PHYTOPLANKTON CHLOROPHYLL *a* CONCENTRATION AND COMMUNITY STRUCTURE OF TWO TEMPORARILY OPEN/CLOSED ESTUARIES

Report to the Water Research Commission

by

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EXECUTIVE SUMMARY

1.1 BACKGROUND AND OVERVIEW OF THE PROJECT

This project investigated phytoplankton chlorophyll *a* (chl-*a*) concentration and community composition along a spatio-temporal scale in two temporarily open/closed estuaries (TOCE's). Primary productivity measurements were also made seasonally over a one year period. In South Africa, TOCE's make up nearly two thirds of the estuaries along the length of the coastline, however little is known regarding benthic and planktonic microalgal composition and trophic function. There is a lack of knowledge on the importance and role of various phytoplankton size fractions in microalgal production and on community structure under different mouth conditions. This work addressed the gaps in our knowledge of phytoplankton temporal and spatial dynamics in temporarily open/closed estuaries.

1.2 STUDY SITE

The Maitland and Van Stadens estuaries were selected as study sites with the aim of determining whether their close proximities would present similar phytoplankton responses to changes in river inflow and mouth condition. The catchments of the two estuaries are adjacent to one another and are of similar size (60 & 90km^2 respectively) but they have different geological histories. The areas of the two estuaries were different with Maitlands measuring 0.12 km^2 and Van Stadens measuring 0.52 km^2 when the mouth is closed and the water level is at maximum height.

1.3 RESEARCH OBJECTIVES

The objectives of the study were:

- To determine variations in phytoplankton chlorophyll *a* concentration in relation to changes in nutrient input following increased river inflow.
- To determine spatio-temporal distribution of the phytoplankton chlorophyll *a* concentration and relate this to water level fluctuations.
- To examine the influence of fluctuating water level on phytoplankton community structure particularly during periods of mouth closure.
- To determine shifts in phytoplankton community structure following changes in nutrient loading brought about by increased water flow.
- To link the information from this study to ongoing regional research on estuarine reserve determinations and other regional, national, and international related research on temporarily closed/open estuaries.

1.4 STUDY APPROACH

The duration of the field study was three years (April 2001 – April 2004). The sampling protocol included quarterly, monthly and daily surveys of physical and chemical parameters, and biological variables. A once-off annual cycle of primary productivity experiments was carried out on a quarterly basis between December 2002 and September 2003 at both estuaries. Daily surveys of physical, chemical and biological variables were carried out at the Van Stadens estuary during quarterly visits.

1.5 SUMMARY OF RESULTS

Phytoplankton Chlorophyll a *and size-fraction concentrations*

Although the two estuaries are close in proximity they showed dissimilar microalgal responses to changes in river inflow and changes in mouth condition. Both estuaries showed an increase in phytoplankton chl-*a* concentration in response to an increase in river inflow, however the magnitude of response was different. Phytoplankton chl-*a* in the Maitland estuary was approximately tenfold more than that in the Van Stadens estuary following a breaching event (1089 and 131 $\mu g.l^{-1}$ respectively). During periods of low river flow, which were associated with the closed mouth phase, both estuaries had the lowest levels of chlorophyll *a*. Throughout the study chlorophyll *a* concentrations in the Van Stadens estuary were consistently lower than those in the Maitland estuary (2.01 and 18.01 $\mu g.l^{-1}$ respectively).

In both estuaries the microphytoplankton group (size fraction > 20 µm) were the most important contributors to phytoplankton chlorophyll *a* concentration making up approximately 53%, whereas the nanophytoplankton and picophytoplankton groups contributed 32 & 15% respectively. Results from this project form part of a PhD thesis (Gama unpubl.). The microphytobenthic studies form part of an MSc dissertation (Skinner 2005).

Nutrients and Nutrient Loading

From these data it is clear that the river is a source of nutrient input to the estuaries, however the nutrient concentrations were low. Phosphate concentrations entering the Maitland estuary were higher than those measured in the Van Stadens estuary. The Maitland estuary had four-fold mean annual total phosphorus concentration compared to the Van Stadens estuary. This form of phosphorus may not be readily available for uptake but may indicate a residual pool of phosphorus that can be exploited by microalgae when converted to a usable form. Total phosphate concentrations in both estuaries were positively related with an increase in river inflow. Although nitrate concentrations in both estuaries did not show such a strong relationship to flow there was an increase in nitrate concentrations with an increase in river discharge. The low concentrations of nutrients entering the Van Stadens estuary were indicative of a relatively undisturbed catchment. Nutrient concentrations measured along the Van Stadens River were never higher than those recorded in the estuary except for ammonium and nitrate concentrations that were higher at a site just below the lower Van Stadens dam. Since there was only one dam release (i.e. for the purpose of sediment removal) during the study period, there were no nutrient effects detected from water entering the river from the dam release.

Nutrient loading determined from the LOICZ budget model showed that the Van Stadens estuary is a sink for both dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP). Based on photosynthesis minus respiration (*p-r*) and nitrogen fixation minus denitrification (*nfix-denitr*) calculations the system was determined to be both heterotrophic as well as denitrifying. This means that nitrogen in the form of N_2-gas could be lost from the estuary into the atmosphere particularly during the closed mouth phase.

Groundwater

During mouth breaching events the level of the water table in the sampling wells fell below 2m from the surface and could not be sampled. However under conditions of extended mouth closure (>4 months) the level of the water table rose to a depth of 1.5m from the surface. Although macronutrients were detected in the groundwater under closed mouth conditions their concentrations were low and probably not a source of nutrients into the water column of the estuary. Dry periods of low river inflow were characterised by low to no nutrient input to the estuaries and macronutrient concentrations declined to their lowest levels in both estuaries studied. Long periods of mouth closure encouraged benthic micro-and macroalgal proliferation. These periods were characterised by increased water-column transparency depicted by low light attenuation coefficients throughout the estuary. Septic tanks used by the Van Stadens Resort are located on the north east side of the resort. They drain into the surf zone and were not considered to be a significant source of nutrient input to the groundwater (Van Stadens Management).

Once-off productivity measurements

Nanophytoplankton production contributed approximately 75% to total phytoplankton production in both estuaries during the spring (open mouth phase) and summer (closed mouth phase) seasons. During the closed mouth phase the micro- and picophytoplankton sized-fractions did not contribute significantly to total phytoplankton production. Nutrient concentrations were low during the however, production was high perhaps indicative of the high rates of turnover linked to close nutrient coupling between the available nutrient pools, bacteria and phytoplankton often exhibited in oligotrophic systems. Support of zooplankton production by the phytoplankton production appeared to be inadequate given the measured grazing rates. Such high secondary production rates are perhaps augmented by microphytobenthic sources.

Community composition

The Van Stadens estuary had chrysophytes as the dominant taxonomic group during the closed mouth phases of the early part of the study in 2001. However, following a breaching event (>2.5 $m^3.s^{-1}$ river discharge) there was a shift in the community structure that favoured one of cryptophytes and dinoflagellates. Intermittent periods of marine water overwash in late spring of 2002 during the closed mouth phase stimulated a bloom (>6.0 $x10^5$ cells ml^{-1}) of small-sized (3-5μm long along main axis) chlorophytes that persisted over a two-month period. This situation had not been observed previously although there had been episodes of seawater entering the estuary at the mouth across the sandbar.

1.6 CONCLUSIONS

1. The Maitland and Van Stadens estuaries are two estuaries adjacent to one another that experience similar climatic conditions, however they demonstrated distinct biological responses. Total phosphates in the Van Stadens and Maitland estuaries were positively related with an increase in river inflow (R^2 = 0.903 and 0.996)

respectively. Although these data have very few sampling points (N=5, the number of times there was flow in the Van Stadens River over a two year period), they indicate a strong pattern of the river as a source of nutrients. Both estuaries were nitrogen limited although on a few occasions under low flow conditions both estuaries showed phosphorus limitation.

2. Floods in the winter of 2002 had different effects on the geomorphology of the two estuaries. There was significant scouring and dune washout of the Maitland estuary compared to a more canalised and depositional effect on the Van Stadens estuary. Episodic freshets that occur in these estuaries are associated with rainfall events in the catchment and those > 3.0 m^3s^{-1} breach the mouth of the estuary. Sustained freshwater inflow will keep the mouth of the estuary open until river discharge falls below 0.8 m^3s^{-1}. Low river inflow keeps salinity in the upper reaches of the estuary oligohaline, however seawater input as overwash can increase salinities to mesohaline levels.

3. From this study it is clear that the Van Stadens and Maitland rivers acted as significant sources of nutrient input into both estuaries although the magnitude of nutrient concentrations entering the estuary were low. Owing to the steep topography and that part of Van Stadens catchment is a nature conservancy the level of nutrient input remained low.

4. Nutrient concentrations entering the Maitland estuary were significantly higher than those measured in the Van Stadens estuary particularly phosphates. Intense farming activities in the Maitland catchment indicate that future development will result in increased nutrient concentrations into the Maitland estuary.

5. The two estuaries showed dissimilar microalgal responses to changes in river inflow and mouth condition. Microalgal chl-*a* concentrations from the Van Stadens estuary were consistently lower compared with that measured from the Maitland estuary. The presence of the two dams in the upper catchment may be instrumental in trapping nutrients coming from the upper part of the catchment area.

6. Phytoplankton chl-*a* in the Maitland was a magnitude higher at times than Van Stadens. The nanophytoplankton size group predominantly drove primary productivity in the water column for most of the periods surveyed in both estuaries suggesting that the major energy pathway was primarily through this size-fraction.

7. This project has demonstrated the effects river inflow has on the phytoplankton chl-*a* concentration, community composition and also a once-off seasonal productivity study along a spatio-temporal scale over a three year period in two temporarily open/closed estuaries in the Eastern Cape. Periods of increased river inflow stimulated phytoplankton chlorophyll *a* and altered phytoplankton community structure by favouring a flagellated community of microphytoplankton. During periods of low river inflow small-sized flagellated cells and diatoms become equally prominent with large heterotrophic cryptophytes and dinoflagellates also present.

8. We can conclude that these estuaries are oligotrophic. When the river flows there is nutrient input. These freshets are also important in keeping the mouth open and maintaining a connection with the marine environment.

9. This study has contributed towards the understanding of microalgal dynamics in TOCE's. These data will be used by the DWAF to set the ecological reserve (freshwater requirements) of TOCE's as required by the NWF.

2. SUMMARY OF NEW KNOWLEDGE GENERATED FROM THE RESEARCH

This study showed that river inflow influenced sediment and nutrient transport. Discharges >3.0m^3s^{-1} introduced suspended matter that limited available light thus limiting phytoplankton production. In addition, discharges of those magnitudes or greater were effective in scouring the estuarine bed. Freshwater pulses initially diluted the estuarine water causing the water to become fresh, however when the mouth was breached the estuary became tidal with marine water penetrating into the estuary. Salinity gradients persisted only as long as there was freshwater inflow and the mouth remained open. Low river inflow maintained the upper reaches fresh while over wash over the sand bars kept high salinities in the lower reaches. These salinity and density gradients were short-lived as wind induced mixing broke up the stratification within a few days.

This study has demonstrated that from the once-off productivity experiments the nanophytoplankton size-fraction was responsible for driving water column production mainly in the spring and summer seasons. Macronutrients entering the estuaries through river inflow are essential in stimulating phytoplankton chlorophyll *a* concentrations especially following an increase in freshwater input. An increase in phosphate and nitrate inputs into the estuaries was positively related to freshwater inflow associated with breaching events. Periods of low river flow (closed mouth phase) did not contribute significantly to ambient estuarine nutrient concentrations. Riverine nutrient concentrations remained as low as estuarine concentrations during the two-year period of river monitoring. Contributions of nutrients from groundwater sources were varied although at times nutrient concentrations in the sampling wells were higher than estuarine concentrations. Microphytoplankton chl-*a* was the size-fraction most stimulated following breaching events and this pattern held true in both temporarily open/closed estuaries studied. This is in sharp contrast to what has been recorded for similar estuaries within the region and elsewhere. This indicates the significance of this size group's contribution to pelagic microalgal production and that not all temporarily open/closed estuaries respond similarly to environmental and biological factors even though they may experience similar hydrological and chemical changes.

Phytoplankton cell densities and community structure were dissimilar in the two estuaries. In the Van Stadens Estuary the phytoplankton community was comprised of dinoflagellates and cryptophytes during the first year of study followed by a shift in the community to small flagellated chlorophytes during the second year of the project. For the same period in the Maitland estuary chlorophytes were the dominant group

particularly in the summer months of 2001. Chrysophytes were the dominant taxa in the spring of 2002. Non-parametric multidimensional analyses based on presence/absence (Van Stadens) and log-transformed (Maitland) species data showed that phytoplankton species composition is influenced by changes in mouth condition. Phosphate concentrations in the Maitland estuary were higher than those measured for the Van Stadens estuary. In contrast nitrogen levels were higher in Van Stadens compared to the Maitland estuary. This means that the Van Stadens catchment is less disturbed by anthropological alterations and is rather poor as a source of nutrients whereas the Maitland catchment receives additional nutrient supply from farming activities. This study has highlighted the significance of river inflow to small temporarily open/closed estuaries to the fact that continued reduction in river inflow will impact microalgal concentrations and alter phytoplankton species composition to the detriment of ecosystem functioning. Anthropogenically induced changes on the catchment will increase nutrient input levels in receiving rivers and estuaries hence negatively influencing phytoplankton community structure with the resultant effects on higher trophic levels.

This study has documented phytoplankton species composition for these two temporarily open/closed estuaries. Future changes in species abundance and composition can be used as indicators of changes in water quality.

3. CAPACITY BUILDING

During the course of this research project, opportunities have been made available for previously disadvantaged individuals to gain experience in scientific field work, laboratory practice and presentation skills, in the form of research projects or as research assistants.

- degree.

Table 3.1. Team members involved in the project between April 2001 and April 2004.

ITEM	PERSONNEL
Field and laboratory assistants:	Mr Ayanda Ntalo, Mr Simtembile Pambuka, Ms Nicola Downey, Ms Zanele Sambokwe
Third year BSc projects:	Ms Zimasa Jika, Ms Zanele Sambokwe, Mr Simtembile Pambuka, Ms Laura Rose, Mr Erich Maletzsky, and Mr Richard Fenwick. For brief summaries see Appendix: Third Year Projects.
Honours projects:	Mr Mawethu Nyakatya, Ms Tracy Skinner For brief summaries see Appendix: Honours Projects.
MSc project:	Ms Tracy Skinner For a summary see Appendix: MSc Study.
PhD project:	Mr Phumelele Gama

Capacity building formed a key feature of this project. The research forms a component of Mr Gama's doctoral studies. In the first and second years of the project Mr M. Nyakatya and Ms T. Skinner successfully completed their research projects and graduated with Honours degrees. Six third year students from historically disadvantaged backgrounds successfully completed their research projects within this project. Ms T. Skinner has completed her MSc and Mr Gama is completing his PhD.

4. RECOMMENDATIONS FOR TECHNOLOGY TRANSFER OF RESULTS

The data from this study have been compared with similar studies within the region and nationally to consider its findings with respect to its contribution to the understanding of microalgal dynamics in temporarily open/closed estuaries. This has highlighted the differences that southern Cape estuaries (e.g. Kasouga, Van Stadens, Maitland) exhibit compared to those that occur in the subtropical (e.g. Mpenjati, Mdloti) biogeographical regions of the coastline.

To date this research has been presented at local and international conferences thus resulting in a transfer of knowledge. A number of scientific papers are planned and a list of abstracts presented and a list of intended publications is included below.

List of abstracts presented at conferences

1. Are environmental factors reliable indicators of phytoplankton response under short-term environmental changes?
2. The effect of an episodic flooding event on filamentous algal composition and abundance in a small temporarily open/closed estuary in the Eastern Cape, South Africa.
3. Seasonal distributions in phytoplankton size-fraction biomass associated with changes in physical and chemical variables in a temporarily open/closed estuary in the Eastern Cape, South Africa.
4. Temporal and spatial variation in microphytobenthic biomass in relation to changes in physico-chemical characteristics of a small temporarily open/closed estuary in the Eastern Cape.
5. Phytoplankton response to physico-chemical changes in a temporarily open/closed estuary.

List of intended publications

1. Seasonal phytoplankton composition, structure and biomass in a temporarily open estuary.
2. Temporal variation in phytoplankton assemblages and pigment composition in small-intermittently open coastal lagoon
3. Response of estuarine macroalgal populations following an episodic flooding event in the Maitland Estuary..
4. The effect of mouth breaching on microphytobenthic biomass in a small temporarily open/closed estuary, Eastern Cape.

5. Microphytobenthic biomass and community structure on different sediment types in a small temporarily open/closed estuary, Eastern Cape.

6. Diatom migration in the surface sediment of a sand dominated small temporarily open/closed estuary, Eastern Cape.

- The results are available to be used in future DWAF reserve (Ecological water requirement) studies. The results will be available to local, regional and national managers, estuarine scientists, teachers and conservation groups to use towards the understanding of how temporarily open/closed estuaries function.
- The data generated from this project forms a base-line data set for the two estuaries studied that can be used in the future by scientists, managers and local authorities in monitoring changes in microalgal community composition over time.

5. RECOMMENDATIONS FOR FUTURE RESEARCH

Fundamental questions still remain to be answered with regard to mechanisms responsible for high levels of biodiversity observed in these small estuaries. These include:

- First, to understand how low levels of primary production are able to support high levels of secondary and tertiary production,
- Second, to examine how biological interactions, at the phytoplankton-zooplankton level, are influenced by different physico-chemical factors,
- Third, to investigate nutrient fluxes (e.g. nitrates, phosphates, & silicates) during periods of high and low river inflow.
- And lastly, to examine how individual plankton communities are structured by changes in mouth condition of the estuary following an increase in river inflow.

This will help develop a plankton trophic food web model for this and possibly other temporarily open/closed estuaries that can be applied across similar estuaries within the region and possibly across other geographic regions. This will improve our understanding of the role of physico-chemically triggered anthropogenic change. Furthermore, it would clarify what these effects would have on the food web structure and overall health of the estuarine ecosystem. Knowledge of changes in trophic interactions with and without major freshwater input (mouth opening, closing and over wash events) will aid decision-making processes related to developments in the catchments of the TOCE's.

6. ARCHIVING OF DATA GENERATED DURING THE PROJECT

All forms of data and related material (i.e. raw and electronic) that has been generated from this project will be archived in the Department of Botany, Nelson Mandela Metropolitan University.

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

Dr SA Mitchell	Water Research Commission (Chairman)
Dr DR du Preez	University of Port Elizabeth
Dr S Sym	University of the Witswatersrand
Dr AJ Boyd	Department of Environmental Affairs & Tourism, Marine Coastal Management (MCM)
Prof JU Grobbelaar	University of the Free State
Prof TH Wooldridge	University of Port Elizabeth
Mr D Hay	Institute of Natural Resources
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GLOSSARY OF TERMS

ANOSIM	Analysis of similarity
CE	Counting efficiency
C:N:P	Carbon:Nitrogen:Phosphorus Ratios
Cpm	Counts per minute
DIC	Dissolved Inorganic Carbon
DF	Degree of Freedom
dpm	Disintegrations per minute
F	*F*-Statistic
$H^{\#}$	*H*-number
in.d.	Internal diameter
IOE	Intermittently open estuary
K_d	Light Attenuation coefficient
Micro	Microphytoplankton
MDS	Multi-Dimensional Scaling
MPB	Microphytobenthos
MS	Mean Sum of Square
Nano	Nanophytoplankton
NO_3^-	Nitrate
NO_2^-	Nitrite
NH_4^+	Ammonium
N:P	Nitrogen : Phosphorus ratio
One-Way ANOVA	One-Way Analysis of Variance
P	Probability level of significance
PAR	Photosynthetically Active Radiation
Pico	Picophytoplankton
SiO_4	Silicate
SRP	Soluble Reactive Phosphorus
SS	Sum of Square
TOCE	Temporarily open/closed estuary
TP	Total Phosphorus
Two-Way ANOVA	Two-Way Analysis of Variance

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1. INTRODUCTION

In South Africa, temporarily open/closed estuaries make up nearly two thirds of the estuaries along the length of the coastline, however little is still known regarding benthic and planktonic microalgal composition and trophic function. Temporarily open/closed estuaries are increasingly coming under threat due to anthropogenic impacts ranging from damming and water abstraction upstream to development and urbanization along estuarine and adjacent coastal floodplains. Such riverine and catchment impacts can account for a vast array of ecological (i.e. alteration of flow and the associated modification of the physico-chemical processes including variation in the availability of inorganic minerals) and economical (i.e. increase or decrease in coastal or estuary property value, loss of habitat for key fish species for the estuary fisheries that use estuaries as nurseries) consequences (Byren and Davies 1989, Davies and Day 1998, Lamberth and Turpie 2001). The Departments of Environmental Affairs and Tourism and Water Affairs and Forestry have both recognized the economic and environmental value of estuaries particularly temporarily open/closed estuaries given that a great number are increasingly settled and developed as nodes for tourism and recreation. Such development initiatives will progressively place undue pressure on the environment requiring sound management and protection.

Several studies have demonstrated the importance of microalgal production in permanently open estuarine ecosystems (Head 1976, Ray *et al.* 1989, Mallin *et al.* 1991). Only recently have studies looked at the role of phytoplankton production and biomass (Bally *et al.* 1985, Froneman 2000a, 2002b, Perissinotto *et al.* 2000, Walker *et al.* 2001) and benthic microalgal biomass characterisation (Nozais et al 2001) in temporarily open/closed estuaries. The present study focused on the production and chlorophyll *a* concentration of the micro- (>20µm), nano- (2.7-20µm), and picophytoplankton (<2.7-1.2µm) fractions and their response to changes in mouth condition. The need to fractionate the phytoplankton community arises from the idea that the phytoplankton assemblages are naturally composed of different forms of algae which are of varying sizes (Wetzel 1983, Bold and Wynne 1981) hence their importance in channelling carbon energy up the food chain is related to the dominant size group present given the prevailing environmental conditions (Kitchell 1992).

Furthermore, it examines the changes in phytoplankton community structure in response to changing mouth condition along temporal and spatial scales. The size of zooplankton standing stock can be a reflection of the availability and magnitude of the production and biomass of microalgae. Herbivorous grazers keep phytoplankton densities in check and are, in turn, controlled by larger predacious zooplankton and smaller fishes that are then preyed upon by large piscivorous fish. The group at the end of this food chain is often the target of fisheries and anglers alike. Selection of phytoplankton by herbivorous grazers has been shown to shape microalgal production, biomass and community structure to a point where trophic energy pathways are altered (Carpenter 1988, Carpenter and Kitchell 1988).

The elimination of phytoplankton palatable to the zooplankton grazers will favour the success of the less preyed upon species switching algal community structure toward non-

palatable forms thus altering the trophic dynamics at the top of the food chain (Lathrop and Carpenter 1992). Information regarding which planktonic microalgal group is the driver of energy during a seasonal cycle will provide significant insight on how certain microalgal groups may influence food web energy dynamics. Therefore, an understanding of 'bottom up' effects (i.e. nutrient loading) would aid in the prediction and management of excess nutrient input into estuaries particularly those classified as sensitive. Moreover, such information can have important implications for management particularly when the presence of specific microalgal groups coincides with the periods of recruitment into the estuary of fish and their larvae.

It should be noted that the aims and objectives presently addressed by this study have been modified from the originally proposed ones (listed below) following the recommendations from the steering committee. In addition the present report emanates from a project originally titled "Phytoplankton primary production and community structure of two temporarily open/closed estuaries" that was funded by the WRC.

The following are abbreviated objectives as per the proposal:

To understand the major energy pathways that are driven by phytoplankton production of various size fractions during periods of mouth breaching and closure.

To examine the influence of fluctuating water level on phytoplankton primary production particularly during periods of low flow

To determine shifts in phytoplankton community structure following nutrient enrichment experiments that will simulate changes in water quality (eutrophication) brought about by increased anthropogenic input.

To determine spatio-temporal distribution of the phytoplankton and relate this to water level fluctuations

To link the information from this study to ongoing regional research on estuarine reserve determinations including other regional, national, and international related research on temporarily closed/open estuaries

1.1. AIMS OF PROJECT

The broad aims of the project were;

- To quantify phytoplankton production and characterise community structure of two temporarily open/closed estuaries along temporal and spatial scales
- To determine how changes in water-volume in the estuary associated with mouth opening and closing would influence phytoplankton production and community structure.

1.2. PROJECT OBJECTIVES

The following are modified objectives as per the steering committee recommendation:

- To determine phytoplankton production in relation to changes in increased river inflow,
- To determine spatio-temporal distribution of the phytoplankton chlorophyll *a* concentration and relate this to water level fluctuations,
- To examine the influence of fluctuating water level on phytoplankton community structure particularly during periods of mouth closure,
- To determine shifts in phytoplankton community structure following changes in nutrient loading brought about by increased water flow,
- To link the information from this study to ongoing regional research on estuarine reserve determinations and other regional, national, and international related research on temporarily closed/open estuaries.

1.3. HYPOTHESES

This investigation tested the following hypotheses that;

- Nanoplankton and microphytoplankton (2.7 – 20μm & >20μm in size along major axis respectively) form the dominant phytoplankton that control production during periods of increased river inflow in temporarily open/closed estuaries,
- Picophytoplankton (1.2 - 2.7 μm in size along major axis) form the dominant phytoplankton group that drive primary production during periods of low river inflow,
- Prolonged periods between breaching events (closed-mouth phase) will favour increased pico and nanophytoplankton biomass and production,
- During periods of mouth breaching microphytoplankton production will decrease beyond levels that can support macro-zooplankton biomass and production,
- Increased nutrient loading associated with high levels of river inflow will support higher nanophytoplankton biomass and production,
- High nutrient concentrations associated with increased river inflow will shift phytoplankton community structure from that dominated by pico- and nanophytoplankton to that dominated by larger (>20 μm) sized microphytoplankton,
- Dinoflagellates will dominate the water column during periods of mouth breaching and low water clarity, while chrysophytes, particularly bacillariophytes (diatoms); will dominate during periods of mouth closure and increased water clarity

The aims, objectives and hypotheses of this study are specifically addressed in Chapters 5, 6 and 8.

2. LITERATURE REVIEW

2.1 Introduction

Geomorphological background

Post Mesozoic tectonic events on the southern African landscape gave rise to the establishment of the eastern escarpment that divided the subcontinent asymmetrically with a westward gradient, the highest point being the Lesotho Highlands in the east (Moon and Dardis 1988). On the eastside of the escarpment east-flowing rivers were formed (Dardis *et al.* 1988). The late Pliocene (2.5 Ma) land mass uplift epoch of the Southern African subcontinent witnessed the development of the African land-surface erosional phase that resulted in the dissection of the coastal hinterland. Changes in the global climate patterns over geological time resulted in marked climate fluctuations that effected changes in sea level creating deep incisions on the land surface resulting in steep gorges presently seen in several areas across the Eastern and South Cape coasts (Moon and Dardis 1988, Lubke and de Moor 1988). The low sea level during the Pliocene and Pleistocene gave rise to deep incisions of the bedrock and the subsequent sea level rise in the Holocene era resulted in the drowning of a number of river valleys and estuaries along the coastline particularly in the South and East Cape coasts (Ramsay 1995).

The development of present fluvial systems has been attributed to climate and the hydrologic regime. Following the regression of the sea level the coastal marine platform, which is evident west of Port Elizabeth to the Tsitsikamma along the Eastern Cape coast, was deeply incised by the rivers that cut through the Table Mountain quartzite forming deep gorges such as those of the Van Stadens, Bloukrans, and Storms Rivers (Lubke and De Moor 1988). The subsequent rise in the sea level during the Holocene epoch initiated a landward retreat of the shoreline consequently modifying the lower reaches of most rivers by drowning river valleys with concomitant infilling of sediment. Unlike the river systems mentioned that formed from deep cuts of the coastal marine platform, the development of smaller fluvial systems along this similar coastal stretch (i.e. Maitland River, Kabeljous River, Seekoei River, etc.) differed in that many of them formed after the formation of the coastal marine platform.

The geology of both the Van Stadens and Maitland catchments is derived primarily from rock of the mid Palaeozoic Era that formed the Table Mountain Group of the Cape Supergroup (Rust 1998). Subsequent sedimentation and depositional processes during the Holocene have resulted in the formations that are present today. Although the Van Stadens and Maitland River catchments occur adjacent to one another their geology and geomorphological formations are varied. The geology and geomorphology of the Van Stadens River date back from the late Miocene to the Pleistocene epoch following the drop in sea level that produced deep incisions of the Table Mountain Quartzite whereas the Maitland River formed following the recent Holocene sea level rise that produced new drainage lines post the development of the coastal marine platform (Dardis *et al.* 1988, Rust 1998, Cooper *et al.* 1999). Dune movement as a result of aeolian sand deposition significantly influences the mouth area of these two estuaries, particularly during periods of strong westerly and southwesterly winds.

Biogeographical Distribution of Temporarily Open / Closed Estuaries

The South African coastline stretches from the Orange River in the west to the Kosi Bay system near the Mozambique border on the east a distance of about 3700 km. Along this coastline are a total of 465 estuaries that receive river flow from the South African interior with most of these emptying into the Indian Ocean, and approximately 72% of those are classified as temporarily open/closed estuaries (Whitfield 2000). South African estuaries are subdivided into three main climatological regions. They include (1) a cool temperate region from the Orange River in the Northern Cape to the Cape Point in the Cape Peninsula; (2) a warm temperate region from Cape Point to the Mbashe River; and (3) a subtropical region from the Mbashe River to the Kosi Bay estuarine system. Apart from a few dry riverbeds along the west coast that run only during periods of significant rainfall, the cool temperate region has approximately seven TOCEs. The warm temperate and the subtropical regions have the majority of the temporarily open/closed estuaries about 86 and 90 respectively (Whitfield 2000). The Maitland and Van Stadens estuaries are both located within the warm temperate region of the South African coastline and their catchments lie adjacent to each other with the latter draining a larger (i.e. 60 & 90 km^2 respectively) surface area.

The Significance of Catchment Size

South Africa is a dry country characterised by a mean annual rainfall of 520mm and the landscape has a few river systems that drain toward the west and a number of river systems emptying mainly to the east (Heydorn and Tinley 1980, Heydorn 1991). One of the major features of temporarily open/closed estuaries is their small catchment size. Catchment size, topography (i.e. relief ratio), type of geology and vegetation cover are critical elements in governing and controlling the amount of precipitation (i.e. rainfall) intercepted, retained and released as surface, subsurface runoff and groundwater following a rainfall event that influence river discharge (Dardis *et al.* 1988). The upper to middle portion of the Van Stadens catchment has high relief ratios characterised by very high gradients indicative of very steep gorges. The Maitland catchment is much more characteristic of undulating hills composed of vegetated fossil dunes of medium to low gradient.

Fluvial systems that drain large catchment areas (e.g. >1000km^2) give rise to the establishment of perennial streams and rivers that produce large permanently open estuarine systems along the coastline. Conversely, small catchments (i.e. <500km^2) drain smaller surface areas resulting in semi-perennial and ephemeral river systems that become active during the wet periods of the year or when significant rainfall events take place. River systems that occur in small catchments are prone to flushing when heavy sporadic rainfall events take place. Because of the high energy conditions generated during peak discharge large amounts of sediment transport occur over short time periods (Hughes and Stone 1987, Moore 1987). During dry periods of the year particularly under low-river flow conditions the estuary is cut off from the sea by a sandbar that forms a barrier at the mouth. The sandbar barrier at the mouth is a resultant combination of two processes namely longshore and cross-shore sediment transport (Cooper *et al.* 1999). Discharge during these periods is normally low <0.3 $m^3.s^{-1}$ and is insufficient to maintain an open mouth condition. However, during periods of high rainfall and increased freshwater runoff, the water level

within the estuary may rise appreciably until it exceeds the height of the sandbar at the mouth leading to a breaching event (Whitfield 1992, Allanson and Baird 1999). Although the bulk of the water in the estuary is riverine, substantial water volume augmentation into the estuary emanates from the sea during the closed mouth condition as marine over-wash across the sandbar.

2.2 Physical and Chemical Characteristics

Climate and hydrodynamics

The arid nature of the southern African subcontinent has meant that climate tends to control and influence river discharge and evaporation rates coupled with imposing certain constraints on the occurrence and growth of estuarine vegetation (Moon and Dardis 1988, Allanson and Baird 1999). Estuaries situated along the warm temperate region experience a varied seasonal pattern of rainfall. Those found in the western and southern Cape coasts can be subjected to an all-year and winter rainfall seasonal pattern whilst those occurring in the Eastern Cape coast tend to experience a bimodal (i.e. spring & autumn) and summer rainfall pattern (Stone *et al.* 1998). The amount of precipitation received influences discharge such that during wet periods river flow can be sustained for prolonged periods, however, evaporation rates during drought often exceed input from river inflow causing some estuaries to become hypersaline.

The frequency and duration of mouth breaching for a number of the small catchment-sized estuarine systems located along eastern and southern Cape coasts varies significantly and can be attributed to the prevailing local atmospheric weather conditions. Major movements of air masses that cover vast distances over land affect climate and weather (Stone *et al.* 1998). However, the associated influence of altitude, mountain orientation and distance from the sea brings about variable changes in weather patterns over shorter distances. These localised changes give rise to patchy distribution of rainfall patterns over very short distances such that even catchments adjacent to one another will receive different amounts of rainfall. Thus, the discharge regime will give rise to variable periods of mouth closure of these estuaries during the dry season effectively isolating them from connecting to the sea. Seasonal patterns of river flow are subject to variable periods of low salinity during wetter times of the year followed by high salinity to hypersaline conditions following extended periods of increased evaporation associated with low river flow.

The processes that control fluvial and marine sediment supply and distribution within estuaries also control hydrodynamic patterns which in turn affect physical, chemical and biological characteristics (Boon 1975, Dyre 1979, Vieira and Chant 1993, Mallin 1994). Sediment supply and transport in estuaries is made available by weathering processes on the catchment and carried down by river flow following rain events. However, the intermittent nature of rainfall occurrence across these regions means that temporarily open/closed estuaries experience sediment transport and deposition during periods of peak discharge. Most of the sediments occurring in these estuaries originates from the marine environment introduced through tidal inflow following subsidence of high river discharge when the mouth is still open with some entering as aeolian deposition (Whitfield and Lubke 1998, Cooper *et al.* 1999). Spring tides that coincide with storm surges, which are a common occurrence along the Eastern and Southern Cape coasts, are responsible for the

additional marine sediment brought in by means of overwash or overtopping across the sandbar (Reddering 1988, Cooper 1990).

Subsequent to breaching of the mouth the water level within the estuary falls very rapidly, often exposing large areas of the benthic substratum that had remained submerged for extended periods during the closed-mouth condition. This period is often characterised by extensive colonisation of the substratum by a productive community of algae, macrophytes and benthic animals (Day 1981, Day *et al.* 1989, Wooldridge and Loubser 1996, Adams *et al.* 1999). During breaching events, usually when flow rates exceed 5 $m^3.s^{-1}$ river conditions prevail and control the flow patterns, which are typified by estuary bed scour and channel modification (Cooper *et al.* 1999). However, when the freshwater inflow declines to approximately 2-5 $m^3.s^{-1}$, a normal estuarine open-mouth phase is established characterised by regular tidal exchange with seawater penetration reaching into the middle and upper reaches. The tidal prism, which is the volume of water exchange between the sea and estuary over a tidal cycle, for a number of these estuaries, however, is small and shallow generally not exceeding 10 m^3 (Whitfield 1992, Schumann *et al.* 1999).

The open-mouth condition is short-lived owing to the competition between wave and flood-tide-driven forces that suspend and deposit sediments from the marine environment against scouring and ebb-tidal currents from river-flow forces. Owing to prevailing seasonal conditions, river-flow forces override under high discharge, however wave forces will close the mouth under increased and sustained wave action. High-energy wave conditions associated with strong coastal-storm surges coupled with low average rainfall and runoff in spring and summer result in an increased frequency of mouth closure for most of the small estuaries. Closure periods can vary from days, months or even years depending on the climatic conditions and rainfall patterns in the catchment areas. Seasonality of these estuaries is best illustrated by the depth and condition or state of the mouth, which is normally reflected in the water level and water column salinity composition.

Irradiance

The term irradiance generally refers to the amount of solar radiation falling on a given area per given time. This quantity often includes all ranges of the solar energy spectrum received on the earth's surface and aquatic bodies (Lobban and Harrison 1997). The portion of the solar spectrum that is of concern and use by photoautotrophic organisms is visible light energy or photosynthetically active radiation (PAR), which spans between the 400 and 700 nm range. Photosynthetically active radiation is important in the productivity of phytoplankton and the depth to which attached plants can grow. Light energy received varies with the time of the year and distance from the equator with a significant amount obtained during the warmer months of the year (Lobban and Harrison 1997). Diel variability, prevailing weather conditions, associated with suspended inorganic and organic matter have been well demonstrated for a number of aquatic environments in altering light quality and quantity with depth (Oertel and Dustan 1981, Bledsoe and Phlips 2000).

The depth at which a 20cm black and white disc disappears and appears when lowered and raised in the water column is referred to as the Secchi depth and has been used to approximate the depth at which light intensity is attenuated to approximately 1% that of the

surface intensity (i.e. compensation depth). Secchi disc measurements are a widely used convenient method of determining light penetration in different types of aquatic bodies (Kirk 1983, Bledsoe and Phlips 2000). Although less accurate, Secchi disc readings offer a quick and efficient way of assessing the level of dissolved and suspended matter attenuating light at depth by absorption. A relation between the Secchi depth *D*, the extinction coefficient of white light and the quantity of suspended particles has been established (Harvey 1955, Perkins 1974). Past studies by Day (1981) and Begg (1984), show that Secchi disc measurements for temporarily open estuaries range from a minima of 0.01m during high river inflow to a maximum of 10m when the mouth is closed. In recent studies on TOCEs turbidity has frequently been measured in nephelometric turbidity units (NTU), which have shown a wide range of values from 0.2-20 NTU during the closed-mouth phase to 60-90 NTU following heavy river inflow that leads to mouth breaching (Cooper *et al.* 1993, Froneman 2002a). When there is river inflow and the mouth is open the level of suspensoids in the water column tend to decrease from the mouth to the upper reaches.

In temporarily open/closed estuaries light attenuation may vary widely particularly with regard to season, time of the day, prevailing weather conditions and suspended matter in the water column. Surface irradiance levels recorded for some TOCEs have ranged from minima of 71 to maximum of 2300 $\mu mol.m^{-2}.s^{-1}$ during closed mouth conditions (Perissinotto *et al.* 2000, Nozais *et al.* 2001). Increased rainfall in the catchment has been positively associated with a reduction in light penetration (i.e. $>K_d$) at depth as a consequence of high-suspended matter from increased runoff (Nozais *et al.* 2001). Due to the low levels of suspensoids and the shallow depth of many of these estuaries during low river inflow under closed mouth conditions light availability at the bottom is generally greater than 30 - 60% that at the surface (Perissinotto *et al.* 2000, Nozais *et al.* 2001, Froneman 2002b). Light attenuation coefficients during these periods are normally low ranging from 1.1 – $3.3m^{-1}$, particularly for estuaries from the subtropical region, that have water-column depths ranging from 1.5 – 4.0m. On the other hand, estuarine systems from the warm temperate region exhibit much lower K_d values under low river inflow and closed mouth conditions (Perissinotto *et al.* 2002). This may be indicative of the type of geology occurring in the catchments that these estuaries drain that contributes to the difference between subtropical and warm temperate estuaries (Cooper *et al.* 1999). The high light at depth coupled with low river inflow and turbulence provide suitable conditions for the establishment of mats of benthic micro- and macroflora.

There are marked increases in turbidity levels with light attenuation coefficients often reaching values of 8.0 to 9.0 m^{-1} following increased river flow with high concentrations of suspended silt and sediment. Increased river flow leads to mouth breaching that can last from weeks to months establishing a connection and interaction with the sea (Perissinotto *et al.* 2000, Nozais *et al.* 2001). During this period, the percentage of surface light intensity reaching the bottom is often less than 0.1% at the sediment/water interface resulting in a small euphotic zone. The reduction in the photic zone can limit phytoplankton growth by significantly reducing the photosynthetic efficiency of microalgae (Day 1981, Cloern *et al.* 1983, Day *et al.* 1989).

Temperature

Estuarine environments are characterised by the mixing between river-borne water and seawater, and as such, temperature influences will be broadly determined by the downstream flow of river water and the flooding marine-borne water modified by solar heating and evaporative thermal loss due to cooling (Day 1981). Seasonality and regional climate are major factors affecting water column temperatures in temporarily open/closed estuaries. River water entering an estuary is normally warmer during the summer seasons while retaining much cooler water temperatures in winter. Consequently, due to the sea's huge thermal buffering capacity including the entrainment of upwelled deep-cooler oceanic waters onto the epipelagic layers, seawater along the coast is generally colder than estuarine water throughout the year. Given that temporarily open/closed estuaries are seasonally cut-off from the sea for extended periods the major source of water input is mainly through river inflow. As a result, during seasonally dry periods, characterised by low flow and calm local weather conditions, changes in water-column and sediment temperatures are affected mainly through solar radiation. Unlike permanently open estuaries TOCEs show higher evaporative loss due to larger surface areas and elevated evapotranspiration by dense emergent and submersed macrophyte stands (Day 1981, Day *et al.* 1989).

Seasonal temperature ranges are most pronounced in the cool-temperate region of the west coast and least in the subtropical region of the east coast. Estuaries located in cool-temperate regions show annual temperatures ranges from 9 – 11^{o}C in the winter and from 24 – 27^{o}C during the summer. In the warm-temperate regions they range from winter lows of 14 – summer highs of 28^{o}C, whereas subtropical ones can range from 18 – 30^{o}C (Day 1981, Lubke and De Moor 1988). Due to the low freshwater inflows especially during seasonally dry periods of the year, temporarily open/closed estuaries show smaller variation compared to permanently open estuaries (Bally *et al.* 1985). However, following strong storm surges along the coast, cooler marine water can be introduced into the isolated estuary over the sandbar leading to strong vertical and horizontal salinity and temperature gradients. Nevertheless these conditions are short-lived persisting for hours to days and can be rapidly destroyed by wind-generated turbulence that homogenises the entire water column. Without further exchange between estuarine water, river inflow and marine water temperature profiles within TOCEs remain vertically and horizontally uniform with minor variations of less than 2^{o}C between lower and upper estuarine reaches.

Salinity

Increased river inflow introduces several important characteristics to the physical and chemical properties of the estuary relative to the magnitude of the discharge (Day *et al.* 1989, Schumann *et al.* 1999). River flows greater than 5.0m^{3}s^{-1} can breach the closed mouth of the estuary and are characterised by strong unidirectional flow of freshwater (Huizinga 1994, 1996). These conditions may persist until the flow dissipates as the flow from the catchment subsides whereupon riverine flows drop to levels that permit estuarine and marine water exchange. Freshwater flood conditions completely reduce salinities rendering the estuary fresh until flood conditions recede. During the open mouth phase TOCEs experience strong horizontal and vertical salinity gradients similar to those typically

observed in permanently open estuaries, and this period is normally characterised by a tidal dominated phase (Perissinotto et al 2000, Froneman 2002a).

Under closed mouth conditions without any appreciable freshwater inflow or marine overwash salinities can vary on temporal scales ranging from days to weeks rather than hours as in open systems where tidal effects are significant. However, when there are strong storm-surges that produce sea-swells >5m in height, they lead to overtopping events resulting in substantial volumes of seawater entering the estuary over the sandbar. Vertical salinity gradients, particularly at the mouth, can vary from hours to days and as the weather-front passes get rapidly eroded by wind-driven mixing. This is quickly followed by uniform salinity conditions throughout the lower reaches of the estuary. Wind energies, particularly during these stormy events, are essential in the distribution of very saline marine water up the estuary through wind-driven and density differential mixing (Day *et al.* 1989).

In areas of high annual rainfall (i.e. western regions of the warm temperate and the subtropical coasts) the continuous freshwater inflow results in salinities dropping to near freshwater conditions (Nozais *et al.* 2001). Conversely, in warm arid regions, especially during drought periods, salinities can reach hypersaline levels (e.g. 60 – 90ppt) with strong impact on the estuarine biota (Whitfield and Bruton 1989). Where estuarine systems are very shallow (i.e. mean depth <1.0m) oligohaline to mesohaline conditions can persist for extended periods of 9 – 12 months with much of the freshwater input coming from dune seepage (Campbell and Bate 1986) and groundwater input (Harvey and Odum 1990).

2.3 Nutrients

The rate at which the activities of biological communities are regulated in natural estuarine waters is governed by the quantity of the chemical content and their associated interactions (Day *et al.* 1989, Allanson and Winter 1999). Several studies have shown the importance of essential nutrient requirements for the sustenance and development of phytoplankton in freshwater and marine environments (Brand *et al.* 1983, Hecky and Kilham 1988). The availability of estuarine chemical constituents is primarily influenced and governed by climate which controls weathering processes (Ollier 1982, Hall 1992) while the supply is limited by fluvial transport which is dependent on the frequency, magnitude and scale of rainfall events in the catchment (Dardis *et al.* 1988, Allanson and Baird 1999). For the most part estuaries along the southern and eastern coasts of South Africa with sustained river inflow derive most of their nutrients from land although a few systems may deviate from this pattern (see Bally *et al.* 1985). In contrast to this type of estuarine system, temporarily open/closed estuaries along this geographical region exhibit varied response patterns. Under closed-mouth conditions macronutrient (NO_2, NO_3, NH_4, PO_4) levels remain very low (i.e. < 1.0 $mg.l^{-1}$) throughout the water column. However, slightly elevated concentrations may be detected in the water column following strong coastal weather-fronts that induce wind-turbulence that re-suspend bottom sediments. Although the dynamics of macronutrient cycling in temporarily open/closed estuaries are poorly understood (Day 1981, Allanson and Winter 1999, Nozais *et al.* 2001), recent work on seasonal (Perissinotto *et al* 2000, Froneman 2002a) and annual (Walker *et al.* 2001) concentrations emanating from research from estuaries in the warm temperate and subtropical regions is beginning to

shed some light on the relative concentrations available for uptake during wet and dry periods of the year.

2.4 Biological Characteristics

Solar radiation, particularly photosynthetically active radiation (PAR) is utilised by photoautotrophic organisms in the process of photosynthesis (Walker 1992, Lobban and Harrison 1997). Photosynthesis is the process by which higher plants, algae, and certain species of bacteria transform and store solar energy in the form of energy-rich organic molecules. Under optimal climatic conditions and given efficient cellular-metabolism, photosynthesis can result in the rapid accumulation of plant matter. Furthermore, the rate at which biomass is built up strongly depends on the prevailing environmental conditions together with the genetic attributes of the organisms concerned (Ramus 1990, Ramus and Rosenberg 1980, Walker 1992). Photosynthesis is influenced by abiotic characteristics (i.e. light, temperature, pH, carbon, water, and nutrients). Consequently, these factors vary the photosynthetic rates at which carbon is 'fixed' and stored. Changes in the photosynthetic rates or fixed carbon allow for the determination of primary production within a given area or volume.

Biologists frequently use primary production as a tool to assess an ecosystem's capacity to support and sustain biodiversity. It is also used for monitoring plant and/or algal community changes within a given habitat over a period of time (Hilmer 1984 & 1990, Mallin *et al.* 1991). In estuarine ecosystems primary producers (i.e. photosynthetic bacteria, microscopic algae, macrophytes, seagrasses, and mangroves) form the base of the food chain that supports aquatic and bird life (Day 1981, Hilmer 1984, Adams *et al.* 1992, Whitfield 1992, Wooldridge and Loubser 1996). Hence, primary production becomes critical in shaping food web dynamics, as it is the main source of energy flow responsible for sustaining high estuarine biodiversity. Abnormally induced changes or disturbances in the relative amounts of production can give rise to rapid shifts in algal and plant biomass, leading to deterioration in water quality, transparency and loss of suitable habitat. Such shifts in algal and plant community structure can lead to further changes in food web dynamics that favour and encourage development of nuisance animal and plant species (Wetzel 1983).

Studies showing high levels of productivity have been carried out in a number of large estuaries of the northern hemisphere (Cloern *et al.* 1983, Cloern 1987, Mallin 1994, Underwood and Kromkamp 1999). Since these estuaries are driven by phytoplankton production light is considered one of the main factors controlling water-column productivity such that system production may be limited by photic depth (Cole and Cloern 1987). In contrast to northern hemisphere estuaries, South African estuaries are on average considerably shallower and therefore tend to have a greater photic depth to mixing depth (Day 1981). Although there have been studies on primary production (Robarts 1976, Henry *et al.* 1977, Dye 1978, Bally *et al* 1985, Hilmer 1984, 1990, Allanson and Read 1995) in some South African estuaries in the cool and warm temperate biogeographic regions, many of these were carried out on permanently open estuaries. There is still a lack of available data on similar systems from other biogeographical regions of the country. There is a paucity of studies on the smaller temporarily open/closed estuaries that span the four

biogeographical regions and make up the majority of estuaries along the South African coastline. Recently, a few studies have looked at the contributory role of microalgal production (Perissinotto *et al.* 2000, Nozais *et al.* 2001, Walker *et al.* 2001, Perissinotto *et al.* 2002) and associated rates of productivity to estuarine primary production (Froneman 2000a, 2002b, Perissinotto *et al.* 2003). These studies are starting to provide a basis for our understanding of the fate of microalgal carbon in ecosystem production. These studies have been on estuaries located in two east coast biogeographical regions the warm temperate (e.g. Nyara and Kasouga estuaries) and the subtropical (Mpenjati and Mdloti estuaries) regions that show completely different climate and hydrological features. The microalgal response patterns in subtropical estuaries are sensitive to the wet and dry seasonal climate pattern particularly when river discharge is elevated during the summer periods. While the availability of macronutrients possibly limits microalgal production in the warm temperate biogeographical region, this is in sharp contrast to subtropical estuaries. Nutrient input through river inflow associated with the interaction with seawater following the opening of the mouth is essential for microalgal production and community structure. Microalgal productivity tends to be higher during open-mouth compared to closed-mouth conditions supporting the importance of river borne nutrient supply for increased productivity (Froneman 2000a, 2002b, Perissinotto *et al.* 2003). Subtropical estuaries however, exhibit an opposite response with increased productivity occurring during the onset of the low river-inflow period depicted by mouth closure. During open mouth conditions flow is generally too high to support any water column production as a result of flushing and short residence time. The varied response patterns of microalgal production to physical and chemical factors in these estuaries illustrates the heterogeneous nature of each estuarine system to the extent that no broad general patterns can yet be drawn as being applicable to all temporarily open/closed estuaries.

Phytoplankton community size-structure and taxonomic composition has been associated with the efficiency of carbon transfer across trophic levels (Mallin *et al.* 1991, 1994, Bledsoe and Phlips 2000). In oligotrophic north temperate estuaries or coastal bays characterised by deeper mean depths and greater mixed depth to photic depth diatoms are the major contributor to taxonomic community structure (Bledsoe and Phlips 2000). Flagellates particularly dinoflagellates form a minor contribution to overall community structure, however in mesotrophic estuaries phytoplankton community composition varies along a temporal scale but the flagellated community (i.e. dinoflagellates, cryptophytes and small-sized flagellated greens) is mostly dominant (Mallin *et al.* 1991). Studies conducted on temporarily open/closed estuaries from the warm temperate region show that they are generally nutrient poor and thus support low microalgal production (Walker *et al.* 2001, Froneman 2002a & b). In contrast, however, when compared to those from the subtropical region like the uMdloti Estuary (Perissinotto *et al.* 2002) they appear to support a higher phytoplankton concentration (Figure 1). According to studies carried out in permanently open estuaries (Margalef 1978, Hilmer and Bate 1991, Snow *et al.* 2000a) diatom abundances are favoured under well-mixed and nutrient replete conditions whereas dinoflagellates species persist following the onset of a halo- and chemocline. Studies in phytoplankton community structure and ecological functioning in South African estuaries are still lacking especially in temporarily open/closed estuaries. Although phytoplankton community structure has been related to the amounts of nutrients transported together with

the rates of flow (Snow *et al.* 2000a, Snow *et al* 2000b), our understanding of the response of species composition to these and other environmental factors is still poor. Effects of environmental changes and influences on phytoplankton production and community structure over annual or even longer temporal scales in temporarily/open closed estuaries are still poorly understood. Rates of phytoplankton production have been recorded for some permanently open South African estuaries and can vary from as low as 13 to greater than 300g.Cm^{-2} yr^{-1} indicative of a wide range of estuarine systems that are reflective of their unique biogeographical localities. Recent productivity studies (Froneman 2002, Perissinotto *et al.* 2003) in temporarily open/closed estuaries have emphasised the importance of small-sized picophytoplankton to total water column production, implicating them as a significant link to secondary and hence tertiary production. However, these studies are based on short temporal periods and do not track annual seasonal cycles.

A number of temporarily open/closed estuaries are characterised as nutrient poor especially those located in the warm temperate region, thus nutrient supply and availability is crucial to phytoplankton population dynamics and production. Aquatic bodies that are nutrient poor have been shown to support small-sized phytoplankton as these microalgae show rapid rates of nutrient uptake and high rates of turnover suggesting that nutrients in short supply are quickly taken up by pico sized phytoplankton owing to their large surface area to volume ratios (Goldman and Gilbert. 1983, Armstrong 1994, Fisher *et al.* 1995, Kirchman 2000). The transference of carbon energy up the food web is not well understood for these systems, hence more studies need to be carried out in temporarily open/closed that span the three South African biogeographical regions in order to better understand these systems.

3. STUDY AREA AND LAND USE

Van Stadens and Maitland Catchment

The Maitland (33^{o} 59'2'' S, 25^{o} 17' 4''E) and Van Stadens (33^{o} 58'1''S, 25^{o}13'20''E) estuaries were selected as the two study sites as they occur close together (Figure 2) in St Francis Bay and have approximately similar catchment sizes (60 & 90km^{2} respectively). The Maitland catchment is primarily farmland with a relatively small portion of land covered by shrub-thicket vegetation near the coastline designated as nature reserve. A number of farm dams occur in the catchment and are used to store water for irrigation and for livestock, mainly dairy cattle. The lower end of the Maitland River, just above the estuary head, has extensive stands of the common reed, *Phragmites australis* and at the estuary head there is a mixture of *Phragmites australis* and bulrush, *Typha capensis* subsp. *capensis.* The middle to lower section of the estuary is characterised by a mixture of emergent and submerged macrophyte plants like *Typha* and *Ruppia cirrhosa*. The maximum depth of the Maitland estuary when the mouth is closed is 2 m whilst the mean depth is approximately 0.9 m. Because of its shallowness and high light transparencies, most of the bottom is covered by mats of filamentous green algae (i.e. *Stigeoclonium* sp., *Oedogonium* sp., and *Spirogyra* sp.) that grow extensively following mouth breaching by lowering the water level thus leading to increased light availability on the sediment.

The Van Stadens catchment is largely covered by shrub-thicket vegetation with some areas covered with forests, however some areas of the catchment are used as farmland primarily for dairy and chicken rearing. A significant portion of the catchment is characterised by very steep gorges of Table Mountain Quartzite that is covered by fynbos in the upper and middle regions with valley-bushveld thicket near the coast. The forestry company Sappi uses a quarter of the upper catchment for silviculture. Two dams have been built on the Van Stadens River with a total capacity of approximately 0.47 x10^{6}m^{3}. Unlike the Maitland estuary that receives reduced light penetration at depth when the estuary is closed, the Van Stadens estuary when full is characterised by high water clarity at depth from the mouth right up to the estuary head. Light reaches the bottom at all depths (approx. 2-3m), however there are no mats of filamentous algae growing on the bottom. Mean estuarine water depth is approximately 3m with a maximum depth of 8m at its deepest point. There are sparse macrophyte stands of *Phragmites* along the length of the estuary increasing slightly at the head of the estuary. Both systems are only open intermittently throughout the year and remain closed for the majority of the time.

As a result of their proximity, the two estuaries occur on similar geologic substrata and therefore tend to share similar catchment vegetation types. Whereas these two estuarine systems may have similar geologic histories there are however, distinct geomorphological differences that distinguish one estuary from the other. It is because of these differences that these two estuarine ecosystems were selected as study sites as this will make them very suitable for comparative study in terms of their physical and chemical attributes including their biological composition. In addition both estuaries are popular tourist destinations for swimming, fishing, and boating and therefore need to be managed for recreational purposes.

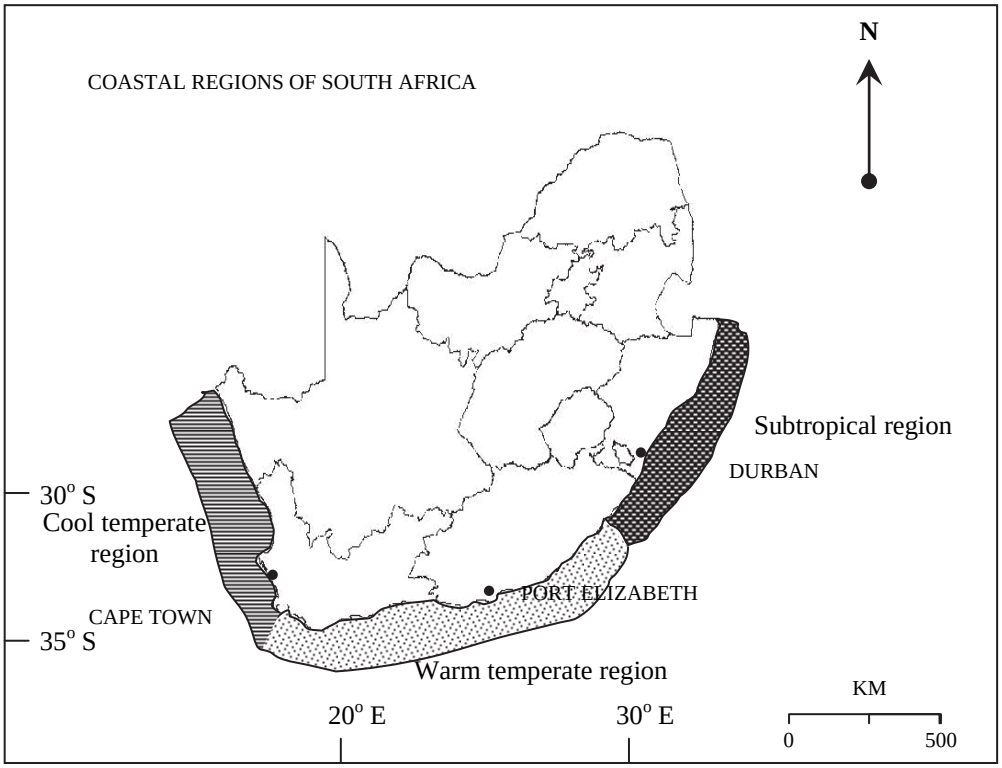


Figure 1. Map of South Africa showing the three geographical regions of the coastline. (Modified after Whitfield 1992).

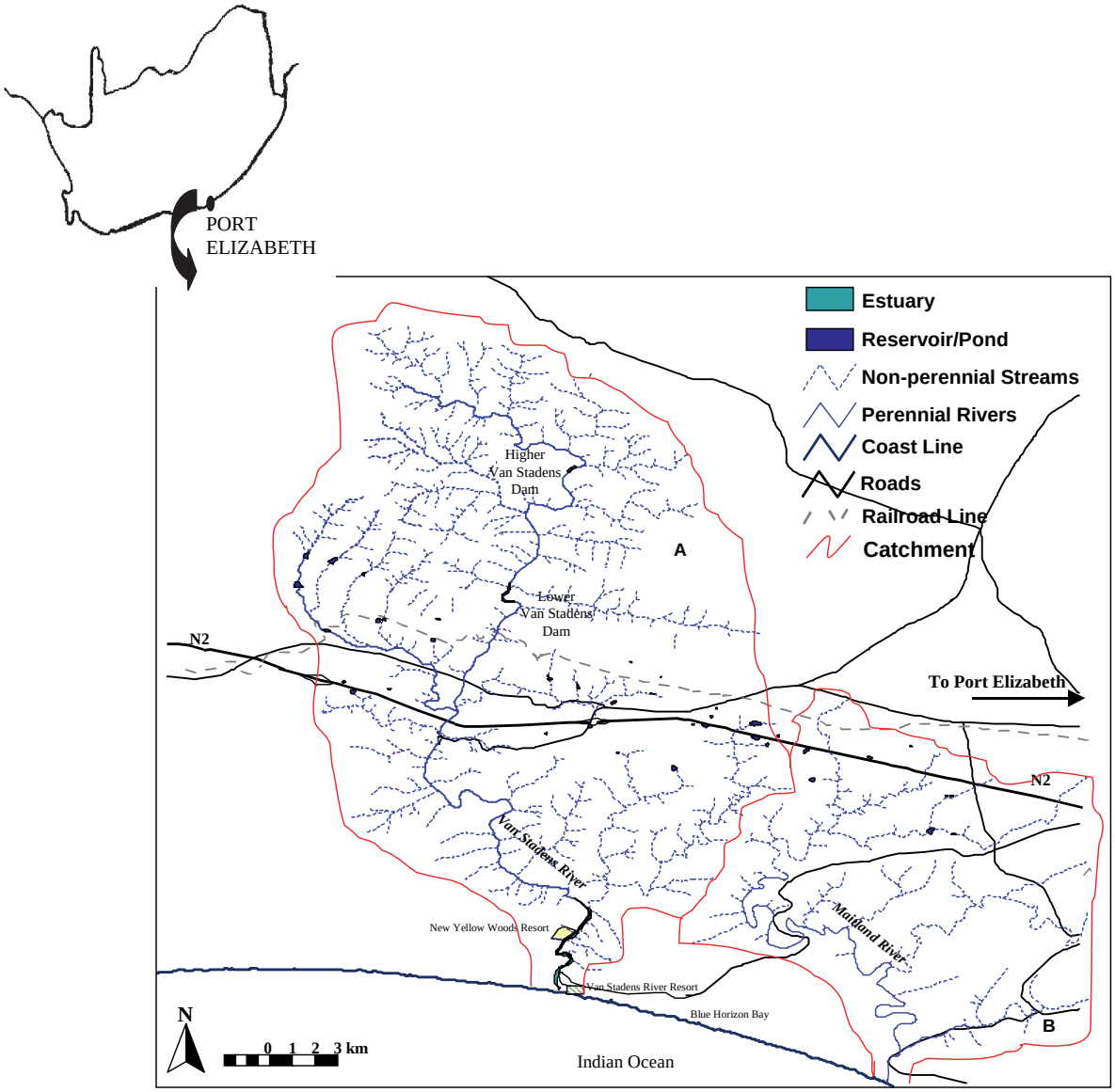


Figure 2. Map of South Africa (insert) and the catchments of the (a) Van Stadens River and (b) the Maitland River.

4. METHODS AND MATERIALS

4.1 Sampling Period and Protocol

The duration of the study was three years from April 2001 to April 2004. The sampling regime included monthly sampling and each seasonal cycle had intensive quarterly surveys that included 5 to 10 day daily sampling. The sampling strategy for monthly sampling attempted to adhere to regularly spaced monthly intervals where possible and when changes in mouth conditions (i.e. mouth breaching event) dictated. Sampling conducted on a quarterly basis was spaced as far as possible at regular 3-month intervals or as the mouth conditions dictated.

4.2 Sampling and Primary Production Assay Methods

4.2.1 Abiotic variables

Physical and Chemical Parameters:

Monthly measurements for incident light using a *LiCor* 1000 Data Logger linked to a *LiCor* 192 4π spherical quantum sensor, light transparency using a 30cm black and white Secchi disk, temperature, conductivity, pH, and salinity were measured using a YSI 650 MDS logger & sonde and were taken at each of the five sampling stations from just below the surface, at 0.25m and subsequently at 0.5m intervals. River discharge was determined on a quarterly basis in the upper, middle and lower parts of the catchment using a (Marsh-McBirney FlowMate Portable electromagnetic flow meter). Replicate estuarine water samples for chemical analysis were taken at all five stations along the length of the estuary just below the surface and 0.3-0.5m above the bottom sediment depending on estuarine water level. The water samples were analysed for the following macronutrients; Silicon as dissolved inorganic silicate (SiO_4), Ammonium (NH_4), Nitrate (NO_3), Nitrite (NO_2), total phosphorus (TP) and soluble reactive phosphorus (SRP) (Table 1). Samples for nutrient analyses were placed in cooler boxes maintained at low (0 - 5^{o}C) temperatures prior to analysis while in transit from the field to the laboratory and during quarterly field surveys samples for nutrient analysis were kept frozen (-20^{o}C) until analysis within a week. All analyses were carried out at the UPE Botany laboratory following standard chemical analyses (Strickland and Parson 1972, Wetzel and Likens 1991). To improve sensitivity of the spectrophotometric optical density readings a 5.0cm borosilicate cuvette was used for nutrient readings as recommended in Wetzel and Likens (1991) and Strickland and Parson (1972) for low concentrations.

Table 1. Measured macronutrients, range of concentrations measured and the detection limits of the selected methods used in the analyses during the study period.

Chemical Constituent	Range ($ug \cdot l^{-1}$)	Detection limit ($ug \cdot l^{-1}$)	Reference
SiO_4	5.1 – 246.3	20.0	Wetzel & Likens 1991
NH_4^+	0.03 – 4.99	0.04	Strickland & Parson 1972
NO_3^-	0.03 - 2.41	0.05	Strickland & Parson 1972
NO_2^-	0.01 – 0.1	0.01	Strickland & Parson 1972
TP	0.05 - 2.99	1.0	Wetzel & Likens 1991
SRP	0.05 – 2.00	1.0	Wetzel & Likens 1991

4.2.2 Biological variables

Phytoplankton Chlorophyll a

Monthly chlorophyll *a* (Chl *a*) analyses were carried out from replicate samples taken from the upper and lower portions of the water column at all stations along the length of the estuary. Replicate water samples were collected in 1-litre opaque polyethylene bottles and placed in cooler boxes on ice until further analysis in the laboratory (~2hr). Water samples were immediately filtered by serially fractionating into micro (>20µm), nano (2.7-20µm), and pico (1.2-2.7µm) size-fractions using a 20µm polyurethane Nitex mesh, Whatman GF/D and Whatman GF/C filter papers. The mesh and filter papers were then placed in vials darkened with black electric tape and filled with 15ml acetone and were allowed to extract for 20-24hr in a dark cold room kept at 4^{o}C. Mesh samples were sonicated for about 20-30min while all other filters were macerated using a mortar and pestle and cleared by filtering through a Whatman GF/C filter paper then read on a GBC650 - UV/VIS (ultraviolet/visible) spectrophotometer. A trichromatic method (Jeffrey and Humphrey 1975) was used to determine chlorophyll concentrations before and after acidification with 1N HCL.

Phytoplankton Taxonomic Identification and Enumeration

In addition to chlorophyll *a,* samples for phytoplankton cell densities were preserved for the determination of phytoplankton community structure. Samples for phytoplankton enumeration were collected in opaque 1-litre polyethylene bottles and immediately preserved with acidified Lugol's solution then returned to the laboratory and settled for a minimum of 72 hrs. Phytoplankton enumeration and identification was undertaken using a haemocytometer under an Olympus BX40F light microscope at 400X magnification. Sub-samples were also taken and preserved in dilute (4%) gluteraldehyde solution for the preparation of permanent mounts. Phytoplankton taxa were identified to the species level where possible, but certainly to the nearest genus using the following identification keys H. Bold & C. Wynn (1981), C. R. Tomas (1997), and W. Prescott (1989).

Microphytobenthic chlorophyll a

Monthly chlorophyll *a* analyses were carried out from samples taken from the upper 10mm of the sediment surface. The microphytobenthic chl-*a* samples were collected using a modified method by Rodriguez (1993) by using a plexi-glass corer (300mm long and 20mm internal diameter). All sediment samples collected were taken at a water depth of 0.5m or less. However, when the estuary reached its maximum water level this rule could not be followed as the water level was greater than 0.5m at some sites. A sample was collected by inserting the corer halfway then gently lifted by covering the underside with one hand while lifting with the other. A rubber plunger was inserted at the bottom of the corer by gently pushing it up until the overlying water was displaced exposing the sediment surface. A knife blade was used to section off the first 10mm of the core and placed into a polyethylene container with 30ml of ethanol. Each sediment sub-sample used to extract chl *a* was weighed in order to express chl *a* concentration in terms grams of sediment. To complete the chlorophyll *a* extraction samples were placed in a dark cold room at 4^{o}C overnight (~24hr) before further analyses were run. Chlorophyll *a* analyses were completed by clearing the samples by filtering them through a Whatman GF/C filter paper and

read on a GBC650 - UV/VIS (ultraviolet/visible) spectrophotometer. From tests run earlier comparing the use of a spectrophotometer and a high performance liquid chromatography (HPLC) they showed that there was a significantly close correlation (N=15, r^2 = 0.935) between results generated from a spectrophotometer and an HPLC such that subsequent benthic sediment chl-*a* analyses were carried out using a spectrophotometer.

4.2.3 ^{14}C Preparation and Analysis Methods

Phytoplankton Primary Production Experiments

In situ phytoplankton productivity studies were carried out in both the Maitland and Van Stadens according to a modified Strickland and Parson ^{14}C method (1972). Phytoplankton productivity experiments were carried out once every season over an annual cycle. Efforts were made to include a particular mouth condition, however this was not always achieved. Subsamples of estuarine water were placed in 250ml biological oxygen demand bottles (BOD) whereupon a known amount of a carbon tracer ($NaH^{14}CO_3$) was added to the samples and then incubated at depth for 4hr. Incubation bottles were suspended at depths corresponding to 30% (bottom) and 80% (top) of the surface radiation at each station. Samples were placed at each site along the length of the estuary. Termination of the experiment was accomplished by filtering samples into three size-fractions microphytoplankton (>20μm), nanophytoplankton (2.7 – 20μm) and picophytoplankton (1.2 - 2.7μm) size-fractions for the determination of productivity of each group.

Determination of dissolved inorganic carbon (DIC)

In determining DIC five (250ml) estuarine water samples were collected from the sites where primary production experiments were conducted in both of the estuaries. These samples were titrated with a 0.1N HCl solution following a modified method by Skirrow (1965). The titration was carried out until the point of inflection (4.0) where upon the added volume of acid indicated the proportion of bicarbonate molecules in solution (~0.5-1.0ml). Dissolved inorganic carbon was then calculated as follows:

$$DIC = V \cdot N \cdot 12 / v'$$

where, DIC = dissolved inorganic carbon (mg l^{-1})
V – volume of acid (ml)
N – normality of acid
12 – conversion from mmol to mg
v′ − volume of sample filtered (l)

Primary Production Assay

In each estuary, 5l of estuarine water from two depths, near the bottom (0.3m) and just below the surface (0.3m) were sampled using an opaque water sampler. The collected water was carefully mixed then poured into a total of 8 X 250ml glass bottles per station and placed in dark cooler boxes until inoculated with ^{14}C isotope. One of the 4 bottles for each depth per station was completely wrapped in heavy-duty aluminium foil to serve as a dark control bottle and a second bottle was used as a control blank, both were processed similarly to the inoculated samples. The bottles were attached to an anchored floatation device that ensured that the bottles were held stationary.

For incubation studies, all bottles were inoculated with 500μl $NaH^{14}CO_3$ to give a specific activity of 3.7MBq ml^{-1} then quickly as possible returned to the depths where the water was collected (Campbell & Bate 1986, Froneman 2000a). Immediately following inoculation and following a 3 – 5 minute equilibration period, a sub-sample of 10ml was taken for the purpose of determining initial radioactivity background levels. From the 10ml sub-sample, 2ml was pipetted into a scintillation vial. To this amount 13ml of Ultima Gold scintillation cocktail was added. The remaining 8ml was filtered and the filter rinsed in 0.5ml concentrated HCl. To this amount, 15ml of Filtercount was added to dissolve the filter. To the filtrate, 1ml of concentrated HCl was added followed by the addition of 10ml of scintillation cocktail. These samples allowed for the determination of 'time zero' background levels, which were deducted from the values of treatment samples.

The sampled water was then allowed to incubate for about 4 hours. At the end of the incubation period bottles were brought up to the surface and quickly placed in dark environmentally controlled boxes then immediately taken to the laboratory for further processing. The incubation was terminated by immediately filtering the water. In order to determine production of different size fractions of phytoplankton, 50ml of water were serially filtered at very low filtration pressures (vacuum <2.5cm Hg) through a 25μm (polypropylene), a 3.0μm glass-fibre filter and 1.2μm Millipore membrane filter (Froneman 2000a). Each filter paper containing labelled phytoplankton and filtrate were treated separately. Following filtering, the filters were quickly rinsed in 0.5 – 1ml concentrated HCl in order to remove any non-biologically labelled carbon. All treatment samples were processed as mentioned above. Disintegration per minute (DPMs) was counted in a Beckman liquid scintillation counter. Disintegrations per minute were converted to daily productivity rates using the following equation:

$$\text{Primary Production (Pp)} = (DPMs \cdot DIC \cdot 1.06) / (DPMi \cdot T) \cdot 1000$$

where, Pp = rate of primary production (mg C m^{-3} h^{-1})
DPMs = sample disintegrations per minute
DPMi = initial disintegrations per minute
DIC = dissolved inorganic carbon (mg l^{-1})
T = incubation time (hours)
1.06 = ^{14}C: ^{12}C-carbon isotope discrimination factor
1000 = conversion from litres to m^3

Volumetric productivity values were converted to areal productivity values by depth integration and dividing by the incubation period (Subba Rao 2002).

All samples were counted using a Beckman liquid scintillation counter using the $H^{\#}$- method of quench correction. This method relies on the shifting of the peak in the energy spectrum of emissions (counts). The more quenching, the more the peak moves to the low energy range of the spectrum. This shift is monitored by the scintillation counter which calculates the $H^{\#}$ as a direct measure of the shift. Using standard quenched samples together with an unquenched standard, a quench correction curve is generally drawn up and used to calculate the counting efficiency of the sample using the $H^{\#}$. These are then counted in the scintillation counter and the counting efficiency of each sample determined using the

disintegrations per minute (dpm) given on the standards and the counts per minute (cpm) determined by the counter. Counting efficiency is given by the following relationship:

$$CE = cpm / dpm$$

where CE – counting efficiency
cpm – counts per minute
dpm – disintegrations per minute

It should be noted here that primary production experiments conducted during the study were carried out once every season over a complete seasonal cycle. The experiments were run on a once-off basis during these periods thus do not account for daily fluctuations in environmental variables normally experienced by microalgae over several days.

4.3 Groundwater Sampling

We monitored ground water seepage by establishing 2 pipes (semi-permanent 'water wells') at 5 sites along the length of the estuary on either side of the estuary where it was possible to gain access. However, the site nearest to the mouth had to be abandoned as it was continuously vandalised. The wells were constructed of 1.5 to 1.75 m long PVC with an internal diameter of 75mm. They extended to the depth of the water table where water samples were collected for analysis of macronutrients (i.e. nitrate-nitrogen, nitrite-nitrogen, ammonia-nitrogen, total phosphorus, and soluble reactive phosphorus). A 2.0m silicon tube (8.0mm in.d.) was used to siphon water from each well and then placed in acid washed 250ml borosilicate glass bottles and kept in a cooler until analysis at the laboratory (within 2hr of sampling). These were analysed as described above for water column macronutrient concentrations.

4.4 River to Sea Sampling

During quarterly visits surveys were carried out along a total of ten sites of the river and estuary from just below the lower Van Stadens dam right down to the mouth of the estuary including samples taken from the sea. Surveys included sampling for physical and chemical parameters as described above. In addition discharge measurements were determined at three points along the river during an open and a closed mouth event following the method of Gordon *et al.* (1992).

4.5 Nutrient Loading (LOICZ-Water Budget Model)

As a result of their small catchment sizes and intermittent river flow a number of rivers emptying into temporarily open/closed estuaries do not have flow gauges that monitor daily stream flows. The Van Stadens is one such estuary. We needed a hydrological model that required little environmental data input in order to determine nutrient loading rates into the estuary t

The Land-Ocean Interactions in the Coastal Zone (LOICZ) biogeochemical budget model is a simple mass balance calculation of specific variables within a defined geographic area over a defined period of time (Gordon *et al.* 1996). The model deals with fluxes of variables such as water, salt, sediment carbon (C), nitrogen (N), phosphorus (P), etc.) The LOICZ Biogeochemical Modelling Guidelines (Gordon *et al.* 1996) promote a single approach for constructing budgets to describe coastal marine environments for purposes of comparison with similar budgets (http://data.ecology.su.se/MNODE).

This approach has three parts to it and include:

1. What is the resident period of water movement through the system of interest?
2. What is total turnover time of nutrient elements (CNP) in the system of interest?
3. What can be inferred about system performance from the differences between the exchange of water and the movement of nutrients?

The theoretical framework and detailed guidelines for calculating flows of water, salt, N and P budgets are found in the LOICZ Biogeochemical Modelling Guidelines on the web page given above. The model used for determining budgets for the Van Stadens Estuary involved the use of a ONE Box Model. This model is normally used when a system of interest has the following general attributes:

a. It is horizontally and vertically uniform (i.e. small freshwater input vs. volume of system).
b. There is thorough mixing of the water column with the vertical discontinuities in salinity and density (i.e. persistent strong wind).

The budget and associated calculations were determined according to the following method.

Water Budget: - this establishes a budget of freshwater inflows and evaporative outflows to balance the water volume in the system.

Data Needs:	rate of river discharge (V_Q), precipitation (V_P), evaporation (V_E), groundwater (V_G) if important, sewage discharge (V_O)
Unit of measure:	$m^3 \cdot d^{-1}$
Calculation:	V_R (residual flux) = V_{out} - V_{in}
	$V_R = V_E - (V_Q + V_P + V_G + V_O)$

Salt Budget: Salt is conserved in the system, therefore the salt flux not accounted for by the salinities used to describe the freshwater flow in the water budget must be balanced by mixing.

Data Needs:	salinity of the system (S_{sys}), salinity of the adjacent ocean (S_{ocn})
Unit of measure:	psu (practical salinity units)
Calculation:	S_R (average salinity at the boundary) = $(S_{ocn} + S_{sys})/2$
	$V_R S_R$ is the salt flux carried by the residual flow

Salt must be conserved so the residual salt flux is brought back to the system through the mixing salt flux across the boundary ($V_X S_X$) via tides, wind and general circulation pattern.

$$V_R S_R = - V_X S_X$$
$$S_X = (S_{ocn} - S_{sys})$$
$$V_X(\text{mixing flux}) = -V_R S_R / S_X$$

N and P Budgets: All dissolved N and P will exchange between the system of interest and adjacent ocean according to the criteria established in the water and salt budgets. Deviations are attributed to net nonconservative reactions of N and P in the system.

Data Needs: concentration of dissolved inorganic N (nitrate, nitrite, ammonium) and P (phosphate) in the system (DIN_{sys}, DIP_{sys}), in the adjacent ocean (DIN_{ocn}, DIP_{ocn}), in the inflowing river water (DIN_Q, DIP_Q). If important in groundwater (DIN_G, DIP_G), some estimate of nutrient loading from sewage or other discharges (DIN_O, DIP_O)

Unit of measure: $\mu mol \cdot l^{-1} = mmol \cdot m^{-3}$

Calculation: $V_Q DIN_Q$ –riverine DIN flux, $V_G DIN_G$ –groundwater DIN flux, $V_O DIN_O$ – sewage DIN flux

$V_R DIN_R$ residual DIN flux

where: $DIN_R = (DIN_{ocn} – DIN_{sys})/2$

$V_X DIN_X$ –mixing DIN flux

where: $DIN_X = (DIN_{ocn} – DIN_{sys})$

$\Delta DIN = flux_{out} – flux_{in}$

$\Delta DIN = -(V_X DIN_X + V_R DIN_R + V_G DIN_G + V_O DIN_O + V_Q DIN_Q)$

Calculations for DIP were determined similarly as above.

Stoichiometric relationships: The assumption made is that non-conservative flux of DIP with respect to salt and water is an approximation of net metabolism (photosynthesis and respiration) at the scale of the system. The non-conservative flux of DIN approximates net nitrogen fixation minus denitrification.

Calculation: (p-r) –photosynthesis minus respiration

(p-r) $= \Delta DIP(C:P)_{part}$

(*nfix-denit*) –N fixation minus denitrification

(*nfix-denit*) $= \Delta DIN – \Delta DIP(N:P)_{part}$

where: $(C:P)_{part}$ and $(N:P)_{part}$ are the ratios of organic matter reacting in system.

For the Van Stadens estuary freshwater flow and nutrient inputs from groundwater and other sources (e.g. sewage) were not considered for the calculations since groundwater flow estimates were not available and there is no direct sewage outflow into the system. Groundwater nutrient concentrations were measured during the study (see Chapter 5, subsection 5.1.2) but were variable and considered negligible and were not included in the budget calculations. Future studies undertaken in the Van Stadens however, should attempt to assess the contribution of groundwater to the water flow budget.

5. RESULTS

5.1 Van Stadens Estuary

5.1.1 Physical and Chemical Parameters

Closed mouth phases of the estuary were characterised by strong horizontal and vertical salinity gradients especially when the closed phase was accompanied by strong overtopping events. Depending on the duration and intensity of the overtopping events these marked stratifications can, however be short-lived as the winds that generate and push the swells over the sand bar creating these pcynoclines also assist in mixing the water column, hence aiding in rapidly eroding them. These very dynamic, hydrologically and chemically driven interactions together with very low river inflow tend to raise the overall water column salinities to levels that range from 18(ppt) in the upper reaches to 20(ppt) in the lower reaches. Although freshwater input from the river is generally low during these closed mouth periods, it appears however, that even flows below $0.1m^3s^{-1}$ can be sufficient to maintain horizontal and vertical stratification for periods ranging from days to several weeks. Periods of heavy river discharge are generally characterised by substantially low water column salinities with values decreasing to below 0.5 (ppt) at the mouth of the estuary (Figure 3b). As the flooding river ebbs a measurable saline water wedge is able to penetrate up into the estuary re-establishing horizontal and vertical stratification. Maintenance of high salinities within the estuary depends on prevailing regional climatic weather patterns, as high marine swells along St Francis Bay and the coastline adjacent to Van Stadens river mouth tend to be associated with low-pressure cells that produce high waves increasing the frequency of overtopping events.

Water column temperatures closely tracked air temperature patterns characterised by high temperatures during the summer and low temperatures in winter (Figure 3b). Water column transparencies in the estuary were high during the early months of the first year of the study except during the months of August, September and November 2001. Those months coincided with periods of high river discharge that resulted in light attenuation coefficients greater than $3.0m^{-1}$ (Figure 3a). The release of water from the lower Van Stadens dam by the Nelson Mandela Metropolitan Municipality in September 2001 released large volumes of water that scoured and carried high levels of suspended sediments resulting in the highest levels of light attenuation observed throughout the study period. In August, during the second year of the study, the mean monthly rainfall exceeded 8mm yet light attenuation remained below $2.0m^{-1}$. Even though the rainfall pattern over the next two years of the study showed peaks of >4mm (mean daily rainfall, SA Weather Service) during autumn and winter months, light transparencies remained high exhibited by low light attenuation coefficients of less than $1.0m^{-1}$ throughout that period.

Large volumes of clear marine water came in through overtopping during the early months of 2003, diluted the freshwater and increased light penetration to the bottom sediments. Increased light penetration during the summer season coupled with low river inflow and a lack of turbulent generated water movement at depth sufficient to shift bottom sediments, permitted growth of extensive mats of a filamentous macroalga and a submersed macrophyte (*Ulva* sp., and *Potamogeton pectinatus*) respectively. Discharge in the Van Stadens estuary was measured during five visits at the upper river sites below the lower Van Stadens dam, the estuary head and at the mouth when it was open.

River flow in March 2002 was at its lowest and insufficient to breach the mouth, however it was adequate to maintain mesohaline conditions throughout the estuary (Figure 3b). Increased river discharge in July 2002 coincided with the first rains in late autumn and early winter such that rainfall measuring >10mm in late winter produced peak flows in September both in the Van Stadens and the Maitland estuaries (Figure 4). High levels of river flow during this period had a marked influence on the sediment bed of the two estuaries as severe scouring and sand deposition was observed particularly at the Maitland estuary (Plates 1 & 2 showing the Van Stadens and Maitland estuaries before and after the flood event).

Following the freshwater inflow sharp declines in salinities were recorded for both estuaries indicating oligohaline conditions. The increased river flow caused the estuary mouth to breach and remained open for six to eight weeks during which time it functioned as a tidal estuary. Low river inflow and an increase in the frequency of strong gale force westerly-winds caused wave movement and the deposition of sediment in the mouth area closing the mouth. When the mouth was closed during summer, increased water level and good light transparency promoted the growth of benthic macroalgae and submersed macrophytes. This resulted in increased oxygen concentrations to saturation levels. Although dissolved oxygen varied over the study period, a One Way ANOVA showed that there were significant differences in dissolved oxygen between months ($F=190.815$, $P<0.001$). Conductivity closely tracked salinity concentrations over the study period with minimum concentrations recorded during the 2002 flood event whereas maximum levels occurred following a nine-month period of mouth closure.

Macronutrient levels in the Van Stadens estuary during the study period generally remained low (phosphates <0.5 $\mu g.l^{-1}$ & nitrogen compounds <1.0 $\mu g.l^{-1}$) with the exception of the periods (August 2001, August to October 2002 and July to August 2003) where river inflow was high (Figures 4a & 5). Estuarine monthly nutrient concentrations were closely related to periods of increased river inflow with above average concentrations recorded during peak discharges in September 2002 and August 2003. A One-way ANOVA test showed that there were significant differences in nutrient levels between months in 2002 ($F=54.486$, $P<0.001$). During the periods of mouth closure (i.e. April and May 2002) phosphate concentrations reached their minimum levels, this was however, not observed for the similar period in the following year although the lowest concentrations for that year were measured in January and February 2003. Nitrate concentrations reached their maximum in May just prior to peak discharges observed in September 2002. The elevated levels of nitrates in May and July were attributed to inputs from the river inflow since discharge rates during that period were above $0.5m^3s^{-1}$. However, when river discharges exceeded $1.0m^3s^{-1}$ nutrients were washed out of the estuary. Periods of low river inflow together with closed mouth conditions were characterised by very low nutrient concentrations. Low rainfall in the latter part of 2003 resulted in low to no flow entering the estuary such that the mouth of the estuary remained closed for over 12 months. During that period nutrient levels (nitrate and phosphates) remained low in sharp contrast to ammonium levels that were higher in early spring and summer (Figure 5).

Plate 1. The Van Stadens Estuary showing the middle reaches A & B, and lower reaches C & D before and after the winter floods of 2002.

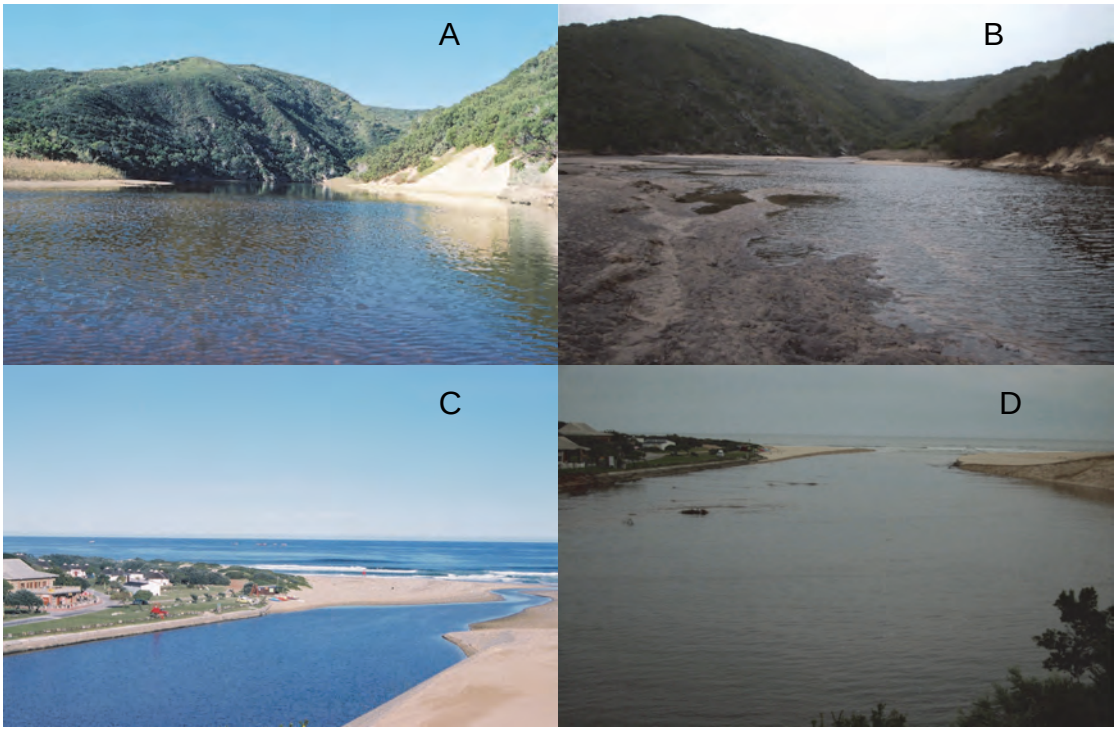
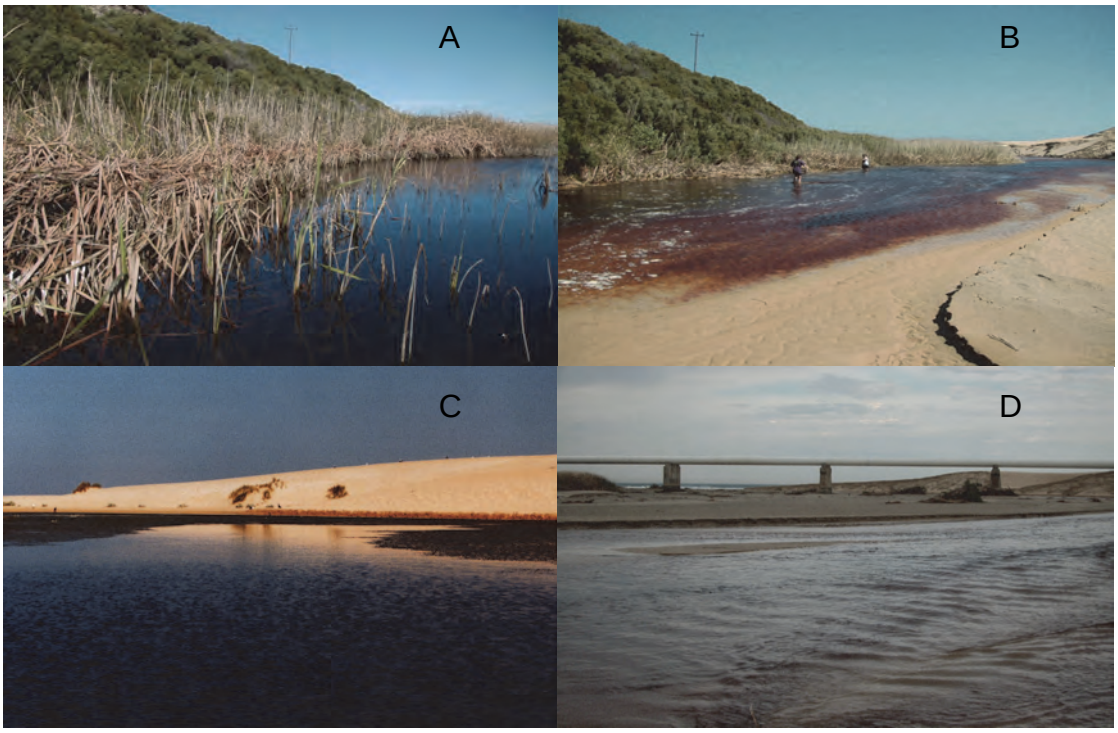


Plate 2. The Maitland Estuary showing the middle reaches A & B, and lower reaches C & D before and after the winter floods of 2002.



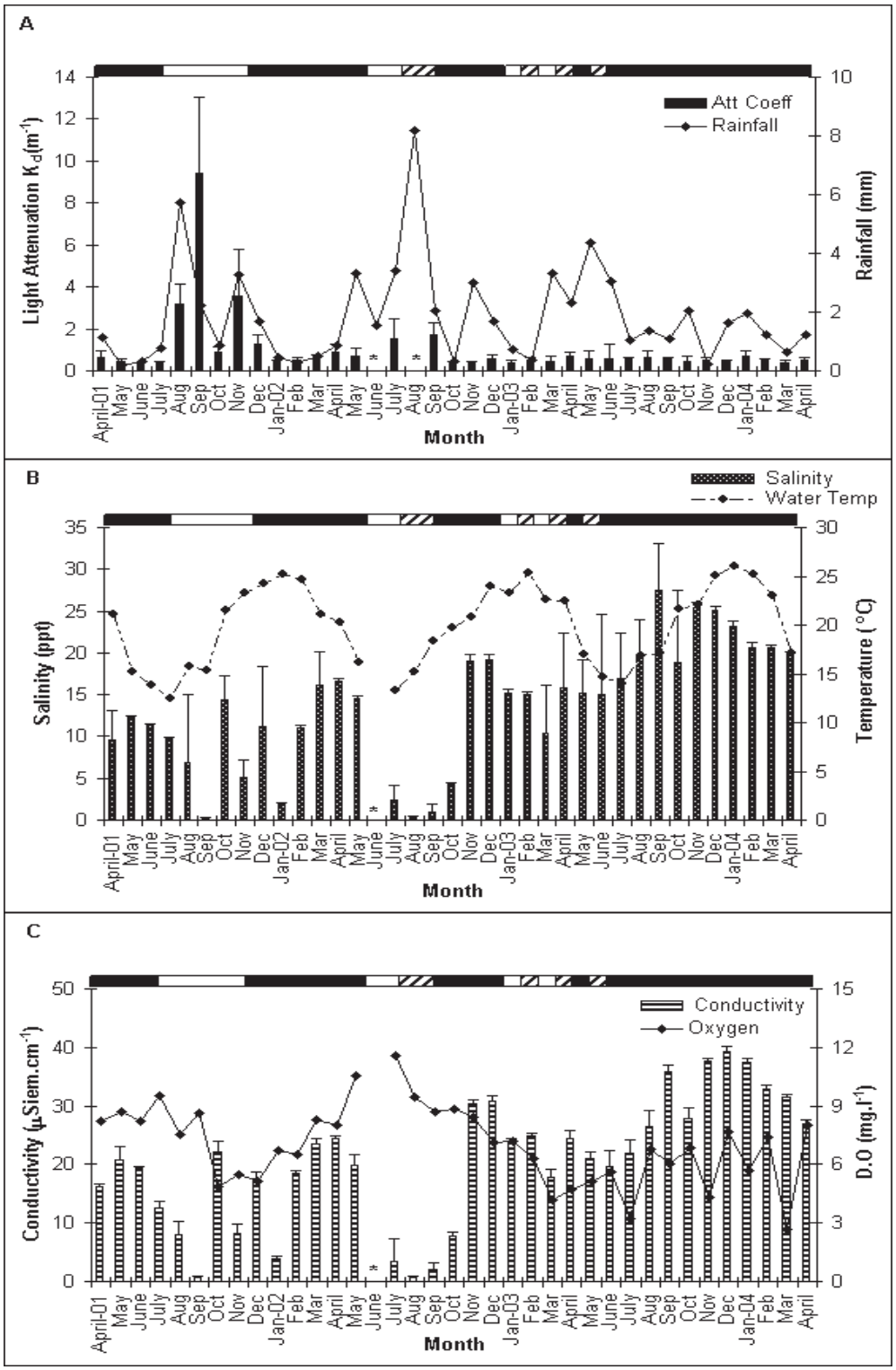


Figure 3 Van Stadens Estuary light attenuation coefficient and rainfall – (a), salinity and water temperature – (b), and conductivity and dissolved oxygen – (c), for the period April 2001 to April 2004. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open and hatched bar = over topping. An * denotes months with no records.

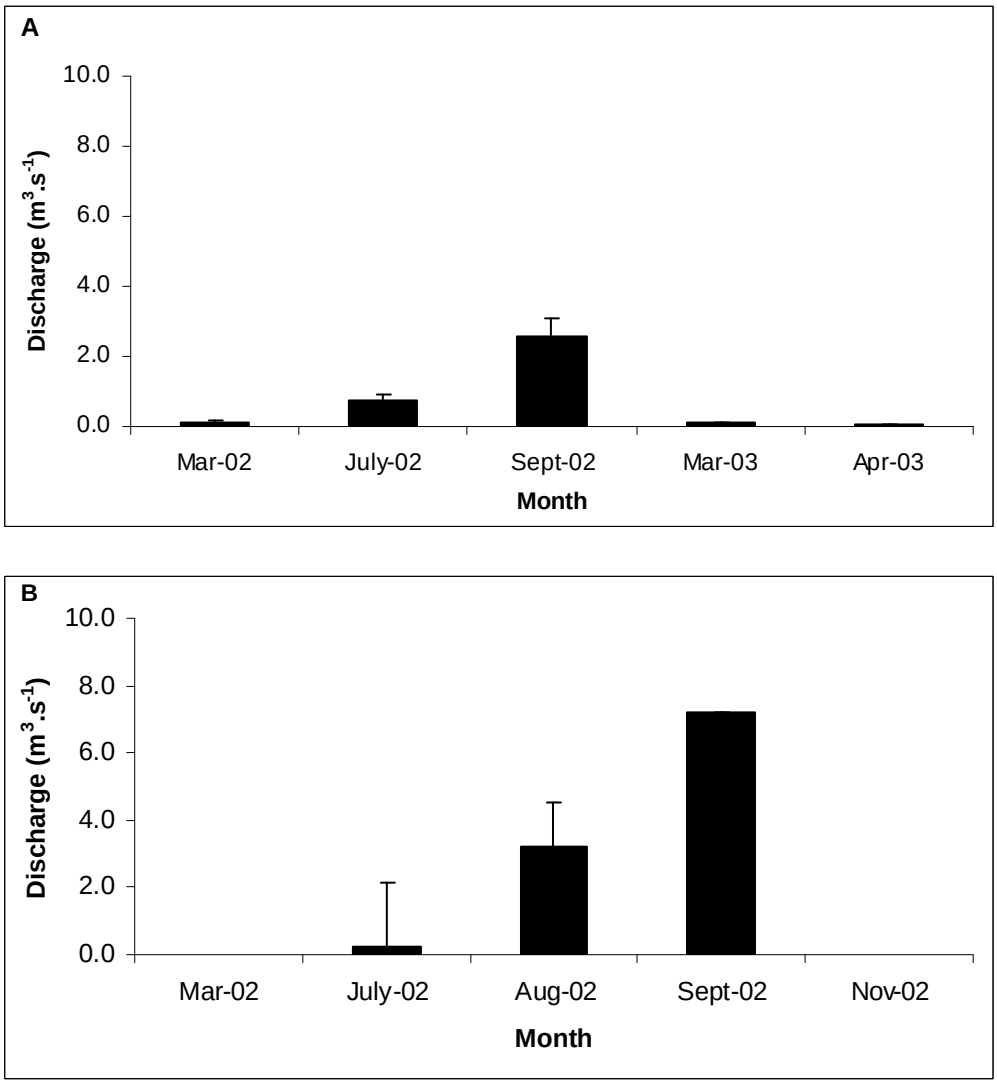


Figure 4. Discharge measurements of stream in-flow taken at (a) Van Stadens and (b) Maitland estuaries between November 2001 and April 2003.

There were no clear differences in nutrient concentrations between the top and bottom layers of the water column or between the upper and the lower reaches of the estuary. As a result of no site and depth differences following a One-way ANOVA test ($P > 0.05$) nutrient data for the sites were vertically integrated and pooled. The even distribution of nutrients in the water column and throughout the length of the estuary suggests a well-mixed estuary even when pycnoclines were well established. Wind induced mixing of the water column seemed to ensure the disruption of any established vertical or horizontal stratification. Results from data taken from daily sample runs showed that even when vertical stratification persisted over several days nutrient concentrations remained similar both vertically and horizontally along the length of the estuary (Figure 5). This means that although strong density differences may exist between the upper and lower part of the water column the availability of nutrients appears however, not to be restricted by this density barrier. Silicon concentrations were significantly higher in the first year compared to the second year of the study ('t'-Test $\alpha=0.05$, $P<0.0083$). The low silicon concentrations in the latter part of the study may have been the result of low rainfall during that period (see Figure 3a.).

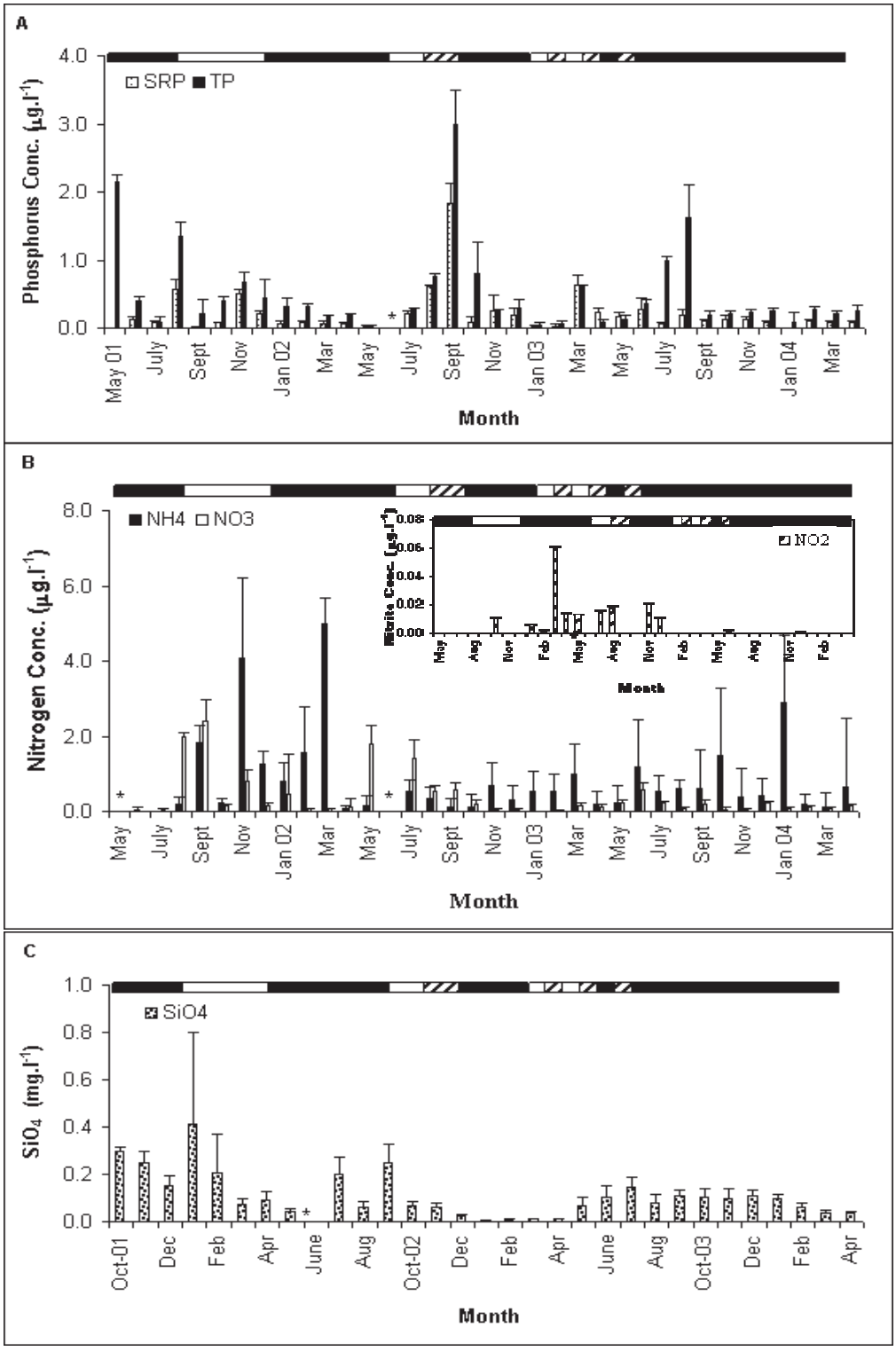


Figure 5. Van Stadens Estuary nutrient concentrations for the period May 2001 to April 2004, (a) total phosphate – TP and soluble reactive phosphate – SRP, (b) ammonium – NH_4 and nitrate – NO_3, and (c) dissolved silicon – SiO_4 from October 2001 – April 2004. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open and hatched bar = over topping. #—denotes values below detectable limits, * not sampled. Insert is nitrite – NO_2 for similar period.

Although generally low and wide-ranging, macronutrient concentrations sampled along the Van Stadens River were higher than those in the estuary particularly following periods of rainfall. Possibly because there is no water release from the dams in the upper catchment and any flow in the river is entirely due to overspill the nature of nutrient input into the estuary will be variable. An area along the river that may be a possible source of nutrients is just below the tall bridge of the N2 national road, where phosphates levels were almost double the levels recorded in the estuary. Nitrate and ammonium concentrations showed elevated levels just below the Van Stadens River lower dam then gradually decreased further down the river until the mouth of the estuary. This does however suggest that nitrogen compounds possibly undergo a series of biogeochemical cycling processes as they are carried down along the river and perhaps may not reach the estuary or very low quantities reach the estuary.

The chemical composition of phytoplankton is defined by the ratio of their chemical make up which normally is given as C106:N16:P1 (Redfield 1958). The proportion of which reflects their physiological requirement that they require from the environment and these are generally referred to as the Redfield ratio (Wetzel 1983, Lobban and Harrison 1997). These values give a gross indication as to the supply and availability of macronutrients particularly nitrogen and phosphorus in the water such that plant or algal growth and development will be limited by the nutrient available in the least amount. Redfield ratios were determined for the Van Stadens estuary. When plotting total dissolved inorganic nitrogen (DIN) to total phosphate (TP) ratios for the study period, it appeared that, except for a few cases in September 2001, March & May 2002, and January 2004, the estuary was always nitrogen limited (Figure 6). Mouth breaching did not appear to greatly influence the ratios except for the period in September 2001. It was only on three occasions during periods of low river inflow that the estuary was phosphorus limited. Although nitrogen levels were generally higher in the adjacent sea (Figure 12), periods of marine overwash did not appear to shift the estuarine N:P ratios. Under closed mouth conditions nitrogen compounds may be augmented by water from the sea during overtopping events and also from the large fish fauna that use the estuary as a nursery (Strydom 2003). The open mouth phase maintains continuous exchange with the marine environment such that nutrient availability is governed by the interaction between fluvial flow and tidal influences.

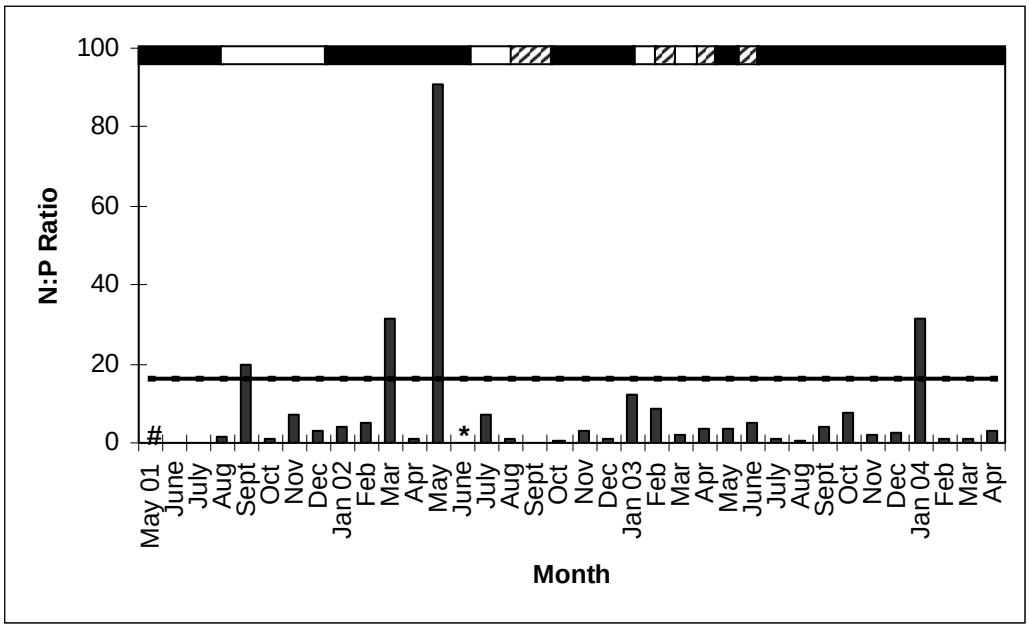


Figure 6. Van Stadens Estuary N:P ratios for the period May 2001 to April 2004. Horizontal line denotes the 16:1 Redfield ratio of Nitrogen & Phosphorus. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open, and hatched bar = over topping. Note: June & July 01 values were 0.08 & 0.24 respectively and September 02 value was 0.22. #—denotes values below detectable limits and *denoted not sampled.

5.1.1.1 Groundwater

Groundwater was sampled when sufficient water was available in the wells sunken along the length of the estuary. Water samples were analysed for dissolved macronutrients (i.e. TP, SRP, NO_3, NO_2, NH_4, & SiO_4) as described in Chapter 4 (Figure 7). Water depth in the estuary influenced the availability of groundwater. Presence of water in the wells was normally indicated by water depths of above 4m at the deepest point (site 1 Figure 2) and of approximately 1.9m at the shallowest point (site 5) of the estuary. On the other hand breaching of the estuary mouth caused substantial loss of water volume in the estuary leading to a reduction in the water depth. This loss in water subsequently led to a reduction in the height of the water table thus decreasing the water in the wells. Extended periods of closed mouth conditions (approx. 60% of the time in 2002) permitted the water table level to increase sufficiently for water to be present in the wells enabling sampling of groundwater. Even though the estuary mouth had been closed for periods ranging from two to three months water in the wells was insufficient for sampling suggesting perhaps, that subsurface flow was generally away from the estuary (influent), although further studies would have to be undertaken to examine this aspect.

Preliminary results of groundwater macronutrients in the first year of monitoring (May 2002 to April 2003) were varied owing to the absence of groundwater during some months. There were no clear indications that groundwater was a source of nutrients into the water column, however groundwater nutrient concentrations were significantly higher than the water column concentrations (Figures 5 and 7) ('t'-test $p<0.05$). When groundwater was available in the wells total phosphorus concentrations remained consistently high, averaging

1.39mg.l^{1} in the first year and 3.73mg.l^{1} in the 2nd year of monitoring during the the periods of mouth closure.

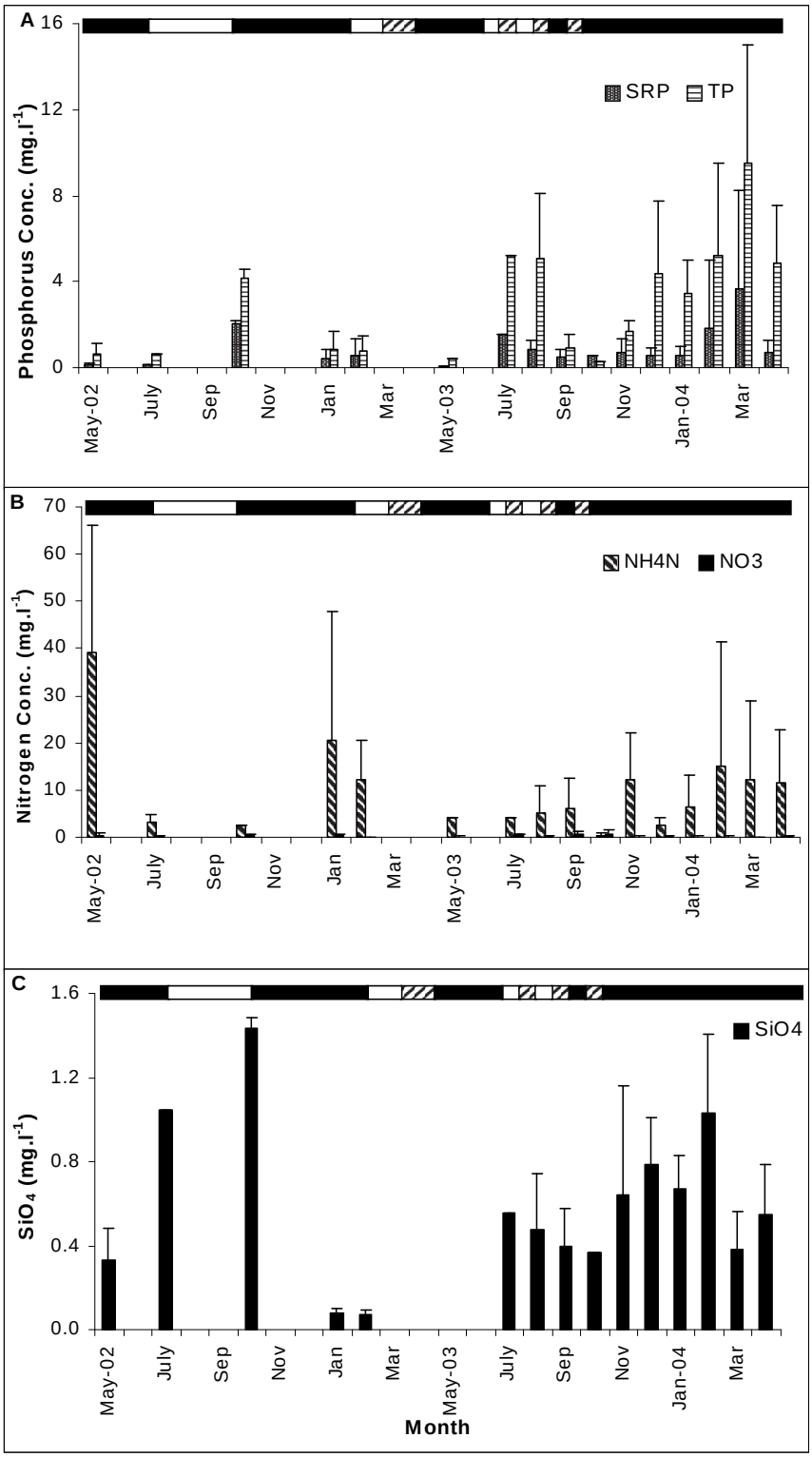


Figure 7. Van Stadens Estuary groundwater nutrient concentrations for the period May 2002 to April 2004, (a) total phosphate – TP and soluble reactive phosphate – SRP, (b) ammonium – NH_4 and nitrate – NO_3, and (c) dissolved silicon – SiO_4. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open, and hatched bar =

over topping. Blank months denote periods not sampled because of insufficient water in the wells.

Groundwater nitrate concentrations remained low (<0.3mg.l^{-1}) throughout the duration of the study showing no variation with the change in mouth condition. In contrast, ammonium levels were always high during periods of low flow and closed mouth conditions (Figure 7b). Groundwater nitrite concentrations were below detectable levels throughout the monitoring period and therefore are not reported here. Following the mouth-breaching event in September 2001 groundwater silicon levels reached the highest levels (1.5mg.l^{-1}) observed over the study period. In 2003 the mouth closed in April and remained closed until December 2004 making it the longest period of mouth closure since the monitoring began. Water in the wells was available as early as July 2003 although water depth in the estuary reached its maximum of approximately 4.5-5.0m in March 2004. Septic tanks used by the Van Stadens Resort establishment are located on the north east side of the resort and therefore drain into the surf zone in the marine environment. They were considered not to be a significant source of nutrient input to the groundwater (Van Stadens Management).

5.1.1.2 River and Estuarine Discharge and Nutrients

River discharge estimates taken every quarter were made along several sites on the Van Stadens River and at the head and mouth of the estuary. Mean river discharge rates during the report period were characterised by high flows during the late winter and early spring coinciding with periods of high rainfall in the catchment. Low flows were recorded during late summer and early autumn. During periods of peak flow (>3.0m^3 s^{-1}), in September, the mouth opened and reached its maximum width of approximately 100m, whereas, at flows less than 1.0m^3 s^{-1} the width ranged between 20-25m. When discharges were 0.1m^3 s^{-1} or less the mouth remained closed or showed a semi-closed state whereby an immeasurable (with present equipment) amount of water would flow out without any seawater entering the estuary (Figure 4a). Wind-generated swells especially during the closed-mouth phase augmented water volumes in the Van Stadens estuary bringing in substantial volumes of saline water that in turn would promote breaching when critical estuarine volumes were reached. These hydrological episodes of overtopping and breaching occur quite rapidly, and are short-lived taking place within hours, particularly overtopping, making it difficult to measure and quantify the volumes of water entering and leaving the estuary.

Since the mouth condition record was kept estuary mouth dynamics have varied over the study period. However, a pattern of mouth breaching and closure emerged during the wet and dry periods of the year. Since the study began the estuary was generally closed for approximately 60%, open for 26% and experienced overtopping for about 14% of the time (Figure 8). Erratic local weather patterns in 2002 contributed to a less predictable frequency of mouth breaching adding to the complex hydrological and ecological functioning of the estuary. Although overtopping accounts for only 14% of the time this event is important in augmenting estuarine water volumes including the introduction of clear saline water and possibly nutrients.

Dam releases were an exception rather that the rule as the only time during the three year study period water was released into the Van Stadens River was for the purpose of scouring the lower dam which took place in September of 2001 (pers. comm. Water Manager Nelson Mandela Metropolitan Municipality). Releases from the dam did not introduce appreciable amounts of macronutrients into the river and estuary. River to mouth surveys were carried out over a two-year period on a quarterly basis. Total phosphorus and soluble reactive phosphorus concentrations from the upper catchment were not much higher than those measured in the estuary during March 2002; however nitrate and ammonium levels were higher when compared with estuarine concentrations (Figure 5). Other quarterly surveys carried out during the study showed similar results. Except for the nutrient and discharge measurements taken during the quarterly surveys there were no other discharge measurements. Therefore, river discharge measurements that resulted in mouth breaching during other periods of the year were not measured.

During quarterly survey intervals when discharge was measured concurrently with nutrients, total phosphates in the Van Stadens and Maitland estuaries were positively related with an increase in river inflow (R^2 = 0.903 and 0.996 respectively). Although these data have very few sampling points (N=5, the number of times there was flow in the Van Stadens River over a two year period), they indicate a strong pattern of the river as a source of nutrients. It further shows that the amount of nutrients entering an estuary is influenced by the magnitude of river inflow. Nitrate concentrations were not strongly influenced by river inflow during the same period although from monthly nutrient concentration measurements nitrate levels were higher following mouth breaching events. Increased freshwater inflow did have a significant influence on mean estuarine nutrient concentrations during periods when the mouth breached. Estimates of river and estuarine discharge together with nutrients measured in the river, estuary and the ocean were used to determine loading rates into the estuary using the LOICZ biogeochemical model (Gordon *et al.* 1996). The calculations were determined only for the Van Stadens estuary since river discharge data were not available for the Maitlands estuary.

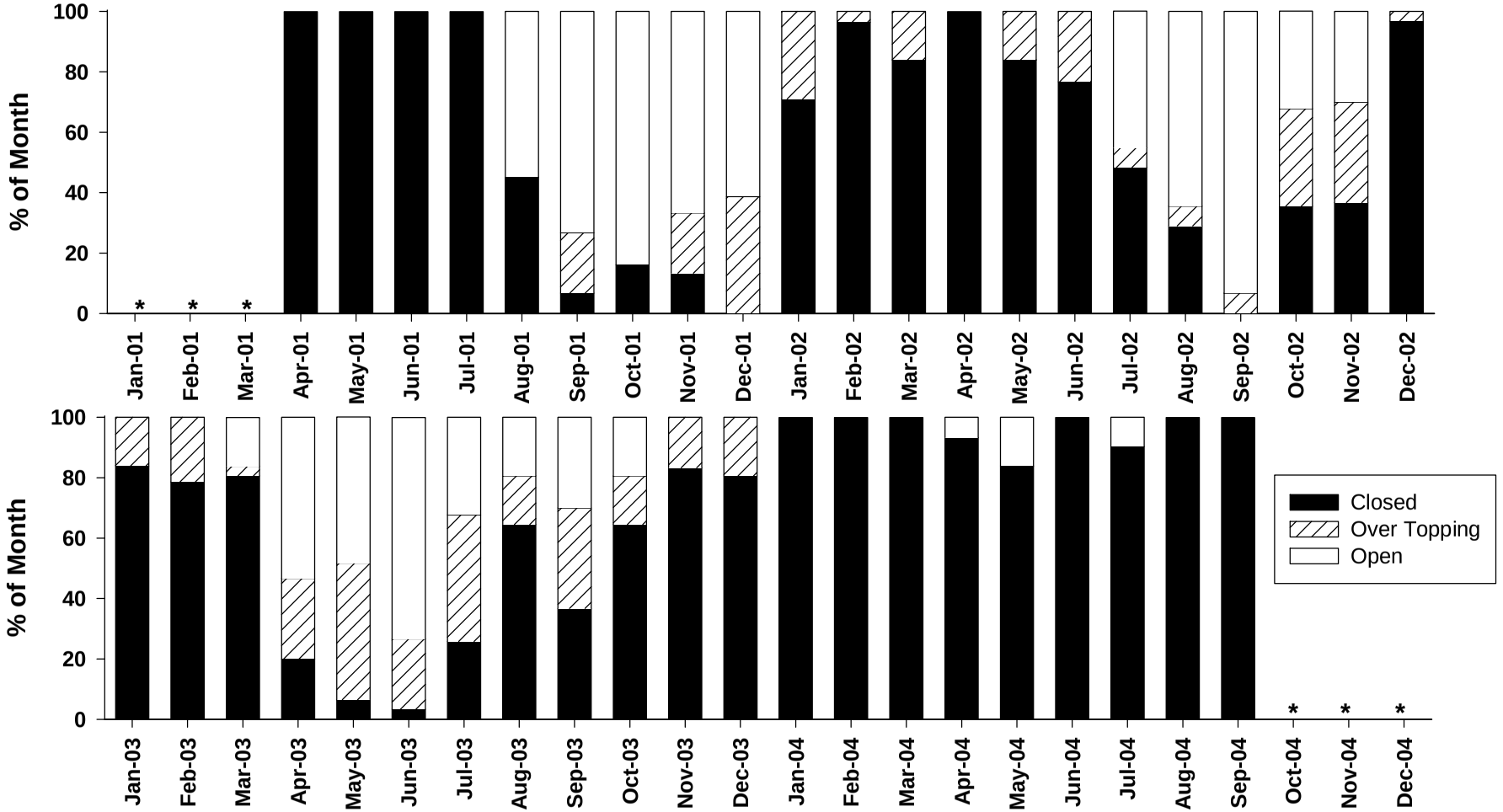


Figure 8. Van Stadens mouth condition from April 2001 to December 2002 (top panel) and January 2003 to September 2004 (bottom panel) expressed as percent of time spent closed (solid bars), over topping (hatched bars) or open (open bars), by month.
A * denotes months with no record.

5.1.1.3 Nutrient Loading (LOICZ Biogeochemical Model)

Water and salt balance

Water and salt budgets are provided for this estuary for an annual cycle and data used are given in Table 2. Rainfall patterns are variable along the Eastern Cape coastal region with rain occurring mostly during the months of March to June, and from August to November. Since the LOICZ biogeochemical water and salt budget model is centred on hydrographic fluxes between the estuary and ocean it is therefore dependent on salinity gradients between the two (Gordon *et al* 1996). Because the estuary is temporarily open/closed, water and salt fluxes and associated calculations were based on an open mouth condition in order to fulfil the model requirement of accounting for mass boundaries between the estuary and the sea.

The results of the water and salt balance are illustrated in Figure 9. The residual water (V_R) and salt fluxes ($V_R S_R$), as well as the exchange flows of salt water (V_X) and salinity [$V_X(S_{ocn}-S_{syst})$] are calculated and averaged over an annual cycle. Since evaporation losses are unknown for the Van Stadens for the model it is assumed that evaporative losses are equivalent to precipitation thus giving it a similar magnitude (-0.304 10^3 m^3 day^{-1}). The negative sign indicates a loss from the system. Residual flow therefore takes on an equivalent magnitude as discharge and the salt-water flux is 82.89 10^3 m^3 day^{-1}. The water exchange time [*t*] of water in the system is approximately 11.2 days. The short water exchange time is due in part to the rapid flushing and emptying of the estuary when increased flows are experienced. However, it does not to account for the often-extended periods of low to no-flow inputs of freshwater. The major input and output terms show that the residual flows are from the system and exchange flows of salt water are into the system meaning that salt is imported into the estuary in order to maintain salinity.

Table 2. Water flux, salinity, and water exchange time for the Van Stadens estuary.

	Freshwater Input (10^3 m^3 day^{-1})			**Residual flow** (10^3 m^3 day^{-1})		**Salinity** (psu)		**Water exchange time** (days)
	V_Q	V_P	V_E	V_R	V_X	S_{syst}	S_{ocn}	τ
Annual means	73.44	0.304	-0.304	-73.44	82.89	13.51	35	11.19

Budgets of non-conservative materials

The Van Stadens River estuary is not subjected to significant point sources of nutrient input. Land use activity in the catchment is mainly dairy and poultry farming on a limited scale. Nutrient input in the form of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) is largely during the wet periods of the year when dry streams begin to run. Although nutrient input takes place during the wet season concentrations remain low. Nutrient concentration values used for determining the budget of non-conservative materials are shown on Table 3. These concentrations were measured from riverine, estuarine and marine water and are based on values averaged over a year.

Table 3. Nutrient concentrations for the Van Stadens River, estuary and the adjacent ocean averaged over an annual cycle.

	DIP (mmol m^{-3})			**DIN (mmol m^{-3})**		
	DIP_{syst}	DIP_{ocn}	DIP_R	DIN_{syst}	DIN_{ocn}	DIN_R
Annual	0.5	0.65	0.58	1.2	0.83	1.02

DIP and DIN balance

The non-conservative DIP (*ΔDIP*) and DIN (*ΔDIN*) fluxes are both negative for the Van Stadens estuary indicating that the system is a sink for these nutrients (Table 4). This is consistent with the observations and measurements of macronutrients SRP, TP, NO_3, NO_2, & NH_4 monitored over a three-year period averaging approximately 0.21, 0.48, 0.38, 0.01, 0.85 mmol m^{-3} respectively. Mouth breaching and overtopping events appear to be of importance as marine nutrients can be imported into the estuary. The results of the non-conservative materials are illustrated in Figure 10 & 11.

Table 4. Non-conservative fluxes of DIP and DIN for the Van Stadens River estuary.

	Δ*DIP*		**Δ*DIN***	
	(mol day^{-1})	(mmol m^{-2} day^{-1})	(mol day^{-1})	(mmol m^{-2} day^{-1})
Annual	-11.43	-1.73×10^{-3}	-136.23	-66.64×10^{-6}

Stoichiometric calculations of aspects of net system metabolism

The apparent net ecosystem metabolism (*p-r*) of the Van Stadens estuarine system, estimated from Redfield stoichiometric calculations (-2.02×10^{-3}mmol C m^{-2} day^{-1}), indicates that it is net heterotrophic (Table 5). Results also show that the difference between nitrogen fixation and denitrification (*nfix-denitr*) is -5.32×10^{-4}mmol N m^{-2} day^{-1}, suggesting that the system is net denitrifying.

Table 5. Apparent net ecosystem metabolism for the Van Stadens River estuary.

	(*p-r*) (mmol C m^{-2} day^{-1})	**(*nfix-denitr*)** (mmol N m^{-2} day^{-1})
Annual	-2.019×10^{-3}	-5.319×10^{-4}

Water and Salt Balances for the Van Stadens estuary

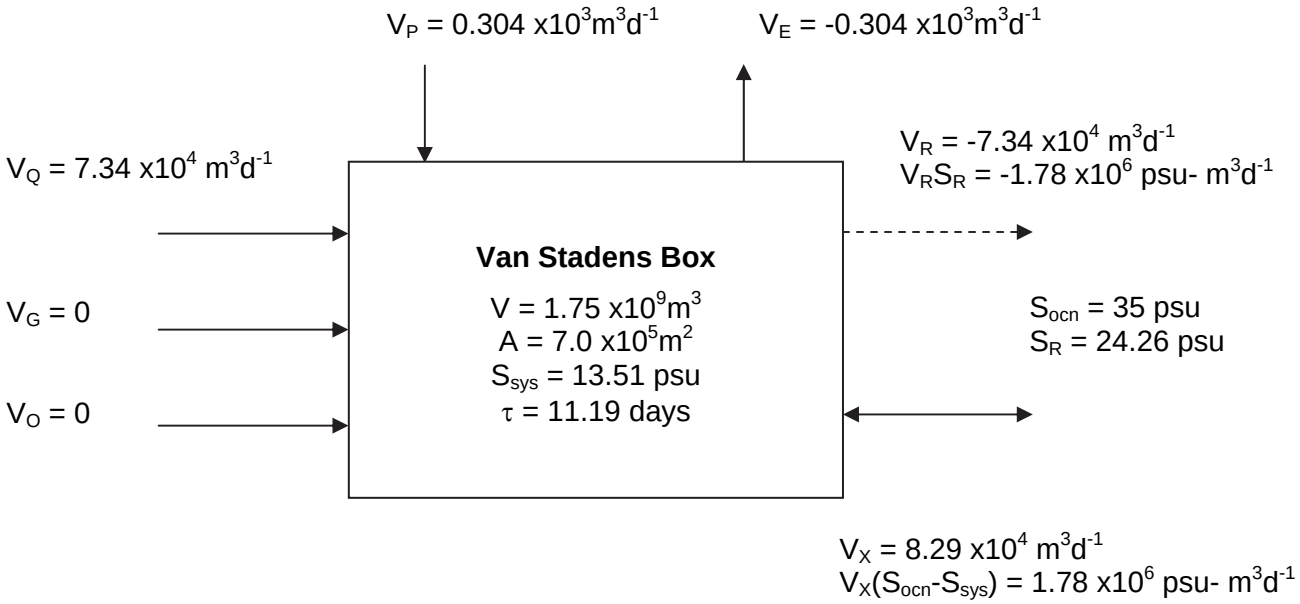


Figure 9. Water and salt budget for the Van Stadens estuary for the period April 2002 – March 2003.

Dissolved Inorganic Phosphorus (DIP) Balance for the Van Stadens estuary

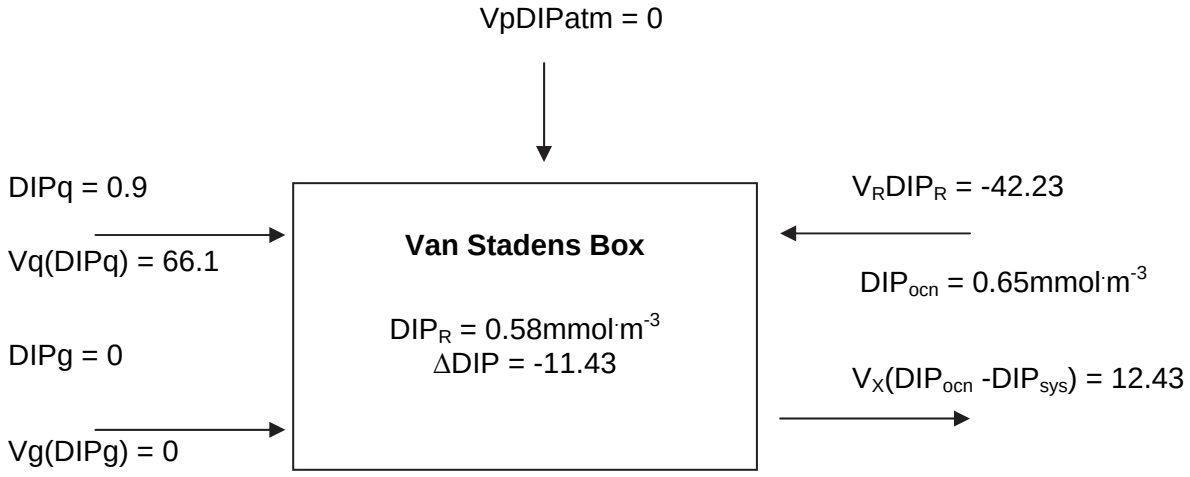


Figure 10. Dissolved inorganic phosphorus budget for the Van Stadens estuary for the period April 2002 – March 2003.

Dissolved Inorganic Nitrogen (DIN) Balance for the Van Stadens estuary

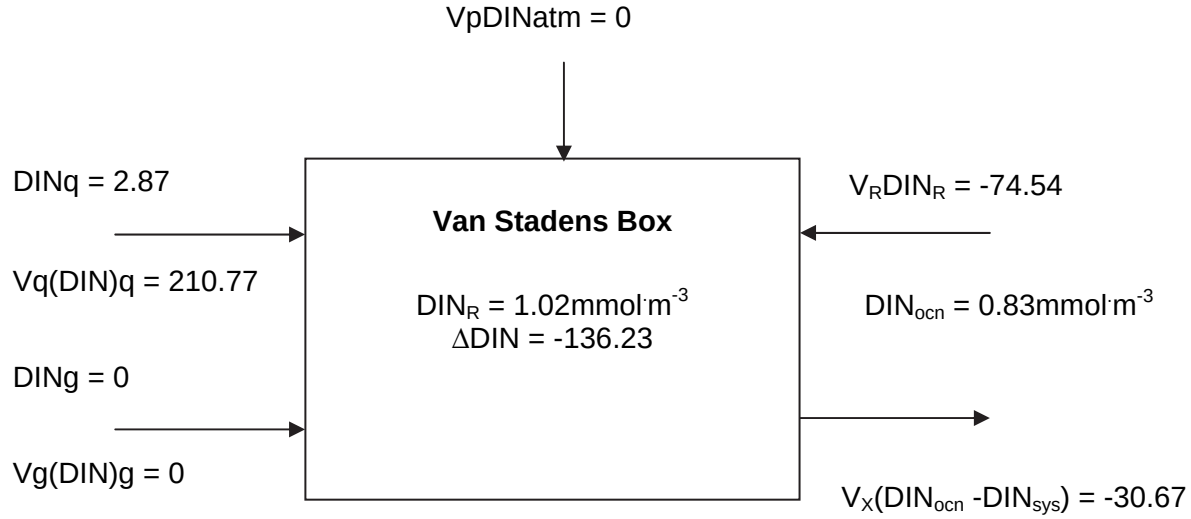


Figure 11. Dissolved inorganic nitrogen budget for the Van Stadens estuary for the period April 2002 – March 2003.

5.1.2 Biological Variables

5.1.2.1 Phytoplankton Chlorophyll *a*

Chlorophyll *a* concentrations throughout the study ranged between 0.8μg.l^{-1} in September 2001 to a peak of 14.2μg.l^{-1} in December 2001. The peak observed in December was the maximum measured for the duration of the study. There were no clear differences between chl-*a* concentrations in the upper and bottom layers of the water column and thus values reported here are averages for the water column. Chlorophyll *a* concentrations recorded in the upper reaches were comparable to those observed in the middle and lower reaches of the estuary. This is indicative of wind-induced circulation and mixing of nutrients throughout the water column and estuary. Between December 2002 and February 2003, total phosphates and dissolved inorganic nitrogen were at their lowest concentrations compared to those of the similar period in the previous year. Although there were water column density differences between top and bottom layers from time to time over the study period, there were no significant differences observed with regard to the vertical distribution of nutrients ($P>0.05$). Moreover, the density differences would be short-lived lasting approximately three to five days depending on prevailing weather conditions. Chlorophyll *a* concentrations responded to elevated nutrient input as a result of increased river flow. This occurred when rainfall was ≥2mm during the study. The microphytoplankton fraction contributed over 60% with the nanophytoplankton group contributing approximately 26% to total chl-*a* during the study period. This suggests that although total chl-*a* concentrations were relatively low throughout the study period, large-sized phytoplankton made up the majority of the chlorophyll *a* fraction (Figure 13). A pairwise multiple comparison procedures (Tukey Test) following a one-way ANOVA showed strong differences (p=102, q=12.074, at $P<0.001$) between nanophytoplankton and microphytoplankton in December 2001.

During the open mouth phase the microphytoplankton group contributed approximately 74% to total chl-*a*, whereas during the closed and overtopping phases they made up approximately ≤55%. The phytoplankton chlorophyll *a* data are in sharp contrast with the primary production data during that period showing that the nanophytoplankton fraction was the most productive group of the three (Figure 14a). Possible explanations for this discrepancy would firstly, be that the nanophytoplankton fraction shows high rates of turnover compared to the other fractions; secondly, this fraction might be the preferred size for grazers hence selective feeding on this group would keep their densities low, yet would have to have high levels of turnover in order to maintain their standing stock; and lastly, the chosen mesh size (25μm) for the production experiments for the retention of the microphytoplankton fraction was not able to retain this size group that would be greater than 20μm (as with the nylon mesh for chlorophyll determination) and yet smaller than 25μm. From the phytoplankton community structure data and cell-size measurements we've been able to determine that approximately 10 – 15% of some of the flagellates and diatoms fall within an 18 - 25μm size range.

In May and July 2001 under low river inflow and closed mouth conditions the picophytoplankton chl *a* fraction constituted either the dominant fraction or was co-dominant with the microphytoplankton size group. Although no statistically significant differences were observed between bottom and top layers of the water column the picophytoplankton showed a general pattern of increased levels in the upper part of the water column. This pattern persisted during the periods of low river inflow until the first mouth-breaching event in August. This was followed by the release of water from the bottom of the lower Van Stadens dam in September that kept the mouth opened for several weeks (Figure 8). Following the initial decline in chl *a* concentrations after the increased freshwater inflow the microphytoplankton were dominant making up over 60% of chl *a* concentrations in the water column by the summer of 2001. This microphytoplankton distributional pattern lasted until the following winter-autumn rains of 2002 that caused extensive flooding and scouring of the estuary. There was a similar sharp decline in chl *a* concentrations following increased inflow as was observed during the previous breaching event, however there was a decrease in the magnitude of the phytoplankton chl *a* compared to 2001. Yet the microphytoplankton displayed a similar spatial and temporal distributional pattern constituting on average over 55% of the phytoplankton chl a size fraction.

The closed mouth condition that began early in December 2002 saw the development of extensive mats of *Ulva* sp. (a benthic green filamentous macroalga) that lasted throughout the summer. During this period water column chlorophyll *a* concentrations never reached the levels observed for the same period of the previous year. This period was also associated with low macronutrient concentrations possibly because of competition for nutrients by benthic filamentous macroalgae that encrusted large areas of the sandy bottom of the estuary. During the prolonged closed-mouth condition in the latter part of 2003 phytoplankton chl *a* size structure had nanophytoplankton and microphytoplankton as co-dominants with both fractions contributing approximately 30 – 40% in terms of chl-*a* with the picophytoplankton making up the difference. During the closed mouth phase of the 2003 summer season the upper and lower portions of the water column were characterised by

uniform distribution of the micro- and nanophytoplankton chl *a* concentrations although concentrations remained lower than for the same period in 2002.

5.1.2.2. Microphytobenthic Chlorophyll *a*

In contrast to the phytoplankton chl-*a* response to increased nutrient input microphytobenthic chl-*a* did not show any changes to increased river flow except during the floods of September 2002 where benthic chl-*a* reached their lowest concentrations (Figure 13c). The highest concentrations were observed in July following several days of overtopping that were associated with low attenuation coefficients throughout that year. An account of the microphytobenthic chlorophyll a concentrations and community structure is presented in the appendix of this report and a detailed write-up is part of an MSc dissertation (Skinner 2005).

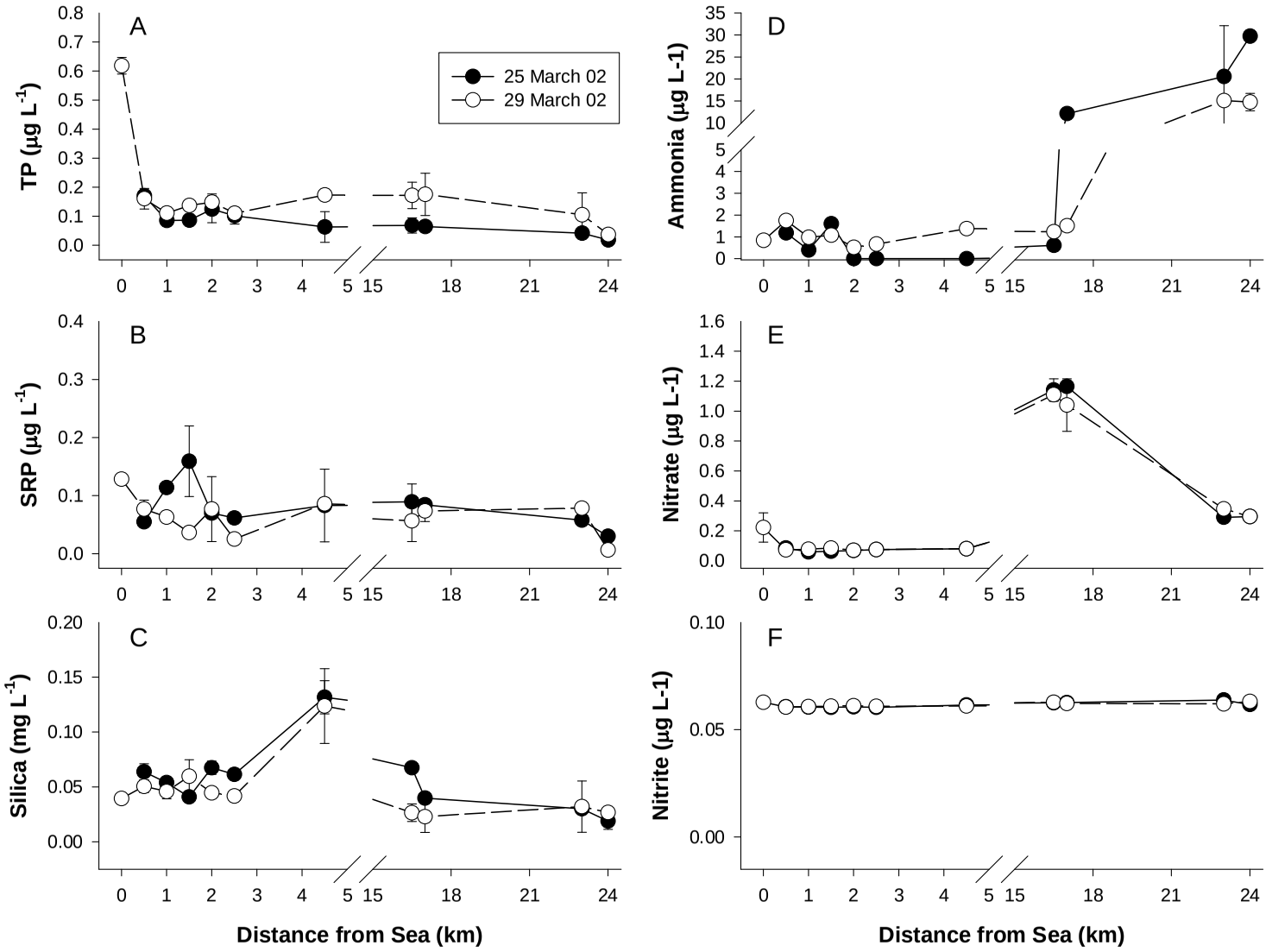


Figure 12. Nutrient data sampled along the Van Stadens River to the mouth including the sea, March 2002. (a) total phosphorus (TP); (b) soluble reactive phosphorus (SRP); (c) silicon – SiO_4; (d) ammonium – NH_4; (e) nitrate – NO_3; (f) nitrite – NO_2. Note: 0=sea.

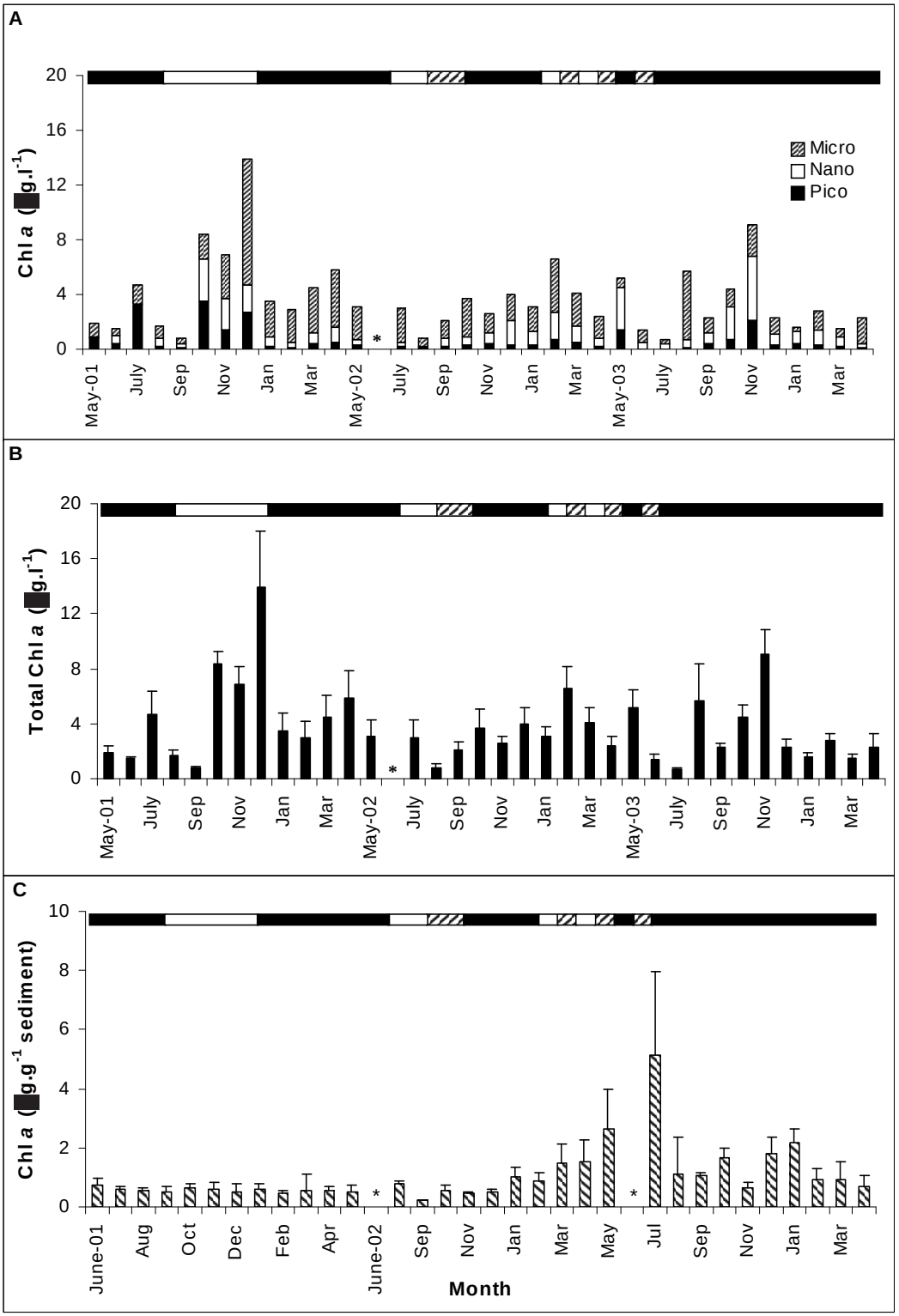


Figure 13. Van Stadens Estuary chl-*a* for the period May 2001 to April 2004. (a) Size fractions: Micro –microphytoplankton (> 20µm); Nano –nanophytoplankton (20 - 2.7µm); Pico –picophytoplankton (2.7 - 1.2µm), (b) total community chl-*a*, (c) microphytobenthic chl-*a*. An * denotes months not sampled. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open, and hatched bar = overtopping.

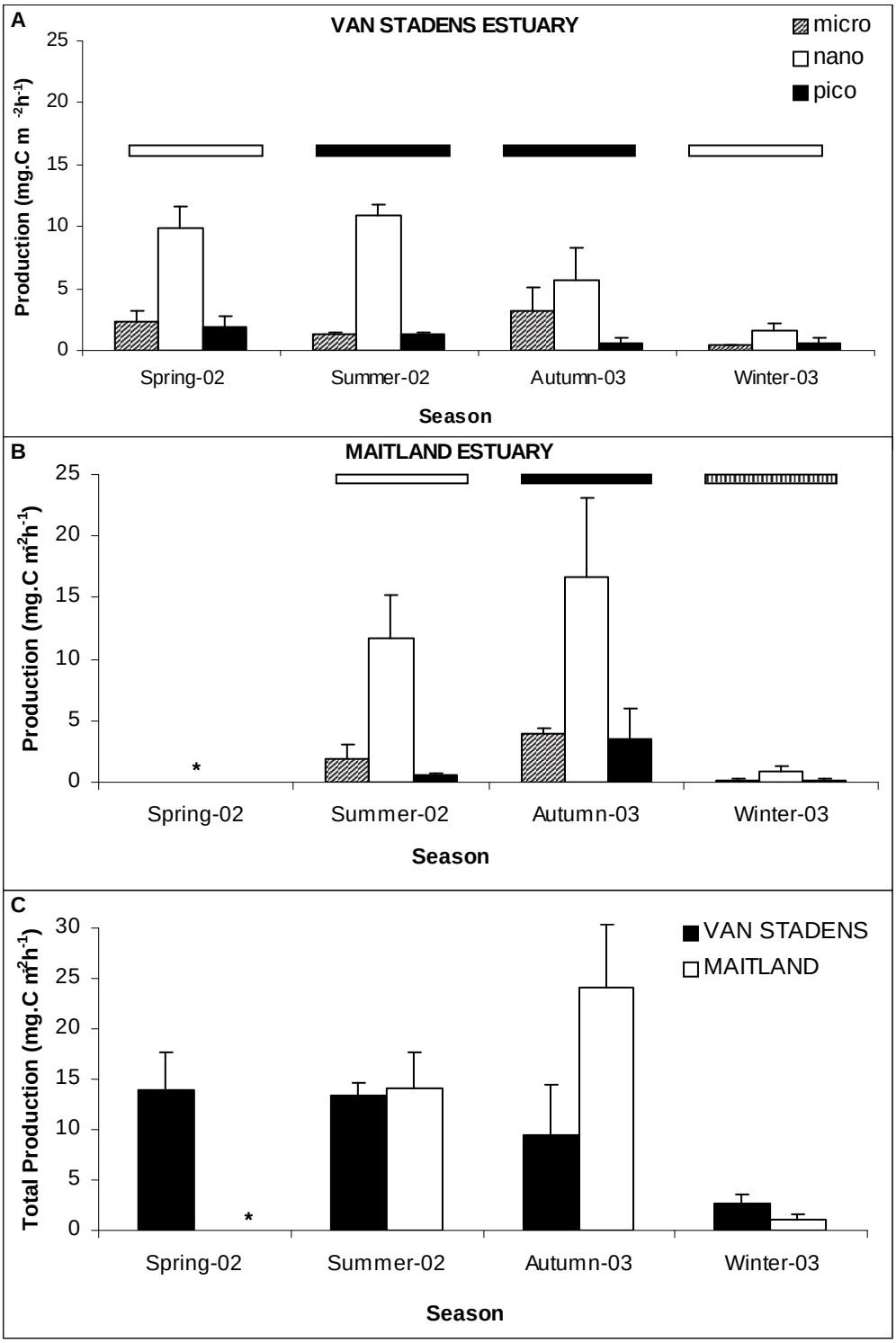


Figure 14. Seasonal primary production of each estuary, (a) Van Stadens production by size fraction, (b) Maitland production by size fraction, where size fractions are: Micro –microphytoplankton (> 20µm); Nano –nanophytoplankton (20 - 2.7µm); Pico –picophytoplankton (2.7 - 1.2µm). (c) Total primary production measured for each estuary. Horizontal bars signify three states of the mouth: solid bar = closed, open bar = open, and hatched bar = overtopping.

The phytoplankton community structure data presented here includes data from April 2001 to December 2002. Phytoplankton cell densities were generally low as expected for these unproductive oligotrophic systems, however two bloom events took place in late spring and early summer of 2002. The highest cell densities 8.09 $x10^5$ cells ml^{-1}, were recorded in December 2002 in the lower reaches of the estuary (Figure 15). Samples from elsewhere in the estuary did not show such high abundances. In November similar densities (5.60 $x10^5$ cells ml^{-1}) of the green algal bloom were present at site 1 (i.e. site nearest the mouth). Two small (3.0 – 5.0μm long along major central axis) flagellated algae responsible for such high cell numbers were *Micromonas* sp. and *Pyramimonas* sp. These algae were present in the middle and upper reaches of the estuary but were not as abundant only making up approximately 10% of the total phytoplankton count for those samples. Flagellated algae tended to make up the bulk of the phytoplankton cell counts throughout the water column and sample dates considered. Preliminary results indicate that during the open mouth phases of 2001, flagellates (i.e. dinoflagellates, cryptophytes and haptophytes) were dominant in the water column. The diatom assemblage was made up of *Nitzschia closterium*, *Melosira nummuloides* and *Diatoma* sp. and was equally distributed along the length of the estuary. However, on occasion the diatoms *N*. *closterium* and *Diatoma* sp. were prevalent in the lower reaches of the estuary. The dinoflagellates *Peridinium* sp., *Gymnodinium* sp. and *Katodinium* sp. contributed over 57% to the total group of flagellates with cryptophytes, haptophytes and euglenophytes making up the rest. The brackish conditions in December following mouth breaching coupled with a rather turbid water column associated with an input of nutrients provided ideal conditions for dinoflagellates to develop. Apart from diatoms the non-flagellated group of greens and cyanobacteria were only rarely represented and when present were found in samples taken during the summer period.

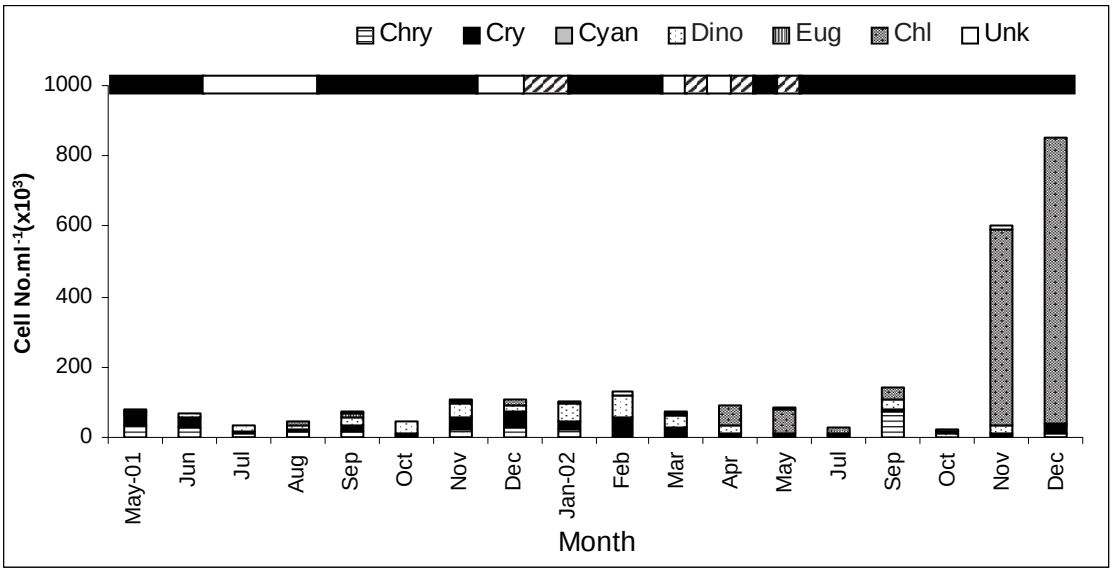


Figure 15. Van Stadens phytoplankton cell densities and community composition for the period May 2001 to December 2002. Phyla enumerated: Chl – Chlorophyta, Chry – Chrysophyta, Cyan – Cyanophyta, Dino – Dinophyta, Eug – Euglenophyta, Rha – Rhaphidiophyta, Unk – other. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open, and hatched bar = overtopping.

Each month was assigned a mouth condition state based on the condition of the mouth when the estuary was sampled. This allocation was used to determine relationships between phytoplankton species and mouth condition. (1) To explain the variability in the distribution of the phytoplankton community structure along a temporal scale a similarity matrix was performed on phytoplankton cell numbers using a Clarke and Warwick Statistical Package Primer (1994). (2) A non-metric multidimensional scaling plot was generated based on the presence/absence of the species data and represented on a two dimensional plot (Figure 16). (3) The plot shows how some months (mouth conditions) have clustered together indicating similarity to one another. A two-way nested ANOSIM analysis test was carried out to test for the differences between months (where months were used as surrogates of mouth condition) averaged across years. Following the test based on the presence/absence of log transformed data for the phytoplankton species resulted in a test statistic with R-value of 0.545 and P=0.002 indicating that approximately 50% of the variability is explained by mouth condition. Although the stress level of the relationship is high (Stress=0.19) it, however illustrates that mouth condition is important in structuring phytoplankton communities within the Van Stadens estuary.

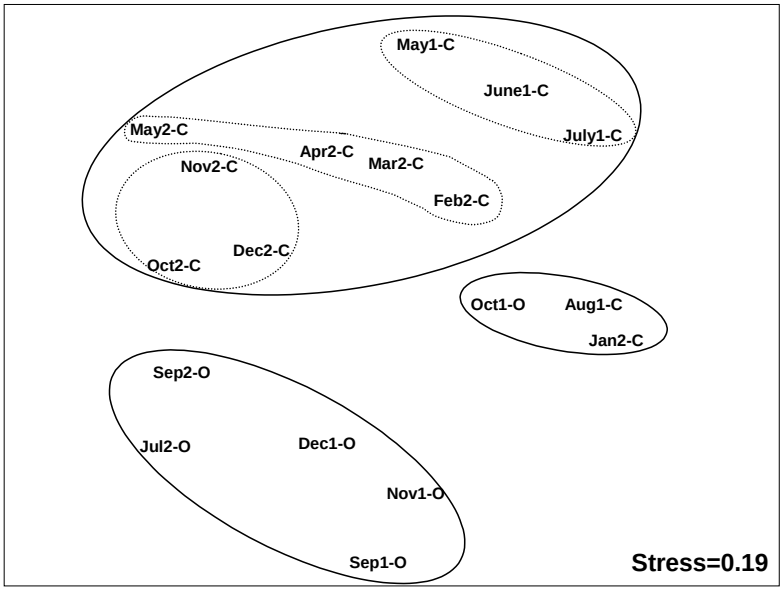


Figure 16. An MDS plot of Van Stadens phytoplankton community for each month and year sampled including state of mouth condition. O = open, C = closed, 1 = 2001, 2 = 2002, Jan-January, Feb-February, Mar-March, Apr-April, Sept-September, Oct-October, Nov-November, Dec-December. Solid-lined circles denote three major groups representing mouth state, dotted-lined circles represent seasonal periods within the closed state periods.

5.1.2.3 Zooplankton Community Structure

Two sites in the deeper lower reaches of the estuary were sampled at dusk for assessing zooplankton densities and community structure using a WP-2 tow net. Data reported here from those tows was pooled to give mean zooplankton densities. Mean total zooplankton densities in the lower reaches were 9278 ind. m^{-3} and *Pseudodiaptomus hessei* comprised

the dominant species with approximately 5819 ind. m^{-3}. *Acartia longipatella* was the second most common zooplankton and consisted of 2698 ind. m^{-3}. Dr Froneman assisted in grazing experiments in the Van Stadens estuary following methods outlined in Froneman (2000a). Community-grazing impact studies were carried out during summer with zooplankton samples taken from the same sites as those used for community structure and taxonomic determinations. Mean community-grazing rates were 8.66mg C m^{-3} d^{-1}; this may represent a moderate to high grazing rate considering that total phytoplankton production in summer was approximately 13.4mg C m^{-3} d^{-1} (Table 6). Zooplankton biomass values represent a fairly high number of grazers suggesting that grazing pressure on phytoplankton is great and may closely control phytoplankton biomass and production. Support of zooplankton production by the phytoplankton production appeared to be inadequate given the grazing rates reported. Such high secondary production rates are perhaps augmented by microphytobenthic sources.

Table 6. Zooplankton taxa and grazing impact rates in December 2002 in the Van Stadens estuary.

Zooplankton Community Structure	Mean of Individuals m^{-3}
Pseudodiaptomus hessei	5819.5
Acartia longipatella	2698.0
Cumaceans	178.5
Mesopodpsis wooldridgei	73.0
Other	509.0
Total	9278.0
Grazing impact rates (mg C m^{-3} d^{-1})	8.7

5.1.3 Once-off Seasonal Primary Production

Primary production experiments were carried out once over an annual seasonal cycle both in the Van Stadens and Maitland estuaries. Production studies were not carried out in the Maitland estuary in the spring of 2002, as this period occurred a couple of weeks following intense floods that took place in July. The floods scoured the estuary flat and washed off sand dunes (Plate 1) such that the water depth at the deepest point of the estuary was <0.1m and discharge was approximately 8.0m^3s^{-1} (see Figure 4). A two-way ANOVA test was performed to test for differences among seasons. There were significant differences detected among seasons and chl-*a* size-fractions as a factor within seasons following a pairwise multiple comparison procedures (Tukey test) (Table 7). Nanophytoplankton productivity was significantly different between both pico- and microphytoplankton in spring and summer (q=4.720, P=0.005 and q=7.697, P<0.001. In winter productivity was considerably lower compared to the other seasons in both estuaries (Figure 14c).

Maitland total phytoplankton productivity was significantly higher than that at Van Stadens during the autumn and showed no significant differences between summer, winter and spring. In both estuaries the nanophytoplankton size-group showed the greatest productivity.

Table 7. Two-way ANOVA results showing the significance of the difference among seasons and phytoplankton size-fractions

Source of Variation	DF	SS	MS	F	P
Fraction	2	25.339	12.670	27.778	<0.001**
Season	3	16.054	5.351	11.733	<0.001**
Season x Fraction	6	7.173	1.195	2.621	0.028*

There was no Season x Fraction interaction. * Not significant. ** Significant *P*-value

Primary productivity experiments were conducted during periods that coincided with a particular phase of the mouth, however we were not able to run experiments for each phase of the mouth within each season as each mouth condition rarely presents itself within a given season. Although the monitored nutrient levels were low during productivity trials levels of production however, were high associated with the nanophytoplankton size group perhaps indicative of the high rates of turnover linked to close nutrient coupling between the available nutrient pools, bacteria and phytoplankton often exhibited in oligotrophic systems (Day *et al.*1989).

5.2 Maitland Estuary

5.2.1 Physical and Chemical Parameters

Unlike the Van Stadens estuary, mouth condition at the Maitland estuary was not monitored on a daily basis, however the monthly record provides a positive representation of how often the condition of the mouth changed. The frequency of open/closed mouth conditions for the Maitland estuary differed from that observed for the Van Stadens estuary. During the study period the mouth was open approximately 26% of the time and was closed for 32% while the majority of the time the mouth was in a semi-closed state whereby estuarine water slowly flows out without interacting with the sea in a tidal fashion. Salinity was generally low never getting above 5 ppt except when there were strong overtopping events that introduced marine water into the estuary (Figure 17a). The estuary is perched and set back from the coastline protected by sand dunes on the west. During overwash events substantial volumes of seawater were introduced into the estuary resulting in elevated salinities. These events were however, short-lived as they rapidly dissipated over short-time periods through dilution from low river inflow and freshwater seepage from the sand dunes or loss of more dense saline water through subsurface seepage across the sand bar. Periods of overwash in late summer of 2002 did not appreciably affect estuarine salinity levels except those of the lower reaches. A breaching event in July connected the estuary with the sea introducing saline water throughout the length of the estuary and producing the highest salinity recorded that year. High rainfall in August resulted in a flood that kept the mouth open for approximately 2 months. Salinity during that period dropped to below 0.5 ppt.

The scouring of the estuary sediment bed floor changed the geomorphology and position of the mouth by removing and deepening the channel therefore improving access of marine water into the estuary. This change had an impact on average salinities following the flood, as they were higher in November and December 2002 possibly as a result of improved saltwater intrusion over the lower sand bar.

Strong westerly winds in February 2003 generated huge swells that brought in seawater raising average salinities to a maximum of 11 ppt. In August of that year huge swells associated with a spring tide introduced large volumes of seawater that produced salinity stratification in the middle to lower reaches of the estuary. In a water column depth of 0.5 m and 0.75 m the middle and lower reaches had salinity ranges from 0.8 to 24.5 ppt and 3.7 to 28.8 ppt respectively. The highest average salinity readings for the study period were in April 2004 following sustained south-westerly winds that introduced marine water that pushed right up to the head of the estuary.

Water-column temperatures tended to track seasonal temperatures with maximum temperatures of 25^{o}C reached during the summer periods and minimum of 12^{o}C attained in winter. The change in water depth influenced average water column temperatures, as they were generally higher following the flood event compared to those of the same period for the previous year (Figure 17b). Summer water-column temperatures in 2002 and 2003 were significantly higher post the winter flood event (ANOVA, $F17, 2.22 = 181.547$ $P<0.001$). The results showed that the flood event in the winter of 2002 completely altered the estuary from one that was lacustrine-like, covered and fringed by submerged and emergent macrophytes to one that was shallow and devoid of all submerged and most emergent vegetation (see Plates 1 & 2). Growth of submerged and floating aquatic plants, particularly in the middle to the upper reaches, reduced light penetration at depth with high attenuation coefficients averaging 1.03 (m^{-1}) in the first year compared with 0.73 (m^{-1}) in the last year of the study. The amount of solar radiation reaching the sediment bottom was improved as a consequence of the reduced water column depth resulting in significantly higher irradiance levels compared with those taken prior to the flood (ANOVA, $F3, 0.168 = 54.486$, $p<0.001$). The river water entering the estuary was stained a dark reddish-brown colour and heavily so after rainfall events. This was indicative of similar humic substances commonly observed in most Southeastern and Western Cape rivers that drain catchments covered in fynbos vegetation (Davies and Day 1998). Attenuation of light at depth was observed particularly in areas clear of submerged and floating aquatic vegetation in the early part of the study. Water column depth was considerably reduced following the floods from a maximum depth of 2.05 m to 0.50 m at the deepest point of the estuary resulting in a greatly improved photic depth.

Dissolved oxygen concentration in the early part of the study were above saturation levels, however by the same time the following year had significantly decreased resulting in anoxia in the upper reaches near the bottom (ANOVA on ranks, $q = 6.599$, $P<0.05$). Following the floods that scoured the estuary, water levels declined and oxygen concentrations improved often approaching super saturation particularly when mats of filamentous green algae developed along the bottom of the upper and middle reaches of the estuary. Water column conductivity tracked the salinity profile over the course of the study period and did not change (Figure 17c).

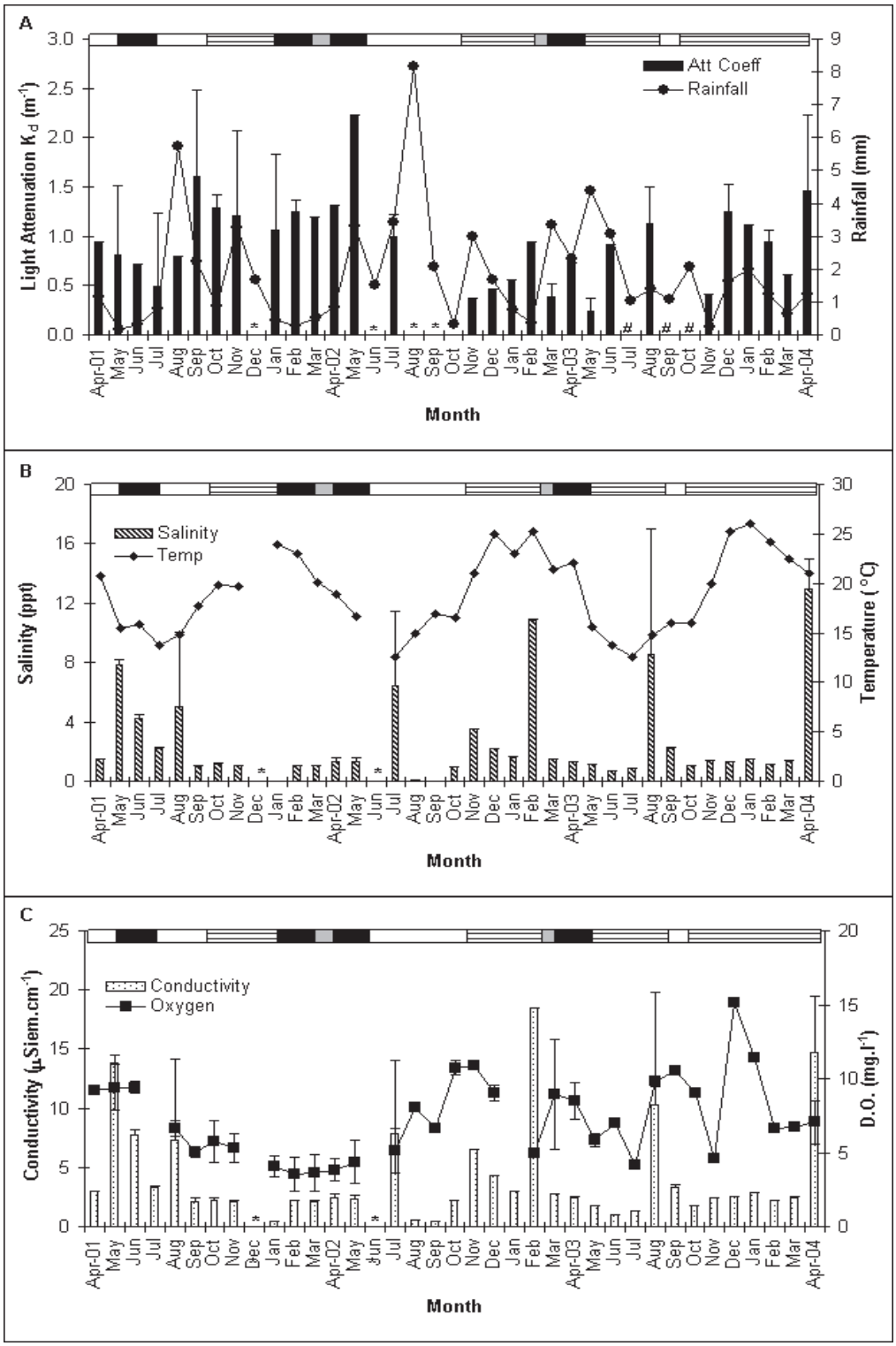


Figure 17. Maitland Estuary attenuation coefficient and rainfall - (a), salinity and temperature - (b), and conductivity and dissolved oxygen - (c), for the period April 2001 to April 2004. Horizontal bar signifies three states of the mouth open bar=open, solid bar=closed, and stippled=overtopping. An *denotes dates measurements not taken.

Discharge measurements for the Maitland estuary were conducted in the second year of the study and the results reported here are from March to November 2002 during periods of river flow. Discharges recorded at the mouth of the Maitland estuary subsequent to the flood event peaked at approximately $8.0m^3s^{-1}$ (Figure 4b). Sand dune movement coupled with long shore current movement along the coastline had produced an elevated sand barrier at the mouth that resulted in the development of a small shallow lagoon-like body of water behind with a maximum depth greater than 2m in the lower reaches. A flood event took place in August that scoured the estuarine bed floor removing emergent, submerged and floating aquatic plants at the same time depositing sediment and dead organic debris. Water depth in the estuary was reduced considerably from a maximum of 2.05m to 0.5m at the deepest point of the estuary. The estuary was subsequently reduced to a small and shallow river-like channel of 20 m at the widest point without much water being retained for about two months after the floods. Increased long-shore sediment transport built up a sand barrier at the mouth that enabled retention of water, however the barrier was readily breached when water accumulated above a water depth of 0.75m.

Nutrient concentrations measured in the Maitland estuary during the study period showed a similar pattern as those observed in the Van Stadens estuary. Apart from the flooding during August and September 2002 that significantly scoured the estuary, previous periods (i.e. 2001) that were associated with high river inflow brought in increased nutrient concentrations that peaked at a maximum of $1.0\mu g.l^{-1}$ for nitrates and $1.2\mu g.l^{-1}$ for total phosphates. During the summer and early part of the autumn season in the first year of the study, phosphate concentrations were variable although generally they remained $\geq 1.0\mu g.l^{-1}$ particularly in the mid to upper reaches of the estuary. Nutrient input during September 2001 was significantly related to increased river flow ($F_{6,\ 0.0867} = 39.155$, $P < 0.001$). On occasions the sampled macronutrients were below detectable levels (Figure 19).

In March 2002 ammonium concentrations peaked at $11.0\mu g.l^{-1}$ representing the highest value recorded throughout the study period (Figure 19b). These high concentrations were associated with the period when dissolved oxygen concentrations were at their lowest together with extensive growth of blue-green algal mats. This was observed in the middle to upper reaches of the estuary covering the bottom sediments while some mats would float to the water surface when dislodged from the bottom. These conditions persisted during the summer well into the winter months of 2002 until scoured out by the floods. In September 2002 an increase of water column phosphate and nitrogen macronutrients was recorded following the flood event with the phosphate concentrations reaching a maximum of $4.0\mu g.l^{-1}$ and for the same period nitrogen levels peaked at $1.0\mu g.l^{-1}$ (Figure 19). The floods scoured and deposited sediments on the estuary bed floor that resulted in a water column with high levels of suspended matter rendering it turbid for several weeks. However, by September the water column showed low levels ($<1.0mg.l^{-1}$) of suspended sediments and was also stained reddish-brown similar to a number of Southeastern and Western Cape rivers and estuaries that drain catchments covered in fynbos vegetation (Day 1981, Lubke and de Moor 1988, Davis and Day 1998). The Maitland estuary was always nitrogen limited except during the late summer of 2002 when the estuary was experiencing

increased levels of anoxia at depth then it may have become phosphorus limited (Figure 18).

By comparison with the previous year nutrient levels during the summer months were much lower after the flood event suggesting that nutrient retention was reduced perhaps as a result of diminished river inflow coupled with low retention of water within the estuary as some water flowed out albeit at extremely low discharge rates. The estuary was in a semi-closed state. Silicon levels were always an order of magnitude higher than other macronutrients monitored over the duration of the study. Periods of overtopping coupled with low river inflows were associated with an increase in dissolved silicon concentrations and during the latter part of the study the mouth remained in the semi-closed state for over 10 months. It was during this period that higher silicon levels were recorded compared to the similar period of the previous year.

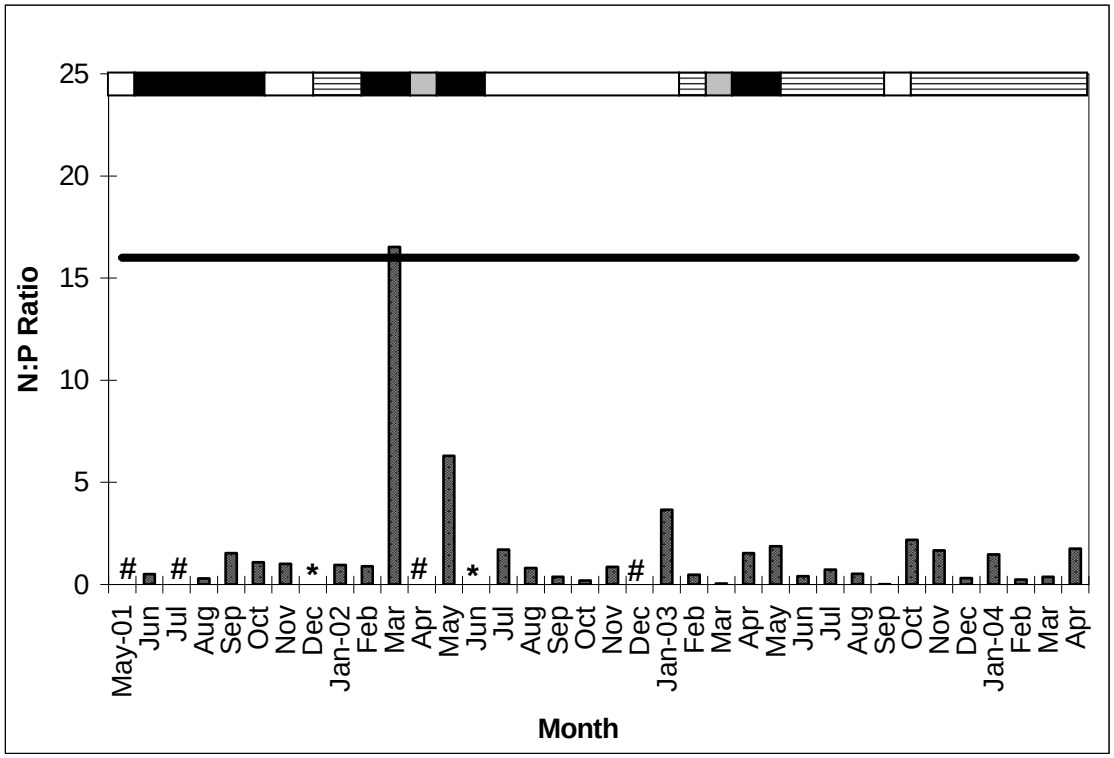


Figure 18. Maitland Estuary N:P ratios for the period of May 2001 to April 2004. Horizontal line denotes the 16:1 Redfield ratio of Nitrogen & Phosphorus. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open, and grey bar = over topping, lined bar = semi-open. A # denotes values below detectable limits, * not sampled.

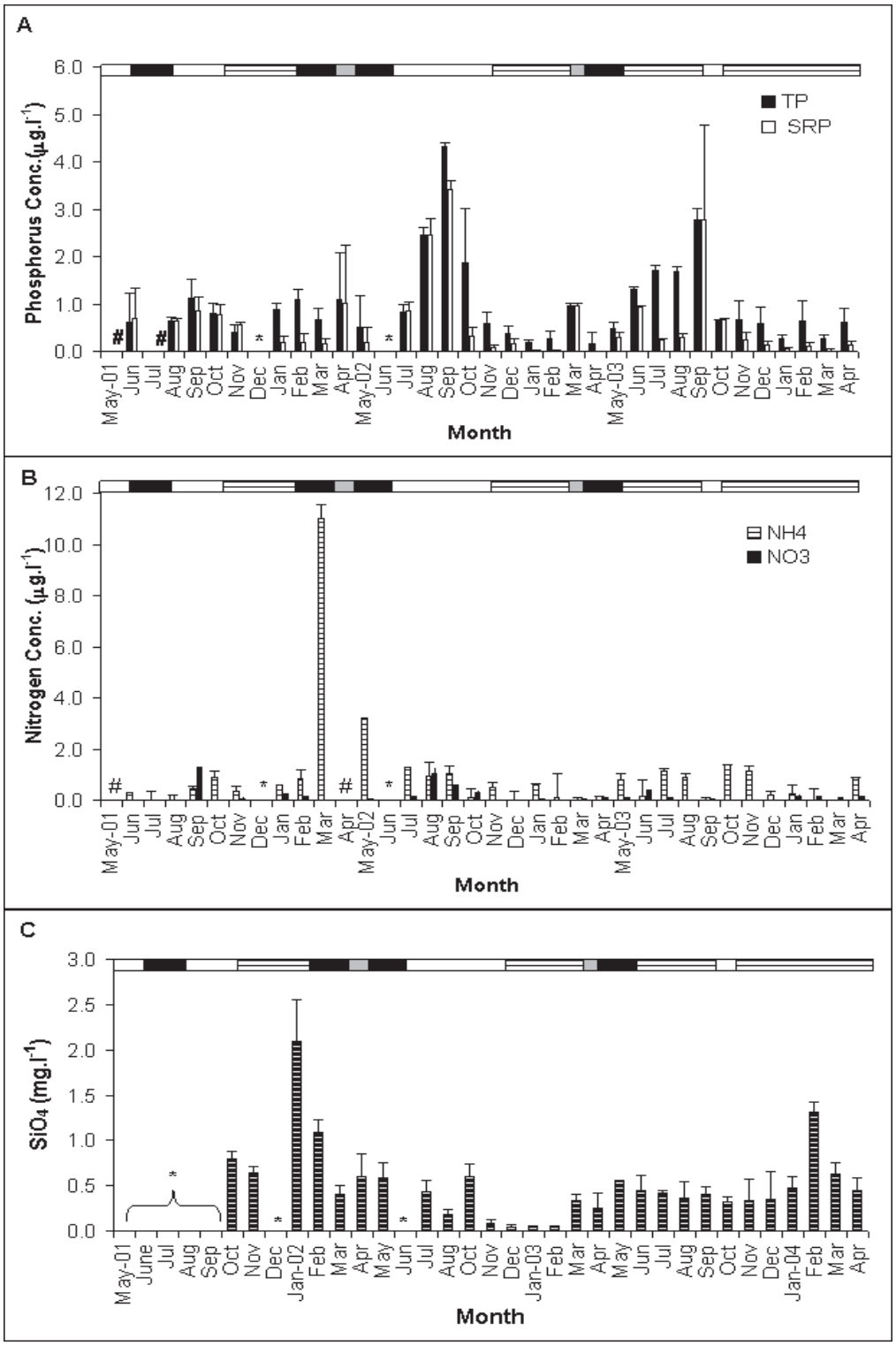


Figure 19. Maitland Estuary nutrient concentrations for the period May 2001 to April 2004,
(a) total phosphate – TP and soluble reactive phosphate – SRP,
(b) ammonium – NH_4 and nitrate – NO_3, and
(c) dissolved silicon – SiO_4 from October 2001 – April 2004
Horizontal bar signifies three states of the mouth:
Solid bar = closed; Open bar = open, and
Grey bar = over topping, Lined bar = semi-open.
A # denotes values below detectable limits and * denotes months not sampled.

5.2.2 Biotic variables

5.2.2.1 Phytoplankton Chlorophyll *a*

The Maitland estuary supported moderate phytoplankton chl-*a* levels in the early part of the study. After a mouth-breaching event in April that lasted until June 2001 chl-*a* concentrations ranged from 9.6 to 15.6μg.l^{-1}. Over the subsequent month river flow decreased to a point where the mouth closed. During the closed mouth phase from June to July the picophytoplankton was the dominant group throughout the estuary particularly in the top layers of the water column. However, just prior to the second breaching event that year total chlorophyll *a* levels had reached a maximum of 22.8μg.l^{-1} with the microphytoplankton having replaced the picophytoplankton making up the majority of the phytoplankton fraction. When the mouth breached following sustained rainfall in the catchment chlorophyll *a* concentrations sharply declined and remained low until November where concentrations peaked at 138μg.l^{-1} with the micro- and nanophytoplankton fractions co-dominant in the water column. The level of the water in the estuary was reduced considerably following the second breaching in 2001 resulting in a shallow water column.

As there were no significant site and depth differences from the chl-*a* data, the results were pooled and are reported as monthly means (Table 8). In the beginning of the summer season river flow subsided such that interaction with the sea gradually diminished until a trickle of estuarine water flowed out (semi-closed mouth phase) throughout summer. The Maitland River completely dried up during the summer isolating the estuary from the river and the sea. The estuary headwaters became overgrown with submersed, floating and emergent macrophytes extending down to the middle reaches. During this period the estuary water was oligohaline as salinity levels were <1.5 (ppt). Phytoplankton chl-*a* concentrations during the summer months of 2002 remained high with both the micro- and nanophytoplankton fractions being the co-dominant groups until the onset of autumn when the pico- and nanophytoplankton comprised the major chl-*a* fractions in the water column.

Table 8. Two-way ANOVA results showing no significant response in phytoplankton chl-*a* to site or depth variables.

Source of Variation	DF	SS	MS	F	P
Site No.	2	5.51	2.75	0.142	0.868
Depth	1	2.34	2.34	0.121	0.729
Site No. x Depth	2	44.61	22.31	1.151	0.320
Residual	111	2151.15	19.38		
Total	116	2203.50	18.99		

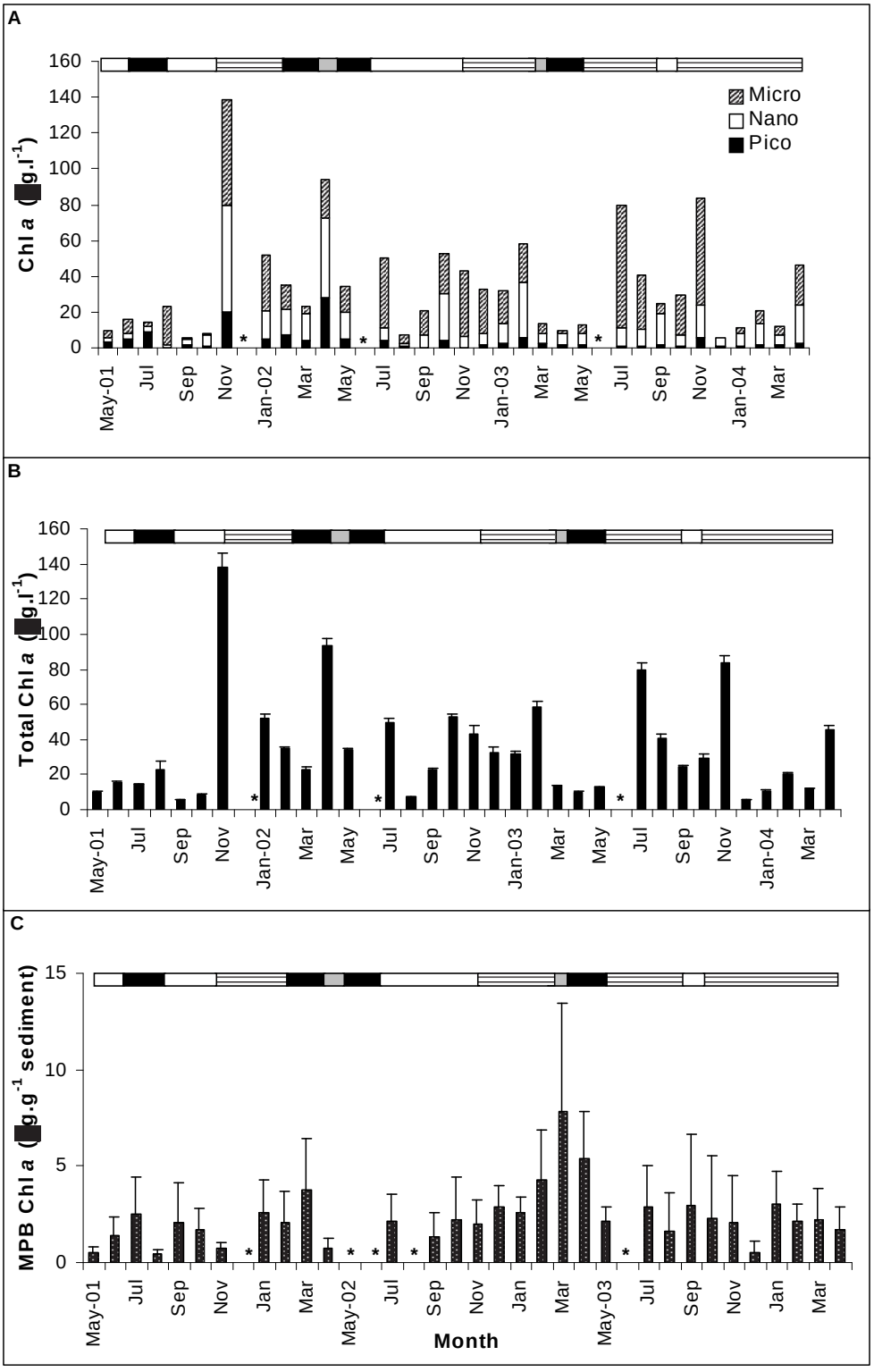


Figure 20. Maitland Estuary chl-*a* for the period May 2001 to April 2004. (a) Size fractions: Micro –microphytoplankton (> 20µm); Nano –nanophytoplankton (20 - 2.7µm); Pico –picophytoplankton (2.7 - 1.2µm), (b) total community chl-*a*, (c) microphytobenthic algal chl-*a*. Horizontal bar signifies three states of the mouth: solid bar = closed, open bar = open, and grey bar = over topping, lined bar = semi-open. An * denotes months not sampled.

Following the increase in river flow in July and the subsequent flood that occurred in late August and early September, the Maitland estuary was transformed into a sandy riverbed with the estuary reduced to a narrow and shallow channel. The reduced water depth meant that samples could only be collected at the surface. Data for phytoplankton chl-*a* taken in September is possibly a combination of dislodged benthic microalgae that had been translocated downstream by the strong flows experienced earlier that month. Taxonomic examination of the water samples taken from the estuary revealed that the majority of the suspended microalgal community was made up of phytoplankton with less than 10% of the community made up of benthic microalgae. Phytoplankton in the Maitland estuary was reduced during the flood period with a mean total chlorophyll *a* of 7.29μg l^{-1}. Although estuarine discharge peaked in September, chlorophyll *a* had decreased to its lowest levels in August and remained low throughout September recovering to a maximum of 53μg.l^{-1} in October. The microphytoplankton fraction comprised the dominant size group during July, August, October and November of the third year of the study except in September when the nanophytoplankton group formed the dominant chl-*a* fraction (Figure 20a). The third breaching event in September of 2003 was short lived as it lasted approximately two weeks. Prior and post that event there were several overtopping incidents associated with an increase in estuarine water depth. By late summer of 2004 water depth had risen to a maximum of 1.0m in the lower reaches. The picophytoplankton comprised the major fraction of the phytoplankton community in the early part of 2004 such that during the closed-overtopping period of the late summer phytoplankton chl-*a* culminated in a bloom in April. The shallow water depth together with a high light environment stimulated the growth of filamentous macroalgae (e.g. *Spirogyra*, *Oedogonium*, *Chara* and *Ulva*) and microphytobenthic algae. These filamentous macroalgae were more prevalent during the summer periods following the floods mostly occurring along the margins of the upper reaches of the estuary.

Following a mouth breaching event the microphytoplankton constituted the dominant fraction of the phytoplankton chl-*a* contributing from as much as 64 - 94% of the total chl-*a*. This pattern held true all through the periods of increased river inflow. However, in October 2002 the nanophytoplankton was co-dominant contributing approximately 49% to total chlorophyll *a*. The picophytoplankton group rarely contributed above 15% except during the first year of monitoring where picophytoplankton made up from 9.0 - 59% of the total chl-*a* in the water column. In late summer of 2003 when the mouth had been closed for over six months the nanophytoplankton fraction began to increase in concentration contributing over 65% to total chlorophyll *a*. This pattern was evident during periods of low river flow associated with mouth closure or when some water just flows over the sand bar.

The phytoplankton community structure was composed mainly of seven taxonomic groups. During the first year of the study cyanophytes comprised the majority of the phytoplankton assemblage with the chlorophytes making up the second major group. Of the cyanophytes recorded during the study filamentous blue-greens, mainly *Anabaena* sp., *Oscillatoria* sp. and a coccoid species of *Chroococcus* sp. were the most common taxa encountered primarily from the middle to the upper reaches of the estuary. Two small flagellated greens, *Nephroselmis* sp. and *Tetraselmis* sp. were the next dominant genera particularly from the lower reaches of the estuary. Diatoms, dinoflagellates and euglenoids did not comprise a

significant proportion of the cell numbers (Figure 21). Euglenoids however, were present following the first breaching event in 2001. A second bloom of cyanobacteria in November of that year was observed and during that period greens and cryptophytes comprised the major group of algae observed throughout the estuary. This pattern of phytoplankton community structure persisted through the summer period with the small flagellated greens becoming the dominant algae. In the winter months of 2002 the phytoplankton community shifted toward a more diverse assemblage made up of chlorophytes, chrysophytes, cryptophytes cyanophytes and euglenoids. Cell densities reached their lowest levels during the winter months, although no samples were taken in June and August of that year. The phytoplankton community had shifted following the floods of 2002 to that made up of mainly the chrysophytes, rhaphidiophytes, chlorophytes and dinoflagellates.

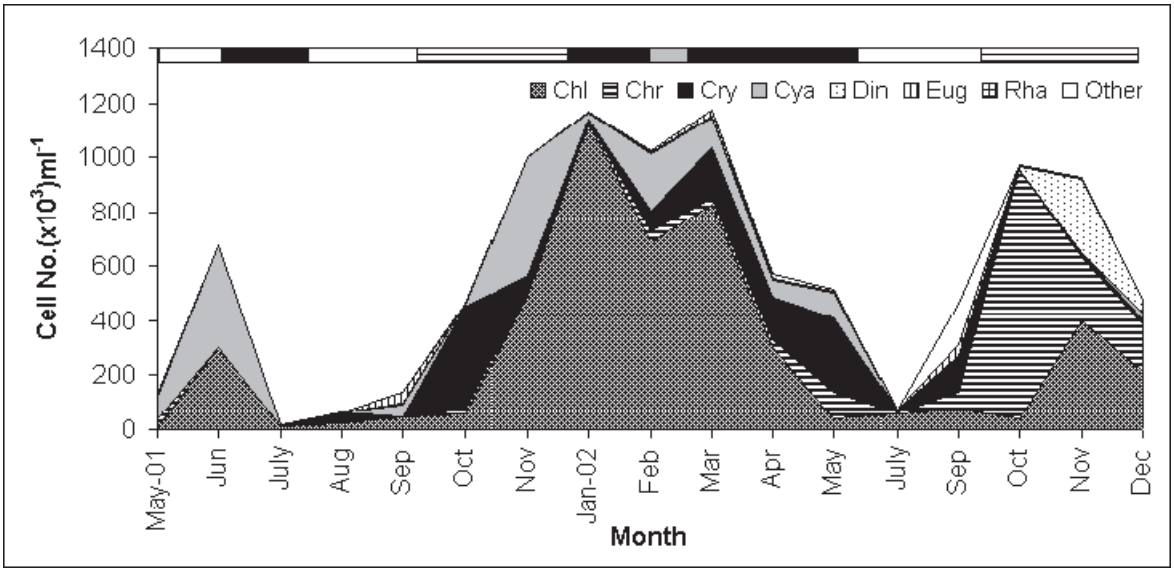


Figure 21. Maitland phytoplankton cell densities and community composition for the period May 2001 to December 2002.
Phyla enumerated: Chl – Chlorophyta, Chry – Chrysophyta, Cryp – Cryptophyta, Cyan – Cyanophyta, Dino – Dinophyta, Eug – Euglenophyta, Rha – Rhaphidiophyta, Unk – other.
Horizontal bar signifies four states of the mouth: Solid bar = closed, Open bar = open, and Grey bar = over topping, Lined bar = semi-open.

A non-metric multidimensional scaling plot was generated based on the presence/absence of the Maitland species data and represented on a two-dimensional plot using a Clarke and Warwick Statistical Package Primer (1994). The plot shows how some dates have clustered together indicating their similarity to one another (Figure 22). A two-way nested ANOSIM analysis test was carried out to test for the differences between months (where months were used as surrogates of mouth condition) averaged across years. Following the test based on log transformed data of the phytoplankton species data resulted in a test statistic with a value of R= 0.401 and P=0.002 indicating that approximately 40% of the variability is explained by mouth condition and the rest possibly by a variable that was not measured. These results indicate that mouth condition can influence the structuring of phytoplankton communities within the Maitland estuary.

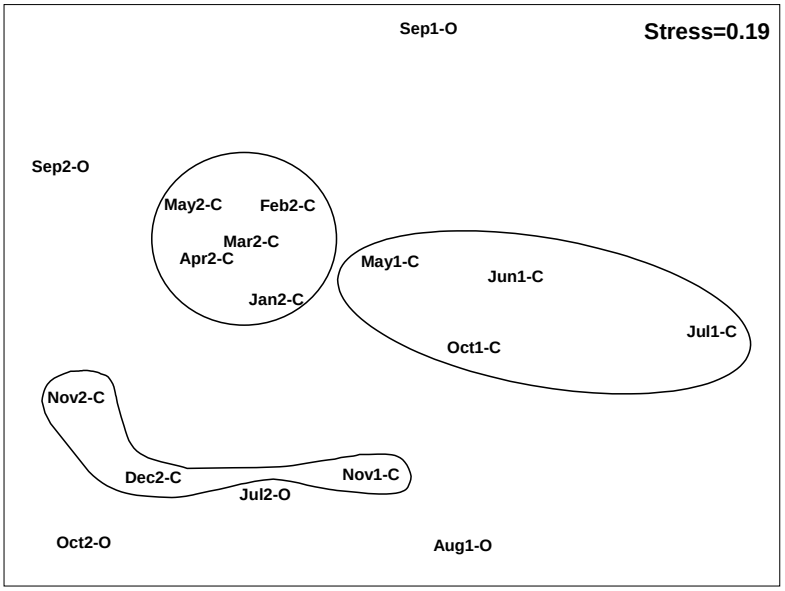


Figure 22. An MDS plot of Maitland phytoplankton community structure by year, month, and mouth condition. 1 = 2001, 2 = 2002; O = open, C = closed. Solid-lined circles denote three major groups of closed mouth state samples by year and seasonal period.

5.2.2.2 Microphytobenthic Chlorophyll *a*

Microphytobenthic (MPB) chl-*a* remained low in the first year of the study. Chlorophyll *a* maximum were reached in March when the mouth had been closed for several months (Figure 20c). It was not until the post flood phase that MPB chl-*a* levels peaked at 8.0μg.g^{-1} sediment. Although the estuarine substrate was scoured during the floods, re-colonisation of the benthic substrate by epipsammic microalgae was rapid such that by the beginning of the following year benthic microalgal chlorophyll *a* had recovered to the highest levels recorded during the study period. Although the MPB chl-*a* was variable it however demonstrated a positive response to periods when the mouth was closed particularly when the estuarine water depth was reduced and the light environment at depth was improved.

6. DISCUSSION

6.1 Abiotic Responses

River flow in the two systems studied is essential in terms of nutrient transport and supply for the stimulation of microalgal production (Perissinotto 2000, Froneman 2002a and 2002b). The hydrological patterns of these two estuarine systems are greatly influenced by local and regional weather patterns since these estuaries respond rapidly to increases in river inflow. Rainfall and subsequent runoff from the catchments is essential in the supply of nutrients to the two estuaries studied. Freshwater inflow did have a significant influence on mean estuarine nutrient concentrations during periods when the mouth breached. These patterns have been observed in several studies on temporarily open/closed estuaries in the region (Perissinotto 2000, Walker *et al.* 2001). Mean phosphate and nitrate input were positively related with increases in river inflow in both estuaries studied. The non-release of water from the two Van Stadens dams has possibly limited the amount of water reaching the estuary. During dry periods, particularly when they last over several seasonal cycles, the Van Stadens River dries up effectively cutting the estuary from the river. These conditions can lead to the estuary becoming hypersaline as evaporative losses outweigh freshwater input coupled with the simultaneous overwash input of marine water. Water from the dams is not released except for the purposes of scouring the dams in order to remove accumulated sediment deposits. This estuary occurs within a semiarid region of the Eastern Cape that receives a mean annual precipitation (MAP) of between 600-800mm therefore any outflow from the dam is only when the dams are at their full capacity, and this is seldom the case. Under closed mouth conditions the water depth in the estuary increased and the euphotic depth exceeded the mixing depth. However, when freshets come through carrying suspended inorganic matter the euphotic depth was reduced to a few centimetres below the surface. Light availability to the phytoplankton under these conditions became limiting even though the nutrient supply was improved. Chlorophyll *a* concentrations recorded following such episodes were at their lowest even after river inflow has subsided. In the literature (Huizinga 1994, 1996), river flows of $5.0 m^3.s^{-1}$ and greater have been used based on hydrological simulation models including observations on some temporarily open/closed estuaries as sufficient to breach the mouth of an estuary. From this study however, we have observed that minimum flows of $3.0 m^3.s^{-1}$ have been sufficient to breach the Van Stadens and Maitland estuary mouths. Such breaches are depended on a number of factors including volume of water in the estuary, height of sand bar across the mouth and elevation of estuary above the mean sea level height.

Unlike the Van Stadens River the Maitland River does not have any Department of Water Affairs and Forestry commissioned dams. However, there are a number of small farm dams that have reduced the flow of water reaching the estuary and have increased the encroachment of aquatic macrophytes along its course. Although not quantified it was observed that during periods following heavy rainfall substantial amounts of organic and inorganic matter are washed down the river and end up either being retained within the estuary or completely washed out to sea. The organic matter could form a source of carbon for the microbial communities in these systems (Day *et al.* 1989). From this study it was observed that phosphate concentrations increased following an increase in river inflow and that were much higher compared to periods when river flow was low. Farming activities in

the Maitland catchment are mainly agriculture, dairy, chicken and pig farming that include fertilising large tracts of land with organic fertilisers. These could serve as sources of nutrients that would potentially increase nutrient inputs in the estuary particularly if such activities are scaled up. Prior to the estuary being scoured by floods, high quantities of filamentous and coccoid cyanophytes comprised a significant proportion of the phytoplankton community for several months. These bloom periods were also associated with the build up of anoxia at depth.

Groundwater as a source of nutrients into the Van Stadens estuary is considered negligible when the mouth is open and the water table is deep. However, when the estuary is full following prolonged closed mouth conditions effluent flow conditions may predominate such that nutrient input can be derived from this source. Nutrients entering in this manner however, appear to be taken up by benthic micro- and macroflora and may not be available for phytoplankton growth. Long periods of mouth closure ensure input of nutrients via groundwater seepage into the estuary encouraging benthic micro-and macroalgal proliferation. During the last year of the study the mouth had remained closed for over 10 months the longest closed-mouth period since the study began. This was apparent from the successive monthly data points obtained indicating a raised water table (Figure 8a, b & c). Prolonged closed-mouth conditions are characterised by increased water-column transparencies with low light attenuation coefficients spanning the entire estuary, which favours benthic micro-and macroalgal growth as well as submersed macrophytes like *Potamogeton pectinus*.

6.2 Biotic Responses

This study investigated phytoplankton primary productivity in two temporarily open/closed estuaries over an annual seasonal cycle. Production estimates from the Maitland estuary were considerably higher during the autumn than those from the Van Stadens although they were not significantly different during other seasonal periods. The nanophytoplankton size-group was the key fraction driving water column production in both the estuaries across all seasons. This finding is attributed to the high rates of turnover exhibited by this group of algae. This however, was in sharp contrast to the chl-*a* results in that from a total of 35 months sampled the microphytoplankton group made up the majority of the chlorophyll *a* size-fractions approximately 57.14% of the time while the nanophytoplankton and the picophytoplankton group made up 17.14 and 8.57% respectively. From a chl-*a* standpoint the microphytoplankton group are a major size-group that is key to the water column microalgal production. This may illustrate as well as emphasise the significant point regarding microalgal studies that the mere sampling of water for chl-*a* may not completely reveal the salient aspects of microalgal metabolism and production of temporarily open/closed estuaries. When comparing these findings to the Maitland chl-*a* results the production data corroborates the chl-*a* data in that of the 33 months sampled over the study period the nanophytoplankton size-group was the major contributor to phytoplankton chl-*a* approximately 47.21% of the time whereas the microphytoplankton were second highest contributor about 41.21% of the time. These results illustrate the dissimilarities between these two adjacent estuaries in terms of microalgal chl-*a* and the rates of productivity within each estuarine system.

Both estuaries showed an increase in chl-a in response to an increase in river inflow, however the magnitude of response was different. Total phytoplankton chl-*a* biomass levels in the Maitland estuary were approximately ten fold more than that in the Van Stadens estuary following a breaching event. Nanophytoplankton production contributed over 75% to total phytoplankton production during the spring (open mouth phase) and summer (closed mouth phase) seasons. Both micro- and picophytoplankton did not contribute significantly to total phytoplankton production during the closed mouth phase. The microphytoplankton were the most important contributors to phytoplankton standing crop making up over 55% of overall biomass, whereas nanophytoplankton showed high levels of turnover surpassing picophytoplankton by approximately 20%. The nanophytoplankton fraction drives primary production year round; especially during the growing seasons (spring & summer) signifying that heterotrophic plankton production in these estuarine systems are mainly supported by this phytoplankton group. Microalgal chl-*a* concentrations from the Van Stadens estuary were consistently lower compared with that measured from the Maitland estuary. This was unexpected since the Van Stadens estuary has a greater catchment area than the Maitland's therefore it would be expected that a larger surface area would deliver more nutrients. The fact that it was not the case is probably explained by the presence of the two dams in the upper catchment that trap any nutrients coming from the upper part of the catchment area.

Unlike nearby regional temporarily open/closed estuaries, picophytoplankton in the Van Stadens and Maitland estuaries are not key contributors to phytoplankton production. Microphytoplankton contributed as much as the picophytoplankton size group to total water column production although during certain seasonal periods of the year the microphytoplankton size-fraction can contribute more. Other regional studies implicate the very small-sized group of the phytoplankton assemblage as the important size-group in these types of estuarine systems (Froneman 2000a, 2002b, Perissinotto *et al.* 2003). Our data suggests that the intermediate and large size fractions can be important to ecosystem production and may also reflect the biogeographical uniqueness of the estuarine system with its associated catchment signatures.

The phytoplankton community structure in both estuaries was represented by between six and seven taxonomic groups. Cell densities for the Van Stadens estuary were low (<200 $x10^3$ cells ml^{-1}) reflecting the oligotrophic nature of the estuary. In contrast in the Maitland estuary cell densities averaged greater than 500 $x10^3$ cells ml^{-1} over a 20 month period. Phytoplankton blooms in November and December 2002 were associated with extended periods of low river inflow and were dominated by small flagellated chlorophytes (e.g. *Micromonas* sp. and *Pyramimonas* sp.). Although these algae had been observed previously their cell numbers had never been greater than 10%. Nutrient concentrations during this period were low. During the same months in the Maitland estuary two small flagellated greens (e.g. *Tetraselmis* sp. and *Nephroselmis* sp.) were the dominant phytoplankton under low nutrient conditions although the species differed. This suggests that although the species may be different and occur in separate estuaries they may respond in a similar manner to environmental factors. During periods of increased river inflow large-sized flagellates dominated the water column possibly responding to an

increased nutrient supply as well as the ability not flocculate with suspended matter (Cuker *et al.* 1991 and Burkholder *et al.*1997).

Under periods of low river inflow that were associated with a clear water phase large flagellated heterotrophic forms of phytoplankton were present with cell abundances greater than 10% particularly dinoflagellates like *Amphidinium* sp. and *Peridinium* sp*.* including cryptophytes like *Cryptomonas* sp. These algae were often equally present in the upper and lower portions of the water column. These algae have been shown to augment their nutritional requirements by actively consuming small photoautotrophic phytoplankton (Bold and Wynn 1985). This suggests that under conditions of low nutrient concentrations algal species that exhibit dual modes of metabolism will succeed. Although the strength of the relationships between mouth condition and species distribution was weak it, however does appear that changes in mouth condition has an influence phytoplankton community structure. This means that any changes in the frequency of natural flooding will possibly alter the regularity of breaching hence affecting phytoplankton community composition.

6.3 Annual Patterns

The Maitland and Van Stadens estuaries were selected as study sites with the aim of determining whether their close proximities would present similar microalgal responses to changes in river inflow and mouth condition. From the data the two estuaries, although close in proximity showed dissimilar microalgal responses to changes in river inflow and changes in mouth condition

Microalgal biomass for the Van Stadens estuary was consistently lower than those from the Maitland estuary, which had higher nutrient input. Both estuaries depend strongly on nutrient input from the river and the frequency with which these events take place has a strong bearing on the ecological functioning of these small temporarily open/closed estuaries. Certainly the hydrodynamic influences do override biotic ones, however the way the biology responds to these physical forcings will determine ecosystem function. The pattern of mouth condition for the two estuaries was varied with the Maitland estuary open more than the Van Stadens. This was the result of the flood that had a greater impact on the Maitlands estuary compared to Van Stadens. A semi-closed mouth condition over 8 months resulted in a continuous flow of estuarine water out to sea yet there was no true connection with the marine water. This was not the case with the Van Stadens estuary, as the sandbar across the mouth is placed high (perched) the water cannot readily flow out to sea. Nanophytoplankton production is certainly the size-fraction driving water column production within the Van Stadens and Maitland estuaries while microphytoplankton is the key size-group responsible for biomass production.

7. SUMMARY OF NEW KNOWLEDGE GENERATED FROM THE RESEARCH

From this study it was clear that river inflow is essential to sediment and nutrient transport, which are critical to estuarine microalgal production. Discharges >3.0m^3s^{-1} are instrumental in introducing suspended matter that limits light availability thus limiting phytoplankton production. In addition discharges of those magnitudes or greater will scour the estuarine bed floor particularly small estuaries similar to the Van Stadens and the Maitland. Freshwater pulses will initially dilute the estuarine water causing the water to become fresh, however when the mouth is breached the estuary becomes tidal with marine water penetrating into the estuary. Salinity gradients persist only as long as there is freshwater inflow and the mouth remains open. Low river inflow and sediment transport along the coast combine to isolate the estuary from the sea. Low levels of river inflow will maintain the upper reaches fresh while overwash over the sand bar will keep high salinities in the lower reaches. These salinity and density gradients are short-lived as wind induced mixing breaks up the stratification generated within a few days.

This study has demonstrated that from the once-off productivity experiments nanophytoplankton size-fraction is responsible for driving water column production mainly in the growing seasons of the annual cycle. Macronutrients entering the estuaries through river inflow are essential in stimulating phytoplankton chlorophyll *a* concentrations especially following an increase in freshwater input. An increase in phosphate and nitrate inputs into the estuaries was positively related to freshwater inflow associated with breaching events. Periods of low river flow (closed mouth phase) did not contribute significantly to ambient estuarine nutrient concentrations. Riverine nutrient concentrations remained as low as estuarine concentrations during the two-year period of river monitoring. Contributions of nutrients from groundwater sources were varied although at times nutrient concentrations in the sampling wells were higher than estuarine concentrations. Microphytoplankton chl-*a* was the size-fraction most stimulated following breaching events and this pattern held true in both temporarily open/closed estuaries studied. This is in sharp contrast to what has been recorded for similar estuaries within the region and elsewhere. This indicates the significance of this size group's contribution to pelagic microalgal production and that not all temporarily open/closed estuaries respond similarly to environmental and biological factors even though they may experience similar hydrological and chemical changes.

Phytoplankton cell densities and community structure were dissimilar in the two estuaries. In the Van Stadens Estuary the phytoplankton community was predominantly comprised of flagellates consisting of dinoflagellates and cryptophytes during the first year of study followed by a shift in the community that was made-up of small flagellated chlorophytes during the second year of the project. For the same period in the Maitland Estuary chlorophytes were the dominant group particularly in the summer months of 2001 that switched to that where chrysophytes were the dominant taxa in the spring of 2002. Non-parametric multidimensional analyses based on presence/absence (Van Stadens) and log-transformed (Maitland) species data showed that phytoplankton species composition is influenced by changes in mouth condition. Phosphate concentrations in the Maitland estuary were higher than those measured for the Van Stadens estuary. In contrast nitrogen levels were higher in Van Stadens compared to the Maitland estuary. This means that the

Van Stadens catchment is less disturbed by anthropogenic inputs and is a poor nutrient source whereas the Maitland catchment receives additional nutrient supply from farming activities. This study has highlighted the significance of river inflow to small temporarily open/closed estuaries to the fact that continued reduction in river inflow will impact microalgal concentrations and alter phytoplankton species composition to the detriment of ecosystem functioning. Anthropogenically induced changes in the catchment will increase nutrient input concentrations in receiving rivers and estuaries hence negatively influencing phytoplankton community structure with the resultant effects on higher trophic levels.

This study has documented phytoplankton species composition for these two temporarily open/closed estuaries. Future changes in species abundance and composition can be used as indicators of changes in water quality.

8. LINKS WITH REGIONAL RESEARCH

- The data from this study have been compared with similar studies within the Eastern Cape region (Perissinotto *et al.* 2000, Walker *et al.* 2001, Froneman 2002a & b), and other coastal provinces (Nozais *et al.* 2001, Perissinotto *et al.* 2002) to consider its findings with respect to its contribution to the understanding of microalgal dynamics in temporarily open/closed estuaries. This work has highlighted the inherent intra- and interregional differences that these estuaries show although some may share similar climate and geographical characteristics.
- Regional studies on temporarily open/closed estuaries (Perissinotto *et al.* 2000, Walker *et al.* 2001, Froneman 2002a & b) have specified the importance of picophytoplankton as the size-fraction that is essential in driving phytoplankton production, which is in sharp contrast to what this study has shown albeit from once-off seasonal productivity experiments. In these studies the picophytoplankton often constituted the majority of the phytoplankton fraction and thus would make up the dominant group in terms of production. Our findings indicate that although this size group can make up the majority of the chl *a* concentration under certain conditions of low river inflow it is the nanophytoplankton group that are responsible for production.
- Findings from this study have been used as a guide in a Rapid Level Assessment of an Ecological Reserve Determination for the Tsitsikamma Estuary, a temporarily open/closed estuary, for the Department of Water Affairs and Forestry (Taljaard *et al.* 2003).

9. CONCLUSIONS

- The Maitland and Van Stadens estuaries are two estuaries adjacent to one another and share similar weather characteristics, however they demonstrated distinct biological responses. Total phosphates in the Van Stadens and Maitland estuaries were positively related with an increase in river inflow (R^2 = 0.903 and 0.996) respectively. Although these data have very few sampling points (N=5, the number of times there was flow in the Van Stadens River over a two year period), they however indicate a strong pattern of the river as a source of nutrients. Both estuaries were determined to be nitrogen limited although on a few occasions under low flow conditions both estuaries exhibited phosphorus limitation.
- Flood events that took place in the winter of 2002 had very different outcomes on the geomorphologies of the two estuarine systems. This was exhibited by significant scouring and dune washout of the Maitland estuary compared to a more canalised and depositional effect on the Van Stadens estuary. Episodic freshets that occur in these estuarine systems are associated with rainfall events on the catchment and those >3.0m^3s^{-1} breaches the mouth of the estuary. Sustained freshwater inflow will maintain the mouth of the estuary open until river discharges fall below 0.8 m^3s^{-1}. Low river inflows keep salinity levels in the upper reaches of the estuary oligohaline, however seawater input as overwash can raise salinities to mesohaline levels.
- From this study it is clear that the Van Stadens and Maitland rivers acted as significant sources of nutrient input into both estuaries respectively although the magnitude of nutrient concentrations entering the estuary were low. Owing to the steep topography and that part of Van Stadens catchment is a nature conservancy the level of nutrient input remains low.
- Conversely, nutrient levels entering the Maitland estuary were significantly higher than those measured in the Van Stadens estuary particularly phosphates. Intense farming activities in the Maitland catchment however, point to the fact that future development in the area by the scaling up of these activities will result in increased nutrient levels into the Maitland estuary.
- The two estuaries showed dissimilar microalgal responses to changes in river inflow and mouth condition. Microalgal chl-*a* concentrations from the Van Stadens estuary were consistently lower compared with that measured from the Maitland estuary. The presence of the two dams in the upper catchment may be instrumental in trapping any nutrients coming from the upper part of the catchment area.
- Phytoplankton chl-*a* in the Maitland was at times tenfold higher compared with that from the Van Stadens. The nanophytoplankton size group predominantly drove phytoplankton productivity for most of the periods surveyed in both estuaries suggesting that in both these estuaries the major energy pathway is primarily through this size-fraction whereupon micro-sized zooplankton grazers graze them.
- This project has demonstrated the effects river inflow has on the phytoplankton chl-*a* concentration, community composition and also a once-off seasonal productivity along a spatio-temporal scale over a three year period in two temporarily open/closed estuaries in the Eastern Cape. Periods of increased river inflow stimulated phytoplankton chlorophyll *a* and altered phytoplankton community structure by favouring a flagellated community of microphytoplankton.

During periods of low river inflow small-sized flagellated cells and diatoms become equally prominent with large heterotrophic cryptophytes and dinoflagellates in the water column.

10. RECOMMENDATIONS FOR FUTURE RESEARCH

Fundamental questions still remain to be answered with regard to mechanisms responsible for high levels of biodiversity observed in these small estuaries. These include:

- First, to understand how low levels of primary production are able to support high levels of secondary and tertiary production,
- Second, to examine how biological interactions, at the phytoplankton-zooplankton level, are influenced by different physico-chemical factors,
- Third, to investigate nutrient fluxes (e.g. nitrates, phosphates, & silicates) during periods of high and low river inflow.
- And lastly, to examine how individual plankton communities are structured by changes in mouth condition of the estuary following an increase in river inflow.

This will help develop a plankton trophic food web model for this and possibly other temporarily open/closed estuaries that can be applied across similar estuaries within the region and possibly across other geographic regions. This will improve our understanding of the role of physico-chemically triggered anthropogenic change. Furthermore, it would clarify what these effects would have on the food web structure and overall health of the estuarine ecosystem. Knowledge of changes in trophic interactions with and without major freshwater input (mouth opening, closing and over wash events) will aid decision-making processes related to developments in the catchments of the TOCE's.

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APPENDIX A:
Third Year Projects
(Z. Sambokwe, Z. Jika, S. E. Pambuka, L. Rose, E. Maletzsky, R. M. Fenwick)

Summary of outputs from student projects

Research projects were made available in the form of third year and honours projects supervised by P. T. Gama. These projects trained students in fieldwork, laboratory and data analysis, and report writing while supplying useful information to the broader study. The third year students and their projects are listed in Table A1.

Table A1: Projects completed by third year students from 2001-2004

Student	Year	Project Title
Z. Sambokwe	2001	Phytoplankton response to increased grazing levels of zooplankton.
Z. Jika	2001	Microalgal production in a fresh water pond.
S. E. Pambuka	2002	Determination of chlorophyll *a* recovered over short and long term extraction periods.
L. Rose	2003	Characterisation of the Van Stadens microphytobenthic biomass in relation to sediment type under different estuary mouth conditions.
E. Maletzsky	2003	Comparison of Maitland and Van Stadens Estuary phytoplankton chlorophyll *a* recovered by elusion, maceration and sonication.
R. M. Fenwick	2004	Effects of nutrient enrichment on phytoplankton biomass from a temporarily open estuary.

Three projects dealt with the response of algal biomass to variation in environmental conditions, the first dealt with the effect of zooplankton on phytoplankton biomass (Sambokwe 2001). The second project, investigated the effect of changes in physico-chemical conditions and mouth condition on microphytobenthic biomass (Rose 2003). And the last, the addition of nutrients in the water column on phytoplankton biomass (Fenwick 2004). Jika (2001) investigated the use of oxygen evolution as an indicator of primary production of phytoplankton in a freshwater system. The two remaining projects investigated various methods of chlorophyll *a* extraction, including extraction time (Pambuka 2003) and various methods of treating filtered samples (Maletzsky 2003).

APPENDIX B: Honours Treatises (Mawethu Nyakatya and Tracy Skinner)

1. Periphyton dynamics in the Van Stadens Estuary: a temporarily open/closed system.

Mawethu Nyakatya submitted his honours project, supervised by Phumelele Gama, in December 2001. The study investigated biomass accumulation and species composition on an artificial substrate over a 5-month period. Glass slides acted as an artificial substrate and were placed at 5 sites approximately 0.5km apart from the lower to upper reaches of the estuary. Slides were suspended in the water column 0.5m above the sediment surface; replicates of the slides were removed after 3 and 5 months in July and September 2001. Chlorophyll *a* concentration was determined by scrapping periphyton from slides, extracting chlorophyll *a* in ethanol and reading the extract using a spectrophotometer. A second scrapping was preserved and used for identification of species and cell counts using a light microscope and haemocytometer.

Results from this study showed highest periphyton chlorophyll *a* concentrations during July 2001 (ranging from 4-35ug.l^{-1}) with biomass increasing from the lower to upper reaches of the estuary. In December 2001 chlorophyll *a* concentrations ranged from 5-20μg.l^{-1} with biomass increasing toward the middle reaches and decreasing at the lower and upper reaches of the estuary. Species identified during this study included *Nitzschia longissima*, *Amphora coffeaeformis*, *Navicula* sp., *Peronia* sp. and *Melosira* sp.

2. Temporal variation in filamentous macroalgal abundance and composition

The honours treatise by Tracy Skinner was submitted in November 2002. This study looked at variation in biomass and species composition of filamentous algae in the Maitland Estuary. Bimonthly sampling was conducted from March-November 2002, three transects were placed across the width of the estuary approximately 0.5km apart, corresponding to the upper, middle and lower reaches of the estuary. Along each of the transects, 10 quadrats (0.01m^2) were randomly placed and all submerged vegetation collected, algae and submerged macrophytes were separated, a quarter of the algae collected was extracted in ethanol and chlorophyll *a* read using both a spectrophotometer and HPLC (high performance liquid chromatography) for biomass determination. The remaining algal sample was preserved and later identified using a light microscope and haemocytometer. Submerged macrophyte biomass was determined by drying and weighing the samples after separating them from other attached material.

Results from this study showed that the mean algal chlorophyll *a* concentrations were 150mg.m^{-2}, while submerged macrophyte dry weight averaged 300g.m^{-2} for the months of March, May and July 2002. The Maitland Estuary experienced a flooding event during September 2002, which resulted in the removal of all submerged macrophytes and filamentous algae. Algal biomass had recovered and increased to 140 mg.m^{-2} by April 2003, while submerged macrophyte increased to only 50g.m^{-2}. Prior to the flooding event *Spirogyra* sp. was the only filamentous algae present, post flooding *Spirogyra* sp., *Cladophora* sp. and *Chara* sp. were present.

APPENDIX C: MSc. Study (Tracy Skinner)

Determination of microphytobenthic biomass and species composition in a small temporarily open/closed estuary

Introduction

The aim of this study was to relate changes in microphytobenthic biomass and community structure to variation in physico-chemical conditions of the Van Stadens Estuary. Microphytobenthos is a collective term used to describe microalgae (diatoms, euglenoids, chlorophytes and cyanophytes) that constitute the benthos. Microphytobenthos may refer to algae that are epilithic, epipsammic or epipelic according to the available substrate.

Microphytobenthos plays an important role in estuaries with regards to sediment stability, nutrient cycling, primary production and more recently has been identified as an indicator of estuarine health. Sediment in an estuary may be stabilised due to the presence of a thin biofilm layer on the surface of the sediment as a consequence of diatoms. A number of studies abroad have shown that diatoms produce extracellular carbohydrate complexes (Staats *et al.* 2001, Underwood and Paterson 1993) that bind the sediment, reduce resuspension of particulate matter (Facca *et al.* 2002) and increase critical erosion shear stress of the sediment (Riethmüller *et al.* 2000).

The exchange of nutrients between the water column and sediment is influenced by microphytobenthic biofilms at the sediment – water column interface. In a study by Krom (1991), he showed that microphytobenthos limit the efflux of nutrients from the sediment due to high assimilation rates of the microphytobenthic layer. In addition, by increasing oxygen levels in the sediment the microphytobenthos can reduce denitrification (Rysgaard *et al.* 1995) in the sediment thus decreasing the transport of phosphorus into overlying water (Granéli and Sundbäck 1985).

Temporarily open/closed estuaries often show microphytobenthic primary production to be higher than that of the phytoplankton while in permanently open estuaries it is the phytoplankton that shows higher productivity (Cahoon *et al.* 1993). Froneman (2002) compared phytoplankton and microphytobenthic biomass under closed, overtopping and open phases of the temporarily open/closed Kasouga Estuary and showed that phytoplankton biomass increased when the mouth of the estuary was open, while microphytobenthic biomass decreased. Throughout the study period microphytobenthic biomass remained higher than phytoplankton biomass reaching up to 130mg Chl-*a* m^{-2} during the closed phase, while phytoplankton biomass peaked at 5mg Chl-*a* m^{-2} during the open phase of the estuary.

Three hypotheses were tested to investigate the structure and functioning of microphytobenthos in the Van Stadens Estuary:

1. The microphytobenthic community in the lower reaches will be dominated by large sized (>50µm) diatoms, and by small-sized (20-50µm) diatoms, chlorophytes and cyanophytes in the upper reaches.
2. Microphytobenthos will decrease by 50-80% immediately following mouth breaching and gradually increase following a prolonged closed phase.

3. Coarse-fine sediment in the lower reaches will yield low biomass concentrations compared to fine-muddy substrate which will yield higher biomass in the upper reaches of the estuary.
4. Diatom migration within the sediment will influence the community structure within the upper 1cm of sediment, with smaller *Navicula* spp. dominating in the morning and being replaced by larger *Cylindrotheca* spp. later in the day.

Materials and Methods

For the purpose of this study, three sampling sites were selected corresponding to the lower, middle and upper reaches of the Van Stadens Estuary. At each of these sites, a line transect 25m in length was placed along the bank of the estuary, the transect had to be moved up or down the bank as water level changed over the sampling period so that the sampling area was submerged by water between 10-50cm in depth. Water column samples were collected adjacent to each transect for nutrient analysis (total phosphate, soluble reactive phosphate, ammonia, nitrate and silica) and *in situ* physico-chemical measurements were recorded (temperature, salinity, conductivity, dissolved oxygen, redox, pH and light).

Samples were randomly collected for the determination of sediment characteristics along a transect at each site. Sediment organic content was determined by drying sediment samples at 105^{o}C and then ashed at 550 ^{o}C (Thornton *et al.* 1999). Grain size distribution was determined by sieving dried sediment (105 ^{o}C) through sieves of mesh sizes 500, 250, 125 and 63µm (Thornton *et al.* 1999). Porewater was extracted along each transect using tensiometers during quarterly community analysis sampling, redox potential of the porewater was recorded and samples were filtered and immediately frozen for nutrient analysis.

Samples for the determination of microphytobenthic biomass and community composition were collected using a 20mm internal diameter corer (Rodriguez 1993) which was randomly placed along the transect. Two cores were taken immediately adjacent to each other and the upper 1cm of sediment was used from one core for biomass determination and the other for community analysis. Chlorophyll *a* was extracted with 30 ml ethanol over a 24-hour period at 5^{o}C, and then the extract was cleared using Schleisher Schuell (GF/C equivalent) filter paper and read using a high performance liquid chromatography (HPLC) (Riethmüller *et al.* 2000). When it was established that a good correlation existed (R^2 = 0.95, n=20) between samples read with an HPLC and a spectrophotometer, subsequent analyses were done using a spectrophotometer (Hillebrand and Kahlert 2002). Samples for community analysis were preserved with Lugol's solution in the field and stored in a darkened environmental chamber at 5^{o}C. The sample was re-suspended and a drop (10-15µl) was placed on a haemocytometer and algal taxa identified to species level where possible under a light microscope at 400x magnification, using available keys.

Sediment Experiment

Sediment was collected from the Van Stadens Estuary and sieved into four different size fractions: coarse sand (CS: 500-1000µm), medium sand (MS: 250-500µm), fine sand (FS: 125-250µm) and very fine sand (VFS: 63-125µm). A total of 15 perspex sediment trays

(Figure 1) with the following dimensions (200mm x 200mm) were designed and subdivided into 16 compartments (50mm x 50mm), the base of each sediment tray was covered by an 80µm mesh to allow interaction between the sediment tray and benthos. Sediment types (CS, MS, FS and VFS) were randomly allocated within each of the sediment trays then were placed in a sheltered area of the Van Stadens Estuary to allow colonisation by microphytobenthos. Weekly sampling was conducted where water column, porewater (as above) and sediment tray samples were taken. Tray sampling involved the removal of three trays each week, each compartment within a tray was sub-sampled where two cores were taken for biomass determination and one core for species identification.

Diatom Migration

Sampling was conducted quarterly during March, June, September and December 2003, samples were taken from one site in the lower reach of the estuary. Cores were taken from the site on an hourly basis from 11am - 2pm, the upper 0-0.5cm and 0.5-1cm of the core sediment was collected and preserved. The diatom frustules were cleaned and mounted as described by Hassle (1978). Diatoms were identified under oil emersion at 1000x magnification using a Zeiss light microscope.

Results

Community Analysis

To test the first hypothesis, addressing microphytobenthic community structure along the length of the estuary, sampling was conducted quarterly during March, June, September and December 2003. Sampling involved the analysis of water column and porewater physico-chemical characteristics, sediment characteristics, core collection for biomass determination (using a spectrophotometer) and community composition (as described above). Over the course of the study the Van Stadens Estuary had three distinct mouth phases open, closed and overtopping. Open conditions were experienced in March and June, overtopping during September and closed during December 2003.

Results from water column, porewater and sediment characteristic data showed that there was little variation between sites, more variation was observed over a temporal scale due to mouth condition (influenced by salinity and sediment grain size structure) and season (influenced temperature). Nutrient concentrations were found to be consistently low during the course of sampling and porewater nutrient concentrations remained higher than that of water column nutrients (Figure 2). Microphytobenthic biomass varied between 0.3-5.7 $\mu g.g^{-1}$ sediment and showed no clear trend over the sampling period and was not related to physico-chemical conditions of the estuary. When comparing chlorophyll *a* concentrations obtained from the spectrophotometer and HPLC a good relationship was found (R^2 = 0.935), for this reason subsequent chlorophyll *a* concentrations were measured using only the spectrophotometer.

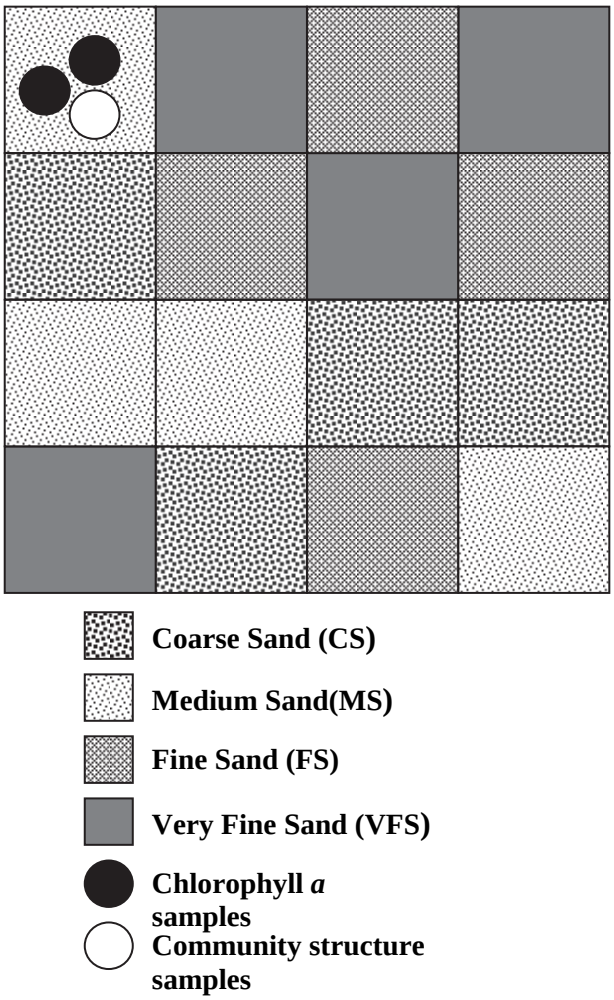

Figure 1 Layout of the sediment trays used to test the effect of sediment grain size on microphytobenthic biomass, showing the random placement of different size fractions and the use of cores to sample for biomass and species identification.

The microphytobenthic community was dominated by diatoms (>90% at all sites on each sampling date) the most abundant species were *Nitzschia closterium*, *Amphora coffeaeformis*, *Pleurosigma aestuarii* and *Navicula gregaria*. Canonical correspondence analysis (CCA) plots were drawn in CANOCO using environmental and species data obtained from each sampling date, a Monte Carlo Permutation test was run on the data. No physico-chemical characteristic was found to significantly influence the community composition between sites due to the low variation between sites. For this reason community data from all sampling trips (total of 49 species) were combined with the physico-chemical data and a CCA plot was drawn (Figure 3), a Monte Carlo Permutation test was run and this showed that the percentage of coarse sand within the sediment had a significant effect on the microphytobenthic community structure (P=0.05, F=1.69).

The first hypothesis was rejected, as there was no significant difference in the community composition at the three sites. Diatoms were dominant in the lower, middle and upper reaches of the estuary due to the lack of longitudinal physico-chemical differences in the estuary at the time of sampling.

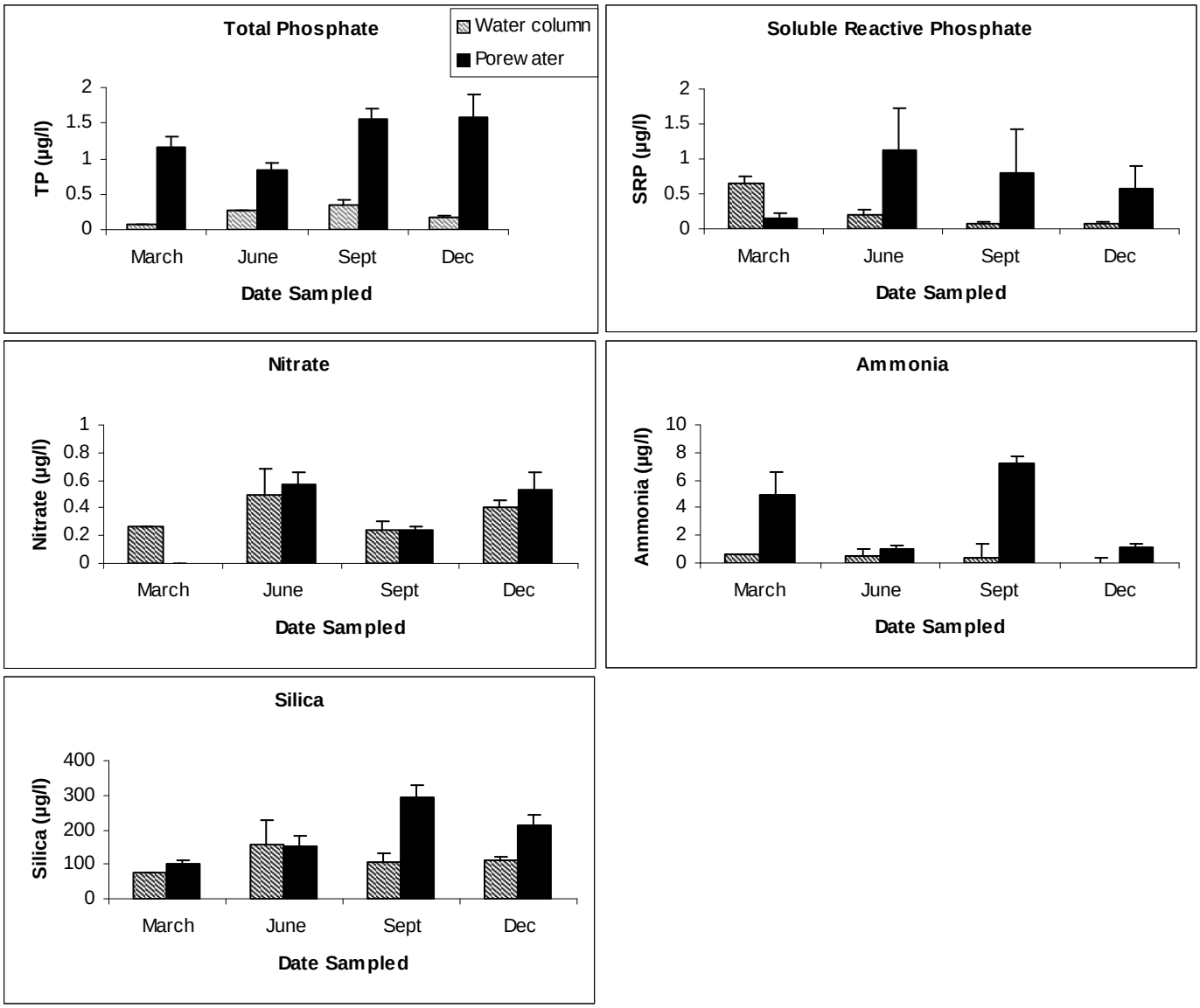

Figure 2 Mean water column and porewater macronutrient concentrations taken during the months March, June, September and December 2003 (mean ± standard error).

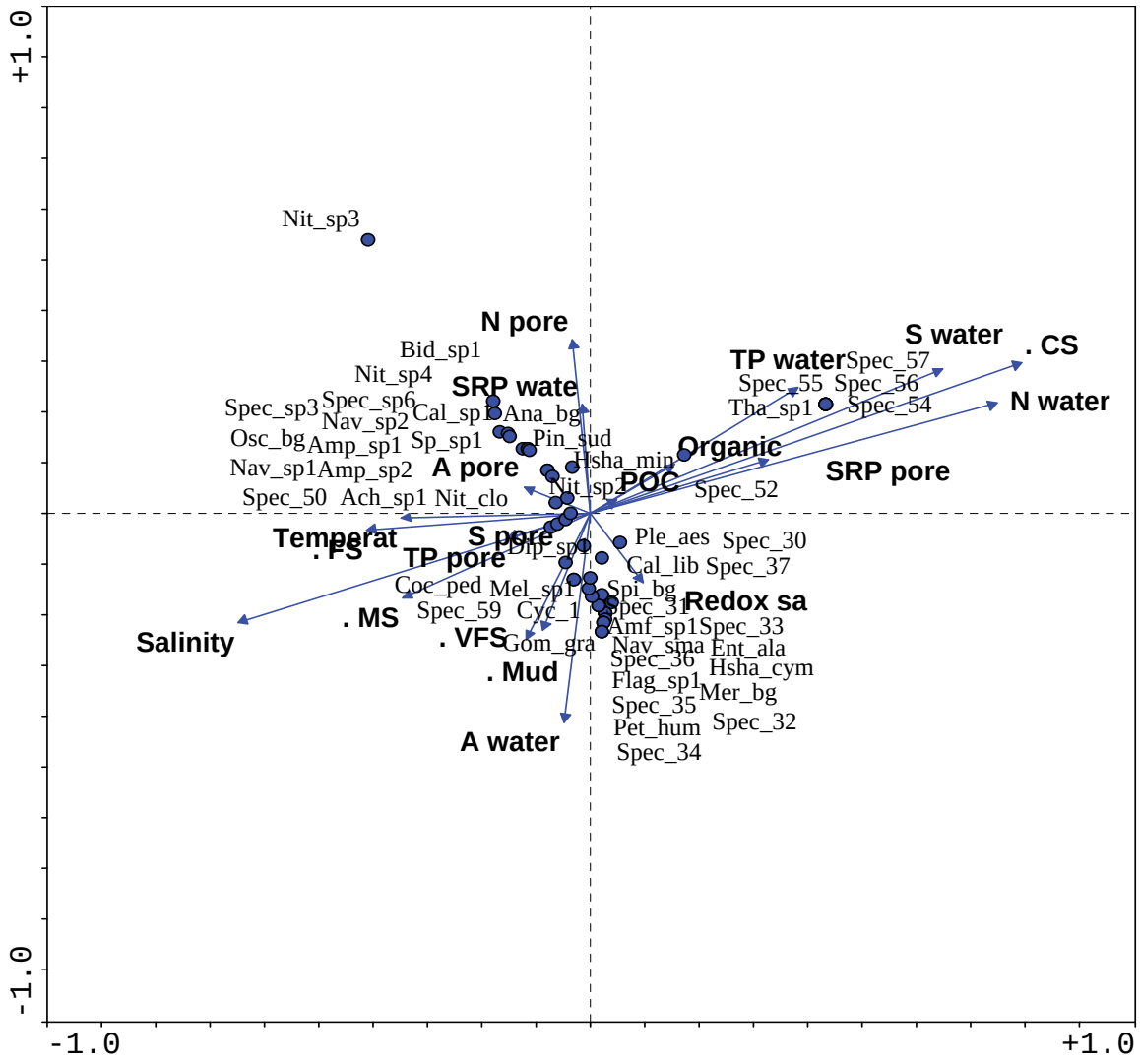

Figure 3 CCA plot of environmental variables and species identified at all sites including all sampling dates (ClySlt – Clay/silt >63µm, VFS – very fine sand 63-125µm, FS – fine sand 125-250µm, MS – medium sand 250-500µm, CS – coarse sand >500µm, Temp – water column temperature, Sal – water column salinity, POC – water column particulate organic content, DOC – water column dissolved organic carbon, SOC – percentage sediment organic content, Redx – sediment Redox potential , and TP, SRP, A, N and S – total and soluble reactive phosphate, ammonium, nitrate and silica porewater concentrations).

Mouth Opening Event

The effect of a mouth opening event on microphytobenthic biomass was investigated during weekly sampling over a five week period following a mouth opening event in October 2003. The mouth of the estuary was open for two days, due to prior overtopping events that filled the estuary with saline water until the mouth breached, and not due to increased inflow of freshwater from the catchment. The result of this was that the estuary was not flushed and scoured as expected, but water was simply drained from the estuary and the water level dropped.

Results showed that on the first sampling date salinity dropped from an average of 30ppt in the lower reaches to 5ppt in the upper reaches, for the remaining sampling dates salinity throughout the estuary averaged 28ppt (Figure 4). Physico-chemical conditions in the estuary showed little variation between sampling dates or sites, with temperature averaging 23 ^{o}C and nutrient concentrations being consistently low.

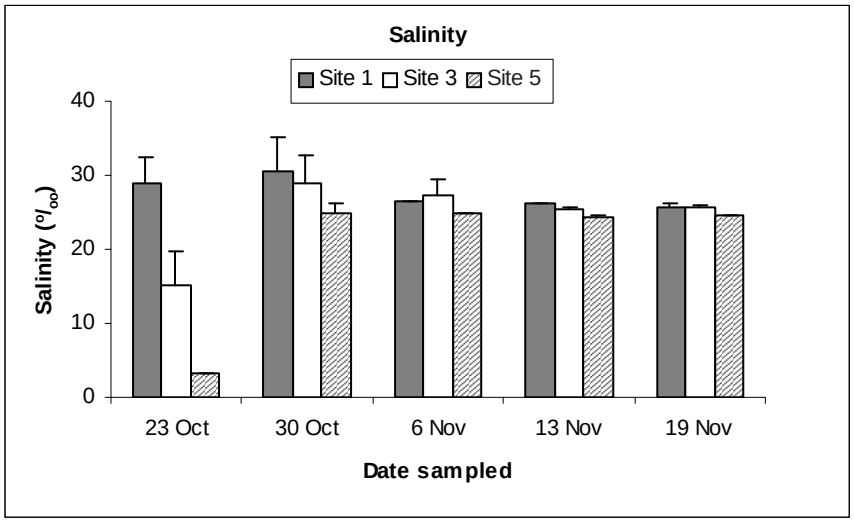

Figure 4 Mean salinity values at weekly intervals following a mouth-opening event during October and November 2003 (mean ± standard error).

Microphytobenthic biomass did not show a marked decline following the mouth opening event, nor was there an increase in biomass following mouth closure over the five-week sampling period (Figure 5). The hypothesis could not be rejected as a mouth opening event of greater magnitude was expected and may show expected results, as have been shown in other South African estuaries (Froneman 2002).

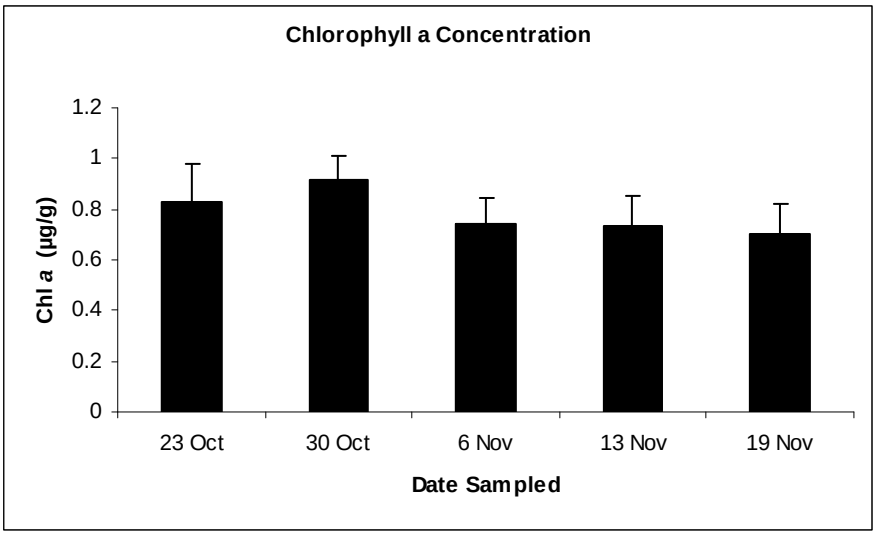

Figure 5 Mean chlorophyll *a* concentrations at weekly intervals following a mouth-opening event during October and November 2003 (mean ± standard error).

Sediment Size Structure and Biomass

The third hypothesis, relating microphytobenthic biomass to sediment size fractions, could not be tested using available field data as there was little difference in sediment size structure between sites. Therefore an *in situ* sediment experiment was designed to test this hypothesis more rigorously.

Results from this study again showed little variation in physico-chemical conditions within the area sampled, salinity and temperature averaged 20ppt and 23^{o}C respectively, and nutrient concentrations remained consistently low. Microphytobenthic biomass on the sediment trays was opposite to that hypothesised, as highest biomass was found on coarse sand, biomass then decreased with a decrease in sediment grain size so that the lowest biomass was found on very fine sand (Figure 6), therefore rejecting the third hypothesis. Species analysis data in the Masters dissertation by Tracy Skinner UPE.

Diatom Migration

An additional study was conducted in order to observe variation in the microphytobenthic diatom community over time.

Results from March 2003 showed that species dominance within the diatom community varied during the time sampled. Species were said to be dominant when accounting for 10% or more of the diatom community. Five species were found to be dominant *Amphora coffeaformis*, *Navicula* sp1., *Nitzschia dissipata*, *Navicula* sp2. and *Amphora arcus*. *Amphora coffeaeformis* showed a clear trend as this species was dominant at 0-0.5cm at 11am and 2pm, but migrates into the lower sediment (0.5-1cm) from 12-1pm, *Navicula* sp2. showed the opposite behavior as these cells migrated to the surface at midday.

Table 1: Species identified and abbreviations used in CCA plot using combined data from March, June, September and December from all sites sampled.

Species	Abbreviation used (CCA)
Achnanthes oblongella	Ach_obl
Amphora sp1.	Amp_sp1
Amphora sp2.	Amp_sp2
Anabaena sp.	Ana_sp
Caloneis africana	Cal_afr
Caloneis liber	Cal_lib
Cocconeis sp.	Coc_sp
Cyclotella sp.	Cyc_sp
Diploneis caffra	Dip_caf
Entomoneis alata	Ent_ala
Euglenoid	Eug_sp
Flagellate	Fla_sp
Gyrosigma prolongatum	Gyr_pro
Licmophora sp.	Lic_sp
Melosira sp.	Mel_sp
Merismopedia sp.	Mer_sp
Navicula crucicula	Nav_cru
Navicula sp1.	Nav_sp1
Navicula sp2.	Nav_sp2
Nitzschia dissipata	Nit_dis
Nitzschia distans	Niz_dis
Nitzschia sp.	Nit_sp
Oscillatoria sp.	Osc_sp
Petroneis humerosa	Pet_hum
Planothidium delicatulum	Pla_del
Pleurosigma aestuarii	Ple_aes
Seminavis sp.	Sem_sp
Spirulina sp.	Spi_sp
Synedra sp.	Syn_sp

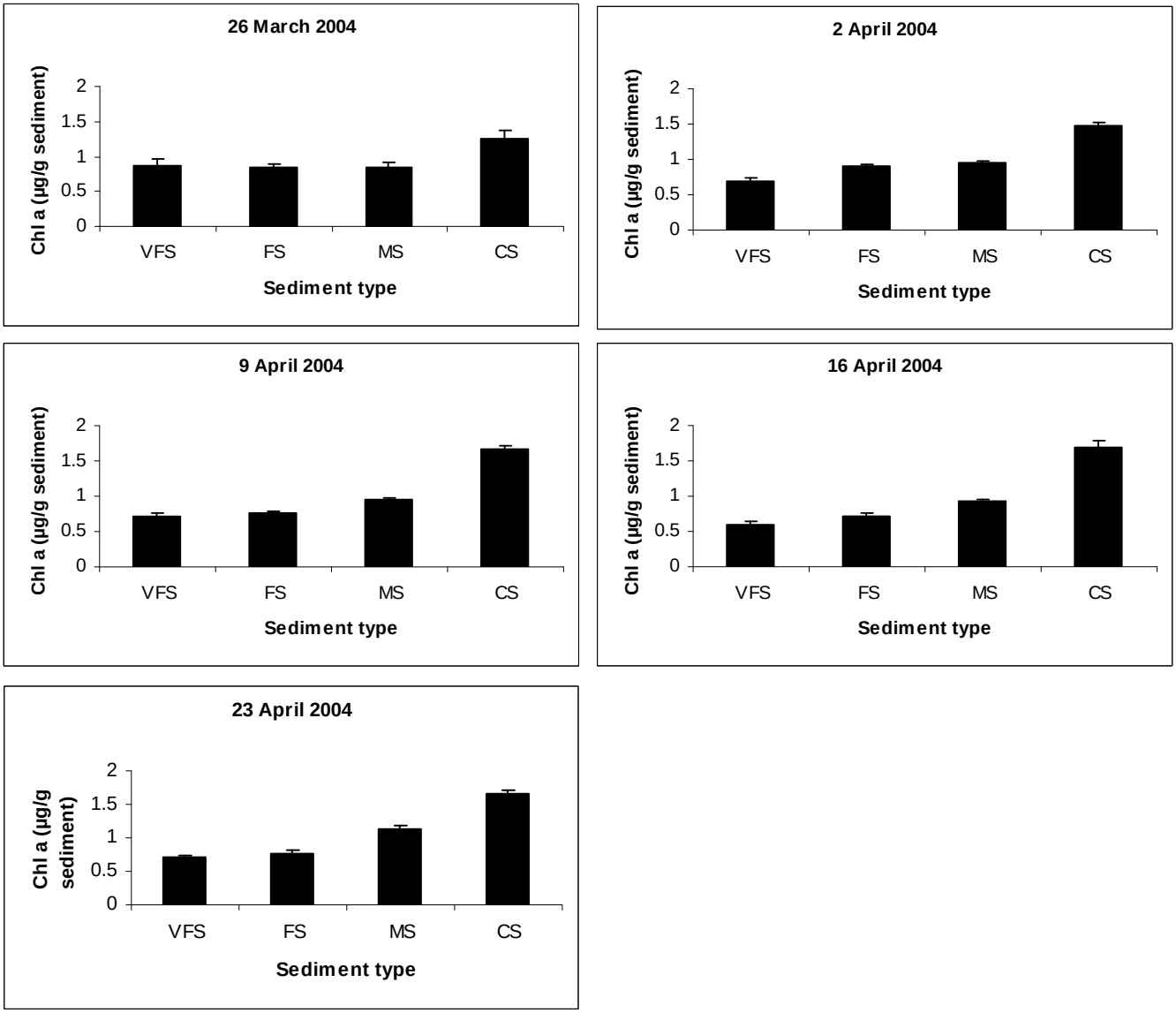

Figure 6 Mean chlorophyll *a* concentration from sediment trays sampled weekly during March and April 2004, showing an increase in biomass from very fine sand (VFS) to coarse sand (CS) (mean ± standard error).

Conclusion

Spatial variation in the microphytobenthic community structure of the Van Stadens Estuary was not explained by the physico-chemical characteristics measured due to the homogenous nature of the estuary. At a temporal scale, community structure was influenced by the percentage of coarse sand present within the sediment. From this study microphytobenthic biomass did not show a significant decrease following a mouth breaching event of low magnitude as previously observed in this estuary a year earlier (Gama unpubl.). Experimental work on the Van Stadens estuarine sediment suggests that microphytobenthos biomass is highest in the coarse sand sediments and least abundant in the very fine sand. Migration studies show that depending on the time of day sampled and time of the year, submersed diatom community composition in the 10mm of the sediment surface varies as a result of light intensity.

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