Phytoplankton Chlorophyll a Concentration and Community Structure in Two Temporarily Open/Closed Estuaries in the Eastern Cape, South Africa

by

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Promoter: Prof JB Adams
DECLARATION

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ABSTRACT

River flow is important in controlling phytoplankton distribution in estuaries. Data on the effect of river inflow on phytoplankton distribution patterns in temporarily open/closed estuaries is lacking. This study investigated the influence of river inflow on size-fractionated phytoplankton biomass (Chl a), community composition and environmental parameters measured monthly over three years in two temporarily open/closed estuaries in the Eastern Cape, South Africa. A once-off primary production study over an annual cycle was completed in the Van Stadens and Maitland estuaries. The study monitored physical, chemical and biological characteristics in both estuaries to examine the effects of changes in environmental factors and river inflow. Daily sampling of physico-chemical and biological variables from river to sea was carried out in the Van Stadens to investigate short-time scale effects of changes in environmental factors and river inflow on the phytoplankton biomass. Five and three stations in the main channel of the Van Stadens and Maitland estuaries respectively were sampled at 0.5 m below the water surface and 0.5 m above the sediment surface for biological and chemical variables and at the surface, 0.25 m and every 0.5 m thereafter for physical parameters. Five stations adjacent to the main channel along the estuary were monitored for groundwater macronutrient concentrations and five additional sites located within the upper catchment of the Van Stadens River were sampled on a quarterly basis over two years.

Both estuaries were characterised by distinct hydrological conditions, an overwash, an open, a closed and a semi-closed mouth phase. Flooding in the Maitland and Van Stadens estuaries in 2001 and 2002 caused sediment scour, altered channel morphology and brought about breaching of the mouth. Flood driven mouth-breaching events occurred three and four times in each of the estuaries during the study. The mouth stayed open 20 – 25% and was closed 60 – 65% of the time. In the Van Stadens the closed overwash mouth condition occurred approximately 10 – 20% of the time while in the Maitland it occurred less with the semi-closed mouth condition occurring 10 – 20% of the time. Incidents related to mouth opening not associated with strong river floods occurred approximately 10 – 15% of the time, although in the Maitland a semi-closed mouth state persisted more frequently than in the Van Stadens Estuary. During flooding events salinity dropped to low levels (< 5 psu) but soon recovered to brackish conditions when river flow was reduced and marine water penetrated deep upstream. Reduction in river flow combined with marine sediment deposition resulted in the closure of the mouth. During closed mouth conditions strong onshore storm surges and spring high tides introduced marine water through overwash that kept salinity high. In both estuaries salinity showed a negative correlation with rainfall ($R^2 = 0.12$), indicative of the strong influence of marine overwash that kept salinity high thus masking the influence of freshwater.

High rainfall in the Van Stadens Estuary caused high levels of turbidity that reduced light penetration at depth. Light attenuation was positively correlated with the high rainfall ($R^2 = 0.26$) suggesting that increased turbidity was linked to rainfall induced discharge. In contrast, in the Maitland Estuary light
attenuation did not show any correlation with increased rainfall possibly because of the reduced water depth and increased euphotic zone following the floods in 2002. High river inflow introduced macronutrients in both estuaries such that dissolved inorganic phosphates (DIP) and dissolved inorganic nitrogen (DIN) concentrations in the Van Stadens Estuary were strongly correlated with rainfall \( R^2 = 0.78 \) and 0.57 respectively. In the Maitland Estuary DIP and DIN concentrations remained significantly higher \( (p < 0.05) \) compared to that in the Van Stadens suggesting that the Maitland catchment contributed greater nutrient input into the estuary and may be associated with farming activities. Phytoplankton chlorophyll \( a \) (Chl \( a \)) ranged from 0.8 – 13.9 \( \mu \text{g} \text{L}^{-1} \) in the Van Stadens and in the Maitland Estuary from 5.3 – 138 \( \mu \text{g} \text{L}^{-1} \) during the 3-year study. During the open mouth condition Chl \( a \) biomass and primary production ranged from 5.4 – 52.9 \( \mu \text{g} \text{Chl} \text{a} \text{L}^{-1} \) and 1.2 – 11.7 \( \text{mg C m}^{-2} \text{d}^{-1} \) in the Maitland and in the Van Stadens from 1.6 – 9.8 \( \mu \text{g} \text{Chl} \text{a} \text{L}^{-1} \) and 1.2 - 14 \( \text{mg C m}^{-2} \text{d}^{-1} \) respectively. Maximum annual primary production in the Maitland and Van Stadens estuaries was 8.8 and 5.1 \( \text{g C m}^{-2} \text{y}^{-1} \) respectively. When the mouth was open in the Van Stadens Estuary the microphytoplankton (> 20 \( \mu \text{m} \)) accounted for > 65% of the Chl \( a \), whereas during closed mouth conditions they accounted for about 55% of the Chl \( a \) biomass. Chlorophytes became the dominant taxon in the dry summer months but were replaced by cryptophytes and dinoflagellates during the wet season. When nutrient concentrations were low during low flow conditions in the Van Stadens Estuary mixotrophic microphytoplankton became an important fraction of the water column together with phototrophic dinoflagellates and cryptophytes. In the Maitland large sized chlorophytes were the dominant taxa in late spring and summer seasons and made up more than 80% of the cell numbers. In the Maitland before the floods in 2002 cyanophytes were the dominant group in late spring contributing more than 75% in cell abundance. Data from the short-term study in the Van Stadens Estuary showed similarities and differences in the Chl \( a \) response to increased river inflow. High river inflow initially reduced Chl \( a \) biomass followed by a recovery period of a couple of days compared to a 8 – 10 week recovery period in studies monitored over seasonal and annual temporal scales. The responses may be dissimilar but help to illustrate that there are similar response patterns to environmental forcing necessary to support phytoplankton biomass at different temporal scales.

This study has demonstrated that flooding events caused by strong river flow cause breaching of the mouth, a reduction in salinity and marked nutrient input. Although the causes of flooding can be similar in both estuaries the resultant effects are varied and can alter the ability of the estuary to retain water. This study was able to demonstrate that the supply of macronutrients from the catchment was strongly correlated with rainfall \( R^2 = 0.67 \) and that phytoplankton growth mainly depended on an allochthonous source of macronutrients although internal supplies could be critical at times in controlling microalgal biomass.
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<td>ICOLL</td>
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1. General Introduction

1.1 Background

Estuaries on the eastern seaboard of the South African coast developed as a result of sea level fluctuations during the Holocene epoch (Lubke and de Moor 1988). They have over time undergone varying degrees of infilling from fluvial and marine sedimentation processes. Temporarily open/closed estuaries are mainly structured by sporadic hydrodynamic influences that occur as floods including strong storm surges from the marine environment. The fluvial processes controlling their form are a result of limited freshwater input from the small catchment areas. The mean annual runoff is low such that river inflow is insufficient in maintaining a permanently open mouth. In the absence of strong river inflow a sediment bar forms across the mouth of the estuary that is periodically scoured when heavy flows are experienced. In temporarily open/closed estuaries sediment deposition is primarily of fluvial origin with limited sediment from the marine environment when open and tidal but may experience sediment input from overwash during strong on-shore sea-storm events. When strong freshets occur normally this type of estuary behaves as a river-dominated system for short periods (Perissinotto et al. 2000). When the mouth is breached the estuary becomes tidal, but soon closes as a result of coastal wave action associated with long-shore sediment transport across the mouth.

In South Africa, temporarily open/closed estuaries (TOCEs), non-tidal or lagoon-like estuaries make up nearly two-thirds of the estuaries found along the length of the coastline. Of these approximately 45% occur in the warm temperate Eastern Cape, 54% in the subtropical Kwa-Zulu/Natal and 1% in the cool temperate Western Cape. However, little is still known regarding planktonic and benthic microalgal composition and trophic function (Day 1981, Whitfield 1992). Temporarily open/closed estuaries are increasingly coming under threat due to anthropogenic impacts ranging from damming, upstream water abstraction to development and urbanization along estuarine and adjacent coastal floodplains. These activities cause a number of ecological (i.e. alteration of flow and modification of physico-chemical processes including variation in the availability of inorganic minerals) and economic (i.e. increase or decrease in coastal or estuary property value, loss of habitat for key fish species that use estuaries as nurseries) impacts (Byren and Davies 1989, Davies and Day 1998, Lambeth 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 TOCEs given that a great number are increasingly settled and developed as nodes for tourism and recreation. These developments will increase pressure on the estuarine environment thus requiring sound management and protection.
Studies carried out abroad (Head 1976, Ray et al. 1989, Mallin et al. 1991) and in South Africa (Hilmer 1984, 1990, Bally et al. 1985, Bate et al. 1986) have demonstrated the importance of microalgal production in permanently open estuaries. Although the size and scale of most estuaries in Europe and North America are not comparable to systems in South Africa there are certain similarities in the physical, chemical and biological processes that determine their ecological functioning (Day et al. 1989). Light transparency, inorganic nutrient input, nutrient residence periods and grazing effects can influence phytoplankton production (Hilmer 1984, Cloern 1987, Mallin et al. 1991, Mallin and Paerl 1994). Dissolved and suspended particulate matter are the principal constituents that absorb surface radiation as it enters the water column impacting directly on light attenuation, hence limiting phytoplankton growth (Smith 1982, Cloern 1987, Cole et al. 1992, Bledsoe and Phlips 2000). Nutrient inputs into estuaries from allochthonous sources have enhanced phytoplankton growth rates and biomass (Pinckney et al. 1999, Örnólfsdóttir et al. 2004). Estuaries occurring near large urban and peri-urban centres that drain extensive catchments, mainly in the northern hemisphere, show significant levels of internal nutrient loading such that primary production is essentially autochthonous, e.g. Chesapeake Bay and Swan Canning studies (Fisher et al. 1992, Gerritse et al. 1998). In a number of US estuarine studies (Cushing 1976, Cloern 1982, Doering et al. 1986) herbivory by zooplankton and macrobenthic fauna have been shown to reduce phytoplankton standing stocks, which influence phytoplankton seasonal patterns. These factors have also been shown to be important in South African permanently open estuaries (Day 1981, Hilmer 1984, Adams et al. 1999, Allanson and Baird 1999, Wooldridge 1999) although their magnitude and significance vary since the dominant processes governing microalgal production are more physical and hydrological than biological (Day 1981, Cooper et al. 1999).

In contrast, there has been little research focusing on TOCEs. Although a growing body of evidence is beginning to emerge regarding the physical, hydrological (Cooper 1988, Slinger and Largier 1991, Cooper et al. 1999, Schumann et al. 1999), chemical (Slinger et al. 1995, Allanson and Winter 1999), and biological (Wooldridge 1994, Perissinotto et al. 2000, Froneman 2002a) features of TOCEs in South Africa there is still a lack of data regarding the ecological functioning of these estuaries. Studies are beginning to look at the role of phytoplankton and benthic microalgal biomass and production (Perissinotto et al. 2000, Froneman 2000a, 2002b, Nozais et al. 2001, and Walker et al. 2001) in TOCEs. Owing to their shallow depth and broad channel that is covered by water when the mouth is closed, TOCEs support a rich benthic flora. As a result the majority of the primary production in these estuaries particularly during periods of mouth closure has been attributed to the microphytobenthos (Adams et al. 1999, Froneman 2002a & b, Perissinotto et al. 2002).
1.1.1 Phytoplankton Research in South Africa with reference to Temporarily Open/Closed Estuaries: Present Understanding

Studies on phytoplankton in temporarily open/closed estuaries (TOCEs) are not well documented for various reasons among which include the lack of capacity in microalgal taxonomy and the tedious nature of preserving, preparation, and identification of microalgal samples (Day 1981). Despite such shortcomings a number of studies have been carried out on TOCEs ranging from the Bot River Estuary in the Western Cape to the Mdloti River Estuary in Kwa-Zulu/Natal in the east (Bally et al. 1985, Nozais et al. 2001). These estuaries span two biogeographical regions (i.e., warm temperate and subtropical) along the South African coast (Whitfield 1994). The biological characteristics measured in these estuaries are summarised in Table 1.1.

Several studies in temporarily open/closed estuaries have used chlorophyll $a$ as a measure of phytoplankton biomass, while a few studies have quantified primary production and phytoplankton community structure using phytoplankton abundance measures. However these studies are the exception rather than the rule highlighting the lack of scientific knowledge on these estuaries. In these studies some have considered estimates of microphytobenthic (MPB) biomass and community structure (Table 1.1). Microphytobenthic biomass distribution patterns are generally uniform throughout the entire estuary particularly during periods of low river inflow, although in other systems peaks have been observed in the middle reaches, which are associated with zones of deposition (Snow and Adams 2007). When the estuary mouth opens following increased river inflow the estuary displays characteristics observed in permanently open estuaries with greater biomass in the upper reaches and less biomass near the mouth. There are only a few reports that have documented studies on benthic microalgal production. The general conclusion is that MPB are less productive than phytoplankton (Perissinotto et al. 2003). Although under closed mouth conditions water transparencies are greatly improved enabling light to reach the bottom. Water column nutrients during these periods are normally low suggesting that the nutrient source is mainly from the benthic sediments, phytoplankton rates of turnover remain high resulting in high productivity for these estuaries (Perissinotto et al. 2003). The group of algae normally responsible for high levels of production are the small-sized picophytoplankton, which often make up the majority size group and to a lesser extent the nanophytoplankton (Froneman 2002a & b).

These studies clearly indicate that chlorophyll $a$ concentrations in TOCEs are influenced mostly by physical and chemical factors. Freshwater inflow into TOCEs is essential in several ways. River discharges greater than 3 m$^3$·s$^{-1}$ are important in that they bring about breaching of the mouth particularly when the level of the water in the estuary is at maximum height (Huizinga 1994, 1996). The scouring
effect of the estuary bed that accompanies discharges of this and greater magnitudes transports and deposits fluvial sediment while redistributing estuarine sediment along the sediment floor (Largier and Taljaard 1991, Largier et al. 1992, Allanson and Read 1995). One of the immediate effects of increased river inflow is flushing and subsequent draining of the estuarine water right out to sea until the peak of the discharge subsides. Under these conditions chlorophyll $a$ concentrations are significantly reduced as the resident phytoplankton community is washed out to sea (Walker et al. 2001, Froneman 2002a). Suspended matter transported by low river inflow to the estuary can limit light penetration at depth often reducing it to the upper 0.1-0.5m of the water column (Day 1981, Cloern 1987). Freshwater inflow into the estuary introduces nutrients that are carried down from the catchment which are responsible for fertilizing the water column hence stimulating growth of the phytoplankton (Cloern et al. 1983, Chan and Hamilton 2001, Taylor et al. 2004).

Studies investigating the support of heterotrophic production in TOCEs have received more attention than that shown to autotrophic organisms (Day 1981, Whitfield 1992). The effects of planktonic grazers on phytoplankton populations in TOCEs indicate that secondary production is generally limited by the availability of prey, therefore an alternative food supply has to be sourced in order to sustain the levels of production often observed for these systems (Perissinotto et al. 2000, Kibirige and Perissinotto 2003). This research does point to a pattern indicative of high zooplankton production supported by low water-column primary production in these types of estuaries. As a consequence, the microbenthic algae have been implicated as the alternative source of carbon necessary to augment the pelagic demand of the zooplankton. Freshwater inflow has also been shown to influence zooplankton production and biomass (Perissinotto et al. 2000, Froneman 2002a). Strong pulses of river inflow can decimate zooplankton-standing stocks from flushing, whereas sustained moderate to low flows bring in macronutrients that stimulate the growth of phytoplankton and increase the availability of prey items for the grazing community. These patterns however, cannot be generalised to other similar estuaries as seen with systems located in the subtropical biogeographical region that show increased zooplankton biomass response during a closed mouth condition (Whitfield 1992, Kibirige and Perissinotto 2003).
Table 1.1 Temporarily open/closed estuaries studied along the South African coast and the biological characteristics measured. * denotes data available, - no data.

<table>
<thead>
<tr>
<th>Estuary</th>
<th>Chl a</th>
<th>Primary Production</th>
<th>Community Structure</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Bot River</td>
<td>*</td>
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<td>Bally et al. 1985</td>
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<td>East Kleinemonde</td>
<td>*</td>
<td>-</td>
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<td>Gama 2007</td>
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<td>Great Brak</td>
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<td>Adams and Bate 1994</td>
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<td>Kabeljous</td>
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<td>Kasouga</td>
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<td>Froneman 2002a, 2002b, 2004</td>
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<td>Maitland</td>
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<td>Present study</td>
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<tr>
<td>Mdloti</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>Nozais et al. 2001</td>
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<tr>
<td>Mhlanga</td>
<td>*</td>
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<td>Thomas et al. 2005</td>
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<tr>
<td>Mngazi</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>Snow and Adams 2007</td>
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<td>Swartvlei</td>
<td>*</td>
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<td>-</td>
<td>Robarts 1976</td>
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<td>Van Stadens</td>
<td>*</td>
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<td>Present study</td>
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1.1.2 Chlorophyll a Size-Fractionation

Coastal and estuarine environments are fast coming under increased pressure from anthropogenic activities in the catchment and adjacent coastal areas and altering the input rates, distribution and movement of nutrients in receiving waters (Malone 1977, Kemp and Boynton 1984, Taylor et al. 2004). Changes in nutrient loading can alter phytoplankton species composition by promoting the growth of species able to rapidly exploit available nutrients plus tolerate the changed environment (Hecky and Kilham 1988, Hansson 1992, Leibold and Wilbur 1992). Such shifts in the phytoplankton community are likely to be carried through the food web with the effect of altering food web dynamics and ultimately the flow of carbon through the pelagic ecosystem. Over several decades studies on phytoplankton size classes have received some attention because of the influence of particle size on phytoplankton population dynamics (Anderson 1965, Durbin et al. 1975, Tada et al. 1999), including the effect particle size has on...
the carbon flow through the pelagic food web by influencing the community structure of higher trophic levels (Jochem 2003, Murrell and Lores 2004). There are only a few studies in South Africa that have fractionated the water column chlorophyll \(a\) content in TOCEs (Froneman 2002b, Perissinotto et al. 2003). Results showed that the pico- and nanophytoplankton fractions were important in terms of biomass particularly during the closed mouth phase. This was also supported by the productivity data with the pico- and nanophytoplankton size groups showing higher rates of production.

The present study focused on the primary production and chlorophyll \(a\) concentration of the micro- (\(>20\) \(\mu\)m), nano- (2.7-20 \(\mu\)m), and picophytoplankton (1.2-2.7 \(\mu\)m) 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 particle sizes (Wetzel 1983, Bold and Wynne 1985), 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, this study examined 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. 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.
### 1.1.3 Primary Productivity

Studies on microalgal primary productivity in TOCEs have often been measured using different methods, therefore precluding direct comparison between estuaries. However, these various measurements provide an insight into general patterns of carbon sequestration and its potential availability to higher trophic levels. Studies on water column primary productivity in TOCEs show that phytoplankton productivity is generally higher than that of the microphytobenthos (Perissinotto et al. 2000), although in the Bot River Estuary phytoplankton annual primary production was found to be similar to that of the microphytobenthos (Bally et al. 1985).

The effects of different hydrological phases (high river inflow or floods, over-wash, and low inflows) on phytoplankton primary productivity were investigated in estuaries in the subtropical (Mpenjati, Mdloti) and the warm temperate (Kasouga, Maitland and Van Stadens) biogeographical regions (Froneman 2002a, Perissinotto et al. 2003, Gama et al. 2005). Water column productivity during the closed phase in the Kasouga was the lowest of all three hydrological phases, ranging between 10.92 and 19.83 mg C m\(^{-2}\) h\(^{-1}\). On the other hand, during the high river inflow phase (flood) the highest phytoplankton production was recorded, ranging between 40.09 and 64.66 mg C m\(^{-2}\) h\(^{-1}\). During this latter hydrological phase the mouth was not breached although the estuary did experience a significant influx of freshwater.

The increase in water column nutrient availability associated with the increased river inflow possibly accounted for the observed high productivity (Froneman 2002a). In contrast to the findings in the Kasouga Estuary, where picophytoplankton contributed the most to water column production during the closed and over-wash mouth phases, in the Van Stadens and Maitland estuaries the nanophytoplankton component was the largest contributor to production during both the open and closed phases.

Except for a few studies conducted in different biogeographical regions along the South African coast, microphytobenthic primary productivity data are lacking for most TOCEs. Benthic primary production recorded for the Bot Estuary (58 g m\(^{-2}\) y\(^{-1}\)) was calculated to be below the average of 67-200 g m\(^{-2}\) y\(^{-1}\) for global estuarine environments reported by Ferguson et al. (1981). However, when considering other systems in South Africa, the level of production for the Bot Estuary was similar to that of other estuaries with sandy sediments (Bally et al. 1985), although estuaries with muddy sediments have recorded higher production (Anandraj et al. 2007).
1.2 Aims

The aims 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 shifts in phytoplankton community structure following changes in nutrient loading brought about by increased water flow,
- To determine spatio-temporal distribution of the phytoplankton chlorophyll $a$ concentration and relate this to mouth condition,
- To examine the influence of mouth condition on phytoplankton community structure particularly during periods of mouth closure,
- To quantify size fractionated primary production over an annual seasonal cycle with a view to establish carbon energy flow patterns in TOCEs and relate it to changes in river flow.

1.3 Hypotheses

This investigation set out to test the following hypotheses that:

- Nano- and microphytoplankton size fractions (2.7 – 20 µm & >20 µm in size along major axis respectively) form the dominant phytoplankton that drive primary production during periods of increased river inflow in TOCEs,
- Picophytoplankton (1.2 - 2.7 µm in size along major axis) form the dominant phytoplankton group that drive primary production during closed mouth periods,
- Prolonged periods of closed-mouth conditions favour increased pico- and nanophytoplankton biomass and production,
- During periods of mouth breaching microphytoplankton production decreases beyond levels sufficient to support macro-zooplankton biomass and production,
- Increased nutrient supply associated with increased levels of river inflow supports higher concentrations of nano- and microphytoplankton biomass and production by shifting phytoplankton community structure from pico- and nanophytoplankton to larger (>20 µm) sized microphytoplankton,
- Dinoflagellates and large-sized flagellated algae 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 improved water clarity.
2. Literature Review

2.1 Introduction

2.1.1 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 (King 1972, 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 1998). 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 eastern coastline particularly along the South and East Cape coasts (Ramsay 1995).

The development of fluvial systems has been attributed to, among other factors, 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 1998). 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) occurred 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 catchment systems occur adjacent to one another their geology and geomorphological formations and subsequent landform development 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 westerlies and south-westerly winds.

2.1.2 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. There are a total of 258 estuaries with a few large systems that drain the interior (Whitfield 2000). Others receive river flow from the immediate coastal zone and most of these empty into the Indian Ocean. Approximately 72% of those estuaries are classified as temporarily open/closed estuaries (TOCEs) (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 Cape Point; (2) a warm temperate region from Cape Point to the Mbashe River; and (3) a subtropical region from the Mbashe River to Kosi Bay. 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. These estuaries mostly occur in the warm temperate and the subtropical region, 127 and 121 systems 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. 30 & 90 km$^2$, respectively) surface area of the two catchments.

2.1.3 The Significance of Catchment Size

South Africa is a dry country characterised by a mean annual rainfall of approximately 520 mm 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 TOCEs is their small catchment size. Catchment size, topography (i.e. relief ratio), type of geology and vegetation cover control the amount of precipitation (i.e. rainfall) intercepted, retained and released as surface, subsurface runoff and groundwater following a rainfall event (Dardis et al. 1988). The upper to middle portion of the Van Stadens catchment has high relief ratios characterised by high gradients indicative of steep gorges. The Maitland catchment has undulating hills composed of vegetated fossil dunes of medium to low gradient.
Fluvial systems that drain large catchment areas (e.g. >1000 km²) give rise to the establishment of perennial streams and rivers that produce large permanently open estuaries along the coastline. Conversely, small catchments (i.e. <250 km²) 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 extreme 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 m³.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 enters the estuary from the sea. This occurs during closed-mouth conditions, as marine over-wash across the sandbar driven primarily by westerly and south-westerly winds that generate strong storm surges, which have a significant impact on the coastline.

2.2 Physical and Chemical Characteristics

2.2.1 Climate and hydrodynamics
The arid nature of the southern African subcontinent and climate influences river discharge and evaporation rates and impose certain constraints on the occurrence and growth of estuarine vegetation (Moon and Dardis 1988, Allanson and Baird 1999). Estuaries in 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 pattern whilst those occurring in the Eastern Cape 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 periods often exceed input from river inflow causing some estuaries to become hypersaline.

The frequency and duration of mouth breaching for small estuaries in the eastern and southern Cape varies significantly and can be attributed to local 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 being connected to the sea. Seasonal patterns of river flow are subject to variable periods of low salinity in the estuary 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, Blaber et al. 1984, Vieira and Chant 1993, Mallin 1994). Sediment supply and transport in these estuaries is primarily made available by weathering processes in the catchment and carried down by surface runoff and by river flow after rainfall events. However, the intermittent nature of rainfall occurrence across these regions means that sediment transport and deposition in TOCEs occurs during periods of peak discharge. A greater portion of the sediments 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 across the sandbar (Reddering 1988, Cooper 1990).

After the mouth of the estuary breaches water level within the estuary falls rapidly, often exposing large areas of the benthos that had remained submerged for extended periods during the closed-mouth condition. The closed mouth period is often characterised by extensive colonisation of the bottom sediments by a productive community of algae, macrophytes and benthic animals (Day 1981, Day et al. 1989, Wooldridge and Loubser 1996, Adams et al. 1999). When breaching events occur, usually when flow rates exceed 5 m$^3$s$^{-1}$ river conditions prevail influencing flow patterns, which are typified by estuary bed scour and channel modification (Cooper 1990). 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. For a number of these estuaries the tidal prism is small and shallow generally not exceeding 10 m$^3$, which means that the volume of water exchange between the sea and estuary over a tidal cycle is minimal (Allanson and Read 1987, Whitfield 1992, Schumann et al. 1999).
The open-mouth condition is short-lived because of wave and flood-tide-driven forces that suspend and deposit sediments from the marine environment. Under high river flow conditions the strong out flowing river water decreases wave forces, however the mouth will close under increased and sustained wave action. Low average rainfall and runoff in spring and summer seasons coupled with high-energy wave conditions from strong coastal-storm surges increase the frequency of mouth closure in these small estuaries (Perissinotto et al. 2000, Froneman 2002a). Moreover, mouth closure periods can vary from days, months or even years depending on the prevailing weather and rainfall patterns in the catchment areas. The seasonality of these estuaries is best illustrated by state of the mouth, which is normally reflected in the water level and environmental conditions of the estuary.

2.2.2 Light, Temperature, Salinity and Nutrients

The portion of the solar spectrum that is of concern and used by photoautotrophic organisms is visible light energy or photosynthetically active radiation (PAR). This radiation spectrum spans between the 400 and 700 nm range and determines productivity of microalgae including affecting the depth to which attached plants can grow. Light energy received at the surface and at depth varies with the time of the year and the 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).

Secchi disc measurements are a widely used convenient method of determining light penetration in different types of aquatic bodies and offer a quick and efficient way of assessing the level of dissolved and suspended matter attenuating light at depth by absorption (Kirk 1983, Bledsoe and Phlips 2000). Past studies by Day (1981) and Begg (1984), show that Secchi disc measurements for temporarily open/closed estuaries range from a minima of 0.01 m during high river inflow to a maxima of 10 m 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 lead to mouth breaching (Cooper et al. 1993, Froneman 2002a). The level of suspensoids in the water column tends to decrease from the mouth to the upper reaches when the mouth is open and there is river inflow.

In temporarily open/closed estuaries, subsurface light attenuation varies in relation to season, time of the day, prevailing weather conditions and suspended matter in the water column. Surface irradiance
recorded for some TOCEs have ranged from 71 to 2300 µmol.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 – 3.3 m\(^{-1}\), particularly for estuaries from the subtropical region, that have water-column depths ranging from 1.5 – 4.0 m. On the other hand, estuaries 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 levels of light at depth coupled with low river inflow provide suitable conditions for the establishment of benthic mats of micro- and macroflora.

There are marked increases in turbidity levels with light attenuation coefficients often reaching values of 8.0 - 29 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 diminished euphotic zone. The reduction in the photic zone can limit phytoplankton growth by significantly reducing the photosynthetic efficiency of the microalgae (Day 1981, Cloern et al. 1983, Day et al. 1989).

Estuarine environments are characterised by the mixing between river-borne water and seawater, and as such, temperature influences are 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 TOCEs. River water entering an estuary is normally warmer during the summer seasons compared to 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 TOCEs are seasonally cut-off from the sea for extended periods the major source of water input is 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 macrophyte stands (Day 1981, Day et al. 1989, Snow and Adams 2007).

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°C in the winter and from 24 – 27°C during the summer. In the warm-temperate regions they range from winter lows of 14 to summer highs of 28°C, whereas subtropical estuaries can range from 18 – 30°C (Day 1981, Lubke and De Moor 1998). Due to the low freshwater inflows especially during seasonally dry periods of the year, TOCEs show smaller variation compared to permanently open estuaries (Bally et al. 1985). However, following heavy 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°C between lower and upper estuarine reaches (Perissinotto et al. 2000, Froneman 2002a). The absence of horizontal salinity gradients in these estuaries can influence the biota by extending the general distribution of species adapted to low salinity when river flow is high or high salinity when river flow is low, particularly during wet or dry periods respectively (Day 1980, Wooldridge 1999).

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 5.0 m³s⁻¹ can breach the closed mouth of the estuary and are characterised by strong unidirectional flow of freshwater (Huiizinga 1994, 1996). These conditions may persist until the flow dissipates as the peak 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). These conditions are however short-lived as they persist for periods ranging between days to weeks. The decrease in river inflow accompanied with sediment transport at the mouth from coastal waves and long shore ocean current drift act in concert to constrict and ultimately build a sand bar that effectively closes off the mouth (Day 1981, Whitfield 1992).
Under closed mouth conditions and in the absence of any appreciable freshwater inflow or marine overwash salinities in the estuary 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 > 5 m in height, they lead to overwash events resulting in substantial volumes of seawater entering the estuary over the sandbar. Vertical salinity gradients, particularly at the mouth, can fluctuate 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, 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 – 90 psu) with strong impact on the estuarine biota (Whitfield and Bruton 1989). Where estuaries are very shallow (i.e. mean depth <1.0 m) 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, Campbell et al. 1991) and groundwater input (Harvey and Odum 1990).

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 regulated by climate which controls weathering processes (Ollier 1984, Hall 1992) while the supply is limited by fluvial transport that 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 however deviate from this pattern (see Bally et al. 1985). In contrast TOCEs along this geographical region exhibit varied response patterns. Open mouth conditions bring in increased levels of macronutrients that remain in the water column for several days following mouth closure. Under closed-mouth conditions macronutrient (NO$_2^-$, NO$_3^-$, NH$_4^+$, PO$_4^{3-}$) levels remain very low (i.e. < 1.0 mg L$^{-1}$) throughout the water column. Nitrogen to phosphorus ratios (considering the 16:1 N: P Redfield Ratios), in these systems suggest that nitrogen is generally the most limiting nutrient under closed mouth conditions switching to phosphorus limitation under open mouth conditions (Perissinotto et al. 2003).
Submerged macrophytes and microphytobenthic algae are often dominant primary producers in TOCEs and are able to take advantage of the abundant nutrient pools in the sediment, under low levels of turbidity and turbulence during stable salinity conditions. However, slightly elevated nutrient concentrations may be detected in the water column following re-suspension of bottom sediments as a result of wind-turbulence caused by strong coastal weather-fronts. Although the dynamics of macronutrient cycling in TOCEs are poorly understood (Day 1981, Allanson and Winter 1999, Nozais et al. 2001), work on seasonal (Perissinotto et al. 2000, Froneman 2002a) and annual (Walker et al. 2001, Gama et al. 2005) 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. Additional studies are necessary that will closely examine the links between nutrient supply and uptake by primary producers and associated biogeochemical cycling between benthic and pelagic zones in these estuaries.

2.3 Biological Characteristics

In estuaries primary producers including photosynthetic bacteria, microscopic algae, macrophytes, seagrasses, and mangroves form the base of the food chain that supports aquatic and bird life that inhabit these ecosystems (Day 1980, 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 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 a decline in the 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 plant and animal species (Wetzel 1983).

Studies showing high levels of productivity have been well demonstrated in a number of large estuaries of the northern hemisphere (Cloern et al. 1983, Cloern 1987, Mallin 1994, Underwood and Kromkamp 1999). A number of these estuaries are driven by phytoplankton production and as such, turbidity has been suggested as 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, in some South African estuaries in the cool (Robarts 1976, Henry et al. 1977) and warm temperate (Dye 1978, Bally et al. 1985, Hilmer 1984, 1990, Allanson and Read 1995) biogeographic.
regions, these were mainly on permanently open ones. 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 TOCEs that span the three biogeographical regions that 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) to total 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 and have been on estuaries located in two east coast biogeographical regions (i.e. warm temperate and subtropical) 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 estuary to the extent that no broad general patterns can yet be drawn as being applicable to all TOCEs.

This is one of the first studies to investigate the response of phytoplankton community structure in TOCEs to changes in river inflow and mouth condition. Phytoplankton community size-structure and taxonomic composition has been associated with the efficiency of carbon transfer across trophic levels (Mallin et al. 1991, Mallin and Paerl 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). TOCEs from the warm temperate region are generally nutrient poor compared to those from the subtropical region and thus appear to support a more diverse phytoplankton assemblage.
Phytoplankton Chlorophyll a Concentration and Community Structure

composed mainly of diatoms and dinoflagellates. 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 of phytoplankton community structure and ecological functioning in South African estuaries are still lacking especially in TOCEs. Although phytoplankton community structure has been related to the amounts of nutrients transported together with the rates of flow (Snow et al. 2000a, 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 along annual or even longer temporal scales in intermittently opened 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 300 gCm$^{-2}$ yr$^{-1}$ indicative of a wide range of estuaries that are reflective of their unique biogeographical localities. Recent productivity studies (Froneman 2002b, Perissinotto et al. 2003) in TOCEs 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 TOCEs 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). Although studies in the uMdloti, uMpenjati, and iNyara estuaries (Perissinotto et al. 2003) and the Kasouga estuary (Froneman 2002b) have demonstrated energy flow pathway links between primary producers and their grazers, no conclusive generalization can be drawn regarding carbon partitioning and processing at lower trophic levels. These studies have shown that the phytoplankton is dominated by pico- (< 3.0 µm) and nanophytoplankton (3.0 – 20 µm) because of low macronutrient input. It is however not clear which size group would be favoured under replete nutrient supply. Studies from the uMhlanga and uMdloti estuaries suggest that increased nutrient concentrations does not appear to affect phytoplankton Chl a size structure as pico- and nanophytoplankton were consistently the dominant fractions during different mouth conditions (Perissinotto et al. 2003, Thomas et al. 2005). Whereas research from the Kasouga has demonstrated a shift in the Chl a size structure under different mouth states as pico- and nanophytoplankton were dominant under closed mouth conditions and microphytoplankton...
became dominant when the mouth was open (Froneman 2002a, Froneman 2006). Froneman (2006) suggested that the microheterotrophs mediate carbon flow to higher trophic levels under low nutrient supply while the mesozooplankton regulates energy flow when nutrients are high. There is still a lack of scientific data on the transfer of energy up the food web including a clear understanding of the selective pressures driving carbon partitioning under different freshwater inflow conditions in TOCEs that span the three South African biogeographical regions.

The influence of river flow on phytoplankton community composition in POEs is well documented (Pinckney et al. 1998, Chan and Hamilton 2001, Gameiro et al. 2004). These studies have demonstrated how variable river flow affects phytoplankton species composition and succession across different spatial and temporal scales (Fisher et al. 1992, Harding 1994). A number of studies in South African POEs have shown that the water column is comprised mainly by flagellates than by diatoms and most of the latter found in the benthos (Hilmer and Bate 1991, Adams and Bate 1994). Planktonic diatoms are generally favoured under well mixed conditions whereas flagellated species, especially dinoflagellates, do well when nutrients are sufficiently available and the water column is stratified (Adams et al. 1999). Monbet (1992) showed that mixing of the water column reduced phytoplankton biomass while stable and stratified conditions supported the increased development of phytoplankton biomass. Information on the effects of river flow on phytoplankton composition and structure in TOCEs is lacking. Only a few studies have reported on the consequences of river flow on phytoplankton community composition in TOCEs (Walker et al. 2001, Gobler et al. 2005, Thomas et al. 2005). TOCEs receive a significant volume of water and supply of macronutrients during periods of flooding. Flooding displaces phytoplankton cells into the marine environment during the initial pulse but water retention returns when flow drops below a critical point that allows for reproduction and biomass accumulation. On the other hand sea water penetration when the mouth is open serves to recruit marine phytoplankton species that become trapped when the mouth closes may cause alteration to phytoplankton species diversity. The hydrodynamic influence of increased river flow and nutrient influx and subsequent changes in salinity from seawater penetration on the phytoplankton community structure is poorly understood.

2.4 Temporarily Open/Closed Estuaries in a Global Context

Temporarily open/closed estuaries make up the majority of estuaries found along the South African coastline. Estuaries that open intermittently to the ocean also occur in several other countries around the world including Australia, Brazil, Mexico, New Zealand, northeast coast of the United States of America, and Uruguay. Studies from the east coast of Australia mainly New South Wales (NSW), have focused on
sediment transport across an estuarine sand bar (Ballock et al. 2004) including exploring methods to standardise criteria of assessment to anthropogenic changes (Haines et al. 2006). Other studies have documented spatial and temporal patterns of macrofauna and fish assemblages in intermittently closed/open lakes and lagoons (ICOLLS) (Young et al. 1997, Griffiths 2001, Dye & Barros 2005, Jones & West 2005). Research by Towney & Thompson (2001) in Western Australia and Everett et al. (2007) from NSW represent some of the few studies undertaken on such estuaries that begin to examine the ecology of primary producers to nutrient changes and mouth state. Studies in Brazil have looked at zooplankton response to sandbar opening (Kozlowsky-Suzuki & Bozelli 2004, Santangelo et al. 2007). In other parts of the world, however such estuaries (e.g. TOCEs, ICOLLS, bar-built, sandbar, & blind estuaries) may not constitute a significant proportion of the total number of estuaries within the coastal length of those countries (e.g. USA) (Gobler et al. 2005) and perhaps these estuaries have not been observed to play an important role in the coastal ecology and the goods and services they provide.

Although a number of studies have been conducted on these estuaries, there is still little known about their ecological functioning and the goods and services they provide local and regional economies (Lamberth and Turpie 2001). In South Africa temporarily open/closed estuaries remain open as a result of peak freshwater inflow, however they close during periods of low flow or drought. A number of these systems are also kept artificially open by dredging to prevent flooding of low lying developed areas (Day 1981, Whitfield 1992, Dye and Barros 2005). The local municipality in Mecox Bay, Long Island periodically carries out artificial breaching of the estuary to enable exchange with the sea (Gobler et al. 2005). Similar management strategies have been widely adopted for such estuaries in a number of countries as a means to mitigate against flooding and to purge the accumulation of nutrients and pollutants that promote the growth of nuisance plant matter (Towney & Thompson 2001, Dye & Barros 2005, Jones & West 2005, Everett et al. 2007). Such artificial tampering with the entrance of these water bodies has revealed varied responses including increasing the salinity levels and altering spatial gradients as well as affecting recruitment of the flora and fauna within these systems (Towney & Thompson 2001, Everett et al. 2007, Santangelo et al. 2007).

Close similarities between Mecox Bay, Wilson Inlet and some South African temporarily open/closed estuaries (SATOCES) can be seen in the physico-chemical factors most influenced by mouth phases (Towney & Thompson 2001, Froneman 2002b, Gobler et al. 2005, Thomas et al. 2005). Input from groundwater and river inflow under closed mouth phases appears to be important both in Mecox Bay and in SATOCES in increasing water-level depth resulting in lowering salinity levels as additional freshwater enters the estuary. Episodes of marine overwash across the sand bar occur in these systems and introduces
marine sediment, clear and cool saline water as well as acting as a source of plankton and nekton (Whitfield 1992, Froneman 2002b, Baldock et al. 2004, Gobler et al. 2005). Recruitment of marine biota in this form into the estuary augments phytoplankton and zooplankton stocks increasing species richness of both planktonic groups particularly in the lower reaches. Differences were seen in the influence of open mouth events on salinity. Generally in SATOCEs increased river inflow introduces freshwater that breaches the mouth and lowers salinity whereas in Mecox Bay mouth open phases were associated with an influx of marine water that increased salinity. However, following mouth closure salinity drops to mesohaline levels in contrast to some SATOCEs that display a gradual increase in salinity due to a reduction in river input, evaporation and marine overwash.

South African temporarily open/closed estuaries are characterised by low phytoplankton biomass and changes in their biomass has been correlated with open and closed mouth phases of the estuary (Perissinotto et al. 2000, 2002, & 2003, Nozais et al. 2001, Froneman 2002a, & b, Gama et al. 2005). Despite the fact that TOCEs or intermittently closed/open lakes and lagoons (ICOLLs) occur in a number of countries including the southern and western coasts of Australia and in the west and northeast coasts of California and New York of the US, there are no studies from these countries in recognized peer-reviewed journals documenting phytoplankton temporal and spatial distributions as a response to the physical and chemical environment imposed by these systems. While studies done in the southeast coasts of Australia in intermittently closed/open lakes and lagoons (Jones and West 2005) and South Africa (Whitfield 1994) have shown the significance of TOCEs/ICOLLs to the production of the fisheries there is still a lack of scientific information about these systems despite being widely distributed in a number of countries around the world. This lack of scientific data from elsewhere around the world is perhaps an indication of the perceived low-level of importance these systems have to the fisheries industry (Jones and West 2005). Mouth breaching in most SATOCEs is natural, mainly from increased river inflow, whereas in Mecox Bay it is largely artificial by dredging. Although the forces driving mouth breaching in these geographically distinct systems are different their effect on phytoplankton Chl a concentrations was similar. Comparable to SATOCEs, phytoplankton Chl a concentrations in Mecox Bay declined initially after mouth breaching then steadily increased reaching a peak level several weeks into the open phase. The high levels of Chl a are maintained for a short time even after the mouth had closed as nutrient pools were high. This was then followed by a decline to minimum Chl a concentrations suggesting that the available pool of nutrients had been exhausted thus limiting growth. Similar Chl a distribution patterns that track changes in mouth conditions are also apparent in SATOCEs although the magnitude of Chl a concentrations varies from one estuary to the next (Perissinotto et al. 2000, 2002, & 2003, Nozais et al. 2001, Froneman 2002a, & b, Gama et al. 2005, Gobler et al. 2005). In Mecox Bay temporal distribution of Chl a appeared to follow a
seasonal pattern with a peak during summer and declining to minimum levels in winter. Although changes in mouth dynamics may have had a stronger influence on the Chl a response there is however a temperature effect. The seasonality response of the Chl a observed in this estuary may be partly due to its geographical positioning as the estuary is situated in the temperate zone and is influenced by the wide range of water-column temperature extremes (5-25°C). Seasonality in SATOCEs is not quite clear cut as seen in cold north temperate systems, a number of these systems occur in warm temperate and subtropical regions that generally have a minimum range of water-column temperatures (Nozais et al. 2001). Hydrodynamic forces appear to be a more dominant feature controlling phytoplankton Chl a distribution patterns along temporal scales than water-column temperatures.

When considering the response of size-fractionated Chl a data in Mecox Bay it is comparable to what has been observed in SATOCEs. Picophytoplankton and nanophytoplankton generally form the dominant size fractions throughout the year although in some SATOCEs microphytoplankton can show dominance immediately following breaching and are then soon replaced by pico and nanophytoplankton (Froneman 2000, Gama et al. 2005). In contrast to Mecox Bay, the phytoplankton community structure in SATOCEs is generally characterised by flagellates and diatoms. The length of time that a mouth remains closed and the frequencies with which that state changes has a bearing on the phytoplankton species composition and size structure. It is clear that mouth state (used as a surrogate of river inflow) has a significant effect on phytoplankton Chl a (biomass) concentration and species composition. The magnitude of freshwater inflow affects the amount of turbulence generated by eroding water-column stability through mixing, increased levels of turbidity from sediment re-suspension, and dilution of seawater. Turbidity caused by water-column mixing will affect the light field available for phytoplankton through continuous circulation of algal cells between the photic and aphotic layers (Cloern 1987).

2.5 Synthesis

The geological and early evolutionary histories of most coastal topographies have characterised the dominant hydrodynamic patterns that produced the geomorphological landscapes observed presently (Moon and Dardis 1988). However, the variable climate induced processes of infilling and scouring over recent epochs have produced differences that physically distinguish one estuary from another. It is these unique physical features that confer distinct inherent physico-chemical and possibly biological properties observed in present day estuaries. This was investigated for two adjacent temporarily open /closed estuaries the Van Stadens and the Maitland estuaries.
Temporarily open/closed estuaries including the Van Stadens and Maitland estuaries are characterised by an extended period of mouth closure. The mouth is periodically breached as a consequence of the magnitude of runoff from the catchment. Catchment size, relief ratio, vegetation cover and the type of geology are critical to water storage capacity of a catchment as this affects runoff and hence influences river discharge. In the Eastern and Southern Cape coasts a number of temporarily open/closed estuaries have small catchments and are located in semiarid or arid regions. This combination of climate and catchment size often means that these estuaries are closed off from the sea for the most part of the year. Periods of isolation from the sea can result in hyposaline or hypersaline conditions as a result of a continuous supply of freshwater (former) or a cut-off of river inflow together with high levels of evaporation (latter). During floods the hydrodynamic influences are pronounced often shaping the estuarine geomorphology through bed scour and sediment deposition (Huizinga 1996, Schumann et al. 1999).

Small temporarily open/closed estuaries such as the Van Stadens and Maitland are subject to significant fluvial and estuarine sediment transport. Large volumes of fluvial sediment are imported into the estuary and exported out to the marine environment following strong floods. Such sediment deposition and removal alters and reshapes estuarine bed morphology by burying old habitats while creating new areas for benthic microalgal colonisation. Formation of a sand bar across the mouth results from a decrease in river flow coupled with the transport of marine sediment from longshore current movements. River flows greater than 1.0 m³ s⁻¹ are critical in maintaining an open mouth condition in these type of estuaries. The isolation of the estuary from the marine environment by the sand bar restricts any movement of water in or out of the estuary therefore limiting any exchange of physical, chemical and biological interaction between the estuary and the marine environment. Fluvial sediments and other associated suspended matter transported during flood conditions (i.e. open mouth) considerably reduce light penetration possibly limiting microalgal production (Cloern 1987, Perissinotto et al. 2003). However, under low flow conditions (i.e. closed mouth) the light field is sufficient to reach the bottom sediments, which supports high benthic microalgal production (Perissinotto et al. 2000, 2002, 2003). During warm periods of the year under breached mouth conditions increased water column temperatures produce stratification that periodically limits chemical and biological exchange between the surface and bottom layers of the water column. These conditions are, however, short-lived particularly when the mouth closes as rapid mixing erodes any stratification formed effectively making the water column homogenous. Thus mixing facilitates the availability and spatial distribution of macronutrients vertically and horizontally throughout the water column and along the length of the estuary. Although nutrient levels in TOCE’s are generally low, especially for the Eastern and Southern Cape estuaries, periodic re-suspension of bottom sediments
can become important in sustaining phytoplankton populations and enhancing primary production (Froneman 2002b, Perissinotto et al. 2003). Microalgal nutrient uptake and nutrient cycling in TOCE’s are poorly understood although it has been suggested (Froneman 2002a, Perissinotto et al. 2002) that a close link between small-sized microalgal primary producers and heterotrophic nanoflagellate grazers exist that may be instrumental in the channelling and processing of carbon up the estuarine food web.

The principal hydrodynamic forces controlling the overall behaviour of temporarily open/closed estuaries globally appears to be similar although the geology, climate and magnitude of these forces may inherently be unique to individual estuaries. Freshets and floods are crucial in sediment bed scour and transport, nutrient input, and estuarine residence time including establishing of a link between the estuary and the sea (i.e. mouth breaching). As water abstraction demands increase upstream for agricultural, industrial and urban use less water is continuously received downstream for regular estuarine channel maintenance and flood plain inundation. The reduction in the amount of the water carried downstream including the frequency and magnitudes of the floods will mean abnormally longer periods of mouth closure (Whitfield & Bruton 1989, Whitfield 1992). The broad implications of artificially and naturally breached TOCEs particularly under increased water abstraction demands investigation to understand the ecological functioning for improved decision making toward better management practises.

It is clear from a number of recent studies that phytoplankton biomass (Chl a) in warm temperate TOCE’s generally show peaks two to eight weeks following a flush of freshwater inflow which is closely associated with an increase in the supply of dissolved inorganic nutrients mainly nitrogen (Froneman 2002a, Gama et al. 2005). Concomitant with the increased freshwater inflow the mouth downstream is breached allowing marine water intrusion to penetrate and reach the upper reaches. This may depend on the gradient of the berm at the time of breaching. Estuaries with steeper gradients at the mouth (i.e. Maitland, Seekoi and Kabeljous) only allow marine water intrusion into the lower reaches, whereas less perched estuaries will have deeper tidal prisms (Whitfield 1994). At the river/estuary interface the mixing of freshwater and marine water produces brackish conditions that are conducive to the proliferation of dinoflagellates (Snow et al. 2000a). Although this phenomenon is common in permanently open estuaries it is short-lived in TOCEs as the physical and chemical factors that produce and sustain such conditions rapidly dissociate and disintegrate resulting in a mixed water column. Thus, spatial distribution of phytoplankton chlorophyll a and species composition in TOCEs generally do not show variation along the length of the estuary. However, the lower reaches might show more marine species near the mouth due to marine overwash whereas at the head of the estuary freshwater species may become more pronounced because of river input. These conditions however, may persist for only short periods as complete water
column and whole estuarine mixing can take place within a few days evidenced by homogeneous salinity levels. Our understanding of environmental and biological factors governing temporal and spatial distribution patterns of phytoplankton biomass and community composition in TOCEs is still poor. Further studies are required to help elucidate the apparent close links between mineral supply and storage in plant matter as well as grazing and regeneration by bacteria (Malone et al. 1988).
3. Study Area

3.1 Van Stadens and Maitland Catchments

The Maitland (33° 59′2″ S, 25° 17′ 4″E) and Van Stadens (33° 58′1″S, 25°13′20″E) estuaries were selected as the two study sites as they occur close together (Fig. 3.1) in St Francis Bay and have approximately similar catchment sizes (60 & 90 km² respectively). The mouth of the Maitland Estuary is approximately 5 km east of the Van Stadens Estuary mouth. The catchment area is the smaller of the two systems. The estuary is very small with an area of about 0.06 km² and total length of 600 m. The estuary is perched and situated behind mobile sand dunes that protect it from strong storm surges. 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. The lower end of the Maitland River, just above the estuary head, has extensive stands of the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. and at the estuary head there is a mixture of *P. australis*, *Typha capensis* (Rohrb.) N.E.Br. and *Juncus* sp. The middle to lower section of the estuary is characterised by a mixture of emergent and submerged macrophyte plants like *T. capensis* and *Ruppia cirrhosa* (Pentag). The maximum depth of the Maitland Estuary when the mouth is closed is 2 m (Station 1, Fig. 3.2) 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 when water level is low and light availability high. The position of the estuary ensures that the estuary remains oligohaline for the majority of the year as it rarely connects with the sea and receives freshwater seepage from the adjacent dunes even when it is cut off from the river during drought periods. Strong floods in July of 2001 scourled the river and estuary removing all the in-channel vegetation except for some along the fringes. The floods deposited sediments in the estuary such that the estuary became shallow with the water level at site 1 reaching a maximum of 0.75 m resulting in a more intermittent mouth closure frequency for the next two years of the study.

The Van Stadens Estuary is located approximately 40 km south of Port Elizabeth. 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 third of the upper catchment for silviculture. Two dams have been built on the Van Stadens River with a total capacity of approximately 0.47 x10⁶ m³. The length of the estuary is 3 km long at maximum water level when it is in a closed-mouth phase and decreases to about 2.5 km when in an
open-mouth condition. Unlike the Maitland Estuary that has reduced light penetration at depth when the estuary is closed, the Van Stadens Estuary is characterised by high water clarity at depth from the mouth right up to the estuary head. Light penetration reaches the bottom at all depths (approx. 2 - 3 m). During extended periods of mouth closure the increased light transparency encourages growth of macroalgae near the mouth and submerged macrophytes in the middle and lower reaches of the estuary. Mean estuarine water depth is approximately 3 m with a maximum depth of 5 m at its deepest point. The upper reaches are fringed by emergent macrophyte stands of Phragmites australis with submerged beds of Potamogeton pectinatus. There are sparse stands of P. australis along the riparian zone of the middle reaches of the estuary increasing slightly toward the head of the estuary. Sediment type is characterised by coarse sand (250-500 µm) in the upper reaches and becoming a mixture of fine sand (150-250 µm) and very fine sand (64-150 µm) from the middle reaches to the estuary mouth. On the eastern bank near the mouth is a resort that caters for recreational activities. The waste and sewerage disposal system that the resort makes use of is a septic tank system which disposes off directly into the sea (Van Stadens Management pers. comm.). At the height of occupancy, mainly during the Easter and Christmas holiday breaks, the resort holds over 1000 individuals. Normally during the dry summer periods when the river flow is low the mouth of the estuary is closed creating a lagoon-like water body that is a favourite for recreation. Under these conditions of mouth closure water levels in the estuary reach their maximum and light penetration is generally greater than the maximum depth. Mats of a green filamentous macroalga (Ulva sp.) colonise the bottom sediments particularly during warmer months.

As a result of their proximity, the two estuaries occur on similar geologic substrata and therefore tend to share similar catchment vegetation types. The estuaries have distinct geomorphological differences that distinguish one estuary from the other. The Maitland sits adjacent to a mobile dune field that carries aeolian sand into the estuary when there are strong onshore westerly winds. On the other hand, the Van Stadens is protected by consolidated dunes covered with vegetation and only the mouth region is exposed to aeolian sand deposition. It is because of these differences that these two estuarine ecosystems were selected as study sites as this will make them suitable for comparative study in terms of their physical and chemical attributes including their biological composition. Both systems are only open intermittently throughout the year and remain closed for the majority of the time. Furthermore, both estuaries are popular tourist destinations for swimming, fishing, and small craft boating and therefore need to be managed for recreational purposes.
Figure 3.1  Map of South Africa (a) and (b) the catchments of the Van Stadens River (A) and the Maitland River (B). Sites 6 – 10 indicate stations visited in the upper catchment of the Van Stadens River and sites 1 - 5 are shown on Figure 3.2.
3.2 Land Use

The Maitland catchment is primarily farmland with dairy farming making up the dominant form of farming practised. Livestock and crop farming are also carried out but make up a very small proportion. A number of farm dams occur in the catchment and are used to store water mainly for irrigation for cattle feed and some crops. Approximately a tenth of the catchment is conserved as a nature reserve and a significant portion zoned as a residential area for smallholding type housing.

The Van Stadens catchment is characterised by steep gorges of Table Mountain Quartzite that are covered by fynbos in the upper and middle regions with valley-bushveld (thicket) near the coast. A significant portion of the catchment is largely covered by shrub-thicket vegetation with some areas covered with coastal forests, however some areas of the catchment are used as farmland primarily for dairy, livestock, chicken and pig farming. Only a small portion of the upper catchment is residential and a majority of that portion used for forestry.

Figure 3.2 Position of the sites visited water column (circles) and groundwater (letters) in the Van Stadens and Maitland estuaries during April 2001 and May 2004. Note: There were no upper catchment and groundwater sites in Maitland River and estuary respectively.
4. General Methods and Materials

4.1 The Rationale Used to Investigate Environmental Effects on Phytoplankton Biomass and Community Structure in a Temporarily Open/Closed Estuary

In order to investigate in detail the influence of river flow on phytoplankton chlorophyll \( a \) concentrations and community structure a study was undertaken in the Van Stadens Estuary. This estuary was the focal point of investigation for a study on seasonal, monthly and daily environmental changes and the effect on the phytoplankton size-structure, biomass and community composition. In order to assess sources of nutrient input into the Van Stadens Estuary river water and groundwater sources were monitored seasonally and where possible after a rainfall event. To determine whether environmental variability would exert similar responses on the phytoplankton biomass and community structure comparative studies were carried out in the Maitland Estuary. This included seasonal and monthly monitoring. This study approach enabled one to determine how TOCEs with adjacent catchments and similar climate conditions would behave with regard to the effects of environmental factors on phytoplankton population dynamics. The river and groundwater sources were not monitored in the Maitland Estuary.

4.2 Physical and Chemical Parameters

Vertical profiles for photosynthetically active radiation (PAR) were taken using a Li Cor 190 4\( \pi \)-Spherical Quantum Sensor connected to a Li Cor 1000 data logger and for temperature, conductivity, salinity, and pH were taken using a WTW CTD just below the surface, at 0.25m and subsequently at 0.5 m intervals, except for PAR that included above the surface measurements. Light transparency was measured using a 30 cm black and white Secchi disk. Environmental parameters (i.e. temperature, conductivity, salinity, redox, and pH) were subsequently (April 2002 to 2004) measured using a YSI 650 Multi-probe sensor. River discharge was determined on a quarterly basis in the upper, middle and lower sections of the catchment using a Marsh-McBirney FlowMate Portable electromagnetic flow meter. Duplicate estuarine water samples for nutrient analysis were taken at five stations along the length of the estuary as indicated above. The samples were analysed for the following macronutrients; nitrogen (\( \text{NH}_4^+, \text{NO}_3^-, \text{NO}_2^- \)) phosphorus (TP, SRP) and dissolved silica (DSi). During monthly surveys water samples for nutrient analyses were placed in cooler boxes maintained at low (0-4°C) temperatures while in transit from the field to the laboratory prior to analysis and during quarterly field surveys samples for nutrient analysis were kept frozen (-20°C) until analysis within a week of collection. Comparisons of samples from previous observations of fresh samples (i.e. samples analysed within 4hrs) versus ones kept frozen over a week did not reveal significant differences (\( t \)-test, \( n=10 \) \( p<0.01 \)). All macronutrient analyses were carried out at the Botany laboratory (NMMU) following standard chemical analysis methods of Strickland and
Phytoplankton Chlorophyll a Concentration and Community Structure

Parson (1972) and Wetzel and Likens (1991). Soluble reactive phosphorus (SRP) determination included the filtered water to react with a composite of molybdate, ascorbic acid and trivalent antimony that produce a blue-coloured complex of which absorbance was measured at 885 nm. Total phosphorus determination included a persulfate digestion step followed by the SRP method mentioned above.

The determination of ammonium followed the phenol-hypochlorite method using nitroprusside as a catalyst and the extinction read at 640 nm in a spectrophotometer as described in Solorzano (1969). For the determination of nitrate and nitrite the cadmium-reduction method was followed as modified by Bate and Heelas (1975). Dissolved silica was determined by using acidic ammonium molybdate then reducing the silicomolybdate complex with sodium sulphite which forms a blue molybdate colour and the extinction read at 700 nm according to the method by Wetzel and Likens (1991). During quarterly surveys river to mouth visits were taken at 10 stations from the Lower Van Stadens dam in the upper catchment to the mouth of the estuary. Physico-chemical characteristics were measured and water quality samples analysed as described above. Five stations were selected for placing duplicate groundwater wells adjacent to the estuary stations approximately 10-15 m from the wetted edge (see Fig. 3.2). The wells were 1.5-2.0 m deep (depending on the depth of water during initial placement) supported with PVC piping that was capped at the bottom with several small holes (<1 mm in diam.) drilled into the bottom 0.5 m of the pipe. A polyethylene cover was placed at the top of the pipes to prevent water and debris from entering. Monthly water samples were collected for macronutrient analysis by lowering a 2 m X 8 mm (inter. diam) silicon tubing until it reached the surface of the water then the overlying water was siphoned into 250 ml borosilicate bottles and placed in cooler boxes maintained between 0-4°C until analysis in the laboratory as described above.

The state of the mouth or its condition was characterised based on the amount of time (e.g. days – weeks) it remained opened following breaching or the time it stayed closed after a sand bar had formed across the mouth of the estuary according to the daily mouth record. This information was obtained from daily observations at the Van Stadens Resort by Mr Du Preez (groundsman manager). Only periodic mouth condition records were available for the Maitland Estuary because there was no one available to do daily observations. The duration of each event was used as a guide to characterise the condition of the mouth for the Van Stadens (Table 4.1), whereas in the Maitland the periodic record of mouth phases served as a basis for depicting mouth conditions during the study period. The percentage of total time a specific mouth state occurred over a given period (e.g. 1-month) exceeded 50%, then that condition during that period was determined to be the predominant mouth condition. Horizontal bars depicting mouth states in subsequent figures throughout the thesis are based on this determination.
Table 4.1  Van Stadens River mouth states including an approximate duration each phase persisted based on a daily record of the mouth condition.

<table>
<thead>
<tr>
<th>Mouth State</th>
<th>Duration</th>
<th>Abbreviation</th>
<th>Comment / Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed mouth condition</td>
<td>1-8 weeks</td>
<td>CMC</td>
<td>Short-term period that the mouth stays in a closed condition</td>
</tr>
<tr>
<td>Open mouth condition</td>
<td>&lt; 5-day</td>
<td>OMC</td>
<td>Short-term period that the mouth stays in an open condition</td>
</tr>
<tr>
<td>Closed/Overwash condition</td>
<td>&lt; 5-day</td>
<td>C/OWC</td>
<td>Closed mouth condition associated with input of marine water over the sand bar</td>
</tr>
<tr>
<td>Closed-mouth-phase</td>
<td>&gt; 2-months</td>
<td>CMP</td>
<td>Long-term period that the mouth stays in a closed condition</td>
</tr>
<tr>
<td>Open-mouth-phase</td>
<td>&gt; 3-weeks</td>
<td>OMP</td>
<td>Long-term period that the mouth stays in an open condition</td>
</tr>
</tbody>
</table>

4.3 Phytoplankton Size-fractionated Chlorophyll a

Monthly chlorophyll a analyses were carried out from water samples taken from the upper and lower portions of the water column at five stations. Replicate water samples were collected using a Students horizontal 3 L sampler (Aquatic Research Instruments) and placed in brown 1 L polyethylene bottles placed on ice in cooler boxes kept at about 4 °C until analysis at the laboratory (within 2 hr from sampling) under dim light. During quarterly surveys chlorophyll a water samples were treated as above except that following filtration, filter papers were folded and placed in aluminium foil and immediately placed in a freezer at ~20 °C until further analysis within a week. Tests run between samples collected and analysed within 24hrs against those frozen and analysed 7 days later did not show evidence of a significant loss in chlorophyll content between the two sets of samples (t-test, n=20 p<0.001 R²=0.965).

For size-fractionated chlorophyll, samples were serially filtered through a 20 µm nitex screen mesh (micro), then a 2.7 µm Whatman GF/D filter paper (nano) and lastly through a 1.2 µm Whatman GF/C filter paper (pico). A few drops of a saturated solution of MgCO₃ were placed in the water during filtering to minimise degradation of the Chl a pigment. Filters were immediately placed in opaque 20ml glass vials containing 15 ml of 90% acetone and placed in a cold room kept at 4°C for 24hrs. Following extraction sample vials with the nitex screen mesh were sonicated for 15-20 min in a bath of cool water to ensure all the cells were ruptured and dislodged from the mesh pores. Light microscope examination at 400X of the
mesh revealed a greater than 95% (n=20) removal of cells from the screen pores. The rest of the samples on filter paper were macerated with a pestle and mortar and cleared by filtering through a Schleicher & Schuell GF52 filter paper (GF/C equivalent). Pigment extinction coefficients were read on a GBC650 - UV/VIS spectrophotometer and calculated according to the trichromatic method for mixed natural phytoplankton communities by Jeffrey and Humphrey (1975).

Trichromatic Method Equations for Mixed Phytoplankton Communities containing Chlorophyll a, b, & c (Jeffrey and Humphrey 1975)

\[
Chl \ a \ (\mu g/l \ or \ mg/m^3) = (C_a)(\nu)/(V)(Z)
\]

where: \( C_a = 11.85 \ E_{664} - 1.54 \ E_{647} - 0.08 \ E_{630} \) and \( E_{664o} = A_{664} - A_{750} \).

\[
Chl \ b \ (\mu g/l \ or \ mg/m^3) = (C_b)(\nu)/(V)(Z)
\]

where: \( C_b = 21.03 \ E_{647} - 5.43 \ E_{664} - 2.66 \ E_{630} \) and \( E_{647o} = A_{647} - A_{750} \), etc. as above.

\[
Chl \ c_1+c_2 \ (\mu g/l \ or \ mg/m^3) = (C_c)(\nu)/(V)(Z)
\]

where: \( C_{c1+c2} = 24.52 \ E_{630} - 1.67 \ E_{664} - 7.60 \ E_{647} \) and \( E_{630o} = A_{630} - A_{750} \).

where: \( \nu \) = volume of extract in ml

\( V \) = volume of water filtered in litres

\( Z \) = length of light path through cuvette or cell in cm

Chlorophyll pigment values reported here mostly consider chlorophyll a pigment concentrations only, although concentrations of other pigments were also measured but were not included in this report.

In addition to chlorophyll a, samples for phytoplankton cell densities were collected similarly to that for chlorophyll samples and preserved for the determination of phytoplankton community structure. Samples for phytoplankton enumeration were collected in opaque 1-litre polyethylene bottles and preserved by adding 1% of the sampling jar volume with acidified Lugol's solution (Wetzel and Likens 1991). These were then placed in a cold room (4 °C) then allowed to settle for a minimum of 72 hrs. The samples were concentrated by gently siphoning the overlying water until a 50 ml volume remained then transferred into 50ml bottles. Samples were enumerated using a Neubauer improved double ruling haemocytometer under.
an Olympus BX40 light microscope at 400X magnification. Cell densities were calculated according to the following equation:

\[
\text{Cell No. ml}^{-1} = \frac{C \times V}{A \times D \times K}
\]

where, \(C\) = number of cells counted  
\(A\) = area of chamber (mm\(^2\))  
\(D\) = depth under cover slip (mm)  
\(V\) = volume of sample settled (ml)  
\(K\) = concentration factor = 20

Phytoplankton from the following taxa Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta, Dinophyta, and Euglenophyta were identified to species level where possible, but certainly to genus level using the following identification keys; Bold and Wynn (1981), Prescott (1962), and Tomas (1997). As a result of the difficulty in enumerating and identifying picophytoplankton under routine light microscopic identification, they were not included in the counts during this study (Sieracki et al. 2004).

According to Sieburth et al. (1978) particle size fractions include picophytoplankton (0.2 – 2.0 µm), nanophytoplankton (2.0 – 20 µm) and microphytoplankton (>20 µm). In this study, particle size fractions of the water column were based on a modified size-fraction scale, where the picophytoplankton only included the 1.2 – 2.7 µm size fraction. Bacterioplankton with a size range of 0.2 - 1.0 µm normally associated with organotrophs and chemotrophs were not included as they are generally characterised as heterotrophic (Davis et al. 1985). Phototrophic prokaryotes included within the picophytoplankton normally have a size range 1.0 – 2.0 µm and were assumed to form part of the photoautotrophic fraction captured in this study (Johnson and Sieburth 1979). Although a significant component of the picophytoplankton may be underestimated (Sieburth et al. 1978) by this method and hence caution should be considered when comparing these data with similar regional studies, it is believed that the results obtained are a realistic reflection of the phytoplankton patterns observed during the course of the study.

### 4.4 Microphytobenthic chlorophyll a

Monthly chlorophyll a analyses were carried out from samples taken from the upper 10 mm of the sediment surface. Samples for the determination of microphytobenthic Chl a biomass were collected using a modified method by Rodriguez (1993) by using a plexi-glass corer (300 mm long and 20 mm internal diameter). All sediment samples collected were taken at a water level 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 higher than 0.5 m at some stations (i.e. 3, 4 & 5). A sample was collected by inserting the corer
halfway into the sediment then gently lifted by covering the underside with a flat object or a hand while simultaneously lifting with the other hand. 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. Care was taken to ensure that there was limited disturbance to the overlying sediment surface so as not to dislodge the upper microphytobenthic layer. A sharp-knife blade was used to section off the first 10 mm of the core and placed into an opaque 50 ml polyethylene container with 30 ml of 95% ethanol. Each sediment sub-sample used to extract chlorophyll a was weighed in order to determine Chl a concentration in terms of grams of sediment. To complete the chlorophyll a extraction samples were placed in a dark cold room at 4°C overnight (~24 hr) before further analyses were run. Chlorophyll a analyses were completed by clearing the samples by filtering them through a Schleicher and Schuell GF52 filter paper then read on a GBC650 - UV/VIS spectrophotometer. Tests were run to compare results from the spectrophotometer with the 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.

4.5 14C Preparation and Analysis Methods

4.5.1 Phytoplankton Primary Production Experiments

In situ phytoplankton productivity studies were carried out in both the Maitland and Van Stadens estuaries according to a modified Strickland and Parson 14C 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 250 ml biological oxygen demand bottles (BOD) whereupon a known amount of a carbon tracer (NaH14CO3) was added to the samples and then incubated at depth for 4 hr. 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 (>20 µm), nanophytoplankton (2.7 – 20 µm) and picophytoplankton (1.2 - 2.7 µm) size-fractions for the determination of productivity of each group.

4.5.2 Determination of dissolved inorganic carbon (DIC)

In determining DIC five (250 ml) 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 (~0.5-1.0 ml). Dissolved inorganic carbon was then calculated as follows: \[ \text{DIC} = V \times N \times 12 / v' \]

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)

4.5.3 Primary Production Assay

In each estuary, 5 L of estuarine water from two depths, near the bottom (0.3 m from sediment corresponding to 30% of surface light intensity) and just below the surface (0.3 m from water surface corresponding to 80% of surface light intensity) were sampled using an opaque water sampler. The collected water was carefully mixed then poured into a total of 8 X 250 ml BOD glass bottles (i.e. 4 bottles for each depth) per station and placed in dark cooler boxes until inoculated with \(^{14}\text{C}\) 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. Bottles were then suspended in the water column at depths equivalent to 30 and 80% surface irradiance based on light attenuation measurements with depth using a Li Cor 190 4\(\pi\)-Spherical Quantum Sensor connected to a Li Cor 1000 data logger. These depths were determined to be generally equivalent to 0.5 m below and above the water and sediment surfaces respectively.

For incubation studies, all bottles were inoculated with 500 \(\mu\)l NaH\(^{14}\text{CO}_3\) to give a specific activity of 3.7 MBq 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 10 ml was taken for the purpose of determining initial radioactivity background levels. From the 10 ml sub-sample, 2 ml was pipetted into a scintillation vial. To this amount 13 ml of Ultima Gold scintillation cocktail was added. The remaining 8 ml was filtered and the filter rinsed in 0.5 ml concentrated HCl. To this amount, 15 ml of Filtercount was added to dissolve the filter. To the filtrate, 1 ml of concentrated HCl was added followed by the addition of 10 ml 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, 50 ml of water were serially filtered at very low filtration pressures (vacuum <2.5 cm Hg) through a 25 µm (polypropylene), a 3.0 µm glass-fibre filter and 1.2 µm Millipore membrane filter (Froneman 2002b). Each filter paper containing labelled phytoplankton and filtrate were treated separately. Following filtering, the filters were quickly rinsed in 0.5 – 1 ml 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:

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

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$^\theta$- method of quench correction (Campbell & Bate 1986). 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$^\theta$ 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$^\theta$. 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: $\text{CE} = \frac{\text{cpm}}{\text{dpm}}$
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.

### 4.6 Data Analysis

The relationships between chlorophyll $a$ concentrations and environmental variables along temporal and spatial scales were analysed by using two and one-way ANOVA (Sokal and Rohlf 1969). Multiple comparisons among pairs of means were performed using Tukey’s significant difference method when a significant ANOVA result occurred. The degree of relationships between major taxonomic groups of phytoplankton was determined using the Bray-Curtis similarities analysis following $4^{th}$-root transformation of counts and displayed along a two-dimensional plot using non-metric multi-dimensional scaling (MDS) ordination (Primer Statistical Software Plymouth Marine Laboratory Clarke and Warwick 1994). Significance of the ordination was tested using ANOSIM analysis as a test statistic to test the significance between $a$ priori groupings, such as changes in phytoplankton species assemblage by sample date or season using calculated similarity matrices. Linear regressions were used to establish relationships between environmental and biological variables.
5. Seasonal Responses of Size-Fractionated Chlorophyll \(a\) and Phytoplankton Assemblages to Freshwater Flow in the Van Stadens Estuary

5.1 Introduction

Estuarine environments are one of the most productive and diverse aquatic environments due to the dynamic nature of the downstream flow of freshwater and tidal flood of marine water (Day et al. 1989). A significant proportion of the primary production sustaining food web dynamics in many permanently open estuaries in the northern hemisphere is attributed to phytoplankton (Cloern 1987, Cole and Cloern 1987, Mallin et al. 1991). Phytoplankton distribution patterns in estuaries are a function of various environmental factors. Spatio-temporal distributions of phytoplankton assemblages in permanently open estuaries are characterised by patchiness attributable to variable freshwater inflow (Day et al. 1989, Mallin 1994, Adolf et al. 2006), turbidity (Cloern 1987, Cole et al. 1992, May et al. 2003) nutrient concentrations (Staver et al. 1996, Pinckney et al. 1998, Örnólfsdóttir et al. 2004, Spatharis et al. 2007), mixing depth and transport processes (Huisman and Weissing 2002) and grazing intensity (Juhl & Murrell 2005), competition and senescence (Pinckney et al. 1998, Sommer et al. 2000). Changes in phytoplankton chlorophyll \(a\) (Chl \(a\)) concentrations are most pronounced under conditions of variable hydrodynamic flow patterns (Mallin 1994, Snow et al. 2000a & b, Chan and Hamilton 2001). It is well recognised in permanently open estuaries (POEs) that river flow serves as a nutrient source (Mallin et al. 1991, Mallin 1994, Piehler et al. 2004), and as a result may support high phytoplankton Chl \(a\) concentrations and cell densities normally over a sustained period of time owing to nutrient availability coupled with retention times sufficient for the increase in microalgal production (Kristiansen 1998, Piehler et al. 2004). This study investigated the relationship between variable weather patterns along the semi-arid coastal plain of the Eastern Cape of South Africa and the supply of nutrients into the receiving estuary together with the frequency of mouth breaching events.

Freshets or episodic river flow in permanently open estuaries can 1) alter channel bed morphology through scouring and sediment deposition (Allanson & Baird 1999), 2) introduce intermittent pulses of nutrients that, in the short-term stimulate phytoplankton production (Perissinotto et al. 2000, Froneman 2002a) and change community structure (Gobler et al. 2005), 3) modify the residence period of the phytoplankton populations by rapidly flushing them out of the system (Mallin 1994) and/or inducing zones of high production along the river/estuarine interface (Snow et al. 2000a). The change in channel morphology induced by heavy flooding often modifies the depth of the water column and creates varying depth profiles along the length of the estuary. Although similar processes take place in temporarily open/closed
estuaries however, the relationship between rainfall events and the frequency of mouth opening is not clear. Strong rainfall events are generally required to trigger a breaching episode. The behaviour and response patterns of some TOCEs to mouth breaching events can vary even when the catchments of adjacent estuaries may share similar weather conditions (Thomas et al. 2005).

Low flow conditions in TOCEs during dry periods are insufficient to maintain an open mouth resulting in the closure of the mouth driven by strong tides and long-shore transport processes that build up a sandbar at the mouth. Under these conditions water behind the sandbar fills up the estuary inundating exposed benthic areas and increasing water volume in the estuary. Nutrient levels during these periods decline to low levels and significantly depress chlorophyll \( a \) concentrations to oligotrophic levels (Froneman 2002a). Some studies have demonstrated that the nanophytoplankton tend to be the dominant size fraction in terms of chlorophyll \( a \) concentration in the phytoplankton community structure (Perissinotto et al. 2000, Gobler et al. 2005). These studies suggested that this size group would form the dominant group under low nutrient conditions because of their small cell size. In addition, following increased river influx nutrient levels improve, yet the same phytoplankton size fraction forms the abundant group. This however, is in contrast with other studies that have found higher Chl \( a \) concentrations associated with the larger-sized microphytoplankton size fraction (Froneman 2002a).

Size-fractionated phytoplankton has been considered to be useful in determining the phytoplankton composition of pelagic ecosystems with the view of understanding energy pathways from primary producers to higher trophic levels (Tada et al. 1999, Caroppo 2000). In South Africa the use of size-fractionated chlorophyll \( a \) in TOCEs has recently gained increased attention in an attempt to understand carbon energy pathway links that will shed light into the food web mechanisms responsible for sustaining production of higher trophic levels (Froneman 2002a, Perissinotto et al. 2003). Although TOCEs form the majority of the estuaries found along the South African shoreline there is still a lack of scientific data regarding the ecological functioning of these systems relative to the permanently open ones. A paucity of information still exists regarding our understanding of the function and role played by phytoplankton including the significance of the phytoplankton community structure to the overall estuarine ecosystem function.

The response patterns of the microflora to irregular nutrient inputs have been shown to generally follow rainfall patterns with chlorophyll \( a \) peaks developing subsequent to a flood event (Gobler et al. 2005, Thomas et al. 2005). In addition, phytoplankton distribution patterns in temporarily open/closed estuaries are also strongly influenced by hydrodynamic flow patterns, however their impact is usually sudden and
short-lived (i.e. 24hr to a couple of days) depending on the magnitude of the runoff from the catchment (Walker et al. 2001, Froneman 2006). Although our understanding of the influence of increased river flow on estuarine microalgae has improved (Froneman 2002a, Nozais et al. 2001, Thomas et al. 2005), our knowledge regarding time periods longer than an annual seasonal cycle is lacking. Recent studies that have examined phytoplankton distribution patterns have mainly been over an annual cycle (Perissinotto et al. 2000, Walker et al. 2001, Froneman 2002a, & b, Thomas et al. 2005). Long-term (i.e. >1 year) influences of river inflow and estuarine geomorphological modifications on phytoplankton production and population dynamics are little understood for these estuaries.

In the Van Stadens Estuary, a TOCE, I tested the following hypotheses, 1) nano- and microphytoplankton size fractions (2.7 – 20 µm & >20 µm along major axis respectively) form the dominant phytoplankton that drive primary production during periods of increased river inflow, 2) picophytoplankton (1.2 - 2.7 µm along major axis) form the dominant phytoplankton group that drive primary production during periods of low river inflow, 3) prolonged periods between breaching events (closed-mouth phase) favour increased pico- and nanophytoplankton biomass and production, 4) during periods of mouth breaching microphytoplankton production decrease beyond levels sufficient to support macro-zooplankton biomass and production, 5) increased nutrient supply associated with increased river flow support higher concentrations of microphytoplankton biomass and production by shifting phytoplankton community structure from pico- and nanophytoplankton to larger sized (>20 µm) microphytoplankton, 6) dinoflagellates and large-sized flagellated algae are dominant in the water column during periods of mouth breaching and low water clarity, while chrysophytes, particularly bacillariophytes (diatoms); are dominant during periods of mouth closure and improved water clarity.

Hypotheses that will be covered in this discussion pertain to those regarding size-fractionated phytoplankton Chl a biomass (e.g. 3, 5 & 6) and those dealing with primary production (1, 2, & 4) will be discussed in Chapter 9, which deals specifically with primary production.

5.2 Methods and Materials

For more details on the methods and materials regarding the measurement and processing of physical, chemical and biological variables refer to the General Methods and Materials Section 4 page (30).
Figure 5.1. 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. Mouth data was compiled from daily mouth observations (Mr Du Preez Van Stadens Resort).
5.3 Results

5.3.1 Physical and Chemical Parameters

There were four dry periods during the study from April – August 2001, January – July 2002, October 2002 – March 2003, and July 2003 – April 2004 including the period prior to the beginning of the study. These periods coincided with mean monthly rainfall of below 20 mm and thus constituted the first- (FCMP), second- (SCMP), third- (TCMP) and fourth- (UCMP) closed-mouth-phases respectively (see Chpt. 4 Table 4.1). Daily mouth records of the Van Stadens Estuary showed that on an annual basis the mouth remained closed for approximately 60 – 65%, open for 20 – 25% and the rest of the time (10 – 20%) experienced marine overwash. During dry periods of the year closed mouth phases were characterised by horizontal and vertical salinity gradients especially when heavy marine overwash events occurred (Fig. 5.1). Depending on the duration and intensity of the overwash events these pycnoclines were short-lived and rapidly eroded by strong wind-induced turbulent mixing of the water column. As a result of the low river inflow (<0.1 m³s⁻¹) during the closed mouth periods water column salinity increased ranging from 18 psu in the upper reaches to 20 psu in the lower reaches. Under these conditions the low freshwater input sustained horizontal and vertical stratification. Dry periods resulted in the mouth remaining closed for extended periods especially during the TCMP when the mouth remained closed for over 12 months (Fig. 5.1).

Rainfall patterns during the study followed a typical seasonal pattern for the region characterised by high rainfall in late winter and early spring with low rainfall during the summer and autumn periods (Fig. 5.2a). There were three peak periods where mean monthly rainfall was >40 mm and during these times the mouth of the estuary breached (Fig. 5.2a). Two of those periods constituted the first- (FOMP) and second-open-mouth-phases (SOMP) whereupon the mouth remained open for over 50% of the time in a month. A third period was characterised by an open mouth frequency of less than 40% and defined as a series of open-mouth-conditions (OMC) (see Table 4.1). Strong rains early in August 2001 were preceded by a dry period that lasted for over a year. These rains resulted in the breaching of the mouth later that month (Fig. 5.2a). A couple of weeks after the mouth was breached riverine discharge at low tide was estimated at 1.5 m³s⁻¹ near the mouth (see Appendix 1, Plate 1 A - E). The estuary mouth remained open for approximately 4 to 5 weeks after which river flow decreased to less than 0.5 m³s⁻¹. This breaching event constituted the first-open-mouth-phase (FOMP). The August rains initiated the first breaching of the mouth, but it was the release from the Van Stadens Dam by the water division of the Nelson Mandela Metropolitan Municipality in September that maintained a longer open mouth phase. The release of the dam water estimated to be ~0.31 x10⁶ m³ introduced fine particulate suspended matter that reduced light
transparencies in the water column. Light attenuation coefficients for a period of about two weeks following the release ranged from 3.21 – 9.40 ($K_d$ m$^{-1}$). Light attenuation during this period was positively correlated with rainfall ($p < 0.05$). By October the mouth closed and in late November to early December there were episodes of marine overwash coupled with intermittent rainfall that augmented estuarine volumes that led to brief OMC incidences. Sporadic rainfall during summer months over the course of the study was sufficient to sustain river flow that maintained a high water level in the estuary. These rainfall events however, were not sufficient to cause breaching of the mouth.

Heavy rains (monthly mean > 80 mm) in early July 2002 caused breaching of the mouth interrupting the second-closed-mouth-phase (SCMP) that had lasted from January – July 2002. These rains followed moderate rainfall (monthly mean ~35 mm) that occurred in May. The breaching event lasted approximately 3 – 4 weeks forming the second-open-mouth-phase (SOMP). In contrast to the open-mouth-phases (i.e. open mouth > 3 weeks) in the first and second year of the study, which were characterised by oligohaline water column conditions, the open-mouth-conditions (i.e. open mouth < 5 day) during the third year (April – June 2003) resulted in a mesohaline (mean 15.3 ±0.5 psu) water column. The low rainfall (monthly mean <20 mm) period from 2003 – 2004 contributed to a long dry season that produced the UCMP. During this period salinity readings reached their highest levels recorded over the study period (Fig. 5.2b). The water column electrical conductivity profile tracked closely that of salinity with lowest readings occurring during periods of high river flow and highest readings under closed mouth conditions, especially during the UCMP. Turbidity levels after SOMP were lower compared with the periods after FOMP with light attenuation coefficients ranging from 1.5 – 1.7 ($K_d$ m$^{-1}$). In the third year light attenuation coefficients reached the lowest levels (mean 0.54 ±0.1 $K_d$ m$^{-1}$) recorded over the three-year period of the study. This period was also associated with a higher frequency of marine overwash events that introduced clear marine water.

The influence of regional seasonal weather conditions over the three years of the study was apparent from water-column temperatures with low and high temperatures occurring during the winter and summer seasons respectively (Fig. 5.2b). High summer temperatures normally peaked in January (mean 25.5 ±0.4 °C) and winter temperatures reached a minimum in July (mean 13.2 ±0.8 °C). Dissolved oxygen (DO) levels did not show seasonality but appeared to be influenced by mouth condition. The SOMP had the highest DO concentrations whereas the UCMP recorded the lowest levels (mean 9.9 mg L$^{-1}$±1.5 S.D., & 5.9 mg L$^{-1}$±1.9 S.D.) respectively. This was however, variable since the FOMP in 2001 and the OMCs in 2003 recorded relatively low DO concentrations (mean 6.3 mg L$^{-1}$±1.7 S.D., & 5.2 mg L$^{-1}$ ±0.4 S.D.) while the FCMP and SCMP both recorded relatively high (mean 8.7 mg L$^{-1}$±0.6 S.D., & 8.0 mg L$^{-1}$±1.6
S.D.) DO levels respectively. The introduction of marine water over the sand bar during closed mouth conditions improved light transparencies in the water column with photon flux densities averaging >500 ±20 μmol m$^{-2}$ s$^{-1}$ at the sediment surface. These conditions encouraged the growth of benthic micro- (e.g. bacillariophytes & cyanophytes) and macroalgae (Ulva sp., Fucus sp. & Sargassum sp.) especially in the lower reaches of the estuary. A one-way ANOVA analysis showed that there was no significant difference between the photic depth ($Z_p$) and total depth ($Z_{tot}$) ($p>0.05$). In the upper reaches submersed (Potamogeton pectinatus) and emergent (Phragmites australis) macrophytes proliferated particularly during the extended dry period of 2003 – 2004. This period was also associated with an increase in estuarine water level since marine water became the major source of water augmenting estuarine water volume while the mouth remained closed.

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 being detected following a two-way ANOVA test ($p>0.05$) nutrient data for the sites were vertically integrated and pooled. On average macronutrient levels in the Van Stadens Estuary generally remained low throughout the study period. This pattern was evident particularly during the dry periods. The periods with peak discharges that caused the mouth to open on three occasions were associated with an increase in macronutrient concentrations. Estuarine monthly nutrient concentrations were closely related to periods of increased river flow with above average concentrations recorded during peak discharges in August, September, & November 2001 and August & September 2002 and also in March & June 2003 (Fig. 5.3). A Spearman’s rank correlation analysis between mean monthly rainfall data and macronutrient concentrations revealed a significant correlation of 0.67 ($p < 0.05$). In July prior to the FOMP in 2001 DIP concentrations reached their minimum levels (<0.10 μg L$^{-1}$), however the increased river flow in August 2001 increased concentrations to levels above 1.0 μg L$^{-1}$. During the same period DIN concentrations were similarly augmented by the increased riverine input, although ammonia concentrations continued to remain above average for the next six months. During September 2002 DIP concentrations reached their highest levels (mean 3.0 ±0.5 μg L$^{-1}$) that coincided with the peak rainfall event a month earlier. A one-way ANOVA test showed that there were significant differences between months in 2002 ($F_{54.486}, p<0.001$).

Following the peak in September DIP concentrations dropped until March 2003 where there was another increase with the SRP fraction making up 50 – 55% of the DIP. This increase followed a spate of moderate rainfall that increased river discharge and opened the mouth. A combination of intermittent rainfall and marine overwash events produced a series of open and closed mouth conditions that sustained
moderate levels of DIP & DIN, mainly as ammonium, culminating in high concentrations by January 2004. Periods of low river flow associated with closed mouth conditions were characterised by very low macronutrient concentrations. Low average rainfall in the late summer (January - March 2002) resulted in very low to almost no river flow entering the estuary. During that period DIP concentrations remained low in sharp contrast to DIN, mainly ammonium concentrations that were significantly higher in early spring and summer ($p < 0.05$) (Fig 5.3). Variability in the DIN and DIP concentrations in the Van Stadens Estuary resulted in shifts in DIN: DIP ratios over the three-year study period. There were no clear seasonal patterns since the DIN: DIP ratios ranged from <1 to 38 between June 2001 and May 2002 then declined and remained below 16 until January 2004 where it reached 31. Except during the FOMP when DIN: DIP ratio was 19, river inflow appeared not to have had a strong influence on the N: P ratios since values remained below 16 during subsequent OMCs. Dissolved silica (DSi) did not display a clear seasonal pattern ($p > 0.05$) over the study period. DSi levels reached a maximum of 290 ±20 µg L$^{-1}$ in October 2001 while the estuary mouth remained opened, but declined to 150 µg L$^{-1}$ when the mouth closed by mid December 2002. In January 2002, during the SCMP under a series of marine over wash episodes, DSi concentrations peaked at 410 ± 390 µg L$^{-1}$ then declined to a low of 40 ±20 µg L$^{-1}$. DSi concentrations were low with values ranging from 5 – 10 ±1 µg L$^{-1}$ during the summer season 2002. Following the series of OMCs in the autumn of 2003 DSi concentrations increased to just above 100 µg L$^{-1}$ and remained unchanged until late summer the following year.

5.3.2 Groundwater and River to Mouth Nutrient Response

Groundwater (GW) was sampled when sufficient water was available to carry out all the water quality analyses for dissolved macronutrients (i.e. TP, SRP, NO$_3^-$, NO$_2^-$, NH$_4^+$, & DSi). Water depth in the estuary was critical in influencing the availability of groundwater. Water depths of above 4m at the deepest point (Station 1 Fig. 3.2) and approximately 1.9m at the shallowest point (Station 5) of the estuary normally indicated presence of water in the wells. On the other hand breaching of the estuary mouth caused loss of water volume in the estuary, which led to a reduction in the water depth and a subsequent drop in the height of the water table resulting in the draining of the water in the wells. Extended periods of closed mouth conditions permitted the water table to rise sufficiently for water to be present in the wells, which enabled sampling. On certain occasions, even though the mouth of the estuary had been closed for more than 2-months, wells were devoid of water. Periods of more than 3-months of mouth closure appeared to be essential in retaining sufficient volume of water in the wells. Groundwater macronutrient concentrations varied owing to the absence of GW in the wells during certain months of the year. There were no clear indications that GW was a source of nutrients into the water column, although GW nutrient concentrations were an order of magnitude higher than water column levels and were significantly different ($p < 0.001$) (Figs. 5.3 & 5.5).
Figure 5.2  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. * denotes no data. Vertical lines (±1 S.E.).
Figure 5.3. Van Stadens Estuary nutrient concentrations for the period May 2001 to April 2004, (A) DIP = total phosphate – TP and soluble reactive phosphate – SRP, (B) DIN = ammonium–NH$_4^+$, nitrate–NO$_3^-$, nitrite–NO$_2^-$ from October 2001 – April 2004. Horizontal bar is described as in Figure 5.2. # Denotes values below detectable limits, * no data. Vertical lines (±1 S.E.).
During periods when the estuary mouth was closed GW TP concentrations were consistently high averaging 1.39, 3.73, and 3.5 mg L\(^{-1}\) in the first, second and third year of monitoring respectively (Fig. 5.5a). Groundwater nitrite concentrations were below detectable levels and are therefore not reported here. Nitrate concentrations remained low averaging less than 0.3 mg L\(^{-1}\) over the study period showing no variation with the change in mouth condition. In contrast, ammonium concentrations were always high during periods of low flow and closed mouth conditions (Fig. 5.5b). Following the SOMP groundwater DSi levels reached the highest levels (\(1.4 \pm 0.05\) mg L\(^{-1}\)) observed over the study period \((p < 0.001)\). Dissolved silica subsequently declined to low levels (\(0.08 \pm 0.02\) mg L\(^{-1}\)) by February. In July of 2003 the mouth closed and remained closed until December 2004 with occasional periods of overwash. During this period DSi levels increased and remained steady above 0.4 mg L\(^{-1}\) then reached a peak of 1.0 mg L\(^{-1}\) by February 2004 (Fig. 5.6). The closed mouth period from July 2003 until December 2004 was the longest period of mouth closure since the monitoring period began. Water depth in the estuary only reached a maximum of approximately 4.5 – 5.0 m at Station 1 in March of 2004. The variability in the presence of well water associated with estuarine water volume had an influence on the DIN and DIP concentrations over the period of study. DIN and DIP ratios ranged from a high of 55 in May then declined to a low of 0.5 in October 2002. In January 2003 DIN: DIP ratios reached a maximum of 16 dropping below 10 in May then remaining at about 2 during the summer months (Fig. 5.6).
Figure 5.5  Van Stadens Estuary groundwater macronutrient 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$_3^-$. Horizontal bar is as described in Figure 5.2. Blank month spaces denote periods not sampled. Vertical lines (±1 S.E.).
Figure 5.6  Van Stadens Estuary groundwater dissolved silica–DSi concentrations for the period May 2002 to April 2004. Horizontal bar is as described in Figure 5.2. Blank month spaces denote periods not sampled. Vertical lines (±1 S.E.).

Figure 5.7  Van Stadens Estuary groundwater DIN: DIP ratios for the period of May 2002 to April 2004. Line denotes the 16:1 nitrogen: phosphorus molar ratios. Horizontal bar as described in Figure 5.2. Blank month areas denote periods not sampled.

River to mouth surveys were carried out from March 2002 – December 2003 on a quarterly basis and at times that closely followed rainfall events. The response pattern of macronutrients over that period was
generally similar hence data reported on here is taken from March 2002 – April 2003. Although generally low and variable, macronutrient concentrations sampled along the Van Stadens River were higher than those in the estuary particularly following periods of rainfall. Dissolved inorganic phosphorus concentrations from the upper catchment were not much higher than those measured in the estuary during March 2002, however dissolved inorganic nitrogen levels were significantly higher (Student’s $t$-test, $p < 0.05$) when compared with estuarine concentrations (Fig. 5.8A & B). The steeply incised nature of the Van Stadens River in the upper part of the catchment (Appendix 1, Plate E & F) meant that most of the surface runoff readily drained and was rapidly channelled through the gorge downstream. The water draining this region of the catchment including below the Dam on occasion had higher ammonium levels compared to sites in the middle of the catchment. The majority of the nitrogen contribution (~90%) into the river water from the upper catchment was ammonium with nitrates contributing the rest of the nitrogen.

In March 2002 when river flow was low (<0.05 cm$^3$ s$^{-1}$) the river did not act as the source of inorganic phosphates, however after the rains in September and in December, although concentrations were much lower, the river contributed significantly to phosphate concentrations (Fig. 5.8 A & C). A similar pattern was also observed with regard to supply of nitrates, although not in similar magnitude, river input of nitrate concentrations did increase after rainfall events. Stations 7 & 8 located 16 & 17 km from the mouth (see Chpt. 3 Fig. 3.2) span the point at which the National Road (N2) crosses the Van Stadens River (Appendix 1, Plate F). At these two sites significantly higher phosphate and nitrate levels ($p < 0.001$) were recorded than at any other river sites including the estuary especially following rain events. During dry periods when river flow was low phosphate concentrations were higher in the estuary particularly in the lower reaches compared to both the upper reaches and higher up in the catchment. This pattern held throughout 2003, although it was variable.
Figure 5.8  Van Stadens River to mouth macronutrient data surveyed quarterly from March 2002 – December 2002. Panels a, c, e, & g = total phosphorus-TP & soluble reactive phosphorus-SRP; panels b, d, f, & h = ammonium-NH$_4^+$, & nitrate-NO$_3^-$. Vertical lines (±1 S.E.).
5.3.4 Phytoplankton Chlorophyll a Concentration, Size-fractions and Community Structure

Chlorophyll a concentrations showed seasonal responses with peaks in Chl a levels occurring in the spring and summer of 2001, autumn and summer 2002 and late spring 2003 (Fig. 5.9a). Except for July 2001, low Chl a concentrations were recorded during winter periods (e.g. July 2002, & 2003). Seasonal periods linked with high river discharge associated with rainfall also reduced Chl a concentrations in the estuary. Chlorophyll a levels ranged between 0.8 µg L\(^{-1}\) (± 0.11 S.D.) in September 2001 and increased to a peak of 13.9 µg L\(^{-1}\) (± 4.1 S.D.) in December of 2001. The peak in Chl a by December was the highest concentration measured over the duration of the study. There were, however, no clear spatial (i.e. horizontal & vertical) differences in Chl a concentrations (Student’s t-test, \(p > 0.10\)) therefore, the values reported here were averaged over the five estuary sites including the upper and lower layers of the water column. The first Chl a peak 8.4 µg L\(^{-1}\) (± 0.9 S.D.) occurred in October 2001 8-10 weeks after the Van Stadens Estuary mouth had breached. Chlorophyll a pigment concentrations over the first-open-mouth-phase FOMP (e.g. October – December) responded positively to the elevated nutrient input resulting from an increase in river flow. The high nutrient influx from increased runoff was correlated with the heavy rainfall in August (>80 mm monthly mean) \(R^2 = 0.68\). The magnitude of water discharge following the rainfall event together with the release of water from the Van Stadens dam kept the estuary mouth open for several weeks. This sustained open mouth phase maintained high Chl a concentrations over three months culminating in a bloom in December. The periods that exhibited high Chl a concentrations (e.g. December’01, February & November ‘03) corresponded strongly with rainfall-associated discharge, although there was a lag in Chl a response (one-way ANOVA \(p < 0.05\)) (Fig. 5.9a). The high river discharge and subsequent flushing following heavy rainfall resulted in marked reduction in phytoplankton Chl a however, these reductions were generally short-lived. Chlorophyll a levels subsequently dropped during the second-closed-mouth-phase (SCMP) then increased forming a minor bloom in April 2002. Despite an increased nutrient load associated with high discharge that caused the mouth to breach in August 2002, Chl a concentrations remained low (< 4.0 µg L\(^{-1}\)). Chl a concentrations did not show significant improvement until the summer of 2003. A combination of mouth opening and marine overwash episodes between January and March 2003 produced a small Chl a peak in February. This was followed by another peak in November during the fourth-closed-mouth-phase (UCMP). In late June 2003 the mouth closed and remained closed until December 2004 and during this time there were a series of intermittent marine overwash events that lasted until the spring of that year. Although the study was completed in April 2004 continued monitoring revealed that no mouth breaching events took place during that year making it the longest period that the mouth stayed closed over the duration of the study.
Figure 5.9  Van Stadens Estuary Chl a for the periods 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, * denotes months not sampled. Horizontal bar signifies three states of the mouth: solid bar – closed, open bar – open, and hatched bar – overwash. Vertical lines (±1 S.E.).

Overall the microphytoplankton fraction contributed over 60% with the nanophytoplankton group contributing approximately 26% and the picophytoplankton fraction making up the rest of the total chl-a during the study period (Fig. 5.9b). In July prior to the FOMP picophytoplankton formed the dominant size fraction contributing > 70% to total Chl a. However, in September after the mouth had remained opened for about a month Chl a levels reached a minimum before increasing sharply in October. During this period the picophytoplankton and nanophytoplankton contributed about 42 & 36% respectively to total Chl a concentration while the microphytoplankton made up the difference. This pattern, however, did not persist when Chl a concentrations peaked in December the microphytoplankton became the dominant size fraction for the next 11-month period and contributed >72%. A pairwise multiple comparison procedure (Tukey Test) following a one-way ANOVA
showed strong differences ($p = 102, q = 12.074$, at $p < 0.001$) between nanophytoplankton and microphytoplankton. During periods associated with the open-mouth-phases the microphytoplankton group contributed the majority $\sim 74\%$ to total Chl $a$, whereas during the closed and marine overwash phases they made up $<55\%$. With the exception of July in 2001, the picophytoplankton did not become a major contributor to total Chl $a$ concentration contributing between $9 \& 14\%$ in both the second and third year periods of the study respectively. On the other hand the nanophytoplankton contributed between $26 \& 41\%$ over the same period. Throughout the prolonged closed-mouth condition in the latter part of 2003 phytoplankton size structure comprised the nanophytoplankton and microphytoplankton as co-dominants with both fractions contributing approximately $30 \sim 40\%$ in terms of chl-$a$ with the picophytoplankton making up the difference. The open-mouth-phase (OMP) in May 2003 was the single occasion where the nanophytoplankton became the dominant size fraction. In contrast, during the UCMP (e.g. June 2003 – April 2004) they were the dominant group for four out of ten months and co-dominant with the microphytoplankton for three and displayed greater abundances for the rest of that period (Fig. 5.9b).

Phytoplankton cell densities were generally low as expected for this unproductive oligotrophic system ranging from $7.78 \times 10^3$ to $849.4 \times 10^3$ cells ml$^{-1}$ ($\pm 0.76 \& 133.4$ S.E.) respectively, however two major bloom events took place in late spring and early summer of 2002. The highest cell densities $8.49 \times 10^5$ cells ml$^{-1}$, were recorded in December 2002 in the lower reaches of the estuary (Figure 5.10). Samples from elsewhere in the estuary did not show such high levels of abundance. Earlier in November similar density levels ($5.60 \times 10^5$ cells ml$^{-1}$) of a green algal bloom were present mostly at site-1 (i.e. 0.5 km from sea), yet similar genera did occur at site-2, however in low numbers ($<1.0 \times 10^3$ cells ml$^{-1}$). Two of the flagellated algal genera included *Micromonas* sp. and *Pyramimonas* sp. ($3 \sim 5 \ \mu \text{m}$ along central axis) in the lower reaches. These algae were also present in the middle and upper reaches of the estuary but were not as abundant and contributed approximately $10\%$ of the total phytoplankton count in the samples. Flagellated algae tended to make up the bulk of the phytoplankton cell counts throughout the water column of most sample dates considered. During the first-closed-mouth-phase (FCMP), a period also characterised as a clear-water-phase with a deep euphotic depth (e.g. April – July 2001), chlorophytes and chrysophytes constituted the dominant taxa at 4 out of 5 sites ($p <0.05$) (Figure 5.10). In the same period phytoplankton cell densities ranged between $3.1$ and $8.1 \times 10^4$ cells ml$^{-1}$ and coincided with a period characterised by low rainfall associated with a deep euphotic depth, and a mesohaline water column. In May TP concentrations were high but subsequently dropped during the FCMP, although low in densities ($< 20\%$) over this period the large-sized dinoflagellates persisted under conditions of very low macronutrient concentrations composed mainly of heterotrophic species. Small-sized ($3\sim 5 \ \mu \text{m}$ diam.) green algae from microscopic counts were observed attached to cellular microfibrilar extensions of the dinoflagellates (e.g. *Amphidinium perculatum, Protoperidinium cerasus*). The presence of these
extensions in a number of the dinoflagellates encountered seems to indicate active preying by this group of phytoplankton on the small algae. From late May to early August phytoplankton cell numbers were low, however the bulk of the phytoplankton included chrysophytes (e.g. *Nitzschia closterium*, *N. longissima*, *Melosira* sp., and *Gyrosigma* sp.) and cryptophytes (e.g. *Chroomonas amphiioxea*, *Cryptomonas* sp. *Rhodomonas* sp.) which were equally dominant. (see Appendix 2 for representative taxa).

Phytoplankton cell numbers reached minimum levels in July 2001, however this was in contrast to the *Chl a* data as pigment concentrations were 4.0 µg L$^{-1}$ during this month. When the mouth breached in August 2001 phytoplankton densities were still low however by November cell numbers had increased to approximately double that during the mouth opening event (Fig. 5.9). In terms of percent composition throughout the FOMP, the phytoplankton assemblage consisted of large-sized (>30 µm diam.) flagellates that included *Cryptomonas ovata*, *Protoperidinium bipes*, *Peridinium* sp., and *Ornithocercus* sp.. The concentrations varied but they persisted long after the mouth was closed in late December extending well into the SCMP. This pattern continued until the onset of autumn when the community shifted and chlorophytes became the dominant phytoplankton group. The chlorophytes remained dominant but were at times co-dominant with the cryptophytes. A second long-lasting mouth breaching phase (e.g. SOMP July – September 2002) began in late July and during this period phytoplankton densities declined to low levels that were comparable to that observed for the similar period in the previous year ($p < 0.05$) and reached a minimum of 29.4 cells x10$^3$ ml$^{-1}$ (±2.3 S.E.) by July 2002.

The third-closed-mouth-phase (TCMP) started in October and lasted until March 2003. Throughout this period the phytoplankton community underwent shifts in composition and size structure. At first nano-sized chrysophytes and cryptophytes were co-dominant, particularly just after the mouth had closed, to that where pico-nano-sized chlorophytes and micro-sized dinoflagellates formed the bulk of the phytoplankton community (Figs. 5.10 & 5.11). The sequence of succession of the phytoplankton community in the second year following changes in mouth condition appeared to repeat some of the patterns observed (e.g. phytoplankton composition and size structure) in the first year of the study although the timing and development of hydrodynamic events and physical factors differed. The rainfall event in April 2003 was of sufficient magnitude to cause breaching of the mouth that produced the third-open-mouth-phase (TOMP April – June 2003). An influx of DIP & DIN from the increased river flow during this period initially supported a high concentration of chlorophytes and dinoflagellates that was completely succeeded by chrysophytes that were composed mainly of small-sized diatoms (~10 x 5 µm along horizontal axis).
Figure 5.10 Phytoplankton cell densities and community composition by taxa for the period May 2001 to December 2003 from the Van Stadens Estuary. Phyla include: Chloro– Chlorophyta, Crypt– Cryptophyta, Chry– Chrysophyta, Dino– Dinophyta, Cyano– Cyanophyta, Eug– Euglenophyta. Horizontal bar is as described in Figure 5.8.
Flagellated chlorophytes, primarily *Micromonas* sp. & *Tetraselmis* sp., became the dominant phytoplankton species in winter as early as July when the mouth closed until late summer although on a few occasions chrysophytes were co-dominant. This period was characterised by a series of episodic marine overwash events that introduced clear saline water that maintained a deep euphotic depth and higher than normal salinity levels (~ 25 psu) (see Fig. 5.2b). Ammonium levels were also uncharacteristically elevated while phosphate concentrations remained low, although TP was consistently and significantly higher than SRP concentrations ($p < 0.05$). When the estuary experienced a prolonged period of mouth closure during the third year of the study, large-sized dinoflagellates and cryptophytes comprised less than 10% of the phytoplankton community. Except for December 2003, this observation was further confirmed by size-structured pigment data where the majority of the pigment contribution was mainly by the nanophytoplankton size-fraction.

A similarity matrix was performed on phytoplankton cell numbers in an attempt to explain the variability in the distribution of the phytoplankton community structure along a temporal scale associated with the status of the mouth (Clarke and Warwick Statistical Package Primer 1994). From the matrix a non-metric multidimensional scaling plot was generated based on the presence/absence of the phytoplankton species data. The result of which was represented on a two dimensional plot (Fig. 5.12). From the plot it is apparent that similarity existed as some dates tended to cluster together. The

![Figure 5.11 Phytoplankton percent contribution by taxa for the period May 2001 to December 2003 from the Van Stadens Estuary. Phyla enumerated included: Chloro–Chlorophyta, Eug– Euglenophyta, Dino– Dinophyta, Cyano– Cyanophyta, Crypt–Cryptophyta, Chry– Chrysophyta. Horizontal bar is as described in Figure 5.8. *Denotes no data collected.](image)
condition of the mouth at the time of sampling was used to link it with the date the sample was taken (e.g. if the mouth was open in May of year-1, then that month period would be coded as May1-O or May2-C for closed mouth in May of year-2). Months that the mouth was closed and when the estuary experienced significant marine overwash were treated as closed mouth periods. A two-way nested analysis of similarity (ANOSIM) was carried out to test for the differences between months of the phytoplankton species data. Following the test based on the presence/absence data for the phytoplankton species resulted in a test statistic with a R-value of 0.545 and P=0.002 indicating that over 50% of the variability could be attributed to mouth condition. The data indicated a good similarity among phytoplankton species exposed to similar hydrodynamic conditions occurring at different temporal scales.

**Figure 5.12** An MDS plot of the Van Stadens Estuary phytoplankton community by month, year and mouth condition. O = open, C = closed, 1 = 2001, 2 = 2002. Solid-lined circles denote three major groups representing mouth condition; dotted-lined circles represent seasonal periods within the closed state periods.

### 5.3.5 Microphytobenthic Chlorophyll a Concentrations

In contrast to the water column Chl a response to increased nutrient input, the microphytobenthic (MPB) Chl a concentrations did not show any significant changes (p < 0.001) to increased river flow in the first year of the study (Fig. 5.13). Pigment concentrations remained below 1.0 µg Chl a g⁻¹ sediment (± 0.1 SE) in the first ten months. The highest Chl a levels were recorded in late July 2003 less than a month before the mouth breached in September. This was after several days of marine overwash that was associated with a water column with low light attenuation coefficients and a deep euphotic depth. Flood conditions in July 2002 and June 2003 resulted in sediment scour that caused
lowest MPB Chl a biomass. After the June 2003 floods MPB Chl a increased sharply (Fig. 5.13). Microphytobenthic Chl a concentrations steadily increased during the summer of 2002 a period characterised by a deep euphotic depth, warm water column temperatures and low river flow. Peak MPB Chl a concentrations occurred in July 2003 just after the mouth had been closed for about a week following the TOMP (April – June 2003). Receding river flow coupled with sediment movement and deposition across the mouth from on-shore strong wave action along the coast accelerated the closure of the mouth in late June early July. The latter conditions persisted during July by introducing clear saline water into the lower reaches of the estuary well after the mouth was closed. Despite an improved water column clarity and a generally stable sediment floor MPB Chl a concentrations never attained the levels recorded in July, yet overall benthic Chl a concentrations were significantly higher the remainder of the third year than those recorded during the first year (p < 0.01) (Fig. 5.13).

![Graph](image)

**Figure 5.13** Microphytobenthic chlorophyll a (MPB Chl a) concentrations taken from the Van Stadens Estuary from June 2001 – April 2004. Horizontal bar is as described in Figure 5.8. *Denotes no data collected. Vertical lines (±1 S.E.).

### 5.6 Discussion

#### 5.6.1 Physical and Chemical Parameters

The frequency of mouth breaching in the Van Stadens occurred about 20 to 25% of the time over the three year study period resulting in three open mouth phases. Rainfall events in the catchment were closely linked to episodes of mouth breaching and were considered essential in influencing mouth condition. Open mouth events resulted from a sufficiently strong magnitude of river flow (> 3 m³ s⁻¹) kept the mouth in an open condition for an extended period giving rise to three open-mouth-phases. The high discharge of fresh water flow affected estuarine circulation patterns by limiting marine tidal
inflow and altering channel geomorphology through scouring and sand deposition. River flood conditions in August 2001 and September 2002 caused breaching of the mouth, reduced salinity levels and introduced fine particulate inorganic suspended matter that reduced the euphotic depth. The suspended sediment load produced highly turbid conditions that suppressed light transparencies and limited the development of phytoplankton and MPB growth. Light reductions from turbidity-induced river runoff have been shown to affect phytoplankton production (Cloern 1987). The combined effects of a reduced euphotic depth coupled with flushing and benthic sediment scour during flood conditions may also explain the reduction in the production and development of both phytoplankton and MPB Chl a biomass (Snow et al. 2000a). The breaching of the mouth in April 2003 did not have a similar effect as previous events. This breaching incident was mainly associated with an estuary that was full (max. water-level at site-1 >5 m) that was triggered by a moderate amount of rainfall (~ 40 mm monthly mean).

The hydrodynamic effects on the physical and chemical variables in the Van Stadens Estuary were pronounced and seemed to influence subsequent physical and chemical attributes. The periodic freshwater flooding over the three year study period lowered water column salinity, but incidents of marine overwash rapidly re-established mesohaline conditions after the mouth closed. Even under moderate durations of mouth closure marine overwash events augmented marine water kept salinity levels high especially during the UCMP. These saline conditions may have created conditions that supported large densities of phytoplankton taxa that are normally associated with the marine environment (e.g. *Tetraselmis marina* and *Micromonas* sp.) (Tomas 1997). On three occasions when the mouth was closed and salinity was high (15-25 psu) chlorophytes (< 5.0 µm diam.) became the dominant taxon throughout the water column. In terms of pigment composition pico- and nanophytoplankton contributed about 40% each to total Chl a biomass in this study, a scenario that has also been observed in other regional studies (Froneman 2002a). Although other studies have attributed the pigment contributions occurring during dry periods to the small-size fractions (e.g. pico- & nanophytoplankton) they have not carried out any taxonomic algal identifications. The Van Stadens study was the first that has made taxonomic identification of the algae that accounted for the significant contribution in Chl a concentrations and community composition.

Apart from the high suspended load introduced by flooding, enhanced macronutrients were also brought into the estuary in this manner. The supply of macronutrients into the Van Stadens Estuary was strongly correlated with rainfall. This was further confirmed from river data that showed elevated macronutrient levels immediately after rain events compared to dry seasonal periods. Macronutrient input into the Van Stadens is dependent on the supply from the catchment and its availability is limited by both the frequency of rainfall events and man-made obstructions (e.g. dams, weirs, & course ways). Physical structures that alter and modify the river channel and bed morphology can influence physico-
chemical characteristics of water in receiving waters downstream (Davies and Day 1998). The Van Stadens Dam located in the upper catchment is critical in controlling the quantity of water received downstream since no water is released except as overspill over the wall of the dam during wet periods. During dry periods macronutrient levels in the river and the estuary became depleted. However, overwash from the sea may have augmented macronutrients supply in the lower reaches as slightly raised concentrations of DIP and DIN were measured when such conditions prevailed.

Ammonium concentrations in the estuary remained high throughout the study period and only declined on two occasions. These were associated with increased river flow. There is evidence that the river was the source of ammonium entering the estuary since high concentrations were always measured in the upper catchment. Although in the middle part of the catchment concentrations dropped markedly suggesting rapid removal of the nitrogen prior to it reaching the estuary. Nitrate and phosphate concentrations measured during high river flow were positively correlated with flooding events. This demonstrates a strong link between catchment derived nutrients and the estuary suggesting that activities in the catchment can affect estuarine processes (Twomey and Thompson 2001, Flemer and Champ 2006). Under closed mouth conditions nutrient concentrations remained low and at times were barely detectable. Under these conditions the source of nitrogen in the estuary was mostly from ammonium since its concentrations were always higher than nitrate except during floods. Groundwater nutrient concentrations were often significantly higher than the water column when the mouth was closed suggesting that another possible route of entry into the water column may be from groundwater. The MPB may have used this available pool of ammonium (Skinner et al. 2006) particularly when the mouth was closed for extended periods (e.g. TCMP & UCMP).

Consistent with regional studies on TOCEs, MPB Chl a biomass was higher than the water column Chl a concentrations. The values measured in this study were comparable with other regional studies (Froneman 2002a, Perissinotto et al. 2002 & 2003). A decrease in the euphotic depth from increased turbidity has been suggested as a reason for reduced MPB biomass (Nozais et al. 2001, Froneman 2002a, Perissinotto et al. 2002). Although light levels were reduced during flood periods in the Van Stadens MPB biomass did not show any correlation with increased turbidity. This suggests that light may not be significantly limiting MPB growth and that other factors such as removal by scour and resuspension or burial from sediment deposition may be involved. Although there were three occasions with floods that scoured the sediment only two resulted in the lowest Chl a concentrations. MPB Chl a concentrations did not show strong temporal or spatial variation possibly suggesting strong resilience and rapid recovery following a disturbance and uniform distribution along the length of the estuary (Widows and Brinsley 2002). In contrast, regional studies have demonstrated temporal and spatial variability (Froneman 2002a, Perissinotto et al. 2002), yet in the Van Stadens this was not the case. Spatial MPB Chl a distribution patterns in the Van Stadens were consistently similar throughout
the length of the estuary suggesting homogeneity in sediment grain-size necessary for the colonisation by the epipsammic diatom community including possible similar water quality characteristics throughout the length of the estuary (Skinner et al. 2005). Significant differences in Chl a biomass were only detected in July 2003 3 – 4 weeks after the floods in June as a possible response to the increased nutrient concentrations and euphotic depth (Perissinotto et al. 2003). The lack of spatial MPB biomass in this study is consistent with other regional studies, although those studies reported variation in Chl a biomass in response to mouth condition, a situation that was not observed in the Van Stadens (Froneman 2002b). The effects of mouth condition on MPB Chl a biomass in the Van Stadens Estuary did not significantly affect Chl a biomass suggesting strong resiliency and rapid recovery following flooding and possibly sustaining strong grazing pressure under closed mouth conditions that effectively maintain MPB Chl a biomass generally at low concentrations (Ford and Honeywill 2002).

With the exception of four occasions when the estuary was P-limited, DIN: DIP ratios in the Van Stadens generally reflected nitrogen limitation throughout the study. Macronutrients associated with river influx did induce P-limitation (Thomas et al. 2005), however decomposition processes of macroalgae and submersed macrophytes under prolonged mouth closure may have encouraged the release of ammonium leading to P-limitation during those periods (Welsh et al. 2000, Caffrey et al. 2002). Nitrogen appears to be limiting in the Van Stadens but its main supply comes from freshwater input and secondarily from the benthos. The availability of the latter N pool to the pelagic algae is perhaps not available for uptake but is taken up by benthic communities (Skinner et al. 2006).

5.6.2 Phytoplankton Chlorophyll a Concentration and Community Structure

The response of phytoplankton Chl a to freshwater influx was as expected. Macronutrient input increased phytoplankton biomass resulting in peaks of Chl a biomass particularly following increases in discharge-associated macronutrient concentrations. The magnitude of Chl a response however was not anticipated, as the highest peak in Chl a occurred several weeks after the breaching event. There was however a gradual increase leading to the bloom in December 2001. There was no other breached induced Chl a peak that matched those levels over the study period. This suggest that perhaps the duration between the periods the mouth stays closed is critically important in determining the level of the phytoplankton response. Prior to the commencement of the study in April 2001 the Van Stadens mouth had remained closed for over 12-months by the time it breached in August. On the other hand it may also mean that the duration the mouth stays open is critical in establishing a continuous link with the terrestrial and marine environments by creating ‘permanent open mouth’ conditions similar to those observed for such estuaries. The FOMP (August – December) was the longest period that the mouth had remained open during this study perhaps supporting the above argument. Although there were other open-mouth-phases (e.g. SOMP & TOMP) these lasted less than 6-weeks.
In contrast to what other studies have suggested, in this study the microphytoplankton were the dominant size group for about 55 & 65% of the time in the first and second year respectively and were co-dominant (~ 43%) with the nanophytoplankton in the last year of the study. Freshwater influx supported the growth and development of larger sized phytoplankton. This increase in nutrient concentration brought about a succession of phytoplankton from nanophytoplankton to larger sized microphytoplankton. These data agree with hypothesis (5) that increased nutrient input from increased river flow support higher biomass concentrations of microphytoplankton. In the Van Stadens the microphytoplankton contributed more Chl a biomass and on more occasions than the other size groups. These data agree with studies carried out regionally in the Kasouga (Froneman 2002b) and overseas in Mecox Bay (Gobler et al. 2005) that have shown high Chl a biomass from micro- and nanophytoplankton associated with an increase in nutrient input. The importance of larger phytoplankton cells to water column biomass production has been demonstrated for coastal systems that frequently experience variable nutrient supply and environmental conditions (Cermeño et al. 2006). The Chl a biomass contribution by picophytoplankton in this study was normally less than that of the other size fractions either during the closed or the open mouth phases. These data therefore, do not agree with hypothesis (3), which states that prolonged periods of mouth closure favour increased pico- and nanophytoplankton biomass. Neither the pico- nor the nanophytoplankton became the dominant size fractions under a prolonged closed-mouth-phase in the Van Stadens Estuary. There was, however, one exception in July 2001 when the picophytoplankton formed the dominant group, but this pattern was never repeated suggesting that the environmental conditions that lead to those observations were unique.

The water column immediately after breaching was dominated by picophytoplankton then nanophytoplankton and finally microphytoplankton. Similar microphytoplankton responses after a flood have been reported in the Kasouga Estuary (Froneman 2002b) Other studies elsewhere have not reported this kind of temporal succession in pigment size structure (Perissinotto et al. 2000, Froneman 2002a & b, Nozais et al. 2001, Thomas et al. 2005). The microphytoplankton size group persisted until December and was very successful in either competitively excluding the other groups or the smaller size groups were possibly grazed. By this time macronutrient (NO₃⁻ & SRP) levels had dropped markedly and were probably insufficient to support the large cells and were thus eliminated in favour of smaller forms (e.g. nanophytoplankton or Micromonas pusilla). Although the duration in the open-mouth-phases and quantity of Chl a biomass was variable over the study period the sequence of succession of the size groups of the phytoplankton remained similar. This suggests that although the magnitude and extent of the hydrodynamic forcing may vary over time the biological response pattern, especially in terms of pigment size fraction stays the same following each perturbation. This idea was further supported by the non-parametric multi-dimensional data analysis. Results from the
multi-dimensional test based on the phytoplankton species presence/absence data indicated that over 50% of the variability observed could be attributed to mouth condition. The distribution patterns of phytoplankton species exhibited shared attributes when exposed to hydrodynamic conditions occurring at different temporal scales. There was, however, a stronger similarity among phytoplankton species experiencing comparable hydrodynamic conditions within the same year of sampling than between different years. These results, thus indicate that mouth condition is important in structuring phytoplankton communities within the Van Stadens Estuary.

During the closed mouth phases when macronutrient concentrations dropped to low levels densities of large-sized mixotrophic dinoflagellates (e.g. *Amphidinium operculatum*, *Protoperidinium bipes*, & *Peridinium* sp. and some tintinnids) comprised a significant proportion of the phytoplankton and zooplankton communities respectively in the water column. Although grazers were not quantitatively accounted for in this study, their presence in phytoplankton samples was noted and appeared to be generally associated with these clear water phases. From this study the dinoflagellates, as part of the large sized phytoplankton, were dominant during increased river flow as well as during the closed mouth phases. The phytoplankton species data partially agrees with hypothesis (6), which states that dinoflagellates and large-sized flagellated algae dominate the water column during periods of mouth breaching and low water clarity, while chrysophytes, particularly diatoms, dominate during periods of mouth closure and improved water clarity. Dinoflagellates did make up a considerable proportion of the phytoplankton species composition during the flood phase when turbidity was high but also made a contribution when attenuation coefficients were low during the closed-mouth phases instead of the diatoms as postulated. At times the dinoflagellates were co-dominant with other large-sized flagellates (e.g. cryptophytes), although cryptophytes did not constitute a significant contribution during the clear water phase. In Mecox Bay, dinoflagellates were also abundant after an open mouth event and diatoms were prevalent during the closed inlet phase (Gobler *et al.* 2005). Diatom contribution during the clear water phase in the Van Stadens was generally short-lived and was normally succeeded by chlorophytes particularly in the second and third year. This suggests that under high nutrients, turbulent and unstable water column conditions large-sized dinoflagellates do well (Burkholder 1992), whereas diatoms grow well under stable and clear water column conditions (Gobler *et al.* 2005, Cermeño *et al.* 2006).

Mixotrophic forms of nutrition have been well demonstrated in estuarine and coastal waters and these sustain some phytoplankton communities under depleted macronutrient conditions (Katechakis and Stibor 2006, Paffenhöfer *et al.* 2007). Mixotrophy in oligotrophic estuaries has not been adequately demonstrated, yet in the Van Stadens Estuary light microscopic observations revealed phagotrophic feeding activities by dinoflagellates and cryptophytes on small green algal cells in samples collected during dry periods of the year (Appendix 2, Plate 1). This mode of nutrition may help explain the
important link between periods of low macronutrient concentrations when mixotrophy becomes important as a source of carbon and other nutrient when light is not limiting and conversely when nutrients are replete from increased river flow which may support photoautotrophy. These two processes may be regarded as ‘famine or feast’ with respect to the availability of nutrients or lack thereof necessary to support phytoplankton biomass that may exist in these estuaries. Although many studies involving mixotrophy have included staining with acridine orange, primulin or 4’,6-diamidino-2-phenylindole (DAPI) in order to confirm presence or lack thereof of pigmented plastids in phagotrophic forms of plankton, light microscopic observations after staining with Calcofluor or Lugol’s solution have also been employed as a relatively rapid method of determining presence of mixotrophy or phagotrophy in dinoflagellates (Lewitus et al. 1999, Olseng et al. 2002).

The results from this study, to the author’s knowledge, have not been demonstrated in TOCEs to date although some studies may have made inferences. Long term monitoring of the Van Stadens Estuary has demonstrated the significance of river flow in supplying nutrients thus regulating phytoplankton community size structure and species composition, which in turn influences food web dynamics. In addition, the magnitude of river flow has an effect on the duration the estuary mouth remains open or closed thus regulating estuarine microalgal biomass production. Conclusive demonstration of these relationships would require experimental investigations to further elucidate the cause and effect responses associated with the manipulation of the hydrodynamics, nutrient supply and individual phytoplankton species. Little is known about TOCEs and their ecological functioning although a large number of these estuaries occur in coastal areas that are experiencing rapid development. Management of these estuaries requires a thorough knowledge and understanding of ecosystem processes. The distribution of these estuaries across broad geographic locations means that they exhibit a range of responses to physical, chemical and biological perturbations.
6. **Daily Response Patterns of Phytoplankton Chlorophyll a Concentrations to Physico-Chemical Changes in the Van Stadens Estuary**

6.1 **Introduction**

Permanently open estuaries (POEs) are generally characterised by river flow that continuously export material to the marine environment and conversely by the intrusion of marine water that floods upstream bringing with it saline water. POEs exhibit a longitudinal salinity gradient with higher salinity near the mouth and lower salinity in the upper reaches. In contrast temporarily open/closed estuaries (TOCEs) show a wide range of salinity conditions that include almost freshwater conditions on one extreme and hypersalinity on the opposite (Allanson and Baird 1999, Whitfield 2000). TOCEs are cut off from the sea for variable lengths of time and only connect during periods of high river discharge by breaching the sand bar at the mouth. During periods when there is a connection with the sea TOCEs function similarly to POEs. However, depending on the magnitude of river flow that maintains the mouth open versus marine sediment transport from long-shore currents that close it, the estuary can experience a wide range of flow, salinity, and nutrient concentrations (Perissinotto et al. 2003, Gobler et al. 2005, Thomas et al. 2006, Snow and Adams 2007). As a consequence of isolation from the sea TOCEs display a diverse range of ecosystem habitats that are inhabited by an assemblage of microflora adapted to different water quality conditions. The upper reaches of estuaries that experience a continuous supply of freshwater tend to support microalgal communities that are similar to those encountered in freshwater environments (Barron et al. 2002), whereas marine-like communities are found occurring near the mouth as these are closely associated with and are influenced by assemblages from the neritic (Seoane et al. 2006).

The frequency and intensity of river flow and saline water intrusion into estuaries depend on the hydrological and geographical features of the particular system. These can include 1) the regularity and magnitude of river discharge, 2) tidal flow related to the regular rise and fall of the tide, and 3) tidal flow associated with storm surges linked with wind speed and direction on upstream movement of sea water (Kristiansen 1998, Malone et al. 1988, Muylaert and Vyverman 2006). It is well established that salinity has a detrimental effect on organisms inhabiting inland saline and coastal environments (Kakinuma et al. 2006, Velasco et al. 2006). It has also be shown that organisms exposed to varying levels of salinity (e.g. low 0.5–5.0 and high 30–35 PSU) exhibit the greatest change in species composition when subjected to fluctuations in freshwater intrusion (Flöder and Burns 2004, Gasinūaitė et al. 2005). Freshwater and marine planktonic organisms that are exposed to these variable conditions undergo severe osmotic stresses (Kies 1997), and as a result show a decline in biomass, diversity and number of species (Flöder and Burns 2004). Studies have demonstrated a decline in phytoplankton densities in populations occurring in either oligohaline or polyhaline waters when subjected to the introduction of increased freshwater by altering the physico-chemical conditions.
in the water column (Kristiansen 1998, and Flöder and Burns 2004). Phytoplankton communities inhabiting these environments tend to be more susceptible or less tolerant to sudden fluctuations in salinity compared with those inhabiting mesohaline habitats (Barron et al. 2002). A rapid change in salinity negatively affects a number of species such that their recovery is inhibited or greatly delayed even when previous water column physico-chemical conditions are re-established. Although adapted species can quickly take their place because of fast growth rates shown by some of these species (Tomas 1997).

Although a number of studies have demonstrated the effects of varying salinity on phytoplankton populations their conclusions have been diverse (Flöder and Burns 2004, Pilkaitytė et al. 2004, Gasinūaitė et al. 2005). Observations of phytoplankton communities under varying salinity have mostly been from experimental studies monitored over a range of temporal scales in POEs (Berges et al. 2002, May et al. 2003, Flöder and Burns 2004, Gómez et al. 2004, Pilkaitytė et al. 2004, Gasinūaitė et al. 2005). Apart from the current study to date there is only one other TOCE study that has investigated daily phytoplankton response patterns to increased river inflow (i.e. used here as a surrogate to approximate varying salinity) (Gama 2007). There is a paucity of information on phytoplankton response patterns to varying salinity conditions in temporarily open/closed estuaries. The episodic nature of discharge associated rainfall that characterises river inflow in temporarily open/closed estuaries offers an opportunity to examine in situ the effects of a range of salinity and river inflow on natural phytoplankton populations over a short-term period. In late spring of 2001 three different conditions of the Van Stadens Estuary were captured over a six-day period that permitted investigation of the effects of varying physico-chemical changes on phytoplankton biomass. A number of studies have shown how water column microalgal size structure is important in determining the energy pathway in the transfer of carbon up the trophic level (Caroppo 2000, Cermeño et al. 2006). Recent studies in regional and international TOCEs have shown that increased river inflow support small sized phytoplankton even though nutrient supply is increased (Perissinotto et al. 2000, Gobler et al. 2005). Other studies have recorded an increase in microphytoplankton following an increased influx of freshwater (Froneman 2002a). The increased supply of nutrients following high river inflow has the potential to stimulate phytoplankton biomass, yet high discharge can also dilute the estuarine water as well as remove phytoplankton cells by flushing them out to sea (Kristiansen 1998, Gameiro et al. 2004).

Variation in salinity in this study was used as a surrogate of the amount of freshwater flow received in the Van Stadens as this estuary is influenced by high river runoff following extended periods of low to no river inflow and by salt water penetration after the mouth has been breached. Depending on the magnitude of river inflow varying levels of salinity are experienced within the estuary which allows for the examination of different salinity and river inflow on phytoplankton (Flöder and Burns 2004,
Gómez et al. 2004). This study, therefore, investigated the short-term (daily) responses of phytoplankton chlorophyll a concentrations (biomass) to varying physico-chemical changes associated with increased river flow under different mouth conditions. The study set out to test the following hypotheses that: 1) increased freshwater flow and subsequent salt water intrusion would initially suppress Chl a biomass even though nutrients are high and salt water intrusion would keep Chl a biomass low even after the mouth closed, and 2) the nanophytoplankton would form the dominant size group throughout the different conditions of the mouth over the short-term period of observation.

6.2 Methods and Materials

Detailed methods and materials used in this study were described in Section 4, General Methods and Materials. The estuary was monitored over six days (28 November – 03 December 2001). The results reported cover all dates except the 02 December. Daily surveys of physico-chemical factors (temperature, light, conductivity, dissolved oxygen, salinity, pH, DIN – ammonium-NH₄⁺, nitrate-NO₃, nitrite-NO₂⁻, and DIP – total phosphorus-TP, soluble reactive phosphorus-SRP), and Chl a concentration as biomass were measured as described earlier however, phytoplankton community composition was not assessed. The daily monitoring afforded the opportunity to track changes in estuarine water level including the condition of the mouth. During this study three mouth conditions were observed (i.e. open, closed and closed/overwash). The open-mouth condition lasted about two days while the closed-mouth persisted for approximately four days accompanied by an overwash event. The stratification coefficient (S) was estimated using the following equation:

\[ S \ (m^{-1}) = \frac{(S_s - S_b)}{D} \]

where \( S_s \) and \( S_b \) are surface and bottom salinity and \( D \) is the depth of the water column taken from Sharp et al. (1986).

6.3 Results

6.3.1 Physico-chemical Parameters

The combination of rainfall and an almost full estuary in November 2001 was sufficient to breach the estuary mouth (Fig. 6.2). The mouth breached four days prior to the commencement of monitoring on the 28th November. A list of physico-chemical parameters measured during the study is listed in Table 6.1. Although variable, salinity between surface and bottom layers of the water column remained low (< 1.5 psu) throughout the estuary during the open mouth condition. The mouth closed three days later due to strong onshore storm surges that closed off the mouth. The storm surge introduced saline water raising salinity to the highest level of 19 psu by Day-6 when the survey period concluded (Fig. 6.1). Following mouth closure there was a significant halocline that was evident along the axial length of the estuary and this persisted over the next couple of days and became more pronounced near the
mouth ($p < 0.001$). The lower reaches displayed strong stratification ($S = 10.5 \text{ m}^{1}$) compared to when the mouth was open ($S = 0.6 \text{ m}^{1}$) four days earlier. There were no significant daily salinity differences when the mouth was in an open condition, however, salinity was significantly higher during the closed-mouth phase compared to the open-mouth phase (one-way ANOVA, $p < 0.05$). There are no flow gauges on the river or at the head of the estuary which precluded monitoring of discharge, attempt were made to estimate flow using an orange which is allowed to float over a known distance (Gordon et al. 1992). This method had to be discarded because of strong winds that impeded the direction of the orange on the surface of the water.

When the mouth was open water column turbidity increased reducing light penetration in the upper reaches. Attenuation coefficients remained high despite the fact that the water column depth had decreased by approximately 50%. A linear regression showed that there was a strong correlation between light attenuation and total Chl a concentration ($R^2 = 0.46$, $y = -1.686x + 15.499$, $n = 5$)

### Table 6.1

Mean values (±1 S.E.) of light attenuation $K_d$ (m$^1$), temperature ($^\circ$C), salinity (psu), and dissolved oxygen (mg L$^{-1}$) measured from the surface (top- 0.0 - 1.0 m) and at depth (bottom- 1.0 - 2.0 m) at five sites over six days during an open (28 - 30$^{th}$ November) and closed (01 - 03$^{rd}$ December 2001) mouth phase. * Depth integrated data (0.0 – 1.0 m). ** No data collected.

<table>
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Phytoplankton Chlorophyll a Concentration and Community Structure
suggesting a possible link with increased water column turbidity when the mouth was open. Light attenuation declined to low levels (mean 1.25 m$^{-1}$ ±0.64 S.E.) following the introduction of clear marine water from overwash after the mouth closed, particularly in the lower reaches of the estuary. Longitudinal and vertical water column temperatures remained uniform throughout the study although bottom layers experienced a slight drop in temperature. Dissolved oxygen concentrations displayed a different response with the surface layers exhibiting near 80% saturation levels, which was in contrast with those measured from the bottom layers at < 30% saturation on the third-day of the study during the open-mouth condition. After the mouth closed oxygen concentrations at depth improved to levels approaching >50% saturation particularly in the middle reaches.

![Figure 6.1](image-url)  

**Figure 6.1** Mean salinity (±1 S.E.) for the Van Stadens Estuary taken over six days. Horizontal line denotes days the mouth remained in an open or a closed mouth condition. *No data sampled.*
Water column conductivity levels, not shown here, closely tracked that of the salinity profile during both open and closed mouth conditions for both surface and bottom layers. pH measurements ranged between 8.3 – 8.8 across the vertical profile of the estuary during the open mouth condition. Data was not collected during the closed mouth phase as a result of instrument malfunction. River inflow during the open-mouth condition introduced considerable amounts of dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) macronutrient concentrations into the estuary (Figs. 6.3 & 6.4). A two-way ANOVA showed no significant differences between TP concentrations in surface and bottom water column layers ($p > 0.065$), however significant differences were detected between concentrations on Day-1 and those measured on Day-6 ($p < 0.05$) (Fig. 6.3a & b). Vertical SRP concentrations were markedly lower than TP concentrations in the first three days and became comparable toward the last few days of the study. Highest TP concentrations over the duration of the study (mean 1.30 µg L$^{-1}$ ±0.2 S.E.) were measured at Station 3 in the bottom layers of the water column on Day-4 following closure of the estuary mouth. Phytoplankton $Chl\ a$ concentrations in the bottom layers of the water column showed a strong negative correlation after a linear regression analysis with TP concentrations by the end of the study ($R^2 = 0.489$, $n = 5$, $y = -29.314x + 26.478$).

In contrast, the highest nitrate concentrations (mean 1.70 µg L$^{-1}$ ±0.04 S.E.) were recorded in the upper reaches (e.g. Stations 4 & 5) throughout the water column and decreased toward the mouth. No clear differences were evident between surface and bottom layers of the water column. Nitrate

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**Figure 6.2** Mean monthly rainfall from April 2001 to March 2002 measured at the Port Elizabeth Airport (South African Weather Bureau). Arrows indicate periods the Van Stadens mouth breached.
concentrations declined during the closed mouth condition with the lowest concentrations measured on Day-6 of the study (Fig. 6.4a & b). Dissolved ammonium concentrations both in the surface and bottom layers of the water column were significantly higher than nitrate levels ($p < 0.001$) and remained uniform during the five days then dropped by Day-6 to levels below 0.5 (µg L$^{-1}$). During the open mouth phase the highest ammonium concentrations occurred from the middle to the lower reaches of the estuary, particularly in the bottom layers of the water column. A linear regression analysis showed a negative correlation between phytoplankton Chl a concentrations and bottom layer DIN concentrations over six-days ($R^2 = 0.321$, $n = 5$, $y = -4.347x + 22.13$).

![Bar charts showing phosphorus concentrations in the top and bottom layers of the water column over six days.](image)

**Figure 6.3** A) Surface and lower (b) total phosphate – TP and soluble reactive phosphorus – SRP concentrations (± 1 S.E.) taken over the 6-Day study period in the Van Stadens Estuary. The horizontal line denotes the days the mouth was open and closed. *No data sampled.
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Figure 6.4  A) Surface and B) lower nitrate – NO$_3^-$ and ammonium – NH$_4^+$ concentrations (± 1 S.E.) taken over the 6-Day study period in the Van Stadens Estuary. The horizontal line denotes the days the mouth was open and closed. *No data sampled.

6.3.2 Phytoplankton Chlorophyll a Concentration
Chlorophyll a concentrations displayed high spatial and temporal variability. Total phytoplankton Chl a concentrations ranged from 2.8 – 20.5 µg L$^{-1}$ in the surface and bottom waters respectively over the duration of the study. Following a two-way ANOVA test there were no significant differences detected between surface and bottom Chl a concentrations ($p > 0.89$) although Chl a levels in the bottom layers appeared to be slightly higher (Fig. 6.5). During the open mouth phase Chl a concentrations peaked at 13.5 µg L$^{-1}$ (± 4.5 S.E.) then declined sharply to a low of 2.8 µg L$^{-1}$ (± 0.5
Phytoplankton Chlorophyll a Concentration and Community Structure

S.E.) on Day-2. Concentrations gradually increased following the closure of the mouth reaching a peak of 14.6 µg L⁻¹ (± 3.1 S.E.) on Day-4. Mean Chl a concentrations measured during the open mouth condition were significantly different to Chl a concentrations measured during the closed mouth phase (paired t-test, p < 0.05) suggesting a strong influence from increased river flow. On Day-1 during the open-mouth condition the microphytoplankton were the dominant size group in the bottom waters contributing >61%, yet in the surface layer the nanophytoplankton comprised over 50% of the Chl a pigment concentration. In the following days in the bottom layers of the water column the microphytoplankton was gradually replaced by the nanophytoplankton while the reverse occurred in the surface layer. Just prior to the mouth closing the nanophytoplankton was the dominant fraction throughout the water column contributing more than 54% to total Chl a concentrations. Following mouth closure the microphytoplankton became the dominant size-fraction for the duration of the study. The picophytoplankton displayed the greatest contribution by Day-6 when water column macronutrient concentrations were at a minimum and contributed in excess of 29% to total Chl a concentrations. Phytoplankton Chl a concentration showed a positive correlation with increased bottom water salinity ($R^2 = 0.439$, n = 5, $y = 0.3725x + 6.7875$) suggesting that water in the bottom layers was probably of marine origin.
Phytoplankton Chlorophyll a Concentration and Community Structure

Figure 6.5 Chlorophyll a concentrations – Chl a (±1 S.E.) (a), and size-fractionated Chl a concentrations from surface (b) and bottom (c) waters measured over 6-days in the Van Stadens Estuary. Microphytoplankton – micro (>20 µm), nanophytoplankton – nano (2.7 – 20 µm), picophytoplankton – pico (1.2 – 2.7 µm). Horizontal line denotes period the mouth was open and closed. *No data sampled.
6.4 Discussion

One of the hypotheses this study set out to test was that increased freshwater flow and subsequent salt water intrusion would suppress Chl $a$ biomass keeping it low even after the mouth closed. From the time the study began during an open-mouth condition vertical and horizontal salinity remained low indicative of freshwater inflow. During this period Chl $a$ concentrations were initially high but then declined to their lowest concentrations. According to the daily mouth records the mouth had breached four days prior to the commencement of the study therefore the peak in Chl $a$ on Day-1 was possibly a response to the earlier flushing of upstream riverine microalgae. Although of species assemblages were not verified, previous studies have shown an increase in microalgae dislodged upstream and transported downstream following flooding (Chan and Hamilton 2001). The subsequent drop observed on Day-2, may reflect the base Chl $a$ concentrations as a result of the physical and chemical destabilisation of the water column from freshwater flow coupled with some bottom salt water penetration upstream (Kristiansen 1998, Gómez et al. 2004). Soluble reactive phosphorus and ammonium concentrations remained unchanged throughout the two layers of the water column and appeared not to be affected by either conditions of the mouth, whereas TP concentrations showed a slight increase, particularly at depth, until the reduction in flow and subsequent closure of the mouth then dropped to low values by Day-6. This suggests that 1) the vertical and horizontal spatial distribution of P and NH$_4^+$ was generally uniform and thus was available for uptake by the phytoplankton, 2) that P was not limiting and the high TP values were a result of increased organic particulate matter settling from the surface layer. In addition, N:P ratios indicated that nitrogen was always the limiting macronutrient throughout the study even though nitrate and ammonium concentrations were high during the open-mouth condition. The closure of the mouth resulted in a drop in NO$_3^-$ and NH$_4^+$ concentrations by the end of the study suggesting a rapid removal from the water column as river supplies became depleted since DIN nutrient concentrations were negatively correlated with Chl $a$ biomass.

The open-mouth phase increased turbidity especially by Day-2. This suppressed light penetration at depth and increased river flow may have contributed to the low phytoplankton Chl $a$ concentrations, since light attenuation was positively correlated with increased turbidity, although other studies have discounted turbidity as a factor limiting phytoplankton production in TOCEs. This suggests that nutrients are possibly the main factor affecting phytoplankton biomass production (Nozais et al. 2001). In a study in Mecox Bay dilution or export out of the estuary was considered a possible factor in the initial reduction of Chl $a$ concentrations when the mouth was open (Gobler et al. 2005). This may have been the case here, however, river flow had been reduced to levels that could not sustain an open mouth condition. In this study however, during the open-mouth condition macronutrient
concentrations remained high while light penetration was considerably reduced and therefore, may have affected phytoplankton biomass production (Cloern 1987, May et al. 2003). An increase in light penetration following the closure of the mouth was associated with a gain in Chl $a$ concentration by Day-4. This increase in Chl $a$ concentration took place under a highly stratified water column condition as surface temperatures and stratification coefficient values were at maximum during this same period. Studies in estuarine and coastal environments have suggested that phytoplankton biomass production might be severely limited under stratified conditions as they are unable to access nutrients trapped below the pycnocline (Malone et al. 1988, Flöder and Burns 2004). In this study however, macronutrient levels were relatively high, especially NH$_4^+$ concentrations, even though NO$_3^-$ and NH$_4^+$ concentrations had declined considerably by the end of the study. This indicates that although strong stratified conditions persisted during this period the vertical distribution of macronutrients was sufficiently adequate to support phytoplankton development in the surface and bottom layers benefiting from a deep photic depth.

River inflow caused breaching and introduced significant quantities of macronutrients (DIP & DIN), which remained high until several days after the mouth was closed. Although these macronutrients were replete throughout the water column it appeared that they were not readily taken up by the microalgae in the short-term as indicated by the low Chl $a$ concentrations. An increase in the penetration of seawater upstream was evident on Day-3 as salinity stratification intensified, particularly in the middle to lower reaches. During this period river flow subsided, which allowed greater penetration of seawater upstream further producing strong spatial (vertical and axial) salinity differences. The intrusion of saline water upstream did not appear to influence phytoplankton Chl $a$ levels or their spatial distribution patterns in the water column especially when two distinct layers of fresh water at the surface and seawater in the bottom became established. In contrast, the highest peaks in Chl $a$ concentrations were displayed during the maximum period of stratification. River flow on the other hand had a strong temporal effect on phytoplankton distribution by decreasing and displacing Chl $a$ concentrations when flow was high during the earlier days of the study followed by a marked recovery when river flow receded by the end of the study.

The second hypothesis tested was whether nanophytoplankton would form the dominant size group during the different conditions of the mouth over a short-time scale of observation. On Day-1 when river flow was high and the mouth was open nanophytoplankton were the dominant group in surface waters, whereas in the bottom waters the microphytoplankton was dominant. Size structure shifted as the nanophytoplankton formed the dominant size-fraction both in the surface and bottom layers of the water column on Day-2 contributing 49 and 46%, while on Day-3 they contributed as much as 66 and 42% respectively to total Chl $a$, although the overall Chl $a$ concentrations remained very low possibly from flushing. During increased river flow larger sized particles were possibly flushed out to sea...
including phytoplankton cells particularly microphytoplankton and large-sized zooplankton. The nanophytoplankton size group, however, fared better during this period as it made up the major fraction of the Chl a pigment concentrations. The success of this small-sized phytoplankton group can be attributed to their size (e.g. < 20µm and greater surface area to volume ratio) and physiology (rapid uptake of nutrients and efficient metabolic processing) in the use of inorganic material (Caroppo 2000, Veldhuis et al. 2005). This physiological attribute can possibly explain their rapid gain in Chl a biomass coupled with a decline in competition from large-sized phytoplankton for similar resources and a possible relaxation from the grazing pressure by microzooplankton (Sommer et al. 2000, Froneman 2004a & 2004b).

The mouth closed on Day-3 due to sediment build-up assisted by strong onshore winds coupled with a reduction in river flow. The nanophytoplankton flourished and were the dominant group possibly exploiting the high macronutrient concentrations. They were subsequently replaced by larger-sized phytoplankton cells in the surface waters. The reduction in river flow and the closing of the mouth increased water level and the photic depth, which possibly allowed for longer residence time for microalgal growth resulting in an increase in the microphytoplankton Chl a concentrations both in the surface and bottom layers of the water column. This pattern continued until the end of the study. The shift in the phytoplankton size structure from nano- to microphytoplankton may be a result of 1) improved ability to remain in suspension by exploiting replete nutrient resources, 2) although normally associated with lower surface area to volume, the microphytoplankton, particularly dinoflagellates and cryptophytes, may have used other mechanisms including ectoenzyme activities (i.e. alkaline phosphatise hydrolysis of phosphate esters) for certain macronutrients (e.g. phosphates) (Burkholder 1992, Dyhrman 2005, Kamjunke et al. 2007), 2) high selective grazing pressure by the microzooplankton following the increase in nanophytoplankton as an abundant source of size specific prey items (Sommer et al. 2000, Froneman 2004a & 2004b).

From this study it was clear that changes in the macronutrients had a significant influence on the phytoplankton Chl a biomass. However, the consideration of whole community Chl a biomass was not sufficient to explain the response patterns of the phytoplankton community. The data showed no clear spatial (vertical and axial) or temporal distribution patterns of total Chl a biomass under different salinity ranging from oligohaline to mesohaline conditions (e.g. open & closed mouth conditions respectively). This can be ascribed to the even distribution of nutrients in the water column. Important relationships emerged, however when the phytoplankton community was separated into various size fractions that corresponded to particle sizes that the grazing planktonic community would generally prey on, which form the main pathways of carbon energy transfer to higher trophic levels. Hydrodynamic forcing and variation in salinity did influence phytoplankton community size structure by promoting nanophytoplankton growth under increased river flow while supporting
microphytoplankton during low flow conditions. This was somewhat contrary to regional studies and elsewhere that have suggested the opposite (Perissinotto et al. 2000, Froneman 2002a & b, Gobler et al. 2005). Consistent with studies in the uMdloti and uMhlanga estuaries (Perissinotto et al. 2003), Thomas et al. 2005) in this study nanophytoplankton were the dominant group when the mouth was open but when the mouth was closed microphytoplankton became the dominant group. This was not in agreement with their findings of nanophytoplankton being the dominant group under closed mouth conditions. The prevalence by nanophytoplankton when the mouth was open was inconsistent with studies from the Kasouga Estuary that found the microphytoplankton as the dominant group (Froneman 2002a & b). It should be noted however, that the frequency and duration of monitoring in these studies spans different time scales to that undertaken in this study and therefore responses may differ. The estuaries identified in the reports mentioned have been characterised as generally oligotrophic and hence are comprised of low phytoplankton Chl a biomass. Furthermore, in these systems the phytoplankton community size structure is also characterised by the dominance of small-sized phytoplankton during low nutrient conditions and large-sized phytoplankton when nutrient supply is high.

Consistent with these observations is that the Van Stadens Estuary has low nutrient and Chl a concentrations and largely depends on external nutrient input mainly from the river. What is inconsistent, however, is that during increased river inflow and high nutrient concentrations the nanophytoplankton was the dominant size group and when flow was reduced and the nutrient levels low the microphytoplankton formed the major fraction in the water column. The discrepancy can possibly be attributed to the fact that in this study observations were conducted on a daily basis, which allowed for close tracking of changes of physical and chemical parameters. In the studies referred to earlier measurements were typically made over longer time scales ranging from months to annual seasonal cycles which possibly missed the accurate response patterns by monitoring at such long temporal scales (Perissinotto et al. 2000, 2003, Froneman 2002a & b, Gobler et al. 2005). Alternative explanations in my results include 1) that the hydrodynamic force of flushing and co-floculation of organic and inorganic suspended sediment material may have eliminated larger-sized particles including the microphytoplankton (Avnimelech et al. 1982, Burkholder 1992) from the water column during high river flow favouring the nanophytoplankton, and 2) the environmental conditions in the water column had not yet reached a settling phase whereby the physical and chemical parameters would be at steady state, therefore they still reflected a very perturbed and dynamic water column although a strong halocline existed yet other physico-chemical parameters displayed homogeneity.

Comparing results from this short-term study with that collected over longer time scales (e.g. seasonal and annual) there were distinct differences and similarities in the Chl a response to increased river inflow in the Van Stadens Estuary. Strong river flow resulted in an initial reduction in Chl a
concentrations. Phytoplankton Chl a recovery appeared to take place over longer periods (e.g. 8-10 weeks) before peak concentrations were reached. In contrast, peak Chl a response took place over a couple of days. Although these responses appear dissimilar they may illustrate similar physical and chemical mechanisms essential in supporting phytoplankton biomass. The amount of time the mouth remains open may be critical in establishing and maintaining a phytoplankton community. In this study the mouth remained open for a few days then closed. When the mouth was open for extended periods peak Chl a concentrations were delayed.

In conclusion, notwithstanding what has been just stated it is quite apparent that short-term investigations in TOCEs are very critical in that they allow for the capturing of accurate events of the natural aquatic environment that would otherwise be missed. The implications of such findings are significant to researchers and conservation managers with regard to the influence of rapid changes in physical and chemical factors and their effect on the microflora. What this study demonstrates is that short-term exposure to variable river influx rapidly reduces salinity and increases nutrients that stimulate phytoplankton biomass only after the mouth has closed. In addition, it shows that nutrients were rapidly depleted possibly from being taken up by the phytoplankton and that if residual supplies of nutrients exceeded uptake rates this might favour conditions that would lead to eutrophication (Flemer and Champ 2006). Changes in mouth condition are one of the important physical features in TOCEs and breaching whether natural or artificial have a profound influence on the phytoplankton. Natural breaching, lowers estuarine water volume, increases the euphotic depth, brings in nutrients that stimulate phytoplankton production and lowers salinity. On the other hand artificial breaching would also lower estuarine volume, but would introduce saline water without much dilution from river water. Higher salinity in the estuary would alter the phytoplankton species diversity favouring that tolerant of high salinity at the expense of brackish species (Flöder and Burns 2004).
7. Seasonal Responses of Size-Fractionated Chlorophyll $a$ and Phytoplankton Assemblages to Freshwater Flow in the Maitland Estuary

7.1 Introduction

Physical and chemical characteristics influencing microalgal spatial and temporal distribution patterns in a temporarily open/closed estuary (TOCE) were identified. Microalgal growth in marine and permanently open estuaries has been attributed to a number of factors including the quality and quantity of light (Falkowski 1981, Cloern 1987), macronutrient availability (Spatharis et al. 2007), advection, mixing and residence time (Eldridge and Sieracki 1993, Chan and Hamilton 2001), competition (Flöder and Burns 2004) and grazing pressure (Sommer et al. 2000, Jochem 2003). In TOCEs (Perissinotto et al. 2000, Froneman 2004a) or bar-built estuaries (Gobler et al. 2005, Santangelo et al. 2007) similar physical and biological properties have been suggested, however our understanding of the magnitude, timing and responses to periodic changes in small, shallow estuaries is still poor.

In order to improve our knowledge of the influences of river flow on phytoplankton in temporarily open/closed estuaries (TOCEs) the Maitland Estuary was monitored for three years. Its catchment is adjacent to the Van Stadens Estuary and this study served as a comparison to that of the Van Stadens to determine if physical, chemical, and biological responses were similar. Selection of the Maitland Estuary was based on the following reasons, 1) its proximity to the Van Stadens Estuary because of a shared geological history and climate conditions, 2) similarity in catchment size (i.e. < 100 km$^2$), vegetation cover and land use patterns, 3) accessibility, which would allow carrying out simultaneous monitoring of both estuaries for the purpose of comparison.

The aim of this study was to capture broad spatio-temporal patterns of the effects of freshwater flow on microalgal biomass ($Chl\ a$) and community structure. This was achieved by conducting a similar monitoring regime concurrently with that carried out in the Van Stadens Estuary. Monthly measurements of physical, chemical and biological variables were carried out in order to test similar hypotheses as those posed for the Van Stadens Estuary. The following hypotheses were tested 1) nano- and microphytoplankton size fractions (2.7 – 20 μm & >20 μm in size along major axis respectively) form the dominant phytoplankton that drive primary production during periods of increased river inflow, 2) 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, 3) prolonged periods between breaching events (closed-mouth phase) favour increased pico- and nanophytoplankton biomass and production, 4) during periods of mouth breaching microphytoplankton production will decrease beyond levels sufficient to support macro-zooplankton.
biomass and production, 5) increased nutrient supply associated with increased river inflow support high concentrations of nano- and microphytoplankton biomass and production by shifting phytoplankton community structure from pico- and nanophytoplankton to larger (>20 µm) sized microphytoplankton, 6) dinoflagellates and large-sized flagellated algae 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 improved water clarity.

Hypotheses that will be covered in this discussion pertain to those regarding size-fractionated phytoplankton Chl a biomass (e.g. 3, 5 & 6) and those dealing with primary production (1, 2, & 4) will be discussed in Chapter 9, which deals specifically with primary production.

7.2 Methods and Materials

Methods and materials used to address the specific components of this study are described in Section 4, General Methods and Materials. Groundwater, river to sea measurements and daily estuarine monitoring was not carried out at the Maitland Estuary. Monthly and quarterly surveys of physicochemical factors (temperature, light, conductivity, dissolved oxygen, salinity, pH, DIN – ammonium, nitrate, nitrite, and DIP – total phosphorus, soluble reactive phosphorus), and biological factors (phytoplankton and microphytobenthic Chl a concentration as biomass including phytoplankton community composition) were measured.

7.3 Results

7.3.1 Physical and Chemical Parameters

Mouth condition at the Maitland Estuary was not monitored on a daily basis, however the monthly record of sampling surveys provides a good 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 flowed out without interacting with sea in a tidal fashion as the estuary was perched above sea level. The Maitland Estuary experienced three open mouth phases (i.e. period of time the mouth was in an open state for ≥ 2-months) and one open mouth condition lasting for less than 2-months. The first-open-mouth-phase (FOMP) began in April – June 2001 and the second-open-mouth-phase (SOMP) lasted from September – November 2001, and third-open-mouth-phase (TOMP) was from July – November 2002. Heavy floods during August 2001 and September 2002 completely altered the geomorphology of the whole estuary. Discharges recorded at the mouth of the Maitland Estuary subsequent to the floods peaked at approximately 8.0 m³s⁻¹. The estuary experienced significant scouring and sediment deposition with the complete removal of submersed, emergent and riparian vegetation. Extensive sand deposition raised the average height of the estuary
above mean sea level (MSL), thus increasing semi-closed mouth conditions. In addition, water depth in the estuary was considerably reduced from a maximum of 2.05 m at the deepest point of the estuary during the first year to 0.5 m by the end of the study in the third year.

Over the 3-yr study salinity ranged from 0 psu in the upper reaches to 16 psu in the lower reaches. In general salinity remained low never reaching above 5 psu except when there were episodes of marine overwash that introduced saline water into the estuary (Fig. 7.1a). During events of overwash substantial volumes of seawater were introduced into the estuary that brought in clear saline water, which resulted in improved light quality at depth and elevated salinity levels. These events were however, short-lived as they rapidly dissipated over short-time periods (e.g. days to weeks). After the breaching related floods in August 2001 average water column salinity changed significantly since levels never exceeded 2 psu. Throughout the study increased salinity (mean 10 ± 2 psu) occurred only on five occasions but this was short-lived lasting only a few weeks. In addition, these marine overwash episodes were generally associated with either onshore wind-driven storm surges or strong spring flood tides. Despite these events and the mouth remaining closed for an extended period of time (e.g. > 6 months) salinity levels in the estuary stayed low ranging between 0 and 4 psu. The highest salinity readings were recorded in April 2004 following sustained onshore southwesterly winds that introduced large volumes of marine water that pushed right up to the head of the estuary.

Water-column temperatures closely tracked seasonal temperatures with a maximum of 25°C during the summer periods and a minimum of 12°C in winter. The change in water depth post September 2002 floods influenced average water column temperatures, as they were generally higher following the flood event compared to the same period for the previous year (Fig. 7.1b). Summer water-column temperatures in 2002 and 2003 were significantly higher post the winter flood event ($F_{17, 2.22} = 181.547, p<0.001$). 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 Appendix 1, Plate 2 A - D). Chlorophyte densities were weakly correlated with warm water column temperatures in spring and summer of 2001 ($R^2 = 0.19, n = 32, y = 0.0069x + 17.683$). The euphotic depth improved considerably with the reduced water column depth. As a result irradiance levels were significantly higher when compared with readings taken prior to the floods (ANOVA, $F_{3, 0.168} = 54.486, p<0.001$). In the first year (2001) of the study dissolved oxygen (DO) concentrations were above saturation levels, but by the following year (2002) had significantly decreased resulting in anoxia at depth in the upper reaches (ANOVA on ranks, $Q = 6.599, p<0.05$). Subsequent to the floods water levels dropped and oxygen concentrations increased often approaching super saturation particularly when mats of filamentous green algae formed on the bottom of the estuary in the upper reaches.
Figure 7.1  Maitland Estuary A) light attenuation coefficient and mean monthly rainfall, B) salinity and water temperature, and C) conductivity and dissolved oxygen for the period April 2001 to 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. Vertical lines indicate ±1 S.E. * denotes no data.
River discharge into the Maitland Estuary followed a seasonal rainfall cycle that breached the estuary mouth in the spring of each year over the 3-year study. High macronutrient input into the estuary was correlated with increased river inflow ($R^2 = 0.36$). Nutrient input during September 2001 and 2002 was significantly related to increased river flow ($F_{6, 0.0867} = 39.155, P <0.001$). Although the mouth breached on four occasions the highest DIP concentrations occurred on two occasions during the peak flood of 2002 and in September 2003 (maximum $4.33 \pm 0.06$ and $2.76 \pm 0.25 \mu g \ L^{-1}$) respectively. In the summer and autumn seasons of the first year total phosphate concentrations were variable and stayed above $1.0 \mu g \ L^{-1}$ but in 2002 and 2003, particularly in the mid to upper reaches, concentrations remained well below $0.5 \pm 0.02 \mu g \ L^{-1}$. DIN concentrations displayed similar patterns with nitrate concentrations contributing the majority of the nitrogen during the flood periods ($p <0.05$). In contrast, ammonium concentrations contributed more to the nitrogen pool during low flow periods (Fig. 7.2b).

In March 2002 ammonium-nitrogen concentrations peaked at $11 \mu g \ L^{-1}$ the highest recorded throughout the study period (Fig. 7.2b). These high levels were associated with low dissolved oxygen concentrations and with extensive blue-green algal mats that covered the bottom sediments in the upper reaches of the estuary. These conditions persisted during the summer and into the winter months until scoured out by the floods in August 2002. The low nitrogen concentrations and higher phosphate levels throughout the study meant that DIN: DIP ratios were N limiting (Fig. 7.3). Exceptionally high ammonium concentrations in late summer 2001 contributed significantly to the nitrogen pool and the system became phosphate limited. In a few cases macronutrients were below detectable limits. After the 2002 floods ammonium concentrations never attained the levels observed during the summer earlier that year.

By comparison with the previous year macronutrient levels during the summer months were much lower following the floods suggesting that nutrient retention was reduced perhaps as a result of diminished river inflow coupled with export from the estuary as continuous outflow occurred although at extremely low discharge rates (i.e. a semi-closed state). Dissolved silicon (DSi) levels in the estuary were always an order of magnitude higher than other monitored macronutrients. Periods of overwash coupled with low river inflows were associated with an increase in DSi concentrations, particularly in the third-closed-mouth-phase (TCMP - November 2003 – April 2004) of the study, when the mouth stayed in the semi-closed state for over 10 months. Higher DSi levels were recorded during this period compared to the similar period of the previous year ($p < 0.001$).
Figure 7.2  Maitland Estuary macronutrient 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 is described in Figure 7.1. Vertical lines indicate ±1 S.E. A # denotes values below detectable level, * no data.
7.3.2 Phyttoplankton and Microphytobenthic Chlorophyll a and Community Structure

There were no differences detected among sites and between depths, so chl-a data were pooled and are reported here as means integrated across sites and depth (Table 7.1). Over the three years of the study phytoplankton Chl a concentrations ranged from 5.3 – 138 µg L\(^{-1}\) and did not follow any seasonal pattern (\(p > 0.60\)) (Fig. 7.4). Although Chl a concentrations did show increases after mouth breaching events, the temporal distribution pattern was generally variable displaying peaks often during low flow periods. The total phytoplankton Chl a concentrations measured during this study were reflective of some of the highest levels measured in a temporarily open/closed estuary generally considered to be oligotrophic. DIN and DIP concentrations ranged between 0 – 11.5 and 0 – 4.5 µg L\(^{-1}\) respectively, indicating a greater supply of phosphorus than nitrogen. The generally higher Chl a concentrations were indicative of the unusually higher phosphate levels although there were no strong correlations between Chl a and macronutrients (DIN & DIP).

In the early part of the study the Maitland Estuary supported low phytoplankton Chl a concentrations, however after the breaching event in September 2001 Chl a levels reached the highest concentrations measured over the three-year period (Fig. 7.4b). During the FOMP (May – June 2001) the microphytoplankton contributed over 40% to total phytoplankton Chl a biomass from samples normally associated with the deeper layers of the water column. When the mouth closed in July the
picophytoplankton succeeded and became the dominant fraction, particularly in the surface layers. Mouth breaching in August drained the estuary dropping the level of the water in the estuary resulting in a very shallow water column (mean < 0.5 m). The depth of the estuary stayed in this manner until April 2004 after the study was concluded. Throughout this period the prevalence of one specific pigment size fraction varied with both the nano- and microphytoplankton equally represented. However, during the open-mouth-phases the microphytoplankton was the dominant size group (~ 71% Chl a) even though at times the response in Chl a concentration lagged for a few weeks before reaching peak levels. On the other hand when the mouth was closed or during periods of marine overwash the nanophytoplankton was the major contributor (> 56% Chl a) to total Chl a biomass. Regardless of the mouth being open during the FOMP the picophytoplankton was co-dominant with the microphytoplankton and reached maximum concentrations in July following mouth closure.

Table 7.1 Two-way ANOVA results showing no response in phytoplankton chl-a to site or depth.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site No.</td>
<td>2</td>
<td>5.51</td>
<td>2.75</td>
<td>0.142</td>
<td>0.868</td>
</tr>
<tr>
<td>Depth</td>
<td>1</td>
<td>2.34</td>
<td>2.34</td>
<td>0.121</td>
<td>0.729</td>
</tr>
<tr>
<td>Site No. x Depth</td>
<td>2</td>
<td>44.61</td>
<td>22.31</td>
<td>1.151</td>
<td>0.320</td>
</tr>
<tr>
<td>Residual</td>
<td>111</td>
<td>2151.15</td>
<td>19.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>2203.50</td>
<td>18.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Microphytobenthic (MPB) Chl a remained low in the first year of the study however increased steadily in the second year then declined to low levels in the third year. Chlorophyll a maxima was reached in March 2003 when the mouth had been closed for over ten months (Fig. 7.5). Prior to the strong floods in September 2002 water level in the estuary had reached a maximum height of 2.0 m at the deepest point near the mouth. The middle to upper reaches were overgrown with submersed species of *Potamogeton pectinatus* and *Ruppia* spp. that considerably reduced PAR at depth (< 50 µmol m⁻² s⁻¹). In the upper sites mats of cyanophytes covered large areas of the sediment and in some areas completely covered submerged plants. It was not until after the flood phase that MPB Chl a levels peaked reaching over 8.0 µg·g⁻¹ sediment (90 mg Chl a m⁻²). Although the estuarine substrate...
was considerably scoured during the September 2002 floods, re-colonisation of the benthos by epipsammic microalgae was rapid such that by the beginning of the following year MPB Chl a had recovered significantly recording the highest levels over the study period ($p < 0.001$). Although the MPB Chl a remained 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 greatly improved.

The phytoplankton community structure was composed of six taxonomic groups. There were four seasonal periods with different taxa of phytoplankton reaching bloom cell densities (i.e. $> 10^5$ cells ml$^{-1}$). In the spring of the first year of the study cyanophytes (blue-greens) and chlorophytes were co-dominant comprising 49 and 43% of the phytoplankton assemblage respectively. In the summer months the chlorophytes succeeded as the major group with the blue-greens and cryptophytes forming the subsequent dominant taxa. In that period two small flagellated greens, *Nephroselmis minuta* and *Tetraselmis* sp. became the dominant genera particularly in the lower reaches of the estuary. Filamentous blue-greens, mainly *Anabaena* sp., *Oscillatoria* sp. and the coccoid *Chroococcus* sp. were the most common taxa encountered primarily from the middle to the upper reaches of the estuary during the warm summer months and these were associated with the low D.O. concentrations measured at those sites. A bloom of chrysophytes comprising two flagellates, *Ochromonas* sp., & *Chryochromulina* sp. and a diatom, *Cyclotella* in the spring of 2002 developed a few weeks after the second-open-mouth-phase (SOMP) and persisted for 6-8 weeks before it was replaced by a chlorophyte bloom in the summer of that year (Fig. 7.6). Although markedly lower in cell abundance compared to the first one a second bloom of chrysophytes occurred in the winter of 2003 following a

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*Figure 7.5*  Maitland Estuary microphytobenthic chlorophyll *a* (*Chl a*) concentrations from May 2001 – April 2004. Horizontal bar as described in Figure 7.1. Vertical lines indicate ±1 S.E. * no data.
mouth breaching event. This bloom was succeeded by a dinoflagellate one (> 103 x10^3 cells ml^-1) that was mainly made up of Gonyaulax sp. an alga not previously encountered in the estuary.

Diatoms, dinoflagellates and euglenoids did not comprise a significant proportion of the cell numbers in the first two years of the study however, euglenoids were present following the first breaching event in 2001 (Fig. 7.6). A second bloom of cyanobacteria in November of that year was observed and during that period greens and cryptophytes comprised the group of algae observed at all of the sites surveyed in 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 and cyanophytes (Fig. 7.7). Cell densities reached their lowest during the winter months, although mention should be made that no samples were taken in June and in August of that year.

Following log-transformed data of the phytoplankton counts a non-metric multidimensional scaling (MDS) plot was generated based on the presence/absence of the Maitland Estuary species data and represented on a two-dimensional plot using a Clarke and Warwick Statistical Package Primer (1994). This is a non-multivariate analysis that considered the presence or absence of phytoplankton species with one variable (e.g. mouth condition). The plot shows how some dates have clustered together indicating their similarity to one another (Fig. 7.8). A two-way nested ANOSIM analysis test was carried out to test for the differences in phytoplankton species composition between months (where months were used as surrogates of mouth condition) and then averaged across years. This revealed a test statistic with a value of R = 0.401 and p =0.002 indicating that approximately 40% of the variability could be explained by mouth condition (Fig. 7.9).
Figure 7.6  Maitland Estuary phytoplankton cell densities and community composition for the period May 2001 to December 2003. Chl – Chlorophyta, Eug – Euglenophyta, Dino – Dinophyta, Cyan – Cyanophyta, Cryp – Cryptophyta, Chry – Chrysophyta. Horizontal bar is described in Figure 7.1.
Phytoplankton Chlorophyll a Concentration and Community Structure

Figure 7.7  Phytoplankton percent contribution by taxa for the period May 2001 to December 2003 from the Maitland Estuary. Phyla enumerated included: Chloro–Chlorophyta, Eug–Euglenophyta, Dino–Dinophyta, Cyan–Cyanophyta, Crypt–Cryptophyta, Chry–Chrysophyta. Horizontal bar is as described in Figure 7.1. *Denotes no data collected.

Figure 7.8  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 sampled by year and seasonal period.
7.4 Discussion

7.4.1 Physical and Chemical Parameters

Water column stratification in the Maitland Estuary was limited to a few occasions that were either associated with increased river inflow and tidal flow once the mouth was open or following strong episodes of marine overwash. Unlike regional TOCEs (e.g. Nyara and Kasouga estuaries) that receive most of their freshwater input from river inflow (Perissinotto et al. 2000, Froneman 2002a) this estuary remained oligohaline over much of the study even when river inflow was low suggesting groundwater and sand dune seepage as a source of freshwater. The high attenuation coefficients during the first year were associated with shading at depth from increased growth of floating and emergent macrophytes (e.g. Ruppia spp., Phragmites australis and Potamogeton pectinatus) that exploited the calm and stable water column conditions, but subsequently declined from possibly self shading and increased growth of epiphytic blue-green algae. Decomposition of organic matter by bacteria on the sediment surface under aerobic conditions is important in the release of organic minerals back into the water column (Clavero et al. 1992, 2000). Some minerals (e.g. phosphates) however, remain unavailable unless conditions become anoxic. Under anaerobic conditions phosphates are readily soluble and become available in the overlying water (Libes 1992, Clavero et al. 2000). In the Maitland the reduction in dissolved oxygen from decomposition of plant matter perhaps led to hypoxia and anoxia at depth in some parts of the upper reaches, which may explain the high phosphate concentrations particularly during late summer months (Clavero et al. 2000). Release of phosphates from anoxic sediments into overlying surface waters has been shown (Blackburn and Henriksen 1983, Libes 1992) as these conditions maintain solubility of metal bound phosphates increasing their flux across the sediment – water interface (Wetzel 1983).

As with a number of TOCEs the introduction of nutrients through increased river inflow followed high catchment rainfall (Perissinotto et al. 2000, Froneman 2002, Gobler et al. 2005). These terrestrial derived nutrients were responsible for high phytoplankton biomass. Phytoplankton Chl a concentrations were elevated after each rainfall episode, although there appeared to be a lag of approximately 4 – 8 weeks before peak Chl a levels were reached. This lag period suggests that some time is required to establish stable water column conditions following a breaching event, for the phytoplankton to respond to the improved pool of available macronutrients (Chan and Hamilton 2001). Consistent with the DIN: DIP ratios phosphate levels were not limiting phytoplankton except in March when the estuary had high internal organic loading from dead and decaying macrophytes and had become hypoxic. Under these conditions ammonium concentrations reached exceptionally high levels. In a number of estuaries low nitrate concentrations have been suggested as limiting phytoplankton growth (Piehler et al. 2004). Nitrate concentrations were consistently low over the three years and only increased following high river inflow, although during periods of mouth closure.
the supply of ammonium into the water column increased augmenting the nitrogen pool. This suggests that the abundant organic matter generated from extensive macrophyte growth during the summer period may have been a source of nitrogen from decomposition processes especially during periods of low flow. Surprisingly however, even after the major flooding event in 2002 that removed most of the submersed and emergent macrophytes, concentrations of ammonium stayed similar to levels measured during the winter months of the first year prior to the spring floods. Bacterial action on decaying and moribund organic matter deposited on the sediment may have increased nutrients on the sediment surface and in interstitial water. This suggests that possibly groundwater seepage through the benthic sediments and bioturbation and re-suspension of mineralised particulate matter on the sediment surface by benthic organisms acted as a source of nitrogen in the form of ammonium input other than from the biogenic decomposition of macrophyte organic matter (Sundbäck et al. 1991, Clavero et al. 2000, Wetzel 2001, Tobias et al. 2003).

During events of overwash substantial volumes of seawater were introduced into the estuary, which elevated the salinity levels. These events were short-lived as they rapidly dissipated within weeks possibly through the dilution from continuous low river inflow (e.g. conditions sustaining a semi-closed state), including perhaps freshwater seepage from the sand dunes, groundwater input and rapid loss of more dense saline water through subsurface seepage across the sand bar. The semi-closed mouth state was a prominent feature over the study period in this estuary as it was the dominant mouth condition. This accounted for the generally low salinity levels mainly during those periods suggesting that a constant low supply of freshwater inflow rapidly diluted any salt water even after strong marine overwash.

7.4.2 Phytoplankton and Microphytobenthic Chlorophyll a and Community Structure

Total Chl a concentrations in the water column were clearly higher post freshwater influx. This is consistent with studies that have been conducted within the geographic region and elsewhere (Perissinotto et al. 2000, Froneman 2002a, & b, Gobler et al. 2005). The difference with these studies however, may be in the response period required to achieve peak levels. In this study Chl a concentrations reached peak densities between two to four weeks following freshwater influx (i.e. freshet). In comparison to other TOCE studies the response period appears to be within the range of 12 days to 4 weeks (Suzuki et al. 2002, Gobler et al. 2005). However, in contrast to some of the regional TOCEs (e.g. uMdloti Estuary) peak Chl a concentrations occurred when the mouth was closed (Perissinotto et al. 2003). The conditions necessary for the establishment of a stable environment for growth and development of phytoplankton Chl a biomass arises when a balance between two physical processes, downstream river flow and tidal inflow, are established which improves water column residence time vital for production (Chan and Hamilton 2001). In the Maitland Estuary such conditions occurred over very short periods of time possibly as a consequence
of a relatively small catchment and estuary that enabled rapid flushing of the system. After the major freshwater pulse has subsided water column conditions are normally re-established in approximately days to two weeks to levels prior to the freshet (Chan and Hamilton 2001, Sellner et al. 2001). The Chl a concentrations measured are higher than that reported for some of the regional TOCEs e.g. the Kasouga and Nyara estuaries (Perissinotto et al. 2000, Froneman 2002b).

Studies on size fractionated Chl a concentrations in estuaries have shown that the pico- and nanophytoplankton fractions form the dominant size groups responsible for most of the water column biomass especially during dry periods (Froneman 2002b, Gobler et al. 2004, 2005). However, others have observed that in either wet or dry periods nanophytoplankton tend to dominate the water column size structure (Perissinotto et al. 2002, Thomas et al. 2005). Although a similar case can be made for the Maitland Estuary, the response was variable. Nanophytoplankton had higher abundances during the low flow summer months in the first year, but in the subsequent dry years they were replaced by the microphytoplankton. These results do not agree completely with hypothesis (3), which states that long periods of mouth closure favour an increase in pico- and nanophytoplankton biomass. In the Maitland Estuary the mouth was closed for approximately 75% of the time and during that time the nanophytoplankton formed the dominant size group for about 38% while the pico- and microphytoplankton were dominant for 1 & 33% of the time respectively. The difference between the percent of time the nano- and microphytoplankton groups were each dominant was not significant (p > 0.05), such that these two groups were considered equally dominant. In addition, there were six out of 27 months when the mouth was closed that the micro- and nanophytoplankton were co-dominant. This suggests that under closed mouth conditions nano- and microphytoplankton can be expected to be the phytoplankton contributing significantly to Chl a biomass. This is in contrast with findings from other studies on TOCE that indicate a dominance or co-dominance of pico- and nanophytoplankton (Froneman 2002a & b, Gobler et al. 2004, 2005). In these studies the prevalence of the small sized phytoplankton was associated with low water column nutrient concentrations which are generally much higher (e.g. range P – 12.4 – 24.8 and N – 11.9 – 350 µg L⁻¹) than that measured in the Maitland. In the Maitland Estuary average macronutrient concentrations were higher (e.g. DIP – 0.93 µg L⁻¹ ±0.15 S.E. & DIN – 0.99 µg L⁻¹ ±0.31 S.E.) than in the Van Stadens Estuary (see Chpt 5) and possibly could account for both phytoplankton groups to coexist under more stable water column conditions when the mouth is closed.

The large-picophytoplankton (e.g. 1.2-2.7µm) never attained sufficient biomass concentrations to become the dominant group except in July 2001 (see Fig. 8.4c). The reason could be that macronutrient concentrations in July had declined to extremely low levels insufficient to support larger cells, thus the picophytoplankton was the size group capable of rapidly taking up available nutrients because of their large surface to volume ratios.
When the mouth was open the microphytoplankton became the dominant group except on two occasions in 2001 when the nanophytoplankton were dominant. During periods of increased river inflow the microphytoplankton group had the highest biomass and at no time did they exhibit co-dominance with other size fractions. These results support hypothesis (5) which states - increased nutrients from increased river inflow support high nano- and microphytoplankton biomass. Although the nanophytoplankton was never co-dominant with the microphytoplankton, the latter size fraction did form the major phytoplankton group under these conditions. The dominance by the microphytoplankton often followed a succession from small-sized fractions to larger phytoplankton.

This pattern confirmed results observed from other studies on TOCEs although in the Maitland Estuary picophytoplankton never developed high biomass concentrations generally reported in these studies. A similar shift in phytoplankton size structure was also observed in the Van Stadens Estuary (present study). This shift in size structure may be attributed to the residual stores of macronutrients that persisted after breaching episodes. This was corroborated by the moderate phosphate and ammonium concentrations measured during the low flow periods that followed the September floods. Another possible reason may be related to internal nutrient cycling from the release of decomposing organic matter left behind after the floods (Clavero et al. 2000).

The Chl a peak in the spring of 2001 coincided with a bloom of green algae (i.e. cell no. > 10^5 ml^-1) that was associated with high and moderate cell densities of blue-greens and cryptophytes respectively. These phytoplankton cell densities occurred after the second mouth breaching event. The positive correlation established between temperature and chlorophytes may explain the success shown by this taxon in spring to early summer of 2001. Warm temperatures have been linked with phytoplankton temporal distribution patterns in estuarine and coastal environments (Fogga 1991). In the summer of 2001 and 2002 chlorophytes reached cell densities >10^6 ml^-1 made up mainly of microphytoplankton and contributed approximately 59% to total Chl a biomass respectively. In terms of percent contribution by cell density chlorophytes contributed about 90% and the species included a desmid Selenastrum sp. and a coccoid green Chlorella sp.. Coccoid greens and desmids have been shown to grow well if nutrients are high and water column conditions are thermally stratified and stable (Fogg 1975, Bold and Wynn 1985). The calm, warm and deep euphotic zone established during the summer periods probably supported the growth of the chlorophytes.

Two chrysophyte flagellates, Chrysochromulina sp and Ochromonas marina, contributed the most to cell numbers producing a bloom after the September floods in 2002. The diatom Cyclotella sp. (see Appendix 2, Plate G), featured in a number of samples collected mainly from the middle and upper reaches during the same period, its high cell densities did not reach bloom (i.e. cell densities > 10^5 ml^-1) proportions. With the exception of that period no comparable blooms were encountered in the
following year. Studies by Estrada et al. (1988) demonstrated that diatoms, particularly centric diatoms grew comparably well with dinoflagellates under conditions with increased nutrients and water column agitation. In the Maitland Estuary dinoflagellate presence was very low in the first year, however moderate densities were measured in the late spring and summer seasons of 2002 and these were related to the phosphate levels found in the middle and lower reaches of the estuary and marine overwash events (Sellner et al. 2001). Although comparably low in cell number to the other taxa dinoflagellate presence seemed to recur quite often following the floods, suggesting that the improved nutrient availability, increased euphotic depth and moderate to low flow conditions provided a suitable environment for their development and growth (Sellner et al. 2001). Dinoflagellates succeed the chrysophytes in the spring and summer of 2002 and on both of those occasions the estuary mouth was open. These results do not agree with hypothesis (6), which states dinoflagellates and large-sized flagellates dominate during periods of increased river inflow and low water clarity and chrysophytes (e.g. diatoms) dominate during periods of low flow. It has been suggested in the literature that diatoms are successful in water column conditions exposed to turbulence or mixing (Margalef 1978, Tilstone et al. 2000). Diatoms in the Maitland Estuary never attained high densities either during the closed-mouth phase or under increased river inflow. The only time diatoms (e.g. Cyclotella spp., Entomoneis alata, Nitzschia acicularis and Neidium spp.) formed high densities (> 200 cells x10^3 ml^{-1}) occurred in July 2003 when the mouth was closed. During this period water column DSi concentrations were moderately high ~500 µg L^{-1}. Increased supply of dissolved silica has been suggested as critical in controlling diatom populations (Tande and Slagstad 1985). The high DSi may have provided adequate supply for the diatom populations under closed mouth conditions. Some of the diatoms encountered in the water column may have been resuspended from the benthos since the depth of the water column during this period was shallow following the 2002 floods. Cyanophyte concentrations decreased and this was possibly associated with a turbulent and mixed environment once the water column depth fell below 0.5 m. Under these dynamic hydrological conditions it becomes unsuitable for the establishment and growth of filamentous and large colonial blue-green algae (Bold and Wynn 1985).

Data from the MDS analysis based on presence/absence of species counts indicated that mouth condition affected phytoplankton distribution patterns. In addition, there were stronger similarities in the phytoplankton assemblages within months of the same season and year exposed to the same physical processes than assemblages under similar hydrological processes occurring in similar months in different years. The timing of these episodic events is crucial with regard to the type of phytoplankton assemblage affected. These results suggest that mouth condition can influence the structuring of phytoplankton communities within the Maitland Estuary by regulating their ability to successfully establish and develop. It is also clear that similar patterns of hydrological processes that take place annually (e.g. rainfall patterns) cannot be used to predict the type of phytoplankton
assemblage encountered. There are few studies that have addressed phytoplankton community structure in TOCEs (Walker et al. 2001, Gobler et al. 2005). This research contributes useful scientific data regarding the response patterns of phytoplankton assemblages to variable river flow, nutrient supply and salinity conditions in TOCEs. Phytoplankton species distributions patterns exhibit a close association with seasonality and hydrodynamic changes. For example, chlorophytes had their highest densities during the summer periods, whereas dinoflagellates bloomed as a consequence of increased river inflow. Cryptophytes did not show any strong response to changes in river flow or changes in salinity conditions, but displayed moderate growth when river flow was high (e.g. September 2001) and when the mouth was closed during the summer months (January – March 2002 and 2003).

Although the Maitland Estuary is situated close to the sea it continuously stays oligohaline supporting estuarine vegetation composed mainly of low salinity tolerant flora or even freshwater species (e.g. *Typha latifolia, Chara sp.*). The small size (0.072 km²) and shallow nature of the estuary makes it susceptible to rapid flushing especially when there have been heavy rains in the catchment. Strong floods experienced in the Maitland Estuary during the study period altered the channel and deposited a significant amount of sediment raising the height of the estuary. These changes possibly affected water retention characteristics of the estuary by limiting accumulation of water even under very low river flow conditions. The Maitland and possibly other similar estuaries that are prone to strong hydrologic forcing may experience recurring physical disturbance that resets the system by scouring and depositing sand. The general hydrodynamic and ecological functioning of the estuary following flood events may impact the size structure and community composition of the microflora shifting it from one composed of blue-greens, cryptophytes and greens to that made up of flagellated chrysophytes, dinoflagellates and chlorophytes. The wide-ranging (e.g. 5.3 – 138.2 µg L⁻¹) and high chlorophyll concentrations measured in the Maitland Estuary suggest that the system is more productive than some of the regional TOCEs e.g. the Nyara and Kasouga estuaries (Perissinotto et al. 2000, Froneman 2002a & b) and the Van Stadens Estuary. Furthermore, the possible land use practises in the catchment are possibly contributing exceedingly more nutrients now than previously. Aspects that were not considered in this study was the influence of zooplankton grazing on structuring phytoplankton assemblages. A number of studies have demonstrated the effects of zooplankton on phytoplankton communities (Mallin et al. 2004, Froneman 2002c, 2006, Paffenröfer et al. 2007). Heterotrophic consumption by nanoflagellates has been demonstrated in coastal and oceanic environments (Sommer and Sommer 2006). The variability in phytoplankton Chl a biomass across spatial and temporal scales in the Maitland Estuary cannot all be attributed to physical disturbances associated with hydrodynamic forcing, biological influences may explain some of the variations observed. Impacts of zooplankton grazing in some regional TOCEs have been higher than in some of the permanently open estuaries particularly during the closed-mouth periods (Perissinotto et al. 2000,
2003, Froneman 2004a & 2004b). Investigations that would examine the role of zooplankton grazing in the Maitland Estuary are lacking and need to be addressed. These studies would help reveal the function and influence of zooplankton grazing on the phytoplankton community under different river flow conditions given the high concentrations of phytoplankton biomass present.
8. **Seasonal Phytoplankton Primary Productivity in the Van Stadens and Maitland Estuaries**

8.1 **Introduction**

Recent productivity studies (Froneman 2002a, Perissinotto et al. 2003) in temporarily open/closed estuaries (TOCEs) have emphasised the importance of small-sized phytoplankton to total water column production, identifying them as a major link to secondary and tertiary aquatic production. The reason behind this is that TOCEs are generally nutrient poor and are characterised by low phytoplankton biomass. These estuaries possess the characteristics of oligotrophic ecosystems and production is mostly driven by the microbial loop (Burkhill et al. 1987, Caroppo 2000, Froneman 2004b). In regions where microbial loop production is prevalent small-sized phytoplankton dominate carbon energy transfer to higher tropic levels through grazing by the microheterotrophs that are in turn grazed upon by larger zooplankton (Froneman 2004b, Ueno et al. 2005).

A number of temporarily open/closed estuaries in the warm temperate region are nutrient poor thus nutrient supply and availability is crucial to phytoplankton population dynamics and production. Most aquatic bodies that are poor in nutrients have been shown to support small-sized phytoplankton since these microalgae show rapid rates of nutrient uptake associated with high rates of turnover. This suggests that nutrients that are in short supply are quickly taken up by picophytoplankton owing to their large surface area to volume ratios (Goldman and Gilbert 1983, Armstrong 1994, Fisher et al. 1995, Kirchman 2000). The transfer of carbon up the food web is not understood for these estuaries. More studies need to be carried out particularly across the three South African biogeographical regions in order to better understand how these systems function.

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 1300 g C m$^{-2}$ yr$^{-1}$ indicative of a wide range of estuaries that are reflective of their unique biogeographical localities e.g. Lake Sibaya in the subtropical, Sundays and Swartkops estuaries in the warm temperate, and the Nahoon River Estuary in the cool temperate, biogeographical regions (Allanson and Hart 1975, Dye 1978, Hilmer 1984, 1990, Bally et al. 1985). Primary production has been measured in a number of estuaries around the world. In an estuarine embayment of the Chesapeake annual production was 139 g C m$^{-2}$ y$^{-1}$ (Mountford 1980), and in the Delaware Estuary production ranged from 36.5 – 657 g C m$^{-2}$ y$^{-1}$ (Pennock and Sharp 1986), whereas in a highly dynamic upwelling system, the Ría de Vigo it ranged from 779 – 7305 g C m$^{-2}$ y$^{-1}$ making it one of the most productive coastal systems (Cermeño et al. 2006). Compared to the systems mentioned South African estuaries seem to span the range of annual production measured in the overseas estuaries (Allanson and Baird 1999).
Temporarily open/closed estuaries form the majority of estuarine types along the South African coastline yet our knowledge of the effects of environmental changes and influences on phytoplankton production and community structure is still poor. Phytoplankton productivity studies carried out in TOCEs (e.g. uMdloti, uMhlanga, and uMpenjati estuaries in the subtropical biogeographical region and Kasouga and Bot River estuaries in the warm and cool temperate biogeographical regions respectively) thus far indicate that water column rates of production are generally higher than the benthos even though the reverse is true regarding biomass (Bally et al. 1985, Froneman 2002a, Perissinotto et al. 2003). Studies in TOCEs have shown that the water column is generally characterised by low algal biomass and high rates of production compared to the high benthic algal biomass and low productivity rates (Froneman 2002a, Perissinotto et al. 2003). Low nutrient concentrations characterise the water column compared to the benthos, hence high nutrient uptake rates have been attributed to the higher production rates observed in the water column than in the benthos (Perissinotto et al. 2003). The aim of this study was to conduct a comparative investigation along an annual seasonal cycle on phytoplankton primary production in two adjacent temporarily open/closed estuaries. This study set out to test three hypotheses by carrying out the experiments in both the Van Stadens and Maitland estuaries. The following hypotheses were tested: 1) nano- and microphytoplankton size fractions (2.7 – 20 µm & >20 µm along major axis respectively) form the dominant phytoplankton that drive primary production during periods of increased river inflow, 2) picophytoplankton (1.2 - 2.7 µm along major axis) form the dominant phytoplankton group that drive primary production during periods of low river inflow, 3) during periods of mouth breaching microphytoplankton production decreases beyond levels sufficient to support macro-zooplankton biomass and production.

8.2 Methods and Materials

Detailed methods and materials used in this study are described in Section 4, General Methods and Materials. Physical parameters were measured as described in the General Methods Chapter and included light, temperature, salinity, pH, conductivity and dissolved oxygen.

8.2.1 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 ¹⁴C 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 2 light and one dark 250 ml biological oxygen demand bottles (BOD) at each depth whereupon a known amount of a carbon tracer (NaH¹⁴CO₃) was added to the samples and then incubated at depth for 4h. Incubation bottles were suspended at depths corresponding to 30% (bottom)
and 80% (top) of the surface radiation at each station. Hourly productivity rates were converted to daily rates by normalizing against hours of daylight. Samples were placed at each site along the length of the estuary (see Fig. 4.2). Termination of the experiment was accomplished by filtering samples into three size-fractions microphytoplankton (>20 μm), nanophytoplankton (2.7 – 20 μm) and picophytoplankton (1.2 - 2.7 μm) for the determination of productivity of each group.

### 8.3 Results

#### 8.3.1 Physico-chemical Parameters

A summary of physico-chemical parameters measured during the productivity experiments over an annual seasonal cycle are presented in Table 8.1. Data were pooled since no significant vertical or horizontal differences were detected following a two-way ANOVA ($p > 0.08$). In both the Maitland and Van Stadens estuaries water column temperatures during summer were warmer than all other seasons with the coldest temperatures recorded in winter. Rainfall induced discharge in the spring breached the mouth and salinity remained low 1.0 (±1.8 psu), whereas under a closed mouth condition during the summer salinity reached a maximum of 19.2 (±0.7 psu). Water column turbidity peaked during the open-mouth condition in the spring, but remained similar throughout the other seasons. Under open mouth conditions during spring in the Van Stadens Estuary a Pearson correlation analysis showed that water column productivity was positively correlated with light and temperature (0.54 and 0.67, $p < 0.05$). However in summer and autumn water column productivity was poorly correlated with light penetration (0.0021), although positively correlated with temperature (0.56 $p < 0.01$) suggestive of the influence of temperature on metabolic processes (Etheridge and Roesler 2005). In the Maitland however, in autumn, water column productivity was negatively correlated with temperature (-0.63 $p < 0.038$). Following the mouth opening events in both estuaries in 2002 water level in the estuary dropped such that primary productivity experiments were integrated over the entire water column as top and bottom samples could not be taken.
Table 8.1  Mean values (±1 S.D.) of light attenuation, temperature, salinity and dissolved oxygen measured during the productivity trials over the four seasonal cycles in the Van Stadens and Maitland estuaries from September 2002 – June 2003. State of mouth condition is noted in parenthesis for the corresponding season.

<table>
<thead>
<tr>
<th>Season &amp; Mouth Condition</th>
<th>Attenuation Coefficient $K_d$ (m$^{-1}$)</th>
<th>Temperature (°C)</th>
<th>Salinity (psu)</th>
<th>D.O. (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Van Stadens Estuary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring (Open)</td>
<td>2.85 (1.6)</td>
<td>18.4 (1.4)</td>
<td>1.0 (1.8)</td>
<td>8.6 (1.7)</td>
</tr>
<tr>
<td>Summer (Closed)</td>
<td>0.58 (0.2)</td>
<td>24.0 (1.4)</td>
<td>19.2 (0.7)</td>
<td>7.1 (1.2)</td>
</tr>
<tr>
<td>Autumn (Closed/Overwash)</td>
<td>0.63 (0.3)</td>
<td>22.6 (0.6)</td>
<td>10.4 (6.3)</td>
<td>4.2 (1.7)</td>
</tr>
<tr>
<td>Winter (Closed)</td>
<td>0.55 (0.8)</td>
<td>14.6 (2.2)</td>
<td>15.1 (9.9)</td>
<td>5.6 (2.8)</td>
</tr>
</tbody>
</table>

| **Maitland Estuary**     |                                          |                  |               |                   |
| Spring (Open)            | 0.00 (0.00)                              | 17.0 (0.3)       | 0.0 (0.0)     | 6.7(0.1)          |
| Summer (Open)            | 0.51 (0.06)                              | 24.9 (0.9)       | 2.3 (0.4)     | 9.0 (1.5)         |
| Autumn (Closed)          | 0.39 (0.01)                              | 21.5 (0.1)       | 1.6 (0.3)     | 8.9 (3.2)         |
| Winter (Closed/Overwash) | 0.91 (0.03)                              | 13.7 (0.3)       | 0.7 (0.0)     | 7.0 (0.1)         |

8.3.2  Seasonal Cycle Phytoplankton Production

Primary production experiments were carried out once over an annual seasonal cycle in the Van Stadens and Maitland estuaries (Fig. 8.1). 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. Production studies were not carried out in the Maitland Estuary in the spring of 2002, as sampling occurred a few weeks after strong floods in August. The floods scoured and washed off sand from the adjacent sand dunes depositing some of the sand in the estuary resulting in a raised sediment floor such that the water depth at the deepest point of the estuary was <0.1m and discharge was approximately 8.0 m$^3$s$^{-1}$. A two-way ANOVA test was performed on the Van Stadens and Maitland data sets 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 procedure (Tukey test) (Table 8.2). Nanophytoplankton production was significantly higher compared to both pico- and microphytoplankton production in spring and summer ($q$=4.720, $P<0.005$ and $q$=7.697, $P<0.001$).
Table 8.2 Two-way ANOVA results from the Van Stadens data showing the significance of the difference among seasons and phytoplankton size-fractions.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>2</td>
<td>25.339</td>
<td>12.670</td>
<td>27.778</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Season</td>
<td>3</td>
<td>16.054</td>
<td>5.351</td>
<td>11.733</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Season x Fraction</td>
<td>6</td>
<td>7.173</td>
<td>1.105</td>
<td>2.621</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

There was no Season x Fraction interaction. * Significant $p < 0.05$. ** Significant $p < 0.01$

Axial productivity patterns were variable although certain size fractions at some stations over the annual seasonal cycle had high levels of production (Figs. 8.2 and 8.3). There were no significant differences among stations within the same season ($p > 0.10$) in the Van Stadens. With the exception of one occasion in the upper reaches of the estuary in the Van Stadens, the microphytoplankton were the second size group that demonstrated high levels of production during the spring season. Microphytoplankton productivity during the spring was significantly different when compared to autumn and winter rates ($p < 0.001$). In the Van Stadens picophytoplankton productivity in the spring was positively correlated with nitrate concentrations and negatively correlated with TP ($R^2 = 0.37$ and $0.70$, $p < 0.05$ respectively). In autumn at Station 2 in the Van Stadens, the microphytoplankton had the highest productivity rates followed by the nanophytoplankton. During this period microphytoplankton productivity was negatively correlated with dissolved inorganic phosphate (SRP + TP) concentrations ($R^2 = 0.59$ and $0.81$, $p < 0.05$ respectively) possibly indicative of high nutrient uptake. During the winter season productivity levels were at their lowest at all sites in comparison to other seasons (Figs. 8.1, 8.2 and 8.3). The nano- and picophytoplankton had the highest and second highest productivity rates respectively at Station 1 in the Maitland and Station 2 in the Van Stadens estuaries, whereas the microphytoplankton had the highest rates of productivity at Station 2 in the Maitland Estuary. Mouth condition in the Van Stadens Estuary appears to have had little influence on water column primary productivity since productivity rates in spring, when the mouth was open, were not significantly different to summer measurements when the mouth was closed ($p > 0.10$). During autumn when the mouth was closed rates of productivity in the Maitland Estuary were significantly higher than those in December when the mouth was closed ($p < 0.05$).

In winter productivity was significantly ($p < 0.001$) lower compared to the other seasons in both estuaries (Fig. 8.3). Total phytoplankton productivity in the Maitland was significantly higher than that at Van Stadens during the autumn ($p < 0.05$). However, there were no significant differences between the other seasons. Similar to the Van Stadens, in the Maitland Estuary the nanophytoplankton size-group showed the greatest rates of productivity across all seasons. Following
a pairwise multiple comparison test significant differences were detected among seasons and chl-\(a\) size-fractions as a factor within seasons (Tukey test) (Table 8.3). In contrast to the Van Stadens, during autumn in the upper reaches of the Maitland Estuary the nanophytoplankton displayed the highest productivity levels (29.7 mg C m\(^{-2}\) h\(^{-1}\), ± 5.2 S.E.). Only during the autumn and winter seasons in the lower reaches did the picophytoplankton size fraction contribute more than the microphytoplankton to total primary production, 31 and 40\% respectively.

Table 8.3  Two-way ANOVA results from the Maitland data showing the significance of the difference among seasons and phytoplankton size-fractions.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>2</td>
<td>19.137</td>
<td>10.760</td>
<td>17.878</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Season</td>
<td>2</td>
<td>15.054</td>
<td>4.135</td>
<td>9.824</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Season x Fraction</td>
<td>4</td>
<td>8.730</td>
<td>1.195</td>
<td>2.712</td>
<td>0.028*</td>
</tr>
</tbody>
</table>

*There was no Season x Fraction interaction. * Significance \(p < 0.05\). ** Significance \(p < 0.01\).

Although the winter season in both estuaries exhibited the lowest productivity rates compared to the other seasons, the middle reaches (Van Staden Stations 2 and 3, Maitland Station 2) seem to have the highest nanophytoplankton productivity levels. The microphytoplankton, particularly in the upper reaches of the Maitland, made the second highest contribution to total primary production (Fig. 8.3). During this period productivity was positively correlated with water column temperature (\(R^2 = 0.34\), \(y = 2.3032x + 13.469\)). Nutrient input to the estuaries took place when there was an increase in river flow. Spring and summer productivity levels did not show any variation but remained similar (\(R^2 = 0.0031\)), although the mouth was open in spring and was closed during the summer months. In contrast, there were significant differences between productivity rates in spring and those during the autumn when the mouth was closed (\(p < 0.05\)) suggestive that temperature might possibly be an important factor influencing production.
Size-fractionated primary production measured at 5-sites along the length of the Van Stadens Estuary during an annual seasonal cycle including the condition of the mouth; a-spring - open, b-summer - closed, c-autumn - closed, d-winter - open. Micro- microphytoplankton (> 20 µm), nano- nanophytoplankton (2.7 – 20 µm), and pico- picophytoplankton (1.2 – 2.7 µm).
Figure 8.2. Size-fractionated primary production measured at 3-sites along the length of the Van Stadens Estuary over an annual seasonal cycle including the condition of the mouth: a-summer - open, b-autumn - closed, c-winter – semi-closed. Micro–microphytoplankton (> 20 µm), nano- nanophytoplankton (2.7 – 20 µm), and pico- picophytoplankton (1.2 – 2.7 µm). Note difference in scale in panel (c).
Figure 8.3  Seasonal size-fractionated primary production for the Van Stadens (A), Maitland estuaries (B) and total production of each estuary (C). Micro – microphytoplankton (> 20 µm), nano- nanophytoplankton (2.7 – 20 µm), and pico- picophytoplankton (1.2 – 2.7 µm). Vertical lines indicate ±1 S.E. Horizontal bars denote mouth condition: solid bar = closed, open bar = open, hatched bar = overwash.
8.4 Discussion

The estimates of phytoplankton primary production from this investigation are in the same range as previous studies on regional TOCEs (Froneman 2002a, Perissinotto et al. 2003). The influence of water temperature, nutrients and light availability in regulating rates of production in estuaries and coastal water is well documented (Falkowski 1981, Campbell and Bate 1986, Berges et al. 2002, Bergmann et al. 2002, Cermeño et al. 2006). The high production rates in summer were possibly a consequence of prevailing environmental conditions in the Van Stadens during that period. Water column temperatures and light penetration in the summer months averaged 24 °C and 0.5 $K_d$ m$^{-1}$ respectively. Following the breaching of the mouth in August water levels in the estuary dropped reaching a minimum in September when productivity trials were carried out. Spring water column productivity rates remained markedly higher than that recorded in autumn and may indicate the phytoplankton response to the spate of increased nutrient input from river flow in enhancing production (Kemp and Boynton 1984, Loureiro et al. 2005).

During the closed mouth phase in the Van Stadens Estuary water level had risen to almost maximum levels, which had allowed for the development of phytoplankton under increased light penetration. Mouth condition did not have a significant effect on primary productivity during this study. This is in contrast with regional studies that have demonstrated increased productivity when the mouth is open (Froneman 2002b). The possible reason for the discrepancy with this study is probably associated with low total available phytoplankton biomass. Chlorophyll a biomass concentrations were considerably lower during the open-mouth phase than during the closed phase, whereas Froneman (2006) recorded highest chlorophyll a levels during an open mouth phase. High chlorophyll a values during an open-mouth condition are not surprising since it was expected as other regional and international studies (Froneman 2002a, Suzuki et al. 2002, Gobler et al. 2005, Santangelo et al. 2007) have shown similar trends as demonstrated by data from the Van Stadens and Maitland estuaries (see Chpts 5 & 7).

Data presented here are comparable with a number of studies that have been performed in TOCEs and elsewhere (Table 8.4). The annual productivity values determined for the Van Stadens and Maitland estuaries (22.8 – 122.3, 10.1 – 210.9 g C m$^{-2}$ y$^{-1}$ respectively) appear to fit within the ranges recorded for the more productive warm temperate TOCEs and some of the permanently open warm temperate systems. These values, although low, do however compare with studies in much larger northern hemisphere estuaries (e.g., Delaware Estuary – Pennock & Sharp 1986, Tomales Bay – Cole 1989 and Neuse River Estuary – Mallin et al. 1991, Ría de Vigo – Cermeño et al. 2006, Chesapeake Bay – Adolf et al. 2006). From these results it is clear that both the Van Stadens and Maitland estuaries display low levels of production and that nutrient supply may limit production. The Maitland Estuary
drains a catchment heavily farmed for beef and dairy products and displays higher nutrient concentrations than the Van Stadens Estuary. This estuary has the potential to have higher levels of production particularly if water column conditions favour greater stability (see Figs. 5.7 and 7.2 Chpt 5 and 7 respectively). Although farms exist in the Van Stadens catchment the majority of the land is natural fynbos and thicket vegetation with the remainder occupied by forestry, therefore nutrient input into the estuary is quite low (DIN 0.02 – 5 and DIP 0.09 – 3 µg L\(^{-1}\)). The low water temperatures in winter in both estuaries may possibly account for the low rates of production observed as productivity was positively correlated with temperature (\(R^2 = 0.34\)).

Table 8.4 Range of primary production values for the Maitland and Van Stadens estuaries including those of published South African temporarily open/closed estuaries for different biogeographical regions.

<table>
<thead>
<tr>
<th>Biogeographic Region</th>
<th>South Africa</th>
<th>Estuary</th>
<th>Production g C m(^2) y(^{-1})</th>
<th>Reference:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub-tropical</td>
<td></td>
<td>uMpenjati</td>
<td>187.5 – 1822.1</td>
<td>Perissinotto et al. 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>uMdloti</td>
<td>26.3 – 1112.5</td>
<td>Perissinotto et al. 2003</td>
</tr>
<tr>
<td>Warm temperate</td>
<td></td>
<td>Kasouga</td>
<td>8.6 – 13.5</td>
<td>Froneman 2002a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Van Stadens</td>
<td>0.95 – 5.1</td>
<td>This Study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maitland</td>
<td>0.42 – 8.8</td>
<td>This Study</td>
</tr>
<tr>
<td>Cool temperate</td>
<td></td>
<td>Bot River</td>
<td>0 – 57.5</td>
<td>Bally et al. 1985</td>
</tr>
</tbody>
</table>

The predominance by the nanophytoplankton at all sites is indicative of the importance of this size group to water column production in both estuaries. This also provides additional evidence of the Van Stadens and the Maitland estuaries as oligotrophic systems in that the small-sized phytoplankton are responsible for the high turnover rates of carbon observed in systems driven by the microbial loop (Caroppo 2000, Froneman 2002b, 2004a, Jochem 2003). However, unlike other typical oligotrophic systems the picophytoplankton does not feature as a significant group, which has been linked with most of the phytoplankton production encountered for these systems (Jochem 2003, Ueno 2005). The lack of the picophytoplankton in these estuaries may be a consequence of rapid removal by grazing of microheterotrophs. The impact of grazing by heterotrophs and mixotrophic algae in TOCEs and other
Phytoplankton Chlorophyll a Concentration and Community Structure

Estuaries has been well demonstrated (Froneman 2000a, 2002a & 2004, Jochem 2003, Perissinotto et al. 2003, Lionard et al. 2005, Katechakis and Stibor 2006, Hlaili et al. 2007, Santangelo et al. 2007). The production experiments in this investigation did not exclude grazers (Dupuy et al. 2007). Although the impact of grazers would be expected to be similar across all treatments, uneven distribution of grazers in containers can cause patchiness within the phytoplankton community increasing variability in the results (Dupuy et al. 2007). The microphytoplankton was the second most important size fraction to phytoplankton production and could be responsible for a considerable proportion of carbon transfer to higher trophic levels. Although macronutrient concentrations were low during productivity trials, levels of production were 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 (Day et al. 1989, Cermeño et al. 2006). The close link between different trophic levels in nutrient poor systems is well documented (Adolf et al. 2006, Cermeño et al. 2006, Dupuy et al. 2007) however data are lacking for TOCEs therefore, future investigations would have to examine those aspects very closely.

Environmental factors (e.g. light and temperature) appear to have had an influence in regulating phytoplankton production in both estuaries (Pearson Correlation 0.54 and 0.67, $p < 0.05$), yet aspects of nutrient limitation and possible grazing pressure require further investigation as this study did not address those points. In the Bot River Estuary production was controlled more by seasonality and available light climate (Bally et al. 1985). Significant temporal patterns were evident and certainly a reduced euphotic depth affected production. The effects of mouth opening on phytoplankton production require additional examination. The once-off productivity trials that were carried out during this investigation were inadequate to effectively capture the events. Similar discrepancies in the effects of mouth condition on production estimates were observed in the Mdloti and the Mpenjati estuaries (Perissinotto et al. 2003).
9. General Discussion and Conclusions

9.1 Hydrodynamic influence on Physical and Chemical Parameters in Van Stadens and Maitland Estuaries

The frequency of mouth breaching in both the Van Stadens and Maitland estuaries occurred about 20 to 25% of the time over 3 years resulting in three and four open mouth phases respectively. The hydrodynamic effects on the physical and chemical variables in these estuaries were pronounced and strongly influenced physical and chemical characteristics. Heavy flooding in August 2001 and September 2002 caused breaching of the mouth, reduced salinity and introduced fine particulate inorganic suspended matter that reduced the euphotic depth. However, in the Maitland Estuary flooding brought about significant changes in the channel geomorphology, such that the height of the sediment floor was raised making the estuary retain less water which increased the frequency of the semi-closed mouth state. Low salinity conditions remained only until river flow was reduced to levels that allowed the tidal prism to reach the upper reaches of the estuary when the mouth was open. The degree to which the estuary mouth was perched above the height of mean sea level also had an influence on the penetration of the tidal prism, for example in the Van Stadens Estuary it could reach 4 km upstream whereas in the Maitland Estuary it only penetrated about 0.25 km. Incidents of marine overwash rapidly re-established mesohaline conditions after the mouth closed. Even under moderate durations of mouth closure marine overwash events kept salinity high. In the Maitland Estuary high salinity from marine overwash events was short-lived often lasting from two-three days to a couple of weeks at most. Freshwater input possibly from the surrounding dunes reduced salinity keeping the estuary oligohaline an average of 2.9 psu over the three years. In contrast, the Van Stadens Estuary generally had mesohaline conditions and salinity averaged 25 psu when freshwater inflow was low and marine overwash occurred. This latter condition is characteristic of some regional (Bally et al. 1985, Perissinotto et al. 2000, Froneman 2002b) and some TOCEs abroad (Twomey and Thompson 2001, Everett et al. 2007).

In the Van Stadens Estuary the supply of macronutrients was strongly correlated with rainfall ($R^2 = 0.67$), whereas in the Maitland Estuary it was not. Although following rainfall events and increased river inflow in the Maitland macronutrient concentrations increased this was not statistically significant. This demonstrates a strong link between catchment derived nutrients and the estuary suggesting that activities in the catchment can affect estuarine processes (Twomey and Thompson 2001, Flemer and Champ 2006). Except on a few occasions, associated with increased river inflow or possibly internally generated sources, nitrogen was limiting in both the Van Stadens and Maitland estuaries. There was evidence that the river acted as a source of ammonium since high concentrations ($p < 0.01$) were always measured in the upper catchment of the Van Stadens River. In contrast, under
closed mouth conditions nitrate concentrations remained low and at times were barely detectable. Under these conditions the source of nitrogen in the estuary was mostly from ammonium since concentrations were normally higher than nitrate concentrations except during floods. Moreover, concentrations in groundwater were often significantly higher when the mouth was closed ($p < 0.01$) suggesting that another possible route of entry into the water column may be from groundwater. It is well accepted that nutrient input into TOCEs is generally allochthonous (Perissinotto et al. 2000, Gobler et al. 2005, Everett et al. 2007, Santangelo et al. 2007). Regional studies that have documented nutrient influx into TOCEs have generally pooled the nitrogen sources as DIN (e.g. nitrate, nitrite and ammonium), but have not considered each nutrient component separately. In order to simplify explanations the same was done in this study, but ammonium was also reported separately. Instances when nitrogen was not limiting (i.e. N:P ratio $> 16:1$) occurred when ammonium contributed over 60-80% to the nitrogen pool. This was the case in both the Van Stadens and Maitland estuaries (see Chpts. 5 & 7, Figs. 5.4 & 7.3) respectively. The role of ammonium as a preferred source of nitrogen for phytoplankton and microphytobenthos is well documented (Zehr and Ward 2002). This aspect of nutrient metabolism for these estuaries is little understood and should be an area of investigation as it could play a crucial role in sustaining phytoplankton metabolic requirements for nitrogen (Zehr and Ward 2002).

In both estuaries macronutrient input was dependent on the supply from the catchment and that availability was limited by both the frequency of rainfall events and man-made obstructions (e.g. dams, weirs, & course ways). Several studies have documented the influence of physical barriers on river flow and its associated effects on the ecology and functioning of riverine and estuarine biota (Reddering and Rust 1990, Davies and Day 1998). These physical structures alter and modify the river channel and bed morphology, which impacts the physico-chemical characteristics of water in receiving waters downstream (Davies and Day 1998). The Van Stadens River has two dams that capture a significant portion $\sim 30-40\%$ of the mean annual rainfall (MAR) (e.g. $0.47 \times 10^6 \text{ m}^3$) while the Maitland Estuary has several farm dams that capture $\sim 40-50\%$ of the MAR. During drought years these rivers have very low flows and can become dry affecting freshwater inflow and water level in the estuaries downstream. In 2001 in the Maitland Estuary the river was cut off from the estuary which limited freshwater inflow and circulation resulting in stagnant waters in the upper reaches. However, during periods of strong storm surges and extreme high water spring tides saline water penetrated into the lower to middle reaches. Although these events were sporadic, in the first year of the study they kept salinity high and possibly maintained a moderate amount of circulation in the lower reaches as the estuary became stratified along its axial length.

Since no water is released from the Van Stadens dam the water that reaches the estuary comes from overspill over the wall of the dam and ephemeral tributaries that flow during wet periods. During dry
periods macronutrient concentrations in the river and the estuary became depleted. TOCEs like the Van Stadens and Maitland estuaries located in semi-arid regions with intermittent rainfall are vulnerable to a reduction in river inflow and the regular supply of macronutrients necessary to maintain estuarine ecological function. Similarly a number of southeastern and western Australian TOCEs or ICOLLs receive intermittent rainfall such that nutrient input into these systems is generally affected by rainfall patterns (Twomey and Thompson 2001, Everett et al. 2007). Water supply into the estuary can be augmented from the sea and is important as it can introduce macronutrients. On a few occasions concentrations of DIP and DIN in the lower reaches of the Van Stadens Estuary were noticeably higher than the middle or upper reaches. This indicates that the marine environment can at times play a crucial role in maintaining a supply of macronutrients, although this will depend on prevailing weather conditions. In addition, evidence from daily monitoring in the summer of 2001 in the Van Stadens Estuary showed how salinity and macronutrient spatio-temporal distribution patterns were rapidly altered from an open to a closed mouth condition (see Chpt. 7 Figs. 7.1-7.3).

9.2 Hydrodynamic influence on Phytoplankton Chlorophyll a Biomass, Primary Production and Community Structure

The response of phytoplankton Chl a to freshwater influx was as expected in both estuaries. Macronutrient input following increased river inflow stimulated phytoplankton biomass resulting in peaks of Chl a associated with each breaching event. The magnitude of Chl a response however varied in each estuary. This was related to the quantity of nutrients received. The Maitland Estuary had higher nutrient concentrations compared to the Van Stadens. Both DIN and DIP levels were 20 – 30% greater in the Maitland Estuary than in the Van Stadens. This difference was possibly responsible for the approximately 10 fold higher Chl a concentrations in the Maitland Estuary. Although macronutrient concentrations were higher in the Maitland Estuary compared to that in the Van Stadens Estuary, they were still characteristic of an oligotrophic water body (e.g. N < 80 & P < 5 µg L⁻¹) (Wetzel 2001). Research from a number of TOCEs shows that these estuaries are generally nutrient poor and largely depend on terrestrially derived nutrients brought in by rainfall induced river inflow (Perissinotto et al. 2003, Froneman 2004a, Gobler et al. 2005, Everett et al. 2007, Santangelo 2007). An increasing number of TOCEs receive elevated macronutrient concentrations from downstream sewage disposal (Thomas et al. 2005, Santangelo et al. 2007). This is placing considerable pressure on the ecological functioning of these systems by altering the natural trophic organisation of the aquatic ecosystem (Day et al. 1989, Allanson and Baird 1999, Piehler et al. 2004).

The highest peaks in Chl a occurred several weeks after the spring floods of 2001 in both estuaries. There were no other breached induced Chl a peaks matching the levels measured in late spring early summer. In both estuaries the duration of the open-mouth phase (e.g. 6 – 8 weeks) seemed critical in
affecting the magnitude of the Chl \(a\) concentrations. This suggests that the duration between the periods the mouth stays closed is critical in determining the level of the phytoplankton response. Although in the Maitland Estuary the duration of an open mouth event in September 2001 was comparable to the one in the following spring of 2002, its effect on Chl \(a\) concentrations was not as pronounced. The length of time the mouth stays open is critical in establishing a continuous link with the terrestrial and marine environments by creating ‘permanent open mouth’ conditions similar to those observed for such estuaries (Mallin and Paerl 1994, Pinckney et al. 1999, Chan and Hamilton 2001).

Previous TOCE studies on microphytobenthos (MPB) have demonstrated that benthic microalgae have higher Chl \(a\) biomass compared to the phytoplankton (Froneman 2002a, Perissinotto et al. 2003). In agreement with these studies microphytobenthic Chl \(a\) in both the Maitland and Van Stadens estuaries were consistently higher that water column biomass. In contrast to the water column Chl \(a\) response to increased nutrient input, the microphytobenthic (MPB) Chl \(a\) concentrations did not show any significant changes \((p < 0.001)\) to increased river flow but appeared to be limited by other factors since high biomass (i.e., > 60 mg Chl \(a\) m\(^{-2}\)) was only achieved after the TOMP. Water clarity is important to ensure sufficient light at depth to support growth (Nozais et al. 2001, Perissinotto et al. 2002).

In contrast to other studies, in this study the microphytoplankton were generally the dominant size group in both the Van Stadens and the Maitland estuaries contributing more than 55% to total Chl \(a\) biomass. The dominance by the microphytoplankton was mostly when the mouth was open but on a number of occasions under closed mouth conditions they contributed the most biomass. In agreement with regional and international studies nutrient input associated with increased freshwater influx supported high biomass of large-sized phytoplankton (Froneman 2002a & b, Gobler et al. 2005). Nano- and picophytoplankton contributions were short-lived and were always succeeded by the microphytoplankton. The Chl \(a\) biomass contribution by picophytoplankton in both estuaries was normally less \((< 20\%)\) than that of the other size fractions either during the closed or the open mouth phases. The importance of microphytoplankton \((> 20\ \mu m)\) to water column biomass production has been well demonstrated for coastal systems that frequently experience variable nutrient supply and environmental conditions (Cermeño et al. 2006). Phytoplankton chlorophyll \(a\) values determined for a number of South African temporarily open/closed estuaries including those for the Maitland and Van Stadens estuaries are listed in Table 9.1. Chlorophyll \(a\) biomass of most estuaries showed that values are generally low. Estimates of phytoplankton production are low values except for two estuaries located in the subtropical biogeographic region that have values comparable with South African permanently open estuaries (Allanson and Baird 1999).
Some comparisons of this study can be made with research conducted overseas on similar estuaries. Rainfall, in a number of TOCEs, bar-built, blind or similar estuaries, is the primary driver of breaching events particularly in regions that have arid to semi arid climate conditions (Elwany et al. 1998, Twomey and Thompson 2001). For instance, in the Van Stadens and the Maitland estuaries scour of the channel bed and mouth dynamics (i.e. opening and closing) was closely associated with rainfall patterns in the catchments that initiated sufficient runoff to cause scour and mouth breaching including nutrient loading. Nutrient supply into a number of TOCEs is primarily associated with an increase in runoff, although the magnitude and degree of availability may vary. Unlike in South African TOCEs, similar estuaries elsewhere can gain their nutrients either from overland runoff, direct precipitation, and groundwater or from the marine environment (Suzuki et al. 2002, Gobler et al. 2005, Gale et al. 2006, Everett 2007, Santagelo et al. 2007). For example, in Mecox Bay, in the U.S.A., nutrient input and availability, derived from groundwater and the sea, was linked to seasonality and was shown to limit phytoplankton biomass and population abundance (Gobler et al. 2005). In contrast, studies from the tropical geographic regions in South America, where nutrient concentrations were considerably higher (i.e. TN > 400 µg L⁻¹ & TP > 48 µg L⁻¹) than those in the Van Sadens and Maitland estuaries, exhibited close links with autochthonous nutrient input coupled with some river inflow (Suzuki et al. 2002, Santagelo et al. 2007). Whereas, in the Van Stadens and the Maitland and in a number of South African TOCEs nutrient input is normally associated with rainfall-induced river inflow and increases in nutrient supplies are closely linked with an increase in phytoplankton biomass and a shift in community composition (Gama et al. 2005, Thomas et al. 2005).

Freshwater inflow into the Van Stadens and Maitland estuaries is mainly from the river, although the Maitland Estuary may receive some freshwater input from groundwater since mean salinity values over a three year period were < 3 psu compared with 14 psu for the Van Stadens. In the oligohaline groundwater fed Iquipari and Imboassica coastal lagoons, in Brazil, salinity values remained below 3 psu until the sand bar was breached. This was normally done artificially and resulted in higher salinity levels thereafter (Suzuki et al. 2002, Santagelo et al. 2007). One of the distinguishing features between the South American estuaries and some Australian ICOLLs and some South African TOCEs is that increases in salinity in the estuary follows sustained open mouth conditions after natural breaching events as well as from marine overwash, which helps to maintain high levels of salinity within the estuary. Although a few estuaries in South Africa have been artificially breached, this is not the norm as seen with similar estuaries in Australia, Brazil, and the northeast US (Suzuki et al. 2002, Gobler et al. 2005, Santagelo et al. 2007, Everett et al. 2007). Incidents of marine overwash in some South African TOCEs are common and play significant hydrodynamic and ecological functional roles through estuarine water volume augmentation, increase in salinity and the recruitment of plant and animal life (Froneman 2006, Anandraj et al. 2007).
<table>
<thead>
<tr>
<th>South Africa</th>
<th>Chlorophyll a (µg L⁻¹)</th>
<th>Production (g C m⁻² y⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bot River</td>
<td>0-6</td>
<td>0-57.5</td>
<td>Bally et al. 1985</td>
</tr>
<tr>
<td>Kasouga</td>
<td>9-21</td>
<td>8.6-13.5</td>
<td>Froneman 2002a</td>
</tr>
<tr>
<td>Maitland</td>
<td>5.3-32.7</td>
<td>0.42-8.8</td>
<td>Present Study</td>
</tr>
<tr>
<td>uMdloti</td>
<td>0.09-8.6</td>
<td>26.3-1112</td>
<td>Perissinotto et al. 2003</td>
</tr>
<tr>
<td>uMpenjati</td>
<td>0.5-11</td>
<td>187-1822</td>
<td>Perissinotto et al. 2003</td>
</tr>
<tr>
<td>Van Stadens</td>
<td>1.4-4.1</td>
<td>0.95-5.10</td>
<td>Present Study</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>International</th>
<th>Chlorophyll a (µg L⁻¹)</th>
<th>Production (g C m⁻² y⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smiths Lake</td>
<td>0.5-4</td>
<td><em>-</em></td>
<td>Everett et al. 2007</td>
</tr>
<tr>
<td>Iquipari Coastal Lagoon</td>
<td>0-500</td>
<td><em>-</em></td>
<td>Suzuki et al. 2002</td>
</tr>
<tr>
<td>Imboassica Lagoon</td>
<td>0-200</td>
<td><em>-</em></td>
<td>Santangelo et al. 2007</td>
</tr>
</tbody>
</table>

Note: * no productivity data available.

The once-off primary production study carried out in both estuaries showed that the nanophytoplankton displayed the highest levels of productivity across all seasons. Changes in the condition of the mouth (i.e. open or closed) did not significantly affect phytoplankton production more so than temperature. Phyttoplankton production in winter was lower than in any other season in both the Van Stadens and Maitland estuaries. The predominance by the nanophytoplankton at all sites is indicative of the importance of this size group to water column production in both estuaries. This supports the idea that the Van Stadens and the Maitland estuaries are oligotrophic systems. The two estuaries have low macronutrient concentrations and primary production is driven by small-sized phytoplankton that display high turnover rates normally associated with ecosystems that feature microbial loop characteristics (Caroppo 2000, Froneman 2002b, 2003, Jochem 2003). However, unlike other typical oligotrophic systems the picophytoplankton does not feature as a significant group, which has been linked with most of the phytoplankton production in these systems (Jochem 2003, Ueno 2005). Productivity data from studies in similar estuaries internationally are presently lacking and make it difficult to compare results from studies conducted in South Africa with those abroad. Evidently this highlights an important gap in our knowledge of similar estuaries from abroad that needs addressing. However, biomass data is available and allows for comparisons to be made.
With the exception of Smiths Lake in eastern Australia, biomass values from the Imboassica and Iquipari Lagoons in Brazil depict a wide range of chlorophyll \( a \) biomass (i.e. 0-200 & 0-500 \( \mu \)g L\(^{-1} \) respectively) suggestive of very productive estuaries. Although, these estuaries may display similar morphometric and possibly similar hydrodynamic changes to mouth opening, they however exhibit different ecological functioning as most are significantly affected by development near their shores and thus have to be artificially open to control for flooding, eutrophication and improved fishery.

Results from the short-term study in the Van Stadens Estuary showed distinct differences and similarities in the \( Chl \ a \) response to increased river inflow with that collected over longer time scales (e.g. seasonal and annual). Strong river flow resulted in a reduction in \( Chl \ a \) concentrations followed by phytoplankton \( Chl \ a \) recovery period of 8-10 weeks. In contrast, the short-term study showed a peak in \( Chl \ a \) in a couple of days. These responses may be dissimilar but may illustrate a similar response pattern to physical and chemical mechanisms essential in supporting phytoplankton biomass. The amount of time the mouth remains open may be critical in establishing and maintaining a phytoplankton community.

The implications of such findings are significant to researchers and conservation managers with regard to the influence of rapid changes in physical and chemical factors and their effect on the microflora. What this study demonstrates is that short-term exposure to variable river influx rapidly reduces salinity and increases nutrients that stimulate phytoplankton biomass only after the mouth has closed. In addition, it shows that nutrients were rapidly depleted possibly from being taken up by the phytoplankton and that if residual supplies of nutrients exceeded uptake rates this might favour conditions that would lead to eutrophication (Flemer and Champ 2006). Changes in mouth condition are one of the important physical features in TOCEs and breaching whether natural or artificial have a profound influence on the phytoplankton. Natural breaching, lowers estuarine water volume, increases the euphotic depth, brings in nutrients that stimulate phytoplankton production and lowers salinity. On the other hand artificial breaching would also lower estuarine volume, but would introduce saline water without much dilution from river water. Higher salinity in the estuary would alter the phytoplankton species diversity favouring that tolerant of high salinity at the expense of brackish species (Flöder and Burns 2004).

The response of phytoplankton communities followed a seasonal temperature pattern in both the Van Stadens and Maitland estuaries and was varied. During the closed mouth phases when macronutrients were low densities of large-sized mixotrophic dinoflagellates (e.g. \textit{Amphidinium operculatum}, \textit{Protoperidinium bipes}, & \textit{Peridinium} sp.) and heterotrophic tintinnids comprised a major proportion of the phytoplankton and zooplankton communities in the water column respectively. Mixotrophic forms of nutrition have been well demonstrated in estuarine and coastal waters and sustain some
photoautotrophic planktonic communities under depleted macronutrient conditions (Katechakis and Stibor 2006, Paffenhöfer et al. 2007). Mixotrophy in oligotrophic estuaries including TOCEs has not been adequately demonstrated, yet in this study phagotrophic feeding activities by dinoflagellates and chrysophytes on small green algal were observed from preserved samples collected during dry periods in the Van Stadens Estuary. Although the observations were qualitative carried out on preserved samples, it is clear that this mode of nutrition may help explain the success of microphytoplankton during periods of low macronutrients to supplement their nutritional requirements (Katechakis and Stibor 2006). Although the Maitland Estuary experienced periods of low concentrations of macronutrients it however did not have large densities of mixotrophic phytoplankton perhaps indicating its slightly fertile characteristic in comparison with the Van Stadens Estuary. There may well be other reasons not addressed by this study that could explain the general absence of this group of phytoplankton in the Maitland Estuary. In addition, from general qualitative assessments the heterotrophic community of tintinnids was relatively absent in most of the preserved samples signalling a possible minor role of this group of zooplankton grazers within the grazing community. A once off zooplankton quantitative assessment at Station 1 during summer in the Van Stadens showed that *Pseudodiaptomus hessii* was the most abundant zooplankton followed by *Acartia longipatella* (5.8 and 2.7 x10³ ind.m⁻³, respectively) (Gama et al. 2005). These two copepod species are wide spread along a number of estuaries in the southeastern coast of South Africa (Froneman 2004a). This however would warrant further quantitative investigations that would elucidate the role of the zooplankton grazing community in structuring phytoplankton in the Maitland Estuary.

Results from MDS analyses that were based on presence/absence of species counts suggest that mouth condition influenced phytoplankton distribution patterns. Furthermore, there were strong temporal effects on phytoplankton species exposed to similar hydrological processes (e.g. breaching or closed/overwash mouth condition) within months of the same season in the same year than species occurring in months of a similar season in different years (e.g. greater similarity between summer phytoplankton species of year-1, than summer species of year-2 when the mouth is either open or closed) (Cermeño et al. 2006). What this suggests is that the frequency and timing of these episodic events (e.g. freshwater flooding or saline water introduction), which can be characterised as disturbances, may trigger a composition of similar functional phytoplankton species but may not necessarily be the same species or composition of species each time there is a disturbance (e.g. high river inflow and macronutrients, turbidity, salinity, and marine water penetration). For instance, in the Van Stadens Estuary when large-sized dinoflagellates featured strongly in the water column they co-occurred with small-sized flagellated chlorophytes, cryptophytes and some diatoms, whereas when large-sized cryptophytes were present they co-occurred with smaller sized flagellated cryptophytes, dinoflagellates and non flagellated greens. Although a similar case can be made for the Maitland Estuary, the species identified were different to those encountered in the Van Stadens Estuary possibly
because of lower salinity. Cyanophytes were a strong feature in the Maitland phytoplankton represented by filamentous and coccoid species (e.g. *Anabaena* sp. & *Chroococcus minutus*) respectively. Further investigations into this response pattern of phytoplankton species composition in both estuaries is warranted in order to understand mechanisms that bring about the shifts and how these changes affect food web transference of carbon.

### 9.3 Conclusion

This study has demonstrated that flooding events caused by strong river flow cause breaching of the mouth, a reduction in salinity and marked nutrient input. The high river flow causes high turbidity that can significantly reduce the euphotic depth thereby limit photosynthesis, which affects phytoplankton growth in both the Van Stadens and Maitland estuaries. Although the causes of flooding can be similar in both estuaries the resultant effects are varied and can bring about a significant change in the geomorphology of an estuary channel by raising the bed floor thereby altering the ability of the estuary to retain water thus increasing the frequency of a semi-closed mouth state. The study was able to demonstrate that the supply of macronutrients from the catchment was strongly correlated with rainfall ($R^2 = 0.67$) and that phytoplankton growth mainly depended on an allochthonous source of macronutrients although internal supplies can be critical at times in controlling microalgal biomass. The high ammonium concentrations in both the estuaries were possibly important in acting as a source of nitrogen when other source of nitrogen became depleted. Macronutrient input from increased river inflow stimulated phytoplankton biomass resulting in significant peaks in *Chl a* that were associated with each breaching event. This investigation demonstrated the significance of the duration of an open-mouth phase that lasted (e.g. 6 – 8 weeks) which was critical in affecting the connectivity to the marine environment and hence the magnitude of *Chl a* concentrations. This study also showed that microphytoplankton can become dominant mostly when the mouth was open but also when the mouth is closed and that this is an aspect that has not been previously demonstrated in the literature i.e. microphytoplankton can contribute a significant amount of biomass during closed mouth conditions. Nanophytoplankton showed the highest levels of productivity across all seasons over all other size fractions and possibly played an important role in the transfer of carbon energy to higher trophic levels in both the Van Stadens and Maitland estuaries. Chlorophytes during the summer seasons became the dominant taxon when the mouth was closed and dinoflagellates because they are mostly marine became the dominant group when the mouth was open. Frequency and timing of river inflow was important in triggering shifts in composition of functional phytoplankton species in successive years. This study showed that although two similar sized estuaries shared a similar climate and geological history their ecological behaviour and response to environmental factors was varied.
10. References


Phytoplankton Chlorophyll a Concentration and Community Structure


Phytoplankton Chlorophyll a Concentration and Community Structure


Appendix 1

Plate 1.1  The Van Stadens Estuary showing the middle reaches A & B, and lower reaches C & D before and after the floods of 2002, E & F are river sites (7, 8 and 9, 10 respectively) in the upper catchment of the Van Stadens River. Note the national road (N2) bridge in panel E.
Plate 1.2  The Maitland Estuary showing the upper to middle reaches facing toward the sea A & B, and lower reaches C & D before and after the floods of 2002. E & F upper to middle reaches, note shallow depth and black stained water.
Appendix 2

Plate 2.1  Taxa identified from the Maitland and Van Stadens estuaries A – *Amphidinium* sp. a mixotrophic dinoflagellate with extended microfibrilar extensions used to capture small phytoplankton. B & C – *Protoperidinium quinquecorne* stained with Lugol’s solution. D – *Cyclotella* sp., E – *Gyrosigma* sp., F – *Navicula longissima*