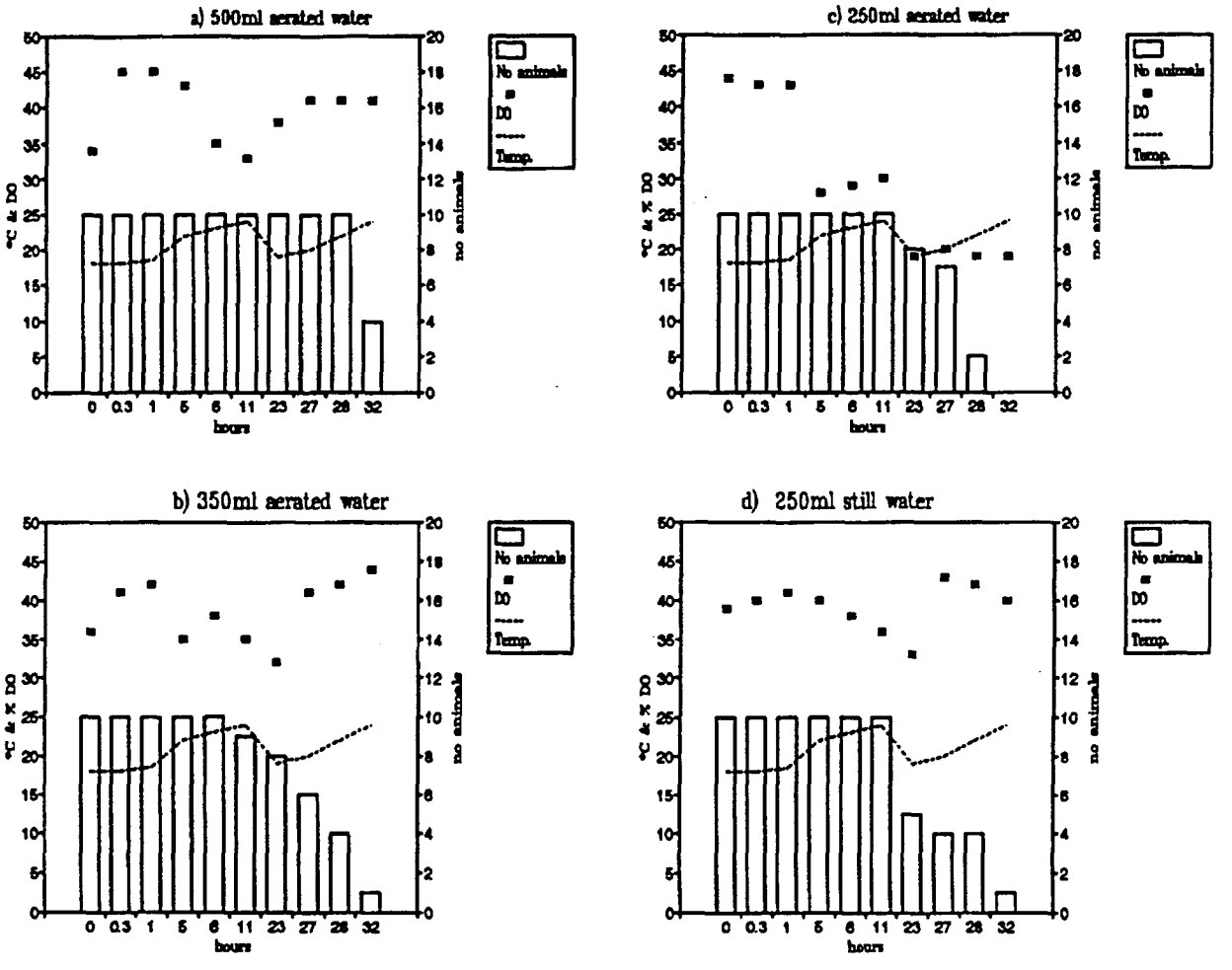

Fig. 3.1.1.2.2 Results of trial to test the effect of aeration on different volumes of water on the survival of *Choroterpes* a) 500ml water (aerated) b) 350ml (aerated) c) 250ml (aerated) d) 250ml (non aerated).

3.1.2 EXPERIMENTAL INVESTIGATION OF SUBSTRATES.(Ch. 3.3.8)

Background

Most riffle dwelling invertebrates perch on substrates which provide both refugia and food. Substrates should be of similar size and shape to produce quantifiable experimental results.

Two experiments were conducted to test the necessity and type of substrate which was to be supplied.

3.1.2.1 THE NECESSITY OF SOLID SUBSTRATE AS OPPOSED TO OPEN SUBSTRATE TESTED IN TURBULENT WATER CONDITIONS.

Aim

To test the suitability of two substrates for *Choroterpes* spp. (Ephemeroptera; Leptophlebiidae).

Method

Three sets of four 500 ml bubblepots were filled with river water and continually aerated:

two sets had foam rubber pads as substrate

two sets had plastic mosquito netting as substrate

one set had no substrate.

The *Choroterpes* nymphs were placed in each replicate set, and the pots checked eight hourly in daytime removing any corpses. The experiment lasted for 4 days.

Results

The laboratory temperature fluctuated between 17°C and 25°C and photoperiod was 11 hours.

The survival rate on foam pads was consistently higher than that on netting. If the survival on netting is compared to that in the control there is no difference.

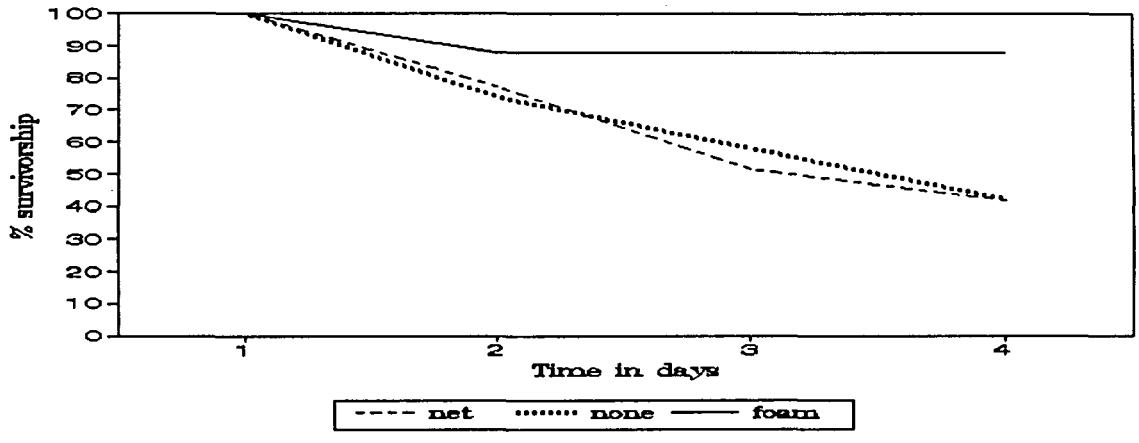

Fig 3.1.2.1 Percentage of survivors in each of three replicates with two types of substrates and a control.

Discussion

For this short period, foam pads as a substrate give better survival rates. It is interesting to note that each time the bubble pots were inspected for dead animals all the specimens were found under the foam pad. This suggests that the foam affords a refuge both from light and from the turbulence of the water.

3.1.2.2 THREE SUBSTRATE TYPES TESTED IN BOTH FLOWING AND TURBULENT WATER CONDITIONS.

Aims

a) To assess the responses of *Choroterpes* spp to three different substrates in both flowing and static water conditions

b) To assess the transport method by which the nymphs were moved form the Kruger National Park.

Method

1) Equipment. Each substrate type was placed into three replicates of both bubblepots and flow-through pots. The substrates tested consisted of foam-rubber pads of 7cm diameter, artificial stones and plastic mesh. Controls contained no substrate. Flow-through pots were made from transparent plastic 500ml bottles with mesh windows inserted on opposing sides. Three mesh sizes (2.0mm, 1.5mm & 1.0mm) were chosen to confine animals of different dimensions. Replicates of these were suspended in the Ciborowski raceways (see Chapter 3.3.2.1). The bubblepots were made from the same size plastic jars as described in Ch.3.3.2.4.
2) Laboratory conditions. The photoperiod was 12 hours and ambient temperature 19°C.
3) Test subjects. *Choroterpes* spp. were collected from the Sabie River and transported to Grahamstown by placing foam pads in aerated insulated boxes (Chapter 3.2) with cooled water. The total transit period (road) was 3 days. On arrival the specimens were moved to raceways and left to acclimatise for one week with detritus and substrate from the Sabie River. The survivors were then sorted into bubblepots and flow-through pots in groups of 10 per container.
4) Food. Finely ground Tetramin was suspended at a ratio of 1 ml/50 ml of water and was fed to the mayflies. Sabie River water was mixed with and later replaced by Palmiet River water.
5) Record keeping. Monitoring took place daily during the week. All dead animals were measured for standard length and preserved
6) Analysis. The results were grouped by weeks. The average number of specimens in all the replicates and the % of the total number of specimens at the start of the experiments was calculated for each treatment.

Results

See Fig.3.1.2.2

The flow through pots were poor containers for experimental purposes as the coarser mesh allowed the animals to escape, so that within the first few days, the original composition of animals in each pot had changed drastically. The smaller mesh clogged up, reducing the flow. Therefore the result in Fig. 3.3.8.2.3b is not an accurate reflection of the intended experimental conditions. The percentage survivals in the bubblepots (Fig.3.3.8.2.3a) show that survival on netting was substantially lower than that on foam pads. 70% of the sample survived for 10 days and 20% for 30 days on foam-pads. In those pots with netting and no substrate a rapid decline in numbers from 70% survival at 5 days to 25% survival by day 10 took place. Even in the raceway (Fig.3.3.8.2.3b) the pots with foam pads retained the largest proportion of animals.

With regard to the transport method, mortalities on arrival were low but larger number died in the first week after the sample was transferred to the raceways. However there was a sufficiently large number of nymphs remaining to stock the experiment with 24 replicates.

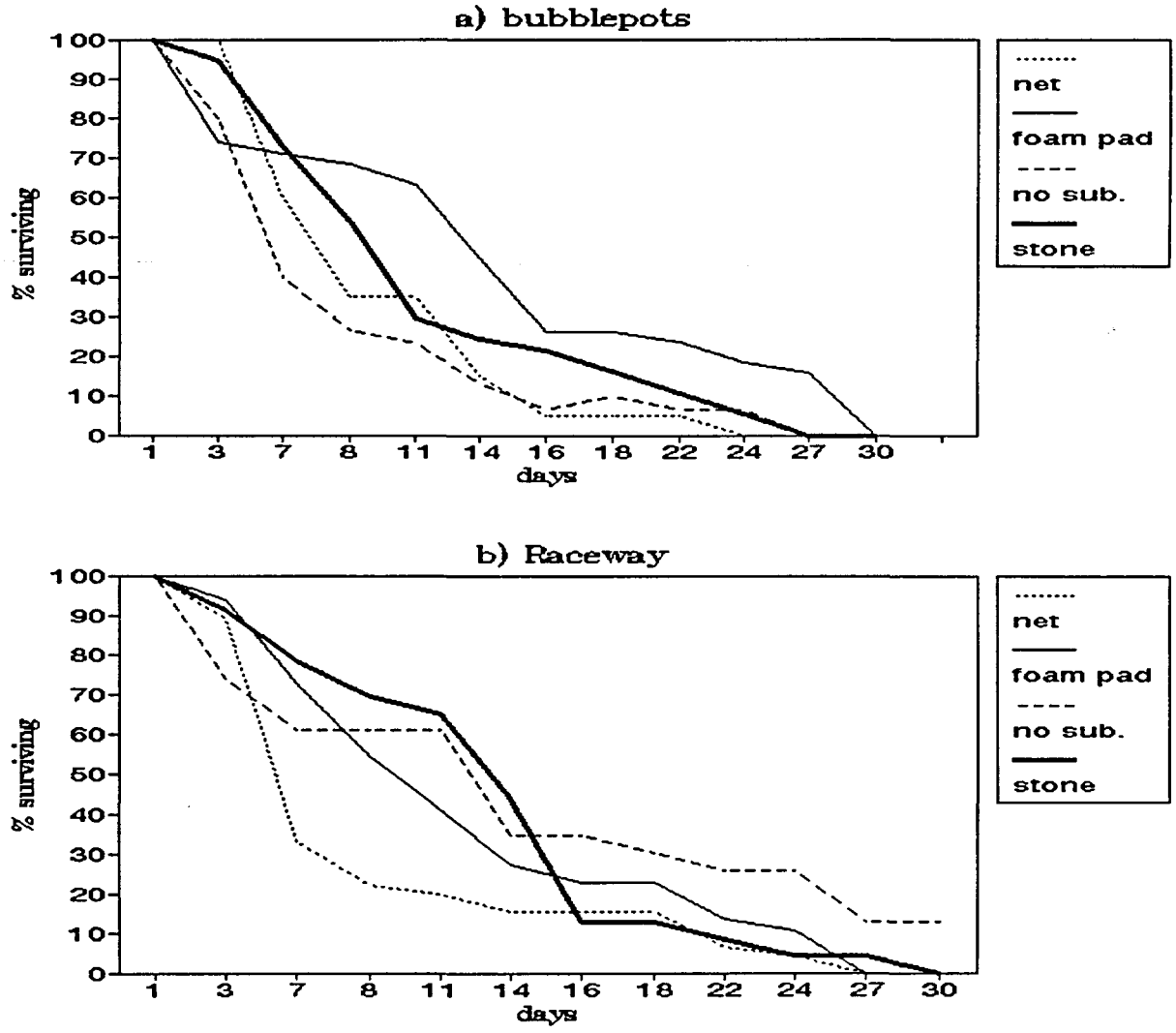

Fig. 3.1.2.2 Percentage of the original sample surviving in all replicates a) in bubblepots and b) in raceway.

Conclusion

Substrate is necessary for the survival of leptophlebiids in bubble pots. The method in which the animals were transported from the Kruger Park ensured survival in excess of a month in the experimental conditions tested.

3.1.3 HYDRAULIC CONDITIONS.

3.1.3.1 COMPARING GROWTH RATES OF LIMPETS IN FLOWING VERSUS STANDING WATER (Ch. 4.2.1.4)

Aim

To compare the growth rate of *Burnupia stenochorias* in flowing water with that of aerated, standing water.

Method

1) Four 50 cm channels, each with approximately equal volumes of water circulating at a known rate and depth within each channel from a common water sump were set up. The water source was Grahamstown tap water, allowed to flow through the system for two days to de-chlorinate.
2) Four bubblepots of known and equal size, each with approximately equal volumes of water and air flow were set up.
3) Both flowing and standing water systems were placed in the C.E. room at temperatures of 19°C (day), 14°C (night) and a photoperiod of 14hrs. The original water levels were maintained with standing tap water no older than four days.
4) Within each pot or channel were placed:
   3 ceramic stones of similar size, covered in periphyton as the source of food
   12 individuals of approximately the same, known size and age (taken from a previous egg-laying experiment).
5) Measurements taken throughout the duration of the experiment were as follows:
   a) initial measurements of length and height of all individuals, to be measured again only at monthly intervals, allowing minimal disturbance or damage to the shells. Dead individuals were removed and measured.
   b) twice daily temperatures were taken to ensure any breakdown in the C.E. room was noted.
   c) initial and thereafter weekly monitoring of TDS, pH and DO levels of the water.
6) Any egg capsules plus the number of egg cases laid were also noted, and removed before those eggs developed and emerged.

Results and Discussion

Table 3.1.3.1 Water conditions within the channels and bubblepots, over time.

Treatment	Max pH	Min pH	Max TDS	Min TDS	Max temp	Min temp
Channels	7,3	6,4	318	165	20°C	17,4°C
Pots	7,8	6,4	326	166	19,6°C	16,6°C

Figs 3.1.3.1.1 and 2 reveal the survival in the channels versus the pots, and the growth rates of the limpets found in the pots only.

In the channels difficulty was found in containing the limpets within each channel despite the fine net covering the holes through which the water flowed. The limpets escaped and landed in the water sump. Each stream should have had a dedicated sump so that any limpet which had moved could be placed back into the correct stream each day. This would still adversely affect growth, as displayed in Section 4.2.1.1. Because of this movement into the sump, the growth rates of the channels cannot be elucidated. Figure 3.3.8.4.2 shows the growth within the pots to have a similar rate, with an adjusted average size after 35 days, when the dead animals (the majority being the larger limpets) were removed. Comparing the survival between bubblepots and channels, it can be seen in Fig 3.3.8.4.1 that the survival in the pots was significantly better.

Many of the limpets had soft shells and a number were found without any shell remaining, unlike the norm, where upon death bacterial and microbial breakdown of the body occurs before the shell dissolves. The water chemistry was obviously unsuitable for continued shell growth despite the level of the pH remaining neutral or slightly alkaline (Table 3.3.8.4). Between monthly counts many limpets disappeared completely suggesting that, (a) they had dissolved entirely between measurements or, (b) more frequent measurements needed to be taken. The experiment was terminated after 3 months because of the above difficulties.

Conclusion

These narrow channels are not suitable for the rearing of *Burnupia* in the laboratory. As a result of the unknown water chemistry and the effect on the shells, more attention has been and will be paid to future experiments in this regard (see section **3.3.6 Water Composition and Hygiene**).

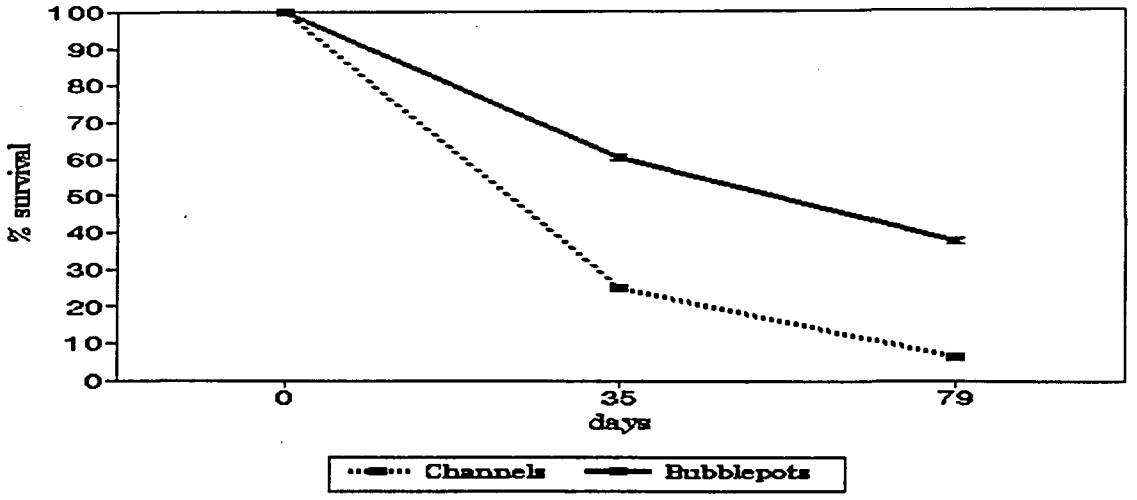

Fig 3.1.3.1.1 Survival of limpets in channels and bubblepots over a three month period.

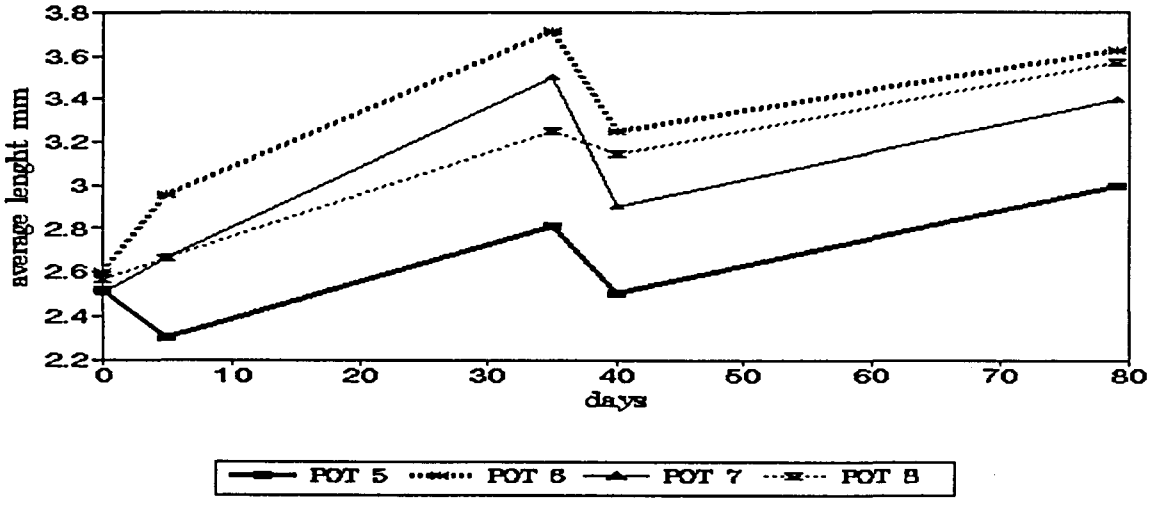

Fig 3.1.3.1.2 Growth of limpets in bubblepots over 79 days corrected for mortalities at 5 and 35 days.

3.1.3.2 INVESTIGATION OF THE SUITABILITY OF SMALL CHANNELS AND BUBBLEPOTS FOR MAYFLIES (Ch.5.2.1).

Aim

To assess whether riffle dwelling leptophlebiid mayflies are obligately rheophyllic and if bubblepots offer a suitable holding facility for experimental and rearing purposes.

Introduction

It was concluded from the previous experiment that the small channels did not provide optimal maintenance conditions for *Adenophlebia auriculata* (Leptophlebiidae) nymphs and therefore bubblepots were tested to ascertain if a better survival rates could be obtained.

Method

1) Four replicates of each treatment were prepared. Treatment consisted of small channels with 5 litre sumps (Chapter 3.3.2.3) and 5 litre bubblepots (Chapter 3.3.2.4.). Each container was supplied with six stones covered in periphyton.
2) The containers were all housed in the laboratory at ambient conditions of light and temperature (13-21°C and 10-12h photoperiod).
3) The specimens were collected from the field and were divided into groups of 17 with size distribution 1.2-1.6 mm HW.
4) Food consisted of periphyton on artificial substrates.
5) Containers were inspected every second day for corpses and shucks, and specimens were measured monthly.

Results

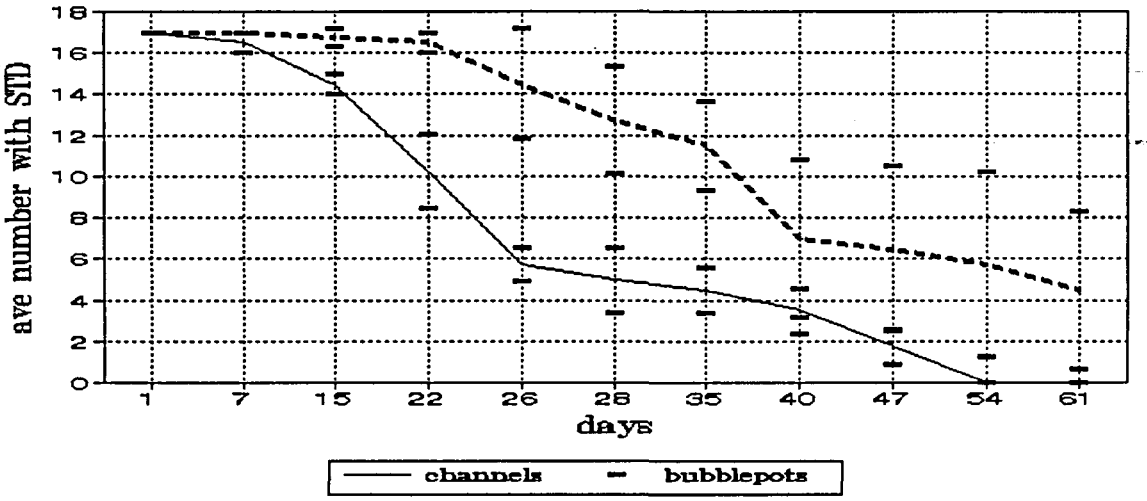

Fig 3.1.3.2 A line graph depicting the survival of nymphs of *A. auriculata* in 5 litre bubblepots or small channels with a 5 litre sump, at ambient laboratory conditions. The triangles represent the total number of adults which emerged during the course of the experiment. The error bars represent standard deviations.

During this trial the nymphs survived for 7 week in the channels and for at least 9 weeks in the pots. When the experiment was discontinued at 9 weeks more than 40% of the nymphs were still alive in 2 of the pots. Of the 68 nymphs which were placed in each set of replicates at the start of the experiment, 37

died in bubblepots and 47 in channels. In two of the bubblepots the water conditions deteriorated visually, becoming murky. In these two pots 15 animals died within four days (days 35-40). The rapid early decline of numbers in the channels can be ascribed to both the emergence of adults and mortalities. Fig. 3.3.8.4 shows that imagoes appeared slightly earlier in the channels than in the pots. Altogether 14 nymphs emerged from the channels and 12 from the pots.

Discussion

The rapid decline in numbers in the channels between days 15 and 26 can be ascribed to both mortalities and emergences. The difference in the condition between the pots and the channels are not only hydraulic (shear force exerted on the nymphs) but also may be due to an insufficient refugia. The temperature regime in the pots is 2-3 °C lower than in the channels due to evaporative cooling, which could account for the earlier emergence of adult in the relatively warmer channels. An ANOVA performed on the replicates proved no significant difference between the replicates in either the channels or the pots (F ratio 0.097, $p>0.05$ for channels and F ratio 1,29 $p=0.2884$) for pots) although the variation in the survival rates in the pots is wider. It is quite clear from Fig 3.3.8.4 that the variation in the sample number in bubblepots is much larger that from those in the channels which could be ascribed to high survival rate (58% and 35%) in two pots. There was a significant difference in the survival rates between channels and bubblepots (ANOVA f ratio 6.7 and $p < 0.05$.) and a multiple range test confirmed the difference.

It can be concluded from the trials that bubblepots are the optimal rearing containers for *A. auriculata* and that they are not obligatory rheophyllics.

3.1.3.3 COMPARING SURVIVAL OF LIMPETS AT HIGH AND LOW DENSITIES IN FLOWING VERSUS STANDING WATER (Ch.4.2.1)

Aim

To test the effects of density and flow on the growth and survival of populations of limpets.

Method

1) Two lots of four channels and 8 bubble pots (described in Section 4.2.4) were set up in the laboratory with each pot and each channel considered a replicate. Each set of 4 channels had one sump. Water was replaced weekly, and topped up with tap water in the case of evaporation occurring.
2) Within each replicate was placed 3 ceramic stones previously allowed to grow periphyton, and a known number of measured limpets, with each set of channels and pots having either a high or a low density of limpets. Limpets that died were replaced with an individual of similar size from a field-caught laboratory culture until the 48th day, to

maintain the original densities.

3) Dissolved oxygen, pH, and TDS were monitored weekly and temperature daily.

4) The trial ran from 19 April to 28 September 1994, a total of 162 days.

Results

Water TDS, pH and temperature varied little between the two sumps and between the eight pots. Generally the pots were 1° or 2°C cooler than the channels, presumably because the turbulence resulting from the aeration reduced temperature. The limpets moved from the channels, despite the presence of fine gauze over the outlets, into the sumps from where they had to be moved back to the channels daily. This continual disturbance has previously been shown (see section 4.2.2) to have a detrimental effect on the growth of the limpets. Algal growth became black and slimy after 3 months (algal growth not identified) in the channels, on the surface of the channels themselves, and was continually scraped off, being considered unsuitable as a food source for the limpets. This did not occur in the pots, where long (>10cm) filamentous green algae grew. Near the end of October both the channels and pots displayed a colourless, slimy gelatinous growth, and it was apparent the limpets were not growing, hence the trial was abandoned.

Table 3.1.3.3. a) F = flowing replicate, P = bubblepots. % loss = loss after the 48th day when replacement was discontinued. No end = numbers of limpets remaining, including spat that had hatched. b. Temperature, pH and TDS are averaged for all pots and channels.

a)

Repl.	F1	F2	F3	F4	F5	F6	F7	F8	P1	P2	P3	P4	P5	P6	P7	P8
No start	108	115	91	109	14	14	14	14	109	105	105	110	17	23	11	13
No end	86	26	58	52	7	2	9	4	37	69	72	72	104	41	32	47
% loss	9.3	14.0	16.5	24.8	28.6	14.3	7.14	14.3	77.0	16.2	19.1	10.9	17.7	4.4	63.6	15.4

b)

Treatment	T.max	T.min	T.ag	pHmax	pHmin	pHavg	TDSmax	TDS min	TDS ag
Channels	22.3	12.0	17.2	8.7	6.4	7.0	453	218	304
Bubble pots	21.3	9.5	15.2	8.6	5.2	7.0	395	227	288

The graphs in Fig 3.1.3.2, on the next page, display the sequential population profile in the experiment. For instance the striped bars at the 3mm position gives an indication of the number and size of limpets remaining from day O, 1mm size group. Similarly the striped bars at position 4 and 5 relate to the animals resulting from the size classes 2 and 3 mm at day 0.

In the channels, (Fig 3.1.3.2 a & b) the numbers of limpets present decline steadily from the initial stocking density. In the bubble pots (c & d) the gain is much larger especially in the pot which started with a low population density.

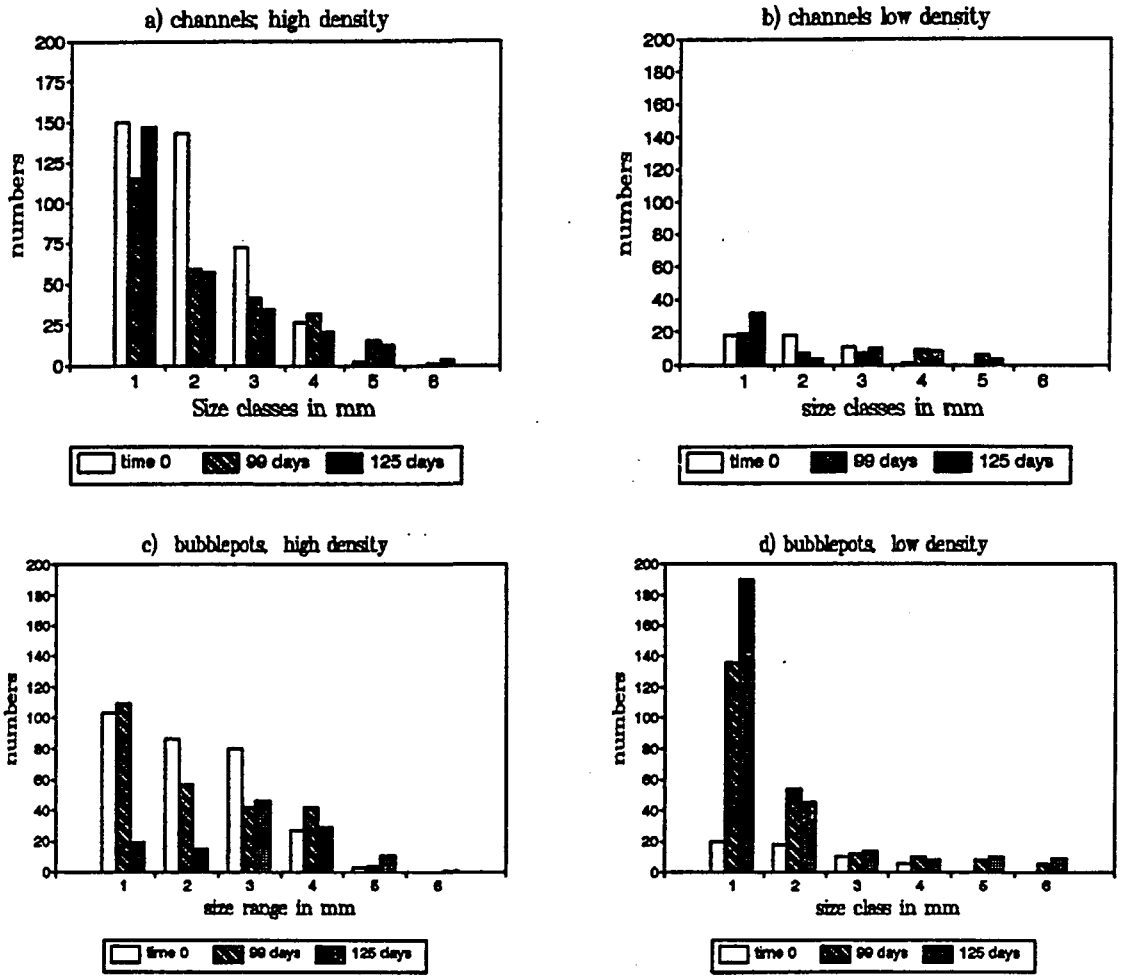

Fig 3.1.3.3 Graphs a-d displays the average size/frequency distribution of limpets. The clear bars indicate the size distribution at the start of the experiment, the diagonally striped bars the size frequency distribution after 99 days which is largely a result if the capsules deposited and the juveniles hatched in the first phase of the experiment and the hatched bars depict the size frequency at the close of the experiment.

Conclusion

More egg capsules were laid in the bubble pots than in the flowing channels. However, those limpets in the channels were highly disturbed, with an apparently unsuitable growth of periphyton. Since this experiment, the use of these channels in testing the effects of flowing water has been abandoned because of the described difficulties. Except for the low population density in the pots, the survival rate did not appear to differ between the flowing and standing waters.

3.1.3.4 CULTURING LIMPETS IN FLOWING VERSUS STANDING WATER (Ch.4.2.1.4).

Aim

To compare the survival of limpets in flowing versus standing, aerated water.

Method

1) Approximately 700 limpets were collected in the field, using anaesthetics and maintained in the laboratory streams with de-chlorinated tap water for a few days to allow for any that died to be removed.
2) Three channel replicates (each a large recirculating stream made of heavy duty PVC piping) and three 10 litre basins were set up in the laboratory under ambient conditions of temperature and light (see Section 3.3.2.3 for details). Tap water was used throughout this experiment, and renewed weekly from a source which had been allowed to stand for two or three days to de-chlorinate.
3) Limpets were then transferred in equal numbers, on tiles which had accumulated a layer of periphyton, into the 6 replicates. An equal distribution of sizes was made, and these varied from 2mm to 4mm in length.
4) Dead limpets were counted and removed, and egg cases were counted daily. TDS, temperature, % oxygen and pH were monitored with each change of water. The experiment was allowed to run for 9 weeks.

Results

See Table 3.1.3.4.1 & 2. and Fig 3.1.3.4.

Table 3.1.3.4.1 Conditions of pH and TDS within the channels and bubblepots.

Rep.	pH max.	pH min.	pH ag.	TDS max.	TDS min	TDS ag.
channel R1	7,6	6,3	7,1	420	119	179
channel R2	7,9	6,7	7,2	245	114	155
channel R3	7,8	6,8	7,2	259	133	159
b.pot B1	7,6	6,2	7,1	296	129	169
b.pot B2	7,9	6,8	7,2	302	114	157
b.pot B3	7,8	6,7	7,3	217	133	161

Temperatures remained the same for each replicate. Max = 22°C, min = 17°C, average = 19°C. % oxygen was approximately 70% for all replicates.

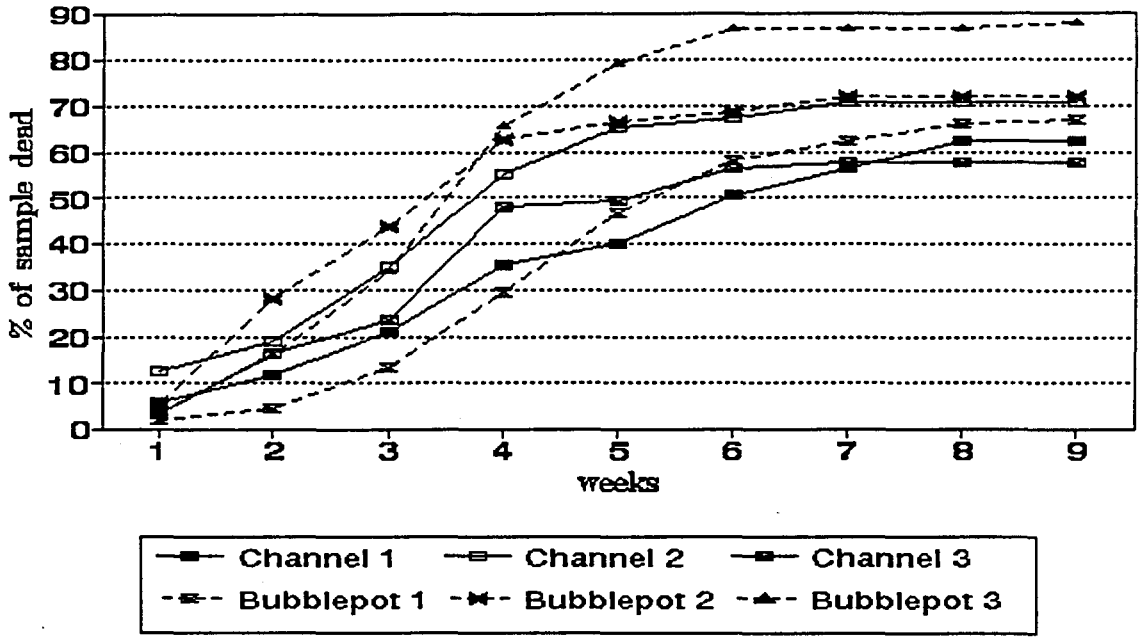

Fig 3.1.3.4 Cumulative mortality of limpets in flowing (Channels 1, 2 and 3) and standing (Bubblepots 1, 2 and 3) waters.

A greater number of egg capsules were laid in the pots; those that hatched were disregarded in the counts of limpets, as they were easy to identify by their size compared to the larger limpets initially placed in the replicates. Hence, % mortality does not include newly laid limpets. The rate of mortality varied considerably, both between pots, between channels and between all the replicates. The average mortality for the channels was 64,5%, for the pots, 75,6%.

Conclusion

In this experiment, the channels gave a greater survival rate than the basins of standing water. More egg capsules were laid in the standing aerated water, however the survival of hatchlings was not monitored.

Table 3.1.3.4.2. R1, R2 and R3 = replicates of flowing channels; B1, B2 and B3 = replicates of standing aerated water; Live = number alive at the start of the experiment; % mortality is accumulative; Dead = number that died weekly; Egg cases = number of egg cases laid weekly.

Replicate	R1	Channel			R2	Channel			R3	Channel		
Week	Live	% Mort.	Dead	Egg cases	Live	% Mort.	Dead	Egg cases	Live	% Mort	Dead	Egg cases
1	85	5.88	5	0	89	12.35	11	0	85	3.53	3	6
2		11.76	5	34		19.10	6	8		16.47	11	65
3		21.18	8	5		34.83	14	64		23.52	6	0
4		35.29	12	0		55.06	18	0		48.23	21	32
5		40.90	4	4		65.17	9	15		49.41	1	
6		50.59	9	4		67.41	2	3		56.47	6	
7		56.48	5			70.78	3			57.65	1	
8		62.35	5							57.65		
9		62.35								57.66		
Totals	85		53	47			63	90			49	103
Replicate	B1	Basin			B2	Basin			B3	Basin		
Week	Live	% Mort.	Dead	Egg cases	Live	% Mort.	Dead	Egg cases	Live	% Mort.	Dead	Egg cases
1	88	2.27	2		89	5.63	5		82	3.67	3	35
2		4.55	2	35		28.09	20	24		15.86	10	80
3		13.64	8	11		43.82	14	34		34.14	15	
4		29.55	14			62.92	17			65.85	26	
5		46.59	15	18		66.29	3	16		79.27	11	
6		57.95	10			68.54	2			86.58	6	
7		62.5	4			71.91	3			86.58		
8		65.99	3							86.58		
9		67.04	1							87.80	1	
Totals	88		59	64	89		69	74	82		72	115

3.1.3.5 THE EFFECT OF TEMPERATURE ON GROWTH RATE, UNDER FLOWING AND STANDING CONDITIONS (Ch.4.2.1.4).

Aim

To compare the growth rate of *B. stenochorias* in flowing water and turbulent standing water at 15°C, 20°C and 25°C.

Introduction

Temperature and food are the most frequently reported factors which affect life history traits in aquatic invertebrates. Temperature directly affects growth by influencing metabolic rates and feeding rates (Giberson and Rosenberg 1992). It also affects food quality and quantity by influencing algal production and growth rates on detritus (Ward and Stanford 1982).

Method

1) Three sets of four channels were set up with approximately equal volumes of water circulating at a known rate and depth within each channel from individual water sumps. The sumps were suspended in water baths heated by aquarium heaters to either 20°C or 25°C. The third set of channels remained at the ambient C.E. room temperature of 15°C. The Grahamstown tap water had been supplemented with various chemicals (see section **3.3.6 Water Composition and Hygiene**).
2) Three sets of four bubblepots of known and equal size, each with approximately equal volumes of water and air flow were set up. Two sets of these were placed in water baths as described above. Lids were placed on the pots to help stabilise temperatures and decrease the evaporation rate.
3) Within each pot or channel was placed 2 ceramic tiles of similar size, covered in periphyton as the source of food, and 20 individuals of known sizes from app 1-3mm shell length.
4) Both flowing and standing water systems were placed in the C.E. room at a temperature of 15°C and photoperiod of 14hrs. The original water levels were maintained with tap water which had been allowed to dechlorinate. Water was changed in all systems every three weeks, each change being supplemented with nutrients as before.
5) Shell length was measured initially and thereafter every two weeks. Dead individuals were removed and measured daily and replaced with new individual as close as possible in size.

Results and Discussion

The aquatic thermoregulators used to maintain the sumps was extremely unreliable and consequently unavoidable temperature fluctuations occurred. The results could not be used.

3.1.3.6 GROWTH OF MAYFLIES IN SMALL CHANNELS (Ch.5.2.1).

This experiment was by Simon Burton as part of a student project.

Introduction

Although the species selected for experimental investigation are all found in riffles and runs in rivers, they may not be obligatory rheophyllics. It is quite difficult to keep small animals confined to artificial channels as the escape route is always open. It was therefore decided to investigate whether well aerated static water would be an suitable alternative to running water. Two experiments were conducted: the growth and survival was tested in channels only; survival was tested in channels and bubblepots.

Aim

a) To test the suitability of the small channels as described in Ch. 3.3.2.3 for experimental purposes

b) To ascertain growth rates of *A auriculata* nymphs in these channels and under ambient and controlled environment room (CER) conditions.

Method

1) Eight small recirculating channels were set up, each with three kaolin stones covered in laboratory-cultured periphyton, which acted as both food and substrate for the nymphs.

2) Four channels were kept in the laboratory under ambient conditions (16°C-20°C and 12-13H photoperiod) while the other four were housed in the CER at 17 °C days and 15 °C nights and 14H photoperiod.

3) Nymphs of all sizes were collected from the field and were returned to the laboratory, measured and placed in groups of ten in the recirculating channels which were checked daily for emergences or shucks while dead nymphs were removed. The nymphal head-widths (HW) were measured weekly.

Results

A. auriculata did not survive well in channels with three stones. A mean of 33% and 32% of the original sample survived three weeks in the CER and laboratory respectively. However, by following individual nymphs for up to four weeks mean absolute growth rates could be calculated for each run, for the CER and the laboratory and for all the nymphs combined. These growth rates are summarised in Table 3.3.8.3. There was no significant difference between the growth rates of nymphs in the CER and in the laboratory ($P > 0.05$).

Table 3.1.3.5.1 Growth rates mm/day (absolute) calculated from survivors in each channel in the laboratory and the CER.

	CER	LAB	
Channel 1	0.0145	0.017	
Channel 2	0.043	0.017	
Channel 3	0.022	0.028	Mean abs. growth
Channel 4	0.014	0.009	for total no.
Mean	0.0228	0.0191	0.0148

Discussion and Conclusions

The low survival rate of nymphs in channels in the laboratory could be explained in two ways:
a) The flow rate in the system may be too rapid. In the Palmiet River the nymphs favour the rocky edges of the stream out of direct current where coarse grained sediment collects under rocks as substratum i.e. that they inhabit low flow areas out of direct current while few smaller nymphs are found in moderate currents (pers.obs.). This observation will be tested statistically after sufficient collections have been made. The channels which are constructed of smooth plastic guttering such that a shallow but fairly strong current flowed over a few smooth rocks most probably did not offer sufficient refuge or foothold for the nymphs. Hence, the nymphs were subjected to fairly strong current leading to large energy expenditure to hold a fixed position s opposed to it being utilized for growth.
b) The algal growth and other food in the channels was not suitable for sustaining the nymphs. The suitability of the periphyton has not been substantially investigated.

The lack of any significant difference in the growth rates between the laboratory and the CER may be due to the mean ambient temperature at that time being similar to the CER temperature setting. However, a growth rate of between 0.019-0.022mm/day means that a nymph could grow from hatching to a maximum size in four months. Temperatures in the field stream fluctuate around 15-24 °C and growth rate has been found to be linked to temperature in invertebrates (see Chapter 5). Similarly if food with a higher protein content than periphyton is supplied the growth rate may increase.

3.1.4 FEEDING TRIALS.

3.1.4.1 TETRAMIN AS DIET FOR *ADENOPHLEBIA AURICULATA* (LEPTOPHLEBIIDAE)(Ch.5.2.2).

Aim

To test the suitability of Tetramin as feed for *Adenophlebia auriculata* (Leptophlebiidae).

Introduction

The first trial was conducted on a number of taxa from the Palmiet River, including a large number of *A. auriculata* the responses of which is reported here. Tetramin was offered as an alternative to natural detritus from the river. This trial took place at the same time as the test for suitable substrates was being conducted and netting was used as substrate.

Method

1) Bubblepots (500ml) with air-stones inserted through the lid were fitted with plastic mesh around the perimeter to provide perches for the test animals. Netting was used as it allowed the animals to be observed without disturbance.
2) The CER with ambience of 12h photoperiod and temperature variation of 12 °C night 20 °C day housed the containers.
3) Field collected nymphs were sorted into the bubblepots and left for forty-eight hours to acclimate. The water was replaced with clean river water and the experimental feeding was started.
4) The Tetramin was finely ground. One ml of ground Tetramin suspended in 50 ml of water and 2.5 ml of this suspension was added to each container. 2.5 ml of natural detritus suspension collected from the river was added to the other pots. The food was replenished every Friday and Monday when the water was replaced with dechlorinated tap water.
5) Monitoring. The containers were inspected daily and the dead animals preserved.

Results

See Fig. 3.1.4.1

After 48 Hours the Tetramin pots had 15, 12, 14 specimens in each replicate and the detritus pots 6, 16, 20 specimens in each replicate due to mortality and all the collected remainders had perished. On the first day of feeding 1ml of Tetramin was added to the relevant pots but by the next morning it was clear that the food supply level had to be reduced as 1 ml caused the water quality to deteriorate.

The survival rate of *A. auriculata* is greatest on Tetramin. 50% survived for five weeks, 30% survived for six weeks and 10% of the population survived for eight weeks. Detritus on the other hand only

sustained 50% of the population for 2-3 weeks with a more rapid decline in numbers.
A regression analysis done on the two sets of data indicated a slope of -5.05 for the population fed on TETRAMIN and a slope of -4.65 for those fed on detritus.

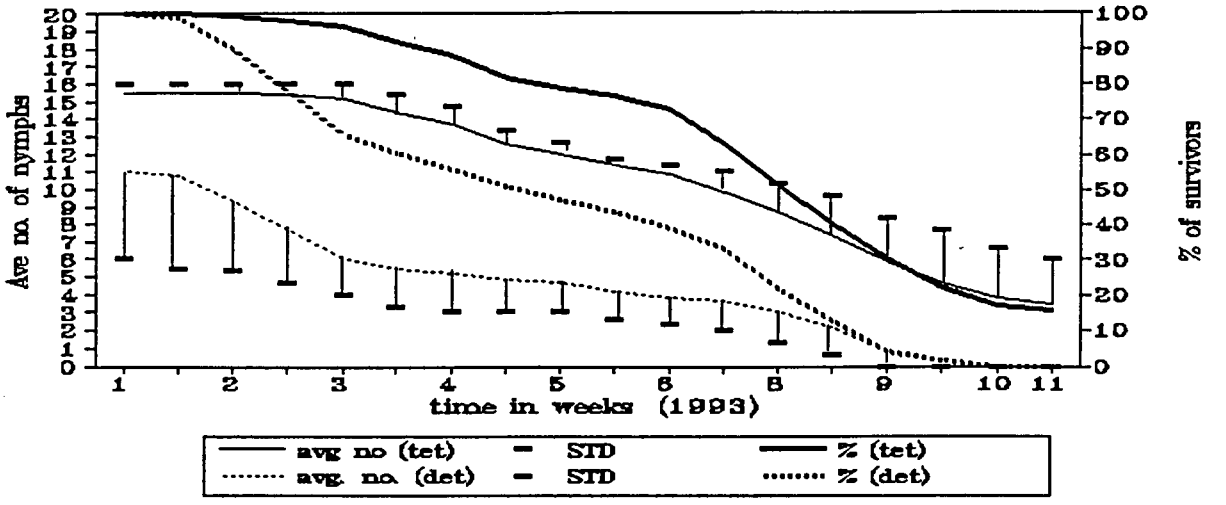

Fig. 3.1.4.1 Line graph depicting the average numbers remaining alive in three replicates (error bars indicate standard deviations) The heavy lines represent the percentage of the total sample surviving. The large variation in the samples fed on detritus is due to the uneven number of nymphs in the replicates.

Conclusion

TETRAMIN appears to be an satisfactory diet for the mayfly nymphs. An experiment will have to ce conducted during which the growth rate of the nymphs when fed on TETRAMIN with have to be compared to those fed on natural diet of leaves and periphyton. A further experiment should also compare the effect of these two diets on the fecundity of the females.

APPENDIX TO CHAPTER 4

Table 4.1. Data pertaining to the growth and survival of the limpets under different temperatures and densities.T = temperature °C; D = density per 500ml pot; Time = days; No = number of limpets per pot; Size mm = average length mm; STD = standard deviation; % CV = percentage coefficient of variation; Max mm = maximum length of limpets present; Min mm = minimum length of limpets present; % Growth = percentage growth per Time interval. (Ch.4.2.1.5)

Treatment	Time	No	% Survivors	Size mm	STD	% CV	Max mm	Min mm	% Growth	Daily % increase
T15D10	1	31	100	1.06	0.20	19.8	2.00	1.00	0	0
	8	25	80.645	1.06	0.22	20.3	2.00	1.00	1.9	0.27
	15	25	80.645	1.51	0.33	21.8	2.40	1.00	41.31	5.90
	22	21	67.742	1.51	0.39	25.6	2.40	1.00	-0.16	-0.023
	29	18	58.065	1.63	0.44	26.9	2.50	1.00	7.8	1.12
	43	14	45.161	2.11	0.52	24.5	3.00	1.20	29.45	4.20
	57	12	38.71	2.51	0.39	15.5	3.10	1.80	19.04	2.72
	71	9	29.032	2.70	0.42	15.6	3.20	1.80	7.64	1.09
	85	8	25.806	2.79	0.48	17.3	3.50	1.80	3.24	0.46
	113	6	19.355	3.03	0.63	20.6	4.10	2.20	8.81	1.24
	120	6	19.355	3.13	0.60	19.0	4.10	2.30	3.30	0.47

T15D20	1	81	100	1.20	0.34	28.4	2.00	1.00	0	0
	8	69	85.185	1.21	0.34	28.5	2.00	1.00	1.05	0.15
	15	62	76.543	1.56	0.31	19.9	2.20	1.00	29.28	4.18
	22	56	69.136	1.61	0.37	23.0	2.50	1.00	3.06	0.44
	29	45	55.556	1.62	0.35	21.3	2.40	1.00	0.60	0.09
	43	36	44.444	1.86	0.41	22.1	2.60	1.00	14.55	1.04
	57	28	34.568	2.29	0.34	14.9	3.00	1.60	23	1.64
	71	26	32.099	2.57	0.44	17.0	3.20	1.50	12.57	0.90
	85	19	23.457	2.60	0.43	16.5	3.20	2.00	1.046	0.07
	113	14	17.284	2.94	0.43	14.6	3.50	2.40	12.91	0.92
	120	12	14.815	2.88	0.46	15.8	3.50	2.00	-1.78	-0.13
T15D20	1	112	100	1.09	0.25	23.1	2.50	1.00	0	0
	8	72	64.286	1.10	0.28	25.0	2.50	1.00	1.366	0.19
	15	65	58.036	1.59	0.30	19.0	2.40	1.00	43.79	6.25
	22	58	51.786	1.52	0.31	20.3	2.50	1.00	-4.22	-0.60
	29	49	43.75	1.60	0.32	20.0	2.30	1.00	5.081	0.72
	43	39	34.821	1.86	0.43	23.1	2.90	1.00	16.66	1.18
	57	31	27.679	2.15	0.57	26.4	4.00	1.20	15.25	1.09
	71	26	23.214	2.39	0.52	21.8	3.60	1.00	11.17	0.80
	85	20	17.857	2.54	0.46	18.2	3.50	2.00	6.135	0.44
	113	15	13.393	2.95	0.64	21.9	4.20	2.20	16.24	1.16
	120	14	12.5	3.01	0.59	19.6	4.10	2.20	2.052	0.14

T20D10	1	42	100	1.12	0.30	27.2	2.00	1.00	0	0
	8	42	100	1.15	0.42	36.9	2.80	1.00	2.766	0.39
	15	42	100	1.87	0.37	19.7	2.80	1.00	62.32	8.90
	22	41	97.619	1.94	0.39	20.2	3.00	1.40	3.876	0.55
	29	37	88.095	2.10	0.52	24.9	3.50	1.40	8.163	0.58
	43	32	76.19	2.60	0.40	15.5	3.50	2.00	23.82	1.70
	57	24	57.143	2.88	0.44	15.1	4.00	2.00	11.03	0.79
	71	11	26.19	2.82	0.54	19.2	3.60	1.80	-2.26	-0.16
	85	10	23.81	3.02	0.55	18.2	4.00	2.00	7.161	0.51
	113	10	23.81	2.87	0.72	25.2	4.20	1.50	-4.97	-0.35
	120	4	9.5238	3.23	0.71	22.0	4.40	2.50	12.37	1.76
T20D20	1	81	100	1.12	0.26	23.6	2.00	1.00	0	0
	8	79	97.531	1.24	0.43	34.5	2.50	1.00	11.03	1.57
	15	74	91.358	1.80	0.36	20.1	2.80	1.00	45.32	6.47
	22	75	92.593	1.82	0.38	21.1	3.20	1.00	1.181	0.17
	29	62	76.543	2.07	0.45	21.9	3.50	1.00	13.27	1.89
	43	54	66.667	2.38	0.35	14.9	3.20	1.60	15.35	1.09
	57	35	43.21	2.67	0.45	16.9	3.60	1.80	11.85	0.85
T20D30	1	128	100	1.08	0.26	24.2	2.50	1.00	0	0
	8	110	85.938	1.16	0.30	26.0	2.50	1.00	7.51	1.07
	15	109	85.156	1.77	0.33	18.6	2.60	1.00	52.84	0.75
	22	95	74.219	1.70	0.33	19.7	2.80	1.00	-4.16	-0.59
	29	86	67.188	1.91	0.37	19.2	3.00	1.20	12.59	1.80
	43	77	60.156	2.19	0.47	21.6	3.50	1.30	14.41	1.02
	57	60	46.875	2.20	0.45	20.6	3.50	1.50	0.67	0.048
	71	18	14.063	2.47	0.33	13.2	3.20	2.00	12.29	0.87
	85	11	8.5938	2.54	0.36	14.2	3.00	2.00	2.594	0.18
	113	10	7.8125	3.11	0.41	13.1	3.60	2.40	22.62	1.62

	120	8	6.25	3.05	0.42	13.7	3.80	2.40	-1.93	-0.14
T25D10	1	42	100	1.06	0.20	18.4	2.00	1.00	0	0
	8	36	85.714	1.26	0.53	42.2	3.00	1.00	19.29	2.75
	15	32	76.19	2.02	0.44	22.0	3.30	1.40	59.73	8.53
	22	29	69.048	2.23	0.57	25.7	4.10	1.00	10.34	1.478
	29	28	66.667	2.45	0.59	24.3	4.50	1.50	9.985	0.71
	43	22	52.381	2.75	0.67	24.4	4.60	1.40	12.06	0.86
	57	19	45.238	3.01	0.53	17.5	4.20	2.00	9.463	0.67
	71	14	33.333	3.33	0.46	13.9	4.00	2.60	10.76	0.77
	85	10	23.81	3.48	0.35	10.0	4.00	2.90	4.549	0.32
	113	7	16.667	3.34	0.42	12.6	4.00	2.60	-3.94	-0.28
	120	3	7.1429	3.53	0.17	4.8	3.70	3.30	5.698	0.81
T25D20	1	79	100	1.09	0.25	22.8	2.00	1.00	0	0
	8	78	98.734	1.33	0.41	30.7	2.50	1.00	21.75	2.98
	15	77	97.468	1.88	0.39	21.0	2.60	1.00	41.72	5.96
	22	69	87.342	2.09	0.43	20.6	3.20	1.50	11.36	1.62
	29	54	68.354	2.22	0.41	18.6	3.00	1.50	6.113	0.43
	43	44	55.696	2.58	0.43	16.9	3.50	1.50	15.88	1.13
	57	34	43.038	2.72	0.43	15.8	3.60	1.50	5.654	0.40
	71	16	20.253	2.92	0.51	17.6	3.60	2.00	7.284	0.52
	85	12	15.19	3.12	0.57	18.4	4.00	2.40	6.781	0.48
	113	7	8.8608	3.56	0.50	14.0	4.10	2.60	14.13	1.01
	120	6	7.5949	3.68	0.51	13.8	4.20	2.70	3.548	0.50

T25D30	1	125	100	1.1	0.24	22.1	2	1	0	0
	8	92	73.6	1.12	0.27	24.1	2.50	1.00	1.779	3.11
	15	84	67.2	1.87	0.29	15.7	2.50	1.00	66.63	0.25
	22	75	60	2.03	0.41	20.1	3.00	1.00	8.927	9.517
	29	68	54.4	2.06	0.40	19.6	3.00	1.50	1.175	1.27
	43	49	39.2	2.27	0.46	20.2	3.60	1.50	10.58	0.08
	57	36	28.8	2.47	0.48	19.6	3.50	1.50	8.742	0.75
	71	21	16.8	2.99	0.49	16.5	3.80	2.00	20.77	0.62
	85	13	10.4	3.00	0.46	15.2	3.50	2.00	0.478	1.48
	113	11	8.8	3.35	0.56	16.6	4.00	2.40	11.52	0.03
	120	3	2.4	3.20	0.59	18.4	4.00	2.60	-4.35	0.82
T25D30	1	128	100	1.04	0.17	16.2	2.00	1.00	0	0
	8	108	84.375	1.05	0.18	17.1	2.00	1.00	1.523	0.21
	15	82	64.063	1.58	0.27	17.4	2.20	1.00	49.93	7.13
	22	63	49.219	1.54	0.30	19.3	2.50	1.00	-2.38	-0.34
	29	56	43.75	1.58	0.36	22.8	2.20	1.00	2.515	0.18
	43	9	7.0313	1.97	0.47	23.8	2.50	1.00	24.73	1.76
	57	6	4.6875	2.35	0.53	22.5	3.00	1.60	19.49	1.39
	71	6	4.6875	2.52	0.37	14.8	3.00	2.00	7.092	0.50
	85	6	4.6875	2.88	0.74	25.8	4.00	2.00	14.57	1.04

APPENDIX 7

RECOMMENDATIONS FOR THE PLANNING OF AQUACULTURE FACILITIES.

The following are a list of basic guidelines and rules for maximizing the probability of success with seawater culture systems. They have been gained from experience.

* Take great care in quantifying the requirements, as they will greatly effect the complexity and cost of the system.
* Consider long-term as well as short-term requirements, even though they are more difficult to quantify.
* Major cost underestimates are more likely to result from necessary but uncounted components and services or increased requirements rather than errors in specific items.
* Consider operating approaches and procedures before the design is fixed. Significant input from operational personnel is needed in the design and construction phases.
* Demands for services (flow rate, compresses air, etc.) usually increase in quantity and quality with time. If possible, provide extra floor space, access to piping, access to drains and provisions for electrical power for anticipatable future retrofits.
* Remember that the key to low risk and high performance systems is large amounts of high quality seawater. In short, very conservative biomass loading relative to the available water quality. If a system is working well, increasing the biomass will increase the risks.
* Anticipate probably failures and plan accordingly to minimize the consequences.
* Provide redundant equipment to back up critical functions in emergencies. Resist temptation to use backup equipment in normal operations just because it is available.
* Responsibilities and decision-making procedures for emergencies should be decided before crisis occur, remembering that they rarely occur at convenient times.
* Do not forget routine maintenance and inventorying necessary spare parts when operations are going well, it is twice as important when things are not going well.
* Be extremely careful in selecting all materials and supplies used in and around seawater systems, because your organisms may be very sensitive and seawater is very corrosive. Do not forget to include the surrounding building and paints, sprays, cleaners, sealers and solvents used near culture organisms.
* Leach all materials in running seawater for at least two weeks before use.
* Take great care with all fittings, pipe and equipment on the suction side of pumps to avoid even the most minute air leak. Supersaturation can easily kill.
* Make the suction-side as large in diameter and as short as possible to minimize suction-side frictional losses.
* Place pressure gauges on both the suction and discharge side of pumps to monitor the condition of the lines and the performance of the pumps. Watch for biofouling, especially on the suction

side. Service regularly.

* Adequately pitch all floors in wet-lab areas towards the drains remembering that concrete may shrink on drying.
* Greatly over-size drains to take high transient flows. Drains can never be too large. Even the largest drains will occasionally clog if not maintained.
* Expect very high suspended solids content in incoming seawater from shallow water intakes during storms or heavy waves.
* Anticipate the need to remove sediment and debris from any parts of the system with low flow velocities.
* Bury or otherwise make inaccessible as little of the system as possible. The inaccessible parts are inevitably the parts you will want to get at later.
* Place all electrical outlets up high, above any unintentional water input.
* Use all ground fault interrupters on all indoor and outdoor electrical outlets. Do not underestimate the electrical hazards associated with seawater.
* Inspect and service intake screens regularly.
* Be careful in locating the intakes. They should not be situated so as to pick up debris, recycle drain water or ever experience breaking waves. Do not underestimate the forces of the sea on intakes and other exposed structures.
* Remember that the piping/processing system and the pumps are highly interactive. Changes in either area will effect the other.
* In wet-lab and mechanical areas use "X" fittings with blanked faces where "L's" and "T's" are needed. This maximizes accessibility for cleaning and future modifications.
* If you are on call, do not place on automatic alarm any functions or events that can wait until normal working hours.
* In a field with so much misleading or incomplete information, data voids, "experts" and variations in conditions, it is worth remembering when dealing with the unknown or uncertain that one test, under the actual conditions to be encountered, may be work a 1000 expert opinions.

REFERENCE

Huguenin JE & E Colt. 1989. Design and operating guide for aquaculture in seawater system. Developments in Aquaculture and Fisheries science 20. Elsevier Amsterdam.

STANDARD LABORATORY ORGANISMS FOR WATER QUALITY STUDIES PROGRAMME

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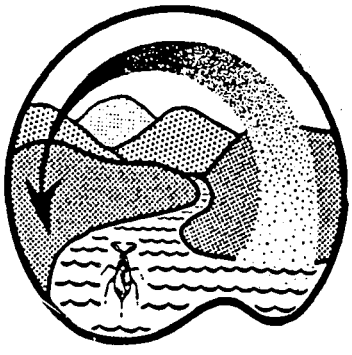

FINAL REPORT TO

THE WATER RESEARCH COMMISSION

WRC Report No. 545/1/97
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EXECUTIVE SUMMARY

The two digit numbers in the summary corresponds to those of the chapters in the main report.

1. INTRODUCTION, BACKGROUND AND MOTIVATION

Aquatic toxicologists in South Africa use standard laboratory organisms, such as *Daphnia pulex*, most of which are from lentic or standing water environments for the determination of pollutant tolerances. The aim of this project is the selection, maintenance and captive breeding of suitable test species from lotic or flowing water habitats for the use in an artificial stream system which is being developed at Rhodes University (Palmer *et al* 1995). At the artificial stream system in the Institute for Water Research, water quality guidelines for the natural environment will be determined using these indigenous taxa. A range of representative taxa should be selected to be tested because responses to the same chemical differ from species to species. Standard test species enable different researchers to produce similar results from a species reared under known circumstances. It has been established that the full consequences of pollution for the aquatic ecosystem can not be determined unless the responses of member species of these communities are assessed. Niederlehner & Cairns (1990) and Stokes (1986) found much diversity in the responses of species to pollutants, which resulted ultimately in the decrease of species richness and composition through reduction in egg production and growth reduction, while primary productivity, decomposition and nutrient processing remained essentially the same.

1.1 AIMS OF THE PROJECT

- To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.
- To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.
- To attempt to establish methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.

1.2 APPROACH IN PLANNING THE PROJECT

The establishment of a laboratory culture method for riverine invertebrates requires the selection of suitable candidate species and the investigation of the responses of the selected species to the variety of abiotic conditions such as temperature and photoperiod which will prevail in a laboratory. The project plan was formulated with the recognition that information about the habitat requirements would dictate the type of container which would offer the best option for laboratory maintenance. Information about the life cycle would dictate the periods when mature animals would be available for laboratory breeding, and the functional feeding group to which the species belongs would determine what type of food should be offered. The effect of other biotic variables such as density and fecundity on the production potential of the programme also has to be investigated. The design of the experimental programme took into consideration which combination of abiotic and biotic factors would have the greatest impact on the growth and survival of the test candidates.

The research programme had a dual nature which overlapped in all areas. The selection of suitable taxa from the lotic habitat and the investigation of the biological and environmental requirements of these species for laboratory maintenance and breeding was closely interwoven with the development of the equipment and methods to maintain macroinvertebrates in aquaculture.

2. SUMMARY OF PROGRESS - SELECTION AND SCREENING OF CANDIDATE SPECIES

2.1 SELECTION CRITERIA

Certain criteria to be considered in the selection of organisms, suitable as subjects for ecotoxicological studies, have been developed by researchers in the field over the years. These can be grouped into several categories.

Sensitivity

♦ *High susceptibility to pollutant stress.*
Different taxonomic groups respond more, or less, sensitively to different pollutants. Therefore we have focused on abundant, widely distributed taxa, that are not present under grossly impaired conditions.

Ecology & Life History Information

♦ *Importance in terms of abundance or productivity, physical community structure or regulatory properties in the system* (ie the role of the chosen species in the ecosystem should be relatively important).

Multi-species test systems should include representatives of producer, regenerator and circulating phases but these taxa do need to be present in sufficient numbers for reliable collection to be suitable for laboratory breeding.

♦ *Wide distribution*

Test results which are obtained from species with a wide distribution are theoretically applicable to a wide range of ecosystems. However, the wide distribution may be an indication of phenotypic plasticity.

Provenance

♦ *Species identification.*

In cases where problems are experienced with identity it is necessary to collect parent stock from a limited range and keep could record of the identifying features.

♦ *The ecology and physiology of the species.*

Information on temporal and spatial variation in life-history features of aquatic invertebrates is essential in planning laboratory schedules for field collections.

♦ *Low genetic and biological variability.*

The importance of low variability in achieving reproducible test results is obvious, but when these results are extrapolated to the responses of wild populations which have naturally more variability the guidelines set may appear conservative.

Maintenance And Breeding

♦ *Easily held or cultured in laboratory for experimental ecotoxicological procedures.*

Herbivores or detritivores such as shredders, grazers or filter feeders are generally easier to maintain than predators or parasites with host requirements.

♦ *Easily sampled* i.e large and robust.

Hierarchy of Considerations

After three years of experience in the selection and maintenance of taxa we believe these should be ranked as follows;

1. Availability in the field and size of the organism.
2. Suitable life history and biology for maintenance in laboratories.
3. Accurate identification.
4. Position and function in ecosystem.
5. Sensitivity to range of test chemicals.

Ideal and achievable aims are seldom the same and compromises in the selection process had to be made in this complex and unpredictable procedure. Unless a field population of the candidate species is easily accessible, replenishing stocks for the early laboratory investigation can become prohibitively expensive. Problems associated with life history styles such as aerial phases can be circumvented in many but not all cases, but if a species has very narrow and specific habitat requirements this may make their culture uneconomic. We have come to the conclusion that a problematic taxonomy is not insurmountable if the identifying features are carefully described. However species complexes from the same habitat must be regarded with caution.

2.2 SCREENING OF ORGANISMS

After several screening trials and from observations during experiments both in this programme and during toxicological investigations conducted by Palmer *et al* (1995) as well as in field collections the following taxa were selected for further investigation. Those taxa which have been subject to detailed investigation are listed below.

Ephemeroptera Leptophlebiidae.

♦ *Choroterpes elegans:* A good candidate, reasonably accessible (Buffalo river, eastern Cape) with wide distribution, of good size, easy to feed and fairly robust, but fairly tolerant as it occurs in lower reaches of rivers. Its temperature range is possibly lower than taxa from upland rivers. Taxonomy well established.

♦ *Adenophlebia auriculata:* This is an excellent candidate, which is easily available, comparatively large and easy to feed. It is found in numerous upland rivers with good water quality so is quite sensitive and widely distributed. Robust with careful handling. Taxonomy is reliable and there are two species in the genus. However the breeding

biology is obscure.

Mollusca Ancylidae

♦ *Burnupia stenochorias:* An excellent candidate and easy to feed, robust with specialist handling and breeds easily in the laboratory. A better candidate than other gastropods as it is more susceptible to external conditions due to the anatomy. All life stages are aquatic.

Three other candidates which have good potential as laboratory candidates are *Thricorythis* spp. (Ephemeroptera); *Cheumatopsyche* spp. (Trichoptera) & Planaria.

3 SUMMARY OF PROGRESS - DEVELOPMENT OF METHODS

3.1 FIELD COLLECTION AND TRANSPORT

Animals should be handled as little as possible during collection and transported in cool well aerated water with pliable solid substrate. If possible, animals should be collected and transported on the same day. It was proved experimentally that touching limpets increased mortality rates and it was decided to use an anaesthetic during field collection and to line all containers in which the limpets are grown with plastic bags so that they could be moved without being handled.

3.2 MAINTENANCE EQUIPMENT

3.2.1 Artificial Streams - Recirculating Channels

Two sizes of recirculating channels of the same basic design were constructed (Fig 3.3.2.1). A plastic or PVC channel containing substrate, through which water is pumped from a sump by submersible aquarium pumps via a horizontal spreader. There are some problems associated with the use of these systems

- difficulty in the maintenance while in use.
- no easy method to allow trapping of adult insects on emergence.
- small juveniles escape easily from the outflow.

3.2.2 Static Water - Bubblepots

Bubblepots are cylindrical opaque or transparent plastic containers of a variety of sizes, equipped with

aerators. These are the most suitable containers for rearing small juveniles. They are easily maintained and transported. It was shown experimentally that one airstone produces the same level of dissolved that oxygen to all volumes of water and temperature and dissolved oxygen are negatively correlated.

3.2.3 Substrate

Ovoid kaolin stoneware substrate (6cm x 4cm x 4cm) have been manufactured to standardize the available algal food growing surface and to provide refugia (Fig 3.3.2.1 c). In addition, periphyton is cultivated on 8cm x 8cm unglazed tiles which provide a more accurately measurable surface of available food. A variety of materials were tested as substrate for mayflies. Observations from all experiments confirmed that solid substrate are essential for the optimal survival of stream macro-invertebrates. For the transport of invertebrates softer substrate such as fine textured plastic foam rubber, plastic sheeting or leaves from the collection site should be offered.

3.2.4 Food provision

The limpet is a grazer and the mayflies are brusher collectors so that both animals collect their food by scraping the surface from stones. Periphyton grown in the laboratory has proved to be an adequate basic food to sustain growth in both species. Degraded leaves, river detritus and TETRAMIN have all proved capable of sustaining mayflies.

3.3 CONCLUSIONS ABOUT METHODS OF LABORATORY MAINTENANCE

In conclusion, the species investigated so far can be adequately housed and fed prior to being made available for experimental purposes. The most important conditions for the successful maintenance and breeding of invertebrates are:

- Correct light conditions for algal growth to ensure good food supply.
- Temperature regulation.
- A good water supply, preferably from a non municipal source, with the ability to regulate its quality and condition.

4. SUMMARY OF RESULTS - INVESTIGATION OF *BURNUPIA STENOCHORIAS*

4.2 EXPERIMENTAL INVESTIGATIONS

4.2.1 OPTIMAL REARING CONDITIONS

4.2.1.1 A Pilot study on suitable laboratory conditions

A pilot study was conducted during which limpets were kept in 500ml bubblepots and were offered three different feed types. It was established that i) the limpets between 4-6mm shell length were sexually mature and laid eggs within 4 weeks while ii) limpets which were between 2-4mm in shell length were not sexually mature but did mature and then laid eggs with in 8 week of the start of the experiment. iii) Spat from both sizes of limpets grew between 0.019and 0.32mm/day on the diet provided. iv) In some containers a second generation of spat was produced. This pilot study showed that it would be possible to cultivate the limpet in the laboratory.

4.2.1.2 Handling requirements

Touching the limpets, either in the laboratory or transporting from the field, has a very negative effect on both their growth and survival. To overcome this not only was the use of anaesthesia investigated as stated above, but a measuring template was devised to enable measurement without removal from the container. We believe that what is lost in accuracy of measurement is gained in the increase in survival. The survivorship of the limpet in the laboratory has not been very high (20%). Initial losses after hatching are high and this is not unexpected as similar mortalities have been reported (Russell-Hunter, 1953).

4.2.1.3 Dietary requirements

Although the specific food requirements of *Burnupia* have not yet been investigated the species which most closely resembles it, *Ancylus fluviatilis* is a microherbivore of epilithic algae, particularly diatoms. *Burnupia* feeds by rasping the stone surface with the radula which is similar to the behaviour reported for *Ancylus*. Artificial food (pellets of *Spirulina*, fishmeal, starch and essential amino acids), natural periphyton and periphyton from an enriched environment all gave the same growth rate for smaller limpets (2-4mm) but these differed in the larger size group (4-6mm) where the artificial food proved to be the better food source and also gave the highest yield of eggs.

The debate as to whether test animals should be fed during ecotoxicological testing to minimise stress was addressed experimentally and it was found that 96 hours (acute test period) with no food did not affect the survival of either adults or sub-adults.

4.2.1.4 Hydraulic requirements

Although the limpets are naturally found in lotic habitats, it is presumed that this is in response to their need for high levels of oxygen within the water body. In the laboratory it has been found that a greater number of eggs are laid in aerated standing than in flowing water (section 4.2.1.5). The suitability of the design of channel used was clearly demonstrated in sections 4.2.1.4 to 4.2.1.7 where small channels (50cm long) consistently gave poor survival, and this was ascribed to the large angle of slope, and the lack of a good foothold on the smooth surface of these channels for the limpets. Large channels (1.5 M Long) gave a higher survival rate than standing, aerated water, although the difference in the survival of hatchlings between the channels and the standing water needs to be investigated further.

4.2.1.5 Temperature effects

At 15°C, 20°C and 25°C steady temperature, the higher temperature regime resulted in a significantly increased growth rate, at densities of up to 30 limpets per 500ml volume of water ($p<0,001$).
Work reported on other snails suggests that a circadian temperature fluctuation, rather than a constant temperature favours all physiological activities. This is a factor which will be taken into consideration when a dedicated laboratory is designed.

4.2.1.5 Density effects

Under high densities intensified grazing pressure and increased output of metabolic wastes may cause a shift in the species composition and succession of the periphyton assemblage (Bronmark 1989). Densities of 10 limpets per 500ml volume of water (230cm^2 available surface area) attained higher growth rates than densities of 20 and 30. The effect of density on fecundity has not yet been investigated but may prove significant. Chernin & Michelson (1957a & b), Wright (1960) and Eisenberg (1966) all showed a negative effect on the mean clutch size with increasing density in other snail species.

4.2.2 REPRODUCTION AND FECUNDITY

4.2.2.1 Breeding biology

As hermaphrodites (Brown, 1980) these limpets are able to produce young without copulating, but copulation has been observed, usually a smaller limpet acting as the male (Bantustan, 1950; pers. obs.). In hermaphrodite animals it is always difficult to determine if self or cross-fertilisation has taken place without genetic investigation. Further it is also often impossible to determine which partner act as female without direct observation. Because of our lack of understanding of the complexities involved in the reproductive style in the early period of the project, the results from the fecundity experiments conducted to date are not clear cut. However some indication of expected reproductive capacity and possible yield of juveniles can be made. Other researchers have also found a large degree of variation, and the difficulty we have experienced in determining individual fecundity is also universal.

The size of limpet at sexual maturity was always greater than 3,4mm shell length

4.2.2.3 Fecundity

The number of capsules and eggs produced by an individual is widely variable. The number of eggs/capsule ranges from 1 to 13. The average number produced by a group of limpets seemed to vary slightly depending on the history of the parents. Mature wild reared limpets laid capsules with an average of 5,75 eggs per capsule when brought into the laboratory. Juvenile limpets (approx. 2,5mm,) reared in the laboratory either as pairs or singly produced on average 3,7 egg/capsule. From August to October 1995 a field sampling programme has been underway and the average number of eggs per capsule has been determined as 5,4.

The numbers of capsules recorded from one pair of limpets in the laboratory also ranged widely. From pairs of field reared adults brought into the laboratory 14-44 (avg 24.7) capsules were recorded and from laboratory reared pairs 1-16 (avg 8.1) per pair was recorded.

More capsules were laid in the aerated, standing water, than in the flowing water channels in the laboratory.

4.2.2.4 Embryology

The embryological development of the eggs has been described and appears to follow a generalised

molluscan pattern in the initial stages but like other Ancylidae does not undergo torsion in the last stages before hatching. *Burnupia* displays direct development, with the young emerging as crawling snails. Hatching success in the laboratory is 91%. The embryonic period is 14 to 17 days, at an average temperature of 19°C. Cooler temperatures extend the time of hatching up to 21 days at 13°C (pers. obs.).

4.3 FIELD INVESTIGATIONS

After 8 months sampling of wild populations we have been able to determine that a small number of eggs are laid throughout the winter, but that there is a build up to peak egg-laying in spring. Growth rates and cohort responses to temperature variations in the field can only be analyzed after several seasonal cycles have been recorded.

4.4 CONCLUSIONS

The greatest advantage that the limpet has for laboratory rearing is, perhaps, the readiness with which it lays eggs in captivity. The most serious bottleneck in the production potential of the limpet is low survivorship of hatchlings but it is believed that enough knowledge has been accumulated to enable a pilot maintenance project to be launched. It appears from preliminary investigations that the limpet may have a similar life history to that of the mayfly.

5. SUMMARY OF RESULTS - INVESTIGATION OF *ADENOPHLEBIA AURICULATA* AND *CHOROTERPES ELEGANS*

5.1.1 INTRODUCTION

Two candidate species from the mayfly family Leptophlebiidae, *Adenophlebia auriculata* and *Choroterpes elegans*, were investigated, primarily in the field with fewer laboratory experiments being conducted than with the limpets. The emphasis on field work was because the life cycle, breeding strategy and mating behaviour of these animals are largely unknown. It is unlikely that laboratory maintenance will involve natural mating and egg laying as is the case with the limpets. However the knowledge of life history patterns gathered will enable optimal use of field collections for sub-adults. Investigation of artificial fertilization will be continued.

Growth rates, moulting frequency and suitable feeds have been investigated in laboratory experiments with

A. auriculata and *C. elegans.*

5.2.1 LABORATORY MAINTENANCE AND REARING CONDITIONS

5.2.1.1 Hydraulic requirements

Habitat requirements such the hydraulic conditions and substrate type was investigated. In this instance it was discovered that solid substrate are an essential prerequisite and that although the species is found most abundantly in riffles and runs it survives the best in static bubbled water in the laboratory.

5.2.1.2 Dietary requirements

It was found that if the natural diet of decaying leaves, detritus and periphyton was supplemented with TETRAMIN, a commercial fish food, optimal conditions for growth were provided if water quality was good.

5.2.1.3 Temperature effects

Rising temperatures depressed levels of dissolved oxygen and mortalities of *Choroterpes* sp. occurred when the DO reached 30 % at 25°C, except in the largest volumes of water where onset of mortality was delayed.

The average instar period for nymphs of *A. auriculata* is inversely correlated to temperature. (Table 6.5.1)

Table 6.5.1. Average instar length of *A. auriculata* at three temperatures in two experiments and overall instar period calculated by ANOVA.

Treatment	25°C	20°C	15°C
Petri-dish	7.7(2.66)	8.8(3.12)	13.5(6.20)
Bubblepot	7.4(3.43)	10.4(4.47)	14.9(6.23)
Overall avg.	7.52	9.24	10.12

Summary of growth rates in response to diet and temperature variation.

Table 6.5.2 Absolute growth rates (mm/day) calculated from all experiments in channels and bubblepots in the laboratory

Experiment no.	Growth rate mm/day	Temperature	Hydraulic/diet conditions
5.2.3	0.0191- 0.0220	19-22°C	Flowing/periphyton
5.2.4	0.0142-0.0277	17-22 °C	Static/ leaves & tetramin
5.2.5	0.0047/0.0085/0.015	15, 20, 25 °C	Static/ leaves
RANGE	0.005-0.03	15-25°C	

5.3 FIELD INVESTIGATIONS

Field populations have been studied over a three year period. *A. auriculata* in the Palmiet river in riffles and runs have a similar size distribution but higher densities were found in runs. Hatchlings have not yet been captured in the field. In 1993 there appeared to be a late autumn/early winter and a late winter/early spring emergence of subimagoes. This was confirmed when a weekly sampling regime was instituted in 1994. In addition a large summer emergence was found which encompassed several weeks in February and March.

Aspects of the life history of the mayfly that still need investigation include; a) accurate measurements of the effect of temperature on growth rate; b) factors which influence fecundity; c) metamorphoses; d) the habitat of hatchling nymphs (< 0.4mm HW.) which may be gravel and sand (de Moor pers comm.) or leaf packs; e) cues which triggers emergence; f) determination of growth rates in the field to verify those obtained in laboratory culture.

5.4 REPRODUCTION AND FECUNDITY

5.4.1 ARTIFICIAL FERTILIZATION

The successful captive mating of mayflies have never been accomplished. It was decided to resort to the well tried piscicultural practice of artificial fertilisation. It was demonstrated that the eggs of *A. auriculata* could be artificially fertilised and that viable offspring resulted. The most successful method involved dissecting the seminal vesicles out of a male imago in a freshly prepared solution of insect Ringers. The

female was then induced to release her eggs into the solution after decapitation by dipping the tip of her abdomen onto the surface of the mixture. This mixture of eggs and sperm was then left for at least 15 minutes before the eggs were transferred to a suitable substrate in river water where they rapidly adhered to the surface. Egg development was fastest at 25 °C. and took 16 -22 days to hatch. However, further work is necessary to perfect the techniques and to increase the hatch rate.

Sufficient numbers of new hatchlings have never been available for experimental investigation of rearing but in the petri-dishes where they hatched they reached the second instar. The smallest sizes (0.2 -.4 mm head width) of field caught nymphs have all survived well in the laboratory (Ch.5.2.).

5.5 CONCLUSIONS

Sufficient supply from laboratory stocks is still not possible as several problems associated with reproduction in captivity and survival of a large proportion of both selected species have not been solved.

6 ACHIEVEMENT OF AIMS

i. *To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.*

♦ Screening was accomplished in the eastern areas of the country, and in addition invertebrate workers country wide were consulted by questionnaire and nine suitable candidates were identified. Of these three species have been investigated to varying degrees.

ii. *To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.*

♦ A pilot programme has been established and methods to feed and house invertebrates are in place. However the pilot project is not yet operational because of difficulties with the long term survival of the limpet and artificial fertilisation and rearing of the hatchlings of the mayfly.

iii. *To attempt to establish methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.*

♦ Until the above problems are solved, a sufficient supply of experimental organisms will not be possible.

However during the course of the project toxicological experiments were supplied with limpets on two occasions and many of the problems associated with handling, transporting, and maintaining the test species were addressed and solved.

Although not stated explicitly in the aims of the project, implicit in the work programme and the development of the methods, has been the investigation of the biology and life history of the two species selected. Knowledge gained about the previously unknown responses of these two species to environmental variables has been significant and will contribute to the understanding of ecosystem function of subtropical rivers.

7 RECOMMENDATIONS FOR FUTURE WORK

Requirements of the cultivation, maintenance and supply of sufficient experimental animals for ecotoxicological experiments in artificial stream laboratory (which is now fully operational):

- Further knowledge of the breeding requirements, and better survival of juvenile *Burnupia*.
- Development of techniques for artificial insemination and maintenance of early instars of *A. auriculata*.
- The investigation of optimal growing conditions for the cultivation of periphyton.
- Increasing the scale of laboratory maintenance facilities to provide more capacity for the maintenance of larger laboratory populations.
- The design and construction of a dedicated laboratory for production and maintenance of experimental populations of invertebrates. (see section 7.1).

7.1 DEVELOPMENT OF A DEDICATED REARING AND MAINTENANCE LABORATORY

Present facilities in the Institute for Water Research are inadequate for the envisaged production of experimental animals. The requirements for a purpose-built facility are as follows:

- Floor space of at least 100 m^2.

- Adequate temperature control, by air-conditioning.
- Adequate natural light, e.g. through sky-lights.
- Periphyton cultivation facilities which are separate from the insect rearing facilities.
- Water purification facilities, e.g. by deionisation.
- Rearing and breeding channels, as well as extensive population holding facilities, with temperature and light control.

Two initiatives are proposed for the second phase of this project, which begins in January 1996:

- A visit to Stroud Water Research Centre, Pennsylvania, where a large amount of mayfly research is conducted and Virginia Polytechnic in Blackenberg where ecotoxicological testing has been ongoing for 20 years, to learn about the conditions described above.
- The engagement of a fund-raiser, to obtain funding for the proposed laboratory.

7.1.1 DEVELOPMENT OF THE REARING FACILITY

The development of this facility should take into consideration the needs which are peculiar to the culture of lotic invertebrates. However, they rely on general principles of aquacultural design which have been developed throughout the world for many years. Huguenin & Colt (1989) produced a guidebook in which much of the available literature on general principles of aquacultural design needs for both sea and freshwater have been summarised (Appendix 6). The requirements which are particular to this project revolve around the provision of the correct feed by the cultivation of periphyton, and the addition of decaying leaves supplemented by TETRAMIN as the basic diet for the mayflies. A subsidiary consideration is the maintenance of large numbers of smaller-sized specimens (those not fully grown) in a holding facility in readiness for use as toxicology test subjects.

Laboratories where invertebrates are cultured on a routine basis should be investigated. Information will be obtained from two sources: Stroud Water Research Centre, Pennsylvania where mayfly culture has been ongoing for at least 20 years; and the Virginia Polytechnic where ecotoxicology work is also of long standing.

Once these investigations are complete plans can then be drawn up for the construction of a dedicated facility in Grahamstown. A fund-raiser will be engaged to obtain funding for the project, and after the plans have been approved, and funding is obtained, the building will proceed. Prof.T.Hecht and Mr.P.Britz at the Department if Ichthyology and Fisheries and Science at Rhodes both have extensive experience in the construction of aquaculture facilities and have agreed to oversee the planning.

The expertise being developed in this and during the earlier project can be applied to other areas of environmental management such as the rehabilitation of damaged rivers and streams. The restocking of rivers with macroinvertebrates will speed their recovery. This was a contributing factor towards the interest in collaboration shown by the Dept. of Nature and Environmental Conservation.

ACKNOWLEDGEMENTS

In a project with a diverse nature such as this one has proved to be, the people who are called on for advice and information come from many professions. We would like to acknowledge the assistance of aquatic biologists country wide, the irrigation engineers and aquaculturists we have consulted. We have been fortunate that in the eastern Cape and particularly at Rhodes University many experts were easily accessible.

To Dr. Jay O'Keeffe and Dr. Tally Palmer, goes our gratitude for creating the opportunity together with the Water Research Commission, to embark on what has been a fascinating and at times frustrating project. Jay and Tally thank you, for firm guidance and loyal support. To our colleagues in the Institute we would like to give thanks for help, support and lots of fun. In particular, I would like to thank Margi Rogers our ever cheerful and unflappable secretary who, together with Ann McKane, helped to keep the finances of the project ordered. To Patsy Goetsch, thank you for encouragement and editorial help and Gaye Youthed for technical support. As project researcher, I would like to thank Heather for bringing better order and structure the project, and Nkosnathi for making and maintaining the equipment as well as for valuable assistance in the field.

Colleagues at Rhodes University who have been ready to give sound advice include Prof. Tom Hecht, Peter Britz, Sarah Radloff and Martin Villet. Horst Kaiser must be singled out, for although he acted as official consultant, for statistical analysis, his input has been enthusiastic and unstinting. He tackled some nasty analytical problems and came up innovative solutions.

One of the other advantages of working at a university is the availability of students eager to find suitable projects every year. I have been fortunate to have had three sterling young people working with me. Thank you; Lisa Horne, Bruce Davison and Simon Burton for your input.

The WATER RESEARCH COMMISSION fulfils an important role in the scientific community of the country by supporting many scientists and giving them the scope to pursue work which is not only of their own interest but of great national importance. We in this project has been extremely fortunate in having Dr. Steve Mitchell as Project Manager. He has been encouraging, supportive and always at hand with guidance. More than anything Steve I want to thank you for having confidence in us, for that has allowed our confidence to grow. Although Dr Peter Reid is no longer with the Commission, we would nevertheless like to acknowledge his contribution, which was often one of grasping the nub of a problem, in wide ranging discussions.

Finally the members of the steering committee of the project must be thanked for their input and time. The members not mentioned already were; Professors MN Bruton, JA Day, IG Gaiger, Dr. KCD Hamman, Mr D Roux and Prof. JHJ van Vuren.

Guide to this report.

Each chapter in this report stands on its own with its own reference list. The appendices are numbered according to the relevant chapter except Appendix 3 which refers to both Chapter 3 and 5. Table 5.5.1 appear in both the Executive Summary and in Chapter 5 (summary).

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CHAPTER 1
OBJECTIVES AND BACKGROUND

Introduction

Aquatic toxicologists in South Africa use standard laboratory organisms for the determination of pollutant tolerances. Most of the invertebrate test organisms used are lentic inhabitants. The aim of this project is the selection, maintenance and captive breeding of suitable test species from lotic habitats for the use in an artificial stream system which is being developed at Rhodes University (Palmer *et al* 1995). Laboratory toxicological bioassays assess the possible effects of proposed new chemical products or the reasons how and why chemicals have affected the target organisms. Ecotoxicology involves the investigation of the fate and effect of toxic agents, as well as the tolerances of test species to these substances, in the ecosystem (De Kruijf 1988). The artificial stream system in the Water Research Institute at Rhodes will be employed in identifying these responses and in doing so, determine water quality guidelines for the natural environment. To test the tolerances of flowing water taxa to the water quality variables this facility needs a reliable and uninterrupted supply of indigenous test organisms from the lotic environment.

1.1 PROJECT AIMS

- To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

- To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.

- To attempt to establish methodologies for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.

1.2.1 TARGETS

GANNT CHART - APPROXIMATE SCHEDULING OF PROJECT TASKS.

YEAR	1993				1994				1995			
QUARTER	1	2	3	4	1	2	3	4	1	2	3	4
1. Literature review	X	X	X	X	X	X	X	X	X	X	X	
2. Life history data		X	X	X	X	X	X	X	X	X	X	X
3. Habitat		X	X	X	X	X	X	X	X	X		
4. Interim report						X						
5. Lab maintenance				X		X	X	X	X	X	X	X
8. Pilot scale rearing						X	X	X	X	X	X	
9. Transport methods				X	X	X	X	X				
10. Final report										X	X	X

1.3 BACKGROUND TO PROJECT

The establishment of biological tolerance criteria for the aquatic environment has become critical in this country with its limited water resources, as ever more pressure is being exerted on this resource through increased utilisation. The full consequences of pollution on the aquatic ecosystem can not be determined unless the responses of member species of these communities are assessed. Examples of the variety of responses from resident species to pollutants are cited here to highlight the need for a representative selection of species to be investigated. Niederlehner & Cairns (1990) found that members of periphyton communities showed a variety of significant responses by slight changes in pH, which often resulted in the biomass remaining the same whilst the community structure and perhaps function changed. This was consistent with previous findings of Stokes (1986) that species richness decreased and composition changed while primary productivity, decomposition and nutrient processing remained essentially the same to a pH of 5.6. From studies on other commonly tested multicellular organisms these authors extrapolated that a pH decline from 6.0 will begin to have adverse effects on insects (chironomids), fish, crustacea (Cladocera) and some amphibians. Six fish species, cladocerans, copepods, rotifers and protozoans were tested at various levels of ammonia exposure by Hermanutz *et al (1987) and revealed widely differing responses to concentration as well as type i.e. reduction in egg production, growth reduction or histological lesions in some organs.*

Petersen et al (1992) state that some ecological concepts are often not considered in ecotoxicology, and discusses their importance in assessing the effects of hazardous substances on aquatic systems at the population, community and ecosystem levels. He suggests that the study of a small group of ecologically related organisms, a guild, can provide more information on the effect of toxic substances than the study of the whole community. A key to this analysis is the recognition of the difference in species sensitivity and it is suggested that the specialist-generalist concept can be used to predict which species will be most affected by toxic substances.

1.3.2 APPROACH IN PLANNING THE PROJECT.

The establishment of a laboratory culture method for riverine invertebrates requires firstly the selection of suitable candidate species and then investigation of the responses of the selected species to the variety of conditions which will prevail in a laboratory. Naturally, considerable variation occurs in temporal and spatial scales of life-history features such as emergence, feeding and growth, and movements and migrations of aquatic insects (Rosenberg & Resh, 1993). Information on the degree of variation which occurs is needed to plan laboratory investigations, and field sampling programmes, for toxicological

experiments. Since experimental toxicology was started concurrently with the breeding programme; and the breeding programme is dependent on "seeding" populations in the field; this project aimed both to provide information on field populations, and on the breeding potential of selected stream organisms. If sampling is done at times when the largest number and/or the most vulnerable stages are readily available, progress of the work will be optimised.
The project plan was formulated with the recognition that information about the habitat requirements would dictate the type of container which would offer the best option for laboratory maintenance. The life cycle would dictate the periods when mature animals would be available for laboratory breeding, and the functional feeding group to which the species belongs would determine what type of food should be offered. The design of the experimental programme took into consideration which of the laboratory variables would have the greatest impact on the growth and survival of the test candidates.

Single species tests are important for establishing the effect of chemicals on growth, reproductive success, behaviour and other biologically important information, and these have been refined over the last 40 years. Although Cairns (1992) has made some progress in the use of multi-species test systems these involve lower orders. We have used the single species approach, but have selected invertebrate taxa from different orders.

1.4 SUMMARY OF PROGRESS

1.4.1 SCREENING OF ORGANISMS

Aim

To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

Results

A protocol for selecting appropriate experimental organisms was developed through reviewing the literature pertaining to the use of artificial streams and associated types of research. Rivers in the Mpumalanga, eastern Cape and western Cape were sampled and faunal complexes returned to the laboratory where the survival of the various species was recorded. From these experiments a short list of suitable species was compiled. At the same time the laboratory culture methodologies were investigated. A questionnaire was circulated to invertebrate biologists at various universities and their responses are recorded in Chapter 2.

1.4.2 MAINTENANCE OF REPRODUCING POPULATIONS

Aim

To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.

Results

In attempting to satisfy this aim a two-pronged approach was used. To be able to design optimal holding and breeding conditions for any species some understanding of the life history, the habitat requirements and the breeding biology is needed. Consequently, the responses of the selected species to laboratory conditions were recorded experimentally, while the ecology and life history were recorded by regular field investigations.

Ancylidae

The limpet *Burnupia stenochorias* was found to reproduce readily in the laboratory. Consequently a number of experiments were conducted to ascertain the responses of this animal. The following areas have been and continue to be investigated.

1) Growth rates
    a) suitability of various hydraulic water conditions.
    b) influence of temperature (15°C, 18-20°C and 25°C)
    c) effects of disturbance
2) Food requirements
3) Reproductive biology
    a) Fecundity and hermaphroditism.
    b) Developmental period and basic embryology.
4) Field studies, initiated in 1995.

It is believed that enough knowledge has been accumulated to enable a pilot maintenance project to be launched.

Leptophlebiidae

Two candidate species from the mayfly family Leptophlebiidae, *Adenophlebia auriculata* and *Euthraulus (Choroterpes) elegans*, were investigated, primarily in the field with fewer laboratory experiments being conducted than with the limpets. The emphasis on field work was because the breeding strategy and mating behaviour of these animals are largely unknown. To date the life history of *A. auriculata* has been monitored, although a direct correlation of environmental cues and breeding has not yet been established. Laboratory maintenance experiments using *A. auriculata* and *Choroterpes*

spp. as subjects, have investigated growth rates, moulting frequency and suitable feeds. It is unlikely that laboratory maintenance will involve mating and egg laying, as the cues for these are unknown. However, a knowledge of life history patterns in the field will enable optimal use of field collections. Investigation of artificial fertilisation will be outlined further.

1.4.3 METHODS

Aim

To develop methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and tests for macro-invertebrate tolerances to various conditions in the experimental stream project.

Results

One of the essential components of successful toxicity testing is reliable survival of control populations. To this end it was important to establish optimal conditions in the experimental systems. In addition, conditions under which maintenance and breeding will be optimal needed investigation. At this stage we have identified the following key parameters: hydraulic conditions, light, temperature and food requirements. To date, control survival is reliably 80 - 90% over 96 hours, however, the sufficient supply from laboratory stocks is still far from possible as several problems associated with reproduction in captivity and life long survival of both selected species have not been solved.

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CHAPTER 2
SELECTION AND SCREENING OF LOTIC INVERTEBRATES SUITABLE FOR LABORATORY REARING AS EXPERIMENTAL ANIMALS

2.1 A REVIEW OF THE SELECTION CRITERIA TO BE APPLIED TO CANDIDATES FOR CAPTIVE REARING FOR ECOTOXICOLOGICAL STUDIES

The choice of a standard laboratory organism for rearing in large numbers should obviously be governed by carefully selected criteria. In many ways these criteria are similar to those that apply to the choice of bio-monitoring indicator species which are utilised to monitor the effects of xenobiotics on the natural community. Ideally, indicator organisms are those species that have narrow and specific environmental tolerances. Conversely, organisms that have wide tolerances for different environmental conditions, and whose patterns of distribution or abundance are only slightly affected by substantial variations in environmental quality, are poor indicators.

The principal underlying assumption in using indicator organisms for water quality assessment is that the abundant presence of the indicator signifies that its physical, chemical, and nutritional requirements are being met. Table 2.1.1 lists the characteristics of "ideal" indicator organisms as proposed by Rosenberg and Wiens (1976) and Hellawell (1986) and the desirable properties for "ideal" test species

suggested by De Kock, de Kruijf & van de Guchte (1988) and the high degree of similarity in the requirements for both biomonitoring and laboratory test procedures is evident. The implications of these criteria highlights the need to have an understanding of the biology of those species which are to be used in toxicology tests.

Table 2.1.1 Desirable selection criteria for indicator species compared to those for laboratory test species. Several criteria can be grouped and are discussed under the same heading.

IDEAL INDICATOR SPECIES (Rosenberg and Wiens (1976) & Hellawell (1986))	IDEAL TEST SPECIES (De Kock, de Kruif & van de Guchte, (1988))
Taxonomic soundness and easy recognition by the nonspecialist. Taxonomic uncertainties will complicate long-term monitoring and between-site interpretation.	Species identification should be reliable.
Cosmopolitan distribution (or distribution involving an ecological analog), which would allow for comparative studies on local, national, and international scales.	Species should be widely distributed.
	Species should have high susceptibility to pollutant stress.
Numerical abundance allows for ease of sampling and for conclusions about quantitative distribution patterns.	Species should be abundant or dominant components of the natural system.
Low genetic and ecological variability (indicators should have relatively narrow ecological demands).	Species should have low genetic and biological variability.
Ecological characteristics should be well known. Background physiological and autecological information should be available widely.	Information from previous ecological or physiological studies should be available as well as abundant ecological data.
Suitable for use in laboratory studies to allow for determination of causality	Species should be easily held or cultured in the laboratory for experimental procedures.
Large body size which would facilitate sampling and sorting.	Species should be easily sampled.
Limited mobility and relatively long life history to allow for ease of integration on spatial and temporal scales.	

2.1.1 SENSITIVITY

♦ *High susceptibility to pollutant stress.*

Over a period of twenty years, investigations at the Virginia University Institute for Environmental and Hazardous Materials Studies have established that naturally derived, unstressed communities of aquatic microbes and macro-invertebrates are composed of species showing a normal distribution of stress tolerance from sensitive to resistant. Cairns (1986) addresses the concept of the "most sensitive species " and the erroneous perception that responses from sensitive species can be extrapolated to a wider array of species at different levels of biological organisation. Firstly, the sensitive species may not be resident in the ecosystem for which management decisions are to be taken and incorrect criteria could be set, and secondly, responses to chemicals are known to vary from species to species. In addition, different taxonomic groups respond more, or less, sensitively to different pollutants. Therefore we have focused on abundant, widely distributed taxa, that are not present under grossly impaired conditions.

2.1.2 ECOLOGY & LIFE HISTORY INFORMATION

♦ *Importance in terms of abundance or productivity, physical community structure or regulatory properties in the system* (ie the role of the chosen species in the ecosystem should be relatively important).

An understanding of ecosystem function and structure is essential in the selection of species to breed for laboratory testing. Therefore representatives from different trophic levels should be selected. Cairns (1975) also suggests that not all species are equally important in the functioning of a system and coined the term "critical species" for those which have the most telling effect. It has been demonstrated several times that the removal of top predators can result in population explosions while the overproduction of lower level producers has resulted in severe population imbalances and the development of pest species.

The distribution of individuals within species follows a recognizable pattern: a few species are represented by many individuals, many species are represented by few individuals, and some species are intermediate (Williams, 1953). Pearson *et al* (1983) proposed using species of intermediate abundance as pollution indicators. He argued that very abundant taxa may be rejected as indicators because they may have opportunistic characteristics such as high reproductive capacity and good dispersal mechanisms, rather than being tolerant to the pollutant which may have made them less suitable. Species need to be present in sufficient numbers for reliable collection.

For ecotoxicological testing, those species which play a key role in the functioning of ecosystems need to be identified. In water bodies these are likely to be the predators, algal grazers and the litter processors as well as decomposers (bacteria and fungi). Most of these are benthic inhabitants.

♦ *Wide distribution*

Test results which are obtained from species with a wide distribution are theoretically applicable to a wide range of ecosystems. However, the wide distribution may be an indication of phenotypic plasticity. Thus, species with restricted distribution abilities could be adversely affected by incorrect tolerance criteria set for their habitats if derived from non-residents.

2.1.3 PROVENANCE

The provenance of parent populations of test animals should be of prime concern and:

♦ *Species identification should be reliable*

In southern Africa this could prove to be a stumbling block as taxonomists are rare and the identification of many taxa is difficult. However, the importance of accurate identification must be of prime concern. In cases where problems are being experienced the necessity of collection parent stock from a limited range is important and the need to establish the identifying features accurately is of prime importance. This information will be of value when the taxonomy is established formally.

♦ *The ecology and physiology of the species should be known.*

Information on temporal and spatial variations in life-history features of aquatic invertebrates is essential in planning laboratory schedules for field collections, for the replenishment of laboratory stocks. The local knowledge base of the variations is rather shallow and narrow; and most indigenous aquatic invertebrates have not been investigated. Guidelines of expected responses may be ascertained from literature if the life histories of related organisms, but this information should always be regarded as indicative of expected emergence patterns and reproductive periods. Once a test organism has been selected, it is important to investigate these aspects of its biology.

Invertebrates may acclimate physiologically or behaviorally to a pollutant but this resistance would not be inherited (Johnson *et al, 1993). Alternatively, they may develop increased resistance by natural selection (genetic adaptation), and Klerks and Weis (1987) found that most populations in polluted areas have some form of increased resistance. Investigations of the same species from related but pristine and altered systems may assist in the identification of vulnerable indicator species.*

♦ *The species should have low genetic and biological variability.* Populations from pristine conditions are ideal stock material as behavioral and biochemical adjustments to foreign influences have not taken place. While the importance of low variability in achieving reproducible test results can be understood, it is when results are extrapolated to the responses of wild populations in field conditions the may be found to be

conservative. Conservative results are appropriate for setting environmentally protective guidelines.

2.1.4 MAINTENANCE AND BREEDING

♦ *Easily held or cultured in laboratory for experimental ecotoxicological procedures.*
Ideally good laboratory culture subjects should reproduce in one medium and not have an aerial phase but this would exclude many aquatic insects, limiting the range of species from the aquatic system to be tested. A parthenogenetic or self fertilising life style would facilitate breeding as complex environmental and behavioural mating cues are then excluded.

Predacious feeding habits are complex to maintain in laboratory situations but omnivores, herbivores or detritivores such as shredders, grazers or filter feeders are generally easier to maintain. Information on feeding biology from the literature can be of assistance in selecting likely candidates.

♦ *Easily sampled.*
If animals are too small or fragile they may disintegrate before their mortalities can be recorded during LC_{50} tests. Sublethal tests on the other hand investigate such processes as osmoregulation, cellular respiration and ventilation, gas transport and haemopeitic systems, and the test species should offer the researchers the option of analysing the affected organs or observing various behavioral responses with reasonable ease. If the species is too small these observation are more difficult to record.

2.1.5 HIERARCHY OF CONSIDERATIONS

What is the hierarchy of considerations in the selection process?
After three years of experience we would rank them as follows;

1. Availability in the field and transportability.
2. Suitable life history and biology for maintenance in laboratories.
3. Classification and relationships.
4. Position and function in ecosystem.
5. Sensitivity to range of test chemicals.

Compromise is inevitable in such a complex and unpredictable procedure. Unless a field population of the candidate species is easily accessible, replenishing stocks for the early laboratory investigation can become prohibitively expensive. Problems associated with life history styles such as aerial phases can be circumvented in many but not all cases, but if a species with very narrow habitat requirements requires highly specific keeping conditions this may make their culture uneconomic. We have come to the conclusion that uncertain taxonomy may not be an insurmountable problem if the identifying features are carefully described. However species complexes from the same habitat must be regarded with caution. The

most important criterion for the first species to be tested is laboratory survival.

2.2 SITES SURVEYED AND SPECIES SCREENED

Introduction

To date, the aquatic species employed in laboratory testing are used mainly due to their ease of culture and well researched life histories. Species used for aquatic toxicology work can be found most commonly among the Pisces and Crustacea, with a few members of the Insecta represented from the order Diptera (Chironomidae). To widen the range of possible test species, communities from riffles and runs of rivers were collected using suitable methods (Chapter 3) and screened for survival under a variety of laboratory conditions. Once this simple screening had been accomplished and some background knowledge had been acquired on the survivorship and responses of the taxa collected, more rigorous and replicated experiments were conducted.

Aim

To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.

2.2.1 SITES SURVEYED

A number of relatively pristine areas were visited to bring back live material from suitable locations for screening; it was presumed that the resident populations would not have become habituated to foreign substances. The following rivers were visited: Buffalo, Nahoon and Kologa Rivers: East London and Stutterheim; Kowie, Berg and Palmiet Rivers: Grahamstown district; Sabie River: Kruger National Park; Kirstenbosch Stream: Cape Town.

2.2.2 AVAILABILITY AND ABUNDANCE OF TAXA

The availability of suitable species from the sites visited is reported in Table 1, Appendix 2. Invertebrate experts country-wide were consulted to gather information and solicit opinions on the suitability and availability of species for use as regional standard taxa. Before compiling a questionnaire several invertebrate experts were interviewed: Prof. Schoonbee (RAU), Mr. Bickerton (CSIR, Stellenbosch) and Dr. Hart (UCN) (culture methods): Dr. King & Prof. Davies and Day (UCT; insect keeping): Prof C. Appleton (UN, Pmb; molluscan culture). The anecdotal information gathered was considered when selecting suitable taxa, and when suitable equipment was designed. The questionnaire was then compiled and circulated to invertebrate workers throughout the country (Results; Table 2.2.2). The questionnaire is appended. (Appendix 2.2).

Table 2.2.2 List of possible suitable candidate species.

GROUP	*SPECIES*	AREA	RESPONDENT
CRUSTACEA	*Paramelita nigricolus* *P. capensis*	W. Cape	Day; Griffiths
	Caridina spp.	Natal	Rayner; Alletson; Hart
	Palaemon capensis	W Cape	Day
	Macrobrachium sp.	E Tvl.	Van Vuren
	Streptochephalus sp.	E Cape	Day
MOLLUSCA	*Bulinus tropicus*	Natal	Schiff; Rayner
	Ferissia sp.	Natal	Schiff; Rayner
	Burnupia sp.	Wide	O'Keeffe
	Lymnaea natalensis	E. Cape	
	Physopsis globosus		
PLANARIA	*Dugesia sp.*		Day
INSECTA Ephemeroptera	*Trichorythus spp.* *Afronurus harrisoni* *Adenoplebia auriculata* *Choroterpes elegans*	Wide	 Alletson; Palmer King
Trichoptera	*Cheumatopsyche afra &* *C. thomasetti*	Wide	Palmer

2.2.3 SCREENING OF SUITABLE TAXA

Introduction

A broad screening programme involves testing suitable maintenance equipment and feed as well as testing the responses of the animals to the provided laboratory conditions, the variation of which must be monitored and recorded. Most riffle dwelling invertebrates are thigmotactic. The substrate are usually the food source as well. As the screening progressed we tested the suitability of various substrates and food supplements as well as the two hydraulic conditions of flowing versus turbulent water (channels or raceways against bubblepots (Appendix 3).

Aims

i. To investigate the survivorship of riverine macro-invertebrates in a range of laboratory conditions in order to ascertain optimal holding conditions.

ii. To ascertain if selected riffle dwellers are obligately rheophylous (require flowing water) and their response to changing levels of temperature and dissolved oxygen (see Chapter 3 appendix 3).

iii. To screen various types of substrates for suitability (see Chapter 3 Appendix 3)

iv. To test the suitability of commercial food as diet of invertebrates as opposed to naturally occurring food from a relatively pristine river.

General methods

Collections were made according to methods described in Chapter 3. During the trials the oval raceways, and a range of bubblepots including 500ml jars were used as experimental containers while plastic netting and foam rubber pads were tested as suitable substrates. Both ground TETRAMIN, and detritus and decayed leaves from the home stream were offered as food. The collected animals were placed in raceways with stones and detritus from the collection site. In bubblepots, stones or plastic mosquito netting or rigid foam-pads were provided as substrate. In Grahamstown the laboratory was air conditioned to a steady 19°C but in the Kruger Park such facilities did not exist.

Faunal complexes from the following rivers were screened:
Buffalo, Kologa, Palmiet (E. Cape) and Sabie (Mpumalanga) rivers.
Detailed experimental reports are in Appendices 2 & 3.

Results

The results in Table 2.2.3 are summarized from six trials and numerous laboratory observations while conducting experiments with other aims. The best survivors among the insecta were Trichoptera (Hydropsychidae, *Cheumatopsyche* sp.) & Ephemeroptera (Trichorythidae, *Thrichorythus* spp. and Leptophlebiidae *Choroterpes & Adenophlebia* spp). Good survival rates were also shown by larval Sphaenidae and Planaria. The cumulative mortalities recorded in the controls of the salinity tolerance experiments conducted in the Kruger Park (Palmer *et al* 1995) support the fact that *Trichorythus* spp and *Choroterpes* spp are both good candidate species for laboratory culture as average mortality seldom

exceeded 10% in raceways. In contrast to the excellent survivorship in raceways, mortalities in excess of 80% were recorded in 500 ml bubblepots with inadequate substrate and temperatures rising (16-28°C) over four hours. Heptageniidae were the worst candidates as they are physically fragile. Freshwater limpets of the genus *Burnupia* showed the best survival rate.

Trials indicated that both raceways and bubblepots provided adequate holding/maintenance containers provided that sufficient oxygen, food and correct substrate is provided. Temperatures should not rise rapidly to above 25°C.

Table 2.2.3 Results of all screening trials summarized.

Poor = <20%; Moderate 20-40%; Good > 40% ; Excellent = 60% survival over 45 days. Container code: BP = bubblepot; RW = raceway; C = channel. Feed code:D = detritus; A = algae; T = tetramin; L = leaves.

ORDER FAMILY	SPECIES	SURVIVAL	TEMP. °C	CONTAINER	FEED
TRICHOPTERA Hydropsychidae	*Cheumatopsyche thomasetti* & *C. afra*	good	19 - 23	RW & BP	D & T
Philopotamidae	*Chimara* sp.	poor	19 - 24	RW & BP	D & T
Leptoceridae	*Leptoceris* sp. & *Oecetis* sp		19 - 24	RW & BP	D & T
EPHEMEROPTERA Leptophlebiidae	*Choroterpes* spp *Adenophlebia auriculata*	good excellent	19 - 24 15 - 25	RW & BP BP+ RW & C	T, D & A T, D, L, A
Baetidae	*Centroptilum*,sp *Afroptilum*,sp *Pseudocloeon*,sp *Beatis* spp.	good (not separately screened)	14 - 23	BP & RW	D & T
Heptageniidae	*Afronurus , sp* *Compsonuriella* spp.	poor	14 - 25	BP	D & T
Trichorythidae	*Trichorythus sp* *Neuroceanis* spp.	excellent (not screened separately)	14 - 24	RW & BP	
Caenidae	*Austroceanis* sp.	moderate	14 - 24	BP	D & T
COLEOPTERA Gyrinidae		poor			
Elmidae		poor			
Dryopidae		poor			
Psephenidae		good	19	RW	A
MOLLUSCA Ancylidae	*Burnupia stenochorias*	excellent	14-25	RW & BP	A
PLANARIA		good	15-23	BP	D & T

Species selected for further investigation.

Once these trials had been concluded the genera that emerged as candidates for further investigation were:

- Trichorythidae and Leptophlebiidae.
    - *Trichorythus.* Although a strong candidate, accessibility is a problem as there are no local stocks. It would be better to perfect the technique of invertebrate aquaculture before investigating this genus. The taxonomy is also unclear.
    - *Choroterpes* A good candidate, reasonably accessible (Buffalo river, eastern Cape) with wide distribution, of good size, easy to feed and fairly robust, but fairly tolerant as it occurs in lower reaches of rivers. Its temperature range possibly higher than taxa from upland rivers. Taxonomy would be easy to establish.
    - *Adenophlebia* This is an excellent candidate, which is easily available, of good size and easy to feed. It is found in numerous upland rivers with good water quality so is quite sensitive and widely distributed. Robust with careful handling. Taxonomy is reliable and there are two species in the genus. However the breeding biology is obscure.
- Baetidae. Suitable for field collection and use in experiments but the identification is difficult and the nymphs are too small.
- Psephenidae. Good candidate but sparsely distributed and nothing is known about the biology other than that adults do not fly to mate.
- Trichoptera have been kept in laboratory by other researchers and the taxonomy good.
    - *Cheumatopsyche sp.* A good candidate on survival ratings but there may be problems with feeding, keeping pupae and the breeding biology is obscure. In this case we also feel that it would be worth investigating once the techniques are established.
- Ancylidae.
    - *Burnupia* An excellent candidate and easy to feed, robust if carefully handled and breeds easily in the laboratory. A better candidate than other gastropoda as it is more susceptible to external conditions due to the exposed foot. It has no aerial stage.
- Planaria. A good candidates and easy to keep as it is robust if rather small. It is widely distributed and always found in riffle collections. The taxonomy may be obscure. There has not been enough time to investigate experimentally. It may be difficult to observe in large experimental streams (Palmer *et al* 1995).

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CHAPTER 3

DEVELOPMENT OF METHODS

Aims:

(i) To investigate and develop methods for collecting live invertebrates from rivers and transporting them from the field to the laboratory and from the breeding/maintenance facility to the testing laboratory of the client.

(iii) To develop practical, versatile receptacles such as pots and channels in which to maintain, breed and move invertebrates at an acceptable cost.

3.1 COLLECTION OF INVERTEBRATES FROM NATURAL WATERS

3.1.1. HANDLING IN THE FIELD

Animals should not be handled during collection. If rocks are rinsed into the containers the least damage is inflicted. Where this is not possible the occupants of movable rocks must be washed into a fine-meshed (10μm) net held immediately downstream from a rock. The rock is lifted rapidly and placed either in the mouth of the net or washed into the collecting bucket. Water and substrate under the rock is then fanned through the net which is emptied into the bucket with extreme care. These nets should be fairly shallow, with a wide mouth. On the bank of the river the collection is then placed into a cooler box with chilled aerated water. Collections can be stored overnight in aerated cooler boxes with foam rubber pads, detritus and leaves from the collection site. The sorting of the undamaged specimens and measuring can then take place the following day. A variable acclimation period (2-5 days) may be necessary before the experimental conditions are instituted. The experimental containers should have a number of specimens excess to that needed to accommodate mortalities. Nevertheless reserves from the remainder of the collection should be kept in similar conditions to those of the experimental containers. The containers should be supplied with detritus and leaves to enable the animals to survive an acclimation period. As soon as the mortality rate stabilizes the animals can be measured and the numbers controlled for the start of the experiment. Animals which are more difficult to remove from rocks can be moved by a small firm paintbrush or the edge of an exposed, washed film. If possible, animals should be collected and transported on the same day.

Sessile limpets are easily damaged during manual removal from rocks. We have found that the technique of anaesthetizing animals in the field by using 2% solution of magnesium sulphate to be effective:

Method

1) Mix 25 litre 2% $MgSO_4$ in a large sturdy plastic bucket using river water.
2) Suspend a slightly smaller net bag in the bucket with a tie around the mouth of the bucket. Quite large rocks can then be completely submerged in the solution.
3) Submerge the sample rock in this solution for a period of 2-5 min. after which the limpets will be comatose.
4) Move the rock and the net bag to a container with river water and wash the animals off the rocks into the bottom of the net.
4) Place the collection in plastic bags filled with well oxygenated river water in insulated containers. The limpets usually recover in 5-10 minutes.

This method was developed by Mrs HI White in the Department of Ichthyology and Fisheries Science (Rhodes) for use in abalone farming. Preliminary investigations have indicated 95% survivorship for specimens collected in this manner. However, long term effects must be investigated experimentally before this could be adopted as a method of choice. A similar effect can be achieved with CO_2 bubbled through

water around the rock, but the logistics of transporting gas cylinders or dry ice have not been investigated.

Plastic bags and sheets can be used to line all the containers in which animals are reared or maintained and will facilitate moving collections without having to handle the specimens too frequently. This procedure has only been used in the last few months and has not been tested rigorously

3.2 TRANSPORT

Introduction

The handling and transport of small aquatic animals in large numbers without damage presents problems which centre around handling, buffeting by the water movement, temperature and oxygen supply. Ideally, cultured animals should be kept in the containers in which they are maintained until they reach the experimental facility, whereupon they have to be transferred with extreme care. Successful transport methods must conform to the following criteria.

Temperature

The temperature should be kept between 8°C and 15°C at all times if possible. A second insulated box with ice taken on collecting trips assists in keeping the collection cool. Animals should be placed in insulated containers immediately after being washed off rocks in the field.

Substrate

Benthic animals require perches but hard objects damage the animals during transport. Finely textured plastic foam appears suitable. Leaf detritus from the collection site also offers suitable perches.

Oxygenation

Oxygen levels should be maintained between 50 and 85% using portable air pumps.

Methods

1) Equip a cooler box with a battery driven aerator pump connected to an air-stone suspended from tubing running through a hole in the lid. Take a portable pump which can run off the cigarette lighter socket in a motor vehicle as backup.
2) For field collecting, commercially available insulated boxes have been modified in the following way. Line the collecting box with a plastic bag of appropriate size and place a thin, fairly rigid layer of foam rubber on the bottom. A deeper layer of foam slightly larger than the internal area of the box is placed on the surface. The bottom layer serves as perch for the animals and the top layer prevents excessive wave action of the water during travel.
3) Small cooler boxes, similarly modified will be useful for the transport of groups of like animals from the culture facility to the testing laboratory.

4) On arrival at the collection site, put river water in the cooler box and add about 250 ml of ice to lower the temperature. The cooler box acts as a store for the animals which are collected into a bucket in the river, and must remain closed. The aerator pump can be started as soon as the animals are added to the cooler box.

5) a) When limpets and snails are collected, a generous layer of leaf detritus from the site, added to the containers, will act as perches as well as facilitating removal from the container, and counting and measuring in the laboratory, without handling the animals.

b) An alternate perch was made for mayflies and stoneflies, consisting of a plastic frame, of the same interior dimension of the container, covered with net and suspended in the container. If water from the collecting buckets is carefully poured through the screen, mayflies find an immediate perch. This method also facilitates sorting.

3.3 MAINTENANCE

Introduction

Frutiger (1984) states that stream-dwelling organisms are physiologically dependent on current and that their behaviour may be seriously affected by being kept in lentic conditions. Consequently, laboratory investigations with running water animals should, in principle, only be carried out under conditions of flow.

Pontash & Cairns (1989) tested the responses of a community of riffle dwellers to four hydraulic conditions. Static and flow through systems with current maintained the largest diversity and numbers of specimens but certain species did well in all conditions. They showed that a microcosm of riffle insect communities can be maintained for up to 30 days with moderate current and minimal flow through with temperature and photoperiod nearly identical to ambient conditions. Adequate quality and quantity of available food proved a limiting factor, and a supplemental source of nutrition may be required during long-term experiments with large numbers. Algae, detritus and decaying organic matter form part of the diet of the macro-benthos. Therefore conditions suitable for the growth and reproduction of algae which form part of the diet of the species to be investigated must be provided (See section 3.3.4.2). Conditions suitable for algal cultivation is discussed in section 3.3.5.2 (Laboratory conditions).

3.3.1 DESCRIPTION OF HOLDING EQUIPMENT

3.3.1.1 Artificial Streams - Raceways

Although all the early trials were conducted in the 'PERSPEX' raceways designed by Ciborowski (1979) (Ch. 2 & 5) these proved unsuitable for long term, uninterrupted use. The channels leaked and the motors failed,

while mobile substrates smaller than 2cm jam the paddle wheel.

3.3.1.2 Artificial Streams - Planted Stream

A small stream in which stream macro- and microphytes were grown on sand, gravel and rocks was constructed in May 1993. This system was modified several times during its existence and tested the feasibility of constructing and maintaining a miniature stream ecosystem indoors. The maintenance of this stream for nearly 2 years demonstrated the importance of the correct light and temperature to the healthy growth of stream plants.

This system consisted of four flow-through pools and a return flow pool. Water was recirculated by submersible aquarium pumps with variable output. The stream substrate consisted of gravel overlaid with sand from the parent stream into which sedges and reeds from the parent stream was planted. These were: Cyperaceae; *Fuirena hirsuta* and *Scirpus proliber* and Juncaceae; *Juncus dregeanus*. Rocks with periphyton and algae from the parent stream were introduced.
Initially the stream was kept in a constant environment room (CER) with a photoperiod of 14 hrs, with an initial temperature regime of 15 °C night and 19 °C day. After a 12 week period during which only artificial light supplied by fluorescent tubes and incandescent globes was available, the plants were etiolated with brown marks on their leaves. Eight Lindner "Linodym" grow lights with additional red spectrum output were installed in the C.E. room which improved the plant condition. This brought the available light in the room up from 12 to 15 micromoles/m^2/sec quanta and from 20.4 to 25.6 PAR (photosynthetic assimilation rate). Pyronometer readings went up from 10.7 to 21.2 micromoles/m^2/sec quanta. The light intensity in the room varied from 13 to 69.0 micromoles/m^2/sec quanta, depending on where the SKYE ELE SKR 100 meter SKR 10 sensor was positioned. According to Mr. B. Sonnenberg of the Botany Department at Rhodes University (pers. comm.) a riverine thicket at midday usually has between 50 to 70 micromoles/m^2/sec quanta. Periphyton and algae cultivated on rocks and artificial substrate thrive under these conditions.

This stream was moved to a west facing laboratory with natural light where it functioned very well.
but was dismantled due to space constraints in March 1995.

3.3.1.3 Artificial Streams - Recirculating Channels

Recirculating systems (Fig 3.3.2.1) consist of a plastic or PVC channel through which water is pumped from a sump by submersible aquarium pumps via a horizontal spreader. Ovoid kaolin stone and small square tiles are placed in the channels for substrates. The sumps are plastic bins varying in size from 5 to 85 litre (Figure 3.3.2.1 a & b). Two scales of channels were constructed. The larger channels consist of 250 mm heavy duty PVC piping which are halved lengthwise and stoppered at both ends. These rest on metal adjustable stands. The smaller channels are 0.5 m lengths of square profile white guttering (Marley) stoppered at both ends.

A variety of makes of submersible aquarium pumps have been tested (Natura, Idra, Aquaclear and Reno). The head required and the pump setting will vary the output, but their range is between 500 and 1000 litres/hour^2. Flow rate can be varied by changing the slope of each channel, by altering the delivery rate of the pumps or by a control valve set into the pipe from the sump.

The small channels with individual 5 litre buckets as sumps are used as replicated experimental systems. The large channels are holding and growing systems for experimental animals and the cultivation of algae and periphyton. These systems are housed in a laboratory with natural light conditions as well as daylight tubes. During the course of the project the experimental systems have been housed in three different localities of which a north west facing laboratory with large windows provided the best growing conditions for algae.

Flow through pots

Individual mayflies sometimes had to be isolated in flowing water for various tests and containers which allowed water to flow through them but kept the mayfly isolated were constructed. The flow-through pots were made from transparent plastic 500ml bottles (similar to those used for the small bubblepots) with mesh windows inserted on opposing sides. Three mesh sizes (2.0mm, 1.5mm & 1.0mm) were chosen to confine animals of different dimensions. The mesh size must be considered when the animals to be placed the pot is selected other wise they escape. In situations where the water has a high suspension load the mesh can clog up very quickly.

Problems encountered with these systems to date include;

a) difficulty in the maintenance while in use.

b) no easy method to allow trapping of adult insects on emergence.

3.3.1.4 Static Water - Bubblepots

Bubblepots are cylindrical opaque plastic buckets with straight or sloping sides and lids, equipped with aerators. A variety of sizes of containers were tested but the most often 500ml bottles, 5 litre buckets and 50cm diameter plastic basins were used. These containers are used in a variety of experiments and the larger containers proved ideal for raising limpet hatchlings and maintaining leptophlebiid mayflies (Fig 3.3.1.1 a).

A small number of cylindrical containers were equipped with a central spiral baffle which created a directed current. These SPIRAL POTS were labour intensive to make and materials to manufacture the baffles were difficult to obtain locally. They were also difficult to clean and maintain while only a small number of animals could be kept in them. They were discarded as an impractical option.

3.3.2 SUBSTRATE PROVISION

Clifford *et al* (1989) tested the substrate preference of benthic macro-invertebrates in natural streams, between rough and smooth tiles as a place on which to perch. It was found that rough tiles were generally preferred to smooth tiles particularly by chironomids. However, Heptageniidae (mayfly) nymphs were found in consistently higher numbers on smooth tiles. This highlights the fact that not only are substrates essential but that they should be of the right quality.

Ovoid kaolin stones (6cm x 4cm x 4cm) have been manufactured to standardize the available food surface and to provide refugia (Fig 3.3.1.1 c). In addition, periphyton is cultivated on 8cm x 8cm unglazed tiles. These provide a more accurately measurable surface of available food, can be used in shallow water, and are practical for monitoring the growth of small invertebrates as they afford a uniformly flat surface for microscopic observation and measurement. Both these types of substrate are pale in colour to facilitate observation of the organisms.

3.3.3 FOOD PROVISION

3.3.3.1 Introduction

The provision of the correct feed is of paramount importance in establishing successful culture systems. Particle size, composition and presentation are all important factors to be considered. Benthic macro-invertebrates have been classified into several functional feeding groups, according to the methods in which food is assimilated (Cummins & Klug 1979). Each of these groups consume a specific type of food in a specific way and the presentation of the food can be as important as the content.

It has been amply illustrated that food quality can contribute significantly to growth rate and therefore to duration of life-cycle, size at maturity and fecundity (Anderson & Cummins 1979; Sweeney *et al* 1986). In the laboratory optimal growth in the shortest period for rearing and reproductive purposes is desirable, while growth retardation, once the correct size for testing purposes has been reached, to maintain adequate stock, is essential. Therefore feeding on optimal diet and the development of this diet often forms the largest portion of aquaculture research project. However the scope of this investigation does not allow for such elaborate investigation and it was therefore decided to test commercially formulated fish food for suitability. Tetramin is such a food and readily available and has been used by other workers to supplement the natural diet of insects. On the other hand it was felt that the cultivation of periphyton as a suitable general feed should not present too many difficulties, so we embarked on a trial and error periphyton growing project.

Food Preferences

Food generally is accepted as a major component of a population's environment and when a population reaches epidemic proportions, food shortages are likely to occur, particularly within our laboratory streams where there may be maximum densities and the problem may be one of quality and not quantity.

Limpets

Little is known about the ability of freshwater pulmonates to select from within the spectrum of algal foods available to them. Work by Calow (1973 b) shows very clearly that *Ancylus fluviatilis*, a close relative of *Burnupia*, which has the same method of rasping action in order to acquire its food, actively seeks out algae in preference to, although also consuming, lichen, detritus and fungi. Calow maintains that in the absence of epilithic algae, lichen may be used but it results in less rapid growth and must be considered as a less rich energy source. In 1973(a) he showed that the preferred algae were the diatoms. *Burnupia* is very likely a micro-herbivore grazer with the same preferences. Small sand grains (mineral particles) are potentially important as they allow greater trituration of food (Hunter 1980), of particular importance in the digestion of diatoms which Calow (1970) suggests are less efficiently assimilated than green filamentous algae due to their high ash content and protective exoskeleton. Eisenberg (1970) showed very clearly with *Lymnaea elodes* that the inverse relationships between snail density and mean size, mean number of eggs per egg mass, as well as total eggs were found to disappear with food additives. Food additives appear to also have improved the survivorship of the snails, and he presents evidence which indicates that the food limitation in this snail is one which probably involved accessory growth factors. In the case of *Burnupia* we have to ask the question - is the algal assemblage provided in the streams sufficient for maximum growth and fecundity?

Mayflies

Cummins & Klug (1979) found that animals that belong to a certain functional feeding group may be unable to digest food which does not form part of their preferred diet. For example, shredders lack an enzyme such as cellulase to digest complete polymers such as cellulose but the semi-digested or hydrolysed polymer may well be digestible. So conditioned or semi-composed leaves with a high microbial fauna are consumed faster than leaves less colonised and faster growth may result. Rosillon (1988) showed experimentally that growth responses of *Ephemerella ignita* to water temperature variations differed according to the content of the food available for that species. Work on the growth rates of the mayfly *Leptophlebia intermedia* on leaves from different trees has shown that the diet can influence adult size and fecundity but not growth rate (Sweeney *et al* 1986). It is therefore important that the diet offered should contain the right proportions of protein, carbohydrate and lipids in a digestible form but also of the correct physical formulation.

3.3.3.2 Periphyton

The multi-specific complex of diatoms, filamentous algae and bacteria which develops on the surface of stones probably forms the most generally acceptable food source for a range of functional feeding groups. It was decided to cultivate periphyton on artificial stone substrates in the channels as a basic food source to be supplemented for the specific needs of macroinvertebrates.

Three aspects concerning the growth of algae (and hence the basic source of food) in the laboratory streams should be considered:

(1) What species of algae do the limpets or mayflies prefer and which will increase growth to a maximum rate.

(2) What species of algae grow in the laboratory streams; how the chemical conditions of the water, the physical form of the substrates, and the light conditions all affect the growth.

(3) What is the interaction between the limpets and the algae, particularly the effect that the grazing action has on algal species composition and water conditions.

Laboratory Conditions

Periphyton distribution in streams is often a mosaic of assemblages on different successional trajectories with the degree of patchiness related to spatial and temporal variation in the environment (Fisher, 1983). Succession within a patch is bounded by the composition of the species pool and the physiological characteristics of the species, and it is influenced by the interaction of factors such as invasion rate, nutrients, light, temperature, current, substrate and herbivory (DeNicola and McIntire 1990). A review of the literature highlighted the complexity of the interaction between environmental variables such as current speed, light intensity, substrate quality and algal species in the colonisation of substrates.

(a) Lamberti *et al* (1989) studied the interactions between algal assemblages and herbivorous snails (*Juga silicula*) and found that a light intensity of 100μmol/m^2/s is ideal for laboratory stream algal growth and 250 snails/m biomass equivalent. Diatoms remain dominant in the system and grazing is sufficient to "control" filamentous and blue-green algae. In their experiment, this light intensity is not high enough (compared to 400μmol/m^2/s) to produce rapid algal growth, causing diatoms to decrease in number. They found that at intensities of less than 100μmol/m^2/s the grazing impact was too great for algal growth to be sustained. Lamberti *et al* (1989) also found that high light intensity gave high algal growth and consequent sloughing of algae to form detritus. This would contribute to changes in the chemical nature of the water as these algae break down, possibly to the detriment of the stream system. Water should then be changed regularly for this reason alone. It appears then that the light intensities recorded in the CER and the laboratory which ranged between 13 and 69μmol/m^2/s are inadequate for good growth.

In a Leptophlebiidae experiment conducted in our laboratory, it was found insufficient change of water caused the rapid build-up of sewage fungus, an indicator of high organic nutrients, which in turn rapidly increased in volume and presumably consumed large amounts of the dissolved oxygen.

(b) DeNicola and McIntire (1990) examined the effect of water flow on the algal assemblage in re-circulating laboratory streams, comparing the colonization of algae on both stones and unglazed clay tiles. At flow rates of 2 litres/min periphyton accumulation was greater on recessed regions behind substrates than on the tops. The higher water velocities above the substrates increased the rate at which algal cells flowed over them, but the relatively unidirectional flow and higher shear velocities

may have inhibited cell attachment. In contrast, the zone behind the substrates had multidirectional flow with lower colonization of algal cells (Munteanu and Maly 1981, Stevenson 1983). McIntire (1966), and Steinman and McIntire (1986) both observed a higher accumulation of biomass in faster currents (38cm/s) than in slower (9cm/s) currents. Studies which show that current flow enhances nutrient uptake, respiration, photosynthesis and growth in algae (Whitford, 1960) support the hypothesis that biomass accumulation should be higher in faster current regimes after initial establishment. Results by DeNicola and McIntire (1990) also showed that assemblages in the different current regimes became more similar over time. The major difference in the species was in the greater abundance of *Stigeoclonium tenue*, a member of the Chlorophyceae (green filamentous) family, on recessed substrates towards the end of the experiment. This genus appears to be dominant in our own laboratory streams (Ms. Josca, Botany Dept, University of Cape Town, 1994).

McIntire (1966) found green filamentous algae increased biomass much faster in slow currents (2 l/min). Faster currents showed a more felt-like dense growth on the substrate, dark green or brownish in colour, with a predominance of diatoms. Whitford and Schumaker (1964) (in McIntire 1966) demonstrated that lotic and lentic species of *Oedogonium* and *Spirogyra* have higher rates of respiration and phosphorus uptake when subjected to a current velocity of 15cm/s than in still water, which led to a greater growth rate.

Steinman and McIntire (1986), in comparing both light (450 and 50$\mu E/m^2/s$ and current velocity (5cm/s and 15cm/s) in laboratory streams, found the seven most commonly found diatoms exhibited different patterns of response to the experimental conditions: *Synedra ulna* and *Fragilaria vaucheriae* had relatively high mean biomasses at the higher light level and lower current velocity; *S. rumpens* var. *familiaris* and *Nitzschia oregona* had greater mean biomasses at both the hight light level and higher current velocity; the mean biomasses of *Nitzschia dissipata* had a negative relationship with light energy and a positive relationship with current velocity; *Nitzschia linearis* had greater biomasses at the lower current velocity while *Achanthes lanceolata* had lower mean biomasses at the lower current velocity. At the end of the experiment (32 days), *Stigeoclonium tenue* formed an extensive reticulation on tiles from the higher photon flux density. In contrast, algal assemblages from the lower photon flux density retained a dense understorey of *Achanthes lanceolata* and an upper layer of larger diatoms. However several species of *Nitzchia* were indifferent to treatment effects, suggesting other factors were important in influencing structure.

Decisions as to how best to provide food for the test species on the basis of light incidence and current velocity, are therefore almost impossible until further studies on both the exact food requirements and the responses of diatoms which can be identified from the species habitat have been completed.

(c) The following literature was helpful in making the decision as to whether clays tiles or artificial stones would be more suitable for the growth of *Burnupia:* Lamberti and Resh (1985) compared introduced tiles and natural substrates for sampling algae and found least variability between tiles.

DeNicola and McIntire (1990) showed the differences between tiles and stones which affected in their colonization by, and the subsequent growth of algae to be the degree of roughness. Colonization began with small diatoms, then the large diatoms, blue-greens, ciliates, and finally green filamentous, with the slower currents encouraging green filamentous on the stones in particular. This latter work clearly indicates that tiles are a more suitable substrate for algal growth for the limpets than artificial stones.

Tuchman and Stevenson (1980), when comparing diatoms communities on clay tile, sterilized rock and natural substrate in a small stream, found clay tiles yielded the least variability between replicate samples. For trial purposes with *Burnupia* this is the ideal means of providing food.

The application of the above mentioned background information, (sections (b) and (c)) to the maintenance and breeding of suitable test species must be investigated experimentally.

In the initial phase of the project the algal colonisation within the recirculating systems was initiated by stones and water brought in from the field collecting sites. A few days elapsed before significant colonisation of the clean artificial stones occurred. The substrates are maintained in conditioned tap water in the recirculating systems.

Initially nutrient enrichment in the form of the mixture given in **section 3.3.6** was used to encourage algal growth to ensure the algae would not be a limiting factor in any of the experiments. However, we later found investigations by Marks and Lowe (1989) confirmed our observations that nutrient enrichment, particularly nitrogen and phosphorus, causes a shift in the algal composition from diatoms to less useable filamentous green algae over a 28 day period. Without any herbivorous activity in the periphyton cultivation streams, experience has shown it necessary to continually scrub the substrates to decrease the amount of filamentous algae.

When used as a food source for *Adenophlebia* and in the initial growth of *Burnupia* the periphyton stones are placed in bubblepots. Aeration provides a high percentage of dissolved oxygen, required by these species and presumably sufficient for the algae. In later stages of growth we envisage the limpets will be transferred to the laboratory streams where they will complete their development, and from where many will be transferred to the ecotoxicological work. Future work on the algal assemblages will be necessary, monitoring the species present through each of these above stages.

Effects of Grazing on the Algae and Water Conditions

DeNicola *et al* (1990) compared substrates grazed by the snail *Juga silicula* with ungrazed substrates ($500/m^2$) in laboratory streams, found in ungrazed streams after, 40 days, the algal assemblage consisted of a thick mat of diatoms and the chlorophyte *Scenedesmus obliquus*, with an overstorey of filaments of the chlorophyte *Stigeoclonium tenue*. In general, introductions of grazers at any stage altered this pattern by

removing biomass, accelerating the replacement of *S. obliquus* by diatoms, and suppressing the growth of filaments. Grazing also reduced the relative abundance of the larger diatom *Nitzschia oregona* but increased the relative abundance of the smaller adnate diatoms *Nitzschia frustulum* var. *perpusilla* and *Navicula minimum.* These results give and indication of the feeding preferences of the snail.

Hunter (1980) evaluated the effects of *Lymnaea, Mysa,* and *Helisoma* on periphyton on tiles in a pond, and found these species were essentially non-selective within their "normal" range of food dimensions (medium to large size diatoms) with massive reductions in living cells, detritus and particulate inorganic matter alike. This had the effect of reducing the quantity of periphyton by reduced dry weight, carbon and chlorophyll *a* per dm^3. In contrast, grazing increased aufwuchs quality as indicated by increased chlorophyll *a* and nitrogen per mg dry weight and by decreased C:N ratio. Grazing at this density (216 snails/m^2) reduced both the abundance and diversity of the attached community from 80 889 individuals/cm^2 and 24 taxa on the controls to 501 individuals/cm^2 and 8 taxa on grazed substrates.

Marks and Lowe (1989), in their work on the interactive effects of snail grazing and nutrient enrichment on periphyton communities, found that nutrient enrichment had a much greater effect on altering community structures than did grazing. Although the snail ratio per unit area was smaller than we would expect to see with *Burnupia,* grazers had little effect on diversity and relative abundance of major classes in low-nutrient communities, although they did alter the species composition. In nutrient-rich communities grazing decreased diversity and caused a shift in relative abundance of the major classes, and encourages the presence of *Stigeoclonium tenue* a filamentous algae and therefor not a food item of choice for grazing snails.

The chemical conditioning of the water by the limpets may affect the growth of the periphyton: they remove ions, amino acids, and oxygen and add ammonia, carbon dioxide, ions, mucopolysaccharides and organic acids. Their grazing action is particularly important in causing plant factors to be released into the medium, organic acids in particular which will acidify the water medium. Thomas, Goldsworthy and Benjamin (1974) showed this very clearly with *Biomphalaria glabrata.*

Ms.P. Josca (University of Cape Town, 1994) confirmed the extreme diversity of the periphyton which had been cultivated in our laboratory streams, including numerous species of pennate diatoms, desmids, cyanophyte and unicellular and filamentous Chlorophytes.

3.3.3.3. Feed Other Than Periphyton.

Choroterpes elegans and *Adenophlebia auriculata* are both classed as brusher collectors (Palmer *et al,* 1993) and therefore have fairly omnivorous feeding requirements. In the field these nymphs are most frequently found under rocks where fine sediment collects and in large leafpacks. It was therefore decided to test ground Tetramin as a suitable food source and offer this in addition to periphyton as an alternative to detritus. When nymphs are kept in large bubblepots, leaf packs are always brought back from the field as a supplement to periphyton. Containers of autumn leaves matured in water for 2-3 weeks have been

documented as suitable feed for a number of mayfly species (Sweeney *et al* 1986). See (Ch.5.2.4) for results.

3.3.4 WATER COMPOSITION AND HYGIENE

Although water from the river of origin of the animals to be investigated is the logical medium for use in transferring the populations from the field to the laboratory and for maintaining populations and conducting experiments, transporting large volumes of water on a regular basis presents problems. When considering a laboratory source of water, we have found tap water allowed better algal growth than distilled water. Total use of dechlorinated tap water, while readily available, may not be equally suitable for non-filamentous algal growth which is the food source in the majority of experiments. In other centres, micro-elements have been added to water to obtain better results in the cultivation of plants and animals. The addition of nitrogen and phosphorus to agar plates dispersed in New Zealand streams substantially increased the algal growth taking place on them (Winterbourn, 1990).

The two sets of chemical additives listed below are considered good nutrient for algal cultivation (pers. com. M. Logie; Microbiology Dept., Rhodes University). Those chemicals listed on the right are made up into a one litre stock solution:

NaHCO	4,20 g/litre	FeCl	0,2mg/l
KNO	0,51 g/l	EDTA	1,8mg/l
MgSO	1,23 g/l	H BO	14,0mg/l
CaCl	0,044 g/l	MgCl	0,8mg/l
KH PO	0,027 g/l	ZnCl	0.1mg/l
		CoCl	0.002mg/l
		CaCl	0.02mg/l

The stock solution was used in our laboratory streams at a rate of 1ml per litre of water, and all other chemicals were used at a dilution of 15% of the volume of tap water. However, both the expense of the nutrients, and the density and type of algae (filamentous Chlorophytes) which grew the most rapidly necessitated discontinuing their use.

Stein (1973) suggests water should be changed every two to three weeks when used to culture both algae and limpets because:

(a) CO_2 is depleted by the algae.

(b) the submersible pumps produce ozone, lethal to the algae.

She reported that the use of black, white, and clear polyethylene material is safe for the culturing of freshwater algae, with no significant leaching of elements into the water.

Water was monitored daily or weekly in the laboratory streams by electronic CIBA CORNING probes which measure temperature, Ph, conductivity and dissolved oxygen (DO). Nutrient analysis has to date not been done on a regular basis as the expertise to do the analyses has not been developed, although the facilities are available.

The necessary chemical composition and balance of water for the growth of algae and invertebrates need to be investigated further by consultation with Prof. Braam Pieterse (Potchefstroom Univeristeit) and some experimentation.

3.3.5 DENSITY

Chernin and Michelson (1957 a & b) studied the effects of population density on *Australorbis (Planorbis) glabratus*, and found that snails maintained under crowded conditions grew more slowly and were less fecund than those in less densely populated aquaria. Wright (1960) showed that there is no single factor which can be held responsible for diminished performance under high densities and suggested three factors may be involved: (1) food, (2) collisions, and (3) chemical pollution. DeNicola et al (1990) demonstrated that grazing by stream invertebrates has an impact on both periphytic species composition and abundance, especially in laboratory streams.

In developing management strategies the effect of density in aqualcultural situations is of great economic interest. Density effects on limpets were investigated and the results are reported in Chapter 4.2.2.2

3.3.6 REQUIREMENTS FOR BREEDING

The art of breeding animals in captivity rests on providing the correct cues in terms of photoperiod and temperature as well as understanding the mating behaviour. Unless the laboratory animals are reared with optimal nutrition, fecundity will be lowered. Care must also be taken to maintain a broadly based gene pool with the regular importation of wild parent stock (De Kock and van Eeden 1981). To date the breeding behaviour of the limpet has been observed (Chapter 4.2.3). The effects of programmed circadian temperature fluctuations on the population dynamics of Burnupia will have to be considered (Appleton *(et al)* De Kock and van Eeden (1986) found that egg production, hatching rate, survival and growth rates were all positively influenced by daily temperature fluctuations. Photoperiod will also have to be considered in its influence on the limpets. The causes and methods to prevent the high mortalities must be investigated. Periphyton seems to provide adequate nutrition but species composition will be investigated to a greater degree.

The successful captive breeding of mayflies has eluded all workers. It was decided to resort to the time honoured piscicultural practice of artificial fertilisation with which we have had limited success. The technique has not been perfected and will need further investigation. Several authors have reported similar experiences (Chapter 5.4). With regard to mayfly hatchlings, sufficient numbers of new hatchlings have never

been available for experimental investigation but the smallest (0.2 -.4 mm head-width) field caught nymphs have all survived well on a diet of decayed leaves and ground Tetramin added to periphyton (Ch.5.2.).

3.3.7 EXPERIMENTAL INVESTIGATION OF EQUIPMENT

(see Appendix 3 for experimental detail)

Five experiments were conducted to determine conditions within the containers and to explore optimal maintenance conditions. The experimental results are reported here under the numbers assigned in Appendix 3.

a) EXPERIMENT 3.1.1.1: One air-stone produces the same level of dissolved oxygen to all volumes of water while temperature and dissolved oxygen are negatively correlated.

b) EXPERIMENT 3.1.1.2: When testing the effect of rising temperature on the survival rate of Choroterpes sp. (Leptophlebiidae) in different volumes of water, it was found that rising temperature depressed levels of dissolved oxygen and mortalities occurred when the DO reached 30 %, after the temperature had peaked at 25°C from 11 hours. In 500ml of water, mortality onset was delayed until 32 hours at which stage in all the other conditions 80 % mortality had occurred.

c) SUBSTRATES; EXPERIMENTS 3.1.2.1 & 2: A variety of materials were tested for suitability as substrates for mayflies. Foamrubber pads, kaolin artificial stones and plastic mosquito netting were tested on *Choroterpes spp.* The survival rate with foam-pads and stones was consistently higher than that with netting, which was as poor as that in the controls where no substrate was supplied. This was true for both running and standing water. Each time a container was inspected all the live specimens were found under the substrate, suggesting that a refuge from light and turbulence is needed. Observations from other experiments, (not conducted to test substrates) confirmed this finding (Ch. 5.2.3).

The method in which the animals were transported from the Kruger National Park ensured survival in excess of a month in the experimental conditions tested. Expected residence time of nymphs in the laboratory can be extrapolated from these results.

d) HYDRAULIC CONDITIONS Although the species selected for experimental investigation are all found in riffles and runs in rivers, they may not be obligate rheophyllic. Conclusions from five of the experiments which were conducted to determine the responses of the two selected species to variation in hydraulic conditions are reported here.

EXPERIMENT 3.1.3.1. The growth rates of *B. stenochorias* in flowing and bubbled water was compared using dechlorinated tap water. In the experimental channels some unknown factor caused early mortality of the limpets and no results for comparison with growth in the pots were available.

EXPERIMENT 3.1.3.2, A & B: Two similar experiment was conducted on *Adenophlebia auriculata* nymphs. The condition in the pots and the channels are not only hydraulic different, resulting in diffrences in the shear forces exerted on the nymphs (excacerbated by insufficient refugia) but the temperature regime in the pots is 2-3 °C lower than in the channels due to evaporative cooling.

3.1.3.2.A: In the first experiment three kaolin stones were provided in both the channels and the bubblepots. The follwing differences were observed;
a) adult emerged sooner earlier in the relatively warmer channels.
b) a rapid decline in numbers in the channels between days 15 and 26 due to both mortalities and emergences. There was no significant difference between the replicates in either the channels or the pots (ANOVA F ratio 0.097, $p>0.05$ for channels and F ratio 1,29 $p=0.2884$) for pots) although the variation in the survival rates in the pots is greater. It is quite clear from Fig 3.1.3.2 that the variation in the sample number in bubblepots is much larger that from those in the channels which could be ascribed to high survival rate (58% and 35%) in two pots.
c) there was a significant difference in the survival rates between channels and bubblepots (ANOVA f ratio 6.7 and $p < 0.05$.) and a multiple range test confirmed the difference.

3.1.3.2.B: In the second experiment the channels contained 6 substrates were tested against 5 litre bubblepots with the same number of substrates. The survival period recorded in this experiment was much higher than in A. Firstly the nymphs survived for at least 7 week in both conditions but longer in bubblepots. Of the 68 nymphs which were placed in each set of replicates at the start of the experiment, 54% died in bubblepots and 69% in channels. The early decline of numbers in the channels was due to both the emergence of adults and mortalities. In Fig. 3.1.1.5 (Appendix 3) altogether 20% of the nymphs emerged from the channels and 18% from the pots. The difference in the survival rates between channels and bubblepots was significant (f ratio 6.7 and $p < 0.05$.).

A. auriculata did not survive well in channels with three stones. A mean of 33% and 32% of the original sample survived three weeks in the CER and laboratory respectively. The low survival rate of nymphs in channels in the laboratory could be due to two reasons. The flow rate in the system may be too rapid or there may have been too few stones in the channels. In the Palmiet River the nymphs favour the rocky edges of the stream out of direct current where coarse grained sediment collects under rocks as substratum i.e. that they inhabit low flow areas out of direct current while few smaller nymphs are found in moderate currents. The smooth plastic of the channels and angle caused a fairly strong current to flowed over a few smooth rocks which most probably did not offer sufficient refuge or foothold for the nymphs. This may have caused large energy expenditure to keep positions.

It can be concluded from the above two trials (APPENDIX 3: 3.1.1.4 A & B) that bubblepots are the optimal rearing container for *A. auriculata* and that they are not obligately rheophilous.

e) DIET EXPERIMENT 3.1.1.5: The suitability of commercial fish food as diet for *A. auriculata* was tested by monitoring survival over a 12 week period. Suspensions of ground TETRAMIN powder and river detritus was offered as food. The survival rate of *A. auriculata* was the best on Tetramin when 50% survived for five weeks, 30% survived for six weeks and 10% of the population survived for eight weeks. Detritus on the other hand sustained 50% of the population for only 2-3 weeks with a more rapid decline in numbers thereafter and all perished after 8-9 weeks. However again it must be pointed out that some losses was due to emergences.

3.3.8 SUMMARY

In conclusion, the three species investigated so far can be adequately housed and fed prior to being made available for experimental purposes. The fact that neither species was obligately rheophilic makes the prospect of rearing small animals to a size suitable for experimental purposes much less intimidating. Bubblepots are easy and cheap to set up, and more easily managed in terms of water quality and food provision. If the pot is lined with a plastic bag before limpet eggs are introduced, it will be easier to move these to channels once they have reached a size larger than 1.5 mm.

Although several groups of limpets and mayflies has been fed on the periphyton cultivated in the laboratory, it is not yet certain that we have achieved optimal growth under experimental conditions. Therefor indications drawn from a literature review that:

a) Light intensity of 100μmol/m^2/s is ideal for laboratory stream algal growth, and at less than 100μmol/m^2/s the grazing impact was too great for algal growth be sustained and
b) Current speed can significantly influence both the species and the rate at which diatoms colonise substrates, will have to be investigated once the dietary preference of *Burnupia* is determined.

Chemical conditioning of the water by the limpets may affect the growth of the periphyton: they remove ions, amino acids, and oxygen and add ammonia, carbon dioxide, ions, mucopolysaccharides and organic acids. Their grazing action is particularly important in causing plant factors to be released into the medium, organic acids in particular which will acidify the water medium. Thomas, Goldsworthy and Benjamin (1974) showed this very clearly with *Biomphalaria glabrata.*

The importance of the correct conditions in which to house a facility in which natural food can be grown was demonstrated to us by the variety of laboratories in which conditions varied considerably which we have had to occupy during the project period. That in itself proved an test of certain type. In our experience to date

the most important conditions for the successful maintenance and breeding of invertebrates are:

1. Correct light conditions for algal growth.
2. A method to exercise thermal regulation.
3. A supply of good quality water, preferably from a non municipal source with the ability to regulate its quality and condition.
4. A manager who is meticulous, aware of the necessity of hygiene yet sensitive to the needs of invertebrates.

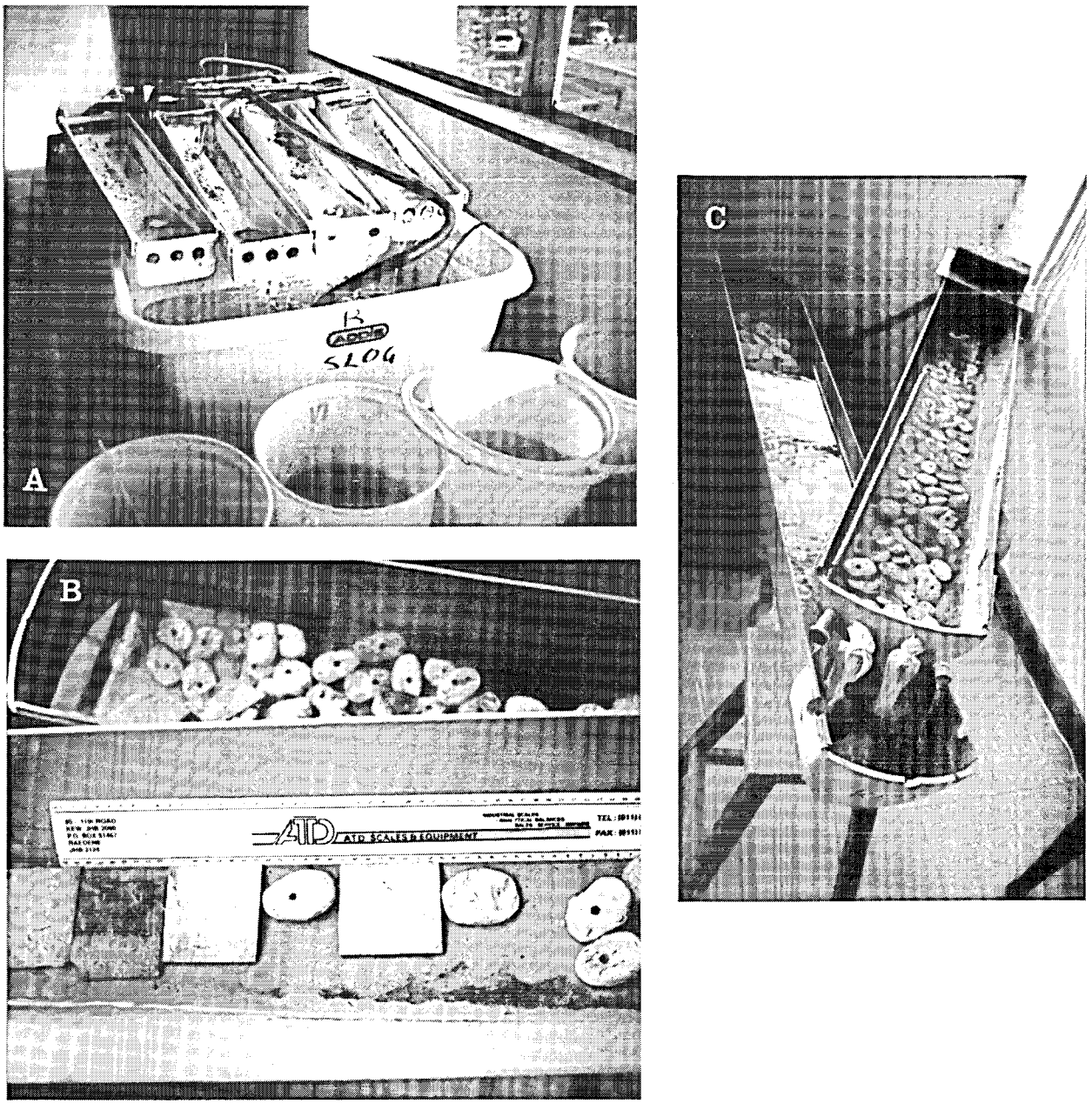

Fig. 3.3.1.1. a) Bubblepots and small recirculating streams. b) Artificial kaolin stones and unglazed tiles for perches and the cultivation of periphyton. c) Large recirculating stream with artificial stones.

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CHAPTER 4

INVESTIGATIONS OF THE LIMPET *BURNUPIA STENOCHORIAS* (MELVILL & PONSONBY).

CONTENTS

4.1 BACKGROUND TO *BURNUPIA STENOCHORIAS* (Pulmonata; Basommatophora)

The freshwater limpet *Burnupia stenochorias* proved to be an excellent survivor in the initial trials to screen riffle and run inhabitants for laboratory culture. It was therefore decided the life history of this species should be investigated and in doing so, attempt to establish optimal conditions for its laboratory culture.

The fresh water pulmonates make up a large proportion of the benthic animal biomass of the margins of larger lakes and rivers, but are particularly well adapted and successful in smaller, more variable aquatic habitats such as ponds, marshes, streams and ditches (Russell-Hunter, 1978). The Ancylidae are worldwide in distribution, comprising 7 genera (Hubendick, 1964), three of which occur in Africa; *Ferrissia* which is cosmopolitan, *Ancylus* with a mainly palaearctic range and *Burnupia* which is restricted to Africa (Brown, 1980). The genus *Burnupia* is found mainly in rivers but also on vegetation in pools and lakes. There are nine nominal species in southern Africa, and Brown (1967,1980) gives some indication of the distribution patterns, and the general systematic state of the genus. A revision of the genus (states Brown 1967) would reveal cases of synonymy among the large number of nominal forms recognised by Walker (1923) and Connolly (1939), and consequently one has to proceed with caution in establishing the identity of any population being investigated.

These limpets seem to have an especially high requirement for oxygen, as they are almost entirely confined to small, stony streams and the shores of lakes which are exposed to considerable wave-action (Brown 1980). Although easily overlooked, members of the Ancylidae are present in most freshwater habitats, those with clean, well oxygenated water being favourable for species of *Burnupia*, while the members of *Ferrissia* are characteristic of stagnant waters (Brown, 1980).

The ancylids have shells analogous to those found in the Capulidae, in that the shell is not conical but is slightly coiled, and there is no metamorphosis of the shell in growth (Russell-Hunter, 1953). The shell of *Burnupia* can reach lengths of up to 10mm, with the apex usually prominent, turned to the right and with rows of radial pits (Brown, 1980). The edge of the shell consists of relatively flexible uncalcified material which enables it to conform to the irregularities of the surfaces to which these limpets attach themselves. Unlike *Patella* and other marine littoral species, there is no "homing" in freshwater limpets and consequently, instead of the rock surface at one particular place becoming shaped to fit the limpet's shell, the shell must be flexible enough to become shaped to the rock surface of many different resting-places (Russell-Hunter, 1953).

The ancylid fresh water limpets represent the most advanced fresh water basommatophorans. They have lost nearly all traces of the pulmonary cavity and, to an even greater degree than the closely related

Planorbidae, have developed well vascularized, extensive neomorphic gills from evaginations of rectal mantle lobes between the foot and the roof of the mantle on the left side of the body. These gills provide an extensive surface area for aquatic gas exchange, allowing the respiration of fresh water limpets to be entirely cutaneous and therefore totally aquatic (Basch, 1963; Hubendick, 1964, 1970; Purchon, 1977; Russell-Hunter, 1978).

In the southern African sub-continent no investigations into the biology of the Ancylidae have been conducted, with one exception: Oberholzer (1963) reported the morphology and histology of *Burnupia mooiensis*. The closest relatives of the *Burnupia* spp which have been extensively investigated are within the genus *Ancylus*, in particular *Ancylus fluviatilis* which occurs widely in Europe and North Africa in lotic freshwater environments. It appears to have many similarities of life history style to *B. stenochorias*. Eggs are laid in capsules of a similar design and in similar numbers (1-13). Roughly 10 to 20 egg capsules are deposited by each individual over the entire life span. The shell sizes attained by populations fall in similar ranges to those measured to date for *B. stenochorias*. Hatched size is between 0.80-1.00 mm shell length with average adult size not exceeding 6.00-8.00 mm. Eggs are laid at night and under stones as has been observed in *B. stenochorias* (Hubendick, 1970; Russell Hunter, 1953; 1961a & 1961b; Geldiay, 1956; Lamberet, 1966). *Ancylus fluviatilis* feeds on periphyton, mostly diatoms and green algae, on smooth stone and rock surfaces. Locomotion takes place on top of the stones and resting periods underneath (Schwenk & Schwoerbel, 1973; Streit, 1981).

Ecology

Although *B. stenochorias* is an inhabitant of streams and is found largely in riffles and runs it has been collected in some pool habitats where very slow flow has been recorded. Appleton (1978) discussed the range of abiotic factors which may limit the distribution of Planorbid snails and states that while qualitatively certain abiotic factors may not be limiting to distribution, quantitatively they may well have some limiting influence. Of these, temperature and current velocity seem to have the largest influence while ionic composition does not seem to have a limiting effect on distribution but rather on levels of abundance.

Current velocity has a marked effect on both *Bulinus* sp. and *Biomphalaria pfeifferei* in that they seldom occur in velocities exceeding 0.3 m/sec. Temperature is the most important limiting factor in standing waters and current velocity in flowing systems where it is allied to rock hardness or erodability. In British streams, calcium ion concentrations of below 3 and 5 mg/l proved to be limiting to the distribution of *Lymnaea pereger* and *Planorbis albus*. High magnesium content on the other hand severely limited egg production of *Biomphalaria* under laboratory conditions. Many such magnesium rich waters occur in S.A. especially in Swaziland and Eastern Transvaal areas. Snails appear to be most abundant in harder waters above approximately 30mg/l of calcium ions. The conditions in local rivers should be correlated to the distribution of *Burnupia spp.*

Population Control and Breeding Strategy

Aldridge (1982) reports on extensive research on density dependant control mechanisms in freshwater snails. Many different mechanisms have been implicated including trophic limitations (van der Steen, 1967; Eisenberg, 1970; Mooij-Vogelaar et al, 1973), pheromone-like substances (Berrie & Visser, 1963; Berrie 1968) metabolite accumulation in the environment (Hiruta & Kawabata 1954) and the frequency of tactile contact between snails (Chernin & Michealson, 1957; Berrie, 1968; Eversole, 1974). In *Heliosoma* increasing adult density had a more marked effect on fecundity than egg density (Eversole, 1974). In *Leptoxis* density of eggs, not adults, affects fecundity and there appears to be a lag time between early egg laying when egg density has no effect and later when *Leptoxis* females respond negatively to high egg density ± 1 week prior to the hatching of the earliest eggs. This suggests the possibility of a mechanism that allows females to track and compensate for variation in egg mortality.
Russell-Hunter (1953) observed in his study on *A. fluviatilis*, that from mid-April to mid-August 10% of adults survived and less than 1% survived to mid-September.

It has been reported by several authors that freshwater pulmonates show remarkable phenotypic plasticity and variation in life cycle patterns. *L. palustris* can have a simple one generation per year pattern with incomplete replacement to a two generation overwintering pattern and a pattern intermediate between these. The degree of plasticity can be correlated to the degree of specialisation with specialised species like *Ferrisa rivularis* showing inter-populational variation on the same scale as *L. palustris* shows on the intra-populational level.

Bondesen (1950) showed that *A. fluviatilis* in Europe produces very large eggs in comparison to other fresh water gastropods of similar size, and he suggested that this contributes to the survival of young under unfavourable conditions. With regard to growth as observed in the field and laboratory it is noteworthy that different embryos within a single capsule may show marked differences in growth rate and these differences may continue long after hatching (Prinsloo & Van Eeden, 1973). This fact can be explored further.

Temperature

Three North American planorbid families have an optimal temperature range of between 24°C-26°C. At higher temperatures growth is faster but egg production is limited. *Lymnaea stagnalis* shows an optimal range around 22°C. Temperature appears to affect the gonad development through impaired tissue development. It could be advantageous to support early growth in elevated thermal conditions and to move the populations to lower temperatures at late juvenile stages. Daily warming and cooling rates have been held to provide stimuli responsible for the observed nocturnal and activity patterns of several pulmonate snails of both aquatic and terrestrial habit.

Streit (1985) reported that assimilation and rates of feed, grazing rate and radular movement are directly

linked to temperature in *Ancylus fluviatilis*. Optimal temperatures for this species appear to lie between 13°C and 19°C. Reduction of the metabolic rate to a winter resting state in this species may occur anywhere between 5°C and 10°C depending on the ecological history of the population, those from colder areas having a lower switch trigger and undergoing complete rest. It is most likely to be in the area of temperature responses that *Burnupia* may show its biggest differences from *A. fluviatilis* because of the relatively elevated temperature regimes in which it has evolved in Africa.

Feeding and Growth

In the various habitats Ancylidae are found, they feed by rasping at rock surfaces with their radulae (Moss, 1980). *Lymnaea palustris* and *L. pereger* feed on aufwuchs with little ability to select diatoms in preference to filamentous algae. with food taking an average of 90 minutes to travel the length of the gut. It was found that radula stroke rate diminished with increasing food richness or density. When feeding on thin coatings of food the snails move forward faster than when feeding on thicker coatings of food. Under the latter circumstances the sideways sweeps of the head increase (Hunter, 1975). Streit (1985) found that low levels of periphyton on substrates were ignored by foraging adults; that feeding rates varied with temperature, with radular rate and area grazed increasing with temperature. However, he found that assimilation rate decreases at high temperatures due to increased metabolic demands. Juveniles have relatively larger teeth than adults which allows them to graze similar grazing areas and feed to adults.

Turner (1926) found that the standard length of *L. pereger* doubled every four weeks over the period of exponential growth which was confirmed by Calow (1981) who found that, in common with other freshwater snails, growth under laboratory conditions is sigmoid with an initial linear phase followed by an exponential phase, after which size increase decelerates to a steady state. As regards the influence of temperature, the optimal temperature for growth and fecundity was found to be between 16°C and 22°C with both growth and egg production rates reducing sharply as temperature increased.

Calow (1973) suggests that variations in diet may affect growth and presents evidence to suggest that a lichenophilous habitat, as opposed to one with a pre-dominance of algae particularly diatoms, may result in reduced growth of *A. fluviatilis*.

Respiration

The respiration of freshwater snails displays an increase in oxygen consumption with increase in temperature but the rate and level of consumption differs with species. However the consumption rates fall in a fairly narrow range and pulmonates and prosobranchs seem to have similar rates of respiration. The incipient limiting or critical level of oxygen supply for freshwater gastropods was investigated by Berg & Ockelmann (1960) and they reported a species specific variety of responses to declining oxygen concentrations.

For example:

Lymnaea auriculata is able to maintain its oxygen consumption in relation to decreasing concentration to about 11% DO after which uptake decreases.
L. pereger uptake decreases with decreasing oxygen concentration particularly below 8% DO.
L. palustris has decreasing uptake with decreasing concentration but there seems to be an increase as the DO level approaches 12-13%, after which uptake decreases again.
Myxas glutinosa maintained uptake to 12 % DO after which it decreases, particularly below 6% DO.
Bythia tentaculata oxygen consumption decreases with oxygen concentration.
Physa fontinalis is able to maintain uptake with decrease in concentration after the initial small decline. Uptake begins to decline at 13% DO with a rapid drop at concentrations below 6% DO.
Valvata piscinalis is the same and rapid decline in uptake start at concentration of 9-10%.
Bithnia leachi consumption is maintained or may increase with decreasing concentration till 13-14% DO whereupon uptake decreases but the decline is not as rapid as in other species i.o.w consumption at all oxygen conditions is relatively close to normal.

Berg et al (1958) demonstrated that the oxygen consumption of *Ancylus fluviatilis* increases 1.3 to 2 times in spring and summer over the winter values and he regards this increase due to sexual activity. Similar results were reported for *Lymnaea pereger* and *L. palustris*.

Reproduction and Fecundity

Like most pulmonates, Basommatophorans are hermaphrodite, apparently produce eggs and sperm simultaneously and are capable of self-fertilisation, but the copulatory organs seem to be designed to promote cross-fertilisation (Brown, 1980). Observations of mating among *B. stenochorias* have been infrequent, although observations have not been made during the nights. Protandrous hermaphroditism can not be deduced from the observations carried out thus far in this study.

In the laboratory Durrant (1976) concluded that specimens of *A. fluviatilis* as small as 4.00 mm in length had active ovotestes and laid egg capsules producing viable spat. It was seen that no spat were produced by individuals smaller than 4.00 mm. Bondeson (1950) and Geldiay (1956) reported that *A. fluviatilis* lays between 1-11 eggs per capsule. Russel-Hunter (1953) showed that the average number of eggs laid by an individual *A. fluviatilis* is 46 contained in 12 capsules while Streit 1976 reported a much higher value of between 66-113.

Russell-Hunter (1953) made the observation that the majority of the adult population die soon after spawning. This observation may hold true for populations of *B. stenochorias*. This semelparous reproductive cycle compared to the iteroparous condition when parents survive reproduction and live to reproduce again (Calow, 1978) may be an adaptation to variable habitats such as found in streams.

Jarne & Delay (1990) examined the effects of self fertilisation and cross fertilisation in *Lymnaea pereger*.

Cross fertilIsation resulted in a larger number of eggs laid, and young hatching and reaching sexual maturity. Jarne *et al* (1991) provided similar evidence with *Bulinus globosus* where selfing snails and snails produced by selfing were less fit in terms of fecundity and the hatchability of their eggs.

Data on other Basommatophora have also provided evidence for an inbreeding depression. Boycott et al (1930) showed that allosperm (*ie* that from the second individual) can be stored and remain viable after copulation, but as it becomes exhausted or dies, the self-fertilisation rate gradually increases (Duncan 1975). Vianey-Liaud (1976) observed a decrease of 40% in the number of eggs laid in *Biomphalaria glabrata* when comparing snails during a grouping period with the same snails one month after isolation. It was suggested that snails had exhausted their allosperm and had progressively switched to self fertilisation. In this case, previous copulations did not prevent an inbreeding depression (estimated by the number of eggs laid).

The possibility of a similar drop in reproductive fitness in *Burnupia* has not yet been examined. However literature indicates that for a general breeding programme, there is a need for continual re-introduction of individuals from an external source to maintain a high reproductive fitness.

Russell-Hunter (1953) observed that in a population of *A. fluviatilis* eggs that hatched at the start of the period of intense spawning had a better chance of surviving the early stages of development than those which hatched from egg masses laid later. This illustrates the phenomenon of competition between individuals where a high mortality rate will result from a dense population.

Toxicity Tests

The need to obtain life history information and apply this information to macroinvertebrate toxicity tests is emphasised by Buikema and Benfield (1979). However, very little work has been accomplished in understanding the life history variables that may affect toxicity test results within snails, and limpets in particular (Wurtz and Bridges 1961; Cairns and Messenger 1974). Examples where life history patterns have been used in determining toxicities are Weir and Walter (1976) and Harman (1974), where immature snails are documented as showing greater sensitivity to toxicants than adults; Flannagan (1974) showed that the influence of a toxicant impairs reproduction through four generations of *Heliosoma trivolvis*. Cocks (1973) and Spronk *et al* (1971) examined the effect of toxicants on the water balance within *Biomphalaria* and *Lymnaea* and Ishak and Mohamed (1975) showed the significant physiological disorders resulting form high doses of copper sulphate in *Biomphalaria alexandrina.* Nduku and Harrison, (1976) demonstrated the detrimental effects on egg production, hatching and shell development due to lowered calcium levels in water. There is a large body of work reporting on the acute and subacute effects of various toxic substances, including halogens, molluscicides, insecticides, fertilizers, copper salts and other compounds on snails, but it is not the intention of this chapter to deal exhaustively with these. No work of an ecotoxicological nature seems to have been completed on the Ancylidae.

4.2 EXPERIMENTAL INVESTIGATION OF *B. STENOCHORIAS*

The investigation to establish optimal conditions for the rearing, handling and breeding of *Burnupia* has been conducted along the following lines, but not necessarily in this sequence:

(4.2.1) Growth and survival;

Pilot study to investigate methods an explore the responses of *Burnupia*
influence of handling
influence of hydraulic conditions (standing with aeration or flowing)
influence of temperature
influence of density

(4.2.2) Reproductive biology; fecundity and hermaphroditism
developmental period and basic embryology

4.2.1. ESTABLISHMENT OF OPTIMAL REARING CONDITIONS - GROWTH AND SURVIVAL.

4.2.1.1 Pilot study on the feeding, growth, survival and reproduction of *B. stenochorias*, conducted by Miss L Horne as a student project.

The project write-up has been shortened.

Aims

To test the effect of three different types of food on the growth and reproduction of *Burnupia stenochorias* under ambient conditions of light and temperature and to assess the survival of the captive population under given laboratory conditions in an effort to establish the suitability of this animal for laboratory culture.

Method

1) Limpets collected from the Blaauwkrantz River were carefully removed from the rocks (to minimize damage to the shells), measured and divided into two size groups with a shell length ranging between 4.0mm to 6.0mm and 2.5mm to 3.5mm.
2) Stones, some of which had been allowed to grow periphyton, were placed into four replicates of two 500ml bubble pots. The stones in the controls (NF) and the artificial food (BR) replicates were scrubbed of all periphyton and sterilized in alcohol. The artificial food was specially formulated by Mr P. Britz from the Department of Ichthyology and Fisheries Science at Rhodes University, for the commercial culture of Abalone. The pellet consists of Spirulina, fish meal, starch and essential amino acids. Periphyton stones were left in ponds at the fish farm for 2-3 weeks to allow the periphyton to grow (FP) or brought back from the river (RF). The 15 limpets in each container were allowed to settle onto the rocks before the air stones were introduced.

Table 4.2.1.1.1. Experimental Design

POPULATION (lg.)			POPULATION (ss.)			FOOD TYPE
Label	Stone volume	Limpet size (mm)	Label	Stone volume	Limpet size (mm)	
NF1	120	4-6	NF2	50	2-4	none
FP1	60	4-6	FP2	75	2-4	Periphyton from fish farm
RF1	50	4-6	RF2	50	2-4	Periphyton from river
BR1	120	4-6	BR2	20	2-4	Artificial food

3) Shell length of specimens were measured monthly using a micrometer eyepiece at a magnification of 120. The spat were counted and a sub-sample of 15 were measured.

4) Containers were kept at ambient temperature throughout the duration of the experiment which lasted from 16 March to 31 August. Water was changed once a week and topped up to the 500ml mark when the level dropped.

Results & Discussion

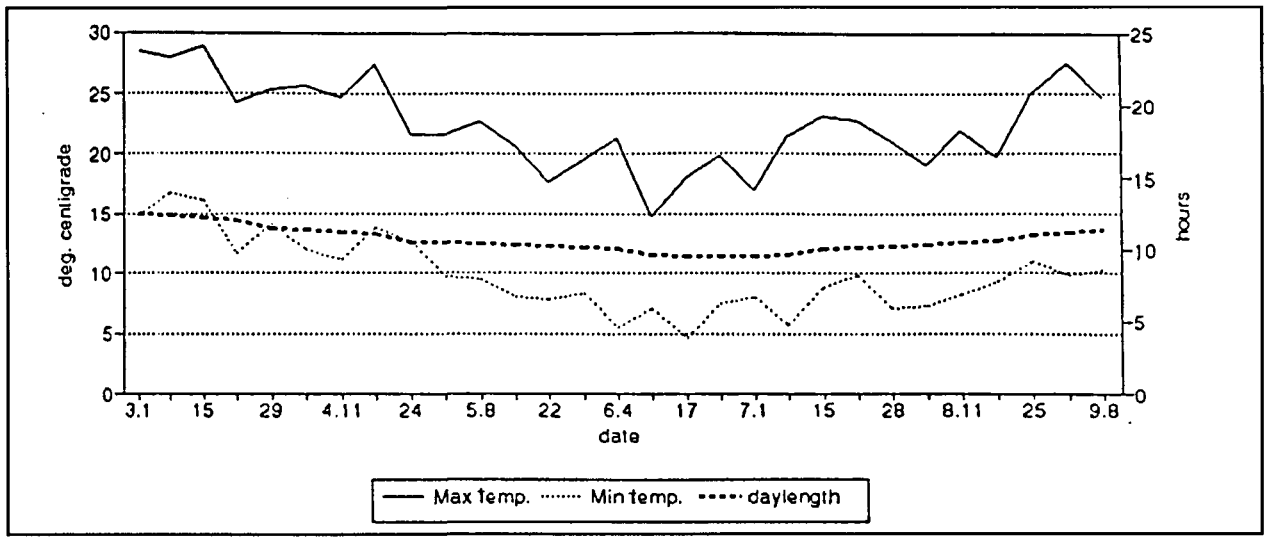


Fig. 4.2.1.1.1 Ambient temperatures and daylength for the experimental period.

In those containers which began with sterile rocks, periphyton grew which provided a food source and enabled the spat to survive and grow. Where artificial food was provided, the periphyton grew more densely than in the others, possibly due to the enrichment of the water by the artificial food. In those containers with natural periphyton the growth became dense and had to be regularly scraped away.

Growth of adults

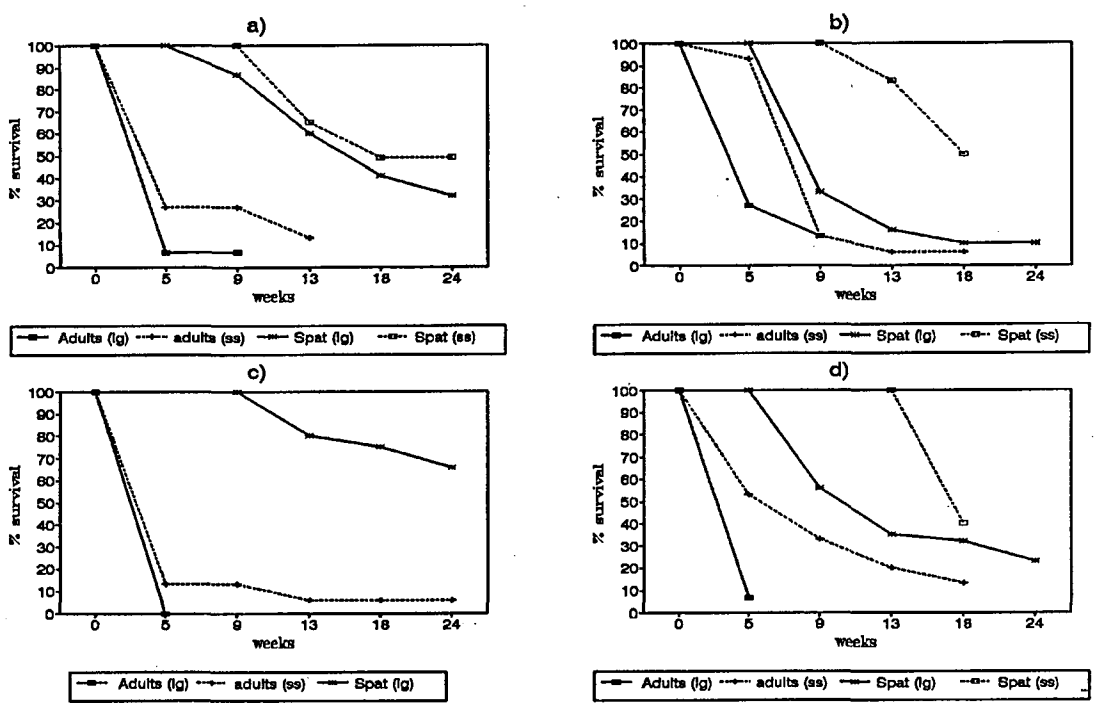


Fig 4.2.1.1.2 a) to d) Graphs to show the percentage survival of limpets under four feeding regimes.

Table 4.2.1.1.2 Average growth rates per day for the adult populations (mm). BR = artificial food; FP = fish farm periphyton; RF = river periphyton; NF = no food 1 = population size range (4-6mm); 2 = population size range (2-4mm).

Feed	Time (Days)	Ave size (mm) at start	Ave size (mm) at end	Growth (mm)	Ave growth rate/day (mm)
BR1	63	3.54	5.0	1.46	0.023
BR2	92	2.24	5.0	2.76	0.030
FP1	63	3.50	3.70	0.20	0.003
FP2	127	2.26	5.0	2.74	0.022
RF1	38	3.68	4.40	0.72	0.018
RF2	127	2.12	4.60	2.48	0.019
NF1	38	3.73	0.00	0.00	0.000
NF2	168	2.32	6.0	3.68	0.022

The low average growth rate of 0.003mm/day which is seen in the FP1 replicate is due to the largest specimens dying in the first month, affecting the average size considerably. The best growth was achieved in containers BR2 and BR1 which were fed on artificial food. The two samples fed on river periphyton showed similar growth rates (0.018-0.019mm/day) while the sample of small animals (FP2) fed on fish farm periphyton, showed slightly better growth approximating that of the larger animals fed on artificial food(0.22 versus 0.23 mm/day). The sample of small animals with no food continued to grow to a maximum length of 6mm after 24 weeks at the rate of 0.022mm/day.

There were not enough available data points for the adult populations and so a linear regression analysis could not be conducted.

Growth of spat

Table 4.2.1.1.3 Average growth rates per day for spat populations (mm). BR = artificial food; FP = fish farm periphyton; RF = river periphyton; NF = no food 1 = large population (4-6mm); 2 = small population (2-4mm).

Feed	Time (days)	Ave size at start (mm)	Ave size at end (mm)	Growth (mm)	Ave growth rate/day (mm)
BR1	125	1.41	4.29	2.88	0.023
BR2	105	1.04	4.44	3.40	0.032
P1	125	1.14	3.94	2.80	0.022
FP2	64	1.20	2.74	1.54	0.024
RF1	125	1.56	3.94	2.38	0.019
RF2	35	1.20	1.85	0.65	0.019
NF1	105	1.40	5.0	3.60	0.034

NOTE: No spat were produced in NF2.

The spat produced by all the adults showed positive growth in all conditions. Best growth rates were obtained on artificial food supplement. Spat in the container which originally had no food had the highest growth rate.

A simple regression analysis showed a fit of 96%, 90%, 97% of the sample to the regression equation (line). Although there appears to be a difference in growth rates, a Kruskal-Wallis analysis of final sizes reached by spat populations in the three food types showed no significant differences.

Reproduction

In Table 4.2.1.1.4 the reproductive gain which was realised in each treatment is laid out. As can be seen the reproductive gain varied considerably between replicates. The adults which were between 4 and 5mm shell length at the start of the experiment died off sooner than smaller limpets and on average produced more young (60/12; 62/10; 91/65) except in the container where no periphyton was supplied where the reverse was true (5/75). The animals in the RF2 containers were smaller than those in the NF2, FP2 and BR2 containers which could be the reason for the two periods of egg laying observed. On the whole the reproductive gain from the N1 generation which were reared under laboratory conditions was lower than that from the wild reared parents.

Table 4.2.1.1.4 Reproductive gain in each treatment. Column 2 = the average size with standard deviations of the 15 limpets at the start of the experiment ; STD = standard deviation; Column 3 = number of survivors which were present when the first spat were observed; Column 4 = average size with standard deviations; Date = date on which spat were observed; the last three columns refer to second (N2) generation spat which appeared in the containers from the N1 generation as well as the average size and the number of the parents present in the container and the date on which observed. 3^1 = first measurement and spat count. 2^2 = second measurement and spat count.

Treatment	Ave size(STD) at start	Survi-vors	Size	N1 Spat	Date	N2 Spat	Date	NI Parent Ave (STD)	No.
NF2	2.9(0.24)	1	7.5	75	31/8				
NF1	4.65(0.62)	0	0	5	18/5				
FP2	2.833(0.25)	2	4.63(0.38)	12	18/5	55	31/8	3.42(0.12)	6
FP1	4.38(0.39)	4	4 .38(0.13)	60	28/4	44	31/8	4.92(0.19)	6
RF2	2.65(0.44)	3^1	5.67(0.42)	10	16/6				
		2^2	5.75(0.25)	200	31/8				
RF1	4.6(0.42)	1	5.5	62	28/4	10	31/8	4.99(0.11)	14
BR2	2.8(0.26)	4	3.25(0.25)	65	16/5	15	18/7	4.29(0.34)	32
BR1	4.433(0.39)	1	6.0	91	28/4	48	31/8	5.36(0.56)	29

The interval between the start of the experiment and the first observation of spat was shorter for the larger adults (5-9 weeks) than for the smaller adults (9-13 weeks). We can deduce that the smaller animals had not reached sexual maturity when the experiment started. We observed that eggs were not laid by individuals under 4mm in length and can therefore suggest 3.5-4.0mm to be the minimum size required for B.stenochorias to reach sexual maturity.

Survival

It is clear from Figures 4.2.1.1 a)-d) that the larger animals died sooner than the smaller adults. However they produced a larger number of offspring except in the no-food situation where one of the smaller adults not only survived for the longest period but also produced the largest number of spat. If the slopes of the lines are compared a rapid mortality in the early period of the experiment can be seen followed by a decrease in the mortality rate. This is unlikely to be a reflection of the density of the spat (presuming the greater the density, the greater the mortality) because in BR1, 91 spat showed a slow decline while 60 spat in FP1 showed a much more rapid decline.

The spat survived for longer than the adults. This is depicted by more gradual slopes in the spat than in the adult

limpets. The spat were hatched under laboratory conditions and thus did not experience any physical disturbance. A possible explanation for the higher mortalities in adults could be due to their reaching the end of their natural life span.

Two Chi-square tests were performed to test if the survival of the populations of spat from the two sizes of parents were different. These tests proved that there was a highly significant relationship between the survivorship of the spat and the three food types in conditions of turbulent water ($X=197.0$; $D.F=9$; $p<0.000010$). The test did not however tell us which food type gave us the greatest survival. However, it can be observed in Fig 4.2.1.2.1 that the spat from the population of larger adults in the container with artificial food had a higher survival rate of 45% compared to 30% and 10% on other food types 13 weeks after hatching. The survival rate of the spat from the larger adults with no food proved to be the highest with a 70% survival.

The average shell length of the spat when first observed was 1.12mm. As egg laying or hatching was not observed it is not possible to determine the early growth rate or the size at hatching. This size is comparable with that reported by Russel-Hunter (1953) for *Ancylus fluviatilis*. In the laboratory, Durrant (1976) concluded that specimens of *A.fluviatilis* had active ovotestes and laid egg capsules producing viable spat at 4.0mm in length. This result correlates favourably with the results obtained on the minimum size required to enable reproduction of *B.stenochorias*.

It has been suggested by various authors that Basommatophorans are hermaphroditic. Städler et al. (1993) observed that *A.fluviatilis* has the ability to self-fertilise but appears to switch to self-fertilisation only when mates are unavailable. This phenomenon of switching from cross-fertilisation to self-fertilisation may explain why in the container with no food there was only one adult individual left in the container after week 13 and after 24 weeks a population of spat was observed. If *B.stenochorias* is able to self-fertilise we can deduce that this population of spat arose from the single adult survivor as the result of self-fertilisation. In the light of later experiment (B:4.2.10) this deduction appears to be correct.

It is evident that the greatest numbers of spat hatched in the container with artificial food. It was also observed (Table 4.1.2.3) that this population had the greatest survival rate and thus it can be postulated that the artificial food provides a better source of nutrition as it is able to sustain a population containing more individuals without a high mortality rate.

Bondesen (1950) shows that *A. fluviatilis* in Europe produces very large eggs in comparison with other fresh water gastropods of similar size, and he suggests that this contributes to the survival of young under unfavourable conditions. With regard to growth as observed in the field and laboratory it is noteworthy that different embryos within a single capsule may show marked differences in growth rate and these differences may continue long after hatching (Prinsloo & Van Eeden, 1973). Russell-Hunter (1953) also observed that in his study on *A.fluviatilis*, from mid April to mid August 10% of adults survived and less than 1% survived to mid September. These results compare favourably to the results obtained in this study (Figs. 4.2.2 a)-d) where *B.stenochorias* fed on artificial food, fish farm periphyton and river periphyton displayed a survival rate of 10% over approximately

the same time period. It must be noted however, that this survival rate was observed from the population of initially smaller sizes (2-4mm) and not of the larger size range (4-6mm). This may be due to the semelparous life history and proves that the larger adults were close to the end of their natural lives.

A species of oligochaete (unknown) was often found between the mantle and the disc of the foot of *B.stenochorias*, probably as a commensal rather than as a parasite. The oligochaete *Chaetogaster limnei* (Baer) was found on the species *Ancylus fluviatilis* (Geldiay, 1956).

As the experiment progressed the shells of *B.stenochorias* became covered with various algal species and limpets were often seen browsing on each other with small ones frequently found on the shells of older limpets.

Conclusion

This investigation proved to be valuable as a pilot study indicating the potential as a species for laboratory culture. Areas which require further investigation became apparent. These include density effects on reproduction and survivorship and responses to food types and other abiotic variables such as the hydraulic regime and temperature. The experimental design will have to be improved to allow for adequate statistical analysis.

4.2.1.2 Growth and Survival in Disturbed and Undisturbed Conditions

Aim

To establish growth rates at a stable temperature and to establish if the measurements taken every two weeks, which disturbed the limpets, had any effect on the survival and growth rates.

Method

1) 300 adult limpets were collected from the Blaauwkrantz River on 13 October, 1993 and held 14h00 photoperiod and 16-23±2°C which were ambient conditions. These limpets were kept in a shallow bubblepot with ample substrate covered with periphyton on which egg cases were deposited.
2) 14 x 5 litre bubblepots were each half filled with water from the river of origin, covering a tile with periphyton growth. Six of the pots were labelled numerically and eight alphabetically.
3) Stones with eggs cases were placed in these pots 12 days after the adult were collected and placed in a CER at 18 °C and 14hOO photoperiod. The population in the pots labelled alphabetically were not measured until they were 94 days old but were counted 2 weekly intervals which meant that they were undisturbed. In order to determine the developmental period the egg cases on the stones were examined every two days in the numbered containers until the hatching was completed. Thereafter a sub-sample of the limpets were removed to be measured and counted every 14 days, until age 139 days.

Results

See Table 4.2.1.2 and Figures 4.2.1.2.1; .2; .3 and .4.

The Analysis of Variance conducted on the average sizes reached by populations in all containers at 94 days showed an F ratio of 22.870, df = 13,339, and $p < 0.0001$ which indicates a significant difference between treatments. A multiple range test conducted on the population size between all containers showed that significant differences appeared between some containers (f=12.123; df 7,216; p<.00001) : grouping containers that showed the smallest differences together resulted in groups ADEJLN; 123; 45; and CD (Fig 4.2.1.2.2). When these groups were tested against each other in the sequences given below, all the comparisons yielded significant differences:

123-ABEJLN; 45-ABEJLN; ABEJLN-C; ABEJLN-D; 123-45; 123-C; 123-D; 45-C.

The sizes achieved showed that those animals that had been disturbed for measuring were smaller (1,4 - 1,8 mm) on average than those which had not been disturbed (2.0 - 2.6 mm).

Mortalities

Regression analyses were conducted between the percentage survival, and age of the population in each container in both treatments using BMDP.

(a) Undisturbed population:

At the 5% level of probability, there was a significant difference between containers when all containers were tested together. F = 3.045; df = 16,27; p value = 0.00523.

Graphs in Fig. 4.2.1.2.4 (a) and (b) show that the slopes of containers A and K are different from the others. The regression slopes of A and K are respectively -9.05 and -25.6 while that of all the other populations are between -14.7 and -17.8. Therefore excluding A and K, F = 1.617; df = 12,21; p = 0.16154. When tested this proved that there was no significant difference between the regression slopes in containers BCEFJLN.

(b) Disturbed population:

The regression slopes ranged between -27 and -19 in all the containers and there was no significant difference between the regressions of the population in these containers (F ratio = 1.308.517 df 10,18 and p = < 0.029762). However when the populations in the two treatments were compared to one another there was a significant difference between the rate of decline (with or without A & K). The mortality rate in disturbed populations had a regression line of -21 and that of the undisturbed populations was -16 (F ratio = 95.517 df=1,28 p value= <0.00001).

Discussion and Conclusion

Physical handling of the limpets has a detrimental effect on both the growth and survival rate of juvenile limpets, between the sizes of 0,6mm and 2,4mm shell length. Minimal handling is therefore necessary when collecting from the field and when measuring the limpets during experiments. Fig. 4.2.1.2.3 shows the difference in the % of the sample surviving after 30 days in undisturbed (a,b) and disturbed (c) pots. The vulnerable nature of the juveniles is demonstrated by the fact that between 70 and 90% of the animals survived in 7 out of 9 containers

in undisturbed while less than 70 % of the animals survived the first 30 days if disturbed. However after the initial period, survival rate stabilized until day 66 and then declined more sharply except in container 1. In the undisturbed containers the high level of survival was sustained until day 45 with the exception of K and F but after that the mortality rate increased. In two containers a total mortality was recorded which could perhaps be ascribed to a pathogen. However if the undisturbed containers are compared to those which were regularly disturbed it is clear that survival was on the average 10% higher when undisturbed.

As mentioned in Chapter 3.1, field handling has been reduced since this experiment by using an anaesthetic, and a template made of transparent photographic film with measured punctured holes ensuring minimal handling when measuring the limpets.

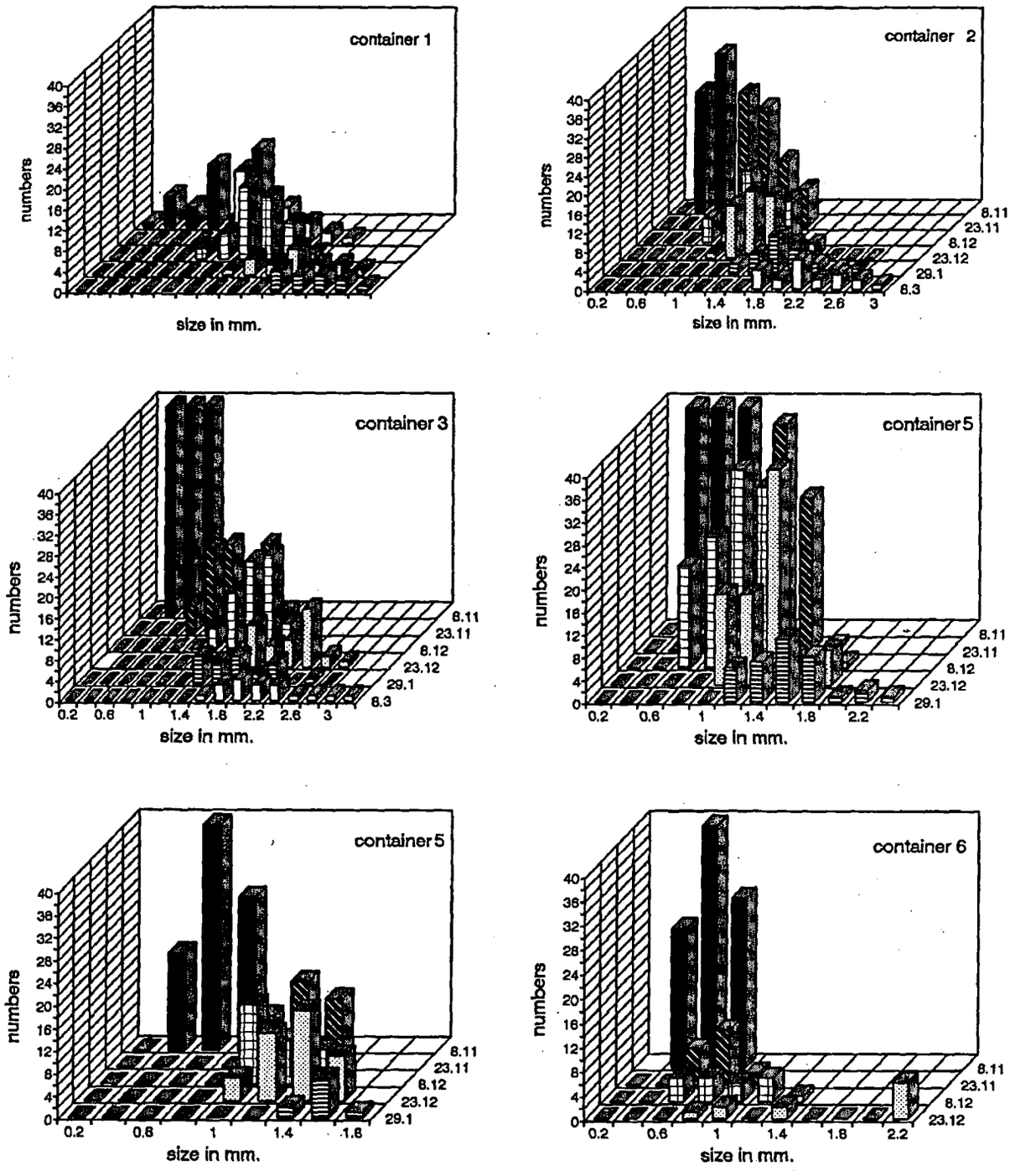


Fig. 4.2.1.2.1 Frequency distribution of the population size at a number of dates during the experimental period. The numbers 3.11 - 8.11 are the dates during which 50% of the eggs had hatched.

Table 4.2.1.2.1. Table showing the numbers in each container at the start of the experiment, after 94 days; the average shell length with standard deviation, achieved in each container and daily growth rate in mm. Disturbed containers are labelled 1-6; undisturbed containers are A-N.

POT	INITIAL NUMBERS	FINAL NUMBERS	AVERAGE SIZE (mm)	STANDARD DEVIATION	GROWTH RATE
1	295	17	1.79	.057	0.018
2	165	23	1.84	.066	0.017
3	247	31	1.75	.056	0.014
4	371	41	1.38	.043	0.0088
5	94	14	1.46	.040	0.009
6	101	11	1.90	.152	0.014
A	85	60	1.96	.040	0.018
B	67	23	2.17	.062	0.019
C	104	23	2.39	.080	0.0237
E	96	21	2.57	.104	0.024
F	94	29	2.00	.071	0.016
J	64	16	2.08	.113	0.021
L	79	30	1.88	.053	0.017
N	98	22	2.05	.042	0.020

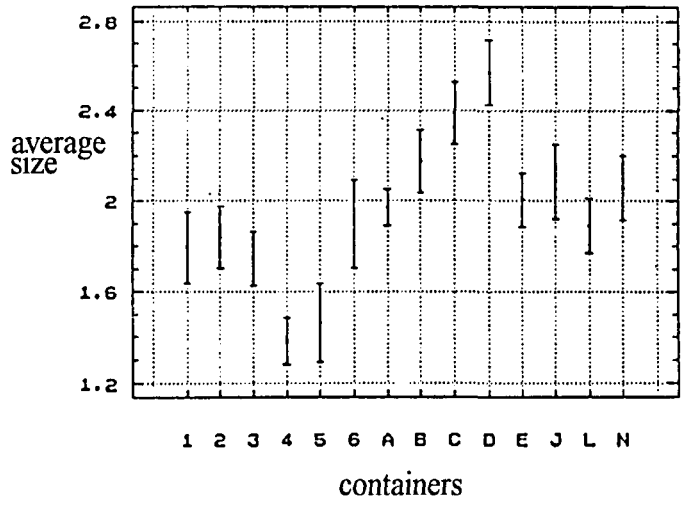


Fig. 4.2.1.2.2 Average size the sample achieved after a period of 94 days in both disturbed (1-6) and undisturbed (A-N) containers.

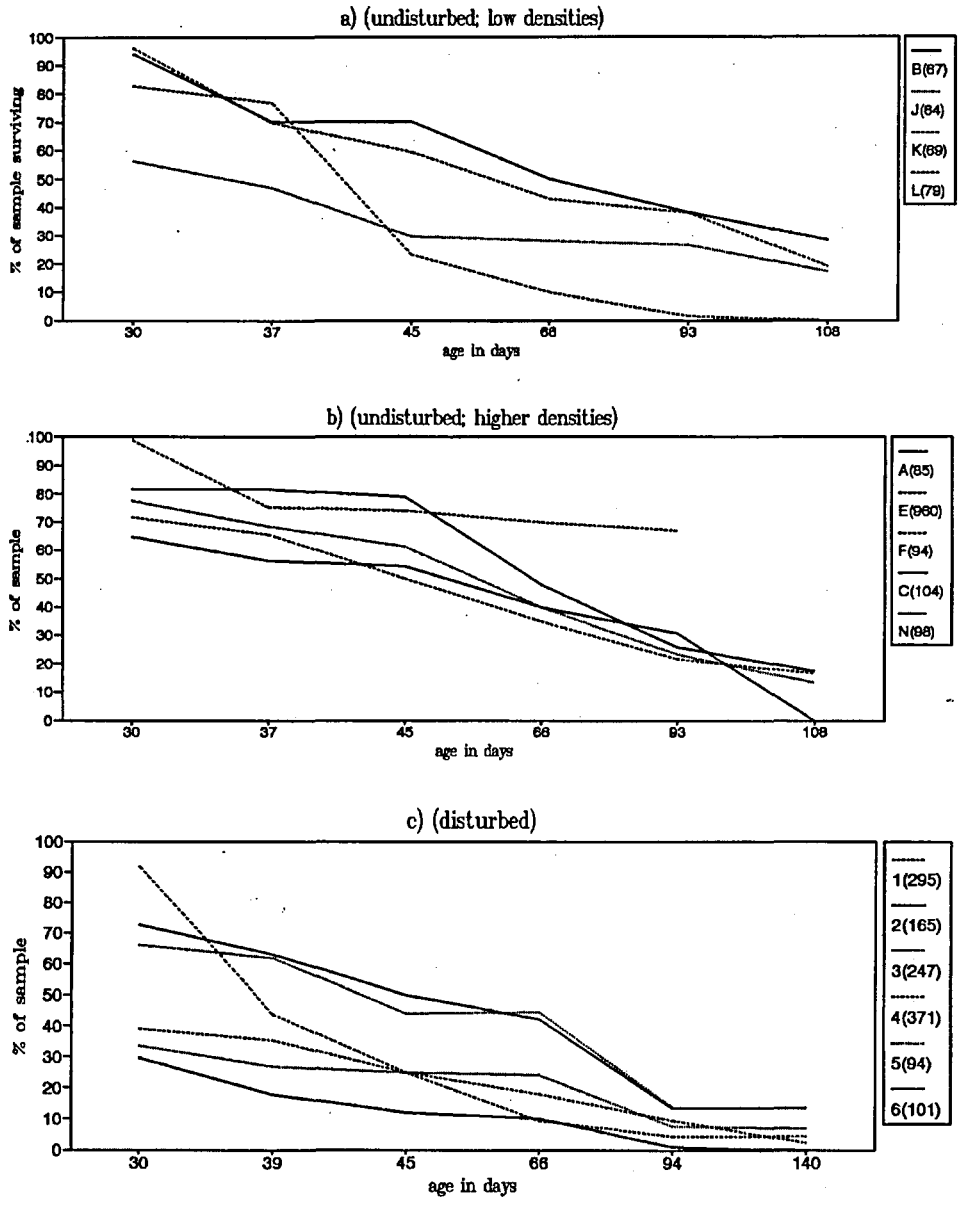


Fig. 4.2.1.2.3 Percentage of sample surviving during the experimental period. The legends indicate the initial size of the sample in each container. (a) and (b) undisturbed containers (c) disturbed samples (removed for measuring)

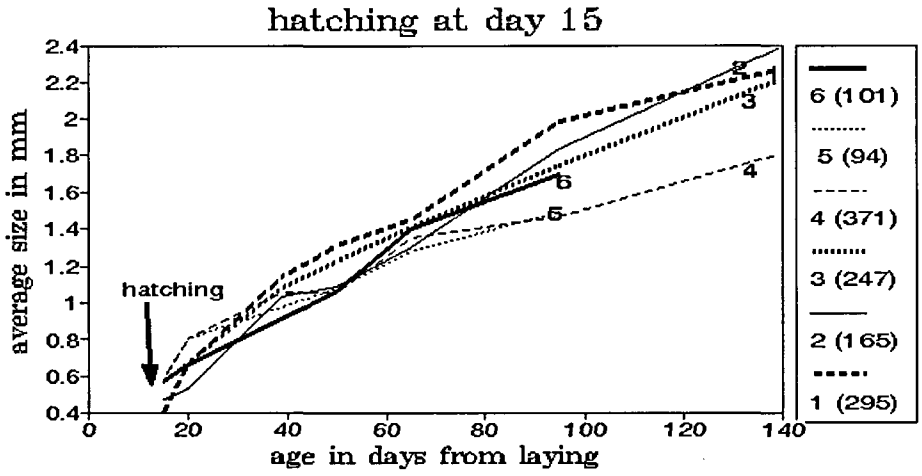


Fig. 4.2.1.2.4 Average growth rate of *B. stenochorias* juveniles over 139 days from deposition Sample size legend

As can be seen all the animals in container 6 had died, and in container 5 three remained.

4.2.1.3 A Comparison of Survival Rates Between Fed and Unfed Populations

Aim

To assess the length of time a given number of adults and sub-adults can survive without food.

The debate as to whether test animals should be fed during ecotoxicological testing is unresolved. The question about the stress caused by lack of food and the attendant change in response to toxins needs to be resolved. This experiment is the first step in gaining insight into this problem. The projected experimental period for toxicology testing is four days.

Method

1) 8 channels, each 50cm in length with approximately equal amounts of water circulating through at a known rate and depth within each channel from a common water sump were set up. The water source was Grahamstown tap water, allowed to floe through the system for 2 days to allow for de-chlorination.
2) 4 channels had 3 ceramic stones of similar size, covered in periphyton, placed in each channel. The source of the stones was from the ongoing laboratory culture of algal growth where stones are held in recirculating water mixed from various sources (riverine and predominately tap water). As these channels are in the same laboratory as this experiment, temperatures did not alter and therefore there was no change in the growth of the periphyton. The stones were visually

assessed to ensure approximately the same amount of periphyton was placed in each replicate.

3) 4 channels had 3 ceramic stones of similar surface area to those of the above, placed in each channel. These stones were scrubbed and dried to ensure there was no periphyton. All channels were treated similarly, to ensure there was no food available.

4) On each stone in all of the 8 channels 2 adult (approx. 2,5mm in length) field-caught limpets of known length and height were placed, particular care being taken not to damage the animals in any way when moving them. The limpets were acclimatised for one week to the laboratory conditions.

5) Twice daily temperatures (at 08h00 and 15h30) and daily levels of TDS, pH and % oxygen were measured.

Results

Water conditions did not differ between all the replicates over the fifteen days. One way analyses were conducted on the two treatment populations at 4,8,12 and 16 days and at no stage were any significant differences detected.

Table 4.2.1.3. Statistical data of ANOVA. Day = number of days after treatment. df = degrees of freedom.

Day	F ratio	df	p value
4	2,6667	2,7	0,1835
8	0,667	2,7	0,6151
12	0,267	2,7	0,8469
15	1,250	2,7	0,3629

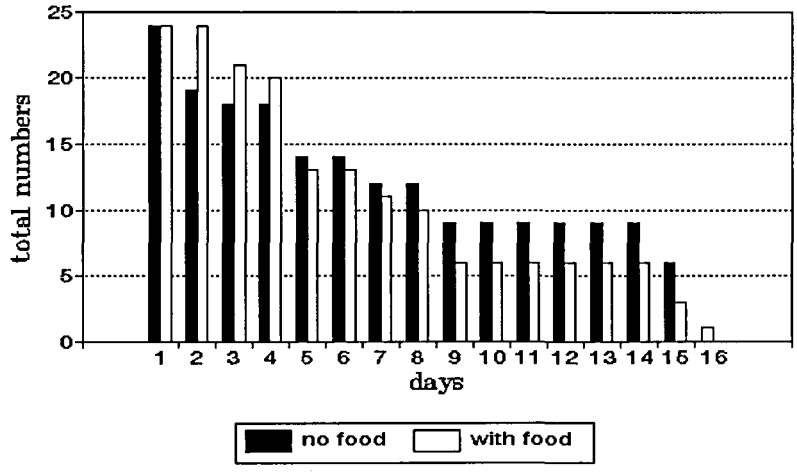


Fig. 4.2.1.3 Total number of limpets remaining in each treatment.

Conclusion

A period of 4 days (96 hours), that period under which ecotoxicological testing is completed, without food does

not appear to affect the survival of adults and sub-adults.

4.2.1.4 Hydraulic conditions

Several trials were conducted to test the responses of the limpets to channels (flowing water) and bubblepots (standing aerated water). Full procedures are reported in Appendix 3, section 3.1.3. dealing with the development of equipment.

4.2.1.4.1 Aim: To compare the growth rate of *B. stenochorias* in flowing water with that of aerated, standing water.

Method

Four 50 cm channels, each with approximately equal volumes of Grahamstown de-chlorinated tap water and four bubblepots of known and equal size, each with approximately equal volumes of water and air flow were housed in the CER at 19°C (day), 14°C (night) and a photoperiod of 14hrs. Within each pot or channel were placed 3 ceramic stones with periphyton as food source and 12 limpets of equal size.

Results and Discussion

Despite the fine net covering the holes through which the water flowed the limpets escaped and landed in the water sump. This made it difficult to asses growth rates of limpets in the channels. Survival in bubblepots (60% at 35 days and 40% at 79 days) was significantly better than in channels (225 at 35 days and 5% at 79 days). In the channels many of the limpets lost their shells or had extremely soft shells, which indicate unsuitable water chemistry despite pH levels which were neutral or slightly alkaline. The experiment was terminated after 3 months because of the above difficulties.

In the bubblepots the daily growth rates achieved in the first 35 days ranged between 0.01mm and 0.03mm and during the subsequent 39 days 0.01mm to 0.02mm at an average temperature of 19°C.

Conclusion

Narrow channels with three stones are not suitable for the rearing of *Burnupia* in the laboratory. More attention will be paid to water composition in future experiments (see section **3.3.6 Water Composition and Hygiene**).

4.2.1.4.2 Aim: To test the effects of density and flow on the survival and reproductive capacity of limpets.

Method

Two sets of four channels, each set with a common sump and 8 bubblepots were housed in the laboratory at an average temperature of 17.2 °C in channels and 15.2°C in pots. Three periphyton stones, and a known number of measured limpets ±100 and ±10, were placed in each channel and bubblepot. Dead limpets were replaced with an individual of similar size from a field-caught sample until the 48th day, to maintain the original densities. Dissolved oxygen, Ph, and TDS were monitored weekly and temperature daily. The trial ran for 162 days over the winter period when day-length declined from 11 to 9 hours and then increased again to 10 hours.

Results

The limpets moved from the channels, into the sumps from where they had to be moved back to the channels daily to maintain the density. This continual disturbance has previously been shown (see section 4.2.2) to have a detrimental effect on the growth of the limpets. The eggs deposited during the experimental period were not removed so that some indication of the reproductive gain in the various conditions was given. The highest gain of 6 hatchlings per adult was achieved in a bubblepot with low density of limpets. In the rest of the containers a negative reproductive gain was recorded.

The mortality in two bubblepots was high (77% & 63.6%). In two channels mortalities in excess of 20% were recorded. In the rest of the channels and bubblepots mortalities ranged between 4 and 19% (ave. 13%)

Conclusion

More egg capsules were laid in the bubble pots than in the flowing channels, especially at low density of limpets. The survival rate did not appear to differ markedly between the flowing and standing waters with the exceptions stated above. However the highest mortalities were recorded in the pots which could indicate a pitfall of pots as rearing equipment, that if a pathogen or any other cause of mortality enters such a confined space the entire sample in that pot is at risk.

From this exercise we therefor conclude that small numbers of adult limpets in a bubblepot will give the greatest numbers of offspring at an average survival rate. If some way could be found to preventing large numbers of limpets from escaping the channels, these would provide adequate facility in which to rear limpets.

4.2.1.4.3 Aim: To compare the survival of limpets in flowing versus standing, aerated water.

Method

About 700 limpets were collected in the field, using anaesthetics and maintained in the laboratory streams with de-chlorinated tap water for a few days to allow for any that died to be removed. Equal numbers of 2mm to 4mm

limpets were then placed on tiles which had accumulated a layer of periphyton, in three replicates of the large recirculating streams and three 10 litre basins, set up in the laboratory under ambient conditions of temperature (22°C-17°C, ave 19°C) and light. Dead limpets were counted and removed, and egg cases were counted daily. TDS, temperature, % oxygen and pH were monitored with each change of water. The experiment was allowed to run for 9 weeks.

Results

A greater number of egg capsules were laid in the pots, (64; 74; 115; ave. 84.3%) than in channels (47; 90; 103; ave. 80) The mortality rate varied considerably, between and within all the replicates. The average mortality for the channels was 64,5%, (71%; 62%; 58%;) for the pots, 75,6% (67% 72%; 88%).

Conclusion

In this experiment, the channels gave a better average survival rate than the pots where more egg capsules were laid.

4.2.1.4.4. Aim: To compare the growth rate of *B. stenochorias* in flowing water and turbulent standing water at 15°C, 20°C and 25°C.

Method. This experiment was conducted in a CER at 15°C. Four water baths were equipped with an aquatic thermoregulator each. Two were maintained at 20 and two at 25 °C. In two baths four five litre sumps were placed from which four channels was supplied. In the other two baths five litre bubble pots were placed. Two further baths were maintained at the ambient temperature of 15°C, one equipped with bubblepots and one with channels and sumps. Twenty limpets of known size were placed in each of the bubblepots and channels and fed on periphyton.

Conclusion

The aquatic thermoregulators used to maintain the water temperature in the sumps of the channels were unreliable and consequently unavoidable temperature fluctuations occurred. The results could not be used. It was therefore decided to conduct an experiment in which only bubblepots were used in an attempt to ascertain the growth rate of the limpets at three temperatures.

4.2.1.5. The Effect of Temperature and Density on Growth.

Aim

To determine the effect of temperature and density on the growth and survival of *B. stenochorias* with a view to determining optimal stocking density and ambient water temperature in a laboratory culture.

Introduction

Temperature and food are the most frequently reported factors which affect life history traits in aquatic invertebrates. Temperature directly affects growth by influencing metabolic rates and feeding rates (Giberson and Rosenberg 1992). It also affects food quality and quantity by influencing algal production and growth rates on detritus (Ward and Stanford 1982). When evaluating growth and development for the purposes of aquaculture, not only should temperature and food be considered but also density. At high densities competitive interaction may affect feeding rates and growth.

Method

1) The Controlled Environment Room was set at a stable 15°C.
2) Egg capsules laid on ceramic stones in the laboratory streams by field-caught *Burnupia* were placed in 500ml transparent plastic jars (called bubble pots) which were filled with 400ml of water, aerated, and allowed to acclimatise to the CT room temperature. A few days after hatching (which was completed by 29 September 1994) many of the spat moved to the sides of the containers. These pots were then manipulated so that there were 12 pots each of 10, 20 or 30 *Burnupia* per pot. The ceramic stones were removed, as were any excess numbers of spat which were kept separately and used to replace any limpets that died in the first two weeks of the experiment. This method ensured no handling of the spats occurred. First measurements were taken on the 13 October 1994 @ 14 days old.
3) Four water baths were used, each a rectangular white plastic container 50cm x 25cm x 25cm deep. In each water bath twelve of these bubble pots were placed, with 4 representatives from each of the densities. Each pot was given a clear soft-plastic lid held in place with an elastic band. Through this lid an aerator was suspended and into each bubble pot was placed one ceramic stone previously allowed to grow periphyton. The water in the bath was then filled and maintained at the same level as that in the bubble pots.
4) Each water bath was maintained at a different temperature, namely ambient 15°C, 20°C and 25°C.
5) The water within each pot was maintained at 400ml with distilled water and replaced every week with aerated dechlorinated calcified tap water (this water was kept aerated in the C.E Room, with crushed shells suspended, acting as the source of calcium). The pH, TDS and were monitored weekly.
6) The *Burnupia* were measured weekly for the first 4 weeks and thereafter fortnightly.

Measurements were taken with a prepared template through the sides of the pots. Any deaths within the first 2 weeks were replaced with individuals from the initial population of hatchlings.

7) The light intensity in the C.E. room was maintained at 15microm/m^2/sec quanta with daylight bulbs to increase the available range wavelength. The photoperiod was 14 hours.

Results

See Table 1, Appendix 4; Table 4.2.1.5. and Fig. 4.2.1.5.

Table 4.2.1.5. BMDP analyses of temperature versus density results. D** refers to density of 10, 20 or 30 limpets per container while T** refers to the temperature at which the experiment was conducted.

ANALYSIS				F RATIO	PROBAB.
at all temps	D10 slope 0,01994	D20 slope 0,01896	D30 slope 0,01820	25,189	<0,001
at temp 15°C	D10 slope 0,0231	D20 slope 0,02236	D30 slope 0,02153	11,417	<0,001
at temp 20°C	D10 slope 0,01822	D20 slope 0,0290	D30 slope 0,01795	30,138	<0,001
at temp 25°C	D10 slope 0,0231	D20 slope 0,02236	D30 slope 0,02153	11,417	<0,0011
T15 vs T20 at all densities	T15 slope 0,01682	T20 slope 0,01926		84,186	<0,001
at all densities	T15 slope 0,01682	T20 slope 0,01926	T25 slope 0,02242	122,121	<0,001
at density 30	T15 slope 0,01663	T20 slope 0,01795	T25 slope 0,02153	44,283	<0,001
D10 vs D20 at all temperatures	D10 slope 0,01994	D20 slope 0,01896		5,165	<0,005

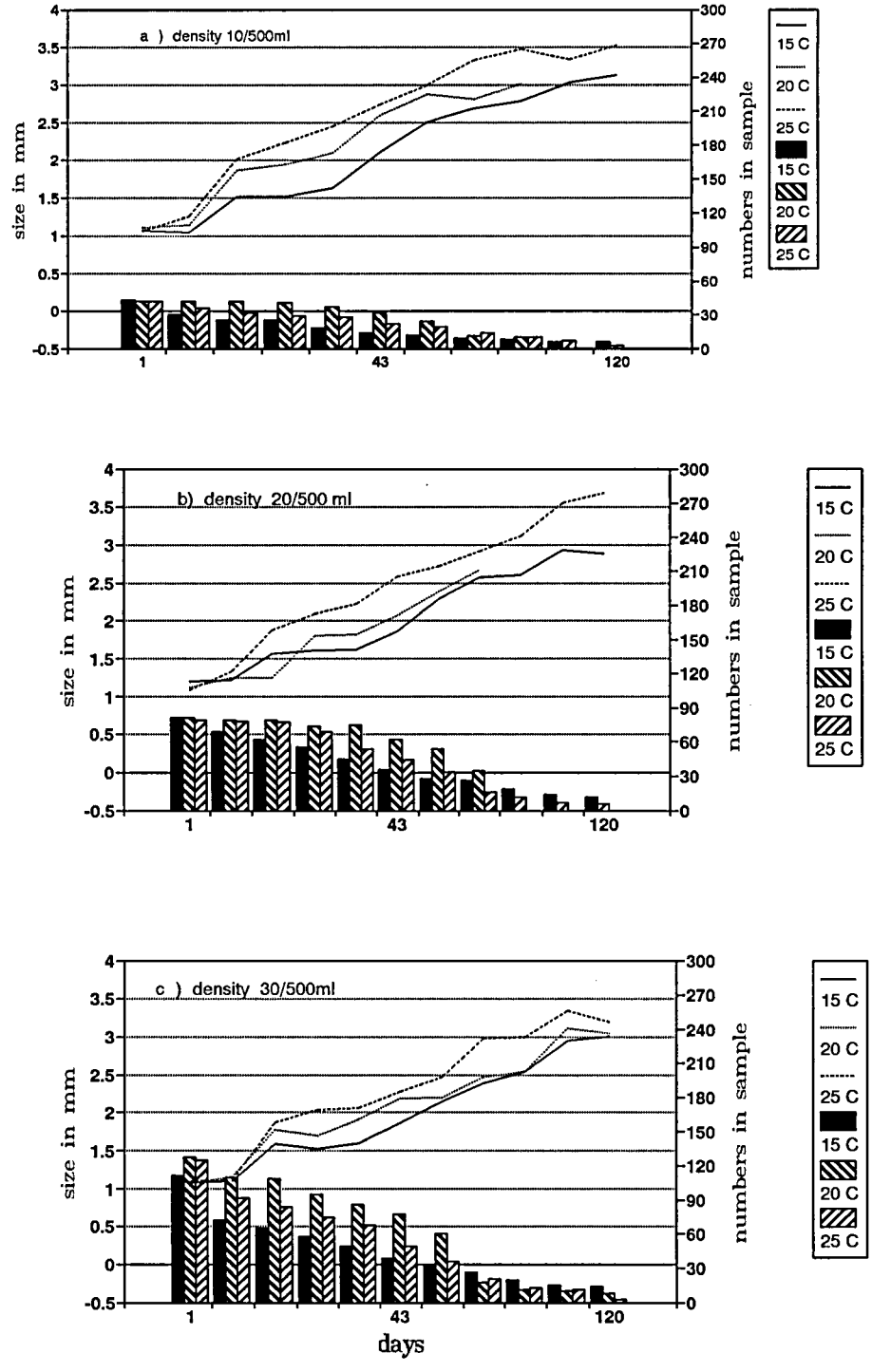


Fig. 4.2.1.5. The average size of *B. stenochorias* in all the replicates in each treatment (lines) and the total number of limpets in each treatment (bars).

Discussion

Temperature

It is generally agreed by ecologists that temperature is one of the most important physical influences of any biotope, in particular of the freshwater biotope (Shiff 1964). The apparent ability of snails to withstand wide variations in temperature has been noted by many workers. However Shiff (1964) points out that many of these inadequately measured the overall conditions of the habitat, and states that over very hot periods water at a depth of 25cm may be 5°C cooler than that at the surface. Mozley (1959) also stressed the importance of microhabitats in which animals may seek refuge from overall conditions. It is thus probable that the apparently wide thermal tolerance shown by aquatic snails may be due to their ability to select the more suitable microhabitats. This opportunity was not afforded by this *Burnupia* experiment.

Population patterns are strongly influenced by temperature, as demonstrated in the genus *Physa* (Duncan 1959, Russel-Hunter 1961(b), Girod 1969, DeWitt 1955, Eckblad 1973 and others). These authors found that differences in the number of generations through different geographical regions is linked to differences not only in food source quality, but also in higher mean growth rates and shorter generation times, all as a result of the influence of their ambient temperature. Similarly, temperature has been directly correlated with growth rate in many freshwater pulmonate snails (McMahon 1975(a). In general growth rates tend to increase with increasing temperature up to a critical temperature (usually near the upper limit) after which growth rate declines. Lambert (1989) showed the rate of individual growth and demographic parameters (survivorship, fecundity and net reproductive rate) of *Lymnaea peregra* are strongly influenced by temperature. The freshwater snail *Indoplanorbis exustus* showed very clearly (Raut *et al* 1992) an optimal temperature (30°C) for growth and number of capsules and eggs produced per week.

Table 4.2.1.5.1 reveals highly significant results ($p<0,001$):

(a) when all densities were combined, increasing temperature increased growth rate.

(b) at a density of 30, increasing temperature increased growth rate.

(c) when comparing temperatures of 15°C and 20°C a higher growth rate was seen at 20°C.

For the analysis of accumulated mortalities as opposed to growth, to see if either temperature or density affected mortality, it was necessary to find the time at which 50% mortality occurred within each replicate, using a detoured probit analysis (STATGRAPHICS). A two way ANOVA using these values at 50% mortalities, revealed that neither temperature nor density was found to have any effect on the time taken to reach the 50% mortality.

In order to calculate maximum ingestion rates for different sizes and temperatures, Streit (1985) showed the speed of rasping is a strong function of temperature, with the least time needed being at 22°C. Similarly the speed of

the radular movements, and hence the amount ingested per unit time, is a function of size, food concentration and temperature, with maximum ingestion at 25°C for the large limpets. When comparing production efficiencies of juveniles and larger limpets, he found the juveniles are better converters with a production efficiency of 83,9% at 10°C, but only 8% at 19°C for the large limpets.

These findings indicate difficulty in assessing the results obtained when comparing the growth rate of *Burnupia* at three temperatures. Duncan (1959) found that temperature was of fundamental importance in affecting the rate of development, and in influencing the sexual maturity and oviposition in *Physa fontinalis*; Streit (1975) showed for *Ancylus maximum* ingestion and assimilation rates were at 25°C, maximum growth and egg laying were at 19°C, maximum value of production/ingestion was at 13°C, and the lower limit of egg laying was between 7°C and 10°C. These results may also suggest that for any large scale production different temperatures may be necessary for the optimisation of the growth of different size groups.

Density

If we were to consider a natural population, and to decide what ultimate mechanism(s) determine the numbers in a population, one view is that they are regulated in a density-dependent fashion, and that this regulation is mediated through intra- and interspecific relationships which result directly from density feedback (Eisenberg 1966). Within this experiment, presuming the only variable within one temperature is density, a density-independent model must predict that convergence would not occur and that high and low density replicates would remain disparate in time. As various density-independent processes acted on the sub-populations, they would fluctuate, but a plot of the log densities with time would give a series of quasi-parallel lines. On the other hand, density-dependent models would predict that high and low density replicates would converge in time, although the level at which they converged would almost certainly be influenced by density-independent processes (Eisenberg 1966).

Chernin and Michelson (1957a & b) studied the effects of population density of *Australorbis glabratus*, and found that snails maintained under crowded conditions grew more slowly and were less fecund than those in less densely populated aquaria. They also found that, whereas a doubling of the number of snails in a given volume of water produced a marked reducing effect on the growth and reproductive potential of the individuals, a similar effect was not obtained by keeping the numbers constant and halving the water volume. In re-assessing these results, Wright (1960) showed that there is no single factor which can be held responsible for poor growth and lowered fecundity under high densities. He suggested three factors involved: (a) food, (b) collisions, and (c) chemical pollution.

(a) FOOD: Density can affect growth rate by limiting available food especially among grazers such as *Burnupia*. The effect of snail grazing on periphyton is manifested in a shift in the species composition and succession of periphyton assemblages (reviewed in Bronmark 1989). Succession of a periphyton assemblage generally proceeds from a monolayer assemblage dominated by small, adnate diatoms, to a more structurally complex community

with stalked diatoms and small or large filamentous algae (Steinman et al, 1987). An increase in grazing pressure will halt succession at an intermediate stage or earlier, relative to the pressure (Cattaneo 1983). Although the food requirements of *Burnupia* have not yet been investigated, Calow (1973, 1975), Schwenk and Schwoerbel (1973) & Streit (1975) found that *Ancylus fluviatilis* is a microherbivore, the preferred food being epilithic algae, particularly diatoms. *Burnupia* exhibits feeding behaviour similar to that reported for *Ancylus*, which is random rasping of the stone surface with the radula. Thus it seems likely that it's food requirements will be similar to *Ancylus*. During this experiment there was no consistent analysis of the periphyton assemblage. Visually there appeared to be very little differences in the replicates, with the presence of filamentous green algae in each replicate. Periodic scrapings of the surfaces of both the ceramic stones and the sides of each container revealed an abundance of both single and filamentous diatom species throughout the replicates, although their relative abundance was not calculated. Visually it appeared that grazing pressure was having very little effect on the periphyton assemblage at these densities. Thomas *et al* (1974), Muller (1983) and Wetzel (1983) remind us that nutrient concentrations contribute to the control of periphyton assemblages. Snails will alter the chemical composition of the environment by removing ions, amino acids, and oxygen, and by adding substances including ammonia, carbon dioxide, ions, mucopolysaccharides and organic acids. They claim that mollusc excrement or defecation increase nutrient availability, which in turn enhance algal productivity. In this way it overrides the effects of high density (and therefore grazing) on periphyton growth.

We conclude, with regard to food within this trial:

(i) initially food was not a limiting factor and is unlikely to have contributed to any differences between the three densities of limpet tested here.

(ii) After approximately 43 days the numbers of limpets in the high relative to the low density replicates had dropped to similar levels (see Fig 4.2.1.5) and thus the feeding pressure was likely to have been similar between all replicates. At this stage the periphyton consisted of a large proportion of filamentous algae. Streit (1985), in his work on *Ancylus fluviatilis*, showed there was an upper limit to the thickness of periphyton layer that this limpet could cope with because of the limitations resulting from the size of the radula teeth, of similar size to *Burnupia* (Oberholzer 1963). The thickness of periphyton is very likely to have been at a level where the limpets were limited in their food uptake, contributing to growth impediment as the trial progressed.

(b) COLLISIONS: Density dependent stimulation of tactile and visual receptors is a second possibility for density effects, suggested by Wright (1960). Close proximity of individuals of the same species with a resulting antagonistic behaviour is seen in a number of the Physidae, for example in *Physella virgata* (Brown, Carman, and Inchausty, 1994) but this appears not to have been recorded in the Ancylidae (Kawata 1993). Under natural conditions *Burnupia* are regularly seen in close proximity to each other on the same rock. Observations have shown that all sizes of individuals move very small distances, seldom even moving from rocks of approximately 500ml in volume, in natural streams (pers. obs.). The confines of the 500ml jars used in this experiment would therefore be an unlikely source of irritation to the *Burnupia* under any of the tested densities.

(C) CHEMICAL POLLUTION: Wright (1960) showed that chemical pollution by the snail *Bulinus forskalii* played a major part in affecting growth and fecundity. In a confined, static environment any chemical inhibitor that is emitted will have a more pronounced effect. Whether this inhibition of growth and survival with *Bulinus* is brought about by a chemical in their waste products or by some specific substance of a pheromonal nature has not been ascertained. Since Chernin and Michelson (1957a and b) were unable to demonstrate by chemical means any important differences in the composition of the water samples taken from the different densities they studied with *A glabratus*, it seems likely the active substance is at a very low concentration (and therefore possibly a pheromone).

This phenomenon of growth inhibition has been demonstrated in various other plant and animal groups. Overcrowding and consequent growth inhibition was reported by Rose (1960) in tadpoles. Growing tadpoles of *Rana pipiens* release more of this substance and become less sensitive to a given concentration of it. Thus large tadpoles will suppress the growth of smaller ones kept in the same container. The substance produced was species specific. Berrie and Visser (1963) showed very clearly with *Biomphalaria sudanica* (Martens) that a chemical is produced by the individuals that at high densities produces a growth inhibition. When artificially increased to just beyond the level that was found in natural pools, this chemical became lethal. They found that the greater the number of individuals, and the larger they grew, the greater the inhibition of growth. These conclusions were somewhat contradicted by the work of Thomas *et al* (1975) who showed that with *Biomphalaria glabrata* (Say) that an increase in growth of juveniles and natality rates was achieved with crowding *ie* by increasing snail numbers or by decreasing the volume available to critical thresholds. Further increase in snail numbers or a decrease in volume available to each snail beyond the optimum levels showed a decrease in growth and natality rates of the snails. In closed systems with equivalent densities of 20 *Burnupia* per 500ml water, they proved conclusively with this species that no inhibitory pheromone was produced by the snails which was toxic to growth or reproduction rates under this density, which appeared to be the optimum condition.

Unlike *Biomphalaria*, however, *Burnupia* is not naturally found in pools and stagnant water (Brown 1980) but under such experimental conditions, it would not be surprising to find that pheromones did in fact have a greater part to play on the growth of individuals than under natural flowing systems. On the basis of this, our results would be difficult to interpret. Initial growth of small individuals would show some variation. Would those larger than the average, within each container, then have a growth inhibiting influence on those smaller, by the production of one or more chemical factors? Examination of each single growth regression curve for high density replicates (graphs not provided) reveals a large disparity in the sizes of limpets surviving in each replicate. Survivorship within the high density replicates was much poorer than in the lower densities (see Fig 4.2.1.5), possibly also partially explained by these growth factors.

Table 4.2.1.5 shows highly significant results ($p<0,001$) when considering the density results:

(a) when combining all temperatures, increasing the density decreased the growth rate.

(b) at 15°C, D10 had a greater growth rate than D20 or D30.

(c) at 20°C a density of 20 had the greatest growth rate.

(d) at 25°C D10 had the greatest growth rate.

(e) when comparing the growth rate at all temperatures of D10 and D20, D10 was higher, but at a less significant value ($p<0,005$).

Conclusion to Section 4.2.1.5.

This experiment suggests that a temperature of 25°C and a density of 10 per 500ml water would attain the greatest growth rate for *Burnupia*. Although this trial was investigating the effects of temperature and density on the growth and survival of limpets, an important factor, when considering the propagation of these animals, is the effect that temperature and density will have on their fecundity. The mean number of eggs per egg case and the viability of those eggs should now be considered, under specified conditions of temperature and density. Chernin and Michelson (1957a and b), Wright (1960) and Eisenberg (1966) all showed in their laboratory results with Basommatophorans that there was a negative effect on the mean clutch size with increasing density. Streit (1985) suggests carbon partitioning between growth and egg production is also a function of temperature, remaining constant for *Ancylus* from 13°C to the lethal temperature. The situation within *Burnupia* needs to be investigated (see section **4.2.2.**).

4.2.2 REPRODUCTION AND FECUNDITY

The fecundity and breeding biology of an animal under given conditions is essential information in the establishment of an aquacultural programme. The family Ancylidae is recognised as hermaphroditic but there is no information in the literature on the reproductive biology of the genus *Burnupia.*

Questions that need addressing include:

a) How many egg-cases and eggs can be expected from a single or pair of adults and what is the range and average number of eggs produced during the period of egg deposition under given conditions? Is the sequence in egg-case deposition similar to that reported for *Ancylus fluviatilis*?

b) What is the duration of the egg-laying period and which cues initiate reproductive activity?

c) Is cross fertilisation essential to reproductive activity and viable eggs?

d) Is reproductive maturity of both male and female gonads sequential or simultaneous and at what age or size does maturity occur?

The following experiments aimed at answering these questions.

4.2.2.1 Egg Development

Aim

To ascertain from each egg capsule laid, the number of eggs within each capsule, the number of spat that emerge, the number of days to emergence and to ascertain embryological development on a daily basis.

Method

1) Adults were collected from the Blaauwkrantz River and conditions at the time of collection were recorded from midstream, the area from where the majority of adults were collected. Riverine water was used as the holding water for the adults, in an aerated basin at 19°C in the CER.

2) Numbered glass microscope slides 2,5cm x 7,6cm were previously prepared by placing in a recirculating stream where they developed a thin layer of green algae.

3) A series of 9 bubble pots (labelled 1-9) were set up. Numbers 4 and 5 were white 5 litre buckets, and the remainder were 500 ml transparent plastic jars. Each bubble pot was kept half-filled with water. A sling of white netting supported a prepared slide on which the adults were placed, in the water. The lids of the small pots were placed loosely on the top in order to reduce the evaporation rate of the water. The water used was from the Belmont River collected at the same time as the adults, and distilled water was used for any replenishment in order to maintain the levels of total dissolved salts. TDS, temperature and pH were monitored twice weekly. After 9 days the water was replaced with Palmiet River water and TDS, temperature and pH were

again noted.

4) One adult between 4,5mm and 6mm in length was placed on each slide. When an adult died it was replaced with either one or a pair of adults of approximately the same size.

5) Daily checks at approximately 08h00 were carried out to monitor newly laid egg capsules. When new capsules were noted the slide was moved to a labelled 500 ml bubble pot equipped as described above, in order to follow the individual development of each egg capsule. The adult in the original bubble pot was placed on a new, prepared slide .

6) The daily development of each egg capsule was recorded on a separate data sheet. Black and white photographs and coloured slides were made throughout the entire development of the capsules. Photographs were taken with a WILD Heerbrugg M400 microscope and magnification was usually 10 x 32 unless stated otherwise in the captions to the photographs.

7) The experiment was considered complete when no further egg laying occurred, and all the young had emerged. Adults were placed in the system on 1 February, the experiment proceeding for three weeks.

Results

See Table 4.2.2.1.

Summary of table

During the experimental period 26 egg capsules were laid of which 4 (15,38%) were accidentally damaged. These will be excluded from the remainder of the summary. From the remaining 22 egg capsules laid, 134 eggs were counted (average = 6,09/ capsule). These eggs yielded 122 spat which is a hatching rate of 91,04%.
The average period from lay date to hatching was 14,9 days (n = 21).

Water conditions

Table 4.2.2.1 Water quality parameters recorded during the experiment.

Parameter	River water	Laboratory water
TDS	506 mg/l	148-591 mg/l
pH	6.8	6.22-7.62
Temperature	23.1°C	19.0-23.4°C

Temperatures varied between 19°C and 23,4°C averaging 21°C in all the containers. There was a small variation between the pots at any one time despite identical ambient conditions. This may indicate that differences in the aeration rates between the pots affect evaporation rates and hence temperature.
Similarly, the turbulence created by the aeration is substantially less in pots 4 and 5 with larger volumes of water than the other smaller pots. Note that a greater number of eggs were laid in these two pots.

Oviposition and embryonic development

Most pulmonates exhibit direct development and emerge as crawling snails. In *B. stenochorias* as for other Ancylidae, the oviduct opens to the left, as seen in Fig.4.2.2.1.3 a) which depicts a copulating pair.

The Basommatophora show extreme variability in the processes preceding oviposition and these appear partly dependant on external factors. Observations have shown that Ancylidae, although hermaphrodite, do copulate, with usually the smaller (younger) individual acting as the male (Bondesen 1950). This has been observed in the artificial streams with *B. stenochorias* and it has been established that some individuals will lay eggs without the benefit of copulation (4.2.2.4). Chain copulation as was seen in Planorbidae and Lymnaeidae species (Duncan 1975) has not been observed with *B. stenochorias*. It has been observed that sexual development can vary from one hermaphroditic individual to another, but usually within the Ancylidae the male organs develop first (Duncan 1975). The sexual development of *B. stenochorias* will be elucidated when stained sections of individuals of known ages are studied in October 1995 (see section **4.2.3**).

Oviposition

Oviposition in freshwater snails is primarily dependant on sexual maturity and water temperature, and since growth and maturation are in turn dependant on temperature, this factor has a major role in controlling reproductive cycles (Duncan 1975). Brown (1967) shows that the distribution of the genus *B. stenochorias* is linked to the warmer regions of South Africa. Temperatures within this experiment varied from 19°C to 23,4°C.

In this experiment eggs were laid between 11h00 and 05h00. The majority of Gastropoda appear to oviposit during this time period although *Ancylus fluviatilis* (Muller) has been known to lay until midday (Bondesen 1950). Oviposition takes place underneath any substrate provided, but egg capsules are also deposited on the surface of the containers.

Table 4.2.2.2 The number of egg capsules deposited during the experimental period. The figures in brackets in the table represent the following: (one capsule)(no. eggs)(no. spat hatched)(no. days to hatching).

Date	Pot 1	Pot 2	Pot 4	Pot 5	Pot 7	Pot 9
2/2		1(1)(1)(14½)	1(8)(8)(13½) 1(8)(8)(13½)			
3/2	1(7)(7)(13½)		1(8)(killed)	1(9)(9)(15½)		
4/2	1(6)(6)(14½)			1(8)(8)(14½) 1(9)(9)(13½)	new adult	new adult
5/2	1(1)(0)					
6/2			new adult			
7/2		new adult		1(8)(8)(13½)		
8/2			1(5)(5)(15½)			
9/2		new pair	mating			new pair
10/2			1(8)(killed) 1(6)(6)(15½)			
11/2			1(5)(5)(17½)		1(5)(5)(14½)	1(4)(2)(15½) 1(3)(3)(14½)
12/2		1(7)(6)(13½)		1(6)(6)(17½)		
13/2				1(1)(1)(16½)		
14/2		dead adult				
15/2		1(5)(killed) 1(2)(killed)		1(6)(6)(15½)		
21/2			1(8)(8)(15½)	1(5)(5)(15½)		

Generally the size of eggs produced relative to the amount of albumen and yolk varies in pulmonate gastropods (Bondesen 1950). In *B. stenochorias* the area of the egg case is slightly smaller than the area of the shell of the fully developed egg. The freshwater pulmonates possess an external membrane, the continuation of which is referred to as the 'egg string', an important phylogenetic character. *B. stenochorias* does not have this feature which confirms it as a member of the family Ancylidae. The terminology of the egg capsules is shown in Fig 4.2.2.1.1.

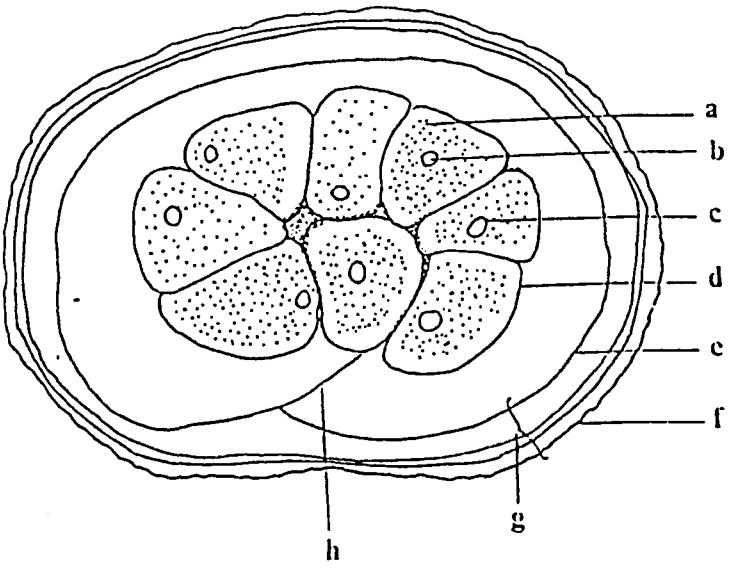


Fig. 4.2.2.1.1. Terminology of the egg capsule structure of *Burnupia stenochorias* (a = albumen, b = yolk (a & b = egg or ovum), c = yolk membrane (primary membrane), d = internal membrane (egg case or secondary envelope), e = internal capsule, f = quaternary envelope, g = external capsule, h = terminal tail).

The capsule of *B. stenochorias* is gelatinous, disc-shaped and slightly convex, 1,3 - 1,8 mm wide and 1,4 - 1,9 mm long. The quaternary envelope (as seen in Fig. 4.2.2.1.1) is unique to the Ancylidae and is an irregular membrane secreted by the foot, covering the capsule itself. This affords protection against parasites and foreign bodies. The resilient nature of the capsule perhaps makes it more suitable for laboratory handling than other non-Ancylidae species. The operculate suture which acts as a preformed line of rupture through which the young will emerge, appears to follow the edge of the capsule but can not be differentiated in our photographs. The capsule appears transparent immediately after oviposition, changing to pale yellow as it matures. The eggs are initially transparent and gradually become darker yellow as embryos develop. Because of the transparency of the structure it is extremely difficult to see with the naked eye on opaque surfaces. Since studying the egg capsules so intensively we have succeeded in observing eggs in the field.

The terminal tail of the internal capsule is closed and turned more or less over the initial point and covered by the external capsule, which indicates that *B. stenochorias* does not turn as it oviposits, a behaviour which is characteristic of most freshwater pulmonates. The eggs are not in a spiral formation as in the Lymnaeidae but are in one layer. The position of each egg relative to the others indicates the order of succession of lay, and when viewed from the upper side, the first egg is at the top, the second egg has its front edge under the rear edge of the first egg, etc. Each egg is of a polygonal shape and varies in number from 1 - 13 per capsule, although in this experiment, the maximum number recorded was nine.

Embryogenesis

The daily recording of the embryonic development revealed variations in the period of time taken for organogenesis and hatching from the capsule. No attempt was made to determine the cause or to quantify the extent of this variation. Development is reported here in 24 hour periods although observations were made more frequently.

The embryonic period consists of two phases, namely cleavage and organogenesis.

Cleavage phase

Hours 0-24; DAY 0-1

Cleavage of the cells take place. Blastulation occurs and micromeres and macromeres are formed. The embryo is uniformly pale yellow and appears to remain the same size.

Hours 24-48; DAY 1-2,

During the gastrulation phase the cells which are formed during the blastulation are differentiated into the three primary germ layers. The divisions of the macromeres and micromeres are not synchronous, and consequently the embryo rotates in the albumen.

Organogenesis phase

DAY 2.5-4.5, Fig 4.2.2.1.2a

There is an increase in the rate of rotation of the embryo. The colour becomes deeper and the shape elongates from its initial circular form. The albumen is slowly absorbed by the embryo so that it is approximately ½ to ⅓ the size of the egg case, and appears as a concentrated grey mass: the albumen is stored in albumen cell complexes which deepen the colour of the embryo's developing digestive organs to a golden yellow. The remainder of the embryo is grey. Differentiation between the digestive area and the podocephalic area is visible. The embryo increases in size in relationship to the egg, which indicates considerable mitotic activity.

DAY 4.5-5.5, Fig. 4.2.2.1.2b

There is a dramatic increase in the size of the embryo in relationship to the egg and a clear difference in colour and form between the digestive region and the podocephallic area. The globular nature of the embryo is changing with the podocephalic area distinct from the remainder of the body.

DAY 6.5, Fig. 4.2.2.1.2c

The embryo tumbles very slowly within the egg case relative to previous days. The tentacular /optic lobes begin differentiating. The albumen continues to decrease in volume.

DAY 7.5, Fig. 4.2.2.1.2d

Tentacular lobes develop and there are visible signs of the eyes. A faint heart pulse is observed in a few individuals.

DAY 8.5 The eyes are red and clearly visible. Heart beat is obvious in the most developmentally advanced specimens with vortices of fluid movement around and within the embryo. Relative to other gastropods, the pulmonates are characterised by a relatively early organogenesis of the radula (Moor, 1983) and in *B. stenochorias* the position of the radula can now be seen in some individuals. Shell development can now be seen clearly, just covering the organs posteriorly and appearing to grow towards the anterior end

to eventually cover the head region. From the ventral side the foot can be seen developing. The albumen has been almost totally absorbed. There is considerable individual variation in developmental rate.

DAY 9.5 The internal membrane begins to collapse. Heart beat and the vortex movement of fluid are both easily observed. Further development of the shell shows the initial laying down of the circular patterns seen in the adult.

DAY 10.5 Fig 4.2.2.1.2e

The shell appears almost complete in the development of its circumference, deepening in colour to a golden brown as the striations develop. The inner egg membrane continues to collapse.

DAY 11.5

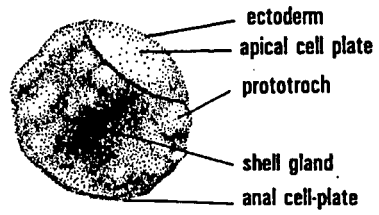
The embryos are all actively moving, bumping into each other. The shell colour deepens, and the rim now completely covers the entire animal. There is a strong heart beat and the radula is moving, while vortices of fluid movement are seen within the body. Remnants of albumen are still visible.

DAY 12.5-13.5

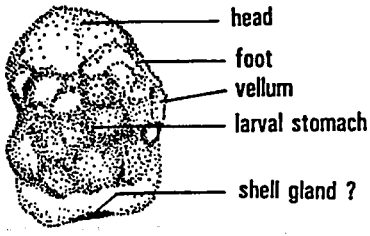
Movement of radula and heart is stronger and the larvae are active, occasionally turning over in their position. Internal membranes can still be seen. During this period the first hatchings occur from the egg cases.

DAY 14.5 The shell becomes more domed in shape. This is the average hatching date for this population. All internal membranes have collapsed.

DAY 15.5 Individuals are immobile prior to hatching from the capsule. Emergence from the capsule occurred in this experiment in the early hours of the morning until approximately 08h15, emerging as crawling snails, as seen with most pulmonates (Fretter and Peake 1975). However, unlike the *Ancylus* species, there is no lid opening; instead the larvae crawl under the external capsule, apparently by nudging head and shell against the edge until it lifts. As each individual emerges (with approximately 10 minutes between each one) it appears easier to move under the capsule and out. There appear to be no egg remains in the capsule.



a)



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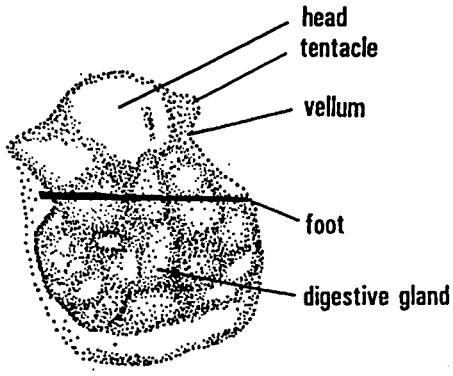

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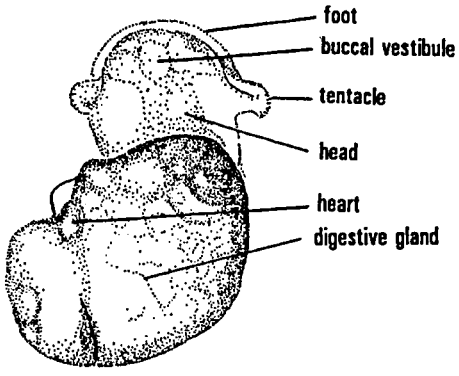

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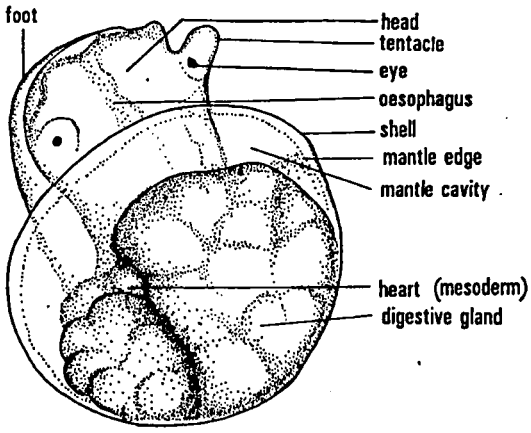

e)

Fig 4.2.2.1.2 a) Embryo at about 96 hours old. Early organogenesis. b) Embryo at 5.5 days, body is elongating. c) In embryos at 6.5 days and d) at 7.5 days the adult morphology can be

discerned.

Discussion

This general overview of the major developmental events which take place in gastropods has been compiled from Raven (1975) and Moor (1983). Observations made during this work is related to their information. The embryos are very small and under available magnification details of the development, especially in the early stages of blastulation, were difficult to distinguish.

At the third division of the cells, spiral cleavage results in the formation of animal micromeres and vegetative macromeres. It has been elucidated in earlier studies that determination of the fate of micromeres and macromeres takes place at the very early stages of blastulation (after the third division). Cleavage gives rise to a coeloblastula with a wide cleavage cavity. Gastrulation takes place by invagination of the archenteron at the vegetative pole. The embryos at 2 days old depict this stage. The eggs of pulmonates in the cleavage stage begin to ingest albumen in all the cells. This was seen during day 2. The albumen is laid down in the ectoplasmic part of the cells in special albumen vacuoles, thought to be by a process of pinocytosis. After gastrulation, the uptake of albumen is more and more restricted to the endoderm. Part of the endoderm develops into the albumen sac, or larval digestive gland, which plays an important part in the uptake and digestion of albumen.

Organogenesis takes place in the following sequence:
The trochophore stage is where the dorsally situated head vesicle, the median apical plate and the ventrolateral cephalic plates are differentiated. During days 3-5 this differentiation became visible in the embryos. From the median apical plate the cerebral ganglia, tentacles and eyes develop. As in most other animals, the nervous system is mainly of ectodermal origin.

Before gastrulation is complete, cells in the dorsal (post trochal) area of the embryo enlarge to form a prospective shell field (Fig.4.2.2.1.2a). This area invaginates to form the shell gland, a narrow pit with a circular opening. The cells which secrete the periostrachum (early shell) are brought close together at the rim of the shell gland and thus the shell is formed with no hole in it. Subsequently the shell gland evaginates again and the shell spreads. The shell producing cells divide mitotically and the shell grows. In gastropods the shell field is saucer-shaped and this is visible in Fig.4.2.2.1.2b. The shell gland gradually changes to the mantle, from the edge of which the principal growth of the shell takes place (Fig.4.2.2.1.2e). The mantle edge therefore also contributes to the development of the mantle cavity.

The lung primordium arises posteriorly as an ectodermal invagination and the mantle cavity later surrounds this invagination and gradually the pneumostome forms. The walls of the pulmonary cavity fold to enclose adjacent blood vessels forming the vascular reticulum of the lung. This development was not observable under binocular microscope.

The foot originates as an ectodermal thickening on the ventral side behind the mouth at an early embryonic stage and only later an outgrowth covered with cilia appears, clearly seen during day 8.

The invagination of the stomadeum (mouth) starts on the ventral side and from there the remainder of the digestive system is formed. Basommatophora have a larval digestive gland, as described above, that disappears at the end of the embryonic period. The development of the radula starts early in embryonic life and by the time hatching takes place several radular teeth have already been lost. The earliest visible signs of the radula were during day 8.
The archenteron is the primordial lumen which later develops into the mid- and hindgut, and in the Ancylidae is lined with giant cells which contain the ingested albumen. The pronephridia consist of 2 - 4 cells with the efferent duct consisting of three cells.

The environmental factors which may influence egg laying include the level of turbulence and the TDS of the water. It was observed but not quantified that in the pots where the bubbling was the most severe, no eggs were laid. Two further interesting points emerged with regard to the TDS levels of water:

(a) pots 10 (8 eggs), 11 (1 egg), 12 (7 eggs), 18 (1 egg) had a TDS of 500. Only pot 18's single egg did not hatch.

(b) No egg capsules were laid where TDS remained below 95.

Future work should investigate the effect of turbulence and TDS levels on egg laying and hatching rate.

4.2.2.2 Capsule Size Distribution (in relation to number of eggs per capsule)

Aims

To establish the range and average size of egg capsules (number of eggs within each) laid by adults collected from the field, and to establish the developmental time from laying to hatching of the same capsules at 17°C.

Method

1) Approximately 300 limpets were collected in the field from the Blaauwkrantz River on 10 October 1993. The population was placed in a 44cm diameter plastic basin with natural stones, as well as artificial substrates which were covered with laboratory grown periphyton. The basin was maintained at ambient light and temperature conditions (16±2°C - 23±2°C, 14h00 photoperiod) with water from the river which was aerated.
2) After 12 days, 18 stones and tiles which had periphyton cover were removed from the basin and placed in six 5 litre bubble pots in the C.E. room at 18 °C and 14h00 photoperiod. The bubble pots, labelled 1 to 6, contained a mix of river water and conditioned tap water. The water was kept at the same level by the addition of both mature tap water and distilled water to maintain TDS and pH at a similar level.
3) Every second day the substrates were examined and the development of the capsule and

eggs noted. Before and after hatching the population in each container was counted and a sub-sample measured under a Nikon stereo-microscope equipped with an eyepiece micrometer. The measurements continued at two week intervals for four months, the last being taken at 139 days. The early counts of the population once the juveniles had started moving around were inaccurate as the animals were small (0.8 - 1.0 mm shell length) and pale in colour which made them difficult to see with the naked eye, particularly once they moved onto the container. Early numbers are therefore extrapolations from later, more accurate, numbers of survivors.

Results

Table 4.2.2.2.1 Water conditions prevailing in disturbed pots.

CONTAINER	1	2	3	4	5	6
Ave TDS	422.3	374.4	332.5	335.1	336.6	381.1
STD	115.7	93.1	87.2	93.8	102.8	100.1
MAX	679	650	650	565	606	602
MIN	248	271	227	220	230	253
Ave pH	7.54	7.45	7.56	7.6	7.53	7.47
STD	0.53	0.50	0.58	0.64	0.60	0.57
MAX	8.23	8.16	8.38	8.48	8.31	8.21
MIN	6.69	6.59	6.62	6.65	6.75	6.76

Table 4.2.2.2.2 Water conditions in undisturbed pots.

CONTAINER	H	I	J	K	L	M	N	A	B	C	E	F
Ave TDS	234.6	255	185.7	209	222.6	314.9	263.35	402.7	396.3	331.5	237.5	182.5
STD	18.62	13.41	195.92	23.52	20.68	97.62	36.36	56.90	62.27	42.11	49.03	195.26
Max.	255	274	739	249	275	453	314	490	555	381	373	700
Min.	210	245	67	171	187	0	201	312	281	242	141	75
Ave pH	7.57	7.32	7.6	7.4	7.4	7.3	7.3	7.36	7.53	7.15	7.66	7.68
STD	0.43	0.40	0.30	0.32	0.32	0.33	0.37	0.38	0.44	0.25	0.64	0.24
Max.	8	7.9	7.9	8	8	8	8	8	8.1	7.5	8.9	8.1
Min.	7.13	7	7.01	7.06	7.07	7	7	7	7	6.9	7.1	7.4

Table 4.2.2.2.3 Number of egg capsules, average number of eggs per capsule and total number of eggs examined in each container.

Container	Egg Capsules	Eggs	Average (std) Eggs/Capsule
1	60	285	4.8 (2.45)
4	51	276	5.4 (2.77)
2	35	184	5.3 (2.27)
3	41	229	5.6 (2.91)
5	13	67	5.2 (2.41)
6	9	55	6.1 (2.92)
TOTALS	209	1096	5.3

Average number of eggs in total number capsule = 5,4 (1-12).

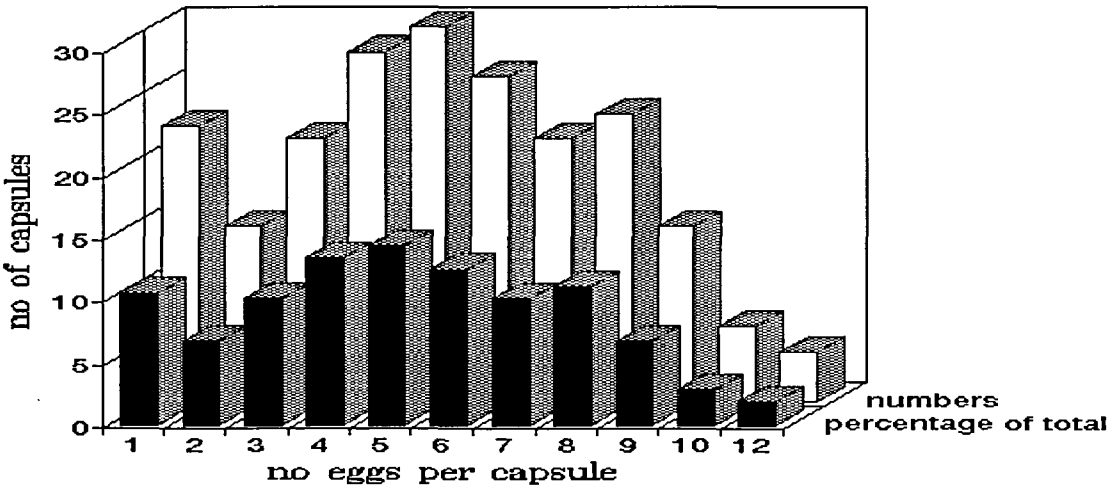

Fig 4.2.2.2. Frequency distribution graph of the range and frequency of size of capsules deposited in late spring to early summer (October 1993).

Initially the capsules are totally transparent, but after an average of six days the eggs within the capsule become visible and can be counted. See Fig 4.2.2.2.2 and Tables 4.2.2.2.3 and 4.2.2.2.4. Note the low number of capsules containing 2 eggs and the relatively larger number containing only one egg (Fig. 4.2.2.3). Although not reflected in the bar graph the largest number of eggs per capsule found was 13.

Developmental period

If one assumes that egg-laying commenced on 11 or 12 October, by 22 October the oldest embryos were visible and hatching commenced between 1 and 3 November, 21-24 days after capture. The results obtained in the section on development showed that the incubation period is 14-17 days but the longest period recorded to date is 19-20 days. This would put the earliest lay-date in this copulation at 15 October. The last possible lay-date, therefore, is 21 October which gives a range in age of 5 days. Most hatching was complete by 8 November. If these eggs were deposited between 19 and 21 October the developmental time was 18 days.

Table 4.2.2.2.4 Dates of developmental events noted in six sample populations of egg capsules. E L-D = earliest lay date; L L-D = latest lay date; E Hatch = date of earliest hatch; E Days = number of days to hatch; 50% H-D = date when 50% hatched; 50% Days = number of days to 50% hatched.

Rep	E L-D	L L-D	E Hatch	E Days	50% H-D	50% Days
1	16.10	21.10	2.11	17	8.11	19
2	15.10	21.10	1.11	17	7.11	19
3	15.10	21.10	1.11	17	8.11	20
4	14.10	18.10	31.10	17	3.11	17
5	16.10	21.10	2.11	13	7.11	18
6	16.10	21.10	1.11	17	8.11	18

Size and age

Because the late embryos are easily observed through the eggcase prior to hatching accurate measurements were obtained for the pre- and post hatching period. When eyes can be seen the embryo is usually between 0.3 - 0.5mm long. Sizes of 14 to 17 day old embryos immediately prior to hatching ranged from 0.32 - 0.72mm. At this stage the shell is golden brown in colour. At hatching sizes range from 0.65 - 0.8mm. At a shell length of 1.2mm, the shell is a golden brown colour. At about this size the shell darkens and the first transparent rim of the newly deposited shell can be observed by 1.3mm.

Conclusion

The number of eggs in each capsule varied from 1 to 12 (occasionally some capsules containing 13 have been noted), with an average of 5,4 per capsule. Developmental time under these conditions of temperature, pH and TDS was on average 18 days.

4.2.2.3 Fecundity in the Laboratory Under Different Temperatures

Aims

To establish the fecundity of *B. stenochorias* under different temperature regimes by determining the number of egg-capsules and eggs a mature adult limpet produces: and to determine if fecundity is affected by short-term temperature changes.

Method

1) 6 x 5litre bubblepots each had a sling of white netting suspended from the rim onto which was suspended a ceramic tile 6cm x 6cm. All tiles had a covering of periphytic growth. Each bubblepot was half filled with water from the river of origin, covering the tile, and was stabilised for two days before the adults were introduced.
2) Experiment (A) had two adults of similar size which had been taken from the natural habitat placed on the tile after their length had been measured. Experiment (B) had four adults of similar size placed on each tile. In both cases adults that died were replaced by similar sized animals in the initial stage of the experiment. When no more adults were available or more than 50% of the adults had died the experiment was terminated.
3) Observations were made every second day. The egg capsules deposited on both the ceramic tiles and the sides of the pots were counted and the number of eggs noted.
4) Both experiments were conducted under three temperature regimes: (A) 19°C, 25°C and 14h00 photoperiod. (B) 15°C, 20°C and 14h00 photoperiod.

Results

Table 4.2.2.3 Summary of results obtained from two experiments to determine the reproductive capacity of *B. stenochorias* in the laboratory.

Treatment	Total adults	Total		Ave eggs /adult	Ave eggs/ treatment	STD.
		Capsules	Eggs			
22°C (amb.)B	23	134	395	17	43	38
15°C B	16	49	184	12	26	11.5
20°C B	17	81	384	23	64	113
20°C(amb.) A	15	34	178	12	25	n/c
19°C A	19	61	335	17	48	n/c
25°C A	9	13	72	8	10	n/c

Despite the use of the sling the limpets occasionally moved onto the sides of the pots where a large number of the eggs were laid. This caused difficulty in determining the number of eggs in each of these capsules. Table 4.2.2.3 shows the number of adults used in each of the three temperature regimes for the

entire duration of the experiment, the total number of eggs produced by these adults in each of the treatments, the average number of eggs per adult per treatment and the standard deviation in each of the treatments.

The possible number of eggs produced by an adult ranged from 4 to 49. As four eggs per adult was clearly an unlikely result in view of the largest possible number these were not included in the frequency distribution but were included in the assessment of the standard deviations.

Discussion

Both fecundity experiments produced inconclusive and widely variable results. The number of cases per adult limpet ranged from one to five but no conclusions could be drawn from this experiment with regard to the fecundity of this species. Further experiments were required before any substantial information and conclusions could be drawn. The cause of the high adult mortality was not determined, but is likely to have been the chemistry of the water. Such premature death of an adult does not give a true reflection of its reproductive capacity.

The findings of Appleton and Eriksson (1984) and de Kock and van Eeden (1986) indicate that *Biomphalaria pfeifferi* would benefit under laboratory conditions from a temperature regime with a daily fluctuation of more than 10°C. Constant temperatures in previous experiments (de Kock and van Eeden, 1981) revealed that the highest values for different population parameters such as natality, survival and growth rates were generally attained at different constant temperatures. With *B. pfeifferi* the lower temperatures, as a rule, favoured longevity while the higher temperatures favoured growth rates and natality. This suggests that a circadian temperature fluctuation within a breeding system for *Burnupia* may favour all physiological activities. The importance of combining the temperatures experienced with day-length hours to give Degree Days, a known physiological activator, is emphasised here. The results of the analyses of the field data of ambient conditions, combined with the data pertaining to size, relative numbers and egg-laying presently being accrued (section **4.2.3**) should be linked to the fecundity experiments.

4.2.2.4 Eggs from Single Limpets

Aim

To determine whether unmated individuals will lay eggs, how many they will lay and the frequency of laying.

Method

1) On 30 September 1994 single limpets taken from the Blaauwkrantz River, from 2,5mm in length, were placed in 500ml bubblepots together with two ceramic stones which had previously been allowed to grow periphyton. The pots were kept at ambient temperature

and light in the laboratory.

2) By 28 November 1994 all limpets had a shell length of 3mm or longer, and the first eggs were laid (not necessarily in all the pots).

Results

See Table 4.2.2.4.

Average capsules per individual (#10) = 6,7. Average eggs per individual (#10)= 20,7

Average laying frequency (# 7) = 5,8 days. Average size at initial laying (length) = 4,4

Average water pH = 6,64 (6,51-7,21) and TDS = 183 (144-228)

Table 4.2.2.4 Summary of the results of the egg-laying capacity of single limpets.
Size = length of each individual from 28 November 1994 until 19 February 1995 at the end of the experiment, or at the time of death; Size laying = size (mm length) at which laying began. Caps = no. of capsules layed. Frequency = average no. of days between each capsule laid, from first day of lay. * = smallest size at which laying occurred. Hatchlings = total number of young which grew to 1mm in length.

Pot no	size mm	Size laying	Date died	Caps	Eggs in capsules	Total no eggs laid	Frequency days	Hatch lings
1	3-4	-	14.2.95	0	-	0	-	-
2	3,6-4	3,6 *	20.1.95	17	1-5 ave.3,6	62	3,6	26
3	3,7-4,3	4	11.1.95	6	1-8 ave.3,8	23	8,3	10
4	3,5	-	12.12.95	0	-	0	-	-
5	3,7-6	5	19.2.95	7	1-6 ave.3,2	22	7,5	22
6	4,4-5	-	14.2.95	0	-	0	-	-
7	4,4-5	4,7	28.1.95	11	1-4 ave.2,3	25	3,2	24
8	3,5-4,6	4,5	19.2.95	4	1-3 ave.1,5	6	6,2	1
9	3,5-4,5	4,5	-	5	1-3 ave.1,75	9	7,2	1
10	4,2-5	4,5	14.2.95	17	1-6 ave.3,5	60	3,6	27

Discussion

Observations have shown *Burnupia* to be greater than 3mm in length when mating, therefore it is unlikely that these limpets had mated before being brought from the field and separated in the laboratory. Water conditions were acidic with low TDS levels when compared to the natural conditions of the Blaauwkrantz River (see Section **4.2.3**): this may have been the cause of premature death for the limpets, thus potentially decreasing the total number of capsules laid per individual within this experiment. Approximately 70%

of the limpets laid, at this time of the year, without mating. Of those that laid, all were 3,6mm or greater in length. There was a great variability in the number of capsules and eggs laid, which has been a consistent observation through all the *Burnupia* experiments.

4.2.2.5 Eggs from Mating Pairs, July 1994

Aim

To determine the number of eggs laid by mating pairs.

Method

1) Large numbers of limpets 3mm in length were collected from the Blaauwkrantz River and maintained in the laboratory streams.
2) Those pairs that were then found mating (11) were carefully removed from the streams, measured for length, and placed, still as mating pairs, in 500ml bubblepots. They were kept in tap water under laboratory conditions of ambient temperature and light.
3) The number of capsules and eggs laid was monitored.

Results

Table 4.2.2.5 Number of eggs and capsules laid by mated pairs. Male = length (mm) of "male" in mating pair, at the time of mating. Female = length (mm) of "female" in mating pair, at the time of mating.

Pair no	No of capsules	No of eggs	Male mm	Female mm
1	44	160	5,15	4
2	24	68	4,3	4,15
3	20	85	4,6	5,3
4	24	130	5	5,7
5	21	92	5,15	5,4
6	30	134	4,15	4,9
7	19	91	4,7	4,6
8	21	84	4,4	4
9	5	16	3,4	4
10	28	98	5	4
11	14	44	3	4
Average	22,7	91,1	4,2	4,55

Average laying frequency = 2,2 days

Water Averages; pH = 6,9 (6,2-7,4); TDS = 135 (119-188); temperature = 17,6°C (14°-23°C)

Discussion

It has often been observed that when moving Ancylidae from natural conditions into the laboratory, Ancylidae will immediately mate and invariably lay eggs (Calow 1978). This was the case here. As can be seen from Table 4.2.2.5 the sizes of the limpets within each mating pair differed considerably. With the exception of one, all the limpets were 3,4mm in length or greater, and as personal observations have shown limpets greater than 3,4 are capable of laying eggs, it seems very likely that many of these limpets had already laid eggs in the field before they were collected, an important factor when considering the total potential offspring of each pair, or each individual. Similarly, many of these limpets could have already mated in the field, before again mating in the laboratory. The effect this has on the fecundity of each limpet is unknown, particularly as the Ancylidae possess the ability to store all allosperm (from a second limpet) in their spermatheca . This will be discussed further when the field and sexual development trial, section **4.2.3**, is completed in 1996.

Nevertheless, the results of this experiment can be compared to that in single limpets (section **4.2.2.4**) and pairs of limpets (section **4.2.2.6**).

4.2.2.6 Eggs from Paired Limpets, March 1995

Aim

To determine the number of eggs laid by a pair of limpets, not necessarily having mated.

Method

1) 25 pairs of small (1,8-2mm length) limpets were collected from Blaauwkrantz River and placed in 500ml bubblepots, together with 2 ceramic stones previously allowed to grow periphyton, and tap water.
2) When a limpet died it was replaced with the same size individual which had been taken from the Blaauwkrantz River at the same time. Water was replaced every week.
3) This is an on-going experiment. The number of capsules and eggs laid are monitored, as well as the occurrence of any observed mating.

Results

For the first 30 days the mortality was 60%. Although each dead limpet was replaced, this did not include sizes greater than 3,5mm, the sizes at which mating occurs (pers. obs.). Any eggs which were produced were either a result of the pair mating, or a result of self-fertilisation. Only 2 of the 25 pairs were observed to have mated (however, observations were only made during office hours). Capsules were first laid on 4 May 1995, 55 days after collection from the field.

Water Averages; pH = 6,7 (6,0-7,47); TDS = 146 (116-201); temperature = 17,4°C (15,1°-19,5°C)

To date, 50% of the limpet pairs have not laid capsules (the data shows the 12 that have laid). The length of time an individual lived after the final replacement at 30 days varied considerably. At 91 days (9 June) 25% of the limpets had died, and this figure was 42% after 102 days (20 June) leaving 21 of the original 25 pairs as single limpets. Although on-going, no eggs have been laid since 20 June 1995.

Table 4.2.2.6 Summary of the results of the egg-laying capacity of pairs of limpets. Size laying = size (mm length) of the smaller of the pair at which laying began. Capsules = no of capsules laid. Frequency days = average no of days between each capsule laid, from first day of lay. * = smallest size at which laying began.

Pair no	Size laying	Capsules	Eggs in capsules	Total no eggs	Frequency days
1	3,7	16	1-5 ave.3,5	53	3,6
2	3,5	9	2-4 ave.3,1	28	3,3
3	3,5	10	2-5 ave.3,8	38	2,1
4	4,2	12	2-6 ave.3,5	41	4,3
5	3,5	1	3	3	-
6	3,5	9	1-4 ave.2,3	21	2,3
7	3,7	6	1-3 ave.3	17	5.2
8	3,6	14	1-5 ave.3	42	1,6
9	3,7	10	1-5 ave.3,1	31	5,4
10	3,4 *	4	3 ave.3	12	3,3
11	4	2	2-3 ave.2,5	5	6,5
12	3,8	4	2-4 ave.3	12	4,5
Total average	3,8	8,1	2,88	25,3	3,8

Average capsules per individual = 8,1. Average eggs per individual = 25,3
Average laying frequency = 3,8 days; Average length at initial laying = 3,8 mm

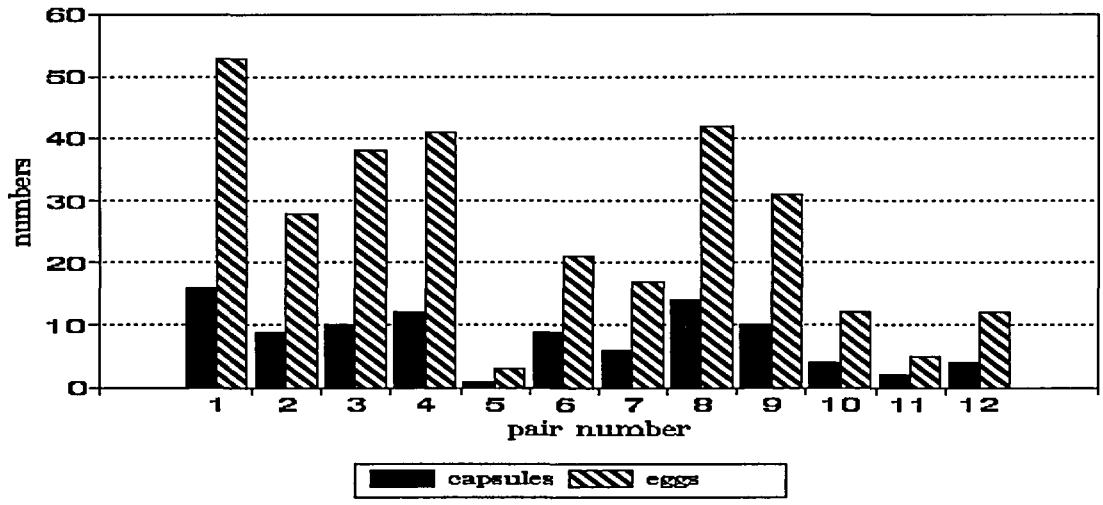

Fig. 4.2.2.6 Graph showing the number of capsules and eggs laid by the mating pairs, March 1995.

Discussion

The pH and TDS values are low when compared to the Blaauwkrantz River values. To date, none of the limpets have grown larger than 4mm, the majority being 3,5mm or less, and it is suggested that these water conditions are having a significantly detrimental effect on both the growth and fecundity (Macan 1961, Dussart 1979). Although the water conditions of the two pair experiments (this and section 4.2.2.5) were very similar, the number of capsules and the number of eggs per capsule are significantly different, that of the mating pairs showing the greater fecundity. This difference may be due to the size of each limpet, the mating pairs being larger; or as a result of decreased fecundity from being reared in the laboratory. The number of eggs per mass in some pulmonates is also related to the quality and quantity of food available to the snails (McMahon *et al* 1974, Hunter 1975, McMahon 1975a): this may also account for differences between the two pairs experiments, where the mating pairs had a natural, more ideal food supply before and during maturation of their reproductive parts, compared to the pairs which had been grown in the laboratory. When they are completed, the results of the field study should therefore be compared to these.

4.3 NATURAL POPULATIONS

Introduction

It is necessary to investigate the natural reproductive cycle of any animal before it can be bred in the laboratory. The patterns of reproduction which emerge from field studies may not necessarily reflect a typical cycle, but may answer many questions, such as: what time of the year, and how often does the species reproduce. These are two vital points when considering rearing in the laboratory. It is appreciated that snails in particular show interpopulation variations in individual growth (Russel-Hunter 1961, Hunter 1975), age and size at reproduction (Brown 1985), fecundity (Browne 1978, Aldridge 1982) and other life history traits (Russel-Hunter 1978, McMahon 1983). There are two possible, non-mutually exclusive explanations, according to Lam and Calow (1989): (i) the differences could be a result of microevolutiuonary adjustments in isolated populations to local selection pressures, in ways that can be predicted by life history theory; (ii) interpopulation divergences might entirely be an expression of developmental plasticity in response to proximate environmental differences.

Very few species within the Ancylidae have been studied in southern Africa and life history variations have not been studied within the genus *Burnupia*. The majority of the *Burnupia* species are found in Africa, south of the Equator, with a small pocket to the north of the Equator (Brown 1980). However, much of the available literature dealing with Ancylidae and other freshwater pulmonates refers to northern hemisphere molluscs living under temperate conditions. Those species closely related to *Burnupia* will provide basic guidelines to various aspects of any investigation into *Burnupia*, but will not provide life history patterns.

Method and Sites

(1) This field study began in February 1995 and is on-going.

(2) Two sites on the Blaauwkrantz River system were chosen because of their easy accessibility. The first, in the Belmont Valley 9 kms from the centre of Grahamstown (referred to as BV), is partially shaded by trees and a steep bank on the north-eastern edge. This site has a bed of small rocks and large boulders (ranging in longest length from 6cm to 41cm) and current speeds ranging to date from 0,32 to 1m/sec . The second site at Manley Flats (called MF) is approximately 4km downstream with grassed banks and considerable light incidence throughout the day. The bed is of fine silt with boulders ranging from 17-38cm longest length, and current speeds ranging to date from 0,06 to 0,42m/sec.

(3) Individuals are measured biweekly to the nearest 0,5mm, and 300 recordings are taken, which includes the number of eggs. At MF these are found on 3 or 4 rocks: at BV many rocks are sampled in order to reach this number. Each rock is lifted out of the water and all individuals are measured *in situ* using a template of washed photographic film into which measured holes have been punched. The rock is then replaced. Biweekly measurements of TDS, $\%O_2$, pH, temperature and current speed are also taken.

(4) 6-10 limpets ranging in size from 2,5mm to the 6mm are removed from outside the measuring sites, and their shells are measured for length, width and height before being preserved using Bouin's fluid, for later mounting in wax, and sectioning in order to monitor the sexual development of cohorts throughout the year. The sectioning of limpets will begin in October 1995, when the development of the penis, the maturation of the ovotestis and the bursa copulatrix will be analyzed.

(5) From August 1995, samples of egg capsules from each site, away from the area of rocks which are measured, are brought back to the laboratory, still attached to the rock, where they remain in aerated water until the number of eggs within each capsule can be counted. These rocks are then returned to the stream.

(6) Three further sites have been chosen which will be monitored in the same way, excluding measurements of sexual development, every 3 months in order to encompass a wider variety of field site conditions.

(7) From October 1995, biomass of periphyton at the MF and BV sites will be estimated using a method similar to that of Lam and Calow (1989), in order to monitor seasonal and site variations. Similarly gut contents will be monitored, to a limited degree because of the lack of specimens which can be removed and dissected from the BV site.

Results

Daily measurements taken during late February at both sites determined that *Burnupia* moved very little from one rock to another, suggesting biweekly measurements would very likely be measuring the same individuals each time, with any recruitment from other rocks upstream or nearby being minimal.

No results have been included from the three sites which will be monitored every three months, as there is insufficient data to date. Neither is there any data showing daylight hours, as this will be considered when all field work has been completed.

Table 4.3.1 Water conditions at the two sites, from February to August 1995.

Site: Blaauwkrantz R.	Factor	Maximum	Minimum	Average
Manley Flats	Temp °C	21,8	9,5	14,1
	TDS	842	284	632
	pH	8,4	5,7	7
Belmont Valley	Temp °C	22,8	9,5	14,1
	TDS	843	401	643
	pH	8,3	5,7	7

Fig 4.3.1 and Fig 4.3.2 show the changes in current flow and water temperatures (monitored at the time

of sampling) at Manley Flats and and Belmont Valley.

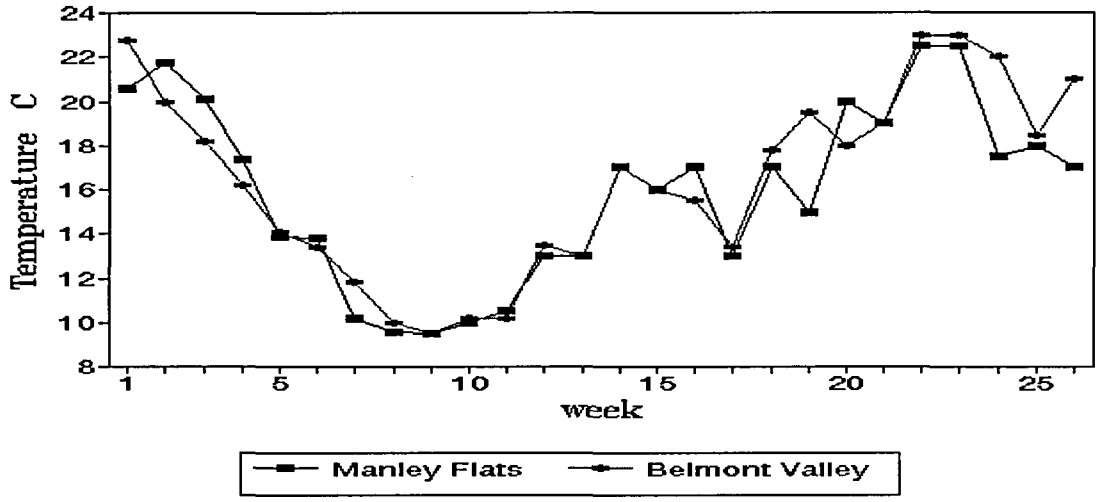

Fig 4.3.1 Changes in temperature (°C) at the Manley Flats and Belmont Valley sites.

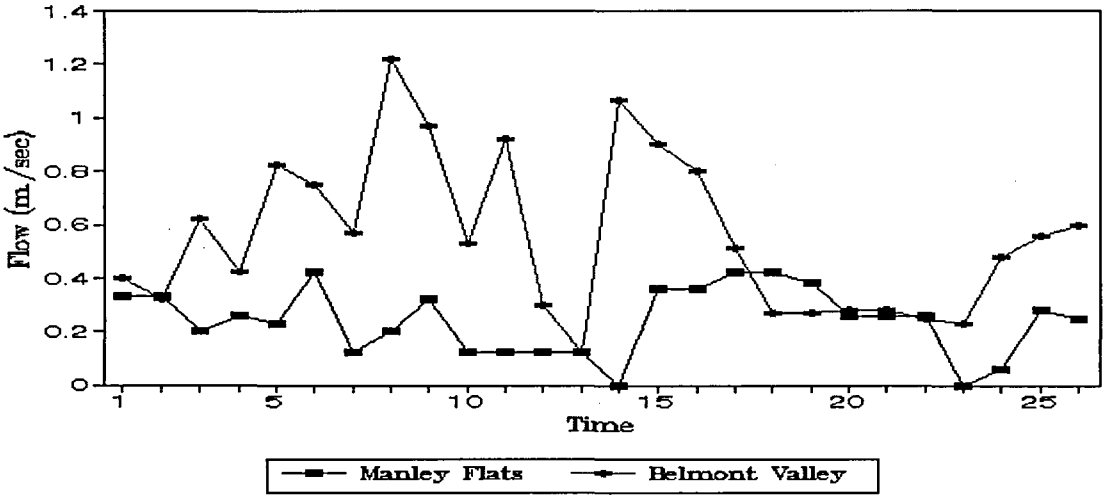

Fig 4.3.2 Changes in flow rate of water at the Manley Flats and Belmont Valley sites.

The results given in Fig 4.3.3 and Fig 4.3.4 show the numbers of limpets found at each site, with each graph depicting the month's samples. Each bar represent the number of limpets measured during the biweekly sampling. The size 0mm indicates the number of egg capsules. Because the samples were taken biweekly, there are three sample dates within the month of March. Only one sample was taken in July, with previous and post sample dates being 23/06/95 and 3/08/95 respectively and this sample is included in the June graph.

No attempt has yet been made to date to identify periphyton species. However, visual observations at the two sites (MF and BV) show a great difference in the algal growth. The MF site has no filamentous growth greater than 2mm on any of the rocks. The epilithic growth appears minimal and is very difficult to detect on any rock in comparison to the BV site, only appearing (dark green) when the river flow dropped considerably after 10 July (see fig 4.3.1). The BV always has growth on every rock, most

having filamentous growth (with filaments up to 60cm in length) and black epilithic, sometimes slimy growth where the limpets are to be found.

The monitoring of eggs within each capsule has proved to be very difficult, mainly because of the inability to magnify the capsules sufficiently in order to differentiate them in the capsule: microscopes cannot be used as the rocks do not fit under the eyepiece. Only scattered results have therefore been accumulated which will be presented in the final write-up of this field trial.

Discussion

When considering the algal growth of the two sites, there is likely to be a difference in the suitability of the food present, with the BV providing less suitable species for the limpets to consume: as previously discussed, the first storey, high diatom content, algal layer is preferred by the Ancylidae (Calow 1973). Filamentous algae are difficult to consume (Calow 1975). When comparing the maximum sizes attained by the limpets at the two sites, it can be seen in the graphs that those at MF are larger than at BV, to date, perhaps due to this difference in food. This will be analyzed in greater detail from October 1995, and should lead to a key factor when considering propagation of limpets in the laboratory.

This preliminary analysis of the sizes attained in the field (plotting frequency bar graphs, or modes and means of successive samples) is of limited biological significance because populations are composed of individuals that belong to different cohorts. Determining the peak times of egg laying, or the number of generations per year using the graphs depicted in fig 4.3.3 is extremely difficult. It can be seen, however, that there are eggs present throughout the sampling period, at both sites, despite the cold temperatures of June and July (interval 8,9 and 10 on graph fig 4.3.2). Observations of copulation, which may give an indication of peak egg-laying periods, are infrequent: personal observations have shown copulation in *Burnupia* occurs during the night and in the early hours of the morning until as late as 09H00, seldom after this time which is when the field samples are taken. The possibility of using polymodal analysis using frequency versus shell height at each collection time will be considered when the field data is complete for at least a 12 month period (Harding 1949, McMahon 1975, Brackenbury 1989). The results, presented as growth curves, should reveal how many cohorts are present through the year. The life span of each cohort in the field will then be seen, as well as the times of egg-laying.

The purpose of monitoring the sexual development of the limpets is to determine any correlation between sexual development and size. The number of eggs per mass has been shown to be directly correlated with size in a number of freshwater pulmonates (Hunter 1972, McMahon 1972, De Witt 1954a and b). Data on the bursa copulatrix and penis will determine at what size an individual can act as a functional female or male respectively. These results can then be linked to those in Section 4.2.2 where egg-laying has been monitored.

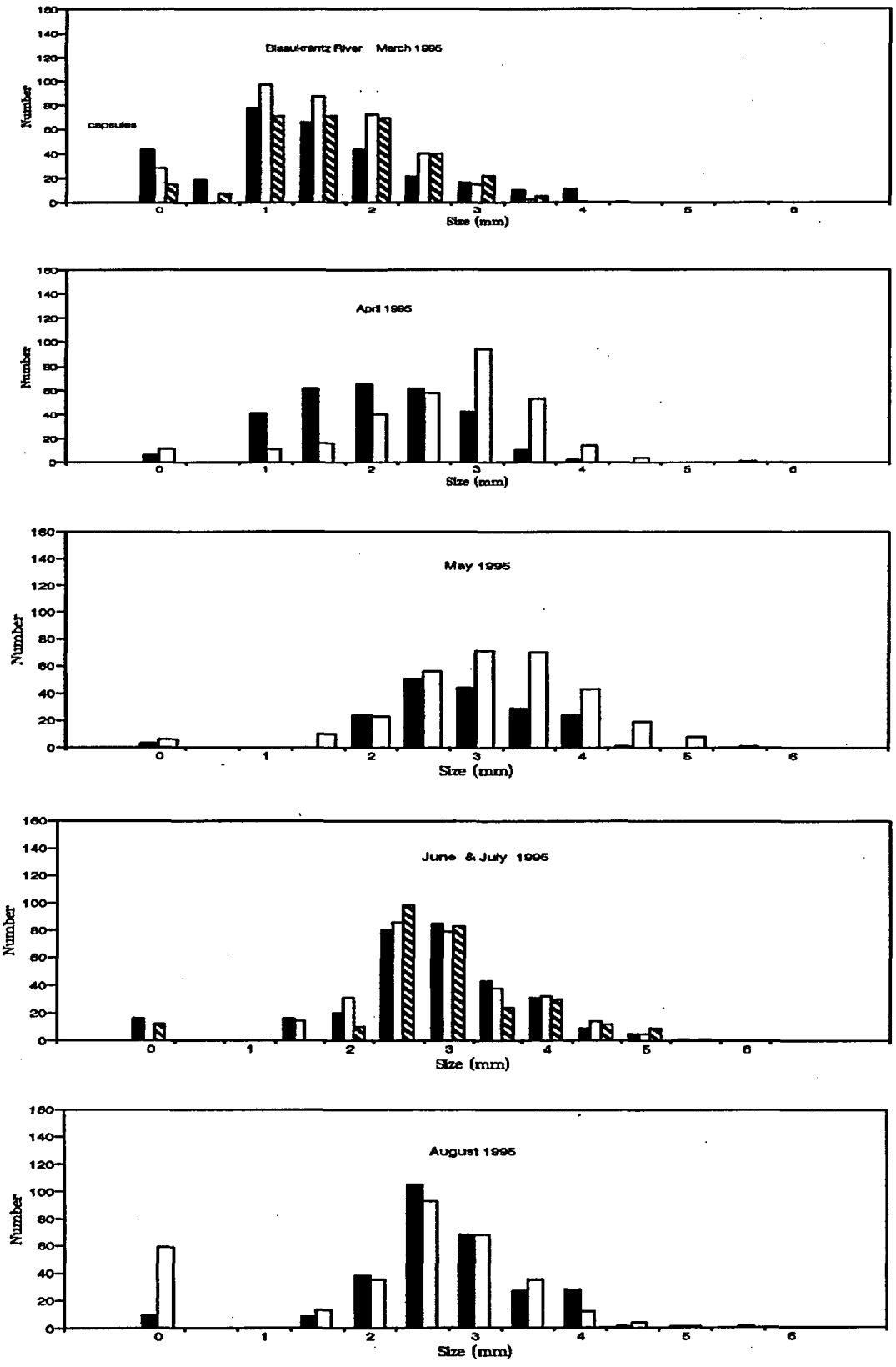

Fig.4.3.3 Sizes and proportional numbers of limpets sampled monthly from March to August 1995 from the Belmont site on the Blaauwkrantz River.

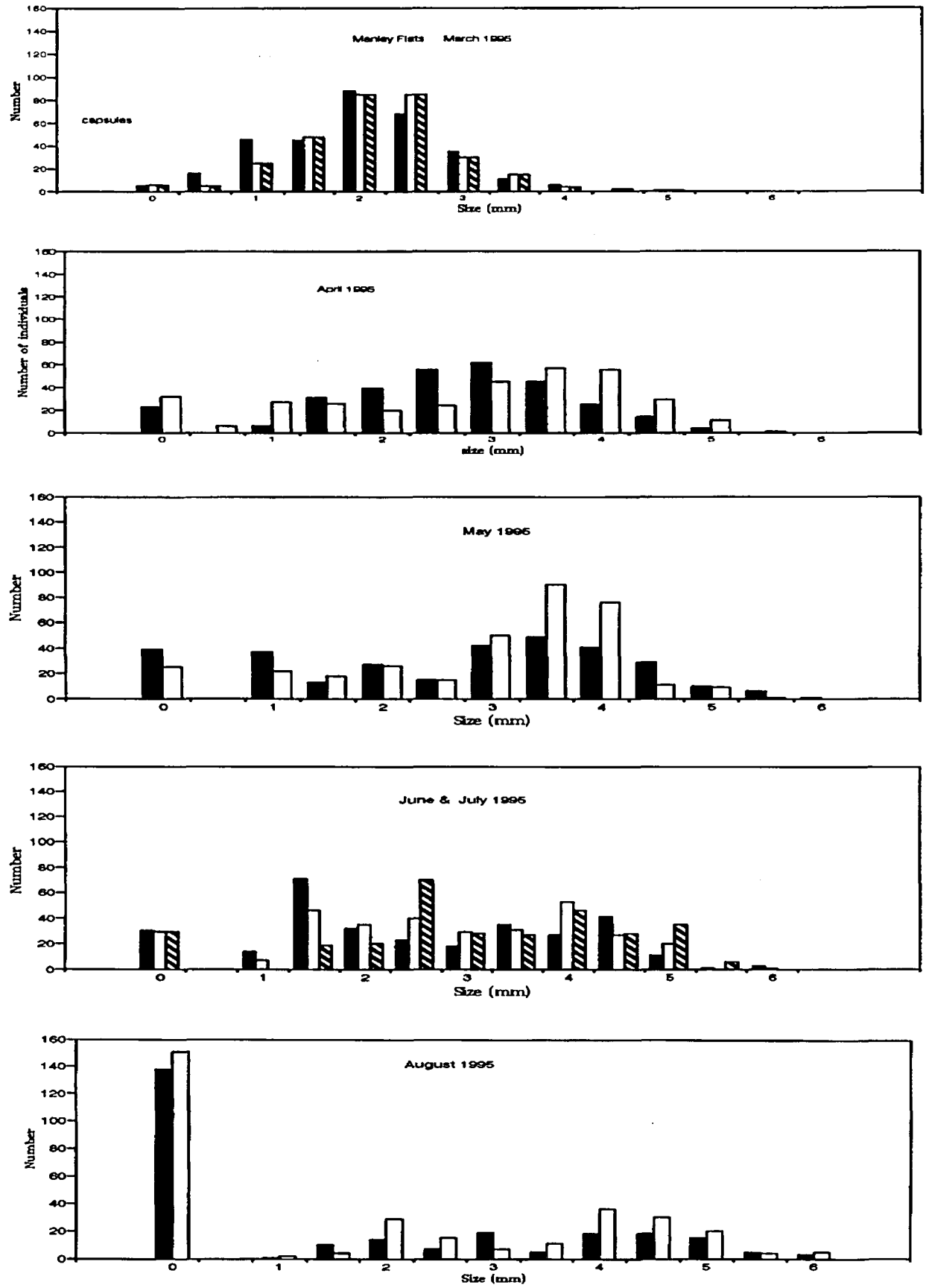

Fig. 4.3.4 Sizes and proportional numbers of limpets sampled monthly from March to August 1995 from the Manley Flats site on the Blaauwkrantz River.

4.4 CONCLUSIONS

With *Burnupia* surviving well in the initial trials it was decided to investigate the life history of this limpet and establish optimal conditions for its laboratory culture. There have been no previous investigations into the biology of any of the African Ancylidae, with the exception of Oberholzer's work (1963) on the morphology and histology of *Burnupia mooiensis*. This lack of any background information on life histories necessitated literature searches further afield, where this information was gained from predominantly the Ancylid *Ancylus fluviatilis*, which is a European and north African species found in lotic freshwater habitats and which appears to have many similarities of life history style to *Burnupia* (Russel Hunter, 1953; 1961; Calow, 1973; 1981; and others). Many of the papers on the bilharzia snails *Biomphalaria* and *Bulinus*, on *Physa*, and on Lymnaeid species which were consulted (although acknowledged as different families with different habitat requirements), revealed valuable information on ecology; population control and breeding strategies; and the effects of temperature, density and food on growth and reproduction.

The attempts to establish optimal rearing conditions revealed the following points:

1) Touching the limpets, either in the laboratory or transporting from the field, has a very negative effect on both the growth and survival.

2) Dietary requirements.
Although the food requirements of *Burnupia* have not yet been investigated, Calow (1973, 1975), Schwenk and Schwoerbel (1973), and Streit (1975) found that *Ancylus* is a microherbivore which prefers epilithic algae, particularly diatoms. *Burnupia* exhibits feeding behaviour similar to that reported for *Ancylus*, which is a rasping action of the stone surface with the radula. Thus, it seems likely that they will have the same food requirements. Optimal feed provision for a laboratory population is always a major part of the development of an aquaculture project. However the cultivation of the most suitable periphyton, under optimal growth conditions of water nutrients and light spectra and intensity, has not been investigated. In an experiment (section 4.2.1.2) with various food sources, artificial food (pellets of *Spirulina*, fish meal, starch and essential amino acids), river periphyton, and fish farm periphyton all proved to be suitable for the survival of the smaller limpets (<4mm), but the artificial food gave better survival rates for those greater than 4mm.
When limpets of all sizes were left for four days without food, that time during which ecotoxicological testing is completed, the survival of either adults or sub-adults was not affected.

3) Hydraulic conditions.
In the laboratory it has been found that a greater number of eggs are laid in aerated standing than in flowing water (section 4.2.1.5), although the possible negative effect of high aeration and consequent turbulence should be considered (section 4.2.2.1). Limpets held in channels made of heavy duty PVC piping proved to have a greater survival rate than standing, aerated water.

4) Water conditions.

As indicated in section 3.3.6, the correct water composition suitable for rearing and holding the limpets has proved to be very difficult to determine. In all experiments the water was considered to be a limiting factor in achieving the optimal survival, growth and reproductive rates and will need careful consideration in the next phase of this project.

5) Temperature.

It is generally agreed by ecologists that temperature plays one of the most important roles in influencing population patterns (Shiff 1964). The strong influence of temperature on the life cycle of *Physa* has been demonstrated by a number of authors (Duncan 1959, Russel-Hunter 1961, Girod 1969, DeWitt 1955, Eckblad 1973 and others), strongly linked to food source. Many freshwater pulmonate snails have their growth rate directly correlated with temperature, increasing up to an optimal or critical temperature, after which growth declines (McMahon 1975). The freshwater snail *Indoplanorbis exustus* showed very clearly (Raut *et al* 1992) an optimal temperature for growth and number of capsules and eggs produced per week. Where our experiments where water baths could be maintained at 15°C, 20°C and 25°C (section 4.2.1.8), increasing temperature increased growth rate, at densities of up to 30 limpets per 500ml volume of water ($p<0,001$).

6) Density.

When considering the breeding of limpets in the laboratory, the effect of density on the growth and fecundity is important. Under high densities, the availability of food may be limiting, or may cause a shift in the species composition and succession of the periphyton assemblage (Bronmark 1989). In the experiment where the effect of different combinations of density and temperature were analyzed (section 4.2.1.8), the density of 10 limpets per 500ml volume of water attained the greatest growth rate for *Burnupia* at temperatures of 15°C and 25°C, being marginally less significant than a density of 20 limpets at 20°C. Although not considered here the effect of density on fecundity may be significant. Chernin & Michelson (1957a & b), Wright (1960) and Eisenberg (1966) all showed a negative effect on the mean clutch size with increasing density.

Various experiments were conducted to further understand the fecundity and breeding biology of *Burnupia*, under given conditions, essential information for any aquaculture programme. The following information has been accumulated (section 4.2.2, Reproduction and Fecundity, and 4.2.3, Natural Populations):

1) *Burnupia* is hermaphrodite. Copulation commences at size greater than 3,4mm in shell length, although eggs have been deposited by limpets reared in isolation. The sectioning of limpets (described in section 4.3) will accurately correlate size of limpet to sexual development.

2) The total development of the eggs has been described (section 4.2.2.1).

3) The number of capsules and eggs produced by an individual is widely variable (section 4.2.2.2 and 4.2.2.3). The number of eggs per capsule varies from 1 to 13; they take 14 to 15 days to hatch from date of lay, under water conditions of average pH > 7,3 (6,6-8,4), average TDS = 280 (185-420), and average temperature 19°C. Cooler temperatures extend the time of hatching up to 21 days at 13°C (pers. obs.). Those limpets that were collected from the streams laid capsules with an average of 5,75 eggs per capsule in the laboratory. Those that were collected at a very small size (approx. 2,5mm, before expected sexual maturity) and reared in the laboratory either as pairs (4.2.2.6) or as singles (4.2.2.4) produced on average fewer eggs per capsule (3,7) than those limpets collected at a larger size from the streams, and allowed to lay eggs in the laboratory (6,1). Generally, water conditions in the laboratory had a pH of <7,0 and an average TDS = 136. It has been found (section 4.3) that the average number of eggs per capsule laid in natural conditions is 5,4 (August to October). The river conditions have an average pH of >7,0 and average TDS of >300. If the water conditions in the laboratory had adversely affected their development and fecundity, this supports the views of Macan (1961) and Dussart (1979) who found that water conditions in the laboratory, using tap water as a source, has a significant effect on both growth and fecundity.

4) The number of capsules laid per limpet pair is estimated to range from 1 to 21.

5) Hatching in the laboratory is at a rate of 91%. They display direct development, with the young emerging as crawling snails.

6) From the field observations and analyses (section 4.3) a clear indication of the cohorts within each year should be obtained. From this, egg-laying times through the seasons will become clear, and when linked to the data relating to Degree-days, it is hoped that this information can be used to obtain optimal rearing in the laboratory.

4.5 REFERENCES

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CHAPTER 5.
INVESTIGATIONS OF THE LEPTOPHLEBIID MAYFLIES *ADENOPHLEBIA AURICULATA* (EATON), 1871 AND *CHOROTERPES ELEGANS* (BARNARD, 1932).

5.1 BACKGROUND

5.1.1 INTRODUCTION

A survey by Mayer & Ellersieck (1986) indicated a global need to develop acute toxicity methods for baetid and burrowing mayflies, caddisflies and stoneflies. We have selected genera from the family Leptophlebiidae as useful mayflies to breed in the laboratory as future toxicity test organisms. The members of this family is widespread and fairly large in size, robust and easy to identify. They therefore fulfil several of the selection criteria (Chap. 2). According to Johnson *et al* (1993) the life history indicators of environmental stress are survival, growth and reproduction. This, to us, emphasises that information about these life history parameters, under natural unstressed conditions, must be available as a baseline against which to judge results from toxicological experiments. Even results from the experimental controls should be judged against this information. The experiments which will determine optimal conditions for laboratory maintenance and reproduction will give much information in the areas mentioned above, but observations of the responses of natural population to field conditions are essential to verify laboratory results which in turn shed light on areas such as growth rates, which are difficult to determine in the field.

5.1.2 EPHEMEROPTERA AND TOXICOLOGY

Mayflies are an important component of stream ecosystems and are recognised indicator species in biomonitoring. Pontasch & Cairns (1991) suggest that mayflies are the group most sensitive to the deleterious effects of effluent (effect of concentration being dependant on species) while chironomid densities increased after exposure to effluent. There is a growing literature of laboratory and field studies in toxicology and ecotoxicology in which a variety of mayfly species have been the subject of investigation. The effects of a wide range of chemicals have been reported but those receiving the most attention seem to be heavy metals.

The effect of copper and zinc from both food (diatoms) and water on the growth and emergence of *Epeorus latifolium* was evaluated in an indoor model stream by Hatakeyama (1989). It was found that the moult interval was nearly double that of the control at the concentrations tested. Frick & Herrmann (1990) experimentally studied the occurrence of aluminum accumulation in nymphs of *Heptagenia sulphurea* at low pH (4.5). Jop (1991) investigated the bioconcentration of cadmium, copper, lead and zinc in various stages of the field collected *Ephemera danica* Muller, *Ephemera vulgata* (L.), *Leptophlebia vespertina* (L.) and *Baetis vernus* Curt from South Poland, while Gerhardt (1992) investigated acute toxicity of cadmium to *Baetis rhodani* & *Leptophlebia marginata* at pH 5 and 7 simultaneously in static (ST) and flow through (FT) systems. He found *L. marginata* to be more tolerant than *B. rhodani* and that both species tolerated Cd better in the ST system than in the FT system, especially at pH 5. Saouter *et al* (1991) tested the bio-accumulation of inorganic mercury in *Hexagenia rigida*, analysing various anatomical regions on a structural and ultrastructural level and found that gills and gut could be transfer routes for mercury absorption, but also target organs for metal accumulation. Cadmium and mercury content in emergent *Hexagenia bilineata* from the Upper Mississippi River was analyzed by Dukerschein *et al* (1992).

The responses which are useful guides to the effects of pollutants have also been investigated. For instance Diamond, *et al* (1992) used *Stenonema modestum* (eastern United States & Canada) in subacute tests and showed that moult production was a more sensitive indicator of pollutant effects than length of organism or width of head capsule measurements. Tests using sodium chloride or silver nitrate and coal mine effluent demonstrated that these methods and end points provide repeatable and relatively sensitive data. Comparisons of scale were reported by Van Wijngaarden (1993) when he evaluated the field results against those from laboratory ecotoxicological research when the response of *Cloeon dipterum* to chlorpyrifos (Dursban) was evaluated in a laboratory toxicity test, in indoor microcosms, outdoor artificial ponds and experimental ditches. It was concluded that the laboratory toxicity tests gave good prediction of acute direct effects. *Cloeon triangulifer* was evaluated as a bioassay organism by assessing life history response and body burden to the exposure of chlordane by Sweeney *et al* (1993) and was judged to be well suited to laboratory testing because it has a relatively short egg and larval stage and can be readily

cultured.

We are in agreement with Buikema & Voshell (1993) that life history information would not only be useful for culturing laboratory test species but also for identifying sensitive life stages and pertinent ecological variables which could modify organism sensitivities. In this manner defensible chronic test methods can be developed. Thus, in planning the investigation of the mayflies, it was decided to concentrate on gathering information on the life history in the field and to amplify that with laboratory investigation.

5.2 LABORATORY MAINTENANCE EXPERIMENTS

5.2.1 DEVELOPMENT OF HOLDING CONDITIONS

Four experiments which are reported in Appendix 3.3.3 were conducted to determine optimal holding conditions for leptophlebiid mayflies. The findings are reported here.

5.2.1.2. Substrate selection

The necessity of and most suitable type of substrate was examined in two experiment with *Choroterpes* as subject. Substrate that were offered included stone, plastic foam, and rigid plastic mesh.
In bubblepots (App.3; Fig.3.3.8.2) the survival on netting was similar to the control where no substrate was offered. if substrate was offered 90% of the samples were still alive after four days. All three the substrates were tested in both flowing and bubbled water for a month and it wad found that 70% of the sample survived for 10 days and 20% for 30 days on foam-pads and stones in contrast to mesh and the controls where survival rate dropped from 70% survival at 5 days to 25% survival by day 10 (App.3; Fig. 3.3.8.2.3)

5.2.1.3 Hydraulic conditions

The suitability of the small channels and bubble pots were tested in two experiments. In the first channels were placed in the CER and the laboratory and in the second channels and bubblepots were placed in the laboratory.
After three weeks in the first experiment 32 and 33% of the sample had died and at 4 weeks 100% whereas 30% of the sample survived for 7 weeks in the channels in the second experiment. However the survival rates in the bubblepots were significantly higher than those in the channels in the second experiment. (ANOVA result f ratio 6.7 $p<0.05$) In two of the replicates 40% of the nymphs were still123

alive after 9 weeks.

Growth rates for individual nymphs were calculated in the first experiment and are summarised in Table 5.2.1.1. There was no significant difference between the growth rates of nymphs in the CER and in the laboratory ($P > 0.05$) as the average temperatures were similar

Table 5.2.1.1. Growth rates calculated from survivors in each channels in the laboratory and CER results (Growth rates in mm/day). Average ambient temperature 19°C.

	CER	LAB
Channel 1	0.015	0.017
Channel 2	0.043	0.017
Channel 3	0.022	0.028
Channel 4	0.014	0.009
Mean	0.023	0.019

Conclusions

An absolute growth rate of between 0.019-0.022mm/day means that a nymph could grow from hatching to a maximum size in four months. Temperatures in the field stream fluctuate around 15-24 °C and growth rate has been found to be linked to temperature in invertebrates.

It can be concluded from the above trials that bubblepots are the optimal rearing container for *A. auriculata* and that they are not obligately rheophilous.

5.2.2 FEEDING TRIALS.

5.2.2.1 Suitability of commercial fish food for survival

In the first six months of the project period, a trial to test the suitability of TETRAMIN as feed for stream invertebrates was conducted. TETRAMIN is a registered fish food consisting of carbohydrate, protein and lipid, with a total energy value of ** kilojoules/ gm. 70% of the nymphs of *A. auriculata* survived for 7 weeks while in the controls which were fed on detritus 25% of the sample survived for 7 weeks (Appendix 3.3.8.5).

5.2.2.2 Growth rates on two feeds

Aim: To assess the growth of the nymphs in the laboratory at ambient conditions, and to determine if TETRAMIN as an alternate to decaying leaves gives a better growth rate.

Introduction

It has been amply illustrated that food quality can contribute significantly to growth rate and therefore to the generation period, size at maturity and fecundity (Anderson & Cummins 1979; Sweeney *et al* 1986). In the laboratory optimal growth in the shortest period for rearing and reproductive purposes is desirable. If, however, the aim is to have large number of animals of a certain size in stock for extended periods, the fact that growth is retarded at low temperatures becomes important. This option can then be exercised once the correct size for testing purposes has been reached, to move the animals into a cooler situation with reduced feeding and in a sense put them in suspension. Feeding on optimal diet to suit each purpose and the development of this diet often forms the largest portion of aquaculture research project. However the scope of this investigation does not allow for an in-depth investigation into diet and it was therefore decided to see if the addition of TETRAMIN as a supplement to periphyton, instead of the natural diet of decaying plant matter (Leaves) would provide an adequate diet.

Materials and Method

1) Eight x 5 litre replicate of bubblepots were furnished with 10 substrates with periphyton grown in the laboratory placed in the bottom. In 4 replicates the nymphs were fed *ad libitum* on decaying leaves from the study site in addition to the periphyton while the other 4 replicates received 0.5ml ground TETRAMIN weekly in addition to the available periphyton. A leaf shape of inert black plastic was placed in these pots to resemble the real leaf.
2) Nymphs of *A. auriculata* were collected in the field and after being sorted, were allowed to acclimate for a week before being measured. The dead animals were replaced from the remainder which had been kept in a large bubblepot with stream detritus and leaves adjacent to the experimental pots. The experiment started two weeks after the collection date. In some containers up to 80% of the

nymphs died and were replaced. Twenty one nymphs between the sizes of 0.4 and 0.8 mm headwidth were placed in each bubblepot. All the nymphs were measured weekly, the water and food were replaced and the number of shucks were recorded.

3) The average head width of each sample was calculated weekly. The absolute growth rate was calculated weekly, and over a period of 47 days which was the period prior to a large number of emergences. Statistical analysis was performed on STATGRAPHICS 7 software package.

Results Fig. 5.2.2.2.1, and 2a) & 2b). The results of this experiment were ambiguous and difficult to interpret. The average rate of growth was difficult to calculate as, beside the mortalities which occurred, a number of adults emerged. Whenever an adult emerges the average size of the remainder of the sample becomes smaller and the growth rate then appears negative or retarded. Survivals had to be calculated taking both the mortalities and the emergences into consideration. Although the experiment lasted for 12 weeks, the average size increases up to day 50 only is depicted (Fig 5.2.2.2.1). To record accurate growth rates, nymphs would have to be reared in singly.

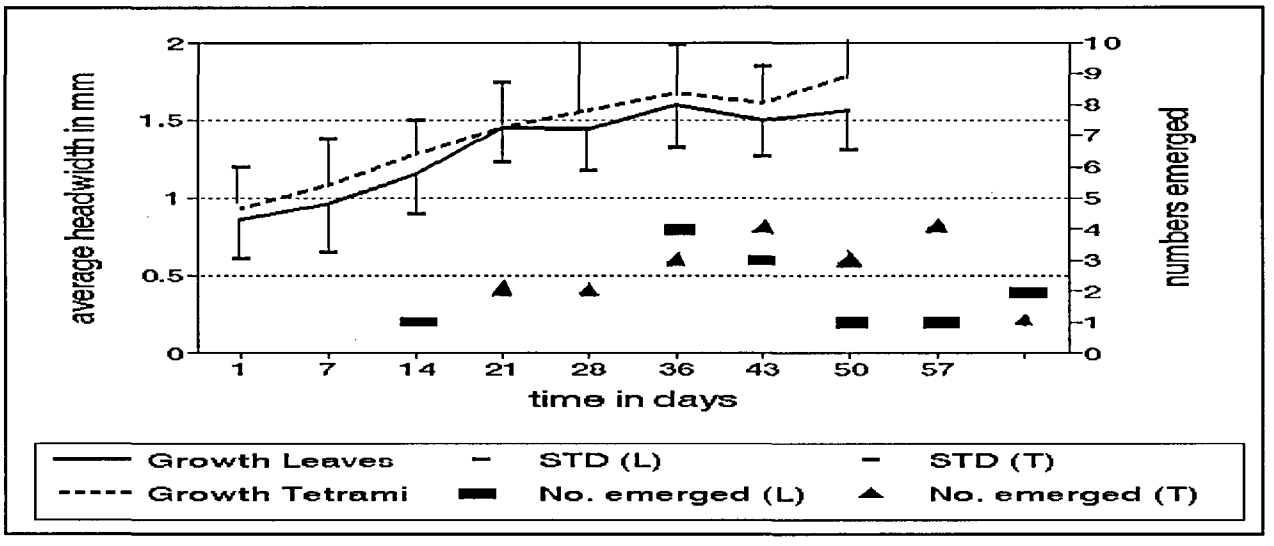

Fig. 5.2.2.2.1 Growth of nymphs of *A. auriculata* in bubblepots fed on decaying leaves or TETRAMIN in conjunction with periphyton. The average headwidth of all the specimens in all the replicates is given with and error bar indicating standard deviation from the mean. The emergences of adults are included in this graph indicated by symbols. The influence of the emerged adults which are no longer a factor in the calculation is reflected by the negative slope of the line between weeks 36 and 43.

A simple regression performed on the two treatments yielded a slope 0.013 for growth on leaves and 0.016 for growth on TETRAMIN. In Fig. 5.2.2.2.1 this is borne out by the difference in the slopes yielded from the line graph constructed by calculating the average size of sample each week.

Table 5.2.2.2.1 Average absolute growth rates mm/day calculated for each week from average headwidth of the total number of nymphs in all replicates. In the last row the growth rates have been calculated for the total number of days given in the left column.

Time, weeks	Growth on leaves, mm/day	Growth on TETRAMIN, mm/day
1	0.0146	0.02
2	0.0267	0.029
3	0.0419	0.024
4	0.004	0.016
5	0.0219	0.0008
7	-0.014	0.024
Average growth over 14 days 21 days 57 days	0.0206 0.0277 0.0142	0.0243 0.0241 0.0157

In Table 5.2.2.2.1 the daily growth rate is calculated on a weekly basis. In those weeks when large animals died or emerged the growth appeared to be negative which is an artifact. Growth rates calculated over a longer period as in the last row of the table during which 7 emergences had taken place in both treatments. The mean growth rate is nearly halved. However it was clear during the course of the experiment that some animals did exhibit no growth and this could be morbidity due to pathogens. There was evidence of fungal growth in pots 8 and 6 both of which were fed on TETRAMIN.

Table 5.2.2.2.2 an ANOVA performed to test differences between all replicates in which the growth achieved by *A.auriculata nymphs* on leaves(L) were tested against that achieved on TETRAMIN(T). The results of multiple range analysis is given below. Each replicate has the treatment type next to the number (7.Leaves).

Method 95% LSD			
Level	Count	LS Mean	Homogeneous groups
7.L	78	1.1864234	X
5.L	132	1.1911134	X
2.T	132	1.2067042	X
4.L	107	1.2979697	-X
6.T	92	1.3334692	-XX
10.L	141	1.3467826	-XX
8.T	117	1.3916301	--XX
9.T	80	1.4675727	---X

An ANOVA comparing the two treatments over time produced an F ratio 24.08 at P= 0.0000 which shows a significant difference between the treatments, despite the fact that there is such a degree of overlap between the replicates.

During the experimental period a total of 63 specimens died and 21 emerged in those containers where leaves formed the diet and 60 died and 24 emerged when TETRAMIN was the diet.

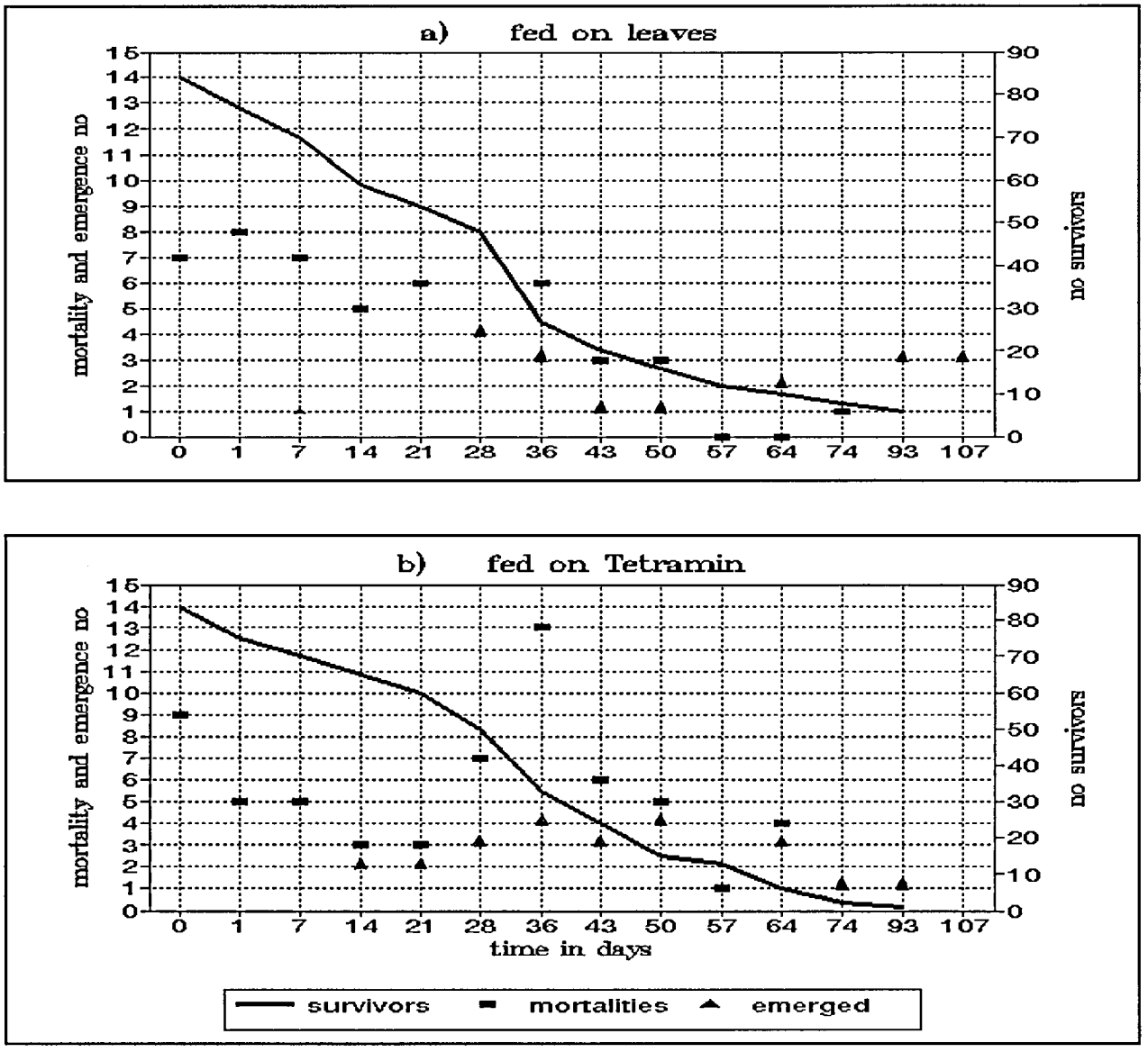

Fig. 5.2.2.2.2. a) & b) Survival, mortality and emergence (total numbers in all replicates) of nymphs of *A. auriculata* in bubblepots when fed on a) leaves and periphyton and b) TETRAMIN and periphyton.

The largest nymph at the start of the experiment (1.2 mm HW) emerged after 2 weeks. Those which were between 1.1 and 1.0 mm HW emerged after 5-6 weeks. The smallest nymphs in this trial was between 0.4 and 0.6 mm HW and the last emergences took place after 93-107 days which was 13-15 weeks on natural food. This gives some indication of the summer growth period which may be found of nymphs in the field. It is notable that the growth period is shorter and that emergences took place earlier in the TETRAMIN treatment.

Conclusion

A supplement of TETRAMIN to the diet of *A. auriculata* nymphs will increase the growth rate. Care has to be taken to maintain water quality as the largest numbers of early deaths were recorded in those pots where TETRAMIN had been added. The results from the experiment using only leaves as feed give a growth rate of 0.013 from the regression and 0.02 if the growth rate over the first 21 days is calculated. This indicates that it may take 10 - 14 weeks for a specimens of HW 0.6mm to reach sub-adult stage in summer conditions. and 14 - 23 weeks to grow from hatching to sexual maturity.

5.2.3 TEMPERATURE EFFECTS

5.2.3.1 Temperature effects on moulting rates.

Aim

To determine instar period for *A. auriculata* at three temperatures.

Mayflies have a number of post-embryonic moults, with estimates of between 10 and 50; however, most are in the range 15-25 (Brittain 1982). The number of instars is also influenced by temperature (Needham & Traver 1972) and has been cited as a useful indicator of pollution stress (Diamond *et al*1992).

Materials and Methods.

Two separate experiments were conducted to determine instar period and the effect of temperature on the moulting frequency.

1. a) Forty five 9 cm petri-dishes each containing a decaying leaf, from leafpacks in which the nymphs were found, were used as containers. To simulate the subdued light under rocks these were stored in brown cardboard boxes.

   b) Fifteen 5 litre bubblepots with approximately 500ml of water were furnished with periphyton stones in the second experiment.

2) All containers were equally divided into CERs at 15°C and 25°C and the laboratory at ambient (17-22°C). Photoperiod was 14h in the CER and 14-13h (ambient for summer) in the laboratory.

3) (a) One animal was placed in each container and if it died it was replaced. Sizes ranged between 0.6 and 1.2.mm HW.

   (b) Three animals of small, medium and large size (range 1.2 - 1.6mm HW) were placed in each container so that shuck size could be correlated to the moulted animal.

4) Containers were observed daily and in (a) the specimens were measured whenever a shuck was observed. Experimental period was 18 week for (a) and 9 weeks for (b).

4) The results are portrayed as a frequency range of instar period.

Results. Fig. 5.2.3.1. a) & b); Fig. 5.2.3.2 and Table 5.2.3.1.

Results.

Instar period.

Table 5.2.3.1. Average instar period at three temperatures for nymphs in the laboratory. The figures in brackets are standard deviations for the average numbers of days between moults.

PETRI-DISHES	**25°C**	**17-22°C (ambient)**	**15°C**
Number of observations	353	373	959
Average no of days (STD.)	7.67(2.66)	8.88(3.12)	13.5(6.20)
BUBBLEPOTS	25°C	20°C	15°C
Observations	266	355	239
Average instar length	7.4(3.43)	10.44(4.47)	14.94(6.23)
Ave(SE) calculated by ANOVA	7.52(0.54)	9.24(0.53)	10.12(0.31)

The average instar period for nymphs of *A. auriculata* at the three temperatures in the two separate experiments and fed on two different but natural diets are within one day of each other except for those experiments which were held at room temperature.

An ANOVA and multiple range analysis for all treatments indicated significant differences between all temperatures for both treatments (f ratio 35.9 $p<0.0000$) but no significant difference between the results of the two experiments (F ratio 3.3 $p=0.0683$).

In Fig. 5.2.3.1(a) instars of three days can be observed for nymphs kept at both 25 and 15 °C. While it may be feasible at 25 °C it is highly unlikely that a three day intermoult would take place at 15 °C due to the reduction in metabolic rate. It is therefore suggested that this brief period can be ascribed to observer error as different observers were used during the long run of the experiment. However stress of capture may cause frequent moulting and can not be altogether discounted. If the outliers of 3 and 4 days are removed a more realistic image emerges.

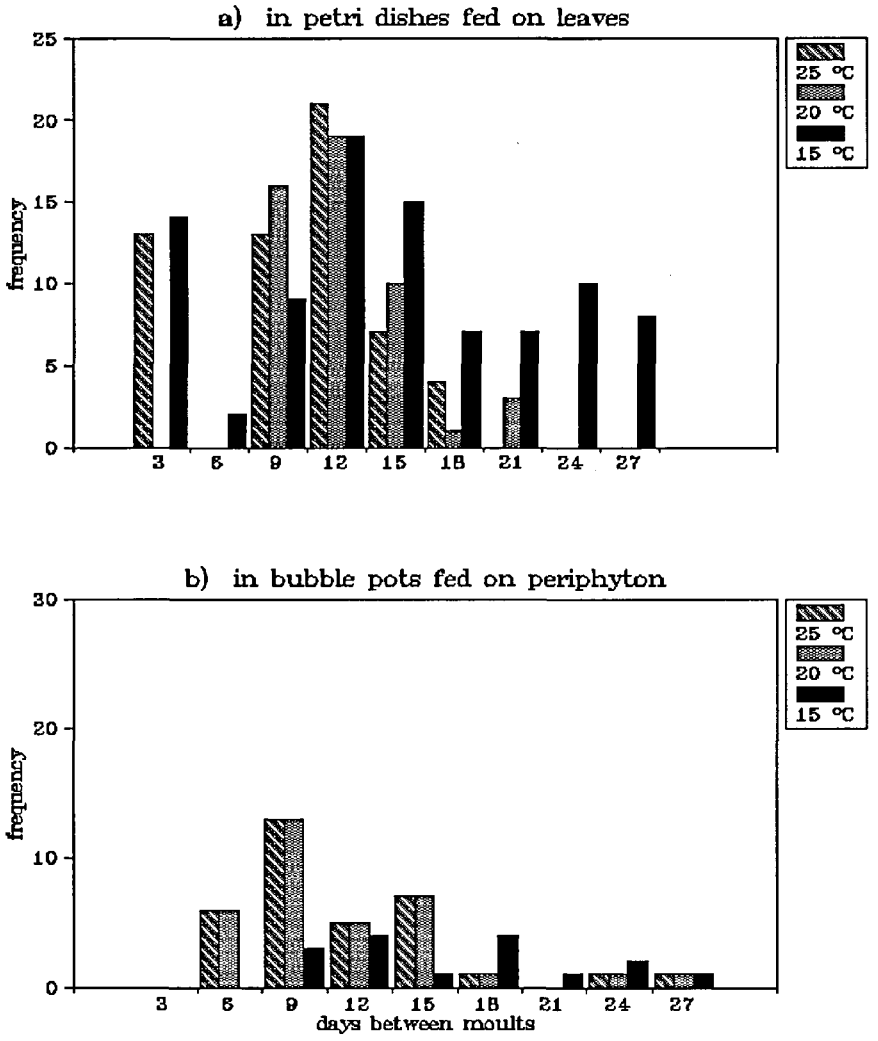

Fig.5.2.3.1 Frequency distribution of instar period for nymphs *A. auriculata* at three temperatures.

a) single nymphs were placed in petri dishes and fed on decaying leaves (b) two or three nymphs placed in 5 litre bubblepots and fed on periphyton.

The range in the number of days between moults is wide but there is a clear trend towards the lengthening of the instar period with lowering of temperature as can be expected. In (a) observations were carried out for a longer period (18) weeks) than for (b) (9 weeks). This was due to the fact that smaller animals were used in experiment (a).

There is a degree of overlap in instar period between all temperatures (6 and 12 days). The present data set is not large enough for this to show clear trends. If the instar length can be correlated with the age and size of nymphs as well as the stage in the experimental period when it occurred more clarity may be obtained for the reason for this overlap.

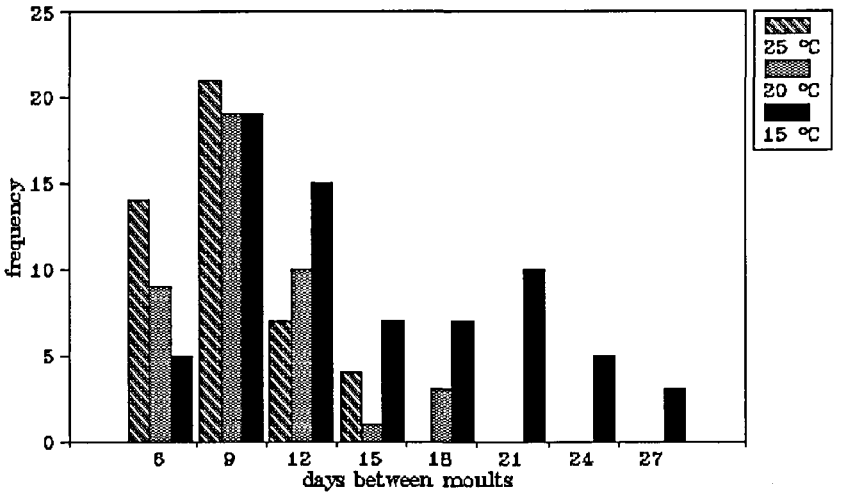

Fig. 5.2.3.2 Frequency distribution of instar periods, with outliers removed, of nymphs of *A.auriculata,* fed on leaves, reared singly in petri dishes.

b) Survival and growth rate in experiment (a)

The survivorship is very different at the three temperatures. At 15°C 3 animals died, at ambient (20°C) temperature 8, while at 25°C, 22 died. The largest number of mortalities occurred 2 weeks after the start of the experiment at 25°C when 12 nymphs died within 7 days.

The daily growth rates of the longest lived animals at each temperature regime were calculated.
At 15 °C 8 nymphs survived for between 70 and 126 days at 20°C 5 animals between 40 and 111 while at 25°C 4 animals between 40 and 93 days. The growth rates recorded for these long lived nymphs were

Table 5.2.3.2. Survival period and daily growth rate of longest lived nymphs, at three temperatures in petri-dishes with leaves as food.

TEMP.	RANGE DAYS SURVIVED	AVE. no. DAYS	GROWTH RATES mm/day	AVE. GROWTH RATE
15°C	(n=8) 128-71	117	0.003-0.008	0.00478
20°C	(n=5) 40-111	74.5	0.008-0.010	0.0085
25°C	(n=4) 40-93	70	0.009-0.022	0.01500

Although there are difference between the growth rates the ranges recorded are large and there was a degree of overlap between the temperatures. Nevertheless, the animals at 15°C grew much more slowly

than at the other two temperatures.

Discussion.

Several authors have found a variation in instar period and Newbold *et al* (1994) has discussed the possibility of a pre-emergence diapause period during which a population of nymphs may be able to synchronise emergence in order to facilitate mating possibilities.

It seems that leptophlebiid nymphs are vulnerable at higher temperatures as high mortality was observed. This could be as a result of oxygen deprivation as the petri-dishes were not aerated.

5.3. FIELD INVESTIGATIONS.

THE LIFE HISTORY OF THE MAYFLY *ADENOPHLEBIA AURICULATA* (EATON) (EPHEMEROPTERA, LEPTOPHLEBIIDAE) IN A SUBTROPICAL FOURTH ORDER STREAM IN SOUTHERN AFRICA.

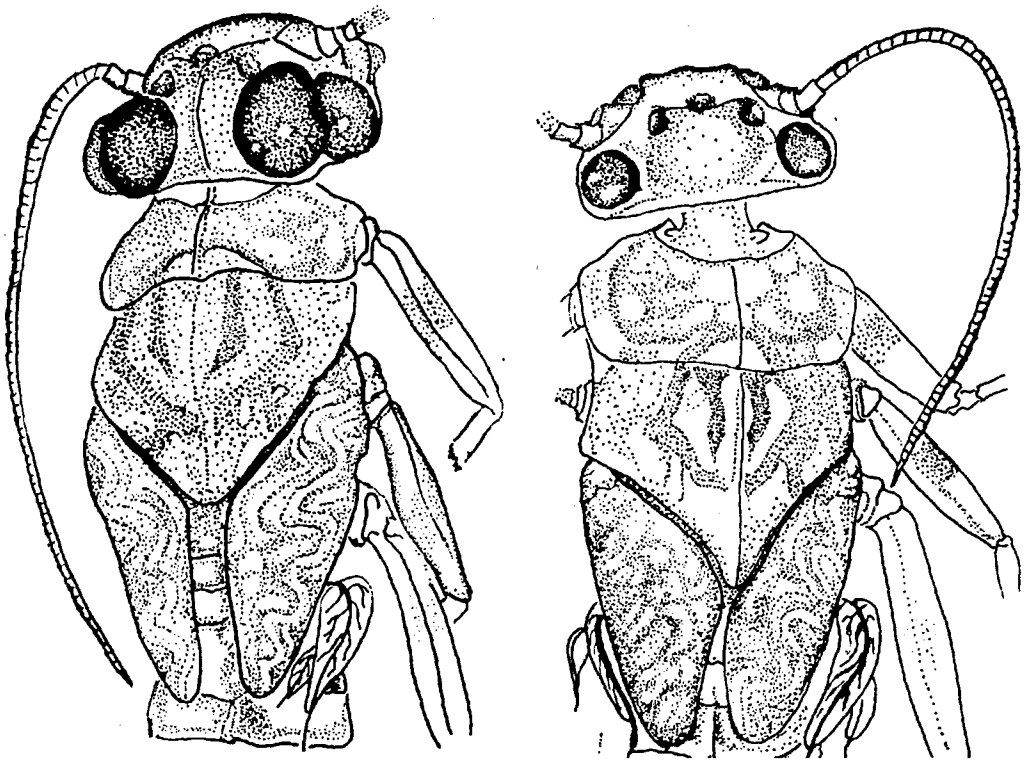

Fig. 5.3.1. a) Male of late instar nymph of *A. auriculata* showing the large eyes and b) female nymph with long wingbuds indicating that they are undergoing metamorphoses.

Introduction

The order Ephemeroptera dates from Carboniferous and Permian times and today their highest diversity is in lotic habitats where they are an important component of the ecosystem. They consume organic matter such as leaves and fine particulate organic matter as well as algae, and this energy in then made available to predators in the system (King *et.al* 1987 a & b). These processes are essential in the upper reaches of rivers where the majority of energy in the system is imported from outside in the form of leaves and detritus.

Mayflies are unique among the insects in that they have two winged adult stages, the subimago and the imago. There is much debate as to whether this is a primitive or derived state (Edmunds & McCafferty 1988).

The presence of long dark wingbuds usually indicates imminent emergence. During this preadult phase males with large double eyes can be distinguished from females which tend to be larger (Fig. 5.3.1) In leptophlebiids the pharate nymph crawls a few centimetres out of the water usually onto a rock or up vegetation and moults into the subimago (Needham & Traver 1972; Edmunds & McCafferty 1988). The subimago may then fly a short distance to cover before resting. Subimagoes are generally not strong fliers and seldom fly far (Edmunds & McCafferty 1988). After 24-48 hours the subimago moults into an imago (Needham & Traver 1972) which differs from the subimago in that the dark, opaque wings with a fringe of fine cilia become clear and reflective and the cerci and forelegs are longer (Needham & Traver 1972; Brittain 1982; Edmunds & McCafferty 1988). The only two functions of adult mayflies are mating and oviposition and the adults have no functional digestive system (Needham & Traver 1972; Brittain 1982; Edmunds & McCafferty, 1988). Hence, adult mayflies seldom survive for more than one week and usually die due to desiccation (Edmunds 1972). The general breeding behaviour of mayflies involves the males forming a swarm, any female flying through the swarm is usually copulated with immediately (Needham & Traver 1972; Brittain 1982; Edmunds & McCafferty 1988). This breeding behaviour therforee requires fairly closely synchronised life cycles and it is uncertain how the loosely synchronised emergence pattern observed in *A. auriculata* allows for successful mating if this was their mating strategy. However we have never observed swarms of adults in the river.

In the Palmiet River in the eastern Cape, the leptophlebiid *Adenophlebia auriculata* is an abundant component of the aquatic macroinvertebrate fauna, and is an important pollution indicator species as it is fairly widespread and inhabits upper reaches of rivers and streams. Detailed information on its response, to laboratory conditions as well as knowledge of its population distribution, seasonality and habitat preference is needed to determine whether *A. auriculata* will be a suitable subject for laboratory culture for ecotoxicological testing.

This population of mayflies has now been investigated since March of 1993. Our sampling programme has been modified as more information on the life cycle has become available.

Aims

1) To determine the seasonal population densities, habitat preferences, life history style (uni/bi/multivoltine) and the peak emergence periods of *A. auriculata* in the Palmiet river by analysing the proportions of the nymphal population in three size classes.
2) To attempt to correlate the rate of growth determined experimentally for *A. auriculata* nymphs to population dynamics in the field and, in doing this, estimate the life cycle.

Environmental influences

Investigations into the environmental factors which govern the life cycle of stream macroinvertebrates are numerous and mayflies, particularly, have received much attention in recent years. The total development time is governed by such external factors as temperature, available food and photoperiod which all influence the expression of the genotype of which temperature may be the most important (Sweeney 1978 & 1984). Investigations have revealed similar traits in large numbers species from similar biomes (Wiggins, 1977; Clifford, 1982; Newbold *et.al,* 1994; Jackson & Sweeney, 1995) while differences in life history is also found between occupants of the same biome (Sweeney & Vannotte, 1981; Jacobi & Benke, 1991; Sweeney, *et al* 1995). This leads one to the conclusion that the genotype will determine the life history style: how the species apportions the available energy between growth, maintenance activities and reproduction, and how the energy made available for reproduction is utilised. The various life history styles which have evolved in similar biomes give insight into the variety of responses which have evolved in response to the same environmental variables.

Sweeney (1978), investigating the metabolism of Isonychia bicolor showed that there is little metabolic compensation or acclimation to thermal fluctuations within this species and that summer and winter generations will exhibit the same metabolic rate and response to thermal changes. It was found that respiration rates of both winter and summer nymphs were the same when measured at 15 °C. Similar responses were recorded from gastropods by Berg *et al* (1958) & Calow (1975). It is therforee not surprising that several studies (Wise, 1980; Giberson & Rosenberg, 1994; Sweeney, *et al*, 1995; Pritchard & Zloty 1994) have revealed that intra-specific voltinism may be affected by the thermal regime of the environment. This results in a uni or semi-voltine life cycle gradually becoming bi-voltine as the thermal regime of the environment increases. Furthermore it has become clear that as the ambient temperature of an aquatic system increases with a concomitant decrease in seasonal fluctuation, the life history styles of the assemblages of invertebrate inhabitants tend toward multivoltinism (Jackson & Sweeney 1995; Jacobi & Benke 1991). Under these circumstances the development speeds up with increase in temperature.

However, adult size and fecundity, which are positively correlated (Anderson & Cummins 1979), and developmental period can be adversely affected by temperatures outside the optimal range for a given species. Sweeney & Vanotte (1978) hypothesised that this is due to a disequilibrium between larval growth rate and the timing of metamorphoses caused by a shift in the energy partitioning.

Materials and Methods

Study site and field data.

1) The study site is a stretch about 0.8km upstream of the confluence of the Berg and Palmiet rivers at the national road and comprises a series of riffles, runs and pools (Wadeson 1993) (Fig 5.3.2). During 1993 a stretch of about 0.5 Km consisting of 2 riffles, 2 runs and a pool is sampled at monthly intervals from February to September. In 1994-95 the stretch which is sampled weekly, is the run where the highest densities of *A. auriculata* were recorded throughout the year in 1993. During the weekly field samples the specimens which were collected were measured as described below and immediately returned to the upstream area of the study site in order to minimise the impact of the investigation. Animals which were captured and returned to the laboratory were collected downstream from the study site.

Water temperature, conductivity and pH were taken weekly and averaged for each quarter (table 5.3.1.) while the level of the stream is also noted. Daily maximum and minimum air temperature as well as rainfall was acquired from the local weather station. Cumulative weekly aerial and aquatic thermal inputs were summed from degree days using the formula (DD=T0-Tmax*dayL/24)*7. Where To = 10°C. 10 °C is chosen from literature as the temperature below which no development may take place but will have to be verified experimentally.

Table 5.3.1. Average water quality information from the Palmiet River 1994-1994.

YEAR	SUMMER			AUTUMN			WINTER			SPRING		
Condition	pH	TDS	°C	H	TDS	°C	pH	TDS	°C	pH	TDS	°C
1994	5.81	68.4	24.8	7.2	84	21	6.0	86	17.6	7.0	71	17.2
1995	6.0	73.5	20.1	7.0	71.3	19.4	8.0	86	12.1	7.0	96	20

Sampling method and measuring techniques.

2) The collecting method followed the method described in Chapter 3. The collection methods for the derivation of quantitative data was for the same collector to collect for a standard time period. These samples were preserved and returned to the laboratory. Rocks for sampling were selected randomly over the entire sample area regardless of size or position unless habitat-specific samples were being taken. Habitat specific samples were taken from rocks in and out of direct current. The sizes rocks sampled were noted. The subimaginal shucks on rocks at the edges of the stream is

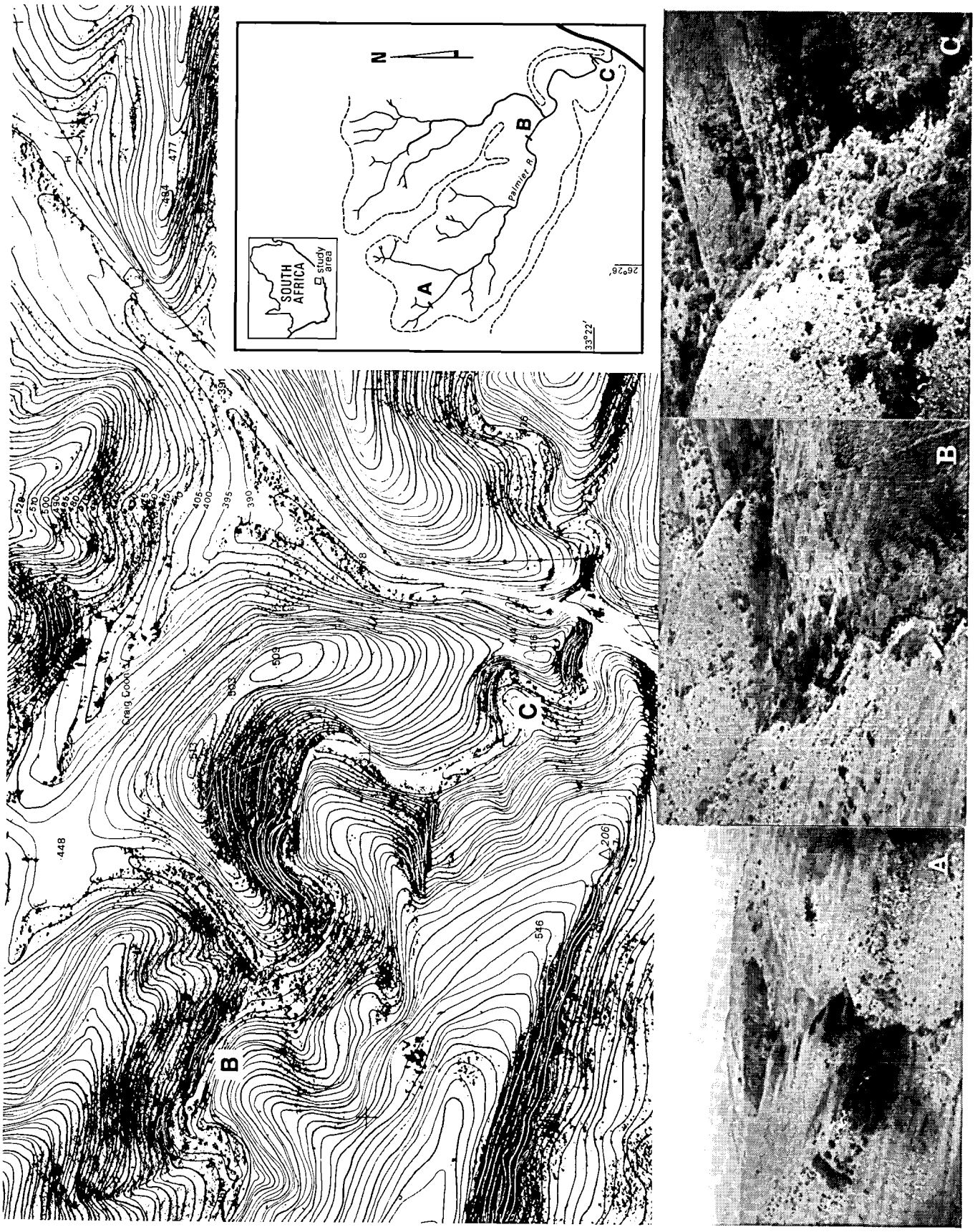

Fig. 5.3.2 Contour map of the Palmiet river Grahamstown, eastern Cape, RSA with aerial views of selected sites; a) headwaters b) second order reach c) 4 or 5th order reach at the study site.

counted each week as were adults, either resting on the edges of the stream, drowned, or caught in spider webs.

3) The dorsal head-widths (HW) across the widest points at the eyes were measured in the laboratory using a calibrated microscope eyepiece at 250 times magnification or on a calibrated 'V' on graph paper. The head widths show a closer correlation to the instar than does body length (Palmer pers.comm.). During the weekly sampling of 1994-95 the head-widths of fifty randomly selected insects were measured in the field. Once measured the insects were placed in a holding bucket to prevent re-sampling and were released into the same site once the sample was complete. Sizes below 1mm HW were not randomly selected but carefully sought as these animals are more difficult to capture. The smallest individuals (<0.04mm HW) were never captured, which leads to an underestimation of the proportion of hatchling nymphs in the population as well as difficulty in estimating hatching period and the duration of the earliest part of the post embryonic period. In this study there is a need to distinguish late instar nymphs from earlier stages. These nymphs are termed subadult nymphs and should not be mistaken or confused with the subimagoes which refer to the winged pre-adult stage.
4) Analysis. The collected measurements were grouped in three size classes. Those below 0.9mm HW were grouped and represent the recruits from the hatchlings of the previous laying. The sizes between 1.0 and 1.6 mm HW were considered the standing stock of the population. The sizes above 1.7mm represent the sub-adult, maturation phase with the development of wing-buds and gonads. The presence of long dark wing-buds usually indicate imminent emergence.

Results

Temporal variation in population.

Change in densities over time.

The changes in density and size frequency distribution of the nymphs in the five habitats in this reach of over a seven month period gives an indication of possible seasonal effects. As stated in the methods section, it must be kept in mind that newly hatched and very small nymphs are not readily captured so that there is always an under estimation of numbers and it is difficult to determine hatch dates.

At monthly sampling intervals in 1993 it is possible to monitor the changes in population structure and to predict possible emergence periods. The most striking aspect of these samples is the presence of all size classes throughout the year (Fig. 5.3.3).

The March sample was small with the largest number of nymphs found in riffle 1. However this was the first sample collected and an element of inexperience may account for the low numbers. In April there was an increase in the numbers of recruits in all sites but most marked in riffle 3. By May the recruits

appeared in larger numbers in run 2, and the standing stock size class numbers had increased markedly at all sites. The first sub-adults appeared at runs 2 & 4. The population profile looks very similar in July except that some larger nymphs were captured in the pool. In August there is a dramatic increase in numbers at site 2, in both the standing stock and the sub-adult size classes. This trend carries through to September, with a decline in numbers of sub-adults.

The overall trend seen in the reach over the 7 month sampling period in 1993 showed a overall increase in the total number of nymphs caught from March to September (late summer to spring) as well as an increase in the density of the nymphs with a drop in density in July (Fig. 5.3.3)

The presence of a strong cohort of fairly large nymphs in September indicated a spring emergence. A fairly synchronous spring or early summer emergence is implied by the sudden appearance of late instars in August, and the appearance of large numbers of shucks in the stream.

At this stage of the investigation we made the following deductions:

1. There may be an extended non synchronous mating/laying period indicated by the presence of small nymphs throughout the year.
2. The nymphs of this species are to be found in runs where the current speed is less than in riffles.

However the information clearly had large areas of uncertainty such as what happens in summer period. We therfore needed more frequent sampling over periods when emergence was to be expected. We hoped to be able to deduce the cues for emergence and flight, and to estimate the duration of the life cycle from this sampling regime.

Consequently, a new sampling regime was devised for 1994, aimed at discovering breeding periodicity and life cycle. At first we sampled weekly at those times when the results from 1993 had led us to expect an adult emergence but by the spring of 1994 it was decided to sample weekly for a period of at least eighteen months and after that to review the data.

Habitat preferences.

There appeared to be a seasonal change in the density distribution pattern of nymphs in the different habitats. In the colder late summer and winter months the nymphs appear to aggregate in the runs, with higher densities in the run with little canopy cover from July. The decrease in densities in July from May in all the habitats indicates a winter emergence of adults (Fig.5.3.4). The appearance of larger numbers of nymphs in the samples in May and September could be an indication of the increase in the nymphal size of the population which makes for easier collection for experimental purposes.

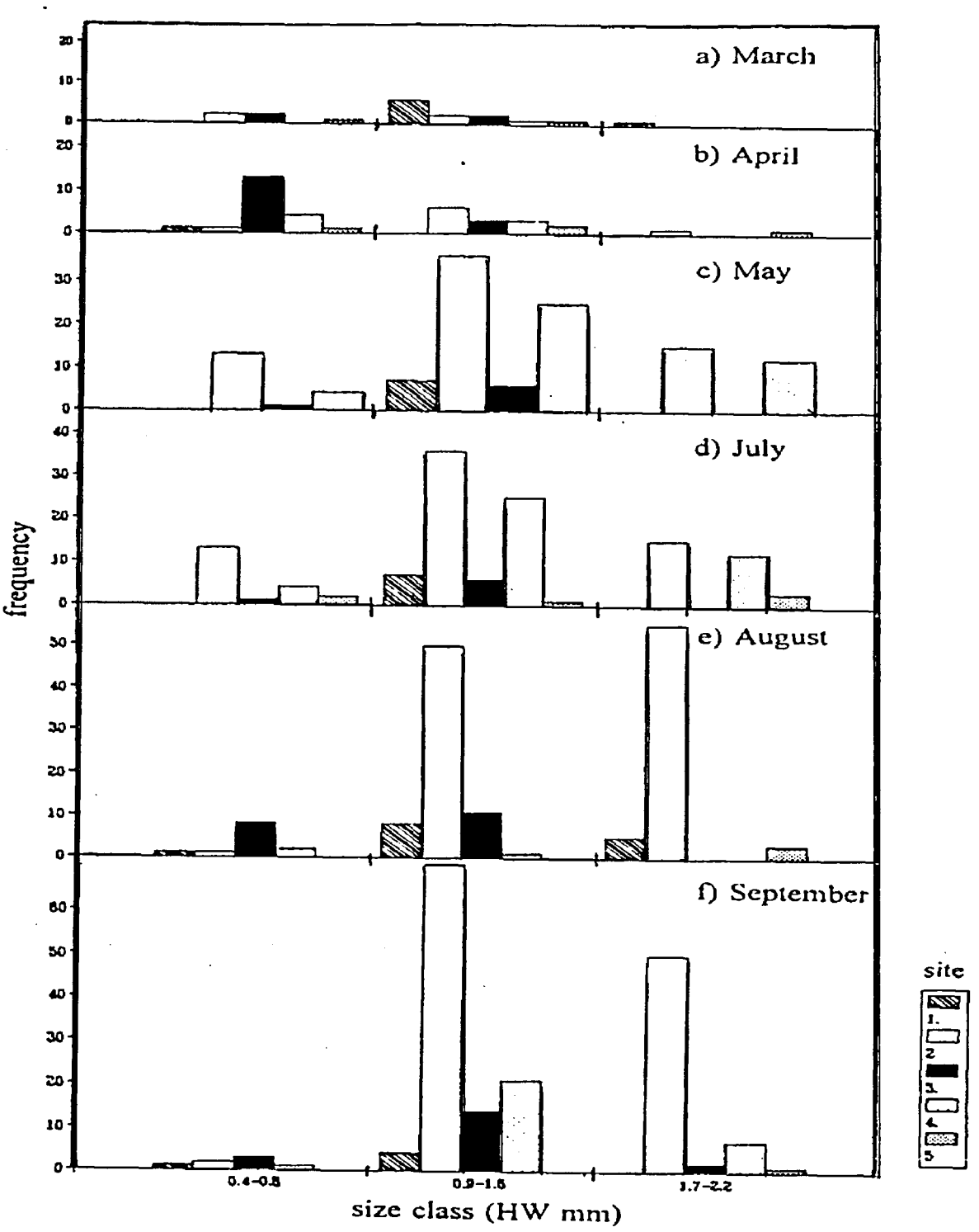

Fig. 5.3.3 Size frequency distribution of nymphs of *A.auriculata* sampled during six months (1993) at five sites in the Palmiet river. The samples have been dived into three size classes and the number of each size class is shown for each site which is coded with a different pattern, i.e. Site 1= riffle; Site 2 = run; site 3 = riffle; Site 4 = run; Site 5 = pool.1. There may be 2 generations per year with eggs being laid in May and September.

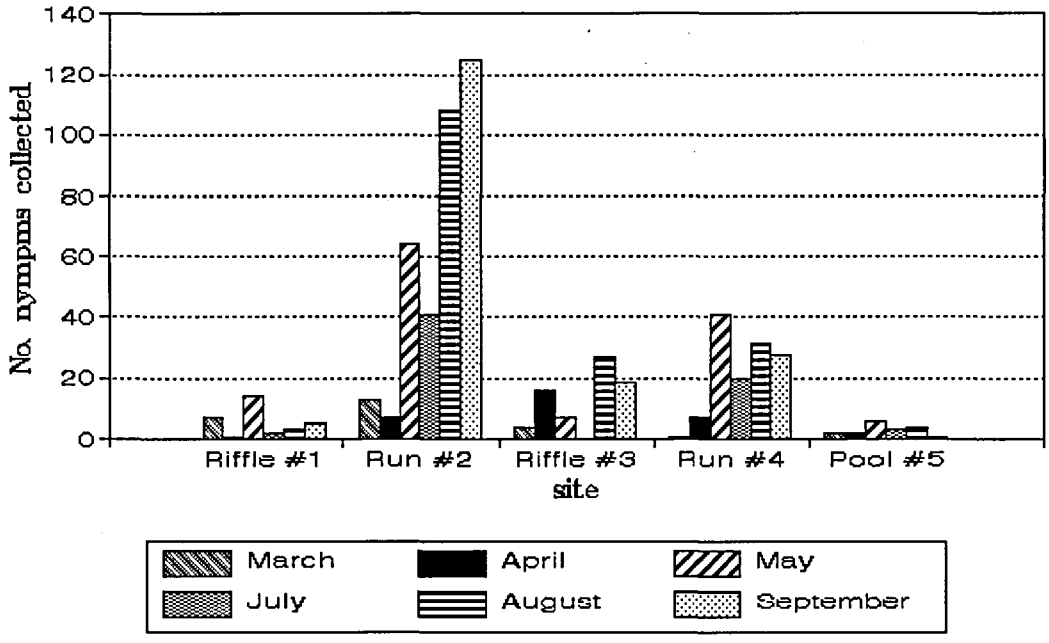

Fig. 5.3.4. Temporal spatial fluctuation of the population *A. auriculata* nymphs in the Palmiet River during 1993, showing numbers of nymphs captured each month at each site. Population densities were also calculated for the area sampled and showed a similar pattern of rise in autumn, followed by a decline in winter with numbers rising again in spring. It can be seen that site 2 and 4 consistently yielded higher numbers of nypmhs.

Temporal population dynamics

We deduce from Fig.5.3.5 that there are several major emergence periods during the year but that a low level of emergences take place at frequent intervals. However, the most notable feature of the population profile is the standing stock of nymphs (1 - 1.59 mm HW) comprising an average of 50% of the samples except for brief periods when the percentage of the standing stock in the sample was below 42% such as in week 45 and 59, 69, 88. Although the numbers of recruits in the samples fluctuate considerably but it is clear from the figure that recruitment continues throughout the year. Similarly ther are always sub-adults nymphs present in all but a few weeks during the year. However this does not indicate that emergences take place throughout the year, as the large nymphs are not necessarily pre-emrgence. In the period before emerging the sub-adult metamorphoses developing wingbuds, testes and ovaries (Fig.5.3.1). We therfore conclude that *Adenophlebia auriculata* is a multivoltine species.

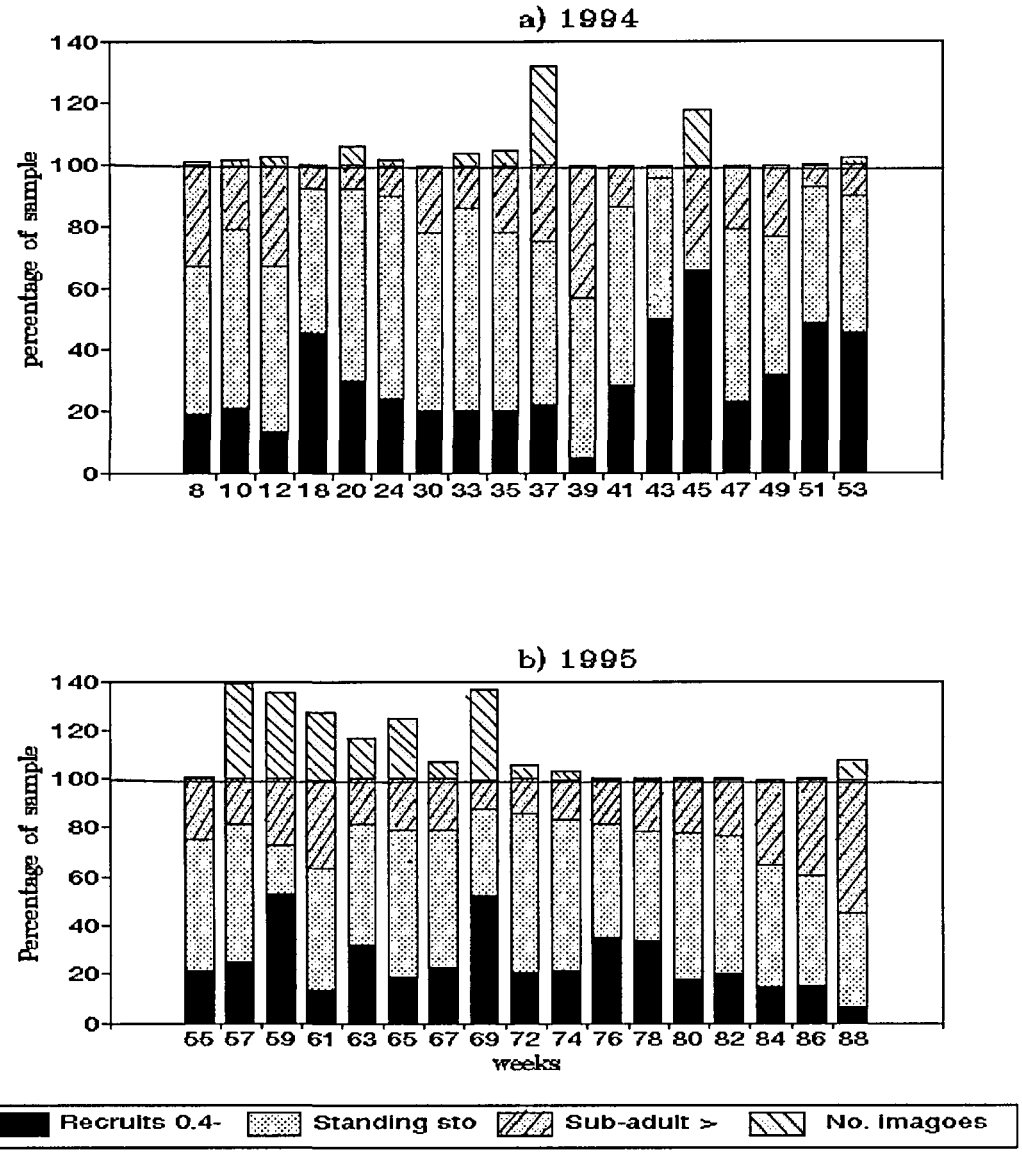

Figure 5.3.5 a & b). Stacked bar graphs depicting the population structure at one site in the Palmiet river over a two year period. The weekly samples are pooled at fortnightly intervals which would encompass an intermoult period of a large number of the population (see Exp. 5.2.3.1. instars between 8 and 15 days). a) Samples for 1994; b) samples in 1995. The X-axis displays weeks from February 1994 to 1995.

It must be noted that January, February, and June 1994 and part of April were not sampled. From August 1994 sampling was as described. The size classes are represented as percentage of the samples but the emerged adults and shucks have been added as numbers in the top line.

The question about the life cycle and the breeding periodicity remains unresolved, for although it is now known when the mating periods are, unless we can determine the period between hatching and sexual maturity we will be unable to deduce if the species is in fact multivoltine, or perhaps bivoltine. The factors which complicate interpretation of the available information are largely due to the scarcity of visible adults and the difficulty in finding the eggs, which makes the distinguishing of cohorts difficult. Further information which has not become apparent are the cues for emergence and the degree of synchronicity.

Firstly, consider the major emergence events. Emergences occurred in late April (weeks 16-18), when 7 adults were caught in the emergence trap and adults were observed at the site and 19 final instar shucks were found on rocks at the site. Further evidence of an emergence in April was the high proportion of final instar nymphs in late March, the very low proportion of recruits. It is possible that emergences were occurring throughout March and April. The results of samples from weeks 63 to 65 indicate a similar pattern for April 1995.

The next major emergence which was tracked occurred in weeks 35-37, (spring) when 5 adults were caught in the emergence trap and 22 final instar shucks were found at the site. The relatively short emergence period is most likely an artifact (Fig.5.3.6) as there was a rainstorm the week following, which could have washed the shucks off the rocks. This deduction is supported by the fact that the number of sub-adult were still high in the nymphal population despite the large emergences the preceding weeks.

Six weeks later a less numerous emergence occurred followed by the major summer emergence which was tracked from weeks 57 through 64. In May, (wk 70), another smallish emergence was recorded. During the winter spring which followed (weeks 72-86) no shucks were recorded and the sub-adults were present, but no maturation was noted. The expected early spring emergence in week 84 did not happen and the first shucks were counted in early October.

To enable interpretation of these data and to determine which emergences are related, mayflies have been reared in the laboratory at ambient temperatures in summer in order to get estimate lifespan. In Experiment 5.2.2 (Fig. 5.2.2.1) it became evident that it takes between 10 and 14 weeks for an animal of 0.6mm HW to reach maturity at 20 °C. From other observation (Exp. 5.4.1.) it has been established that the eggs hatch in 17 to 20 days (2-3 weeks). It has been estimated that a hatchling can reach 0.6 mm HW in 4-6 weeks. This estimate was obtained indirectly when a leaf pack with no nymphs in it was kept in a bubblepot and six weeks later small nymphs were found in the container, suggesting that leaf-packs may be the area to search for eggs.

Given all this information it can tentatively be inferred that the lifecycle of *A. auriculata* during summer is between 18 and 24 weeks. The spring emergence seems to give rise to the adults which emerge in

summer and the autumn emergence gives rise to those adults of the October emergence (Fig 5.3.5 (a & b)). Estimated growth rates were used to calculate possible generation periods of 14 to 23 weeks.

To display environmental variation and the changes in the sub-adult portion of the samples Fig. 5.3.6 was developed by the addition of recorded water temperature and average degree-days for the relevant interval after which the rainfall was overlaid on the numbers of sub-adults and imagoes. The most obvious fact that emerges from this is the difference in period between the autumn/late summer emergences and the spring/early summer flights (22 weeks versus 28 weeks). This may be related to the decline in the amount of heat energy available. The number of sub-adults trapped appears also to be correlated with the amount of rain.

To test the hypothesis that it is the total amount of available energy which governs the generation period, the degree-days were summed over the two apparent generation times (Fig 5.3.7), and these figures overlaid on the preadult nymphal and adult numbers. The similarity in the amount of summed energy which brought a generation to maturity becomes apparent.

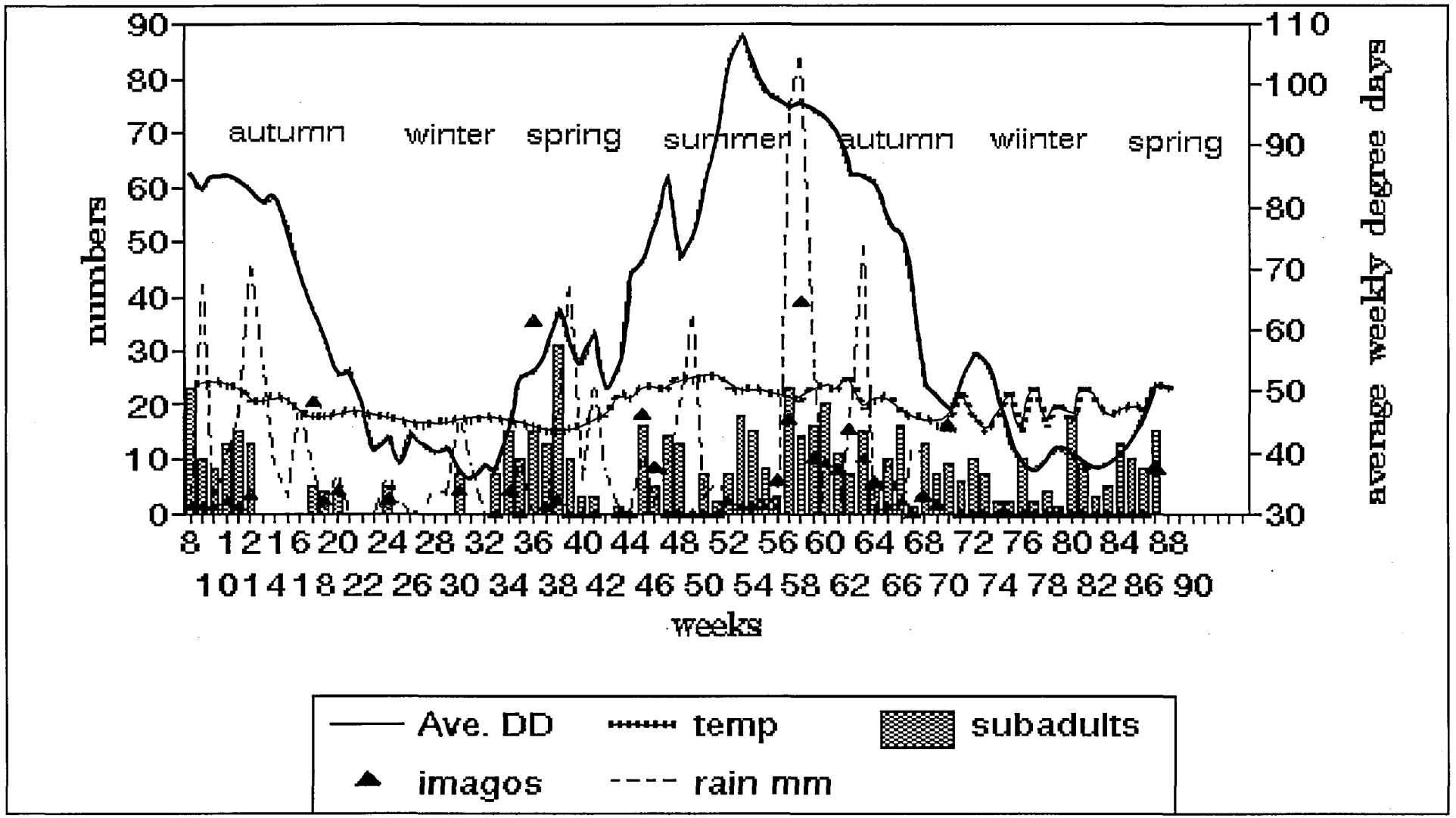

Fig. 5.3.6 The ambient environmental parameters (1994-95) in the Grahamstown district and average weekly temperatures in the Palmiet river overlaid on the sub-adult nymphal groups.

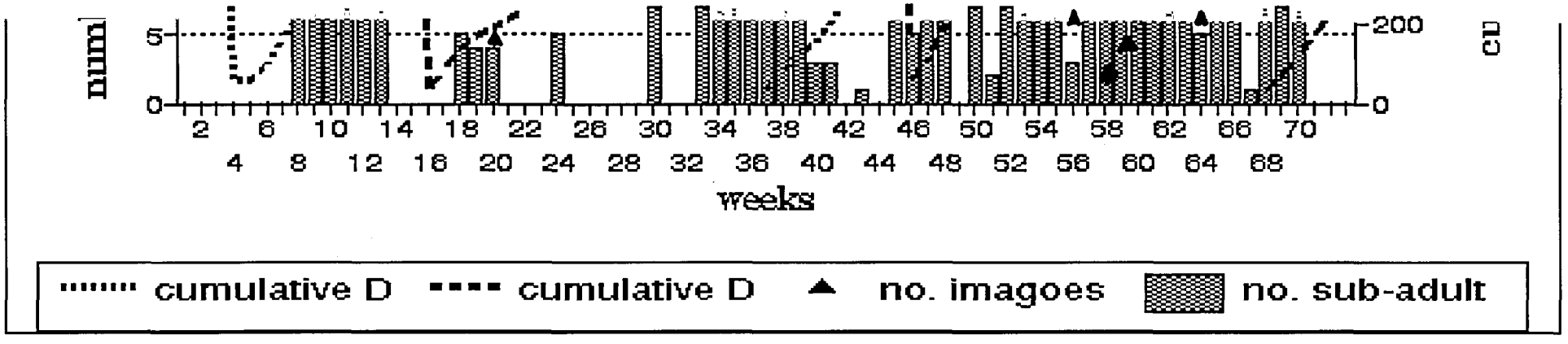

Fig. 5.3.7. Degree-days (DD) summed over the proposed lifespan of nymphs of *A. auriculata* in the Palmiet River, superimposed over bars representing subadults and triangles representing imagoes.

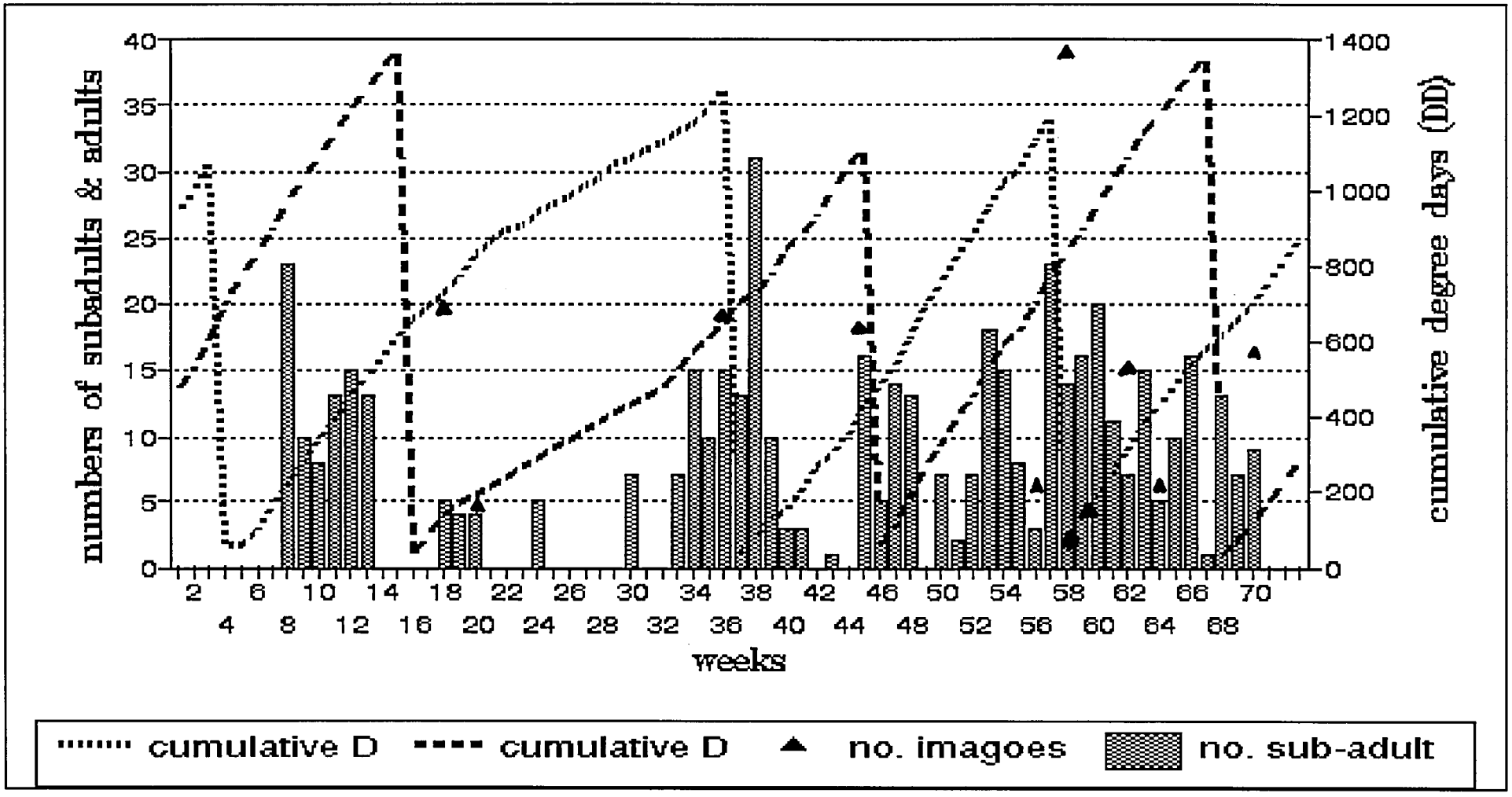

Fig. 5.3.7. Degree-days (DD) summed over the proposed lifespan of nymphs of *A. auriculata* in the Palmiet River, superimposed over bars representing subadults and triangles representing imagoes.

Discussion

Sampling method.

In designing a field sampling protocol the dilemma is to choose a sampling procedure which will yield the most comprehensive and representative insight into the dynamics of the life history. The problem areas which we encountered were an adult phase which is not only short lived but sparsely distributed, with poorly documented behaviour and an apparent overlap in size classes. We concur with Malicky (1989) when he questions the validity of using the captured aerial phases of aquatic insect to deduce quantitative measures such as secondary production. To counter this problem, the presence or absence of the adult and subimaginal shucks in the surrounds of the stream was used as an indication of emergence. However, an absence of the shucks could not be taken as an indication that no emergence activity was taking place as rain and rising river levels could wash the shucks off the rocks. We have concluded that the condition of the pre-adult nymphs will have to be more carefully documented and that perhaps a third size class will be created to separate nymphs with wingbuds from those of the same size but not in metamorphoses.

Cues for population changes

Studies have shown that flooding in unstable rivers, changes in water temperature and pH, pollution and food availability are amongst the factors that affect survival and distribution of other leptophlebiid mayflies (Collier & Winterbourn, 1990; Dudgeon, 1989; 1990; Graesser, 1988; Hall *et al*, 1988; Jowett *et al*, 1991; King *et al*, 1988; Scrigmeour *et al*, 1988; Scrigmeour and Winterbourn, 1989; Scrimgeour, 1991). During the period of investigation several flooding periods occurred when the river level rose dramatically and sampling was somewhat difficult. It was found that the numbers which were required for the sample could be collected in the detritus which collects at the edges of high water.

Temporal population dynamics and emergence periodicity

Brittain (1976) has shown that another leptophlebiid, *Leptophlebia vespertina,* in the northern hemisphere, has an adult emergences in late autumn and early winter. However it has become increasingly clear that the voltinism of aquatic insects in temperate areas are quite different from those in sub-tropical areas. This is largely due to the fact that temperature seems to be the major controlling factor influencing emergence periodicity as previous studies have shown (Brittain, 1976,1982, Sweeney *et al*;1978, Newbold *et al* 1994, Giberson & Rosenberg, 1994).

At monthly sampling intervals during 1993, it was possible to monitor the changes in population structure and to predict possible adult emergence periods for *A. auriculata* in spring and autumn. This complemented the findings of Sweeney (1978) who showed that *Isonychia bicolor* winter generations underwent a fairly synchronous emergence in the first ten days of spring in response to higher water temperatures in Pennsylvania. That the emergence in spring is fairly synchronous, is implied by the

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General conclusions

The introductory study of *A. auriculata,* in the Palmiet River suggested that the population distribution of nymphs in similar habitats (riffles and runs) are similar in size distribution but that higher densities were found in runs and that density of the size class 0.6-1.6 mm HW also fluctuated during the year. It has been impossible to estimate the size of hatchling as they are difficult to capture. This sampling monthly sampling regime over a seven month period indicated a possible late autumn/early winter and a late winter/early spring emergence of subimagoes. This was confirmed when the sampling regime was more frequent and extended over the summer period when a large summer emergence was found. The fact that emergences occur between these major events makes the analysis of the captured samples to determine the life cycle extremely complex. Laboratory growth trials which have been conducted give some indication of the growth rate but this cannot be taken as absolute until confirmed by field growth

trials. Temperature and food availability experiments will be conducted to examine the responses of this species to these variables.

Questions remaining include; a) what triggers emergence; b) what is the exact influence of temperature on growth rate; c) factors which influence fecundity and metamorphoses; d) do the hatchling nymphs (< 0.4mm HW.) inhabit the gravel and sand (de Moor pers comm.) or leaf packs as we suspect.

The correlation of environmental cues {photic = (day length and moon phases), thermal = (water temperature and degree-day) and partial pressure} with emergence periodicity requires a data set which covers more than one year to ensure replication of seasonal variations. Only if similar responses occur over a number of seasons, can conclusions be drawn about the cause and effect of climatic control on the life history of this mayfly.

5.4 ARTIFICIAL FERTILISATION

Aim

To attempt to artificially fertilize eggs in the laboratory and study the embryological development.

5.4.1 BACKGROUND

In the family Leptophlebiidae, female imagos lay eggs in suitable habitats by descending to the water and releasing a few eggs at a time when the tip of their abdomen is dipped into the water (Needham & Traver 1972; Brittain 1982). The eggs are covered with fine spring-like hairs which uncoil on contact with water and become adhesive (Needham & Traver 1972). The eggs presumably adhere to the first solid object that they come in contact with. The time taken for the eggs to hatch is strongly influenced by temperature. Most mayfly eggs may hatch in two to three weeks but those laid in cold streams (<10 °C) may diapause over winter (Needham & Traver 1972; Wise, 1980 and Brittain, 1982). The effect of temperature on hatching also appears to be linked to the natural environmental regime of the species.

Suter & Bishop (1989) incubated eggs of *Atalophlebia australis, Nousia inconspicua, N. fuscula & Baetis soror* from South Australia under constant temperature conditions in the laboratory (Range 4-24 °C). Embryonic development occurred at all temperatures, but hatching did not occur below 12 °C for *A. australis,* below 15 °C for *N. inconspicua* or below 5 °C for *N. fuscula & B. soror*. Photoperiod length had no effect on the incubation period. The hatch rate of eggs of *Potamanthus formosus* which was 34-53% over a temperature range of 15-30 °C, dropped abruptly below 15 °C to almost zero at 13°C (Watanabe,1992). Brittain & Campbell(1991), incubated the eggs of *Coloburiscoides* sp. in the laboratory at constant temperatures at 5°C intervals between 5 °C and 30 °C. Hatching success was high (> 80%) at temperatures between 10 °C and 25 °C. No eggs hatched either at 5 °C or 30 °C. Artificially fertilized eggs had low (< 10%) hatching success. Beside temperature, the pH of the water in which the species evolved must be considered when developing methods of artificial incubation. Rowe *et al* (1988) reared *Leptophlebia cupida, Habrophlebia vibrans, Stenonema femoratum & Baetis flavistriga* at pH levels (4.0, 4.5, 5.0, and 6.5) in the laboratory and the proportion of eggs undergoing eclosion did not vary with pH. Hatching rate was affected at the three lower pH values for *H. vibrans, & B. flavistriga* but was unaffected in the other three species which occur in low pH waters. Punzo & Thompson (1990) investigated the combined effects of pH and temperature on hatching and hatchling survival of *Caenis diminuta & C. hilaris* and found high mortality at pH 3.5 over a temperature range of 10-30 °C with best hatching and survival success 20 °C over a pH range of 4.0-7.2.

5.4.2 AN EXPLORATION OF METHODS TO ACHIEVE ARTIFICIAL FERTILIZATION FOR *A. AURICULATA.* S Burton (investigator) & E H Haigh (supervisor)

Aim

To achieve artificial fertilisation of the eggs of a leptophlebiid mayfly.

Introduction

At present most animals used in laboratory experiments are collected from the field. This is not only time consuming but may lead to over-sampling in the field. Hence, an artificial breeding program in the laboratory would be invaluable with regard to saving time and maintaining numbers in the field.

Artificial fertilization of egg of Ephemeroptera has been reported by several authors as part of a programme of investigating growth rates and life histories. Sweeney (1978) reports combining the sperm and eggs (*Isonychia bicolor*) directly on a glass slide without any other solution, leaving the mixture together for 5 minutes and the washing the eggs into a container of filtered stream water. Giberson & Rosenberg (1992a) on the other hand strip the egg from female sub imagoes into Yeagar's solution and then macerate the terminal segments of the males and place these with the eggs for 10 minutes. They also report that the fertilized eggs of *Hexagenia limbata* could be stored at 8 °C for extended periods and will nevertheless develop normally when returned to higher temperatures.

Material and Methods

Late or final instar nymphs were collected from a site removed from the field study site and returned to the laboratory and placed in bubble pots in a CER. Large rocks were placed in the bubble pots to provide crawl out area for the emergence of the sub imagoes. The time taken for the final instar nymphs to emerge could be reduced by placing them in a warm CER (20-23 °C). Subimagoes were removed from the bubble pots and placed in ventilated jars until they moulted into imagoes.

Fertilization

A number of methods were attempted to effect artificial fertilization. These included:

a) pinning a male and removing the head and then attempting artificial copulation with a female by holding their genitalia together. This method has proved successful with mosquitoes (WHO Report 1975).

b) dissecting the eggs out of a female in insect Ringers, Yaeger's solution and a mixture of insect Ringers and Yaeger's solution immediately dissecting out the seminal vesicles of the male and mixing the sperm and eggs. Both sperm and eggs were examined microscopically to check viability.

c) dissecting a male in insect Ringers and then stimulating a female to release its eggs onto the sperm by removing its head and dipping the tip of its abdomen in the Ringers above the sperm.

d) fertilization was also attempted between;

i. a male imago and a female subimago,

ii. between a male subimago and a female imago and

iii. between male and female subimagoes to determine whether the subimagoes contained viable eggs or sperm.

Eggs were then left in the sperm/Ringers mix for 5, 10, 15, 20 and 30min before being transferred onto ground glass slides in stream water to determine the minimum time that the eggs and sperm should be mixed to yield a sufficiently high fertilization rate.

Incubation

In the first series of experiments the newly fertilized eggs were placed in 500ml bottles of stream water with no lid containing an air stone with a nozzle to create a current and left at ambient temperature. In the second series of experiments the newly fertilized eggs were placed on ground glass slides in petri dishes of stream water and incubated at either 17 °C or 25 °C in CERs. Giberson (pers. comm.), found this method of artificial fertilization succeeded. Small samples of roughly 15-20 eggs were removed from the egg/sperm mix and transferred to small bottles. Eggs were also removed immediately after being placed in the petri dishes of water, every 24 hours for a week and thereafter once a week. All eggs were preserved in formaldehyde for microscopic examination. Drawings were made of the egg development.

Results

Microscopic examination (1000X) showed that the sperm were motile and were still viable after being dissected out of the seminal vesicles, handled with a pipette and transferred to a Ringers solution. The eggs were ellipsoid and approximately 200 μm by 90 μm in size. Fig 5.4.1.(a) shows the surface, shape and size of the eggs prior to fertilization. Hence, examination under 100X magnification was sufficient for observation of embryological development. Due to the three dimensional nature of the eggs examination was difficult at any higher magnification. The eggs are translucent white in colour and are covered with many small boss-like protuberances. After submergence in water a coat of fine 'hairs' is evident so that the eggs look similar to ciliated protozoans. These 'hairs' enable the eggs to adhere to surfaces as smooth as glass.

The first series of experiments (a) accomplished fertilization but development did not take place. Within 24h of fertilization a perivitelline space was evident in the majority of eggs (Fig. 5.4.1 b)) but no further development occurred. This perivitelline space did not form in eggs that were not fertilized in a control experiment and these eggs broke down after a period of approximately 2 weeks.

Fertilization was also accomplished in the second series of experiments (b & c) and development followed.

The length of time that the sperm and eggs were in contact influenced the proportion of fertilized eggs with approximately 50% fertilized after 5min, 70% fertilized after 10min, 80% fertilized after 15min and 85% fertilized after 20 and 30min. Hence, in all of the following experiments in the second series the sperm and eggs were left together for 15min as a fertilization rate of 80% was thought to be sufficient. It was also found that subimagoes did contain viable sperm or eggs and fertilization rates were no different when male and female imagoes, a male imago and a female subimago, a male subimago and a female imago and male and female subimagoes were used for the fertilization experiment. Nymphs that managed to break totally free from the chorion were regarded as having hatched.

As was found by Giberson (pers. comm.) the hatch rate of artificially fertilised eggs was less than 15%. Further, eggs hatched in only three of the experiments. All three successful experiments involved a male imago and a female imago. The eggs in the first two were fertilized by dissection of both imagoes and yielded very low hatch rates. Only 5% and 6% hatched in the first and second successful experiments respectively. In the third successful experiment (c) the female imago was not dissected but induced to release her eggs as described above. In this experiment a hatch rate of approximately 15% occurred. Those eggs that did hatch hatched between 16 and 22 days at 25 °C. No eggs incubated at 19 °C have hatched but many appeared to still be developing at the termination of this trial.

Fig 5.4.1. (b-g) shows the sequence of development over time. Once again the perivitelline space formed in approximately 24 hours. After five days a small embryo was visible as a dark area in the egg that had displaced some of the yolk. This increased in size and length and a head area, a thoracic area and an abdominal area were evident on the tenth day. After 14 days the embryo's began to resemble mayfly nymphs with a head, three thoracic segments and a number of abdominal segments. An eye patch, antennae and legs were also visible. Fig. 5.4.1.(f) shows a hatched egg with the hole in the chorion clearly visible. In all hatched eggs the hole was a longitudinal slit orientated near one pole of the egg. The first instar nymphs were approximately 350 μm long and resembled the later instars except for their pentagonal shaped heads lack of filamentous gills; simple tarsi and three equally sized ocelli (Fig.5.4.2 a) These nymphs were able to swim and crawl and fed by brushing the bottom of the petri dishes. Gills began to develop in the later instars and these instars were more active (Fig.5.4.2 b). Only two replicates of each experiment were performed due to a lack of available adults.

Discussion

Artificial Fertilization

The reasons for the failure of the first series of artificial fertilization experiments are not clear. Perhaps the containers in which the eggs were placed were not suitable (too deep, not sufficient oxygen). It was observed that algae soon covered the eggs on the slides which would not occur in the high current areas

in the stream where the eggs would settle.

The second series of experiments during which the eggs were incubated in shallow petri dishes was more successful. Algal growth was suppressed by covering the petri dishes with a semi-transparent lid, thus reducing the light intensity. In her successful experiments with a different species of mayfly, Giberson (pers.comm.) used Yeager's solution. Yeager's solution, however, triggered the adhesive 'hairs' prematurely and resulted in the eggs not adhering when placed in water. Thereafter, a fresh insect Ringers solution and a combination of the two were used and both proved successful. The reasons for the high fertilisation rate and low hatch rates are not clear and can only be determined with further experimentation. Many eggs could have succumbed to bacteria or other microorganisms so it may be necessary to use filtered stream water. Further, 25 °C may have been too high and the eggs may have been oxygen deprived. A possible method for this would be to fertilise a large number of eggs as described above and 'seed' them onto a fine mesh screen in still water. Once the eggs adhere which takes a few minutes, a current can be directed over the screen to ventilate the eggs. A detailed study of the development has not been accomplished at this stage as insufficient numbers of eggs and hatchlings were available.

Conclusion

It has been demonstrated that the eggs of *A. auriculata* can be artificially fertilised in the laboratory and viable offspring can be obtained. The most successful method involved dissecting the seminal vesicles out of a male imago in a fresh solution of freshly prepared insect Ringers. The female can then be induced to release her eggs into the solution by decapitation and dipping the tip of her abdomen onto the surface of the mixture. This mixture of eggs and sperm is then left for at least 15 minutes before the eggs are transferred to a suitable substrate in river water. Egg development is fastest at 25 °C. However, further work is necessary in this field to increase the hatch rate.

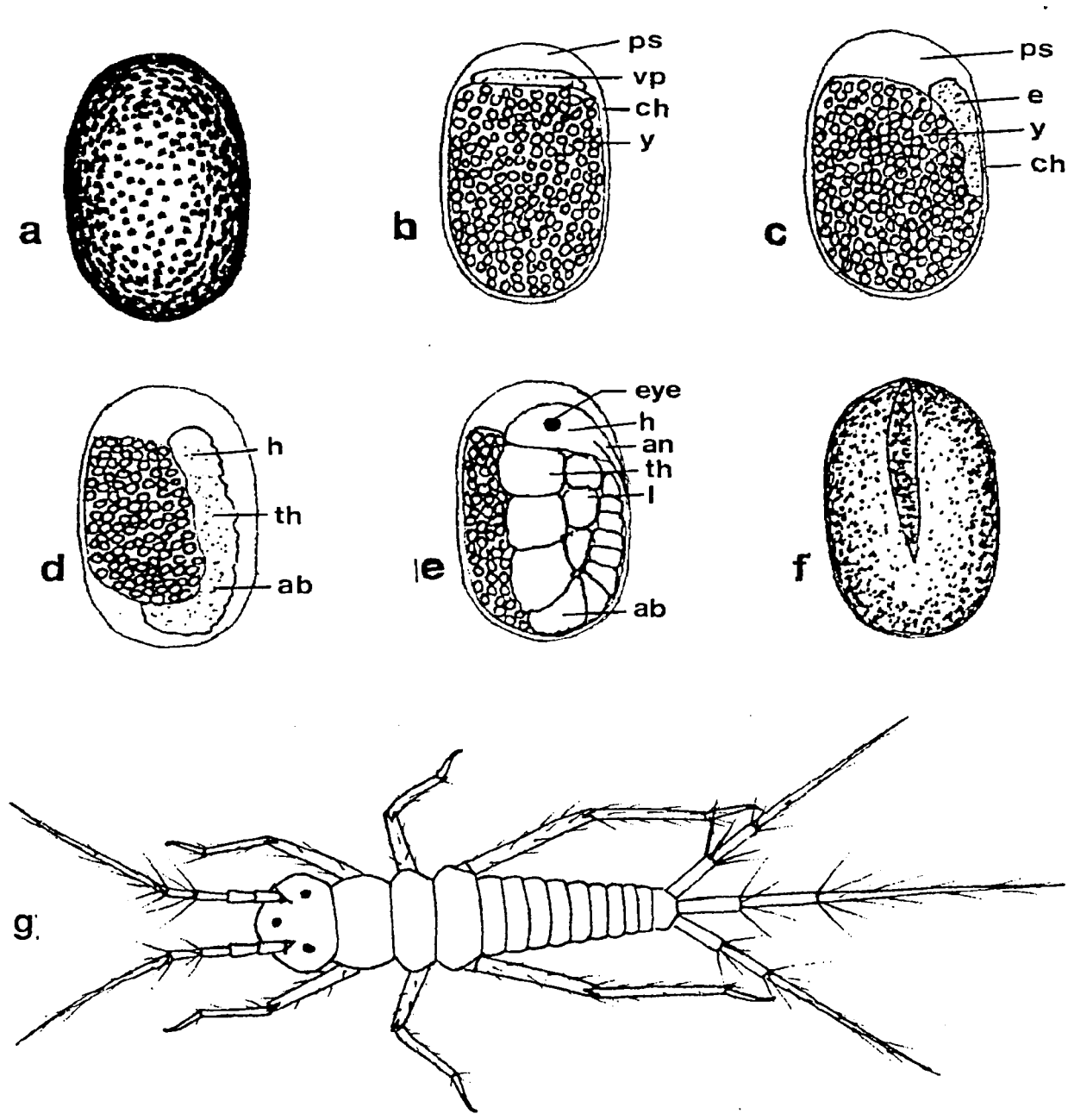

Fig. 5.4.1. Embryonic development of *A. auriculata* after artificial fertilization. (a) Surface of egg prior to fertilization; (b) fertilized egg with perivitelline space, 24 hours; (c) early embryological development, five days; (d) embryo with head, thoracic and abdominal regions, 10 days; (e) late embryological development, 14 days; (f) chorion of hatched egg, 16 days; (g) Newly hatched first instar nymph. ch- chorion; vp- ventral plate; ps- perivitelline space; y- yolk; e- embryo; h- head; th- thorax; ab- abdomen; an- antennae; l- leg. (Interpretation based on an embryological study of a heptageniid mayfly by Needham & Traver (1972))

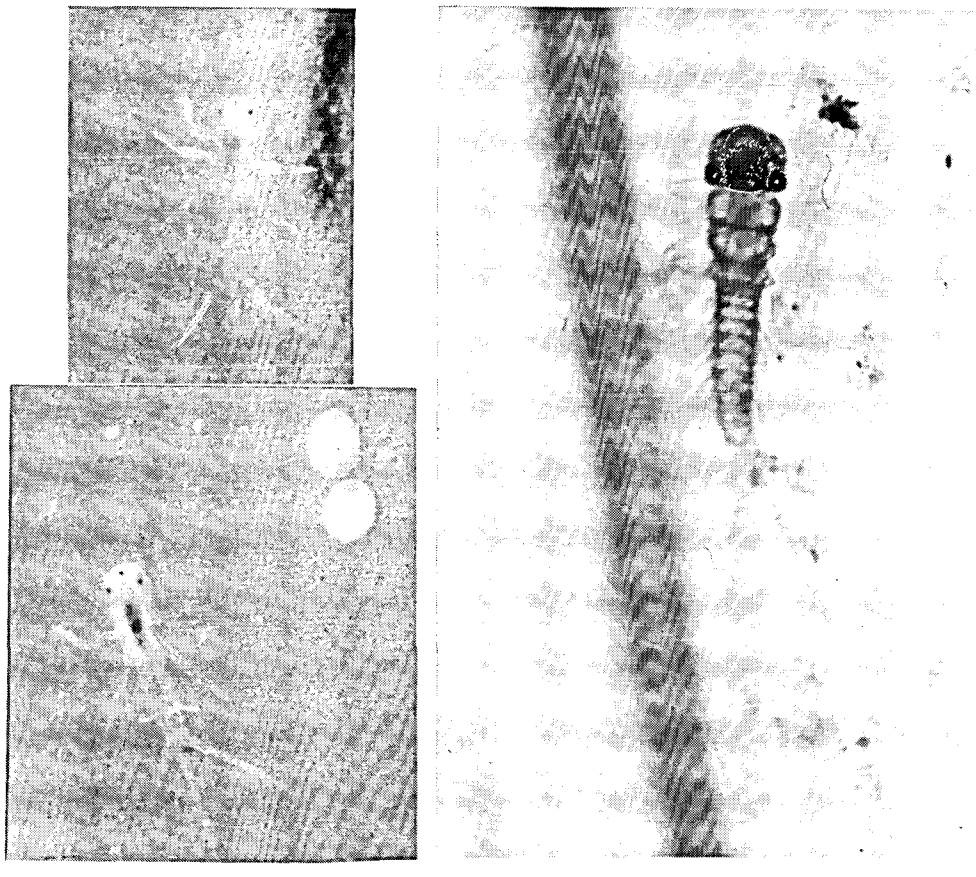

Fig. 5.4.2. Photomicrograph of nymphs of *A. auriculata* which were hatched from artificially fertilized egg. a) first instar nymph b) second instar nymph with two gills. (Mag.x 3200) c) Field caught nymph of the smallest size which is found in collections.

5.5 SUMMARY.

When a new field of research is broached the planning of the protocols is similar to a journey into unknown territory. Maps are available in the form of guidelines from previous work in related areas and established methods which can be adapted, but the topography of the landscape is unknown and the pitfalls are only discovered through experience.

In the case of an invertebrate aquaculture project the methods employed in pisciculture have offered useful guidelines as far as methods are concerned, and the literature of basic research on aquatic invertebrate biology has offered ample information to indicate the life history style which may be encountered in the selected candidate. Principles which were to be heeded in the execution of the project were found in the review of literature on the use of stream macroinvertebrates in ecotoxicology.

According to Johnson *et al* (1993), the life history indicators of environmental stress are survival, growth and reproduction. This was an indication that information about these life history parameters, under natural unstressed conditions, must be compiled and utilized as a baseline against which to judge results from toxicological experiments. Buikema & Benfield (1979) reviewed a hundred publications on toxicology and found that 50% used no life history information in the analysis of the results. A survey by Mayer & Ellersieck (1986) indicated a global need to develop acute toxicity methods for baetid and burrowing mayflies, caddisflies and stoneflies because these orders form an important component of stream ecosystems and are recognised indicator species in biomonitoring. Pontasch & Cairns (1991) suggest that mayflies are the group most sensitive to the deleterious effects of pollutants.

The experimental work on *A.auriculata* was planned in such a manner as to give information on their responses to:

a) Habitat variables such as hydraulic conditions and substrate type. It was discovered that solid substrate was an essential pre-requisite and, that although the species is found most abundantly in riffles and runs, it survives better in static aerated water than in experimental channels. This concurs with observations in the field that *A.auriculata* is seldom captured in the full current but tends to be found under stones on the edges of runs where detritus collects.

b) Diet and temperature variables. In this investigation it was found that the natural diet of decaying leaves and periphyton could be successfully supplemented by the addition of TETRAMIN, a commercial fish food, and provide optimal conditions for growth provided that good water quality was maintained. Daily growth rate in response to diet and temperature variation was calculated and is given in table 5.5.1 below. The average instar period for nymphs of *A. auriculata* was negatively correlated with temperature and the range was wide. The average instar period calculated was 7.5 days at 25°C; 10.44 days at 20°C and

15 days at 15°C. The shortest instar in all cases was 4 days and the longest 27 days.

Table 5.5.1 Growth rates (mm/day) pre sub-adult nymphs calculated from all experiments in channels and bubblepots in the laboratory

EXPERIMENT NO.	AVE. GROWTH RATE MM/DAY	TEMPERATURE CONDITIONS	HYDRAULIC COND. & DIET
5.2.1	0.019-0.023	19-22°C	Flowing; periphyton
5.2.2	0.014-0.042	17-22 °C	Static; leaves TETRAMIN
5.2.5	0.005 0.009 0.015	15 °C 20 °C 25 °C	Static, leaves
RANGE	0.005-0.04	15-25°C	

Buikema and Benfield (1978) discussed the importance of using both life cycle and life history information in designing and assessing toxicity tests. For many reasons, different life stages, such as immatures and gravid females, have been found to be more sensitive to toxicants. Reproductive impairment and moulting frequency are good measures of stress. Therforee, if the selected test species is to become a successful laboratory animal, its responses to test conditions should be accurately interpretable against adequate natural life history information. To fulfil some of these requirements, the field population was studied over a three year period. We determined that *A. auriculata* probably has a multivoltine life cycle. There was a major emergence period from February throughout March and April at ever decreasing numbers. Then in May, after a hiatus, another brief emergence was recorded followed by a relatively short emergence period in spring. During winter and summer no emergences were recorded and the sub-adults present in the samples showed no visible signs of metamorphoses.

Using the estimated experimental growth rates to calculate possible generation periods, a figure of 14 to 23 weeks is reached for *A. auriculata.* The spring emergence could give rise to the adults which emerge in late summer and the autumn emergence may give rise to late spring adults.

A captive breeding programme is essential to have sufficient numbers of test subjects of a guaranteed quality and genetic. Given the current level of knowledge about the breeding behaviour and cues for *A. auriculata,* natural reproduction in the laboratory is not yet feasible, so methods for artificial fertilisation were investigated. The most successful method involved dissecting the seminal vesicles out of a male

imago in freshly prepared insect Ringers solution. The female was induced to release her eggs into the solution by decapitation and dipping the tip of her abdomen onto the surface of the mixture. This mixture of eggs and sperm is left for at least 15 minutes before the eggs are transferred to a suitable substrate in river water. Egg development is fastest at 25 °C and viable offspring of *A. auriculata* resulted.

Further investigations of this species should include:

a) A detailed experimental investigation of artificial fertilisation techniques and methods to increase hatch rate.
b) Rearing of hatchlings.
c) Determination of growth rates and generation periods in the field.
d) Field investigations of mating behaviour.

The completion of this work should enable us to rear several other mayfly species from similar habitats and with similar food requirements in the laboratory. These may include *Choroterpes elegans* from the Buffalo River and other *Choroterpes* species. This genus has already passed the first test of survival as has *Trichorythus* spp. from the Sabie River (Kruger Park).

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CHAPTER 6
SUMMARY OF RESULTS

Introduction

The sustained cultivation of an organism in an artificial environment is dependant on several related factors, primarily the provision of a suitable environment. The design of the environment should be as closely modelled as possible to the natural environment in which the organism occurs; using those environmental influences considered to be the most important, including adequate uninterrupted food supply; the correct ambient temperature and photoperiod to optimise growth and fecundity; and in the case of aquatic organisms, water with the correct nutrient balance. Literature surveys may obviate the need for extensive and time consuming experimental investigation and results from short term research projects provide salient parameters. Once the abiotic factors and habitat are selected, the impact of other biotic factors such as density effects can be determined.

In this project the habitat requirements, some of the dietary requirements, the responses to temperature variation and a description of the natural environment of both species have been addressed.

6.1. SUMMARY OF RESULTS

The selection process for species to be maintained in laboratory systems was determined by the needs of the artificial stream project, in their investigation of tolerances of lotic macro-invertebrates to water quality variables. This lead to the investigations into the biological and environmental requirements of those species selected, and the necessary equipment and methods necessary for maintaining them.

6.1.1. SCREENING OF ORGANISMS.

There are certain criteria to be considered in the selection of organisms in determining their suitability as subjects for ecotoxicological studies. For the purposes of both the Artificial Streams Project and the Standard laboratory Organisms Project, the hierarchy of considerations in the selection process were ranked as follows;

1. Availability in the field and size of the organism. A field population of the candidate

species must be easily accessible, so that stocks for early laboratory experiments can be replenished.

2. Suitable life history and biology for maintenance in laboratories. Problems associated with aerial phases can be overcome. Narrow habitat requirements would necessitate specific, possibly expensive, holding conditions and would, therefore, be undesirable. So too would very specific dietary requirements.
3. Accurate identification.
4. Sensitivity to a range of test chemicals.
5. Position and function in the ecosystem.

Several screening trials, observations during toxicological investigations conducted by Palmer *et al* (1995), and field collections contributed to the identification of 18 possible taxa, found among the Crustacea, Mollusca, Insecta and Platyhelminthes. Those taxa which have been subject to detailed investigation as a result of this selection process are:

- Ephemeroptera; Leptophlebiidae.
  Euthraulus (Choroterpes) elegans and *Adenophlebia auriculata:*
- Mollusca; Ancylidae
  Burnupia stenochorias:

Further genera which have potential as laboratory candidates are *Tricorythis* sp. (Ephemeroptera); *Cheumatopsyche* spp. (Trichoptera) & Planaria. Among the Crustacea, an amphipod, *Paramelita* sp., is already used as a test subject at the University of Cape Town and *Caridina* sp. has been successfully bred at the University of Natal.

6.1.2 METHOD DEVELOPMENT

The conditions which were a prerequisite for maintenance and breeding of macroinvertebrates from rivers were investigated, and the following key parameters were identified: hydraulic conditions and water quality, substrate, diet and temperature.

With regard to collection and transport, animals should be handled as little as possible during collection and transported in cool, well aerated water with substrates. Two forms of maintenance equipment were tested, namely recirculating channels of two sizes, and cylindrical plastic bubblepots of various sizes. These proved to be the most suitable containers for rearing small juveniles of both the limpets and the mayflies. Observations from all experiments confirmed that solid substrates are essential for the optimal survival of the selected stream macro-invertebrates. However, for their transport, softer substrates such as fine textured plastic foam rubber, plastic sheeting or leaves from the collection site should be offered.

With regard to dietary requirements, the limpet is a grazer and the mayflies are brusher collectors: both animals collect their food by scraping the surface of stones. Periphyton grown in the laboratory has proved to an adequate basic food to sustain growth in both species, with degraded leaves, river detritus and TETRAMIN also suitable for mayflies.

In conclusion, the species investigated to date can be adequately housed and fed prior to being used for experimental purposes in the Artificial Streams Project. The most important conditions for the successful maintenance, and particularly breeding, of invertebrates are:

1. Correct light conditions for algal growth to ensure good food supply.
2. Adequate thermal regulation.
3. A good water supply, preferably from a non municipal source with the facility to regulate its quality and condition.

6.1.3 INVESTIGATION OF SELECTED SPECIES

In attempting to satisfy the second aim of the project (to develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions) a two-pronged approach was used. To be able to design optimal holding and breeding conditions for any species some understanding of the life history, the habitat requirements and the breeding biology is needed. Consequently, the responses of the selected species to laboratory conditions were recorded experimentally, while the ecology and life history were recorded by regular field investigations.

6.1.3.1 Laboratory Maintenance and Rearing Conditions

Hydraulic requirements

It would appear that neither species is obligately rheophillic as both species showed good survival in bubblepots. It was clear that the provision of substrates is essential in both hydraulic conditions. Hatchlings of limpets survive better in bubblepots, mainly because they are easily swept from channels. The limpets laid a greater number of eggs in aerated, standing rather than in aerated, flowing water.

Dietary requirements

Burnupia is a microherbivore which feeds by rasping the stone surface with the radula. It has been adequately maintained on laboratory cultured periphyton (see Chapter 4). It does not require feeding during short experiments (96 hours, acute test period: see Section 4.2.1.3). The mayflies are collector gatherers and it was found that the natural feed of decaying leaves, detritus and periphyton could be supplemented by TETRAMIN, which provided optimal conditions for growth.

Temperature

Temperature effects

Both species responded in a predictable manner to various, constant temperatures (15°C, 20°C and 25°C) with increased growth rates, and the mortalities of the limpets did not appear to be significantly affected. Mayflies responded with significantly shortened instar periods but with increased mortalities at 25°C.

Density effects

The best survival was attained at a density of 20 limpets at 20°C. This can then be regarded as the optimal stocking density. However, the effect of this density and temperature on the fecundity is unknown, and it has been stated by many authors that temperature which rises above certain optimal limits has a detrimental effect on the fecundity (section 4.2.2)

Handling requirements

Handling adversely affects the growth and survival of limpets. Anaesthetics must be used when collecting from the field, and a measuring template during laboratory experiments. Mayflies are more robust and can be easily handled with a small camel hair paintbrush.

6.1.3.2 Biology of selected species

Breeding biology of *Burnupia stenochorias* .

As hermaphrodites (Brown, 1980) these limpets are able to produce young without copulating, but copulation has been observed, with the smaller limpet acting as the male. Copulation takes place at night and early in the morning, with eggs being laid at night under stones. The size of limpet at time of egg-laying was always observed to be at greater than 3,4mm shell length. Field observations for eight months have determined that a small number of eggs are laid throughout the winter, but that a substantial increase in egg-laying occurs in the months of September and October.

Embryology

Burnupia displays direct development, with the young emerging as crawling snails. Hatching success in the laboratory is 91%. The embryonic period is 14 to 17 days at a temperature of 19°C. Cooler temperatures extend the time of hatching up to 21 days at 13°C (pers.obs.).

Fecundity

The number of eggs per capsule ranges from 1 to 13. Those limpets reared in the wild laid between 14-44 (ave 24.7) capsules per pair with an average of 5,75 eggs per capsule when brought into the laboratory. Juvenile limpets (approx. 2,5mm) reared in the laboratory either as pairs or singly produced on average 3,7 egg/capsule and 1-16 (ave 8.1) capsules per pair.

Breeding biology of *A. auriculata*

The breeding biology of *A. auriculata* is unknown and literature searches indicate no captive mating has ever been described. However it was demonstrated that the eggs could be artificially fertilised, producing viable hatchlings.

Field investigations

The field population has been studied over a three year period in the Palmiet River where the largest numbers of *A. auriculata* are found in riffles and runs. The size distribution of nymphs is similar in these two habitats but higher densities occur in runs. Hatchlings have not yet been captured in the field. There are major emergences in late autumn/early winter, late winter/early spring and a large summer emergence over several weeks in February and March. Size distributions of nymphs over a twelve month period indicates overlapping cohorts. Laboratory growth trials have given an indication of the growth rate and therefore possible generation period in laboratory conditions.

6.2 COMPLETION OF AIMS

i. *To screen riverine organisms from different regions of southern Africa, in order to identify suitable species for laboratory maintenance.*

♦ Screening was accomplished in the eastern areas of the country. Invertebrate researchers countrywide were consulted by questionnaire and eighteen candidates suitable for laboratory cultivation were identified. Of these, three species were investigated further.

ii. *To develop a pilot programme to maintain reproducing populations of these selected standard organisms under laboratory conditions.*

♦ A pilot programme has been established and methods to feed and house these chosen invertebrates are in place. Problems do, however, exist concerning long term survival of the limpet (through more than two generations) and the artificial fertilisation and rearing of hatchlings of the mayfly.

iii. *To attempt to establish methods for the sufficient supply of suitable taxa for a range of experimental purposes which would include toxicity testing and test for macroinvertebrate to various conditions in the experimental stream project.*

♦ Until the problems referred to in (ii) are solved, the sufficient supply is not yet feasible. However during the course of the project toxicological experiments were supplied with limpets on two occasions and several problems associated with handling and transporting the test species were addressed and solved.

6.3 CONCLUSION AND DISCUSSION

We believe that the new knowledge gained about responses of these two species to environmental variables has been significant, contributing to the understanding of ecosystem function of subtropical rivers.

It is believed that enough knowledge has been accumulated to enable a sustainable pilot maintenance project to be launched. From the figures obtained about growth rate, expected survival and density effects recorded, the productive potential of each of the large channels or bubblepots can be calculated, despite the areas of uncertainty which remain.

Aspects of the life history of the mayfly that still need investigation include;
a) the exact influence of temperature on growth rate and field growth rates;
b) fecundity and factors which influence it;
c) metamorphoses;
d) the abode of the nymphs less than 0.4mm HW.;
e) cues which trigger emergence.
f) techniques for artificial fertilization and hatchling rearing, the most important aspect to be investigated.

With regard to the limpet, the high mortality experienced in the laboratory must be overcome. It is suspected that both the water supplied to the limpets in the laboratory, to date, and the inadequate supply of the correct diatom combination, could be the causes. Detailed investigation of the diet should be completed.

CHAPTER 7
RECOMMENDATIONS FOR FUTURE WORK

Given the problems highlighted in Chapter 6, the sufficient supply of test species to the stream laboratory on an onoging basis is not yet possible. The bottlenecks we have identified are reproduction in captivity of the mayfly and life long survival of a large proportion of both selected species.
We estimate that the further requirements for the cultivation, maintenance and supply of sufficient experimental animals for ecotoxicological experiments to artificial stream laboratory can be listed as follows:

- Further knowledge of the cues for breeding, and better survival of juvenile *Burnupia.*
- Development of techniques for artificial insemination and maintenance of early instars of *A. auriculata.*
- The investigation of optimal growing conditions for the cultivation of periphyton.
- Increasing the scale of laboratory maintenance facilities to provide more capacity for the maintenance of larger laboratory populations.
- The design and construction of a dedicated laboratory for production and maintenance of experimental populations of invertebrates.

7.1 DEVELOPMENT OF A DEDICATED REARING AND MAINTENANCE LABORATORY

Present facilities in the Institute for Water Research are inadequate for the envisaged production of experimental animals. The requirements for a purpose-built facility are as follows:

- Floor space of at least 100 m^2.
- Adequate temperature control, by air-conditioning.
- Adequate natural light.
- Periphyton cultivation facilities which are separate from the insect rearing facilities.

- Water purification facilities, e.g. by ultraviolet irradiation and filtration.

- Rearing and breeding channels, as well as extensive population holding facilities, with temperature and light control.

Two initiatives are proposed for the second phase of this project, which begins in January 1996:

- A visit to Stroud Water Research Centre, Pensylvania, where a large amount of mayfly research is conducted and Virginia Polytechnic in Blackenberg where ecotoxicological testing has been ongoing for 20 years, to learn about the conditions described above.

- The engagement of a fund-raiser, to obtain funding for the building of a proposed laboratory.

7.2 OBJECTIVES FOR THE SECOND PHASE OF THE PROJECT.

The objectives of the proposed project can be divided into three main aims:

1) To complete the experimental and field investigations on the selected test species:
Burnupia stenochorias. Field studies of natural reproduction and laboratory studies on specific feeding requirements and handling techniques wil be executed.
Adenophlebia auriculata. The laboratory studies on artificial fertilization, and the rearing of hatchlings will be continued.

2) To scale up the experimental techniques developed to a larger production level. To test the suitability of various biological filtration systems at this larger scale to ensure optimal water quality. To optimise the culture of selected diatom species present in periphyton.

3) The investigation of design features and costing for a laboratory dedicated to the large-scale production of the selected species.

7.3 PROPOSED WORK PROGRAMME.

7.3.1 *BURNUPIA STENOCHORIAS.*

a) The field investigation currently being conducted into the reproductive biology of the limpet, should be completed by August 1996. Fortnightly observations which involve the measurement of 300 individuals and the recording of environmental variables, are made at two sites on the Blaauwkrantz River.

b) To identify the onset of sexual maturity by sectioning individuals of known age and size collected through the year will be completed by September 1996. This will link individual size to sexual maturity.

c) Investigation of the dietary requirements of the limpet will be initiated in January 1996, followed by the investigation of fecundity in response to diet.

d) Analysis of the results will be conducted during the experimental period but final interpretation will be possible at the completion of the experimental work.

Mrs Heather Davies-Coleman will be responsible for this work with support from and supervision by Mrs. Haigh and Prof. O'Keeffe.

e) Experiments to test handling methods using two anaesthetics will be conducted by Mrs Haigh in collaboration with Mrs HI White of the Department of Fisheries Science and Ichthyology, Rhodes University.

f) The identity of the various members of the genus *Burnupia* is not clear because shell shape which is the major identifying feature varies not only between species, but also between sites, depending on current speed and type of substratum (Brown, 1980). To identify species with certainty it is necessary to investigate the genetic basis of variations among populations and taxa. This would form the basis for postgraduate thesis. The uncertainty in this area is a problem with the development of the limpet as a country wide test species

7.3.2 *ADENOPHLEBIA AURICULATA.*

a) The current field investigation of this species in the Palmiet river will be completed by May 1996.

b) Experimental work on artificial fertilization and rearing of the hatchlings will be ongoing in 1996 and early 1997.

c) The influence of temperature on growth and fecundity will be investigated as part of the rearing programme.

Mrs Lil Haigh will be responsible for the investigations of the mayfly.

7.3.3 PERIPHYTON.

The investigation of the identity of the component periphyton species and optimal water quality conditions for the growth of periphyton will be conducted in collaboration with Prof. Braam Pieterse at Potchefstroom University and possibly with Prof. Grobbelaar from University of the Free State during 1997.

7.3.4 DEVELOPMENT OF THE REARING FACILITY

A preliminary agreement has been reached with Dr Anton Bok of the East Cape Department of Nature and Environmental Conservation to conduct a trial at Amalinda fish hatchery in East London, where an existing indoor pond which appears to be suitable for the maintenance of invertebrates on a large scale is situated. Water of excellent quality. This trial will enable us to monitor the interior environmental conditions in a structure of this type for an extended period and establish the suitability of this design. It is foreseen that the commissioning of the trial will be undertaken by Mrs Haigh but the monitoring will be carried out by staff at the hatchery under her supervision.

Once these investigations have been completed plans can then be drawn up for the construction of a dedicated facility in Grahamstown. A fund-raiser will be engaged to obtain funding for the project. Prof. T. Hecht and Mr. P. Britz at the Department of Ichthyology and Fisheries and Science at Rhodes both have extensive experience in the construction of aquaculture facilities and have agreed to oversee the planning phase.

7.4 REFERENCES

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HUGUENIN, J.E. & COLT, J. 1989. Design and Operating Guide for Aquaculture Seawater Systems. Elsevier Science Publishers, Amsterdam.

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APPENDIX 2

2.1 INVESTIGATIONS INTO THE IDENTITY AND LOCATION OF SUITABLE TAXA.

Table 2.1.1. Collecting trips undertaken.

DATE	RIVER AND LOCATION
January, September & October 1993	Buffalo River; East London -Maden Dam
February 1993 to date	Berg & Palmiet Rivers; Grahamstown district
March 1993 to date	Belmont Valley stream; Grahamstown
July - August 1993	Sabie River; Kruger National Park
August 1993	Nahoon river; East London
March 1994	Kologa & Buffalo River; Stutterheim

Table 2.1.2. Availability of suitable species from the sites visited over the period of the project to date. Abundance has not been quantified. XX indicates that sufficient numbers (at least 180) of animals of similar size in good condition were collected during a two to three hour period for a replicated experiment (s/s = small sizes).

LOCALITY	SPECIES	TIME /DATE	ABUNDANCE		
			Good	Fair	Poor
PALMIET (Grahamstown)	Leptophlebiidae, Baetidae Trichoptera	DECEMBER -MARCH			X
		MARCH - MAY	XX		
		MAY - JULY		X	
		AUGUST - DECEMBER	XX		
BLAAUWKRANZ stream Grahamstown	Ancylidae	AUGUST - NOVEMBER	XX	X	
		JANUARY - APRIL			X s/s
	Baetidae	AT ALL TIMES	XX	X	
BUFFALO upper reaches	Leptophlebiidae Baetidae, Trichoptera Ancylidae	JANUARY		X	
		MARCH - APRIL	XX		
BUFFALO lower reaches (East London)	Leptophlebiidae	SEPTEMBER	X		
KOLOGA river	Ephemeroptera many spp. Ancylidae Trichoptera	APRIL		X	
SUNDAYS river, Carlisle bridge	Baetidae Trichoptera			X	

2.1.1 CONSULTATION WITH OTHER RESEARCHERS

Invertebrate experts country-wide have been consulted to gather information and solicit opinions on the suitability and availability of species for use as regional standard taxa. A questionnaire was compiled and circulated to invertebrate workers throughout the country. Those which were returned, have been analyzed and the table below is the result.

Several people were interviewed during the past year. These include; At RAU Prof. Schoonbee and Dr. Bickerton at the CSIR (Stellenbosch) on the culture of Crustacea. Drs Day and King and their staff and Prof. Davies and his co workers at UCT. Consultation was also held with Prof C. Appleton from UCN on the subject of molluscan culture. The anecdotal information thus gathered, was used to help during the designing of the equipment and in selecting suitable taxa. Questionnaire appended (APPENDIX 2a)

Table 2.1.3. List of possible suitable candidate taxa.

GROUP	*SPECIES*	AREA	RESPONDENT
CRUSTACEA	*Paramelita nigricolus* *P. capensis*	W. Cape	Day; Griffiths
	Caridina spp.	Natal	Rayner; Alletson
	Palaemon capensis	W Cape	Day
	Macrobrachium sp.	E Tvl.	Van Vuren
	Streptochephalus sp.	E Cape	Day
MOLLUSCA	*Bullinus tropicus*	Natal	Rayner
	Ferissia sp.	Natal	Rayner
	Burnupia sp.	Wide	O'Keeffe
	Lymnaea natalensis	E. Cape	
	Physopsis globosus		
Planaria	*Dugesia sp.*		Day
INSECTA Ephemeroptra	*Trichorythus spp.* *Afronurus harrisoni* *Adenoplebia auriculata* *Choroterpes elegans & other spp.*	Wide	Alletson; Palmer
Trichoptera	*Cheumatopsyche afra &* *C. thomasetti*		

2.2 SCREENING TRIALS FOR THE SELECTION OF TAXA SUITABLE FOR LABORATORY CULTIVATION.

Screening of faunal complexes involved placing collections in a variety of laboratory conditions and noting their responses and survival under laboratory conditions. Screening was carried out in the Eastern Cape and in the Kruger Park (Mpumalango) where fauna from the Sabie River was screened. Samples were placed in raceways, bubble pots and flow-through channels and observed.

2.2.1 BUFFALO RIVER FAUNA IN RACEWAY

In January 1993 the first field collection was made from the Buffalo river. The opportunity was also used to investigate collection and transport methods on an informal basis. The collected samples were transported in cooler boxes with leaves and detritus. The collected animals were placed in a raceway after being sorted in the laboratory. Stones and detritus from the collection site was placed in the raceway. The laboratory was air conditioned to a steady 19°C. The survival of the captive population was monitored every third day until the air conditioning failed. Rising temperature rendered conditions unsuitable for continued work and resulted in all the animals dying after three weeks.

Results

The following deductions were made from the observations of the species in the raceway.

a) Ephemeroptera. Several families of mayflies were represented in the collection but only Leptophlebiidae (*Adenophlebia & Choroterpes)* were present in significant numbers. Members of these families were regularly found on or under the rocks positioned closest to the paddle wheel where the water had the greatest amount of turbulence.

b) Coleoptera. A small number of Psephenidae larvae survived and these grazed on the surface of the greenest rocks in the straight of the raceway. However these were the first to die when the temperature rose.

c) Mollusca. Freshwater limpets of the family Ancylidae (*Burnupia*) showed the best survival rate and were found in the straight and on the sides of the raceway.

2.2.2 PALMIET RIVER FAUNA IN RACEWAY and SMALL BUBBLEPOTS

Aim

To discover if directional current is essential for the survival of animals found in riffles and runs in rivers. Artificial channels require expensive construction while containers such as basins and buckets are readily available at any supermarket shelf, can easily be equipped to provide static aerated water with readily available aquarium equipment.

Materials and methods

A collection was made in the Palmiet/Berg River east of Grahamstown and kept under similar conditions

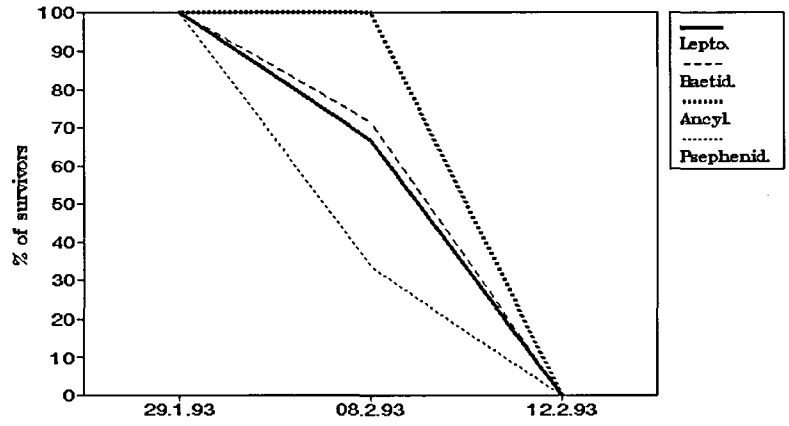

Fig. 2.2.1 % survivors in raceway during 1993.

to those described in 2.2.1 above. An emergence cage was constructed for the raceway to trap emerged adults.

Fifty-four Leptophlebiidae, 31 Baetidae, 9 Trichoptera, 7 Platyhelminthes and 3 Chironomidae were brought back from the field and placed in the raceway.

Three 500ml plastic bottles were equipped with air-stones and pebbles. Two to three *Adenophlebia auriculata* mayfly nymphs were placed in each of these bottles.

Results

Breakdowns of equipment such as failure of the air-conditioning and the burnout of the paddle well motor contributed to the death of the experimental animals.

As can be seen from Fig. 2.3.2.1, 57% of the Baetidae and 60% of the Leptophlebiidae survived for three weeks. The reduction in numbers of the mayflies can largely be ascribed to adult emergences. Altogether 14 (42%) Baetidae and 4 (7.5%) Leptophlebiidae emerged. Of the mayflies placed in the bubblepots 80% survived for four weeks and two of these emerged.

This simple holding exercise gave an indication that both raceways and bubblepots could be good holding facilities provided that sufficient food is available. The survival rate obtained in small bubblepots prompted an investigation into the suitability of this method for long term maintenance and feeding. Several trials were conducted.

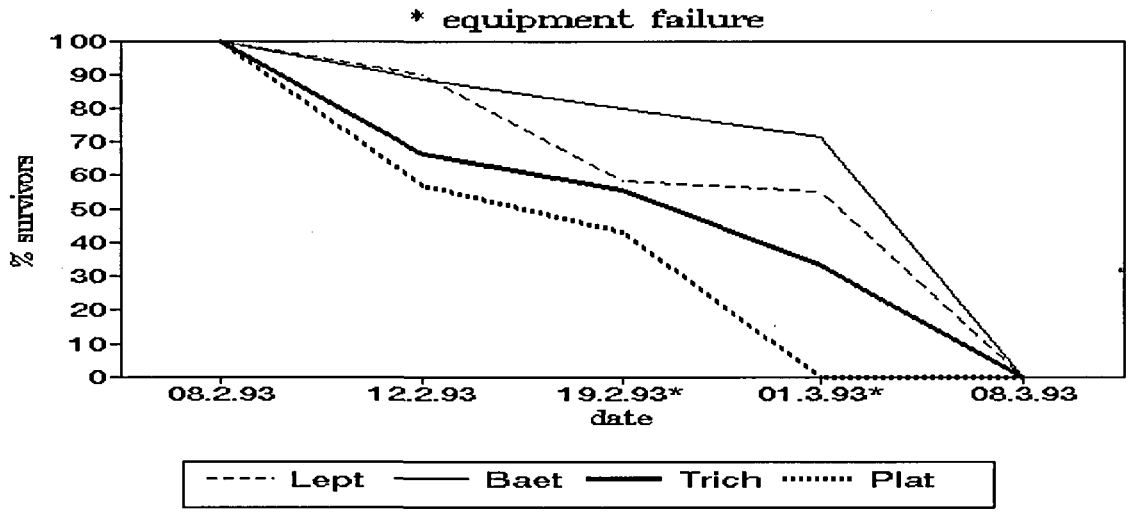

Fig. 2.2.2 % survivors of fauna from the Palmiet river Grahamstown in raceway.

2.2.3 SCREENING OF INVERTEBRATE FAUNA FROM THE SABIE RIVER (MAPUMALANGA)

Aim

To screen the most abundant taxa of riffles in the Sabie River in the Kruger National Park for suitability to captive maintenance under laboratory conditions. The survival response to different feeds and a variety of substrates was assessed.

The following trials were conducted.

1. To test the suitability of commercial food for feeding of invertebrates from a relatively pristine river.
2. Response of fauna to DO levels (see Ch. 2 & App. 2).
3. Screening of various substrates for suitability (see Ch 2 & App. 2).

Materials and methods

This was the general experimental protocol which was followed in the Kruger National Park

Sets of three or four replicates of 500ml bubblepots were used. River water was used in all cases. The pots were aerated by electrical aquarium air-pumps. No temperature or daylight control was attempted. The controls contained no food or substrate.

Families and genera used in the experiment:

ORDER	FAMILY	GENERA & SPECIES
Trichoptera	Hydropsychidae	*Cheumatopsyche thomasetti* & other spp.
	Philopotamidae	*Chimara* sp.
ORDER	FAMILY	GENERA & SPECIES
Trichoptera	Leptoceridae	*Leptoceris* sp., *Oecetis* sp.
Ephemeroptera	Leptophlebiidae	*Choroterpes* spp.
	Baetidae	*Centroptilum* sp., *Afroptilum* sp., *Pseudocloeon* sp., *Baetis* spp.
	Heptageniidae	*Afronurus* spp., *Compsonuriella* sp.
	Tricorythidae	*Thricorythus* sp., *Neuroceanis* sp.
	Caenidae	*Austrocaenis* sp.
Coleoptera	Gyrinidae	
	Elmidae	
	Dryopidae	

2.2.4 SURVIVAL TEST OF TAXA KEPT IN BUBBLEPOTS, FED ON TETRAMIN AND DETRITUS SEPARATELY AND MIXED

Aim

To test the suitability of commercial food for feeding of invertebrates from a relatively pristine river.

Method

1) Two screening exercise was attempted but were attempted to screen fauna in the Sabie river. Replicates of bubblepots were supplied with a variety of feed as given below.
2) Tetramin : 1 mg in 50ml of water; 5ml of suspension in each 500ml bottle.
   Detritus : suspension from collection site, 5ml in each 500ml bottle.
   A mixture of detritus and Tetramin suspension as above (D & T): 5ml in each 500ml bottle.
   No food was (no fd) added to the control.
3) Air supply to the pots was evenly regulated. The containers were monitored daily. The ambient photoperiod was 11H00.

Results

Ambient and water temperature ranged between 14-25°C.

Mortalities were extremely high in the first two trials. Only after foam rubber pads were tested as an alternative substrate to netting did survival rates improve.

The results which are of primary interest are the survival rates of the various taxa. However, as far the diet offered is concerned, the smallest percentage of all taxa survived in Tetramin and the largest percentage in the control with no food (Fig.2.3.3.1). Baetidae did best on detritus and Leptophlebiidae on

mixed food while the Tricorythidae fared best under all circumstances.

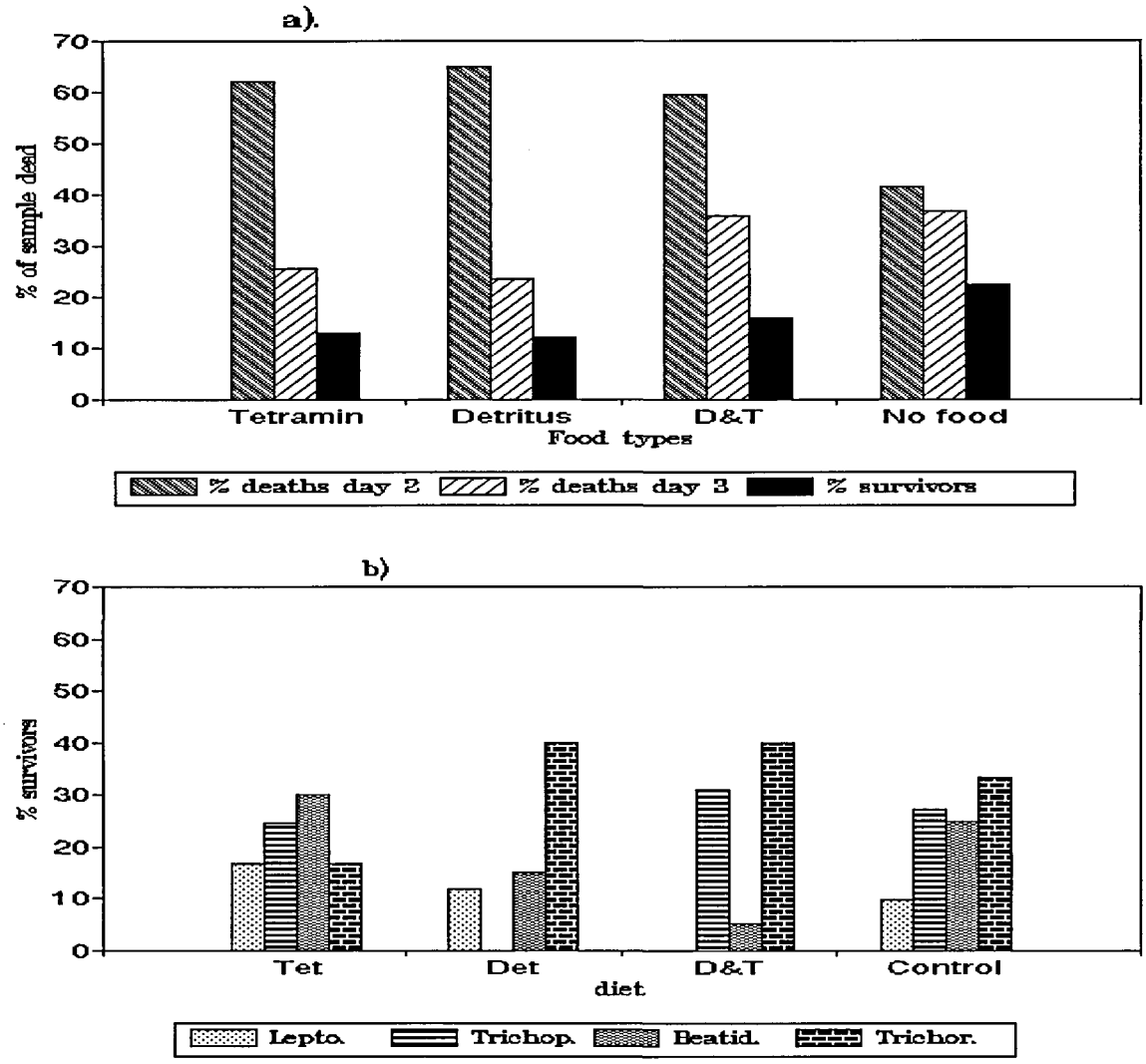

Fig.

Fig. 2.2.4 Bar graphs showing (a) the percentage of the population that died and survived during this period (b) Percentage survivals of each population in all the replicates in each food treatment.

Conclusions

1) The best survivors of all the taxa which were screened in all the experiments conducted in the Kruger National Park were TRICHOPTERA (Hydropsychidae, *Cheumatopsyche* sp.); EPHEMEROPTERA, (Trichorythidae, *Thrichorythus* spp., and Leptophlebiidae, *Choroterpes* spp.).

2) No conclusions could be drawn about the suitability of the food as these experiments as survival was not long enough to make such deductions. The only deduction which can be made from these observations is that invertebrates should not be offered any food when they are returned from the field but allowed to acclimate in water from the environment only.

3) The 500ml bubblepot with netting as substrate is not a suitable container. Netting provided inadequate refuge from the turbulence in the pots, which could have damaged the specimens. Test with foam pads substantiated this conclusion.

4) High water temperatures could have affected the metabolic rate and the available oxygen.

APPENDIX 3

Appendix 3 contains trials conducted to ascertain the effects of various types of equipment and the conditions created in these. Abbreviated result are reported in Chapters 3, 4 and 5.

3.1 DEVELOPMENT OF EQUIPMENT AND METHODS. (Ch. 3.3.8)

Several tests were conducted to establish the conditions in and the suitability of equipment for experimental and holding conditions. A brief report on each experiment is given here.

3.1.1 DISSOLVED OXYGEN

3.1.1.1 TEST OF THE LEVELS OF DISSOLVED OXYGEN DELIVERED BY AIRSTONES IN VARYING VOLUMES OF WATER AND AT FLUCTUATING TEMPERATURES

Aim. To ascertain if airstones will delivers the same amount of oxygen despite a variation in water volume.

Method.
Two replicates each of 1, 2,3 and 5 litres of water were supplied with the same size and make of airstone from a central supply. The temperature and dissolved oxygen levels were measured regular intervals. The containers were moved between two CER one set to fluctuate between 15 - 20 °C and one at 25 °C.

Results
The air-stones appear to aerate the water to the same level despite the different volumes. Temperature appears to have a marked negative correlation with the amount of dissolved oxygen in the water, with corresponding peaks and valleys between rising temperature and falling DO levels.

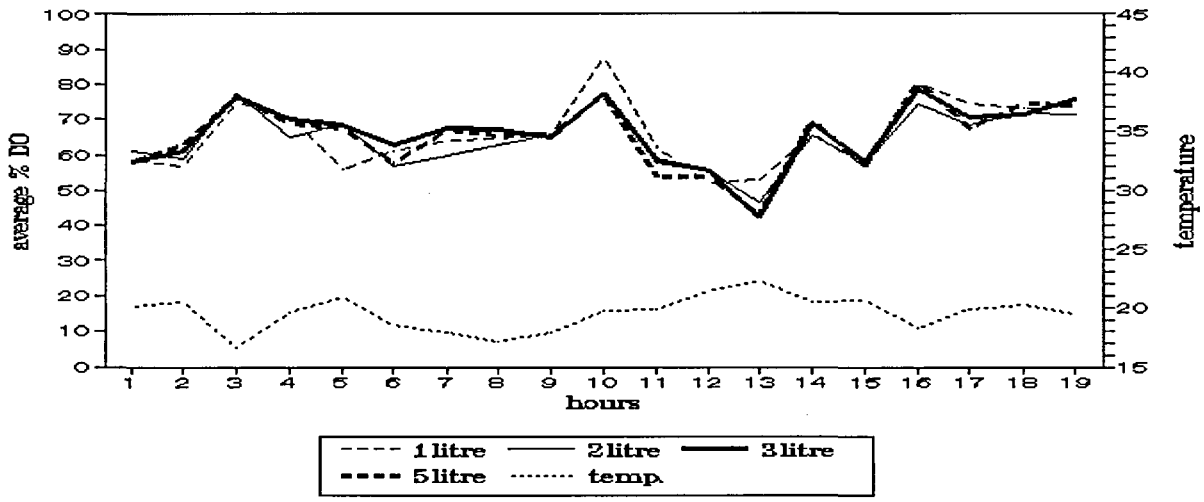

Fig 3.1.1.2.1 Average temperatures in four volumes of water at two temperature regimes.

3.1.1.2 INTERACTION BETWEEN WATER VOLUME, DISSOLVED OXYGEN LEVEL TEMPERATURE AND THE RESPONSE OF *CHOROTERPES* SPP. TO THESE VARIABLES.

As it has been established that temperature and levels of dissolved oxygen are related the next test was to establish the effects of these variable on the survival of mayflies. The levels of dissolved oxygen in riffles and runs of rivers are generally quite high and therefor could be a limiting factor in the survival of rheophyllic animals in static water.

Method

1) Eight 500 ml bubblepot in replicates of two containing with 500 ml, 350 ml and 250 ml of aerated river water and controls with 250 ml non-aerated water were prepared and furnished with plastic netting as substrate.
2) Ten *Choroterpes* nymphs were placed in each bubblepot.
3) Pots were checked hourly during the day but not at night for 36 hours.

Results

Mortalities started occurring as the temperature rose and after 32 hours, 80% mortality was reached.

The most obvious difference in amount of DO is between the 250ml of aerated and static water as can be expected. In the 250ml non-aerated water, the oxygen levels started dropping as soon as the ambient temperature rose and as time progressed overnight oxygen levels dropped down to 30%. This did not occur in any of the other replicates. In 250ml non aerated pots mortalities occurred in the middle of the second day when DO levels had been below 20% for four hours. This would indicate that *Choroterpes* sp. has the ability to respire at relatively low DO levels for short periods. In all other volumes of water DO remained at similar levels but mortality was different. The smallest volume, 250ml aerated water,

showed the most rapid early mortality which may be ascribed to the higher turbulence in the container due to a smaller volume of water. The next most rapid mortality was at 350ml. In 500ml volume of water, which had both the highest levels of DO and the least turbulence, the best survival was evident. Unfortunately only the ambient temperature was recorded so direct temperature effects can not be examined. However the water temperatures were generally 1°C lower than ambient.

This test reveals:

a) The resilience to low DO levels for short periods by *Choroterpes*

b) The possible effect of turbulence on survival of mayflies

c) The essentiality of aeration for the maintenance of constant levels of dissolved oxygen in standing water for the long-term survival of mayflies.

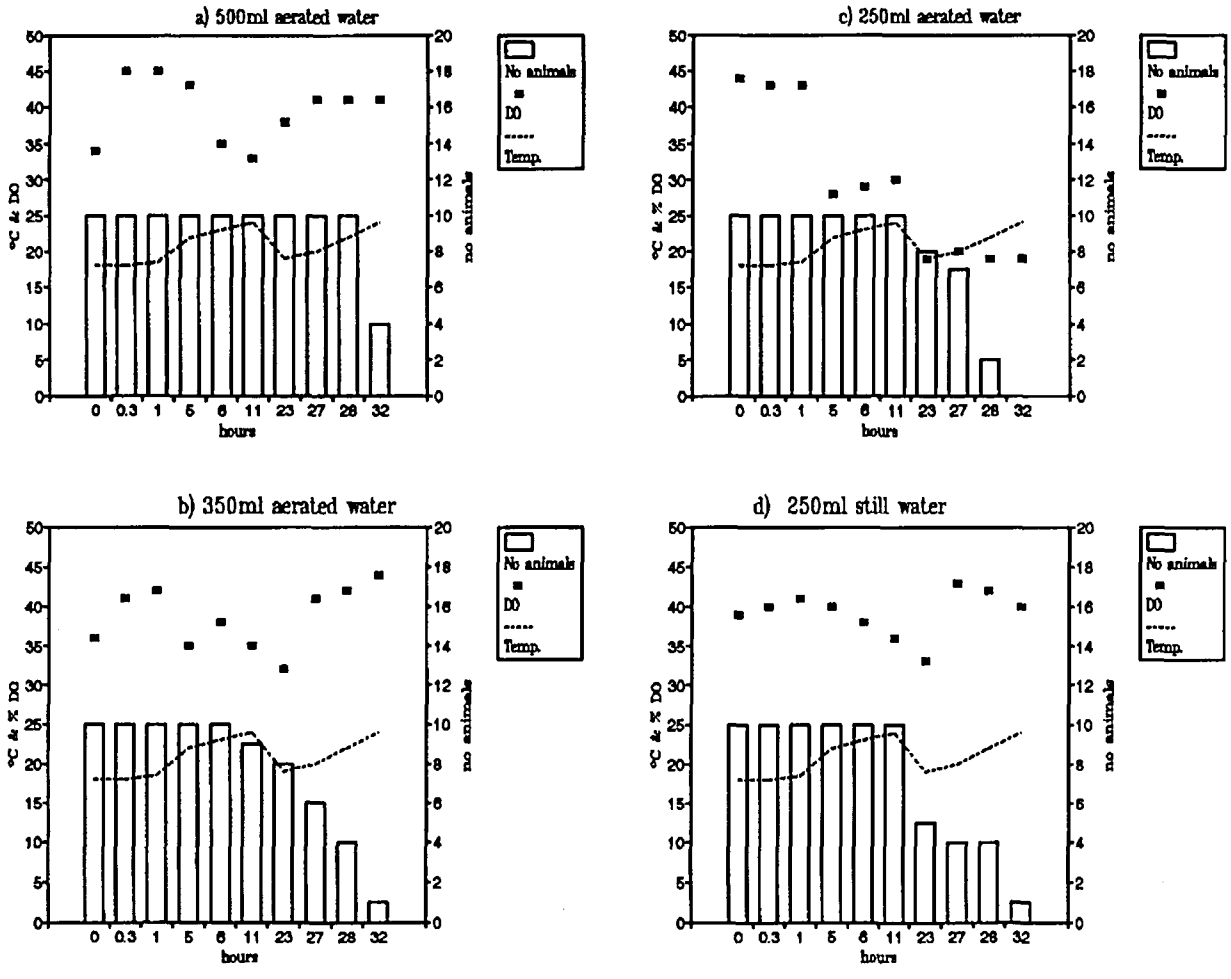

Fig. 3.1.1.2.2 Results of trial to test the effect of aeration on different volumes of water on the survival of *Choroterpes* a) 500ml water (aerated) b) 350ml (aerated) c) 250ml (aerated) d) 250ml (non aerated).

3.1.2 EXPERIMENTAL INVESTIGATION OF SUBSTRATES.(Ch. 3.3.8)

Background

Most riffle dwelling invertebrates perch on substrates which provide both refugia and food. Substrates should be of similar size and shape to produce quantifiable experimental results.

Two experiments were conducted to test the necessity and type of substrate which was to be supplied.

3.1.2.1 THE NECESSITY OF SOLID SUBSTRATE AS OPPOSED TO OPEN SUBSTRATE TESTED IN TURBULENT WATER CONDITIONS.

Aim

To test the suitability of two substrates for *Choroterpes* spp. (Ephemeroptera; Leptophlebiidae).

Method

Three sets of four 500 ml bubblepots were filled with river water and continually aerated:

two sets had foam rubber pads as substrate

two sets had plastic mosquito netting as substrate

one set had no substrate.

The *Choroterpes* nymphs were placed in each replicate set, and the pots checked eight hourly in daytime removing any corpses. The experiment lasted for 4 days.

Results

The laboratory temperature fluctuated between 17°C and 25°C and photoperiod was 11 hours.

The survival rate on foam pads was consistently higher than that on netting. If the survival on netting is compared to that in the control there is no difference.

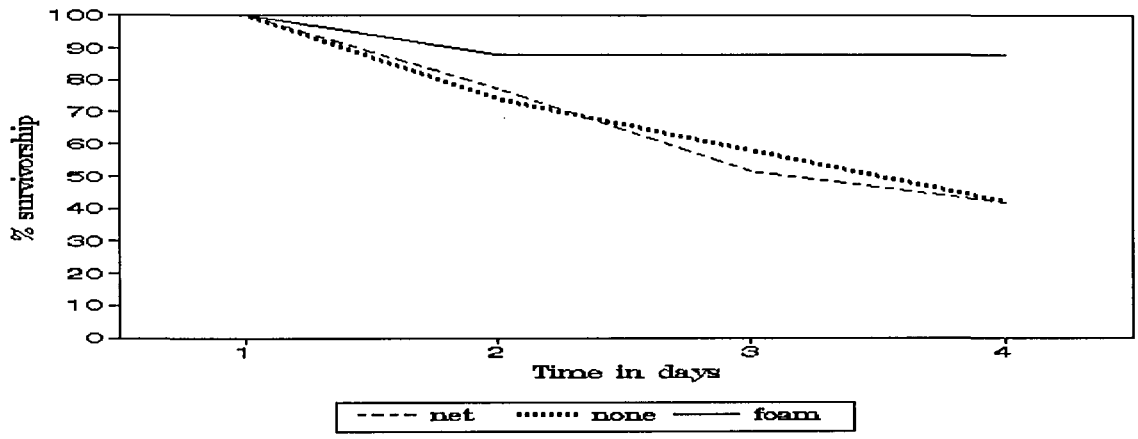

Fig 3.1.2.1 Percentage of survivors in each of three replicates with two types of substrates and a control.

Discussion

For this short period, foam pads as a substrate give better survival rates. It is interesting to note that each time the bubble pots were inspected for dead animals all the specimens were found under the foam pad. This suggests that the foam affords a refuge both from light and from the turbulence of the water.

3.1.2.2 THREE SUBSTRATE TYPES TESTED IN BOTH FLOWING AND TURBULENT WATER CONDITIONS.

Aims

a) To assess the responses of *Choroterpes* spp to three different substrates in both flowing and static water conditions

b) To assess the transport method by which the nymphs were moved form the Kruger National Park.

Method

1) Equipment. Each substrate type was placed into three replicates of both bubblepots and flow-through pots. The substrates tested consisted of foam-rubber pads of 7cm diameter, artificial stones and plastic mesh. Controls contained no substrate. Flow-through pots were made from transparent plastic 500ml bottles with mesh windows inserted on opposing sides. Three mesh sizes (2.0mm, 1.5mm & 1.0mm) were chosen to confine animals of different dimensions. Replicates of these were suspended in the Ciborowski raceways (see Chapter 3.3.2.1). The bubblepots were made from the same size plastic jars as described in Ch.3.3.2.4.
2) Laboratory conditions. The photoperiod was 12 hours and ambient temperature 19°C.
3) Test subjects. *Choroterpes* spp. were collected from the Sabie River and transported to Grahamstown by placing foam pads in aerated insulated boxes (Chapter 3.2) with cooled water. The total transit period (road) was 3 days. On arrival the specimens were moved to raceways and left to acclimatise for one week with detritus and substrate from the Sabie River. The survivors were then sorted into bubblepots and flow-through pots in groups of 10 per container.
4) Food. Finely ground Tetramin was suspended at a ratio of 1 ml/50 ml of water and was fed to the mayflies. Sabie River water was mixed with and later replaced by Palmiet River water.
5) Record keeping. Monitoring took place daily during the week. All dead animals were measured for standard length and preserved
6) Analysis. The results were grouped by weeks. The average number of specimens in all the replicates and the % of the total number of specimens at the start of the experiments was calculated for each treatment.

Results

See Fig.3.1.2.2

The flow through pots were poor containers for experimental purposes as the coarser mesh allowed the animals to escape, so that within the first few days, the original composition of animals in each pot had changed drastically. The smaller mesh clogged up, reducing the flow. Therefore the result in Fig. 3.3.8.2.3b is not an accurate reflection of the intended experimental conditions. The percentage survivals in the bubblepots (Fig.3.3.8.2.3a) show that survival on netting was substantially lower than that on foam pads. 70% of the sample survived for 10 days and 20% for 30 days on foam-pads. In those pots with netting and no substrate a rapid decline in numbers from 70% survival at 5 days to 25% survival by day 10 took place. Even in the raceway (Fig.3.3.8.2.3b) the pots with foam pads retained the largest proportion of animals.

With regard to the transport method, mortalities on arrival were low but larger number died in the first week after the sample was transferred to the raceways. However there was a sufficiently large number of nymphs remaining to stock the experiment with 24 replicates.

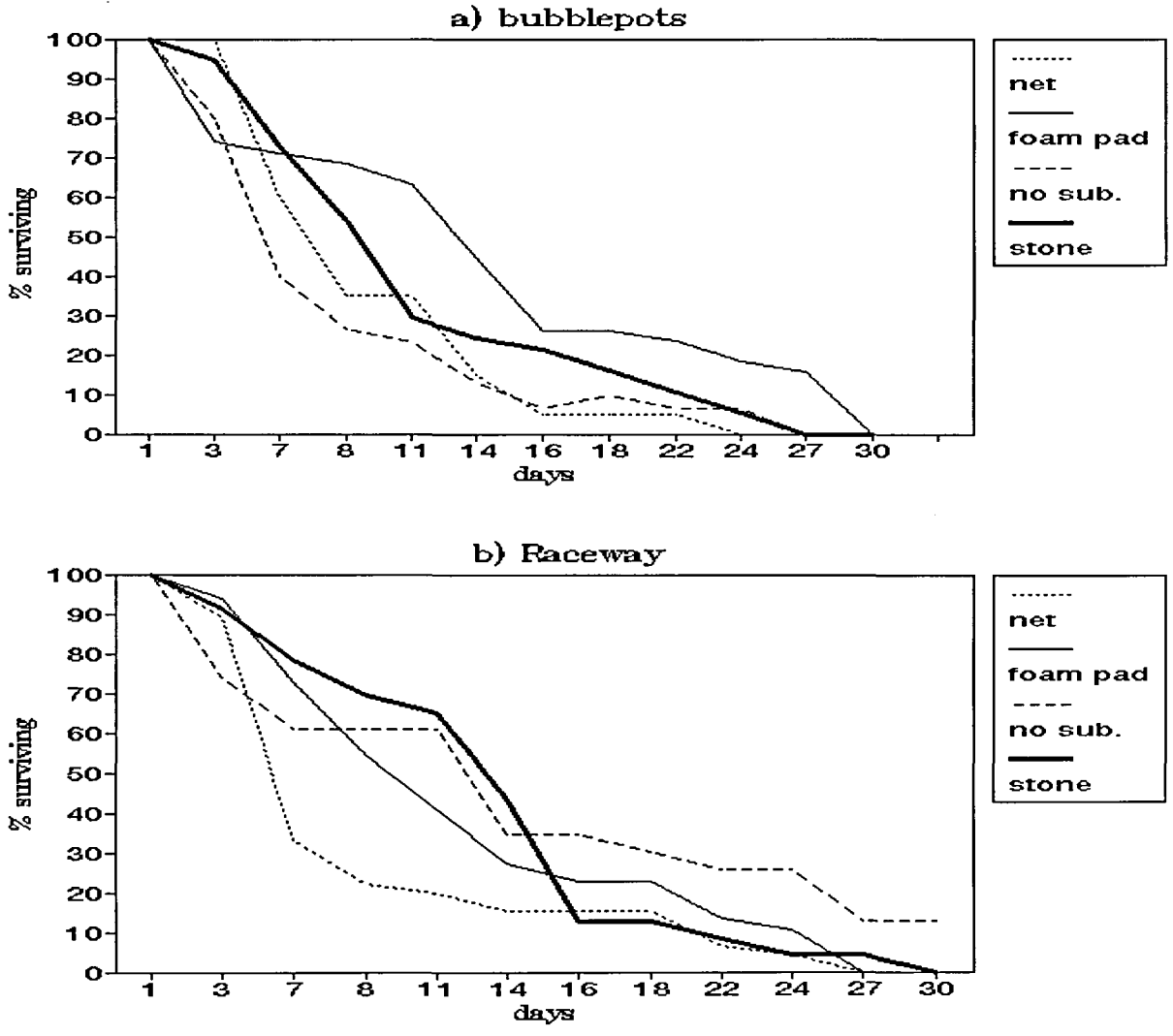

Fig. 3.1.2.2 Percentage of the original sample surviving in all replicates a) in bubblepots and b) in raceway.

Conclusion

Substrate is necessary for the survival of leptophlebiids in bubble pots. The method in which the animals were transported from the Kruger Park ensured survival in excess of a month in the experimental conditions tested.

3.1.3 HYDRAULIC CONDITIONS.

3.1.3.1 COMPARING GROWTH RATES OF LIMPETS IN FLOWING VERSUS STANDING WATER (Ch. 4.2.1.4)

Aim

To compare the growth rate of *Burnupia stenochorias* in flowing water with that of aerated, standing water.

Method

1) Four 50 cm channels, each with approximately equal volumes of water circulating at a known rate and depth within each channel from a common water sump were set up. The water source was Grahamstown tap water, allowed to flow through the system for two days to de-chlorinate.
2) Four bubblepots of known and equal size, each with approximately equal volumes of water and air flow were set up.
3) Both flowing and standing water systems were placed in the C.E. room at temperatures of 19°C (day), 14°C (night) and a photoperiod of 14hrs. The original water levels were maintained with standing tap water no older than four days.
4) Within each pot or channel were placed:
   3 ceramic stones of similar size, covered in periphyton as the source of food
   12 individuals of approximately the same, known size and age (taken from a previous egg-laying experiment).
5) Measurements taken throughout the duration of the experiment were as follows:
   a) initial measurements of length and height of all individuals, to be measured again only at monthly intervals, allowing minimal disturbance or damage to the shells. Dead individuals were removed and measured.
   b) twice daily temperatures were taken to ensure any breakdown in the C.E. room was noted.
   c) initial and thereafter weekly monitoring of TDS, pH and DO levels of the water.
6) Any egg capsules plus the number of egg cases laid were also noted, and removed before those eggs developed and emerged.

Results and Discussion

Table 3.1.3.1 Water conditions within the channels and bubblepots, over time.

Treatment	Max pH	Min pH	Max TDS	Min TDS	Max temp	Min temp
Channels	7,3	6,4	318	165	20°C	17,4°C
Pots	7,8	6,4	326	166	19,6°C	16,6°C

Figs 3.1.3.1.1 and 2 reveal the survival in the channels versus the pots, and the growth rates of the limpets found in the pots only.

In the channels difficulty was found in containing the limpets within each channel despite the fine net covering the holes through which the water flowed. The limpets escaped and landed in the water sump. Each stream should have had a dedicated sump so that any limpet which had moved could be placed back into the correct stream each day. This would still adversely affect growth, as displayed in Section 4.2.1.1. Because of this movement into the sump, the growth rates of the channels cannot be elucidated. Figure 3.3.8.4.2 shows the growth within the pots to have a similar rate, with an adjusted average size after 35 days, when the dead animals (the majority being the larger limpets) were removed. Comparing the survival between bubblepots and channels, it can be seen in Fig 3.3.8.4.1 that the survival in the pots was significantly better.

Many of the limpets had soft shells and a number were found without any shell remaining, unlike the norm, where upon death bacterial and microbial breakdown of the body occurs before the shell dissolves. The water chemistry was obviously unsuitable for continued shell growth despite the level of the pH remaining neutral or slightly alkaline (Table 3.3.8.4). Between monthly counts many limpets disappeared completely suggesting that, (a) they had dissolved entirely between measurements or, (b) more frequent measurements needed to be taken. The experiment was terminated after 3 months because of the above difficulties.

Conclusion

These narrow channels are not suitable for the rearing of *Burnupia* in the laboratory. As a result of the unknown water chemistry and the effect on the shells, more attention has been and will be paid to future experiments in this regard (see section **3.3.6 Water Composition and Hygiene**).

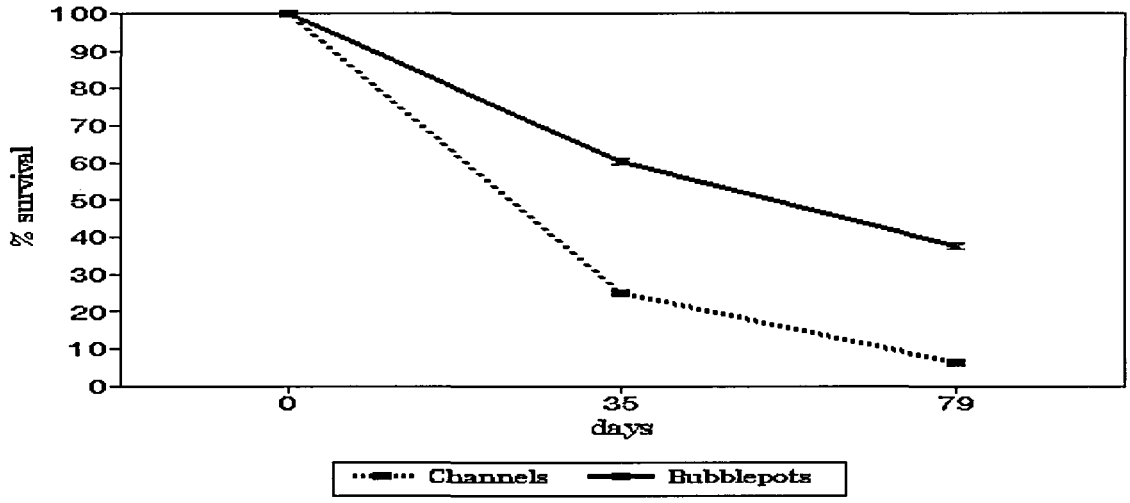

Fig 3.1.3.1.1 Survival of limpets in channels and bubblepots over a three month period.

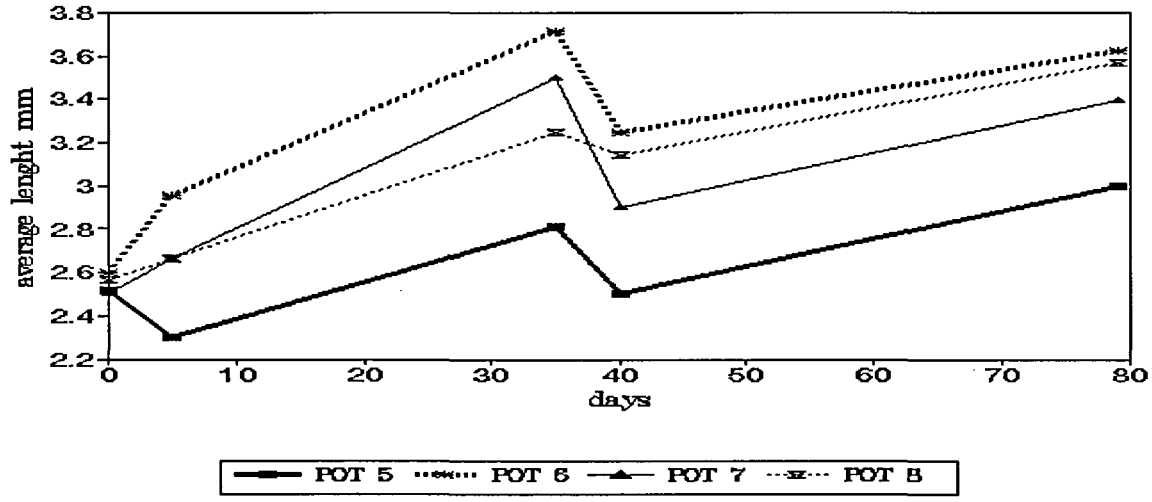

Fig 3.1.3.1.2 Growth of limpets in bubblepots over 79 days corrected for mortalities at 5 and 35 days.

3.1.3.2 INVESTIGATION OF THE SUITABILITY OF SMALL CHANNELS AND BUBBLEPOTS FOR MAYFLIES (Ch.5.2.1).

Aim

To assess whether riffle dwelling leptophlebiid mayflies are obligately rheophyllic and if bubblepots offer a suitable holding facility for experimental and rearing purposes.

Introduction

It was concluded from the previous experiment that the small channels did not provide optimal maintenance conditions for *Adenophlebia auriculata* (Leptophlebiidae) nymphs and therefore bubblepots were tested to ascertain if a better survival rates could be obtained.

Method

1) Four replicates of each treatment were prepared. Treatment consisted of small channels with 5 litre sumps (Chapter 3.3.2.3) and 5 litre bubblepots (Chapter 3.3.2.4.). Each container was supplied with six stones covered in periphyton.
2) The containers were all housed in the laboratory at ambient conditions of light and temperature (13-21°C and 10-12h photoperiod).
3) The specimens were collected from the field and were divided into groups of 17 with size distribution 1.2-1.6 mm HW.
4) Food consisted of periphyton on artificial substrates.
5) Containers were inspected every second day for corpses and shucks, and specimens were measured monthly.

Results

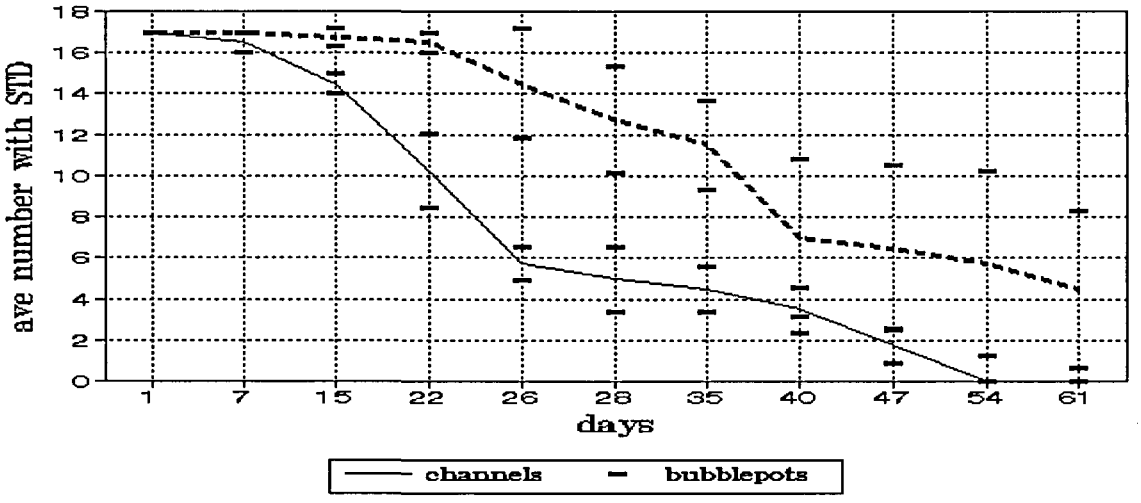

Fig 3.1.3.2 A line graph depicting the survival of nymphs of *A. auriculata* in 5 litre bubblepots or small channels with a 5 litre sump, at ambient laboratory conditions. The triangles represent the total number of adults which emerged during the course of the experiment. The error bars represent standard deviations.

During this trial the nymphs survived for 7 week in the channels and for at least 9 weeks in the pots. When the experiment was discontinued at 9 weeks more than 40% of the nymphs were still alive in 2 of the pots. Of the 68 nymphs which were placed in each set of replicates at the start of the experiment, 37

died in bubblepots and 47 in channels. In two of the bubblepots the water conditions deteriorated visually, becoming murky. In these two pots 15 animals died within four days (days 35-40). The rapid early decline of numbers in the channels can be ascribed to both the emergence of adults and mortalities. Fig. 3.3.8.4 shows that imagoes appeared slightly earlier in the channels than in the pots. Altogether 14 nymphs emerged from the channels and 12 from the pots.

Discussion

The rapid decline in numbers in the channels between days 15 and 26 can be ascribed to both mortalities and emergences. The difference in the condition between the pots and the channels are not only hydraulic (shear force exerted on the nymphs) but also may be due to an insufficient refugia. The temperature regime in the pots is 2-3 °C lower than in the channels due to evaporative cooling, which could account for the earlier emergence of adult in the relatively warmer channels. An ANOVA performed on the replicates proved no significant difference between the replicates in either the channels or the pots (F ratio 0.097, $p>0.05$ for channels and F ratio 1,29 $p=0.2884$) for pots) although the variation in the survival rates in the pots is wider. It is quite clear from Fig 3.3.8.4 that the variation in the sample number in bubblepots is much larger that from those in the channels which could be ascribed to high survival rate (58% and 35%) in two pots. There was a significant difference in the survival rates between channels and bubblepots (ANOVA f ratio 6.7 and $p < 0.05$.) and a multiple range test confirmed the difference.

It can be concluded from the trials that bubblepots are the optimal rearing containers for *A. auriculata* and that they are not obligatory rheophyllics.

3.1.3.3 COMPARING SURVIVAL OF LIMPETS AT HIGH AND LOW DENSITIES IN FLOWING VERSUS STANDING WATER (Ch.4.2.1)

Aim

To test the effects of density and flow on the growth and survival of populations of limpets.

Method

1) Two lots of four channels and 8 bubble pots (described in Section 4.2.4) were set up in the laboratory with each pot and each channel considered a replicate. Each set of 4 channels had one sump. Water was replaced weekly, and topped up with tap water in the case of evaporation occurring.
2) Within each replicate was placed 3 ceramic stones previously allowed to grow periphyton, and a known number of measured limpets, with each set of channels and pots having either a high or a low density of limpets. Limpets that died were replaced with an individual of similar size from a field-caught laboratory culture until the 48th day, to

maintain the original densities.

3) Dissolved oxygen, pH, and TDS were monitored weekly and temperature daily.

4) The trial ran from 19 April to 28 September 1994, a total of 162 days.

Results

Water TDS, pH and temperature varied little between the two sumps and between the eight pots. Generally the pots were 1° or 2°C cooler than the channels, presumably because the turbulence resulting from the aeration reduced temperature. The limpets moved from the channels, despite the presence of fine gauze over the outlets, into the sumps from where they had to be moved back to the channels daily. This continual disturbance has previously been shown (see section 4.2.2) to have a detrimental effect on the growth of the limpets. Algal growth became black and slimy after 3 months (algal growth not identified) in the channels, on the surface of the channels themselves, and was continually scraped off, being considered unsuitable as a food source for the limpets. This did not occur in the pots, where long (>10cm) filamentous green algae grew. Near the end of October both the channels and pots displayed a colourless, slimy gelatinous growth, and it was apparent the limpets were not growing, hence the trial was abandoned.

Table 3.1.3.3. a) F = flowing replicate, P = bubblepots. % loss = loss after the 48th day when replacement was discontinued. No end = numbers of limpets remaining, including spat that had hatched. b. Temperature, pH and TDS are averaged for all pots and channels.

a)

Repl.	F1	F2	F3	F4	F5	F6	F7	F8	P1	P2	P3	P4	P5	P6	P7	P8
No start	108	115	91	109	14	14	14	14	109	105	105	110	17	23	11	13
No end	86	26	58	52	7	2	9	4	37	69	72	72	104	41	32	47
% loss	9.3	14.0	16.5	24.8	28.6	14.3	7.14	14.3	77.0	16.2	19.1	10.9	17.7	4.4	63.6	15.4

b)

Treatment	T.max	T.min	T.ag	pHmax	pHmin	pHavg	TDSmax	TDS min	TDS ag
Channels	22.3	12.0	17.2	8.7	6.4	7.0	453	218	304
Bubble pots	21.3	9.5	15.2	8.6	5.2	7.0	395	227	288

The graphs in Fig 3.1.3.2, on the next page, display the sequential population profile in the experiment. For instance the striped bars at the 3mm position gives an indication of the number and size of limpets remaining from day O, 1mm size group. Similarly the striped bars at position 4 and 5 relate to the animals resulting from the size classes 2 and 3 mm at day 0.

In the channels, (Fig 3.1.3.2 a & b) the numbers of limpets present decline steadily from the initial stocking density. In the bubble pots (c & d) the gain is much larger especially in the pot which started with a low population density.

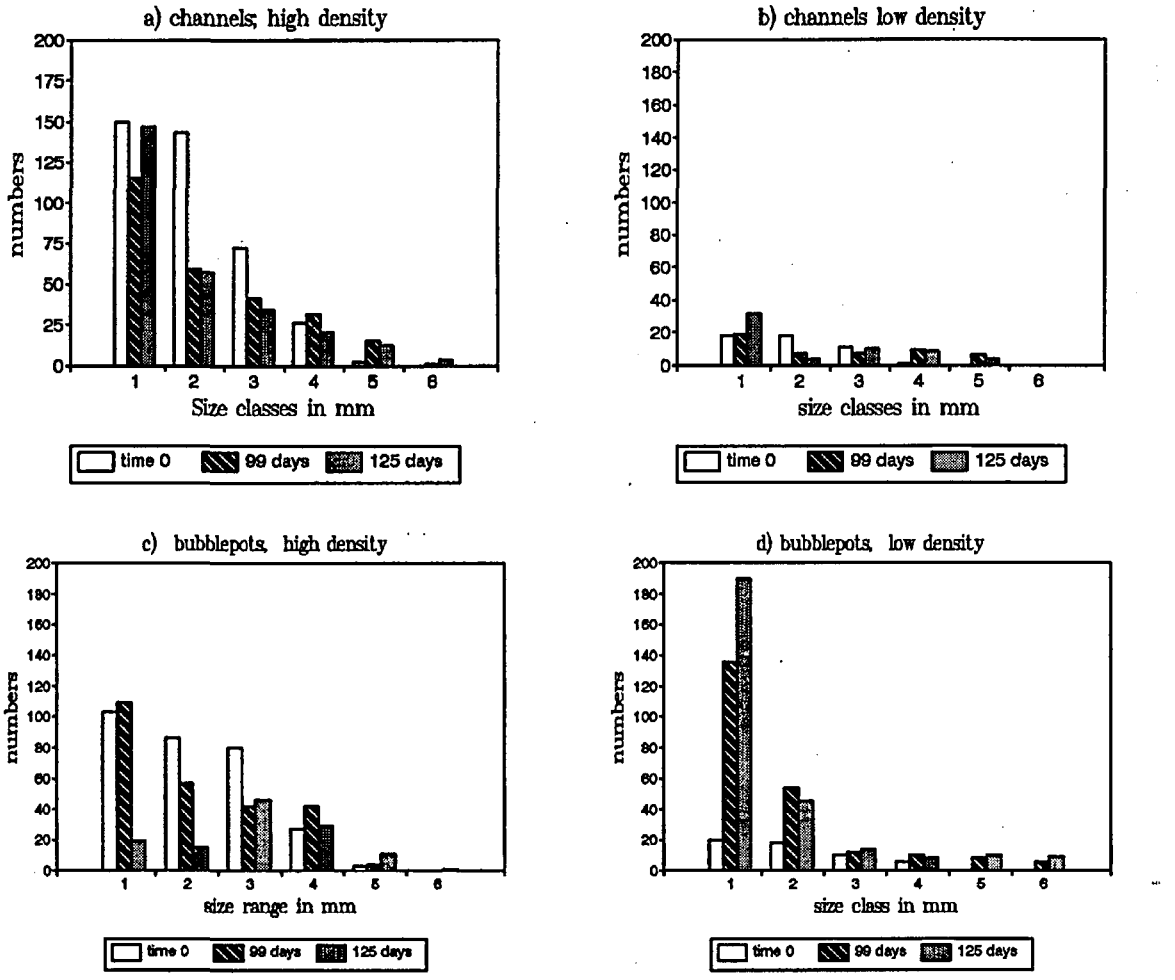

Fig 3.1.3.3 Graphs a-d displays the average size/frequency distribution of limpets. The clear bars indicate the size distribution at the start of the experiment, the diagonally striped bars the size frequency distribution after 99 days which is largely a result if the capsules deposited and the juveniles hatched in the first phase of the experiment and the hatched bars depict the size frequency at the close of the experiment.

Conclusion

More egg capsules were laid in the bubble pots than in the flowing channels. However, those limpets in the channels were highly disturbed, with an apparently unsuitable growth of periphyton. Since this experiment, the use of these channels in testing the effects of flowing water has been abandoned because of the described difficulties. Except for the low population density in the pots, the survival rate did not appear to differ between the flowing and standing waters.

3.1.3.4 CULTURING LIMPETS IN FLOWING VERSUS STANDING WATER (Ch.4.2.1.4).

Aim

To compare the survival of limpets in flowing versus standing, aerated water.

Method

1) Approximately 700 limpets were collected in the field, using anaesthetics and maintained in the laboratory streams with de-chlorinated tap water for a few days to allow for any that died to be removed.
2) Three channel replicates (each a large recirculating stream made of heavy duty PVC piping) and three 10 litre basins were set up in the laboratory under ambient conditions of temperature and light (see Section 3.3.2.3 for details). Tap water was used throughout this experiment, and renewed weekly from a source which had been allowed to stand for two or three days to de-chlorinate.
3) Limpets were then transferred in equal numbers, on tiles which had accumulated a layer of periphyton, into the 6 replicates. An equal distribution of sizes was made, and these varied from 2mm to 4mm in length.
4) Dead limpets were counted and removed, and egg cases were counted daily. TDS, temperature, % oxygen and pH were monitored with each change of water. The experiment was allowed to run for 9 weeks.

Results

See Table 3.1.3.4.1 & 2. and Fig 3.1.3.4.

Table 3.1.3.4.1 Conditions of pH and TDS within the channels and bubblepots.

Rep.	pH max.	pH min.	pH ag.	TDS max.	TDS min	TDS ag.
channel R1	7,6	6,3	7,1	420	119	179
channel R2	7,9	6,7	7,2	245	114	155
channel R3	7,8	6,8	7,2	259	133	159
b.pot B1	7,6	6,2	7,1	296	129	169
b.pot B2	7,9	6,8	7,2	302	114	157
b.pot B3	7,8	6,7	7,3	217	133	161

Temperatures remained the same for each replicate. Max = 22°C, min = 17°C, average = 19°C. % oxygen was approximately 70% for all replicates.

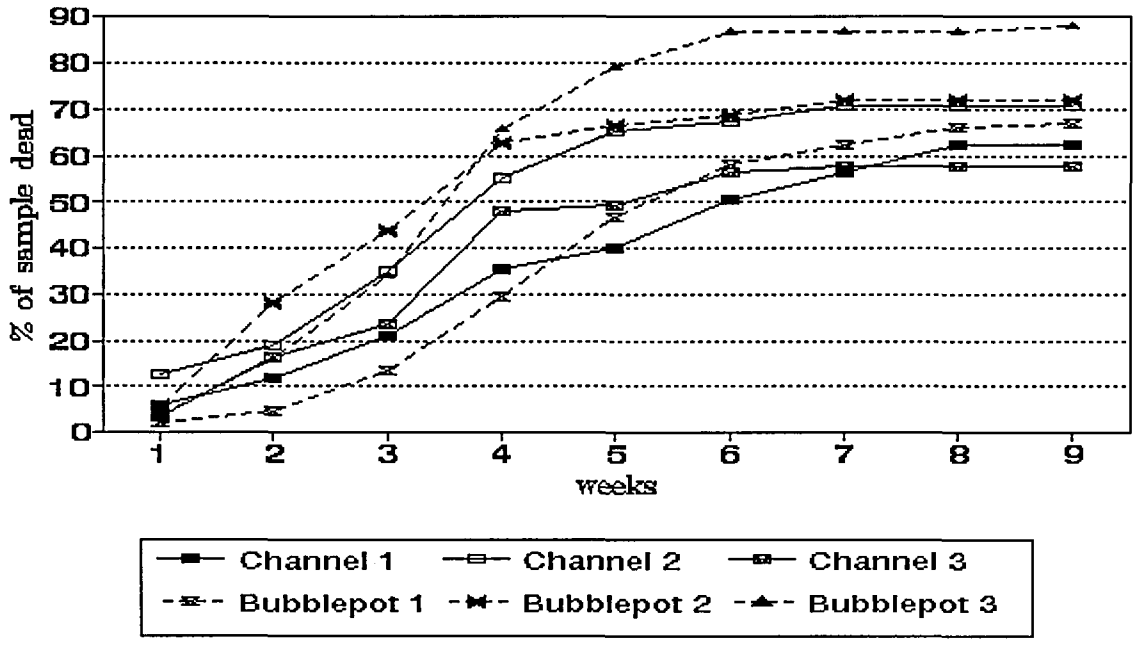

Fig 3.1.3.4 Cumulative mortality of limpets in flowing (Channels 1, 2 and 3) and standing (Bubblepots 1, 2 and 3) waters.

A greater number of egg capsules were laid in the pots; those that hatched were disregarded in the counts of limpets, as they were easy to identify by their size compared to the larger limpets initially placed in the replicates. Hence, % mortality does not include newly laid limpets. The rate of mortality varied considerably, both between pots, between channels and between all the replicates. The average mortality for the channels was 64,5%, for the pots, 75,6%.

Conclusion

In this experiment, the channels gave a greater survival rate than the basins of standing water. More egg capsules were laid in the standing aerated water, however the survival of hatchlings was not monitored.

Table 3.1.3.4.2. R1, R2 and R3 = replicates of flowing channels; B1, B2 and B3 = replicates of standing aerated water; Live = number alive at the start of the experiment; % mortality is accumulative; Dead = number that died weekly; Egg cases = number of egg cases laid weekly.

Replicate	R1	Channel			R2	Channel			R3	Channel		
Week	Live	% Mort.	Dead	Egg cases	Live	% Mort.	Dead	Egg cases	Live	% Mort	Dead	Egg cases
1	85	5.88	5	0	89	12.35	11	0	85	3.53	3	6
2		11.76	5	34		19.10	6	8		16.47	11	65
3		21.18	8	5		34.83	14	64		23.52	6	0
4		35.29	12	0		55.06	18	0		48.23	21	32
5		40.90	4	4		65.17	9	15		49.41	1	
6		50.59	9	4		67.41	2	3		56.47	6	
7		56.48	5			70.78	3			57.65	1	
8		62.35	5							57.65		
9		62.35								57.66		
Totals	85		53	47			63	90			49	103
Replicate	B1	Basin			B2	Basin			B3	Basin		
Week	Live	% Mort.	Dead	Egg cases	Live	% Mort.	Dead	Egg cases	Live	% Mort.	Dead	Egg cases
1	88	2.27	2		89	5.63	5		82	3.67	3	35
2		4.55	2	35		28.09	20	24		15.86	10	80
3		13.64	8	11		43.82	14	34		34.14	15	
4		29.55	14			62.92	17			65.85	26	
5		46.59	15	18		66.29	3	16		79.27	11	
6		57.95	10			68.54	2			86.58	6	
7		62.5	4			71.91	3			86.58		
8		65.99	3							86.58		
9		67.04	1							87.80	1	
Totals	88		59	64	89		69	74	82		72	115

3.1.3.5 THE EFFECT OF TEMPERATURE ON GROWTH RATE, UNDER FLOWING AND STANDING CONDITIONS (Ch.4.2.1.4).

Aim

To compare the growth rate of *B. stenochorias* in flowing water and turbulent standing water at 15°C, 20°C and 25°C.

Introduction

Temperature and food are the most frequently reported factors which affect life history traits in aquatic invertebrates. Temperature directly affects growth by influencing metabolic rates and feeding rates (Giberson and Rosenberg 1992). It also affects food quality and quantity by influencing algal production and growth rates on detritus (Ward and Stanford 1982).

Method

1) Three sets of four channels were set up with approximately equal volumes of water circulating at a known rate and depth within each channel from individual water sumps. The sumps were suspended in water baths heated by aquarium heaters to either 20°C or 25°C. The third set of channels remained at the ambient C.E. room temperature of 15°C. The Grahamstown tap water had been supplemented with various chemicals (see section **3.3.6 Water Composition and Hygiene**).
2) Three sets of four bubblepots of known and equal size, each with approximately equal volumes of water and air flow were set up. Two sets of these were placed in water baths as described above. Lids were placed on the pots to help stabilise temperatures and decrease the evaporation rate.
3) Within each pot or channel was placed 2 ceramic tiles of similar size, covered in periphyton as the source of food, and 20 individuals of known sizes from app 1-3mm shell length.
4) Both flowing and standing water systems were placed in the C.E. room at a temperature of 15°C and photoperiod of 14hrs. The original water levels were maintained with tap water which had been allowed to dechlorinate. Water was changed in all systems every three weeks, each change being supplemented with nutrients as before.
5) Shell length was measured initially and thereafter every two weeks. Dead individuals were removed and measured daily and replaced with new individual as close as possible in size.

Results and Discussion

The aquatic thermoregulators used to maintain the sumps was extremely unreliable and consequently unavoidable temperature fluctuations occurred. The results could not be used.

3.1.3.6 GROWTH OF MAYFLIES IN SMALL CHANNELS (Ch.5.2.1).

This experiment was by Simon Burton as part of a student project.

Introduction

Although the species selected for experimental investigation are all found in riffles and runs in rivers, they may not be obligatory rheophyllics. It is quite difficult to keep small animals confined to artificial channels as the escape route is always open. It was therefore decided to investigate whether well aerated static water would be an suitable alternative to running water. Two experiments were conducted: the growth and survival was tested in channels only; survival was tested in channels and bubblepots.

Aim

a) To test the suitability of the small channels as described in Ch. 3.3.2.3 for experimental purposes

b) To ascertain growth rates of *A auriculata* nymphs in these channels and under ambient and controlled environment room (CER) conditions.

Method

1) Eight small recirculating channels were set up, each with three kaolin stones covered in laboratory-cultured periphyton, which acted as both food and substrate for the nymphs.
2) Four channels were kept in the laboratory under ambient conditions (16°C-20°C and 12-13H photoperiod) while the other four were housed in the CER at 17 °C days and 15 °C nights and 14H photoperiod.
3) Nymphs of all sizes were collected from the field and were returned to the laboratory, measured and placed in groups of ten in the recirculating channels which were checked daily for emergences or shucks while dead nymphs were removed. The nymphal head-widths (HW) were measured weekly.

Results

A. auriculata did not survive well in channels with three stones. A mean of 33% and 32% of the original sample survived three weeks in the CER and laboratory respectively. However, by following individual nymphs for up to four weeks mean absolute growth rates could be calculated for each run, for the CER and the laboratory and for all the nymphs combined. These growth rates are summarised in Table 3.3.8.3. There was no significant difference between the growth rates of nymphs in the CER and in the laboratory ($P > 0.05$).

Table 3.1.3.5.1 Growth rates mm/day (absolute) calculated from survivors in each channel in the laboratory and the CER.

	CER	LAB	
Channel 1	0.0145	0.017	
Channel 2	0.043	0.017	
Channel 3	0.022	0.028	Mean abs. growth
Channel 4	0.014	0.009	for total no.
Mean	0.0228	0.0191	0.0148

Discussion and Conclusions

The low survival rate of nymphs in channels in the laboratory could be explained in two ways:

a) The flow rate in the system may be too rapid. In the Palmiet River the nymphs favour the rocky edges of the stream out of direct current where coarse grained sediment collects under rocks as substratum i.e. that they inhabit low flow areas out of direct current while few smaller nymphs are found in moderate currents (pers.obs.). This observation will be tested statistically after sufficient collections have been made. The channels which are constructed of smooth plastic guttering such that a shallow but fairly strong current flowed over a few smooth rocks most probably did not offer sufficient refuge or foothold for the nymphs. Hence, the nymphs were subjected to fairly strong current leading to large energy expenditure to hold a fixed position s opposed to it being utilized for growth.

b) The algal growth and other food in the channels was not suitable for sustaining the nymphs. The suitability of the periphyton has not been substantially investigated.

The lack of any significant difference in the growth rates between the laboratory and the CER may be due to the mean ambient temperature at that time being similar to the CER temperature setting. However, a growth rate of between 0.019-0.022mm/day means that a nymph could grow from hatching to a maximum size in four months. Temperatures in the field stream fluctuate around 15-24 °C and growth rate has been found to be linked to temperature in invertebrates (see Chapter 5). Similarly if food with a higher protein content than periphyton is supplied the growth rate may increase.

3.1.4 FEEDING TRIALS.

3.1.4.1 TETRAMIN AS DIET FOR *ADENOPHLEBIA AURICULATA* (LEPTOPHLEBIIDAE)(Ch.5.2.2).

Aim

To test the suitability of Tetramin as feed for *Adenophlebia auriculata* (Leptophlebiidae).

Introduction

The first trial was conducted on a number of taxa from the Palmiet River, including a large number of *A. auriculata* the responses of which is reported here. Tetramin was offered as an alternative to natural detritus from the river. This trial took place at the same time as the test for suitable substrates was being conducted and netting was used as substrate.

Method

1) Bubblepots (500ml) with air-stones inserted through the lid were fitted with plastic mesh around the perimeter to provide perches for the test animals. Netting was used as it allowed the animals to be observed without disturbance.
2) The CER with ambience of 12h photoperiod and temperature variation of 12 °C night 20 °C day housed the containers.
3) Field collected nymphs were sorted into the bubblepots and left for forty-eight hours to acclimate. The water was replaced with clean river water and the experimental feeding was started.
4) The Tetramin was finely ground. One ml of ground Tetramin suspended in 50 ml of water and 2.5 ml of this suspension was added to each container. 2.5 ml of natural detritus suspension collected from the river was added to the other pots. The food was replenished every Friday and Monday when the water was replaced with dechlorinated tap water.
5) Monitoring. The containers were inspected daily and the dead animals preserved.

Results

See Fig. 3.1.4.1

After 48 Hours the Tetramin pots had 15, 12, 14 specimens in each replicate and the detritus pots 6, 16, 20 specimens in each replicate due to mortality and all the collected remainders had perished. On the first day of feeding 1ml of Tetramin was added to the relevant pots but by the next morning it was clear that the food supply level had to be reduced as 1 ml caused the water quality to deteriorate.

The survival rate of *A. auriculata* is greatest on Tetramin. 50% survived for five weeks, 30% survived for six weeks and 10% of the population survived for eight weeks. Detritus on the other hand only

sustained 50% of the population for 2-3 weeks with a more rapid decline in numbers.
A regression analysis done on the two sets of data indicated a slope of -5.05 for the population fed on TETRAMIN and a slope of -4.65 for those fed on detritus.

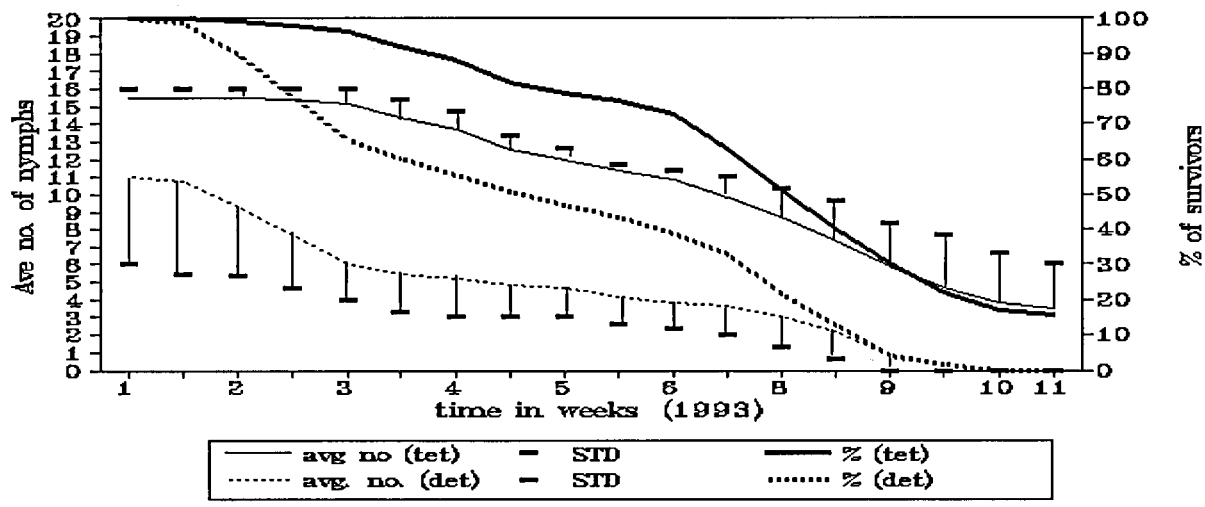

Fig. 3.1.4.1 Line graph depicting the average numbers remaining alive in three replicates (error bars indicate standard deviations) The heavy lines represent the percentage of the total sample surviving. The large variation in the samples fed on detritus is due to the uneven number of nymphs in the replicates.

Conclusion

TETRAMIN appears to be an satisfactory diet for the mayfly nymphs. An experiment will have to ce conducted during which the growth rate of the nymphs when fed on TETRAMIN with have to be compared to those fed on natural diet of leaves and periphyton. A further experiment should also compare the effect of these two diets on the fecundity of the females.

APPENDIX TO CHAPTER 4

Table 4.1. Data pertaining to the growth and survival of the limpets under different temperatures and densities.T = temperature °C; D = density per 500ml pot; Time = days; No = number of limpets per pot; Size mm = average length mm; STD = standard deviation; % CV = percentage coefficient of variation; Max mm = maximum length of limpets present; Min mm = minimum length of limpets present; % Growth = percentage growth per Time interval. (Ch.4.2.1.5)

Treatment	Time	No	% Survivors	Size mm	STD	% CV	Max mm	Min mm	% Growth	Daily % increase
T15D10	1	31	100	1.06	0.20	19.8	2.00	1.00	0	0
	8	25	80.645	1.06	0.22	20.3	2.00	1.00	1.9	0.27
	15	25	80.645	1.51	0.33	21.8	2.40	1.00	41.31	5.90
	22	21	67.742	1.51	0.39	25.6	2.40	1.00	-0.16	-0.023
	29	18	58.065	1.63	0.44	26.9	2.50	1.00	7.8	1.12
	43	14	45.161	2.11	0.52	24.5	3.00	1.20	29.45	4.20
	57	12	38.71	2.51	0.39	15.5	3.10	1.80	19.04	2.72
	71	9	29.032	2.70	0.42	15.6	3.20	1.80	7.64	1.09
	85	8	25.806	2.79	0.48	17.3	3.50	1.80	3.24	0.46
	113	6	19.355	3.03	0.63	20.6	4.10	2.20	8.81	1.24
	120	6	19.355	3.13	0.60	19.0	4.10	2.30	3.30	0.47

T15D20	1	81	100	1.20	0.34	28.4	2.00	1.00	0	0
	8	69	85.185	1.21	0.34	28.5	2.00	1.00	1.05	0.15
	15	62	76.543	1.56	0.31	19.9	2.20	1.00	29.28	4.18
	22	56	69.136	1.61	0.37	23.0	2.50	1.00	3.06	0.44
	29	45	55.556	1.62	0.35	21.3	2.40	1.00	0.60	0.09
	43	36	44.444	1.86	0.41	22.1	2.60	1.00	14.55	1.04
	57	28	34.568	2.29	0.34	14.9	3.00	1.60	23	1.64
	71	26	32.099	2.57	0.44	17.0	3.20	1.50	12.57	0.90
	85	19	23.457	2.60	0.43	16.5	3.20	2.00	1.046	0.07
	113	14	17.284	2.94	0.43	14.6	3.50	2.40	12.91	0.92
	120	12	14.815	2.88	0.46	15.8	3.50	2.00	-1.78	-0.13
T15D20	1	112	100	1.09	0.25	23.1	2.50	1.00	0	0
	8	72	64.286	1.10	0.28	25.0	2.50	1.00	1.366	0.19
	15	65	58.036	1.59	0.30	19.0	2.40	1.00	43.79	6.25
	22	58	51.786	1.52	0.31	20.3	2.50	1.00	-4.22	-0.60
	29	49	43.75	1.60	0.32	20.0	2.30	1.00	5.081	0.72
	43	39	34.821	1.86	0.43	23.1	2.90	1.00	16.66	1.18
	57	31	27.679	2.15	0.57	26.4	4.00	1.20	15.25	1.09
	71	26	23.214	2.39	0.52	21.8	3.60	1.00	11.17	0.80
	85	20	17.857	2.54	0.46	18.2	3.50	2.00	6.135	0.44
	113	15	13.393	2.95	0.64	21.9	4.20	2.20	16.24	1.16
	120	14	12.5	3.01	0.59	19.6	4.10	2.20	2.052	0.14

T20D10	1	42	100	1.12	0.30	27.2	2.00	1.00	0	0
	8	42	100	1.15	0.42	36.9	2.80	1.00	2.766	0.39
	15	42	100	1.87	0.37	19.7	2.80	1.00	62.32	8.90
	22	41	97.619	1.94	0.39	20.2	3.00	1.40	3.876	0.55
	29	37	88.095	2.10	0.52	24.9	3.50	1.40	8.163	0.58
	43	32	76.19	2.60	0.40	15.5	3.50	2.00	23.82	1.70
	57	24	57.143	2.88	0.44	15.1	4.00	2.00	11.03	0.79
	71	11	26.19	2.82	0.54	19.2	3.60	1.80	-2.26	-0.16
	85	10	23.81	3.02	0.55	18.2	4.00	2.00	7.161	0.51
	113	10	23.81	2.87	0.72	25.2	4.20	1.50	-4.97	-0.35
	120	4	9.5238	3.23	0.71	22.0	4.40	2.50	12.37	1.76
T20D20	1	81	100	1.12	0.26	23.6	2.00	1.00	0	0
	8	79	97.531	1.24	0.43	34.5	2.50	1.00	11.03	1.57
	15	74	91.358	1.80	0.36	20.1	2.80	1.00	45.32	6.47
	22	75	92.593	1.82	0.38	21.1	3.20	1.00	1.181	0.17
	29	62	76.543	2.07	0.45	21.9	3.50	1.00	13.27	1.89
	43	54	66.667	2.38	0.35	14.9	3.20	1.60	15.35	1.09
	57	35	43.21	2.67	0.45	16.9	3.60	1.80	11.85	0.85
T20D30	1	128	100	1.08	0.26	24.2	2.50	1.00	0	0
	8	110	85.938	1.16	0.30	26.0	2.50	1.00	7.51	1.07
	15	109	85.156	1.77	0.33	18.6	2.60	1.00	52.84	0.75
	22	95	74.219	1.70	0.33	19.7	2.80	1.00	-4.16	-0.59
	29	86	67.188	1.91	0.37	19.2	3.00	1.20	12.59	1.80
	43	77	60.156	2.19	0.47	21.6	3.50	1.30	14.41	1.02
	57	60	46.875	2.20	0.45	20.6	3.50	1.50	0.67	0.048
	71	18	14.063	2.47	0.33	13.2	3.20	2.00	12.29	0.87
	85	11	8.5938	2.54	0.36	14.2	3.00	2.00	2.594	0.18
	113	10	7.8125	3.11	0.41	13.1	3.60	2.40	22.62	1.62

	120	8	6.25	3.05	0.42	13.7	3.80	2.40	-1.93	-0.14
T25D10	1	42	100	1.06	0.20	18.4	2.00	1.00	0	0
	8	36	85.714	1.26	0.53	42.2	3.00	1.00	19.29	2.75
	15	32	76.19	2.02	0.44	22.0	3.30	1.40	59.73	8.53
	22	29	69.048	2.23	0.57	25.7	4.10	1.00	10.34	1.478
	29	28	66.667	2.45	0.59	24.3	4.50	1.50	9.985	0.71
	43	22	52.381	2.75	0.67	24.4	4.60	1.40	12.06	0.86
	57	19	45.238	3.01	0.53	17.5	4.20	2.00	9.463	0.67
	71	14	33.333	3.33	0.46	13.9	4.00	2.60	10.76	0.77
	85	10	23.81	3.48	0.35	10.0	4.00	2.90	4.549	0.32
	113	7	16.667	3.34	0.42	12.6	4.00	2.60	-3.94	-0.28
	120	3	7.1429	3.53	0.17	4.8	3.70	3.30	5.698	0.81
T25D20	1	79	100	1.09	0.25	22.8	2.00	1.00	0	0
	8	78	98.734	1.33	0.41	30.7	2.50	1.00	21.75	2.98
	15	77	97.468	1.88	0.39	21.0	2.60	1.00	41.72	5.96
	22	69	87.342	2.09	0.43	20.6	3.20	1.50	11.36	1.62
	29	54	68.354	2.22	0.41	18.6	3.00	1.50	6.113	0.43
	43	44	55.696	2.58	0.43	16.9	3.50	1.50	15.88	1.13
	57	34	43.038	2.72	0.43	15.8	3.60	1.50	5.654	0.40
	71	16	20.253	2.92	0.51	17.6	3.60	2.00	7.284	0.52
	85	12	15.19	3.12	0.57	18.4	4.00	2.40	6.781	0.48
	113	7	8.8608	3.56	0.50	14.0	4.10	2.60	14.13	1.01
	120	6	7.5949	3.68	0.51	13.8	4.20	2.70	3.548	0.50

T25D30	1	125	100	1.1	0.24	22.1	2	1	0	0
	8	92	73.6	1.12	0.27	24.1	2.50	1.00	1.779	3.11
	15	84	67.2	1.87	0.29	15.7	2.50	1.00	66.63	0.25
	22	75	60	2.03	0.41	20.1	3.00	1.00	8.927	9.517
	29	68	54.4	2.06	0.40	19.6	3.00	1.50	1.175	1.27
	43	49	39.2	2.27	0.46	20.2	3.60	1.50	10.58	0.08
	57	36	28.8	2.47	0.48	19.6	3.50	1.50	8.742	0.75
	71	21	16.8	2.99	0.49	16.5	3.80	2.00	20.77	0.62
	85	13	10.4	3.00	0.46	15.2	3.50	2.00	0.478	1.48
	113	11	8.8	3.35	0.56	16.6	4.00	2.40	11.52	0.03
	120	3	2.4	3.20	0.59	18.4	4.00	2.60	-4.35	0.82
T25D30	1	128	100	1.04	0.17	16.2	2.00	1.00	0	0
	8	108	84.375	1.05	0.18	17.1	2.00	1.00	1.523	0.21
	15	82	64.063	1.58	0.27	17.4	2.20	1.00	49.93	7.13
	22	63	49.219	1.54	0.30	19.3	2.50	1.00	-2.38	-0.34
	29	56	43.75	1.58	0.36	22.8	2.20	1.00	2.515	0.18
	43	9	7.0313	1.97	0.47	23.8	2.50	1.00	24.73	1.76
	57	6	4.6875	2.35	0.53	22.5	3.00	1.60	19.49	1.39
	71	6	4.6875	2.52	0.37	14.8	3.00	2.00	7.092	0.50
	85	6	4.6875	2.88	0.74	25.8	4.00	2.00	14.57	1.04

APPENDIX 7

RECOMMENDATIONS FOR THE PLANNING OF AQUACULTURE FACILITIES.

The following are a list of basic guidelines and rules for maximizing the probability of success with seawater culture systems. They have been gained from experience.

* Take great care in quantifying the requirements, as they will greatly effect the complexity and cost of the system.
* Consider long-term as well as short-term requirements, even though they are more difficult to quantify.
* Major cost underestimates are more likely to result from necessary but uncounted components and services or increased requirements rather than errors in specific items.
* Consider operating approaches and procedures before the design is fixed. Significant input from operational personnel is needed in the design and construction phases.
* Demands for services (flow rate, compresses air, etc.) usually increase in quantity and quality with time. If possible, provide extra floor space, access to piping, access to drains and provisions for electrical power for anticipatable future retrofits.
* Remember that the key to low risk and high performance systems is large amounts of high quality seawater. In short, very conservative biomass loading relative to the available water quality. If a system is working well, increasing the biomass will increase the risks.
* Anticipate probably failures and plan accordingly to minimize the consequences.
* Provide redundant equipment to back up critical functions in emergencies. Resist temptation to use backup equipment in normal operations just because it is available.
* Responsibilities and decision-making procedures for emergencies should be decided before crisis occur, remembering that they rarely occur at convenient times.
* Do not forget routine maintenance and inventorying necessary spare parts when operations are going well, it is twice as important when things are not going well.
* Be extremely careful in selecting all materials and supplies used in and around seawater systems, because your organisms may be very sensitive and seawater is very corrosive. Do not forget to include the surrounding building and paints, sprays, cleaners, sealers and solvents used near culture organisms.
* Leach all materials in running seawater for at least two weeks before use.
* Take great care with all fittings, pipe and equipment on the suction side of pumps to avoid even the most minute air leak. Supersaturation can easily kill.
* Make the suction-side as large in diameter and as short as possible to minimize suction-side frictional losses.
* Place pressure gauges on both the suction and discharge side of pumps to monitor the condition of the lines and the performance of the pumps. Watch for biofouling, especially on the suction

side. Service regularly.

* Adequately pitch all floors in wet-lab areas towards the drains remembering that concrete may shrink on drying.
* Greatly over-size drains to take high transient flows. Drains can never be too large. Even the largest drains will occasionally clog if not maintained.
* Expect very high suspended solids content in incoming seawater from shallow water intakes during storms or heavy waves.
* Anticipate the need to remove sediment and debris from any parts of the system with low flow velocities.
* Bury or otherwise make inaccessible as little of the system as possible. The inaccessible parts are inevitably the parts you will want to get at later.
* Place all electrical outlets up high, above any unintentional water input.
* Use all ground fault interrupters on all indoor and outdoor electrical outlets. Do not underestimate the electrical hazards associated with seawater.
* Inspect and service intake screens regularly.
* Be careful in locating the intakes. They should not be situated so as to pick up debris, recycle drain water or ever experience breaking waves. Do not underestimate the forces of the sea on intakes and other exposed structures.
* Remember that the piping/processing system and the pumps are highly interactive. Changes in either area will effect the other.
* In wet-lab and mechanical areas use "X" fittings with blanked faces where "L's" and "T's" are needed. This maximizes accessibility for cleaning and future modifications.
* If you are on call, do not place on automatic alarm any functions or events that can wait until normal working hours.
* In a field with so much misleading or incomplete information, data voids, "experts" and variations in conditions, it is worth remembering when dealing with the unknown or uncertain that one test, under the actual conditions to be encountered, may be work a 1000 expert opinions.

REFERENCE

Huguenin JE & E Colt. 1989. Design and operating guide for aquaculture in seawater system. Developments in Aquaculture and Fisheries science 20. Elsevier Amsterdam.