TADPOLES AS BIO-INDICATORS OF STREAM QUALITY: A BASELINE STUDY

by

A CHANNING

Department of Biochemistry
University of the Western Cape
Private Bag X17
Bellville, 7535, South Africa

WRC Report No. 718/1/98
ISBN 1 86845 390 1

Executive Summary

TADPOLES AS BIO-INDICATORS OF STREAM QUALITY:
A BASELINE STUDY

by

A. CHANNING

Department of Biochemistry
University of the Western Cape
Private Bag X17
Bellville, 7535, South Africa

1998

Project Leader: Professor A. Channing

BACKGROUND AND MOTIVATION

This project deals with monitoring water quality using tadpoles. Tadpoles are naturally present in almost all drainages, and provide a low-cost alternative to chemical analyses for routine surveys and monitoring. Local communities should be able to use this approach as a first step in quality control of natural streams.

OBJECTIVES

The objectives of the research are:

1) To determine the diversity of tadpoles in the major catchments in South Africa.

2) To provide a user-friendly method of identification of tadpoles.

3) To place tadpole collections in a major museum, from which working reference collections can be drawn.

5) To determine the sensitivities of certain tadpoles to pollutants like heavy metals and agricultural chemicals.

ACHIEVEMENT OF OBJECTIVES

The objectives as set out above have been met as follows:

1) The detailed distribution maps to 36 species have been compiled and the seasonality of the 36 species has been determined

2) A well-illustrated identification key is available

4) The tadpole collection will be placed in the Port Elizabeth Museum

5) The sensitivity of platanna tadpoles was determined to nine important agricultural chemicals, using the well-established FETAX procedure.

This study shows that tadpoles can be easily identified, and that they are naturally available in most drainages.

The use of tadpoles as clean water indicators should be emphasized both to the professional and to local communities and schools.

SUMMARY OF RESULTS

A total of 36 species of tadpoles occur in streams and drainages.

The list includes 2 species of *Afrixalus* (spiny reed frogs), 4 species of *Bufo* (toads), 2 species of *Cacosternum* (dainty frogs), 1 species of *Capensibufo* (mountain toad), 6 species of *Heleophryne* (ghost frogs), 2 species of *Hyperolius* (reed frogs), 1 species of *Natalobatrachus* (Boneberg's frog), 1 species of *Phrynobatrachus* (puddle frog), 1 species of *Poyntonia* (montane marsh frog), 1 species of *Ptychadena* (grass frog), 5 species of *Rana* (river frogs), 5 species of *Strongylopus* (stream frogs), 3 species of *Tomopterna* (sand frogs), 2 species of *Xenopus* (platannas).

There are representatives in permanent streams in the wet areas, and in temporary streams in the drier areas. Essentially, tadpoles are found in streams during the rainy season, when pollutants are entering the water.

The sensitivity of platanna tadpoles was determined using the standard FETAX procedure, to the following pesticides: *Bacillus thuringiensis* (insecticide), Chlorthalonil (fungicide), Deltamethrin (pyrethroid), Dichlorvos (organophosphorus insecticide), Fenthion (organophosphorus insecticide), Imidacloprid (systemic insecticide), Isazofos (organophosphorus insecticide), Mancozeb (fungicide), and Simazine (herbicide).

Tadpole sensitivity

Pesticide	LC_{50} (mg/l)	EC_{50} (mg/l)
Isazophos	724	0.25
Imidacloprid	17.4	10
Dichlorovos	39.4	0,5
Chlorothalinil	0,09	0,02
Mancozeb	3.08	0,03
Bacillus t.	163.2	0.02
Deltamethrin	0.19	0.006
Fenthion	2.61	0.002

RECOMMENDATIONS FOR FURTHER RESEARCH

This baseline study has shown that the tadpole approach to monitoring water is both practical and economical. Further research should include:

1) A long term study, using caged tadpoles at selected sites along streams that are recognized as high-risk. There are streams draining agricultural areas in the Western Cape that would be ideal test sites.

2) Determination of tadpole sensitivity to other selected agrichemicals. This would allow an evaluation of pesticides that are actually used in an area. This would tie in with recommendation (1).

Acknowledgements

This report is the result of a project funded by the Water Research Commission, file K5/718. The project is titled:

"Tadpoles as bioindicators of stream quality: a baseline study"

The Steering Committee responsible for this project consisted of the following persons:

Dr S. A. Mitchell	Water Research Commission (Chair)
Mr M. E. Mosia	Water Research Commission (Secretary)
Mr H. Braack	National Parks Board
Ms D. R. Drinkrow	South African Museum
Dr L. H. Du Preez	University of the Free State
Dr P. Goetsch	Rhodes University, IWR
Prof N. I. Passmore	University of the Witwatersrand
Prof R. Christie	University of the Western Cape
Dr L. Minter	University of the North
Mr D. S. v d Merwe	Water Research Commission
Mr S. Jooste	Department of Water Affairs

The assistance of the steering committee and the financing of the project by the Water Research Commission is acknowledged.

Projects like this with a large geographical scale are only possible with the active help of many individuals. The author would like to extend his thanks to all who assisted in the field.

Special thanks are offered to Dr Mark Wright, who generously provided samples of the common groups of pesticides, used in the laboratory study.

This project would not have been possible without the constructive advice of the chairperson, Dr S. A. Mitchell.

Dr D. E. van Dijk kindly made figure 36 available. Dr Van Dijk's contributions to the study of tadpoles over many decades, provided the background to this study.

The distribution maps were contructed from the literature, field notes and museum records. The following made available records of

their museum holdings: C. W. Myers (American Museum of Natural History), W. E. Duellman and J. Simmons (Museum of Natural History, University of Kansas), Roy W. McDiarmid (Division of Amphibians and Reptiles, National Museum of Natural History), C. J. McCoy (Carnegie Museum of Natural History), J. P. Rosado (Museum of Comparative Zoology, Harvard University), Dr Hans-Wilhelm Koepcke (Zoologisches Museum, Universität Hamburg), Dr W. Böhme (Zoologisches Forschungsinstitut und Museum Alexander Koening), A. Resetar (Field Museum of Natural History), G. Schneider (Museum of Zoology, The University of Michigan), W. Haacke (Transvaal Museum, Pretoria), W. R. Branch (Port Elizabeth Museum), E. Baard and A. De Villiers (Western Cape Department of Nature Conservation), M. Cherry and D. Drinkrow (South African Museum).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

TERMINOLOGY

Some ecotoxicology terminology

EC_{50} (malformation) - The median effective concentration
MCPA - 2-methyl-4-chlorophenoxy acetic acid
NOAEL - No observed adverse effect level
NOEC - No observed effect concentration
TI - The teratogenic index where TI = LC_{50}/EC_{50}
LC_{50} - The concentration of pollutant at which half of the organisms are killed

Taxonomy

Amphibian taxonomy has become stabilized over the last 30 years, due initially to the work of Poynton (1964). Since that date there has been ongoing discovery of new species, and the realization that many cryptic species have been confused. In order to keep this report as useful as possible, a list is given of the current names (Frost 1985) of the species reported on here, plus older synonyms. One of the problems with southern African frog taxonomy has been the difficulty of rationalizing the taxonomy described separately for west Africa, East Africa, and southern Africa. Dubois (1992) suggested some solutions to this problem, by proposing new generic groupings. His proposals are included in the list that follows, as they are certain to be utilized in future.

Table 1 Current and alternative species names

Current binomial	Synonyms	Dubois' proposals
Afrixalus fornasinii		
Afrixalus spinifrons	*Afrixalus bracycnemis*	
Bufo gariepensis		
Bufo gutturalis	*Bufo regularis*	
Bufo maculatus	*Bufo pusillus*	
Bufo rangeri		
Cacosternum namaquense		
Cacosternum nanum		
Capensibufo tradouwi	*Bufo rosei*, *Bufo tradouwi*	
Heleophryne hewitti		

Current binomial	Synonyms	Dubois' proposals
Heleophryne natalensis		
Heleophryne orientalis	*Heleophryne purcelli*	
Heleophryne purcelli		
Heleophryne regis		
Heleophryne rosei		
Hyperolius marmoratus		
Hyperolius semidiscus		
Natalobatrachus bonebergi		
Phrynobatrachus natalensis		
Poyntonia paludicola		
Ptychadena mossambica		
Rana angolensis		*Afrana angolensis*
Rana dracomontana		*Afrana dracomontana*
Rana fuscigula		*Afrana fuscigula*
Rana vertebralis		*Amietia vertebralis*
Strongylopus fasciatus	*Rana fasciata*	
Strongylopus grayii	*Rana grayii*	
Strongylopus hymenopus	*Rana hymenopus*	
Strongylopus springbokensis		
Strongylopus wageri	*Rana wageri*	
Tomopterna cryptotis	*Pyxicephalus cryptotis*	
Tomopterna delalandii	*Pyxicephalus delalandii*	
Tomopterna marmorata	*Pyxicephalus marmoratus*	
Xenopus laevis		
Xenopus muelleri		

Terminology used in the identification key to stream dwelling tadpoles

Beaks	Robust keratinized structures used for cutting food (also called rostrodonts)
Countershading	A tail that is darker above, and lighter below
Elygium	An outgrowth of the pupil in high altitude species that protects the eye from sunlight
Oral papillae	Small sensory outgrowths that surround the mouth. They occur in one or more rows
Papilla gap	A distinct break in a row of papillae, that may occur on the upper or lower rows of papillae.
Tail muscle	The muscular part of the tail to which the upper and lower fins are attached.
Teeth	Small, dark, complex scraping structures that usually occur in rows above and below the mouth (also called keratodonts)
Tubercle	A small wart-like bump on the skin
Vent	The end of the gut, usually positioned on a short tube at the base of the tail

1. INTRODUCTION

South Africa is not a water-rich country. In order for optimum use to be made of a scarce resource, care has to be taken that water sources are not contaminated, for example with agrichemicals. Contaminated rivers could have a negative effect on crops irrigated from the source, on humans utilizing the source for domestic purposes, and on the broader ecosystem.

Biomonitoring is the use of living organisms to indicate contamination. Laboratory analyses are expensive and sampling is by definition not a continuous process. The use of aquatic organisms has the advantages that they are permanent or long term residents, and can be monitored quickly and economically. Although invertebrates are widely used as biomonitors, tadpoles have the potential to become important organisms in this area. Frogs play a major role in natural pest control, and are an important link in the food chain. The tadpoles are present in large numbers when streams are flowing after the rains, and are therefore key organisms for monitoring run-off and spray drift from aerial applications of pesticides.

Tadpoles have the potential to be useful as part of a low-tech monitoring strategy. If tadpoles cease to be present in streams they have always inhabited, this should be regarded as an early warning of pollution. Tadpoles are sensitive to many pollutants, and are an ideal organism for monitoring studies.

The aims of the present project are:

1) To determine the diversity of tadpoles in the major catchments in South Africa

This was determined by sampling in the field, and by reference to herpetological collections in some of the major museums worldwide.

2) To provide a user-friendly method of identification of tadpoles

An illustrated identification key is presented, that is easy to use.

3) To place a tadpole collection in a major museum

A sample of tadpoles will be placed in the Port Elizabeth Museum.

4) To determine sensitivities of certain tadpoles to pollutants like heavy metals and agricultural chemicals

A series of laboratory experiments using the common platanna,and making use of the internationally accepted FETAX procedue, was adopted. A number of agrichemicals were tested.

The list of catchments is taken from O'Keefe (1985): For the purposes of this study, the following classification of drainages was used: Limpopo, Incomati, Gariep/Vaal, Tugela, Great Fish, Gamtoos, Keurbooms, Goukamma, Gourits, Breë, Berg and the Olifants.

2. LITERATURE REVIEW

This chapter reviews only recent literature. The field is large, and new publications may exceed 100 per week. I have further confined the review to pertinent areas that have a bearing on this project.

2.1. Tadpole Identification

Southern African tadpoles are still not completely known. The first comprehensive synthesis was by Van Dijk (1966), who provided characters, keys and illustrations to many tadpoles. Other descriptions of African tadpoles, and keys include the work of Van Dijk (1972), Amiet & Schiøtz (1974), Channing (1986), Channing & Boycott (1989), and Lambiris (1987, 1989).

2.2. Pesticides and river systems

Globally, there is a relationship between socioeconomic indices and the accumulation of hydrocarbons in vegetation (Calamari et al 1995), with the richer countries producing more waste. Many pesticides find their way into near-surface aquifers (Kolpin et al 1996, Southwick et al 1995, Tasli et al 1996). Proper drainage of the soil can reduce the amount of herbicide loss as runoff by up to 50% (Bengtson et al 1995). Tillage also affects the movement of

herbicide into groundwater (Smith et al 1995). Sources of pollution can affect both freshwater and marine sources. Refinery effluent has been shown to affect both marine and freshwater species (Bleckman et al 1995).

MCPA residues left in developing forest after spraying for weeds are at detectable and significant levels (Eronen et al 1979). The ecotoxicology of dioxins and related compounds has been reviewed by Grimwood & Dobbs (1995).

Once pesticides get into South African rivers, they can be effective for up to 20 km from the source (for *Bacillus thuringiensis*) and up to 50 km for the organophasphate temephos (Palmer et al 1996). Palmer & Palmer (1995) tested insecticides in the Orange river, and concluded that doses of 1.2-2.4 mg/l of temephos could be considered safe, provided that the recommended dosages are adhered to, and that certain sections of the river are left untreated as refugia for sensitive taxa. This will be discussed further below.

Finches are a major pest in South Africa, and Roux et al (1995) report on the effect of using fenthion to control finches on water quality.

Dams can have a beneficial effect in polluted river systems, by improving the quality of water downstream (Palmer & O'Keeffe 1990)

2.3. Invertebrate sensitivity

Studies of the sensitivities of invertebrates to pollutants form a large body of literature. The following brief paragraph serves only as an introduction to this field, and is not intended as a representative survey.

Aquatic invertebrates are particularly sensitive to organic and other compounds leaching into the water (Assmuth et al 1995). Fluoride is an example of a long term pollutant affecting invertebrates (Camargo & Lapoint 1995). Molluscs accumulate radionuclides (Fransevitch et al 1995). Metal salts have been implicated in restricting the growth of ciliate populations (Janssen et al 1995). The herbicide hexazinone negatively affects stream invertebrates (Kreutzwasser et al 1995), and groundwater

pollution is known to change the composition of invertebrate communities (Notenboom et al 1995).

2.4. Fish sensitivity

Fish are common in most drainages, and provide some clues as to expected sensitivity of tadpoles to pesticides. A common pollutant is nitrite derived from fertilizer. Grass carp (*Ctenopharyngodon idella*) have an LC_{50} of 2.5 mg/l. (Alcarez & Espina 1994), while juveniles are more sensitive, with an LC_{50} of 0.25 mg/l (Alcarez & Espina 1995). Fish are generally more sensitive at higher temperatures and ph to the common herbicide Roundup (Bidwell & Gorrie 1995). Different life stages of zebrafish show different acute and long term effects when exposed to mixtures of pesticides (Ensenbach & Nagel 1995). Minnows have, however, been shown to become more tolerant of fluoranthene during a laboratory study (Diamond et al 1995a, 1995b, 1995c). The effect of insecticide on young trout is greatly enhanced if combined with a second pesticide (Paul & Simonin 1995). Combinations of herbicides like 2,4-D and atrazine with insecticide increase the toxicity of the insecticide (Kao et al 1995).

2.5. Pesticides and tadpoles

The carbamate insecticide ZZ-aphox causes structural changes to many organs of tadpoles kept at 20-140 mg/l for 9 weeks (Honrubia et al 1993). Insecticides like ZZ-aphox and folidol cause skeletal malformaions in tadpoles (Alvarez et al 1995). Even herbicides like MCPA have a deleterious effect on development (Bernadini et al 1996).

It has been shown that the carrier in Roundup, a widely used herbicide, is more harmful than the glyphosate active ingredient (Bidwell & Gorrie 1995).

Clearly, active ingredients and carriers need to be tested separately. The fungicide TPT decreases the swimming and feeding times of tadpoles, resulting in small size and longer times to metamorphosis (Semlitsch et al 1995).

Tadpoles have been shown to be sensitive to 8 ppm of the insecticide fenthion, and to 2.4 mg/l of the herbicide triclopyr (Berrill et al 1994). Even short term exposure (24 h) to fenthion

can result in tadpole paralysis. This insecticide has a natural half-life of 24-48 h, but even at low concentrations of 2.5 mg/l, the 24 h exposures can compromise the tadpole by increasing the risk of predation while it is paralysed (Berrill et al 1995). *Bufo* tadpoles show interrupted development when exposed to 47.3 mg/l of malathion, but they show no observable effects at 0.47 mg/l (De Llamas et al 1985).

Rana tadpoles show a decreased activity in low concentrations of the synthetic pyrethroid esfenvalerate, down to 1.3 mg/l, at which concentration the 96 h LC_{50} was 7.29 mg/l. Higher temperatures increase the effects (Materna et al 1995). Dieldrin has an LC_{50} (96 h) of 40.4-49.5 mg/l for *Xenopus laevis* but 8.7-30.3 mg/l. for *Rana catesbeiana* and 71.3 mg/l for *Rana pipiens* (Schuytema et al 1991). Guthion has a 96 h LC_{50} of 2.94 mg/l for *Xenopus laevis* (Schuytema et al 1995).

The synergistic action of pairs of chemicals can be considerably more severe than either alone. Future work needs to examine pairs of agrichemicals that are in use in the same watershed.

Two glycoalkaloids used together show synergistic activity on develpmental toxicity in *Xenopus* embryos (Rayburn et al 1995). **Tadpoles of *Xenopus laevis* can survive 2 mg/l of the fungicide nabam, and 1 mg/l of the herbicide diquat with no harmul effects. But if the tadpoles are placed in the two together, deleterious changes are recorded** (Anderson & Prahlad 1976).

Xenopus laevis tadpoles, exposed to 1, 2, and 10 mg/l atrazine, showed delayed development, and reduced body weight, while exposure to 0,1 and 0,025 mg/l of nonylphenol increased the number of females. It has been suggested that delayed development, low body weight and skewed sex ratio imply poor recruitment for a threatened amphibian species (Blandin & Ramsdell 1995). Long term and short term exposure to lead causes teratogenesis in *Xenopus laevis* (Sobotka & Rahwan 1995). Nitrites from ammonium nitrite fertilizer are toxic to tadpoles . The LC_{50} (96 h) is 13.9-39.3 mg/l. These values are frequently exceeded in agricultural areas around the globe (Hecnar 1995).

Frogs are recognized as important indicators of the health of aquatic systems (Boyer & Grue 1995, Bunn 1995). Toxicants can affect frog populations by 1) affecting disease susceptibility, 2) retarding growth, 3) affecting escape behaviour, 4) affecting reproductive developement, and 5) by directly increasing mortality (Carey & Bryant 1995). A comparative toxicity data base for amphibians has been suggested as a means of assessing toxicity risks (Linder et al 1990).

Tadpoles can respond to pollution by changes in their erythrocytes (Chen & Xia 1995).

Caged tadpoles of *Rana temporaria* have been used to monitor pesticide run-off or spray drift. They show mortality, deformities, reduced rates of metamorphosis and reduced growth (Cooke, 1981).

Tadpoles and eggs have also been transplanted to ponds as an experimental monitoring procedure (Freda & McDonald 1993).

In the Western Cape, naturally occuring acidic blackwater is toxic to *Xenopus laevis* tadpoles, but is tolerated by *Xenopus gilli* . Bog water has been shown to be toxic to other tadpoles (Saber & Dunson 1995).

Different pollutants affect different systems in tadpoles. The summary above shows that red blood cell number, neuronal activity, cell division, fertilization, and a disruption in the normal biochemical pathways that lead to growth, are some of the various effects caused by different substances.

Mercuric chloride affects both fertilization and larval develpment in the river frog *Rana heckscheri* (Punzo 1993).

Xenopus laevis tadpoles are considered a pest in fish farms, and chemical control has been proposed (Theron et al 1992).

Detergents have even been shown to be affecting tadpoles at a site in Russia (Trubetskaya 1994).

Long-term changes in mountain stream frog communities in the mountains of Brazil are attributed to environmental deterioration (Weygoldt 1989).

2.6. Accumulation of pesticides

Chlordane has been shown to accumulate in catfish through the food chain (Murphy & Gooch 1995). Organochlorine pollutants that accumulate in fish are related to the size of the animal. Larger fish accumulate relatively more pesticide (Abd-Allah 1994). Heavy metal pollution is influenced by the presence of humic substances and water hardness (Panttinen et al 1995). Tadpoles have been used to assay for metal pollution, as they accumulate metals (Sparling & Lowe 1996).

Despite an awarness of environmental pollution, pesticides are still accumulating in the top predators like the birds of prey in Africa (Crick 1990). It is known that organochlorine pollution reduces the success of breeding in comorants (Dirksen et al 1995). In Lake Michigan on the other hand, PCBs are approaching stable concentrations in fish (Stow et al 1995) suggesting that the input of PCBs is not increasing. The river Seine has fish contaminated by PCB, pesticides, and heavy metals (Chevreuil et al 1995).

The breakdown of MCPA, (2-methyl-4-phenoxy acetic acid) a common herbicide, has been examined by Bernadini et al (1995). Photolysis is an important agent for degradation (Bourgine et al 1995).

2.7. Atrazine - a major concern

Atrazine and related compounds make up a significant percentage, around 12%, of pesticide sales in the USA (Arnold et al 1995). It is recognized as a hazard to the environment. Atrazine moves through soil (Beck et al 1995) and finds its way into streams and lakes (Bleeker et al 1995, Ng et al 1995). The maximum allowable atrazine concentration for aquatic life in Quebec is 2 mg/l (Laroche & Gallichand 1995).

Atrazine affects the kidney of rainbow trout, even at very low concentrations (Oulmi et al 1995), and affects the ability of crab blood to carry oxygen (Prasad et al 1995).

Work is being done on the natural mechanisms in the soil that trap and degrade atrazine (Anderson & Coats 1995, Entry et al 1995, Mirgain et al 1995, Ro & Chung 1995, Rocha 1995). Natural wetlands are able to cope with a certain amount of atrazine (Chung

et al 1996). Much of this breakdown is associated with grasslands (De Prado et al 1995). Increased microbial activity, produced by the addition of sewerage sludge, (Barriuso et al 1995) or dairy manure (Entry & Emmingham 1995) helps in the degradation of atrazine. On the other hand, effluent may increase the rate of transport of atazine into the water (Graber et al 1995), and salinity affects the loss of atrazine from a water body (Hall et al 1995). The gene responsible for the dechlorination of atrazine in *Pseudomonas* has been cloned (DeSouza 1995). The pollution of water is seasonal (as expected) in parts of Croatia (Gojmerac et al 1996). Even marine phytoplankton is affected (Bester et al 1995).

Atrazine has been shown to be volatile (Foster et al 1995), and this volatiliy is influenced by surface litter (Gish et al 1995). Atrazine is particularly harmful when metals like copper are also present (Gustavson & Wangberg 1995).

Related compounds like simarzine may be more or less harmful in different applications, and care needs to be taken to ensure that related alternative active ingredients are recognized when field trials are carried out.

2.8. FETAX

The FETAX procedure (Frog Embryo Teratogenesis Assay - *Xenopus*) has been positively evaluated as a short-term test for developmental effects (Sabourin & Faulk 1987).

A number of biological assays have been developed for organic compounds in water (Guzella & Mingazzini 1994). The types of malformations and effects that have been observed are detailed by Bantle et al (1991). The natural reproductive cycle of *Xenopus* has been examined by Berk (1938). Fetax has been successfully used for assessing pesticide mixtures in the environment (Dawson & Wilke 1991).

Although most tests are of short duration, it has been suggested that long-term experiments, covering many generations, would be useful (Horne 1995). A comparison between fixed-dose procedures and conventional up-and-down LD_{50} has been discussed by Lipnick et al (1995). The results of toxicity tests like these can be used as input to determine the norms for deriving national guidelines (MacKay et al 1995).

2.9. Other tests

Water quality can be biomonitored using a variety of techniques. The standard literature is not reviewed here. A Biotic Index has been suggested using asemblages of macroinvertebrates, for which sensitivity data is available (Chessman 1995). This approach might also be useful in tadpole-based studies. Hugueny et al (1996) discussed the use of an IBI (Index of Biotic Integrity) which considers factors such as the number of species, the number of intolerant species, the number of species in different taxonomic groups, the percentage of individuals from dominant species, especially resistant species, the number of individuals, the percentage of hybrids, and the percentage of anomalies or diseased individuals.

Developmental toxicity is widely used to measure pollutants (Narotzky & Kavlock 1995).

Population genetics has been suggested as a water quality indicator (Fore et al 1995, Gillespie & Guttman 1993). If allozyme frequencies shift significantly there is a potential loss of individuals with sensitive genotypes. This will reduce variability and make the remaining individuals more susceptible to subsequent stress (Guttman 1994).

Another approach is to simulate a stream ecosystem in the laboratory, and to use this system to determine community responses to pesticides (Schmitz & Hagel 1995, Gruener & Watzin 1996, Sparling et al 1995, Van den Brink et al 1995). A similar approach has also been applied in an outdoor situation (Juttner et al 1995).

Rapid enzymatic tests are described by Burbank & Snell (1994). An enzyme-based assay has been suggested as a rapid toxicity test in invertebrates (Moffat & Snell 1995). Blood enzyme activity is already used to test for lead pollution in fish (Nakagawa et al 1995). Other rapid assessment approaches using macroinvertebrates have been proposed (Rech et al 1995), and rapid tests compared with standard tests by Toussaint et al (1995).

Behavioural changes are a useful indicator of toxicity (Moser et al 1995). Fish behaviour, for example, has been used as an indicator of toxicity (Lorenz et al 1995).

2.10. Present research

The literature review indicates that biomonitoring is a widely used component of ecosystem management. Tadpoles are present in drainages during the rainy season, when run-off and spray drift can be expected to contaminate streams and rivers. There are many different ways to assay streams. Invertebrates, particularly insect larvae, are widely used, along with commercially important fish like trout. Tadpoles produce results which have the potential to extrapolate easily to other terrestrial vertebrates. A number of different testing procedures have been developed, but the FETAX system has been approved by the American Society for Testing Materials, and is used world-wide. The FETAX test is based on the common South African platanna, *Xenopus laevis* . This means that the results obtained by FETAX anywhere in the world are directly applicable to local natural situations. Although any tadpoles present in the water bodies can be used, it was decided to use only *Xenopus* for this base-line study, to be able to compare the results with other studies.

3. DISTRIBUTION AND AVAILABILITY OF TADPOLES

3.1. Methods

3.1.1. Distribution

The drainage systems that were recognized are indicated in figure 1.

This is based on a CSIR publication (O'Keeffe 1985), that was the result of a conference on the conservation status of South African rivers.

The drainage areas recognized are not equally sized, but represent natural distribution areas for amphibians. No attempt has been made to relate frog distribution to the size of the catchment.

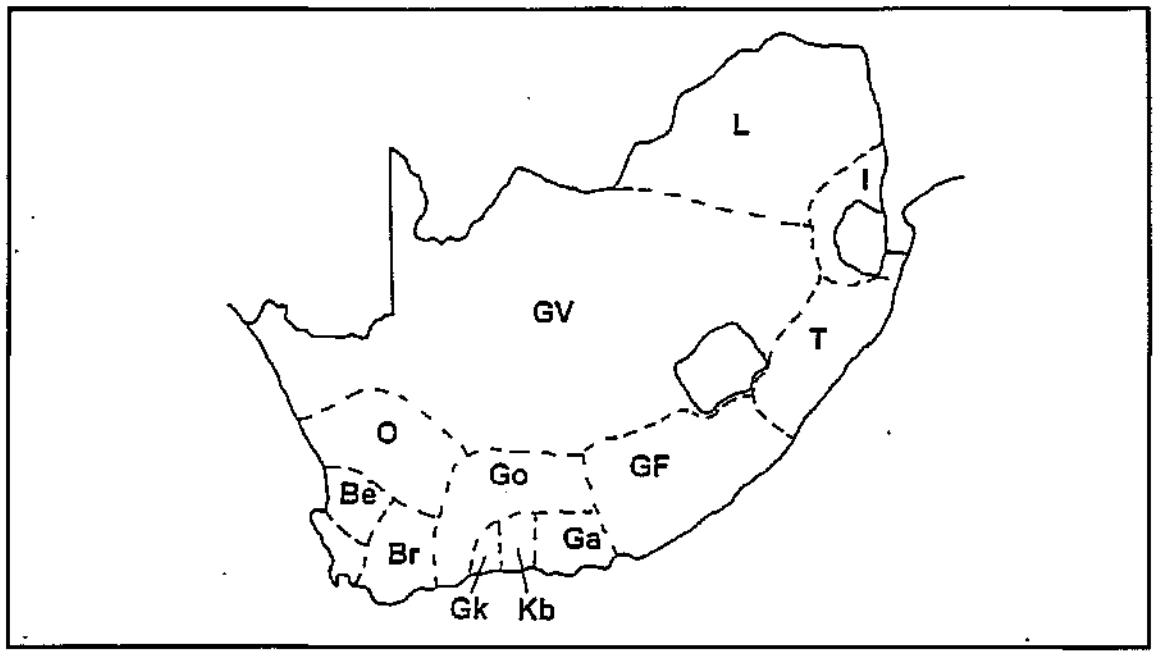


Figure 1. Map of the drainage systems used in this study. L - Limpopo, GV - Gariep/Vaal, I - Incomati, T - Tugela, GF - Great Fish, Ga - Gamtoos, Kb - Keurbooms, Gk - Goukamma, Go - Gourits, Br - Breë, Be - Berg, O - Olifants.

Field trips were made to the major drainage systems in order to confirm the presence of tadpoles. These trips took place during 18-20 August 1995, 16-30 October 1995, 12-22 December 1995, 11-23 January 96, 1-9 April 96, 3-10 May 1996, 2-18 July 1996, 17-23 September 1996, 4-11 November 1996, 21-29 February 1996, 19-25 March 1997, 26-28 April 1997.

Tadpoles were preserved in 10% formalin, and will be deposited in the Port Elizabeth Museum.

In addition, museum records from the major collections in South Africa, Europe and the United States were used to compile the distribution records. The distributions are presented as symbols covering one degree longitude by one degree latitude.

3.1.2. Availability

The availability of tadpoles was compiled from this study, from published literature, and from field notes.

3.2. Tadpole diversity in major drainages

3.2.1. Distribution

The following distribution maps are arranged alphabetically.

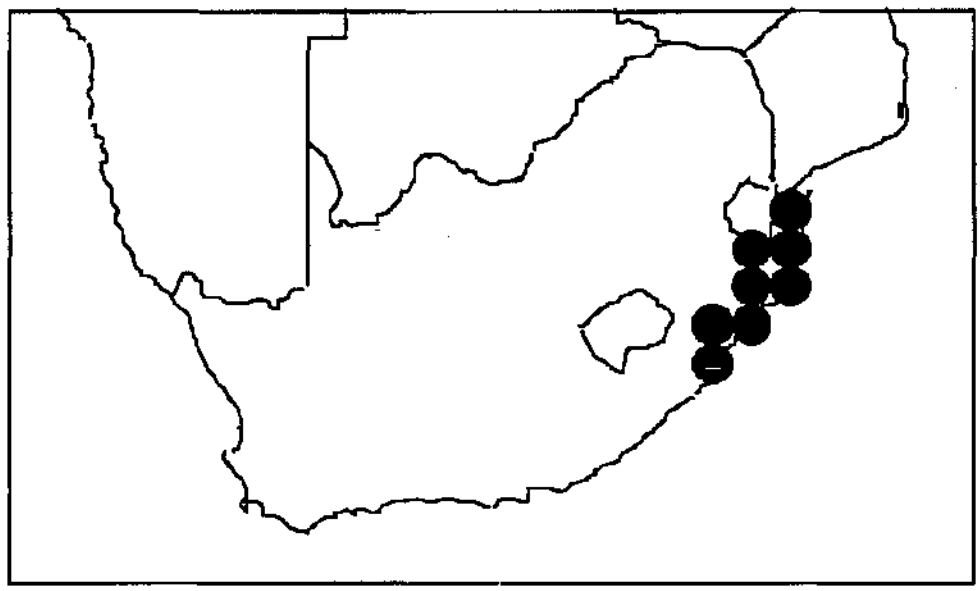


Fig 2. The distribution of *Afrixalus fornasinii*

This species has tadpoles in lowland streams and rivers. Often the tadpoles are found in shallow water amongst emergent vegetation.

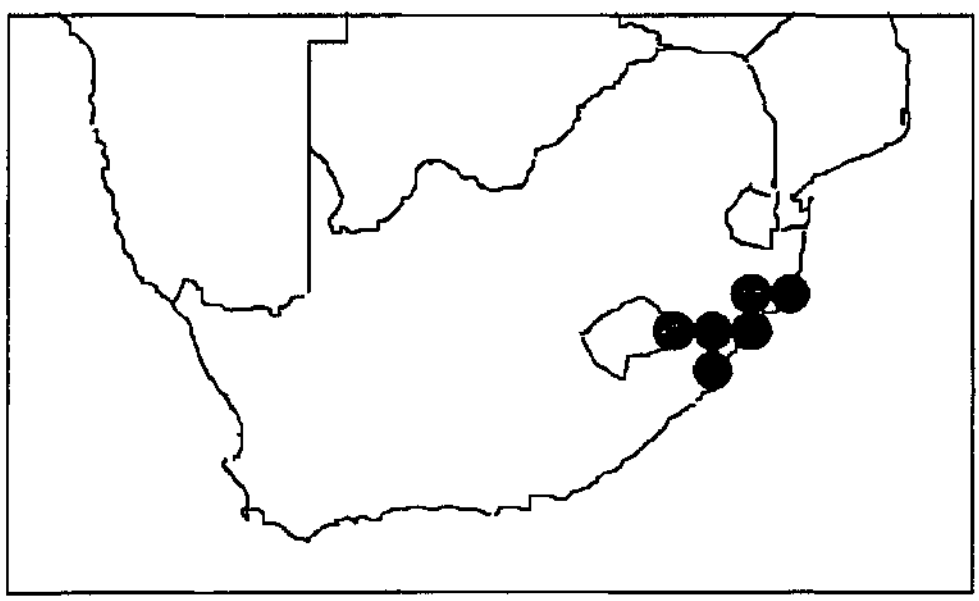


Fig 3. The distribution of *Afrixalus spinifrons*

The tadpoles are found in slow flowing streams amongst vegetation

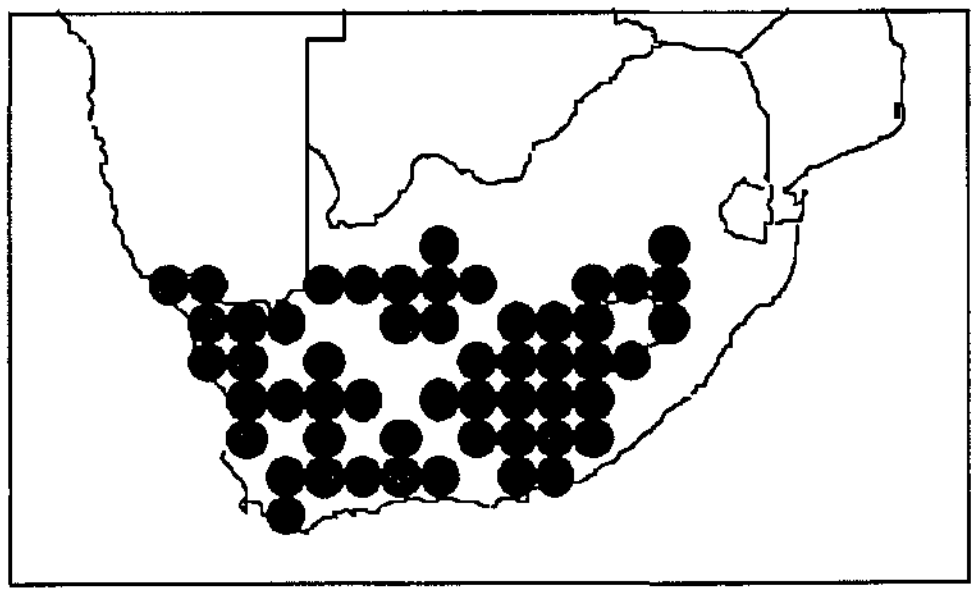


Fig 4. The distribution of *Bufo gariepensis*

This widespread species has tadpoles which can be found in small and large streams. Typically the water is shallow and not well vegetated.

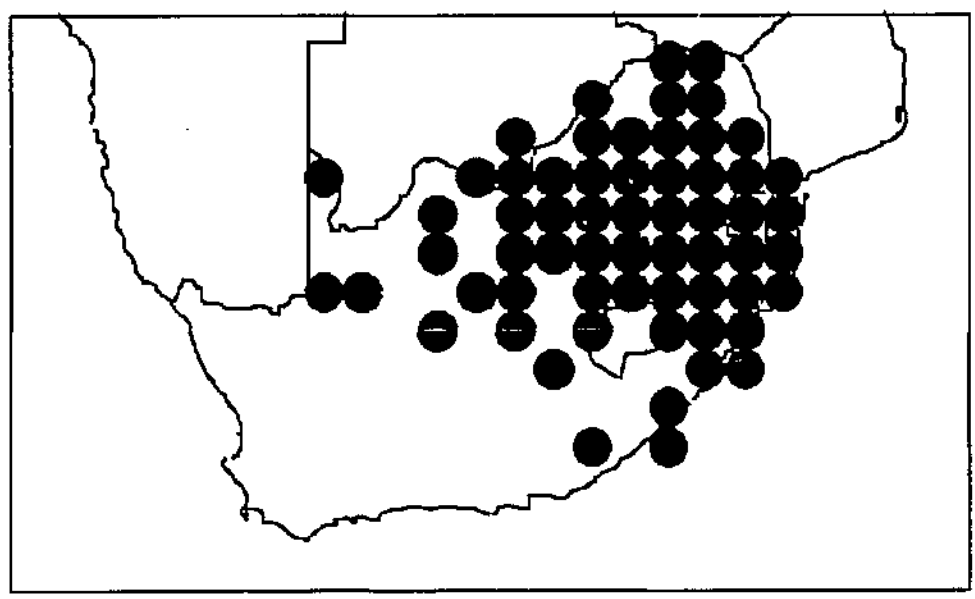


Fig 5. The distribution of *Bufo gutturalis*

The tadpoles of the common toad are gregarious and can be found in large numbers at the bottom and edge of muddy streams.

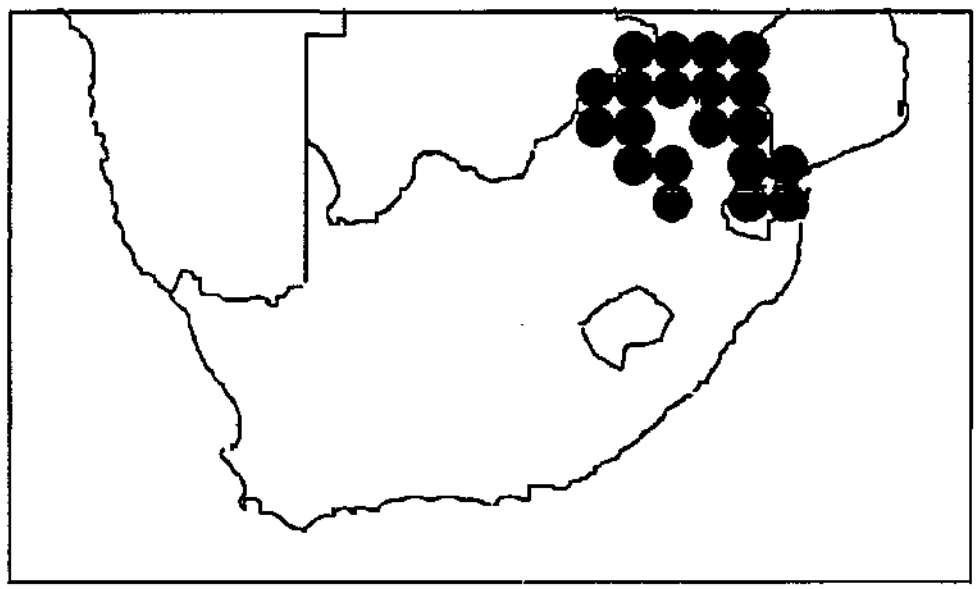


Fig 6 *Bufo maculatus*

The tadpoles are found in shallow backwaters of large rivers.

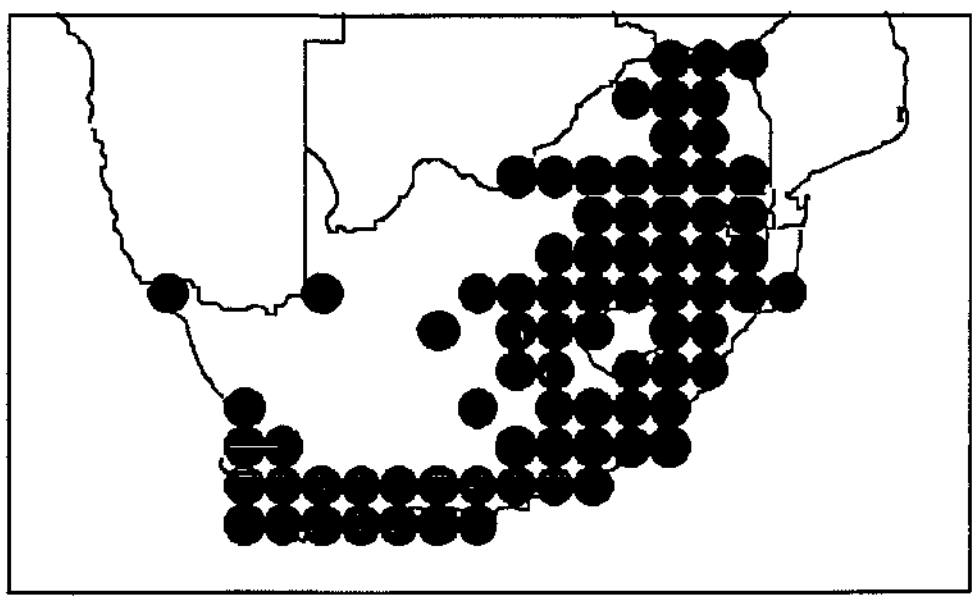


Fig 7. The distribution of *Bufo rangeri*

The tadpoles are ubiquitous in almost all flowing water, where they prefer shallow edges.

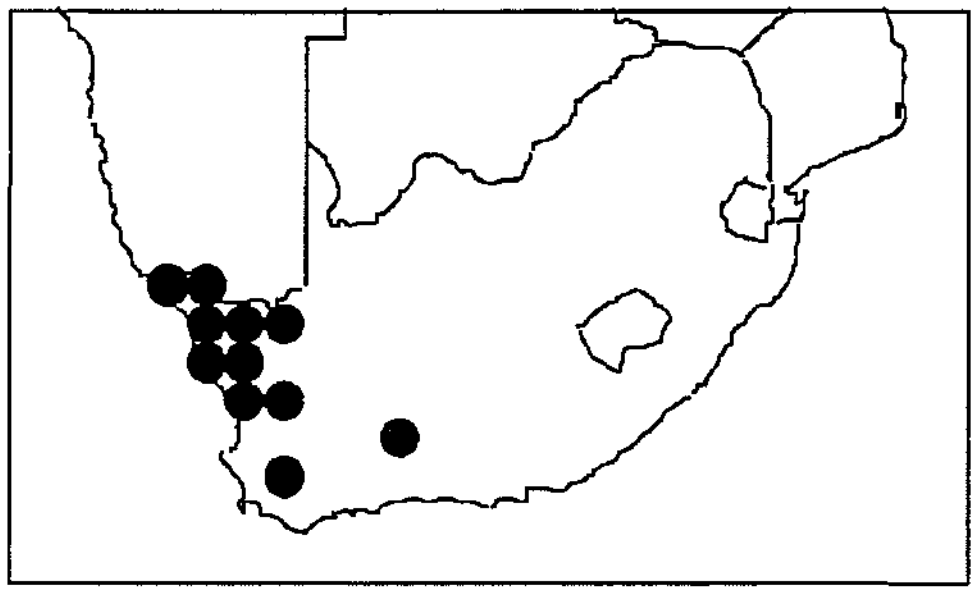


Fig 8. The distribution of *Cacosternum namaquense*

The tadpoles are found along sandy stream bottoms, often remaining in pools formed when the river stops flowing.

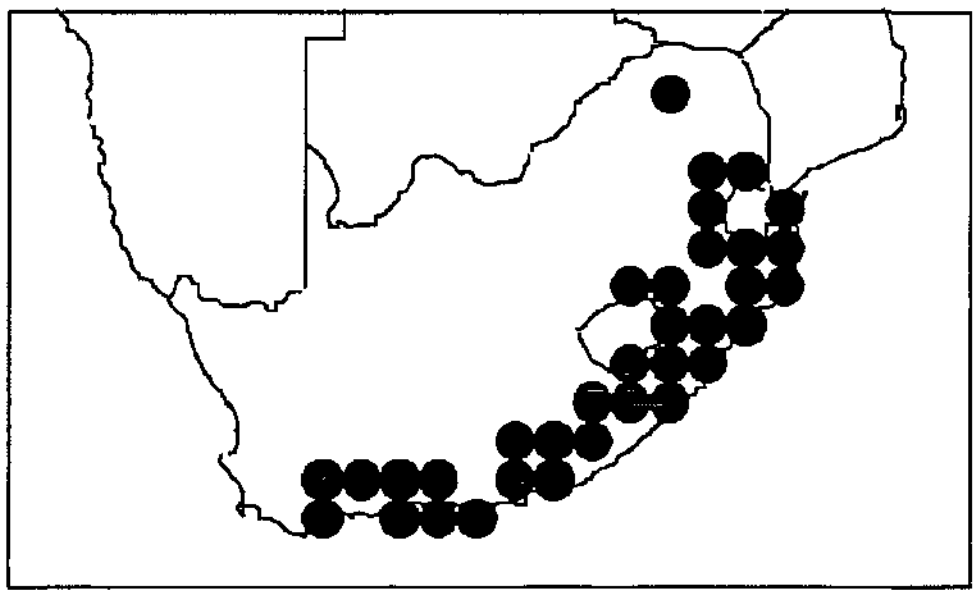


Fig 9. The distribution of *Cacosternum nanum*

The tadpoles of this species are found in slow-flowing drainages, usually associated with grass and other emergent vegetation.

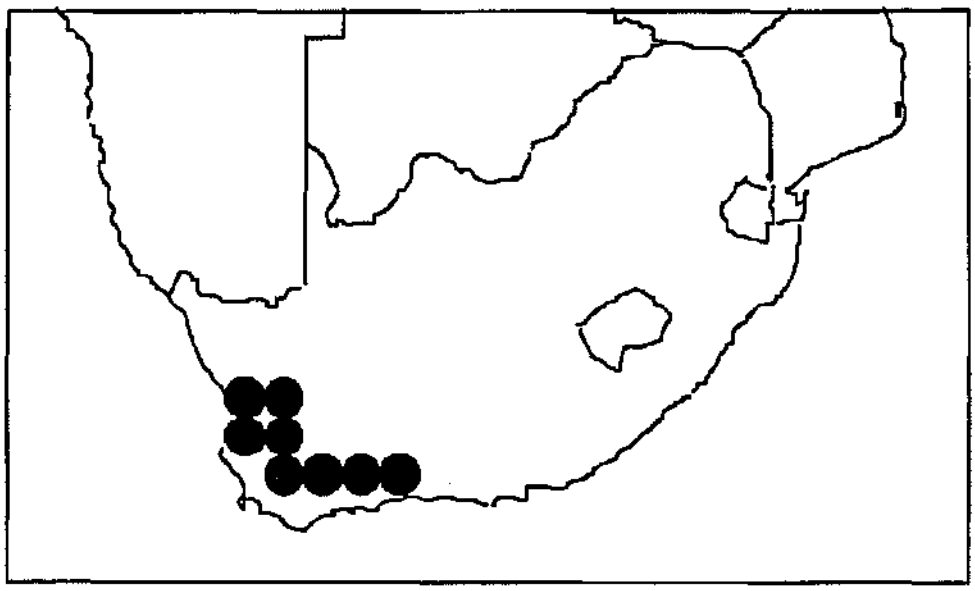


Fig 10. The distribution of *Capensibufo tradouwi*

The tadpoles occur at high altitude, in seepages and slow flowing streams draining the Cape Fold mountains

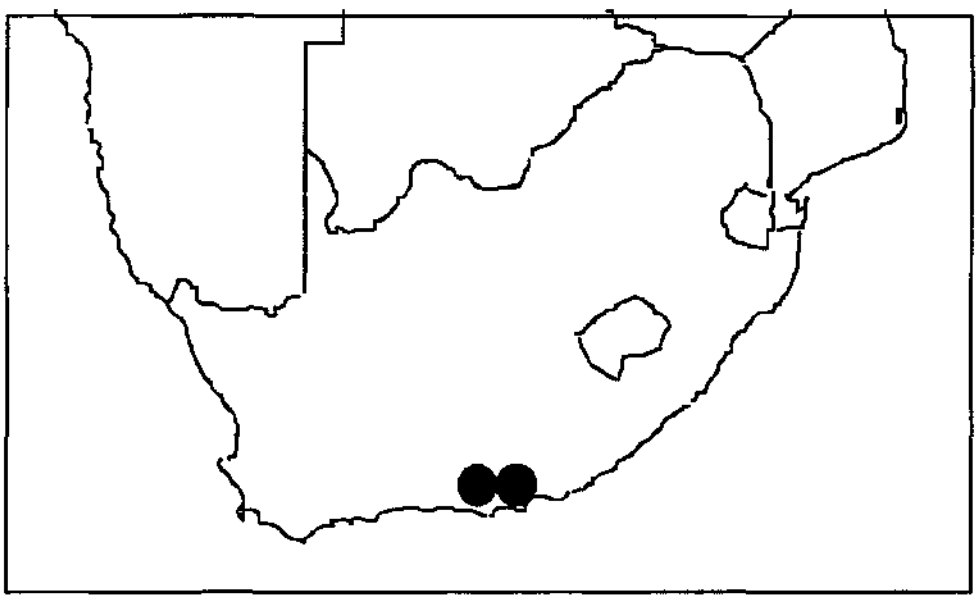


Fig 11 *Heleophryne hewitti*

The tadpoles of this endangered species are restricted to short stretches of fast flowing streams in the Elandsberg Mountains.

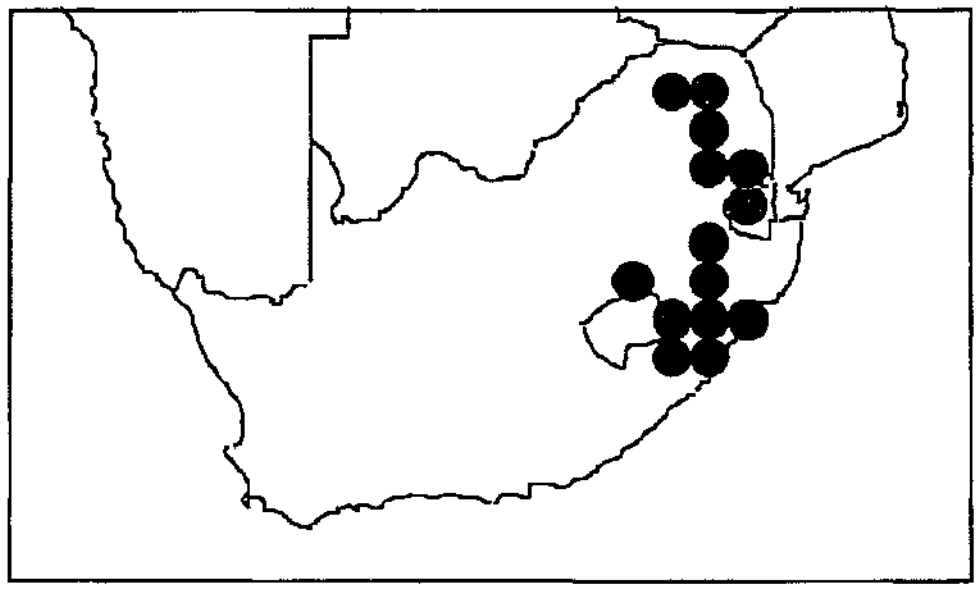


Fig 12. The distribution of *Heleophryne natalensis*

The tadpoles can be found in almost all fast flowing streams, from 3000m down almost to sea level. They prefer well-aerated rivers with water falls and rapids.

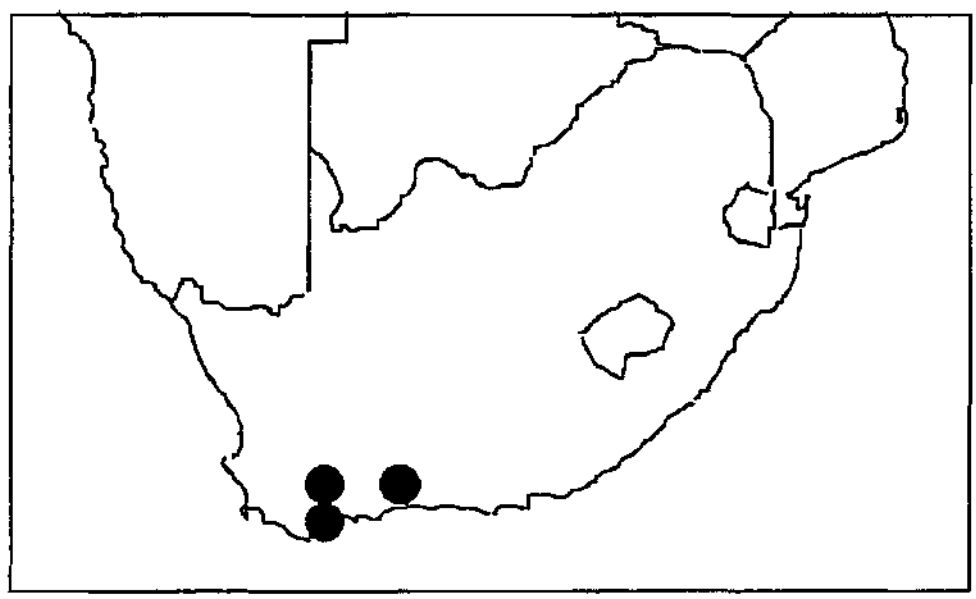


Fig 13. The distribution of *Heleophryne orientalis*

The tadpoles are restricted to the fast flowing streams of the Cape Fold Mountains.

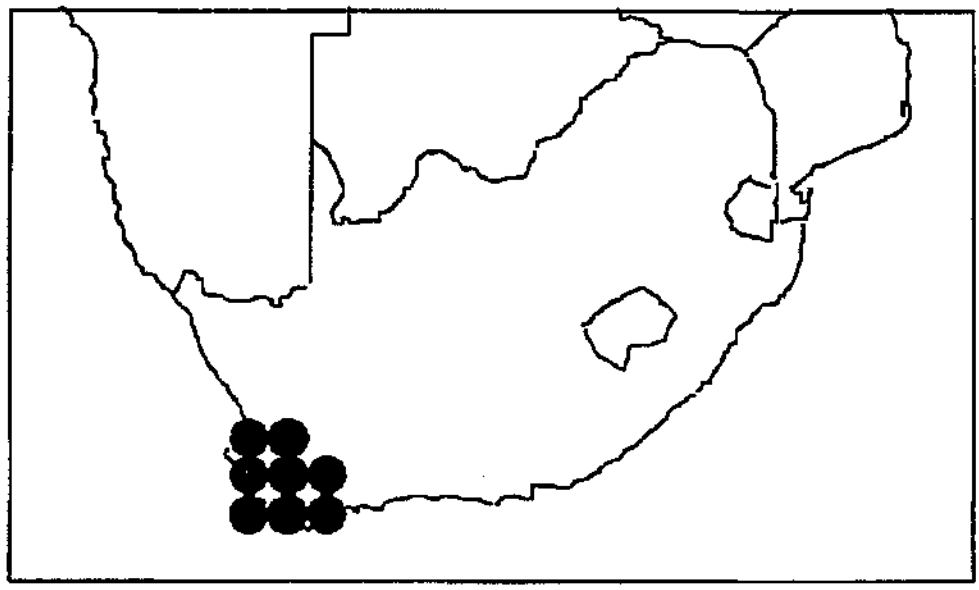


Fig 14. The distribution of *Heleophryne purcelli*

The tadpoles are restricted to fast flowing streams on the western Cape Fold Mountains.

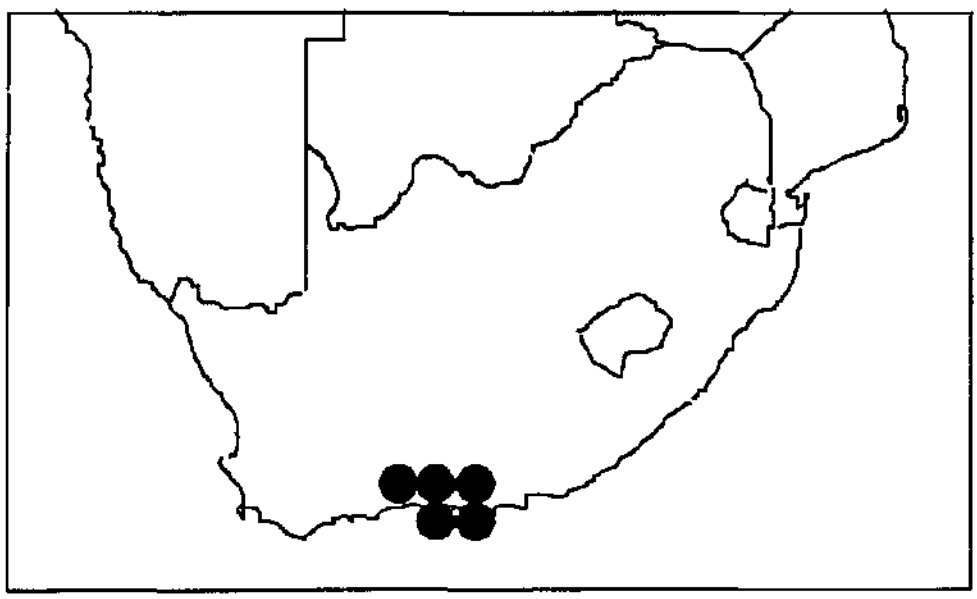


Fig 15. The distribution of *Heleophryne regis*

The tadpoles are restricted to the fast flowing streams of the Cape Fold Mountains.

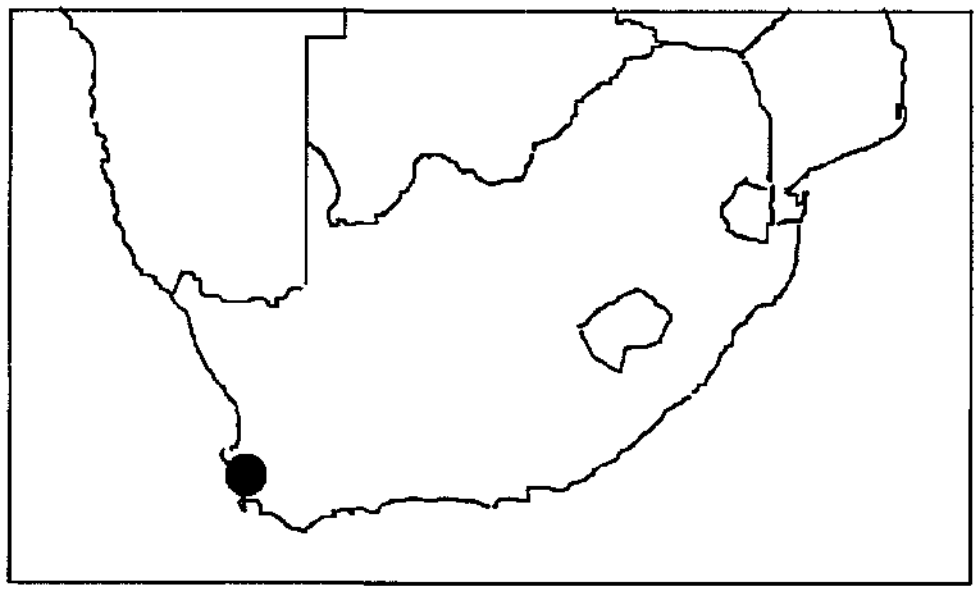


Fig 16. The distribution of *Heleophryne rosei*

The tadpoles of this endangered species are found only in short stretches of streams on the eastern side of Table Mountain on the Cape Peninsula.

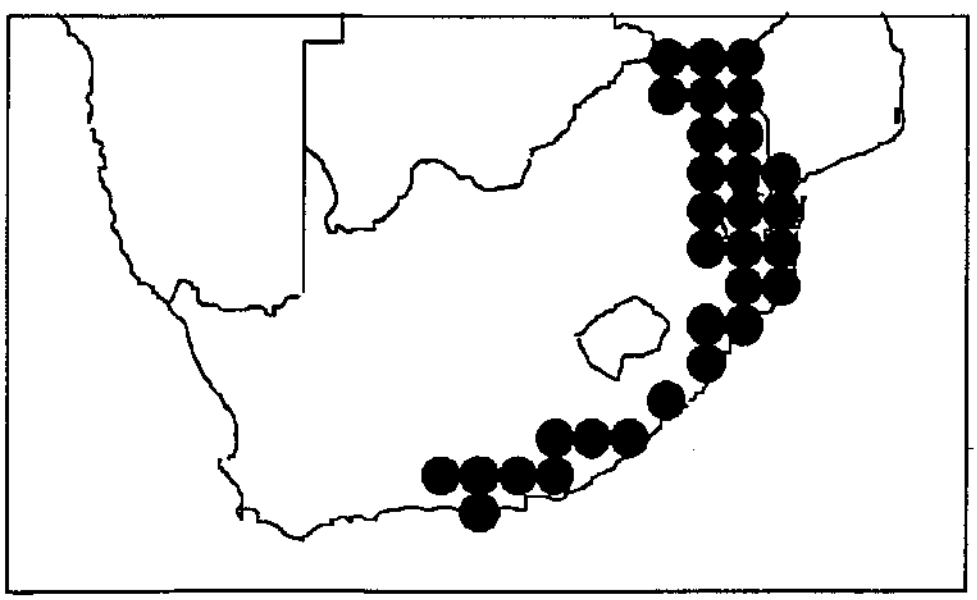


Fig 17. The distribution of *Hyperolius marmoratus*

The tadpoles are found in deep and shallow water that is well vegetated with emergent reeds or grass.

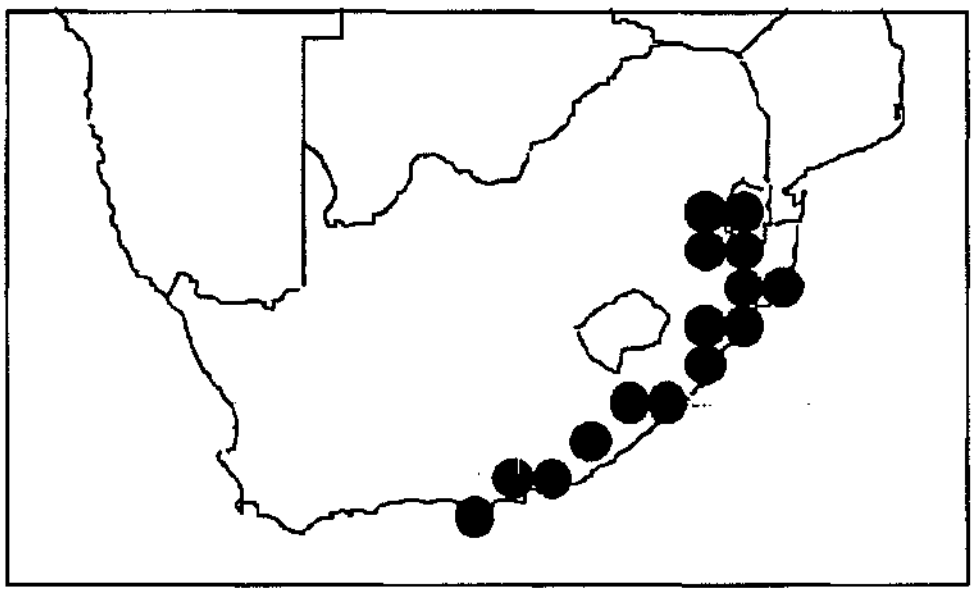


Fig 18. The distribution of *Hyperolis semidiscus*

The tadpoles are found in deep and shallow water that is well vegetated with emergent reeds or grass.

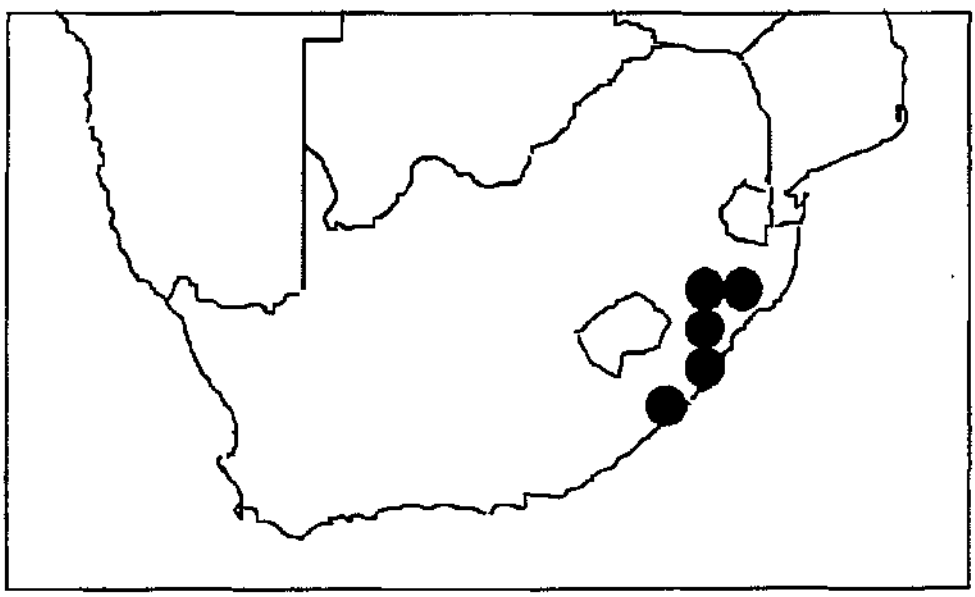


Fig 19. The distribution of *Natalobatrachus bonebergi*

The tadpoles are found only in small streams in forest, or remnants of forest.

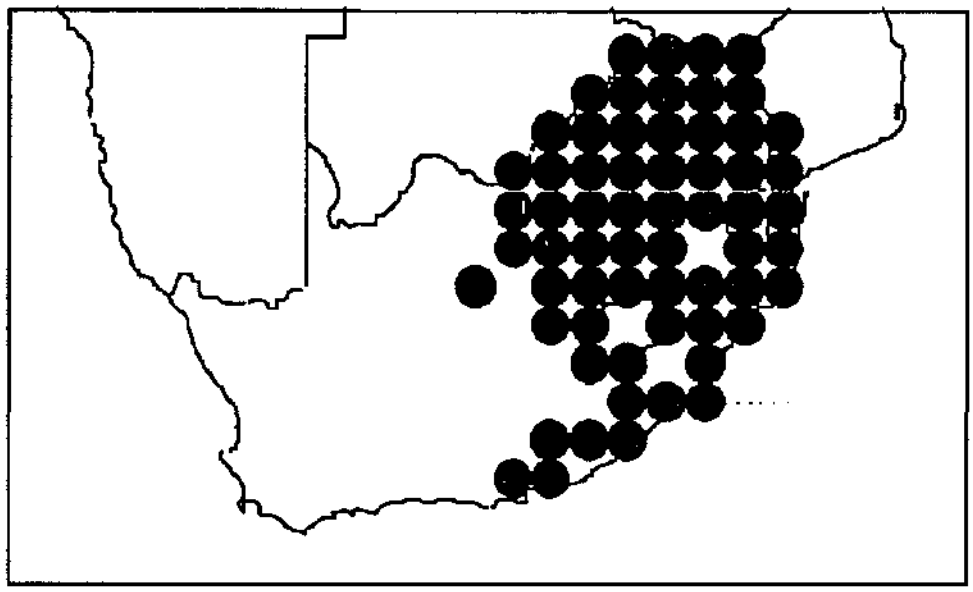


Fig 20. The distribution of *Phrynobatrachus natalensis*

The tadpoles are found in almost any slow flowing water. Often the streams are turbid.

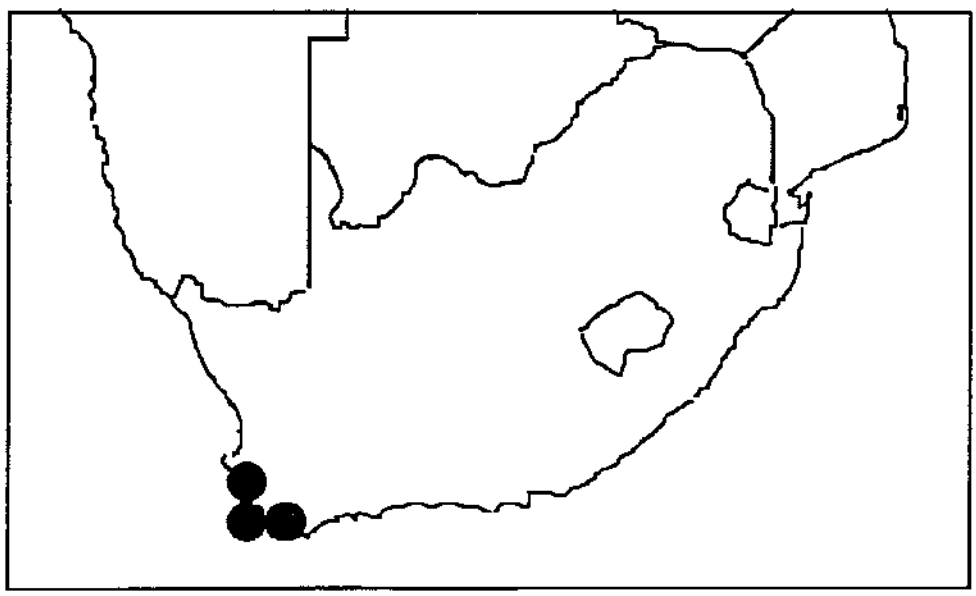


Fig 21. The distribution of *Poyntonia paludicola*

The tadpoles occur in slow flowing seepages on the slopes of the Cape Fold Mountains.

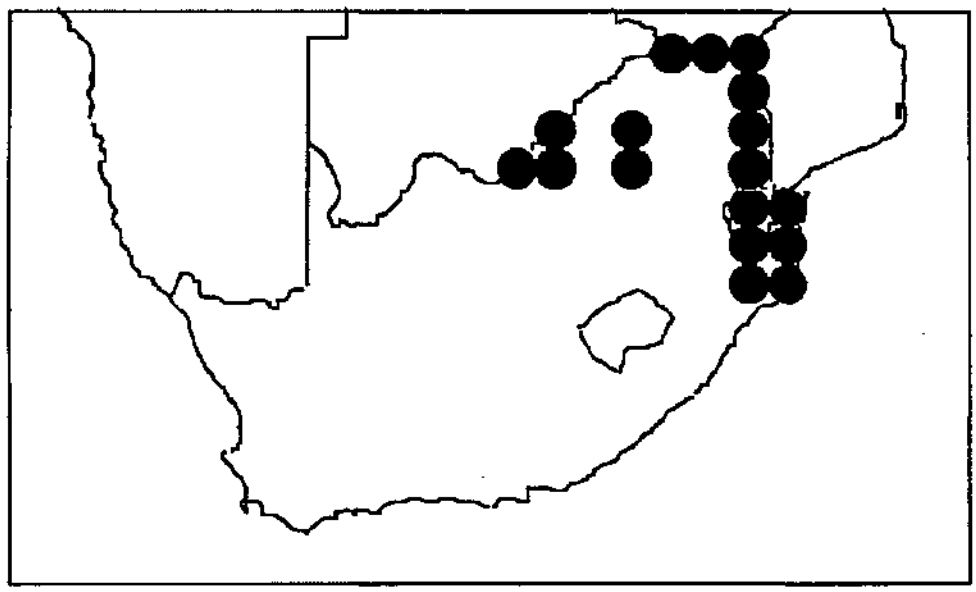


Fig 22. The distribution of *Ptychadena mossambica*

The tadpoles can be found in shallow grassy water, often at the edge of wide, slow flowing sections of drainage.

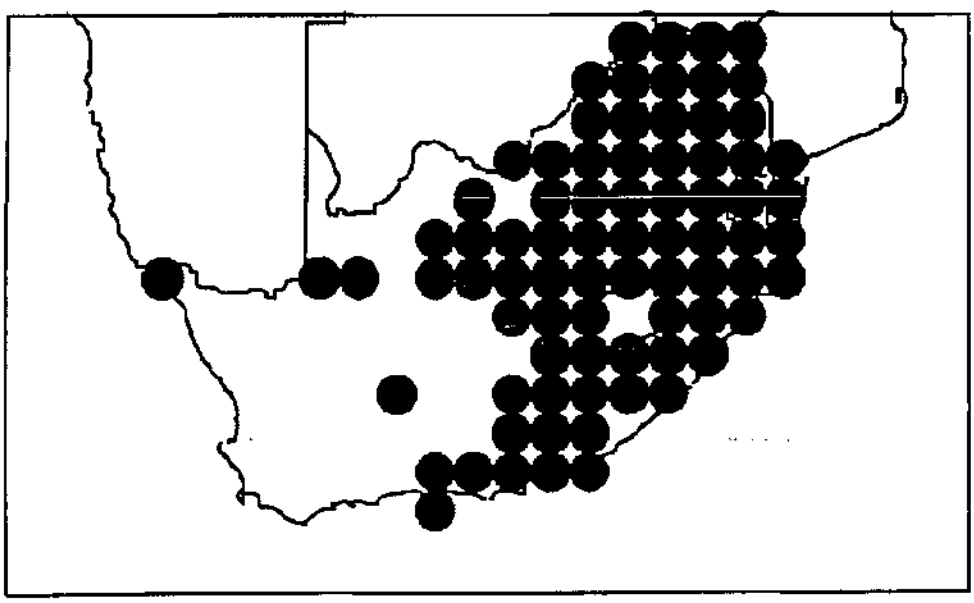


Fig 23. The distribution of *Rana angolensis*

The tadpoles are found in both small and large rivers.

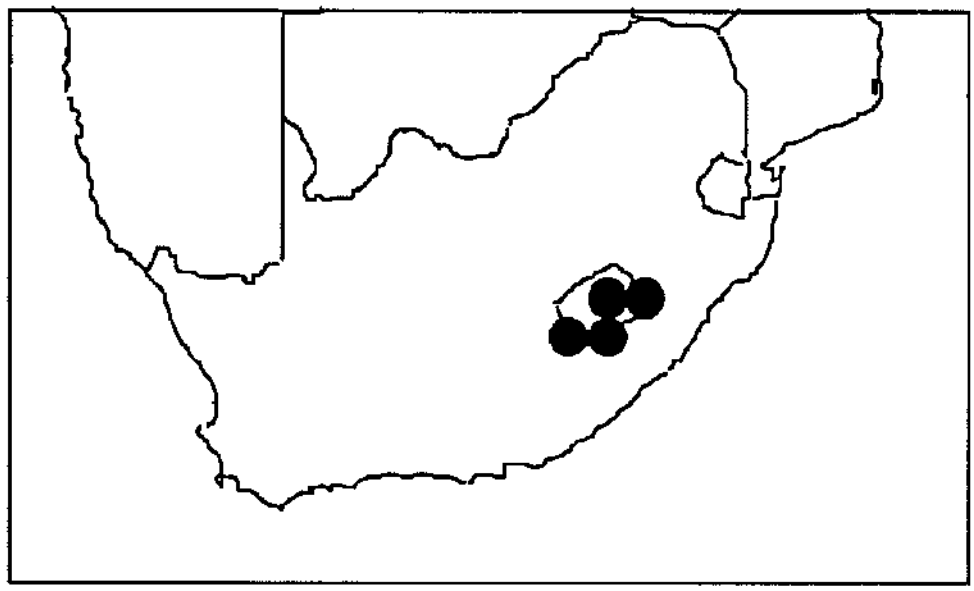


Fig 24. The distribution of *Rana dracomontana*

The large tadpoles of this species occur in the upper reaches of the Gariep river drainage, at altitudes of up to 3000m.

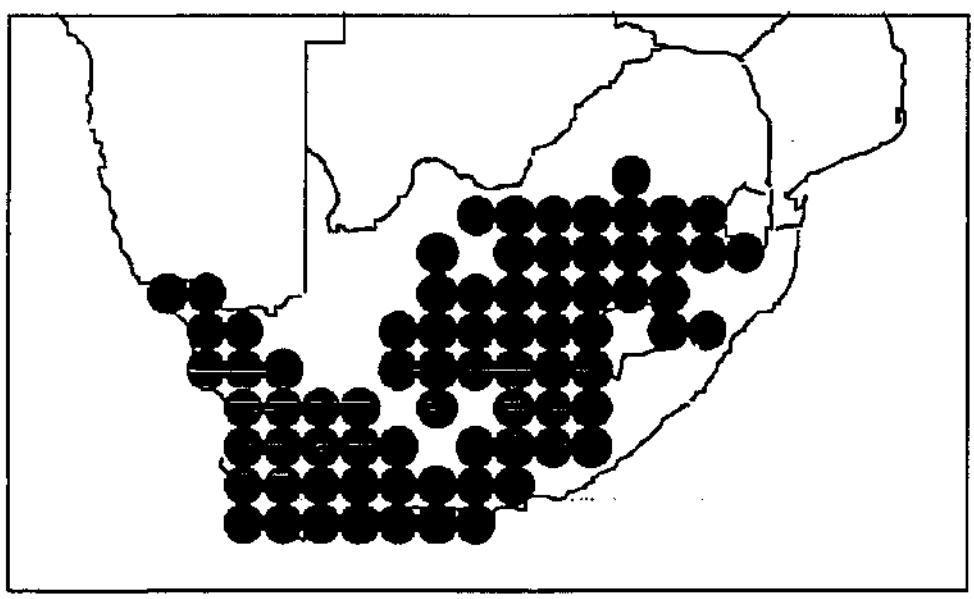


Fig 25. The distribution of *Rana fuscigula*

The robust tadpoles of this species occur in a wide range of streams, from slow flowing water at sea level, to high altitude torrents.

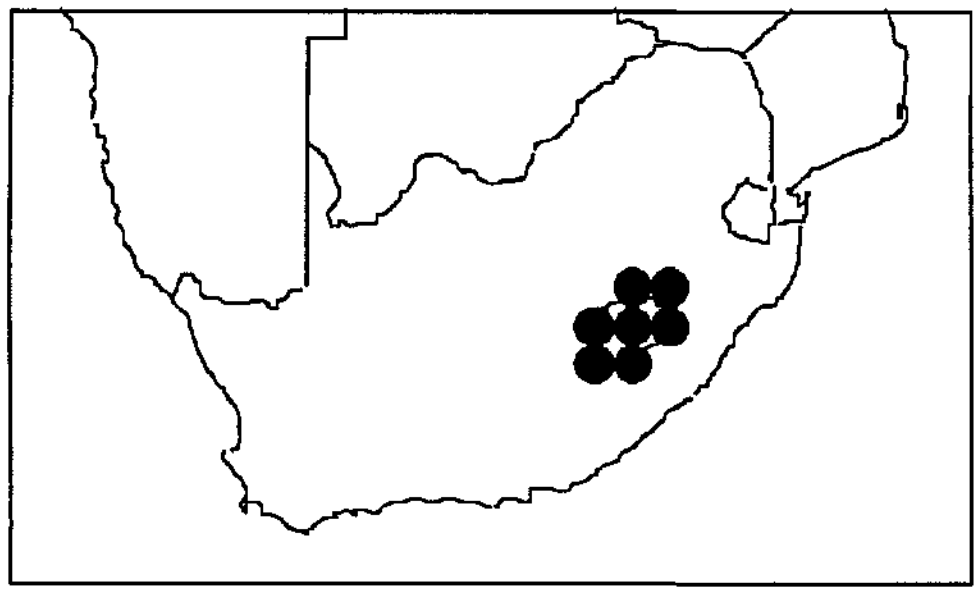


Fig 26. The distribution of *Rana vertebralis*

The tadpoles of this species are found in fast flowing water draining the Drakensberg, from 1500m to the top of the mountain range.

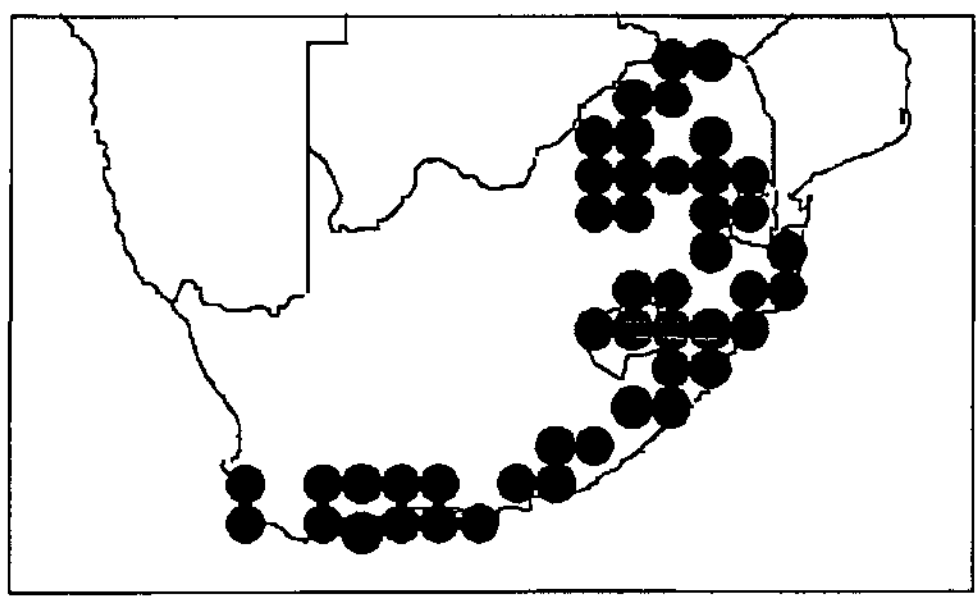


Fig 27. The distribution of *Strongylopus fasciatus*

The tadpoles of this species occur at all altitudes in smaller streams.

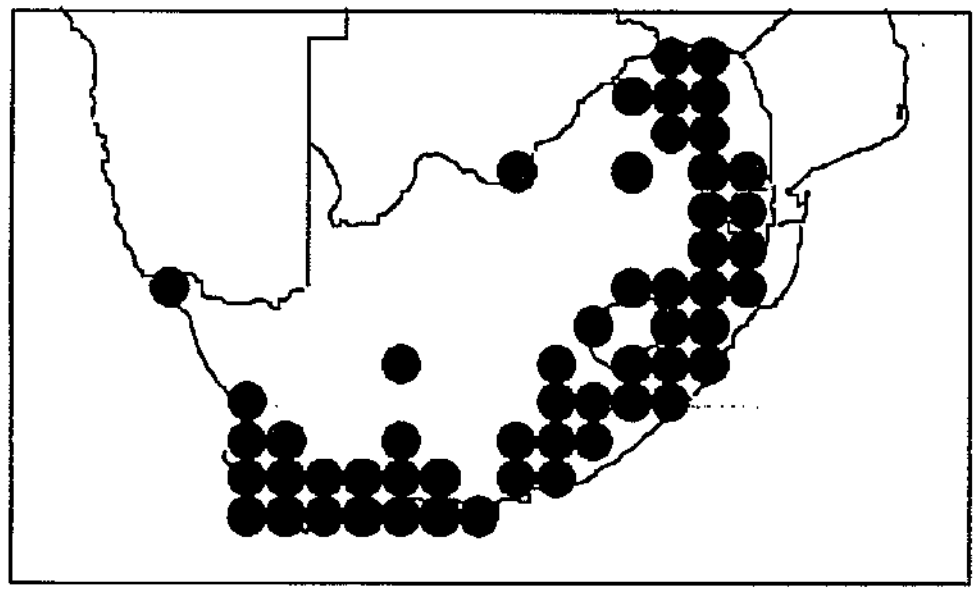


Fig 28. The distribution of *Strongylopus grayii*

This species has tadpoles found in smaller streams, often in turbid and disturbed situations.

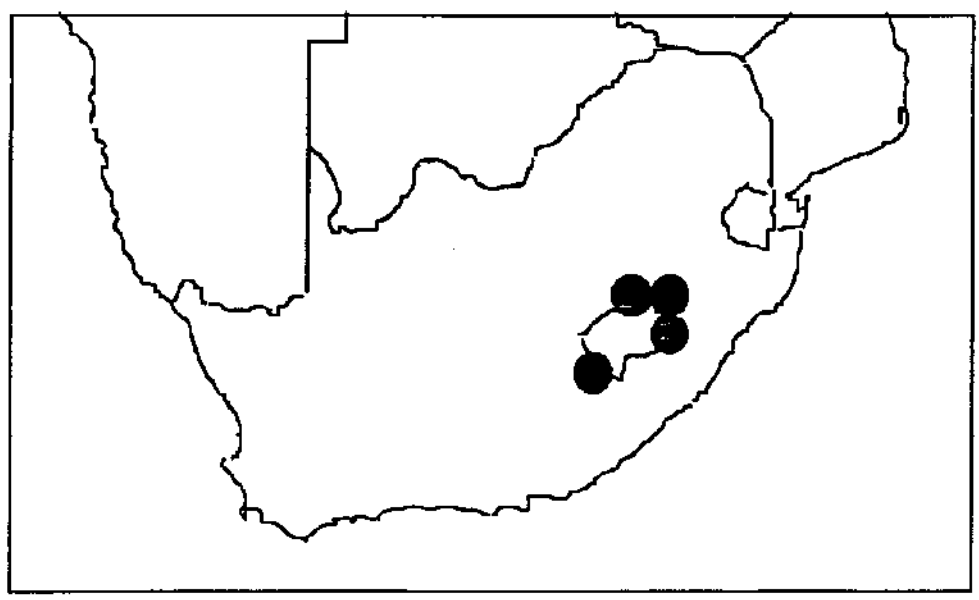


Fig 29. The distribution of *Strongylopus hymenopus*

The tadpoles are restricted to high altitude streams draining the Drakensberg mountains.

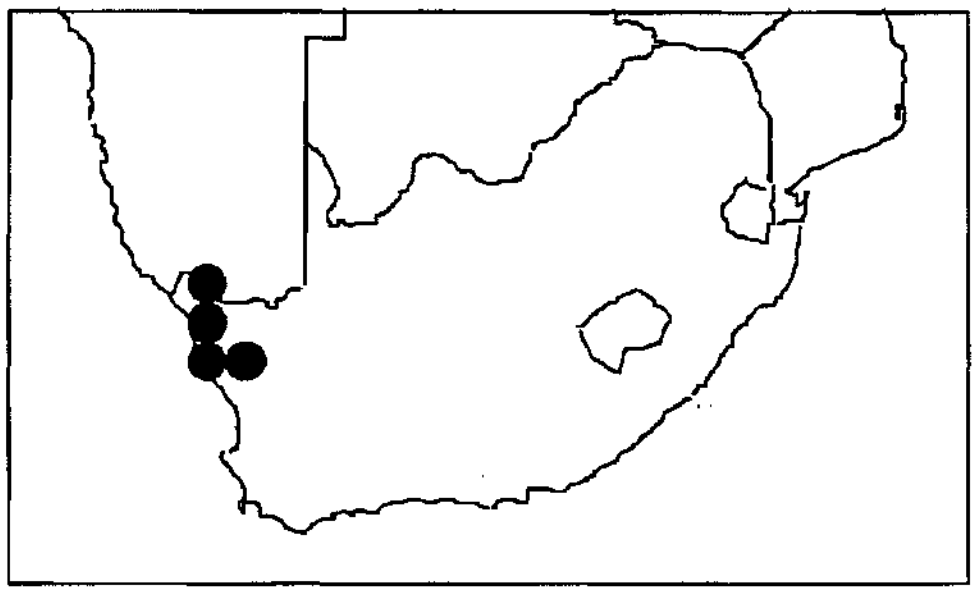


Fig 30. The distribution of *Strongylopus springbokensis*

The tadpoles of this species are found in streams fed by springs, and in pools left after the rivers stop flowing.

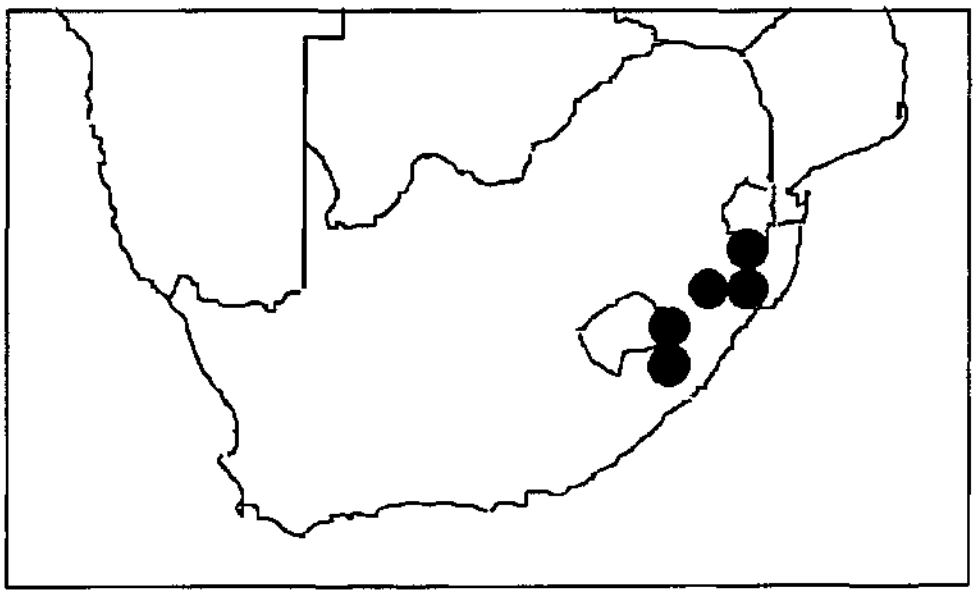


Fig 31. The distribution of *Strongylopus wageri*

The tadpoles of this species are found in forest streams and in high altitude grasslands that were once forested.

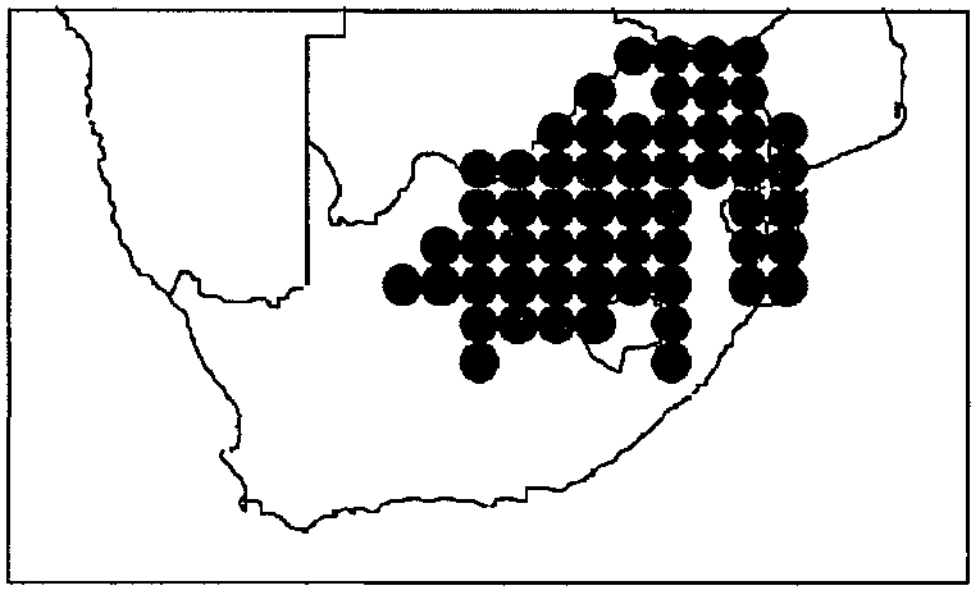


Fig 32. The distribution of *Tomopterna cryptotis*

The tadpoles are found in all drainages where the water is slow flowing. They are associated with muddy substrates.

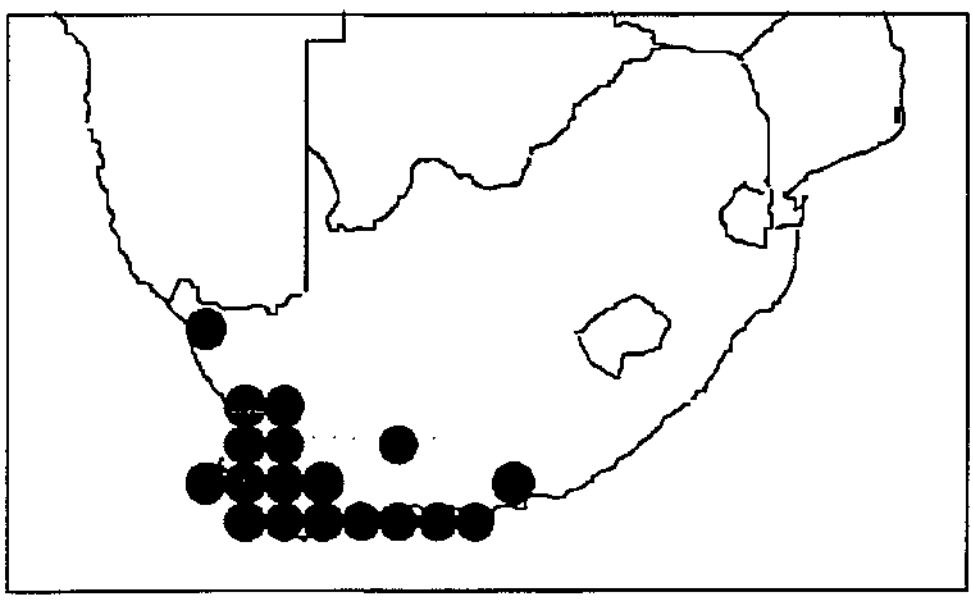


Fig 33. The distribution of *Tomopterna delalandii*

The tadpoles are found in all drainages where the water is slow flowing. They are associated with muddy or gravel substrates.

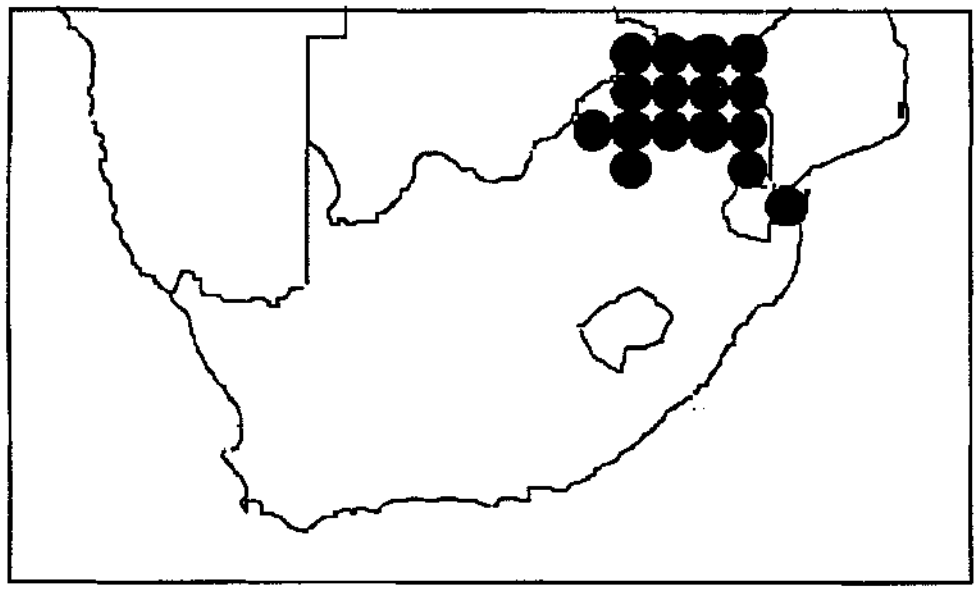


Fig 34. The distribution of *Tomopterna marmorata*

The tadpoles are found in all drainages where the water is slow flowing. They are associated with muddy substrates.

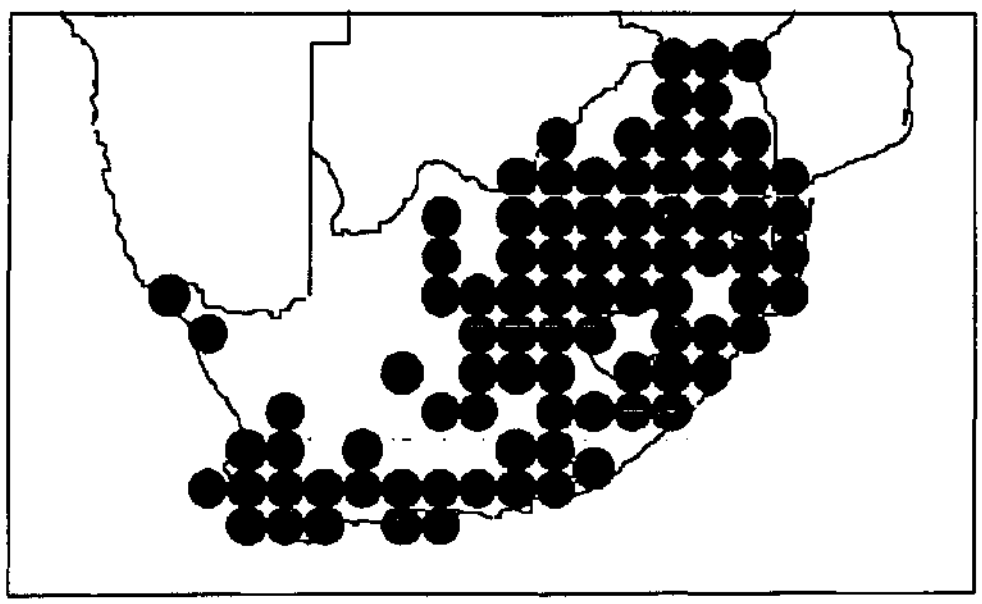


Fig 35. The distribution of *Xenopus laevis*

The tadpoles are gregarious, occurring in schools. They prefer deeper water that is clear.

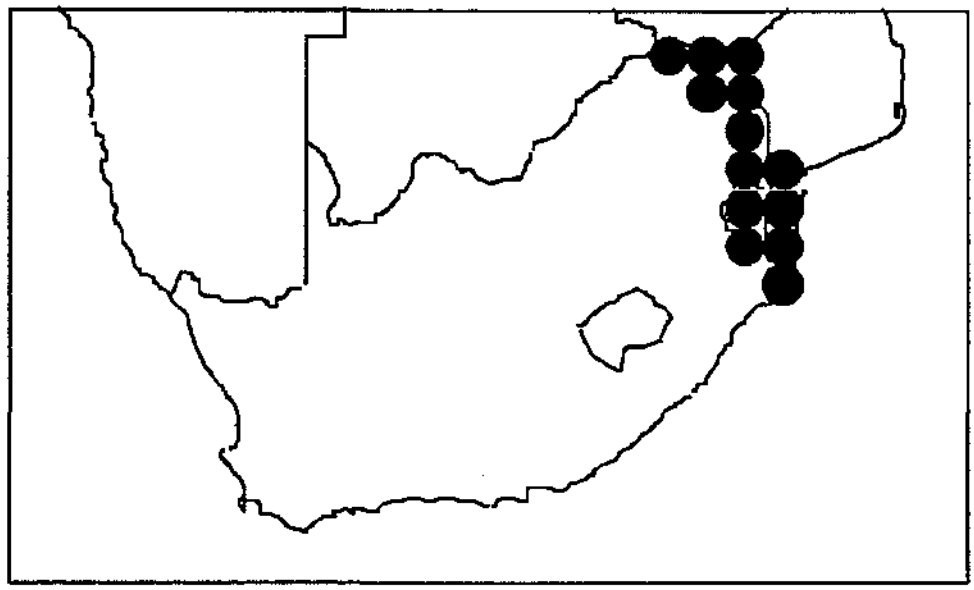


Fig 36. The distribution of *Xenopus muelleri*

The tadpoles are gregarious, occurring in schools. They prefer deeper water, and have been found in both turbid and clear habitats.

3.2.2. Seasonal availability

The presence of tadpoles in streams is dependent on the permanance of the water. For example, perennial mountain streams may have tadpoles all year, with peaks after the rains, once the stream flow has subsided. Annual streams tend to have peaks of tadpole numbers following every major flow. For the purposes of easy analysis, known and expected tadpole presence in streams is summarized in two-month periods in Table 3.

3.3 River system diversity

The classification of the drainage systems follows the map "The Conservation Status of South African Rivers" by J. H. O'Keeffe (1985).

Table 2a. Tadpoles in river systems

RIVER SYSTEM / SPECIES	Limpopo	Incomati	Orange / Vaal	Tugela	Great Fish	Gamtoos
Afrixalus fornasinii	-	-	-	P	-	-
Afrixalus spinifrons	-	-	-	P	-	-
Bufo gariepensis	-	-	P	P	P	P
Bufo gutturalis	P	P	P	P	P	-
Bufo maculatus	P	P	-	-	-	-
Bufo rangeri	P	P	P	P	P	P
Cacosternum namaquense	-	-	-	-	-	-
Cacosternum nanum	P	P	P	P	P	P
Capensibufo tradouwi	-	-	-	-	-	-
Heleophryne hewitti	-	-	-	-	-	P
Heleophryne natalensis	P	P	-	P	-	-
Heleophryne orientalis	-	-	-	-	-	-
Heleophryne purcelli	-	-	-	-	-	-
Heleophryne regis	-	-	-	-	-	-
Hyperolius marmoratus	P	P	-	P	P	P
Hyperolius semidiscus	-	P	-	P	P	P
Natalobatrachus bonebergi	-	-	-	P	-	-
Phrynobatrachus natalensis	P	P	P	P	P	-
Poyntonia paludicola	-	-	-	-	-	-
Ptychadena mossambica	P	P	-	P	-	-
Rana angolensis	P	P	P	P	P	P
Rana dracomontana	-	-	P	-	-	-
Rana fuscigula	-	-	P	P	P	P
Rana vandijki	-	-	-	-	-	-
Rana vertebralis	-	-	P	P	-	-
Strongylopus fasciatus	P	P	P	P	P	P
Strongylopus grayii	P	P	P	P	P	P
Strongylopus hymenopus	-	-	P	P	-	-
Strongylopus wageri	-	-	-	P	-	-
Tomopterna cryptotis	P	P	P	P	-	-
Tomopterna delalandii	-	-	-	-	P	P
Tomopterna marmorata	P	P	-	-	-	-
Tomopterna natalensis	P	P	P	P	-	-
Tomopterna tandyi	P	P	P	P	P	-
Xenopus laevis	P	P	P	P	P	P

Table 2b. Tadpoles in river systems

SPECIES \ RIVER SYSTEM	Keur-booms	Goukamma	Gourits	Breë	Berg	Olifants
Afrixalus fornasinii	-	-	-	-	-	-
Afrixalus spinifrons	-	-	-	-	-	-
Bufo gariepensis	P	P	P	P	P	P
Bufo gutturalis	-	-	-	-	-	-
Bufo maculatus	-	-	-	-	-	-
Bufo rangeri	P	P	P	P	P	P
Cacosternum namaquense	-	-	-	-	-	P
Cacosternum nanum	P	P	P	P	-	-
Capensibufo tradouwi	-	-	-	P	P	P
Heleophryne hewitti	-	-	-	-	-	-
Heleophryne natalensis	-	-	-	-	-	-
Heleophryne orientalis	-	P	-	-	-	-
Heleophryne purcelli	-	-	-	P	P	P
Heleophryne regis	P	P	-	-	-	-
Hyperolius marmoratus	P	P	P	-	-	-
Hyperolius semidiscus	-	-	-	-	-	-
Natalobatrachus bonebergi	-	-	-	-	-	-
Phrynobatrachus natalensis	-	-	-	-	-	-
Poyntonia paludicola	-	-	-	P	-	-
Ptychadena mossambica	-	-	-	-	-	-
Rana angolensis	P	P	P	-	-	-
Rana dracomontana	-	-	-	-	-	-
Rana fuscigula	P	P	P	P	P	P
Rana vandijki	-	-	P	-	-	-
Rana vertebralis	-	-	-	-	-	-
Strongylopus fasciatus	P	P	P	P	P	-
Strongylopus grayii	P	P	P	P	P	P
Strongylopus hymenopus	-	-	-	-	-	-
Strongylopus wageri	-	-	-	-	-	-
Tomopterna cryptotis	-	-	-	-	-	-
Tomopterna delalandii	P	P	P	P	P	P
Tomopterna marmorata	-	-	-	-	-	-
Tomopterna natalensis	-	-	-	-	-	-
Tomopterna tandyi	-	-	-	-	-	-
Xenopus laevis	P	P	P	P	P	P

Table 3. Seasonality of tadpoles

Two-month period / SPECIES	J/F	M/A	M/J	J/A	S/O	N/D
Afrixalus fornasinii	√ √ √	√			√	√ √ √
Afrixalus spinifrons	√ √ √	√			√	√ √ √
Bufo gariepensis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Bufo gutturalis	√ √ √	√			√	√ √ √
Bufo maculatus	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Bufo rangeri	√ √ √					√ √ √
Cacosternum namaquense			√	√ √ √	√	√ √ √
Cacosternum nanum	√ √ √				√ √ √	√ √ √
Capensibufo tradouwi				√ √ √	√ √ √	
Heleophryne hewitti	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Heleophryne natalensis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Heleophryne orientalis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Heleophryne purcelli	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Heleophryne regis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Hyperolius marmoratus	√ √ √	√			√ √ √	√ √ √
Hyperolius semidiscus	√ √ √	√			√ √ √	√ √ √
Natalobatrachus bonebergi	√ √ √	√ √ √				√ √ √
Phrynobatrachus natalensis	√ √ √	√ √ √			√ √ √	√ √ √
Poyntonia paludicola			√ √ √	√ √ √	√ √ √	
Ptychadena mossambica	√ √ √				√ √ √	√ √ √
Rana angolensis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Rana dracomontana	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Rana fuscigula	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Rana sp A	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Rana vertebralis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Strongylopus fasciatus	√ √ √	√ √ √			√ √ √	√ √ √
Strongylopus grayii	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
Strongylopus hymenopus	√ √ √	√ √ √	√ √ √			√ √ √
Strongylopus wageri	√ √ √	√ √ √				√ √ √
Tomopterna cryptotis	√ √ √	√ √ √			√ √ √	√ √ √
Tomopterna delalandii			√ √ √	√ √ √	√ √ √	√ √ √
Tomopterna marmorata	√ √ √	√ √ √				√ √ √
Tomopterna natalensis	√ √ √	√ √ √			√ √ √	√ √ √
Tomopterna tandyi	√ √ √	√ √ √			√ √ √	√ √ √
Xenopus laevis	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √

The three possible ticks within each two-month interval represent, early, mid and late presence. Note that some species may breed in both summer and winter in different parts of the country.

4. IDENTIFICATION OF TADPOLES

The keys and nomenclature are based on the work of Van Dijk 1966, and Lambiris 1987. Tadpoles can be identified using behavioural cues, although for the beginner the details of the overall body proportions, and the arrangement of tooth rows and other mouthpart details, allow a confident identification to be made.

4.1. Preparation of material

With experience it is trivial to make identifications of most of these stream species of free-swimming individuals. Most tadpoles are too small to be positively identified without the use of a hand lens or stereo microscope, at least for those with limited experience. In order to be able to see all the structures, it is important that the tadpoles are preserved in formalin (10%), and then kept for reference purposes in a 5% solution. Tadpoles should never be preserved in 70% ethanol, as they deteriorate rapidly.

For further analyses of DNA or allozymes, it is necessary that the tadpoles be collected and preserved appropriately. Allozyme analyses require fresh material, or material that is frozen and kept in liquid nitrogen in the field. For DNA studies, it is possible to use either frozen material, or tissue preserved in alcohol. In order to prevent contamination with the DNA of food organisms, only a portion of the tail should be used. The rest of the tadpole can be preserved in formalin as a voucher specimen.

4.2. Characters used

The characters used are explained under "terminology" above. The following diagram of the mouthparts of a tadpole (Fig 37), taken from Van Dijk 1966, illustrates the diagnostic features. There is growing recognition that features present inside the buccal cavity are often very diagnostic. However, I believe that it is unnecessary to explore those features here.

4.3. Key to stream tadpoles

Refer to figure 37 for mouthpart nomenclature, and the definitions of terminology above.

The keys consist of a series of choices. Start at 1a/1b, and select the statement that best fits the tadpole you are identifying. Each choice will either lead you to a new pair of statements indicated by a numeral, or to a species identification.

An example of one species of tadpole from each genus is illustrated in figs 38-51.

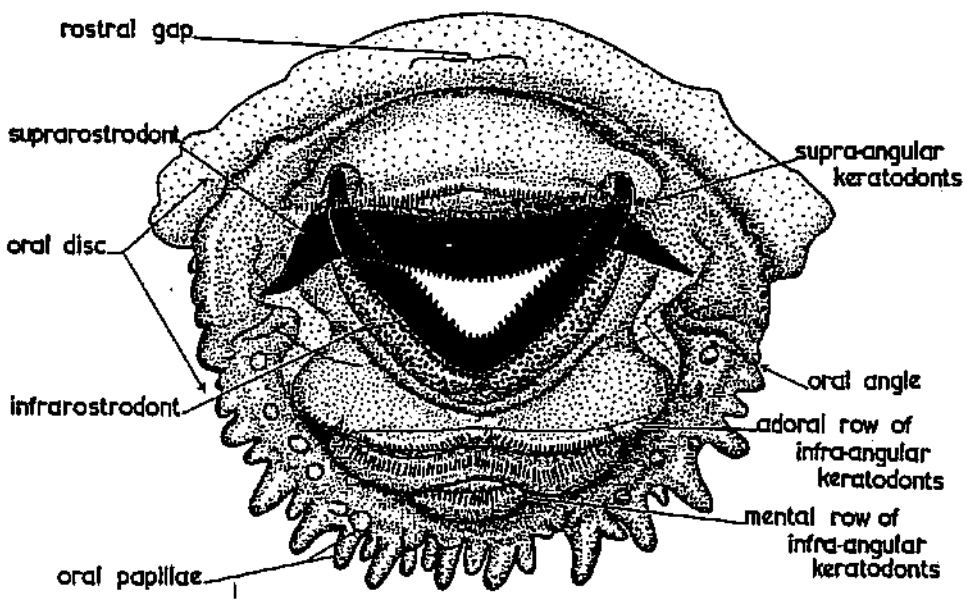


Fig 37. Diagram of the mouthparts of a typical tadpole (After Van Dijk 1966). In the following key, the terminology has been simplified as follows:

Terminology after Van Dijk	Terminology in present key
Rostral gap	Gap in upper papillae
Supra-angular keratodonts	Upper tooth rows
Infra-angular keratodonts	Lower tooth rows
Infrarostrodont	Lower beak
Suprarostrodont	Upper beak

Figure 38. A *Capensibufo* tadpole

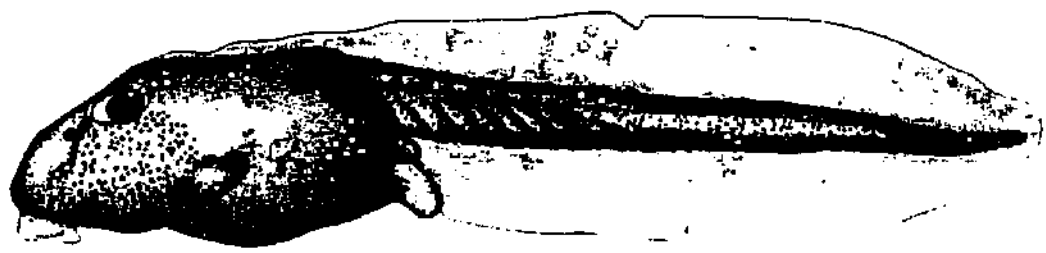


Figure 39. A *Bufo* tadpole

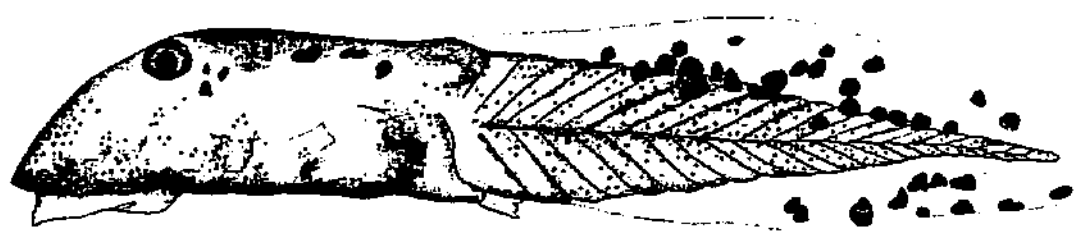


Figure 40. A *Heleophryne* tadpole

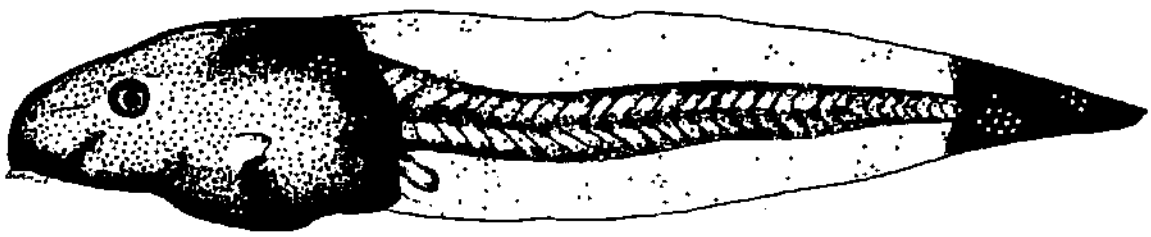


Figure 41. A *Hyperolius* tadpole

Figure 42. An *Afrixalus* tadpole

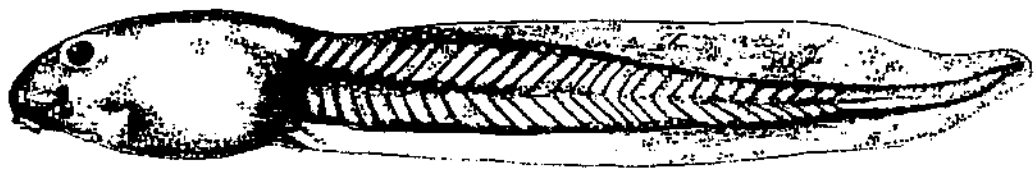


Figure 43. A *Poyntonia* tadpole

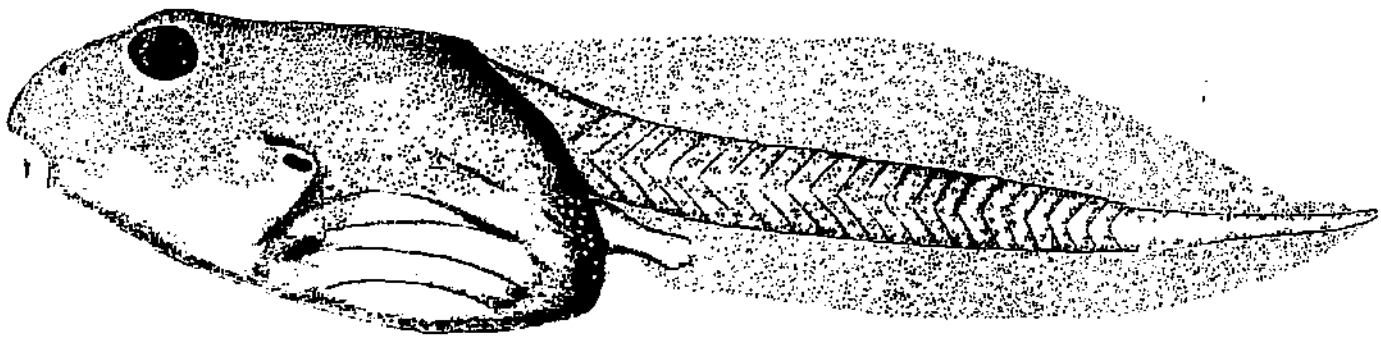


Figure 44. A *Phrynobatrachus* tadpole

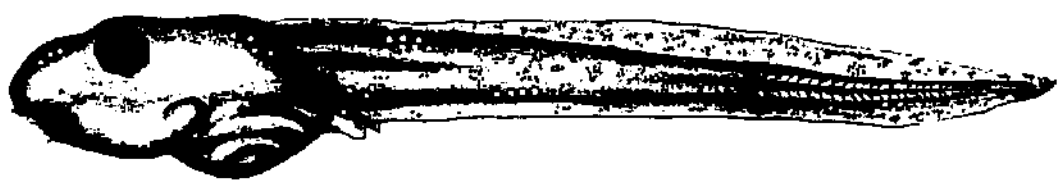


Figure 45. A *Ptychadena* tadpole

Figure 46. A *Tomopterna* tadpole

Figure 47. A *Rana* tadpole

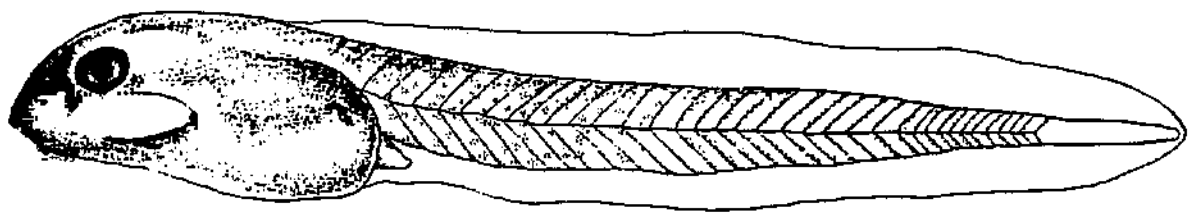


Figure 48. A *Natalobatrachus* tadpole

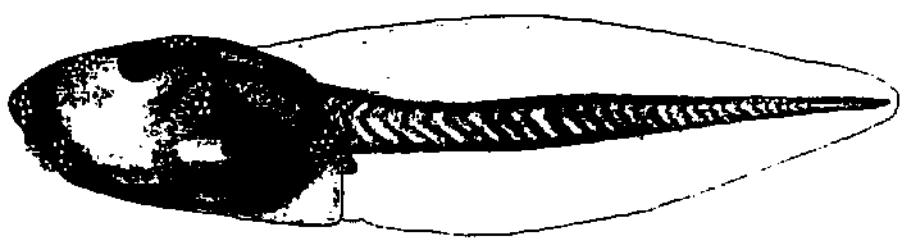


Figure 49. A *Cacosternum* tadpole

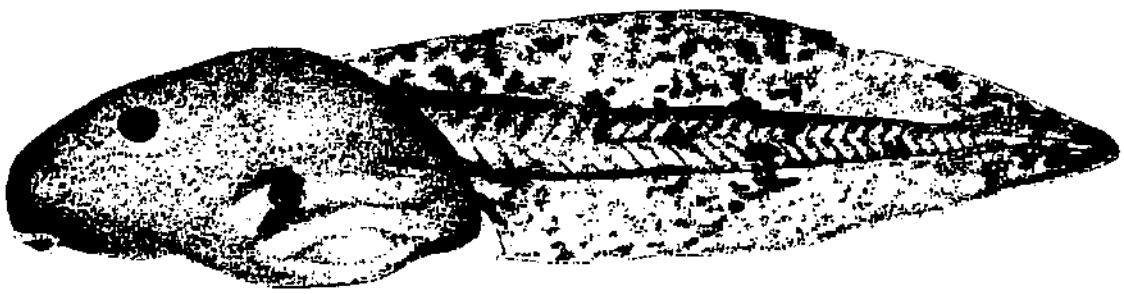


Figure 50. A *Strongylopus* tadpole

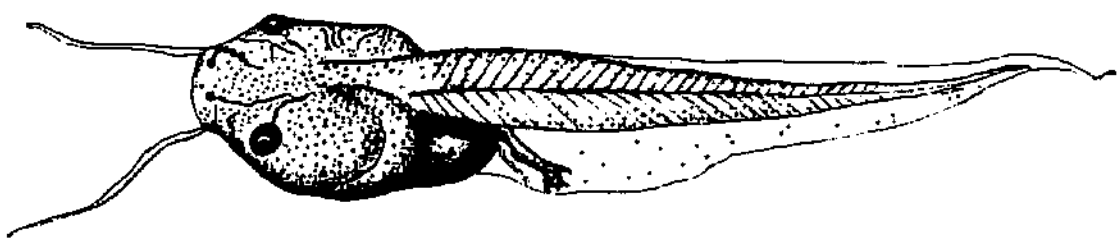


Figure 51. A *Xenopus* tadpole

IDENTIFICATION KEY TO STREAM DWELLING TADPOLES IN SOUTH AFRICA

1a Oral papillae present, and usually also teeth and beaks (fig 36) 2

1b. No papillae, beaks or teeth ***Xenopus laevis***

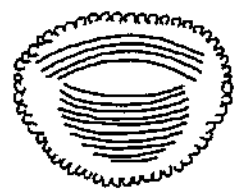


Fig 52

2a. Upper and lower papillae gaps absent, multiple rows of teeth present (fig 52) 9

2b. Upper gap in papillae present 3

Fig 53

3a. A broad lower gap in the oral papillae present, vent in the middle of the fin edge (fig 53) 5

3b. A lower gap absent, or very narrow if present. The vent is on the right side of the fin 4

Fig 54

4a One upper row of teeth, or upper teeth absent (fig 54) 12

4b Two or more rows of upper teeth 15

5a Tail more than twice as long as the body (fig 55) ***Capensibufo tradouwi***

5b Tail less than twice as long as body 6

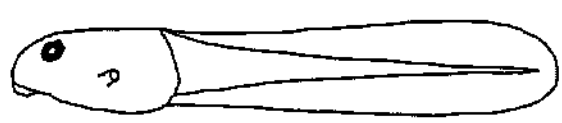


Fig 55

6a Tail nearly uniformly deep along its length 7

6b Tail with distinct convex curvature, below, and above 8

Fig 56

7a Tail countershaded, pale below (fig 56) ***Bufo maculatus***

7b Tail uniformly pigmented (fig 57) ***Bufo gariepensis***

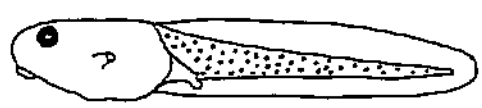


Fig 57

8a Pigmentation over tail muscles confined to upper 2/3 in front (fig 58) ***Bufo rangeri***

Fig 58

8b Pigmentation over tail muscles covers more than 3/4 in front (fig 59) ***Bufo gutturalis***

Fig 59

9a Found only on Table Mountain on the Cape Peninsula ***Heleophryne rosei***

9b Found on mountain ranges elsewhere 10

10a Known from Rode, through the KwaZulu-Natal midlands escarpment, and along the Drakensberg mountains and western Swaziland to north-eastern South Africa ***Heleophryne natalensis***

10b Restricted to the mountains of the western and southern Cape 11

11a Restricted to four rivers in the Elandsberg range, the Geelhoutboom river, Martins river, Klein river and Diepkloof river. ***Heleophryne hewitti***

11b Found east of Montagu along the Langeberg mountains to the Gouritz river valley. ***Heleophryne orientalis***

11c This species is known from the mountains of the southern, south-western, and western Cape, excluding Table mountain, ***Heleophryne purcelli***

11d This frog is known from the mountains east of the Gouritz river valley, from the Huis river in the west, to the Kareedouw mountains in the eastern Cape. ***Heleophryne regis***

12a One upper row of teeth (fig 60) 13
12b No upper rows of teeth 14

Fig 60

13a A dark tail tip present (fig 61)
Hyperolius marmoratus
13b No dark tail tip present
Hyperolius semidiscus

Fig 61

14a One row of lower teeth present (fig 62)
Afrixalus fornasinii
14b Lower tooth rows absent
Afrixalus spinifrons

Fig 62

15a Vent on the right; not continuous with the lower edge of the fin (fig 63) 24
15b Vent continuous with the lower edge of the fin (fig 64) 16

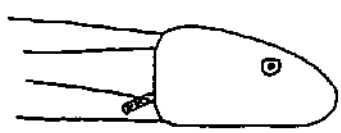


Fig 63

16a One (sometimes two) upper rows, and two lower rows of teeth 17
16b Not as above 18

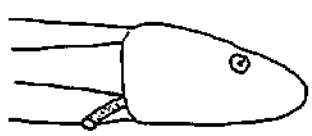


Fig 64

17a Numerous white-tipped tubercles covering top and sides of trunk (fig 65)
Poyntonia paludicola
17b Top and sides of trunk smooth
Phrynobatrachus natalensis

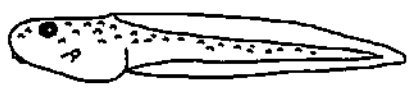


Fig 65

18a Three or more upper rows of teeth, and three or more lower rows of teeth 19
18b Two lower rows of teeth
Ptychadena mossambica

19a Lower beak deep and pigmented to its base (fig 66) 26
19b Lower beak pigmented along its edge only (fig 67) 20

Fig 66

Fig 67

20a Tail not as high as the body and not more than 5/3 the length of the head and trunk. A spur develops besides the fifth toe
Tomopterna **(all species)**

20b Tail as high or higher than body, and more than 5/3 the length of the head and trunk. (fig 68). No spur on foot 21

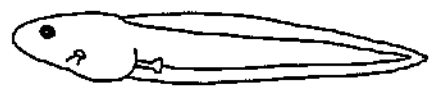


Fig 68

21a Elygium present in eye (Fig.69)
Rana dracomontana

21b No elygium present in eye 22

Fig 69

22a Four rows of lower teeth present
Rana vertebralis

22b Three rows of lower teeth 23

23a Dorsal fin arises steeply out of the body (fig 70) ***Rana fuscigula***

23b Dorsal fin forms a long curve from the body to the tip ***Rana angolensis***

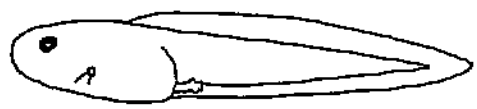


Fig 70

24a Distance between lateral edges of nostrils less than distance between inner margins of eyes (fig 71) 25

24b Distance between lateral edges of nostrils greater than distance between inner margins of eyes
Natalobatrachus bonebergi

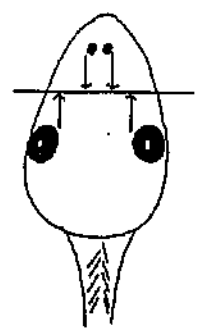


Fig 71

25a Four upper tooth rows
Cacosternum namaquense

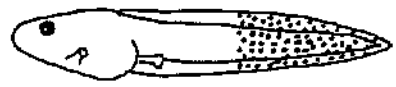
25b Three upper tooth rows
Cacosternum nanum

26a Tail height exceeds body height
Strongylopus fasciatus

26b Tail not higher than body 27

27a Eyes without elygium 29

27b Elygium present (fig 69) 28



72

28a The back fifth of the tail is dark (fig 72)
Strongylopus wageri

28b The tail is not darker towards the tip
Strongylopus hymenopus

29a Two undivided rows of lower teeth
Strongylopus springbokensis

29b Three undivided rows of lower teeth
Strongylopus grayii

5. SENSITIVITY TO PESTICIDES

5.1. FETAX procedure

The FETAX procedure (Frog Embryo Teratogenesis Assay - *Xenopus*) was developed in 1983 by Dr J. Dumont and his team at the Oak Ridge National Laboratory. It is useful for screening for the potential developmental toxicity of particular chemicals, or of environmental samples. Briefly, it consists of exposing early embryos of *Xenopus laevis* , the common platanna, to a dilution series of the test material for 96 hours. Three important comparative values can be obtained from the results. 1) The concentration at which 50% of the embryos fail to develop (LC_{50}), 2) the effective concentration at which 50% of the embryos show developmental abnormalities (EC_{50} malformation), and 3) the minimum concentration to inhibit growth (MCIG).

5.2. Test materials

5.2.1. Bacillus thuringiensis

5.2.1.1. Description and commercial use

This is a bacterium that produces an endotoxin used as an insecticide. The bacterium is ingested by the target insect, and the endotoxin damages the lining of the gut. The insects stop feeding and starve to death (Tomlin 1994). It is highly specific against insects, and used for example on stone fruit, vines, tomatoes and olives. Three different subspecies are used. It is used to control blackflies *Simulium* in the Gariep river.

5.2.1.2. Ecotoxicology

Although apparently harmless to man and other mammals, in water the known fish ecotoxicology LC_{50} (96 h) for guppies is >156, and for gobies >400 mg/l.

5.2.1.3. Environmental fate

Persistence is short, with a half life of the spores only 10 h, due to UV sensitivity. In soil, insecticidal activity has a half life of 20 to 500 d, depending on the bacterium's ability to obtain nutrients.

5.2.2. Chlorthalonil

5.2.2.1. Description and commercial use

This is a fungicide that is non-systemic. It is used to control fungal diseases in a range of crops, including stone fruit, strawberries, pawpaws, bananas and mangoes.

5.2.2.2. Ecotoxicology

Known fish ecotoxicology: LC_{50} (96 h) for rainbow trout 49, bluegill sunfish 62, channel catfish 44 ppb (mol wt 265.9)

5.2.2.3. Environmental fate

In aquatic soil the half life is up to a few days.

5.2.3. Deltamethrin

5.2.3.1. Description and commercial use

This is a pyrethroid insecticide. It is a non-systemic insecticide with contact and stomach action. It is used on almost all crops against a range of insects.

5.2.3.2. Ecotoxicology

Known fish ecotoxicology: LC_{50} (96 h) for rainbow trout 0.91, and bluegill sunfish 1.4 mg/l. It is reported not to be toxic to fish under natural conditions. It is considered not to represent a hazard to aquatic fauna in normal field use (Tomlin 1994).

5.2.3.3. Environmental fate

This compound adsops strongly onto soil, and does not leach into water. It is degraded by microbial action. The half-life under field conditions is less than 23 d.

5.2.4. Dichlorvos

5.2.4.1. Description and commercial use

Classified as organophosphorus, this is an acaricide and insecticide. It is a cholinesterase inhibitor with a rapid knockdown. For this reason it is widely used on a range of crops to control for example flies, mosquitoes and spider mites.

5.2.4.2. Ecotoxicology

Known fish ecotoxicology: LC_{50} (96 h) for rainbow trout 930, golden orfe 450 mg/l.

5.2.4.3. Environmental fate

Reported as non-persistent, with rapid decomposition to phosphoric acid and CO_2. The half life in biologically active water systems is less than 1 d.

5.2.5. Fenthion

5.2.5.1. Description and commercial use

This is an organophosphorus insecticide. It has contact, stomach and respiratory action. It is used on a very wide range of crops to control, for example, fruit flies, leafhoppers and cereal bugs, and also in public health situations to control insect pests likecockroaches and mosquitoes.

5.2.5.2. Ecotoxicology

The LC_{50} (96 h) for bluegill sunfish is 1.6, rainbow trout 0.87, and for golden orfe 2.7 mg/l.

5.2.5.3. Environmental fate

The half life is only 1 d. However, the major aerobic metabolites are fenthion sulfoxide and fenthion sulphone, both of which also have insecticidal properties.

5.2.6. Imidacloprid

5.2.6.1. Description and commercial use

This is a systemic insecticide, that acts on the central nervous system, to cause irreversible blockage of acetylcholine receptors. It is used on soil, to treat seeds, and sprayed on leaves. It is applied on cereals, maize, potatoes, citrus and stone fruit.

5.2.6.2. Ecotoxicology

Known fish ecotoxicology: LC_{50} (96 h) for golden orfe 237, rainbow trout 211 mg/l.

5.2.6.3. Environmental fate

No half life has been determined, partly due to the complex chemistry involved in degradation.

5.2.7. Isazofos

5.2.7.1. Description and commercial use

This is an organophosphorus, used as a nematicide and insecticide. It is a known cholinesterase inhibitor. The product is applied to the soil, and is used for example, to protect citrus, maize, vegetables and sugarcane.

5.2.7.2. Ecotoxicology

Known fish ecotoxicology: bluegill sunfish LC_{50} (96 h) 0.01, carp 0.22, trout 0.008-0.019 mg/l.

5.2.7.3. Environmental fate

It is not known to accumulate in mammals. The half life in water is 10 d.

5.2.8. Mancozeb

5.2.8.1. Description and commercial use

This fungicide is classified as a alkylenebis(dithiocarbamate). It is used to control many fungal diseases in a range of field crops, by spraying or by seed treatment.

5.2.8.2. Ecotoxicology

Known fish ecotoxicology: LC_{50} (48 h) for goldfish 9.0, rainbow trout 2.2, catfish 5.2, carp 4.0 mg/l.

5.2.8.3. Environmental fate

It degrades by hydrolysis, oxidation and photolysis. The half life in soil is 6-15 d.

5.2.9. Simazine

5.2.9.1. Description and commercial use

Classified as 1.3.5-triazine, this herbicide is closely related to atrazine. It acts by accumulating in the apical meristems and leaves, where it is an inhibitor of the photosynthetic electron transport system. It is used on many crops to control annual grasses and broad-leaved weeds.

5.2.9.2. Ecotoxicology

Known fish ecotoxicology: LC_{50} (96 h) for bluegill sunfish 90, rainbow trout >100, crucian carp >100, guppies 49 mg/l.

5.2.9.3. Environmental fate

This compound has a low solubility in water. The half life in soil is 70-110 d.

5.3. Methods

5.3.1. FETAX solution

All experimental dilutions and controls were based on FETAX solution: This is made up by adding the following per litre of deionized water: (ASTM 1991)

625 mg NaCl
96 mg $NaHCO_3$
30 mg KCl
15 mg $CaCl_2$
60 mg $CaSO_4.2H_2O$
75 mg $MgSO_4$

The final pH should be between 7.6 and 7.9.

5.3.2. Stock solutions

Stock solutions of all test materials were made up to standard spraying concentrations. This is based on 500 l of water used to carry the recommended dosage per Ha. The test materials, dosage per Ha, and equivalent amount per 50 ml stock is presented in Table 4.

Table 4. Experimental stock solutions

Test material	Dosage per Ha (of active ingredient)	Amount per 50 ml
Bacillus thuringiensis	750 g	75 mg
Chlorthalonil	2 l	200 µl
Deltamethrin	100 ml	10 µl
Dichlorovos	500 ml	50 µl
Fenthion	500 ml	50 µl
Imidacloprid	200 ml	20 µl
Isazophos	2 l	200 µl
Mancozeb	750 g	75 mg
Simazine	5 l	5 ml (*See note)

*Simazine was adjusted to a dosage of 50 l per Ha, as it showed no effects at lower dosages.

5.3.3. Test animals

Breeding pairs were wild-caught before each experiment, and released afterwards. The male and female were allowed to acclimate for 24-48 h in individual containers.

The animals were brought to reproductive readiness by primer injections of HCG (Human Chorionic Gonadotropin), trade name Pregnyl. The male was given 150 iu, and the female 250 iu. After 48 h the pair were given booster injections and placed together in a dark container. The booster injections were 200 iu for the male, and 300 iu for the female.

The frogs lay eggs in small groups, and attach these to the side and bottom of the container. The eggs are harvested 10 hours after the pair are placed together

5.3.4. Procedure

This is an abbreviated description of the modified FETAX procedure used. The complete description is available in ASTM: E 1439 "Standard Guide for Conducting the Frog Embryo Teratogenesis Assay - Xenopus (FETAX). For each test material a stock solution was made up that is equivalent to a typical spray application, based on 500 l of solution per Ha. The stock concentrations were determined by calculation, not by analysis. A series of 20 ml, 1:2 serial dilutions were prepared in FETAX solution, plus two controls. 10 eggs were placed in each glass dish. The starting eggs were between stages 8 and 11.

The experiment was allowed to run for 96 h at 24°C, or until the controls had reached stage 46 if the temperature varied slightly. The experiment was replicated. After 96 h, the embryos were scored for survival before being fixed in formalin. The embryos were then measured, and checked for malformations.

5.4. Results

Bantle et al (1991) in their "Atlas of Abnormalities" illustrate the kinds of malformations that can be expected in embryos of *Xenopus laevis* . Mortality data are typically analysed using either the probit or Spearman-Karber methods. The data generated in this study did not fit the assumptions or data requirements of probit analysis. LC_{50}, and the 95% confidence limits, were determined

using the parametric Spearman-Karber method (Hamilton et al 1977). EC_{50} is the effective concentration at which development of tadpoles is affected. TI is the teratogenic index where TI = LC_{50}/EC_{50}. It is a measure of the developmental influence of the tested compound, in the absence of obvious lethal effects

The LC_{50} values and their confidence limitsas well as EC_{50}, and TI are summarized in Table 5.

Table 5. Summary of tadpole toxicity measures

Test material	LC_{50} (mg/l)	Lower 95% (mg/l)	Upper 95% (mg/l)	EC_{50} (mg/l)	TI
Bacillus thuringiensis	163.2	126.0	211.3	0.02	8160
Chlorthalonil	0.09	0.07	0.1	0.02	4.5
Deltamethrin	0.19	0.16	0.24	0.006	31
Dichlorovos	39.4	33.9	45.7	0.5	78
Fenthion	2.61	1.86	3.68	0.002	1305
Imidacloprid	17.4	14.6	20.6	10.0	1.74
Isazophos	724	467	1122	0.25	2896
Mancozeb	3.08	2.47	3.84	0.03	102

5.4.1. Bacillus thuringiensis

5.4.1.1. Malformations

Some typical developmental abnormalities are illustrated in fig. 73.

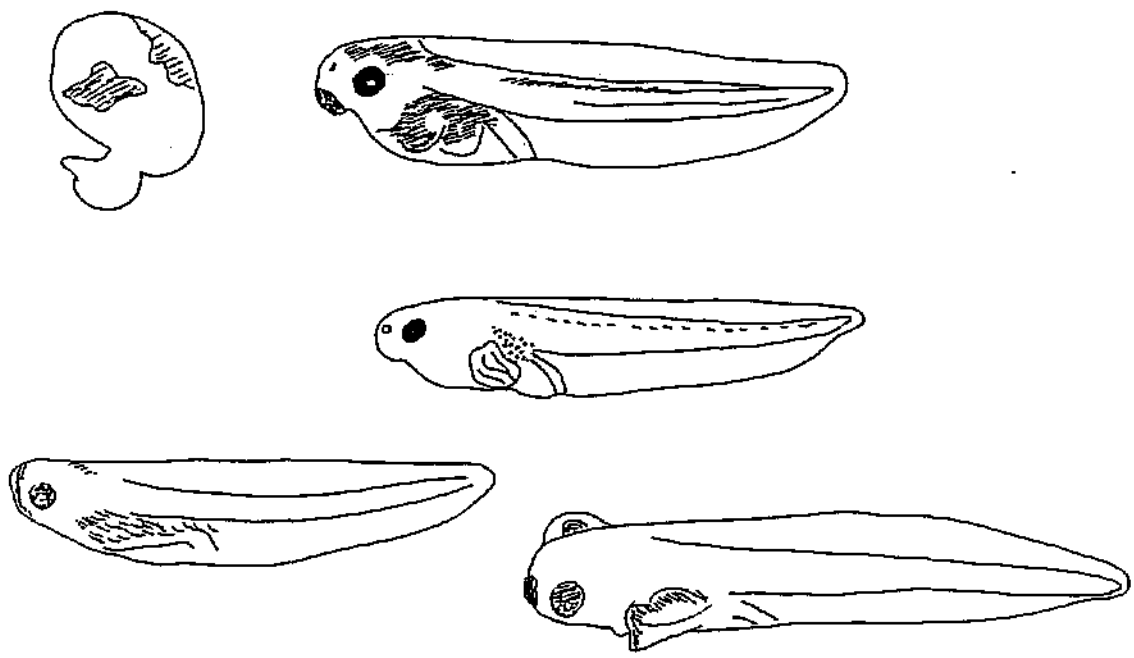


Figure 73. Illustrations of some abnormal development caused by *Bacillus thuringiensis*. The sketches are not all to the same scale. A control is in the centre. Typical abnormalities include a failure of the eggs to develop, abnormal eyes, and tail bent dorsally.

5.4.2. Chlorthalonil

5.4.2.1. Malformations

Some typical developmental abnormalities are illustrated in fig 74.

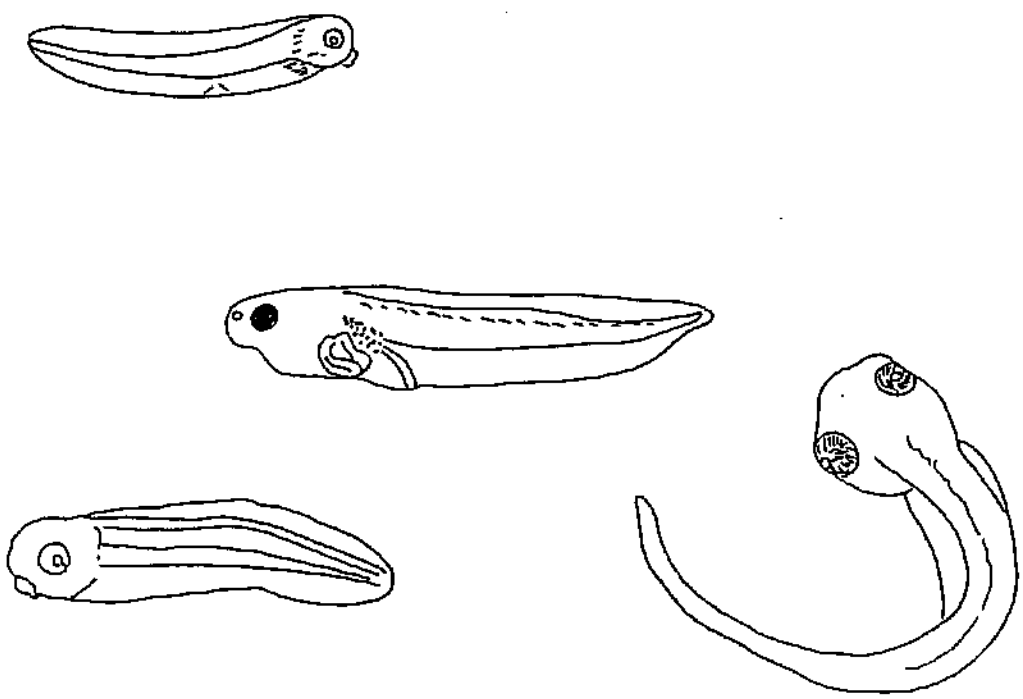


Figure 74. Some abnormalities caused by chlorthalonil. Sketches not to scale. A control tadpole is in the centre. Typical abnormalities include the tail bent up or down, and severe sideways twisting of the body.

5.4.3. Deltamethrin

5.4.3.1. Malformations

Some abnormalities associated with tadpole development in deltamethrin are illustrated in fig 75.

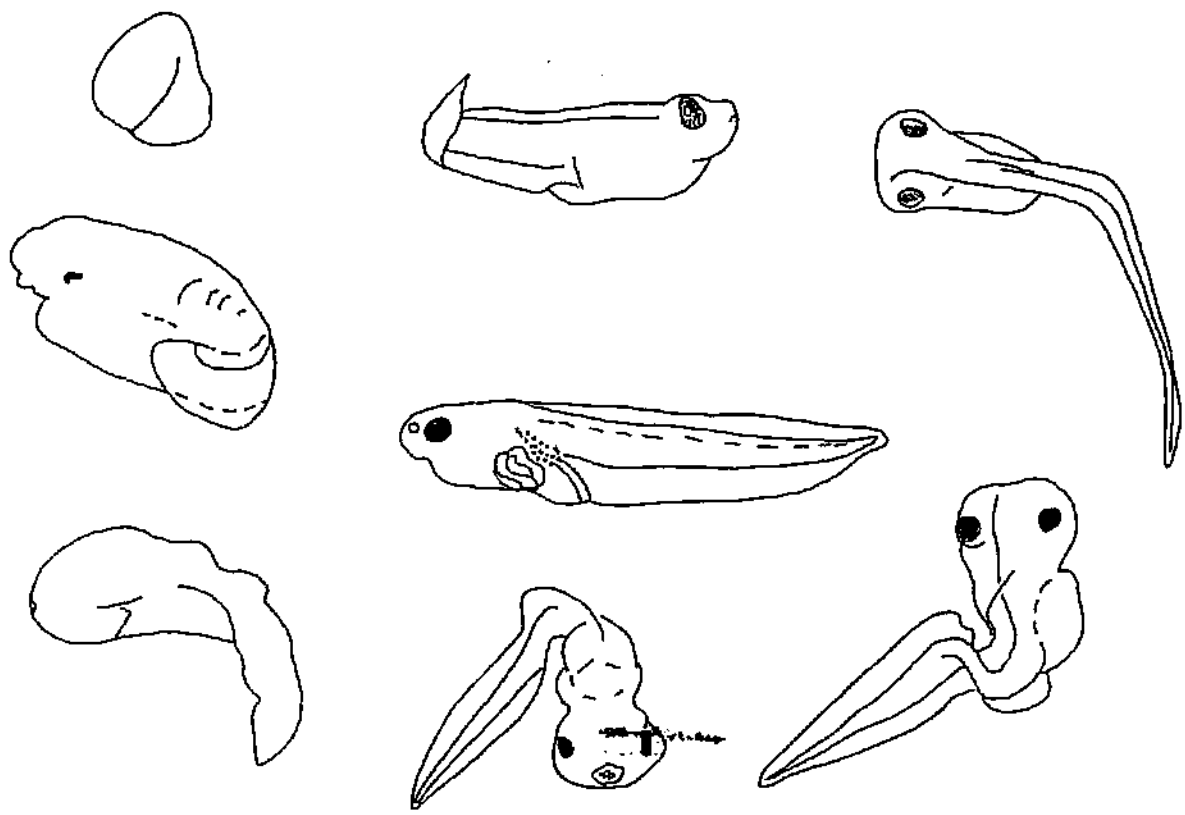


Figure 75. Some abnormalities associated with deltamethrin. The sketches are not to scale. A control tadpole is in the centre. Typical abnormalities include bent or twisted tails, and a failure of the early embryo to develop.

5.4.4. Dichlorvos

5.4.4.1. Malformations

Some abnormalities associated with tadpole development in dichlorovos are illustrated in fig 76.

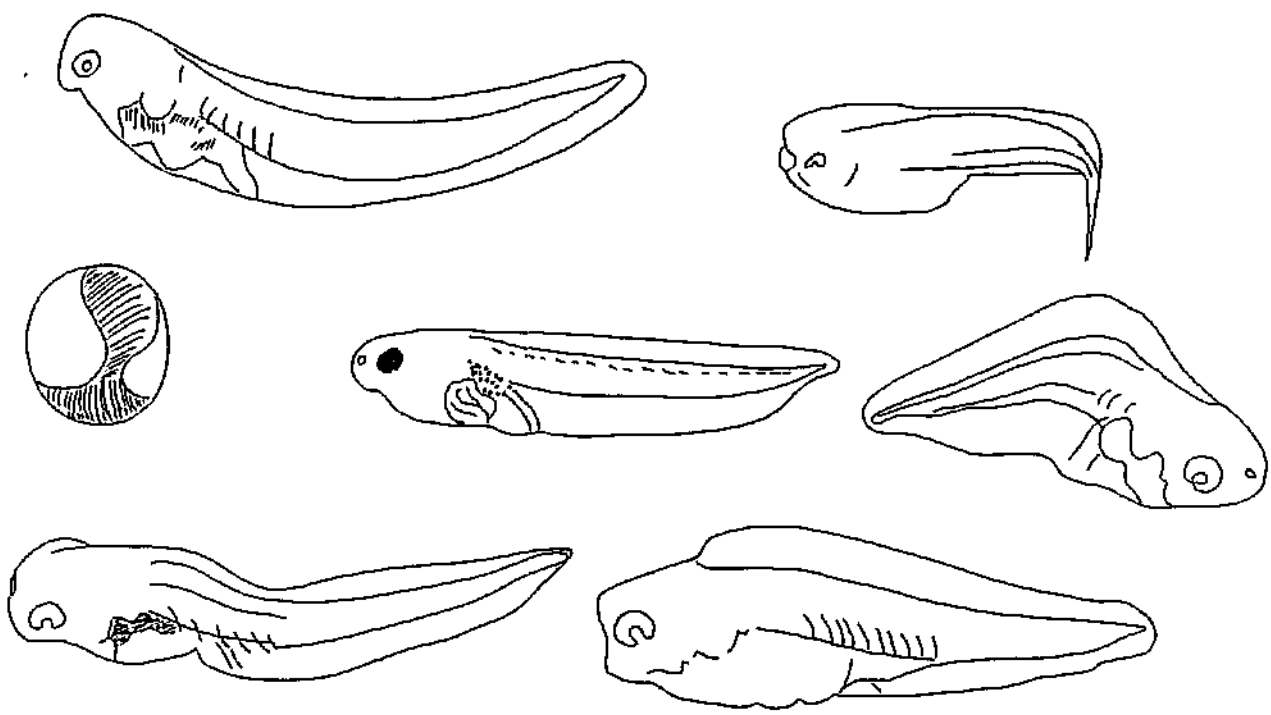


Figure 76. Some abnormalities associated with tadple development in dichlorovos. Sketches not to scale. A control tadpole is in the centre. Typical malformations include bent tails, edema, partial eye development and head shape.

5.4.5. Fenthion

5.4.5.1. Malformations

Some typical developmental abnormalities are illustrated in fig 77.

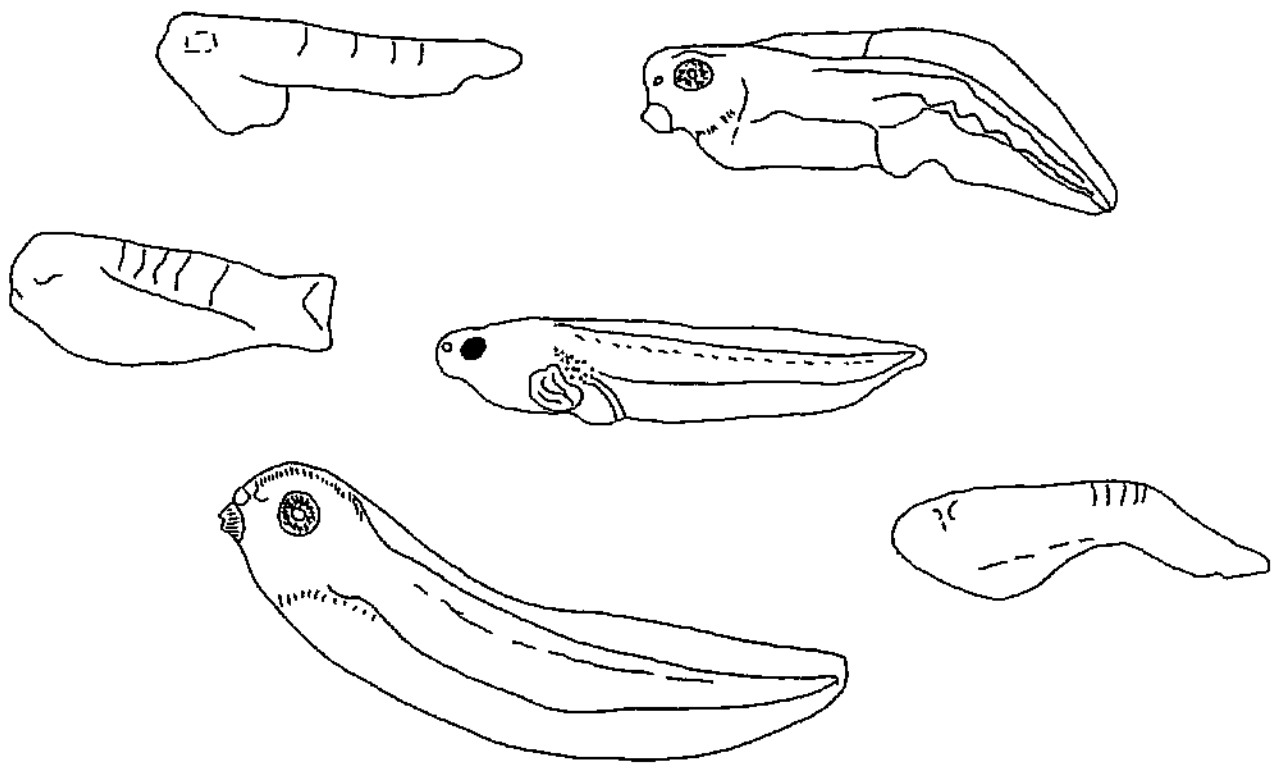


Figure 77. Some developmental abnormalities of *Xenopus* tadpoles associated with fenthion. The skeches are not to scale. A control tadpole is in the centre. Typical malformations include the failure of the head to develop, corrugated tails, and arched tails.

5.4.6. Imidacloprid

5.4.6.1. Malformations

Some developmental abnormalities associated with imidacloprid are illustrated in fig 78.

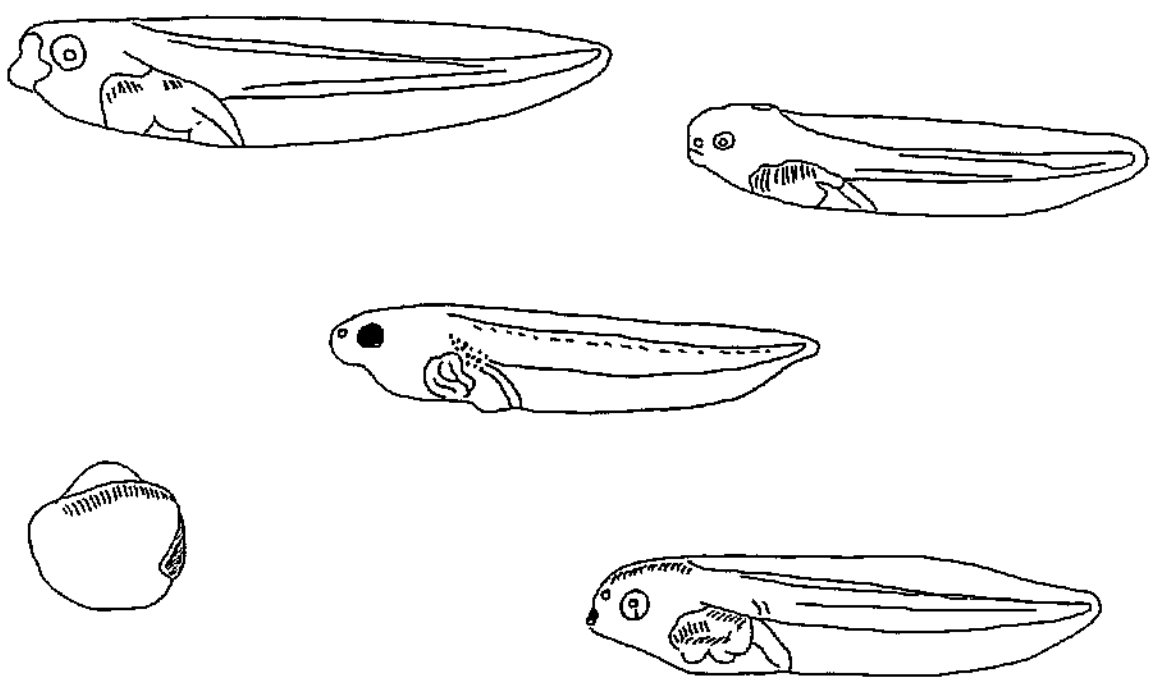


Figure 78. Some developmental abnormalities associated with imidacloprid. The sketches are not to scale. A control tadpole is in the centre. Typical malformations include inhibited egg development, a failure of the mouth to develop, and lack of pigment in the eye.

5.4.7. Isazofos

5.4.7.1. Malformations

Some developmental abnormalities associated with isazophos are illustrated in fig 79.

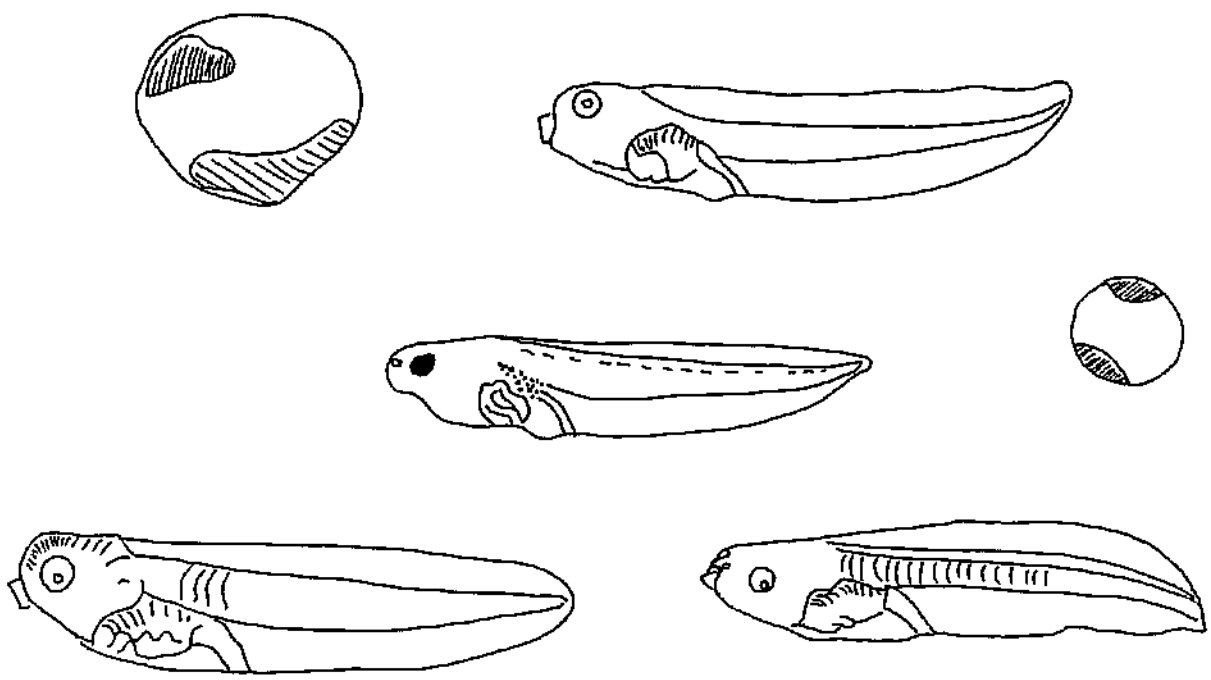


Figure 79: Some abnormalities associated with isazophos. The sketches are not to scale. A control tadpole is in the centre. Typical abnormalities include lack of eye pigment, tail bent up or down, and mis-shapen head.

5.4.8. Mancozeb

5.4.8.1. Malformations

Some malformations associated with tadpole development in mancozeb are illustrated in fig 80.

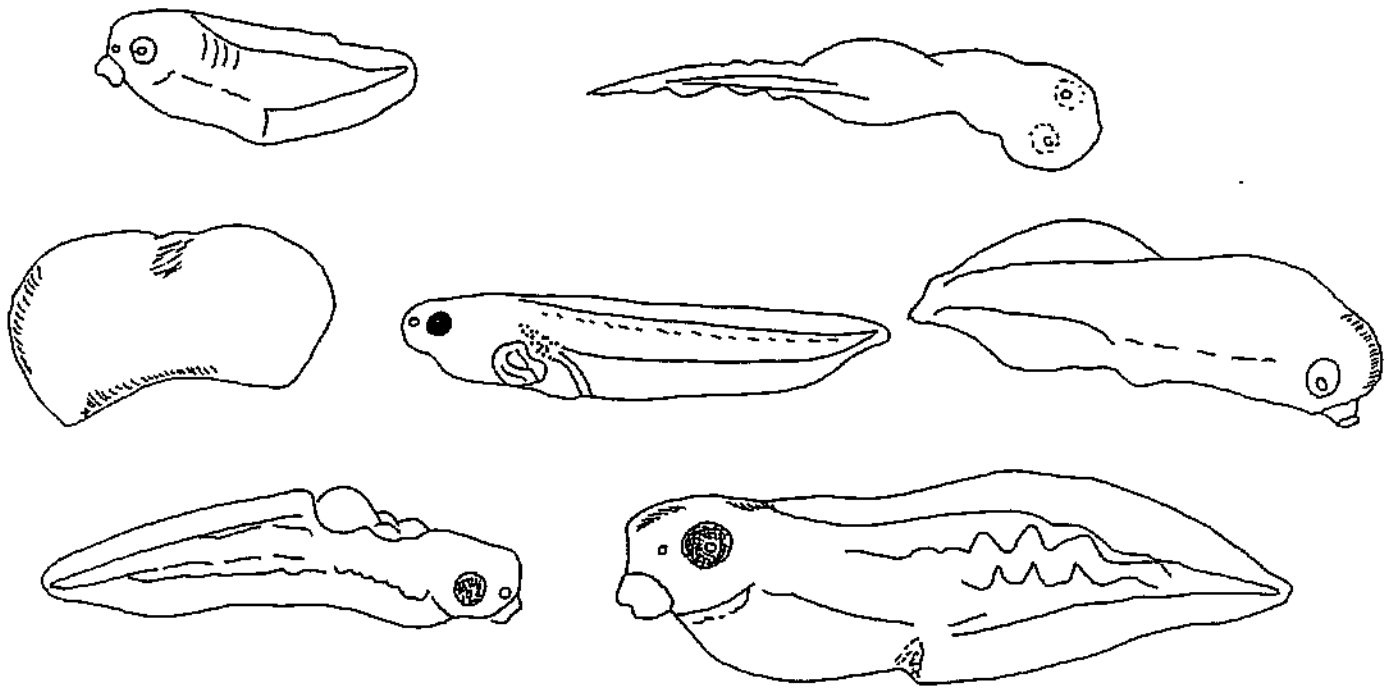


Figure 80. Illustrations of some developmental abnormalities associated with mancozeb. The sketches are not to scale. A control tadpole is in the centre. Typical malformations include edema, lack of tail development, corrugated tail, and a gap in the vertebral column.

5.4.9. Simazine

The herbicide simazine was found to only have a slight effect on tadpoles, when tested at a concentration 10 times more than normal applications. For the purposes of this base-line study, I regard simazine as harmless to *Xenopus* tadpoles.

6. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1. Teratogenic Index

TI = LC50/EC50. This is a measure of developmental hazard. Values higher than 1.5 signify a greater potential for embryos to be malformed in the absence of significant embryo mortality. The values for TI in this study range from 1.74 (moderately hazardous) to 2896 (extremely hazardous).

Tadpole sensitivity values are not included in the information supplied with these pesticides, but this study shows that tadpoles are very sensitive.

6.2. Comparison with literature values for tadpoles

Table 6 summarizes the measured concentrations at which tadpoles show sensitivity (see literature review for sources).

Table 6. Summary of literature LC_{50} values

Pesticide	Taxon	LC_{50} (mg/l)
Carbamate	Xenopus	0.02-0.14 (9 weeks)
Fenthion	Xenopus	8 sensitive
Fenthion	Xenopus	2.5 paralysis
Triclopyr	Xenopus	2.4 sensitive
Malathion	Bufo	47.3 slow develop.
Malathion	Bufo	0.47 no effect
Esfenvalerate	Xenopus	LC_{50} mg/l
Esfenvalerate	Xenopus	1.3 low activity
Dieldrin	Xenopus	LC_{50} 40.4-49.5
Dieldrin	Rana catesbeiana	LC_{50} 8.7-30.3
Dieldrin	Rana pipiens	LC_{50} 71.3
Guthion	Xenopus	LC_{50} 2.94
Nitrite	Xenopus	LC_{50} 13.9-39.3

6.3. Realization of objectives

The objectives of the research were:

1) To determine the diversity of tadpoles in the major catchments in South Africa.

2) To provide a user-friendly method of identification of tadpoles.

3) To place tadpole collections in a major museum, from which working reference collections can be drawn.

5) To determine the sensitivities of certain tadpoles to pollutants like heavy metals and agricultural chemicals.

These objectives have all been met, except that the laboratory study was confined to agricultural pesticides, as heavy metals have been examined in other countries, and those results are applicable here.

6.4. Contribution to the field

This study has demonstrated that it is both feasable and possible to use *Xenopus* tadpoles in laboratory studies of the effects of pesticides. The tadpoles are available during the sason when water is flowing into streams and rivers from agricultural land. Monitoring can be done either using natural populations of tadpoles, or by introducing free-living or captive populations into target drainages. Similar studies have been successfully carried out in ponds in fruit-growing areas of eastern Canada (Harris, pers com.)

Besides the immediate effect observed when tapoles are killed or prevented from developing, there is the potential for genetic damage, either directly to the DNA, or through serious reduction in the genetic variation of a population, by pollution from agrichemicals.

6.5. Future research

It is suggested that this research area has shown the potential for low-tech monitoring. The next phase should include a study over a year or more of caged tadpoles in a target river, in order to determine if the "natural" flow of pesticides can be detected. This could be combined with further FETAX studies of the pesticides actually in use in the area.

The broad survey of tadpole availability completed in the present study, should be extended to detailed studies of a few target drainages, to cover both the species and seasonality, and also the overlap of species spatially and temporally.

It is important that pairs of chemicals that might occur in drainages at the same time be tested for synergistic activity. The carriers of herbicides need to be tested as well, as there is clear evidence that these may be more harmful to animals than the active compounds.

6.6. Recommendations

The work reported on here, and the recommendations that follow, should be of interest to the scientific community, water management auhorities, the Department of Environmental Affairs, the agri-industry and small farmers.

6.6.1 Recommendation 1.

That tadpoles be recognized as important, endangered and useful components of drainages, and that further research be supported.

6.6.2 Recommendation 2

That the present study be followed up by a long term experiment using caged tadpoles in a drainage with significant potential for agricultural pollution.

6.6.3 Recommendation 3

That the tadpole key and drawings be made available to others wishing to make use of this approach.

7. REFERENCES

Abd-Allah A.M.A. Residue levels of organochlorine pollutants in fish from Abu-Quir bay and Idku lake, Alexandria, Egypt. Toxicological & Environmental Chemistry. 4465-71; 1994.

Alcaraz G.; Espina S. Effect of nitrite on the survival of grass carp Ctenopharyngodon idella (Val.), with relation to chloride. Bull. Environ. Contam. Toxicol. 5274-79; 1994.

Alcaraz, G.; Espina, S. Acute toxicity of nitrite in juvenile grass carp modified by weight and temperature. Bulletin of Environmental Contamination and Toxicology. 1995 Sep; 55(3): 473-478.

Altig R.; Christensen M.T. A bibliography of tadpole biology. Smith Herp Infor Ser. 311-33; 1976.

Altig R.; Pace W.L. Scanning electron photomicrograph of tadpole labial teeth. J.Herpetol. 8247-251; 1974.

Alvarez R.; Honrubia M.P.; Herraez M.P. Skeletal malformations induced by the insecticides ZZ-aphox and Folidol during larval development of Rana perezi. Arch. Environ. Contamin. Toxicol. 28: 349-356; 1995.

Amiet, J.-L. & A. Schiotz. Voix d'Amphibiens camerounais. III Hyperoliinae: genres Leptopelis. Ann. Fac. Sci. Cameroun. 1974: 131-163.

Anderson, R.-J., & K. V. Prahlad. Synergistic effects of herbicide and fungicide on Xenopus laevis embryos. Amer. Zool. 15: 791. 1976.

Anderson T.A.; Coats J.R. Screening rhizosphere soil samples for the ability to mineralize elevated concentrations of atrazine and metochlor. J. Environ. Sci.Health. B30(4): 473-484; 1995.

Arnold S.M.; Hickey W.J.; Harris R.F. Degradation of atrazine by Fenton's reagent: condition optimization and product quantification. Environm. Sci. & Technology. 29(8): 2083-2089; 1995.

Assmuth, T.; Penttila, S. Characteristics, Determinants and Interpretations of Acute Lethality in Daphnids Exposed to

Complex Waste Leachates. Aquatic Toxicology. 1995 Feb; 31(2): 125-141.

ASTM (American Society for Testing and Materials). E 1439-91. Standard Guide for Conducting the Frog Embryo Teratogenesis Assay - Xenopus (Fetax). 1991.

Bantle J.A.; Dumont J.N.; Finch R.A.; Linder G. Atlas of abnormalities. A guide for the performance of FETAX. 1-68;

Barriuso, E.; Calvet, R.; Houot, S. Field study of the effect of sewage sludge application on atrazine behaviour in soil. International Journal of Environmental Analytical Chemistry. 1995; 59(2-4): 107-121.

Beck A.J.; Lam V.; Henderson D.E.; Beven K.J.; Harris G.L.; Howse K.R.; Johnston A.E.; Jones K.C. Movement of water and the herbicides atrazine and isoproturon through a large structured clay soil core. J. Contaminant Hydrology. 19237-260; 1995.

Bengtson, R. L.; Carter, C. E.; Fouss, J. L.; Southwick, L. M.; Willis, G. H. Agricultural drainage and water quality in Mississippi delta. Journal of Irrigation and Drainage Engineering - ASCE. 1995 Jul; 121(4): 292-295.

Bernadini G.; Bolzacchini E.; Orlandi M.; Presutti C.; Rindone B. The reactivity and fate of a phenoxyacetic herbicide: 4-chloro-2methylphenoxyacetic acid (MCPA) in the environment. Wat. Sci. Tech. 32(9-10): 205-209; 1995.

Bernadini G.; Spinelli O.; Presutti C.; Vismara C.; Bolzacchini E.; Orlandi M.; Settimi R. Evaluation of the developmental toxicity of the pesticide MCPA and its contaminants phenol and chlorocresol. Enivronm. Toxicol. & Chem. 15(5): 754-760; 1996.

Berrill, M.; Bertram, S.; Pauli, B.; Coulson, D.; Kolohon, M.; Ostrander, D. Comparative sensitivity of amphibian tadpoles to single and pulsed exposures of the forest-use insecticide fenitrothion. Environmental Toxicology and Chemistry. 1995 Jun; 14(6): 1011-1018.

Berrill, M.; Bertram, S.; Mcgillivray, L.; Kolohon, M.; Pauli, B. Effects of Low Concentrations of Forest-Use Pesticides on Frog Embryos and Tadpoles. Environmental Toxicology and Chemistry. 1994 Apr; 13(4): 657-664.

Bert, T. M.; Arnold, W. S. An empirical test of predictions of two competing models for the maintenance and fate of hybrid zones: Both models are supported in a hard-clam hybrid zone. Evolution. 1995 Apr; 49(2): 276-289.

Bester, K.; Huhnerfuss, H.; Brockmann, U.; Rick, H. J. Biological effects of triazine herbicide contamination on marine phytoplankton. Archives of Environmental Contamination and Toxicology. 1995 Oct; 29(3): 277-283.

Bidwell J.R.; Gorrie J.R. Acute toxicity of a herbicide to selected frog species. Report for Western Australian Dept. of Environmental Protection. (10pp): 1995.

Blandin D.A.; Ramsdell H.S. Developmental delay and inhibition of metamorphosis caused by atrazine in tadpoles. Center for Environmental Toxicology and Technology Dept Environm. Health, Colorado State University. 1995.

Blaustein L.; Margalit J. Priority effects in temporary pools: nature and outcome of mosquito larva-toad tadpole interactions depend on order of entrance. J. Anim. Ecol. 6577-84; 1996.

Bleckmann, C. A.; Rabe, B.; Edgmon, S. J.; Fillingame, D. Aquatic toxicity variability for fresh- and saltwater species in refinery wastewater effluent. Environmental Toxicology and Chemistry. 1995 Jul; 14(7): 1219-1223.

Bleecker M.; DeGloria S.D.; HutsonJ.L.; Bryant R.B.; Wagenet R.J. Mapping atrazine leaching potential with integrated environmental databases and simulation models. J. Soil & Water Conservation. 50(4): 388-394; 1995.

Bourgine, F. P.; Chapman, J. I.; Kerai, H.; Duval, J. L.; Green, J. G.; Hamilton, D. The degradation of atrazine and other pesticides by photolysis. Journal of the Chartered Institution of Water and Environmental Management. 1995 Aug; 9(4): 417-423.

Boyer, R.; Grue, C. E. The need for water quality criteria for frogs. Environmental Health Perspectives. 1995 Apr; 103(4): 352-357.

Bunn, S. E.. Biological monitoring of water quality in Australia: Workshop summary and future directions. Australian Journal of Ecology. 1995 Mar; 20(1): 220-227.

Burbank S.E.; Snell T. Rapid toxicity assessment using esterase biomarkers in Brachionus calcyflorus (Rotifera). Environmental Toxicology & Water Quality. 9171-178; 1994.

Calamari, D.; Tremolada, P.; Notarianni, V. Relationships between chlorinated hydrocarbons in vegetation and socioeconomic indices on a global scale. Environmental Science & Technology. 1995 Sep; 29(9): 2267-2272;.

Camargo, J. A.; Lapoint, T. W. Fluoride toxicity to aquatic life: A proposal of safe concentrations for five species of palearctic freshwater invertebrates. Archives of Environmental Contamination and Toxicology. 1995 Aug; 29(2): 159-163.

Carey, C.; Bryant, C. J. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environmental Health Perspectives. 1995 May; 103(Suppl. 4): 13-17.

Channing, A. A new species of the genus Strongylopus Tschudi from Namaqualand, Cape Province, South Africa (Anura: Ranidae). Ann. Cape Prov. Mus. (nat Hist) 16: 127-135. 1986.

Channing, A. & R. C. Boycott. A new frog genus and species from the mountains of the southwestern Cape, South Africa (Anura: Ranidae). Copeia 1989: 467-471. 1989.

Chen, J. J.; Xia, Y. C. Induction of micronuclei in erythrocytes of frog tadpoles by city sewage and its application in environmental monitoring. Munawar, M.; Chang, P.; Dave, G.; Malley, D.; Munawar, S.; Xiu, R. Aquatic Ecosystems of China: Environmental and Toxicological Assessment. PO Box 97747/2509 GC the Hague/Netherlands: S P B Academic Publ; c1995: 95-101. (Ecovision World Monograph Series).

Chessman, B. C. Rapid assessment of rivers using macroinvertebrates: A procedure based on habitat-specific sampling, family level identification and a biotic index. Australian Journal of Ecology. 1995 Mar; 20(1): 122-129.

Chevreuil M.; Carru A.; Chesterikoff A.; Boet P.; Tales E.; Allardi J. Contamination of fish from different areas of the river Seine (France) by organic (PCB and pesticides) and metallic (Cd, Cr, Cu,

Fe, Mn, Pb and Zn) micropollutants. The science of the total environment. 16231-42; 1995.

Chung, K. H.; Ro, K. S.; Roy, D. Fate and enhancement of atrazine biotransformation in anaerobic wetland sediment. Water Research. 1996 Feb; 30(2): 341-346.

Cooke, A.S. Tadpoles as indicators of harmful levels of pollution in the field. Environ. Poll. (A) 25: 123-133. 1981.

Crick, H. Poisoned prey in the heart of Africa. New Scientist 24 Nov. 39-42.

Dawson, D. A. & T. S. Wilke. Evaluation of the Frog Embryo Teratogenesis Assay: Xenopus (FETAX) as a model system for mixture toxicity hazard assessment.

De Llamas M.C.; de Castro A.C.; de D'Angelo A.M.P. Cholinesterase activities in developing amphibian embryos following exposure to the insecticides dieldrin and malathion. Arch. Environ. Contam. Toxicol. 14(2): 161-166; 1985.

De Prado R.; Romera E.; Menendez J. Atrazine detoxification in Panicum dichotomifolium and target site Polygonum lapathifolium. Pesticide Biochem. & Physiol. 521-11; 1995.

De Souza M.L.; Wackett L.P.; Boundy-Mills K.L.; Mandelbaum R.T.; Sadowsky M.J. Cloning, characterization, and expression of a gene region from Pseudomonas sp. Strain ADP involved in the dechlorination of atrazine. Appl. Environ. Microbiol. 61(9): 3373-3378; 1995.

Desouza, M. L.; Wackett, L. P.; Boundymills, K. L.; Mandelbaum, R. T.; Sadowsky, M. J. Cloning, characterization, and expression of a gene region from Pseudomonas sp strain ADP involved in the dechlorination of atrazine. Applied and Environmental Microbiology. 1995 Sep; 61(9): 3373-3378.

Diamond S.A.; Oris J.T.; Guttman S.I. Adaptation to fluoranthene exposure in a laboratory population of flathead minnows. Environ. Toxicol. & Chem. 14(8): 1393-1400; 1995a.

Diamond S.A.; Oris J.T.; Guttman S.I. An inexpensive minnow egg incubation and toxicant exposure system. Environ. Toxicol. & Chem. 14(8): 1387-1388; 1995b.

Diamond, S. A.; Oris, J. T.; Guttman, S. I.. A system for conducting flow-through toxicity tests with larval fish. Environmental Toxicology and Chemistry. 1995c 14(8): 1389-1391.

Dirksen S.; Boudewijn T.J.; Slager L.K.; Mes R.G.; van Schaick M.J.M; de Voogt P. Reduced breeding success of cormorants (Phalacrocorax carbo sinesis) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. Environmental Pollution. 88119-132; 1995.

Dubois, A. Notes sur la classification des Ranidae (Amphibiens Anoures). Bull. mens. Soc. linn. Lyon. 61: 305-352. 1992.

Ensenbach, U.; Nagel, R.. Toxicity of complex chemical mixtures: Acute and long-term effects on different life stages of zebrafish (Brachydanio rerio). Ecotoxicology and Environmental Safety. 1995 Mar; 30(2): 151-157.

Entry J.A.; Donnelly P.K.; Emmingham W.H. Atrazine and 2,4-D mineralization in relation to microbial biomass in soils of young-, second- and old growth riparian forests. Applied Soil Ecology. 277-84; 1995.

Entry J.A.; Emmingham W.H. The influence of dairy manure on atrazine and 2,4- dichlorophenoxyacetic acid mineralization in pasture soils. Can. J. Soil Sci. 75379-383; 1995.

Erasmus A. List of wetland-related expertise in South Africa. Publ. Dept. Environment Affairs and Tourism. 1994.

Eronen L.; Julkunen R.; Saarelainen. MCPA residues in developing forest ecosystem after aerial spraying. Bull. Environm. Contam. Toxicol. 21791-798; 1979.

Fore, S. A.; Guttman, S. I.; Bailer, A. J.; Altfater, D. J.; Counts, B. V. Exploratory analysis of population genetic assessment as a water quality indicator .1. Pimephales notatus. Ecotoxicology and Environmental Safety. 1995 Feb; 30(1): 24-35.

Foster P; Ferrari C.; Turloni S. Environmental behaviour of herbicides. Atrazine volatalization study. Fresenius Envir Bull. 4256-261; 1995.

Frantsevich, L. I.; Pankov, I. V.; Ermakov, A. A.; Kornyushin, A. V.; Zakharchuk, T. N. Molluscs as indicators of environmental

pollution by radionuclides. Russian Journal of Ecology. 1995 Jan; 26(1): 47-52;.

Franzmann, P. D.; Dobson, S. J. The Phylogeny of Bacteria from a Modern Antarctic Refuge. Antarctic Science. 1993 Sep; 5(3): 267-270.

Freda J.; McDonald D.G. Toxicity of amphibian breeding ponds in the Sudbury region. Can. J. Fish Aquat. Sci. 501497-1503; 1993.

Ganzhorn J.U.; Paffrath D. Patterns in air pollution model for the physical basis for olfactory navigation in pigeon homing. J. f Ornithologie. 136159-165; 1995.

Gillespie, R. B.; Guttman, S. I. Correlations Between Water Quality and Frequencies of Allozyme Genotypes in Spotfin Shiner (Notropis spilopteris) Populations. Environmental Pollution. 1993; 81(2): 147-150.

Gish T.J.; Sadeghi A.; Wienhold B.J. Volatilization of alachlor and atrazine as influenced by surface litter. Chemosphere. 31(4): 2971-2982; 1995.

Gosner K.L. Systematic variations in tadpole teeth with notes on food. Herpetologica. 15203-210; 1959.

Graber, E. R.; Gerstl, Z.; Fischer, E.; Mingelgrin, U. Enhanced transport of atrazine under irrigation with effluent. Soil Science Society of America Journal. 1995 Nov; 59(6): 1513-1519.

Grimwood M.J.; Dobbs T.J. A review of the aquatic ecotoxicology of polychlorinated dibenzo-p-dioxins and dibenzofurans. Environmental Toxicology and Water Quality. 1057-75; 1995.

Gruessner B.; Watzin M.C. Response of aquatic communities fron a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. Environm. Toxicol. & Chem. 15(4): 410-419; 1996.

Gulve, P. S. Distribution and extinction patterns within a northern metapopulation of the pool frog, Rana lessonae. Ecology. 1994 Jul; 75(5): 1357-1367.

Gustavson K.; Wangberg S. Tolerence induction and succession in microalgae communities exposed to copper and atrazine. Aquatic Toxicology. 32283-302; 1995

Guttman S.I. Population genetic structure and ecotoxicology. Environmental Health Perspectives. 102(Supplement 12): 97-100; 1994.

Guzzella, L.; Mingazzini, M. Biological assaying of organic compounds in surface waters. Water Science and Technology. 1994; 30(10): 113-124.

Hall, L. W.; Ziegenfuss, M. C.; Anderson, R. D.; Tierney, D. P.; Spittler, T. D.; Lavin, L. The influence of salinity and sediment on the loss of atrazine from the water column. Chemosphere. 1995 Aug; 31(3): 2919-2944.

Hamilton, M. A., Russo, R. C. & Thurston, R. V. 1977. Trimmed Spearman-Karber methods for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11: 714-719. Correction 12: 417 (1978).

Hamilton, S. J.. Hazard assessment of inorganics to three endangered fish in the Green River, Utah. Ecotoxicology and Environmental Safety. 1995 Mar; 30(2): 134-142.

Hecnar, S. J. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. Environmental Toxicology and Chemistry. 1995 Dec; 14(12): 2131-2137.

Honrubia M.P.; Herraez M.P.; Alvarez R. The carbamate insecticide ZZ-aphox induced structural changes of gills, liver, gall-bladder, heart and notochord of Rana perezi tadpoles. Arch. Environ. Contam. Toxicol. 25184-191; 1993.

Horne M.T.; Dunson W.A. Effects of low pH, metals and water hardness on larval amphibians. Arch. Envion. Contam. Toxicol. 29500-505; 1995.

Hose, J. E.; Guillette, L. J. Defining the role of pollutants in the disruption of reproduction in wildlife. Environmental Health Perspectives. 1995 May; 103(Suppl. 4): 87-91.

Hugueny, B., S. Camara, B. Samoura & M. Magassouba. Applying an index of biotic integrity based on fish assemblages in a West African river. Hydrobiologia 331: 71-78. 1996.

Hylander, L. D.; Silva, E. C.; Oliveira, L. J.; Silva, S. A.; Kuntze, E. K.; Silva, D. X. Mercury levels in Alto Pantanal: A screening study. Ambio. 1994 Dec; 23(8): 478-484.

Janssen M.P.M.; Oosterhoff C.; Heijmans G.J.S.M.; Van der Voet H. The toxicity of metal salts and the population growth of the ciliate Colpoda cucculus. Bull. Environ. Contam. Toxicol. 54597-605; 1995.

Juttner I.; Peither A.; Lay J.P.; Ormerod S.J. An outdoor mesocosm study to assess ecotoxicological effects of atrazine on a natural plankton community. Arch. Environ. Contam. Toxicol. 29435-441; 1995.

Kao, L. M.; Wilkinson, C. F.; Brattsten, L. B. In-vivo effects of 2,4-D and atrazine on cytochrome P-450 and insecticide toxicity in southern armyworm (Spodoptera eridania) larvae. Pesticide Science. 1995 Dec; 45(4): 331-334.

Kerley G.I.H.; Knight M.H.; De kock M. Desertification of subtropical thicket in the astern Cape, South Africa: are there alternatives? Environ. Monit. & Asess. 37211-230; 1995.

Kolpin, D. W.; Thurman, E. M.; Goolsby, D. A. Occurrence of selected pesticides and their metabolites in near-surface aquifers of the midwestern United States. Environmental Science & Technology. 1996 Jan; 30(1): 335-340.

Kreutzweiser, D. P.; Capell, S. S.; Sousa, B. C. Hexazinone effects on stream periphyton and invertebrate communities. Environmental Toxicology and Chemistry. 1995 Sep; 14(9): 1521-1527.

Lambiris A.J.L. Description of the tadpole of Strongylopus hymenopus (Boulenger, 1920) (Amphibia: Anura: Ranidae) and a key to described southern African tadpoles of the genus. Ann.Natal Mua. 28(2): 455-462; 1987.

Lambiris, A. J. L. A review of the amphibians of Natal. Lammergeyer 39: 1-210.

Laroche, A. M.; Gallichand, J. Analysis of pesticide residues in surface and groundwater of a small watershed. Transactions of the ASAE. 1995 Nov; 38(6): 1731-1736.

Levy, J.; Chesters, G.. Simulation of atrazine and metabolite transport and fate in a sandy-till aquifer. Journal of Contaminant Hydrology. 1995 Nov; 20(1-2): 67-88.

Linder, G., Barbitta, J. & T. Kwaiser. Short-term amphibian toxicity tests and paraquat toxicity assessment. In. W. G. Landis & W. H.

van der Schalie (eds) ASTM STP 1096. Aquat. Tox. Risk Assess. 13: 189-198.

Lipnick R.L.; Cotruvo J.A.; Hill R.N.; Bruce R.D.; Stitzel K.A.Walker A.P.; Chu I.; Goddard M.; Segal L.; Springer J.A.; Myers R.C. Comparison of the up-and-down conventional LD50 and fixed-dose acute toxicity procedures. ed. Chem. Toxic. 33(3): 223-231; 1995.

London, L. & J. Myers. General patterns of agrichemical usage in the southern region of South Africa. S. Afr. J. Sci. 91: 509-514. 1995.

Lorenz, R.; Spieser, O. H.; Steinberg, C. New ways to ecotoxicology: Quantitative recording of behaviour of fish as toxicity endpoint. Acta Hydrochimica et Hydrobiologica. 1995 Sep; 23(5): 197-201.

Lowell, R. B.; Culp, J. M.; Wrona, F. J. Toxicity testing with artificial streams: Effects of differences in current velocity. Environmental Toxicology and Chemistry. 1995 Jul; 14(7): 1209-1217.

MacKay, H. M., Roux, D. J., Ashton, P. J., Van Vliet, H. R. & S. Jooste. The development of South African water quality guidelines for the natural aquatic environment. Wat. Sci. Tech. 32: 293-299. 1995.

Materna E.J.; Rabeni C.; La Point T.W. Effects of synthetic pyrethroid insecticide esfenvalerate, on larval leopard frogs (Rana spp.). Environmental Toxicology and Chemistry. 14(4): 613-622; 1995.

Miller, M. S.; Doxtader, K. G.. Atrazine impacts on shortgrass prairie microcosms. Journal of Range Management. 1995 Jul; 48(4): 298-306.

Mirgain, I.; Green, G.; Monteil, H.. Biodegradation of the herbicide atrazine in groundwater under laboratory conditions. Environmental Technology. 1995 Oct; 16(10): 967-976.

Moser V.C.; Cheek B.M.; MacPhail R.C. A multidisciplinary approach to toxicological screening: III Neurobehavioral toxicity. J. Toxicology & Environm. Health. 45173-210; 1995.

Muldoon, M. T.; Stanker, L. H. Polymer synthesis and characterization of a molecularly imprinted sorbent assay for

atrazine. Journal of Agricultural and Food Chemistry. 1995 Jun; 43(6): 1424-1427.

Myers, J. L.; Wagger, M. G.; Leidy, R. B.. Chemical movement in relation to tillage system and simulated rainfall intensity:. Journal of Environmental Quality. 1995 Nov; 24(6): 1183-1192.

Nakagawa H.; Nagakawa K.; Sato T. Evaluation of erythrocyte 5-aminoevulinic acid dehydratase activity in the blood of carp Cyprynus carpio as an indicator in fish with water lead pollution. Fisheries Science. 61(1): 91-95; 1995.

Narotsky M.G.; Kavlock R.J. A multidisciplinary approach to toxicological screening: II. Developmental toxicity. J. Toxicology & Environmental Health. 45145-171; 1995.

Naudin, S.; Garric, J.; Vindimian, E.; Bray, M.; Migeon, B.; Vollat, B.; Lenon, G. Influence of the sample preservation mode to assess the chronic toxicity of an industrial effluent. Ecotoxicology and Environmental Safety. 1995 Feb; 30(1): 54-62.

Ng H.Y.F.; Gaynor J.D.; Tan C.S.; Drury C.F. Dissipation and loss of atrazine and metolachlor in surface and subsurface drain water: a case study. Wat. Res. 29(10): 2309-2317; 1995.

Notenboom, J.; Serrano, R.; Morell, I.; Hernandez, F. The phreatic aquifer of the "Plana de Castellon" (Spain): Relationships between animal assemblages and groundwater pollution. Hydrobiologia. 297(3): 241-249.1995.

Noy-Meir I. Desert ecosystems: environment and producers. Ann. Rev. Ecol. & Syst. 425-51; 1973.

O'Keeffe, J. H. (ed). The conservation status of South African rivers. S. Afr. Nat. Sci. Prog. Rep. 131. CSIR, Pretoria

Oulmi, Y.; Negele, R. D.; Braunbeck, T. Segment specificity of the cytological response in rainbow trout (Oncorhynchus mykiss) renal tubules following prolonged exposure to sublethal concentrations of atrazine. Ecotoxicology and Environmental Safety. 1995 Oct; 32(1): 39-50.

Palmer, R. W. & J. H. O'Keeffe. Downstream effects of impoundments on the water chemistry of the Buffalo River (Eastern Cape), South Africa. Hydrobiologia 202: 71-83. 1990.

Palmer, R. W. & A. R. Palmer. Impacts of repeated applications of *Bacillus thuringiensis* var *israelensis* De Barjac and Temephos, used in blackfly (Diptera: Simulidae) control, on macroinvertebrates in the middle Orange River, South Africa. S. Afr. J. Aquatic Sci. 21: 35-55. 1995.

Palmer R.W.; Edwardes M.; Nevill E.M. Timing of larvicide treatments for the control of pest black flies (Diptera: Simuliidae) in a semi-arid environment in South Africa. J. Vector Ecology. 21(1): 48-59; 1996a.

Palmer R.W.; Edwardes M.; Nevill E.M. Downstream carry of larvicides used in the control of pest black flies (Diptera: Simulidae) in the Orange River, South Africa. Journal of Vector Ecology 21: 37-47 (1995).

Paul, E. A.; Simonin, H. A. Comparison of the toxicity of a synergized and non-synergized insecticide to young trout. Bulletin of Environmental Contamination and Toxicology. 1995 Sep; 55(3): 453-460.

Pawlisz, A. V.; Peters, R. H.. Effects of sublethal exposure on lethal body burdens of narcotic organic chemicals in Daphnia magna. Environmental Science & Technology. 1995 Mar; 29(3): 613-621.

Penttinen, S.; Kukkonen, J.; Oikari, A. The kinetics of cadmium in Daphnia magna as affected by humic substances and water hardness. Ecotoxicology and Environmental Safety. 1995 Feb; 30(1): 72-76.

Persicani, D.; Gasparetti, G.; Siro, P.; Bonvini, M. Measurement and simulation of atrazine and alachlor leaching into two field soils. Journal of Contaminant Hydrology. 1995 Aug; 19(2): 127-144.

Phipps G.L.; MattsonV.R.; Ankley G.T. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. Arch. Environ. Contam. Toxicol. 28281-286; 1995.

Plenet S. Freshwater amphipods as biomonitors of metal pollution in surface and interstitial aquatic systems. Freshwater Biology. 33127-137; 1995.

Punzo, F. Effect of Mercuric Chloride on Fertilization and Larval Development in the River Frog, Rana heckscheri (Wright) (Anura,

Ranidae). Bulletin of Environmental Contamination and Toxicology. 1993 Oct; 51(4): 575-581.

Rayburn, J. R.; Friedman, M.; Bantle, J. A. Synergistic interaction of glycoalkaloids alpha-chaconine and alpha-solanine on developmental toxicity in Xenopus embryos. Food and Chemical Toxicology. 1995 Dec; 33(12): 1013-1019.

Resh, V. H.; Norris, R. H.; Barbour, M. T. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Australian Journal of Ecology. 1995 Mar; 20(1): 108-121.

Ro, K. S.; Chung, K. M. Atrazine biotransformation in wetland sediment under different nutrient conditions .2. Aerobic (vol A30, pg 121, 1995). Journal of Environmental Science and Health Part A - Environmental Science and Engineering & Toxic and Hazardous Substance Control. 1995; 30(7): 1641; ISSN: 1077-1204.

Roux, D.; Jooste, S.; Truter, E.; Kempster, P. An aquatic toxicological evaluation of fenthion in the context of finch control in South Africa. Ecotoxicology and Environmental Safety. 1995 Jul; 31(2): 164-172.

Sabourin, T. D. & R. T. Faulk. Comparative evaluation of a short-term test for developmental effects using frog embryos. In: McLachlan, J. A., R. M. Pratt & C. L. Marken (eds). Developmental Toxicology: Machanisms and Risk. Banbury Report #26. Cold Spring Harbour Laboratory N.Y. pp 203-215. (Discussion 215-223). 1987.

Sampath K.; Elango P.; Roseline V. Effect of carbaryl on the levels of protein and amino acids of common frog Rana tigrina. J. Environ. Biol. 16(1): 61-65; 1995.

Schmidt, W. F.; Hapeman, C. J.; Waters, R. M.; Johnson, W. E. NMR and molecular mechanics of alachlor conformation and conformers: Implications to the mechanism of environmental photodegradation. Journal of Agricultural and Food Chemistry. 1995 Jun; 43(6): 1724-1729.

Schmitz A.; Nagel R. Influence of 3,4 dichloroaniline (3,4-DCA) on benthic invertebrates in indoor experimental streams. Ecotoxicology and Environmental Safety. 3063-71; 1995.

Schmitz, A.; Nagel, R. Influence of 3,4-dichloroaniline (3,4-DCA) on benthic invertebrates in indoor experimental streams. Ecotoxicology and Environmental Safety. 1995 Feb; 30(1): 63-71.

Schuytema, G. S.; Nebeker, A. V.; Griffis, W. L. & K. N. Wilson. Teratogenesis, toxicity and bioconcentration in frogs exposed to Dieldrin. Arch. Environ. Contam. Toxicol. 21: 332-350. 1991.

Schuytema, G. S.; Nebeker, A. V.; Griffis, W. L. Comparative toxicity of guthion and guthion 2S to Xenopus laevis and Pseudacris regilla tadpoles. Bulletin of Environmental Contamination and Toxicology. 1995 Mar; 54(3): 382-388.

Semlitsch, R. D.; Foglia, M.; Mueller, A.; Steiner, I.; Fioramonti, E.; Fent, K. Short-term exposure to triphenyltin affects the swimming and feeding behavior of tadpoles. Environmental Toxicology and Chemistry. 1995 Aug; 14(8): 1419-1423.

Skender, L.; Karacic, V.; Bosner, B.; Prpicmajic, D. Assessment of urban population exposure to trichloroethylene and tetrachloroethylene by means of biological monitoring. Archives of Environmental Health. 1994 Nov; 49(6): 445-451.

Sobotka J.M.; Rahwan R.G. Teratogenesis induced by short- and long-term exposure of Xenopus laevis progeny to lead. J. Toxicology & Environmental Health. 44469-484; 1995.

Solomon K.R.; Baker D.B.; Richards R.P.; Dixon K.R.; Klaine S.J.; la Point T.W.; Kendall R.J.; Weisskopf C.P.; Giddings J.M.; Giesy J.P.; Hall L.W.; Williams W.M. Ecological risk assessment of atrazine in North American surface waters. EnvironmentalToxicology and Chemistry. 15(1): 31-76; 1996.

Southwick, L. M.; Willis, G. H.; Johnson, D. C.; Selim, H. M. Leaching of nitrate, atrazine, and metribuzin from sugarcane in southern Louisiana. Journal of Environmental Quality. 1995 Jul; 24(4): 684-690.

Sparling D.W.; Lowe T.P.; Day D.; Dolan K. Responses of amphibian populations to water and soil factors in experimentally-treated

aquatic macrocosms. Arch. Environ. Contam. Toxicol. 29455-461; 1995.

Sparling, D. W.; Lowe, T. P. Metal concentrations of tadpoles in experimental ponds. Environmental Pollution. 1996; 91(2): 149-159.

Stow, C. A.; Carpenter, S. R.; Eby, L. A.; Amrhein, J. F.; Hesselberg, R. J. Evidence that PCBs are approaching stable concentrations in Lake Michigan fishes. Ecological Applications. 1995 Feb; 5(1): 248-260.

Sved, D. W.; Roberts, M. H. A novel use for the continuous-flow serial diluter: Aquatic toxicity testing of contaminated sediments in suspension. Water Research. 1995 Apr; 29(4): 1169-1177.

Tasli S.; Patty L.; Boetti H.; Ravanel P.; Vachaud G.; Scharff C.; Favre-Bonvin J.; Kaouadji M.; Tissut M. Persistence and leaching of atrazine in corn culture in the experimental site of La Cote Saint Andre (Isere, France). Arch. Environ. Contam. Toxicol. 30203-212; 1996.

Theron, J.; Hoffman, L. C.; Prinsloo, J. F. Aspects of the Chemical Control of Xenopus laevis Tadpoles Under Laboratory Conditions. South African Journal of Wildlife Research. 1992 Dec; 22(4): 110-113.

Tomlin, C. (ed) 1994. The Pesticide Manual, incorporating the Agrochemicals Handbook. 10th edition. i-xxxv, 1-1341. The British Crop Protection Council and the Royal Society of Chemistry.

Toussaint M.W.; Shedd T.R.; van der Schalie W.H. A comparison of standard acute toxicity tests with rapid-screening toxicity tests. Environ. Toxicol. & Chem. 14(5): 907-915; 1995.

Trubetskaya, E. A. Experimental investigation of embryonic and larval survival rate of two species of frogs in an environment polluted with detergents. Russian Journal of Ecology. 1994 May; 25(3): 219-224.

Vail, G. D.; Hickman, M. V.; Schreiber, M. M. Soil temperature and water effects on dissipation of commercial and starch encapsulated atrazine formulations. Weed Science. 1995 Oct; 43(4): 555-560.

van den Brink P.J.; van Donck E.; Gylstra R.; Crum S.J.H.; Brock T.C.M. Effects of chronic low concentrations of pesticides chlorpyrifos and atrazine in indoor freshwater microcosms. Chemosphere. 31(5): 3181-3200; 1995.

Verhaar, H. J. M.; Busser, F. J. M.; Hermens, J. L. M. Surrogate parameter for the baseline toxicity content of contaminated water: Simulating the bioconcentration of mixtures of pollutants and counting molecules. Environmental Science & Technology. 1995 Mar; 29(3): 726-734.

Weygoldt P. Changes in the composition of mountain stream frog communities in the Atlantic mountains of Brazil: frogs as indicators of environmental deteriorations? Studies on Neotropical Fauna and Environment. 243(4): 249-255; 1989.

Whiteman, H. H.; Howard, R. D.; Whilten, K. A. Effects of pH on embryo tolerance and adult behavior in the tiger salamander, Ambystoma tigrinum tigrinum. Canadian Journal of Zoology. 1995 Aug; 73(8): 1529-1537.

Whitton, B. A.; Kelly, M. G. Use of algae and other plants for monitoring rivers. Australian Journal of Ecology. 1995 Mar; 20(1): 45-56.

Woinarski J.C.Z.; Price O.; Faith D.P. Application of a taxon priority system for conservation planning by selecting areas which are most distinct from environments already reserved. Biological Conservation. 76147-159; 1996.

Wolf S.A.; Nowak P.J. A regulatory approach to atrazine management: Evaluation of Wisconsin's groundwater protection strategy. J. Soil and Water Cons. 51(1): 94-100; 1996.

Wong P.T.S.; Dixon D.G. Bioassessment of water quality. Environmental Toxicology and Water Quality. 109-17; 1995.

Woube, M. Environmental degradation along the Blue Nile river basin. Ambio. 1994 Dec; 23(8): 519-520.

Wright, I. A.; Chessman, B. C.; Fairweather, P. G.; Benson, L. J. Measuring the impact of sewage effluent on the macroinvertebrate community of an upland stream: The effect of different levels of taxonomic resolution and quantification. Australian Journal of Ecology. 1995 Mar; 20(1): 142-149.