PRIMARY PRODUCERS AS SINKS FOR NITROGEN AND PHOSPHORUS IN THE GREAT BRAK ESTUARY

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WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND

Estuaries are productive ecosystems and are of economic, recreational and aesthetic value (Scharler & Baird, 2005). There are three major areas of concern that estuarine managers face; changes in river flow, eutrophication and sedimentation (Day *et al*. 1989). Poor river catchment management has increased suspended sediment and nutrient loads entering estuaries, eutrophication (defined as enhanced primary productivity due to nutrient over enrichment) being one of the consequences. Subsequently, a build-up of organic matter can result in low oxygen conditions due to microbial decomposition, negatively affecting organisms within the estuary and making the estuary aesthetically unsuitable for human use.

The Great Brak Estuary is a Temporarily Open/Closed Estuary (TOCE) on the southern Cape coast that frequently experiences blooms of filamentous green macroalgae (*Ulva intestinalis* and *Cladophora glomerata*), particularly around Die Eiland in the lower reaches during closed mouth conditions. The high macroalgal cover (>50%) and wet mass (634 to 755 g m^{-2}) prior to the dam release in September 2007 is indicative of an estuary in a poor ecological state according to the EU Water Framework Directive. Results from recently completed Ecological Freshwater Reserve Determination study of the estuary (2008) highlight four contributing factors that may have caused the macroalgal growth;

1) *Reduced river flow*; Afforestation, direct abstraction and damming of river water have reduced flow into the estuary by 56%.

2) *Elevated nutrient loads*; the banks of the estuary support extensive residential development (some of which use septic tanks), agriculture and commercial areas. The significant accumulation of organic material (as a result of anthropogenic activities and accumulated plant litter) together with longer residence times (less freshwater flushing) has enhanced the influence of re-mineralisation processes. As a result, the dissolved organic nitrogen is dominated by the total ammonia-N fraction.

3) *Reduced frequency and intensity of floods* (including the damming effects of bridges and their abutments); large floods and the natural breaching of the estuary mouth are necessary to limit the ingress of marine sediment through the estuary mouth. The hydrological data indicates that the magnitude and occurrence of major floods (1:50 years and higher) has been reduced by 10% to 15%, while medium term floods (1:10 and 1:20 years) have been reduced about 20%. This also means that the potential for

flushing of sediments during such floods has been reduced somewhat. In addition to the reduced intensity of floods, the bridge in the town of Groot Brak has the largest damming effect of the five bridges within the estuary, which is worsened by clogging of the bridge openings by debris such as tree trunks during flood events.

4) *Altered mouth dynamics;* the Great Brak Estuary is breached artificially to prevent flooding of low-lying development on the Island. The estuary is currently breached at approximately 2.0 m MSL. Natural breachings used to occur at levels between +3.0 and +3.5 m MSL. This would have resulted in maximum outflow velocities of between 200 and 500% greater than present, resulting in larger loads of sediment being flushed out of the estuary – particularly the lower reaches.

RATIONALE

A common perception of TOCEs is that they always experience water quality issues. This view is likely to become more entrenched as the human population living around the coast increase, forming dense nodes of development at estuary mouths. This leads to increases in the concentration of nutrients, the incidents of sewage spills, the abstraction of freshwater and the frequency of estuary mouth closure. In many cases the water in the estuary could become a serious health risk and could also stimulate the growth of nuisance macrophytes or macroalgae blooms. These impacts, to mention a few, have a serious impact on the ecosystem services provided by estuaries. To maintain a healthy state requires continuous intervention in order to mitigate the impacts of human activities and to adapt use to changing needs and circumstances.

In the last few years the Council for Scientific and Industrial Research (CSIR) have been developing a proposed generic framework for estuary management plans (EMP) together with the CAPE Estuaries Programme (CSIR, 2009). With regard to the Great Brak Estuary, the information gained from this project is likely to add to the Situation Assessment and Evaluation of the planned EMP. More importantly, approximately 70% of estuaries in South Africa are temporarily open/closed estuaries, a number are experiencing similar problems as the Great Brak, and these are likely to benefit from the proposed management plans developed from this study.

OBJECTIVES AND AIMS

AIM 1

Identify the sources and determine the loads of nitrogen and phosphorus entering the estuary; through point source discharge (e.g. river, sea and storm drains), diffuse discharge (e.g. groundwater seepage from septic tank overflow and golf course irrigation water), atmospheric deposition (rain water) and remineralisation from organic material trapped in the sediment.

AIM 2

Measure the flux of nutrients between the water column and the benthos.

AIM 3

Determine the biomass and cover of macrophytes and macroalgae in the estuary.

AIM 4

Measure the nitrogen and phosphorus content in living plant material; this combined with the biomass/cover will provide an accurate estimate of how much N and P is trapped in the macrophytes and macroalgae.

AIM 5

Describe the environmental conditions in the estuary that favour macroalgal blooms.

AIM 6

Provide recommendations to be included in the Great Brak Estuary Management Plan.

AIM 7

Compare results from the Great Brak Estuary, an estuary dominated by macrophytes and macroalgae, to estuaries dominated by phytoplankton (e.g. the permanently open Sundays Estuary).

METHODOLOGY

The Great Brak Estuary is located on the south coast of South Africa between Mossel Bay and George. The semi-arid catchment (192 km^2) receives a fairly evenly spread amount of rainfall throughout the year with slight peaks in spring and autumn. However, the area is subjected to droughts and occasional flooding and the recorded annual run-off varies from as little as 4.3×10^6 m^3 to as large as 44.5×10^6 m^3 (CSIR 1983). The Wolwedans Dam with a capacity of 23×10^6 m^3 is located three kilometres upstream of the estuary. The average annual volume of flow to the estuary has been considerably reduced during the last decades by afforestation, direct abstraction and damming from 36.8×10^6 m^3 under natural condition to 16.3×10^6 m^3 at present. The Great Brak Estuary is about 6.2 km long with a high tide area of 0.6 km^2 and a tidal prism of 0.3×10^6 m^3. The estuary mouth is bounded by a low rocky headland on the east and a sand spit to the west. Immediately inland of the mouth the estuary widens into a lagoon basin containing a permanent island that is about 400 x 250 m in size. The lower estuary is relative shallow (0.5 to 1.2 m deep) with some deeper areas in scouring zones near the rocky cliffs and bridges. The middle and upper estuary is less than 2 m deep, with some deeper areas between 2 and 4 km from the mouth varying between 3 and 5 m deep. The mouth of the Great Brak Estuary generally closes when high waves coincide with periods of river flow. Artificial breaching is practised at the Great Brak Estuary to prevent flooding of low lying properties. River flow data are available from a DWA gauging station K2H006-A01 that is located 2.1 km from the head of the estuary. There are a number of advantages in selecting the Groot Brak as a study site;

1) The Wolwedans Dam is located near to the head of the estuary. Any planned releases from the dam are communicated through CSIR Stellenbosch and the impacts of the release can be closely monitored.

2) There is a flow gauge located at the head of the estuary and the flow rates are frequently updated on the DWA hydrology website. This means that the loads of nutrients entering the estuary in river water can be accurately measured.

3) The estuary has been well monitored by the CSIR for a few decades and a Resource Directed Measures study of the estuary was completed in 2008. As a result, the estuary is one of the most data-rich systems in the country.

4) The C.A.P.E. Estuaries Program is in the process of developing an Estuary Management Plan for the Great Brak Estuary. The information gained through this study is likely to

highlight the sources of nutrients in the estuary and propose management plans to mitigate the effects.

There were 10 sampling sessions, based on a quarterly sampling regime, of the estuary starting September 2010 to July 2012. Two 24-hour benthic flux experiments were conducted in March 2012 and July 2012. Samples were collected from 11 sites located along the length of the estuary, starting in the sea at the mouth of the estuary and at the DWA weir at the head of the estuary. An estimate of the load of atmospheric deposition from rain events was based on rainwater collected in a rain gauge located at the town of Groot Brak and analysed for nutrients.

Samples collected from 1 m depth intervals at each site in the estuary were filtered and chemically analysed in the laboratory using manual determinations as described in Parsons *et al*. (1984) for total oxidised nitrogen (NO_3-N + NO_2-N), phosphate measured as dissolved inorganic phosphate (DIP) and phytoplankton chlorophyll *a*. Ammonium (measured as NH_4-N) was measured by the manual determination method described in Grasshoff *et al*. (1983). Unfiltered water, sediment and plant material were digested and analysed for total nitrogen (TN) and total phosphorus (TP) using persulphate digestion procedures (APHA 1998). TN and TP after mineralisation with potassium persulphate were determined as nitrate and reactive-P respectively.

Salinity, temperature, pH and dissolved oxygen were measured throughout the water column at each site using a Hanna multiparameter probe.

Four benthic chambers based on the design described by Switzer (2004) were deployed at a site within the lower estuary, where macroalgal blooms frequently occur in winter and in summer. The sampling schedule for the benthic chambers was designed to measure day flux, night flux, and 24-hour flux. *In situ* water conditions (salinity, pH, dissolved oxygen and temperature) were measured using Hanna and YSI multiparameter probes.

RESULTS AND DISCUSSION

The Great Brak Estuary is a located on the South Coast of South Africa and is 6.2 km in length. A dam built just 3 km upstream from the estuary has severely affected the natural flow to the estuary, and has reduced the amount of freshwater flow to the estuary by as much as 56%. This in turn has led to reduced flushing, accumulated organic matter and degradation in the water quality. Associated changes in the physico-chemical characteristics primarily included dissolved oxygen, NH_4^+, and SRP. The oxygen saturation data showed that saturated conditions (+6 mg l^{-1}) were not the general state in the estuary. On the

contrary, hypoxic and even anoxic conditions were rather common. The average NH_4^+ concentration in the estuary averaged about 7 µM and increased with depth to about 12 µM at 2 m depths. Concentrations >45 µM were found in February and April 2011 in the deepest section of estuary, 3.4 km from the mouth. Soluble reactive phosphorus (SRP) was generally below 1 µM in the water column for most months, and had peaks at 1.0 km and 3.4 km in the bottom water. Increases in these nutrients were most likely due to low dissolved oxygen and increased remineralisation of organic matter.

Changes in the submerged macrophytes and macroalga were mostly influenced by mouth state and river inflow. During the closed phase the filamentous macroalgae *Cladophora glomerata* had an area cover ranging from 3000 to 6000 m^2 while *Zostera capensis* and *Ruppia cirrhosa* covered areas ranging from 2000 to 3500 m^2 and 1500 to 2900 m^2 respectively. After the artificial breach, water drained out of the estuary leaving the alga stranded on the marshes and as the flood tide entered the macroalgae was once again redistributed. The alga was then able to utilise the available nutrients in the water column and expand its area cover from 35000 m^2 in February 2011 to 64000 m^2 in March 2011. However, after a large flood event in June 2011, *C. glomerata* had effectively been flushed from the system while the seagrasses responded positively extending their area cover. By comparing the artificial breach with the natural breach, and their effect on the estuary, an important observation was highlighted. By increasing the current allocated freshwater reserve, and using a larger volume of water to breach the mouth artificially, better scouring of sediment and associated organic matter from the estuary could be achieved. This would enable better oxygenation of the water column, reduce remineralisation and minimise algal blooms.

Fluxes of inorganic nutrients (NH_4^+, TOxN [NO_3^- + NO_2^-], SRP) as well as total N and P across the sediment-water interface were investigated within the estuary. This was done in order to quantify the exchange of N and P across this interface so that the potential contribution of sediments to the water column nutrient budget could be determined. The results show that dissolved oxygen concentrations in all incubations during both the summer and winter deployments decreased over the incubation period. Correspondingly there was a decrease in TOxN concentrations, suggesting an uptake from the water column. Decreases in TOxN could also be due to uptake by denitrification. The NH_4^+ and SRP efflux in all incubations during both summer and winter indicate that the sediment in the lower Great Brak Estuary was acting as a source of these inorganic nutrients. The TN and TP followed a similar trend to NH_4^+ and SRP. If the estuary remains closed for a prolonged period

(12 months) with an associated increased organic load present on the benthos, the rate of effluxes from the sediment of N and P would increase.

The relative importance of the submerged macrophytes and microalgae to other sources was quantified in the nutrient budget and it was shown that they play an important role in nutrient storage and subsequent cycling. The submerged macrophytes and macroalgae took up to 30% TN and 38% TP from the water column during the closed phase. However, the sediments were the highest contributors of TN and TP to the system and contributed about 30% of the TN and 40% TP toward the nutrient budget. The river and precipitation contributed less than 3% of the TN and TP input. It has been previously believed that the sediments of South African TOCEs did not have the necessary organic stock to fuel subsequent production. Two important findings arose from this work; the sediment does have the necessary organic stock to fuel production, and that the submerged macrophytes have a significant contribution to nutrient cycling. Nutrient budgets often account for the vegetation within an estuary by relying on their C:N:P ratios. This often leads to underestimates of N and P storage because luxury uptake is common within macrophytes. This is the first detailed account of including macrophytes in a nutrient budget using actual tissue N and P concentrations.

Aim 1

The sediment constantly contributed about 30% of the TN and 40% TP of nutrient transferred to the water column during the closed mouth state. During the closed phase, sediments are therefore the highest contributors of TN and TP to the system. The river and precipitation contributed less than 3% of the TN and TP input. Under the closed mouth state it is unlikely that the nutrients in river water passing the head of the estuary at the weir will reach the lower end of the system and it is therefore expected that the lower reaches become benthic driven with respect to nutrient input.

When the mouth opened under strong flow conditions the Great Brak Estuary acted as a source of nutrients to the sea. This is evident from the magnitude of the loads of N and P coming into and flowing out of the system. The percentage contribution of the river increased from less than 3% of the TN and TP under the closed phase to about 50% and 40% contribution respectively under the open phase. Similarly, there was an increase in the percentage contribution of the error component, which might mainly be associated with an increase in the flow from diffuse sources in the catchment.

Aim 2

Dissolved oxygen concentrations in all incubations during both the summer and winter deployments decreased over the incubation period. Correspondingly there was a decrease in TOxN concentrations, suggesting an uptake from the water column. Decreases in TOxN could also be due to uptake by denitrification. The NH_4^+ and SRP efflux in all incubations during both summer and winter and indicates that the sediment in the Great Brak Estuary was acting as a source of these inorganic nutrients. The TN and TP followed a similar trend to NH_4^+ and SRP.

Aim 3

The dry mass of *Cladophora glomerata* remained stable throughout the study period, even though its area cover decreased during the open mouth state. During the open mouth state the most dominant submerged macrophytes (*Zostera capensis* and *Ruppia cirrhosa*) increased in both area cover and dry mass.

The area cover of *Cladophora glomerata* increased from 23375 m^{-2} in June 2010 to 58339 m^{-2} in August 2010. After the bloom collapsed (August to December 2010) the submerged macrophytes extended their area cover; *Ruppia cirrhosa* ranged from 22913 to 29945 m^{-2} and *Zostera capensis* from 20529 to 37481 m^{-2}. Directly after a freshwater pulse in February 2011 the macroalgae increased its area cover from 36013 m^{-2} to 64336 m^{-2} in March 2011. The bloom was subsequently flushed out of the estuary by the flood in June 2011. Area cover was converted to biomass to determine nutrient load by measuring the biomass of a known core diameter of samples collected from 100% surface cover stands of submerged macropytes and macroalga.

Aim 4

The relative importance of the submerged macrophytes and microalgae to other sources was quantified in the nutrient budget and it was shown that they play an important role in nutrient storage and subsequent cycling. The submerged macrophytes and macroalgae took up to 30% TN and 38% TP from the water column during the closed phase. However, the sediments were the highest contributors of TN and TP to the system and contributed about 30% of the TN and 40% TP toward the nutrient budget. The river and precipitation contributed less than 3% of the TN and TP input.

Aim 5

Of the three estuary states (freshwater dominated, marine dominated and closed mouth) occurring in TOCEs as described by Snow and Taljaard (2007), the closed phase represents a relatively stable state. The closed mouth phase is characterised by having little to no river inflow at the head of the estuary and the formation of a sandbar at the mouth that prevents tidal exchange with the estuary and sea. In small shallow estuaries like the Great Brak, the closed mouth state eventually reverts to a well-mixed (due to wind turbulence) brackish system, with no distinct salinity gradient or stratification. Flushing times depend on the duration of mouth closure which can range from days to years (Taljaard *et al*. 2009 a). This state therefore presents one of the most favourable environments for the growth of *Cladophora glomerata.* Changes in the submerged macrophytes and macroalgae were mostly influenced by mouth state and river inflow.

Aim 6

The C.A.P.E. Estuaries Program is in the process of developing an Estuary Management Plan for the Great Brak Estuary. Once published, the information gained through this study is likely to highlight the sources of nutrients in the estuary and propose management plans to mitigate the effects.

Aim 7

Based on Biggs (1996) strong base flow and open mouth conditions typically favour a top-down control of primary producer growth; i.e. in permanently open estuaries with a stable base flow, factors such as flooding, turbidity, saline intrusion, nutrients in the water column, etc. support a phytoplankton driven system (e.g. Sundays, Swartkops and Gamtoos estuaries). In contrast, low flow conditions and mouth closure can support the bottom-up control of primary producers; i.e. greater light availability, limited nutrients in water column, more stable environment. These conditions are likely to support the growth of opportunistic filamentous macroalgae).

CONCLUSIONS

Temporarily Open Closed Estuaries generally have low river inflow, a long water residence time due to weak flushing, and prolonged mouth closure (Whitfield 1992, Taljaard *et al*. 2009 b) which makes this type of estuary vulnerable to nutrient enrichment and the accumulation of organic matter (Newton and Mudge 2005, Human and Adams 2011). In the closed mouth state in the Great Brak, it is unlikely that nutrients in river water passing the

head of the estuary at the weir will reach the lower end of the system while the estuary remains closed. It is therefore expected that in the lower reaches the nutrients will be supplied from the benthos, i.e. the system will become benthic driven.

After prolonged mouth closure water levels rise and the large salt marshes die back and submerged macrophytes and macroalgae become the dominant plant components within the estuary. As these macrophytes go through their life-cycle i.e. growth, reproduction and death, they act as sources and sinks for N and P within the estuary, and may be causing an accumulation of organic matter in the benthos. Moreover, the presence of the opportunistic macroalgae, *Cladophora glomerata,* which has a much shorter life-cycle than the submerged macrophytes, most likely leads to an even greater accumulation of organic input. The resultant organic load more than likely then fuels subsequent growth i.e. the remineralised organic matter in the form of DIN and DIP which support the next, or current, cycle of growth for the submerged macrophytes. In this study in the Great Brak, from the time the mouth closes until it reopens, *C. glomerata* stored 9406 kg TN and 2176 kg TP. If the mouth of the estuary were to remain closed for a whole year, the estimated storage in the alga could be as high 17288 kg TN and 4675 kg TP. The submerged macrophytes clearly play a significant role in removing and cycling N and P from the water column.

Apart from the cycling of nutrients between the submerged macrophytes and macroalgae, the benthos within the estuary acted as a major source of N and P to the water column. Throughout the closed mouth state the benthos contributed about 30% of the TN and 40% of the TP transferred to the water column. Some studies on ICOLLs in Australia showed that the benthos was the most important source and sink of N and P to and from the water column and could potentially contribute as much as 3 to 4 times the catchment discharge annually (Smith *et al*. 2001, Palmer *et al*. 2002, Spooner and Maher 2009). During benthic chamber deployments, the oxygen within the chambers decreased over the incubation period as a result of respiration. As conditions become anoxic, the major processes occurring in the benthos that lead to the availability of NH_4^+ in the water column are DNRA and remineralisation of labile organic matter. The benthos of the Great Brak, had a net efflux of NH_4^+, SRP, TN and TP and acted as a source of N and P to the water column. This ensured that there was always N and P available for the macrophytes. This is especially important under prolonged closed mouth conditions that cause a build-up of organic matter from decaying macrophytes fauna. The build-up of organic matter in the Great Brak Estuary has been exacerbated by the manner in which it is not flushed since the construction of the large Wolwedans Dam further upstream.

It has been previously believed that the benthos of South African TOCEs did not have the necessary organic stock to fuel subsequent production (Taljaard *et al*. 2009 b). Two important findings arose from this work; the benthos of the Great Brak Estuary does have the necessary organic stock to fuel production and the submerged macrophytes make a significant contribution to nutrient cycling. Nutrient budgets globally, such as the LOICZ models, took the macrophytes within the estuary into account by using their C:N:P ratios and some don't account for the vegetation at all. An inherent shortcoming of this type of modelling approach is that nutrient storage in the vegetation may actually be underestimated because plants often store more N and P than what is presented in a nutrient ratio. This is the first detailed study that accounts for the nutrient storage in macrophytes by using actual measured data thereby emphasising just how important the submerged macrophytes and macroalgae are to a nutrient budget of an estuary. It also becomes clear that it is critical to rely on actual measurements of nutrient storage when dealing with nutrient budgets because the contribution of any macrophyte in an estuary or other type of water body is large and cannot simply be ignored. This research has shown that the submerged macrophytes and macroalgae play important roles in storing and subsequently re-cycling nutrients within the Great Brak Estuary. It has also shown that, in the closed mouth state, the benthos becomes the major source of nutrients to the water column and supplies a similar percentage of N and P to that which gets stored in the submerged macrophytes and macroalgae.

RECOMMENDATIONS FOR FUTURE RESEARCH

A decade ago Switzer (2003) stated that the majority of estuaries in South Africa did not seem to be experiencing problems associated with increased nutrient loads (e.g. eutrophication), which had affected many estuaries in the Northern Hemisphere. As a result, South African estuary research has been less concerned with understanding the processes that govern N and P cycling in these oligotrophic estuaries, than it has been with understanding problem-specific processes such as freshwater flow requirements (Adams *et al*. 2002). Switzer (2003) further warned that if South African estuaries remain unstudied with respect to the processes that govern N and P cycling, it will be possible for modern estuary science to forfeit the opportunity to obtain valuable information on how these estuaries function prior to a time when they suffer from eutrophication. Howarth *et al*. (2002) stated that the human alteration of nutrient cycles was not uniform over the earth and that the greatest nutrient cycle changes were concentrated in areas of greatest population and agricultural production. Therefore, the estuaries of South Africa may not have escaped the problems associated with increased nutrient loads in so much as they have delayed it with their moderate pace of industrialisation, limited use of fertilisers and limited population

densities living on the estuarine littoral and in the catchments. Clearly, South African estuaries have reached the point where they are becoming eutrophic. It is recommended that further investigation on the biogeochemical cycling of South African estuaries be undertaken before they become hypertrophic. This would enable a baseline data source that will become extremely important to the country's water resource managers as they are faced with ongoing development and economic growth. In order to ensure sustainable development and use of an estuary, it is important to know in which state an estuary resided before it became eutrophic. This would provide resource managers and decision makers with the necessary information when decisions are to be made on sustainable use of estuary resources. The Resources Directed Measures (RDM) relies heavily on predicting the state of an estuary in the past, the present and in the future, under different flow scenarios and information on the internal nutrient cycling of estuaries would be beneficial to the RDM methods of determining the health of an estuary.

The findings on the Great Brak Estuary have shown that during the closed phase, relative to "other sources", the benthos contributed the largest source of N and P to the system. This occurred mainly because the flushing mechanism has been removed in this estuary. Similar conditions may arise in other small estuaries in South Africa and in other parts of the world because the demand for freshwater for agriculture, industry and domestic use appears endless. Snow (2008 b) has emphasised that the greatest uncertainties in terms of the biogeochemical or water quality characteristics and processes in TOCEs, particularly those in the cool- and warm-temperate zones, are related to the levels of inorganic and organic nutrients in these systems. Further research is therefore urgently required from a variety of different TOCEs across climatic regions in order to determine how different sediment types in South African TOCEs function with regard to benthic nutrient fluxes, as well as the role of detritus, if we are to understand biogeochemical water quality changes. Because the South African coastline extends across three different climatic regions (subtropical, warm temperate and cool temperate; Figure 2.1) each having different geological composition, it would be rather important to determine how these estuaries function with regard to benthic fluxes over seasonal cycles. This information would bring further clarification on whether or not the estuaries across the climatic regions containing different sediment composition are similar or different to one another, and also provide information on whether or not the different sediment types act as sources or sinks of N and P to their specific water columns. This information is particularly important for the estuaries along the coast which have not yet been heavily impacted by anthropogenic influences, unlike the Great Brak. Not only will it provide necessary information that will aid management, but it will also provide insight into how South African estuaries behaved in their natural state. Finally in conjunction with this

research, it is also recommended that research be conducted on the effect that fauna have on benthic nutrient fluxes. There has been no research in South African TOCEs that have investigated the role that fauna have to play in nutrient cycling. Further investigation on fauna, particularly macrofauna on the benthos of TOCEs need to be studied. It has been shown in other parts of the world that macrofauna enhance benthic reactivity and increase the efficiency of solute mass transfer between the water column and benthos (Kristensen and Hansen 1999, Webb and Eyre 2004). They clearly have an important role to play in influencing the release of nutrients from the benthos.

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TABLE OF CONTENTS

1 INTRODUCTION AND OBJECTIVES 1
2 LITERATURE REVIEW 4
2.1 Introduction to South African Temporarily Open/Closed Estuaries 4
2.2 The cycling of N and P in estuaries 8
2.2.1 Limiting nutrients in the aquatic environment 8
2.2.2 Nitrogen: A microscopic view 8
2.2.3 Phosphorus 12
2.3 Nutrient transformation in the benthic realm 14
2.3.1 Role of primary producers in nutrient cycling 16
2.3.2 Benthic microalgae 17
2.3.3 Macroalgae 17
2.3.4 Submerged macrophytes 21
2.3.5 The effect of grazers 24
2.3.6 The role of decomposition in nutrient cycling 26
2.4 The eco-physiology of *Cladophora* 27
2.4.1 Substrate 27
2.4.2 Hydrodynamics 28
2.4.3 Light 30
2.4.4 Temperature 30
2.4.5 Nutrient availability 31
2.4.6 Reproduction and propagation 32
2.5 Nutrient cycling: A South African context 33
3 THE STATE OF WATER QUALITY IN THE GREAT BRAK ESTUARY AND ITS EFFECT ON THE SUBMERGED MACROPHYTES AND MACROALGAE 34
3.1 Introduction 34
3.2 Materials and methods 36
3.2.1 Study site 36
3.2.2 Physico-chemical characteristics 39
3.2.3 Submerged macrophtyes and macroalgae 41
3.2.4 Data analysis 42
3.3 Results 42
3.3.1 Physico-chemical 42
3.3.2 Submerged macrophytes and macroalgae 59
3.4 Discussion 61
3.5 Conclusion 67
4 THE BENTHIC REGENERATION OF N AND P FROM THE SEDIMENT OF THE GREAT BRAK ESTUARY 69
4.1 Introduction 69
4.2 Materials and methods 71
4.3 Results 72

4.4 Discussion 81
4.5 Conclusion 85
5 THE ROLE OF SUBMERGED MACROPHYTES AND MACROALGAE IN NUTRIENT CYCLING IN THE GREAT BRAK ESTUARY SOUTH AFRICA: A BUDGET APPROACH 87
5.1 Introduction 87
5.2 Materials and methods 89
5.2.1 Data sources 89
5.2.2 Budget overview 91
5.3 Results 93
5.3.1 Total nitrogen (TN) in the dominant submerged macrophytes and macroalga 94
5.3.2 The relationship between the submerged macrophytes and macroalgae as sources and sinks of phosphorus (TP) 99
5.4 Discussion 107
6 GENERAL DISCUSSION AND CONCLUSIONS 113
6.1 Synopsis 113
6.2 Implications for management 114
6.3 Limitations to the study 117
6.4 Future research 119
7 REFERENCES 121
8 APPENDICES 156

LIST OF FIGURES

Figure 2.1 Distribution of biogeographical regions along the South African coast as well as locations of some estuaries (Schumann *et al*. 1999)..... 5

Figure 2.2 The nitrogen cycle showing the chemical forms and key processes involved in the biogeochemical cycling of nitrogen (Herbert *et al*. 1999)..... 9

Figure 3.1 Map of the Great Brak Estuary on the south coast of South Africa..... 37

Figure 3.2 Water level and mouth state of the Great Brak Estuary for the sampling period September 2010 to July 2012. (Open blocks = open mouth conditions and closed blocks = closed mouth condition)..... 38

Figure 3.3 Water level and flow volume for the period November 2010 to July 2011. Open phases are represented by open blocks and closed phases by solid blocks. 38

Figure 3.4 Water temperature along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line..... 44

Figure 3.5 Salinity along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line..... 45

Figure 3.6 Dissolved oxygen concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line..... 47

Figure 3.7 pH along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line. 48

Figure 3.8 Ammonium concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line. 50

Figure 3.9 Soluble reactive phosphorus concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line. 51

Figure 3.10 Total oxidized nitrate concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line. 52

Figure 3.11 Total nitrogen concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line..... 54

Figure 3.12 Total phosphorus concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line..... 55

Figure 3.13 Particulate nitrogen concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line. 57

Figure 3.14 Particulate phosphorus concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line. 58

Figure 3.15 A) Dry mass and B) area cover of submerged macrophytes and macroalgae in the Great Brak Estuary 60

Figure 3.16 A) Total nitrogen and B) total phosphorus per 100 g of submerged macrophytes and macroalga in the Great Brak Estuary..... 61

Figure 4.1 Light and dark benthic chambers deployed at the Great Brak Estuary. 71

Figure 4.2 Temperature during incubation in (A) summer (March 2012) and (B) winter (July 2012). Shaded areas represent evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 73

Figure 4.3 Dissolved oxygen during incubation for (A) summer (March 2012) and (B) winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 73

Figure 4.4 Flux of TN from the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 74

Figure 4.5 Flux of TN from the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 75

Figure 4.6 Flux of TP out of the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 76

Figure 4.7 Flux of TP out of the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations) 76

Figure 4.8 Flux of NH_4^+ from the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 77

Figure 4.9 Flux of NH_4^+ from the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 78

Figure 4.10 Flux of TOxN out of the water column over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 79

Figure 4.11 Flux of TOxN out of the water column over a 27 hour cycle in winter (July 2012). The shaded area represents the evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 79

Figure 4.12 Flux of SRP out of the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 80

Figure 4.13 Flux of SRP out of the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations). 81

Figure 5.1 Bathymetric survey of the lower Great Brak Estuary below the N2 Bridge. 89

Figure 5.2 The water level in relation to volume for the entire estuary. 90

Figure 5.3 Conceptualised box model for water balance in the Great Brak Estuary. 92

Figure 5.4 Total N stored in submerged macrophytes and macroalga in the Great Brak Estuary (■ = closed mouth; □ = open mouth). 97

Figure 5.5 Total P stored in submerged macrophytes and macroalga in the Great Brak Estuary (solid bar = closed mouth; clear bar = open mouth). 101

Figure 5.6 Total storage of TN and TP in the submerged macrophytes and macroalga within the Great Brak Estuary (■ = closed mouth; □ = open mouth)..... 103

Figure 5.7 Total contribution of (A) TN and (B) TP of each component to the Great Brak Estuary (■ = closed mouth; □ = open mouth). The acronym net SMAM stands for the net mass of submerged macrophytes and macroalga. .. 104

LIST OF TABLES

Table 4.1 Total flux of TN from the sediment of light and dark chambers. 74

Table 4.2 Total flux of TP from the sediment of light and dark chambers. 75

Table 4.3 Total flux of NH_4^+ from the sediment of light and dark chambers. 77

Table 4.4 Total flux of TOxN in light and dark chambers. 78

Table 4.5 Total flux of SRP from the sediment of light and dark chambers. 80

Table 5.1 Water balance for the Great Brak Estuary. 94

Table 5.2 Load of TN (kg) in each component of the submerged macrophytes and macroalga (TN stored = ΔC(kg) at time 0+ΔC). 96

Table 5.3 Total nitrogen budget for the Great Brak Estuary. 98

Table 5.4 Load of TP (kg) in each component of the submerged macrophytes and macroalga. 100

Table 5.5 Total phosphorus budget for the Great Brak Estuary 102

Table 5.6 Estimated load (kg) of total nitrogen and phosphorus in each component after a year of mouth closure. 106

Table 5.7 Estimated load (kg) of total nitrogen and phosphorus in each component of the submerged macrophytes and macroalga after a year of mouth closure. 106

LIST OF ABBREVIATIONS

Coefficient of Variation of Annual Flow	Cv
Council for Scientific and Industrial Research	CSIR
Department of Water Affairs	DWA
Dissimilatory Nitrate Reduction to Ammonium	DNRA
Dissolved Inorganic Nitrogen	DIN
Dissolved Inorganic Phosphorus	DIP
Dissolved Organic Matter	DOM
Dissolved Organic Nitrogen	DON
Dissolved Organic Phosphorus	DOP
Dissolved Reactive Silicate	DRS
Intermittent Open and Closed Lakes and Lagoons	ICOLLS
Land Ocean Interactions in the Coastal Zone	LIOCZ
Micro grams per litre	µg l^{-1}
Micromol quanta per square metre per second	micromol m^{-2} s^{-1}
Micromolar	µM
Millimol per square metre per day	mmol m^{-2} d^{-1}
Millimolar	mM
Milligrams per litre	mg l^{-1}
Particulate Inorganic Phosphorus	PIP
Particulate Nitrogen	PN
Particulate Organic Nitrogen	PON
Particulate Organic Phosphorus	POP
Particulate Phosphorus	PP
Permanently Open Estuaries	POEs
Resources Directed Measures	RDM
Soluble Reactive Phosphorus	SRP

Submerged Macrophytes and Macroalgae	SMAM
Temporarily Open/Closed Estuaries	TOCEs
Total Nitrogen	TN
Total Oxidized Nitrate	TOxN
Total Phosphorus	TP

1 INTRODUCTION AND OBJECTIVES

Human activities that change the processes influencing nutrient cycling and transformation in coastal ecosystems (e.g. waste loading and impacts on hydrodynamics) can impact on the ecological services provided by these ecosystems, not only locally but also on larger (regional) scales (Taljaard *et al*. 2007). Nutrients enter the coastal environment through various pathways. They may be derived externally from a catchment (river and/or groundwater), upwelling processes (oceanic transport) or through atmospheric input (Nixon *et al*. 1996). *In situ* chemical, biochemical, and biological processes also contribute to the nutrients in the coastal environment. A distinctive feature of shallow estuaries is the active presence of benthic primary producers, represented by microalgae, macroalgae and rooted macrophytes. This is opposed to oceanic systems where phytoplankton is the only type of primary producer (Krause-Jensen *et al*. 2012). Because these benthic primary producers are present in shallow estuaries, they are able to make a significant contribution to total primary production (McGlathery *et al*. 2001, Öberg 2006, Krause-Jensen *et al*. 2012).

A common physical feature of estuaries is the shallow water column. This is often well mixed and, along with its associated benthic primary producers results in tight coupling between benthic and pelagic processes (Flindt *et al*. 1999, Krause-Jensen *et al*. 2012). It follows that the production of organic matter occurs either at the sediment interface via benthic microalgae, macroalgae and rooted macrophytes or in the pelagic via phytoplankton (Flindt *et al*. 1999). As a result of mixing and re-suspension events, an efficient vertical transport of organic and inorganic matter is achieved, essentially integrating the pelagic and benthic food webs, and biogeochemical processes. Some authors (Eyre and Balls 1999, Roy *et al*. 2001, Dagg *et al*. 2004, McKee *et al*. 2004) describe this pathway as having complex inter-relationships between the water column and bottom sediment constituents, and refer to this as ‘pelagic-benthic coupling’. Since estuaries bridge the gap between the coastal shelf and rivers, nutrients passing from a river catchment may be greatly modified through filtration and transformation before they enter the sea (Eyre 1994, Ferguson *et al*. 2004). The extent of nutrient transformation is linked to the hydrodynamic and chemical regime within a specific estuary. In some instances such as flood events or high flow periods, estuaries merely act as conduits for catchment flows to the near shore and coastal shelf. In such cases the cycling and transformation of catchment-derived nutrients largely occur at sea (Taljaard *et al*. 2007).

Water has become a scarce resource in South Africa because of overpopulation. As a result, both the quantity and quality of available water is threatened. The Great Brak Estuary is a problem area because it is a very popular holiday destination and a large area within the estuary (Die Eiland) has many houses built on an island in the middle of it near the mouth. These houses discharge water including septic tank waste into the estuary and a large amount of water from upriver is required to both keep the mouth open and to flush out nutrients emanating from both Die Eiland and pollution from the town of Groot Brak.

For many years there has been a problem caused by a very dense growth of macrophytes believed to be exacerbated by nutrients arising from both the town and Die Eiland houses. The Wolwedans Dam was built to supply the town of Mossel Bay which grew rapidly after the construction of a liquid fuel from sea gas facility. The greater water demand from Mossel Bay and the estuary itself resulted in the Great Brak Ecological Water Requirement Study recently completed for the Department of Water Affairs (South Africa). This study concluded that further studies were needed to determine the loads of nitrogen (N) and phosphorus (P) flowing through the estuary and to determine how effective the blooms of macroalgae are at trapping and removing N and P from the system. Understanding this aspect has become critical in view of the increased water demand from Mossel Bay that has caused a decrease in ecological water to the estuary. A reduction in river inflow to the estuary translates to more frequent and extended closed mouth conditions, which because of the reduced flushing in turn causes more nuisance algae that impact on both the ‘sense of place’ and water quality of the estuary.

Artificial breaching is practised in this estuary to prevent the flooding of low lying properties on Die Eiland in the lower reaches. The result is that the estuary is artificially breached at lower water levels than natural, adversely affecting the hydrodynamics in the estuary because it limits the required sediment scouring during breaching events. This has been compounded by the effect of reduced floods from the catchment, which are now attenuated by the Wolwedans Dam, and has also led to the gradual accumulation of organic material, nutrients and fine sediment in the deeper parts of the estuary. A decrease in flood intensity has also resulted in a reduction in the normal flood ‘resetting’ effect on the estuary. The result is an estuary with longer residence time, a shallower water column in the lower reaches and more available nutrients. These factors have led to a favourable environment for the growth of nuisance macroalgae (*Cladophora glomerata* (Linnaeus) Kützing).

Hypotheses:

- The estuary acts as a sink for nitrogen and phosphorus during the closed mouth phase.
- The submerged macrophytes and macroalgae play a quantitatively significant role in removing N and P in the estuary.
- The flux of nutrients from the sediment supplies most of the N and P required by the submerged macrophytes and macroalgae for growth.

Research aims:

- To determine the biomass and cover of macrophytes and macroalgae in the estuary.
- To identify the sources and determine the loads of nitrogen (N) and phosphorus (P) entering the estuary (point source, diffuse discharge, atmospheric deposition, remineralisation of organic material trapped in the sediment).
- Measure the flux of nutrients between the water column and the benthos.
- Measure N and P contents in living plant material. These combined with the biomass/cover will provide an estimate of the amount of N and P that is trapped in the macrophytes and macroalgae.
- Describe whether the environmental conditions in the estuary favour macroalgal blooms.
- Provide recommendations to be included into the Great Brak Estuary Management Plan.

2 LITERATURE REVIEW

2.1 Introduction to South African Temporarily Open/Closed Estuaries

South Africa's estuaries fall into three biogeographic zones (Figure 2.1), which have been described by Whitfield (1992) and Schuman *et al*. (1999). They are found in a range extending from the subtropical on the southeast coast, to the cool temperate on the west coast and the warm temperate between these extremes on the south coast (Schumann *et al*. 1999). The origins of these biogeographic zones are primarily due to the different oceanic regions off the coast of South Africa (Schumann *et al*. 1999). The two major currents influencing the coastal waters are the south-flowing Agulhas Current and the north-flowing Benguela Current (Harrison 2004). The Agulhas Current is tropical in origin and therefore has warm water but as it flows south it moves offshore and coastal sea temperatures decline. Average surface sea temperatures off Durban (KwaZulu-Natal) exceed 22°C while off Port Elizabeth (Eastern Cape) they are below 19°C (Lutjeharms *et al*. 2000). The west coast is influenced by the cold Benguela Current where sea surface water temperatures average between 13 and 15°C and off-shore winds result in upwelling during the summer season (Harrison 2004). Due to South Africa's variable climate, rainfall patterns in the different regions vary greatly. The climate in the cool temperate region ranges from semi-arid along the west coast, receiving prolonged periods of low or no rainfall interspersed with short flash rain events, to Mediterranean (seasonal winter rainfall) along the south western coast. Rainfall in the warm temperate region along the south coast is bimodal with slight peaks in spring and autumn, while the subtropical region along the east coast receives largely summer rainfall (Davies and Day 1998). The size and shape of the catchment, as well as the climatic region, determine the river inflow an estuary receives. The size and shape of the catchment in particular control the magnitude and flow distribution of runoff (Reddering and Rust 1990). Catchment size in South Africa varies greatly, from those that are larger than 10 000 km^2 to others that are smaller than 1 km^2. Most of the smaller catchments occur along the warm temperate and subtropical region, while the very large catchments are common in the cool temperate region (Reddering and Rust 1990).

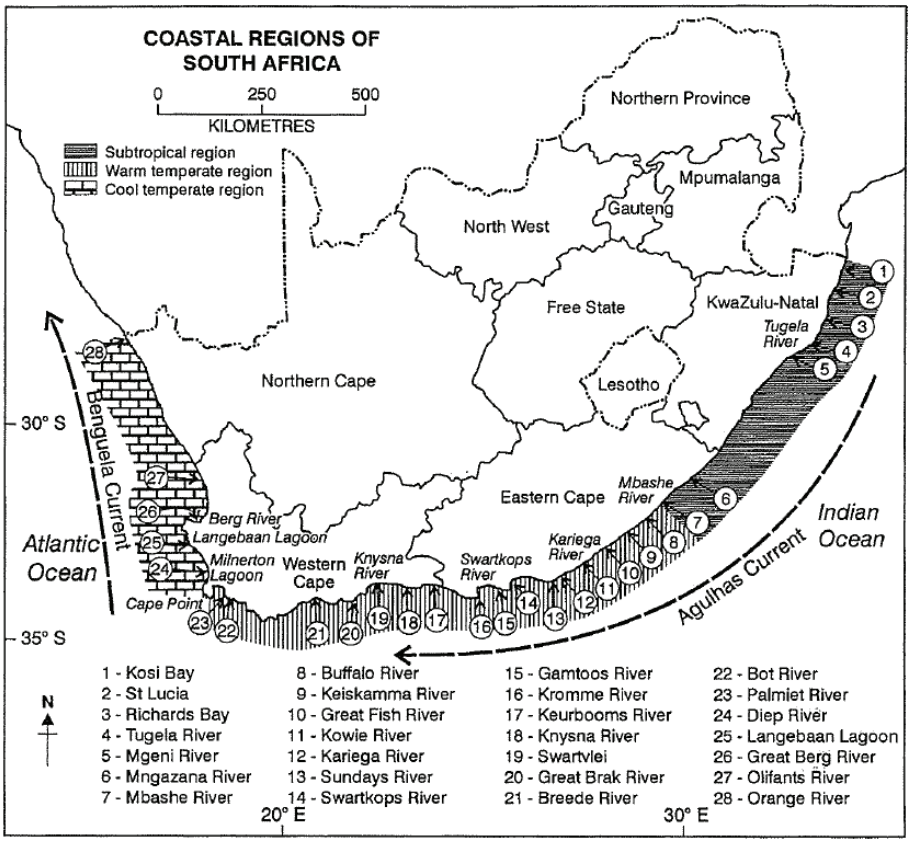

Figure 2.1 Distribution of biogeographical regions along the South African coast as well as locations of some estuaries (Schumann *et al*. 1999).

The South African Coast is about 3000 km long and is dominated by strong winds and a high wave regime throughout most of the year (Packham and Willis 1997). There are 465 estuaries, or 377 functional estuaries, along the coast and by definition there are only two real river mouths, with less than twenty percent of the estuaries being defined as 'permanently open' (Cooper *et al*. 1999, Van Niekerk and Turpie 2012). There are five main types of estuarine systems in southern Africa: Permanently Open Estuaries (POEs), Temporarily Open/Closed Estuaries (TOCEs), river mouths, estuarine lakes and estuarine bays. Temporarily Open/Closed Estuaries, the focus of this study, comprise 75% of these estuaries and generally consist of small microtidal estuaries which remain open to the sea for short periods (Whitfield 1992). They are characterised by having small river catchments less than 500 km^2 (Whitfield 1992). Sand barriers at the mouth of most estuaries limit the input of marine sediment, thereby creating a low-energy central basin that acts as a sediment trap (Radke *et al.* 2004) and promotes the deposition of floccules. Similar estuaries are also found in Australia where they are variously known: barred-estuaries, barrier-built estuaries, Intermittent Open and Closed Lakes and Lagoons (ICOLLs), intermittently open estuaries and intermittently closed estuaries (Griffiths 2001, Roy *et al*. 2001). Lagoons with

ephemeral inlets are relatively common in areas with alternating wet and dry seasons, and occur along the Pacific Coast of the Americas, in Africa and India (Flores-Verdugo *et al*. 1988, Ranasinghe and Pattiaratchi 2003, Gobler *et al*. 2005, Kraus *et al*. 2008). Similar estuaries are also found on the southeastern coasts of Brazil and Uruguay (Conde *et al*. 1999, Bonilla *et al*. 2005). Since TOCEs have a relatively small catchment (less than 500 km^2) (Whitfield 1992) they are greatly influenced by hydrodynamics and factors like the quality of inflowing river water.

The major factors which influence nutrient cycling patterns and storage in estuaries across climatic regions are the timing and magnitude of hydrologic events and the ability to trap nutrient loads (Eyre 2000). The annual run off in the temperate northern hemisphere (North America and Western Europe) is much less variable compared to the annual run off in the temperate regions of the southern hemisphere (Australia and South Africa) (Eyre 2000). Consider the coefficient of variation of annual flow (Cv) as an example. In Western Europe and North America the coefficient of variation of annual flow is 0.29 and 0.35 respectively, but is relatively high (0.7) for both South Africa and Australia. The temperate northern hemisphere estuaries are typically also much larger two layered systems which vary from well mixed to partially stratified. These systems receive regular loading from the catchment (usually during spring) and have a high nutrient retention efficiency which means that a large amount of the nutrients get trapped and later become available to fuel subsequent production (Erye 2000, Taljaard *et al*. 2009 a). In contrast, the smaller systems in the southern hemisphere, such as the TOCEs of South Africa and the ICOLLS in Australia, can vary from completely flushed freshwater dominated systems during periods of high runoff to completely marine dominated systems during periods of low or no river inflow (Largier and Taljaard 1991, Taljaard *et al*. 2009 a). TOCEs in South Africa have even more variable states and vary from completely flushed open freshwater dominated systems to closed freshwater (Mdloti and Mhlanga) and hypersaline systems (Seekooi) under low or no river inflow. In these estuaries, nutrient loading is highly variable and nutrient retention efficiency is low.

The three dominant hydrodynamic states in which TOCEs can exist namely, the open mouth state, the semi-closed mouth state and the closed mouth state, have been described in detail by Snow and Taljaard (2007). A brief description of the major physico-chemical changes associated with these states will be briefly discussed.

In the open mouth state, inflowing water from the river to the estuary is sufficiently high to keep the mouth open to the sea. This state then allows seawater intrusion during high tides, and maintains freshwater in the upper reaches of the estuary. The location of the freshwater front within the estuary is largely a function of the volume of river inflow. Since the mouth is open a longitudinal salinity gradient exists in the estuary and the position of the halocline depends on the rate of inflowing river water. Vertical stratification can also develop, but this occurs mainly in systems where the middle and upper reaches have greater depths. The water column is expected to be well oxygenated (above 6 mg l^{-1}) during this state because there is good water exchange through tidal flushing and river flow.

During the semi-closed mouth state, seawater intrusion occurs only at spring high tides, mainly because of low river inflow and an increased berm height. The berm however, is not so high as to prevent estuary water from draining to the sea. A strong longitudinal salinity gradient develops due to low freshwater input that is restricted to the surface of the estuary, trapping more saline water in the deeper areas. The estuary eventually reverts to a homogeneous brackish water body as a result of wind mixing. The duration of stratification is dependent on the strength of wind mixing forces, the depth of the estuary and river inflow. If vertical stratification develops it may inhibit proper aeration of bottom waters. Bottom waters may become low in dissolved oxygen (< 3 mg l^{-1}) depending on the organic load and the duration of stratified conditions. When vertical stratification is broken down via wind mixing forces, and the water column becomes well mixed, oxygenated water can be introduced into the bottom layers. The deeper pools within the estuary that are effectively cut off from the surface waters, due to strong vertical stratification persisting with depth may still have low dissolved oxygen concentrations.

In the closed mouth state, there is little to no river inflow into the estuary and the berm has developed high enough to completely prevent water drainage from the estuary to the sea. Overwash from the sea into the estuary can occur but is dependent on the height of the berm and conditions at sea. The salinity throughout the water column is homogeneous. Since there is no marked stratification, wind mixing maintains good oxygenation within the water column. Depending on factors like organic loading, low oxygenated water persists in the deeper more stagnant pools. When overwash does occur, it results in seawater intrusion into the bottom water, and the low oxygenated bottom water can become replaced by well oxygenated seawater. The extent of this depends on the volume of seawater entering the system and the estuary's bathymetry. If the estuary receives a continuous flow of freshwater

it may result in the system gradually becoming fresher, whereas if there is no freshwater input the system may become more saline, or even hyper saline in some instances.

2.2 The cycling of N and P in estuaries

2.2.1 Limiting nutrients in the aquatic environment

Limiting nutrients in coastal ecosystems are primarily nitrogen (N) and phosphorus (P). Iron has been considered a limiting element in some systems however it is usually not limiting in river dominated systems (Dagg *et al*. 2004). The major forms of nitrogen in aquatic ecosystems include: dissolved nitrogen (N_2) and nitrous oxide gases (N_2O); dissolved inorganic nitrogen (DIN), i.e. ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-); dissolved organic nitrogen (DON) i.e. urea and uric acid; and particulate organic nitrogen (PON), i.e. in detritus. The main forms of phosphorus (P) in aquatic ecosystems are: dissolved inorganic phosphate (DIP), i.e. $H_2PO_4^{3-}$ and HPO_4^{2-}, dissolved organic phosphorus (DOP); particulate (insoluble) inorganic phosphate (PIP), adsorbed onto cohesive sediment or organic particles; and particulate organic phosphorus (POP), i.e. in detritus. Silica in coastal ecosystems is mainly present as: inorganic silicate (dissolved reactive silicate – DRS); particulate biogenic silica (Conley 1997) – biogenic silica in coastal ecosystems is usually associated with phytoplankton (diatom) and radiolarian (animal) growth. The inorganic nutrient concentrations in an estuary are largely a function of the concentrations in the source waters, i.e. the river and the sea, as well as any physical (e.g. evaporation) or biochemical processes (e.g. biological uptake and remineralisation) that occur within the estuary. Estuarine vegetation also plays an important role in nutrient cycling and transformation, in addition to a number of other *in situ* physical and geo- and biochemical processes (Taljaard *et al*. 2007). Within estuaries, primary producers constitute an array of different floral types which are typically subdivided into microalgae (phytoplankton, benthic microalgae or microphytobenthos and epiphytes), submerged macrophytes (including seagrasses), macroalgae, (emergent macrophytes) reeds and sedges, salt marsh and mangroves.

2.2.2 Nitrogen: A microscopic view

Nitrogen occurs in many oxidation states (-5 to +3) and, as such, can act as a reducing agent or oxidant in the decomposition process of organic matter (Alongi 1998). Because of its many oxidation states, the cycling of nitrogen is more complicated than that of

other elements and involves a series of oxidation reduction reactions mediated by microbial populations (Goeck 2005). Nitrogen gas (N_2) comprises up to 79% of the earth's atmosphere, yet this reservoir is unavailable directly to plants and animals. In order to gain insight into the cycling of nitrogen in the coastal environment it is imperative to understand how the individual microbial mediated processes that control the nitrogen cycle function (Herbert 1999).

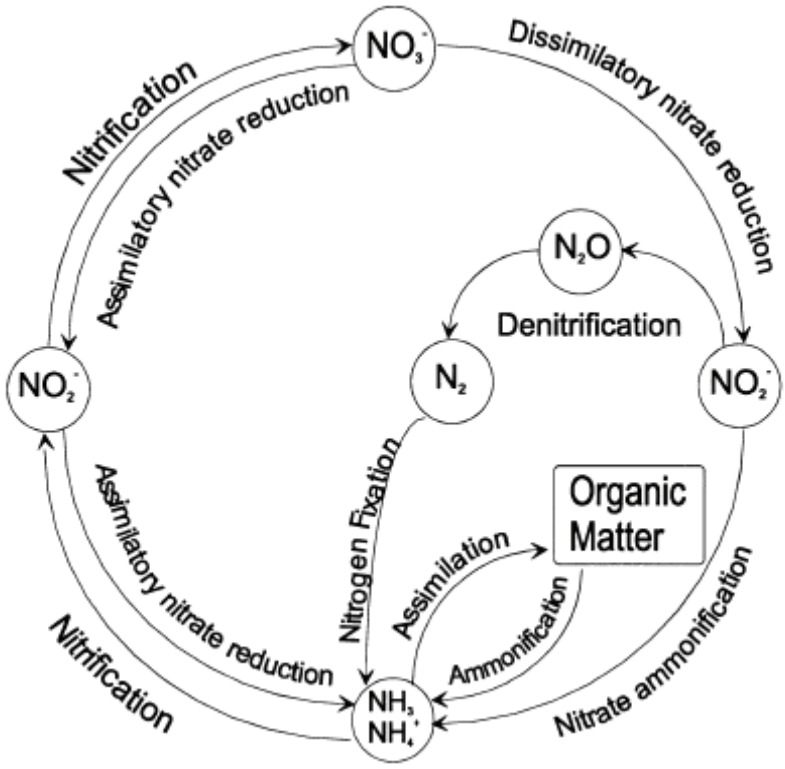

Figure 2.2 The nitrogen cycle showing the chemical forms and key processes involved in the biogeochemical cycling of nitrogen (Herbert *et al*. 1999).

Nitrogen fixation (Figure 2.2) introduces 'new' nitrogen into the biogeochemical cycles of ecosystems (Galloway *et al*. 1996). Nitrogen fixation is the reduction of nitrogen gas to ammonia (Taljaard *et al*. 2007). The reaction can be written as:

$$N_2 + 3\ H_2 \rightarrow 2\ NH_3$$

The process is catalysed by the enzyme nitrogenase and is carried out by both aerobic and anaerobic prokaryotes (Taljaard *et al*. 2007, Prescott *et al*. 2008). Under aerobic conditions a wide range of bacteria (*Azotobacter, Azospirillum*) contribute to this process (Prescott *et al*.

2008). Under anaerobic conditions the most important nitrogen fixers are members of the genus *Clostridium* (Prescott *et al*. 2008). Reductive processes are extremely oxygen sensitive and must occur under anaerobic conditions even in aerobic microorganisms. For example the free living diazotrophs such as *Azotobacter* sp. require oxygen to grow but have the ability to protect their nitrogenase enzyme from oxygen damage. There are also the photosynthetic cyanobacteria (*Cylindrospermum*) which have heterocysts (i.e. cells that lack the oxygen generating steps of photosynthesis and serve as a physical barrier for protecting the nitrogenase enzyme) and also those that lack heterocysts, for example *Oscillatoria*, which fix nitrogen in the dark under anaerobic conditions within the bacterial mat (Herbert 1999, Taljaard *et al*. 2007, Prescott *et al*. 2008).

Nitrogen fixing bacteria also exist in symbiotic relationships with plants. These associations include *Rhizobium* and *Bradyrhizobium* with legumes. In this case oxygen bound to root nodules housing the bacteria are supplied at rates that will not affect the nitrogenase activity (Taljaard *et al*. 2007, Prescott *et al*. 2008). A symbiotic relationship exists between the water fern *Azolla* and the heterocyst producing cyanobacteria *Anabaena.* The nitrogenase enzyme reaction is energetically costly and among the photosynthetic bacteria this energy demand is met by photosynthesis, while for the heterotrophic and chemolithotrophic bacteria the demand is met by the oxidation of organic matter or redox reactions respectively (Taljaard *et al*. 2007). These organisms therefore require light, in the case of the photosynthetic bacteria, unstable organic matter for the heterotrophs, electron donors for the chemolithotrophs and anoxic conditions (Paerl and Pinckney 1996). In estuaries and coastal sediments nitrogen fixation is generally considered to be low due to organic matter being a limiting factor (Herbert 1999, Paerl *et al*. 1987, Taljaard *et al*. 2007). It has been suggested by Howarh *et al*. (1988) that the reason estuaries and other coastal water bodies are nitrogen limited is in part due to the low rates of nitrogen fixation.

Nitrification is the aerobic process of ammonium ion (NH_4^+) oxidation to nitrite (NO_2^-) and the subsequent nitrite oxidation to nitrate (NO_3^-) (Figure 2.2) (Prescott *et al*. 2008). *Nitrosomonas europea* (which occurs in soil, sewage, freshwater and marine habitats), *Nitrosococcus oceanus* (obligatory marine) and *Nitrosospira briensis* (occurs in the soil) oxidizes NH_4^+ to NO_2^- and *Nitrobacter winogradskyi* (which occurs in soil, freshwater and marine habitats) and *Nitrococcus mobilis* (occurs in marine habitats) oxidizes NO_2^- to NO_3^- (Prescott *et al*. 2008). The processes are generally summarised by Berounsky and Nixon (1985) in the equations:

$NH_4^+ + OH^- + 1.5\ O_2 \rightarrow H^+ + NO_2^- + 2\ H_2O$ (ammonium oxidation)

$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ (nitrite oxidation)

In shallow coastal marine systems, such as TOCEs and ICOLLs, *Nitrosomanas* spp. and *Nitrobacter* spp. are the primary organisms responsible for the two nitrification steps respectively. Within coastal marine systems there are a number of biological and physio-chemical factors that influence and regulate nitrifying activity (Herbert 1999). These include temperature, pH, salinity, light, macrofaunal activity, and the presence of rooted macrophytes and inhibitory compounds, as well as the concentrations of NH_4^+, O_2 and dissolved CO_2 (Joye and Hollibaugh 1995). Laboratory studies on the effect of temperature on the growth of nitrifying bacteria isolated from temperate environments have shown that optimum growth occurred between 25-35°C and that below 15°C growth rates decreased sharply, inferring that maximum growth rates should be observed during summer while lowest growth should occur during winter (Herbert 1999, Spooner 2005).

Temperature however, also affects O_2 solubility, and, coupled with increased benthic respiration during summer means downward diffusion of O_2 is limited to the top 1-2 mm of the sediment (Herbert 1999, Spooner 2005). This means that in organically rich sediment the limiting factor for nitrifying activity may be oxygen rather than temperature (Herbert 1999). Since nitrifying bacteria are obligate anaerobes they are confined to the top few millimetres in the sediment, because of the downward diffusion of oxygen which is typically around 1-6 mm depending upon sediment type, organic matter, temperature and degree of bioturbation (Revsbech *et al*. 1980, Herbert 1999). Hence there is competition for oxygen between the nitrifying bacteria and other heterotrophs (Herbert 1999).

Denitrification is a dissimilatory process where heterotrophic bacteria utilise nitrate as a terminal electron acceptor in respiration. The major end products of denitrification include nitrogen gas (N_2) and nitrous oxide (N_2O), although nitrite can also accumulate (NO_2^-) (Figure 2.2) (Herbert 1999, Spooner 2005, Prescott *et al*. 2008). This process has been illustrated by Seitzinger (1988) as:

$NO_3^- \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$

Denitrification is a key process in the nitrogen cycle as it functions to decrease the availability of nitrogen to primary producers in the soil and allows its gaseous end products to diffuse into the atmosphere (Nixon *et al*. 1996, Nowicki *et al*. 1997, Seitzinger 1988, Yoon and Benner 1992). It also functions as a preventive measure against the accumulation of anthropogenic nitrogen sources and, as such, aids in the control of eutrophication in shallow coastal systems (Nixon *et al*. 1996, Nowicki *et al*. 1997, Seitzinger 1988, Yoon and Benner 1992). Within the heterotrophic bacteria there are several taxonomic groups that have the ability to denitrify. The facultative anaerobes, such as *Pseudomonas denitrificans*, which can still grow in the presence of oxygen, but under oxygen depleted conditions are able to maintain respiratory activity by using nitrate as a terminal electron acceptor. This bacterium produces nitrogen gas as the end product of nitrate respiration (Dunn *et al*. 1980, Macfarlane and Herbert 1982, Prescott *et al*. 2008). The coupling of nitrification (aerobic process) to denitrification (anaerobic process) has been recognised as playing a vital role in the biogeochemistry in coastal marine systems. Nitrification generates an important source of nitrate for denitrifying bacteria and once nitrate is utilised in benthic microbial respiration, nitrogen is released as nitrogen gas and, to a lesser extent, nitrous oxide, both species are unavailable for biological uptake and are lost from the system to the atmosphere until fixation occurs (Herbert 1999).

Nitrate ammonification, or dissimilatory nitrate reduction to ammonium (DNRA) as the name implies, is the dissimilatory reduction of nitrate directly to ammonium (Figure 2.2). It is an anaerobic process which is carried out by fermentative bacteria such as *Goebacter metallireducens*, *Desulfovibrio* sp. and *Clostridium* sp. present in highly reduced soil (Dunn *et al*. 1980, Hasan and Hall 1977, Prescott *et al*. 2008). Unlike denitrification where nitrogen is lost from the ecosystem, DNRA retains nitrogen in an accessible state (NH_4^+) within the soil (Herbert 1999, Matheson *et al*. 2002).

2.2.3 Phosphorus

Phosphorus has no volatile forms and has a slower and much simpler cycle than nitrogen (Holtan *et al*. 1988). The phosphorus cycle has been described as ‘one way traffic’ from rock deposits to the water and sediment (Holtan *et al*. 1988). The movement of dissolved reactive phosphorus in the cycle is of ecological importance because it becomes biologically available for aquatic plants (Nixon *et al*. 1980). Phosphorus is almost exclusively present as phosphate PO_4^{-3} in natural environments. It is generally present in association with other ions, such as H^+, Mg^{2+} and Ca^{2+} in aqueous complexes, while it occurs with Ca^{2+}

and F^- in calcium-phosphate minerals like carbonatefluorapatite (CFA), hydroxyapatite and fluorapatite and with Fe^{2+} and Fe^{3+} in Fe-phosphate minerals (Slomp 2012).

The formation of CFA takes place authigenically (*in situ* in the sediment) hence it is called 'authigenic Ca-P', while hydroxyapatite is known as 'biogenic Ca-P' because it is derived from fish bones and scales (Slomp 2012). Detrital apatite is the collective term given to other apatite minerals derived from mechanical weathering on land. Phosphate may also adhere to Fe oxyhydroxide and carbonate surfaces (Ruttenberg 2004). The loosely adsorbed P is often designated as 'exchangeable P'. Phosphorus may become available to the biosphere (mobilization of P) through a number of reactions such as organic matter degradation, desorption, and mineral dissolution reactions (Slomp 2012).

Sources of phosphorus to the coastal zone

The primary origin of phosphorus is geological. There are three broad groups of phosphate deposits, namely apatite [Ca_5 $(PO_4)_3$ (F, Cl, OH], apatite deposits of igneous and metamorphic origin, sedimentary phosphorites and guano related deposits (Holtan *et al*. 1988). Apatite is the most abundant primary P mineral and is the ultimate source of all bioavailable P 'reactive P' (Slomp 2012). Its dissolution is largely a function of acid exposure from soil microbes and fungi (mycorrhiza) in symbiosis with other plants (Hoffland *et al*. 2004). Apart from chemical exposure, physical distortion of the crystal lattice by fungal hyphae may also cause dissolution (Bonneville *et al*. 2009). P transferral from terrestrial to aquatic systems occurs via diffuse pathways, namely subsurface or groundwater flow, surface runoff and through direct inputs into surface waters (point sources) (Sharpley *et al*. 1995, McDowell *et al*. 2003). The delivery of P, most of which is particulate inorganic phosphorus (PIP), is derived from detrital and authigenic apatite minerals and P bound to Fe oxides, to the coastal zone occurs primarily through riverine transport. Fe-bound P can be remobilized within estuaries and coastal sediments via salinity induced desorption (Froelich 1988, Van der Zee *et al*. 2007) and increased reduction of the Fe-oxide minerals in reducing sediments due to a higher availability of sulfate (Caraco *et al*. 1989). In addition, riverine P can include particulate and dissolved organic P (POP and DOP) as well as dissolved inorganic P (DIP).

Another source of phosphorus, mainly in the form of DIP to coastal waters, arrives through submarine groundwater discharge (Slomp 2012). Slomp and Van Cappellen (2004) suggest that because most aquifers retain phosphorus, submarine groundwater discharge will only

act as an important source where the groundwater flow rate is high, such as on the volcanic island of Hawaii and regions with karstic aquifers. Only a minor fraction of phosphorus is derived from the atmosphere, but Migon and Sandroni (1999) have found that it can be of significant importance in oligotrophic areas without river or groundwater inputs.

Anthropogenic sources of phosphorus have increased throughout the past few centuries and have greatly modified coastal nutrient cycling worldwide (Slomp 2012). These changes are associated with increased production of organic matter and changes in nutrient supply ratios (N:P and N:Si) that influence water quality (e.g. eutrophication and hypoxia) of receiving water bodies. Hypoxia results in an increased availability of P to the water column because it reduces the burial of P that is bound to Fe oxides and organic matter (Ingall *et al*. 1993). Conley *et al*. (2002) found seasonal hypoxia to cause sediment in the Baltic Sea to act as an internal source of P and, according to Vahtera *et al*. (2007), an internal source of P can lead to a system becoming locked in a self-sustaining eutrophic state.

The main uses of phosphate are fertiliser and industrial chemical production (Holtan *et al*. 1988). Fertilisers are applied to crops in to order promote optimum growth yields. However, fertilisers are often granulated in order to prevent rapid fixation with the soil. The result is a large proportion of phosphate in an unavailable form that ultimately gets released as dissolved reactive phosphorus. Complication arises in finding the correct balance between fertiliser application and uptake by crops. The result is an excess application of fertiliser which in turn contributes to the terrestrial load entering marine systems (Heaney *et al*. 2001). Although the United States of America and northern Europe have implemented actions to reduce inputs of terrestrial phosphorus, there are still many countries where excessive amounts of fertiliser are applied to agricultural land (Vitousek *et al*. 2009, Slomp 2012). Furthermore, even in areas where fertiliser application has decreased, there is still a loss of P from the land through erosion and surface runoff. Untreated wastewater containing P also still remains a global problem (Slomp 2012).

2.3 Nutrient transformation in the benthic realm

The transformation of nutrients in the benthic realm is a very important component of shallow water systems such as estuaries, lagoons and the continental shelf (Pratihary *et al*. 2009). Estuaries and coastal systems do not only receive nutrients from sources such as river inflow, land runoff and atmospheric deposition, but also from the sediment underlying these systems, making such a system very productive (Pratihary *et al*. 2009). Amazingly, the

continental systems occupy only about 10% of the global oceanic area, yet 30 to 50% of marine primary production occurs in these regions (Romankevich 1984, Walsh 1991) which consequently also sustain about 90% of the fisheries resource (Pratihary *et al*. 2009). The sediments of estuaries have higher concentrations of organic matter than the open ocean and the organic carbon in these sediments can be a significant sink for atmospheric CO_2 (Santschi *et al*. 1990). Nutrient transport between the sediment and water column occurs mainly as molecular diffusion (Li and Gregory 1974), macrobenthic activities (Kristensen 1985), sediment re-suspension (Hammond *et al*. 1977), transport through bubble tubes (Klump and Martens 1983) and advective transport of porewater (Marinelli *et al*. 1998). Primary producers often have diel patterns which cause an efflux of nutrients during the dark and uptake in the light (Eye and Ferguson 2002).

Plant communities along the coast are among the most productive in the world with large amounts of nutrients (nitrogen and phosphorus) passing through them (Banta *et al*. 2004). Nutrients which are assimilated during plant growth are immobilized temporarily into living biomass and detritus until they are mineralised through grazing or decomposition. Microbial remineralisation of organic matter can be a major recycling pathway for biogenic elements (Matheson *et al*. 2002). During decomposition of organic matter, oxygen and other terminal electron acceptors are consumed and inorganic nutrients are remineralised (Hopkinson *et al*. 2001). Regenerated nutrients from the benthos can be variable in both space and time. In Chesapeake Bay, Boynton and Kemp (1985) found sediments supplied between 13% and 40% of the N required for primary producers, while Cowan and Boynton (1996) calculated that benthic regeneration of N and P contributed from 0-93% and 0-168% demand respectively in this estuary. Sediment regeneration in the Georgia Bight contributed to only 11% of the N demand for primary production (Hopkinson 1987), while in Mobile Bay, sediments contributed from 0-94% N (mean 36%) and 0-83% P (mean 25%) needed for primary production (Cowen *et al*. 1996). This is similar to what Pratihary *et al*. (2009) found in the Mandovi Estuary, where sediments supplied 24% N and 12% P for primary production. Mortazavi *et al*. (2012) estimated that sediments contributed from 0-18% N and 0-9% P required for water column primary producers at Magnolia River, and between 0-38% N and 0-49% P at Mid Bay. Benthic nutrient regeneration was able to meet up to 75% of phytoplankton demand (Billen 1978) and is indicative that benthic nutrient regeneration may be important in regulating spatial and temporal patterns of productivity in overlying water. For example, seasonal input of organic matter from the catchment resulted in a gradual release of inorganic nutrients from sediments at times of the year when primary production was most nutrient limited (Boyton and Kemp 2000, Hopkinson *et al*. 2001). Although benthic

regenerated nutrients are variable spatially and temporally, nutrient input from underlying sediment can contribute substantially to the pelagic nutrient inventory and has the potential to drive water column primary production in shallow systems (Callender and Hammond 1982, Jensen *et al*. 1990, Koho *et al*. 2008). It is clear that these sediments play a significant role in the biogeochemistry of such systems. The absence of these sediments from shallow systems would indicate that primary production would be solely dependent on pelagic regeneration and hence no more productive than the open ocean (Pratihary *et al*. 2009). Several methods exist for measuring the benthic flux of nutrients and the most common ones are *in situ* incubation (Hammond *et al*. 1999, Jahnke *et al*. 2000, Pratihary *et al*. 2009) and intact core incubation (Hopkinson *et al*. 2001, Sundback *et al*. 2003). *In situ* benthic chamber experiments have been found to be the most sensitive method for accurate measurements of benthic flux in estuarine systems (Hammond *et al*. 1985).

2.3.1 Role of primary producers in nutrient cycling

Benthic photoautotrophy plays a key role in regulating nutrient cycling where most of the shallow benthic realm lies within the photic zone (McGlathery *et al*. 2004). Here production by seagrasses, macroalgae and benthic microalgae typically exceed that of phytoplankton (Borum and Sand-Jensen 1996). Following nutrient enrichment in estuaries there is a shift in the dominance of primary producers, commonly from rooted macrophytes (such as seagrasses) and perennial macroalgae to fast growing nuisance green macroalgae and phytoplankton (Sand-Jansen and Borum 1991, Durate 1995, Valiela *et al*. 1997). In heavily polluted (eutrophic) shallow estuaries a change to the phytoplankton dominated state can be expected (Durate 1995, Valiela *et al*. 1997), although this may not affect the total production of the estuary (Borum and Sand-Jansen, 1996). McGlathery *et al*. (2004) state that because primary producers influence nutrient cycling and transformation, they play an important role, as shallow estuaries are buffer zones between land and sea. For example, the uptake and retention of nutrients in plant biomass, the burial of organic matter and the effects of autotrophic metabolism on bacterially mediated processes, all influence nutrient cycling pathways. If there is variation in the rates and pathways of these nutrients due to changes in primary producer communities, this will eventually determine the fate and retention of nutrients and their path to the open ocean (McGlathery *et al*. 2004).

2.3.2 Benthic microalgae

Benthic microalgae can alter the sediment flux of nutrients from the sediment to the water column. A 'filter' effect results when benthic microalgae densities and other biota are orders of magnitude greater than the water column (McGlathery *et al*. 2004). Through this 'filter' effect, assimilation of nutrients by benthic microalgae in the top few millimetres of sediment is able to change the efflux of remineralised nutrients to the overlying water reducing their availability to bacteria, phytoplankton as well as free floating macroalgae (Sundback *et al*. 2000, Anderson *et al*. 2003, Tyler *et al*. 2003). Benthic microalgae can also cause an influx (from water column to the sediment) of nutrients to the sediment when the sediment nutrient sources are insufficient to meet their demands for growth (McGlathery *et al*. 2004). "The filter effect is mediated by both microalgal assimilation and photosynthetic oxygenation of the sediment surface, which in turn creates dynamic chemical and biological gradients" (McGlathery et al. 2004).

2.3.3 Macroalgae

It has been widely accepted that shallow coastal waters may become predominately occupied by ephemeral and epiphytic macroalgae (Valiela *et al*. 1997, Karez *et al*. 2004, McGlathery *et al*. 2007). This is mainly because of the increasing availability of inorganic nutrients namely nitrogen and phosphorus. Common bloom forming species belong to the foliose or uniserate filamentous green algae genera *Ulva*, *Enteromorpha* and *Cladophora* (Fletcher 1996, Raffaelli *et al*. 1998) although occasional red and brown uniserate filamentous algae have been reported (Valiela *et al*. 1997). McGlathery *et al*. (2004) state that the major physiochemical factors within an algal mat, influencing nutrient cycling at the sediment water interface, are variations in light, nutrients, oxygen and pH. These factors are linked to algal photosynthesis, respiration and decomposition and influence nutrient cycling at the sediment water interface and, perhaps, even deep into the sediments. Macroalgae at the ecosystem scale, are capable of storing a significant quantity of nutrients. It is not uncommon for macroalgae growing in highly enriched water to achieve peak biomass of 0.5 kg dry mass m^{-2} and for canopy heights to exceed 0.5 m (McGlathery *et al*. 2004). For example, nitrogen stored in the peak macroalgal biomass had a similar magnitude as the annual nitrogen load from the watershed of Waquoit Bay Massachusetts (Valiela *et al*. 1997). The presence of macroalgae in systems can enhance sediment nutrient cycling, due to the input of organic matter (Tyler *et al*. 2001, 2003). Increased mineralisation rates result in a build-up of NH_4^+ within the mat where light does not penetrate (McGlathery *et al*. 1997).

This can affect the porewater NH_4^+ concentration as deep as 13 cm in the sediment (McGlathery *et al*. 2004). Organic bound nutrients which are recycled can become an important source of nitrogen to sustain macroalgal production, especially in areas with limited tidal exchange (Trimmer *et al*. 2000 a). An increase in sediment NH_4^+ can occur because of the local extinction of bioturbating infauna caused by anoxic conditions. When these species are present there is an increase in oxygen diffusion into the sediments which stimulate nitrification rates (Rysgaard *et al*. 1995, Hansen and Kristensen 1997, Webb and Eyre 2004).

There is evidence that suggests that macroalgae covered sediments are a net sink of inorganic nutrients (Tyler *et al*. 2001, 2003). However, macroalgae can also play an important role in regulating the flux of organic nutrients across the sediment and water column. For example, Tyler *et al*. (2003) observed that *Ulva lactuca* successfully inhibited the flux of urea and dissolved free amino acids from the sediment to the water column. Macroalgae was a net source of organic nitrogen, but a net sink for dissolved inorganic nitrogen (DIN) throughout the year. They found that the macroalgae sequestered urea and dissolved free amino acids, and that of the total nitrogen uptake, 22% was released as dissolved combined amino acids. Over a seasonal growth cycle it is common for macroalgae at the beginning of the growing season to be a net sink of nutrients and near the end of summer to become a net source of nutrient. This is because productivity decreases due to self-shading within mats and high temperatures increasing respiration (McGlathery *et al*. 2004, Higgins *et al*. 2008). Periods of oxygen supersaturation can occur when there are high rates of macroalgal metabolism and oxygen depletion (anoxia) throughout the water column can occur after senescence (Valiela *et al*. 1992, Boynton *et al*. 1996, Gubelit and Berezina 2010). Macroalgal anoxic events tend to be episodic and occur during the warm summer months when production declines. These ‘boom and bust’ cycles of high production and senescence are typical of eutrophic waters (Valiela *et al*. 1997, McGlathery *et al*. 2004).

In a similar way to benthic microalgae, the accumulation of benthic macroalgae can also function as a temporary ‘filter’ for dissolved nutrients by assimilating nutrients from the underlying sediment and decreasing their availability in the water column (Thybo-Christesen *et al*. 1993, McGlathery *et al*. 1997, Tyler *et al*. 2001). Since the photic zone lies within the upper few centimetres of the macroalgal mat, they are capable of moving the oxic-anoxic layer from the sediment into the mat (Krause-Jensen *et al*. 1999, Astill and Lavery 2001). The effectiveness of removal of dissolved inorganic nutrients from the sediment depends on the stability of macroalgae, biomass and productivity. Although free floating macroalgae are

patchy and unstable, Astill and Lavery (2001) found that nutrient and oxygen gradients develop quickly as the beds expanded, suggesting the 'filter' function may even occur in dynamic environments. The concentration of nutrients in the sediments of shallow systems are often more than several orders of magnitude greater than the water column (even in enriched waters), and are therefore considered to have a significant role in the productivity of macroalgae (McGlathery *et al*. 2004). Nutrient turnover studies (Thybo-Christesen *et al*. 1993) in bays have shown that the underlying sediment constitutes a valuable source of nutrients for the growth of macroalgae. Following the collapse of macroalgal bloom in Hog Island Bay, the release rates of organic and dissolved inorganic nitrogen was sufficient to completely mineralise the algal biomass (650 g dry mass m^{-2}) within 13 days. The recycled plant bound nutrients may then stimulate the metabolism and growth of phytoplankton and bacteria (Valiela *et al*. 1997, McGlathery *et al*. 2004).

Tyler *et al*. (2003) also found that the efflux of DIN and urea from the underlying sediments of macroalgal mats was able to support 27 to 75% of the algal N demand. The N flux from the sediment of a subtropical oligotrophic bay was sufficient to meet the algal N demand for growth as well as maintain its persistence in the bay (Stimson and Larned 2000). Sundback *et al*. (2003) found that during spring months, the nutrient efflux from the sediment to the overlying water could contribute to a substantial share of the nutrient demand for early macroalgal growth. When they compared N demand (based on biomass measurements) with measured effluxes of DIN and DIP during the period that that coincides with the onset of macroalgal growth, it indicated that benthic nutrient regeneration contributed from 15 to 125% of the N demand and 16 to 73% of the P demand. According to McGlathery *et al*. (1997) the efflux nutrients from underlying sediment are consumed by the bottom layer of the macroalgal mat, while diffusion of nutrients from the water column is able to meet the N demand of the light-saturated algae in the top layers. The decomposition of algal and other labile (unstable) organic matter could be a major source of nutrients for the early growth of macroalgae and suggests that shallow systems that favour opportunistic macroalgae may function as self-supporting systems through benthic nutrient regeneration (Sundback *et al*. 2003, Pratihary *et al*. 2009).

Generally, the factors which decrease macroalgal productivity also decrease nutrient uptake and reduce the filter effect. These factors include self-shading within the lower layers of a dense mat during summer, and decreased water clarity as a result of high phytoplankton biomass or suspended sediment (McGlathery *et al*. 2004). They singly or in combination often result in the collapse of a macroalgal bloom in the late summer months, the end

product of which leads to the release of plant bound nutrients to the water column. Light limited macroalgae often display a diurnal pattern of nutrient release where nutrients diffuse from the sediment through the mat to the water column in the dark (McGlathery *et al*. 1997). This occurs because algae which are grown under low light lack carbon reserves and are nitrogen saturated. They are therefore dependant on recent photosynthate to build amino acids (McGlathery *et al*. 2004).

In shallow temperate estuarine systems macroalgal blooms take place mainly in spring and autumn (Hubas and Davoult 2006). Cummins *et al*. (2004) have observed that there had been an increase in their frequency and persistence within these systems. These observations are considered to be early indicators of eutrophication in coastal waters as a result of nutrient loading (Valiela *et al*. 1992, McGlathery *et al*. 2007, Teichberg *et al*. 2010). The primary abiotic factors, light and nitrogen availability, are described by McGlathery and Pedersen (1999) to be the dominant cause of macroalgal productivity in temperate estuarine waters. Agricultural and urban activities lead to great variation in these parameters because the level of input resulting from these activities varies (McGlathery *et al*. 1997). The macroalgae found in these environments have mechanisms that ensure a balance between resource variability and growth (McGlathery *et al*. 1997).

Within estuarine systems, nutrient variability is common, and availability may differ seasonally as well as over short time periods (Ramus and Venable 1987). Nutrients contributing to the enrichment process in estuaries also vary. Dissolved inorganic nitrogen (DIN) appears to be the main nutrient in some lagoon environments, while in others the nitrogen is accompanied by inputs of phosphorus (Nixon *et al*. 1986). Macroalgae therefore need to be able to adjust their carbon and nitrogen metabolism as well as their soluble reactive phosphorus (SRP) uptake over these time scales in order to optimize their growth and proliferation in estuarine systems (Menendez 2005). Opportunistic macroalgae are capable of uptake, assimilation and storage of large amounts of nitrogen in areas of high nitrogen loading, leading to low water column concentrations of nutrients (Peckol *et al*. 1994, Aveytua-Alcázar *et al*. 2008). Waquoit Bay Massachusetts has become dominated by two species associated with eutrophication, *Cladophora* spp. and *Gracilaria tikvahiae,* resulting from nitrogen loading via ground water (Peckol *et al*. 1994). Dominance of opportunistic species in areas undergoing eutrophication has been attributed to their successful procurement and storage of inorganic nutrients, high growth rates, and high tolerance to a wide range of temperatures and salinities (Fong and Zedler 1993, Kamer and Fong 2000, Naldi and Vairoli 2002, Lartigue and Sherman 2005, Aveytua-Alcázar *et al*. 2008). Some

macroalgae are intolerant of elevated NH_4^+ (above 40 µM) while others grow in almost full strength sewage (Chang *et al*. 1982). Macroalgal blooms under eutrophic conditions are commonly associated with ecosystem changes. Blooms often out-compete slower growing macrophytes like seagrasses and larger perennial macroalgae (Durate 1995, Valiela *et al*. 1997, Valdemarsen *et al*. 2010, Rasmussen *et al*. 2012). The trophic ecology of the system may also be affected when macroalgae out-compete other primary producers such as the microphytobenthos through shading effects, and directly influence the energy flow in the system (Hubas and Davoult 2006). Estuarine systems are known to become anoxic because of the depletion of oxygen resulting from dense growth of macroalgae and produce toxic compounds like hydrogen sulphide from their subsequent decomposition (Bolam *et al*. 2000, Nedergaad *et al*. 2002). These effects lead to decreases in the infauna species richness and biomass (Cardoso *et al*. 2002, Cummins *et al*. 2004, Berezina and Golubkov 2008).

2.3.4 Submerged macrophytes

Primary production in seagrass meadows in temperate and tropical habitats is, to a large extent, regulated by nitrogen and phosphorus (Short 1987, Hemminga *et al*. 1994, Perez *et al*. 1994, Pedersen 1995). The predominant carbonate nature of the sediments in the tropics and low water column nutrient concentrations have important consequences on the nutrient limitation of seagrasses in these habitats (Holmer *et al*. 2001 a). Seagrasses from tropical water bodies are mainly limited by phosphorus for two reasons (Holmer *et al*. 2001 a). Firstly the rate of nitrogen fixation is usually higher in warmer water (Welsh *et al*. 1996) and secondly phosphate ions (PO_4^{3-}) tend to bind to the carbonate sediments reducing the amount of P available for growth (Udy and Dennison 1996). However, Terrados *et al*. (1999) found the phosphorus content of seagrass tissue in tropical waters to be high and the nitrogen content low suggesting nitrogen to be the limiting nutrient. This implies that further studies are required in order to understand the nutrient limitation in tropical seagrasses (Holmer *et al*. 2001 a). Seagrasses are capable of taking up nutrients with both leaves directly from the water column, and roots from the interstitial water in the sediment (Stapel *et al*. 1996, Gras *et al*. 2003, Nielsen *et al*. 2006). In temperate waters seagrasses obtain most of their nutrients from the sediment using their roots, however leaf uptake can also occur and in the tropics may be even more important because of the low sediment nutrient concentration (Fourqurean *et al*. 1992, Stapel *et al*. 1996, Jensen *et al*. 1998).

Remineralised organic matter present in the sediment is the main source of nutrients available for root uptake by seagrasses (Holmer *et al*. 2001 a). A number of electron

acceptors are available for mineralisation in marine sediments, of which oxygen is an important electron acceptor. However, it is rapidly consumed and is usually only present in the top few centimetres of the sediment (Revsbech *et al*. 1986, McGlathery *et al*. 2004). About 50% of organic matter oxidation occurs under anaerobic conditions (Canfield 1993) but the release of oxygen from the roots of seagrasses can significantly influence the oxygen availability in the sediment (McGlathery *et al*. 2004). For example, Pedersen *et al*. (1998) found that oxygen loss from the roots of *Cymodocea rotundata* was able to contribute to 10% of total sediment oxygen consumption. The electron acceptors nitrate, iron and sulphate are important for mineralisation occurring in the deeper layers of the sediment (Canfield 1993). In tropical sediments the concentration of nitrate is low and denitrification does not play a major role in the decomposition of organic matter (Kristensen *et al*. 1998). Although it appears that sulphate reduction may be of more importance as relatively high rates have been found in seagrass sediments (Blackburn *et al*. 1994, Holmer *et al*. 1999), suggesting that sulphate reduction may make a significant contribution to remineralisation and nutrient availability in tropical seagrass sediments (Holmer *et al*. 2001 a).

Leakage of dissolved organic matter and oxygen from the roots of seagrasses can significantly influence the cycling of nutrients in the sediment. Both these processes are photosynthetically driven (McGlathery *et al*. 2004). Aerobic respiration in the root rhizosphere occurs when the oxygen produced in the photosynthetic leaves is transported to the roots via the lacunal system, a well-developed series of air channels that make up 60% of the total plant volume (McGlathery *et al*. 2004). The oxygen not consumed by the roots is eventually released into the rhizosphere. This creates an aerobic zone in the otherwise anaerobic environment. The release of DOM (typically simple organic compounds that are the products of photosynthesis) by the plant roots takes place simultaneously with aerobic respiration (Ziegler and Benner 1999). Moriarty *et al*. (1986) found that 11% of root exudates, a result of recent photosynthate from *Halodule wrightii*, were released within 6 hours into the root rhizosphere.

Root exudates by plants have been linked to bacterial productivity. Other evidence indicating that root exudates influence bacterial activity are the subsurface peaks in bacterial processes, as well as seasonal and light induced variation in bacterial activity in the sediments where root biomass is highest (Blaabjerg *et al*. 1998). The sulphur reduction rates in a *Zostera marina* bed varied through a diel cycle and were significantly higher in the dark phase indicating that sulphate reducers were fuelled by photosynthetic products released by the macrophytes (Blaabjerg *et al*. 1998). The release of oxygen into the rhizosphere by the

plant roots of species such as *Potamogeton* and *Zostera* formulates oxic and anoxic interfaces which stimulate nitrification, which produces nitrate that is able to enhance denitrification (Cornwell *et al*. 1999, Hansen *et al*. 2000). The stimulation is thought to be linked to the variation in oxygen release and the growth cycle of seagrasses.

The seagrasses, *Cymodocea rotundata* and *Thalassia hemprichii,* released organic compounds from the roots to the rhizosphere at relatively high rates indicating that they supply enough organic carbon to supply the entire sediment mineralisation (Holmer *et al*. 2001 a). In previous work, Holmer *et al*. (2001 b) measured a much lower release rate of 60 mmol C m^{-2} per day in a nearby *Enhalus acoroides* bed compared to the 136 mmol C m^{-2} per day rate they measured with *Cymodocea rotundata.* Holmer *et al*. (2001 a) believe that the excess root release may enrich the porewater and sediment organic matter and that seagrass is a source of DOC (dissolved organic carbon). This is consistent with other findings (Middelboe *et al*. 1998, Ziegler and Benner 1999, Holmer *et al*. 2001 b) where seagrass beds were a source of DOC to the water column. Welsh (2000) found high rates of nitrogen fixation in the root rhizosphere which he believed to be due to the presence of root exudates. However, Boschker *et al*. (2000) found benthic microalgal production to be the major carbon source for sediment microbes and that macrophyte derived organic matter only constituted a limited source of carbon. Seagrasses are able to utilize CO_2 and HCO^-_3 for photosynthetic carbon reduction (Invers *et al*. 2001). The dissolved inorganic carbon (in mM concentration) in seawater is approximately three orders of magnitude greater than nitrogen and phosphorus (Lee and Dunton 2000).

While there are a number of elements necessary for the growth of seagrasses, nitrogen and phosphorus are the most common nutrients which limit growth (Romero *et al*. 2006). Both root and leaf uptake of essential nutrients have been observed in seagrasses (Pedersen *et al*. 1997, Gras *et al*. 2003, Nielsen *et al*. 2006). The inorganic nitrogen sources available to seagrasses include NH_4^+ and NO_3^- within the water column and NH_4^+ in the sediment. It has been observed that there is a higher uptake of NH_4^+ as opposed to NO_3^-, within the leaves of seagrasses (Short and McRoy 1984, Terrados and Williams 1997, Lee and Dunton 1999). NO_3^- assimilation is also influenced by the availability of photosynthate and carbon, making it energetically expensive (Lara *et al*. 1987, Turpin 1991). So the uptake of reduced forms of nitrogen, i.e. NH_4^+, would be of greater benefit to seagrasses. Phosphorus is available to seagrasses as PO_4^{-3}, and is present in both the water column and the sediment. The concentration of nutrients in the water column in seagrass dominated habitats is generally low (< 3 µM NH_4^+ and NO_3^-, < 2 µM PO_4^{-3}) (Hemminga *et al*. 1995, Ruiz and

Romero 2003, Lee *et al*. 2005, Adams 2008). Conversely the sediments have higher nutrient concentrations, and typically range from 20 to 1000 µM NH_4^+ and > 20 µM PO_4^{-3} (Touchette and Burkholder 2000, Lee *et al*. 2005). Hence, some authors consider the sediment to be the most important source of nutrients for seagrasses (Iizumi and Hattori 1982, Short and McRoy 1984, Zimmerman *et al*. 1987), but other studies have shown that in comparison to root uptake, a greater affinity for nutrient uptake occurred at the leaves (Pedersen *et al*. 1997, Lee and Dunton 1999, Alexandre *et al*. 2011).

Due to the occurrence of seagrasses in habitats of low water column nutrient concentrations, it is believed that seagrasses may have adapted to assimilating nutrients under low nutrient conditions. This may indicate that saturation could occur at lower concentrations within leaves, while saturation within roots may occur at much higher concentrations (Stapel *et al*. 1996, Lee and Dunton, 1999). The nutrient uptake kinetics depicts seagrass adaptation as growing in low water column, high sediment nutrient conditions. Even though there are large differences in sediment and water column concentrations, many authors have come to the consensus that a more or less equal acquisition of nutrients occur from the sediment and the water column (Iizumi and Hattori 1982, Short and McRoy 1984, Zimmerman *et al*. 1987, Pedersen and Borum, 1992, Stapel *et al*. 1996, Lee and Dunton, 1999). For example, a 55% sediment NH_4^+ contribution to *Zostera marina* growth was found by Iizumi and Hattori (1982), while Zimmerman *et al*. (1987) found a 60% contribution. Similar findings for *Thalassia testudinum* were observed by Lee and Dunton (1999), where root uptake from the sediment accounted for 50% DIN and leaf uptake the remainder. Within the Great Brak Estuary, the dominant submerged macrophytes consist of *Zostera capensis* Setchell and *Ruppia cirrhosa* (Petagna) Grande, where the water column DIN and SRP concentrations generally range between 7 to 14 µM and 1.5 to 3.2 µM, respectively (DWA 2009).

2.3.5 The effect of grazers

The importance of grazers in nutrient cycling and storage depends on how much primary production they consume. As consumption increases, the quantity of primary production that remains stored as primary producer biomass decreases (Cebrian 2004). Therefore severe grazing has the ability to maintain low levels of producer biomass, and the contribution of grazers to nutrient cycling through faeces production and exudation also increases as a larger portion of primary production is consumed (Cebrian and Durate 1994, Cebrian 2004). Excreta from herbivores are usually much richer in nutrients than detritus from producers, and are therefore decomposed much faster. This means that the potential

for nutrient cycling in the system can significantly increase depending on the percentage primary production consumed and excreted (Cebrian 2004). Studies indicate that the intensity of herbivory on primary producers is variable, ranging from negligible to approximately 100% consumption in microphytobenthos (Baird and Ulanowicz 1993), macroalgae (Ferreira *et al*. 1998) and seagrasses (Valentine *et al*. 2000).

Cebrian (2004) compiled data from previous literature sources, in an attempt to observe trends in the extent and control of herbivory within and between communities of microphytobenthos, macroalgae and seagrasses. His findings point out that indeed herbivory on benthic producers are variable within each community type, and that they may remove small or large percentages of primary production from the benthic primary producers. However, there was a tendency for percentage consumption to be higher in microphytobenthos and macroalgae (c.a. 40%) than in seagrass communities. Most of the seagrass communities lost only 10% of production to herbivores. Although a large variability is found within each community type, microphytobenthos and macroalgae lose a greater portion of production than seagrasses.

Many observations indicate that eutrophication occurring in pristine shallow waters causes the replacement of seagrass with green filamentous macroalgae (Burkholder 2007, Martinez-Lüscher and Holmer 2010, Valdemarsen *et al*. 2010, Rasmussen *et al*. 2012). A change in the structure of these habitats could have negative consequences for many organisms. Some organisms spend their entire life cycle within seagrass meadows and others use the meadow only for certain stages during their life cycle (Cebrian 2004). For example, many of the temporary residents in seagrass meadows consist of juvenile fish, many of which are associated with commercial and recreational value (Heck *et al*. 2003). During recruitment the juveniles find food and shelter in seagrass meadows, and upon development to a certain stage they swim to deeper water. It is clear that seagrasses play an important role as nursery areas for many fish species (Cebrian 2004). Anoxic conditions resulting from macroalgal overgrowth has been well documented. Within these dense mats, photosynthesis is normally restricted to the top few layers because of intense self-shading (Cebrian 2004). As oxygen is intercepted in the top few layers, only a small percentage diffuses downward and is rapidly depleted by the shaded bottom layers. Furthermore, bacterial activity within macroalgal mats exacerbates the depletion of oxygen leading to hypoxic or anoxic conditions (Worcester 1995). Other changes associated with algal blooms is the production of harmful sulphides and ammonia which are particularly harmful to the organisms that inhabit the algal mat (Cebrian 2004).

2.3.6 The role of decomposition in nutrient cycling

Nutrients assimilated during plant growth are temporarily immobilised into living tissue and detritus. These nutrients eventually end up mineralised either through grazing or decomposition (Banta *et al*. 2004). The decomposition of organic matter can be slow and may vary between decades or centuries if the material is 'resistant' to decomposition. The associated nutrients in organic matter then become stored for longer periods. Whether detritus-bound nutrients are mineralised and recycled quickly, or whether they become stored in slowly degradable detritus over longer time scales, is controlled by the rates and patterns of decomposition of plant detritus (Banta *et al*. 2004).

The percentage of net primary production lost through grazing and decomposition differs among primary producers (Durate 1995). Slow growing macrophytes such as seagrasses and macroalgae lose a smaller proportion of net primary production to grazers than fast growing micro- and macroalgae, hence the organic matter produced by slower growing macrophytes ends up as detritus. Decomposition studies (Schmidt 1980, Valiela *et al*. 1985, Buchsbaum *et al*. 1991) on various primary producers have shown that detritus from phytoplankton and fast growing macroalgae degrade faster than slow growing, perennial macroalgae and seagrasses. This suggests that nutrients are recycled much faster in the former groups (Banta *et al*. 2004). Nutrient loss through mineralisation may occur faster than organic matter decomposition (net mineralisation) or may be lost more slowly than organic matter decomposition due to nutrient assimilation by microflora (net immobilization during decomposition) (Banta *et al*. 2004). According to Banta *et al.* (2004) evaluation of mineralisation of detritus bound nutrients should be founded on changes within the N and P pools, and not changes in biomass.

Decomposition of detritus can be viewed in stages (Aber and Melillo 2001). Firstly the rapid leaching phase in which labile matter (i.e. low molecular weight sugars and amino acids) are abiotically lost from the newly dead cells. This is followed by a slower loss of material via microbial activity, i.e. decomposition. Since the quality and susceptibility of compounds to microbial breakdown found in the detritus of plant litter differ, decomposition may occur at different rates. For example, labile matter is decomposed much faster than high molecular weight sugars and cellulose, which in turn decompose faster than lignin (Banta *et al*. 2004). Refractory compounds which are 'resistant' to microbial breakdown may also be present within the plant litter and result in prolonged decomposition rates (years to decades). The decomposition and mineralisation rates of detritus vary greatly between different plant

groups because of the different structural components of these plants (Banta *et al*. 2004). Excessive growth of *Ulva rigida* (4500 g m^{-2}) caused large scale decomposition, leading to anoxia in the water column and sediments in the Venice lagoon (Bona 2006). Berezina and Golubkov (2008) observed that the decomposition of *Cladophora glomerata* within the shallow areas of Neva Estuary resulted in anoxic conditions and high concentrations of SRP (12 µM) within the water column. These concentrations were 2 to 3 times higher than those at the beginning of algal growth in the study area, and this may have been as a result of rapid P release during algal decomposition. Significant decomposition of *Cladophora glomerata* occurred 15 days into the Paalme *et al*. (2002) *in situ* field experiment. They state that temperature was a key factor controlling the decomposition rate, achieving a maximum rate of 65% after thirty days of deployment *in situ* during summer, while no loss of tissue was recorded during the spring deployment. It becomes clear that the fast turnover time of labile organic matter, present in macroalgae, leads to an additional supply of remineralised inorganic nutrients that potentially become available for recycling within the water column (Berezina and Golubkov 2008).

2.4 The eco-physiology of *Cladophora*

In recent years, algal blooms of *Cladopora glomerata* have become prevalent in the Great Brak Estuary and compete with other submerged macrophytes (*Zotera capensis* and *Ruppia cirrhosa*) for light, space and available nutrients. In order to gain insight into why this macroalgae is so successful in occupying any freshwater or marine habitat it comes into contact with, it is necessary to understand its eco-physiology. The genus *Cladophora* has a range of representatives which are dispersed worldwide, often found dominating the benthos of fresh and marine waters (Dodds and Gudder 1992, Higgins *et al*. 2008). *Cladophora* is a filamentous chlorophyte with varied degrees of branching (Van den Hoeck 1982). It is generally an attached benthic alga, although sometimes it occurs as free floating mats and as loose masses on soft substrates. Representatives of this genus can be found in waters ranging from oligotrophic to eutrophic habitats (Dodds and Gudder 1992). Some factors related to distribution and abundance are discussed below:

2.4.1 Substrate

Cladophora glomerata is capable of colonising an array of different substrate types, and preferentially colonises solid substrates (Higgins *et al*. 2005). It has been observed as epiphytic (attached to plants), epilithic (attached to rock) and epizoic (attached to animals)

(Higgins *et al*. 2008). In the Great Brak Estuary it grows in the shallow intertidal, bethically attached to the sediment as well as seagrasses and rock (Adams 2008). Rock attachment is most common in marine species and some form masses which float to the surface of saltmarsh pools or lie loose on the sediment bottom (Dodds and Gudder 1992). Freshwater species have been found attached to fish and clams (Curry *et al*. 1981) and have also been reported occupying rice fields (Pantastico and Suayan 1973) and occur as epiphytes on *Potamogeton pectinatus* in a brackish lake (Howard-Williams and Allanson 1981). The inclination of hard substrates was said to be an important factor for colonisation by Konno (1985), who found that the dominance of marine *Cladophora wrightiana* decreased at an inclination of greater than 120°. Dodds and Gudder (1992) consider that this could be due to the fact that there is less light beneath an overhang. Some species exist as loose aggregates or balls on soft substrates in both marine and freshwater habitats (Niiyama 1989). Others have been found growing in a community with cyanobacteria and stromatolites. By the indirect precipitation of magnesium calcite and the trapping sediment these communities actually build the substrate on which *Cladophora* occurs (Dodds and Gudder 1992).

2.4.2 Hydrodynamics

The success of *Cladophora* can be attributed to its ability to withstand the sheer stress found in benthic regions of rivers and intertidal rocky habitats (Dodds 1991). *Cladophora* has a tough thallus and is flexible allowing flow through and around it. Under low current velocities the thallus spreads out and becomes more streamlined as flow increases. Streamlining may be a general adaptation to flow and has been observed in other macroalgae (Dodds and Gudder 1992). *Cladophora* mainly resides outside the boundary of the diffusion gradient in the area where there is mostly turbulent transport (outside the area dominated by molecular diffusion). There are, however, short tufts which are present within the diffusion boundary (Dodds and Gudder 1992). Transport of materials to and away from the thallus is not constrained by molecular diffusion because of the fact that there can be significant current inside tufts attached to rocks (Dodds 1991). Hydrodynamics to a lesser extent can influence the biomass of *Cladophora* found on a particular substrate. Weak correlations were found between abundance and rock size in a river (Dodds 1991). Dodds and Gudder (1992) suggested that these correlations occurred because of the fact that larger rocks were less likely to be over turned by floods and hence their upper surfaces were subjected to fewer disturbances than smaller rocks (Dodds and Gudder 1992). Sand-Jansen *et al*. (1989) identified that flooding decreased *Cladophora* biomass in rivers. The resistance

to abrasion by filamentous algae relates to its ability to withstand hydrodynamic disturbance. Waves in tidal pools affect the biomass of these algae by breaking off fragments from thick loosely attached mats. The tolerance ranges to surf exposure differ between species, but it is not clear whether these differences are related to flexibility of thalli, stronger hold fasts or other factors (Dodds and Gudder 1992). Complete submersion in rivers and estuaries allows for greater growth than that which occurs in an intertidal rocky shore (Oertel 1991).

Water velocity and photosynthetic rates have also been studied by Pfiefer and McDiffett (1975). They found that there was an increase in the photosynthetic rate of *Cladophora* with an increase in the water velocity from 0 to 2.1 cm s^{-1} and the photosynthetic rate doubled when increased to a further 8.0 cm s^{-1}. Water velocities above 8.0 cm s^{-1}, however, led to decreased rates in photosynthetic performance (Dodds 1991). The transport of CO_2 into the tufts may relieve C depletion by increasing CO_2 availability while at the same time lowering the dependence of HCO_3^- as a C source. The increased photosynthetic rate results from an increased transport of material within the current which enhances algal productivity (Whitford 1960, Dodds 1989). The decrease in photosynthesis above 8.0cm s^{-1} may be related to the streamlining effect as a result of higher current speeds. As tufts become more compact from higher currents, shelf-shading may increase, or transport of material into and away from algae becomes restricted which leads to lower photosynthetic rates. Biomass related to photosynthetic rate has also been studied by Pfiefer and McDiffett (1975) where they found a decrease in photosynthetic rate with an increase in biomass per unit area. They believed the lower photosynthetic rate was due to individual filaments becoming more tightly packed as a result of denser growth of the algae. This led to increased self-shading and decreased transport of material.

The morphology of *Cladophora* in relation to hydrodynamics has been discussed by several authors (Van den Hoek 1964, 1982, Whitton 1970, Gordon *et al*. 1980). Increased wave energy leads to more pronounced branching of filaments in marine algae and cells can become shorter with more turbulence (Van den Hoek 1964, 1982). Similar increased branching occurs in freshwater species with a higher water velocity, along with a decrease in the angle of branches from the main axis (Whitton 1970). ‘Lake balls’ are spherical aggregations of *Cladophora* as result of water motion. Both vertical and horizontal rotations are required for the formation of ‘lake balls’. However, it is unlikely that this is an active biological process because water motion can also cause similar aggregations of nonliving material (Gordon *et al*. 1980, Dodds and Gudder 1992).

2.4.3 Light

Physiological measurements done by Graham *et al*. (1982), as part of the study on growth of nuisance algae in the Great Lakes, established that optimum rates for photosynthesis occurred between 300 and 600 µmol quanta m^{-2} s^{-1}. They also found a net positive photosynthesis when temperature exceeded 5°C and light was above 35 µmol quanta m^{-2} s^{-1}. Similar findings by Lester *et al*. (1988) were obtained for Lake Michigan, where optimum photosynthesis occurred between 345 (July) to 1125 (August) µmol quanta m^{-2} s^{-1}. Compensation points were 44 and 104 µmol quanta m^{-2} s^{-1} respectively. The estuarine *Cladophora albida,* had a net positive photosynthesis rate above 25 µmol quanta m^{-2} s^{-1}. Photosynthetic saturation occurred at 100 µmol quanta m^{-2} s^{-1} at 12°C and at 750 µmol quanta m^{-2} s^{-1} at 30°C, with no observed photoinhibition (Gordon *et al*. 1980).

2.4.4 Temperature

Cladophora seems to die off in the middle of summer in many rivers and lakes (Higgins *et al*. 2005). This is thought to be due to an inability of the alga to maintain dominance above 23°C (Wong *et al*. 1978). Although in some freshwater species, photosynthesis can occur over a short period at temperatures of up to 35°C with optimum rates at 27°C (Dodds and Gudder 1992). The marine species *Cladophora albida* has a maximum photosynthetic oxygen production at 30°C (Gordon *et al*. 1980). Graham *et al*. (1982) found a correlation between temperature and net photosynthetic oxygen production which decreased above 25°C, indicating that the summer die off is related to temperature in Lake Huron. However, other studies have found temperature not to influence midsummer die-off, for example *Cladophora glomerata* had maximum photosynthetic production between 28 and 31°C in Lake Michigan, and Mantai (1987) suggests that in Lake Erie the algae's photosynthetic oxygen production and dark respiration acclimates to the seasonal temperature highs, allowing growth to continue. Dodds and Gudder (1992) believe further studies of photosynthesis sustained at high temperatures are needed to determine the effect of temperature on growth. They point out that there are several factors that may explain why high temperatures cause summer die-offs in some cases but not in others. Firstly, growth responses to temperatures are species dependant. Secondly, interaction may occur between photosynthetic response to irradiance and temperature (Graham *et al*. 1982), an inhibitory temperature at one light level may allow growth at another light level. Thirdly, low nutrients associated with high temperatures (Muller 1983), and fourthly grazers may lower biomass in late summer. Temperature also influences the geographic distribution of

Cladophora (Van den Hoeck 1982, Cambridge *et al*. 1990). Distribution on smaller spatial scales in the intertidal zone may also be a function of temperature (Dodds and Gudder 1992).

2.4.5 Nutrient availability

Cladophora growth has been associated with eutrophication in both marine and freshwater habitats (Lapointe and O'Connell 1989, Lavery *et al*. 1991, Higgins *et al*. 2005, Berezina and Golubkov 2008, Gubelit and Berezina 2010). Examples of this are: eutrophication in Bermuda (Lapointe and O'Connell 1989), in Western Australia (Gordon *et al*. 1980, Lavery *et al*. 1991), in the Neva Estuary, in the Gulf of Finland, marine fish farms (Ruokolahti 1988), in wetlands (Richardson and Schwegler 1986) and in streams and rivers (Sand-Jensen *et al*. 1989, Dodds 1991). In South Africa, other than the Great Brak Estuary, *Clodaphora glomerata* has been found to occur in the oligotrophic East and West Kleinemonde estuaries (TOCEs), as well as the permanently open Mngazana Estuary. There have been no blooms recorded in these estuaries, and this thought to be due to the oligotrophic status of these estuaries (Prinsloo 2012). Excessive growth in freshwater has been related to the addition of phosphorus (Higgins *et al*. 2008). *Cladophora* blooms can also occur in habitats where nitrogen is the limiting nutrient (Lapointe and O'Connell 1989, Dodds 1991). The biomass of *Cladophora* has been found not to correlate with nutrients and is thought to be due to the influence of light (i.e. the algae adjusting for changes in growth rates driven by variations in light) (Lorenz and Herdendorf 1982, Cheney and Hough 1983). Uptake of phosphorus by *Cladophora* has been under considerable study. Some species in both marine and freshwater habitats are able to survive with low ambient extracellular phosphate concentrations (Dodds and Gudder 1992, Higgins *et al*. 2005). For *Cladophora glomerata* half saturation uptake constants around 50-250 µg P l^{-1} (Auer and Canale 1982), 15-86 µg P l^{-1} (Lohman and Priscu 1992) and 8-15 µg P l^{-1} (Wallentinus 1984) have been reported. The half saturation constants for growth of *C. glomerata* are within the ranges reported for uptake which was saturated at 2 mg l^{-1} (Rosemarin 1982). The half saturation constant for uptake by marine *Cladophora albida* ranged from 2 to 55 µg l^{-1} (Gordon *et al*. 1981). *Cladophora* is also capable of producing a phosphatase enzyme, which could allow it to take advantage of dissolved organic phosphorus (Lin 1977).

Critical cell concentrations for N (1.10%) and P (0.06%) have been identified for *Cladophora glomerata* (Dodds and Gudder 1992). If the algal tissue concentration falls below the critical nutrient concentration for a specific nutrient, it is presumably limited by said nutrient. The

ranges of N and P tissue concentrations for *C. glomerata* have been reported as 0.83-4.89% N and 0.04-0.54% P. Critical cell concentrations for the marine *C. albida* has been reported as 1.5% N and 0.05% P (Gordon *et al*. 1981). Salinity can restrict the range of some species of *Cladophora* (Thomas *et al*. 1990). While other species have a broad salinity tolerance range, for example *C. rupestris* which is found in intertidal rock pools and has a range of 5 to 30. The success of most species of *Cladophora* occurring in the intertidal zone suggests that they have a broad salinity tolerance range. The marine *C. rupestris* tolerates considerably higher salinity than the freshwater *C. glomerata* (Dodds and Gudder 1992).

2.4.6 Reproduction and propagation

There is little information on the ecology of reproduction and propagation of *Cladophora* (Dodds and Gudder 1992). *Cladophora* has been observed to have a diplohaplotonic life history (Van den Hoeck 1963, 1982) while in some species sexual reproduction has never been observed, only asexual reproduction by biflagellate or quadraflagellate zoospores (Whitton 1970, Van den Hoeck 1982). The formation of akinetes by some species has been documented (Bold and Wynne 1985) but the there is still limited understanding regarding the factors that form akinetes and their ecological importance. High temperatures, vitamin limitation and a shortened photoperiod have been shown by Hoffman and Graham (1984) to be primary factors which stimulate zoosporogenesis. There are several hundred spores present within each zoosporangium, and upon release they attach their flagella to a hard substratum (Whitton 1970). When favourable conditions arise germination and vegetative growth can occur rapidly, resulting in upright filaments and branching from the main filament (Whitton 1970). According to Van den Hoeck (1963) growth is typically acropetal where apical cells elongate vertically and sub apical cells elongate horizontally. Therefore, the youngest branches are present nearest the apex. *Cladophora glomerata* more than often remains as akinete cells, tightly adhered to its substratum during unfavourable conditions, and proceeds to grow vegetatively as soon as conditions become favourable (Higgins *et al*. 2008). Some authors (Bellis and McLarty 1967, Whitton 1970, Higgins *et al*. 2005) have observed a two node growth cycle for *C. glomerata* in lakes and rivers. They indicate that it grows fastest or blooms in midsummer, followed by detachment of filaments (sloughing) and then has a low growth period and blooms once again in autumn. The autumn bloom may be smaller or larger than the summer bloom. Rosemarin (1982) measured a maximum specific growth rate of 0.8 d^{-1} *in vitro*, but Choo *et al*. (2002) state that these high specific growth rates are seldom realized in natural

settings because of factors like suboptimal environmental conditions, self-shading and reductions in dissolved gas and nutrient exchange within canopies.

2.5 Nutrient cycling: A South African context

There have been very few studies related to submerged macrophytes and macroalgae and the cycling of nutrients in South African systems, and none whatsoever in TOCEs. Howard-Williams and Allanson (1981) studied the cycling of P in a submerged macrophyte (*Potamogeton pectinatus*) bed in Swartvlei, an estuarine lake. Their results showed that the exchange of SRP between *Potamogeton* beds in the littoral zone and open waters was low and attributed this to the effective cycling of nutrients within the beds by components such as periphyton, *Cladophora,* filter-feeders and sediment. They considered the slow exchange between the littoral zone and the open water, to be an important physical factor which contributed to the 'closed' cycling of P in the beds. The work of Tibbles *et al.* (1994) on N fixation associated with *Zosetra* beds in Langebaan Lagoon showed that the highest rate of N fixation occurred in beds with the highest organic matter content. They found that fine muddy sediments supported significantly higher nitrogenase activity compared with sandy sediments, largely due to the quantity of organic matter present.

The most recent studies related to nutrient cycling in South African estuaries include two PhD theses by Switzer (2003) and Goeck (2005). Switzer (2003) worked on the permanently open Knysna estuary, where he found that *in situ* benthic flux of sediment closest to the mouth of the estuary, was more active in the production of NH_4^+, and consumed more TOxN from the water column when compared to sediment further upstream during every season. While Goeck (2005) studied the nutrient flux of the permanently open Swartkops and Mgazana estuaries and compared them to the Sylt-Romo Bight in Germany. She found that sediment in Mngazana acted as a sink for nutrients in summer and winter, reflected by the uptake from the water column. She further found that the estuary was autotrophic during summer and changed to heterotrophic during winter. The Swartkops Estuary was heterotrophic throughout the study period, but more so in autumn and winter, because of large respiration rates dominating net production rates. The sediment acted as a sink for TOxN and SRP and as a source of NH_4^+ to the overlying water. The Sylt-Romo Bight was generally heterotrophic and changed to autotrophic during summer. She concluded that temperature was a primary factor influencing fluxes to varying extents within the systems studied, and the presence and abundance of macrofauna resulted in a significant difference in exchange rates between the different systems.

3 THE STATE OF WATER QUALITY IN THE GREAT BRAK ESTUARY AND ITS EFFECT ON THE SUBMERGED MACROPHYTES AND MACROALGAE

3.1 Introduction

The three main threats to estuaries as recorded by Russell *et al*. (2006), Painting *et al*. (2007) and Manning *et al*. (2007) are changes to river flow, eutrophication and accelerated rates of sedimentation. This along with overpopulation along the coast has led to a decrease in the quantity of freshwater entering estuaries, and many ecological stresses (Flemer and Champ 2006, Russell *et al*. 2006, Snow and Adams 2006, Snow and Adams 2007). While the world average annual rainfall is 860 mm annum^{-1}, the semi-arid regions of the world receive much less rainfall per annum, and in conjuction with a rise in the population, the natural supply of water no longer reaches estuaries (Otieno and Ochieng 2004). This has resulted in the construction of reservoirs and diversion schemes in order to meet the needs of agriculture, industry and domestic use (Otieno and Ochieng 2004). When the inflow of water is severed from rivers with small catchments, estuaries in turn will be, deprived of freshwater, at risk of mouth closure, become more resistant to scouring or become marine dominated (Allanson 2001, Snow and Adams 2007). Since TOCEs have a relatively small catchment (usually less than 500 km^2) (Whitfield 1992) and remain closed off from the sea for prolonged periods, they are greatly influenced by anthropogenic effects such as altered hydrodynamics and degradation in the quality of inflowing river water (Snow and Taljaard 2007). These systems often do not have the ability to cope with excessive nutrient loading and reduced flow.

In 1989 the Department of Water Affairs (DWA) built a 70 m high and 270 m long dam with a capacity of 23 x 10^6 m^3. It was built 3 km upstream of the estuary head and has led to a reduction in freshwater flow which has resulted in the system having predominantly closed mouth state (Slinger *et al*. 2005). The normal rate of flow to the estuary has been considerably reduced during the last decades as a result of afforestation, direct abstraction and the dam from 36.8 x 10^6 m^3 under natural condition to 16.25 x 10^6 m^3 at present. An Environmental Impact Assessment conducted on the dam after construction concluded that reduced flow could be mitigated by artificially breaching the mouth while simultaneously releasing freshwater from the dam to improve the scouring of sediment. The recommended freshwater release was to be 2 x 10^6 m^3 annum^{-1}. In response to this a mouth management plan was developed and the mouth is artificially breached during spring and summer of each year (Slinger *et al*. 2005). Prior to the development of the dam the mouth of the estuary

remained open for fairly long periods (36 -70% of the year) (Huizinga and Van Niekerk 2003). After the construction of the dam during the period 1992-2000, there was still a significant overflow of freshwater into the estuary which kept the mouth open (Huizinga and Van Niekerk 2003) mainly because there was no abstraction of freshwater from the dam in the early post-construction years. However, since abstraction commenced, the mouth of the estuary is now breached only once or twice a year depending on whether or not the allocated volume of water is available (Huizinga and Van Niekerk 2011).

In order to maintain growth, seagrasses require inorganic carbon and nutrients. Because seagrasses can use both CO_2 and HCO_3^- (Invers *et al*. 2001, Lee *et al*. 2007) it is highly unlikely that they would become carbon-limited. The nutrients that commonly limit seagrass growth are nitrogen and phosphorus (Duarte 1990, Romero *et al*. 2006). The inorganic nitrogen forms accessible in the water column to seagrasses are NO_3^- and NH_4^+, while in the sediment NH_4^+ is the predominantly available form. The major phosphorus form is PO_4^{-3} and is found in both the sediment and the water column (Lee *et al*. 2007). Before the construction of the Wolwedans Dam in 1989, the submerged macrophytes (*Ruppia cirrhosa* and *Zostera capensis*) coexisted with the macroalgae *Caulerpa filiformis*, in the water area below Die Eiland Bridge (Morant 1983). These submerged macrophytes occur at elevations less than 0.9 m above MSL and would be present during both open mouth tidal conditions and closed mouth conditions (Adams 2008). However this has changed over the past three decades. The alga *Cladophora glomerata* has grown profusely in the shallow intertidal areas in the lower reaches of the estuary and has become a nuisance to the local residents. Some authors (Valiela *et al*. 1997, Cloern 2001, Blomster *et al*. 2002, McGlathery *et al*. 2007) have declared that the occurrence of opportunistic macroalgae in shallow waters is increasingly common worldwide and is considered to be a response to eutrophication.

The effects of a reduction in the amount of freshwater entering estuaries has been well documented in South Africa and other parts of the world (Slinger and Largier 1990, Largier and Taljaard 1991, Adams and Talbot 1992, Adams *et al*. 1992, Grange and Allanson 1995, Whitfield and Wooldridge 1994, Bate *et al*. 2002, Coates and Guo 2003, Gobler *et al*. 2005, Snow and Adams 2006, Riddin and Adams 2008, Domingues *et al*. 2011, Ortegon and Drake 2011). Special emphasis has been placed on the dependence of these small estuaries on the freshwater flow which serves in conjunction with tidal exchange to flush sediment and water from the estuary (Allanson and Read 1995). This chapter aims to investigate environmental conditions within the estuary and describe the states in which the estuary resides.

3.2 Materials and methods

3.2.1 Study site

The Great Brak Estuary (34°03′23″S; 22°14′18″E) is about 6.2 km long and is located on the south coast of South Africa, drains a forested, semiarid catchment area of 192 km^2. The catchment receives more or less equal amounts of rain throughout the year with slight peaks in spring and autumn. However, the area is subjected to droughts and occasional flooding and the recorded annual run-off varies from as little as 4.3 x 10^6 m^3 to as large as 44.5 x 10^6 m^3 (DWA 2009). The estuary has a high tide area of 0.6 km^2 and a tidal prism of 0.3 x 10^6 m^3 (DWA 2009). The mouth of the estuary is bounded by a low rocky headland on the east and a sand spit on the west. Immediately inland of the mouth the estuary widens into a lagoon basin containing a permanent island about 400 x 250 m in size. The lower estuary is relative shallow (0.5 to 1.2 m deep) with some deeper areas in scouring zones near the rocky cliffs and bridges. The middle and upper estuary is less than 2 m deep, with some deeper areas between 2 and 4 km from the mouth varying between 3 and 4 m deep (Figure 3.1).

The mouth of the Great Brak Estuary generally closes when high waves at sea coincide with periods of low river flow. The mouth of the Great Brak Estuary is breached artificially at 2 m msl. The properties on Die Eiland occur at elevations around 2 m msl within the estuary flood plain (DWA 2009). This prevents the estuary from being breached at higher water levels (> 3 m msl), and as a result limits the amount of sediment removed from the estuary in the lower reaches. The estuary was sampled from September 2010 to July 2012. The mouth was closed during September 2010, November 2010 and April 2011, and was open in February 2011 as well as from June 2011 to July 2012 (Figure 3.2). The mouth of the estuary was artificially breached in February 2011 with a volume of 0.3 x 10^6 m^3 and closed a week later (Figure 3.3). A 1:100 year flood with volume close to 3 x 10^6 m^3 breached the mouth naturally in June 2011 flushing water and sediment out of the estuary and scouring the mouth down to 0 m mean sea level.

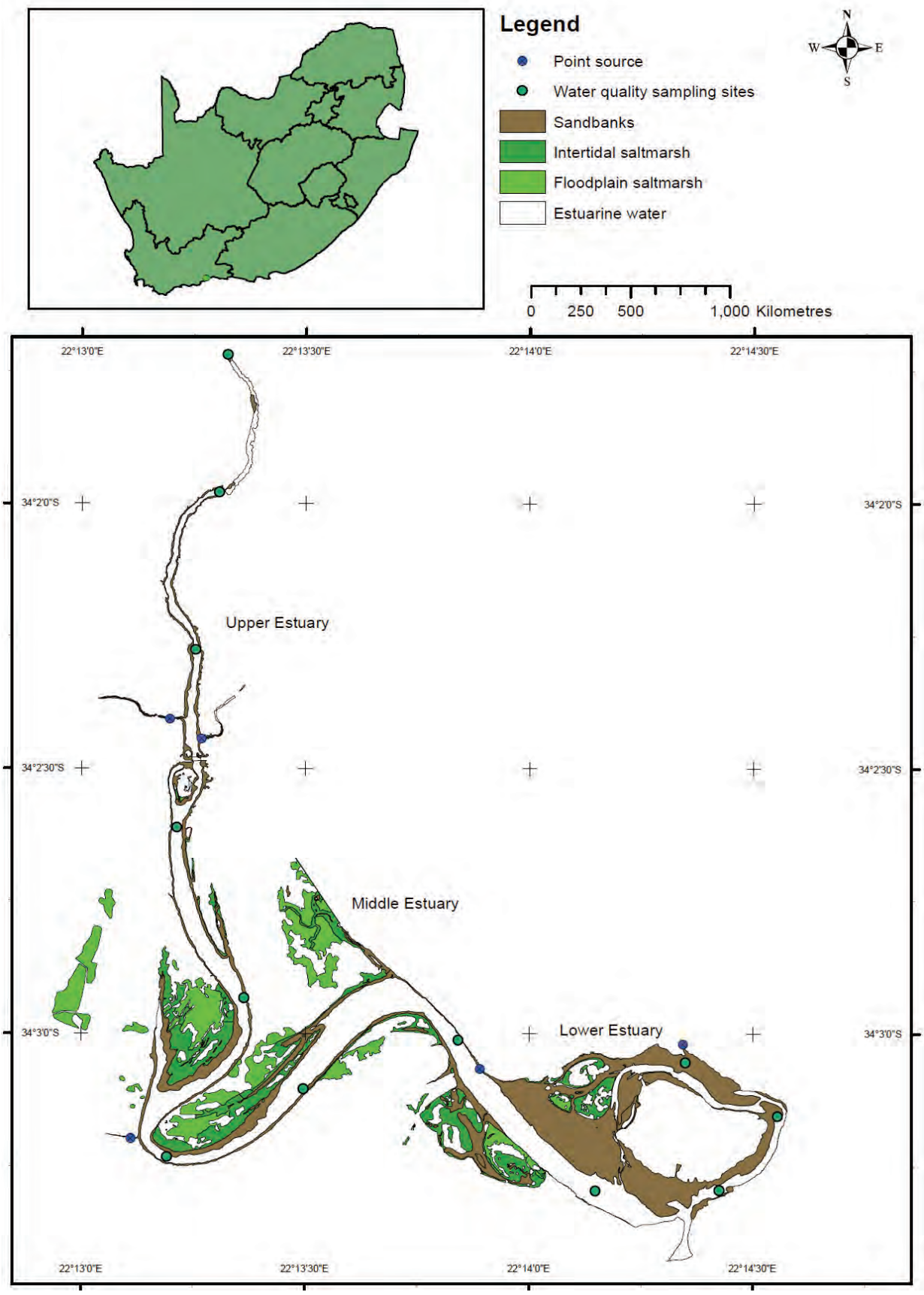

Figure 3.1 Map of the Great Brak Estuary on the south coast of South Africa

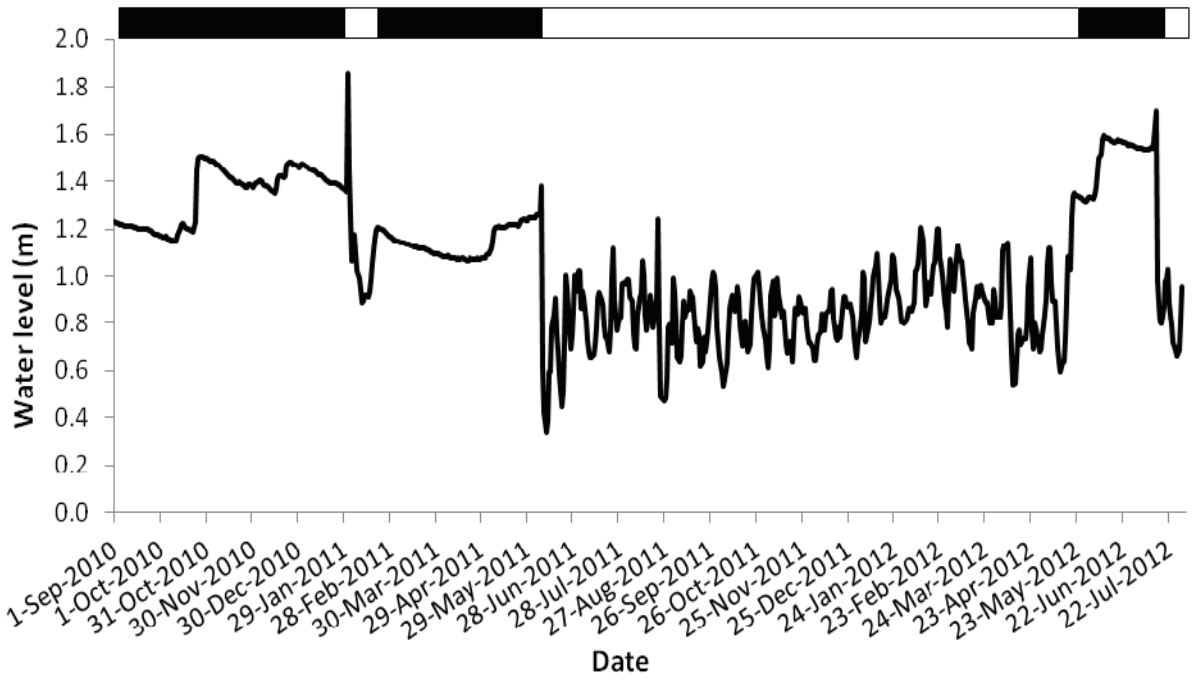

Figure 3.2 Water level and mouth state of the Great Brak Estuary for the sampling period September 2010 to July 2012. (Open blocks = open mouth conditions and closed blocks = closed mouth condition).

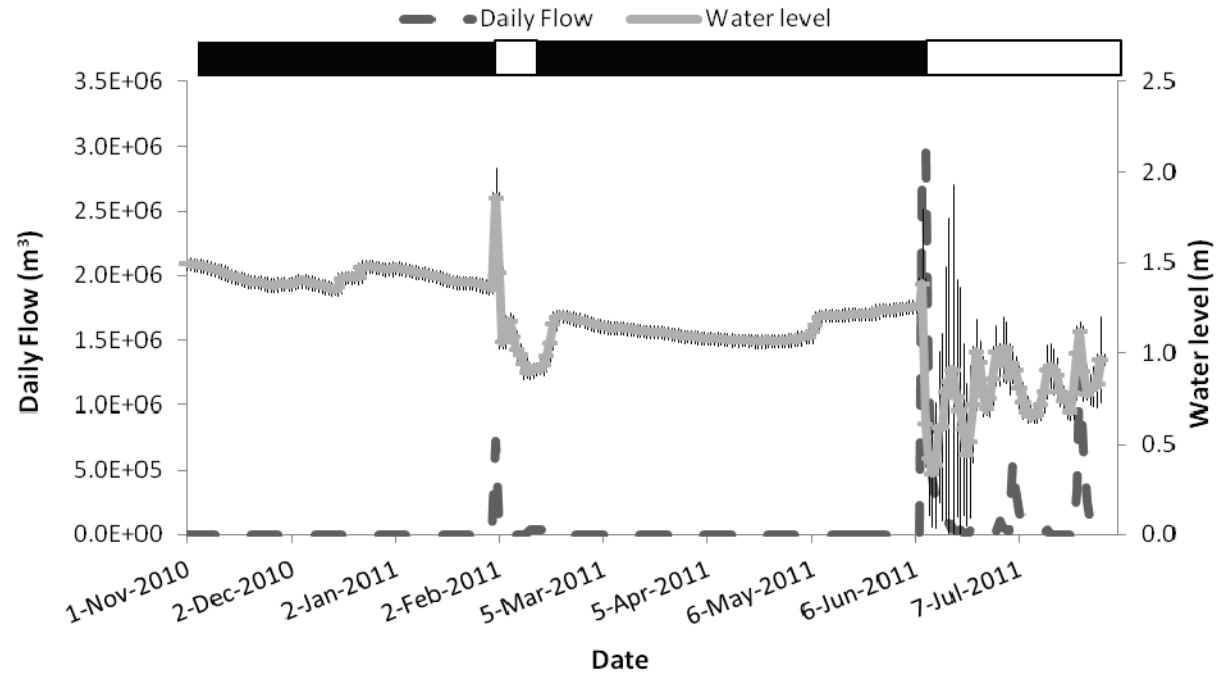

Figure 3.3 Water level and flow volume for the period November 2010 to July 2011. Open phases are represented by open blocks and closed phases by solid blocks.

3.2.2 Physico-chemical characteristics

Salinity, temperature, dissolved oxygen and pH were measured with a Hanna multiprobe at each of the sampling stations (Figure 3.1) at depths of 0.5 m intervals from the surface to the bottom. Water samples for nutrient analysis were collected from the main channel at the surface and at 1 m depths throughout the water profile (Figure 3.1; green dots). The water was filtered through 0.45 µm syringe filters and frozen.

Ammonium (NH_4^+)

Filtered samples for ammonium (NH_4^+) were analysed using standard spectrophotometric methods (Parsons *et al*. 1984). A 10 mM stock solution was made by dissolving 0.535 g of NH_4Cl in 1 litre of deionised water. To make up a 73 µM solution, 0.73 ml of the stock was diluted in a 100 ml. The following standards were then made from the 73 µM solution: by taking 1.5, 3, 6, 12, 24, 48 and 96 ml; concentrations of, 1.10, 2.19, 4.38, 8.76, 17.52, 35.04 and 70.08 µM were made. Only standard curves with $r^2 = 0.99$ were used throughout the analysis.

A pipette was used to transfer 2.5 ml of sample into glass vials, after which 0.1 ml of phenol solution (20 g phenol dissolved in 200 ml 95% ethanol) was added. The mixture was shaken and 0.1 ml sodium nitroprusside was added (1 g sodium nitroprusside to 200 ml deionised H_2O), which was followed by the addition of 0.25 ml oxidizing solution. The oxidizing solution consisted of the 10 ml alkaline reagent (a mixture of 20 g sodium citrate and 1 g NaOH in 100 ml deionised H_2O) and 2.5 ml of sodium hypoclorite solution. After the addition of the oxidizing solution the vials were covered and allowed to stand in the dark for at least 1 hour. Each of the samples and standards were read on a spectrophotometer at 640 nm wave length.

Soluble reactive phosphorus (SRP)

Filtered soluble reactive phosphorus samples were analysed using standard spectrophotometric methods (Parsons *et al*. 1984). A 6 mM stock solution was made by dissolving 0.816 g of KH_2PO_4 in 1 litre of deionised H_2O. A 1.2 ml of the stock was diluted to a 100 ml volumetric flask to make a 72 µM solution. The following standards were then

made from the 72 µM solution: by taking 1.5, 3, 6, 12, 24, 48 and 96 ml; concentrations of, 1.08, 2.16, 4.32, 8.64, 17.28, 34.56, and 69.12 µM were made.

A pipette was used to transfer 2.5 ml of sample into glass vials, after which 0.25 ml of mixed reagent was added. The mixed reagent consisted of a solution of 5 ml of ammonium molybdate (15 g ammonium molybdate in 500 ml deionised H_2O), 12.5 ml of sulphuric acid (70 ml sulphuric acid in 450 ml deionised H_2O), 5 ml ascorbic acid (1.35 g ascorbic acid in 25 ml deionised H_2O) and 2.5 ml potassium antimony tartrate (0.34 g potassium antimony tartrate in deionised H_2O). Each of the samples and standards were read on a spectrophotometer at 885 nm wave length.

Total oxidised nitrogen (TOxN: NO_3^- + NO_2^-)

The detection of total oxidized nitrate was done according to the reduced copper cadmium method as described by Bate and Heelas (1975). The copper cadmium was prepared by washing the cadmium powder with a 100 ml 5% HCl solution. After which the cadmium was rinsed with distilled water. A 500 ml 0.5% $CuSO_4.5H_2O$ solution was then used to rinse the cadmium until it changed from a silver grey to dark grey colour. Immediately after the colour change the dark grey cadmium was rinsed with a 500 ml 0.007 N HCl containing 0.005 M Na_2EDTA solution. Washing was repeated several times and the supernatant containing precipitated copper was decanted until the copper cadmium changed to a silver grey colour. A 5 mM stock solution was made by dissolving 0.51 g of KNO_3 in 1 litre of deionised H_2O. To make up a 144 µM solution, a 2.88 ml of the stock was diluted to a 100 ml in a volumetric flask. The following standards were then made from the 144 µM solution: by taking 1.5, 3, 6, 12, 24, 48 and 96 ml; concentrations of, 2.16, 4.32, 8.64, 17.28, 34.56, 69.12 and 138.24 µM were made.

A pipette was used to transfer 3 ml of sample into glass vials, after which 2 ml of buffer solution (24.4 g l^{-1} NH_4Cl) adjusted to pH of 9.6 was added. Approximately 2 g of copper cadmium was added to the each of the vials and the mixture was agitated for 20 min to ensure all N present was converted to NO_3^-. A 1 ml sample was then withdrawn from each vial, and placed into another glass vial. To each of these vials, 1 ml sulfanilimide solution (1 g sulfanilimide mixed with 100 ml of 1.5 N HCl) and 1 ml diamine hydrochloride solution (0.02 g N1-naphthyl-diamine hydrochloride added to 100 ml deionised H_2O) were added. Each of the samples and standards were read on a spectrophotometer at 540 nm wave length.

Unfiltered water samples were collected for the simultaneous detection of total nitrogen (TN) and phosphorus (TP) and was analysed using the persulphate digestion as described by Grasshoff *et al*. (1983). To each 5 ml water samples and standards, a 1 ml oxidizing agent was added. The oxidizing agent was composed of two parts. Firstly a 0.357 mol l^{-1} sodium hydroxide solution was made (3 g sodium hydroxide in 200 ml water). Secondly 90 ml deionised H_2O was added to 5 g potassium persulphate and 3 g boric acid. 10 ml of the sodium hydroxide solution was then added to 90 ml of the potassium persulphate and boric acid solution mixture, to make up the oxidizing agent. After the addition of the oxidizing agent, the samples were digested in an autoclave at 120°C for 1.5 hours. After the samples cooled, the detection of TN and TP occurs by following the protocol described for TOxN and SRP. The only change is the concentration of the standard series for TN. 5 ml of the 5 mM stock was diluted to a 100 ml volumetric flask to make a 250 µM solution. By taking 5, 10, 20, 40, and 80 ml of the 250 µM stock, concentrations of, 12.5, 25, 50, 100, and 200 µM were made.

Particulate total nitrogen and phosphorus

Additional samples were collected for the determination of particulate nitrogen and phosphorus. Samples of estuary water (500 ml) were collected from 1 m depth intervals and filtered through Whatman GFC filters. These filters were digested using the persulphate digestion method (Grasshoff *et al*. 1983). The filters were added to a 50 ml oxidizing solution. The oxidizing solution consisted of 11.25 g potassium persulphate, 4.75 g sodium hydroxide and 6.75 g boric acid dissolved in 1 l of deionised H_2O. The same method of detection for TN and TP as described above was then followed. All samples were analysed at the Physiology Laboratory of the Nelson Mandela Metropolitan University Botany Department (NMMU South Campus).

The contour profiles (Figures 3.4 to 3.14) for all physico-chemical characteristics were created with Golden Software, Grapher version 6. Later versions of the software do not perform the Krigging function adequately in order to generate proper contours.

3.2.3 Submerged macrophtyes and macroalgae

A PVC corer (Ø = 10.5 cm) was used to extract submerged macrophyte samples at several sites. Six replicates were taken at each site. The samples were stored in a cool

environment while being transported to the laboratory. The plants were dried for 48 hours at 60ºC and the dry mass (g dm m^{-2}) determined using an electronic scale. Above and below ground tissue content of nitrogen and phosphorus were determined for the various macrophyte species. Whole plants (with leaves, rhizomes and roots) or macroalgae (thallus) were harvested and cleaned of sediment in freshwater for 2 to 3 min. The dried tissue was finely ground, and analysed with the persulphate digestion method for the simultaneous detection of total nitrogen and total phosphorus (Grasshoff *et al*. 1983). 0.05 g tissue was added to a 50 ml oxidizing solution and the same method of detection for TN and TP as described above was then used.

Submerged macrophytes and macroalga (*Zostera capensis, Ruppia cirrhosa* and *Cladophora glomerata*) area cover were digitized on a monthly basis and the area was calculated from aerial and ground truth/verified photographs using ArcGis software version 10.

3.2.4 Data analysis

The data was statistically analysed using Statisica Software Version 10. A Shapiro Wilks test for normality was used to determine if the data were parametric or non parametric. When the data showed a non parametric distribution a Kruskal-Wallis ANOVA for significant difference was performed. If the data had a normal distribution a one way ANOVA and Tukey HSD test was performed. When two variables were compared, a T-test or Mann-Whitney U Test was used to analyse data.

3.3 Results

3.3.1 Physico-chemical

Temperature in the estuary followed a seasonal trend with average spring/summer months (November 2010, February 2011 and April 2011) significantly warmer (20-24°C) than the winter months (June-July 2011) (13-16°C) (Figure 3.4) ($H = 164.05$, $n = 193$, $p ,< 0.05$).

The salinity in all months was significantly higher ($H = 143.33$, $n = 279$, $p < 0.05$) than July 2011 (Figure 3.5), mainly because of the incoming freshwater input from the river and catchment. The estuary had just received 55 mm of rainfall. During the closed mouth phases

in September 2010, November 2010 and April 2011 the salinity was generally well distributed as a result of little freshwater input at the head of the estuary and no seawater intrusion at the mouth. After the artificial breach in February 2011 a longitudinal salinity gradient developed in the estuary, with saline water (25-28) around the mouth and brackish water (12-15) extending from the middle to upper reaches (Figure 3.5). In November 2011, the estuary was in a marine dominated state because of marine water intrusion during a spring high tide. The salinity in March 2012 was saline around the mouth extending up to 2 km, whereas stratification occurs from about 2 to 5.5 km, and July 2012, had a similar trend, with salinity ranging between 18 and 26, from the mouth and up to 3 km, and the middle and upper reaches fresh.

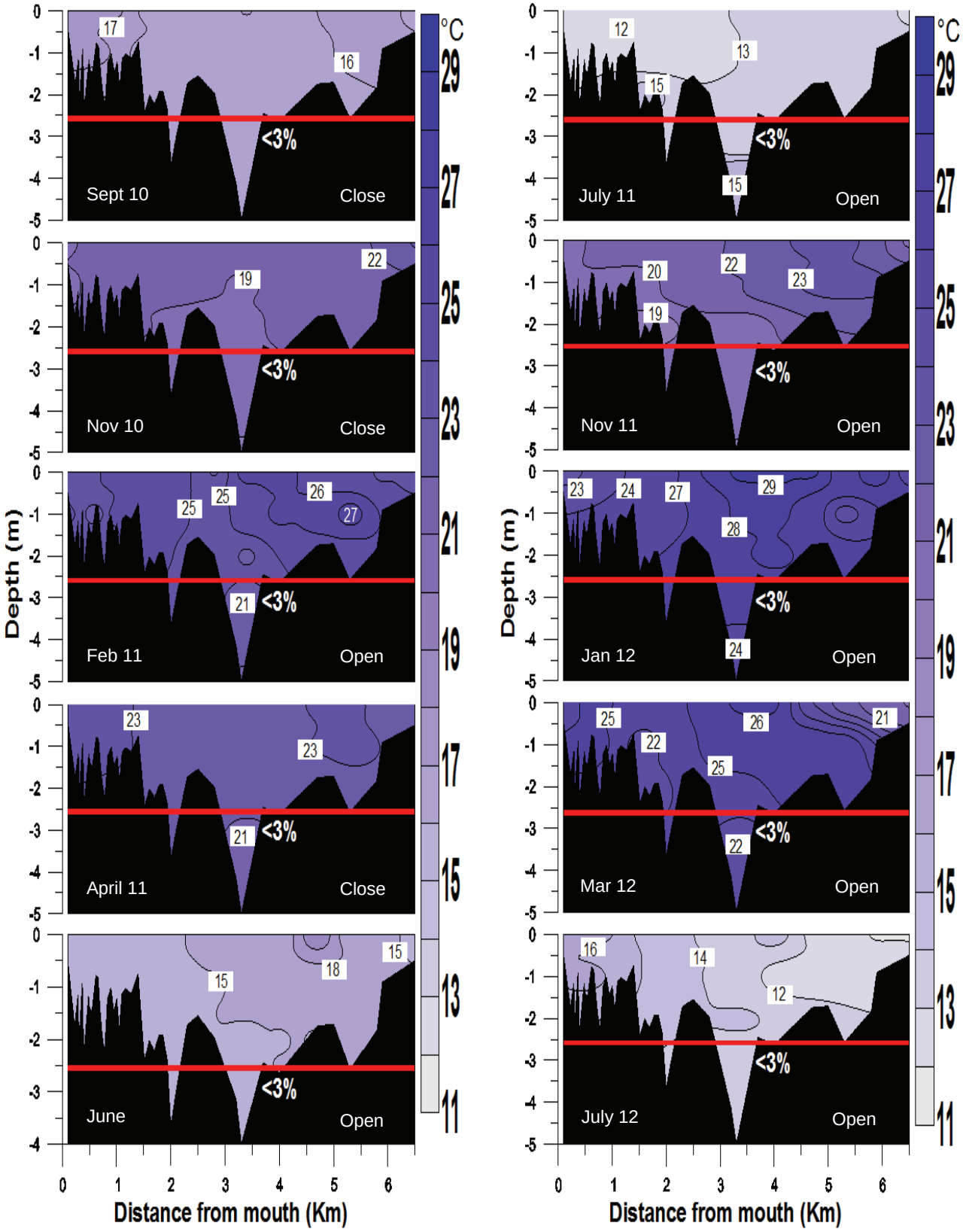

Figure 3.4 Water temperature along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

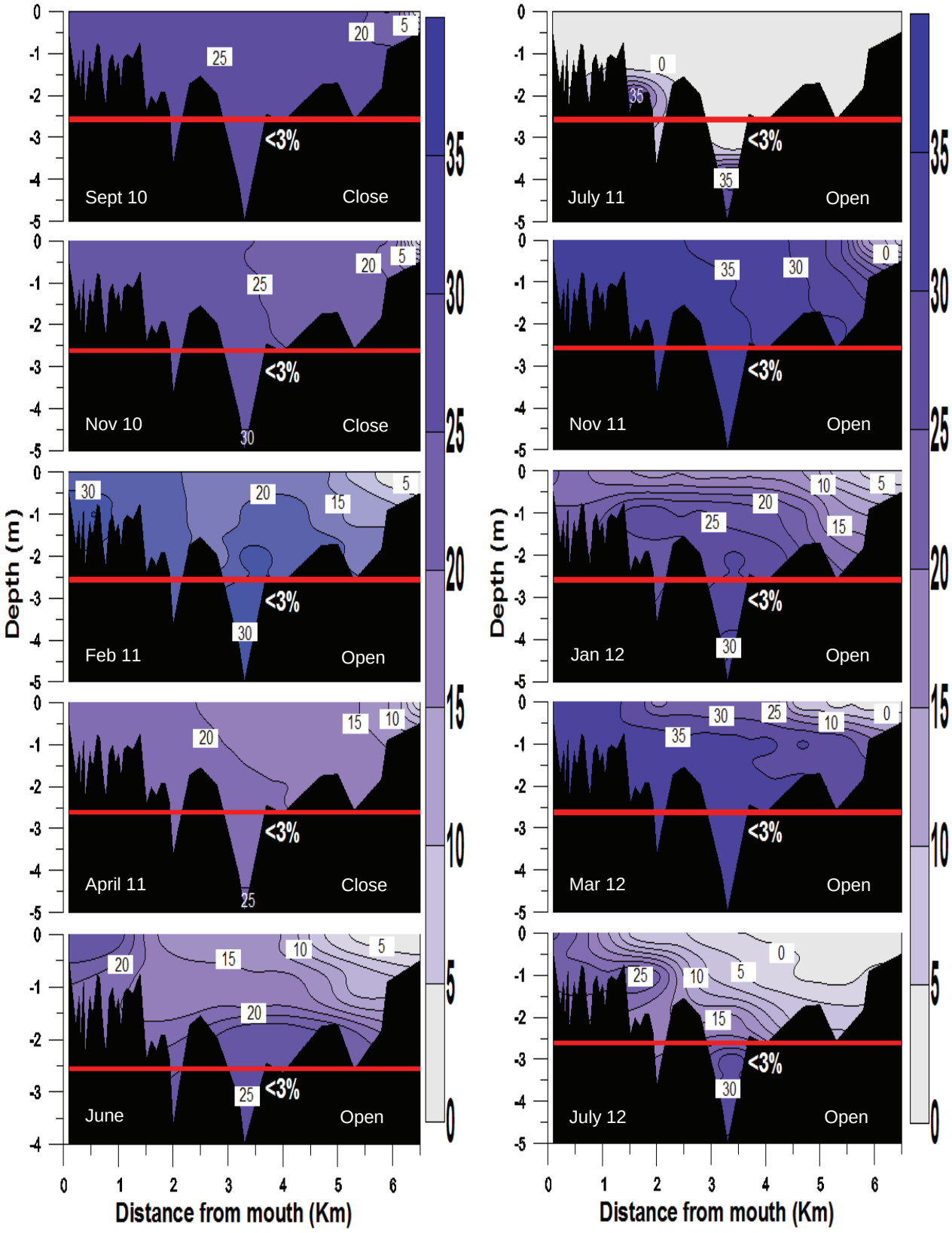

Figure 3.5 Salinity along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

Dissolved oxygen concentration in September 2010, November 2010, and November 2011, was significantly higher ($H = 235.64$, $n = 439$, $p < 0.05$) than the dissolved oxygen concentration in February 2011, April 2011, January 2012 and March 2012 (Figure 3.6). This occurred because during February 2011 the mouth of the estuary was artificially breached, but eventually closed only a week later. The sudden closure of the mouth led to DO levels dropping within the water column, as there was no inflow of water from either the river or the sea. Similarly by April 2011 the DO concentrations dropped even further as there had been no change in the mouth state of the estuary (Figure 3.6). The DO concentration in June 2011 was significantly higher ($H = 160$, $n = 279$, $p < 0.05$) than April 2011 and January 2012, due to a 1:100 flood naturally breaching the mouth in June 2011, which introduced well oxygenated water into the estuary. Similarly the dissolved oxygen was significantly higher ($H = 160$, $n = 279$, $p < 0.05$) in July 2011 than all other months as a result of the strong incoming freshwater flow oxygenating the entire water column. The significantly lower DO concentrations for January 2012 and March 2012 ($H = 235.64$, $n = 439$, $p < 0.05$) may have occurred as a result of lower freshwater pulses entering the estuary in combination with respiration processes depleting DO concentrations (Figure 3.6).

All months, excluding January and July 2012, had a significantly higher ($H = 198.00$, $n = 438$, $p < 0.05$) pH than July 2011 (Figure 3.7). This was mainly because during July 2011 the estuary was in a freshwater dominated state and the pH ranged from 6.5 to 7.7. Similarly the months of September 2010 to June 2011 as well as November 2010 had a pH that was significantly higher than January, March and July 2012 ($H = 198.00$, $n = 438$, $p < 0.05$) because the system received a relatively large freshwater pulse from the river during these months.

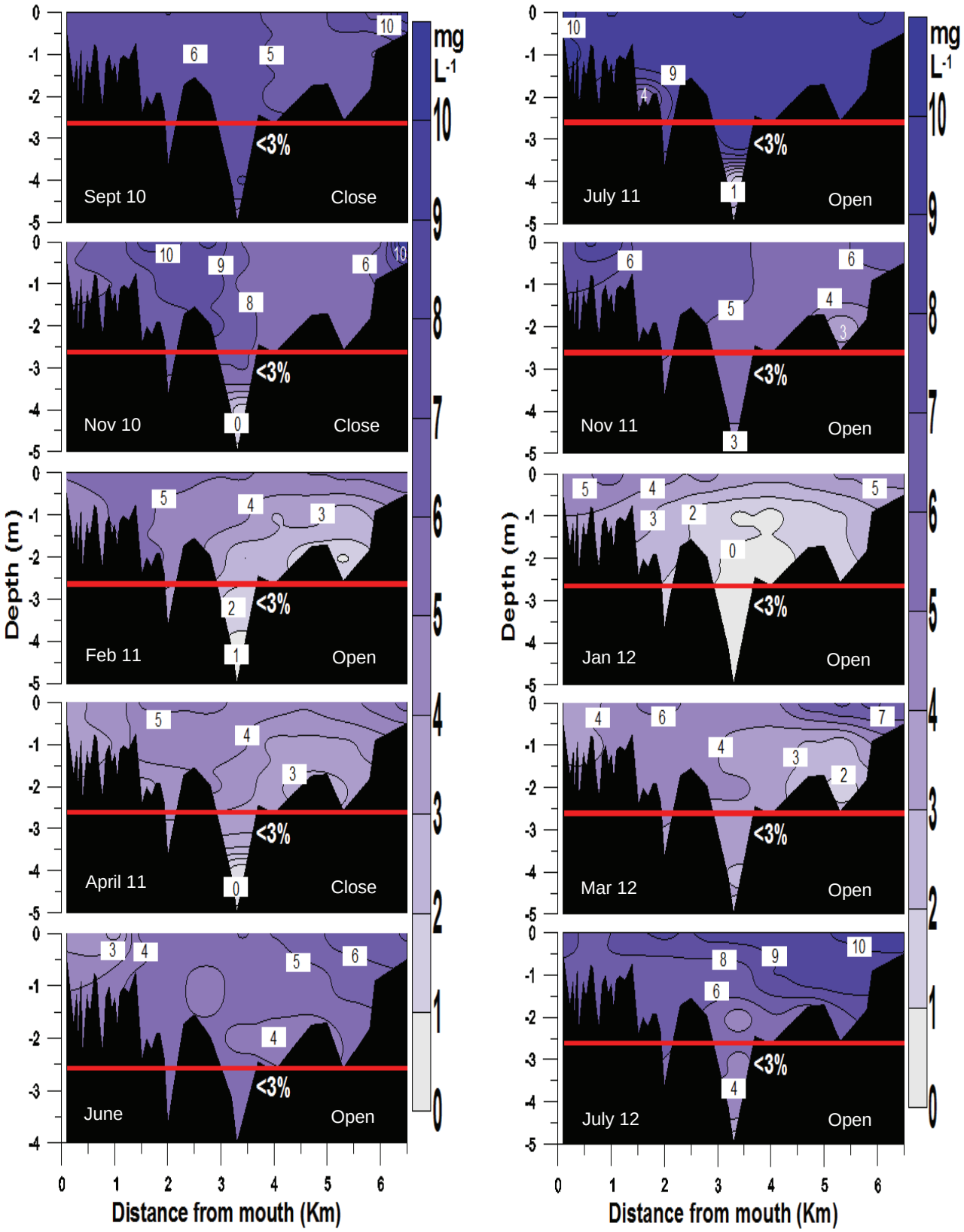

Figure 3.6 Dissolved oxygen concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

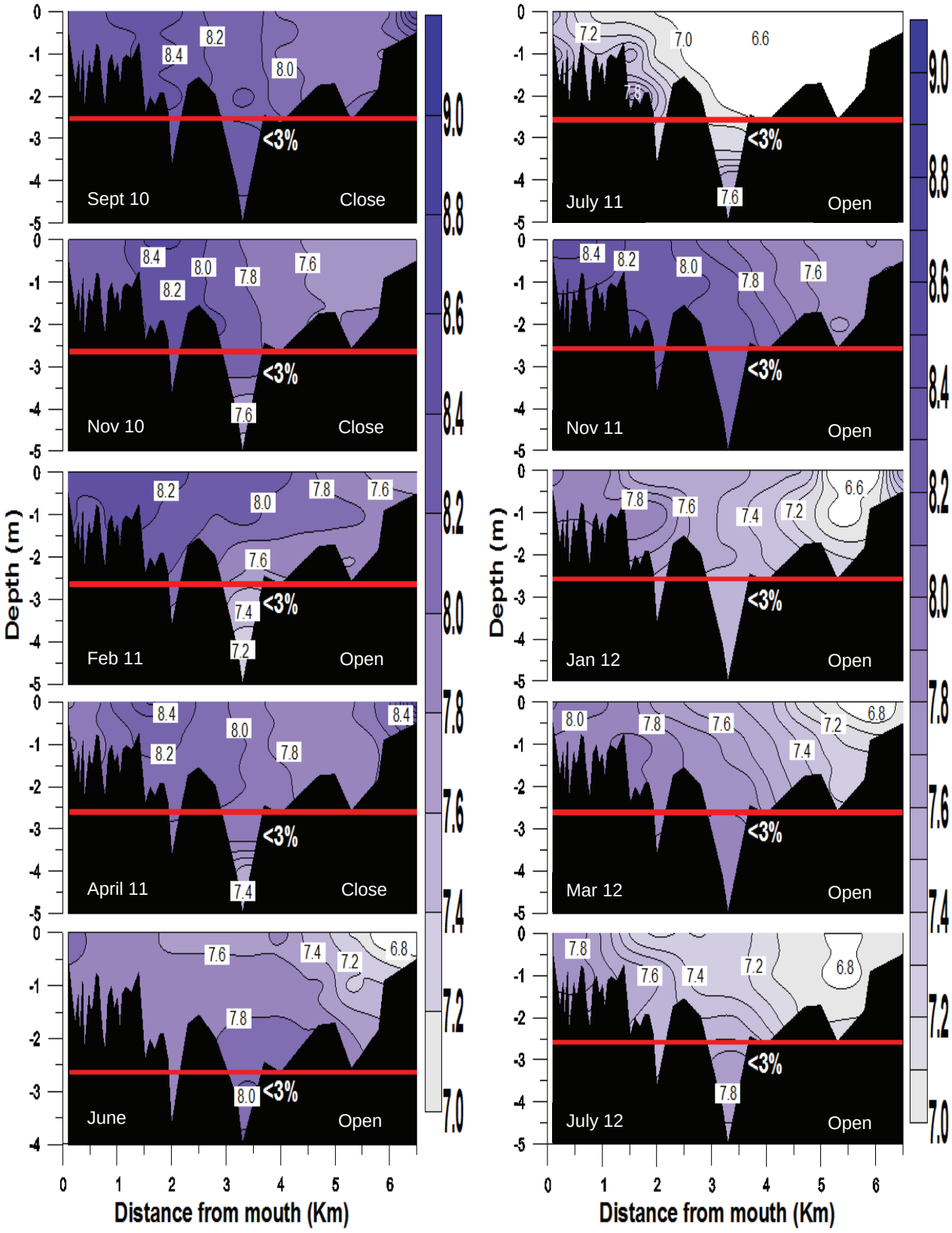

Figure 3.7 pH along the length of the Great Brak Estuary**.** Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

Ammonium concentration in September and November 2010 was generally low throughout the water column with concentrations reaching 7 µM in the bottom water at 3.4 km (Figure 3.8). After the artificial release of freshwater from the dam in February 2011 the NH_4^+ concentration ranged between 5-10 µM in the water column and became significantly greater ($H = 42.34$, $n = 69$, $p < 0.05$) in the bottom water (30 µM) at 3.4 km. A month after mouth closure in April 2011 the surface water ranged between 5-10 µM while the deeper reaches were greater than 45 µM (i.e. 110 µM) (Figure 3.8). The NH_4^+ concentration decreased to less than 5 µM in the deeper sections in the middle of the estuary and ranged between 15-20 µM in the upper reaches (4.7-6.5 km) after the flood in June 2011. July 2011 had strong freshwater flow and the NH_4^+ concentration ranged between 5 and 10 µM. November 2011, January 2012, March 2012 and July 2012 the mouth of the estuary was still open and tidal exchange with bottom waters (5-12 µM) led to a well diluted system (Figure 3.8). June and July 2011 were significantly higher ($H = 328.1$, $n = 759$, $p < 0.05$) in NH_4^+ than all other months. All other months had significantly higher NH_4^+ than September and November 2010 ($H = 328.1$, $n = 759$, $p < 0.05$) (Figure 3.8).

Soluble reactive phosphorus was low, generally below 1 µM in the water column for most months, and had peaks at 1.0 km and 3.4 km in the bottom water (Figure 3.9). Except for January and March 2012, July 2011 was significantly higher in SRP than all other months ($H = 205.97$, $n = 510$, $p < 0.05$). This was mainly due to the incoming flow from catchment introducing SRP into the estuary.

TOxN concentration throughout the estuary water column was generally below 5 µM for all months, and only July 2011 with its associated strong flow had concentration around 20 µM (Figure 3.10) in the surface waters indicative of the catchment acting as the main source of nitrate to the estuary. The TOxN (nitrate + nitrite) in June and July 2011 as well as July 2012 was significantly higher ($H = 562.33$ $N = 771$, $p < 0.05$) than all other months. Similarly, September 2010 and January 2012 had significantly higher ($H = 562.33$, $N = 771$, $p < 0.05$) TOxN concentrations than November 2010, February 2011, April 2011 and March 2012.

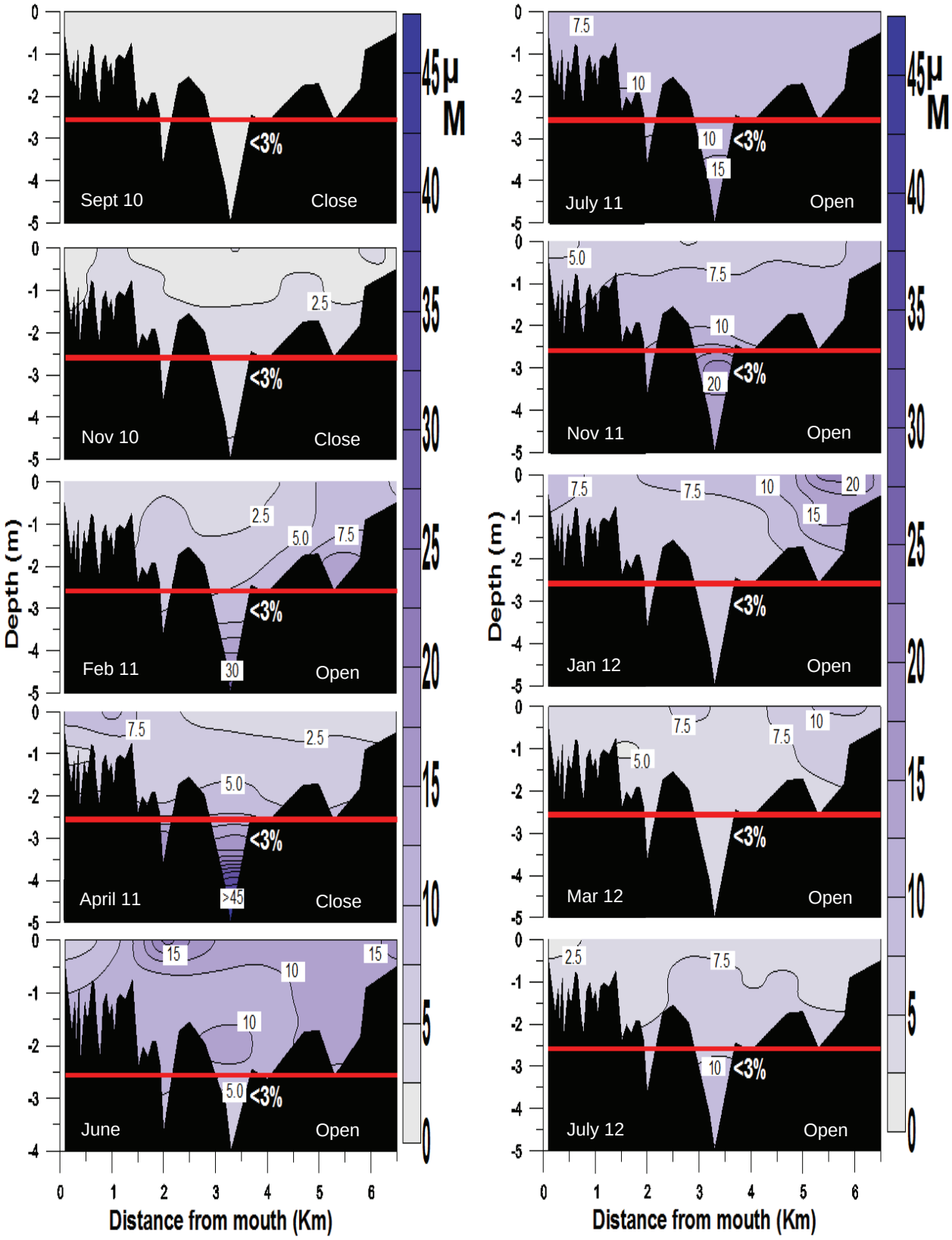

Figure 3.8 Ammonium concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

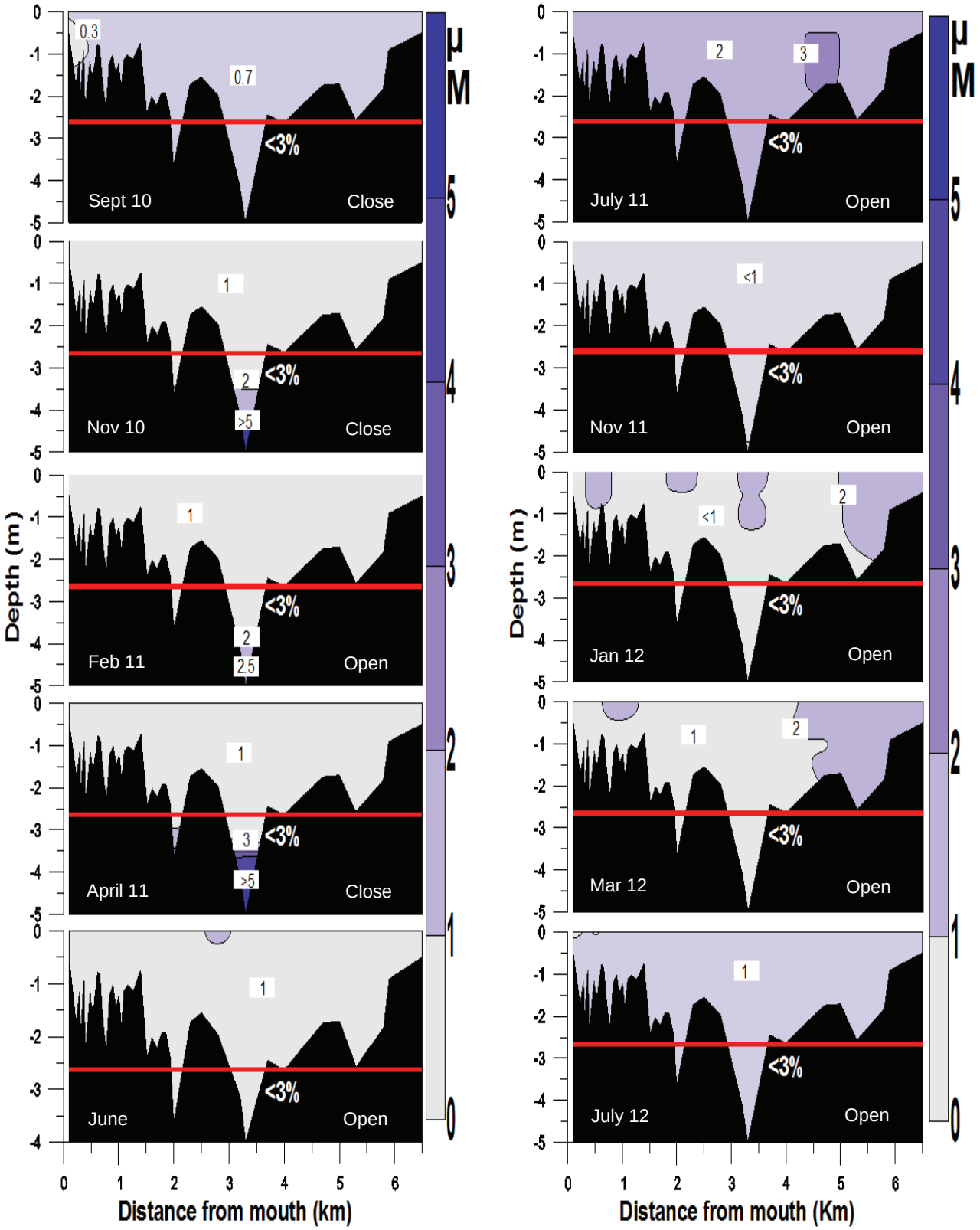

Figure 3.9 Soluble reactive phosphorus concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

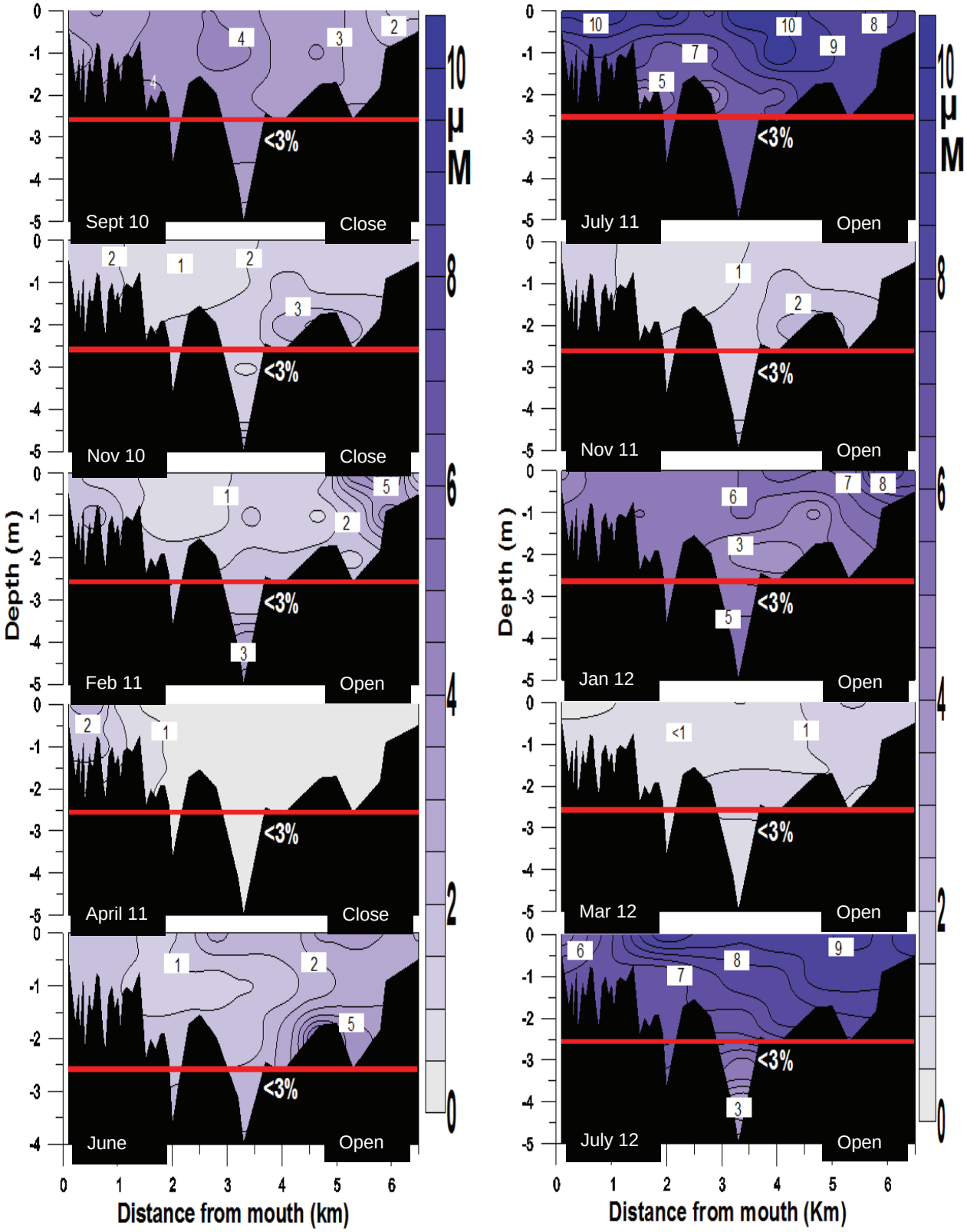

Figure 3.10 Total oxidized nitrate concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

Total nitrogen in July 2011, January 2012, March 2012, and July 2012 were significantly higher ($H = 168.74$, $n = 498$, $p < 0.05$) than all other months and was probably due to incoming flow from the river and catchment which caused a large quantity of suspended organic matter to be present in the water column (Figure 3.11). The TN concentration was generally below 0.5 mg l^{-1} (35.7 µM) throughout the estuary water column in September and November 2010 and April 2011, as there was no inflow into the estuary. There was a higher TN concentration < 1.4 mg l^{-1} (100 µM) at the head of the estuary during the freshwater release from the dam in February 2011 and the flood in June 2011. Because of the strong flow that occurred in July 2011 it led to TN concentrations greater than 1 mg l^{-1} (71 µM) throughout the water column (Figure 3.11). During November 2011 the mouth was open and tidal and this resulted in a well diluted system. However although the mouth was open and tidal in January 2012 there was still a strong freshwater flow present and concentrations again increased above 1 mg l^{-1} (71 µM).

July 2011 had a significantly higher TP concentration than all other months ($H = 191.32$, $n = 498$, $p < 0.05$) (Figure 3.12). Strong river flow, which was strongly stained, appeared to introduce high loads of dissolved and suspended organic matter into the system. The TP generally followed the same trend as the SRP in the estuary, with the deeper anoxic site at 3.4 km having higher TP concentration (Figure 3.12). TP generally ranged between 0.01-0.07 mg l^{-1} (0.32-2.25 µM) in the surface waters for all months except July 2011 and January 2012. These months had higher TP concentration in their surface water because of freshwater flow from the river and catchment introducing particulate and dissolved organic matter into the estuary. The bottom water followed a similar trend to the SRP and was greater than 0.15 mg l^{-1} (5 µM) TP at 3.4 km during November 2010, February and April 2011 as well as July 2012 (Figure 3.12). This is related to anoxia and a lack of flushing occurring in the system during these months.

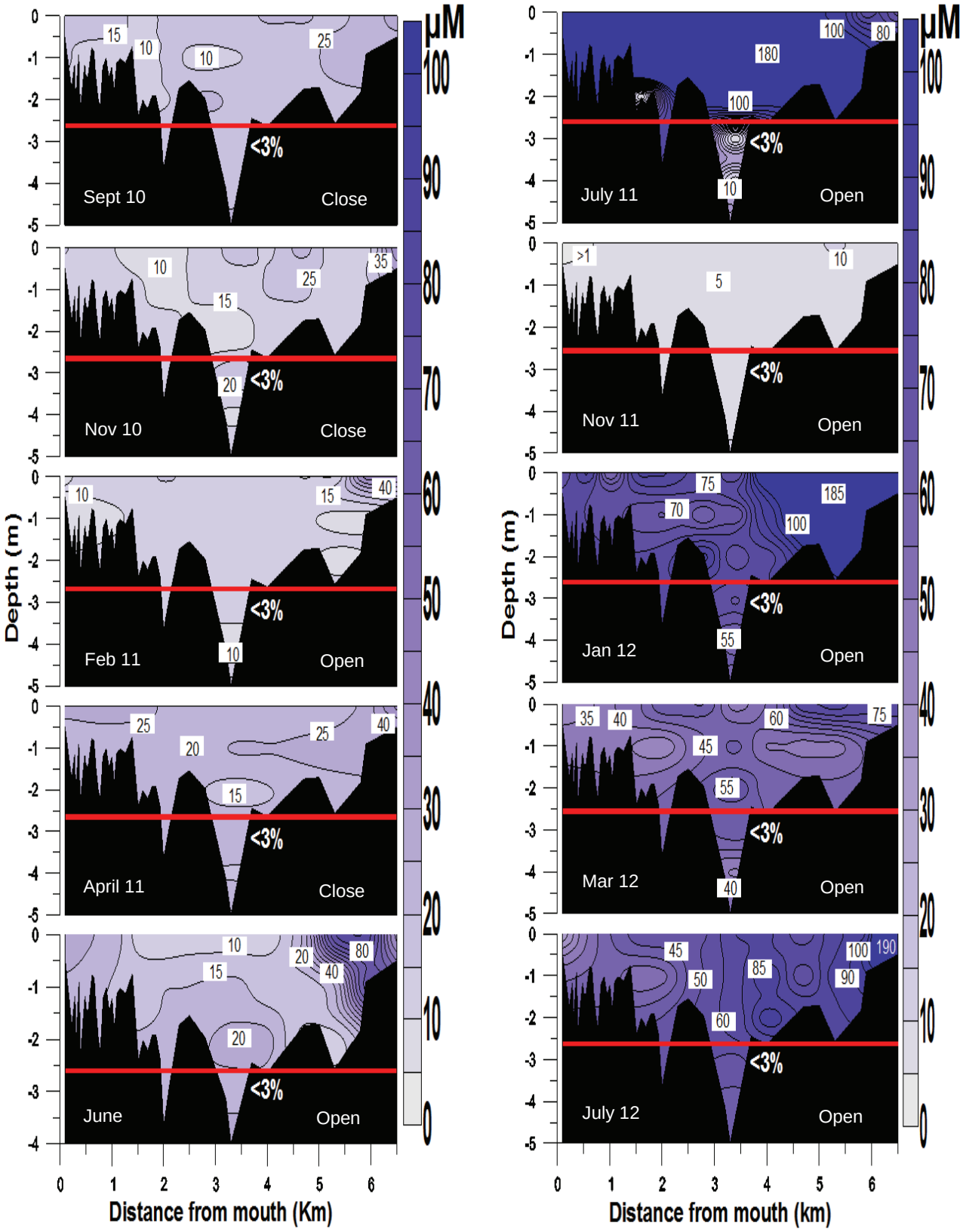

Figure 3.11 Total nitrogen concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

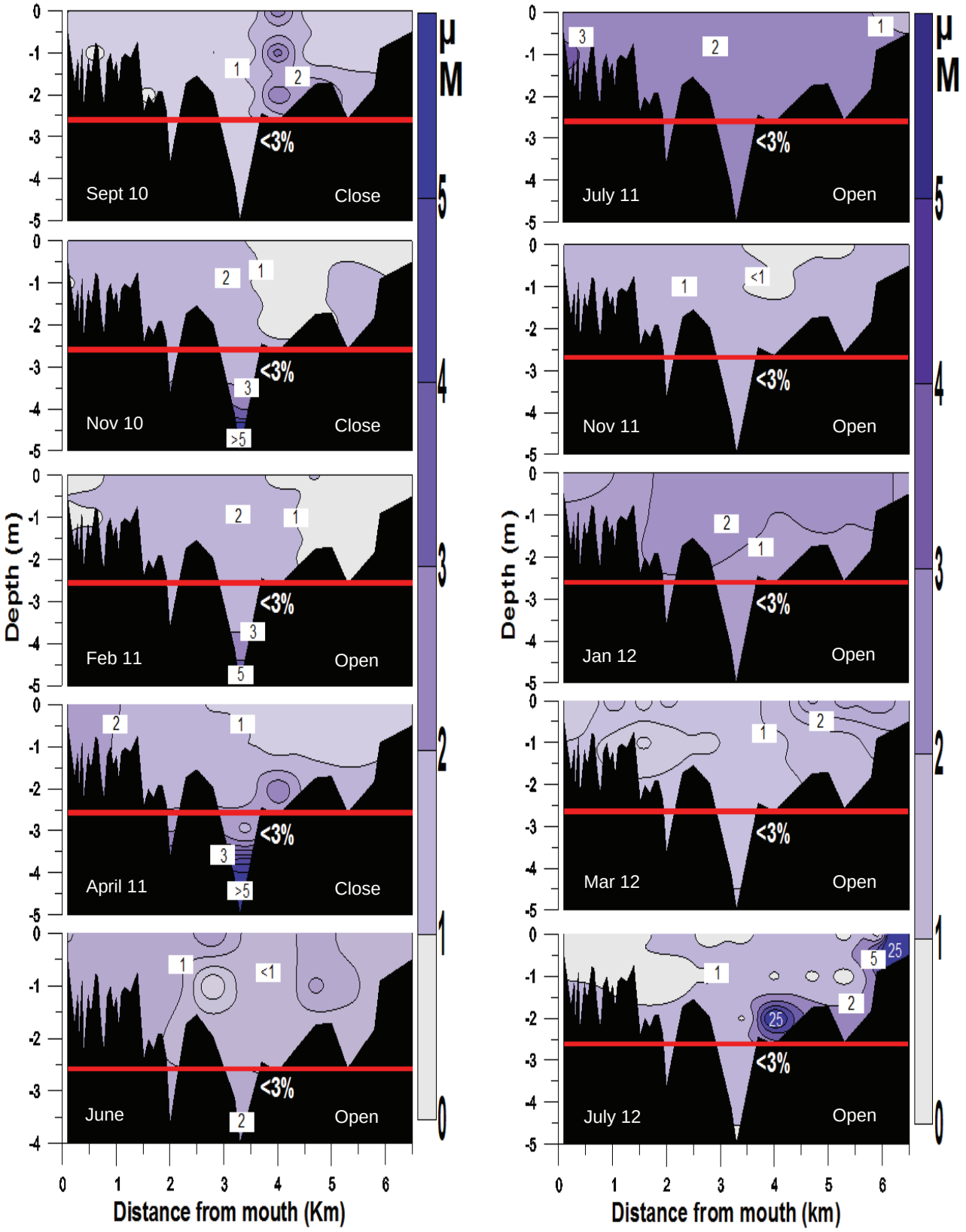

Figure 3.12 Total phosphorus concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

Because the surface area is flat and wide in the lower reaches (0 to 2 km), and narrow toward the upper reaches (2 to 6 km), the larger volume lies above the red line and < 3% of the volume occurs below 2.5 m depth.

The particulate nitrogen (PN) concentration in November 2010 was relatively high, ranging from 30 to 90 µM, but increased to a concentration of 250 µM in February 2011 (Figure 3.13). This occurred because the incoming freshwater that was released from the upstream dam, introduced suspended particulate matter into the estuary. Consequently these months were significantly higher ($H = 373.1$, $n = 642$, $p < 0.05$) in particulate nitrogen than all other months except for January 2012. A similar pulse of freshwater was introduced into the estuary during January 2012 (20 to 100 µM), which subsequently also became significantly higher in PN than the rest of the sampled months ($H = 373.1$, $n = 642$, $p < 0.05$) (Figure 3.13). The particulate nitrogen concentration in April 2011 was significantly higher than June 2011 and July 2012 ($H = 373.1$, $n = 642$, $p < 0.05$). The mouth of the estuary had remained open for only a week after the freshwater release in February 2011, as a result there was still a great deal of particulate nitrogen present within the estuary in April 2011.

The initial particulate phosphorus (PP) November 2010 ranged from 10 to 20 µM and was significantly higher than all other months except for June 2011 and January 2012 ($H = 315.29$, $n = 646$, $p < 0.05$) (Figure 3.14). The latter months also had significantly higher concentrations ranging from 10 to 20 µM and 8 to 44 µM respectively, which is attributed to the incoming freshwater from the river and catchment introducing particulate and dissolved organic matter into the estuary.

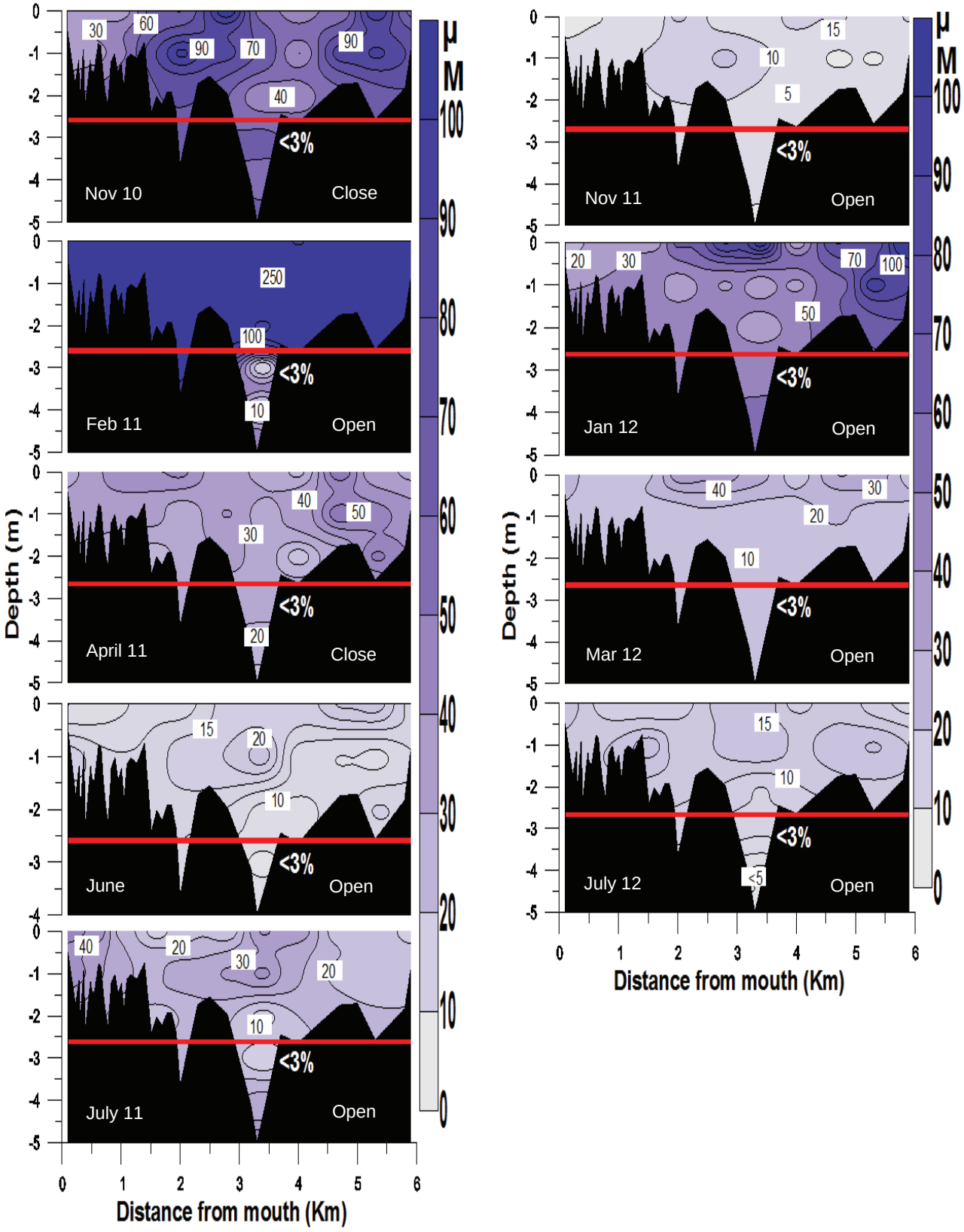

Figure 3.13 Particulate nitrogen concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

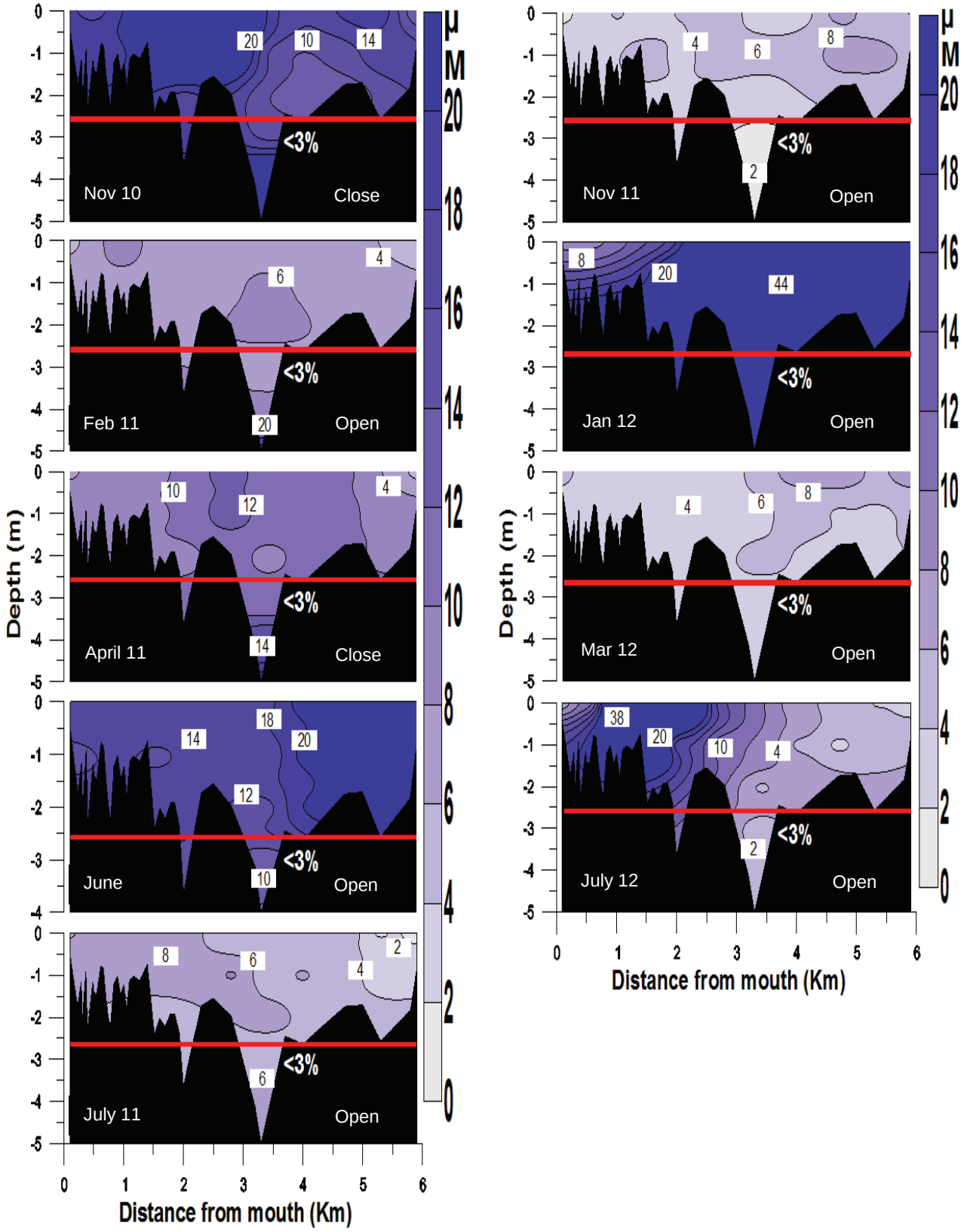

Figure 3.14 Particulate phosphorus concentration along the length of the Great Brak Estuary. Note: < 3% of the estuary volume falls below 2.5 m depth as indicated by the red line.

3.3.2 Submerged macrophytes and macroalgae

There was no significant difference ($F = 1.00$, d.f. = 5, $p > 0.05$) in the biomass of *Cladophora* over the sampling period (Figure 3.15 A). The dry mass of the algae was similar throughout the study period, even though its area cover decreased over the open mouth state. The biomass of *Ruppia* for all months was significantly higher ($F = 9.63$, d.f. = 7, $p < 0.05$) than that in September and November 2010 (Figure 3.15 A). It appears that the *Ruppia* biomass increases under open mouth conditions (June 2011 to July 2012). The *Ruppia* biomass in March 2012, was also significantly higher ($F = 9.63$, d.f. = 7, $p < 0.05$) than July 2012. The biomass of *Zostera capensis* in February 2011 and March 2012 was significantly higher ($H = 25.11$, $n = 33$, $p < 0.05$) than the biomass in September 2010, November 2010 and July 2011. During the open mouth state the submerged macrophytes (*Zostera capensis* and *Ruppia cirrhosa*) appear to increase in both area cover and dry mass (Figure 3.15 A and B).

The area cover of *Cladophora glomerata* increased from 23375 m^{-2} in June 2010 to 58339 m^{-2} in August 2010. After the bloom colapsed (August to December 2010) the submerged macrophytes extended their area cover, *Ruppia cirrhosa* ranged from 22913 to 29945 m^{-2} and *Zostera capensis* from 20529 to 37481 m^{-2} (Figure 3.15 B). Directly after a freshwater pulse in February 2011, the macroalgae increased in surface area cover from 36013 m^{-2} to 64336 m^{-2} in March 2011. The bloom was subsequently flushed out of the estuary by the flood in June 2011.

The TN concentration in *Cladophora* during February 2011, April 2011 and January 2011 was significantly higher ($H = 46.\ 26$, $n = 63$, $p < 0.05$) than September and November 2010 (Figure 3.16 A). The TN in *Ruppia* during April 2011, June 2011 and January 2012 was significantly higher ($F = 43.81$, d.f. = 7, $p < 0.05$) than September 2010, November 2010 and February 2011. The TN in *Zostera* appeared to be stable and only January 2012 TN concentration was significantly higher ($H = 21.44$, $n = 51$, $p < 0.05$) than in March 2012 (Figure 3.16 A).

The TP in *Cladophora* during February 2011, January 2012 and July 2012 was significantly higher ($H = 46.74$, $n = 63$, $p < 0.05$) than September and November 2010 (Figure 3.16 B). The TP concentration in *Ruppia* during March and July 2012 was significantly higher ($F = 23.66$, d.f. = 7, $p < 0.05$) than all other months. February, April and June 2011 also had significantly higher ($F = 23.66$, d.f. = 7, $p < 0.05$) tissue TP concentration than September

and November 2010. The TP concentration in *Zostera* during March and July 2012 was significantly higher ($F = 164.23$, d.f. = 7, $p < 0.05$) than all other months. February, April and June 2011 also had significantly higher ($F = 164.23$, d.f. = 7, $p < 0.05$) tissue TP concentration than September and November 2010 (Figure 3.16 B).

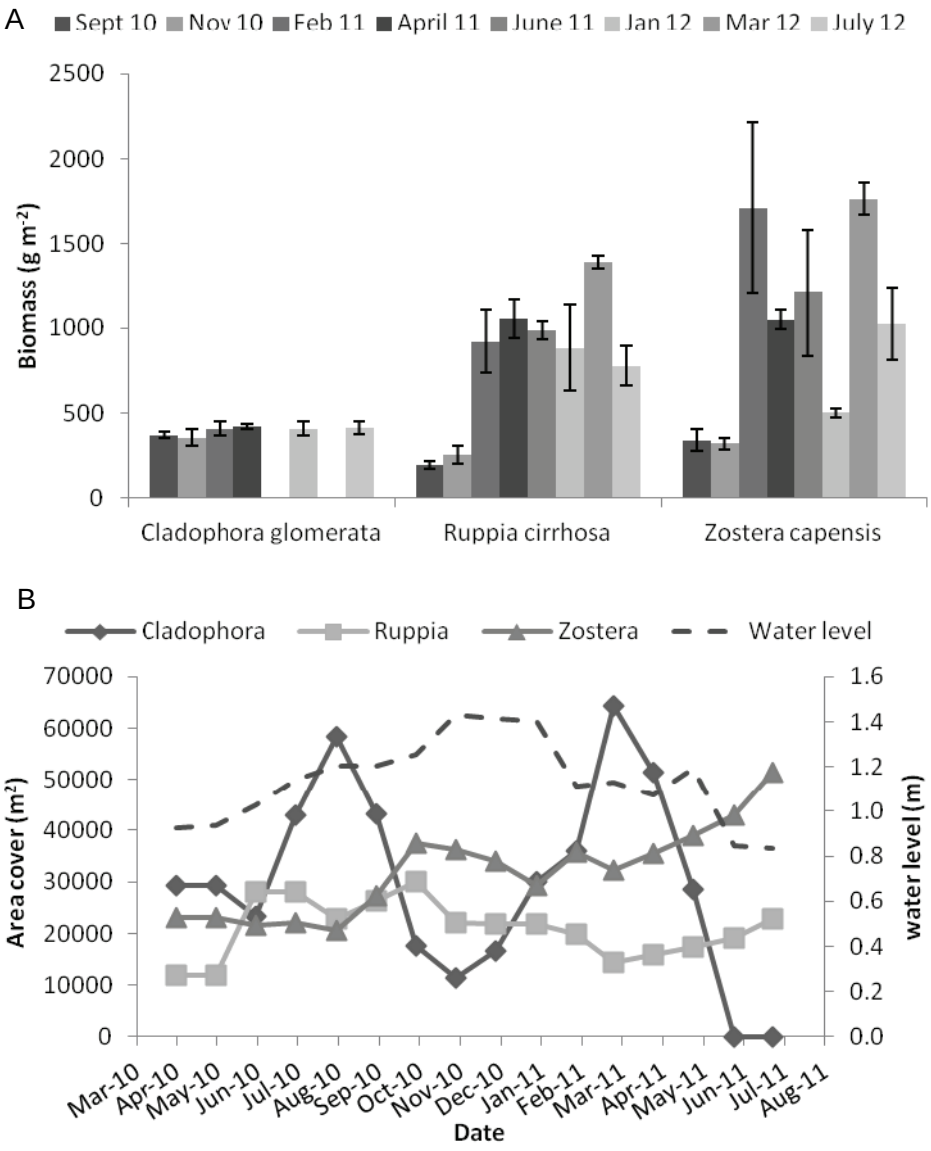

Figure 3.15 A) Dry mass and B) area cover of submerged macrophytes and macroalgae in the Great Brak Estuary

The results indicate that there was an increase in the TN and TP concentration within *Cladophora* after the artificial breach in February 2011 (Figure 3.16 A and B). After *Cladophora* was flushed out of the estaury, the *Ruppia* TN and TP concentration increased in the months which followed (Figure 3.16 A and B). The TN in *Zostera* remianed

approximately stable throughout the sampling period, and only the TP tissue concentration increased steadily after the breach.

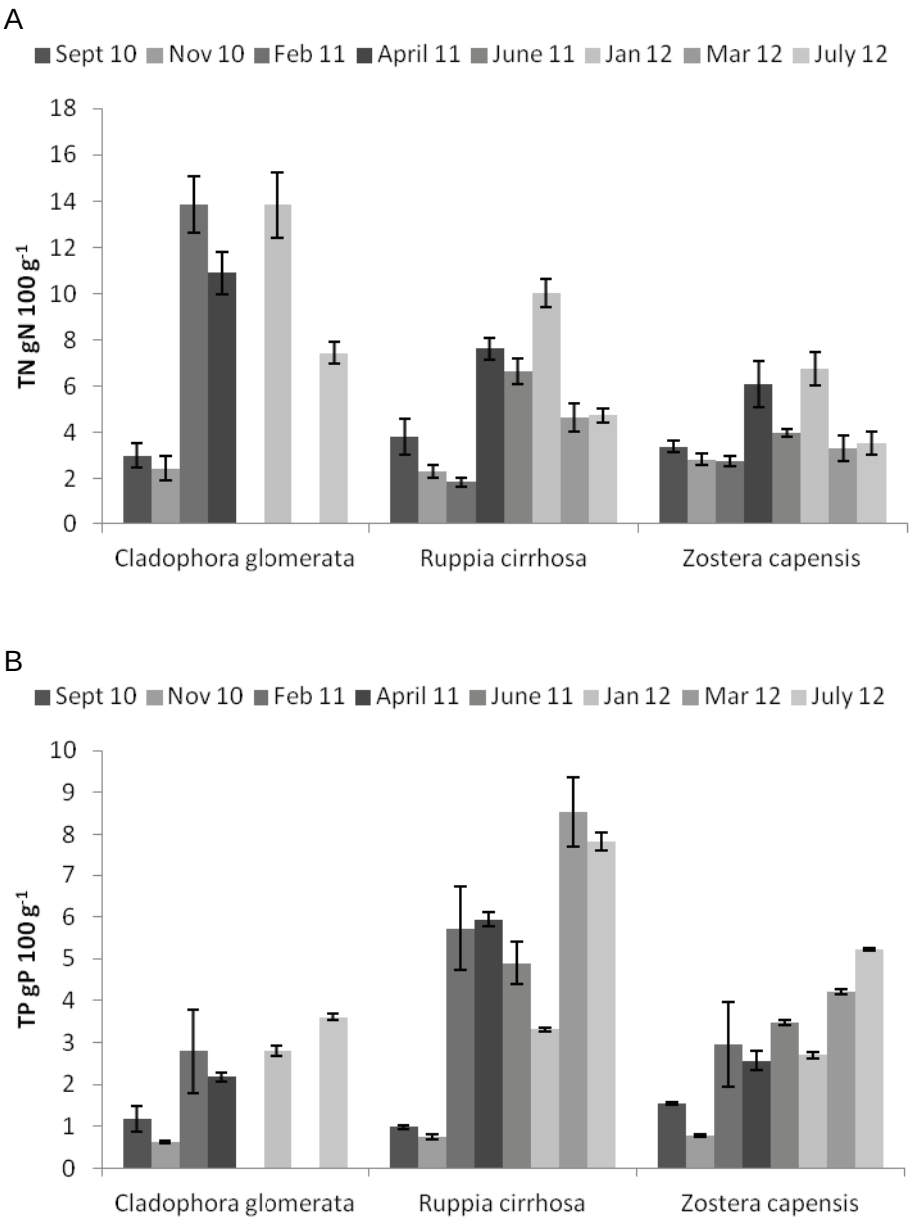

Figure 3.16 A) Total nitrogen and B) total phosphorus per 100 g of submerged macrophytes and macroalga in the Great Brak Estuary

3.4 Discussion

The objective of the chapter was to investigate the physico-chemical characteristics within the estuary and the effects on the submerged macrophytes and macroalga. An

important aspect of the discussion revolves around the effect the dam has had on the functionality of the estuary. Water temperatures followed a distinct seasonal pattern in the Great Brak Estuary and measurements have shown that summer temperatures are generally higher (20-24°C) than winter temperatures (13-16°C). Water temperature variations in TOCEs are usually a function of seasonal trends in atmospheric temperature (Snow and Taljaard 2007, Whitfield *et al*. 2008). In July 2011 the estuary water column had a low pH range, which was primarily due to a strong river inflow. The pH of estuarine water is naturally influenced by the inflowing water sources, being both the river and the sea. Seawater pH is known to range between pH 7.9 and 8.2, while that of river water is usually a function of catchment characteristics. For example, rivers draining Table Mountain quartzite are usually rich in humic acids, originating from typical vegetation found in these soils, and are characterised by low (~4) pH levels. However, as a result of the strong buffering capacity of seawater, pH levels in estuarine waters are usually within the range 7.0-8.5 (Snow and Taljaard 2007).

The artificial breach resulted in a longitudinal salinity gradient with saline water (25-28) around the mouth and brackish water (12-15) extending from the middle to upper reaches. During the closed mouth state (November 2010 and April 2011), the estuary once again became homogenous as salinity levelled out. In small, shallow systems the closed mouth state eventually reverts to a well-mixed (due to wind turbulence) brackish system, with no distinct salinity gradient or stratification (Taljaard *et al*. 2009 b). The estuary became stratified during its open phases in June 2011 (flood) and January 2012 with a wedge of low salinity water (10-15) present in the surface waters and the more saline water (30-34) still being trapped in the deeper bottom water. Stratification is a common phenomenon in estuaries that have tidal exchange (Taljaard *et al*. 2009 b, Lee *et al*. 2011, Gonzalez-Ortegon and Drake 2011) and the Great Brak is a typical example of a South African estuary that may exhibit all types of stratification, namely highly stratified, partially stratified and well mixed as explained by Schumann *et al*. (1999). Of importance is that after the flood ‘new’ sea water replaced the old trapped estuarine water in the bottom sections and indicates that the system had been well flushed.

The oxygen saturation data (Figure 3.6) showed that saturated conditions (>6 mgl^{-1}) were not the general state in the estuary. On the contrary, values below 6 mg l^{-1} were rather common. The indications from these data appear to show that both fresh water and sea water exchange is required to retain a medium to high oxygen concentration. The interpretation here seems to be that even in shallow systems, mouth closure causes oxygen

levels to decline. The decomposition of organic matter under the relatively high temperatures found in the Great Brak results in a rapid consumption of oxygen that cannot be replaced by normal diffusion from the atmosphere and water exchange or a greater extent of turbulence is required.

The Great Brak Estuary is a blackwater system, which, in its natural state, would have been oligotrophic (< 3 µM TOxN and SRP) and the dissolved oxygen concentration would have been greater than 6 mgl^{-1} (Snow 2008 a). The occurrence of the opportunistic macroalgae *C. glomerata*, which had not previously been recorded in the Great Brak Estuary (Morant 1983), indicates that the estuary is showing signs of eutrophication. Even though the water column nutrient concentration still falls within that of an oligotrophic system, the estuary has symptoms of eutrophication. It follows that an estuary's nutrient status cannot be determined by simply measuring the water column nutrients. Such is the case in other studies (Morris and Virnstein 2004, Burkholder *et al*. 2007, Human and Adams 2011) where simple measurements of the water column concentrations alone were more than often unreliable as indicators of nutrient enrichment since nutrients are taken up rapidly by macrophytes or adsorbed to particulate sediments. Still there have been changes in the nutrient concentrations associated with the dam construction, and will be expanded on in this discussion.

The average TOxN over the whole sampling period was generally below 5 µM. However, in July 2011 and 2012 the values rose to 20 and 10 µM respectively. This was due to incoming freshwater from the dam and a number of diffuse sources along the estuary, respectively. In July 2011, 55 mm of rain had fallen which introduced nitrate into the estuary. Similar findings from the Colne Estuary in the UK by Thornton *et al*. (2007) showed that peaks in winter nitrate and nitrite concentrations were associated with winter rainfall and subsequent runoff from the surrounding land, which flushed nitrate from the catchment soils. The increase in TOxN in the system in February and July 2011 were due to incoming freshwater from the dam. During July 2011, 55 mm of rain had fallen on the estuary introducing nitrates into the estuary via the dam and diffuse sources.

The dissolved inorganic nitrogen in the water column was mainly composed of NH_4^+ which is similar to the findings of Taljaard and Slinger (1993) and DWA (2009) on the Great Brak Estuary and Spooner and Maher (2009)'s work in the ICOLL, Corunna Lake in Australia. In these studies they state that this is primarily due to the longer residence time of the water body and the accumulation of organic matter. NH_4^+ is the major inorganic nutrient released

during remineralisation (Matheson *et al*. 2002). The average NH_4^+ concentration in the estuary was generally around 7 µM in the water column and increased with depth to about 12 µM at 2 m depths. Higher concentrations (>45 µM) in February and April 2011 were found at the deep hole at 3.4 km upstream. The higher NH_4^+ in the bottom water (> 2 m depth) may be linked to low dissolved oxygen which coupled with a rise in temperature lead to increased remineralisation of organic matter. Rising water temperatures are known to stimulate increased rates of remineralisation that causes greater ammonium release from the sediments which can support higher primary production (Vouve *et al.* 2000). Snow and Taljaard (2007) described the estuary as frequently having high NH_4^+ and dissolved inorganic phosphorus (DIP) concentrations in the deeper pools of the middle and upper reaches that usually coincided with anoxic conditions resulting from organic decomposition and long residence times. They state that the higher concentrations were probably associated with remineralisation processes and that this condition was present even under the open mouth state when vertical stratification caused 'trapping' of the bottom waters. The increase in NH_4^+ in February 2011 in the surface waters was caused by the release of freshwater from the dam. This is similar to DWA (2009) findings where they state that low oxygenated subsurface flow releases of freshwater from the dam also contribute to the rising NH_4^+ concentrations in the water column as opposed to oxidized nitrogen forms. Regular seasonal flooding would usually prevent significant accumulation of organic inputs in other systems, but the Wolwedans Dam attenuates this flooding, effectively removing this re-setting mechanism from the Great Brak Estuary. The peaks in SRP in the bottom water are also associated with the long residence time and organic matter accumulation in the estuary. SRP was below detectable limits within the estuary water column before a reduction of freshwater inflow occurred (DWA 2009).

The average TN concentration was 35.7 µM during the closed mouth states and increased to an average TN concentration of about 71 µM during the open mouth states. The associated increase in TN was due to strong river flow, which was tannin stained, and appeared to introduce high loads of dissolved and suspended organic matter into the system. The TP generally followed the same trend as the SRP in the estuary. Similarly the particulate nitrogen (PN) and particulate phosphorus (PP) ranged from 20 to 250 µM and 10 to 44 µM respectively, during the freshwater pulsed states of the estuary. The river appears to be a source of TN and TP to the estuary when there is flow from the dam, as is evident with the higher TN and TP in the water column concentrations during the June 2011 floods. McKee *et al*. (2000) reported that most of the nitrogen (74%) and phosphorus (84%) load

entered the estuary during one month when flooding occurred in the catchment in Richmond River Estuary.

During the closed phase the filamentous macroalgae *C. glomerata* had an area cover ranging from 3000 to 6000 m^2 while *Z. capensis and R. cirrhosa* covered an area of 2000 to 3500 m^2 and 1500 to 2900 m^2, respectively (Figure 3.17 B). The macroalgae out-competes the submerged macrophytes, *R. cirrhosa* and *Z. capensis.* This is mainly due to the calm sheltered conditions in this estuary and the available nutrients. Opportunistic macroalgae are capable of taking up, assimilating and storing large amounts of nitrogen in areas of high nitrogen loading. This leads to low water column concentrations (Peckol *et al*. 1994). Macroalgal blooms under eutrophic conditions are commonly associated with some form of ecosystem change. Algal blooms often out-compete slower growing macrophytes like *Z. capensis* and *R. cirrhosa* and larger perennial macroalgae (Durate 1995, Valiela *et al*. 1997, Lomstein *et al*. 2006, Martinez-Lüscher and Holmer 2010, Valdemarsen *et al*. 2010, Rasmussen *et al*. 2012). The trophic ecology of the Great Brak system may also be affected when macroalgae out-compete other primary producers such as the microphytobenthos through shading and directly influence the normal energy flow in the system (Hubas and Davoult 2006). Estuarine systems are known to become anoxic because of the depletion of oxygen resulting from dense growth of macroalgae that produce toxic compounds like hydrogen sulphide from their subsequent decomposition (Bolam *et al*. 2000, Nedergaad *et al*. 2002, Berglund *et al*. 2003).

C. glomerata appears to follow a seasonal cycle during the closed mouth state (Figure 3.17 B) whereby growth occurs during the autumn and winter months and die back occurs during the spring summer months. As expected this has been observed elsewhere in rivers and lakes by other authors (Wong *et al*. 1978, Graham *et al*. 1982) who indicate that the die-off seems to occur in the middle of summer. They attributed this to the inability of the alga to maintain dominance above 23°C. Although some studies have found temperature not to influence midsummer die-off, for example *C. glomerata* had maximum photosynthetic production rate between 28°C and 31°C in Lake Michigan. Mantai (1987) suggested that in Lake Erie the algae's photosynthetic oxygen production and dark respiration acclimates to the seasonal temperature highs, allowing growth to continue. Dodds and Gudder (1992) point out that there are several factors that may explain why high temperatures cause summer die-off in some cases but not in others. Firstly, growth response to temperature is species dependent. Secondly, interaction may occur between photosynthetic response to irradiance and temperature (Graham *et al*. 1982), i.e. an inhibitory temperature at one light

level may allow growth at another light level. Thirdly, low nutrients associated with high temperatures (Muller 1983) may be inhibitory and fourthly grazing may reduce biomass in late summer.

Soon after its seasonal die-off which usually occurs during the spring to summer months, *C. glomerata* attained its highest recorded area cover in March 2011 (autumn). The growth of the alga in the Great Brak occurs from late autumn and reaches a peak area cover in the winter months. It is clear that the onset of growth was prompted by another factor other than its seasonal cycle. The growth response coincided with the artificial release of freshwater from the dam. The flow from the dam was not sufficiently strong to flush water, sediments and the alga out of the estuary and the mouth closed after only a week. As water levels rose the alga was deposited onto the marsh areas. After breaching occurred water drained out of the estuary leaving the alga stranded on the marshes and as the flood tide entered the macroalga was once again redistributed. The alga was then able to utilise the available nutrients in the water column and expand its area cover from 35000 m^2 in February 2011 to 64000 m^2 in March 2011. Although not significantly higher, the dry mass of *C. glomerata* exceeded that of both *R. cirrhosa* and *Z. capensis* during the closed mouth state in September and November 2010. Similar results measured as percentage cover (< 50% area cover) and wet mass (634-755 g m^2) were reported in (DWA 2009). The high biomass and expansion of the macroalgae displays a deteriorated ecological state of the estuary (Scanlan *et al*. 2006, Gubelit and Berezina 2010).

After the estuary mouth had been flushed and remained open (June 11 to July 2012) the dry mass of the submerged macrophytes increased considerably (Figure 3.17 A) as they extended their area cover within the lower reaches of the estuary. Although *R. cirrhosa* is prone to desiccation (Riddin and Adams 2008), tidal action and the bathymetry of the lower estuary is deep enough to provide a suitable habitat for the expansion and growth of the submerged macrophyte during the open phase. The dry mass of the algae was similar throughout the study period, even though its area cover decreased under the prolonged open mouth state. The persistence of *C. glomerata* within the lower reaches of the estuary is attributed to its ability to withstand the shear stress found in benthic regions (Dodds 1991). *C. glomerata* has a tough thallus and is flexible allowing water to flow through and around it. Under low current velocities the thallus spreads out and becomes more streamlined as flow increases. Streamlining may be a general adaptation to flow and has been observed in other macroalgae (Dodds and Gudder 1992). Another factor contributing to its successful occupancy of the lower reaches in the estuary is the continuous availability of NH_4^+ in the

estuary which more than likely favours the growth of the nuisance algae. Studies have found that the highest uptake rates of NH_4^+ occur within the lower part of the algal mat (Thybo-Christensen *et al*. 1993) and high irradiance can increase uptake to as high as 900 µM NH_4^+ $m^{-2}h^{-1}$ (McGlathery *et al*. 1997).

The natural breach of the estuary mouth caused by the flood in June 2011 effectively flushed water and scoured a sufficient amount of sediment from the estuary effectively 're-setting the estuary'. McKee *et al*. (2000) studies on the shallow bar built Richmond River Estuary also concluded that the process of flood scouring was likely to be the cleansing mechanism responsible for maintaining water quality on an annual basis. As a consequence of the floods, *C. glomerata* had been washed out of the system. The resistance to abrasion by filamentous algae relates to its ability to withstand hydrodynamic disturbance. Sand-Jansen *et al*. (1989) identified that flooding decreased *C. glomerata* biomass in rivers. Other studies (Durate *et al*. 2002, Suzuki *et al*. 2002, Bonilla *et al*. 2005) have also concluded that exchange with the ocean in coastal lagoons washes out organic matter which would otherwise cause eutrophication and anoxia. These studies point out that if isolation occurs for long periods (months to years) it would favour the development of blooms. According to Oertel (1991) complete submersion of the algal thallus in rivers and estuaries allows for greater growth than that which occurs in an intertidal rocky shore. An increase in nutrients and blooms of filamentous cyanobacteria and diatoms occurred in Imboassica lagoon Brazil after 15 months of mouth closure (Melo 2001). Another example was a phytoplankton bloom that occurred in the subtropical Indian River Lagoon USA after a residence time of 1 year (Badylak and Phlips 2004). A similar condition now exists in the temporarily open closed Great Brak Estuary where prolonged residence time and the recycling of nutrients from the sediment make the lower reaches an ideal environment for the prolific growth of the alga.

3.5 Conclusion

The post flood estuary condition was similar to pre-dam conditions as the natural breach (3×10^6 m^3) with a prolonged flow volume flushed the estuary of sediment and organic matter. This supported a strongly tidal and high oxygenated environment, suppressing the release of remineralised nutrients from bottom sediments. During the artificial breach the maximum water level was capped at 2 m, a fixed volume of 0.3×10^6 m^3 was released and the mouth was scoured to 0.9 m above mean sea level (Figure 3.3). The maximum water level of a natural breach can exceed 3.5 m (Huizinga and Van Niekerk 2003), scouring the mouth region to mean sea level (Figure 3.3) resulting in a tapering off of

flow for months following the event. In contrast to the artificial breach (0.7 x 10^6 m^3) the natural breach did not have an associated *C. glomerata* bloom thereafter. The Great Brak is one of the first examples of an estuary that may function as a result of freshwater releases in conjunction with mouth manipulation, to mimic natural breaching conditions. However in order for this process to be effective a greater quantity of water needs to be made available to the estuary.

The ecological reserve determination set out by DWA (2009) indicated that freshwater releases of 5 x 10 m^3 per annum should be released when the Wolwedans Dam was > 90% full, 3.3 x 10 m^3 per annum when it reached between 80 and 90%, 2.2 x 10 m^3 per annum when water levels reached between 70 and 80% and 1.0 x 10 m^3 per annum when water levels drop < 70%. However, to date there have been no large releases of freshwater from the dam (5 x 10 m^3 and 3.3 x 10 m^3) and appears to be unlikely to occur even though the DWA has accepted the reserve allocation. Reserve allocations of around 2 x 10^6 m^3 per year are insufficient to flush and 'reset' the estuary. Taljaard *et al*. (1993) reported that a total of 2.77 x 10^6 m^3 was enough to ensure full flushing of resident saline water from the estuary. We estimate that 3.5 x 10^6 m^3 of water should be allocated to the reserve and that the 2.77 x 10^6 m^3 should be used to flush the system of sediments and organic matter and the remaining 0.73 x 10^6 m^3 can be used to maintain open mouth tidal conditions. Furthermore the flood tide introduces adequate seawater into the bottom water as far upstream as 4.7 km to flush the deeper sections of the estuary. This evidence serves to indicate the direction the small microtidal estuaries of South Africa and the world are heading. Given the rise in population and ever increasing demand for fresh water, available water to estuaries will become an even scarcer resource, leaving TOCEs in the closed mouth state more frequently and for longer (period-years). It is imperative that the allocated water requirement to estuaries is well researched and documented, and encompasses all facets of biology so that adequate management actions can be made in order to maintain the health of the entire estuary ecosystem. The water quality data as well as the biomass area cover data collected for the submerged macrophytes and macroalgae (February 2011 to July 2012) were used to construct the nutrient budget in Chapter 5. The fundamental conditions that favour algal blooms in the shallow waters of the lower estuary are the prolonged residence time of the water body during the closed mouth state and the availability of remineralised nutrients. Without effective flushing the estuary will continue to experience macroalgal boom and bust cycles.

4 THE BENTHIC REGENERATION OF N AND P FROM THE SEDIMENT OF THE GREAT BRAK ESTUARY

The aim of this chapter is to investigate the flux of inorganic nutrients (NH_4^+, TOxN [NO_3^- + NO_2^-], SRP) as well as total N and P across the sediment-water interface in the estuary. This is in order to quantify the exchange of N and P across this interface so that the potential contribution of sediments to the water column nutrient budget can be determined.

4.1 Introduction

Estuaries are the confluence of river and sea; where freshwater drained from the land mixes with oceanic water to form highly productive systems. Conceptually, land and ocean derived materials are processed in different compartments within an estuary, such as the water column and shallow light-limited subtidal and intertidal sediments (Magalhaes *et al*. 2002). The transformation of these materials is dependent on several parameters, i.e. the rate of external input, the recycling and removal efficiency by biological, chemical and physical processes, as well as the hydrodynamics (e.g. residence times) of the estuary (Seitzinger 1990, Balls 1994, Sakamaki *et al*. 2006). Continued modification of coastal environments, in particular the rise in inorganic and organic nutrient loading, has led to large scale eutrophication of many estuaries around the world (Jickells 1998). The exchange of nutrients between the sediment and water interface (benthic pelagic coupling) of intertidal and subtidal areas of estuaries is capable of playing two important, but opposing roles (Magalhaes *et al*. 2002). It has been found that regenerated nutrients that are fluxed into the water column are able to supply most of the N and P required for phytoplankton primary production (Rizzo 1990, Cowan *et al*. 1996). In contrast, large amounts of inorganic nutrients have been removed from the overlying water column by bacterial mats (Teague *et al*. 1988, Ogilvie *et al*. 1997). Since nutrient loads, which would otherwise be discharged into coastal waters, are taken up by primary producers and removed from the water column they act as a biological control of coastal eutrophication. As the processes of removal and production occur simultaneously, the net direction of nutrient flux will depend on which is the dominant process (Magalhaes *et al*. 2002).

Nixon (1981) and Kemp *et al*. (1992) stated that high rates of primary production can occur in shallow estuaries as a result of effective recycling and retention within benthic and pelagic processes. The release of regenerated nutrients (N and P), in particular remineralised

phytodetritus from the sediments to the water column in shallow water systems, are subsequently utilised by the primary producers (Jensen *et al*. 1990, Koho *et al*. 2008). Ultimately, the inorganic N form that becomes available to primary producers depends on the type of bacteria present as well as the state of oxygenation. Under anoxic conditions NO_3^- is reduced to gaseous N_2 (denitrification) by heterotrophic bacteria in the sediment, leading to a loss of N through the water column and resulting in a loss of bioavailable nitrogen from the estuary (Jorgensen and Sorensen 1988, Herbert *et al*. 1999). Alternatively, processes such as dissimilatory nitrate reduction to ammonium (DNRA) and ammonia oxidation (anammox) also occur under anoxic conditions and result in a bioavailable form of N released to the estuary (Matheson *et al*. 2002, Brandes *et al*. 2007, Prescott *et al*. 2008). The nitrification of NH_4^+ to NO_2^- to NO_3^- occurs under oxic conditions by nitrifying bacteria present in the sediment, which can subsequently be released to the water column. Similarly, the flux of phosphate from the sediment is also affected by oxygen as well as soil redox potential where the release of PO_4^{3-} (or SRP) occurs under hypoxic or anoxic conditions (Koop *et al*. 1990, Slomp 2012).

Benthic pelagic coupling is influenced by three factors, namely the depth of the water column, temperature and mixing events. It is believed that benthic pelagic coupling may be more pronounced in shallow water systems than in deeper coastal regions since a larger fraction of phytodetritus is able to reach the bottom sediment (Hargrave 1973, Nixon 1981) that can then be remineralised into a more available form for primary producers (Oviatt *et al*. 1986, Jensen *et al*. 1990). Taljaard *et al*. (2009 b) believed that *in situ* regeneration of inorganic nutrients through biochemical processes (e.g. remineralisation) in South African TOCEs does not forms a significant supply of inorganic nutrients to the water column. However, they also stated that further studies were warranted to confirm their findings. When water column temperatures increase, the rate of remineralisation increases and results in more NH_4^+ being released from the sediment that in turn can support higher rates of primary production (Vouve *et al*. 2000). However, it has also been noted that the contribution of N from the sediment to phytoplankton demand may be less important than other sources of N during periods of high primary production (Hopkinson 1987). Mixing events may disturb benthic pelagic coupling by resuspension of either surface sediment particles or nutrient-rich porewater (Porter *et al*. 2010) that can result in a shift from net heterotrophy to net autotrophy in the water column in just a few days after a mixing event (Lawrence *et al*. 2004). Remineralised N from sediments is frequently in the inorganic form and as a result is readily taken up by primary producers (Boynton *et al*. 1995).

4.2 Materials and methods

The analytical methods for the detection of nutrients have been described in chapter 3.2.2. Benthic flux chambers provide a simple method of investigating nutrient flux *in situ*. It has been demonstrated in benthic studies of estuaries that nutrient concentrations in the overlying water are proportional to the nutrient flux occurring at the sediments (Dollar *et al*. 1991, An and Joye 2001, Switzer 2003). In this study two periods in 2012 were selected for benthic chamber deployment, one during the summer and the other in winter. The summer deployment represents long daylight hours and elevated water temperatures and the winter deployment represents cooler temperatures and reduced daylight hours.

Figure 4.1 Light and dark benthic chambers deployed at the Great Brak Estuary.

The chamber is composed of acrylic material (Figure 4.1). A total of two light and two dark chambers were deployed during each experiment to determine the flux of nutrients across the sediment-water column interface. The chambers were inserted an average depth of 15 cm into the sediment and covered an area of 0.15 m^2. The total volume of the chamber is approximately 40 L and has an average volume of 37 L of water when deployed. Samples were collected by syringe through nylon tubing inserted into the chamber and replaced by ambient water enclosed in a submerged collapsible plastic bag attached to the outer wall of

the chamber. In order to mimic mixing, water was sucked into a 50 ml syringe and pushed back into the chamber 10 times just prior to a sample being taken. Care was taken not to disturb the sediment. The sampling tube was kept bubble-free before drawing each sample. Sampling started immediately after deployment of the chamber, i.e. at time 0. Chambers were deployed for 24 hours and sampled every 1 hour for TN, TP, NH_4^+, TOxN and SRP in summer. Based on the summer results the decision was taken to draw samples every three hours during the winter sampling session.

Benthic flux was calculated as follows (Dollar *et al*. 1991):

$$F = V (C_t – C_o) / (A \times T)$$

Where V = volume (l) of water inside the benthic chamber over the sediment at initial deployment, C_o and C_t = the concentrations (µM) of nutrient before and after time T (hrs), and A = area (m^2) of sediment enclosed in chamber. As per convention, negative flux denotes flux from overlying water to sediment (loss of nutrient from the water column) and positive flux (efflux) denotes flux from sediment to overlying water. NO^-_3 influx rate was considered as the benthic denitrification rate.

4.3 Results

The temperature for all incubations during the summer deployment (March 2012) ranged between 25 and 30°C (Figure 4.2 A) and was significantly higher ($U = 1.00$, $p < 0.05$) than the temperature during the winter deployment (June 2012) that ranged between 12 and 15°C (Figure 4.2 B).

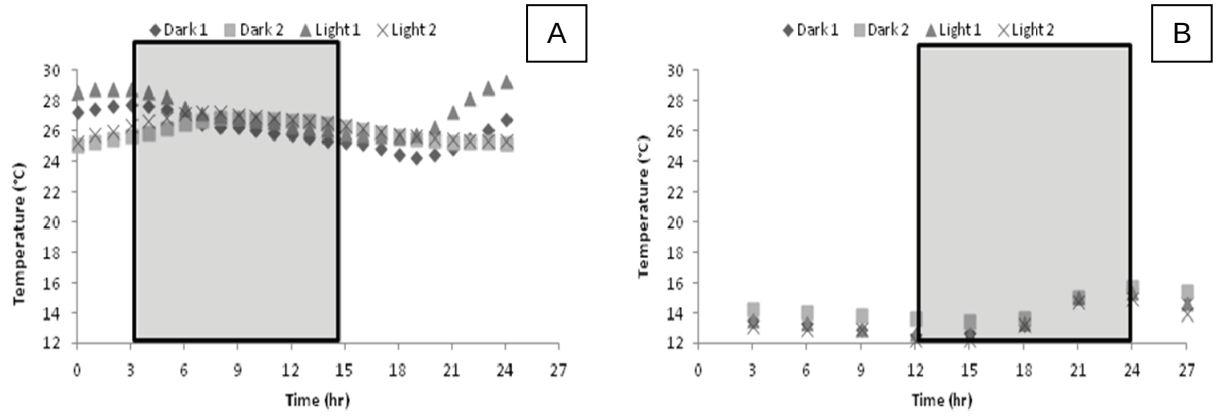

Figure 4.2 Temperature during incubation in (A) summer (March 2012) and (B) winter (July 2012). Shaded areas represent evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

Dissolved oxygen during summer decreased steadily over the 25 hour cycle for all incubations except for Light 2 (Figure 4.3 A), which had a slight increase in dissolved oxygen during the light period. A similar decreasing dissolved oxygen concentration pattern over time was observed for the incubations in winter (Figure 4.3 B). However, the averaged dissolved oxygen concentration in July was significantly higher than that of March ($U = 300.5$, $p < 0.05$).

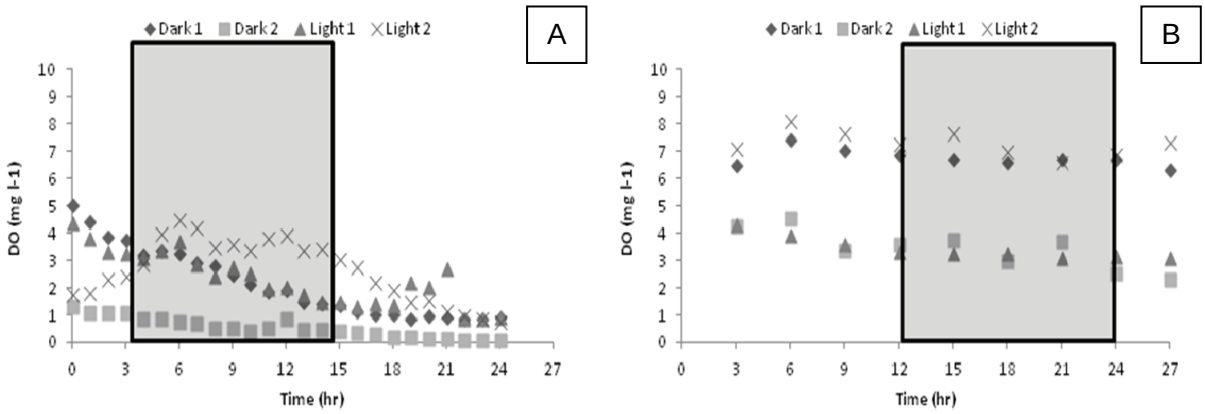

Figure 4.3 Dissolved oxygen during incubation for (A) summer (March 2012) and (B) winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

Total nitrogen concentration in both the light and dark during summer increased slightly over the 25 hour period. The concentration in the dark chambers increased from 50 µM to 65 µM and increased from 50 µM to 60 µM (Figure 4.4) in the light. In winter the concentration in the light increased from 60 µM to 80 µM (Figure 4.5) and the concentration in the dark

increased from 50 µM to 80 µM. Thus, the average flux of TN (2.30 mmol m^{-2} d^{-1}) during winter was significantly higher than summer (1.51 mmol m^{-2} d^{-1}) ($U = 6174$, $p < 0.05$) (Table 4.1).

Table 4.1 Total flux of TN from the sediment of light and dark chambers.

	Summer (March 2012)	Winter (July 2012)
	F mmol m^{-2} d^{-1}	F mmol m^{-2} d^{-1}
Light 1	1.20	1.45
Light 2	0.92	2.78
Dark 1	1.69	2.03
Dark 2	2.24	2.95
Average	1.51	2.30

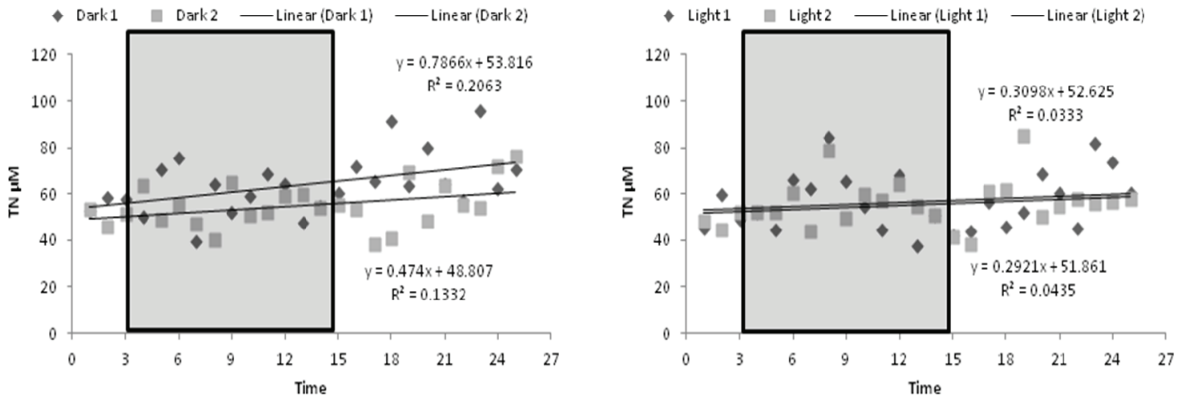

Figure 4.4 Flux of TN from the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

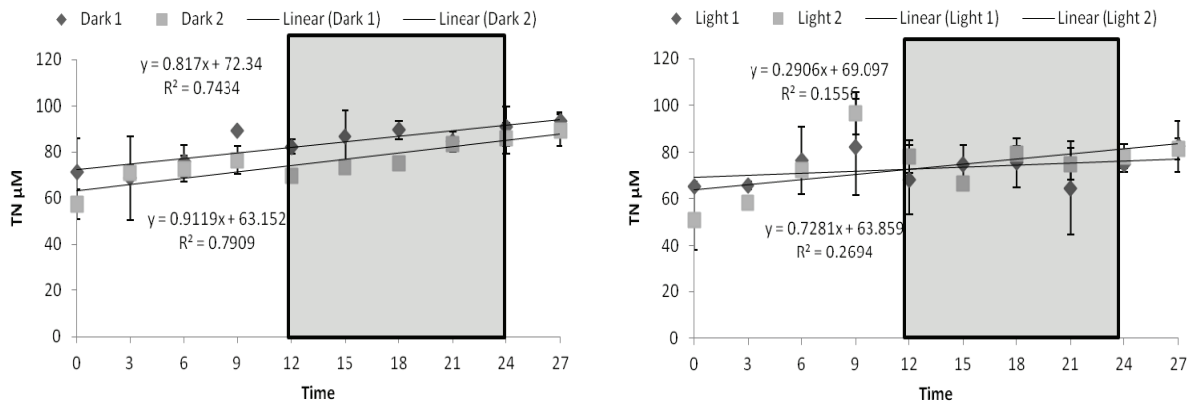

Figure 4.5 Flux of TN from the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

During summer (Figure 4.6), TP in Light 2 was significantly higher than in Light 1 and similarly Dark 2 also had significantly higher TP concentration than Dark 1 ($H = 101.09$, $n = 250$, $p < 0.05$). The TP concentration was also significantly higher in Light 2 when compared to Dark 1. Similarly, Dark 2 was significantly higher in TP than Light 1 ($H = 101.09$, $n = 250$, $p < 0.05$). There were no significant differences in the TP concentration during winter ($F = 2.76$, d.f. = 3, $p > 0.05$) (Figure 4.7). The average flux of TP from the sediment in summer (0.12 mmol m^{-2} d^{-1}) was significantly higher than the flux in winter (0.05 mmol m^{-2} d^{-1}) ($U = 2509$, $p < 0.05$) (Table 4.2).

Table 4.2 Total flux of TP from the sediment of light and dark chambers.

	Summer (March 2012)	Winter (July 2012)
	F mmol m^{-2} d^{-1}	F mmol m^{-2} d^{-1}
Light 1	0.06	0.03
Light 2	0.22	0.01
Dark 1	0.10	0.03
Dark 2	0.10	0.14
Average	0.12	0.05

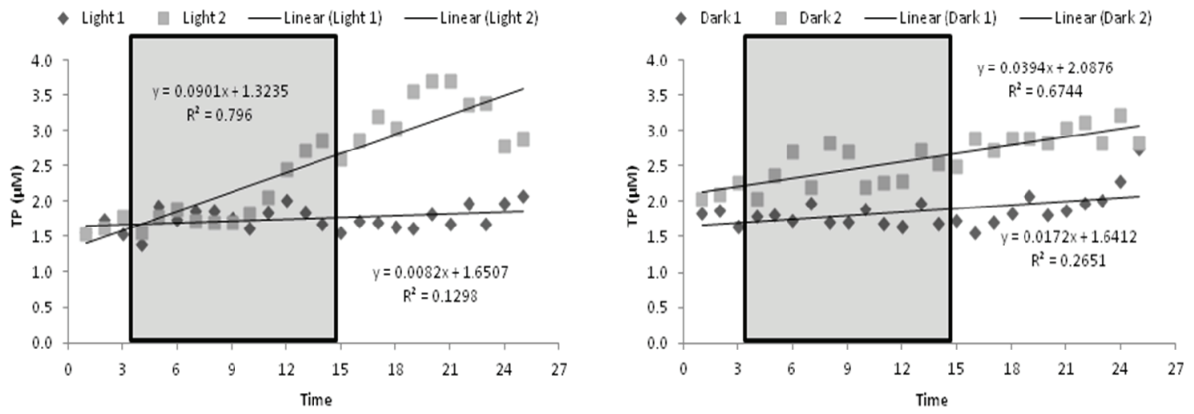

Figure 4.6 Flux of TP out of the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

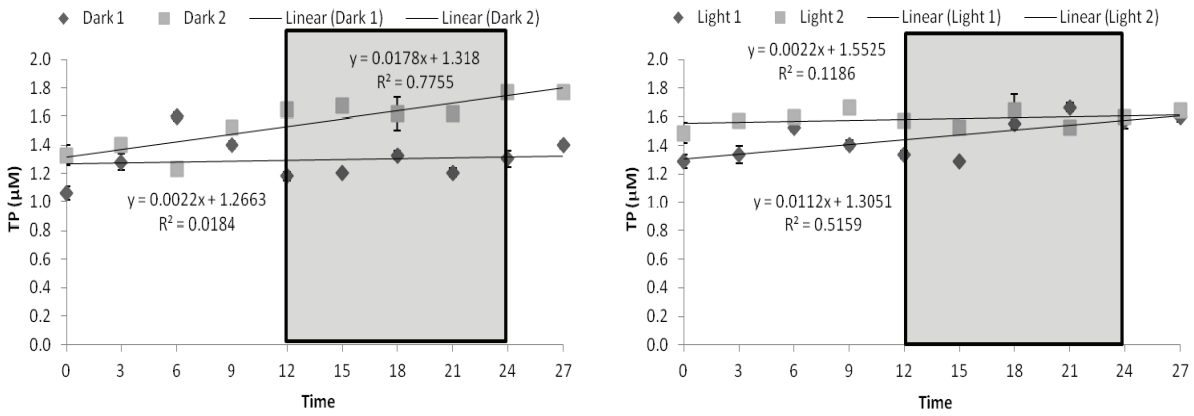

Figure 4.7 Flux of TP out of the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations)

Average NH_4^+ concentrations in both dark incubations during summer were significantly higher than the light incubations ($F = 20.10$, d.f. = 3, $p < 0.05$), with concentrations in Dark 1 and 2 increasing from 1 µM to12 µM and 1.2 µM to 7 µM respectively, while that of Light 1 and 2 increased from 1 µM to 4 µM and 1.5 µM to 3 µM respectively (Figure 4.8). During winter the average NH_4^+ concentration in the Dark 1 increased from 4 µM to 7µM and was significantly lower in concentration than Dark 2, which increased from 4 µM to 11µM ($H = 17.17$, $n = 120$, $p < 0.05$) (Figure 4.9). The average NH_4^+ concentration in Dark 2 was also significantly higher than Light 2, the latter increasing from 3 µM to 6µM ($H = 17.17$, $n = 120$, $p < 0.05$) (Figure 4.9).

The average flux of NH_4^+ was always from the sediment into the water column in both the summer and winter deployments. Although the average flux of NH_4^+ from the sediment was slightly higher in summer (0.61 mmol m^{-2} d^{-1}) than winter (0.50 mmol m^{-2} d^{-1}) (Table 4.3), there was no significant difference (U = 5.00, p > 0.05) between the two periods.

Table 4.3 Total flux of NH_4^+ from the sediment of light and dark chambers.

	Summer (March 2012)	Winter (July 2012)
	F mmol m^{-2} d^{-1}	F mmol m^{-2} d^{-1}
Light 1	0.40	0.31
Light 2	0.35	0.27
Dark 1	1.00	0.93
Dark 2	0.70	0.48
Average	0.61	0.50

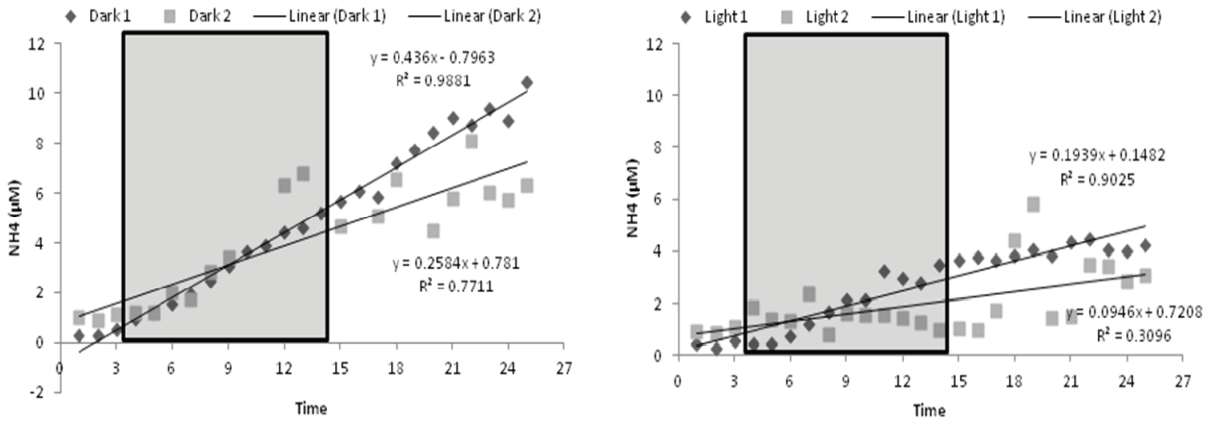

Figure 4.8 Flux of NH_4^+ from the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

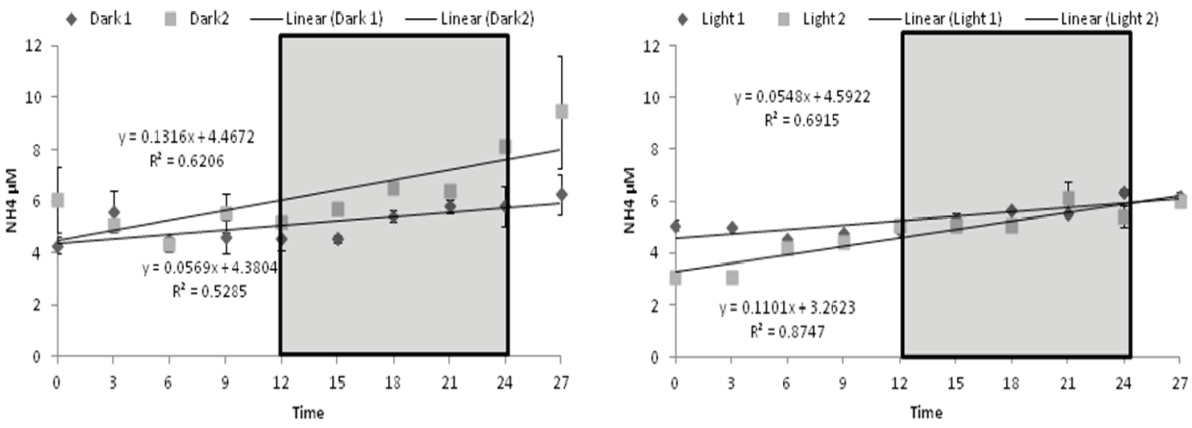

Figure 4.9 Flux of NH_4^+ from the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

The concentration of TOxN during summer (March 2012) in both dark chambers decreased from 6 µM to 4 µM and in both light chambers from 8 µM to 3 µM (Figure 4.10). The TOxN in both dark and light chambers decreased over the winter (July 2012) incubation period (Figure 4.11) from approximately 10 µM to 5 µM.

In all instances the average flux of TOxN (Table 4.4) was directed from the water column to the sediment, averaging 0.24 mmol m^{-2} d^{-1} during summer and 0.09 mmol m^{-2} d^{-1} during winter. However no significant difference was found between the two periods ($U = 4$, $p > 0.05$). The direction of flux may also be an indication of denitrification, and or phytoplankton uptake.

Table 4.4 Total flux of TOxN in light and dark chambers.

	Summer (March 2012)	Winter (July 2012)
	F mmol m^{-2} d^{-1}	F mmol m^{-2} d^{-1}
Light 1	-0.05	0.02
Light 2	-0.16	-0.10
Dark 1	-0.55	-0.03
Dark 2	-0.19	-0.27
Average	-0.24	-0.09

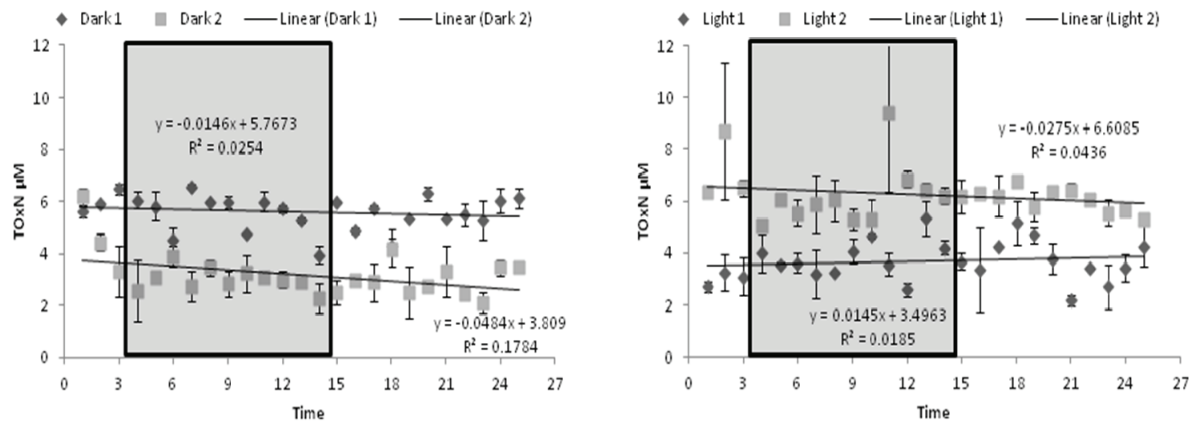

Figure 4.10 Flux of TOxN out of the water column over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

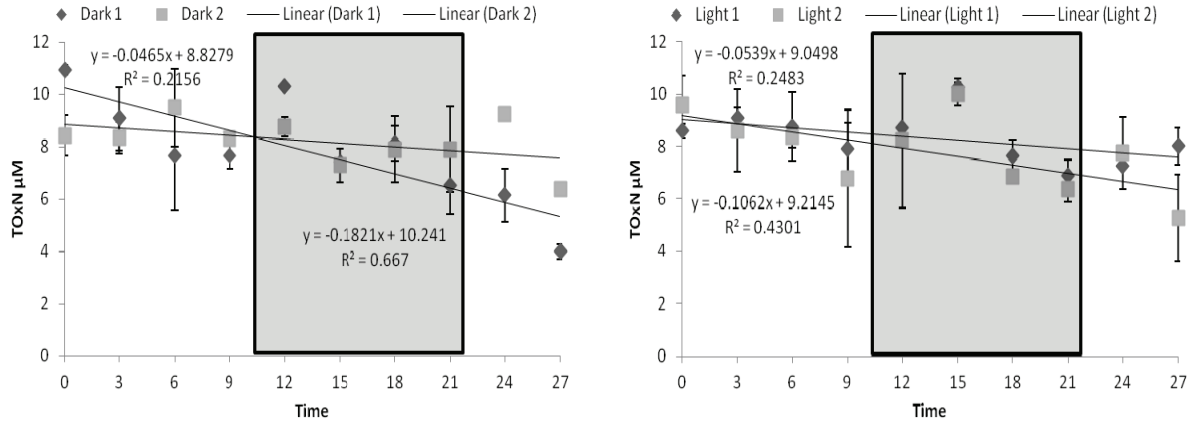

Figure 4.11 Flux of TOxN out of the water column over a 27 hour cycle in winter (July 2012). The shaded area represents the evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

During the summer deployment (March 2012), Light 2 ranged from 0.92 to 2.2 µM and was significant higher in SRP ($H = 41.38$, $n = 250$, $p < 0.05$) concentration than Light 1 (0.91-1.21 µM). Similarly, Light 2 was significantly higher ($H = 41.38$, $n = 250$, $p < 0.05$) in SRP than both dark chambers (0.91-1.21 µM) (Figure 4.12). In winter Dark 2 had a SRP range from 0.40 to 0.45 µM and was significant higher than ($H = 68.43$, $n = 120$, $p < 0.05$) Dark 1 (0.23-0.37 µM) (Figure 4.13). Light 2 (0.27-0.37 µM) was significantly higher in SRP than Light 1 (0.27-0.37 µM).There were also significantly higher SRP concentrations in both dark chambers when compared to the light chambers ($H = 68.43$, $n = 120$, $p < 0.05$) (Figure 4.13).

The average flux of SRP in summer was 0.043 mmol m^{-2} d^{-1} and was significantly higher ($T = -4.47$, d.f. = 3, $p < 0.05$) than the average efflux of 0.010 mmol m^{-2} d^{-1} in winter (Table 4.5).

Table 4.5 Total flux of SRP from the sediment of light and dark chambers.

	Summer (March 2012)	Winter (July 2012)
	F mmol m^{-2} d^{-1}	F mmol m^{-2} d^{-1}
Light 1	0.031	0.011
Light 2	0.064	0.008
Dark 1	0.043	0.013
Dark 2	0.036	0.005
Average	0.043	0.010

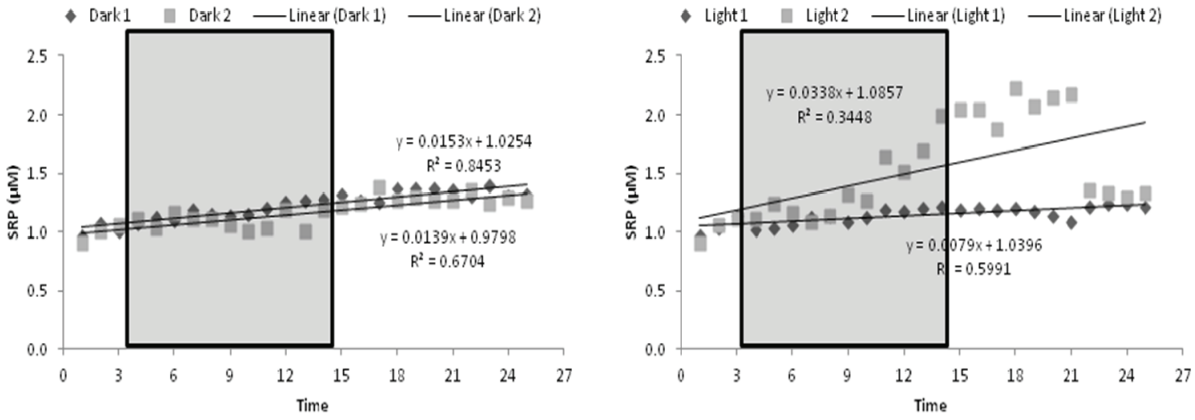

Figure 4.12 Flux of SRP out of the sediment over a 25 hour cycle in summer (March 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

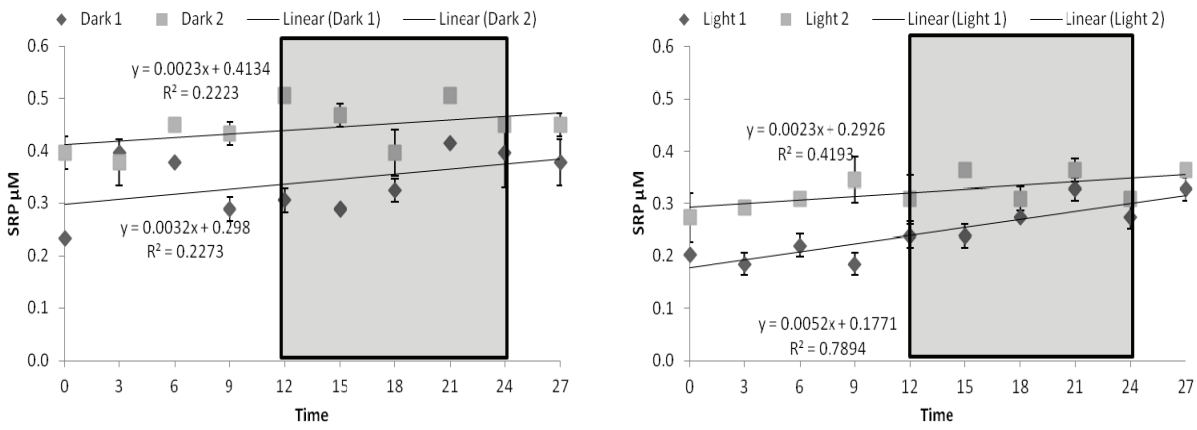

Figure 4.13 Flux of SRP out of the sediment over a 27 hour cycle in winter (July 2012). The shaded area represents evening hours (Dark 1 and 2 are dark incubations and Light 1 and 2 are light incubations).

4.4 Discussion

Temperature in the chambers increased during the day and decreased during the night during both the summer and winter deployments, although the average temperature in summer was significantly warmer than in winter. This was also found in the East Kleinemonde Estuary by Taljaard *et al*. (2008) who stated that these findings illustrated that temperature in the estuary water column is largely a function of atmospheric temperature. Except for one light chamber in summer (March 2012) showing an increase in dissolved oxygen concentration during daylight hours, dissolved oxygen concentrations in all chambers during both the summer and winter deployments steadily decreased over the incubation period. This pattern has also been observed by Sundby *et al*. (1986) in Gullmarsfjorden, Revsbech *et al*. (1988) in Aarhus Bay and recently by Pratihary *et al*. (2009) in the Mandovi Estuary in India. Similar findings were also obtained by Berelson *et al*. (1998), where they reported that there was no tendency for oxygen uptake rates to decrease during daylight hours of incubation. Furthermore, Nicholson *et al*. (1996) did a series of deployments (each deployment consisted of three light and three dark chambers) and found that in 18 out of 24 deployments there was no difference between dark and light chamber oxygen fluxes, all decreasing over the incubation period. While six of the chambers showed evidence of oxygen production during daylight hours there was always a net oxygen decrease over the 24 h cycle.

Total oxidized nitrogen (TOxN) concentrations in the water column decreased over the incubation period in both summer and winter. Similar findings were reported for the Mandovi Estuary (Pratihary *et al*. 2009). They found that the benthic TOxN flux decreased in most of the periods sampled and attributed this to a combination of low nitrification rates and the sediment acting as a sink for NO_3^-. This also partly explained the higher NH_4^+ concentrations that they found. Such results were obtained by Pedersen *et al*. (1999) as they observed an increase in NH_4^+ efflux with a simultaneous decrease in NO_3^- flux that was attributed to a lack of oxygen penetration into the sediment. The findings of Spooner and Maher (2009) illustrated that the sediment acted as a constant source of NH_4^+ throughout the year.

The results of this study displayed NH_4^+ efflux in all chambers during both summer and winter. The decreasing TOxN trend observed during both periods may also be due to other processes such as denitrification, where NO_3^- is converted to $N_{2(g)}$ and the dissimilatory nitrate reduction to ammonium (DNRA) whereby NO_3^- is transformed directly into ammonium by microbes present in highly reduced sediment (Herbert *et al*. 1999, Brandes *et al*. 2007, Prescott *et al*. 2008). Under conditions of anoxia, dissimilatory nitrate reduction to ammonium potentially competes with denitrification for oxidized inorganic nitrogen (NO_3^- or NO_2^-) (Thornton *et al*. 2007, Spooner and Maher 2009) and generally rates of denitrification decrease with increasing organic carbon loads (Heggie *et al*. 1999). A consequence of DNRA is that it produces nitrogen in a bioavailable form through the reduction of oxidized inorganic nitrogen to NH_4^+, which may be directly assimilated by microorganisms and plants (Thornton *et al*. 2007). However, it is more likely that increases in NH_4^+ are due to remineralised organic matter present on the sediment surface. NH_4^+ is the major inorganic nutrient released during remineralisation (Matheson *et al*. 2002).

The results in this study suggest that sediment of the Great Brak Estuary acts as a source of NH_4^+ and partly as a sink for TOxN. The DIN is mainly composed of NH_4^+ as a result of processes such as DNRA and remineralisation of organic matter present in the sediment. Although our results showed no significant difference in the efflux of NH_4^+ during both periods, other authors have indicated that seasonal temperature do influence fluxes. Takayanagi and Yamada (1999) indicate that NH_4^+ efflux increases as bottom water temperatures increase because of increased microbial activity. Jahnke *et al*. (2005) noted that the dissolved NH_4^+ fluxes from the sediment increase to sustain a summer maximum and dropped off sharply as bottom water began to cool. The higher NH_4^+ efflux may also be related to the low oxygen concentration which when coupled with a rise in temperature leads to increased remineralisation of organic matter. Rising water temperatures increase

remineralisation rates that cause greater ammonium release from the sediments and support higher rates of primary production in the water column (Vouve 2000, De Vittor *et al*. 2012).

The average SRP flux into the water column in summer was 0.043 mmol m^{-2} $d^{-1}$and 0.010 mmol m^{-2} d^{-1} in winter. These effluxes are relatively low when compared to those found by Pratihary *et al*. (2009) where the efflux ranged from 0.12 mmol m^{-2} d^{-1} in winter to 0.24 mmol m^{-2} d^{-1} in summer. The lower SRP effluxes could be occurring because SRP may be forming complexes with the bottom sediments thereby retaining most of the SRP in a bound form. Heggie *et al*. (1999) observed that the sediments in Australian Intermittently Open Closed Lagoons (ICOLLs), that are similar to South African TOCEs, retain phosphorus by the formation of iron hydroxide complexes (similar complexes are formed with aluminium) in the oxic zones. Upon depletion of oxygen in our chambers there was an increase in the efflux of SRP. This was expected because under depleted oxygen conditions the hydrous iron oxides become reduced and SRP is released leading to higher concentrations of SRP in the water column (Spooner and Maher 2009). Froelich (1988) showed that SRP exhibited adsorption and desorption to clay particles as a result of their natural oxide coatings. He further illustrated this as a two-step process with an initial fast reaction of sorption or desorption occurring in minutes to hours, while the slow reaction can take days to months. According to him the fast reaction accounted for 50-90% of the response for most natural clays and oxides, with a maximum sorption occurring at pH levels of 4.8-6. Froelich (1988) observed that the dominant P form adsorbed to the surface of clay particles at this pH range was $H_2PO_4^-$ and upon exposure to higher salinity water (that has higher pH) the P ion was released. Given that water that drains from the catchment area into the Great Brak Estuary has a pH of about 5 and mixes with higher salinity water within the estuary, it is expected that the P bound to sediments are processed as described by Froelich (1988). Although the SRP efflux was low, there was consistent efflux of SRP during both deployment periods, following a similar trend to NH_4^+. Results indicate that the flux of SRP was more pronounced during summer than in winter. This is mainly linked to the difference in temperature since higher temperatures will result in a greater release of SRP during the decomposition of organic matter (i.e. anoxia is more likely to occur). Remineralisation varies significantly with season and is probably in response to changing bottom temperatures and organic matter inputs (Jahnke *et al*. 2005).

A large component of the TN and TP in the chambers is composed of organic N and P. This is evident from the low inorganic N and P concentrations found during the incubation period. The total TN and TP are composed of both the inorganic and organic fraction, so it stands to

reason that an increase in either component would lead to an increase in the TN. There was only a slight increase in TN and TP within the chambers over the deployment periods. This efflux of TN and TP was probably due to an efflux of inorganic nutrients NH_4^+ and SRP during the incubation period. The availability of organic N and P serves as a consistent source for the processes of DNRA and remineralisation to NH_4^+ and SRP. The sources of DON and DOP on the benthos of shallow water systems are primarily derived from the benthic and pelagic primary producers (Pedersen *et al*. 1999). Nutrients that are assimilated during plant growth are immobilized temporarily into living biomass and detritus until they are mineralised through grazing or decomposition. Evidence of DON release from the sediment can be inferred from the studies of Enoksson (1993) and Pedersen *et al*. (1999). Both experiments showed that after the addition of diatom cells and *Zostera marina* leaves respectively to the sediment, DON was released after the first few days of incubation. They suggested that the released DON was due to leaching of plant and algal storage compounds after autolysis of the cells. Pedersen *et al*. (1999) further pointed out that because leaching is a temporary occurrence, hydrolysis and mineralisation products contribute more to the DOM efflux with time. Nonetheless, the supply of organic-rich detritus to the sediment coupled with low dissolved oxygen within the bottom water during prolonged closed mouth condition acts as a source for inorganic and dissolved organic N and P to the water column.

Another important biological component that also has to be considered is the effect of macrofaunal species on the benthic flux of nutrients and dissolved oxygen from the sediment. Observations during 24 hour sampling of the chambers in the Greak Brak indicate that there are numerous groups of fish and crustaceans within the shallow waters of the estuary. There have been no detailed studies conducted in TOCEs that link the sediment nutrient flux to the overlying water column in South African estuaries. Studies from other parts of the world (Aller 1988, Hansen and Kristensen 1998, Kristensen and Hansen 1999, Webb and Eyre 2004) have found that macrofauna enhance sediment reactivity and increase the efficiency of solute mass transfer between the water column and sediments, a process termed bioturbation. *Trypaea australiensis* (formally *Callianassa australiensis*) increased sediment oxygen demand by 80% and approximately 15% was used for respiration by the shrimp while the remainder was used for oxidation reactions and microbial respiration (Webb and Eyre 2004). Macrofaunal activities were found to increase dissolved oxygen consumption due to the enhancement of oxidation reactions like sulphide and pyrite oxidation, nitrification and increased respiration by macrofauna and microbial communities (Kristensen *et al*. 1991, Pelegri *et al*. 1994, Paterson and Thorne 1995).

Webb and Eyre (2004) showed that the presence of *Trypaea australiensis* in sediments of the Brunswick River estuary was able to contribute to 76% efflux of $N_{2(g)}$ from total denitrification occurring in the sediments. Kristensen *et al*. (1985) and (1991) found similar contributions of 66% and 52% respectively of $N_{2(g)}$ efflux from sediments in their macrofaunal studies. Others (Vetter and Hoppkinson 1985, Lillebo *et al*. 1999, Lavrentyev *et al*. 2000) have shown that the stimulation of denitrification was linked to an increased supply of NH_4^+ in macrofaunal burrows from animal excretion, the accumulation of organic matter and increased remineralisation rates due to enhanced microbial activity. Webb and Eyre (2004) found a mean net efflux of NH_4^+ (0.969 mmol m^{-2} d^{-1}) in sediments containing *Trypaea australiensis* and concluded that the shrimp contributed a significant supply of NH_4^+ to the water column and as such, probably stimulated autotrophic productivity. Correspondingly, they also found a net efflux of PO_4^{-3} from sediment containing *Trypaea australiensis.* They and other authors (Carlton and Wetzel 1988, Paterson and Thorne 1995) believe that because the shrimp is able to tolerate low oxygen concentrations within their burrows, they may be stimulating localised anoxic releases of PO_4^{-3} in their burrow linings and this, together with excretion, results in a net efflux of PO_4^{-3} from the sediment. As such, the presence of *Trypaea australiensis* also represents a net source of phosphate to the water column, which may contribute to primary productivity (Webb and Eyre 2004). Although the contribution of macrofauna to nutrient cycling did not form part of the scope of this research, they clearly have an important role to play since they are able to enhance the flux of nutrients out of the sediments.

4.5 Conclusion

Temporarily Open Closed Estuaries are characterised by low river inflow, weak flushing and long residence time resulting in prolonged mouth closure (Whitfield 1992, Taljaard *et al*. 2009 b). These characteristics make TOCEs vulnerable to nutrient enrichment and a build-up of organic matter (Newton and Mudge 2005, Human and Adams 2011). Studies on ICOLLS showed that benthos were the most important sources and sinks of N and P to the water column and could potentially contribute as much as 3-4 times the catchment discharges (Smith *et al*. 2001, Palmer *et al*. 2002, Spooner and Maher 2009). In the case of the Great Brak, respiration from the organisms in the benthos (benthic respiration) seems to be the major metabolic process occurring during the incubation period with a net flux of oxygen toward the benthos. This study showed that the benthos had a net efflux of NH_4^+, SRP, TN and TP and acted as a source of N and P during both study periods. Organic matter introduced either via the catchment or internally from the pelagic and benthic

primary producers, ensures a sufficient organic load to fuel growth. This ensures that there is always N and P available for the macrophytes present within the estuary. This is especially so under prolonged closed mouth conditions. The build-up of organic matter in the Great Brak Estuary has been exacerbated by the manner in which it is not flushed since the construction of the Wolwedans Dam upstream. The construction of the dam has led to a reduction in river flow. This means that there has been a significant base flow reduction that would have flushed the estuary regularly. While water from the dam is made available to breach the mouth, it is often not sufficient to flush the estuary. This seems to be causing an accumulation of both sediment and organic matter within the estuary. This continues until the system gets flushed by a major flood. If the estuary remains closed for a prolonged period (>12 months), with an increased organic load present on the benthos, the associated rates of effluxes of N and P would increase. In order to reduce the organic load to the system better flushing methods or more importantly, an increase in base flow, is needed to reduce residence times of water in the estuary.

5 THE ROLE OF SUBMERGED MACROPHYTES AND MACROALGAE IN NUTRIENT CYCLING IN THE GREAT BRAK ESTUARY SOUTH AFRICA: A BUDGET APPROACH

5.1 Introduction

Nutrient budgets must quantify the sources and sinks of the materials in question. Budgets are useful tools, indicating from where these materials are derived (sources), where they get depleted (sinks) and whether any changes have occurred (e.g. the conversion of N from one form to another via biological processes). The primary limiting nutrients in the marine environment are nitrogen and phosphorus (Robson *et al*. 2008). They are required for both primary production and the microbial pathway of the food web. All other biota depend on the food and habitat provided by the primary producers in the system, and therefore depend on well-functioning nutrient cycles. Hence a nutrient budget is necessary in order to understand the sources and losses of nutrients in a system. Robson *et al*. (2008) considered that the fate of nutrients from anthropogenic sources entering estuaries is dependent on complex interactions between hydrodynamics and biological processes that determine whether or not nutrients are retained or exported; and that it is highly probable that in shallow waters with weak tidal flushing nutrients may be retained within the estuary. Nutrient input from underlying benthos can contribute substantially to the pelagic nutrient inventory and has the potential as the only source driving water column primary production in shallow systems (Callender and Hammond 1982, Jensen *et al*. 1990, Koho *et al*. 2008). Boynton and Kemp (2000) and Hopkinson *et al*. (2001) state that when primary production is low, seasonal inputs of organic matter from the catchment, which end up in the estuary benthos, can provide a source of remineralised inorganic nutrients. It is clear that these sources play a significant role in the biogeochemistry of such systems and, according to Pratihary *et al*. (2009), if these sources were absent from shallow systems, then primary production would be solely dependent on pelagic regeneration or fresh sources from water inflow.

Although well studied in some other parts of the world there is a paucity of information on mass balance approaches in South Africa, and particularly so in the case of TOCEs. Various models with differing complexity have been developed for estuaries. One such model was the LOICZ biogeochemical model which has become common in the literature over the past three decades with more than 200 systems of the global coastal zone assessed so far (Buddemeier *et al*. 2002). Most of these, though, were macrotidal systems that are permanently open to the sea. This approach is too broad and calculates the nitrogen budget

from DIP and the molar C:N:P ratio of the reacting organic matter, typically from the dominant primary producers. The purpose of the LOICZ was to gain understanding of how coastal bodies exchange nutrients with the sea. TOCEs can remain closed off from the sea for prolonged periods and need a model that is better suited to their specific functionality. However, a recent study by Giordani *et al*. (2008) assessed the robustness of the LOICZ model in shallow water systems and found that to some extent it can represent a wide range of trophic conditions which usually occur in shallow coastal systems. They warn though that the simplifications that have been introduced to apply the LOICZ to data poor systems such as the use of unique C:N:P ratios and dissolved inorganic phosphorus relationships, should be believed with caution in shallow environments. Frequent closed mouth conditions as a result of reduced freshwater inflow causes a lack of flushing which results in long residence times which make TOCEs especially vulnerable to water quality changes (Human and Adams 2011). Informed decisions on management of such systems are possible only if their nutrient and hydro dynamics are properly understood and the relationship between catchment and estuarine processes are quantified (Smakhtin 2004).

The aim of this chapter was to determine the load of nitrogen and phosphorus in the submerged macrophytes and macroalgae of the estuary and to gain a better insight into the cycling of these nutrients between the benthos and water column. A nutrient budget for the estuary was constructed in order to quantify the contribution of the submerged macrophytes and macroalgae relative to other contributing sources of N and P. Nutrient budgets often account for the vegetation component by relying on C:N:P ratio of the dominating macrophytes. This is an inherent shortcoming because the vegetation may actually be storing more N and P than what is presented in the ratio. This is the first detailed account which incorporates vegetation into a nutrient budget and does not rely solely on C:N:P ratios. There are, however, many other emergent vegetation types other than just submerged macrophytes and macroalgae, i.e. salt marsh, reeds and sedges as well as grazers that influence the nutrient budget within estuaries and need to be taken into account when considering the complete budget. The redfield ratio was used to estimate the component, net denitrification (Kalnejais *et al*. 1999, Emily *et al*. 2005, Robson *et al*. 2008). There are few studies that have investigated the interactions between anthropogenic inputs, benthic biogeochemistry and submerged macrophytes and macroalgae within South Africa.

5.2 Materials and methods

5.2.1 Data sources

In order to measure the bathymetry of the entire estuary, the sampling effort was split into the lower estuary (below the N2 Bridge) and the middle to upper estuary (above the N2 Bridge). The bridges crossing the estuary effectively constrain the bathymetry of middle and upper estuary. In contrast, the bathymetry of lower estuary around the island is much more dynamic (**Figure 5.1**); sand is deposited at the mouth of the estuary from wind and wave deposition and floods have a strong scouring effect. The Department of Water Affairs (DWA) performed bathymetric profiles of the estuary in 2006. It was therefore decided that a full bathymetric survey would be conducted on the dynamic lower reaches of the estuary, in case any changes had occurred since the last survey.

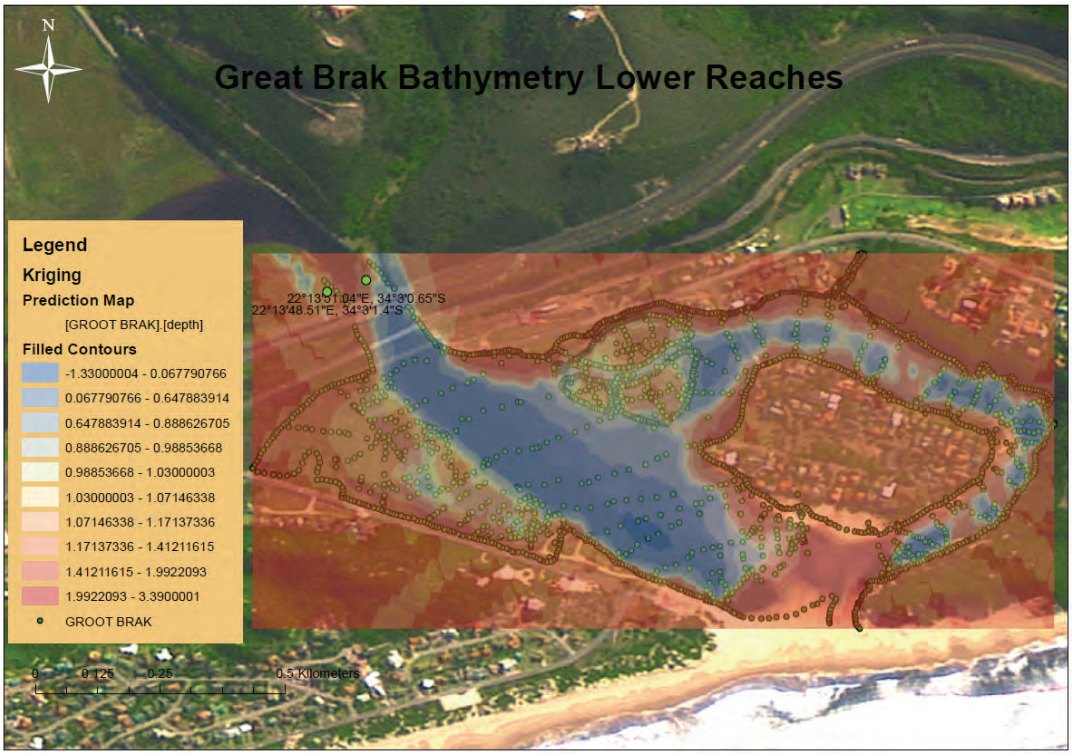

Figure 5.1 Bathymetric survey of the lower Great Brak Estuary below the N2 Bridge.

There is a permanent water level recorder (K2H004) located on the railway bridge in the lower estuary, which is vital when determining the volume of the estuary. The volume of water at any given water level could then be calculated for the entire estuary. The CSIR

water volume at a 2 m msl for the entire estuary was 1 005 800 m^3 and the lower reaches was nearly half that (455 591 m^3) (**Figure 5.2**).

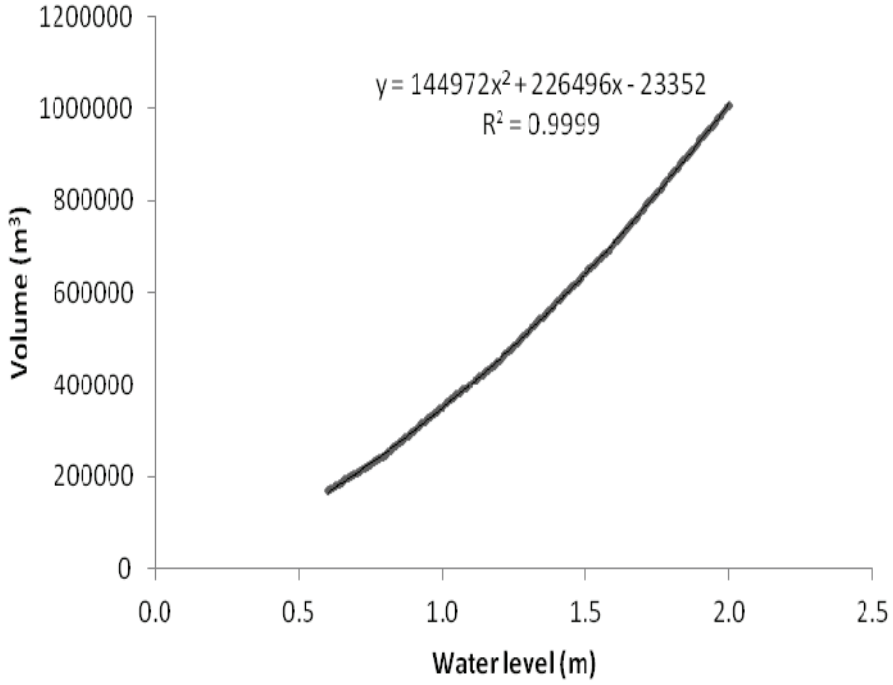

Figure 5.2 The water level in relation to volume for the entire estuary.

In addition to water level data, flow data were sourced from a gauging station (K2H002) at the head of the estuary. These stations are maintained by the DWA, South Africa. Evaporation data were also obtained from the nearby DWA Hartenbos Sewerage works station. Rainfall and temperature data were sourced from Weather SA.

The TN and TP water samples collected from the Great Brak Estuary were combined with the flow data and used to determine the load of N and P within the water column and river. The data were analysed as described previously (Chapter 3.2.2). Benthic chambers were deployed in order to estimate the flux of TN and TP from the sediment of the Great Brak Estuary and analysed as described in Chapters 3.2.2 and 4.2. The analysis of TN and TP within the submerged macrophyte and macroalgae tissues was processed as described in Chapter 3.2.3. Macrophyte area cover was digitised and calculated from aerial, ground truthed photographs using ArcGIS software version 10. The combined nutrients and area cover provide an accurate estimate of the nutrient load contained in the macrophytes.

5.2.2 Budget overview

Water Budget

Assessment of an accurate water budget is dependent on having reliable estimates of each component. The water balance used in this study considered the estuary as a reservoir because an estuary that is blocked off from the sea by a sand bar is effectively a reservoir, with the sand bar acting as a restricting wall (Smakhtin 2004). The main water balance accounting components in this model include inflow, rainfall on the estuary surface, and evaporative losses. The estuary water balance may then be described by the following equation: Input – Output = Δ Storage / time.

$$(V_r + Vs + V_p) - (Ve + Vs) = \Delta S/t \qquad \text{(Equation 5.1)}$$

Where inputs consist of:

- Flow from the river V_r in m^3 ($m^{-3}s^{-1}$ x 60 s x 60 min x 24 hours x no. of days),
- Flow from diffuse sources V_s in m^3 (solved after all other variables have been derived), and
- V_p is the precipitation onto the surface of the estuary in m^3 ((mm day^{-1}/1000) x no. of days x area cover m^2).

Outputs consist of:

- Evaporative losses Ve (m^3) (mm day^{-1} x 1000 x no. of days x area cover m^2) and
- Seepage through the berm Vs (m^3).

Unlike the perched estuaries on the east coast of South Africa where seepage through the berm is a prominent feature and cannot be excluded (Lawrie *et al*. 2010), the estuaries on the south coast lose very little water during the closed phase (Van Niekerk pers comm.) and therefore seepage was eliminated from the water budget. Change in storage (ΔS/t) represents the change in the volume of water within the study area per unit time. A salinity budget was not considered because the estuary is relatively well mixed in it is closed.

The water budget can be presented as a box model (Figure 5.3) with Inputs – Outputs = ΔS/t

or: $(V_r + V_s) – (V_{pnet}) = \Delta S/t$ (Equation 5.2)

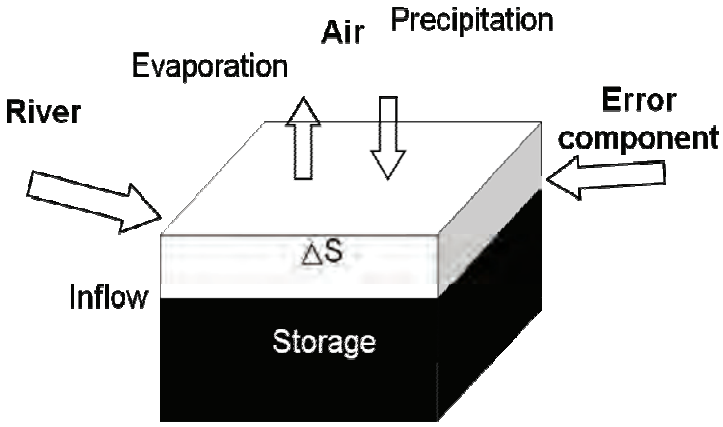

Figure 5.3 Conceptualised box model for water balance in the Great Brak Estuary.

Nutrient budget

The total nitrogen in the estuary can be written as:

$$TN = \Delta((V_rC_N + V_sC_N + VpCN) + (RN) – (\text{net } Dn + S_{PT} + S_{PN} + S_{CN} + S_{ZN} + S_{RN}))/t$$

(Equation 5.3)

Where:

- V_rC_N (kg) is the load in the river flow, (volume m^{-3} x (mg l^{-1} x $10^6/10^3$), i.e. kg m^{-3})
- V_pC_N (kg) is the precipitation (m day^{-1} x area cover m^2) x kg m^{-3}
- R_N (kg) is the release of TN from bottom sediment, (kg m^{-2} x area cover m^2)
- net D_n is denitrification less nitrogen fixation (kg), (volume m^{-3} x kg m^{-3})
- S_{PT} is the storage in the particulate component, (volume m^{-3} x kg m^{-3})
- S_{CN} represents storage of N in *Cladophora glomerata* (kg), (kg m^{-2} x area cover m^2)
- S_{ZN} is storage in *Zostera capensis* (kg), (kg m^{-2} x area cover m^2)

- S_{RN} is storage in *Ruppia cirrhosa* (kg), (kg m^{-2} x area cover m^2), and

- V_sC_N is the error component – also referred to as 'other sources' – which is a combined unknown and includes diffuse loads, groundwater, inputs and outputs from other vegetation as well as fauna, of TN and TP. This component then represents the immeasurable error in the budget. Note that the error component was calculated after derivation of all other terms in the equation. This term includes all unknown variables and is not of significance to the purpose of this study, but is required in order to indicate the value of other variables that could not be measured.

Estimates of net denitrification were calculated using stoichiometric analysis (Kalnejais *et al*. 1999, Emily *et al*. 2005, Robson *et al*. 2008). In this approach two assumptions are made: (i) changes in dissolved inorganic phosphorus (DIP) stores within the estuary that are not attributable to inflows or tidal exchanges, are due to biological kinetics and, (ii) biological uptake of dissolved inorganic nitrogen (DIN) adheres to the Redfield nutrient ratio. The expected change in inorganic nitrogen stores, ΔDIN_{exp}, in the absence of denitrification and nitrogen fixation, would therefore be equivalent to ΔDIP multiplied by the Redfield ratio (16 mol N:1 mol P). Differences between ΔDINexp and the observed change in DIN (ΔDINobs) are attributed to net denitrification (net Dn) and estimated as:

$$\text{net Dn} = \Delta DIN_{exp} - \Delta DIN_{obs} / \Delta t = 16\,(DIP) - \Delta DIN_{obs} / \Delta t \qquad \text{(Equation 5.4)}$$

Similarly the nutrient budget for TP can be written as:

$$TP = \Delta\left((V_rC_P + V_sC_P + V_pC_P) + (R_P) - (S_{PT} + S_{PP} + S_{CP} + S_{ZP} + S_{RP})\right)/t \qquad \text{(Equation 5.5)}$$

with subscript P replacing N in Eq. (2) to denote identical processes but without contributions due to denitrification.

5.3 Results

The estuary water volume increased by 36 962 m^3 (Table 5.1) from 17 February 2011 (mouth closure) to 17 March 2011 (1 month later). Of this change in estuary storage volume, the river contributed 19 051 m^3, a net loss of water via evaporation occurred (- 71 101 m^3) and what was left was 89 011 m^3 (un-measurable sources) to balance the equation. Similar

calculations can then be made for the months that follow (17 April 2011 and 17 May 2011). It should be noted that net evaporation, as well as the "other sources" component calculated in the budget, is an overestimate. However, these estimates will not affect the objective of the project which was to calculate the load of the N and P trapped inside the submerged macrophytes and macroalgae. Note that "other sources" include: diffuse source, groundwater as well as that which is not accounted for in the budget, a combination of all unknowns to balance all known inputs. There is a marked increase in the volume of the "other sources" component during the open phase and this is mainly due to river outflow through the mouth of the estuary. The raw data and calculated volumes appear in Appendix A, Tables 1 to 3.

Table 5.1 Water balance for the Great Brak Estuary.

Mouth State	Months after closure	Estuary volume (m^3)	Δ Storage (m^3)	River (m^3)	*Net precipitation* (m^3)	*Error component* (m^3)
Closed	0	376249				
	1	413211	36962	19051	-71101	89011
	2	387335	-25876	12784	-59788	21129
	3	464421	77086	25795	-11844	63135
Open	4	301675	-162746	8048485	57018	-8268249
	5	296634	-5040	2100212	-7004	-2098248

5.3.1 Total nitrogen (TN) in the dominant submerged macrophytes and macroalga

Please refer to the appendix section for the raw data and calculated load of TN trapped in the different submerged macrophytes and macroalgae (Appendix A, Tables 11 and 12).

A month after mouth closure, a total storage of 5473 kg TN was trapped within the submerged macrophytes and macroalga; *Ruppia cirrhosa, Zostera capensis* and *Cladophora glomerata.* During the first month after mouth closure there was an uptake of 1328 kg TN from the estuary by *C. glomerata,* while the submerged macrophytes lost 781 kg TN (*Z. capensis* 381 kg TN and *R. cirrhosa* 400 kg TN) (Table 5.2, Figure 5.4). This is reflected

as a net uptake of 547 kg TN from the estuary water column (i.e. a net output from the water, or a net uptake (storage) in the macroalga).

The total N in the submerged macrophytes and macroalga was 6020 kg TN, with 662 kg TN being released to the estuary upon the decomposition of *C. glomerata,* while *Z. capensis* and *R. cirrhosa,* incorporated 182 and 89 kg TN (271 kg TN) into their tissue. This resulted in 391 kg TN being released into the water column (i.e. a net input to the water column) as the resultant decomposition of *C. glomerata* was greater than the combined uptake of *R. cirrhosa* and *Z. capensis* (Table 5.2, Figure 5.4).

After the third month of mouth closure there was a total of 5629 kg TN within the submerged macrophytes and macroalga. Further decomposition of *C. glomerata* released 1176 kg TN to the estuary, and *Z. capensis* and *R. cirrhosa* took up 201 and 98 kg TN from the estuary respectively and once again caused a net input of 878 kg TN to the estuary water column (Table 5.2, Figure 5.4).

By month 4 (June 2011 flood) *C. glomerata* had been flushed out of the estuary (1470 kg TN) and, the submerged macrophytes *Z. capensis* and *R. cirrhosa,* incorporated 221 and 108 kg TN into their tissue respectively (Table 5.2, Figure 5.4). Because the mouth was still open a month later and *C. glomerata* had been flushed out of the estuary, *Z. capensis* and *R. cirrhosa* were the only vegetation present in the water column and they continued to take up 485 and 237 kg TN respectively. This led to a net uptake (storage in the vegetation) or net output of 722 kg TN from the water column (Table 5.2, Figure 5.4).

Table 5.2 Load of TN (kg) in each component of the submerged macrophytes and macroalga (TN stored = ΔC(kg) at time 0+ΔC).

Mouth State	Month	TN (kg)	*Cladophora glomerata*	*Zostera capensis*	*Ruppia cirrhosa*	Total
Closed	1	**TN Stored**	1981	2204	1289	5473
		TN released		381	400	781
		TN taken up	1328			1328
		Net output				548
	2	**TN Stored**	3308	1823	889	6020
		TN released	662			662
		TN taken up		182	89	271
		Net input				391
	3	**TN Stored**	2648	2005	978	5629
		TN released	1176			1176
		TN taken up		201	98	298
		Net input				878
Open	4	**TN Stored**	1470	2206	1075	4751
		TN released	1470			1470
		TN taken up		221	108	328
		Net input				1142
	5	**TN Stored**	0	2426	1183	3609
		TN released	0			0
		TN taken up		485	237	722
		Net Output				722

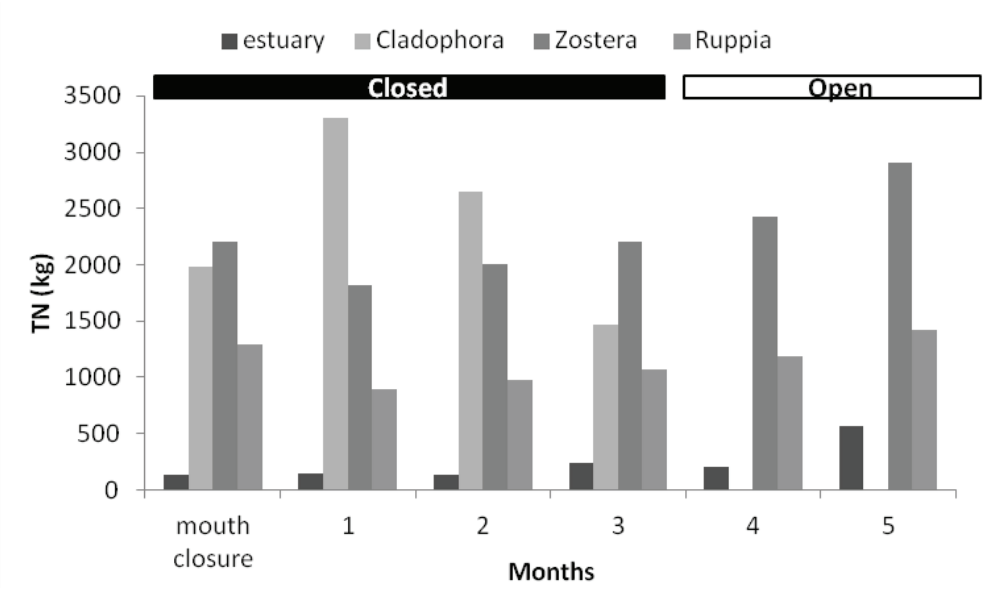

Figure 5.4 Total N stored in submerged macrophytes and macroalga in the Great Brak Estuary (■ = closed mouth; □ = open mouth).

Total nitrogen budget for the Great Brak Estuary

The calculated information from the different Tables 4 to 7, 11 and 12, in Appendix A, was used to construct the nitrogen budget for the Great Brak Estuary. There was an increase of 13 kg TN in the estuary water column one month after closure (Table 5.3). The main inputs to the estuary consisted of benthos (532 kg TN) and particulate matter (348 kg TN) (Table 5.3). The major outputs from the estuary were, the storage in the submerged macrophytes and macroalga (547 kg TN) and "other sources" (347 kg TN). Denitrification was calculated from stoichiometric estimation using the redfield ratio, and resulted in a loss of 3 kg TN to the atmosphere.

During the closed mouth state the main contributors to the estuary were the benthos and the submerged macrophytes and macroalgae, which respectively contributed 578 and 391 kg TN during month 2 and 544 and 878 kg TN during month 3. The only major output arose from "other sources" (Table 5.3). The large output from "other sources" was expected because of the presence of diffuse sources and groundwater which are inputs to the system, but are taken up and stored within the vegetation (salt marshes, reeds and sedges) and fauna of the estuary. The estuary thus acts as a sink for N and P under closed mouth conditions.

The mouth of the estuary was breached in June 2011 by a 1:100 year flood and the accuracy of the nutrient budget decreased for the open phase of the estuary because factors such as tidal mixing and salinity needed to be considered. After the flood the major inputs consisted of the river, the benthos and the submerged macrophytes and macroalga, which contributed 17441, 625 and 1142 kg TN respectively (Table 5.3). Since there was still a considerable outflow through the mouth, the major inputs to the system consisted of the river (5213 kg TN) and the benthos (559 kg TN) while the major output was the submerged macrophytes and macroalga (722 kg TN) (Table 5.3). The larger output from "other sources" during the open phase (19535 and 4610 kg TN) most probably occurs as a result of flow from the catchment increasing diffuse loads, which eventually flow out of the mouth.

Table 5.3 Total nitrogen budget for the Great Brak Estuary.

	Closed mouth			**Open mouth**	
Months from start	1	2	3	4	5
Input (kg)					
River	29	14	33	17441	5213
Release from the benthos	532	578	544	625	559
Precipitation	1	2	10	28	2
Particulate	348	39		270	
Submerged macrophytes and macroalga		391	878	1142	
Output (kg)					
Submerged macrophytes and macroalga	547				722
Denitrification	3	3	2	1	5
Particulate			126		74
Error component	347	1028	1230	19535	4610
Δ Estuary (kg)	13	-9	107	-31	363

5.3.2 The relationship between the submerged macrophytes and macroalgae as sources and sinks of phosphorus (TP)

Please refer to Appendix A (Tables 11 and 12) for the calculated load of TP trapped in the different submerged macrophytes and macroalgae. The P followed a similar trend to that of N in terms of storage, where P became incorporated into the submerged macrophytes and macroalgae. A month after mouth closure, a total of 2991 kg TP was stored within the submerged macrophyte and macroalgae component. Of this total, the opportunistic macroalgae *C. glomerata* gained 307 kg TP, while *Z. capensis* and *R. cirrhosa* lost 267 and 307 kg TP (574 kg TP) to the water column respectively (Table 5.4, Figure 5.5). This resulted in the water column gaining 267 kg (net input).

Two months after mouth closure the submerged macrophytes and macroalgae had 2725 kg TP in their tissues. *C. glomerata* started to decay and lost 153 kg TP, whereas *Z. capensis* and *R. cirrhosa* took up 128 and 68 kg TP (196 kg TP). This resulted in a net storage of 43 kg TP within the submerged macrophytes (i.e. a net output, because the accumulated storage of *Z. capensis* and *R. cirrhosa* is greater than the loss to the water column by *C. glomerata*) (Table 5.4, Figure 5.5). Three months of after mouth closure, further decomposition of the macroalgae resulted in a loss of 272 kg TP while the accumulated uptake of the submerged macrophytes was 216 kg TP, resulting in a net input to the water column of 57 kg TP (Table 5.4, Figure 5.5).

A flood that occurred in June 2011 (4 months after mouth closure) washed all the *C. glomerata* (340 kg TP) out of the system, effectively re-setting the estuary. During this month the submerged macrophytes took up 237 kg TP from the water column (Table 5.4, Figure 5.5). Following the breach, the mouth had remained open and tidal, and the submerged macrophytes responded well to this state as their area cover increased, more TP was stored in their tissue, i.e. further increases of 340 and 182 kg TP for *Z. capensis* and *R. cirrhosa* respectively (net output of 522 TP).

Table 5.4 Load of TP (kg) in each component of the submerged macrophytes and macroalga.

Mouth State	Month	TP (kg)	*Cladophora glomerata*	*Zostera capensis*	*Ruppia cirrhosa*	Total
Closed	1	**TP Storage**	447.19	1544.05	989.00	2980.24
		TP release		266.72	307.04	573.76
		TP uptake	299.84			299.84
		Net Input				273.92
	2	**TP Storage**	747.03	1277.33	681.96	2706.32
		TP release	149.41			149.41
		TP uptake		127.73	68.20	195.93
		Net Output				46.52
	3	**TP Storage**	597.62	1405.06	750.16	2725.84
		TP release	265.61			265.61
		TP uptake		140.51	75.02	215.53
		Net Output				50.08
Open	4	**TP Storage**	332.01	1545.57	825.18	2702.75
		TP release	332.01			332.01
		TP uptake		154.56	82.52	237.08
		Net Output				94.93
	5	**TP Storage**	0.00	1700.12	907.70	2607.82
		TP release		340.02	181.54	521.56
		TP uptake	0.00			
		Net Output				521.56

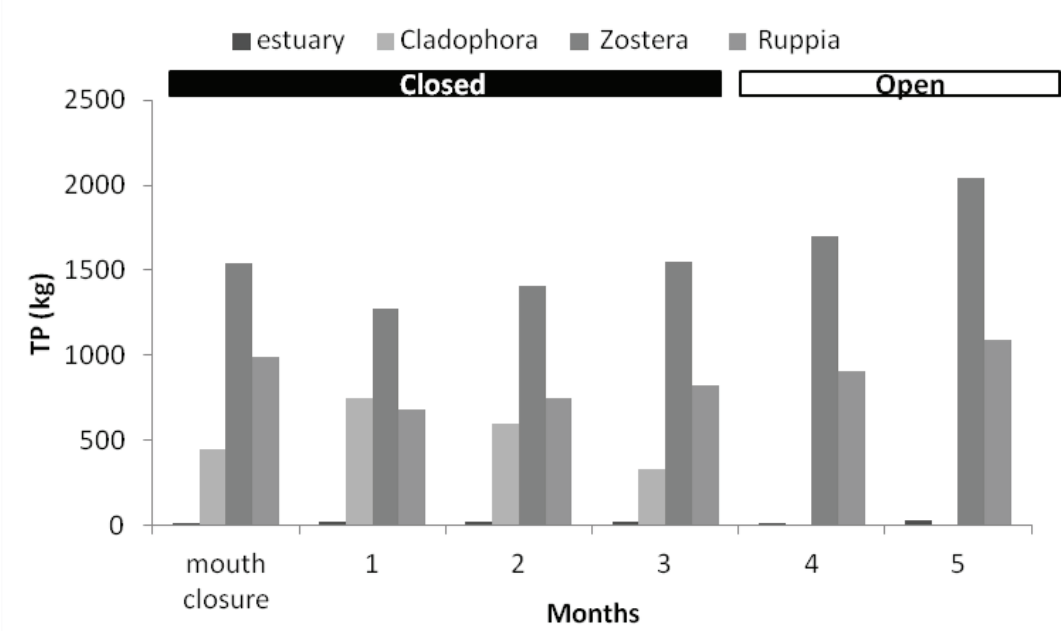

Figure 5.5 Total P stored in submerged macrophytes and macroalga in the Great Brak Estuary (solid bar = closed mouth; clear bar = open mouth).

The total phosphorus budget for the Great Brak Estuary

Please refer to Tables 7 to 12, in Appendix A, for the calculated loads in the phosphorus budget. The main inputs of TP after the first month of mouth closure occurred from the benthos and the submerged macrophytes and macroalga that released 81 and 267 kg TP respectively (Table 5.5). A balance of 305 kg TP was then calculated after all other terms were derived. This output is what is stored in the components of the "other sources". During the closed phase the resulting input from the benthos during months 2 and 3 was 89 and 74 kg TP respectively (Table 5.5). The major output during month 2 was the submerged macrophytes and macroalga component that stored 43 kg TP, while "other sources" stored 25 and 137 kg TP during months 2 and 3 respectively (Table 5.5).

After the 1:100 floods which breached the mouth of the Great Brak Estuary the major inputs to the estuary changed. There was an increase in the TP load from the river to 865 kg TP, while the benthos contribution remained stable around 76 kg TP (Table 5.5). Due to the strong flow, the contribution from the river increased markedly, which was balanced by a much larger contribution (1021 kg TP) from "other sources". A month later the river was still flowing strongly and had an input of 95 kg TP while the benthos had an input of 86 kg TP (Table 5.5). The contribution from "other sources" remained large (813 kg TP) because of the tidal open mouth conditions and probably a noticeable increase in the diffuse source

contribution during flooding. The estuary then acts as a source of TP to the sea during the open mouth phase.

Table 5.5 Total phosphorus budget for the Great Brak Estuary

	Closed mouth			**Open mouth**	
Months	1	2	3	4	5
Input (kg)					
River	2	1	4	865	95
Sediment	81	89	74	76	86
Precipitation	0.02	0.05	0.20	0.33	0.04
Particulate			5		124
Submerged macrophytes & macroalga	267		57	103	
Output (kg)					
Submerged macrophytes & macroalga		43			522
Particulate	40	22		33	
Error component	305	25	137	1021	813
Δ Estuary (kg)	4	1	2	-9	13

It should also be noted that the submerged macrophytes *R. cirrhosa* and *Z. capensis* are capable of assimilating N and P directly from the sediment, and this was not accounted for in the budget. This method of uptake would decrease the “other sources” component contribution of both TN and TP to the nutrient budget. In the same way other plant species, like salt marshes would also lower the contribution of TN and TP of the “other sources” component. Estuarine fauna have their own associated inputs and outputs that would add to and subtract from the nutrient budget. These contributions did not form part of the scope of this research but need consideration and closer study if an accurate nutrient budget is warranted.

The total storage of TN and TP (Figure 5.6) within the submerged macrophytes and macroalga appeared to be stable over the closed mouth phase (mouth closure to month 3), and ranged from 4700 to 6000 kg N, and 2700 to 2980 kg P. The reason for this is when

there is a decline in the TN or TP within the one component there is an increase in the other. Overall submerged macrophytes and macroalga conserved the TN and TP within the system. During the open phase there was a drop in the total storage of TN which was mainly attributed to the absence of *C. glomerata,* after it had been flushed out of the system.

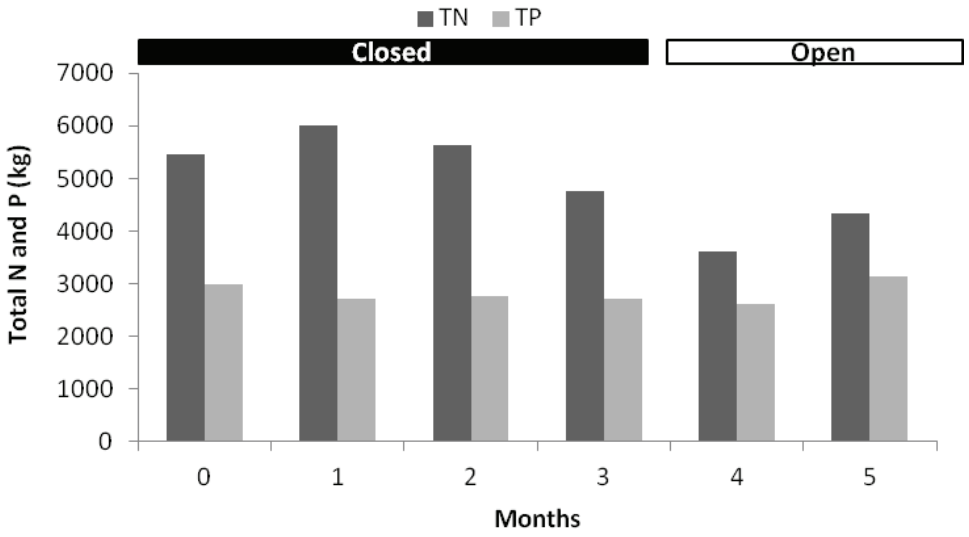

Figure 5.6 Total storage of TN and TP in the submerged macrophytes and macroalga within the Great Brak Estuary (■ = closed mouth; □ = open mouth).

The percentage contribution of each component toward the nutrient budget

During the closed phase, the "other sources" component (or error component) accounted for 20 to 50% of the contribution to the N budget (Figure 5.7 A). This falls within the range of what was expected that would not be accounted for in the budget, because there were a number of sources (unmeasured groundwater, unobserved point sources, salt marsh, reeds, sedges and fauna) that constitute the "other sources" component. The main contributors to the budget then are the benthos, ranging from 20 to 30%, followed by the submerged macrophytes and macroalga component that had a similar percentage contribution. The river, denitrification and precipitation components only made up about 3% of the TN contribution to the budget (Figure 5.7 A).

The TP percentage contribution from "other sources" changed from 43% in the first month to 13% and 50% in months two and three, respectively (Figure 5.7 B). The benthos contributed 12% in the first month and 50% and 27% in months two and three. The submerged

macrophytes and macroalga ranged from 20 to 40% in the TP budget, while the river and precipitation accounted for less than 3%. Particulate material ranged from 3 to 15% during the closed phase (Figure 5.7 B).

The river and “other sources” percentage TN contribution increased substantially (Figure 5.7 A) after the mouth had been flushed open naturally (45 and 50%). Similarly the TP contribution of these inputs also increased to about 40% (Figure 5.7 B). The associated increases were due to increased inflow from the catchment following heavy rain.

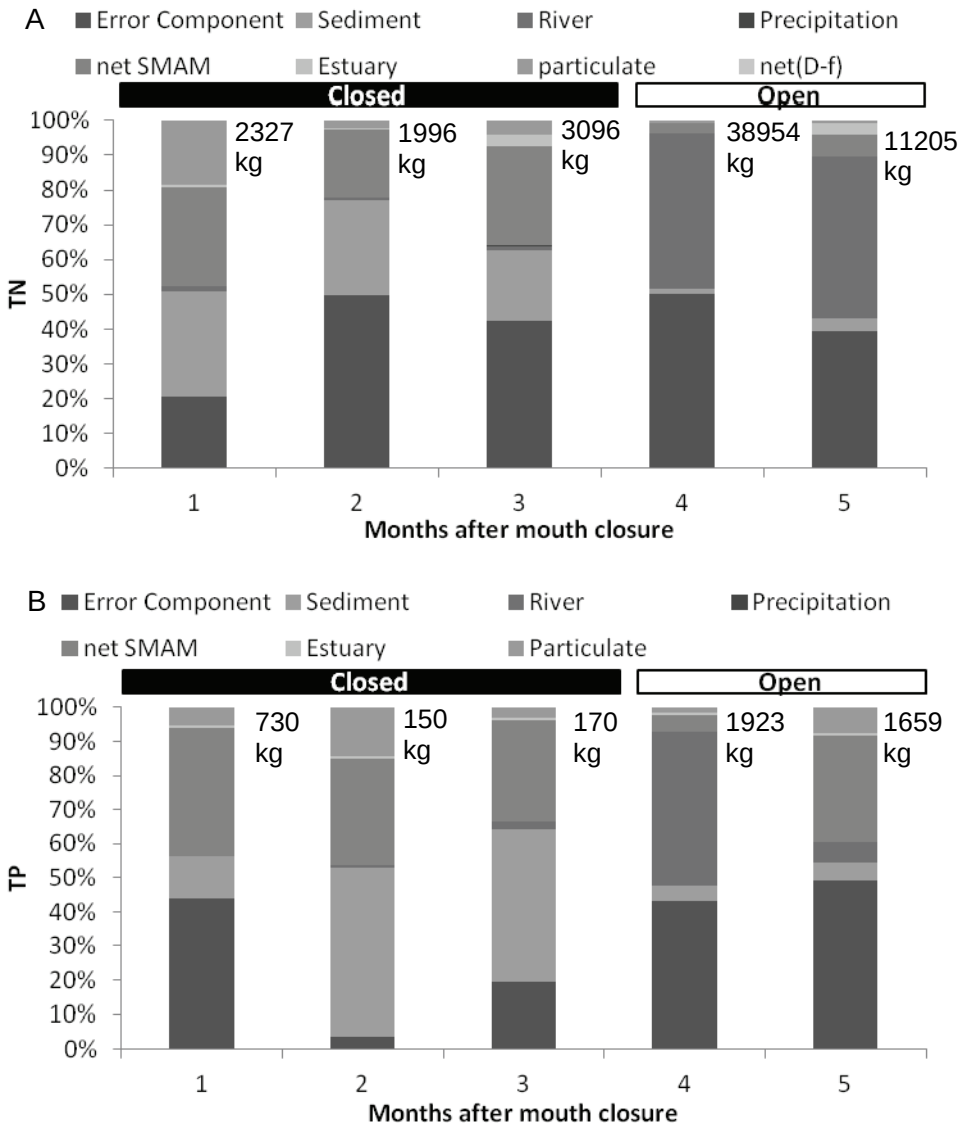

Figure 5.7 Total contribution of (A) TN and (B) TP of each component to the Great Brak Estuary (■ = closed mouth; □ = open mouth). The acronym net SMAM stands for the net mass of submerged macrophytes and macroalga.

Mouth closure for one year

Assuming that the estuary behaves similarly over the period of one year as it did over the three months following mouth closure, calculations can be made to predict what could happen if the mouth were to remain closed for a period of one year. It is not uncommon for the Great Brak Estuary to remain closed for prolonged periods. Recently the estuary remained closed for months (7th July 2009 - 1st February 2011) due to a severe drought in the region. This implies that there is a linear increase from month to month for inputs and outputs and this may very well not be the case during a year closure period. The submerged macrophytes and macroalgae component was estimated on real area cover data collected for a year (April 2010 to April 2011). This information is shown in Appendix A (Tables 13 to 18).

After a year of closure there was a standing stock of 31351 kg TP within the submerged macrophytes and macroalga (Table 5.6 and Table 5.7). From this, 5348 kg TP (of which 950 kg was *C. glomerata,* 1166 kg was *Z. capensis* and 2080 kg was *R. cirrhosa*) was released to the estuary, and 4196 kg TP (of which 1151 kg was *C. glomerata,* 1761 kg was *Z. capensis* and 2436 kg was *R. cirrhosa*) was taken up during the year. This resulted in a net gain of 1151 kg TP to the system. Inputs to the system consisted of the river (27 kg TP), the benthos (975 kg TP), precipitation (2 kg TP), particulate material (202 kg TN) and “other sources” (52 kg TP) (Table 5.6 and Table 5.7).

The TN trapped within the submerged macrophytes and macroalga was 61992 kg TN (Table 5.6 and Table 5.7). During the course of the year 12871 kg TN was taken up by the submerged macrophytes and macroalga (of which 3863 kg was *C. glomerata,* 6180 kg was *Z. capensis* and 2828 kg was *R. cirrhosa*) and 10739 kg TN was released to the estuary (of which 2861 kg was *C. glomerata,* 5352 kg was *Z. capensis* and 2527 kg was *R. cirrhosa*). This resulted in net uptake into the submerged macrophytes and macroalga of 2132 kg TN. Loads to the system included the river, benthos, precipitation, particulate material and “other sources” that contributed 301, 6614, 54, 87 and 4928 kg TN respectively (Table 5.6 and Table 5.7). The system lost 33 kg TN to the atmosphere through denitrification.

Table 5.6 Estimated load (kg) of total nitrogen and phosphorus in each component after a year of mouth closure.

	Total nitrogen	**Total phosphorus**
Months closed	12	12
Input (kg)		
River	301.23	26.61
Release from benthos	6614.09	975.31
Precipitation	53.56	2.36
Particulate	87.19	202.14
Output (kg)		
Submerged macrophytes & macroalga	2132.10	1151.80
Other sources	4928.03	52.31
Net denitrification	33.15	
Δ Estuary (kg)	37.19	2.33

Table 5.7 Estimated load (kg) of total nitrogen and phosphorus in each component of the submerged macrophytes and macroalga after a year of mouth closure.

	Cladophora	***Zostera***	***Ruppia***	**Total**
Total N Storage	17288	29389	15315	61992
TP release	2861	5352	2527	10739
TP uptake	3863	6180	2828	12871
Net output				2132
Total P Storage	4676	12033	14643	31351
TP release	950	1166	2080	4196
TP uptake	1151	1761	2436	5348
Net output				1152

5.4 Discussion

The aim of the current study was to determine the load of nitrogen and phosphorus trapped in the estuary, and to gain a better insight into the cycling of nutrients between the benthos, water column and submerged macrophytes and macroalgae within the Great Brak Estuary. The benthos constantly contributed about 30% to the TN and 40% to the TP of nutrient transferred to the water column during the closed mouth state. During the closed phase, the benthos was therefore the highest contributor of TN and TP to the system. The river and precipitation contributed less than 3% of the TN and TP. Under the closed mouth state it is unlikely that the nutrients in river water passing the head of the estuary at the weir will reach the lower end of the system and it is therefore expected that the lower reaches become benthic driven with respect to nutrient input. Pinon-Gimate *et al*. (2012) noted that 'new' nutrients arriving from external inputs were not the only sources that controlled macroalgal growth and that recycling from the benthos also played a major role. Research on the growth of *Ulva* spp. in two harbours on the southern coast of England by Trimmer *et al*. (2000 a) supported these findings. They established that inputs of external nutrients were responsible for the spring growth of the algae, but it was unlikely that these inputs caused the summer peaks in algal biomass and rapid turnover. They rather attributed the summer growth to intense recycling (ammonification) of organically bound N within the benthos, together with lower rates of denitrification. This would have then provided a sustainable and sufficient N supply for the macroalgae.

When the mouth opened under strong flow conditions the Great Brak Estuary acted as a conduit of nutrients to the sea. This is evident from the magnitude of the loads of N and P coming into and flowing out of the system. The percentage contribution of the river increased from less than 3% of the TN and TP under the closed phase to about 50% and 40% contribution respectively under the open phase. Similarly, there was an increase in the percentage contribution of the "other sources" component, which might mainly be associated with an increase in the flow from diffuse sources in the catchment. McKee *et al*. (2000) found a similar pattern in the shallow bar-built Richmond River Estuary, where most of the nitrogen (74%) and phosphorus (84%) load entered the estuary during one month when flooding occurred in the catchment. They further indicated that during the floods the estuarine basin was completely flushed of brackish water and the majority of the nutrient load passed directly through the estuary and less than 2.5% nitrogen and 5.4% phosphorus were retained in the sediments.

There have been several studies that have reported changes in community composition following nutrient enrichment (Emery *et al*. 2001, Pennings *et al*. 2002, Leston *et al*. 2008). In some systems nutrient enrichment caused phytoplankton to dominate (Taylor *et al*. 2005) while others had macroalgal dominance (Valiela *et al*. 1997, Cloern 2001). Primarily nutrient enrichment stimulates the production of planktonic microalgae and opportunistic macroalgae at the expense of seagrasses and perennial macroalgae. The latter are eventually eliminated through competition for available light and other eutrophication effects such as anoxia (Valiela *et al*. 1997, Cloern 2001, McGlathery *et al*. 2007, Valiela and Bowen 2007). Recently the lower reaches of the Great Brak Estuary have become covered with the macroalga *C. glomerata* and a competitive relationship exists between this macroalga and the submerged macrophytes *R. cirrhosa* and *Z. capensis* (Figure 3.16 B). Under the closed mouth state *C. glomerata* out-competes the two submerged macrophytes. This is thought to be due to the available nutrients and calm sheltered conditions in the estuary. Similar findings were presented by Martinez-Lüscher and Holmer (2010), Valdemarsen *et al*. (2010) and Rasmussen *et al*. (2012) where blooms out-competed the slower growing seagrasses. This occurs primarily because opportunistic macroalgae are capable of uptake, assimilation and storage of large amounts of nitrogen and phosphorus (Peckol *et al*. 1994). Higgins *et al*. (2008) suggests that fast growing *C. glomerata* mats have great capacities for nutrient and CO_2 gas exchange and that they even have the potential to significantly alter biogeochemical cycling. Furthermore, the adverse effects of eutrophication on seagrasses are enhanced in sheltered environments with frequent and high nutrient loading, reduced tidal flushing and fluctuating temperatures (Maier and Pregnall 1990).

Of the three estuary states (freshwater dominated, marine dominated and closed mouth states) occurring in TOCEs as described by Snow and Taljaard (2007), the closed phase represents a relatively stable state. The closed mouth phase is characterised by having little to no river inflow at the head of the estuary and the formation of a sandbar at the mouth that prevents tidal exchange with the estuary and sea. In small shallow estuaries like the Great Brak, the closed mouth state eventually reverts to a well-mixed (due to wind turbulence) brackish system, with no distinct salinity gradient or stratification. Flushing times depend on the duration of mouth closure and can range from days to years (Taljaard *et al*. 2009 a). This state therefore presents one of the most favourable environments for the growth of *C. glomerata.*

Throughout the closed mouth state there was a cycling of nutrients and alternate growth phases between the macroalga and the submerged macrophytes. For the duration of the

macroalgal growth phase *C. glomerata* took up and stored 1328 kg TN and 307 kg TP from the water column, acting as a sink for nitrogen and phosphorus as it expanded its area cover and out-competed the submerged macrophytes. According to Higgins *et al*. (2008) *C. glomerata* acts as a nutrient sink during its growth phase, whereby it removes large amounts of carbon, nitrogen and phosphorus from the water column. A month after acquiring its densest area cover (Figure 3.16 B) the macroalga started to decompose, losing 662 kg TN and 153 kg TP, and becoming a source of nitrogen and phosphorus to the water column. Simultaneously during this time *Z. capensis* took up 182 kg TN and 128 kg TP while *R. cirrhosa* assimilated 89 kg TN and 68 kg TP and extended their area cover within the estuary. Furthermore, the overall load of TN and TP remained relatively stable (Figure 5.6) over the closed mouth phase and ranged from 2700 to 2980 kg TP, and 4700 to 6000 kg TN. This occurred because when there was a decline in the TP or TN within the one component, there was an increase in the other. Observations by Leston *et al*. (2008) provide evidence that seagrasses would re-establish in the absence of opportunistic macroalgae. They noted that a simultaneous recovery of *Zostera noltii* Hornem. occurred in the absence of the green macroalgae (*Ulva* spp.) in the Mondego Estuary in Portugal.

From the time of mouth closure until the mouth opened again, *C. glomerata* stored 9406 kg TN and 2176 kg TP (Figure 5.4 and Figure 5.5). If the mouth of the estuary were to remain closed for the period of a year, however, the estimated storage in the algae would likely increase to 17288 kg TN and 4675 kg TP (Table 5.7). Zertuche-González *et al*. (2009) showed that a standing crop of *Ulva* spp. covering an area of 431 ha (4310000 m^2), was an important sink for C, N, and P during the upwelling months, storing up to 28000 kg TN and 1900 kg TP respectively and was a likely source of these elements when the plant biomass decayed later in the year. They state that, since *Ulva* biomass was not harvested, it was reasonable to assume that the released nitrogen and phosphorus were readily available for subsequent uptake by other primary producers, or entered the decomposers food web. Similarly, after two months of *C. glomerata* decomposition in the Great Brak Estuary it is most likely that the released nutrients became available to the other submerged macrophytes in the system leading to the observed increase in their area cover. Several authors have also found that upon decomposition, *C. glomerata* acts as source of nutrients to the water column (Paalme *et al*. 2002, Berezina and Golubkov 2008, Higgins *et al*. 2008, Lemely *et al.* in press). For example, Berezina and Golubkov (2008) recorded a significant increase, up to 11.6 µM SRP in the water column directly after *C. glomerata* senescence, while Paalme *et al*. (2002) found nitrogen release rates up to 50% over a 14 day period (both aerobically or anaerobically). Phosphorus release was dependent on dissolved oxygen and

under anaerobic conditions 50% P was lost in 7 days, whereas under aerobic conditions 40% P was lost over 30 days. Lemely *et al.* (in press) showed an earlier onset of decomposition by *C. glomerata* (three days into their experiment) compared to the submerged macrophytes *Z. capensis* and *R. cirrhosa,* within the Great Brak Estuary.

It has been well established that as oxygen becomes depleted there is an increase in the rate of remineralised nutrients from the benthos (Trimmer *et al*. 2000 b, Brandes *et al*. 2007, Pratihary *et al*. 2009, Spooner and Maher 2009). This occurs mostly during the closed phase where anoxia in the bottom water leads to remineralisation. The open phase supports a tidal and more oxygenated environment, and partially suppresses the release of remineralised nutrients from benthos. During the open phase, freshwater inflow and tidal conditions lead to well diluted nutrient concentrations in the lower reaches. In addition, the water movement seems to prevent *C. glomerata* from re-establishing. On the other hand, the *R. cirrhosa* and *Z. capensis* flourish under these conditions mainly because of the increased availability of nutrients and light in the absence of *C. glomerata's* shade effect on the submerged macrophytes (Perez *et al*. 1994).

Several studies (Pedersen and Borum 1992, Bocci *et al*. 1997, Van Katwijk *et al*. 1997) have indicated that submerged macrophytes appear to be adapted to nitrogen-poor surroundings as they are able to maintain high production rates with relatively low nitrogen availability within the water column. Submerged macrophytes are able to incorporate inorganic nitrogen such as ammonium and nitrate through both their leaves and roots, and also have a mechanism of conservation where older leaves act as nutrient sinks which afterwards are translocated to more actively growing and nutrient demanding tissues (Pedersen and Borum 1992, Lee and Dunton 1999, Touchette and Burkholder 2000, Gras *et al*. 2003, Nielsen *et al*. 2006, Lee *et al*. 2007, Alexandre *et al*. 2011). It has also been shown that the preferred inorganic nutrient for uptake in submerged macrophytes is ammonium rather than nitrate (Terrados and Williams 1997, Lee and Dunton 1999, Touchette and Burkholder 2000). Uptake via the roots has been considered the primary mechanism of ammonium uptake because of the higher concentration of ammonium in the sediment porewater compared to that of the water column (Short and McRoy 1984, Zimmerman *et al*. 1987, Touchette and Burkholder 2000, Lee *et al*. 2005, Kaldy 2006). Recent experiments done on *Z. noltii* confirm the importance of ammonium as the major inorganic nutrient for uptake; however it also showed that the leaves were the preferred site for ammonium uptake (Alexandre *et al*. 2011). Since ammonium is the major form of dissolved inorganic nitrogen (DIN) present in the estuary water column as well as in the sediment porewater, it is readily available for

uptake by *R. cirrhosa* and *Z. capensis.* Clearly the continuous availability of ammonium meets the submerged macrophytes growth requirements in the absence of *C. glomerata.*

Conclusion

Under closed mouth conditions the estuary appears to become a benthic driven system, with a major contribution of N and P from the benthos. During this time there is an accumulation of organic matter as submerged macrophytes go through their life cycles. Moreover, because *C. glomerata's* life cycle occurs faster than the other submerged macrophytes (Lemely *et al.* in press), it may acquire peak biomass or maximum area cover several times before the mouth re-opens. After each 'boom and bust cycle' there is an increase in the accumulation of organic matter within the detritus pool in the benthos of the estuary. This then fuels subsequent growth, i.e. the remineralised organic matter in the form of DIN and DIP that support the next, or current, cycle of growth for the submerged macrophytes, that is, until conditions once again become favourable for the establishment and growth of *C. glomerata*. By maintaining open mouth conditions with a steady base flow for prolonged periods, better water quality will be promoted in the estuary and blooms of *C. glomerata* will be minimised. A previous recommendation made in the Ecological Water Requirements Study conducted on the Great Brak Estuary (DWA 2009), was the clearing of alien invasive species from the catchment. This might lead to an increased flow to the dam. Another recommendation was the construction of a waste water treatment works for the town of Great Brak, which could reduce nutrient loading to the system (DWA 2009). This waste water treatment plant was constructed (Pasveer plant) and discharges treated waste via a *Phragmites australis* (Cav.) Trin. ex Steud. filled watercourse into the Great Brak Estuary. Although this plant treats sewerage there is still suspicion that raw sewerage flows into the estuary from the side channels, during high flow events in the upper reaches. However, these recommendations did not address the problematic macroalgal blooms in the lower reaches. It is therefore suggested that at least harvesting the macroalgae from the estuary would lead to better water quality, encourage submerged macrophyte recovery and eliminate the excess nutrients (~17000 kg N, 4600 kg P if the mouth remained closed for a year) from the detritus pool in the system after macroalgal decay. This would partially alleviate eutrophication in the estuary.

It is important that the relationship between the different types of macrophytes is fully understood so that informed management decisions can be made. For instance, it is known that the submerged macrophytes such as *Z. capensis* and *R. cirrhosa* are adapted to grow in oligotrophic waters. If, therefore, a successful management plan for the estuary is developed

and implemented that leads to rehabilitation of the water quality (from eutrophic to oligotrophic) and successful removal of the nuisance algae occurs, the submerged macrophytes would respond positively. Considering that aboveground tissues are more sensitive to light than belowground tissues (Perez *et al*. 1994) and their importance to nutrient absorption and photosynthesis, submerged macrophytes must suffer greatly from the presence of *C. glomerata* mats. The positive reaction of the submerged macrophytes in a rehabilitated estuary should have a positive effect that will cascade through the entire trophic system since submerged macrophytes are used by many marine species as food, nursery areas and as shelter from predators.

Taljaard *et al*. (2009 b) believed that the benthos of South African TOCEs did not have the necessary organic stock to fuel subsequent production. Their rationale was that the smaller, shallow systems (e.g. the East Kleinemonde Estuary, a small TOCE in the warm temperate region) can be effectively flushed (or re-set) of accumulated organic matter during seasonal high flows, in contrast to larger, deeper and wider estuaries that will require much higher river inflows for effective flushing, resulting in a build-up of benthic organic matter. Because this flushing mechanism has been removed through the construction of the Wolwedans Dam in the Great Brak River catchment, the benthos has become an important source of nutrients. This research has shown that the submerged macrophytes and macroalga play an important role in storing and subsequent recycling of nutrients within the Great Brak Estuary, and that under the closed mouth state, the benthos becomes the major source of nutrients to the water column and meets most of the submerged macrophytes' and macroalga growth requirements.

6 GENERAL DISCUSSION AND CONCLUSIONS

6.1 Synopsis

Temporarily Open Closed Estuaries generally have low river inflow, a long water residence time due to weak flushing, and prolonged mouth closure (Whitfield 1992, Taljaard *et al*. 2009 b) which makes this type of estuary vulnerable to nutrient enrichment and the accumulation of organic matter (Newton and Mudge 2005, Human and Adams 2011). In the closed mouth state in the Great Brak, it is unlikely that nutrients in river water passing the head of the estuary at the weir will reach the lower end of the system while the estuary remains closed. It is therefore expected that in the lower reaches the nutrients will be supplied from the benthos, i.e. the system will become benthic driven.

After prolonged mouth closure water levels rise and the large salt marshes die back and submerged macrophytes and macroalgae become the dominant plant components within the estuary. As these macrophytes go through their life-cycle, i.e. growth, reproduction and death, they act as sources and sinks for N and P within the estuary, and may be causing an accumulation of organic matter in the benthos. Moreover, the presence of the opportunistic macroalga, *C. glomerata,* which has a much shorter life-cycle than the submerged macrophytes, most likely leads to an even greater accumulation of organic input. The resultant organic load more than likely then fuels subsequent growth, i.e. the remineralised organic matter in the form of DIN and DIP which support the next, or current, cycle of growth for the submerged macrophytes. In this study in the Great Brak, from the time the mouth closes until it reopens, *C. glomerata* stored 9406 kg TN and 2176 kg TP. If the mouth of the estuary were to remain closed for a whole year however, the estimated storage in the algae could be as high 17288 kg TN and 4675 kg TP. The submerged macrophytes clearly play a significant role in removing and cycling N and P from the water column.

Apart from the cycling of nutrients between the submerged macrophytes and macroalgae, the benthos within the estuary acted as a major source of N and P to the water column. Throughout the closed mouth state the benthos contributed about 30% of the TN and 40% of the TP transferred to the water column. Some studies on ICOLLs in Australia showed that the benthos was the most important source and sink of N and P to and from the water column and could potentially contribute as much as 3-4 times the catchment discharge on a per annum basis (Smith *et al*. 2001, Palmer *et al*. 2002, Spooner and Maher 2009). During

benthic chamber deployments, the oxygen within the chambers decreased over the incubation period as a result of respiration. As conditions become anoxic, the major processes occurring in the benthos that lead to the availability of NH_4^+ in the water column are DNRA and remineralisation of labile organic matter. The benthos of the Great Brak, had a net efflux of NH_4^+, SRP, TN and TP and acted as a source of N and P to the water column. This ensured that there was always N and P available for the macrophytes. This is especially important under prolonged closed mouth conditions that cause a build-up of organic matter from decaying macrophytes, crustaceans and other faunal species. The build-up of organic matter in the Great Brak Estuary has been exacerbated by the manner in which it is not flushed since the construction of the large Wolwedans Dam further upstream.

It has been previously believed that the benthos of South African TOCEs did not have the necessary organic stock to fuel subsequent production (Taljaard *et al*. 2009 b). Two important findings arose from this work; the benthos of the Great Brak Estuary does have the necessary organic stock to fuel production and the submerged macrophytes make a significant contribution to nutrient cycling. Nutrient budgets globally, such as the LOICZ models, took the macrophytes within the estuary into account by using their C:N:P ratios and some don't account for the vegetation at all. An inherent shortcoming of this type of modelling approach is that nutrient storage in the vegetation may actually be underestimated because plants often store more N and P than what is presented in a nutrient ratio. This is the first detailed study that accounts for the nutrient storage in macrophytes by using actual measured data thereby emphasising just how important the submerged macrophytes and macroalgae are to a nutrient budget of an estuary. It also becomes clear that it is critical to rely on actual measurements of nutrient storage when dealing with nutrient budgets because the contribution of any macrophyte in an estuary or other type of water body is large and cannot simply be ignored. This research has shown that the submerged macrophytes and macroalgae play important roles in storing and subsequently re-cycling nutrients within the Great Brak Estuary. It has also shown that, in the closed mouth state, the benthos becomes the major source of nutrients to the water column and supplies a similar percentage of N and P to that which gets stored in the submerged macrophytes and macroalgae.

6.2 Implications for management

The construction of the Wolwedans Dam has had a significant impact on the functioning of the estuary. This has been a reduction in flow and the attenuation of an otherwise significant base flow that would have flushed the estuary on a regular basis. Even

though water from the dam is made available to breach the mouth, the present allocation is insufficient to flush the estuary adequately. This has led to a significant accumulation of sediment and organic matter within the lower reaches of the estuary (DWA 2009), which only gets flushed out to sea by “major” floods. In order to reduce the organic load in the system better flushing mechanisms or, more importantly, an increase in base flow, is needed to reduce residence times of water in the estuary. This would also eliminate the accumulation of inorganic nutrients from the decomposing organic matter in the system. It was shown that after the flood the condition of the estuary was similar to pre-dam conditions as the natural breach (3×10^6 m^3) (Figure 3.3) with a prolonged flow volume sufficiently flushed the estuary of sediment and organic matter. This supported a strongly tidal and well oxygenated environment, which presumably suppressed the release of remineralised nutrients from the benthos. This study has shown that a volume of around 2×10^6 m^3 per year is insufficient to flush and ‘reset’ the estuary and that the reserve should be increased to about 3.5×10^6 m^3 of water. This would enable some 2.77×10^6 m^3 of water to be utilised to flush the estuary with the remaining 0.73×10^6 m^3 to maintain open mouth conditions post breach. After the estuary has been flushed and open mouth conditions prevail, the flood tide introduces adequate seawater to the bottom water to as far up as 4.7 km which flushes the deeper sections of the estuary. By maintaining open mouth conditions with a steady base flow for prolonged periods (weeks), there will be better water quality in the estuary and blooms of *C. glomerata* will be minimised.

Management should focus on minimising anthropogenic impacts that possibly contribute to bloom formation. Bushaw-Newton and Sellner (1999), for example, showed that reductions in chemical and nutrient inputs that had been legislated successfully did reduce algal growth. The Pasveer waste water treatment plant was constructed for the town of Great Brak in order to reduce nutrient loading to the estuary in the upper reaches (DWA 2009). Another recommendation was the clearing of alien invasive species from the catchment (DWA 2009). This would result in increased flow to the estuary. However, these strategies are seldom fully implemented because they counter the nation’s main stream of economic development. The Mossgas plant (PetroSA) for example, receives more freshwater (4.8×10^6 m^3 per year from 1990 to 1995, and increased to 5.6×10^6 m^3 per year by 2000) (Huizinga and Van Niekerk 2003) from the Wolwedans Dam and is clearly considered to be more important than the Great Brak Estuary which has received about 2×10^6 m^3 per year freshwater allocation. Because the previous recommendations did not fully address the eutrophication problem in the system, a further recommendation for harvesting the macroalga from the estuary is suggested. This would likely lead to better water quality in the estuary, by eliminating the

excess nutrients from the benthos after macroalgal decay and partially alleviates eutrophication in the estuary. Harvesting the macroalga could also be beneficial both to agriculture as a source of fertiliser and as a social upliftment programme. The poor community in and around the town of Great Brak could be compensated to harvest the macroalgae as part of an implementation programme. The programme could serve as a platform to educate people on the importance of estuaries and how to sustainably use the estuary's resources. Inevitably, it is the people who use the estuary on a regular basis, who impact the system. They use the estuary for recreation, bait collection and fishing, all of which have their associated adverse impacts. The education of the community on the important roles that an estuary plays might change the way they view the system, and by changing their views ultimately it might be possible to change their behaviour.

A secondary benefit could potentially be to commercialise the harvested algal material. A number of strategies have been implemented to use macroalgal blooms either as fuel, processed as food, dehydrated and mixed into poultry and aquaculture feed and mixed with waste to be composted (Charlier *et al*. 2007, Li *et al*. 2010, Ye *et al*. 2011). Investigation on the use of macroalgae in water purification and nutrient cycling was performed (Morand *et al*. 2006). In this process, there is a step that requires the methanisation and hydrolysis of the macroalgae, which requires a large digester that ultimately nullifies the economic return on investment (Schramm 1991, Morand *et al*. 2006). According to Ye *et al*. (2011), of all the various approaches to commercialise macroalgae, the most economically viable is the use of algae as fertiliser. In 2008 in China, a large fertiliser line which would consume 10 000 tons of fresh *Ulva prolifera* O. F. Müller per day was built. It led to a large number of products being produced and sold worldwide (Ye *et al*. 2011). The elemental composition of decomposing *Pilayella littoralis* (L.) Kjellm and *C. glomerata* was determined by Lill *et al*. (2012). They found a high concentration of nitrogen, phosphorus and potassium within the algal material and concluded that the algae would be best suited for agricultural crops like potatoes that require fertiliser rich in potassium. They also calculated that the amount of nitrogen in the harvested algae would be enough to fulfil the recommendations for N fertiliser (100 kg ha^{-1} $year^{-1}$) that should annually be applied to an area of agricultural land, and found that the other nutrients were also close to the recommended application. In the Great Brak the potential storage of N and P in *C. glomerata* is substantial, 17 000 and 4 000 kg respectively per year. For this reason it might also be used as fertiliser. Another factor to take into account is the presence of heavy metals because Lill *et al*. (2012) found nickel to be present in large quantities, while the quantity of most other heavy metals was relatively low. Therefore, before decisions can be made on the use of macroalgae from the Great Brak

Estuary as fertiliser it is recommended that heavy metal content determinations are made first.

Successful removal of the nuisance alga would also be advantageous to the submerged macrophytes in the estuary. The overgrowth of macroalga within the estuary shades submerged macrophytes from the necessary light required for photosynthesis. The conditions in the estuary would also be improved through the removal of the alga by indirectly eliminating anoxia and toxic hydrogen sulphide releases when the *C. glomerata* blooms decompose (Bolam *et al*. 2000, Nedergaad *et al*. 2002). The associated effects of algal blooms have also been known to decrease infauna species richness and biomass (Cummins *et al*. 2004, Berezina and Golubkov 2008). The positive reaction of the submerge macrophytes in a rehabilitated estuary should have a positive effect that will cascade through the entire trophic system, since submerged macrophytes are used by many marine species as food, nursery areas and as shelter from predators.

The Great Brak is one of the first examples of an estuary that may function as result of freshwater releases in conjunction with mouth manipulation that mimic natural breaching conditions. This research serves to indicate the direction in which the small microtidal estuaries of South Africa and the world are heading. As the population increases and their associated activities which require freshwater, less water becomes available to estuaries and TOCEs will remain closed for longer (years). It is imperative that the allocated water requirement to estuaries is well researched and documented and encompasses all facets of biology so that adequate management actions can be made in order to maintain the health of the entire estuary ecosystem.

6.3 Limitations to the study

Attempts to measure the diffuse sources at various sites in the Great Brak (see Appendix B) proved to be logistically impractical. It was not possible to collect samples at all 9 sites throughout the duration of a freshwater pulse into the estuary. A minimum of three samples at each of the 9 diffuse sources would have been required during each freshwater pulse:

- At the beginning of a freshwater pulse in order to capture the largest concentration of nutrient entering the estuary,
- At the midpoint of the pulse,
- At the end of a pulse, to capture the end point concentration.

Groundwater was collected from individual piezometers placed at different intervals along a gradient at various sites in the Great Brak. This was done in order to determine if nutrient concentrations decreased along a gradient towards the estuary mouth. The results were variable (Appendix B), and difficulties arose when trying to convert concentrations to loads, because there were no data revealing the volume of groundwater being recharged to the estuary. The decision was taken that these parameters would merge with the “other sources” component of the nutrient budget, which is a combination of other variables that could not be measured, namely contributions from other flora and fauna.

The “other sources” component is a combined unknown and includes diffuse loads of TN / TP, in the groundwater, inputs and outputs from other vegetation as well as fauna. Hence this “other sources” component was calculated by difference after derivation of all other terms in both equations 2 and 3 (i.e. the N and P budgets). This term displays all unknown variables and is not of significance to the purpose of this study, but is required in order to indicate the balance of other variables which could not be measured.

It should also be noted that the submerged macrophytes *R. cirrhosa* and *Z. capensis* are capable of assimilating N and P directly from the sediment, and this was not accounted for in the budget. The value of this uptake would decrease the contribution of both TN and TP in the “other sources” component in the nutrient budget. In the same way other plant species, like salt marshes must also have reduced the contribution of TN and TP from the “other sources” component. Fauna have their own associated inputs and outputs that would add to and subtract from the nutrient budget. These contributions did not form part of the scope of this research but need consideration and closer study if a more accurate nutrient budget is warranted.

Another limitation to the study was the calculation of nitrogen fixation and denitrification. It was decided to use a well published method, namely, stoichiometric analysis (Kalnejais *et al*. 1999, Emily *et al*. 2005, Robson *et al*. 2008). In this approach two assumptions are made: (i) changes in dissolved inorganic phosphorus (DIP) within the estuary that are not attributable to inflows or tidal exchanges, are due to biological kinetics and, (ii) biological uptake of dissolved inorganic nitrogen (DIN) adheres to the Redfield nutrient ratio. The expected change in inorganic nitrogen, ΔDIN_{exp}, in the absence of denitrification and nitrogen fixation, would therefore be equivalent to ΔDIP multiplied by the Redfield ratio (16 mol N:1 mol P). Differences between ΔDIN_{exp} and the observed change in DIN (ΔDIN_{obs}) are attributed to net denitrification (net Dn) and estimated as::

$$\text{net Dn} = \Delta DIN_{exp} - \Delta DIN_{obs} / \Delta t = 16\,(DIP) - \Delta DIN_{obs} / \Delta t$$

6.4 Future research

A decade ago Switzer (2003) stated that the majority of estuaries in South Africa did not seem to be experiencing problems associated with increased nutrient loads (e.g. eutrophication), which had affected many estuaries in the Northern Hemisphere. As a result, South African estuary research has been less concerned with understanding the processes that govern N and P cycling in these oligotrophic estuaries, than it has been with understanding problem-specific processes such as freshwater flow requirements (Adams *et al*. 2002). Switzer (2003) further warned that if South African estuaries remain unstudied with respect to the processes that govern N and P cycling, it will be possible for modern estuary science to forfeit the opportunity to obtain valuable information on how these estuaries function prior to a time when they suffer from eutrophication. Howarth *et al*. (2002) stated that the human alteration of nutrient cycles was not uniform over the earth and that the greatest nutrient cycle changes were concentrated in areas of greatest population and agricultural production. Therefore, the estuaries of South Africa may not have escaped the problems associated with increased nutrient loads in so much as they have delayed it with their moderate pace of industrialisation, limited use of fertilisers and limited population densities living on the estuarine littoral and in the catchments. Clearly, South African estuaries have reached the point where they are becoming eutrophic. It is recommended that further investigation on the biogeochemical cycling of South African estuaries be undertaken before they become hypertrophic. This would enable a baseline data source that will become extremely important to the country's water resource managers as they are faced with ongoing development and economic growth. In order to ensure sustainable development and use of an estuary, it is important to know in which state an estuary resided before it became eutrophic. This would provide resource managers and decision makers with the necessary information when decisions are to be made on sustainable use of estuary resources. The Resources Directed Measures (RDM) relies heavily on predicting the state of an estuary in the past, the present and in the future, under different flow scenarios and information on the internal nutrient cycling of estuaries would be beneficial to the RDM methods of determining the health of an estuary.

The findings on the Great Brak Estuary have shown that during the closed phase, relative to "other sources", the benthos contributed the largest source of N and P to the system. This occurred mainly because the flushing mechanism has been removed in this estuary. Similar

conditions may arise in other small estuaries in South Africa and in other parts of the world because the demand for freshwater for agriculture, industry and domestic use appears endless. Snow (2008 b) has emphasised that the greatest uncertainties in terms of the biogeochemical or water quality characteristics and processes in TOCEs, particularly those in the cool- and warm-temperate zones, are related to the levels of inorganic and organic nutrients in these systems. Further research is therefore urgently required from a variety of different TOCEs across climatic regions in order to determine how different sediment types in South African TOCEs function with regard to benthic nutrient fluxes, as well as the role of detritus, if we are to understand biogeochemical water quality changes. Because the South African coastline extends across three different climatic regions (subtropical, warm temperate and cool temperate; Figure 2.1) each having different geological composition, it would be rather important to determine how these estuaries function with regard to benthic fluxes over seasonal cycles. This information would bring further clarification on whether or not the estuaries across the climatic regions containing different sediment composition are similar or different to one another, and also provide information on whether or not the different sediment types act as sources or sinks of N and P to their specific water columns. This information is particularly important for the estuaries along the coast which have not yet been heavily impacted by anthropogenic influences, unlike the Great Brak. Not only will it provide necessary information that will aid management, but it will also provide insight into how South African estuaries behaved in their natural state. Finally in conjunction with this research, it is also recommended that research be conducted on the effect that fauna have on benthic nutrient fluxes. There has been no research in South African TOCEs that have investigated the role that fauna have to play in nutrient cycling. Further investigation on fauna, particularly macrofauna on the benthos of TOCEs need to be studied. It has been shown in other parts of the world that macrofauna enhance benthic reactivity and increase the efficiency of solute mass transfer between the water column and benthos (Kristensen and Hansen 1999, Webb and Eyre 2004). They clearly have an important role to play in influencing the release of nutrients from the benthos.

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8 APPENDICES

APPENDIX A

There is a permanent water level recorder (K2H004) located on the railway bridge in the lower estuary. Data were used to determine the estuary bathymetry. The volume of water at any given water level could then be calculated for the entire estuary (Figure 5.2).

Table A1. Water level in relation to volume for the entire estuary from 0.6 to 2 m.

Water level (m)	Volume (m^3)	Water level (m)	Volume (m^3)	Water level (m)	Volume (m^3)	Water level (m)	Volume (m^3)	Water level (m)	Volume (m^3)	Water level (m)	Volume (m^3)
0.60	168650	0.87	281822	1.14	422695	1.41	586405	1.68	766120	1.95	968350
0.61	172477	0.88	287040	1.15	427912	1.42	592710	1.69	773610	1.96	975840
0.62	176305	0.89	292257	1.16	433139	1.43	599015	1.70	781100	1.97	983330
0.63	180132	0.90	297475	1.17	438347	1.44	605320	1.71	788590	1.98	990820
0.64	183960	0.91	302692	1.18	443565	1.45	611625	1.72	796080	1.99	998310
0.65	187787	0.92	307910	1.19	448782	1.46	617930	1.73	803570	2.00	1005800
0.66	191615	0.93	313127	1.20	454000	1.47	624235	1.74	811060		
0.67	195442	0.94	318345	1.21	460305	1.48	630540	1.75	818550		
0.68	199270	0.95	323562	1.22	466610	1.49	636845	1.76	826040		
0.69	203097	0.96	328780	1.23	472915	1.50	643150	1.77	833530		
0.70	206975	0.97	333997	1.24	479220	1.51	649455	1.78	841020		
0.71	210752	0.98	339215	1.25	485525	1.52	655760	1.79	848510		
0.72	214580	0.99	344432	1.26	491830	1.53	662065	1.80	856000		
0.73	218407	1.00	349650	1.27	498135	1.54	668370	1.81	863490		
0.74	222235	1.01	354867	1.28	504440	1.55	674675	1.82	870980		
0.75	226062	1.02	360085	1.29	510745	1.56	680980	1.83	878470		
0.76	229890	1.03	365302	1.30	517050	1.57	687285	1.84	885960		
0.77	233717	1.04	370520	1.31	523355	1.58	693590	1.85	893450		
0.78	237545	1.05	375737	1.32	529660	1.59	699895	1.86	900940		
0.79	241372	1.06	380955	1.33	535965	1.60	706200	1.87	908430		
0.80	245300	1.07	386172	1.34	542270	1.61	713690	1.88	915920		
0.81	250517	1.08	391390	1.35	548575	1.62	721180	1.89	923410		
0.82	255735	1.09	396607	1.36	554880	1.63	728670	1.90	930900		
0.83	260952	1.10	401825	1.37	561185	1.64	736160	1.91	938390		
0.84	266170	1.11	407042	1.38	567490	1.65	743650	1.92	945880		
0.85	271387	1.12	412260	1.39	573795	1.66	751140	1.93	953370		
0.86	276605	1.13	417477	1.40	580100	1.67	758630	1.94	960860		

The monthly river flow was calculated by multiplying the flow ($m^3\ s^{-1}$) by 60 seconds, 60 minutes, 24 hours, and the number of days in the month was then added. These data appear in the accumulated river volume column in Table A2. The water surface area cover of 578335 m^2 was used to convert rainfall and evaporation data from mm/day to m^3, in all calculations. The accumulated monthly volumes shown in Table A2 were used to calculate the water volume of the estuary, river, precipitation and evaporation in Table A3.

Table A2. The calculated volumes for river inflow, precipitation and evaporation used in the water budget.

Date	River flow ($m^3 s^{-1}$)	River Volume (m^3)	Accumulated River Volume (m^3)	Rainfall (mm d^{-1})	Rainfall ($m^3 d^{-1}$)	Accumulated Volume (m^3)	Evaporation (mm d^{-1})	Evaporation ($m^3 d^{-1}$)	Accumulated Volume (m^3)
2/17/2011	0.02	2114	3073198	0.80	463	382858	4.17	2412	1520691
2/18/2011	0.02	1610	3074808	0.00	0	382858	2.92	1689	1522380
2/19/2011	0.01	1153	3075961	0.00	0	382858	2.83	1637	1524017
2/20/2011	0.01	1017	3076978	0.00	0	382858	4.67	2701	1526718
2/21/2011	0.01	960	3077938	1.20	694	383552	4.17	2412	1529129
2/22/2011	0.01	916	3078854	0.00	0	383552	3.17	1833	1530963
2/23/2011	0.01	864	3079718	0.00	0	383552	5.67	3279	1534242
2/24/2011	0.01	794	3080512	0.00	0	383552	6.67	3857	1538099
2/25/2011	0.01	738	3081250	0.00	0	383552	6.17	3568	1541668
2/26/2011	0.01	711	3081961	0.00	0	383552	4.00	2313	1543981
2/27/2011	0.01	720	3082681	0.00	0	383552	5.00	2892	1546873
2/28/2011	0.01	601	3083282	0.00	0	383552	6.00	3470	1550343
3/1/2011	0.01	599	3083881	0.00	0	383552	3.33	1926	1552268
3/2/2011	0.00	428	3084310	0.00	0	383552	6.00	3470	1555739
3/3/2011	0.01	498	3084808	0.00	0	383552	5.33	3083	1558821
3/4/2011	0.01	446	3085254	0.00	0	383552	4.67	2701	1561522
3/5/2011	0.00	315	3085569	0.20	116	383667	5.00	2892	1564414
3/6/2011	0.00	137	3085706	0.00	0	383667	5.00	2892	1567305
3/7/2011	0.00	59	3085765	1.80	1041	384708	4.33	2504	1569809
3/8/2011	0.00	362	3086127	0.00	0	384708	2.67	1544	1571354
3/9/2011	0.01	677	3086804	0.20	116	384824	4.00	2313	1573667
3/10/2011	0.01	738	3087543	0.60	347	385171	5.00	2892	1576559
3/11/2011	0.01	781	3088323	0.60	347	385518	3.67	2122	1578681
3/12/2011	0.01	787	3089110	0.00	0	385518	3.67	2122	1580804
3/13/2011	0.01	717	3089827	0.00	0	385518	4.67	2701	1583504
3/14/2011	0.01	705	3090531	0.00	0	385518	5.00	2892	1586396
3/15/2011	0.01	617	3091149	0.00	0	385518	7.00	4048	1590444
3/16/2011	0.01	508	3091656	0.00	0	385518	5.33	3083	1593527
3/17/2011	0.01	593	3092249	2.40	1388	386906	4.00	2313	1595840
3/18/2011	0.01	446	3092695	0.20	116	387022	4.00	2313	1598154
3/19/2011	0.00	302	3092997	0.00	0	387022	3.33	1926	1600079
3/20/2011	0.01	490	3093487	0.00	0	387022	3.00	1735	1601814
3/21/2011	0.00	367	3093854	0.00	0	387022	4.33	2504	1604319
3/22/2011	0.00	363	3094217	0.80	463	387484	3.00	1735	1606054

3/23/2011	0.00	324	3094541	0.00	0	387484	4.00	2313	1608367
3/24/2011	0.00	319	3094860	0.00	0	387484	3.67	2122	1610489
3/25/2011	0.00	314	3095174	0.00	0	387484	5.00	2892	1613381
3/26/2011	0.00	309	3095483	0.00	0	387484	6.00	3470	1616851
3/27/2011	0.00	304	3095788	0.00	0	387484	5.33	3083	1619934
3/28/2011	0.00	299	3096087	0.00	0	387484	4.33	2504	1622438
3/29/2011	0.00	295	3096382	0.20	116	387600	4.00	2313	1624751
3/30/2011	0.00	290	3096671	2.40	1388	388988	5.33	3083	1627834
3/31/2011	0.00	285	3096956	2.00	1157	390145	4.33	2504	1630338
4/1/2011	0.00	280	3097236	0.00	0	390145	1.83	1058	1631396
4/2/2011	0.00	275	3097511	0.00	0	390145	3.00	1735	1633131
4/3/2011	0.00	410	3097921	0.20	116	390260	2.00	1157	1634288
4/4/2011	0.00	346	3098267	0.20	116	390376	4.33	2504	1636792
4/5/2011	0.00	392	3098659	0.00	0	390376	5.00	2892	1639684
4/6/2011	0.01	439	3099098	0.80	463	390839	3.50	2024	1641708
4/7/2011	0.01	485	3099583	0.00	0	390839	3.67	2122	1643830
4/8/2011	0.01	743	3100326	0.00	0	390839	4.67	2701	1646531
4/9/2011	0.01	648	3100974	0.00	0	390839	3.67	2122	1648654
4/10/2011	0.01	454	3101427	0.00	0	390839	2.33	1348	1650001
4/11/2011	0.01	512	3101939	0.00	0	390839	2.67	1544	1651545
4/12/2011	0.01	545	3102484	0.00	0	390839	2.33	1348	1652893
4/13/2011	0.01	555	3103040	0.00	0	390839	2.00	1157	1654050
4/14/2011	0.01	566	3103605	0.00	0	390839	3.33	1926	1655976
4/15/2011	0.01	437	3104042	0.00	0	390839	3.33	1926	1657901
4/16/2011	0.01	547	3104589	4.80	2776	393615	3.67	2122	1660024
4/17/2011	0.01	444	3105033	0.00	0	393615	4.00	2313	1662337
4/18/2011	0.01	605	3105638	0.00	0	393615	4.00	2313	1664651
4/19/2011	0.01	543	3106180	0.00	0	393615	2.67	1544	1666195
4/20/2011	0.00	427	3106608	0.20	116	393730	2.67	1544	1667739
4/21/2011	0.00	364	3106971	0.00	0	393730	5.00	2892	1670631
4/22/2011	0.00	259	3107230	0.00	0	393730	4.67	2701	1673331
4/23/2011	0.00	288	3107518	0.00	0	393730	4.00	2313	1675645
4/24/2011	0.00	317	3107835	1.40	810	394540	4.00	2313	1677958
4/25/2011	0.00	346	3108181	0.20	116	394656	2.33	1348	1679306
4/26/2011	0.00	391	3108572	3.00	1735	396391	4.50	2603	1681908
4/27/2011	0.01	560	3109131	1.00	578	396969	5.67	3279	1685187
4/28/2011	0.01	542	3109673	0.00	0	396969	2.50	1446	1686633
4/29/2011	0.01	523	3110196	0.00	0	396969	1.00	578	1687211

4/30/2011	0.01	512	3110708	0.00	0	396969	1.67	966	1688177
5/1/2011	0.01	479	3111187	0.00	0	396969	2.00	1157	1689334
5/2/2011	0.01	611	3111798	12.20	7056	404025	2.00	1157	1690491
5/3/2011	0.01	950	3112749	0.20	116	404140	1.67	966	1691456
5/4/2011	0.01	950	3113699	0.00	0	404140	1.17	677	1692133
5/5/2011	0.01	790	3114489	2.20	1272	405413	2.17	1255	1693388
5/6/2011	0.02	1357	3115846	5.00	2892	408305	2.76	1596	1694984
5/7/2011	0.03	2806	3118652	25.00	14458	422763	2.76	1596	1696580
5/8/2011	0.04	3349	3122001	0.20	116	422879	2.76	1596	1698177
5/9/2011	0.02	1440	3123441	0.00	0	422879	2.92	1689	1699865
5/10/2011	0.01	1110	3124550	0.00	0	422879	1.67	966	1700831
5/11/2011	0.01	994	3125544	0.00	0	422879	2.33	1348	1702179
5/12/2011	0.01	930	3126474	0.00	0	422879	2.33	1348	1703526
5/13/2011	0.01	897	3127372	0.20	116	422994	2.00	1157	1704683
5/14/2011	0.01	864	3128236	0.00	0	422994	1.33	769	1705452
5/15/2011	0.01	864	3129100	0.20	116	423110	1.67	966	1706418
5/16/2011	0.01	864	3129964	7.00	4048	427158	1.33	769	1707187
5/17/2011	0.01	864	3130828	0.40	231	427390	1.33	769	1707956
5/18/2011	0.01	798	3131626	0.00	0	427390	1.83	1058	1709015
5/19/2011	0.01	798	3132424	0.20	116	427505	2.67	1544	1710559
5/20/2011	0.01	704	3133128	0.00	0	427505	2.33	1348	1711906
5/21/2011	0.01	691	3133819	0.00	0	427505	1.33	769	1712675
5/22/2011	0.01	700	3134519	0.00	0	427505	1.00	578	1713254
5/23/2011	0.01	613	3135132	0.20	116	427621	2.33	1348	1714601
5/24/2011	0.01	626	3135759	3.40	1966	429587	2.33	1348	1715949
5/25/2011	0.01	1054	3136813	9.80	5668	435255	1.33	769	1716718
5/26/2011	0.01	1273	3138086	2.00	1157	436412	1.33	769	1717487
5/27/2011	0.01	1091	3139177	0.00	0	436412	1.50	868	1718355
5/28/2011	0.01	792	3139969	0.00	0	436412	1.83	1058	1719413
5/29/2011	0.01	778	3140747	0.00	0	436412	2.67	1544	1720957
5/30/2011	0.01	662	3141409	4.40	2545	438956	3.00	1735	1722692
5/31/2011	0.01	926	3142335	0.00	0	438956	2.40	1388	1724080
6/1/2011	0.01	826	3143160	0.00	0	438956	1.50	868	1724948
6/2/2011	0.01	749	3143909	0.00	0	438956	1.50	868	1725815
6/3/2011	0.01	762	3144671	0.00	0	438956	1.65	954	1726770
6/4/2011	0.01	806	3145477	0.20	116	439072	1.65	954	1727724
6/5/2011	0.01	970	3146447	0.00	0	439072	1.55	896	1728620
6/6/2011	0.01	950	3147397	0.00	0	439072	1.17	677	1729297

6/7/2011	0.01	950	3148348	42.60	24637	463709	1.00	578	1729875
6/8/2011	31.02	2680322	5828670	72.20	41756	505465	1.25	723	1730598
6/9/2011	34.13	2949262	8777932	11.60	6709	512173	1.38	798	1731396
6/10/2011	13.82	1194202	9972133	0.20	116	512289	2.13	1232	1732628
6/11/2011	5.19	448746	10420879	0.00	0	512289	1.50	868	1733496
6/12/2011	2.86	247318	10668197	0.20	116	512405	1.00	578	1734074
6/13/2011	1.89	163042	10831239	0.00	0	512405	1.00	578	1734652
6/14/2011	1.45	125521	10956760	0.00	0	512405	1.33	769	1735421
6/15/2011	1.05	90796	11047556	1.00	578	512983	1.17	677	1736098
6/16/2011	1.00	86068	11133624	3.00	1735	514718	1.50	868	1736966
6/17/2011	0.53	45689	11179313	0.00	0	514718	2.25	1301	1738267
6/18/2011	0.38	32833	11212146	0.00	0	514718	1.83	1058	1739325
6/19/2011	0.41	35552	11247698	0.60	347	515065	2.50	1446	1740771
6/20/2011	0.22	19407	11267105	0.00	0	515065	2.50	1446	1742217
6/21/2011	0.13	11313	11278418	0.00	0	515065	1.75	1012	1743229
6/22/2011	0.23	19869	11298287	0.00	0	515065	1.83	1058	1744287
6/23/2011	1.07	92208	11390495	1.60	925	515990	1.00	578	1744866
6/24/2011	0.99	85712	11476207	2.60	1504	517494	0.50	289	1745155
6/25/2011	0.46	39436	11515644	0.40	231	517725	0.50	289	1745444
6/26/2011	0.19	16076	11531720	0.00	0	517725	0.83	480	1745924
6/27/2011	0.16	14056	11545776	0.20	116	517841	1.33	769	1746693
6/28/2011	0.34	29650	11575426	0.00	0	517841	1.17	677	1747370
6/29/2011	0.39	33347	11608772	0.20	116	517957	2.00	1157	1748526
6/30/2011	0.29	24857	11633630	3.60	2082	520039	2.83	1637	1750163
7/1/2011	1.21	104365	11737994	0.00	0	520039	2.10	1215	1751378
7/2/2011	0.73	63062	11801056	6.00	3470	523509	2.88	1666	1753043
7/3/2011	0.44	37672	11838728	8.80	5089	528598	3.00	1735	1754778
7/4/2011	0.33	28664	11867392	16.00	9253	537852	2.25	1301	1756080
7/5/2011	6.76	584427	12451819	0.20	116	537967	2.00	1157	1757236
7/6/2011	4.15	358316	12810135	0.00	0	537967	2.00	1157	1758393
7/7/2011	1.90	163996	12974131	0.00	0	537967	2.67	1544	1759937
7/8/2011	1.13	97837	13071968	0.00	0	537967	1.67	966	1760903
7/9/2011	0.76	65970	13137938	0.00	0	537967	1.00	578	1761481
7/10/2011	0.50	42900	13180838	0.00	0	537967	1.00	578	1762060
7/11/2011	0.20	17638	13198476	0.00	0	537967	1.00	578	1762638
7/12/2011	0.11	9434	13207910	0.00	0	537967	1.67	966	1763604
7/13/2011	0.10	8220	13216129	0.00	0	537967	2.33	1348	1764951
7/14/2011	0.09	7807	13223936	0.00	0	537967	1.50	868	1765819

7/15/2011	0.46	39498	13263434	0.00	0	537967	1.67	966	1766785
7/16/2011	0.15	13034	13276469	0.00	0	537967	1.33	769	1767554
7/17/2011	0.04	3056	13279525	0.00	0	537967	1.67	966	1768520

The water volume was calculated by using the following formula:

$$\text{Water volume} = V_2 - (V_2 - V_1) / (y_2 - y_0) * (y_3 - y_2)$$

Where: V_2 and V_1 are the volumes of water (m^3), and $y_{0,1,2}$ are the different water levels (m) on the specific date at the water level recorder.

Table A3. Water volume estimation based on water level, as well as inputs from the river, precipitation and evaporation.

Date	2/17/2011	3/17/2011	4/17/2011	5/17/2011	6/17/2011	7/17/2011
Row number of this date	779	807	838	868	899	929
Y0 (m)	1.05	1.12	1.07	1.21	0.90	0.89
Y1 (m)	1.05	1.12	1.07	1.22	0.91	0.90
Y2 (m)	1.06	1.13	1.08	1.22	0.91	0.90
V0 (m^3)	375737	412260	386172	460305	297475	292257
V2 (m^3)	380955	417477	391390	466610	302692	297475
Result of interpolation (m^3)	**376249**	**413211**	**387335**	**464420**	**301675**	**296634**
River (m^3)	3073198	3092249	3105033	3130828	11179313	13279525
		19051	**12784**	**25795**	**8048485**	**2100212**
Rainfall input (m^3)	382858	386906	393615	427390	514718	537967
Evaporation (m^3)	1520691	1595840	1662337	1707956	1738267	1768520
Rainfall - evaporation (m^3)	-1137834	-1208934	-1268722	-1280567	-1223549	-1230552
		-71101	**-59788**	**-11844**	**57018**	**-7004**

The change in volume within the estuary from month to month can then be calculated as well as contributions from the river, net precipitation and “other sources” and is displayed in Table A4. The “other source” was calculated by solving for the unknown. This table appears in Chapter 5, Table 5.1 Water balance for the Great Brak Estuary.

In order to convert the concentration in each component from mg l^{-1} to kg m^{-3}, a conversion factor of 0.001 was used. This factor arose from converting mg to kg (divide by 1000 000), and conversion of dm^3 (or l) to m^3 (multiply by 1000) (Table A4).

Table A4. Indicates how the different total nitrogen components were converted to kg m^{-3}.

		Total Nitrogen (mg l^{-1})				Total Nitrogen (kg m^{-3})			
	Month	Estuary	River	Rainfall	Particulate	Estuary	River	Rainfall	Particulate
Mouth closure		0.3391	1.7573	0.4111	1.50	0.00034	0.00176	0.00041	0.00150
Closed mouth	1	0.3396	1.2899	0.4111	0.52	0.00034	0.00129	0.00041	0.00052
	2	0.3402	0.8226	0.4111	0.46	0.00034	0.00082	0.00041	0.00046
	3	0.5149	1.7188	0.4111	0.65	0.00051	0.00172	0.00041	0.00065
Open mouth	4	0.6896	2.6151	0.4111	0.11	0.00069	0.00262	0.00041	0.00011
	5	1.9262	2.3493	0.4111	0.36	0.00193	0.00235	0.00041	0.00036

By multiplying the calculated volumes (Tables A2, A3) and concentrations (Table A4) of the various parameters, the loads of the different components were determined (Table A5).

Table A5. Load of total nitrogen (kg) in estuary, river and precipitation.

Mouth	Months	Date	Days	Estuary volume (m^3)	Estuary V*C (kg)	Δ Estuary Volume (kg)	River (m^3)	River V*C (kg)	Precipitation (m^3)	Precipitation V*C (kg)
Closed	Mouth closure	2/17/2011		376249	127.58					
	1	3/17/2011	28	413211	140.34	12.76	19051	29.03	4048	1.66
	2	4/17/2011	31	387335	131.77	-8.58	12784	13.50	6709	2.76
	3	5/17/2011	30	464421	239.14	107.37	25795	32.78	33775	13.88
Open	4	6/17/2011	31	301675	208.05	-31.09	8048485	17440.87	87329	35.90
	5	7/17/2011	30	296634	571.39	363.34	2100212	5213.18	23249	9.56

A limitation to the study was the calculation of nitrogen fixation and denitrification. It was decided to use a well published method, namely, stoichiometric analysis (Kalnejais *et al*. 1999, Emily *et al*. 2005, Robson *et al*. 2008). In this approach two assumptions are made: (i) changes in dissolved inorganic phosphorus (DIP) stores within the estuary that are not attributable to inflows or tidal exchanges, are due to biological kinetics and, (ii) biological uptake of dissolved inorganic nitrogen (DIN) adheres to the Redfield nutrient ratio. The expected change in inorganic nitrogen stores, ΔDINexp, in the absence of denitrification and nitrogen fixation, would therefore be equivalent to ΔDIP multiplied by the Redfield ratio (16 mol N:1 mol P). Differences between ΔDINexp and the observed change in DIN (ΔDINobs) are attributed to net denitrification (net Dn) and estimated as: net Dn = ΔDINexp – ΔDINobs /Δt = 16 (DIP) – ΔDINobs/ Δt with the resultant concentration in mg l^{-1}. The conversion from mg l^{-1} to kg m^{-3} was done in the same way as described for Table A4. By multiplying the volume (m^3) by with the concentration (kg m^{-3}), the resultant mass of N (kg) lost through denitrification in any given month is obtained.

Table A6. Displays the calculated net denitrification using the stoichiometric analysis.

Days	SRP (mg l^{-1})	DIN (mg l^{-1})	net Dn (mg l^{-1})	net Dn (kg m^{-3})	Volume in estuary (m^3)	net (Dn) V*C (kg)
28	0.022	0.122	0.002	0.000002	376249	**0.59**
31	0.022	0.122	0.008	0.000008	413211	**3.16**
30	0.024	0.122	0.009	0.000009	387335	**3.36**
31	0.019	0.183	0.004	0.000004	464421	**1.77**
30	0.016	0.183	0.002	0.000002	301675	**0.69**
31	0.049	0.243	0.018	0.000018	296634	**5.23**

The contribution from the benthos (Table A7) was calculated by multiplying the concentration released (mmol m^{-2} day^{-1}) by a conversion factor of 8.10, which includes the number of days in the month as well as the water surface area (578335 m^2). This factor also includes conversions from mmol to kg (i.e. (14/1000000) x 578335 x days). The calculation accounts for the atomic mass of nitrogen (14) and the 1000 000 accounts for the conversion to kg.

Table A7. The flux of total nitrogen from the benthos of the estuary.

Days	28	31	30	31	30	31
Total nitrogen (mmol m^{-2} d^{-1})	2.345	2.302	2.240	2.489	2.302	1.513
Total nitrogen (kg)	531.6	577.8	544.8	624.7	559.2	379.8

In order to convert the concentration in each component from mg l^{-1} to kgm^{-3}, a conversion factor of 0.001 was used. This factor arose from the division of 1000 000 (mg to kg), and the multiplication of 1 000 (l to m^3) (Table A8).

Table A8. Indicates how the different total phosphorus components were converted to kg m^{-3}.

		Total Phosphorus (mg l^{-1})				Total Phosphorus (kg m^{-3})			
	Month	Estuary	River	Rainfall	Particulate	Estuary	River	Rainfall	Particulate
Mouth closure		0.0411	0.0800	0.0052	0.23	0.0000411	0.00008	0.0000052	0.00023
Closed mouth	1	0.0473	0.0950	0.0097	0.30	0.0000473	0.000095	0.0000097	0.00030
	2	0.0535	0.1100	0.0052	0.38	0.0000535	0.00011	0.0000052	0.00038
	3	0.0483	0.1750	0.0069	0.31	0.0000483	0.000175	0.0000069	0.00031
Open mouth	4	0.0431	0.0400	0.0008	0.58	0.0000431	0.00004	0.0000008	0.00058
	5	0.0886	0.0500	0.0023	0.17	0.0000886	0.00005	0.0000023	0.00017

By multiplying the calculated volumes (Tables A2, A3) and concentrations (Table A8) of the various parameters, the loads of the different components were determined (Table A9).

Table A9. Load of total phosphorus (kg) in estuary, river and precipitation.

	Months	Date	Days	Volume Estuary (m^3)	Estuary V*C (kg)	Change in Estuary (kg)	River (m^3)	River V*C (kg)	Precipitation (m^3)	Precipitation V*C (kg)
Closed mouth	Mouth closure	2/17/2011		376249	15.46					
	1	3/17/2011	28	413211	19.53	**4.08**	19051	**1.67**	4048	**0.03**
	2	4/17/2011	31	387335	20.71	**1.18**	12784	**1.31**	6709	**0.05**
	3	5/17/2011	30	464421	22.44	**1.73**	25795	**3.68**	33775	**0.20**
Open mouth	4	6/17/2011	31	301675	13.02	**-9.42**	8048485	**865.21**	87329	**0.33**
	5	7/17/2011	30	296634	26.27	**13.25**	2100212	**94.51**	23249	**0.04**

The contribution from the benthos (Table A10) was calculated by multiplying the concentration released (mmol m^{-2} d^{-1}) by a conversion factor of 8.10, which includes the number of days in the month as well as the water surface area (578335 m^2). This factor also includes conversions from mmol to kg (i.e. (30.97/1000000)*578335*days). The calculation accounts for the atomic mass of phosphorus (30.97) and the 1000 000 accounts for the conversion to kg.

Table A10. The flux of total phosphorus from the benthos of the estuary.

Days	28	31	30	31	30	31
Total nitrogen (mmol m^{-2} day^{-1})	0.1610	0.1610	0.1371	0.1608	0.1608	0.1608
Total nitrogen (kg)	**80.74**	**89.39**	**73.69**	**76.15**	**86.40**	**89.28**

Since there was no significant difference (statistics in Chapter 3.2.2) found in the TN and TP concentration within the submerged macrophytes and macroalgae tissue for the months (February - July 2011), the average TN and TP concentration was used in order to calculate the average mass N and P per square metre (Table A11).

Table A11. The total nitrogen and phosphorus within the biomass of the submerged macrophytes and macroalgae.

	Total phosphorus			Total nitrogen		
	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average mass ($g\ m^{-2}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average mass ($g\ m^{-2}$)
C. glomerata	415.5	0.029	11.9	415.5	0.124	51.4
	409.4	0.036		409.4	0.138	
	415.6	0.022		415.6	0.124	
	421.5	0.028		421.5	0.109	
Z. capensis	1321.5	0.030	39.6	1321.5	0.043	56.5
	1707.1	0.030		1707.1	0.027	
	1048.2	0.026		1048.2	0.061	
	1209.2	0.035		1209.2	0.040	
R. cirrhosa	952.3	0.050	47.5	952.3	0.065	61.8
	920.6	0.057		920.6	0.019	
	1052.9	0.059		1052.9	0.076	
	883.4	0.033		883.4	0.100	

By using the mass per square metre (Table A11) and multiplying that with the digitised area cover data, the mass of TN and TP within the submerged macrophytes and macroalgae was calculated (Table A12).

Table A12. The mass of total nitrogen and phosphorus (kg) within the submerged macrophytes and macroalga.

Total phosphorus									
Date	*C. glomerata*			*Z. capensis*			*R. cirrhosa*		
	Area cover (m^2)	Mass (g)	Mass (kg)	Area cover (m^2)	Mass (g)	Mass (kg)	Area cover (m^2)	Mass (g)	Mass (kg)
2/1/2011	38513	458183	**458.18**	38990	1544048	**1544.05**	20840	989003	**989.00**
3/1/2011	64336	765394	**765.39**	32255	1277327	**1277.33**	14370	681965	**681.96**
4/1/2011	51469	612316	**612.32**	35480	1405060	**1405.06**	15807	750161	**750.16**
5/1/2011	28594	340175	**340.18**	39028	1545565	**1545.57**	17388	825178	**825.18**
6/1/2011	0	0	**0.00**	42931	1700122	**1700.12**	19127	907695	**907.70**
7/1/2011	0	0	**0.00**	51518	2040146	**2040.15**	22952	1089234	**1089.23**
Total nitrogen									
Date	*C. glomerata*			*Z. capensis*			*R. cirrhosa*		
	Area cover (m^2)	Mass (g)	Mass (kg)	Area cover (m^2)	Mass (g)	Mass (kg)	Area cover (m^2)	Mass (g)	Mass (kg)
2/1/2011	38513	1980483	**1980.48**	38990	2203575	**2203.57**	20840	1288681	**1288.68**
3/1/2011	64336	3308393	**3308.39**	32255	1822926	**1822.93**	14370	888607	**888.61**
4/1/2011	51469	2646714	**2646.71**	35480	2005218	**2005.22**	15807	977468	**977.47**
5/1/2011	28594	1470397	**1470.40**	39028	2205740	**2205.74**	17388	1075215	**1075.21**
6/1/2011	0	0	**0.00**	42931	2426314	**2426.31**	19127	1182736	**1182.74**
7/1/2011	0	0	**0.00**	51518	2911577	**2911.58**	22952	1419283	**1419.28**

By using the information from Table A12, the storage of TN and TP in the different submerged macrophytes were calculated and displayed in Chapter 5; Table 5.2 and Table 5.4.

All the calculated information from the different tables were then used to construct the nitrogen and phosphorus budgets for the Great Brak Estuary and are displayed in Chapter 5; Table 5.3 and Table 5.5.

Mouth closure for a year

In order to calculate the contribution of the submerged macrophytes and macroalgae to the nutrient budget for the period of 1 year, real area cover data were used from the period of April 2010 to April 2011. The average tissue concentration of and biomass for any given season was used to calculate the specific mass of N and P in the submerged macrophytes.

Table A13. The average biomass and tissue nutrient of the submerged macrophytes and macroalgae.

	Summer		Autumn		Winter		Spring	
	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)
C. glomerata	415.400	0.138	415.400	0.109	415.400	0.074	415.400	0.027
Z. capensis	1103.755	0.047	1406.990	0.047	1116.695	0.217	329.740	0.031
R. cirrhosa	1804.040	0.059	1223.610	0.061	883.070	0.057	225.755	0.031
TN	Mass ($g\ m^{-2}$)		Mass ($g\ m^{-2}$)		Mass ($g\ m^{-2}$)		Mass ($g\ m^{-2}$)	
C. glomerata	57.32		45.27		30.86		11.27	
Z. capensis	52.37		66.34		242.60		10.20	
R. cirrhosa	107.06		75.01		50.07		6.93	

By using the information from Table A13, the mass of TN in the submerged macrophytes and macroalgae was calculated for each of the months throughout the year (Table A14).

Table A14. The mass of TN in the submerged macrophytes and macroalga throughout the year.

Date	*C. glomerata*			*Z. capensis*			*R. cirrhosa*		
	Area (m^2)	Mass (g)	Mass (kg)	Area (m^2)	Mass (g)	Mass (kg)	Area (m^2)	Mass (g)	Mass (kg)
Apr-10	29327.38	1327903	1327.90	22993.61	1525387	1525.39	11791.93	884481	884.48
May-10	29174.71	1320990	1320.99	22993.61	1525387	1525.39	11791.93	884481	884.48
Jun-10	23374.59	721439	721.44	21679.45	5259478	5259.48	28011.84	1402555	1402.55
Jul-10	43005.02	1327316	1327.32	22136.68	5370403	5370.40	28011.84	1402555	1402.55
Aug-10	58339.33	1800598	1800.60	20528.87	4980344	4980.34	22912.82	1147247	1147.25
Sep-10	43126.10	486381	486.38	27362.11	279243	279.24	26358.46	182682	182.68
Oct-10	17674.66	199337	199.34	37481	382511	382.51	29945.35	207542	207.54
Nov-10	11428.03	128887	128.89	36382.18	371297	371.30	22198.62	153852	153.85
Dec-10	16587.07	950857	950.86	34122.36	1787096	1787.10	21769.82	2330890	2330.89
Jan-11	29947.57	1716751	1716.75	29361.5	1537755	1537.75	21769.82	2330890	2330.89
Feb-11	36013.26	2064467	2064.47	35828.1	1876431	1876.43	19839.94	2124258	2124.26
Mar-11	64336.33	2913059	2913.06	32254.89	2139776	2139.78	14370.13	1077865	1077.86
Apr-11	51469.07	2330447	2330.45	35480.38	2353753	2353.75	15807.15	1185651	1185.65

After the mass of tissue nitrogen had been determined for each of the months, it was then possible to calculate the change of TN from month to month. The sum of the individual changes from month to month then represents what was taken up from the estuary water column throughout the year (i.e. storage exceeds loss to the water column) (Table A15). A simplified table of these changes was displayed in Chapter 5; Table 5.7.

Table A15. The contribution of TN to the nitrogen budget for the period of a year.

Total N				Change in TN			
Period	*C. glomerata*	*Z. capensis*	*R. cirrhosa*	Period	*C. glomerata*	*Z. capensis*	*R. cirrhosa*
Apr-10	1327.90	1525.39	884.48	Apr-May	6.9	0.0	0.0
May-10	1320.99	1525.39	884.48	May-Jun	599.6	-3734.1	-518.1
Jun-10	721.44	5259.48	1402.55	Jun-Jul	-605.9	-110.9	0.0
Jul-10	1327.32	5370.40	1402.55	Jul-Aug	-473.3	390.1	255.3
Aug-10	1800.60	4980.34	1147.25	Aug-Sep	1314.2	4701.1	964.6
Sep-10	486.38	279.24	182.68	Sep-Oct	287.0	-103.3	-24.9
Oct-10	199.34	382.51	207.54	Oct-Nov	70.5	11.2	53.7
Nov-10	128.89	371.30	153.85	Nov-Dec	-822.0	-1415.8	-2177.0
Dec-10	950.86	1787.10	2330.89	Dec-Jan	-765.9	249.3	0.0
Jan-11	1716.75	1537.75	2330.89	Jan-Feb	-347.7	-338.7	206.6
Feb-11	2064.47	1876.43	2124.26	Feb-Mar	-848.6	-263.3	1046.4
Mar-11	2913.06	2139.78	1077.86	Mar-Apr	582.6	-214.0	-107.8
Apr-11	2330.45	2353.75	1185.65				
Sum	17288.43	29388.86	15314.95	Sum	-1002.54	-828.37	-301.17
Sum Growth			**61992.2**	Sum Growth			**-2132.08**

The same calculations were then made for the determination of TP to the phosphorus budget.

Table A16. The average biomass and tissue nutrient (TP) of the submerged macrophytes and macroalga.

	Summer		Autumn		Winter		Spring	
	Average biomass ($g\ m^{-2}$)	Mass within 1g ($g\ g^{-1}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)	Average biomass ($g\ m^{-2}$)	Mass within 1g biomass ($g\ g^{-1}$)
C. glomerata	415	0.028	415	0.022	415	0.036	415	0.009
Z. capensis	1104	0.028	1407	0.034	1117	0.044	329	0.012
R. cirrhosa	1804	0.046	1224	0.072	883	0.064	225	0.009
TP	mass ($g\ m^{-2}$)		mass ($g\ m^{-2}$)		mass ($g\ m^{-2}$)		mass ($g\ m^{-2}$)	
C. glomerata	11.58		9.05		14.99		3.73	
Z. capensis	31.15		47.67		48.63		3.84	
R. cirrhosa	83.46		88.65		56.16		1.95	

Table A17. The mass of TN in the submerged macrophytes and macroalgae throughout the year.

	C. glomerata			*Z. capensis*			*R. cirrhosa*		
Date	Area (m^2)	Mass (g)	Mass (kg)	Area (m^2)	Mass (g)	Mass (kg)	Area (m^2)	Mass (g)	Mass (kg)
Apr-10	29327.38	265581	265.58	22993.61	1096240	1096.24	11791.93	1045361	1045.36
May-10	29174.71	264198	264.20	22993.61	1096240	1096.24	11791.93	1045361	1045.36
Jun-10	23374.59	350524	350.52	21679.45	1054317	1054.32	28011.84	1573236	1573.24
Jul-10	43005.02	644901	644.90	22136.68	1076553	1076.55	28011.84	1573236	1573.24
Aug-10	58339.33	874853	874.85	20528.87	998361	998.36	22912.82	1286859	1286.86
Sep-10	43126.10	161231	161.23	27362.11	105111	105.11	26358.46	51472	51.47
Oct-10	17674.66	66078	66.08	37481.00	143982	143.98	29945.35	58477	58.48
Nov-10	11428.03	42725	42.72	36382.18	139761	139.76	22198.62	43349	43.35
Dec-10	16587.07	192238	192.24	34122.36	1062842	1062.84	21769.82	1816995	1816.99
Jan-11	29947.57	347082	347.08	29361.50	914551	914.55	21769.82	1816995	1816.99
Feb-11	36013.26	417381	417.38	35828.10	1115972	1115.97	19839.94	1655919	1655.92
Mar-11	64336.33	582612	582.61	32254.89	1537779	1537.78	14370.13	1273920	1273.92
Apr-11	51469.07	466089	466.09	35480.38	1691557	1691.56	15807.15	1401312	1401.31

The change of TP from month to month was calculated (Table A18), and the sum of the individual changes represents what was taken up from the estuary water column throughout the year (i.e. storage exceeds loss to the water column). A simplified table of these changes was displayed in Chapter 5, Table 5.7.

Table A18. The contribution of TP to the nitrogen budget for the period of a year.

Total phosphorus				Change in TP			
Period	*C. glomerata*	*Z. capensis*	*R. cirrhosa*	Period	*C. glomerata*	*Z. capensis*	*R. cirrhosa*
Apr-10	265.58	1096.2	1045.4	Apr-May	1.4	0.0	0.0
May-10	264.20	1096.2	1045.4	May-Jun	-86.3	41.9	-527.9
Jun-10	350.52	1054.3	1573.2	Jun-Jul	-294.4	-22.2	0.0
Jul-10	644.90	1076.6	1573.2	Jul-Aug	-230.0	78.2	286.4
Aug-10	874.85	998.4	1286.9	Aug-Sep	713.6	893.3	1235.4
Sep-10	161.23	105.1	51.5	Sep-Oct	95.2	-38.9	-7.0
Oct-10	66.08	144.0	58.5	Oct-Nov	23.4	4.2	15.1
Nov-10	42.72	139.8	43.3	Nov-Dec	-149.5	-923.1	-1773.6
Dec-10	192.24	1062.8	1817.0	Dec-Jan	-154.8	148.3	0.0
Jan-11	347.08	914.6	1817.0	Jan-Feb	-70.3	-201.4	161.1
Feb-11	417.38	1116.0	1655.9	Feb-Mar	-165.2	-421.8	382.0
Mar-11	582.61	1537.8	1273.9	Mar-Apr	116.5	-153.8	-127.4
Apr-11	466.09	1691.6	1401.3				
Sum	4675.49	12033.27	14642.49	Sum	-200.51	-595.32	-355.95
Sum Growth			**31351.3**	Sum Growth			**-1151.8**

The contributions made from the river, benthos, precipitation, particulate component and denitrification are averaged loads for the closed mouth state, which were then multiplied by 12 in order to simulate 1 year of closure. The estimated load of TN and TP of the different components were displayed in Chapter 5, Table 5.6.

APPENDIX B

Diffuse Sources of Nutrients

The NH_4^+ concentration in October point source 5 (creek at caravan park) was significantly higher ($H = 16.33$, $n = 30$, $p < 0.05$) than Sites 1 (creek from golf course) and 2 (N2 stormwater drain). June 2011 had no significant differences in NH_4^+ ($p < 0.05$) between the different sites. July 2011 Site 5 was significantly higher ($H = 12.56$, $n = 15$, $p < 0.05$) in NH_4^+ than all other sites (Figure B1). Site 5 in March 2012 was significantly higher ($H = 13.17$, $n = 15$, $p < 0.05$) in NH_4^+ than Site 2. June 2011 had a significantly higher NH_4^+ than October 2010 and March 2012.

In October 2010, Site 5 had significantly higher TOxN ($H = 13.38$, $n = 27$, $p > 0.05$) than Sites 1 and 2 (Figure B1). In June 2011, Sites 1, 2 and 3 had significantly higher TOxN ($F = 12.39$, d.f. = 3, $p < 0.05$) than Site 4. In July 2011, Site 5 had significantly higher TOxN ($F = 11.29$, d.f. = 3, $p < 0.05$) than Sites 1 and 2. Site 4 was also significantly higher in TOxN than Site 2 ($p < 0.05$). March 2012 there was no significant difference between sites ($H = 10.43$, $n = 15$, $p < 0.05$). There was no significant difference in TOxN concentration between the different months ($H = 7.02$, $n = 66$, $p < 0.05$).

October 2010, Site 5 was significantly higher in SRP ($H = 22.05$, $n = 30$, $p < 0.05$) than both Sites 1 and 3 (Figure B1). June 2011, Site 3 had a significantly higher ($H = 10.46$, $n = 12$, $p < 0.05$) SRP concentration compared to Site 1. July 2011, Site 4 was significantly higher in SRP ($H = 10.53$, $n = 12$, $p < 0.05$) than Site 1. March 2012 Site 5 was significantly higher in SRP than all other sites ($F = 30.35$, d.f. = 3, $p < 0.05$). Site 4 was also significantly higher in SRP than Sites 1, 2, and 3 ($F = 30.35$, d.f. = 3, $p < 0.05$). The SRP in March 2012 was significantly higher ($H = 39.53$, $n = 69$, $p > 0.05$) than all other months. Similarly July 2011 had a significantly higher ($H = 39.53$, $n = 69$, $p > 0.05$) SRP than October 2010 and June 2011.

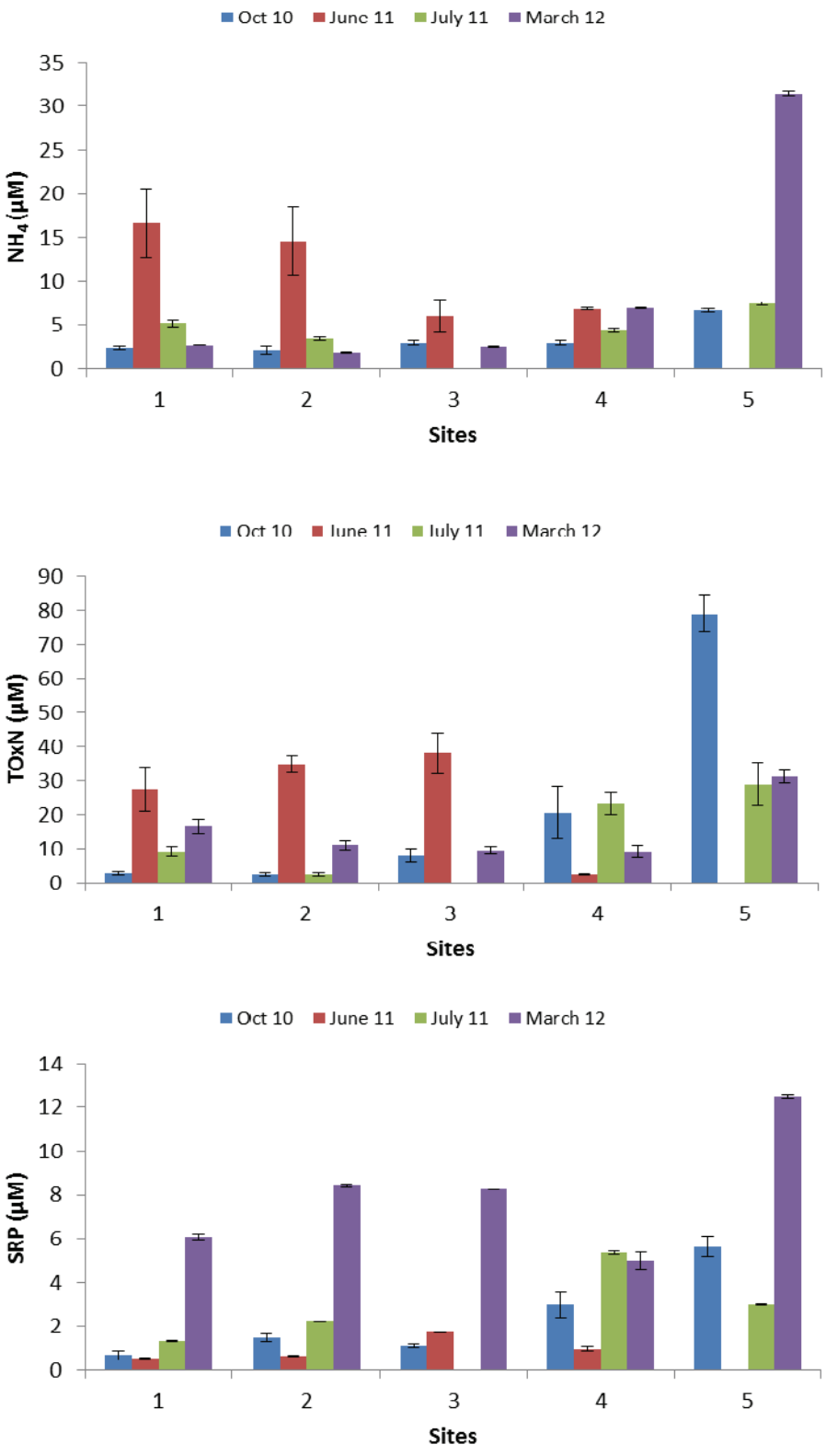

Figure B1. Inorganic diffuse source inputs around the Great Brak Estuary.

July 2011 had no significant difference between the five sites (H = 9.23, n = 15, p > 0.05) The TN at sites 2 an 5 March 2012 were significantly higher than all other sites (F = 30.35, d.f. = 4, p < 0.05). June and July 2011 had significantly higher (H = 24.48, n = 66, p < 0.05) TN concentrations than October 2010 and March 2011.

July 2011, sites 4 an 5 was significantly higher in TP than the other sites (F = 130.44, d. f. = 4, p < 0.05) The TP at sites 2 an 5 March 2012 were significantly higher than all other sites (F = 30.35, d.f. = 4, p < 0.05) (Figure B2). March 2012 was significantly higher (H = 33.80, n = 66, p < 0.05) in TP than all other months. October 2010 as well as July 2011 had significantly higher (H = 33.80, n = 66, p < 0.05) TP than June 2011 (Figure B2).

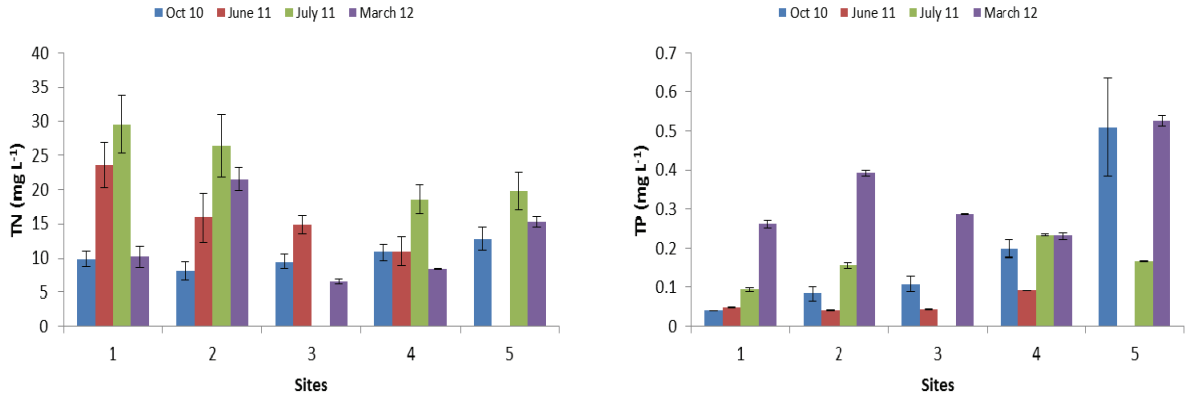

Figure B2. Total nitrogen and total phosphorus input from diffuse sources around the Great Brak Estuary.

Groundwater (piezometer nutrient concentrations)

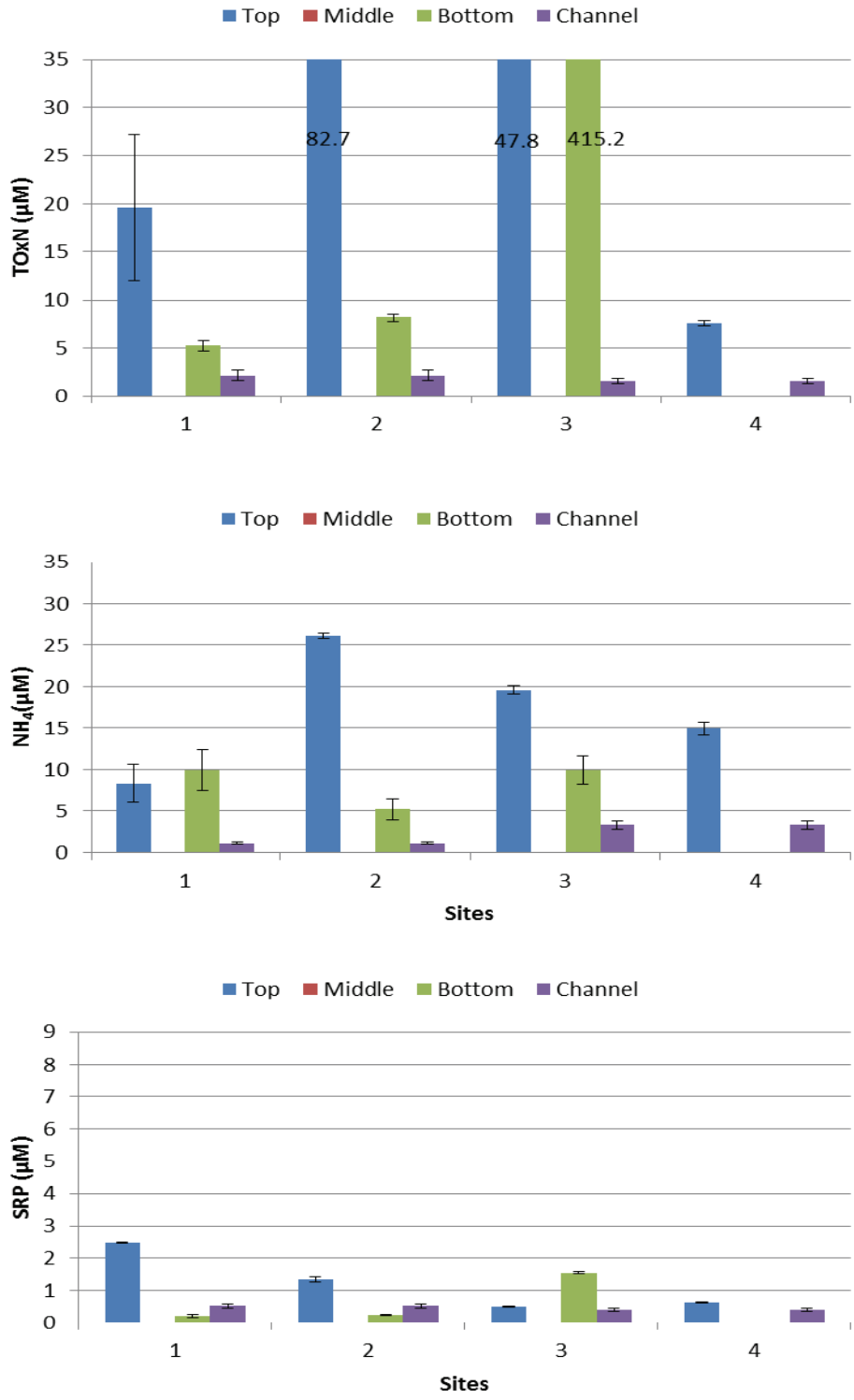

Figure B3. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary, February 2011.

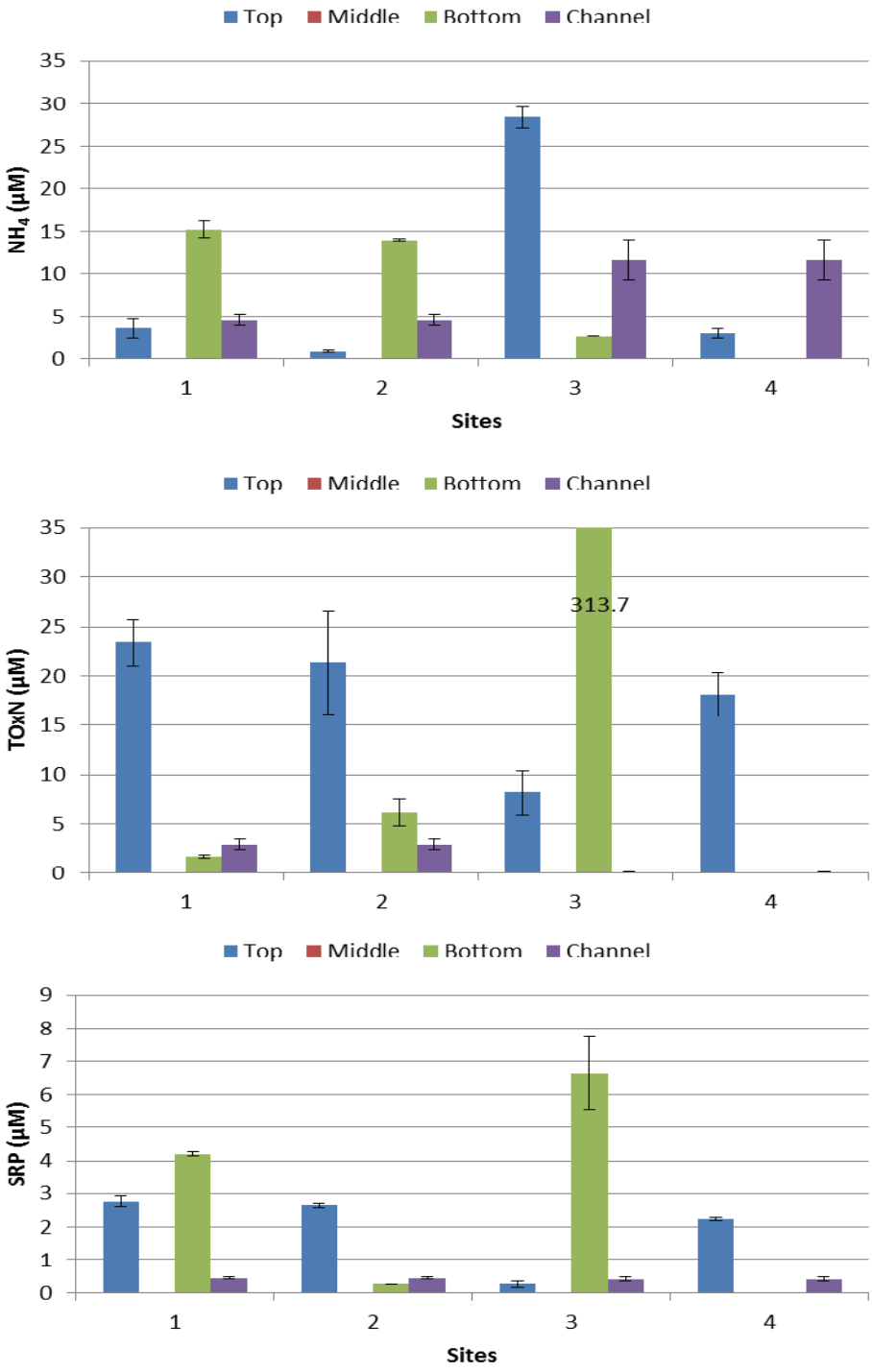

Figure B4. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary, April 2011.

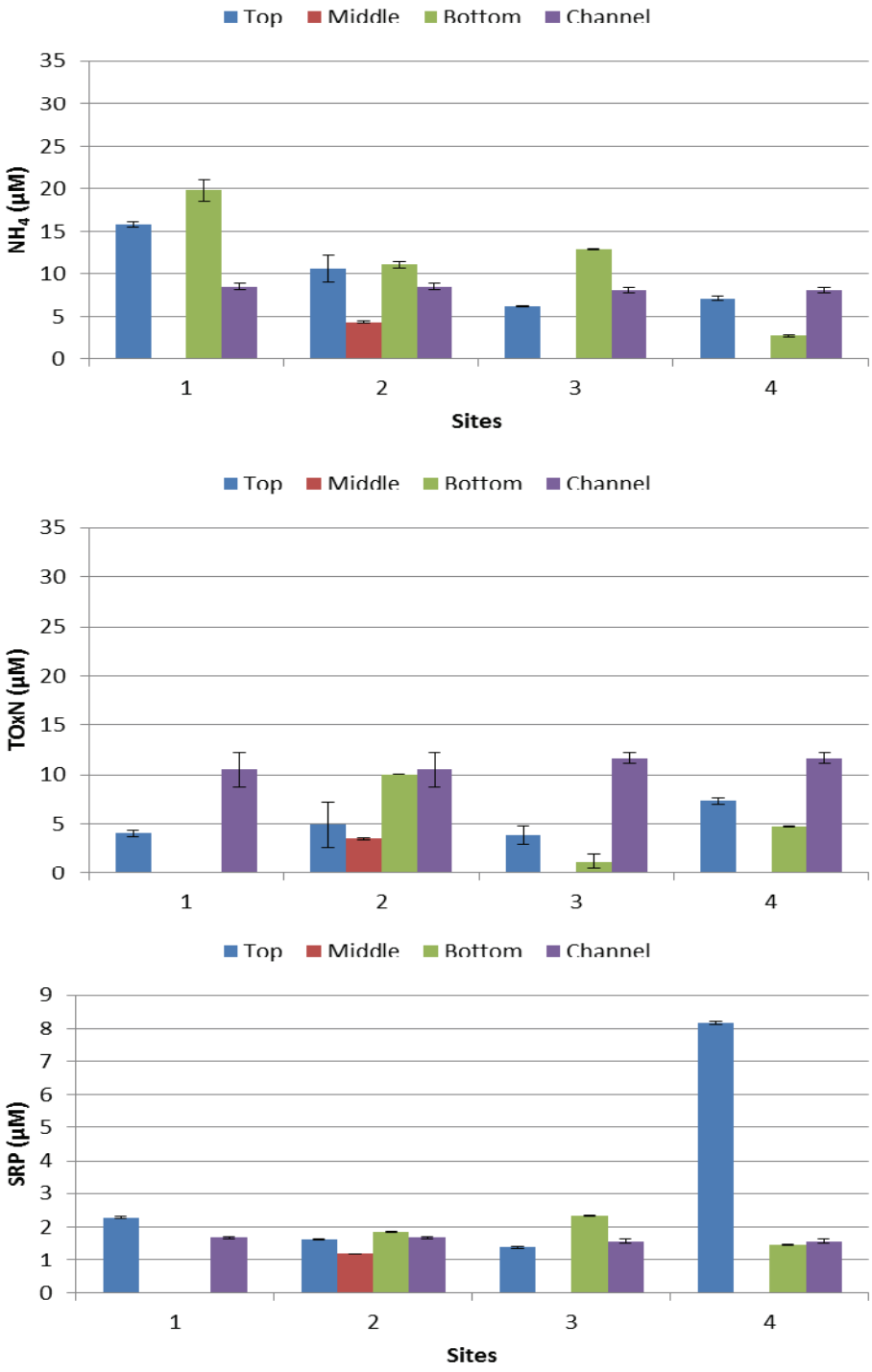

Figure B5. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary, July 2011.

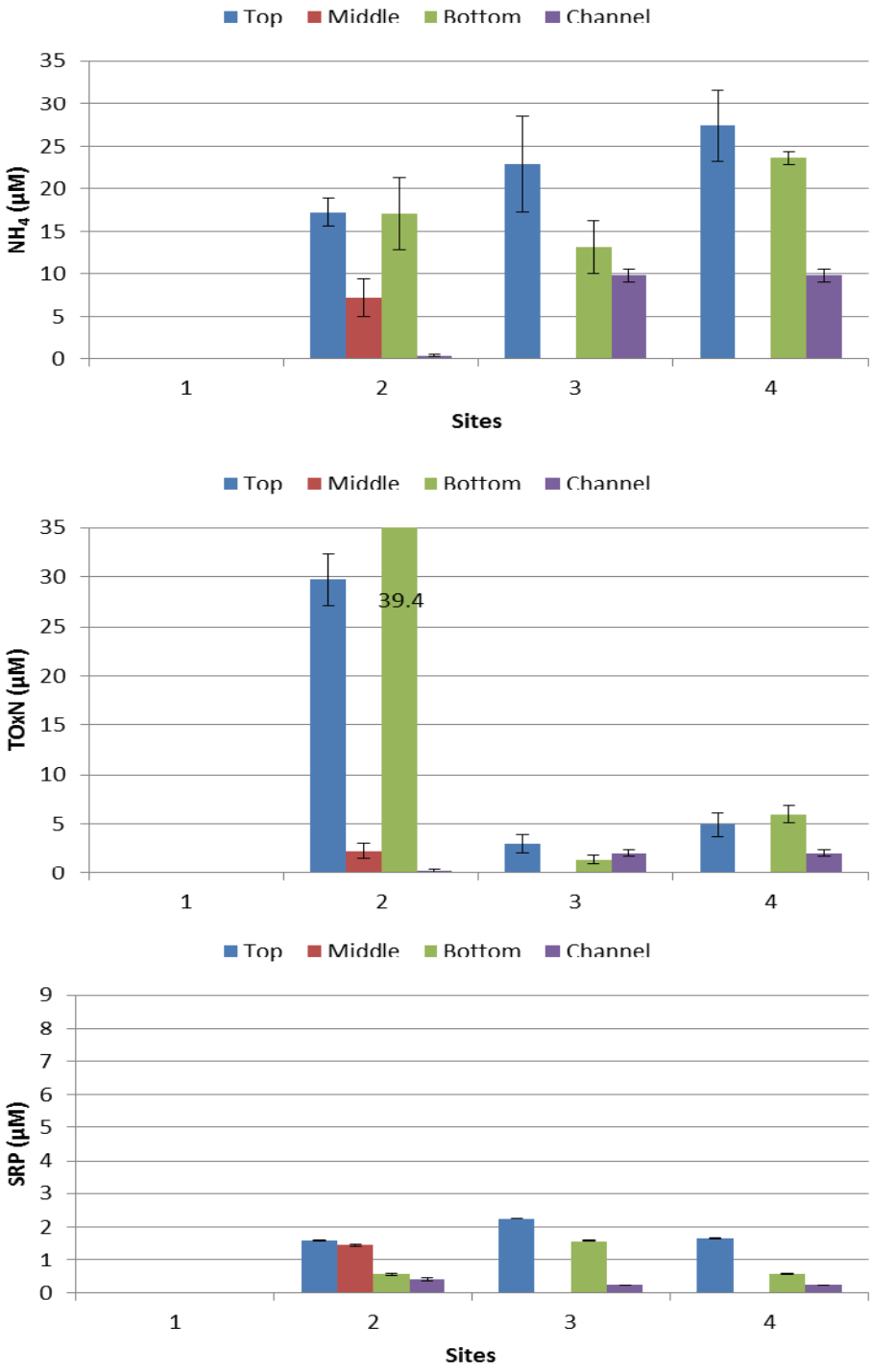

Figure B6. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary, November 2011.

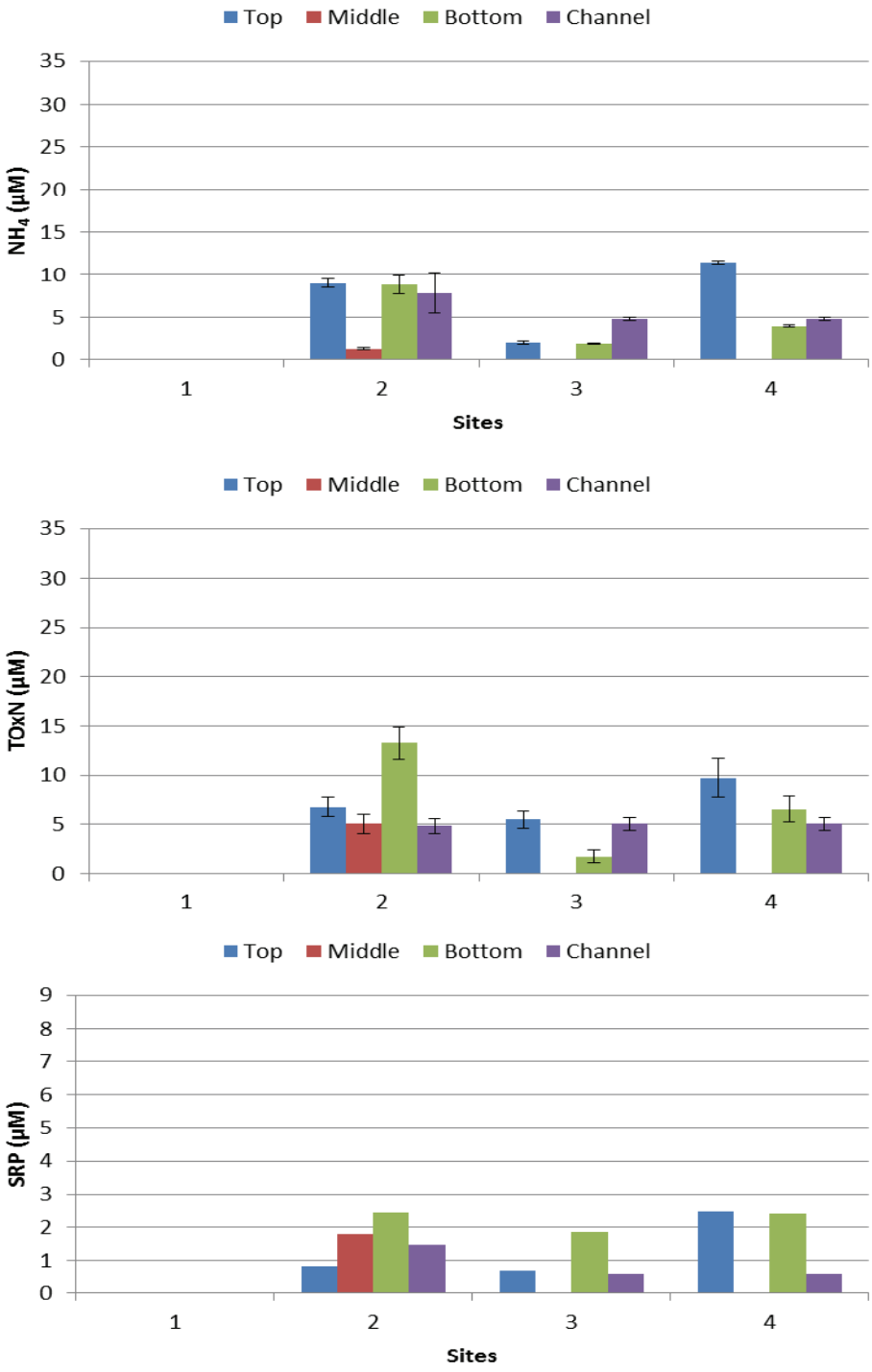

Figure B7. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary, January 2012.

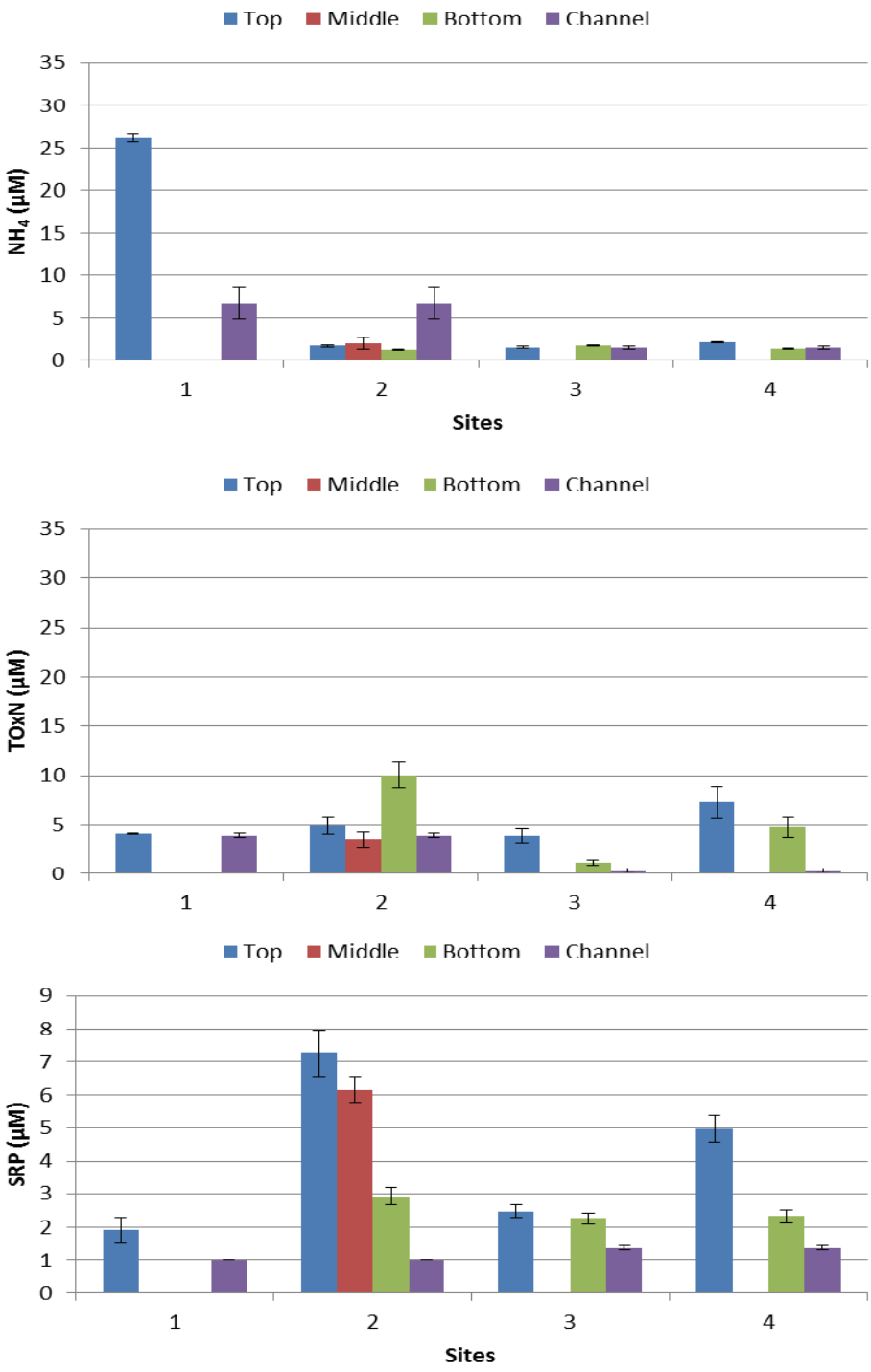

Figure B8. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary March 2012.

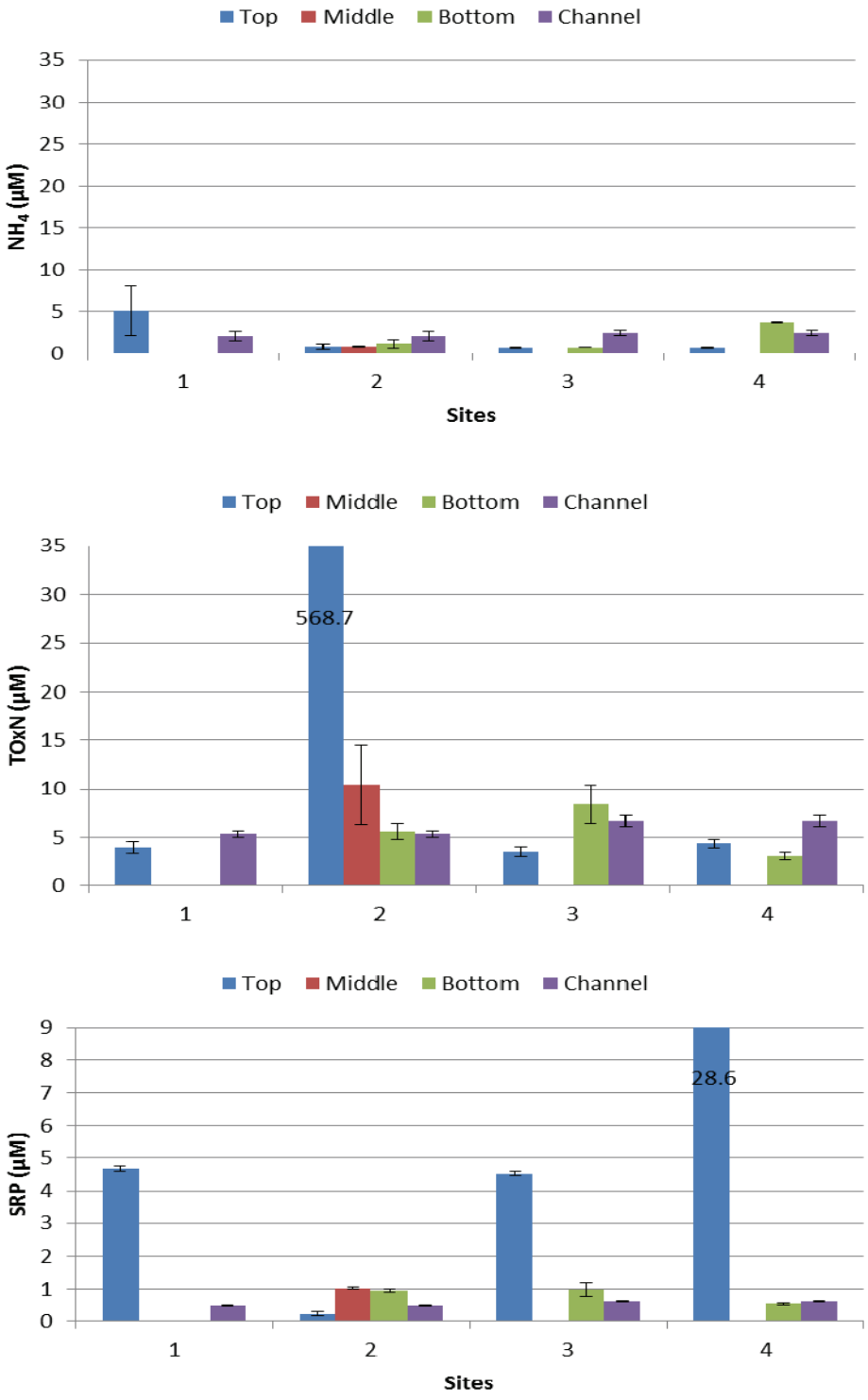

Figure B8. Inorganic nutrients in the groundwater around Die Eiland in the Great Brak Estuary, July 2011.