The role of water column and benthic communities in the spatial and temporal production and uptake of nutrients in controlling the trophic status of the Knysna River Estuary, South Africa.

by

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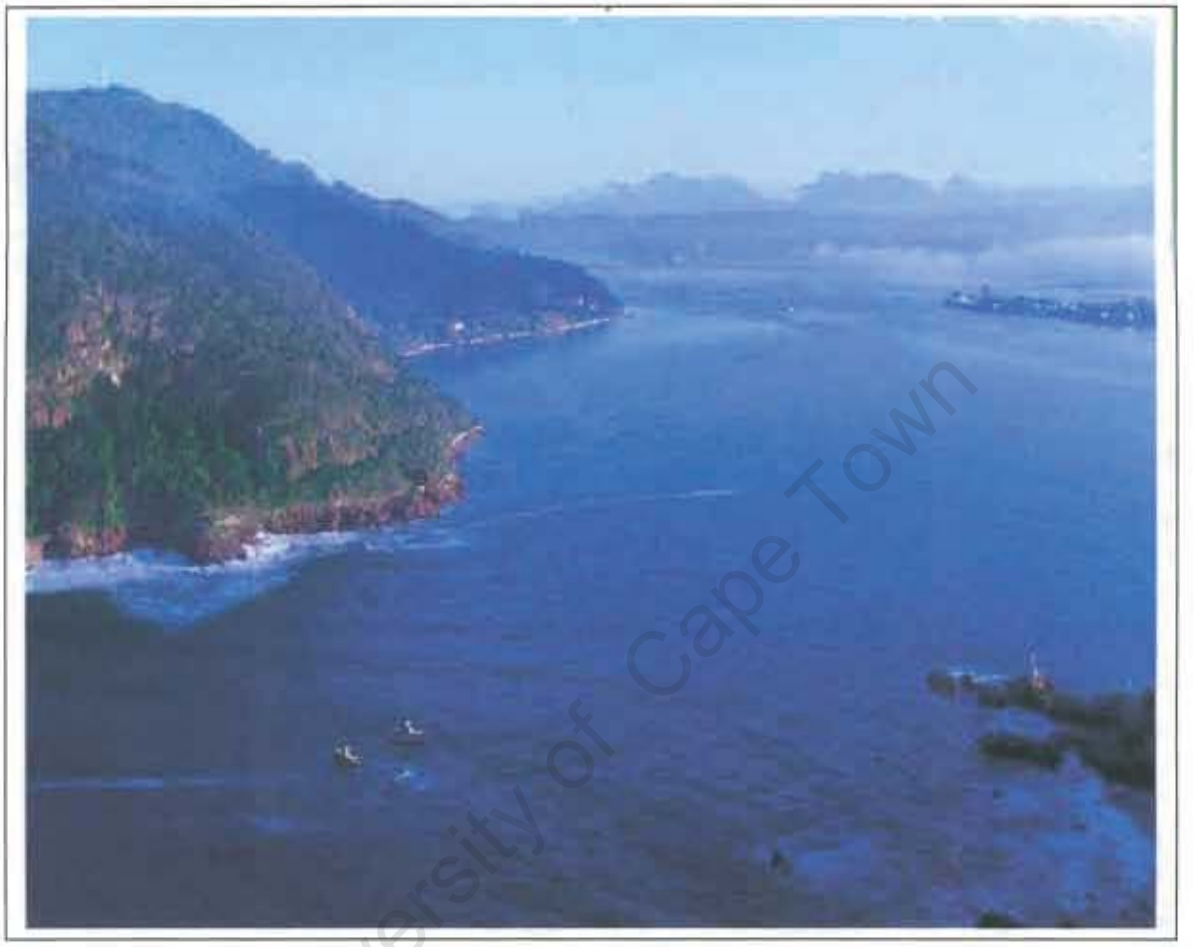

Fishing boats gather in the Knysna Heads, early morning.

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Abstract

The Knysna Estuary is the largest estuarine ecosystem in the warm temperate coastal region of South Africa. The estuary remains oligotrophic (chlorophyll-*a* < 5 mg l^{-1}) despite increases in anthropogenic input of nitrogen and phosphate associated with the rapidly increasing urbanization of its littoral and catchment.

This dissertation relies on original data, which includes the measuring of concentrations of ammonium, nitrates (nitrate and nitrite), dissolved inorganic phosphate and urea in the water column quarterly for one year at 21 stations. It estimates the contribution to these nutrients from rivers, storm drains and rainfall entering the estuary during this year by monitoring concentration and flow in these source waters. Seasonal, tidal and post-flood patterns of nutrient concentration in the water column are assessed with respect to nutrient loading to determine if variations in concentration are the result of loading or processes in the estuary.

Microbial (including phytoplankton) uptake of ammonium, nitrates and urea within the water column were determined spatially over the estuary each quarter, with 191 *in situ* ^{15}N incubation experiments. Spatial and temporal variations in water column uptake rates were compared against variation in concentration of these nutrients seasonally, spatially, and post-flood to assess the response of the uptake rates to changes in nutrient concentrations.

In situ benthic flux chambers were deployed over a 2-month period in winter and a 2-month period in summer at 7 locations to determine the seasonal benthic flux of ammonium, nitrates and dissolved inorganic phosphates between the sediments and the water column. Benthic flux experiments were spatially explicit, to determine the variation in flux in 4 distinct regions of the estuary, and during an upwelling event, which increased nitrate concentrations 10-fold. These experiments demonstrated that sediments closer to the mouth of the estuary

were more active in the production of ammonium, and consumed more nitrates (nitrate and nitrite) from the water column when compared to sediment further upstream in the estuary during every season.

These benthic flux experiments also allowed the examination of the flux of these nutrients during low oxygen conditions, simulating the conditions of closed mouth type estuaries. These experiments were conducted under positive redox conditions. The low dissolved oxygen conditions within these benthic chambers resulted in significantly higher production of ammonium when compared to similar experiments where the dissolved oxygen levels remained in the range typically found in a tidally flushed estuary such as Knysna.

It was determined that urea comprised a significant portion of the nitrogen in the water column during each quarter surveyed. There was a notable increase in the quantity of urea, which entered the Knysna estuary after a spring storm. In the days following this storm, the urea concentration in the estuarine waters increased 4-fold.

Urea contributed significantly to the nitrogen removed from the water column by microbial uptake during all quarterly surveys. However, urea accounted for more than half of the nitrogen taken up by the water column during the summer when nitrogen uptake was at a maximum.

The events and processes studied in this dissertation extended understanding of the regulation of nutrients within the estuary. It was found that a combination of events and processes, which orchestrated the nutrient status of the estuary in such a manner that it remained oligotrophic. Many of these findings can be applied to other oligotrophic estuaries, and offer an explanation for the general oligotrophic character of South African microtidal estuaries.

Introduction

The input of nitrogen (N) and phosphate (P) to any estuary, from external and internal sources, in amounts that exceed what an estuary can typically uptake and utilize will eventually result in nutrient over-enrichment problems, which include increased signs of eutrophication. Many estuaries in North America and Europe (the Northern Hemisphere) have experienced nutrient over-enrichment problems due to increased nutrient loads (Nedwell et al., 1999; Howarth et al., 2002) while the majority of estuaries in South Africa have yet to suffer from this change.

Modern South African research on microtidal estuaries has been principally concerned with the reduction of fresh water to its estuaries (*vide* Adams et al., 2002), while little account has been taken of the internal processes of these estuaries, which control the nutrient flux within them. Examples of these internal processes include the spatial and temporal variations of the benthic flux, as well as the response of the benthic sediments to changes in nutrient concentration of the overlying waters. The exception to this was Taylor (1992), that considered the flux of carbon, nitrogen and phosphate from a salt marsh and macrophyte bed to the Kariega Estuary. Outside of this study the history of South African estuary research has been lacking in research, which considered the role of internal processes and variations in nutrient concentrations in the water column that govern nutrient flux from the sediments. These internal processes received little attention, because the decline in the trophic status of South African estuaries has not paralleled those of the Northern Hemisphere.

Prior to the 1970's estuary research in South Africa and the Northern Hemisphere followed a similar course. This early research of South African estuaries was interested in descriptions of the estuaries features and inventories of the biology in each estuary (Day, 1952). The enduring influence of this

research increased understanding of the relationships between the biota and the diversity of environments found in South African estuaries.

Into the 1970's, South Africa expanded its investigations into how estuarine biota adapted to the diverse and changing physical environments found in its estuaries, and investigated the role of estuaries on fishery populations. Such findings were summarized in a review by Day (1981). In the preface of this review the author pointed out that recent interest in estuarine research increased, due to "world-wide alarm" at the effects of pollution and the decline of marine fisheries. This review revealed that estuary pollution (specifically the increase in nutrient loads in South African estuaries) was not yet a concern in South African estuarine research.

Beginning in the early 1980's, the concerns of estuary research changed rapidly in the Northern Hemisphere. Decades of fertilizer usage, and increases in phosphate inputs resulting from detergents, resulted in over-enrichment of many estuaries. These estuaries showed signs of eutrophication, and public distress at this change was the catalyst for increased research into the processes that control nutrients in these estuaries (Nixon, 1981).

Through the 1980's and the 1990's, South African estuary research was problem-oriented. The major research topics of this period included the environmental stresses that regulated macrophyte growth (e.g., salinity tolerance and distribution), sedimentation and mouth closures and hydrological studies with respect to circulation and riverine inflow into estuaries. Estuarine Health Indexes (EHI) for South African estuaries were developed during this period. However, Morant & Quinn (1999) criticized these as overly reliant upon fish populations and geomorphology, and absent of detailed assessments of water quality. They warned that the over-simplicity of these EHI could lead to a loss of information vital to the management of South African estuaries. In the Northern Hemisphere

during the 1990's, the advance made in understanding the processes that control N and P cycling in estuaries proved central in maintaining, or improving the trophic status of these estuaries (Howarth et al., 1995; Nixon et al., 1996; Nedwell et al., 1999). Much of this research was accomplished by studying high-nutrient, eutrophic estuaries, while there was a relative lack of information on low-nutrient, oligotrophic estuaries (Nedwell et al., 1999).

The study of processes that regulate N and P cycling in a South African oligotrophic estuary had two distinct benefits. First, it provided knowledge of these processes in an area of the world that remained largely unstudied. Second, it provided a baseline understanding of how South African estuarine processes functioned, prior to a period when they become impacted by increased nutrient loads.

The Knysna Estuary was chosen for this study as it is the largest tidal estuary among the 465 estuaries found along the 3000 km coastline of South Africa. It is a warm temperate estuary on the south coast, with cool temperate estuaries found on the west coast, and subtropical estuaries on the east coast of South Africa (Allanson et al., 1999). The work of Largier et al. (2000) demonstrated that Knysna Estuary had three regions, which displayed individual hydrological characteristics. These three regions included an embayment with strong tidal flushing, a lagoon region with limited tidal and river mixing, and an upper estuary region that resembled a stratified river mouth estuary. Therefore, examples of the physical environments found in many of the estuaries of South Africa could be found in the Knysna Estuary. Information from detailed spatial studies of the Knysna Estuary with regard to these hydrological divisions therefore could be applied to the many other South African estuaries to advance the understanding of how processes that controlled N and P functioned.

This thesis is presented in three parts. In part I, the physical characteristics of the Knysna Estuary, hydrology and relevant features of the catchment and adjacent ocean are described. This information came from literature on the Knysna Estuary and was augmented further by field research. This section provides the detailed background necessary for subsequent discussions on processes that affect the water chemistry of the estuary in the context of the environment of the catchment area and adjacent ocean.

Part II introduces the status of dissolved nitrogen and phosphorus nutrients in the water column. Patterns of nutrient concentration were identified from the results of a one-year water column nutrient monitoring program. This program established the variation in concentration of these nutrients in the water column across temporal and spatial scales. To assess the short-term variation of these nutrients, the impact of a spring storm on nutrient concentrations and loading from nutrient inputs were considered during the 4 days following this storm. This data set provided valuable information on the response time of the estuary when it was subjected to nutrient pulse loading following this storm.

Part III evaluates the role of the water column and benthic communities in the production and uptake of nutrients that control the trophic status of the Knysna Estuary. It considers the impact on the water column and benthic communities from changing nutrient loads due to tidal and seasonal events, processes in the catchment basin and nutrient pulse loading. This section assesses the utilization of nutrients within the Knysna Estuary from *in situ* ^{15}N incubation experiments in the water column. The benthic flux of nutrients was measured using *in situ* incubation chambers. With this information fundamental questions are answered pertaining to how the uptake rates and benthic flux of this oligotrophic estuary compared to other well-studied estuaries of various trophic status, and how these processes function to maintain the trophic status of the Knysna Estuary.

Part IV is an overall discussion of how the Knysna Estuary functions in regards to its changing nutrient loads, effects of increasing sedimentation and the increasing urbanization of its littoral. Part IV considers implications for the ecological management of the Knysna Estuary. This section also provides an opportunity to compare Knysna Estuary to estuaries outside South Africa with regard to the measured values of water column uptake and benthic flux of nutrients, which are often considered responsible for over-enrichment problems such as eutrophication.

The information contained in this dissertation allowed a range of questions to be discussed, which are essential in understanding the processes that control nutrients in oligotrophic estuaries. The most important of these questions is: Does a low-nutrient oligotrophic estuary have different water-column uptake rates and the benthic nutrient fluxes, than estuaries subjected to decades of increased nutrient loading?

Part I: The Knysna River Estuary

1.1 The Knysna Estuary, a modern description

The Knysna Estuary is located at latitude 34.1 south and longitude 23.0 east on the southern coast of South Africa. It is a drowned river valley estuary with a meandering S-shape lying in a south-east to north-west orientation. Due to the strong tidal flow through its rocky headlands, the mouth remains perennially open to the sea. Tidal intrusion into the Knysna Estuary extends upstream 18 kilometers as measured along its main channel, where it is limited by the weir on the Charlesford farm (hereafter known as the Charlesford weir).

Downstream from the Charlesford weir the Knysna Estuary is narrow and river-like in appearance with steep tree lined banks. The average depth in these first 3 kilometers is 1 m at low water spring tide (LWST), although several deeper river scour holes can be found. The tidal range of this upper estuary area is reduced by a series of rocky shelves that laterally bisect the estuary downstream of the Charlesford weir. The Old Drift is the most conspicuous example of these rocky shelves that reduce the tidal range in the upper reaches of the estuary. It is 3.4 kilometers downstream of the Charlesford weir. The Old Drift is a manmade shelf of stones used as the primary crossing for wagon and foot traffic, which crossed the Knysna Estuary prior to the construction of bridges.

Downstream of the Old Drift the Knysna Estuary retains its shallow depth of 1 m at LWST while its banks flatten into intertidal wetlands and flood plains. The main channel of the Knysna Estuary begins 600 m upstream of the road bridge of the national highway number 2 (hereafter known as the N2 bridge). The N2 bridge spans 100 m across the Knysna Estuary from its western bank. The remainder of the national highway number 2 (hereafter known as the N2) is supported

across the estuary to the east for 700 m on earthen embankment built upon intertidal mudflats.

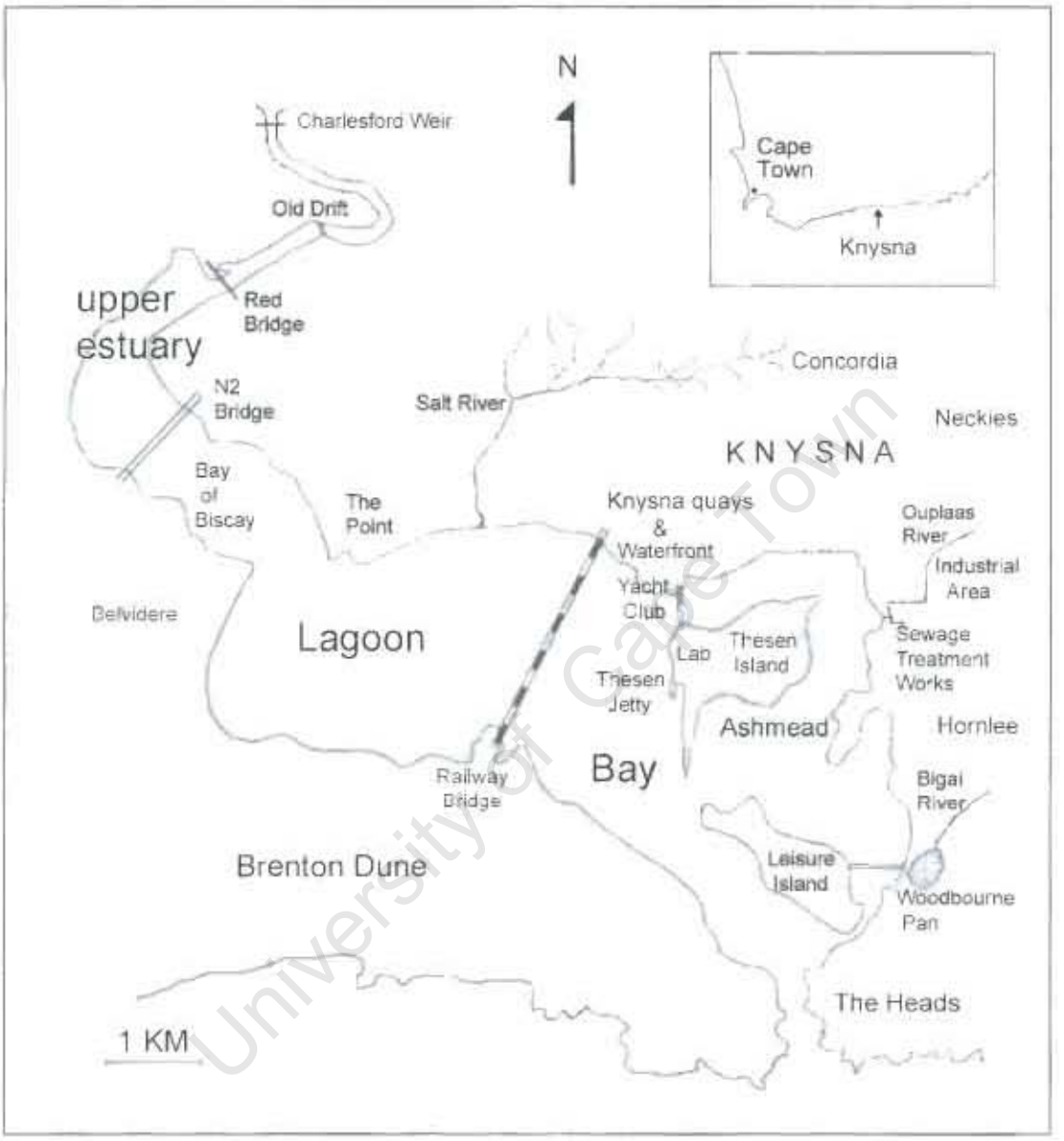


Figure 1.1. The Knysna Estuary including the location of rivers, geographic features, manmade developments and the 4 regions of the estuary (upper estuary, Lagoon, Bay and Ashmead).

The main channel is marked with navigation buoys that are maintained by the National Parks Board. While the beginning of this channel is narrow and bordered by mudflats on either side as it passes under the N2 bridge, it widens to 600 m and deepens to an average depth of 2m at LWST as it enters an area known locally as the Bay of Biscay. The village of Belvidere on the right and a point bar on the left, known as The Point, form the downstream end of the Bay of Biscay and constrict the downstream flow into narrow channels at LWST. For these next 4 kilometers, the main channel narrows to a minimum width of 50 m due to the extensive mudflats and shallows to a minimum of 1 m at LWST. The Knysna Estuary obtains its maximum width in this area, and is defined by a series of intertidal mudflats stabilized by *Zostera capensis* with shallow intervening channels (Figure 1.2).

Figure 1.2. The Knysna Estuary at LWST from the Brenton Dune, with the town of Knysna on the far shore. In this wide middle region of the Estuary, shallow channels separate numerous tidal mudflats.

The main channel widens and becomes deeper where the Salt River enters the Knysna Estuary from the left, 12 kilometers downstream of the Charlesford weir. The main channel passes under the railway bridge 1 kilometer downstream of the entry of the Salt River. The railway line crosses the Knysna Estuary from the town of Knysna on the left to a point bar of the Brenton peninsula on the right, a distance of 1900 m. Earthen embankments were constructed on the intertidal mudflats for 1350 m to support this railway line, while the remaining 550 m is spanned by three bridges. One kilometer downstream of the railway bridge the main channel and surrounding intertidal mudflats empty their waters into an embayment that is deep enough to allow the passage of vessels with a draft of 3 meters.

This natural embayment is the only one of its kind among the estuaries of South Africa (Whitfield, 1992). This embayment was used as a shipping port and proved crucial to the early economic development of Knysna (Tyson, 1971). A left branch of the main channel at the Thesen jetty serves the Knysna waterfront area (hereafter known as the Waterfront). The Waterfront consists of the Knysna Yacht club and residential and commercial developments of the town of Knysna. The Waterfront and its associated small boat harbor were constructed in the 1990's by dredging of intertidal mudflats and supratidal wetlands.

The Ashmead channel (hereafter known as Ashmead) branches to the left off the main channel between the southern end of Thesen Island and northern end of Leisure Island. This area is an extensive system of tidal flats, similar to the tidal flats around the Railway Bridge, but bordered by the islands and the town of Knysna. The Ashmead, although shallow, is navigable by shallow draft boats (<1m) for a distance of 3.2 kilometers from the main channel. Beyond this distance it shallows rapidly across mudflats terminating at the causeway to Thesen Island, which was built in 1910. This causeway was breached by 2 culverts in March 1984 to increase circulation to the upper reaches of the

Ashmead channel (Reddering, 1994). In December of 2002, a bridge was built on this causeway to further increase circulation to the Ashmead.

The final downstream flow of the Knysna Estuary runs between Leisure Island and the rocky tip of the Brenton Dune before it enters the sea. The rocky headlands at the permanently open mouth, known as the Knysna Heads or The Heads, insure tidal flushing by the largest tidal prism of any South African estuary.

1.2 Geomorphology of the Knysna Estuary

The Outeniqua Mountain range delineates the northern boundary of the southern coast of South Africa in the Knysna area. An intensive folding of the land that followed the break up of Gondwanaland formed these east-west trending mountains. The sediments of this area comprise Cretaceous Enon beds that rest on and against Table Mountain Sandstone, often along a fault contact (Tyson, 1971). The unequal erosion rates of these two types of sediments created the diverse geological structures of the Knysna area.

The Knysna river valley was eroded into Cretaceous and Tertiary sediments during the period of coastal emergence and submergence, and was assisted by runoff rates that were much higher than those of modern day. Seismic activity that resulted in down faulting may have assisted in the formation of this valley (Tyson, 1971). Exposed beds of enon conglomerate are evidence of down faulting, and can be seen on the shores of the Knysna Estuary along the northeast and southwest shoreline of the Bay of Biscay. In the Phantom Pass area, near the head of the estuary, exposed beds of enon conglomerate reach 180 m (Tyson, 1971). These features provide modern reminders of the violent geological history that formed the Knysna river valley. When sea level returned

to its modern day level, some 5000 years before present, the valley filled with alluvium resulting in the modern drown river valley of the Knysna Estuary (Tyson, 1971).

Tyson (1971) lists 4 main physiographic divisions of the land in the Knysna catchment basin: the Outeniqua Mountains, the foothill plateau, the coastal platform, and the coastal embayment. The Outeniqua Mountains rise to 1440 m (Mt. Spitskop) form the northern boundary of the Knysna catchment basin. The foothill plateau at 335 - 396 m is a narrow shelf on these mountain slopes, and is most easily recognized as the site of the Millwood gold fields in the upper reaches of the Knysna catchment basin. The coastal platform comprises the largest portion of the catchment basin and is the site of the majority of the agricultural land in the Knysna area. The coastal platform was cut during the Cainozoic, when sea level was 180 - 240 m above the present level. The coastal embayment was formed by dune building during the Quaternary as a result of glacio-eustatic changes in sea level. The Brenton dune is an example of the coastal embayment in the Knysna area. This dune reaches an elevation of 215 meters and it comprises most of the south and southwest shoreline of the Knysna Estuary.

1.3 Soils and vegetation of the catchment area

The Knysna catchment area once accommodated indigenous forest (representative of Afromontane forest of the continent) from its mountain slopes to the coast. The original Afromontane forest of the Knysna area was decimated during the 1800's due to a program of intensive logging that began in 1777 and ran mostly unabated for the next 162 years, ending in 1939. What remains of this indigenous forest is largely confined to the mountain slopes and gorges, which were considered too steep to access (Breitenbach, 1974). Once the

indigenous forests were cleared, large tracts of land were planted as forestry plantations with pines and eucalyptus. These extensive forest plantations currently occupy a third of the catchment area, 133 km^2.

During the past 20 years there has been substantial use of fertilizers in the forestry industry and on the many small cattle farms in the area. The transport of these fertilizers into the Knysna Estuary with the runoff from the catchment area directly influences the nutrient concentrations of the estuary. These processes are examined as an integral part of this study.

The soils of the southwest cape are referred to as the Cape Supergroup soils. They comprise layers of hard sandstone and softer shale, which were deposited over a period of 110 million years. The oldest group of sediments of these Cape Supergroup soils is the Table Mountain Group, which includes Table Mountain Sandstone (Cowling and Richardson, 1995).

The Soil Classification Working Group of the Department of Agricultural Development, South Africa in 1991 classified the soils of the Knysna catchment area as Tsitsikamma Form. The surface layer of this soil form, the Orthic A horizon (depth to 30 cm), is described as a surface horizon that does not qualify as an organic, humic, vertic or melanic topsoil, although it is darkened slightly by organic matter. Below this layer is the E horizon (depth 30 – 40 cm), which is paler in color as it has undergone a net removal of colloidal matter (e.g., iron oxides and organic material). This E horizon rests upon the B horizon with placic pan (depth 40 – 60 cm), which is considerably less permeable to water as it is high in clay content (Figure 1.3) (Soil Classification Working Group, 1991). The B horizon with placic pan will hereafter be referred to as the clay layer. The capacity of this soil form to promote high runoff from the catchment following heavy rainfall is examined in section 1.6.

Figure 1.3. A cross-sectional view of the Tsitsikamma soil form found in the Knysna catchment area. The clay layer found at depths greater than 40 cm limits the penetration of surface water. From, Soil Classification A Taxonomic System For South Africa (Soil Classification Working Group, 1991).

1.4 Water quality as a result of these soils

The long exposure time of soils derived from Table Mountain Sandstone results in calcium (e.g. lime) depleted sediments in the Knysna catchment area. These sediments have a low carbonate content and runoff that contains acidic colloidal organic material (i.e. humic acid) (Reddering, 1994). This humic acid stains the waters of the Knysna River a brown tea color, while maintaining a pH of

approximately 5. The introduction of Knysna River water results in lowered pH near the head of the estuary and establishes a pH gradient throughout the Knysna Estuary (Allanson et. al, 2000a). It follows that events of strong river inflow affect this pH gradient by increasing the volume of low pH water entering at the Charlesford Weir.

1.5 Weather patterns affecting the Knysna Estuary

The Knysna area lies to the east of the Cape winter rainfall region and to the west of the summer rainfall region of South Africa and frequently experiences rainfall during every month of the year. Hughes and Görgens (1981) stated that weather patterns of this area were dominated by the passage of cold fronts along the southern coast of South Africa, which originated in the South Atlantic. Additionally, the Outeniqua Mountains exert a considerable influence on the local weather, acting as a barrier to the inland penetration of shallow weather systems and produce frequent but highly localized orographic mist and rainfall.

Tyson (1971) recorded an average annual rainfall of 722 mm in Knysna over a span of 51 years, prior to 1969. This figure corresponds well with analysis of all available data dating back to 1900 on the entire catchment area, showing the 700 mm mean annual isohyet laterally bisecting the Knysna Estuary (Hughes and Görgens, 1981).

Total rainfall recorded by National Parks Board offices at Thesen Jetty were 564 mm in 2000 and 571 mm in 2001. However, these two years have different distribution patterns of rainfall. The year 2000 had a much longer dry period when 5 consecutive months passed with less than 25 mm of rainfall in each month. The year 2001 provided a much more evenly distributed rainfall, with

only 2 consecutive months passing with less than 25 mm of rainfall in each month. These rainfall records show summer as the peak rainfall period with relatively drier winter periods. This pattern is consistent with historical records of rainfall. The strongest rainfall events in each of these years occurred in early summer. In 2000, 70 mm of rain fell on December 8 and in 2001, 65.4 mm of rain fell on November 4.

The wind patterns throughout the year are affected by the positions of high and low pressure systems and passing cold fronts. Wind directions vary between southwest and southeast for the majority of summer, while passing cold fronts in the winter result in more frequent periods of west winds.

The strong southeast wind pattern that most frequently occurs in the mid to late summer has been studied in detail by Schumann (1999 & 2000). This wind pattern results in coastal upwelling events that introduce plumes of cool water into the Knysna Estuary. During summer the anticyclone ridges south of the continent set up a gradient with the interior low that drives these strong southeast winds. The southeast winds along this coast during the summer period are limited to 2 to 6 days, and result in cooler waters entering the Knysna Estuary during flood tides (Schumann, 1999 & 2000). These pulses of cooler water entering the estuary are of interest to this study as this water is higher in nitrates than the ambient water of the Knysna Estuary.

1.6 Knysna River flow and catchment runoff

This section explores the relationship between rainfall in the catchment and the resulting changes in riverine input into the Knysna Estuary throughout the year. Subsequent sections make use of this information to calculate loading of nutrients on spatial and temporal scales. The runoff from the mountainous

Knysna catchment area after periods of frequent rainfall was exacerbated by the soil composition that features a clay layer near the surface (refer to section 1.3). The rainfall patterns, the mountainous catchment area and the soil composition are some of the factors that contributed to the variable flow rates of the Knysna River.

The total catchment area of the Knysna basin is 400 km^2 as provided by GIS data from the South African Forestry Company Limited (SAFCOL) Kruisfontein plantation office, Knysna. The Knysna River upstream of the Charlesford weir drains a total of 332.3 km^2 of this larger total catchment area as measured by Reddering (1994). This river meanders 60 kilometers from its source in the Outeniqua Mountains to the Charlesford weir, where it reaches its maximum flow (Figure 1.4). However, it was only possible to estimate rather than measure this flow, as there was no gauging weir at the Charlesford weir during this study.

The river flow rate at the Charlesford weir was estimated using data from gauging weir K5H002 (years 2000 & 2001), 22 kilometers upstream of the Charlesford weir on the Knysna River. The Department of Water Affairs and Forestry maintained this flow gauge (Figure 1.5), and the records of mean daily flow rate during this period. This gauging weir recorded the flow from an area of 133 km^2 or 40% of the catchment area, which drained into the Knysna River upstream of the Charlesford weir. During 2000 and 2001, the Knysna Municipality Water Treatment Facility made daily abstractions of water from the Knysna river and its main tributary, the Gouna river, to satisfy the water requirements of the town. Estimates of flow rate of the Knysna River at the Charlesford weir were calculated in $m^3 s^{-1}$ by multiplying daily flow rates at gauging weir K5H002 by 2.5, less daily abstraction. This multiplier of 2.5 less daily abstraction was used to estimate the flow of the Knysna River where it entered the Knysna Estuary, as the river flow was not gauged at this point.

Hereafter, all calculations that require the flow rate of the Knysna River (i.e. net flow rate) will be obtained by this method.

Figure 1.4. The Knysna catchment area includes the locations of gauging weir K5H002, water abstraction points, Knysna and Salt Rivers (shown with major tributaries), Gouna River, Knysna Estuary, Charlesford weir, Millwood and the Jubilee Creek sampling site, marked with "X".

Figure 1.5. Gauging weir K5H002 on the Knysna River in the mountainous catchment area (refer to Figure 1.4 for location).

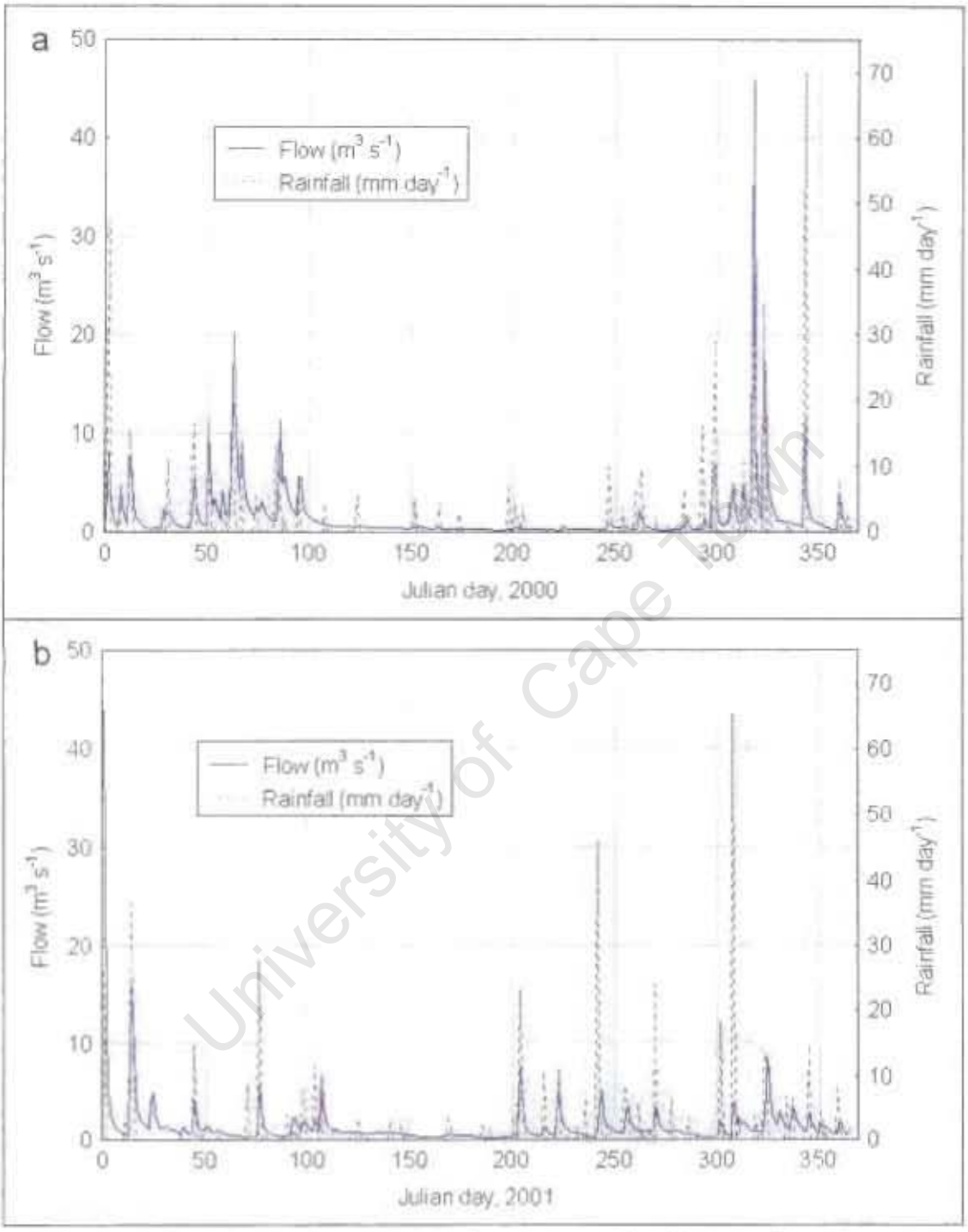
The annual runoff of the Knysna River that enters the Knysna Estuary, was 4.9 x 10^7 m^3 in 2000 and 4.2 x 10^7 m^3 in 2001. These were years when the annual precipitation was approximately 80% of the average annual precipitation of 700 mm as recorded by Hughes and Görgens (1981). These volumes compare well with the annual runoffs calculated by Haw (1984) for annual precipitation totals of 900 mm to 1050 mm that resulted in annual runoffs of 5.9 x 10^7 m^3 to 7.4 x 10^7 m^3.

Largier et al. (2000) reported the mean annual runoff of Knysna catchment was 10^8 m^3, however it is possible that this value is erroneous. The method used by Largier et al. (2000) mistakenly used the entire total catchment basin (400 km^2) as the catchment of the Knysna River. Therefore, flow rate from gauging weir K5H002 was multiplied by 3 to estimate the flow rate at the Charlesford weir. Further, their estimate makes no correction for the water abstracted by the Knysna Municipality, and does not state for which years this mean annual runoff

were based. For these reasons, *inter alia*, their estimate of mean annual runoff was not used.

The water abstracted by the Knysna Municipality was 5% of the annual runoff in 2000 and 6% in 2001. However, since water was abstracted daily, the greatest reduction of river flow into the estuary was during periods of low river flow or the dry season. The dry season is defined by an average flow less than 1 $m^3 s^{-1}$ at the Charlesford weir for 60 days or more. In 2000 the abstractions by the Knysna Municipality accounted for the removal of 17% of river flow during the dry season, with a maximum daily abstraction of 40% during this period. In 2001 these abstractions accounted for the removal of 14% of river flow during the dry season, and a maximum daily abstraction of 37% during this period. The daily flow rates were plotted with daily rainfall amounts for years 2000 (Figure 1.6a), and 2001 (Figure 1.6b).

The smaller rivers and storm drain flows that entered the Knysna Estuary lacked the benefit of gauged measurements and these flow rates were calculated manually when necessary. Manual determinations of Knysna River flow rate at the Charlesford weir were made 6 times during the years 2000 and 2001. This allowed a comparison of the manual determination method with the mean daily flow rate estimated with data from gauging weir K5H002. Manual determinations were the product of the cross sectional area of the river as it spilled over the Charlesford weir and the estimated flow velocity of that cross sectional area. Flow velocity was estimated by timing floating debris over the final meter the Knysna River flowed over the Charlesford weir. Measurements of flow velocity were repeated several times and the mean of these measurements was accepted as valid. These manually measured flow rates were compared using student's t and no significant difference ($P>0.05$) to the estimated flow rates was found (Table 1.1).



Figures 1.6a & b. The estimated daily flow rates of the Knysna River for years 2000 and 2001 as calculated from gauging weir K5H002 data, and daily rainfall as recorded at National Parks Board offices on Thesen Jetty.

Table 1.1. The flow rate of the Knysna River at the Charlesford weir measured manually and estimated from mean daily flow as recorded at gauging weir K5H002 less water abstractions ($m^3 s^{-1}$).

Date	manually	estimated *via* K5H002 data
27/9/00	0.08	0.24
29/10/00	0.65	0.59
15/11/00	3.13	3.36
20/02/01	0.26	1.38
19/03/01	3.12	3.20
24/07/01	2.98	4.88

The peak flow rates of the Knysna River did not coincide with the peak rainfall events in either year (Figures 1.6a & b). For example, on November 13, 2000 (Julian day 318) the flow rate of the Knysna River was 46.0 $m^3 s^{-1}$, representing the maximum flow rate of that year. However, the rainfall total for the 24 hours preceding this flow was 39 mm, far less than the maximum daily rainfall of the year 2000.

The relationship between catchment moisture status and the proportion of rainfall that became runoff was the main characteristic used by Hughes (1983) when he modeled direct runoff from Knysna and its adjacent catchment areas. Therefore, environmental factors in addition to rainfall amount, including moisture content of the soil above the clay layer at a depth of 60 cm (i.e., the catchment moisture status) contributed to the pattern of runoff observed after heavy rainfalls in 2000 and 2001. In consideration of this, the flow on November 13, 2000 was preceded by a period of frequent rainfall that increased the catchment moisture status, with 57 mm of rainfall evenly spread over the 3 weeks prior to this date. Additional strong rainfall resulted in this maximum river flow rate of 46.0 $m^3 s^{-1}$.

However, the maximum yearly flow rate of 44.0 $m^3 s^{-1}$ on January 1, 2001 (Julian day 1) was not explained by an increase in catchment moisture as only 11 mm of rainfall was recorded in the 3 weeks prior to this date, and 26 mm of rainfall was recorded at the National Parks Board offices in the 24 hours prior to this flow. Hughes and Görgens (1981) have shown that the upper catchment receives higher annual rainfall than the Knysna Estuary, and rainfall patterns are different in the upper catchment area. Indeed, higher rainfall amounts in the upper catchment compared to those recorded on the littoral of the Estuary seem the likely cause of this peak flow in 2001.

Evidence of dry soil conditions that restrict runoff from the catchment area after strong rainfall can be seen on December 8, 2000 (Julian day 343) and November 4, 2001 (Julian day 308). These were the highest rainfalls for each year, where 70 mm and 65.4 mm of rain fell respectively, however the river flow rates following these rainfalls were 11.2 $m^3 s^{-1}$ and 4 $m^3 s^{-1}$. Prior to each of these rainfall events there was a three-week period of low rainfall that contributed to the low moisture content of the soil.

In consideration of the volume of fresh water that the Knysna Estuary received from the Knysna River, regardless of rainfall, two distinct flow regimes were apparent in each year (Figure 1.6a & b). In both 2000 and 2001 there were many periods when the flow rate of the Knysna River was less than 1.0 $m^3 s^{-1}$, but only one period in each year when this low flow rate persisted for longer than 60 days. Hence, periods that have flow rates less than 1.0 $m^3 s^{-1}$ for longer than 60 days will hereafter be referred to as dry season, and all days that do not meet this criteria will hereafter be known a wet season.

These observations allowed 2000 and 2001 to be divided into one dry season, beginning and ending in each year. Wet seasons that began in one year and

terminated in the next will be referred to as the wet season of the year that it began. For example, the wet season that began in 2000 and terminated in 2001 will be referred to as the wet season of 2000. The dry season of 2000 was from April 15 (Julian day 106) to September 17 (Julian day 261), and in 2001 from April 26 (Julian day 116) to July 22 (Julian day 203).

1.7 Human occupation of the Knysna catchment

The littoral of the Knysna Estuary first came to the attention of the Dutch East India Company in 1668; prior to this it was inhabited by the Outeniqua and San tribes of indigenous people. In 1809, George Rex, an official of the British government at the Cape, settled the town of Knysna and by the time of his death in 1839 his property encircled the whole of the estuary and included adjacent forest areas. By 1855, Knysna had a population of 60 European adults (Tyson, 1971).

Reliable arteries of transportation, good roads and a railway, were key to the development of the Knysna area. The railway was completed from Cape Town to George in 1907, and extended into Knysna in 1925 (Tyson, 1971). The causeway that would later carry the N2 Bridge was completed in 1949 (Reddering, 1994), with completion of the N2 following in 1972.

Knysna experienced an upsurge in population growth during the 1880's as a result of a minor gold rush in the local mountains. This gold rush centered near a settlement at Millwood 30 kilometers northwest of Knysna, on the foothill plateau of the Outeniqua mountains. However, when the limited gold sources were mined out after a few years, the populations dispersed. It would only be in the later half of the 1900's that Knysna would again see rapid expansion of its population (Figure 1.7).

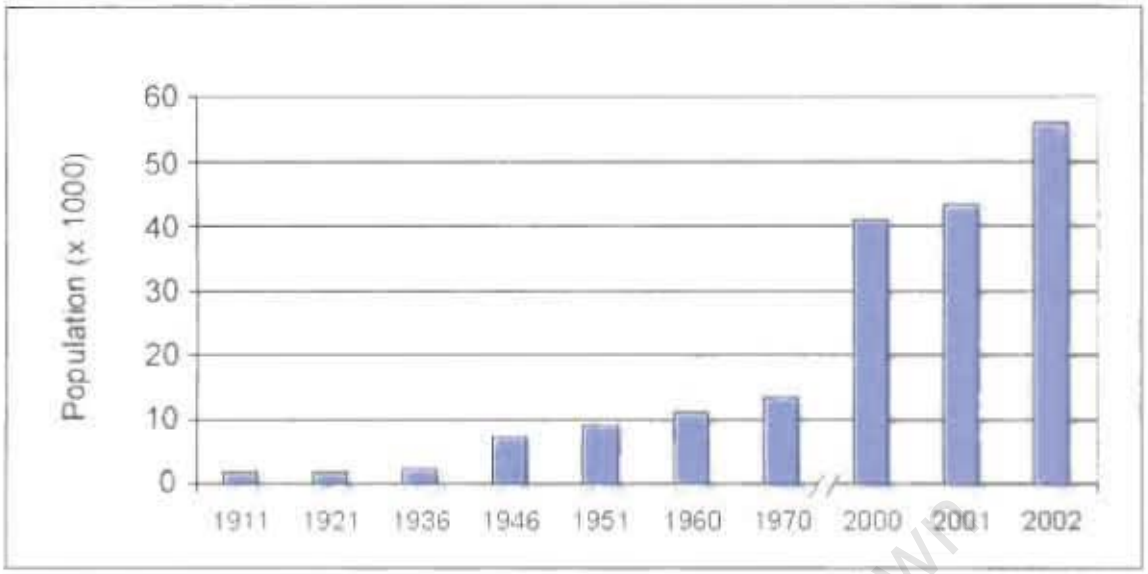


Figure 1.7. The population of Knysna (from Tyson, 1971, voter census 2000, and Knysna Municipality data 2001 and 2002).

The population of Knysna has increased rapidly over the past decade. A portion of this increase was due to the relocation of people, which occurred after the apartheid policies of the former National Party Government were terminated in 1994. This resulted in the expansion of informal housing settlements that existed on the hills surrounding the town of Knysna, and creation of new settlements in the Knysna area. Currently, these settlements are known as the townships of Concordia (north of Knysna), Neckies (northeast of Knysna) and Hornlee (east of Knysna). Many homes in these townships lack proper sewerage systems, and rely on communal pit toilets for sanitation. The impact of these open sewerage systems on the nutrient loading of the estuary will be discussed in subsequent sections.

The population of Knysna reached a peak over the summer holiday season during December and January of both 2000 and 2001. Data on the number of visitors and residence during this period were not available from Knysna

municipal sources (e.g. Tourist Information Center, Knysna City Hall). Nevertheless, it was estimated the population of the town increased 4-fold, exceeding 200,000 persons during this period in each of the years of this study. This estimate was reached after interviews with persons from municipal facilities, and long-term residences of the area, and personal observations from living in Knysna during three holiday seasons.

This surge in population placed an increased strain on the use of the Estuary's resources, which included fishing, bathing and boating during this time period. This increased use of the Estuary and increased population of the town of Knysna was sufficient to be a problem for the local estuarine managers. For example, Bruce Berwick, operations manager of the Knysna Sewage Treatment Works, recorded the highest effluent processing volumes of the year, in excess of 90% of the treatment facilities capacity, during this period. It follows that anthropogenic inputs of nutrients to the Estuary were greatest during this time period. This information coupled with the recent growth in the population of Knysna (refer to figure 1.7), forewarns of a future threat to the water quality of the Estuary.

1.8 Sampling stations

Methods

Stations were established in the Knysna Estuary and around its perimeter at rivers, storm drains and the outflow of the Knysna Sewage Treatment Facility. All measurements and monitoring activity discussed in the subsequent sections occurred at these stations. The configuration of these stations introduced here and listed in Appendix C.

Stations with prefix 'M' along the main channel (M stations) were located at alternate National Parks Board (NPB) navigational buoys. The navigational buoys were numbered from buoy 1 upstream of the N2 Bridge to buoy 28 near the Heads. Station M1 was at buoy 1 and every other buoy provided the sequential M stations. For example, M2 was at buoy 3 and so on through buoy 27, which was M14. The distance between M stations was about 800 m. Station M15 was not located at a NPB navigational buoy. It was established and relocated in front of the Knysna Yacht Club with hand-held Global Positioning Satellite instrument (GPS) (Figure 1.8).

Stations with prefix 'X' (X stations) were located at the NPB buoys between the buoys designated as M stations. Station X15 was at NPB buoy Y1 (Figure 1.8). Stations K1 - K10 in the upper estuary were established and relocated with GPS. Due to the shallow water depths in the upper estuary these stations were sampled twice by kayak, on September 29, 2000 and March 3, 2001.

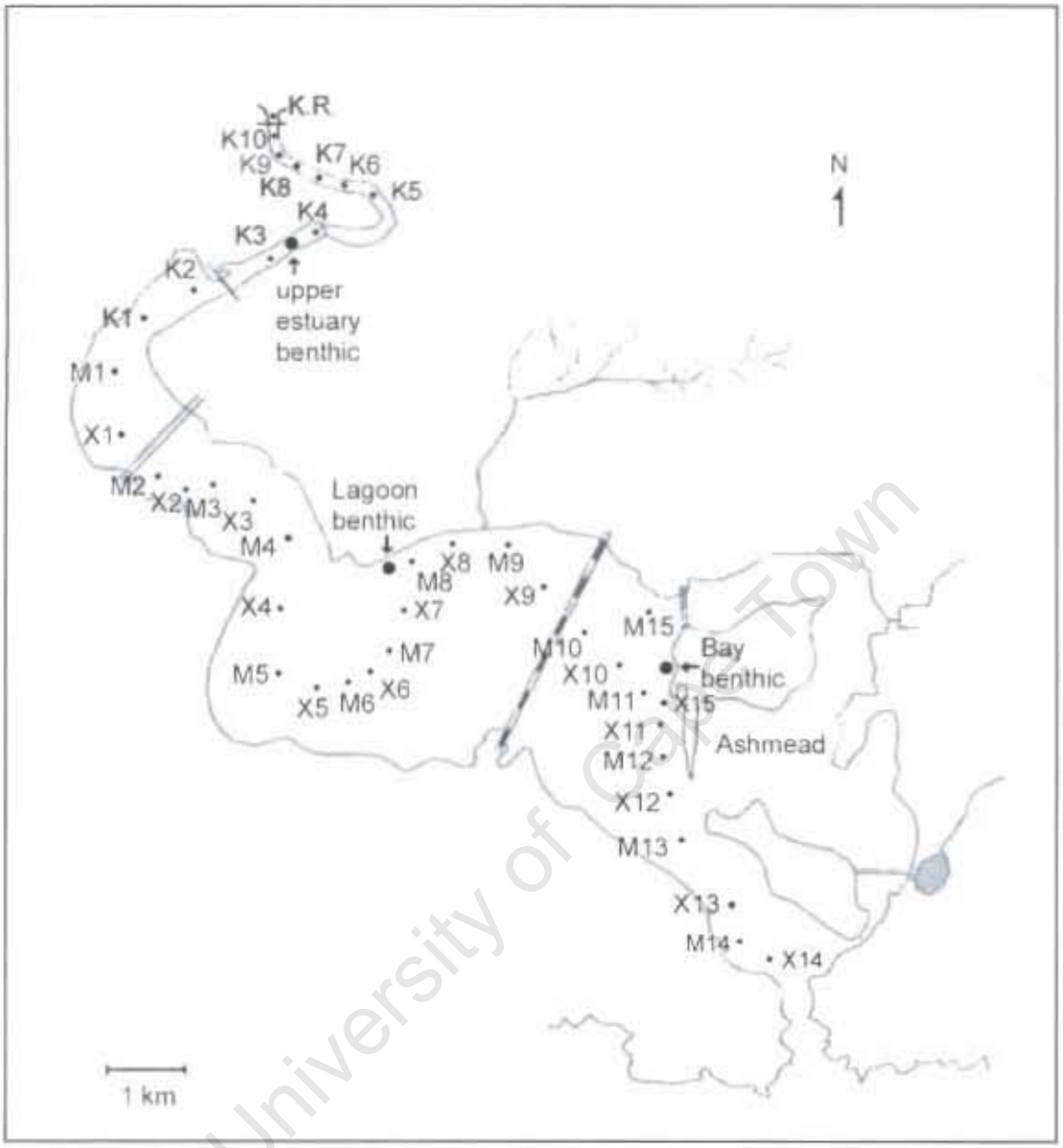


Figure 1.8. The Knysna Estuary indicating the water-column stations of the main channel (M1 – M15 & X1 – X15), the upper estuary region (K1 – K10), the Knysna River (K.R.) and the benthic experiments sites in the upper estuary, Lagoon and Bay.

Stations A3 - A9 in the Ashmead were established in mid-channel at intervals of approximately 500 meters (Figure 1.9). These stations were located where

possible at fixed channel marker post or buoys in the Ashmead, or stations were established and relocated with GPS. Station A1 was sampled from the shore at the culverts that ran under the causeway to Thesen Island. Station A2 was sampled once during high tide by kayak, but water depth at this site was too shallow for the site to be sampled regularly. Station A10 was located 50 m north of the Leisure Island small boat harbor. Stations with the prefix 'S' (S stations) were point sources of water entering the Knysna Estuary. Thirty culverts, storm drains, streams and rivers were located and labeled S1 – S30.

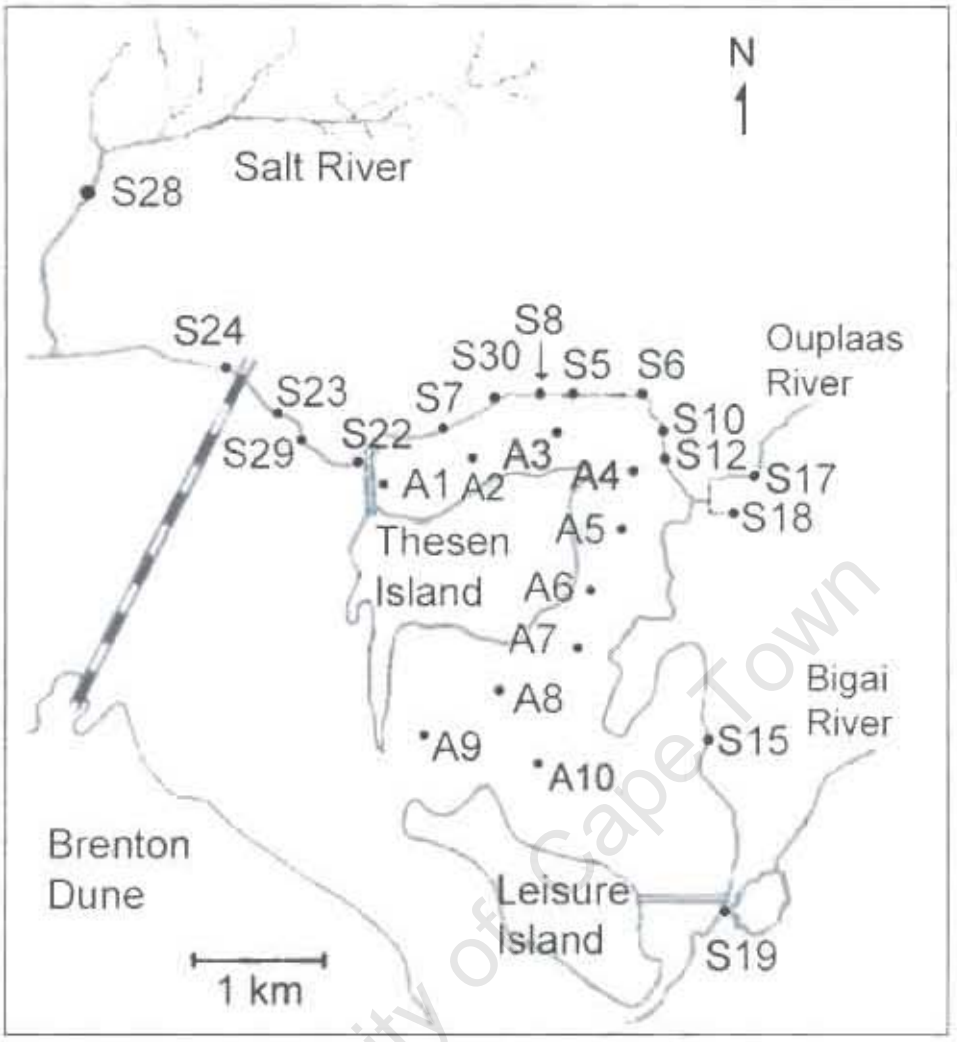


Figure 1.9. The water-column stations in the Ashmead (A1 – A10) and runoff stations. Stations labeled with prefix S are storm drains, culverts and rivers.

The use of NPB navigational buoys in the main channel or fixed channel markers in the Ashmead provided fixed locations at which sampling stations were established. Therefore, a sampling station could be rapidly relocated without the use of a GPS when the station corresponded to one of these locations. The ability to rapidly locate a sampling station was important to the water column monitoring survey method, which will be discussed in Section 2.3. The number of S stations were determined by the number of measurable point sources entering the Estuary and will be discussed in more detail in Section 2.2.

1.9 Hydrography

The Knysna Estuary

Largier et al. (2000) recorded that the Knysna Estuary was dominated by a semi-diurnal M_2 tide with a microtidal range of 0.4 m to 2.0 m. This agreed with Grindley (1985) which described the Knysna Estuary as microtidal, having mean neap and spring tide ranges of 0.5 m and 1.8 m respectively, with astronomical tides resulting in a tide range of 2.18 m.

The hydrographic characteristics of the Knysna Estuary were studied in detail from 1996 - 1998 and published by Largier et al. (2000). In addition to other data, this study utilized 79 longitudinal conductivity, temperature and depth (CTD) surveys at stations along the main channel to characterize the longitudinal thermohaline structure of the estuary. This characterization of the thermohaline structure allowed Largier et al. (2000) to divide the Knysna Estuary into three contiguous basins, the outer basin, the middle basin and the inner basin.

Largier et al. (2000) described the outer basin as a bay with salinities near that of the ocean, generally above 34 ppt and residence times of less than 4 days. The waters of this middle basin had a typical salinity range of 30 - 34 ppt and residence times ranging from 4 to 14 days. The salinity of the inner basin varied between 0 – 30 ppt and residence times from 14 to 18 days. They also noted that the residence times in the inner basin were strongly influenced by river inflow, and exhibited stratification and estuarine circulation. It should be noted that these residence times that were referenced in Largier et al. (2000) used the fraction of freshwater method (Dyer, 1997) based on 3 days in April of 1996 when freshwater inputs from the Knysna River had been less than 1 m^3s^{-1} for several months.

To simplify the text of this dissertation, the outer basin will hereafter be referred to as the Bay, the middle basin as the Lagoon, and the inner basin as the upper estuary. The name Knysna Estuary or term Estuary will be reserved to describe the tidal system in its entirety.

Largier et al. (2000) noted that the boundaries between the Bay and Lagoon, and between the Lagoon and upper estuary were not fixed, they expanded or contracted with changing tidal conditions and river flow in a concertina fashion. For example, the Bay regime contracted during neap tides while the Lagoon regime expanded during these tides. The upper estuary regime extended downstream of the N2 bridge during periods of increased river inflow, and contracted during periods of prolonged drought. However, to simplify this complex association of regions the embankments built to support the railway bridge and the N2 bridge were regarded by Largier et al. (2000) as general boundaries between these three regions.

With regard to the distribution of stations, the boundary between the upper estuary and the Lagoon was set at station M3, where the narrow channel of the upper estuary widens into the Bay of Biscay (refer to Figures 1.1 & 1.8). The upper estuary includes station M3 and all upstream stations. The boundary between the Lagoon and Bay was set at station M10 where the shallow channels and intervening mudflats, characteristics of the Lagoon, were bisected by the railway bridge. Stations M4 – M10 were in the Lagoon region. The Bay was all stations seaward of the Lagoon region.

The Ashmead

The Ashmead was largely ignored by Largier et al. (2000), and no attempt was made to distinguish its hydrological characteristics from those of the Bay.

Likewise, the investigation of water temperature changes in the Knysna Estuary by Schumann (2000) did not account for water temperature changes in the Ashmead in comparison to those of the Bay. This shallow arm of the Knysna Estuary has, however, several physical characteristics that immediately distinguish it from the Bay, and warrant that it was considered as a fourth region of the Estuary for the purposes of this study.

The Ashmead is shallow compared to the Bay and accommodates 40% (3.6×10^3 m^2) of the mudflats found in the Knysna Estuary (refer to appendix A). The Ashmead had a tidal prism of 3.3×10^6 m^3 with a spring tidal range of 1.8 m. The Ashmead had an exchange volume of 92% during this spring tidal range, while the remainder of the Estuary had an exchange volume of 53% at this tidal range.

A further feature, which distinguished this secondary tidal channel from the main system, is its thermal regime. Temperatures at three stations in the Ashmead (A3, A5 & A7) and three stations in the Bay (X11, X13 & X14) for comparison were recorded every 8 minutes by *StowAway TidbiT* temperature loggers (Onset computer corporation, Bourne Massachusetts, USA) with a tested accuracy of 0.5 °C, moored 1 m subsurface (refer to Figure 1.8 & 1.9). The duration of these deployments overlapped for 159 days during the summer and fall (November 30, 2000 – May 18, 2001) referred to here as the summer temperature, and for 102 days during the winter and spring (June 26, 2001 – October 6, 2001) referred to here as winter temperature. The average summer temperature in the Ashmead (21.1 °C) was significantly higher ($p<0.05$) than the average summer temperature in the Bay (19.0 °C). The average winter temperature in the Ashmead (15.7 °C) was also significantly higher ($p<0.05$) than the average winter temperature in the Bay (15.4 °C). The salinity from 48 water samples from the Bay and 48 samples from the Ashmead taken over the course of one year under similar conditions revealed no significant difference ($p>0.05$) in the salinity between the two regions. Although insolation was not measured in this study, the heating of

incoming tidal flow as it passes across mudflats exposed daily to the suns rays at low tide were the likely cause of higher water temperatures in the Ashmead as compared to the Bay.

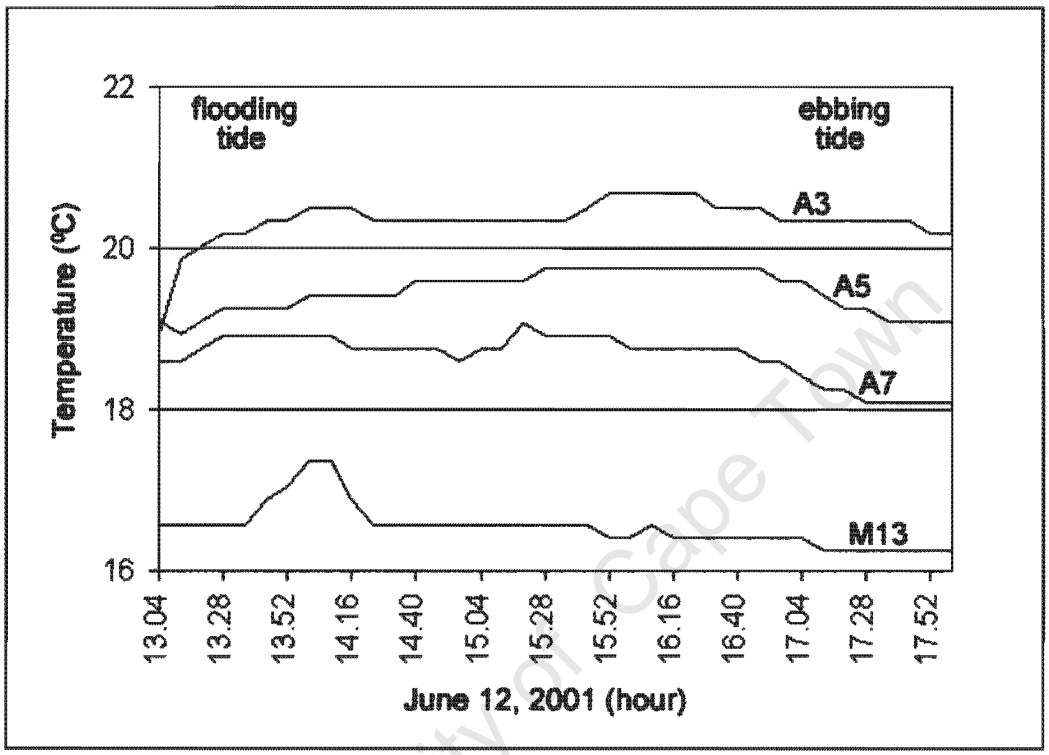


Figure 1.10. Higher water temperatures of the Ashmead (A3, A5 & A7) compared to the Bay (M13) on a winter afternoon with an incoming tide (after 13:00 hours). The decreases in temperatures after 17:00 follow sunset.

In summary, based on the higher average temperatures and physical characteristics of the Ashmead, which included shallower depths and more tidal mudflats, that differentiated it from the adjacent Bay, the Ashmead was considered as a fourth region in addition to the Bay, Lagoon and upper estuary. The boundaries defining Ashmead were a line 300 m south of station A9, the shortest distance between Thesen and Leisure islands, and the causeways to Thesen and Leisure Islands (refer to Figure 1.9).

Tidal prism

Subsequent sections of this text will require detailed information on exchange volumes in the four regions, and tidal prisms of the Knysna Estuary. Thus, it was necessary to calculate tidal prism using the cross sectional analysis method cited in Largier et al. (2000) and described in Schumann et al. (1999). These methods have been repeated with finer resolution, so that the exchange volume and additional hydrological characteristics of each region of the Knysna Estuary can be accurately described.

Station positions, taken with GPS (tested accurate to ca. 10 m), were transposed onto 1:10,000 aerial lithograph photos of the Estuary taken in 1989. Lines were sketched on the aerial photos to describe the current channel and sandbar locations from observations and field measurements. These aerial photos were matched against the SA Navy chart SAN 1021. This chart, made in 1976, was the most recent on the Knysna Estuary and included surveyed details from the railway bridge to the Heads. The Estuary upstream of the railway bridge remained uncharted. Marker (2000) has documented the movement of sandbars and channels in the Knysna Estuary that have occurred over periods as brief as four years. Therefore, given the vintage of the chart and the unsettled nature of the sandbars and channels, the aerial photos were considered to be the most up to date reference available.

Lines were drawn on each aerial photograph perpendicular to the main navigational channel at each station, and the boundaries of the Ashmead and the Bay, spanning the Estuary at HWST. By this method, the estuary was divided into 45 blocks. For most of these blocks, HWST surface area was calculated by multiplying mean width and length of each block. The surface area at LWST was calculated in similar manner.

The surface area of irregularly shaped blocks were calculated by reducing them to geometric shapes, the sum of which represented the total surface area of the block. This was necessary for the most of the Ashmead, and at those blocks in the main channel that contained pronounced bends in their channels.

The mean channel depth of each block was established by soundings, rounded to the nearest 0.1 m, taken at intervals of approximately 50 m along the navigational channels during slack low water of spring tides on April 13 - 15, 2002. These measurements were confirmed against archived CTD data taken during similar tidal conditions.

The average spring tidal range is 1.8 m (Grindley, 1985) until limited by shoals in the Ashmead channel between stations A5 and A1 (refer to Figure 1.9), and at the main estuary upstream of the Old Drift to the Charlesford weir (refer to Figure 1.1). The tidal range in these regions was determined by the difference between high and low water levels as measured by same day CTD cast where available, as well as soundings during high and low spring tide conditions, and debris lines in the upper estuary, upstream the Old Drift.

LWST volume (V_{low}) of the Estuary was estimated to be the sum of the channel volume of each block. Where the channel volume of each block at LWST had dimensions of, mean channel width (w), mean channel depth (d) and block length (l). Thus,

$$V_{low} = w \times d \times l$$

HWST volume (V_{high}) was estimated by adding tidal range (t) to d in the above equation, and adding the volume of the water over the tidal flats. The volume of

water over the tidal flats at HWST was considered to be an asymmetric triangle with surface area (s), and depth (t). Thus,

$$V_{high} = [w \times (d + t) \times l] + [(0.5 \times t) \times (s)]$$

The tidal prism (p) was calculated as the difference between volume at high and low water during spring tide with a tidal range of 1.8 m for the main channel (Figure 1.11) and the Ashmead (Figure 1.12).

Previous estimations of the tidal prism of Knysna Estuary are 19×10^6 m^3 (Grindley, 1985), and 18×10^6 m^3 (Harcourt-Baldwin, 1996). The method described above yielded a tidal prism of 18.5×10^6 m^3, falling between these earlier estimates. Data for these calculations can be found in Appendix A.

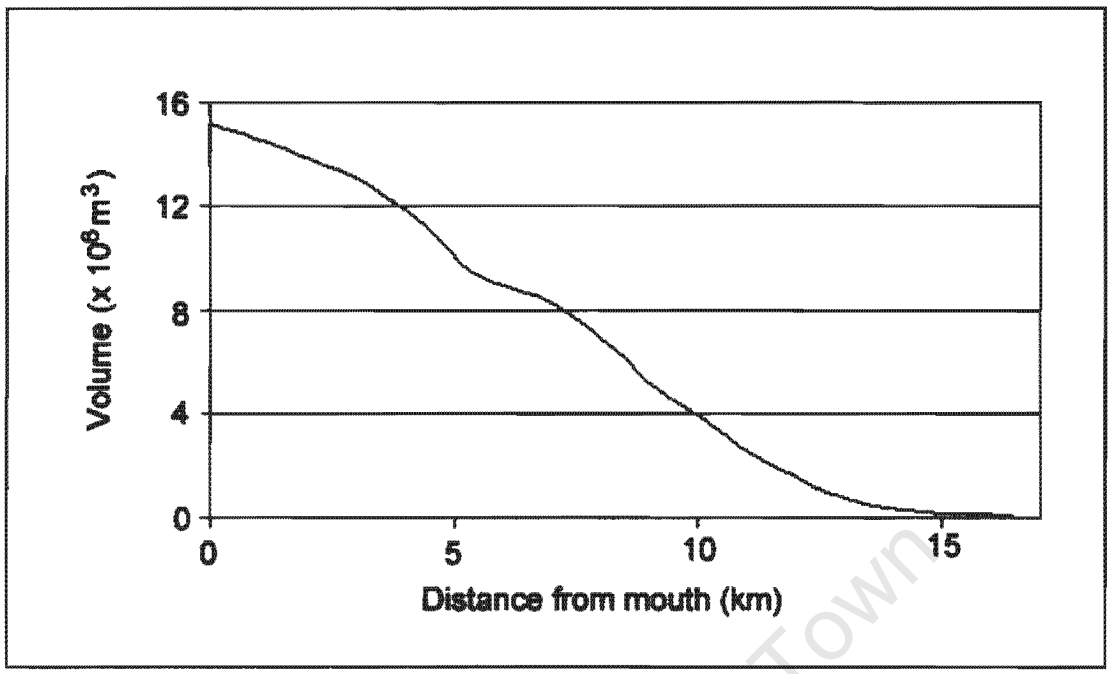


Figure 1.11. The tidal prism of the main channel (without the Ashmead). The upper estuary (>11 kilometers from the mouth) received a very small portion of the tidal prism.

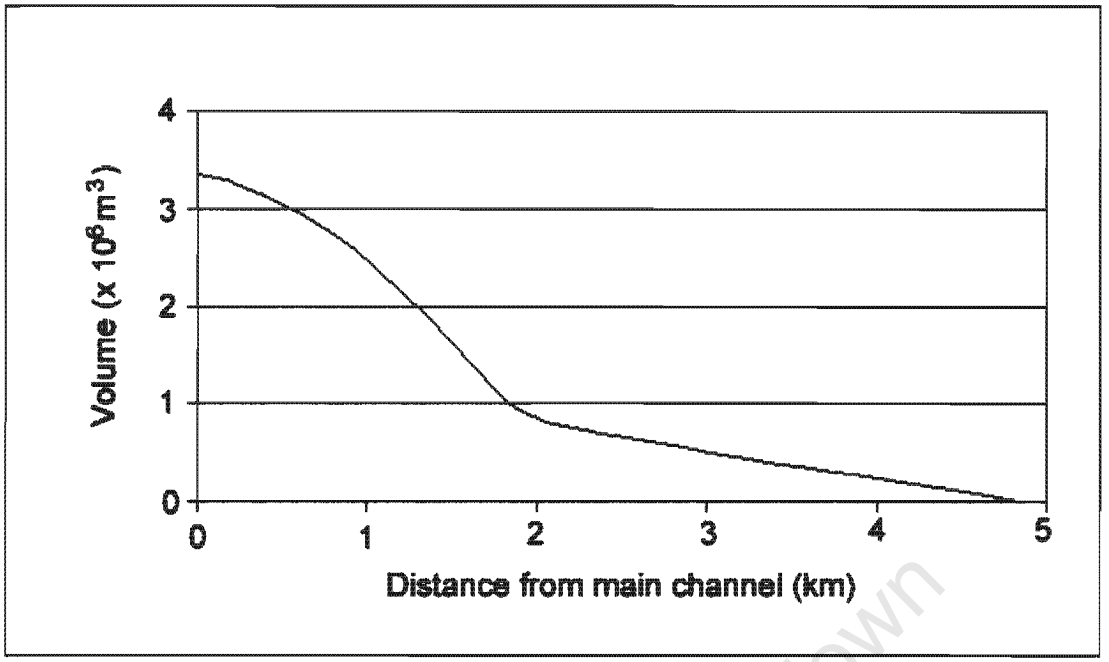


Figure 1.12. The tidal prism of the Ashmead channel. The reduced tidal range in the upper Ashmead (> 2 kilometers from the main channel) and narrower width of the Ashmead in this area reduced the volume of this tidal prism.

1.10 Water temperature variation in the Knysna Estuary

The coastal shelf in the extensive Knysna-Wilderness embayment is shallow reaching a depth of 100 m at a distance of 10 kilometers offshore (Tyler, 1971). Schumann (1999) has demonstrated, with moored thermistor arrays and CTD profiles, a close relationship between water temperature and wind in this coastal region. His study showed that winds with a westward component (i.e., southeast winds) resulted in a rapid upwelling, as fast as 6 hours, of the ocean water column.

Schumann (2000) showed that water temperatures in Knysna Estuary can decrease rapidly as a result of flood tides, which bring into the estuary the cooler

water associated with these upwelling events. The middle to late summer was the period when these extreme changes of water temperature were likely to occur. Schumann (2000) sited specific examples of upwelling in February 1998 and April 1995 when decreases of 10 °C or greater occurred in less than three hours. In winter, the coastal waters were isothermal and temperature decreases of this magnitude did not occur following the occasional southeast wind events.

The pulses of cooler water associated with upwelling that entered the estuary during summer were much higher in nitrates (nitrate + nitrite) than the ambient estuarine water they replaced. For example, an upwelling event that began on February 27, 2002 resulted in a 7-fold increase in nitrate concentration over the average summer concentration of this nutrient (i.e., from 2.5 μmol L^{-1} to 17.5 μmol L^{-1}). These pulses of nitrate resulted in a considerable increase in the quantity of nitrate available to estuarine processes that utilize this nutrient.

As discussed earlier (section 1.8, *The Ashmead*) temperature loggers were deployed at 1 m subsurface in the Ashmead and Bay. The temperature loggers were also deployed 1 m subsurface at stations further upstream in the Estuary, as part of another study. These temperature loggers recorded temperature every 8 minutes for approximately 18 months in 2000 and 2001 and the overall pattern of temperature changes in the Knysna Estuary recorded was largely an extension of the pattern reflected in the longer data set (1994 – 1998) of Schumann (2000). These data demonstrated that temperature decreases of approximately 10 °C occurred in every year that measurements were made, including 2000 and 2001 (Figure 1.13).

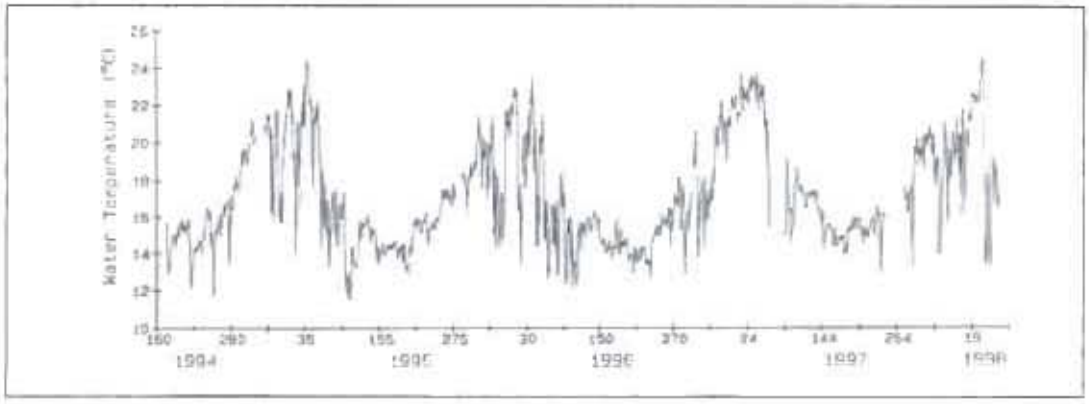


Figure 1.13. Water column temperature records of the Knysna Estuary from 1994 – 1998, from Schumann (2000, Figure 5). The sudden decreases in temperature of 10 °C or more can occur in hours, they were associated with southeast winds that occurred in the middle to late summer, and were consistent for every year measured.

In addition to providing a continuation of data on long-term cycles of temperature change in the Estuary, the temperature loggers illustrated the water temperatures associated with the individual experiments and events, which are discussed in subsequent sections. For example, the upwelling event that began on February 27, 2001 had a direct influence on an experiment that will be discussed in Part III (Figure 1.14). This upwelling event was similar in magnitude to those described by Schumann (2000) where temperatures decreased 10 °C in 3 hours (Figure 1.13).

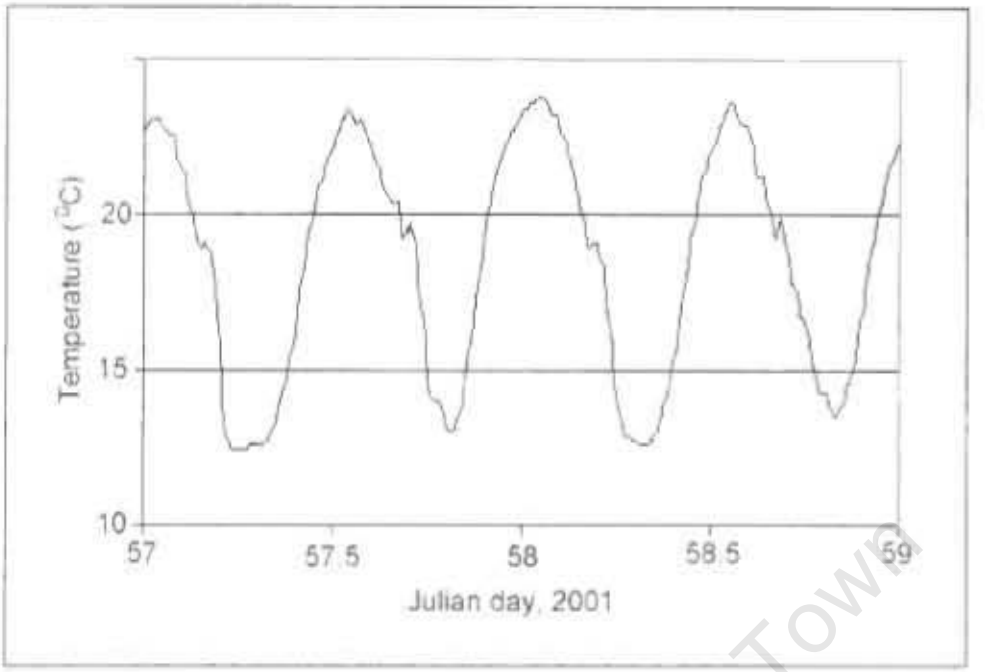


Figure 1.14. Water temperatures recorded at station X11 in the Bay show the intrusion of colder water associated with the incoming tides of February 28th and 29th, 2001. These temperature decreases were the result of upwelling outside the mouth caused by prolonged southeast winds.

In summary, the hydrographic characteristics of the Knysna Estuary are well understood (Schumann 1999 & 2000; Largier et al., 2000; this dissertation). These studies described the Knysna Estuary as a warm temperate estuary with several distinctive properties. It has the largest tidal prism of any South African estuary. It has extensive tidal mudflats and the only deep-water embayment of any South African estuary (Day et al., 1952). Upwelling events during the summer in the adjacent ocean introduces pulses of cool, nitrate-rich water with the flooding tide. These cooler pulses of water primarily affected the Bay and Lagoon regions. However, due to its limited tidal prism the impact of this cooler water on the upper estuary is minimal. While these hydrographic properties of the Knysna Estuary are not shared by all the open estuaries of the warm temperate zone along the South African coast, they provide an insight into the

characteristics of an immature (Reddering and Esterhuysen, 1984) microtidal estuary that contains many of the features found in other estuaries along the South African coast.

Part II: Water-Column Chemistry, the status of nitrogen and phosphate nutrients.

Grindley (1985) summarized the earliest measurements of water column nutrients (N and P) along the Knysna Estuary. The work was limited to 2 surveys of 10 stations along the main channel of the Estuary during August, 1976 and April, 1977. This introductory investigation identified seasonal and spatial patterns of variation of nitrogen nutrients, while phosphate concentrations remained consistent. It did not provide reasons for the variation in nitrogen compounds, and suggested the buffering action of the bottom sediments as cause for the stable phosphate concentrations.

It was an aim of Allanson et al. (2000a) to investigate the extent to which the nutrient chemistry of the water column had changed since 1976 with special reference to the effects of droughts, floods and tidal exchange (hydrological conditions). The study also investigated the contribution of nutrients from anthropogenic sources, and the role these contributions played by changing nutrient concentrations of the Estuary through loading. Although the spatial sampling schedule of the study was extensive (35 water column stations), the irregular intervals at which these samples were taken did not provide a comprehensive pattern of seasonal variation in nutrient concentrations. Nevertheless, the study made considerable progress in demonstrating the strong connection that existed between the hydrological conditions of the Estuary and the impact that the hydrology has on the variance of nutrient concentrations.

From examination of these earlier studies it became clear that a high-frequency sampling schedule, which provided concentrations of water column nutrients at consistent intervals for a period of not less than one year was required to establish the patterns of seasonal and annual variations. However, as stated by Cloern and Nichols (1985), the significance of seasonal and annual variations

cannot be assessed without comparing these to the variability on shorter time scales. Therefore, nutrient concentrations over short time scales (e.g., spring and neap tidal cycles) and during single events (e.g., storms, periods of drought and upwelling events) were incorporated into this sampling schedule. Since these patterns of nutrient variability could be affected by the contribution of nutrients from inputs into the Estuary, external sources of nutrient input (i.e., point sources and rainfall) were monitored in conjunction with this field schedule. The hydrological conditions of the Estuary (i.e. tides, flows, temperature) were closely monitored during all sampling to ensure that the interpretation of the variations could be made in the context of the physical environment in which the samples were collected.

2.1 Water sampling and subsequent analysis

Methods

The Thesen Environmental Studies Laboratory, hereafter referred to as the lab, was located on the shores of the Bay near Thesen Jetty (refer to figure 1.1). Water samples were chemically analyzed at the lab, by manual determinations as described in Parsons et al. (1984), for nitrate (NO_3-N, measured as NO_2-N after reduction), nitrite (measured as NO_2-N), phosphate measured as dissolved inorganic phosphate (DIP) and chlorophyll-*a*. Urea and ammonium (measured as NH_4-N) were measured by the manual determination method described in Grasshoff et al. (1983). The nitrates, NO_3-N and NO_2-N when combined, will hereafter be referred to as NO_x-N. The concentration of these nutrients is expressed in micromoles per liter ($\mu mol\ L^{-1}$). Salinity was measured in the majority of these samples, and chlorophyll-*a* was measured in over half the samples.

Water samples were collected in acid cleaned 1000-ml Schott glass bottles (Schott scientific glass, Parkersburg, West Virginia, USA) triple-rinsed with ambient water prior to sample collection, and transported to the lab inside insulated box for analysis within 2 hours. The exception to this was NO_x-N analysis for which samples were frozen at –10 °C for analysis within 2 weeks, in accordance with Parsons et al. (1984). The samples collected for chlorophyll-*a* determination were filtered immediately upon returning to the lab through Whatman glass fiber filters (GFF) and extracted in cold 90% acetone overnight, in accordance with Parsons et al. (1984). Salinity was measured by a YSI salinity meter (model 80, YSI, Yellow Springs, Ohio, USA) with a resolution of 0.1 ppt or taken from CTD (Sea-Bird-19, Sea-Bird Electronics, Bellevue, Washington, USA) cast made at the station during sampling. The determined precision, expressed as the percent coefficient of variation, for NH_4-N = 6.0%, NO_x-N = 1.8%, DIP = 4.4% and urea = 3.6%.

Results

The waters of the Knysna Estuary were oligotrophic with chlorophyll-*a* levels seldom exceeding 5 $\mu g\ L^{-1}$ (Grindley, 1985, Allanson et al., 2000a, Appendix B) thus it was not expected that phytoplankton alter the concentration of the measured nutrients within the 2 hour period between collection and analyses. However, as a precaution, all samples taken after November 11, 2000 were filtered through Whatman GFF within 1 hour of collection.

To test if filtration caused a change in the nutrient concentrations of the samples, 15 samples collected on February 9, 2001 from stations M1 – M15 were filtered through Whatman GFF immediately after collection, and compared to their unfiltered complement. When these samples were tested by a paired sample t-test they demonstrated no significant difference ($p>0.05$) in filtered and unfiltered samples for nutrients NH_4-N, NO_x-N and urea. DIP tested 21% lower,

a significant difference ($p<0.05$) for filtered samples as opposed to their unfiltered complements.

2.2 Nutrient inputs into the Knysna Estuary

Nutrient inputs from specific locations, rivers, storm drains and effluents from the Knysna sewage treatment works (STW) will be collectively referred to hereafter as nutrients from point sources (refer to Figure 1.9). Allanson et al. (2000a) demonstrated the important contributions of "new" nutrients (N and P) that these inputs bring to the Knysna Estuary. In addition to these point source inputs of nutrients, the non-point source contribution of nutrients from rain falling directly on the surface of the Estuary was measured. These nutrient inputs are introduced in this section, prior to presenting the results of the water column monitoring program in section 2.3, as the role of loading from these nutrient inputs will be examined in that section.

The input of nutrients from an incoming source (e.g., river or storm drain) with units of grams hr^{-1} is referred to as the contribution of nutrients. The terms loading and nutrient loading were reserved to describe the impact nutrient inputs have on a cubic meter of water, where units are given in grams hr^{-1} m^{-3}, or are used to describe the impact of nutrient inputs on a region of the Estuary or the entire Estuary. The units of measure used in this section were weight (e.g., grams) rather than molar units to aid the estuarine managers in visualizing the quantity of these inputs into the Knysna Estuary.

To determine the amount of nutrients introduced from point sources, stations were established to monitor the quantity and nutrient concentrations (NH_4-N, NO_x-N, DIP and urea) of the water entering the Knysna Estuary from these sources. Stations on rivers and the outflow of the STW were established far

enough upstream as to exclude contamination of the flow by tidal intrusion as evidenced by their salinities. Storm drains with outfalls above the height of HWST were monitored at their terminus. With the exception of the flow from station S7, any contamination by tidal intrusion as evidenced by salinity above 2.8 ppt negated these samples from further analysis. The salinity at station S7 was never measured lower than 5.8 ppt, however it is important to include data from S7 in this analysis as it drained the central portion of the town of Knysna.

Nutrient inputs via Ashmead from the sewage treatment works

The daily flow of the STW effluent was measured by a flow meter maintained by the STW as the effluent exited a series of settling ponds upstream of station S18. The flow of water in a storm water culvert on the north side of the STW facility, referred to hereafter as the north flow, was measured manually at station S17 (refer to Figure 1.9). The north flow consists of storm water drainage from the industrial area and the Ouplaas River. This river drains the informal housing development of Neckies, which lacks a sewer system (Bruce Berwick, operations manager of Knysna STW, personal communication) (refer to Figure 1.1).

The north flow and STW flow combined into a single flow that drained into the Ashmead channel across the tidal mudflats between stations A4 and A5. The irregular cross-sectional shape of this single flow across tidal mudflats, and its submergence at HWST prevented the option of measuring flow and sampling at this location. Therefore, the north flow and STW flow were measured individually and the combined flow are hereafter referred to as STW effluent, or STW effluent that enters the Ashmead.

The hourly flow rate that exited the STW was calculated by dividing the recorded daily flow by 24 on the days when samples were taken. However, during a storm

on August 30, 2001 the outflow from the STW was measured manually at the outflow culvert near station S18. This measurement represented the maximum hourly flow rate during this storm, as the hourly flow calculated by daily flow divided by 24 substantially underestimated the value.

Samples were taken from the north flow and STW flow at locations immediately upstream of where these two channels combined. From the initial samples, it was observed that nutrient concentrations in these channels changed along their length. This change in nutrient concentrations was probably due to the biogeochemical actions of the sediments and macrophytes that line the north flow and STW outflow channels. Therefore, samples taken at the terminus of the north flow and the STW flow represented the most reasonable measurement of the concentration of nutrients in the STW effluent that entered the Ashmead.

The concentration of each nutrient in the STW effluent that entered the Ashmead was determined by their weighted-average with respect to individual flow from the north flow (V_A) and the STW flow (V_B).

Thus,

$$\frac{[(C_A)\times(V_A)] + [(C_B)\times(V_B)]}{(V_A + V_B)}$$

Where C_A and C_B are concentrations of nutrients in flows V_A and V_B, respectively.

The STW effluent that entered the Ashmead was monitored four times during the course of the fieldwork from August 3, 2000 until August 30, 2001. These samples represented dry season, early and late wet season and during heavy rainfall. The contribution rates calculated in grams hr^{-1} are given in Table 2.1.

Table 2.1. The contribution of N and P (grams hr^{-1}) into the Ashmead from the STW outflows. Note the marked increase in all nutrients during the heavy rainfall of 30/8/2001.

Date	Sampling conditions	NH_4-N	NO_x-N	DIP	urea
3/8/2000	mid dry season	138	426	769	98
3/11/2000	early wet season	124	185	584	31
20/2/2001	late wet season	63	156	1181	38
30/8/2001	during heavy rainfall	838	4791	5618	200

Nutrient inputs from rainfall

Paerl et al. (2002) showed that the atmospheric deposition of nitrogen (AD-N) can be responsible for over 30% of the introduced N in some east coast estuaries of North America, with 5% of AD-N falling directly upon the surface of these estuaries. This study showed that AD-N was a significant source of N enrichment for N-limited estuarine systems downwind of anthropogenic emissions of reduced inorganic forms of N, and that the conveyance of these emissions to estuaries from hundreds of kilometers away was possible. Knysna was less than 500 kilometers east of the city of Cape Town and less than 300 kilometers west of the city of Port Elizabeth, therefore the possible contribution of N from these distant sources has been considered as an integral part of this study.

The constraints of this study did not allow for an investigation into the contribution of AD-N in the rain falling in the higher elevations of the catchment area. It was assumed that the AD-N in the catchment contributes to the total inorganic N entering the Estuary from the Knysna River. The contribution of AD-N to

estuaries or the coastal waters in South Africa has not been published prior to this study, therefore, no comparison can be made between these data and published data on AD-N in South Africa.

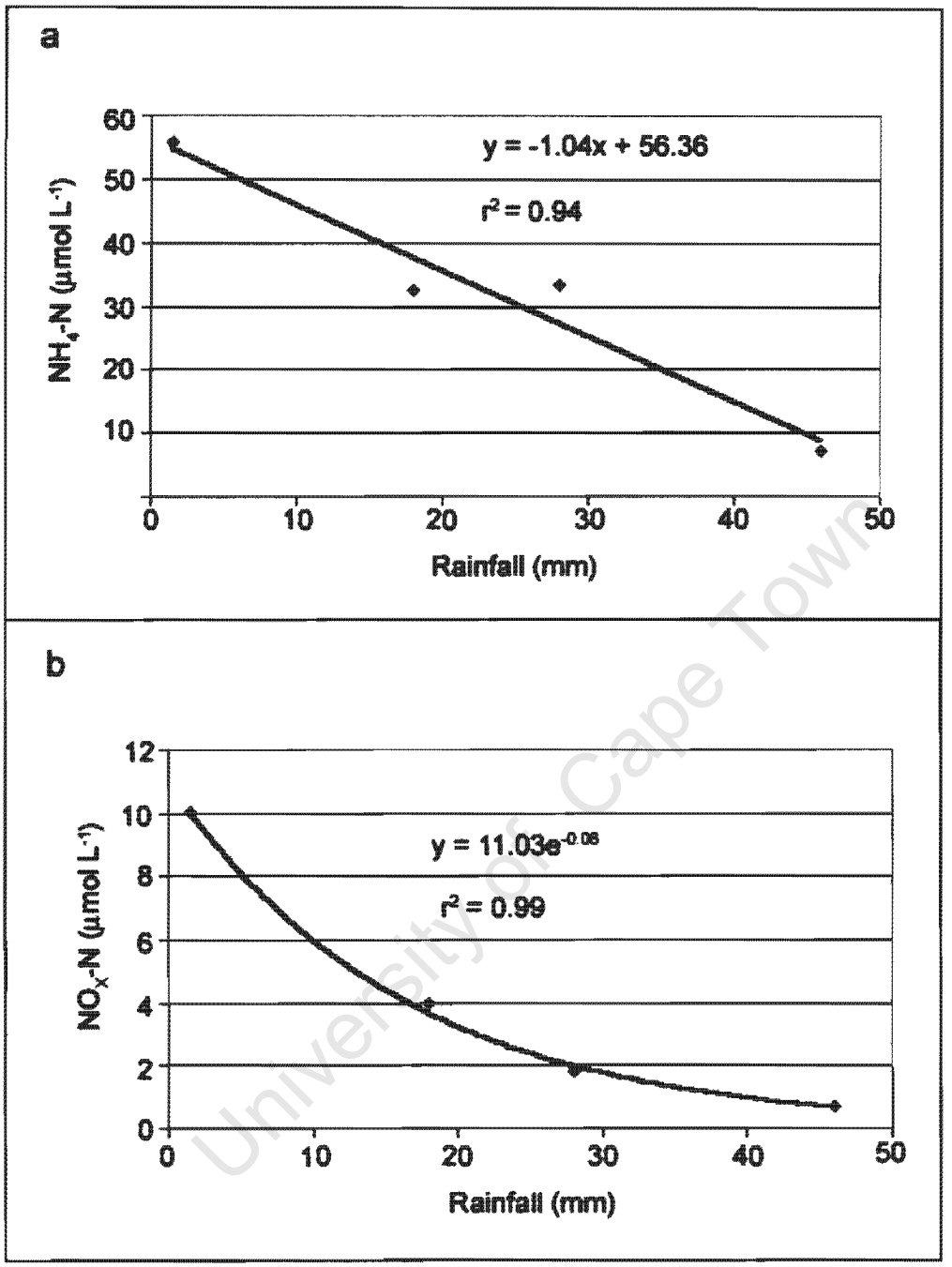
Rainfall samples were collected in acid washed glassware during 4 rainfall events (February 15, 2001; July 1 & 28, 2001; August 30, 2001) at locations on the shore of the Knysna Estuary. It was assumed that the concentration of nutrients measured did not vary between these different locations, remaining constant over the surface of the Estuary. These 4 rainfall events covered a range of rainfall totals from 1.5 mm to 46 mm. The rainwater samples were transported to the lab and immediately analyzed for NH_4-N, NO_x-N, DIP and urea.

The concentration of NH_4-N and NO_x-N in the collected rainwater samples varied with the amount of rain. As a general pattern, the lower the amount of rainfall from the storm the higher the concentrations of NH_4-N and NO_x-N in the samples. The concentrations of DIP and urea in the collected rainwater samples were negligible, therefore they were not considered in this study as nutrients introduced by atmospheric deposition.

Non-linear regression analysis of these data (rainfall vs. N concentration) showed that NO_x-N followed an exponential curve, $y = 11.03e^{-0.06x}$, with a strong coefficient of determination, $r^2 = 0.99$ (Figure 2.1a). The data for NH_4-N was best described by a negative linear regression, $y = -1.04x + 56.36$, where $r^2 = 0.94$ (Figure 2.1b). The values of the slope equations given for NH_4-N and NO_x-N, where x was rainfall amount and y was the nutrient concentration in that rainfall amount, were used to estimate the concentration of NH_4-N and NO_x-N in rainfall amounts from 1.5 mm to 46 mm. Rainfall of 1.5 mm had an NH_4-N concentration of 54.8 μmol L^{-1} and a NO_x-N concentration of 10.1 μmol L^{-1}, and the rainfall of 46 mm had an NH_4-N concentration of 8.6 μmol L^{-1} and a NO_x-N concentration of

0.7 μmol L^{-1}. Rainfall amounts of less than 1.5 mm and more than 46 mm were considered to have a concentration of NH_4-N and NO_x-N equal to these values, respectively.

By this method, the concentration of NH_4-N and NO_x-N in each rainfall recorded at the National Parks Board offices on Thesen Jetty was calculated. The quantity of rain that falls upon the surface of the Knysna Estuary was the product of rainfall amount and the surface area of the estuary at HWST, 1.69 x 10^7 m^2 (refer to appendix A). The contribution of NH_4-N and NO_x-N as a result of rainfall in the dry seasons of 2000 and 2001 and the wet season of 2000 were calculated (Table 2.2). The yearly estimate of AD-N from rain falling directly on the surface of the Knysna Estuary was 327 kg N km^{-2} yr^{-1}. When compared to estimates of AD-N falling directly on the surface of the Sippewissett Marsh, Massachusetts, 785 kg N km^{-2} yr^{-1} (Valiela and Teal, 1979) and Nueces Estuary, Texas, 895 kg N km^{-2} yr^{-1} (Brock, 2001), this estimate was expected given the relative lack of industrialization in South Africa.



Figures 2.1a &b. The concentrations of NH_4-N and NO_x-N in 4 different rainfalls. The best-fit curves as determined by linear regression are shown with the coefficient of determination (r^2). The regression equations provided the formulas used to calculate NH_4-N and NO_x-N concentrations during rainfalls between 1.5 mm and 46 mm.

Table 2.2. The contribution of N from NH_4-N and NO_x-N (10^3 grams) as a result of rain falling directly upon the surface of the Knysna Estuary, during dry and wet seasons (duration given in days).

	NH_4-N	NO_x-N
Dry Season 2000 (87 days)	948	151
Dry Season 2001 (155 days)	312	31
Wet Season 2000 (219 days)	3,250	440

Nutrient inputs from rivers

The rivers that drain into the Knysna Estuary are the Knysna River, the Salt River, the Bigai River and the Ouplaas River. The flow of the Salt, Bigai and Ouplaas Rivers hardly qualify them to be considered more than streams by conventional terminology, however, they will be referred to as rivers herein for simplification of the text. The Knysna River is the parent-river of the Knysna Estuary, its flow and catchment area have been extensively discussed in Part I. The Salt River, drains a basin of 15.3 km^2, the largest of the smaller basins discharging directly into the estuary (Reddering, 1994). The Salt River flow was measured at a concrete culvert, approximately 1 kilometer upstream of the point where the Salt River entered the Knysna Estuary. The Salt River flowed continuously during the course of this study with a minimum flow of 1.6×10^{-3} m^3 s^{-1} measured on February 20, 2001 (refer to Figures 1.8 & 1.9).

The Bigai River enters the Woodbourne Pan before it flows onward to the Bay on the east side of the Leisure Island. The Woodbourne Pan was regularly affected by tidal intrusion from the Bay therefore its flow was not measured regularly. During a strong rainfall on August 8, 2001 salinities of 0.8 ppt were measured in

the outflow from the Woodbourne pan as it spilled into the Bay and flow was measured at 1 $m^3 s^{-1}$. This was the only measurement of the contribution of nutrients from the Bigai River that was recorded during the course of this study.

As mentioned earlier (refer to section 2.2) the Ouplaas River comprises a portion of the north flow of the STW. Its contribution of nutrients, which included those from the STW flow, to the Estuary *via* the Ashmead, are given in Table 2.1. The minimum flow measured at station S17 was 6 x 10^{-3} $m^3 s^{-1}$ on February 20, 2001, which included runoff from the industrial area. To attain accurate flow measurements of these rivers, stations were selected at locations where these rivers flowed across level concrete surfaces. Water samples were taken in conjunction with all flow measurements and analyzed for NH_4-N, NO_x-N, DIP and urea.

The contribution of each nutrient from a river is a product of flow and nutrient concentration. The contribution of the nutrients measured is given for the Knysna River in Table 2.3, and the Salt River in Table 2.4. A comparison between these two tables showed that the nutrient contribution from the Knysna River was substantially higher than that from the Salt River. However, the nutrient contribution from the STW effluent (refer to Table 2.1), often exceeded the contribution of the nutrients from the Knysna River. When nutrient contributions were considered by this method, and given the much higher flow of the Knysna River, it is an unexpected result that the contribution of nutrients in a primarily man-made flow should, at times, be exceeded by the contribution of nutrients from the parent-river of the Knysna Estuary.

Table 2.3. The contribution of N and P (grams hr^{-1}) from the Knysna River. * = lost sample.

Date	Sample conditions	NH_4-N	NO_x-N	DIP	urea
29/9/2000	late dry season	5	0.7	0.9	15
27/10/2000	early wet season	18	249	11	153
15/11/2000	wet season post-storm	256	1645	84	1796
19/3/2001	wet season high-flow	219	677	29	684
20/2/2001	wet season low-flow	22	13	5	56
24/7/2001	early wet season	166	651	23	*

Table 2.4. The contribution of N and P (grams hr^{-1}) from the Salt River. * = lost sample.

Date	Sampling season	NH_4-N	NO_x-N	DIP	urea
8/11/2000	mid dry season	0.5	0.1	0	0.8
15/11/2000	wet season, storm	1	0.7	0.2	4
20/2/2001	wet season, low flow	0.8	0.2	0	0.4
24/7/2001	early wet season	11	66	3	*

Nutrient inputs from storm drains

The flow from storm drains into the Estuary following rainfall was short-lived due to the small catchment areas that the storm drains serve. During periods of little or no rainfall the drains were dry and hence the contribution of nutrients into the Knysna Estuary from storm drains was negligible during these relatively dry periods. In order to measure the contribution of nutrients that the storm drains

can deliver to the Estuary, measurements must be made during a substantial rainfall event.

During the evening hours of August 29, 2001 a storm began and in the following 15 hours, 46 mm of rain fell over the Knysna area with the majority of rain falling in the morning hours (08:00 to 10:30). Flows were measured and samples were obtained from all storm drains and rivers that flowed during this period on the morning of August 30, 2001. These included 10 that drain into the Ashmead, and 5 that drain into Bay. For the subsequent discussions of this storm, the north and south flows from the STW were considered individually unless otherwise stated. The peak flow rate contribution of nutrients from these storm drains is represented in Table 2.5.

The data presented in Table 2.5 clearly reflected a difference in the contribution of nutrients from the Bigai River, STW south flow, north flow and golf course, and the contribution of nutrients from the remaining storm drains. The quantity of nutrients from the Bigai River to the Bay during this storm was perhaps the most unexpected result of this data set. The flow of this river measured at the peak of the storm was 1 $m^3 s^{-1}$. In comparison, the Knysna River contributed considerably less NH_4-N, NO_x-N and DIP to the Estuary when a flow of 3.4 $m^3 s^{-1}$ was measured after an early wet season storm (refer to Table 2.3, 15/11/2000). The quantities of nutrients contained in the Bigai River, especially the quantity of NO_x-N in this flow may reflect an inadequately managed sewerage system in its catchment area.

The data in Table 2.5 provides an opportunity to compare the contribution of nutrients from the informal housing settlement of Neckies, which drained into the north flow, with that of Hornlee, which drained into the Bigai River. The contribution of nutrients from the north flow was considerably less than that from the Bigai River, as would be expected given that the north flow was 0.15 $m^3 s^{-1}$,

or 15% of the flow measured in the Bigai River during this storm. However, urea comprised a much higher proportion of the overall nutrients in the north flow as compared to the Bigai River. The amount of urea in a volume of water from the north flow was approximately 4 times higher than was found in the same volume of water from the Bigai River. This difference may be due to the use of pit toilets in Neckies, as opposed to the toilets of Hornlee that were connected to a plumbed sewage system.

The contribution of nutrients from the STW during the peak of this storm was considerable. The quantities of NO_x-N and DIP in the outflow from the STW settling ponds (station S18, Table 2.5) were an indication of overflow from settling ponds, which flowed into the Ashmead. The flow from the Knysna Golf Course (station S15, Table 2.5) was 0.13 $m^3 s^{-1}$ and the contribution of nutrients, other than urea, from this flow was similar to that of the north flow. The contribution of urea from the golf course outflow was about half that from the north flow.

Reddering (1994) described 4 small catchment cells, with areas ranging from 0.7 to 1.8 km^2, which drain the urban area of Knysna to the north littoral of the Ashmead and the adjacent Bay (the north section of Knysna). These smaller catchments were considered jointly here, and the first nine stations listed in Table 2.5 represent their drainage. Stations S10, S12, S17 and S18 in Table 2.5 represent the drainage of the Ouplaas catchment (7.7 km2) and stations S15 and S19 in Table 2.5 represent the drainage of the Bigai catchment (9.9 km^2) (Reddering, 1994). The Ouplaas and Bigai catchments drained to the east littoral of the Ashmead. If the data in Table 2.5 are divided according to these 3 hydrological divisions it becomes apparent that the larger catchments of the Ouplass and Bigai added considerably more nutrients to the Ashmead than the combined smaller catchment basin that drain the north section of Knysna. This was probably due to the lack of proper sewage facilities in the Ouplaas and Bigai

catchments, while the sewage system in the north section of Knysna was well established.

Table 2.5. The contribution of N and P in grams hr^{-1} from storm drains and rivers (underlined) during strong rainfall. The samples were taken during the climax of a storm that precipitated 46mm in 15 hours ending on 30/8/2001.

Station	NH_4-N	NO_x-N	DIP	urea
S24, Railway bridge	5	113	12	9
S23, Kada stream	66	96	17	7
S29, Oyster Catcher culvert	13	26	6	2
S22, Thesen island causeway	61	239	20	17
S7, end of Union street	5	38	1	2
S30, Ashmead wetland	76	272	15	9
S8, jetty outflow pipe	0.4	0.2	0.1	0
S5, ramp outflow pipe	10	41	2	5
S6, east end of walkway	55	196	18	9
S10, George Rex Pipe 2	2	13	1	0.6
S12, Kathy Park stream	1	0.5	0.3	0.4
S17, north flow (Ouplaas River)	266	309	149	126
S18, STW south outflow	572	4482	5468	74
S15, golf course outflow	322	212	136	59
S19, Bigai River	1613	5392	818	216

Total nutrient loading

An estimation of the total annual nutrient load entering the Knysna Estuary was obtained by calculating the daily average nutrient loads from each source (e.g., rainfall, rivers, storm drains, sewage and ocean)(see Figure 2.2). The daily average nutrient loads are the averages of all measurements made at these sources during the dry and wet season. These daily averages were multiplied by 146 day in the wet season and 219 day in the dry season to provide an annual estimate of nutrient loads. The total DIN input during the wet season was 9,524 kg and the DIN input during the dry season was 4,723 kg. The DIP input for the wet season was 6,414 kg and the DIP input during the dry season was10,118 kg. The total annual load of DIN was 14,247 kg and the total annual load of DIP was 16,532 kg.

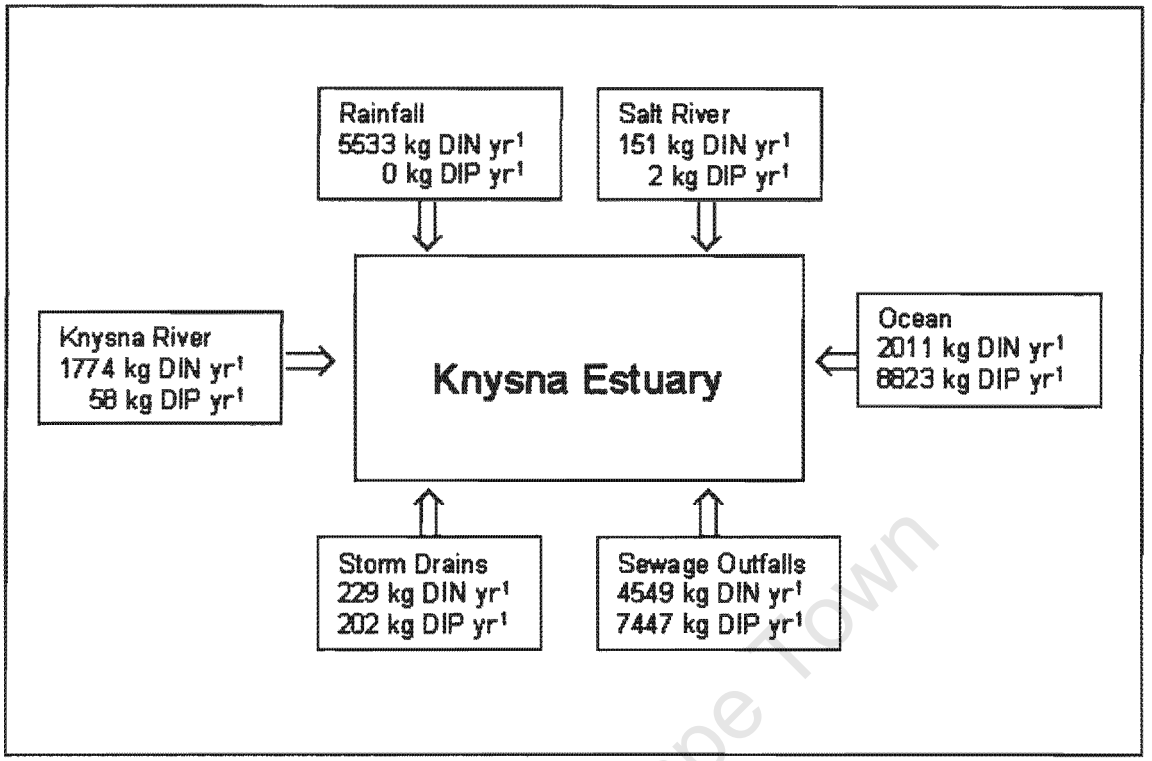


Figure 2.2. Annual loading of DIN and DIP from rivers, rainfall, storm drains, sewage outfalls and ocean. The nutrient loading from the ocean are net values of these nutrients calculated from Switzer and Waldron (2002).

The estimation of net nutrient loading from the ocean in Figure 2.2 (input less output) was calculated from Switzer and Waldron (2002). This paper used the non-conservative mixing properties of salt to calculate the water exchange budget of Knysna Estuary with the adjacent ocean. This water exchange budget and measurements of nutrient concentrations in both the Estuary and ocean during this period provided the estimates of net nutrient exchange.

Allanson et al. (2000a) hypothesized that the quantity of nutrients in the tidal prism eclipsed the contributions of all other sources of nutrients introduced into the Knysna Estuary. His paper stated that the Estuary with a tidal prism of 18.5 x

10^6 m^3 introduced and removed a substantial amount of nutrient during each tidal cycle.

An attempt to calculate the exchange of nutrients with the sea from water volume exchange (e.g., tidal prism) and average nutrient concentrations was made during this study and found to be unrealistically high. The loading amounts estimated by this method were and order of magnitude higher than those found in Switzer and Waldron (2002). It appears that the data available (water volume exchange *via* tidal prism and average nutrient concentrations) were too crude to provide an accurate estimate. An attempt to use data collected by a single bottom-mounted Acoustic Doppler Current Profiler in the Knysna Heads to estimate water volume exchange failed, due to the variable bathymetric structures and substrates found in this compound channel (personal communication, Charles Coughran, Scripps Institution of Oceanography).

The sampling method use by Valiela and Teal (1979) may provide data detailed enough to calculate the net nutrient exchange with the ocean from direct measurements. However, the cost of executing the daily measurements of nutrients and flow across the mouth of the Estuary was prohibitively expensive.

2.3 The water column monitoring surveys

It was the aim of the water column monitoring surveys to determine the patterns of nutrient concentrations in the Estuary with regard to the seasonal (i.e., dry and wet season) and tidal scales (i.e., high and low, neap and spring) for each region (i.e., upper estuary, Lagoon, Bay, Ashmead). A secondary aim was to analyze the tidal scale patterns of nutrient concentration with the intention of determining if these patterns were affected by the input of nutrients from point sources (i.e., loading). The Ashmead was chosen for this investigation, as it was the probable

region to reflect the impact of loading due to its low volume relative to other regions (e.g., 295,000 m^3 at LWST) and high input of nutrients.

For this water column monitoring survey 14 stations along the main channel (stations M1 – M14, Figure 1.8) and seven stations along the Ashmead (stations A3 – A9, Figure 1.9) are referred to as the core stations. The core stations were sampled during same day slack high and slack low water at spring and neap tides. This sampling schedule was repeated every quarter (about three months), beginning in June 2000 and terminating in February of 2001, and resulted in the collection of 336 samples. To ensure that water samples were representative of slack water conditions, all collections were made within one hour along the main channel and a half an hour along the Ashmead during slack tides (i.e., semi-synoptical sampling). Attempts to collect samples at stations other than these core stations within these time limits were not successful, therefore, results from additional stations were excluded from the analysis of the water column monitoring survey. When quarterly survey data were analyzed by month, it was named for the month in which the data collections began.

The water samples were collected 0.5 m below the surface, as CTD surveys taken in conjunction with sample confirmed earlier findings by Largier et al. (2000) and Allanson et al. (2000a) that the water column was well mixed in the Lagoon, Bay and Ashmead regions. The CTD surveys at stations M1 – M3 displayed stratification in the water column during low tides of the wet season, confirming earlier findings of Largier et al. (2000) and indicated that these stations were representative of the upper estuary.

The nutrient concentrations of NH_4-N, NO_x-N, DIP and urea from samples collected at the core stations were divided with respect to the regions in which they were collected. Samples collected in June and August 2000 represented dry season 2000 and samples collected in October 2000 and February 2001

represented wet season 2000. The 4 slack high tides (i.e., 2 spring and 2 neap) that were sampled in each season were averaged to represent high tide concentrations. Similarly, the 4 slack low tides sampled represent average low tide concentrations of each nutrient. When the figures of this section refer to high tide and low tide they refer to the average of all high tides and all low tides sampled, unless otherwise stated in the caption of the figure. This temporally and spatially comprehensive sampling schedule, with a high number of replicate samples taken during each period, made it possible to interpret these data in respect of seasonal and tidal patterns of nutrient concentrations.

The data analysis was represented graphically as whisker plots with mean concentration as the center point and 1 standard deviation as the whisker. Statistica software version 4.5 was used to create all whisker plots. Statistical comparisons were made by ANOVA (by paired sample t-test) also using Statistica software version 4.5, where $p<0.05$ was considered significant.

Ammonium

The NH_4-N concentrations for Lagoon, Bay and Ashmead regions were significantly higher ($p<0.05$) at low tide as opposed to high tide during both the dry and wet seasons. The Ashmead demonstrated the most striking example of this difference, with increases of 1.3 and 1.6 $\mu mol\ L^{-1}$ between high and low tide for the dry and wet seasons, respectively. There was no significant difference ($p>0.05$) between high and low tides in the NH_4-N concentrations of the upper estuary region during either wet or dry season (Figures 2.3 & 2.4).

The impact of nutrient loading from the STW was investigated as a possible cause for the higher average NH_4-N concentrations in the Ashmead measured at

low tide. The contribution of NH_4-N from the STW in grams hr^{-1} under various sampling conditions are given in Table 2.1, with the maximum contribution of NH_4-N (838 grams hr^{-1}) occurring during the storm of August 30, 2001. However, even at the maximum loading rate that was measured in this study, the NH_4-N input from the STW to the Ashmead is negligible as demonstrated in the following exercise:

If the maximum contribution of NH_4-N (838 grams hr^{-1}) from the STW is applied to the Ashmead during slack water LWST for one hour, when the volume of in the Ashmead was at its minimum of 295,000 m^3. The maximum loading rate that results is 2.8×10^{-3} grams hr^{-1} m^{-3}, or an effective increase in concentration of 0.2 μmol hr^{-1} L^{-1}. This number should be viewed with the caveat that it refers to the simulated impact of the peak loading from the STW during the lowest volume of the Ashmead. The volume of the Ashmead does not remain at this low value for more than a 30 minutes as the tide changes. If this calculation is repeated using the maximum volume of the Ashmead (e.g., 3.3×10^6 m^3 at HWST) the resulting loading rate is 0.018 μmol hr^{-1} L^{-1}.

These loading calculations were made to emphasize that even under conditions where the contribution of NH_4-N reached a maximum, their impact on the Ashmead was negligible. When this same calculation was made using 138 grams hr^{-1}, the dry season NH_4-N contribution rate from Table 2.1, and the LWST volume of 295,000 m^3, the loading rate was 0.03 μmol hr^{-1} L^{-1}. This loading rate did not produce a measurable increase in the concentration of NH_4-N in the Ashmead. Table 2.6 contains the expected rates of loading from the contribution of nutrients under various conditions. Table 2.6 demonstrates that the loading from the STW does not have the ability to increase the NH_4-N concentrations of the Ashmead, at a tidal scale, by the amounts measured during the dry season (Figure 2.3) or the wet season (Figure 2.4).

If the data represented in Figures 2.3 and 2.4 were considered seasonally, where seasonal concentration was the combined high and low tide concentrations, there was a significant increase ($p<0.05$) in NH_4-N concentration from dry to wet season in the upper estuary, Lagoon and Ashmead. There was no significant difference ($p>0.05$) in this seasonal change for the Bay. Since, the loading of NH_4-N to the Ashmead did not demonstrate the direct ability to increase NH_4-N concentration at a tidal scale. It was improbable that loading was responsible for this increase at a seasonal scale given the high exchange volumes of the Ashmead (e.g., 92% at spring tide). The exchange time in the upper estuary was considerably longer than in the Ashmead, and estimates vary depending on the method used. The method used by Largier et al. (2000) (i.e. the Dyer method) recorded a flushing time of 18 days for the upper reaches of the Estuary, while Switzer and Waldron (2002) recorded a water exchange time of 177 days for the upper estuary region. Given the slower water exchange times in the upper estuary, the loading of NH_4-N from the Knysna River should comprise a greater portion of the increase. However, it was unlikely that the increases in NH_4-N were completely the result of NH_4-N loading from the Knysna River.

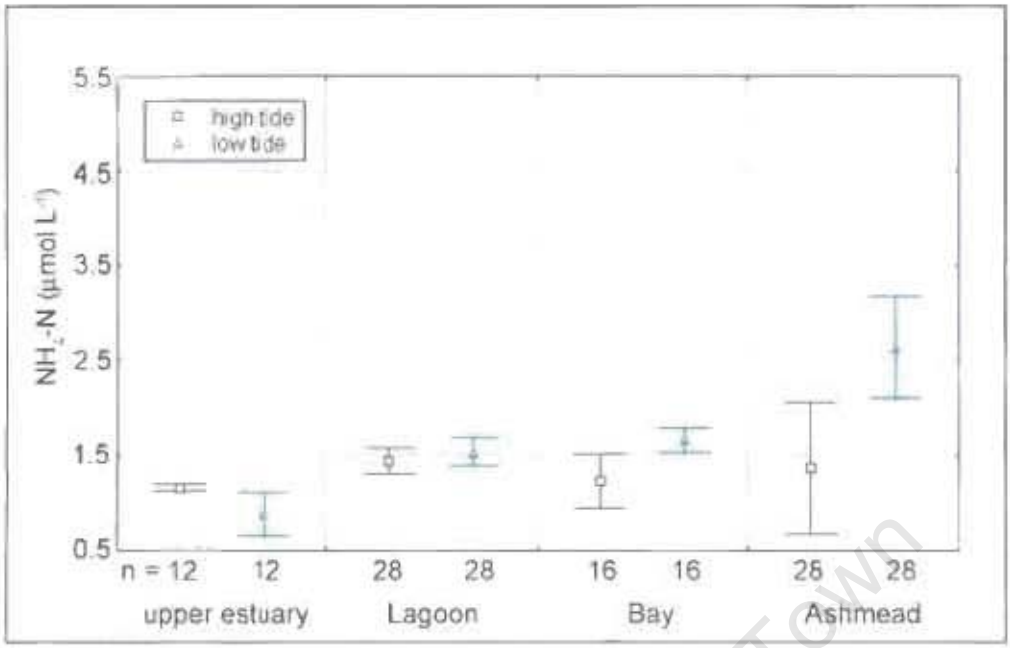


Figure 2.3. The NH_4-N concentrations of the water column in each region during high and low tide of the dry season (n = sample size at each tide).

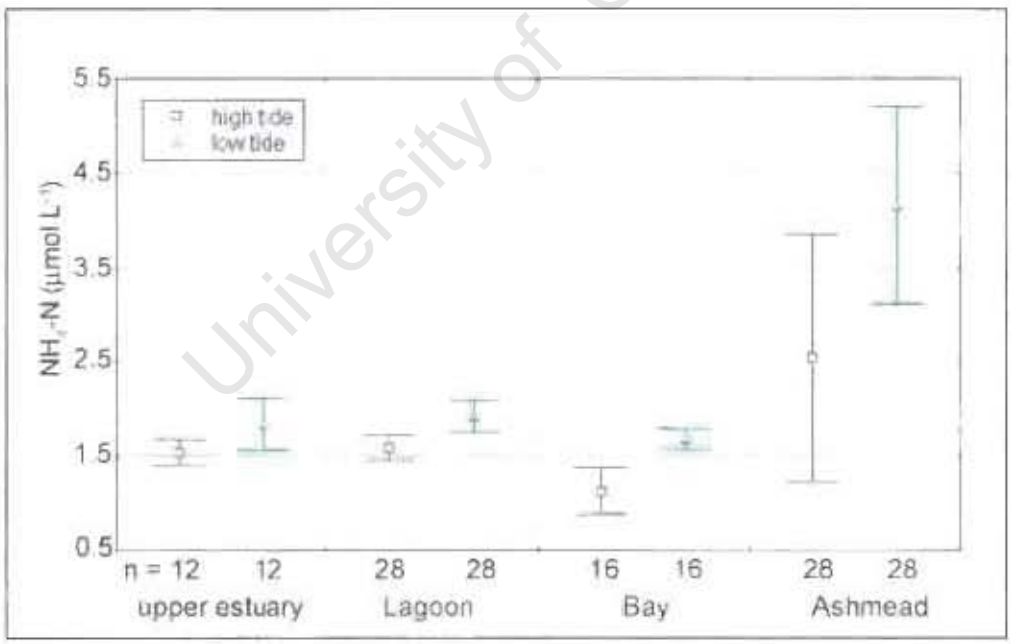


Figure 2.4. The NH_4-N concentrations water column in each region during high and low tide of the wet season (n = sample size at each tide).

Table 2.6. The expected increases in nutrient concentrations ($\mu mol\ hr^{-1}\ L^{-1}$) due to STW effluent entering the Ashmead during LWST volume for one hour. These values represented the maximum (yet limited) influence the STW effluent could impose on the LWST water volume of the Ashmead. At HWST volumes the loading effect of STW effluent entering the Ashmead is nil.

Date	Sampling Conditions	NH_4-N	NO_x-N	DIP	urea
3/8/2000	mid dry season	0.0	0.0	0.1	0.0
3/11/2000	early wet season	0.0	0.0	0.1	0.0
20/2/2001	late wet season	0.0	0.0	0.1	0.0
30/8/2001	at heavy rainfall	0.2	1.2	0.6	0.0

Nitrates

The high tide NO_x-N concentrations were significantly higher ($p<0.05$) in the Lagoon, Bay and Ashmead regions as opposed to the low tide concentrations during the dry season (Figure 2.5). The high tide NO_x-N concentrations in the upper estuary during the dry season showed no significant difference ($p>0.05$) from the low tide concentrations. There were no significant differences ($p>0.05$) between the high tide and low tide concentrations of NO_x-N during the wet season in any of the regions (Figure 2.6).

The loading of NO_x-N in the Ashmead during the dry season from point sources was not believed to be the reason for the increase in concentration of NO_x-N from low to high tide. The contributions of NO_x-N into the Ashmead during the dry season were similar in magnitude to the contributions of NH_4-N. Hence the loading calculations were not repeated for NO_x-N, as these calculations for NH_4-N demonstrated that the impact of loading was far below that which was required to increase the concentration of a nutrient in the Ashmead over half a tidal cycle (ca. 6 hours).

The relationship between tidal range and increases in concentrations of NO_x-N during the dry season was investigated by comparing the average NO_x-N concentrations at high tides to concentrations at low tides for spring and neap tides in the Lagoon, Bay and Ashmead (Table 2.7). This analysis demonstrated that during higher tidal ranges (i.e., spring tides) the increase in NO_x-N concentration between low tide and high tide was higher than these increases measured for neap tides in the Ashmead and Lagoon, but not for the Bay.

Since the water column of the adjacent ocean was well mixed during the dry season (refer to section 1.10), upwelling events were not expected to be a source of NO_x-N in the water entering the Ashmead from the Bay during this

season. However, due to the lack of data on NO_x-N concentrations of the incoming water during these surveys of the Ashmead, it can not be eliminated as a source of the increase in NO_x-N concentrations measured from low to high tide.

Table 2.7. The increases in average NO_x-N concentration during the dry season from low tide to high tide. Units equal $\mu mol\ L^{-1}$ half tidal $cycle^{-1}$.

	neap tides	spring tides
Ashmead	1.5	3.5
Bay	2.3	1.5
Lagoon	0.3	1.3

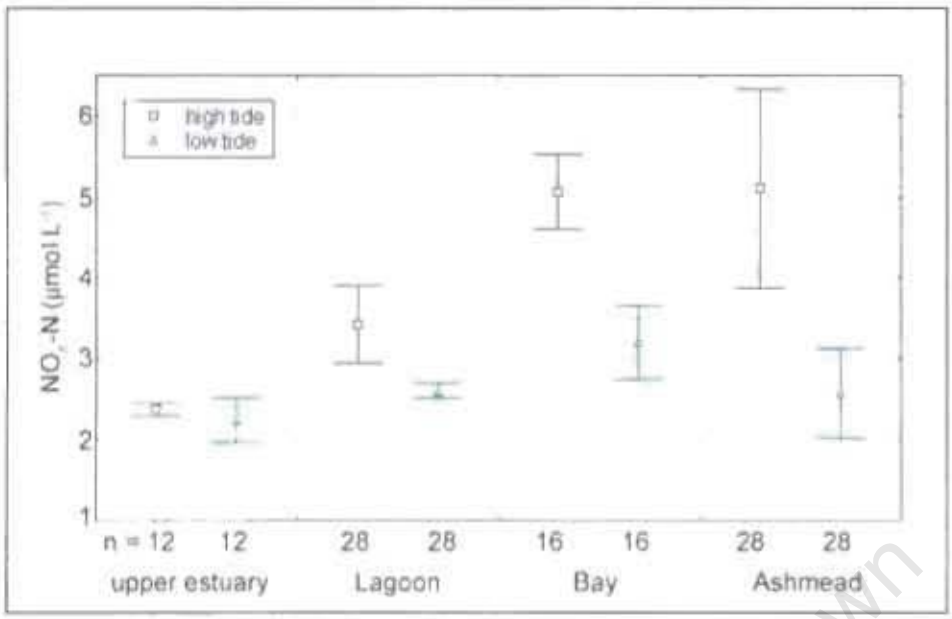


Figure 2.5. The NO_x-N concentrations of the water column in each region during high and low tide of the dry season (n = sample size at each tide).

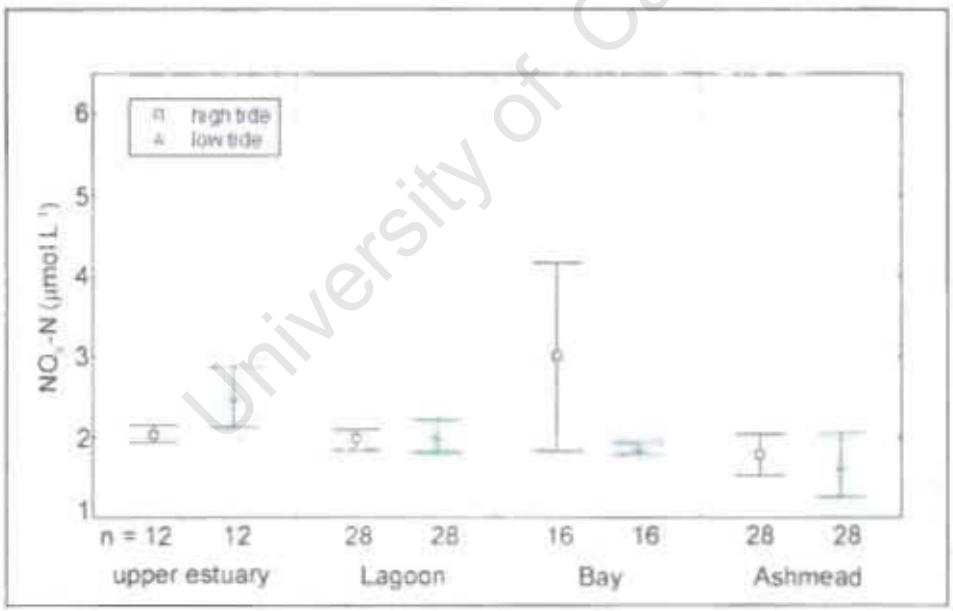


Figure 2.6. The NO_x-N concentrations of the water column in each region during high and low tide of the wet season (n = sample size at each tide).

The relationship of decreasing NH_4-N concentration and increasing NO_x-N concentrations during the dry season over half a tidal cycle indicated that these two events were linked. The relationship between the decrease in NH_4-N and the increase in NO_x-N concentration on a tidal scale is not new to estuarine science. The pattern was first documented in Knysna Estuary on a seasonal scale by Grindley (1985) and this pattern was commonly observed in many oxygenated aquatic sediments (Billen, 1982; Nedwell et al., 1982; Jenkins and Kemp, 1984; Boynton and Kemp, 1985). However, this high-frequency method of sampling the water column has provided data that indicated for the first time, that processes internal to the Knysna Estuary were responsible for the variations in its concentration of NH_4-N and NO_x-N on a tidal scale.

It was hypothesized that processes internal to the Ashmead were responsible for the increase in NO_x-N concentration between high and low tides in the dry season. To a lesser extent these same processes were responsible for the increases in NO_x-N measured in the Lagoon and Bay regions between low tide and high tide during the dry season.

Dissolved inorganic phosphate

The most striking difference in high and low water concentrations of DIP in these surveys was found in the Ashmead, where average low tide concentrations were significantly higher than those of average high tide during both dry and wet seasons (Figures 2.7 and 2.8). Differences between high and low tide concentrations of DIP were not great in the upper estuary, Lagoon or Bay during either the dry or the wet season. Statistically, there were significant differences in the Lagoon region during the dry season and in the upper estuary and Bay during the wet season, between high and low tides, however these changes were small compared to the changes seen in the Ashmead (Figure 2.8).

The pattern of higher concentrations of DIP in the low tide surveys of the Ashmead was similar to that seen for NH_4-N. These DIP data confirmed the earlier work of Allanson et al. (2000a) that demonstrated this pattern for DIP in 1995 and 1997 in the Ashmead. The authors concluded that this pattern was the result of effluent from the STW increasing the concentrations of DIP in the Ashmead, with this effect becoming more pronounced at low tide. However, patterns of DIP concentrations in the Ashmead, as in the case of NH_4-N, were not explained by the loading from the STW (refer to Table 2.6). In comparison, concentrations of DIP in the upper estuary, Lagoon and Bay were stable.

A storm on November 13, 2000 resulted in the average daily flow of the Knysna River cresting at 46 $m^3 s^{-1}$. The salinity in the Estuary as measured on November 14, 2000 showed that a uniform salinity gradient was established along the main channel from station M1 (0 ppt) to station M14 (28 ppt). When DIP was plotted along the salinity gradient (Figure 2.9a) two findings emerge. First, there was an increase in DIP concentration as the salinity increase. Second, under these conditions, the sea was the main source of DIP as indicated by the concentrations of DIP at salinities greater than 20 ppt, which through the mixing of river and sea water approached the DIP concentrations found in the adjacent ocean (0.8 – 1.0 $\mu mol\ L^{-1}$). When DIP concentration was plotted against salinity for the February 2001 HWST survey (Figure 2.9b), during a period when the Knysna River flow was less than 2 $m^3 s^{-1}$, DIP concentrations at stations M1 – M4 range from 1.6 – 1.8 $\mu mol\ L^{-1}$ indicated a possible source of DIP near this area.

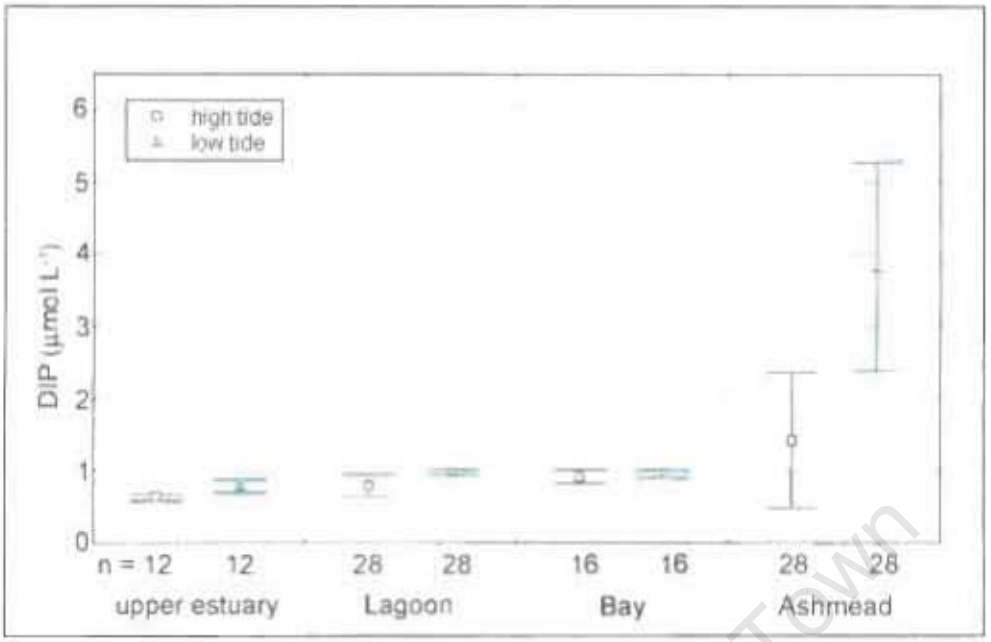


Figure 2.7. The DIP concentrations of the water column in each region during high and low tide of the dry season (n = sample size at each tide).

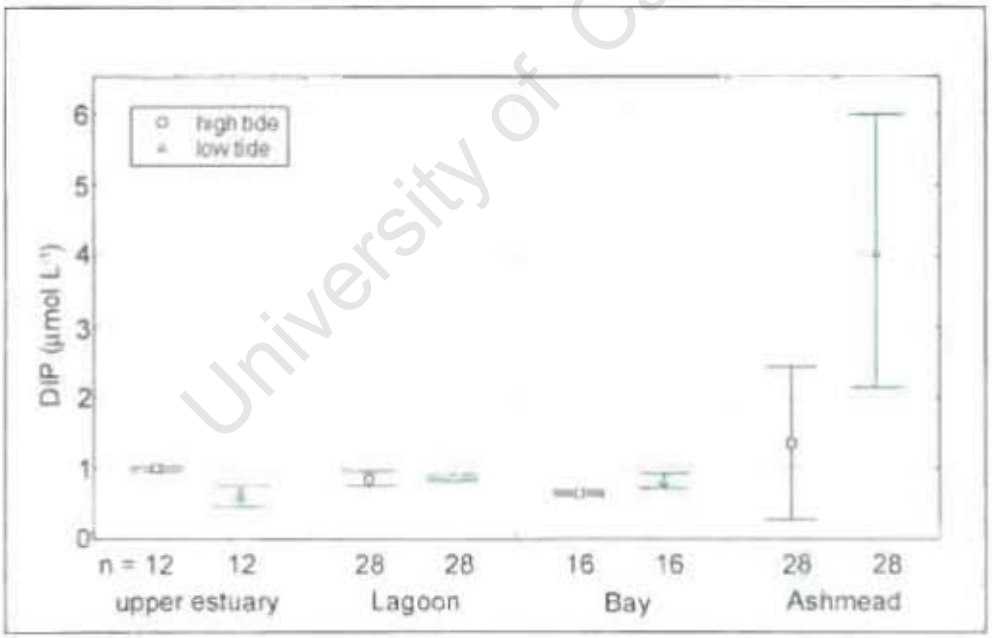


Figure 2.8. The DIP concentrations of the water column in each region during high and low tide of the wet season (n = sample size at each tide).

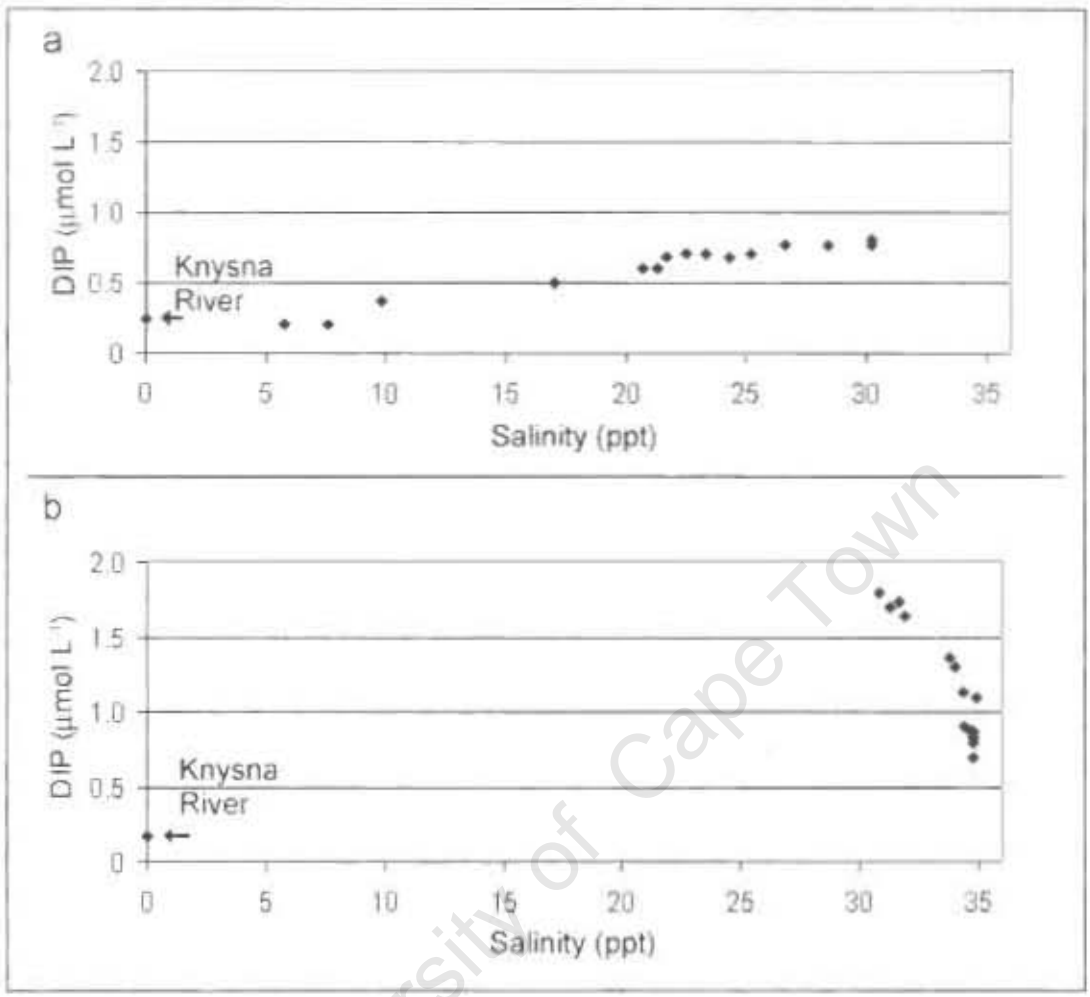


Figure 2.9 DIP concentrations in the Knysna Estuary. (a) DIP concentrations plotted with salinity after the storm of November 13, 2001. (b) Later in the summer these same stations, Knysna River and M1 – M14, were sampled during the February HWST survey and represented salinities typical of the Estuary. This survey indicates a source of DIP during low flow (< 2 $m^3 s^{-1}$) of the Knysna River in the wet season.

As further evidence that the Knysna River was not the direct source of DIP to the estuarine waters, the upper estuary stations K1 – K10 were surveyed on September 29, 2000 after a river flow crested at 5.7 $m^3 s^{-1}$. Salinities of less than

1 ppt were measured at stations K4 – K10 and the DIP concentrations at these stations averaged 0.2 μmol L^{-1}. This survey confirmed that during periods of strong river flow the stations in the upper estuary displayed DIP concentrations similar to those of the Knysna River. However, these same stations displayed an average DIP concentration of 2.3 μmol L^{-1} when surveyed during a low flow period of less than 0.5 m^3 s^{-1} on March 13, 2001. These surveys and data discussed subsequently indicate an internal source of DIP in the upper reaches of the Knysna Estuary.

The DIP concentration for the February HWST survey in the upper estuary and Lagoon demonstrated average DIP concentrations that were not explained by conservative mixing of their respective end-member waters, a pattern that was not seen during similar surveys of the previous October (Figure 2.10). If the water of the upper estuary during HWST was a mixture of upper estuary and Lagoon water at LWST, both having DIP concentrations < 0.8 umol, then mixing of these two waters did not explain the average HWST concentration of 1.8 μmol L^{-1} (n = 3). Likewise, the average concentration of 1.2 μmol L^{-1} (n = 6) measured at HWST in the Lagoon exceeded the DIP concentration found in the flooding tide, as the average DIP concentration in the Bay during the February HWST survey was 0.8 μmol L^{-1}. These data provided evidence that the internal source of DIP was limited to the upper estuary and Lagoon regions, and the increases of DIP from this source were greatest during the higher tidal ranges of spring tide.

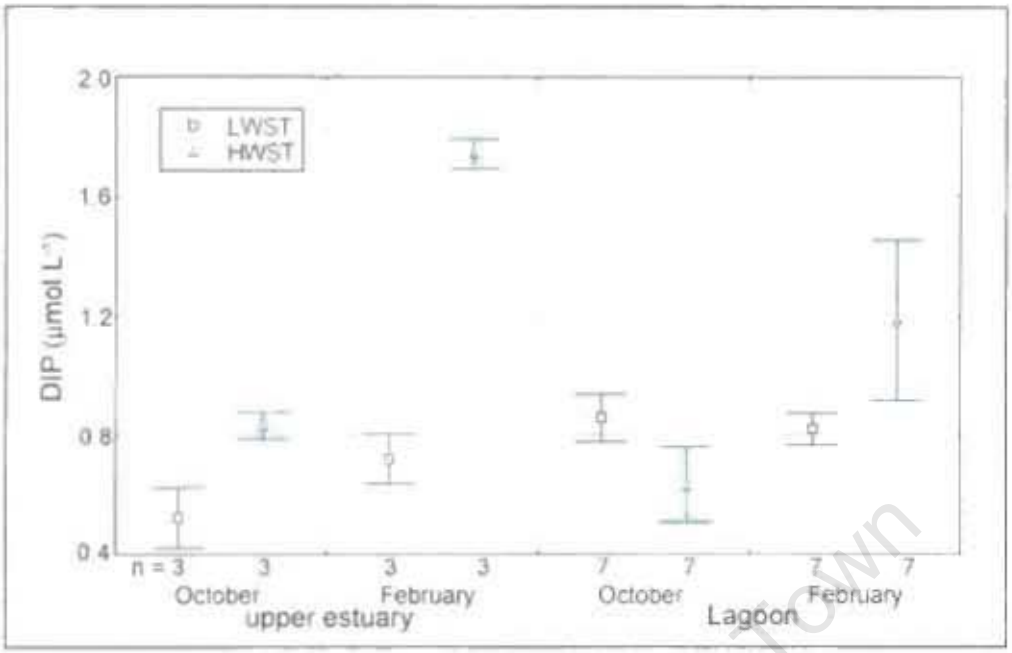


Figure 2.10. The changes in DIP concentration in the upper estuary and Lagoon from the survey data of October 2000 and February 2001. Note the magnitude of increase in DIP from LWST to HWST during February in both regions. Similar changes were not found during the LWST and HWST surveys the previous October.

The February 2001 total rainfall was 18 mm, while the previous 3 months of rainfall ranged between 81 and 99 mm per month. During January two high river flow events resulted in average daily flows of 44 and 16 $m^3 s^{-1}$. The periods of high river flow and sustained high flow during wet months brought a fresh supply of sediment to the Estuary from the Knysna River (Allanson et al., 2000a). It follows that the upper estuary and Lagoon received the majority of these sediments.

Froelich (1988) recorded that sediments with high clay content can bind P in environments where the pH was less than 6, and release this bound P when pH increased beyond this value. The Knysna River water pH was approximately 5

(refer to section 1.4), and during periods of high river flows this low salinity water occupied the upper estuary and Lagoon regions (Largier et al., 2000 and this dissertation). It was suspected that sediments introduced to the Estuary from river water during high flow events were a source of DIP in the upper estuary and Lagoon regions. However, the transportation of sediments were not considered as the cause of DIP increases measured during low tides in the Ashmead as those increases were measured in dry and wet seasons. In addition, the distance between the Ashmead and the head of the Estuary puts it beyond the influence of fluvial sediments originating from the Knysna River.

The data introduced in this section have identified two separate patterns of increasing DIP concentrations, (1) the increase in DIP concentrations at LWST primarily affecting the Ashmead and (2) increase in DIP concentrations in the upper estuary and Lagoon following strong river flows of the wet season 2001. Clearly, additional investigations were required to resolve the mechanism behind these sources of DIP enrichment, and will be addressed in section 3.5.

Urea

When urea concentrations in the water column were considered with respect to tidal state they did not display a trend analogous to the previously discussed nutrients. Urea concentrations were therefore analyzed with respect to the seasons in which the samples were collected. The difference in urea concentrations between wet and dry seasons in each region displayed a notable pattern, with urea concentration significantly higher ($p<0.05$) during the wet season as opposed to the dry season in the Lagoon, Bay and Ashmead (Figure 2.11).

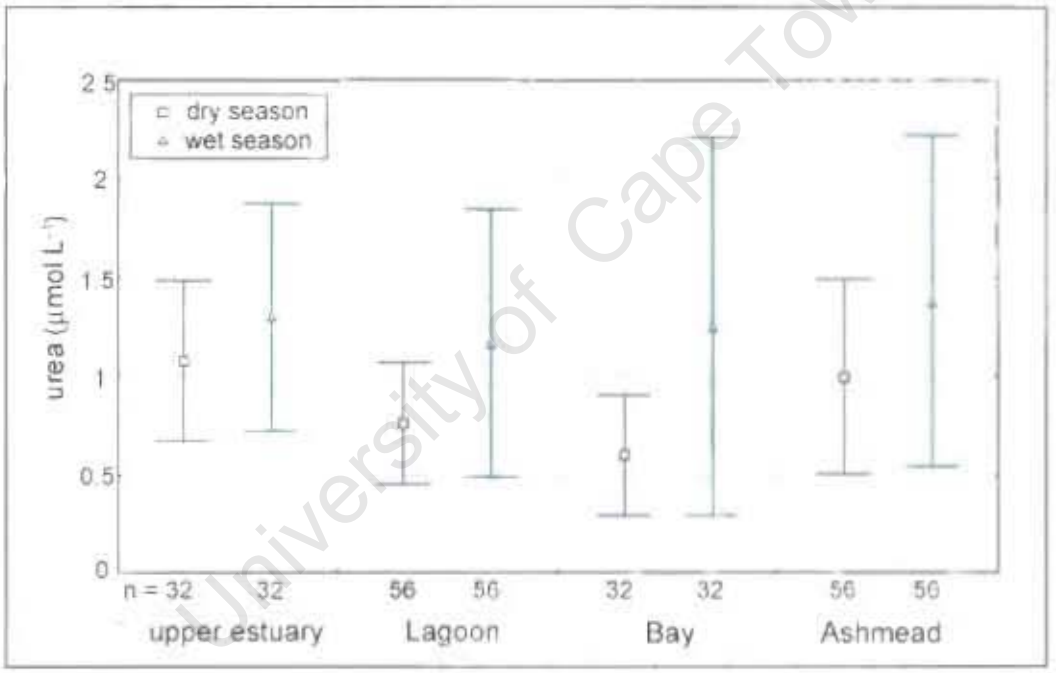
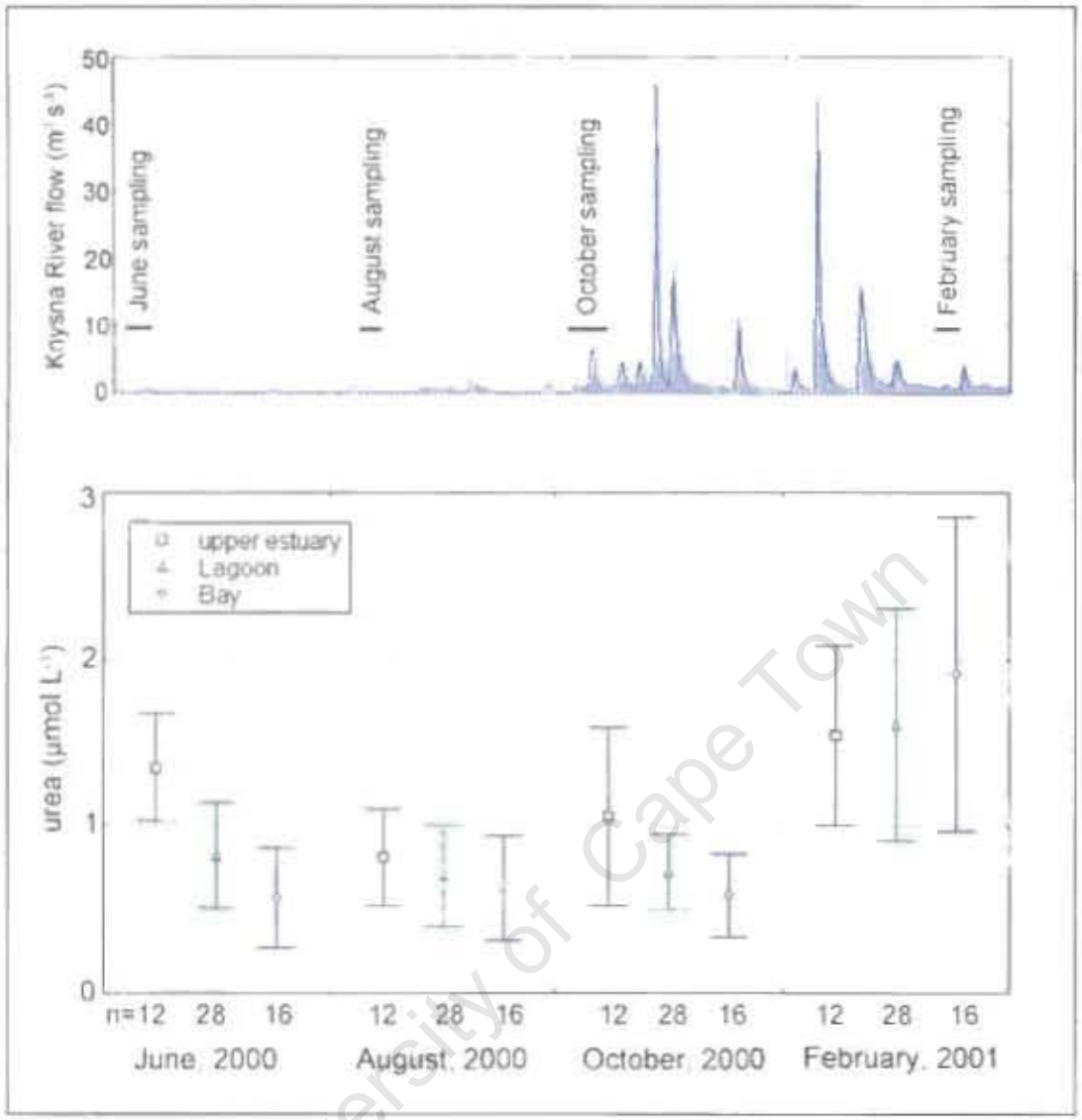


Figure 2.11. The urea concentrations of all regions with respect to dry and wet seasons (n = sample size at each tide).

This preliminary analysis shows that the variations in urea concentration may be best understood by investigating the impact of urea loading on the Knysna Estuary. For example, the changes in urea concentration after a storm of

November 13, 2000 resulted in a 4-fold increase in urea concentrations at stations M1 – M14 as compared to urea concentrations measured at these stations 2 to 4 weeks prior to the storm. This increase in urea concentration was considered to be due, in part, to the runoff of fertilizers used during springtime in beef and dairy cattle farming operations, which entered the Estuary in the flow of the Knysna River (refer to section 2.4). Additionally, this increase in urea may be augmented by the input of urea from pit toilets, which serve as sewage facilities in many informal housing settlements surrounding Knysna. The effluent from these pit toilets drains to the Estuary during the rainfalls of the wet season (refer to section 2.2). The impact of this loading, and subsequent similar urea loading events resulting from the higher flow rates of the wet season, were responsible for a portion of the higher concentrations of urea in the Lagoon and Bay. The relationship between the higher urea concentration measured during February 2001 surveys and the higher flow rates of the wet season is illustrated in Figure 2.12.

The peak in urea concentration during the summer months in Knysna Estuary was similar to the pattern of increases in urea concentration measured in Chesapeake Bay (Lomas et al., 2002). The long-term data set utilized by Lomas et al. (2002) allowed the comparison of urea concentrations measured over 6 years in the surface waters, showing a distinct maximum in the summer. The comparison over 7 years in the deep water of the Chesapeake Bay showed a maximum concentration of urea during spring, and represented an approximate 3-fold increase over the mean urea concentration of the winters of these years. Although the mean urea concentration (winter and summer) that was reported in Lomas et al. (2000) was roughly half of those concentrations measured in Bay region of Knysna Estuary, the magnitude of increase in urea concentration from winter to summer was similar, approximately doubling from winter to summer.



Figures 2.12. Urea concentrations in the Knysna Estuary. The high urea concentrations in the upper estuary, Lagoon and Bay during February were partially the result of urea introduced during the strong flows of the Knysna River during the wet season.

While the investigation of variations in urea concentrations with respect to the season, and specifically the impact of urea loading during wet season has been revealing it does not account for all the variations that were measured. The urea concentrations in the upper estuary during the dry season were significantly

higher ($p<0.05$) than the urea concentrations in the Lagoon and Bay during the dry season (Figure 2.11). This indicated that a source of urea was maintaining these higher concentrations in the upper estuary, as opposed to the Lagoon and Bay, throughout the dry season. However, the loading of urea from the Knysna River, using an average flow of 1 $m^3 s^{-1}$ and contribution rate of 15 grams $hour^{-1}$ (measured on September 29, 2000) amounted to zero when diluted to the average volume of the upper estuary of 3.2 $x10^6$ m^3. Likewise, urea concentrations in the Ashmead during the dry season were significantly higher ($p<0.05$) than the Bay (Figure 2.11), and estimates of urea loading of the Ashmead during the dry season did not account for these higher concentrations.

The patterns of variation in urea concentrations within the Estuary were not completely discerned by analysis with regard to tidal state or the impact of loading. It is likely that these data did not indicate all the processes that were responsible for urea loading within the Estuary.

2.4 The effects of a spring storm

The effects of rainfall and the specific impacts of the storm of November 13, 2000 have been introduced in previous sections. This storm is described in more detail here. Understanding the patterns of nutrient concentration in the Estuary following this event was essential in assessing the biological and chemical responses of the Estuary during flood events, which, because of changes in land use in the catchment introduced significant quantities of nutrients of anthropogenic origin.

The Knysna Estuary, in common with all South African estuaries, experiences long periods of low flow during the dry season (Allanson et al., 2000a, Largier et al., 2000, this dissertation). When floods occur, they flush the Estuary with river

water and the Estuary is essentially "reset". Haw (1984) noted that flood events might serve to cleanse the Estuary of marine-dominated organisms that intrude into the middle and upper reaches during sustained dry periods. Similarly, areas of the Estuary characterised by limited circulation receive riverine sources of N and P during these flood events, which may be important in stimulating processes that maintain the trophic status of the Estuary.

The first substantial rainfall of the wet season of 2000 was 29 mm of rain in 24 hours that ended on October 25 at 08:00 as measured at the National Parks Board offices on Thesen Jetty, with a total of 35.5 mm accumulating over the previous 48 hours. Water samples taken at stations M1 – M14 approximately 12 hours after the storm ended, showed nutrient concentrations and salinity remained near pre-storm levels (refer to Appendix B). Flow rates of the Knysna River, as a result of this first rain of the wet season, increased to approximately 6 $m^3 s^{-1}$ for the two days following the storm. Although this amount of rain appeared substantial after many months of dry conditions, it only served to wet the land and had little affect on the flow of the Knysna River or on the Estuary.

The second rainfall of the wet season 2000 was 39 mm in 24 hours ending 08:00 on November 13, 2000. After this storm, the flow of the Knysna River increased to 45.9 $m^3 s^{-1}$ on November 14, 2000, indicating that the catchment moisture status had remained high from the previous rainfall, which resulted in a much larger portion of this rainfall becoming runoff (refer to section 1.6). This 18-fold increase in flow of the Knysna River was greater than the average of daily flows from the previous 10 days (2.5 $m^3 s^{-1}$) and had a measurable impact on the salinity and nutrients of the Knysna Estuary.

Urea, NH_4-N, NO_x-N and DIP concentrations were monitored during LWST at stations M1 – M15 at 12, 36 and 84 hours following the storm. These data were then compared to baseline data for the nutrients measured as part of the

quarterly sampling surveys discussed previously. Baseline data represent conditions in the Knysna Estuary 2 to 4 weeks prior to the storm of November 13, 2000. The concentration of each of these nutrients was a result of their particular origin and can be best explained by considering each nutrient individually.

Urea

The greater than 4-fold increase in urea concentration in the Estuary after this storm was due in part to the runoff of urea-based fertilizers used on the pasturelands of the catchment area (Figure 2.13). The use of urea-based fertilizers was a method of enriching grazing lands on cattle farms of the Knysna catchment area. Cattle farmers spread these urea fertilizers in granular form on top of their fields after the first substantial rains of the spring have fallen. For example, Charlesford Farm, bordering the Knysna Estuary both upstream and downstream of the Charlesford weir with 70 hectares of pasture lands purchased 5000 kg of urea-based fertilizer during early spring of 2000 (Jacques van Hystein, fertilizer sales Tuinroete Agri, personal communication). If this urea-based fertilizer was spread on these pasturelands after the storm of October 25, 2000, the following storm on November 13, 2000 could have washed a portion of it into the tributaries of the Knysna River or directly into the Estuary where these pasturelands border the upper estuary. Similarly, cattle farms upstream of the Charlesford Farm could have contributed to the quantity of urea entering the Estuary during this time. The use of urea-based fertilizers was not restricted to the cattle farms of the Knysna catchment basin but was also used on golf courses and residential properties in and around Knysna.

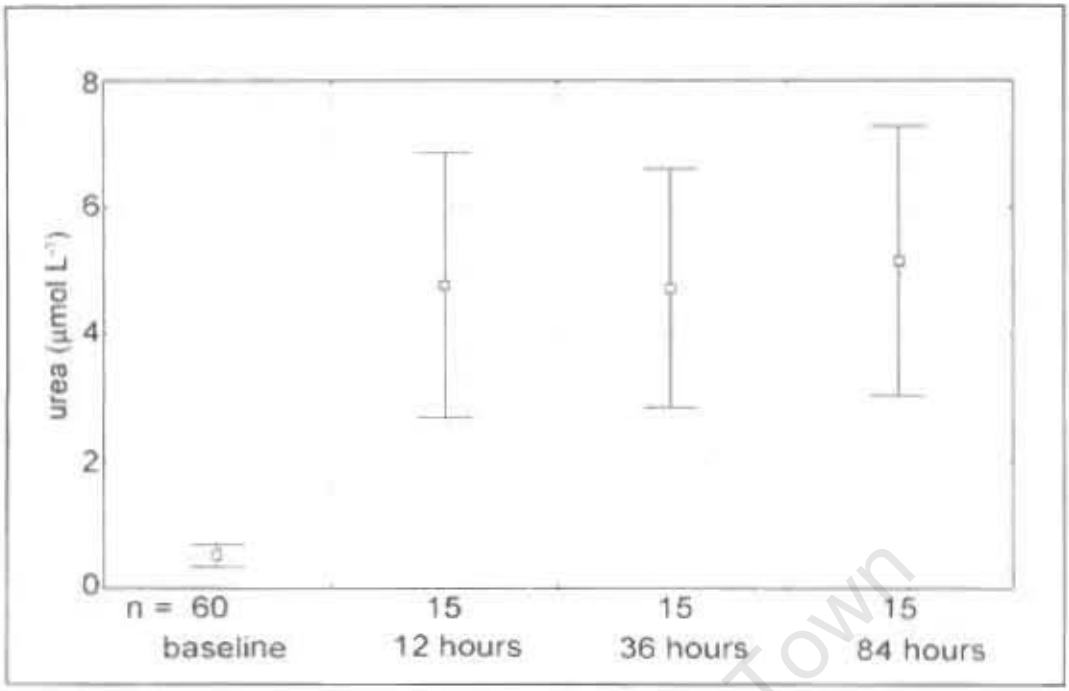


Figure 2.13. The urea concentrations in the Knysna Estuary 12, 36 and 84 hours after storm of November 13, 2000 and baseline values of urea taken 2 to 4 weeks prior to the storm.

The amount of any nutrient needed to increase the concentration within the Knysna Estuary by the amount witnessed after this storm can be calculated by multiplying the amount of increase in the concentration above baseline values by volume of the Knysna Estuary at the time samples were taken. The amount of urea required to increase the LWST concentration in the Knysna Estuary by 4 µmol L^{-1}, the increase seen post rainfall event, was approximately 1500 kg.

The concentration of urea in the Knysna River when measured 48 hours after the rainfall event was 5.8 µmol L^{-1}. It was assumed that the concentration of urea was higher during the initial runoff period when flow of the Knysna River peaked during the first 24 hours after the storm. If the urea concentration were approximately twice that which was measured 48 hours after the storm, or

12 μmol L^{-1}, then the average daily flow of 46 m^3 s^{-1} on November 13, 2000 would result in a one-day loading of 1200 kg of urea to the Estuary.
The Salt River was sampled 48 hours after the November 13, 2000 storm and the urea concentration was 14.4 μmol L^{-1}, with a flow of 4.9 x 10^{-3} m^3 s^{-1}. Other storm drains sampled on November 15, 2000 displayed concentrations of urea ranging from 10.2 to 18.1 μmol L^{-1} (refer to Appendix B). The increase in urea in the Salt River and storm drains in the town of Knysna may be the result of urea-based fertilizers used on residential and commercial properties, and the effluent washed downstream from pit toilets in the informal housing settlements within the Knysna town area.

In summary, the increase in urea concentration in the Knysna River during and after this storm resulted in a proportion of the urea increase in the Knysna Estuary. Additionally, the Salt River and storm drains in the Knysna area would have an impact on this urea loading through increased urea concentration in their respective runoff waters during and after this storm. Non-point sources, such as runoff from pastureland, fertilized lawns and drainage from septic tanks bordering the Estuary can also be expected to add to the urea concentration during periods of high rainfall. However, the urea increases associated with these sources may be confined to a period following this first storm of the season, and additional sources of urea would, therefore, be required to sustain the high concentrations of urea measured in the Estuary during February (refer to Figure 2.12). This subject will be considered again in the final discussion (Part IV).

Ammonium

The NH_4-N increases throughout the Knysna Estuary were not easily explained by the contribution of NH_4-N provided by the Knysna River (Figure 2.14). To

raise the concentration by 4 μmol L^{-1} during LWST, the change seen 36 hours after the storm required the addition of 756 kg of NH_4-N in the Knysna Estuary. Even if the NH_4-N concentration in the Knysna River at peak flow were assumed to be twice that of the concentration that was measured 48 hours after the storm, this contribution can only account for 280 kg of NH_4-N.

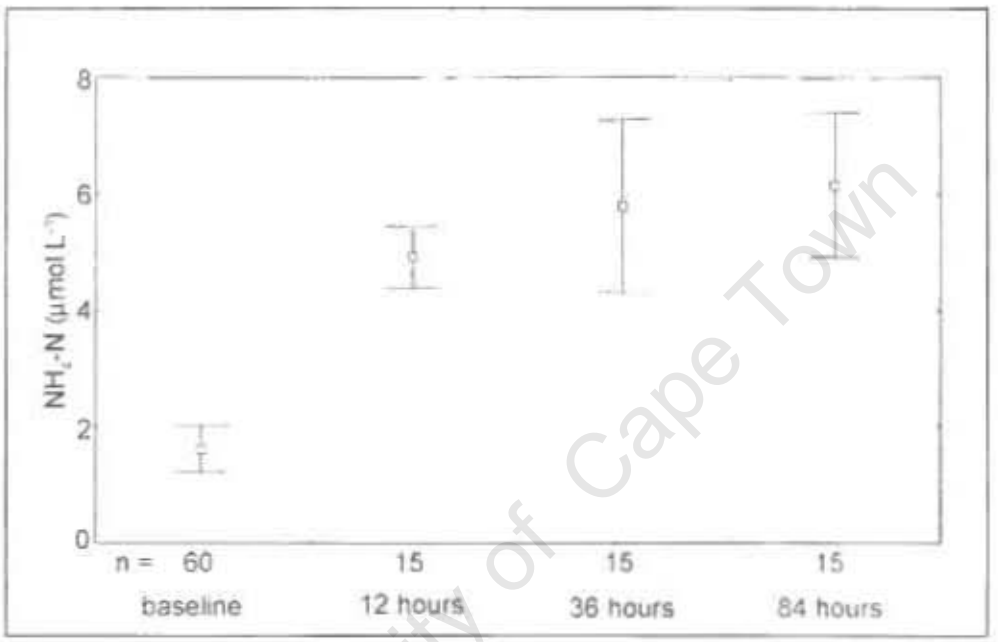


Figure 2.14. The NH_4-N concentrations in the Knysna Estuary 12, 24 and 36 hours after storm of November 13, 2000 and baseline values taken 2 to 4 weeks prior to the storm.

It was likely that the increase in NH_4-N was due, in part, to the breakdown of the labile urea molecule once it had reached the Knysna Estuary. Berg et al. (2001) stated that, urea is rapidly mineralized in both the water column and sediments preceding further hydrolysis to NH_4-N. The breakdown of each kg of urea would result in 2 kg of NH_4-N. It is also conceivable that other sources of NH_4-N, which were not measured in this study, were responsible for the additional loading witnessed after this storm. For example, NH_4-N secretions (e.g., stress

response) from the three million oysters that were cultivated in the Knysna Estuary during this time period, and secretions of NH_4-N from other biological sources (e.g., dense populations of the mudprawn, *Upogebia africana*) could provide an intrinsic source of NH_4-N to the Estuary (Professor B. Allanson, personal communication).

Nitrates

The highest measured NO_x-N concentration in the Knysna River followed increased flow rates at the beginning of the wet season in both 2000 and 2001. The concentration of NO_x-N in the Knysna River at 48 hours after this storm, which was not the peak flow of the storm flow, was still remarkably high at 10.4 μmol L^{-1}.

A study by Louw and Scholes (2002) showed that forest plantation soils of the Mpumalanga escarpment of South Africa have their highest production of NH_4-N during the winter (i.e. dry season). This study also showed a correlation between production of NO_x-N and NH_4-N in these forest soils, as NH_4-N is oxidized to NO_x-N in the absence of uptake by plants. It follows that NO_x-N concentration in these soils would be at or near a maximum during the first rainfalls of the wet season. Their study also showed that in the absence of plant uptake or microbial immobilization, NO_x-N can move rapidly in the downward flow of water through the soil profile, and the mobility of NO_x-N is promoted by the low anion exchange capacity of most South African forest soils. The combination of high concentrations of NO_x-N in these soils and its mobility and capacity for runoff was likely the cause of the high concentration of NO_x-N measured in the Knysna River during the initial runoff of the wet season.

The concentration of NO_x-N in the Knysna Estuary did not peak until 84 hours after the storm (Figure 2.15). The 84-hour post storm concentration of NO_x-N represented an increase of 1134 kg over baseline concentrations. However, since the NO_x-N concentrations increased over a period of 3 days after the storm, only reaching the peak concentration on the third day, two possible hypotheses can be examined. First, the increase in NO_x-N concentration was from the oxidation of the abundant NH_4-N. This process termed nitrification will be discussed in Part III, when the utilization of these nutrients will be considered. Second, the increase in NO_x-N concentration in the Estuary could be due to a delayed release of NO_x-N from the soils of the forest, which may be slower that which was measured for NH_4-N or urea.

Loading from the Knysna River the first day after storm would account for all of this loading or approximately 1100 kg if the NO_x-N concentration were assumed to be twice the concentration measured at 48 hours after this storm. Both debris left by the receding flow and observations by the occupants of Charlesford farm indicate that the flow rate at the Charlesford Weir was twice as high during the first 24 hours after the storm. Therefore, the loading would have been twice that of the loading that was calculated by flow and concentration data taken 48 hours after the storm. However, since the NO_x-N concentration was not measured until 48 hours after the storm it can not be assumed to be constant during the initial 48 hours.

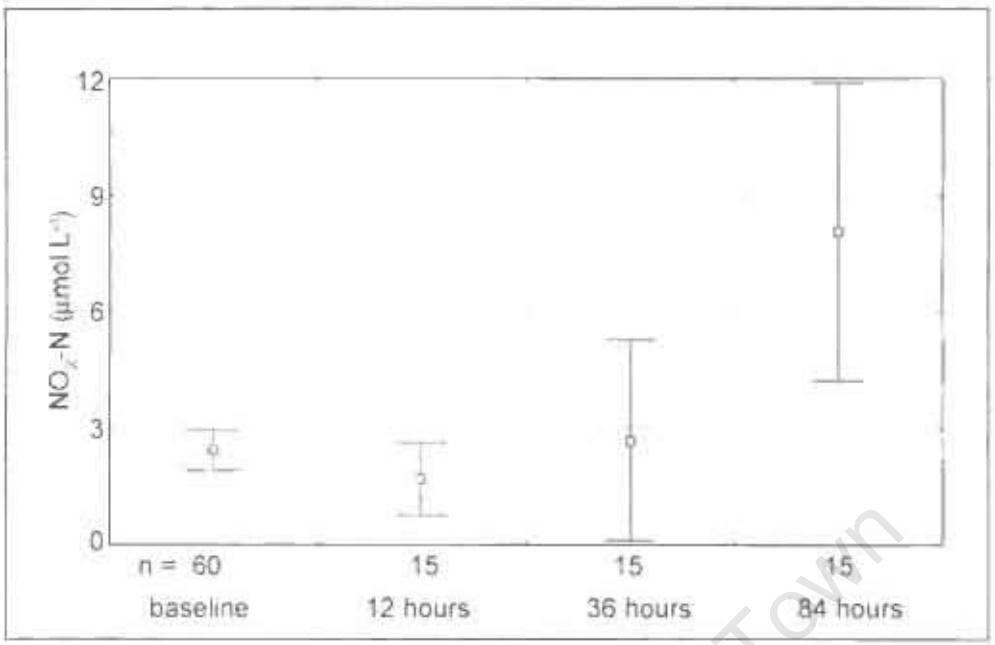


Figure 2.15. The NO_x-N concentrations in the Knysna Estuary 12, 36 and 84 hours after storm of November 13, 2000 and baseline values of NO_x-N taken 2 to 4 weeks prior to this storm.

At these concentrations, NO_x-N displays near-conservative behavior with respect to the salinity gradient (Figure 2.16). This indicated that riverine water and its associated NO_x-N passes rapidly through the Estuary after this flood, so that benthic and water column processes, which affect the uptake of this nutrient, did not affect its concentration. This was essentially the same conclusion reached by Balls (1994), who showed that rapidly flushed low-turbidity estuaries were more likely to demonstrate conservative mixing behavior with respect to phosphate and nitrates. This information supports the theory that a delayed release of NO_x-N from forest soils following this storm, rather than nitrification, is most likely responsible for the peak NO_x-N concentration being reached on the third day after the storm.

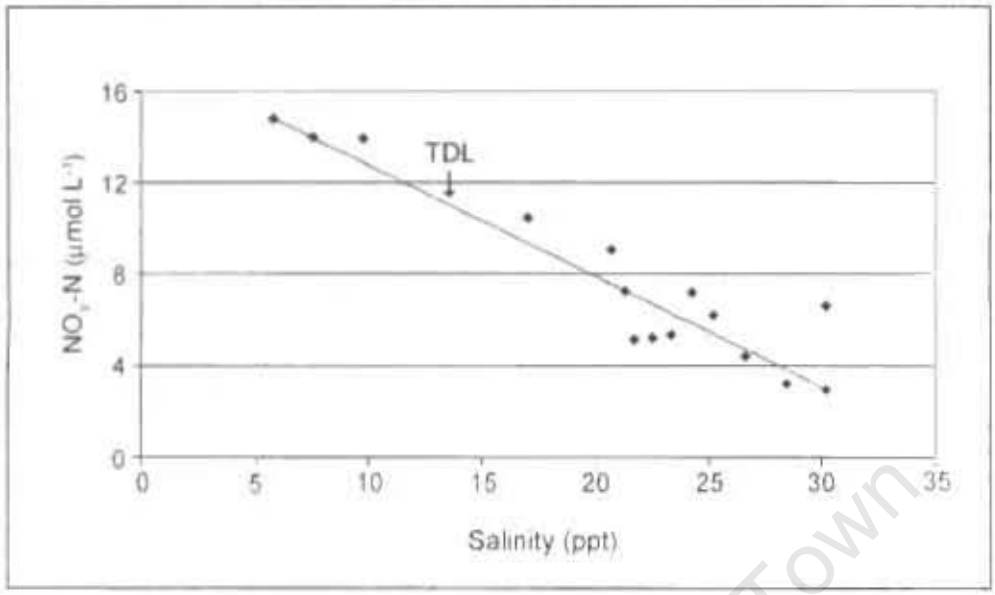


Figure 2.16. The near-conservative behavior of NO_x-N is shown by the close match with the theoretical dilution line (TDL) during the survey of stations M1 – M15, 84 hours after the storm of November 13, 2000.

Dissolved Inorganic Phosphate

In contrast to the other nutrients measured after the storm of November 13, 2000, DIP concentrations decreased (Figure 2.17). The DIP concentration in the Knysna River on November 13, 2000 was 0.3 μmol L^{-1}, lower than the baseline concentration of 0.8 μmol L^{-1} in the Estuary. The inflow of the Knysna River represented an input of 4.4×10^6 m^3 during the first 36 hours. This volume represented one third of the 13.2×10^6 m^3 tidal volume of the main channel during LWST. Calculating a weighted-average with respect to volume for DIP in the main channel using these values gave an expected concentration of 0.6 μmol L^{-1}. This concentration was equal to the measured value of 0.6 μmol L^{-1} 36 hours after the storm (refer to Figure 2.9a).

These results suggested that during a period of high flow of the Knysna River, the DIP concentration showed near-conservative behavior with respect to salinity. Therefore, the internal processes that may add to, or take away from the concentration of this nutrient were near zero or very small during this time.

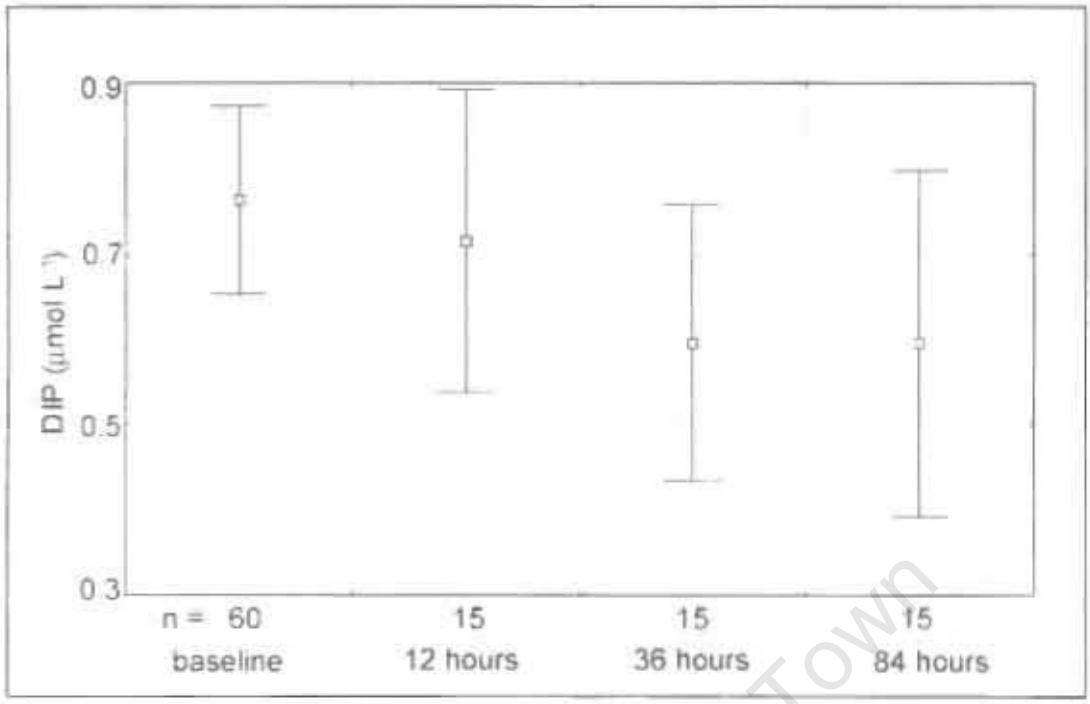


Figure 2.17. The concentration of DIP at stations M1 – M15, as measured 12, 36 and 84-hours after storm of November 13, 2000 and baseline values measured 2 to 4 weeks prior to the storm. The DIP concentration decreased in the days after the storm due to dilution with low-phosphate river water.

Part III: The Utilization of Nutrients

Grindley (1985) noted a decreasing gradient of NO_x-N concentration from the mouth to the head during an August survey of ten stations along the main channel of Knysna Estuary in 1976. When the same survey was done the following April this gradient had reversed. No reason was given for this seasonal change and he suggested a further investigation into its cause.

Allanson et al. (2000a) was the next study that comprehensively addressed nutrients and their variation in the Knysna Estuary. This study found concentrations of NO_x-N and DIP were significantly higher in the Ashmead than in the rest of the Knysna Estuary. They examined the causes for these higher concentrations, by monitoring the inflow of nutrients through storm water drains and STW effluent. This study implicated tidal dilution of effluent from the STW as the cause of decreased concentrations of DIP and NO_x-N at high tide in the Ashmead as opposed to low tide. They also found a seasonal variation of NH_4-N and NO_x-N in the Ashmead, with NH_4-N concentration significantly higher ($p<0.05$) in the summer than in the winter and NOx-N significantly higher ($p<0.05$) in winter as opposed to summer. They implicated increased flow of effluent in the summer as the reason for this pattern.

In their final conclusions Allanson et al. (2000a) revealed that loading played a localized role in nutrient variation in the water column of the main channel. However, it was tidal flushing by the substantial tidal prism coupled with mineralization and utilization within the sediments that prevented the buildup of nutrients in the water column. While this conclusion suggested that the role of benthic flux affected the nutrient concentrations in the Estuary, no experimental investigation was made into these fluxes, or of phytoplankton uptake of the nutrients.

The seasonal pattern described in Part II of higher NH_4-N concentrations in the summer, and higher NO_x-N in the winter is consistent with data from Grindley (1985) and Allanson et al. (2000a). However, as demonstrated earlier, nutrient variations in the Ashmead were not explained by the loading of effluent from STW and storm drains, even during periods of heavy rainfall (refer to section 2.3). And aside from the storm of November 13, 2000, when post-storm concentrations of NO_x-N and DIP showed near-conservative behavior with respect to salinity, nutrient variations in the upper estuary, Lagoon and Bay were, similarly not explained by loading.

Despite the evidence that loading was not responsible for the higher concentrations of NH_4-N, NO_x-N and DIP in the Ashmead, as a general trend the highest average concentrations of these nutrients were measured in this region. Therefore, it was suspected that the source of these nutrients was their flux from the benthic sediments of the Ashmead. The benthic flux of nutrients was not limited to the Ashmead, and was expected to have an influence on the nutrient variations measured in the other regions of the Estuary. In addition to the benthic flux of nutrients, the uptake of nutrients by the phytoplankton community of the Estuary was investigated to determine if uptake by phytoplankton was affected by changes in nutrient concentrations over an annual cycle.

Resolving the role of water column uptake of nutrients and benthic flux of nutrients in the Knysna Estuary will advance the understanding of the observed nutrient variations in the Knysna Estuary. The measurement of benthic fluxes in the upper estuary, Lagoon and Bay regions provided a means of comparing benthic flux in these regions against the Ashmead, and against each other. The purpose of this comparison, was similar to the purpose stated by Boynton and Kemp (1985); to determine if observed benthic nutrient fluxes could be explained in terms of sediment and water column properties along the Estuary.

There are many nutrient budgets prepared for estuaries in North America and Europe, and none of these studies resulted in a closed budget with respect to nutrient production, input, uptake and export (Nixon et al., 1996). Hence, it was not the goal of this section, or this dissertation, to sum these individual processes (e.g., benthic flux, phytoplankton uptake) to the net differences in nutrient concentrations witnessed in the tidal and seasonal surveys of the previous section. The goal of this phase of the study was to quantify the phytoplankton uptake of ammonium, nitrate and urea and the benthic fluxes of NH_4-N, NO_x-N and DIP, both spatially and temporally. This information was then used to make comparisons between the four regions of the Estuary with the purpose of discussing how differences in nutrient concentrations affect phytoplankton uptake and benthic flux.

3.1 Phytoplankton uptake of nutrients

It was the goal of the experiments and subsequent analysis in this section, to measure net phytoplankton uptake, inclusive of microbial uptake (referred to as phytoplankton uptake) during the dry season (i.e., winter) and the wet season (i.e., summer), and to derive general patterns of spatial and temporal nutrient uptake by the phytoplankton. An equally important purpose of this section was to investigate the patterns of changing nutrient uptake by phytoplankton with regard to the changes in the concentrations of nutrient in the Estuary, seasonally, and following the storm of November 13, 2000.

The term dry season and winter are used interchangeably in this section, and similarly the terms of wet season and summer. This was done as the measurements of phytoplankton uptake rates were accomplished within days of the water column nutrient surveys (refer to section 2.3), and therefore are representative of summer and winter conditions with respect to the other data in

this dissertation. It should be noted, that wet season and dry season as defined by the flow rates of the Knysna River (refer to section 1.6) may, in other years, not be representative of winter and summer conditions.

Methods

The uptake of ammonium (NH_4^+), nitrate (NO_3^-) and urea by phytoplankton was measured using ^{15}N tracer techniques and *in situ* incubations as described by Dugdale and Goering (1967). Water was drawn from one meter subsurface using a bilge pump lowered over the side of the boat with its outflow connected to a length of tygon tubing. This method is commonly used in studies of water column organisms and phytoplankton communities (*vide* Amend and Shanks, 1999). At each station, three separate one-liter glass Schott bottles were rinsed with the subsurface water, and then filled with 1000 ml of this water. A quantity of ^{15}N labeled NH_4^+, NO_3^- or urea, prepared in accordance with Waldron and Probyn (1991) hereafter referred to as a spike, was added to each one-liter glass Schott bottle. A sample of the subsurface water was taken to the lab and immediately analyzed for concentrations of these nutrients, providing the ambient concentration of these nutrients prior to the addition of the spike.

The amount of spike was chosen in an attempt to increase the ambient concentration of each nutrient by 10 to 20%. This range was targeted recognizing that over-enrichment could lead to a phytoplankton bloom within the bottle (Trevor Probyn, personal communication). This was achieved by estimating the concentration of each nutrient based on the most recent measurements in the area. The actual percent enrichment of the sample, due to the addition of the spike, was exceeded for NO_3^- where the average enrichment was 25% for the dry season and 37% for the wet season. The average enrichment of NH_4^+ in these experiments approximated the targeted range with 14% enrichment in the dry

season experiments and 12% in the wet season experiments. Urea was lower than this targeted range with enrichment averaging 9% during dry season and 7% during wet season.

After addition of the spike, the glass Schott bottles were suspended in the water column at one meter subsurface for approximately 6 hours. Incubations were terminated by filtration through pre-combusted Whatman GF/F glass fiber filters (500 °C for 16 hours), and the duration of the incubation was recorded. The filters were immediately frozen at –10 °C and later dried at 50 °C for 16 hours. Dried filters were returned for storage at –10 °C and a representative sample of each filter was analyzed with a Finnigan Mat 252 dual inlet stable light isotope ratio mass spectrometer located at the Archaeology Department, University of Cape Town. The mass spectrometer provided the atom percent N and the N peak area of each sample.

The particulate nitrogen (PN) of each sample was estimated from its N peak area, evaluated against the N peak areas of known standards. For example, the percent of PN in the standards and the quantity of standard were known, and the quantities of standards were chosen to provide similar N peak areas as the samples. The standards were analyzed in conjunction with the samples, by running one standard for approximately every 10 samples that were analyzed by the mass spectrometer. The best-fit curve to a plot of N peak area to PN of the known standards, allowed linear regression analysis to be used to determine the PN of each sample. The PN of each sample was corrected against the volume of water filtered, and the quantity of filter that was analyzed.

The specific uptake of each sample was determined as atom percent N of the sample divided by the product of aqueous atom percent N that the spike represented in the incubation and the duration of incubation. The absolute

uptake of each sample was the specific uptake divided by the PN of each sample.

The schedule of these incubation experiments followed a similar pattern to the nutrient monitoring schedule. With the exception of August 2000 at least one incubation experiment was attempted in each region near spring tides, and neap tides during each quarter of the year. Since incubations began in the morning and terminated in the afternoon, the incubations began near slack low on the spring tides, and near slack high on neap tides. This was done in an attempt to average out any changes that may have occurred due to differences in nutrient concentration in the region between slack high and low water.

Results

The quarterly results from ^{15}N incubation experiments were grouped into dry and wet season in accordance with the parameters described in the water column monitoring, section 2.3. The average absolute uptake rate (referred to as uptake rate or uptake) for each nutrient was derived for the upper estuary, Lagoon, Bay and Ashmead (Table 3.1). The results of these ^{15}N incubation experiments provided an overall uptake of an undefined phytoplankton community with a low biomass (typical chlorophyll-*a* < 5 $\mu g\ L^{-1}$). These results do not make reference to specific species uptake or regeneration of nutrients within the water column.

As noted earlier, the Ashmead often displayed nutrient concentrations significantly different from the other regions. Therefore, for comparison of the phytoplankton uptake rates of the Ashmead with the remaining regions of the Estuary, the uptake rates of the upper estuary, Lagoon and Bay were averaged, and referred to as the main channel in this section. The comparison between dry

and wet season average uptake rates for each region was made by paired t-test, where $p<0.05$ was considered a significant change (refer to Table 3.1).

The ^{15}N incubation experiments revealed significant increases ($p<0.05$) in the uptake of NH_4^+, NO_3^- and urea during the wet season (i.e., summer conditions) as compared with the dry season (i.e. winter conditions) in the main channel. While the average uptake of NH_4^+ significantly increased ($p<0.05$) in the Ashmead from dry to wet season, there was no significant ($p>0.05$) difference in the average uptakes of NO_3^- or urea in the Ashmead between dry and wet seasons (refer to Table 3.1).

There was a significant increase ($p<0.05$) in the uptake of urea by the phytoplankton in the Bay and main channel during the wet season as opposed to the dry season. The Ashmead showed a small increase in urea uptake from dry to wet season, which was not significant ($p>0.05$). As shown in section 2.3 (refer to Figure 2.10) there was a significant increase ($p<0.05$) in the urea concentration in the Lagoon, Bay and Ashmead during the wet season. There was no significant change ($p>0.05$) in the concentration of urea in the upper estuary between these seasons.

Table 3.1. Average phytoplankton uptake rates for the dry and wet seasons (μmol N L^{-1} $hour^{-1}$). Values for the main channel (underlined) are the average of values determined for the upper estuary, Lagoon and Bay regions. The difference between the dry and wet seasons (change) is indicated as S, significant ($p<0.05$) or NS, not significant ($p>0.05$).

	Dry Season	Wet Season	change
NH_4^+:			
upper estuary	0.11 (n=8)	0.43 (n=7)	S
Lagoon	0.09 (n=5)	0.41 (n=9)	S
Bay	0.12 (n=8)	0.49 (n=7)	S
main channel	0.11 (n=21)	0.44 (n=23)	S
Ashmead	0.20 (n=6)	0.34 (n=19)	S
NO_3^-:			
upper estuary	0.02 (n=7)	0.06 (n=6)	S
Lagoon	0.01 (n=6)	0.03 (n=7)	NS
Bay	0.03 (n=6)	0.04 (n=4)	NS
main channel	0.02 (n=19)	0.04 (n=17)	S
Ashmead	0.05 (n=7)	0.03 (n=17)	NS
urea:			
upper estuary	0.15 (n=8)	0.33 (n=6)	NS
Lagoon	0.12 (n=6)	0.21 (n=7)	NS
Bay	0.13 (n=8)	0.43 (n=4)	S
main channel	0.14 (n=22)	0.31 (n=17)	S
Ashmead	0.13 (n=6)	0.19 (n=17)	NS

Day and night uptake

In addition to the schedule above, uptake rates were measured during the day and night of May 21, 2000 at two stations (M11 and X11). These ^{15}N incubation experiments were completed at the middle of the day and middle of the night, beginning at 10:00 and 22:00 and terminated four hours later, and tested uptake under light and dark conditions.

NH_4^+, NO_3^- and urea average uptake rate decreased during the night (Table 3.2). The reductions in uptake rate for NH_4^+ and NO_3^- during dark incubations were similar to those reported in Dugdale and Goering (1967) for temperate waters, where dark uptake for NH_4^+ was a reduction of 74% with respect to the light uptake of NH_4^+, and the reduction for NO_3^- was 90%. These data show that the majority of phytoplankton uptake of NH_4^+, NO_3^- and urea occurred during the day.

Table 3.2. Light vs. dark water column uptake of nutrients (μmol N L^{-1} $hour^{-1}$) and the percentage of reduction in this uptake during night incubations.

	Day	Night	reduction
NH_4^+	0.37	0.21	43%
NO_3^-	0.18	0.02	89%
urea	0.48	0.33	30%

Post-storm uptake

The increase in nutrient concentrations witnessed in the main channel as a result of runoff from the November 13, 2000 storm provided an opportunity to examine the changes in uptake rates and nutrient concentrations, during this short-term pulse event. Water samples taken 2 to 4 weeks prior to the storm provided average pre-storm nutrient concentrations for NH_4-N, NO_x-N and urea. After the storm, concentration of these nutrients reached peak levels at different times (refer to section 2.4). For this analysis, pre-storm concentrations were compared to the maximum concentrations reached post-storm.

The ^{15}N incubation experiments accomplished 2 to 4 weeks prior to the storm provided average pre-storm uptake rates of these nutrients along the main channel. Similar ^{15}N incubation experiments were done 72 hours after the storm, providing the average post-storm uptake of these nutrients by the phytoplankton (Table 3.3). These phytoplankton uptake rates were representative of average conditions in the main channel with an equal dispersal of stations in the upper estuary, Lagoon and Bay regions during the pre- and post-storm measurements.

The post-storm uptake rates for NH_4^+, NO_3^- and urea showed a significant decrease ($p<0.05$) when compared to the pre-storm uptake rates of these nutrients in the main channel of the Estuary. It is interesting that these decreases in uptake rates were associated with significant increases ($p<0.05$) in the concentrations of NH_4-N, NO_x-N and urea (Table 3.3).

Table 3.3. Main channel average nutrient concentrations ($\mu mol\ L^{-1}$) and average phytoplankton uptake rates in ($\mu mol\ N\ L^{-1}\ hour^{-1}$) before and after the storm of November 13, 2000. (n = sample size)

	Concentration	Uptake
ammonium pre-storm	1.6 (n = 56)	0.36 (n=10)
ammonium post-storm	5.6 (n=14)	0.26 (n=5)
nitrate pre-storm	2.5* (n=56)	0.06 (n=10)
nitrate post-storm	4.0* (n=14)	0.01 (n=4)
urea pre-storm	0.6 (n=56)	0.29 (n=9)
urea post-storm	4.8 (n=14)	0.15 (n=5)

*measurement includes nitrite (ca. 5% of total NO_3^- + NO_2^-)

Discussion

The concentration of NO_x-N decreased during the wet season (refer to section 2.3), and it is of interest that the average phytoplankton uptake rate of NO_3^- increased significantly ($p<0.05$) in the main channel during this period when the availability of this nutrient was less. While it was not the goal of this study to investigate the reasons behind this, it would seem that there was some requirement for NO_3^- within the phytoplankton community that dictated a minimum amount of its uptake. This is in agreement with other studies (Dugdale and Goering, 1967; Glibert et al., 1982; Dortch, 1990), which discuss a minimum threshold of required uptake of NO_3^-. The review by Dortch (1990) discusses phytoplankton uptake studies that have shown an increase in demand for NO_3^- when supplies are low. This literature stresses that phytoplankton species

composition affects the preference of nutrients, however species composition data was not available for the Knysna Estuary during these uptake experiments.

As discussed earlier (refer to section 2.3), the increase in average urea concentration from winter to summer in Knysna Estuary was of similar magnitude to the increase measured in Chesapeake Bay, approximately doubling from winter to summer. Likewise, the seasonal trend in urea uptake by phytoplankton in Knysna Estuary was similar to the pattern of urea uptake by phytoplankton in Chesapeake Bay as reported by Lomas et al. (2002). Lomas et al. (2002) reported a mean urea uptake rate in the surface waters during summer for Chesapeake Bay of approximately 0.3 μg-at N L^{-1}. This value is equal to the average urea uptake for the main channel during wet season as reported in Table 3.1.

Although there were similarities between Chesapeake Bay and Knysna Estuary with respect to urea, one substantial difference was the higher proportion of measured nitrogen uptake that urea represented in the Knysna Estuary. Lomas et al. (2002) stated that "Annual mean Bay-wide urea uptake rates have fluctuated around 18% of measured nitrogen uptake (sum of NO_3^-, NH_4^+, and urea uptake)". The annual urea uptake for Knysna Estuary was approximately 40% of the measured nitrogen uptake, and exceeded 50% during the summer. Thus, in support of the statement by Lomas et al. (2002), that the planktonic community of Chesapeake Bay is either geared to use urea or can rapidly acclimate to use urea when it is available, the same appears to hold true of Knysna Estuary.

Valiela (1991) recorded that phytoplankton nutrient uptake rates increase as ambient nutrient supply increases, however uptake will not increase indefinitely and will become constant at some higher concentrations as the discrete uptake sites on the cell membranes of available phytoplankton become saturated at

these higher nutrient concentrations. Valiela (1991) concluded that the relation of ambient nutrient supply and growth of phytoplankton cells is at least a three-step process: ambient nutrient concentration determines uptake, this uptake leads to a certain cell content of the nutrient (i.e., internal nutrient content) and cell content sets the growth rate.

Therefore, the nutrient uptake rates of the phytoplankton community of the Knysna Estuary would be expected to increase uptake in response to increases in nutrient concentrations, if the cell uptake sites were not saturated, the internal nutrient content was not satisfied, and growth rate increased. For example, if phytoplankton cells become saturated by these nutrients during a period of quickly increasing nutrient concentrations (i.e., floods) then uptake will reach a constant level, until growth rate of the community increases the available cells. However, in addition to the biological requirements for phytoplankton growth, physical variables such as the dilution of estuarine water with riverine water during flood events may lower their biomass during these floods. A rapid decrease in fresh water flushing time in the Estuary due to increased river flow would be expected to decrease phytoplankton biomass, and decrease nutrient uptake by reducing the amount of phytoplankton cells available for nutrient uptake. Unfortunately, measurements of chlorophyll-*a* concentrations pre- and post-storm were not available for this comparison.

The post-storm nutrient uptake data (Table 3.3) demonstrated a decrease in uptake following an increase in concentration of those nutrients. This data supports a scenario where the physics of the Knysna Estuary regulated the phytoplankton uptake during strong inflow events. It is possible that the increase in river flow to the Estuary associated with this flood reduced the biomass of phytoplankton, and therefore uptake of these nutrients decreased due to a reduction in the available cells. A stress response from the rapid decrease in salinity could also be a factor in the decrease in uptake of these nutrients.

A recent study by Berg et al. (2001) in the Gulf of Riga in the Baltic Sea provided a comparison for nutrient uptake rates by phytoplankton under similar pre- and post-storm conditions. This study measured the uptake of these nutrients prior to, and 4 days after a plume of nutrient-rich water entered the Gulf from the Daugava River. They refer to these data as pre-plume and post-plume. They found that uptake of NO_3^- increased significantly ($p<0.05$) following a 3-fold increase in its concentration from pre- to post-plume. The uptake of NH_4+ also increased significantly ($p<0.05$) following a doubling of its concentration. However, neither the uptake nor the concentration of urea showed a significant change pre- to post-plume.

In comparison to the data of the Gulf of Riga study, the Knysna Estuary demonstrated a contrary result with regard to the uptake of NH_4+ and NO_3^- pre- and post-storm. The uptake of NH_4+ and NO_3^- and urea decreased significantly ($p<0.05$) in post-storm samples (refer to Table 3.3) despite post-storm increases in their concentrations similar to those measured in the Gulf of Riga study.

The differences between this study and that of Berg et al. (2001) were due to the different characteristics of the two estuaries. The Gulf of Riga was less saline and deeper than the Knysna Estuary. The November 13, 2001 storm in the Knysna catchment area resulted in runoff that lowered the average salinity (taken from stations M1 – M15) from 33 ppt to 21 ppt in this shallow estuary in the three days following the storm, indicating a large displacement of high saline waters with river water. Hence, a significant portion of the phytoplankton biomass associated with this saline water may have been flushed out of the Estuary during this post-storm period of fresh water influx. Salinity in the Gulf of Riga varied from 2.2 to 5.8 ppt during their study, and study station depths varied from 2.5 to 20 m along the transect affected by the plume of river water. Although there was no mention of large volumes of water displaced along this transects it

is not likely that the plume of river water monitored in their study displaced phytoplankton biomass to the same extent as in the Knysna Estuary. This was essentially due to the higher water volume of the Gulf of Riga in contrast to Knysna Estuary.

The Gulf of Riga had an active phytoplankton community as shown by its chlorophyll-*a* concentrations, ranging from 23 to 42 $\mu g\ L^{-1}$ in the area of the Daugava River. These concentrations were an order of magnitude higher than those typical of the Knysna Estuary. The active phytoplankton community of the Gulf of Riga would allow a more rapid uptake of nutrients when subjected to rapid increases in nutrients. However, the increases in uptake following increases in concentrations of nutrients were not paralleled in the Knysna Estuary, as the physics governing these two systems are vastly different.

3.2 Sediments characteristics, including redox potentials

The measurement of sediment characteristics of the Estuary is introduced in this section, as they are considered later in the text when discussing variations found in benthic flux of nutrients over the different regions of the Estuary. Benthic flux measurements were taken at specific sites, which are referred to as benthic chamber sites throughout this section. This discussion, however, is restricted to a "qualitative comparison" of sediment characteristics and the impact that these characteristics may have on the benthic flux of nutrients over the regions of the Estuary.

Measurement of sediment characteristics was made during the summer (January and February) and winter (June and July), in concert with the benthic chamber deployments (refer to section 3.3). Comparisons are made between sand and *Zostera capensis* beds (referred to as *Zostera* beds); it is important to note that

while *Zostera capensis* was the predominant subtidal macrophyte (Maree, 2000), other algae species were contained in the *Zostera* beds, especially in the upper estuary region.

Other studies (Rysgaard et al., 1995; Nedwell and Trimmer, 1996; Ogilvie et al., 1997; Trimmer et al., 1998) have measured sediment characteristics in combination with benthic flux of nutrients, and found these measurements useful in interpreting the variations observed in benthic fluxes. For example, Rysgaard et al. (1995) showed a strong correlation between some variables (e.g., benthic microalgae, bioturbating infauna) and benthic flux. However, it was not the aim of this work to measure sediment characteristics in the hope of drawing a strong correlation between them and the variations in benthic nutrient flux. As this was a first-time study of the benthic flux of nutrients using *in situ* benthic flux chambers in a South African estuary. The resources available for this study did not make possible fine-scale measurements of sediment characteristics taken in concert with benthic flux measurements.

Sediments of the Knysna Estuary

The increase in public concern over accretion of sediments in the Knysna Estuary has followed the urbanization of the town, and the development of its catchment, over the past several decades. As Reddering (1994) stated with regard to the Knysna Estuary, "When a concerned observer then sees sediment entering the system, his/her perception is that undesirable and irreversible change is imminent." The outcome of this concern has lead to research efforts dedicated to understanding the process of sedimentation in the Estuary. While the results of these studies have done little to curb the increasing urbanization pressures on the Estuary (refer to section 1.7), they have stimulated research efforts that have produced a rich base of literature with regard to the sedimentation processes which formed the Estuary, and continue to affect it.

For example, the work of Reddering and Esterhuysen (1984), was one such valuable source of information, and included extensive measurements of particulate organic material in the sediments of the Estuary. While patterns of total particulate organic material contained in the sediments were well established in the Estuary careful consideration must be given to which portion of this organic material was biologically reactive within the sediments of the Estuary.

Reddering and Esterhuysen (1984) recorded that the floor of the modern Knysna Estuary was made up of unconsolidated sediments, mostly aeolian sand believed to originate from the erosion of the Brenton Dune. The size distribution of this sand depends on the flow regime and availability of sediment, with courser sandy sediments of marine origin near the mouth. The sand of the subtidal channels was course grain, becoming finer grain on the intertidal and supratidal mudflats.

Further, Reddering and Esterhuysen (1984) stated that the supply of mud to the Knysna Estuary was limited and influxes were concentrated in areas adjacent to Mesozoic deposits where transport was facilitated by runoff. A fluvial fan, rich in mud, extended throughout the entire upper estuary region into the upstream portion of the Lagoon region. The sediments in the Lagoon region were primarily aeolian in origin. In the Bay region, the subtidal sediments were aeolian sand and coarse-grained skeletal carbonate material. The sediments of the Ashmead were a combination of aeolian and alluvial sand and mud, with the fraction of mud in the Ashmead increasing in the intertidal from station A6 to A1 (refer to Figure 1.9), while the subtidal channel was largely comprised of sand. Throughout the Knysna Estuary, much of the intertidal mudflats and supratidal mudflats were stabilized by macrophyte beds and contain much of the finer grain sediments.

Sediment analysis, methods

Reddering and Esterhuysen (1984) divided the Knysna Estuary into regions similar to those used herein and did so to describe the distribution of skeletal carbonate and organic matter in the sediments. They analyzed 31 to 56 samples in each of their 4 regions along latitudinal transect lines, providing an average organic material content for the sediments in each region.

In order to complement these previous measurements, a sediment sample from each of the benthic deployment sites was analyzed in the lab. Sediment cores 20 cm deep were taken at each site. Samples were homogenized and a 50 ml aliquot was taken from each sample. As only organic material was of interest, this aliquot was acidified with hydrochloric acid until effervescence stopped. This step was performed to drive off calcium carbonates (Nedwell and Trimmer, 1996) which may introduce error in the measurement of the organic material (Oliver et al., 2001). The aliquots were dried at 105 °C for approximately 16 hours until they displayed a consistent texture when homogenized. Twenty grams of each dried sample was ignited at 500 °C in a muffled furnace for 6 hours then weighed. This method as outlined in Head (1985) gives a measure of total particulate organic material.

Results

The sediment analysis from the benthic chamber deployment sites revealed a higher organic material content at the benthic chamber sites of the upper estuary and Lagoon than at the sites of the Bay and Ashmead (Table 3.4). These data were similar to the findings of Reddering and Esterhuysen (1984), that showed the organic material content of the sediments increased near the head of the Estuary (Table 3.5).

Table 3.4. The total particulate organic material (expressed as a percent of sediments) from benthic deployment sites.

	% organic material
upper estuary (sand only)	7.3
upper estuary (macrophytes)	5.1
Lagoon (sand only)	6.2
Lagoon (macrophytes)	6.9
Bay (sand only)	1.9
Bay (macrophytes)	2.1
A3	1.8
A5	1.9
A6	2.8
A7	2.3

Table 3.5. The average total particulate organic material by percent, of the surface sediments and at a depth of 50 cm in each region. Data are from Tables 3.1 and 3.2 in Reddering and Esterhuysen (1984).

	Surface	- 50 cm
upper estuary	5.1	6.3
Lagoon	2.2	2.9
Bay	3.4	2.5
Ashmead	4.3	2.3

Discussion

Reddering and Esterhuysen (1984) concluded that higher average organic material content in the Ashmead and Bay surface sediments were the result of marine input, while the higher organic material content in the upper estuary was derived from riverine input. The Lagoon region, between these two sources of organic material, quickly exhausted the limited supply of organic material it received and therefore displays the lowest organic material content at the surface in any of the regions. The pattern described by the measurements of organic material at the benthic chamber sites (Table 3.4), was comparable to these measurements of the surface sediments (Table 3.5), with the exception of the Lagoon region.

It seems in opposition to this point that the organic material at the benthic chamber sites in the Lagoon was the highest for all the benthic chamber sites measured. Data from 1984 revealed that the transect line, which bisected the Lagoon region very near the Lagoon benthic chamber site, displayed an average surface organic material of 1.9%, average bottom organic material was 2.3%, and the sample taken near this position contained only 0.8% organic material (Reddering and Esterhuysen, 1984). These values were considerably lower than the organic material content of 6.2% and 6.9% recorded at the Lagoon benthic chamber site in 2002 (refer to Table 3.4). There may be several reasons for this disparity.

First, as described by Allanson et al. (2000a) large amounts of suspended solids were introduced to the Estuary *via* the Salt River, approximately 1 kilometer east of the Lagoon benthic chamber site (refer to Figure 1.8). They recorded that a major contribution of suspended sediments, which were introduced to the Estuary over the previous 4 years (1996 – 1999), came from the failed Simola golf estate venture in the Salt River catchment area. The flooding during the

spring of 1996 eroded a substantial portion of the golf course development, introducing considerable amounts of sediment to the Salt River and the Estuary. Marker (2000) noted that following the 1996 flooding, the greatest influx of yellow sediment (cover sands) entered the Estuary *via* the Salt River. Now, sediments of a lighter color cover many of the intertidal areas and *Zostera capensis* beds of the Estuary, near the area where the Salt River enters the Estuary (Figure 1.2).

The second reason for this disparity may be due to different sampling methods, intervening floods and hydrological events. By measuring the top 20 cm of sediment at the Lagoon benthic chamber sites, the sample may contain humates in addition to organic material buried since the work of Reddering and Esterhuysen (1984).

The pattern of organic material content of sediments at a depth of 50 cm, as measured by Reddering and Esterhuysen (1984), may be due to humates buried in the sediments (Table 3.5). Humates precipitate out of the water column as the river water mixes with seawater, contributing to the organic material in the sediments of the upper estuary and Lagoon (Professor Brian Allanson, personal communication). The Knysna River draining 83% of the catchment was the major source of humates to the Estuary (refer to section 1.6). The contribution of humates entering the Estuary from the Salt River that drains less than 4% of the catchment was negligible. The contribution of humates to the organic material in the sediments of the Bay and Ashmead regions was expected to be less than in the upper estuary and upstream portion of the Lagoon.

Humates are biologically refractory and only slowly biodegraded (Billen, 1982). The slow degradation of humates derived from the Knysna River, which precipitated onto the sediments of the upper estuary and the upstream portion of the Lagoon, would be buried prior to depletion by biological activity. It would follow that periods of flood would result in increased rates of deposition, and

burial of these humates in these regions. However, the method used for analysis of organic material (i.e., the loss on ignition) does not provide insight into what component of the organic material was from humates.

Several studies have discussed the premise that the breakdown of organic material in sediments results in the flux of inorganic nutrients from sediments (Nixon, 1981; Billen 1982). More recent studies (Boynton and Kemp, 1985; Hunt et al., 2000) have indicated that the elemental composition of the available organic matter (e.g., carbon to nitrogen ratio) is a more important consideration than the quantity of organic matter in sediments with regard to the regeneration of inorganic nutrients. Nedwell et al. (1999) recorded that the amount of available organic matter tends to be only a small fraction (<1%) of the total organic matter present. Moreover, the consideration of the organic carbon component of the organic material present may not provide sufficient information to estimate the flux of nutrients from sediments. For example, the highly detailed study of Nedwell and Trimmer (1996) did not show a strong correlation between organic carbon content of sediments and an increase in flux of ammonium from these sediments.

The measure of organic material by "loss on ignition" method (Head, 1985) provided an overall measure of the total particulate organic material contained in sediments. This method does not reflect the elemental composition of these sediments, or the portion of organic material that was utilized in the flux of inorganic nutrients from these sediments. Therefore, caution will be used, and should be used in the future, when considering these data on spatial patterns of organic material content in the sediments to any spatial trends in benthic fluxes within the Knysna Estuary.

Oxic layer variations and expected changes in fluxes

Nutrient flux between the water column and the sediments are controlled by the vertical concentration gradient of each of the solutes (Nedwell et al., 1999), such that: a) The ammonium concentration is usually higher in the anoxic sediments than in the overlying water due to the mineralization of organic material, and therefore the expected direction of flux is from these sediments to the overlying water. b) The nitrate concentration is usually higher in the water column than in the immediate surface of the sediments (sediment to water interface), accordingly, its flux is into the sediments (Nedwell et al., 1999).
Nitrifying bacteria in the oxic layer produce nitrate through the oxidation of ammonium, a process known as nitrification (Hansen et al., 1981; Nedwell et al., 1999). A constant source of ammonium and oxygen is required for nitrification (Henriksen et al., 1981; Hansen et al., 1981). Nitrate concentrations peak in the middle of the oxic layer as opposed to its boundaries, and therefore diffusion is both upward toward the water column (if allowed by the vertical concentration gradient), and downward toward the anoxic sediments (Brown and McLachlan, 1990; Nedwell et al., 1999). Ammonium in the anoxic layer must pass through the oxic layer to diffuse into the overlying water and if nitrification is sufficiently intense in the oxic layer, the net diffusion of ammonium from the anoxic layer to the overlying water may be blocked (Nedwell et al., 1999).

In summary, the depth of the oxic layer impacts on the flux of ammonium from sediments to the overlying water and deep oxic layers will potentially produce more nitrate than shallow oxic layers, impacting on the flux of nitrate to overlying waters. Essentially, the deeper the oxic layer, the more nitrate produced, and the greater the diffusion gradient between the sediment and the overlying water, assuming comparatively low-nitrate conditions in the overlying water.

The depth of oxic layers in estuaries has been shown to vary seasonally, decreasing in summer as benthic respiration rates peak, and increasing in the winter when reduced benthic respiration rates allow oxygen to penetrate into the sediments (Nedwell et al., 1999). Biological factors can increase the depth of an estuarine oxic layer, these include benthic algal biofilms, burrowing activity of infauna (bioturbation) and high concentrations of dissolved oxygen in the overlying water (Risgaard-Petersen et al., 1994; Rysgaard et al., 1995; Nedwell et al., 1999).

The intertidal zone of Knysna Estuary has abundant infauna. Hodgson et al. (2000) discussed the high variation in the density and biomass of *Upogebia africana*, a mud prawn common in the intertidal zone from the Leisure Island to the Red Bridge. Other, larger infauna common to the Estuary include; the bloodworm *Arenicola loveni*, a bristle-worm which can grow to 80 cm, and the pencil bait, *Solen capensis*, a bivalve mollusc which can grow to 16 cm. All of these species dig burrows, and filter feed on water pumped through the burrows. The expected result of this would be an increase in oxic layer depth at or near the location of these burrows.

Allanson et al. (2000a) found significantly lower dissolved oxygen concentrations in the upper estuary region as a result of biological oxygen demand. This paper also pointed out that increases in dissolved oxygen concentrations are associated with lowered water temperatures as a consequence of upwelling outside the mouth.

The growth of algae was common in the Ashmead channel and has been observed to develop during winter, blanketing the intertidal zone. Densities increase in June and reaching a peak by late August before dissipating rapidly in September. This algae has been identified as *Enteromorpha intestinalis* and *Cladophora rugulosa* (Professor Brian Allanson, personal communication).

Grindley and Snow (1983) mentioned the growth of *Enteromorpha* and *Ulva* associated with the *Zostera capensis* beds near the sewage treatment works outflow on the littoral of Ashmead.

In summary, the Knysna Estuary contains many of the variables (e.g., infauna activity, algae), that have been correlated with increases in oxic layer depth in other estuaries.

Core redox measurements, methods

Oxic layer depths were determined by measuring the redox potential (Eh) of sediment cores taken during each benthic chamber deployment. Cores were taken in an acrylic tube, 500 mm long by 25 mm wide, with a wall thickness of 2.5 mm. This tube was perforated by 3 mm holes at a spacing of 5 mm (center to center). These holes were covered by clear packaging tape during the coring.

The cores were taken by pressing this tube into the sediment between the 4 deployed chambers and capping the top of the core tube with a rubber bung prior to removing it from the sediment. Once removed from the sediment a bung was also fitted into the other end of the tube and the core was immediately transported back to the lab in an insulated box.

A silver/silver chloride reference electrode and a calomel electrode were connected to a voltage meter (Top Tronic model TBM810, Brymen Technology Corporation, Taiwan) capable of measuring to +/- 500 mV with an accuracy of 0.06% at the range used. The silver/silver chloride reference electrode was taken from a pH meter (Orion model 420, Orion, Boston, Massachusetts, USA). The calomel electrodes were constructed in the lab in accordance with Bohn (1971), tested against Light's solution with an EV potential of 476mv (Eaton et

al., 1995 p. 2-75) and found to be accurate to <1% error. The platinum stem of the calomel electrode was 12 mm in length and less than 3mm in diameter. This platinum stem was cleaned in *aqua regia* solution and rinsed with distilled water prior to use to ensure minimum loss of accuracy due to resistance.

The cores were analyzed within two hours of collection. The silver/silver chloride reference electrode was inserted into the top of the acrylic tube in the water overlying the core. A small insertion was made in the packaging tape covering each hole prior to the immediate insertion of the platinum stem of the calomel electrode. Depth and voltage were recorded. This procedure was repeated from the bottom of the core to the top (Figure 3.1).

Figure 3.1. Measurement of the redox potential from a sediment core taken from the site of a benthic flux deployment.

It was found that the voltage reading increased incrementally if the platinum electrode was allowed to rest in the hole. It is assumed this was due to the introduction of oxygen in otherwise anoxic sediments. To minimize error associated with this constraint, the first reading of voltage was recorded immediately after the insertion of the platinum electrode.

Results

The measurements of voltage were plotted against their respective depths, and the depth of the oxic layer was determined as the point of inflection in the curve (Billen, 1982). Generally, the change in redox potential (Eh) was at 200 mV or greater, and corresponded to a color change in the core (Billen, 1982; Nedwell et al., 1982). Sediment cores changed sharply in color from dark gray or black in the lower portion, to a lighter gray or brown in the positive upper portion at or near the point of inflection in the profile. The color change was well defined in most cores and was at or near the change in Eh. When this color change was not well defined, the change in Eh was equally indistinct, and the oxic layer depth was difficult to determine. The results of these core redox measurements are listed in Table 3.6.

Table 3.6. Depth of oxic layer (mm) as determined by change in redox potential (Eh) in the sediment cores. Data rounded downward to nearest 5 mm increment. none = no apparent Eh change.

	Summer	Winter
A3	110*	10
A5	none	none
A6	105*	15
A7	15	no data
Bay-sand	10	10
Bay-*zostera*	5	30
Lagoon-sand	none	30
Lagoon-*zostera*	10	none
upper estuary-sand	65*	95*
upper estuary-*zostera*	105*	105*

* = change in Eh not well defined

The general trend to these data suggested that most main channel sites exhibit a deeper oxic layer during the winter (Table 3.6). Initially, the data for Ashmead appears contrary to this statement. However, upon closer examination of the Ashmead summer data, it is doubtful that the values for oxic depths of 110 and 105 mm are valid, as the Eh change in the cores was not well defined. These abnormally high values could be the result of cores taken at or near the burrows of infauna.

At site A5 there was no indication of a change in the Eh greater than 100 mV in the winter and 50 mV in the summer (Table 3.6). This indicated an oxic layer at LWST deeper than the core depth, and was due to oxygen penetrating the sediment deeper than the core depth. Site A5 was the shallowest of sites tested in the Ashmead, and although deployments were made during neap tides the site became supratidal during some LWST conditions. The infauna that were prevalent in these shallow Ashmead sites may also be a reason for the apparent irregularities in the measurements of oxic depth.

The sand in the Bay displayed a stable oxic layer depth with regard to season while the *Zostera capensis* beds in the Bay, during winter, displayed an increase in oxic layer depth (Table 3.6). A comparison of the Lagoon between winter and summer is not possible due to lack of data. It is noteworthy that the supratidal deployment in the Lagoon *Zostera* bed during the winter displayed no indication of a change in Eh, this reinforcing the observation that A5 displayed an oxic layer deeper than the cored depth due to its supratidal location.

The upper estuary sites demonstrated a consistently deeper oxic layer than other regions. This oxic layer is uniform between the summer and winter in the *Zostera capensis* beds, while slightly deeper during the winter in the sand sediments. Changes in Eh and changes in color within these cores were not well defined, and made the determination of a precise oxic layer depth difficult. This

was thought to be due to the light fluvial sediments in this region, which presented less resistance to oxygen transport, and allowed the introduction of oxygen during the coring process. It is likely that substantial error was introduced as a result of the coring, transportation and analysis of these lighter fluvial sediments.

Discussion

It was apparent from the measurements of oxic layer in the cores taken at the benthic chamber deployment sites that oxic layer depth varied spatially and seasonally. The affects of these changes had a direct influence on the fluxes of nutrients from the benthos. Increased oxic layer depths reduced denitrification, while stimulating production of NO_3^- from nitrification (An and Joye, 2001; Rysgaard et al., 1995). Seasonal and diurnal variation in nitrification and denitrification in shallow water sediments were explicable, in part, by changes in oxygen penetration into the sediments attributable to microalgal growth (Christensen et al.,1990; Risgaard-Petersen et al.,1994).

There were two conclusions from these studies that were immediately applicable to this study. First, the oxic layer depth plays a crucial role in ratio of ammonium to nitrate flux from the sediment, with deeper oxic layers enhancing the production of nitrate *via* nitrification. Secondly, that oxic layer depth was variable, increasing with benthic algae activity or growth, activity of infauna, and seasonal increases in dissolved oxygen in the overlying water column.

In summary, the deployment of benthic flux chambers were scheduled with regard to season, winter and summer, when environmental factors affecting oxic layer depth were expected to be at their extremes. The measurements of oxic

layer depths were made to investigate the relationship, if any, between changing oxic layer depths and the measured nutrient fluxes.

3.3 Benthic Flux Chambers

Benthic flux chambers provide a simple method of investigating nutrient flux *in situ*. It has been demonstrated in estuary benthic studies that nutrient concentrations in the overlying water are proportional to the nutrient fluxes occurring at the sediments (Dollar et al., 1991; An and Joye, 2001).

There are many different designs of benthic flux chamber. The benthic flux chamber design required for Knysna Estuary had to have specifications contingent on a single operator, and an unsophisticated field lab situation. The design was required to provide information on the nutrient changes in the water overlying the sediment for a minimum of 24 hours. The equipment was designed to be easily transportable and deployable by the single operator using only snorkel diving. Finally, the benthic flux chambers deployed had to remain hidden from view from persons passing along the shoreline and in boats.

The chamber design used in the Knysna Estuary was based on the benthic flux chamber design described in An and Joye (2001), which contained the majority of features required. This design was used in a study in Galveston Bay, Texas and found comparable to modeled results and incubated core samples with regard to nutrient flux. The field site conditions (e.g., sediments, temperature, depth, salinity and deployment duration) at Galveston Bay were similar to those found in Knysna Estuary.

The design described in An and Joye (2001) and the chambers constructed for this study did not include an internal stirring mechanism common in many benthic

chamber designs. There is evidence in the literature suggesting that internal stirring mechanism affect nutrient flux in benthic chambers. Glud et al. (1996) demonstrated that the intensity of advective pore water transport is a function of sediment permeability and stir rate within benthic chambers. This study also found that unstirred chambers demonstrated diffusion of a barium dye similar to measured and modeled results. Huettel and Gust (1992) found that sediment permeability in stirred chambers should be carefully considered in chamber design as pressure gradients as small as 1 Pa drive advective porewater transport. The consequences of advective porewater transport include altering the depth of the oxic layer and the release of anoxic porewater into the chamber. Both of these conditions affect biological and geochemical processes in the sediment that alter the flux of nutrients measured in this study (refer to section 3.2).

Benthic chamber design

The benthic flux chambers were constructed of transparent acrylic cylinders with a length of 500 mm, an internal diameter (ID) of 190 mm, and a wall thickness of 5 mm. One end of the cylinder was capped with a circular flat sheet of transparent acrylic with a thickness of 5 mm. The rim of this cap extended 10 mm beyond the edge of the cylinder (Figure 3.2).

An acrylic tube, the diaphragm mount, 25mm ID, 50 mm in length was centered 50 mm below the cap through the wall of the chamber. This exterior portion of this tube served as a mount for an attached diaphragm, a large balloon secured with a tie-wrap, which expanded compensating for the volume of water removed by sampling.

The sampling port, a 160mm length of tygon tubing with an ID of 3 mm, was inserted through the chamber wall 155 mm below the cap. The tygon tube of this sampling port extended 95 mm into the chamber so samples were drawn near the center point of the chamber. The exterior end of the tube was fitted with an one-way aquarium check valve to prevent the back flow of ambient water into the chambers during sampling. The external end of this sampling port was plugged with a conical plastic golf tee between sampling times, as a further precaution against leakage.

All acrylic to acrylic surfaces were joined with acrylic glue and sealed on the exterior edge with a bead of silicone glue to prevent leakage. The sampling tube of tygon where it passed through the chamber wall was sealed with a bead of silicone glue.

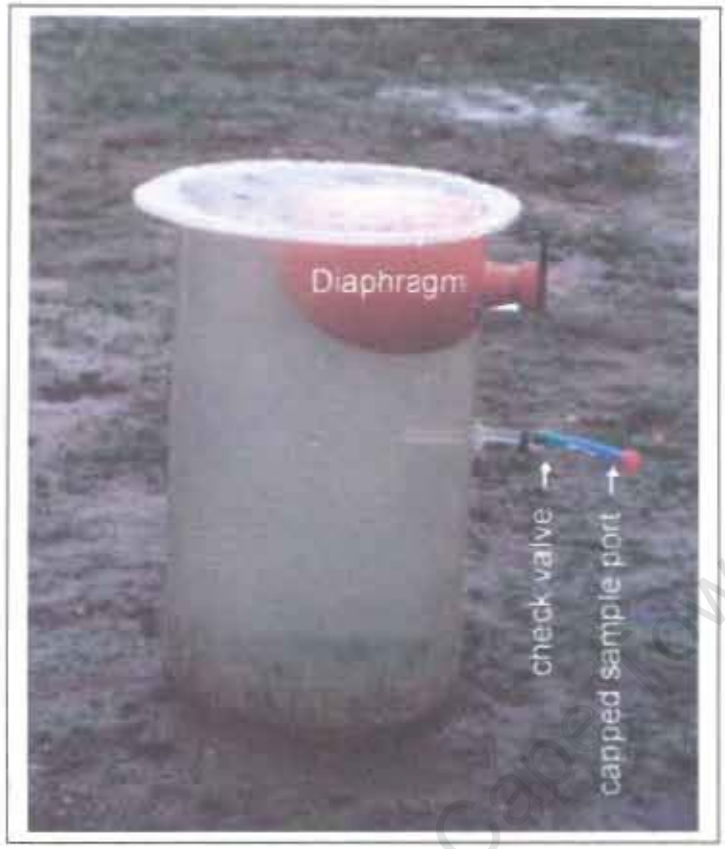


Figure 3.2. A benthic chamber deployed on mud sediments of the intertidal, at low tide conditions showing the capped sample port with one-way check valve and the expansion of the diaphragm after 200 ml of water was drawn.

Site selection for benthic chamber deployments

The objectives in choosing sites for deployment of the benthic chambers were, to compare nutrient fluxes between regions, and to compare flux differences within each region. The first objective was met by collecting fluxes representative of the upper estuary, Lagoon, Bay and Ashmead. The second objective required that the structural differences within each region, that could be measured (e.g., change in oxic layer depth between sand and *Zostera* sites), were identified. In

the upper estuary, Lagoon and Bay there are two benthic environments that the chambers could be deployed in, those with macrophytes (e.g., *Zostera capensis*) and those without (e.g., sand). The deployment of benthic flux chambers in these regions either contained macrophytes, predominantly *Zostera capensis*, and are referred to hereafter as *zostera* sites, or were on sand devoid of macrophytes and are referred to hereafter as sand sites. Further considerations in selecting these sites were security of the benthic chambers from theft or vandalism, accessibility, unobstructed light exposure and limited contamination from outfalls and rivers.

Deployment sites in the Ashmead were limited, due to water depth, to the navigable channel. Macrophytes were ubiquitous on the bottom of this channel, mostly *Caulerpa filiformis* and *Zostera capensis*. Therefore, comparison between sites in the Ashmead with regard to *Zostera* and sand sites was not possible. However, comparisons of fluxes spatially along the Ashmead were of interest given the input of the sewage treatment works between station A4 and A5 (refer to Figure 1.9).

Figure 3.3. The Ashmead, in the vicinity of stations A4 and A5. This view is from the northeast littoral of Ashmead, toward the south. The cliffs of the Knysna Heads at the mouth of the Estuary are visible in the upper left side of this picture.

There was evidence from the water column survey data that nutrient fluxes in the Ashmead vary spatially (refer to section 2.3). The NH_4-N and DIP concentrations measured in the water column at low water, slack tide often showed the highest concentrations near stations A5 and A6 (Figure 3.4).

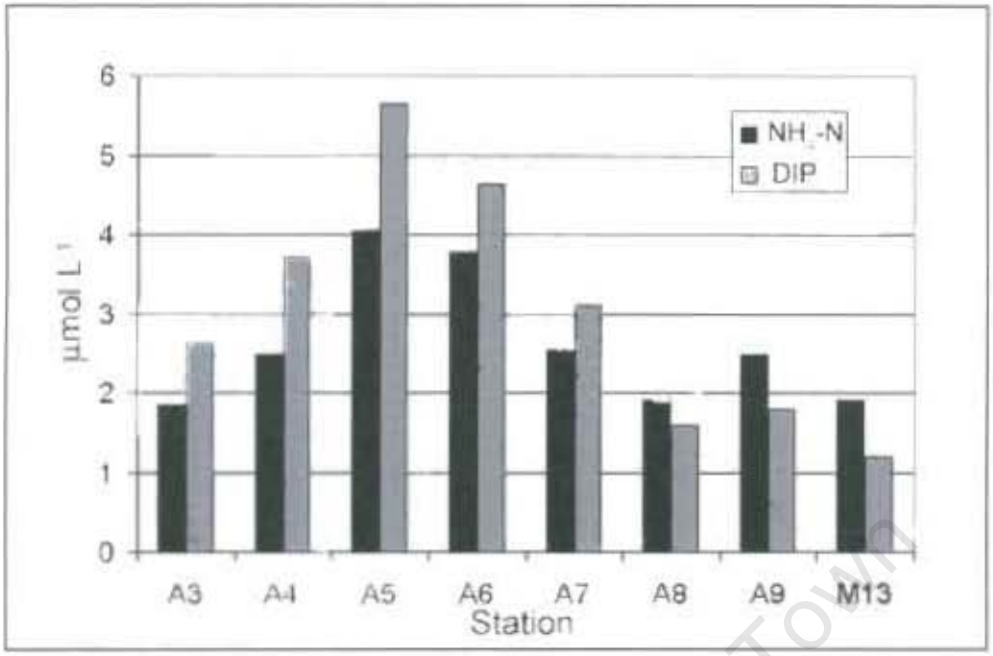


Figure 3.4 Nutrient concentrations in the Ashmead (A3 – A9) and Bay (M13) during LWNT in June 2000. Note the higher concentrations of NH_4-N and DIP in Ashmead at stations A5 and A6.

From these observations it was suspected that the benthic flux of nutrients was the cause of the higher NH_4-N and DIP concentrations which were measured during low tide conditions in the Ashmead. Thus, the proposed null hypothesis that was to be tested by the benthic chamber deployments in the Ashmead: The interaction between tidal conditions and sediment biological activity was not responsible for spatial variations in the flux of NH_4-N and DIP.

To test this hypothesis, benthic chambers sites were selected at A5 and A6. Sites A3 and A7 were chosen as nearby locations (i.e., 500 m separation between sites) of similar depth that were representative of Ashmead sites, which did not display the higher concentrations of NH_4-N and DIP in the overlying water observed at sites A5 and A6. The benthic chambers were deployed in the subtidal, near the edge of the navigable channel in the Ashmead, as a precaution

against damage by passing boats. If there were no significant difference ($p>0.05$) in the fluxes of these nutrients at stations A5 and A6 with respect to stations A7 and A3, this null hypothesis would be accepted.

Benthic chamber deployments, methods

Two periods were selected for benthic chamber deployments, one during winter of 2001 and the other in summer of 2002 (Table 3.7). The winter deployment period was representative of low water temperature and short daylight hours. The summer deployment period represented long daylight hours and high water and air temperatures.

Table 3.7. The deployment schedule of benthic chambers during winter, 2001 and summer, 2002.

	winter, 2001	summer, 2002
A3	26/6/01	18/2/2
A5	25/7/01	5/2/02*
A6	12/6/01	20/2/02
A7	4/7/01	4/3/02 *
Bay, sand site	18/7/01; 25/9/01	27/2/02*
Bay, *zostera* site	6/6/01	24/1/02
Lagoon , sand site	22/6/01	2/2/02*
Lagoon, *zostera* site	22/7/01 #	25/2/02
upper estuary, sand site	17/6/01	28/1/02
upper estuary, *zostera* site	1/7/01	13/2/02*

= intertidal deployment * = PM deployment

Each deployment consisted of four benthic chambers inserted into the sediment at each station to a depth of 190mm determined by a line etched into the chamber wall. This depth was chosen to limit the exchange of porewater outside the chamber, with that contained within the walls of the benthic chambers while allowing the chamber to be pressed into the sediments by a single diver. The limited exchange of porewater outside the chamber with porewater from inside the chamber was demonstrated by the small increase in diaphragm volume (<500 ml), when benthic flux chambers deployed in the intertidal sediments in the Lagoon were exposed during low water conditions (refer to Figure 3.2). Deployment depths were chosen so that the chambers remained submerged throughout the period of the deployment, with the exception of the Lagoon intertidal deployment during winter. Water depths at the deployment sites ranged from one to four meters.

The water column height inside the chambers was 310 mm. The internal volume was 9 liters covering approximately 284 cm^2 area of sediment (refer to Figure 3.2). A minimum of one meter separated the chambers when deployed to prevent shading by neighboring chambers.

The chambers were deployed without the diaphragms in place to minimize any exchange of water from above the sediment with porewater. Chambers were left open for 15 minutes before the diaphragms were attached allowing the chambers to flush with ambient water. The sampling tube was cleared by drawing 20 ml of water into a clean 60 ml syringe before it was plugged. This procedure of clearing the sample tube was repeated prior to each sampling.

The chambers were rinsed with ambient water prior to each deployment, and rinsed with fresh potable water after each deployment. New diaphragms were used for each deployment, and rinsed thoroughly with ambient water prior to their use. All syringes and tubing were rinsed with distilled water between each

sampling, and replaced if worn or discolored. Samples collected were transported to the lab in an insulated box and analyzed for NH_4-N, NO_xN and DIP. All glassware used in the collection of samples, and subsequent analysis was acid-washed in 10% v/v 1M hydrochloric acid.

Three test deployments using four chambers were preformed prior to the planned deployment schedule. These test deployments consisted of all the initial steps of a deployment outlined above and included sampling from each chamber after it was sealed. Triplicate samples of ambient water were taken while the chambers flushed. In addition to refining the deployment techniques, these test deployments established that there was no significant difference ($p<0.05$) between the nutrient concentrations within the newly deployed chambers and the ambient water collected while the chambers settled. Therefore, water samples collected at the depth of the chambers during deployments represented the nutrient concentrations in the chambers at time zero (T_0).

Water samples were collected from the benthic chambers with a 60 ml syringe after clearing the sampling port. Samples were transferred upon surfacing from the dive to acid-washed 100 ml glass jars. Within 20 minutes of collection the samples were filtered through GF/F Whatman filters. The sample jars were sealed with gas tight lids after collection and remained sealed when not being filtered or analyzed. Samples were placed in an insulated box and transported to the lab. Analysis for NH_4-N, NO_x-N and DIP began within two hours of collection.

The sampling schedule for the benthic chambers during winter was designed to measure day flux, night flux, and 24-hour flux. Hence, chambers were deployed at sunrise in the winter (08:00) and sampled 9 hours later at sunset (17:00). The following day, chambers were sampled at sunrise and at sunset, prior to recovering the chambers. Thus each chamber was sampled a total of four times during a 33-hour deployment.

The summer sampling schedule retained the same objectives as established in winter, day flux, night flux and a 24-hour flux. Sample times for summer were at sunrise (07:00) and at sunset (19:00) for two days resulting in 4 sampling during a 36-hour deployment. Summer dissolved oxygen (DO) concentrations were regularly lower than during the winter, and displayed a diurnal pattern with higher DO concentrations in the evening. Therefore, half of the summer deployments began at sunset when ambient DO concentration was at or near maximum (refer to Table 3.7).

Oxygen measurements in the deployed chambers:

Oxygen concentrations in the benthic chambers were measured *via* the Winkler titration method (Parsons et al., 1984), and expressed as units of mg L^{-1}. A sample of approximately 55 ml was drawn from the chamber into a graduated syringe through a watertight manifold connected to the chamber sampling port (refer to Figure 3.2). This manifold was constructed of tygon tubing, plastic aquarium plumbing T-shaped fittings and a one-way check valve that prevented any portion of the sample from flowing back into the chamber (Figure 3.5). Two syringes connected to this manifold delivered separately 0.6 ml of manganous chloride and alkaline iodide into the sample as it was drawn from the chamber. The syringe was then removed from the manifold and sealed underwater before being transported back to the lab for analysis. The samples were titrated against sodium thiosulfate solution, where the standardization factor of this solution was determined prior to each batch of samples. Field tests in estuarine waters using this manifold method to measure dissolved oxygen agreed well with measurements made with a YSI dissolved oxygen meter (model 55, YSI, Yellow Springs, Ohio, USA).

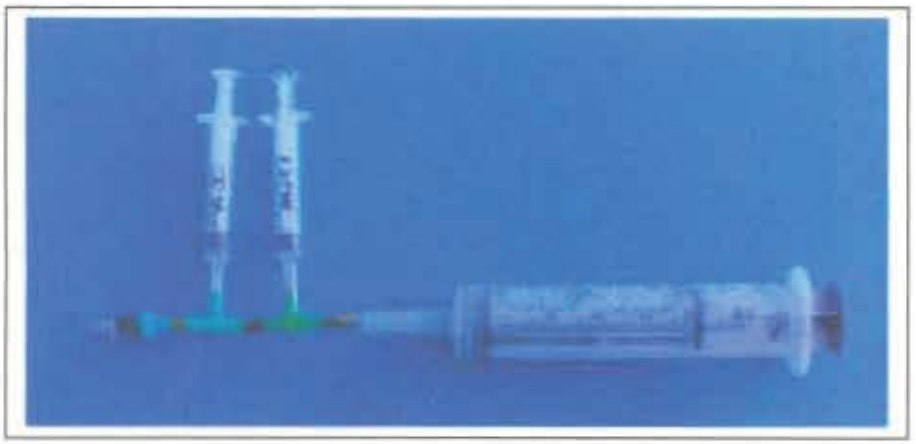


Figure 3.5. Manifold and syringe device used to draw water samples from benthic flux chambers, and adds alkaline iodide and manganous chloride for the determination of oxygen concentration.

Oxygen concentrations were measured in the winter experiments at the termination of each deployment, when the final samples were taken. During the summer experiments oxygen concentrations within the chambers were measured when each chamber was sampled at 12-hour intervals.

In order to assess patterns of oxygen production and respiration within the chambers during the winter, a benthic chamber deployment was carried out during September 2001, while temperatures and light conditions remained near winter conditions. The sampling times of this experiment were the same as winter deployments, and oxygen levels were monitored at each sampling. Results confirmed that the mean oxygen level in the 4 chambers remained near starting levels with an increase of 6% during the first day, a 6% decrease from initial concentration after 24 hours, and samples taken after 33 hours displayed a 22% decrease from initial oxygen concentration.

Dollar et al. (1991) used similar benthic flux chambers in Tomales Bay, and oxygen levels of 1.4 mg L^{-1} or less were considered near anoxic conditions.

which influence changes in nutrient and oxygen flux. While in Galveston Bay, a study that used both *in situ* benthic flux chambers of a similar design to this study and modeled nutrient fluxes, showed that oxygen production from photosynthesis was essential to prevent ammonium accumulation within the sediments and overlying water (An and Joye, 2001).

Three deployments, one each in the upper estuary, Lagoon and Bay regions, during the summer displayed low oxygen concentrations - less than or equal to 2.5 mg L^{-1} in two or more of the benthic flux chambers. The lowest ambient dissolved oxygen concentration, which was measured in the Estuary, was 3.4 mg L^{-1}. The fluxes of NH_4-N in these deployments were notably higher than the fluxes of NH_4-N at these sites when the oxygen concentrations remained greater than 2.5 mg L^{-1}, in all chambers, throughout the duration of the deployments. From these observations, it was concluded that any deployment with an oxygen concentration less than 2.5 mg L^{-1} in two or more of the chambers represented a deployment with low oxygen conditions within the chambers and resulted in higher than expected fluxes of NH_4-N and DIP.

These deployments provided a unique opportunity to obtain data on how estuarine sediments of Knysna Estuary can be expected to behave under low oxygen conditions, with regard to the fluxes of NH_4-N and DIP. While these low oxygen deployments will not be included in the subsequent "standard deployment" analysis, they are discussed at the end of this section in a separate analysis of fluxes under low oxygen conditions.

3.4 Nutrient fluxes in chambers

The nutrient flux (F) per unit area of the benthos was calculated with the equation described by Dollar et al. (1991):

$$F = V (C_t - C_o) / (A \times T)$$

Where V = volume of water inside the benthic chamber over the sediment at initial deployment, C_o and C_t = the concentrations of nutrient before and after time T, and A = area of sediment enclosed in chamber.

The fluxes are described in units of mmol m^{-2} day^{-1}, unless otherwise stated in the text or the figures. These units were chosen to represent the flux, as the method to measure flux was similar in magnitude (i.e., two samples taken during each 24-hour period). Further, an analysis of the available data from the deployments that lasted longer than 24 hours revealed that fluxes during the second day of the deployment were often significantly higher ($p<0.05$) than the fluxes of these nutrients on the first day of the deployment. This was especially true for deployments during the summer, when oxygen concentrations in the chambers were notably lower on the second day than on the first.

Each deployment consisted of 4 benthic flux chambers and the average of the fluxes of NO_x-N, NH_4-N and DIP were plotted on whisker-plots, where the middle point is the average of these values and the whisker represents 1 standard deviation. Nitrate and nitrite were represented together as NO_x-N for these benthic flux chamber results, as NO_2^- concentrations typically represented 5 - 10% of the NO_x-N in these samples.

In general, NO_x-N concentrations in the chambers showed a linear decreased, while NH_4-N and DIP increased linearly (figure 3.6). Although the ranges of

these increases and decreases changed from winter to summer this general pattern held true. Comparisons are made in the subsequent section with regard to differences between regions (spatial variations in fluxes) and between winter and summer (seasonal variations in fluxes). Data on benthic fluxes and changes in nutrient concentrations within the benthic chambers for all deployments are listed in appendix D.

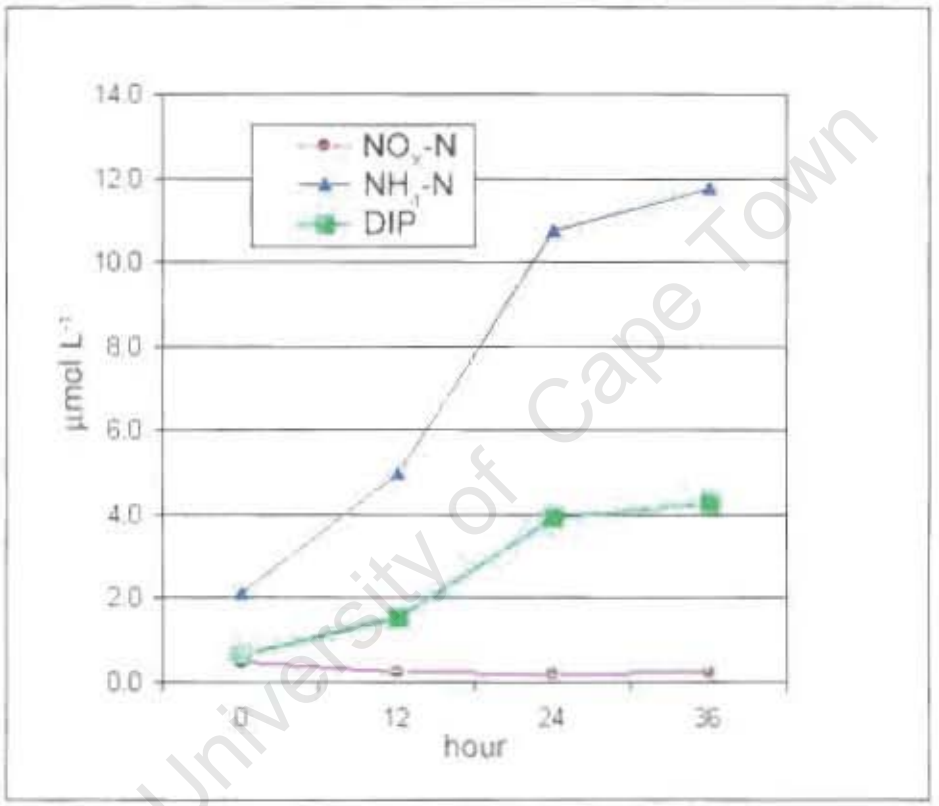


Figure 3.6. Average changes in nutrient concentrations within benthic flux chambers (n=4) during the February 21 – 22, 2002 deployment at station A6. These increases in NH_4 and DIP and decrease in NO_x concentrations were typical of deployments during the summer in the Ashmead.

Summer site differences in fluxes

The goal of this section was to compare the fluxes between the 4 different sites in the Ashmead; and between sites in the upper estuary, Lagoon and Bay regions (e.g., sand vs. *Zostera*). All summer deployments in the Ashmead ran for 36 hours, with the exception of the deployment at A5, which was terminated after the 24-hour measurements were made due to an impending storm. This allowed a comparison between these sites of 24-hour (day^{-1}) fluxes for NH_4-N, NO_x-N and DIP. A comparison between sites in the upper estuary, Lagoon and Bay regions during the summer was not possible as there was only one deployment in each of these regions that was representative of fluxes under well-oxygenated conditions.

The 24-hour NO_x-N fluxes between the Ashmead sites were not significantly different ($p>0.05$) with the exception of A7, which was significantly different ($p<0.05$) from the other NO_x-N fluxes in the Ashmead (Figure 3.7). In deployments A3, A5 and A6 the initial concentration of NO_x-N was 0.7, 0.7 and 0.5 $\mu mol\ L^{-1}$ respectively, typical of summer NO_x-N concentrations in the Ashmead (refer to Appendix B). In the A7 deployment the initial concentration of NO_x-N was 2.9 $\mu mol\ L^{-1}$, and was the result of an upwelling event introducing water with a higher than expected NO_x-N concentration during the time of this deployment.

A further example of the higher NO_x-N fluxes was found in the Bay deployment of February 27, 2002. In this deployment, the initial NO_x-N concentration was 17.5 $\mu mol\ L^{-1}$. This was due to a sustained southeast wind event resulting in coastal upwelling that introduced higher NO_x-N concentration in the bay region with the flood tide. This was the highest concentration of NO_x-N within the water column recorded during the deployment of a benthic flux chamber experiment. This

deployment ran successfully for 36 hours and the average NO_x-N concentration in the four benthic flux chambers fell to 7.9 μmol L^{-1}, at the 36-hour sampling. The NO_x-N fluxes associated with this change in concentration were the highest witnessed during the benthic flux chamber experiments (Figure 3.7).

These two sites, A7 and Bay, represented the most downstream sites along the main channel and the Ashmead, and were thus the sites most likely to be exposed often to higher NO_x-N concentrations as a result of upwelling events in the ocean. The higher initial NO_x-N concentrations at both of these sites provided an opportunity to examine the affect of higher initial NO_x-N concentrations on the benthic fluxes.

Upwelling events, in addition to increasing the NO_x-N concentrations, lowered temperatures and increased dissolved oxygen concentration in the estuarine water. As a result, both of these deployments, A7 in the summer and the Bay site on February 27, 2002, had high initial oxygen concentrations of 8.6 and 7.3 mg L^{-1}, respectively. Additionally, temperatures during the initiation of the deployments were lower than most other summer deployments, and near slack flood tide more closely resembled temperatures of the winter deployments (refer to Figure 1.14). During the course of the A7 deployment temperatures ranged from 14.9 to 20.7 °C, and during the Bay deployment temperatures ranged even lower, from 13.1 to 15.5 °C.

Taylor (1992) used a laboratory experiment in which cored sediments from the Kariega Estuary (about 300 kilometers east of Knysna Estuary) were subjected to concentrations of nitrates varying from 1.8 to 25 μmol L^{-1}, and temperatures ranging from 16 to 25 °C. He found that nitrate flux was controlled by the increase in availability of nitrates in the overlying water, rather than differences in temperature. In support of the findings of Taylor (1992), these benthic chamber

deployments demonstrated that when high NO_x-N concentrations existed in the overlying water the NO_x-N fluxes into the sediment were also high, and temperature did not appear to affect these fluxes.

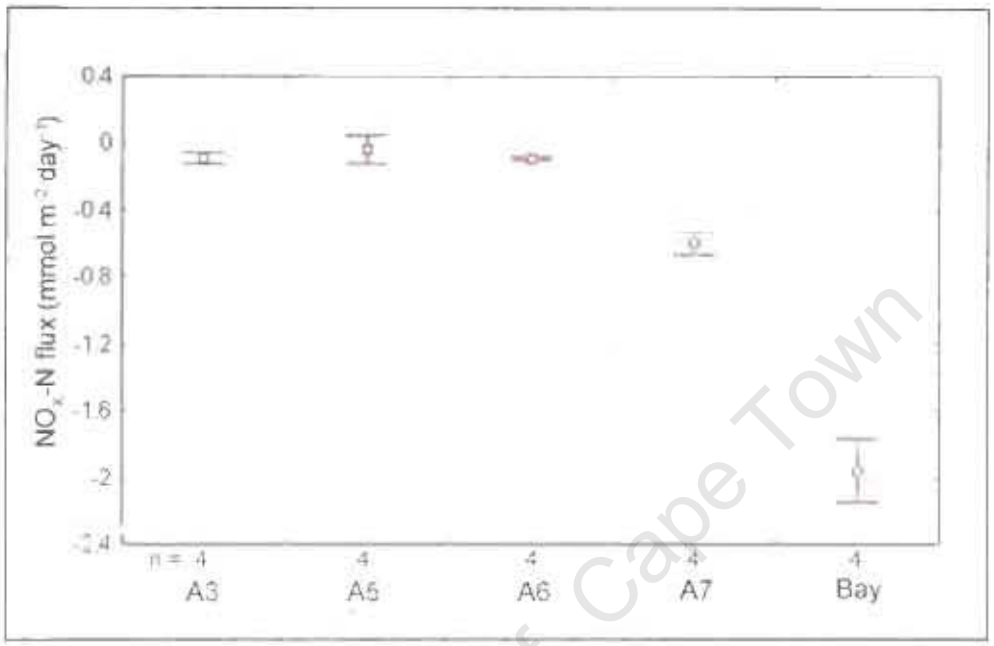


Figure 3.7. NO_x-N fluxes for the Ashmead benthic sites during summer and the Bay site on February 27, 2002. Note the lower flux for A7 and the Bay site. This was due to higher initial concentrations of NO_x-N for the A7 and the Bay deployments as a result of coastal upwelling that elevated NO_x-N into the Knysna Estuary.

The NH_4-N fluxes at site A6 during summer were significantly higher ($p<0.05$) than those measured at the other deployment sites in the Ashmead. Similarly, the DIP fluxes at site A6 during the summer were significantly higher ($p<0.05$) than the DIP fluxes at other sites in the Ashmead. There was no significant difference ($p>0.05$) in the fluxes of NH_4-N or DIP between sites A3, A5 and A7 (Figure 3.8). The reason for higher fluxes of NH_4-N and DIP at site A6 is not immediately apparent. Interestingly, the organic material content was higher at

this site than at the other sites in the Ashmead (refer to Table 3.4). Diatomaceous mats on the sediment surface have been observed at this location during summer (Professor Brian Allanson, personal communication). While there was some indirect evidence to suggest that the sediments at site A6 are more biologically active than other sites in the Ashmead during summer, this pattern did not hold true for the winter (Figure 3.11). Thus, the earlier null hypothesis was confirmed by these benthic flux chamber experiments.

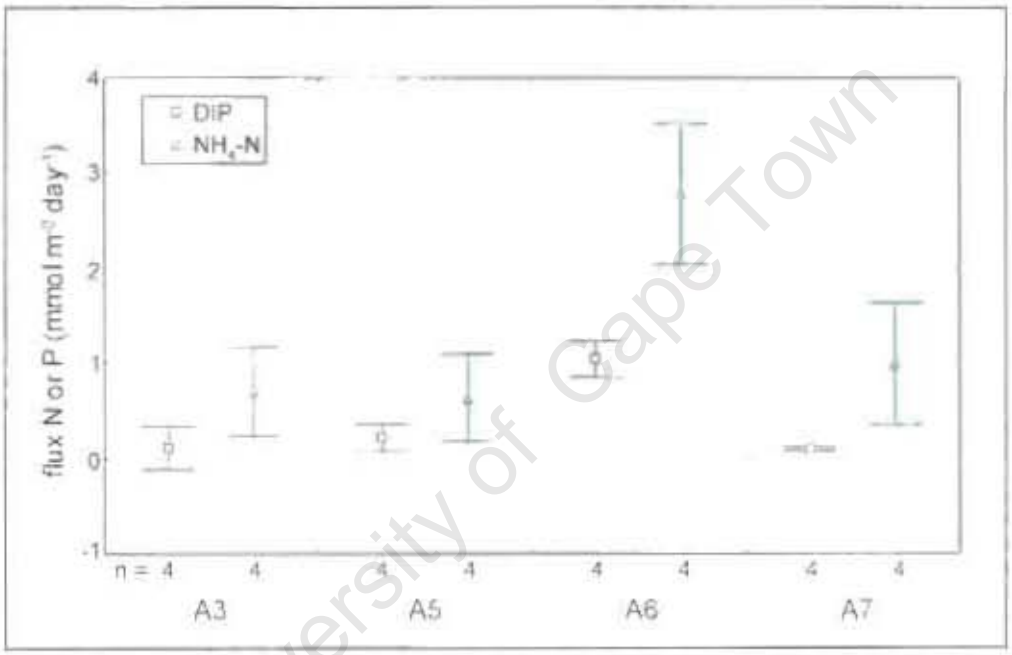


Figure 3.8. Significantly higher ($p<0.05$) benthic fluxes of NH_4-N and DIP were measured at A6, as opposed to other benthic chamber sites in the Ashmead during summer.

Winter site differences in fluxes

The NO_x-N benthic net fluxes in the Ashmead during winter were compared at 9 hours and 24 hours in an attempt to determine if nitrification at these sites significantly increased the NO_x-N concentrations in the overlying water. The fluxes of NO_x-N at 24 hours showed no significant difference ($P>0.05$) between sites. However, the average of 24-hour NO_x-N fluxes at site A5 was positive (0.3 mmol m^{-2} day^{-1}), while the remaining sites in the Ashmead showed average 24-hour NO_x-N fluxes of zero or negative values (Figure 3.9). The comparison in NO_x-N fluxes at 9 hours showed that site A5 was significantly higher ($P<0.05$) than at sites A3 and A6 but not significantly higher ($P>0.05$) than at site A7. Sites A3, A5 and A7 showed an average flux of NO_x-N at 9 hours that was positive (Figure 3.9), indicating a vertical concentration gradient that directed NO_x-N into the overlying water from the Ashmead sediments during winter.

It was hypothesized (refer to section 2.3) that nitrification was the source of increased NO_x-N concentrations measured on a tidal scale in the Ashmead during the winter. These 24-hour average fluxes showed a positive flux of NO_x-N at site A5, and the 9-hour fluxes show positive flux at 3 out of the 4 sites. However, they did not convincingly demonstrate that NO_x-N flux was actively increasing NO_x-N concentrations in the overlying water. These data did not directly indicate that NO_x-N flux was occurring in the Ashmead at quantities sufficient to account for the magnitude of NO_x-N concentration changes measured from LWST to HWST in June, 2000 (refer to Table 2.7). However, these results indicate that a gradient existed during winter to produce a positive flux of NO_x-N at most of the sites tested during the first 9 hours (first day). It was assumed that nitrification in the deeper oxic layer, found during the winter, was the cause.

It is likely that nitrification was not spatially consistent in the Ashmead sediments, and was more prevalent in the intertidal areas of the Ashmead than the subtidal areas. The constraints of this experimental design limited the deployment of the benthic flux chambers to the subtidal channel of the Ashmead. Site A5 was the shallowest of the sites in the Ashmead, and unlike the other sites in the Ashmead the sediments at site A5 were intertidal for a day or two at LWST conditions. Brown and McLachlan (1990) recorded that sediments higher in the intertidal zone of exposed beaches have deeper oxic layer compared to sediments at the swash line. The measurement of oxic layer depths at these sites in the Ashmead (refer to Table 3.8) indicated no apparent change in Eh at site A5 during the winter or the summer measurements, and was inconclusive as to the depth of the oxic layer at this site.

Changes in the NO_x-N fluxes at a temporal scale (hours) may have played an important role in the patterns observed in fluxes of NO_x-N in the Ashmead during the winter deployments. An and Joye (2001) showed that modelled nitrification rates in suboxic sediment may vary considerably during a 24-hour cycle. Thus, averaging these fluxes across 24 hours or even 9 hours may fail to illustrate the maximum NO_x-N concentrations that were obtained in these chambers.

In summary, the results of these benthic flux chamber experiments did not provide convincing evidence that nitrification resulted in an increase in NOx-N concentration in the Ashmead during winter, equal in magnitude to the increases measured during the winter of 2000 (refer to Table 2.7). They did suggest that a positive concentration gradient for NO_x-N existed during the winter from the sediment to the overlying water, and that this gradient was stronger in the intertidal than it was in the subtidal sites of the Ashmead. Future studies should consider an experimental design, which is not limited to static chambers or to the subtidal areas of the Ashmead. For example, the Plexiglas flume described in

Wolaver et al. (1983) could prove a useful design for testing this hypothesis further.

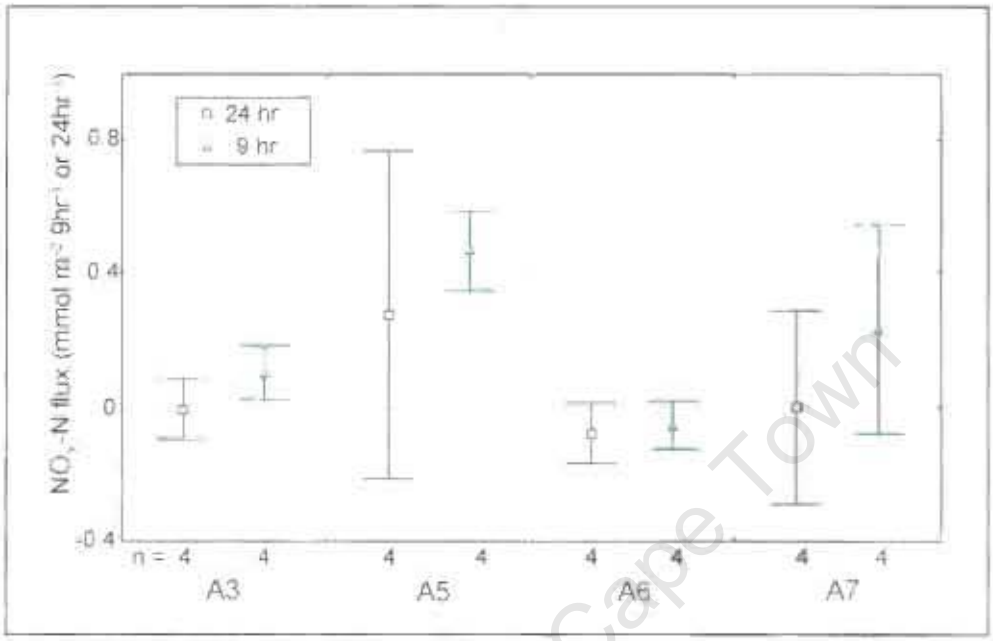


Figure 3.9. The benthic fluxes of NO_x-N were significantly higher ($P<0.05$) at A5 at 9 hours, compared with sites A3 and A6 in the Ashmead. There was no significant difference ($p>0.05$) in the flux of NO_x-N for any of the sites measured at 24 hours.

The NO_x-N fluxes in the sand and *Zostera* sites in the upper estuary, Lagoon and Bay regions during winter are given in Figure 3.10. The fluxes of NO_x-N in these regions were similar to the fluxes of NO_x-N measured at the stations in the Ashmead during the winter (refer to Figure 3.9). However, as discussed earlier in this section, the initial NO_x-N concentration of the benthic flux experiments will primarily control the flux of this nutrient. The changes in the fluxes of NO_x-N between these location (e.g., sand or *Zostera*) at each site must be considered in relation to the initial concentration of this nutrient. Therefore, the lowest NO_x-N fluxes were expected in the Bay sand and *Zostera* sites, which had some of the

highest initial concentration of NO_x-N (4.0 and 2.1 μmol L^{-1}, respectively) of all sites tested in the upper estuary, Lagoon or Bay (Figure 3.10).

Interestingly, due to a miscalculation of the tidal range at the time of deployment the sand site in the Lagoon region was exposed for 3 hours during slack low tide (e.g., intertidal). However, there was no significant difference ($p<0.05$) between the NO_x-N flux at this intertidal site and the Zostera site in the Lagoon during winter. This information is consistent with the discussion that initial NO_x-N concentration is the most important factor controlling NO_x-N flux.

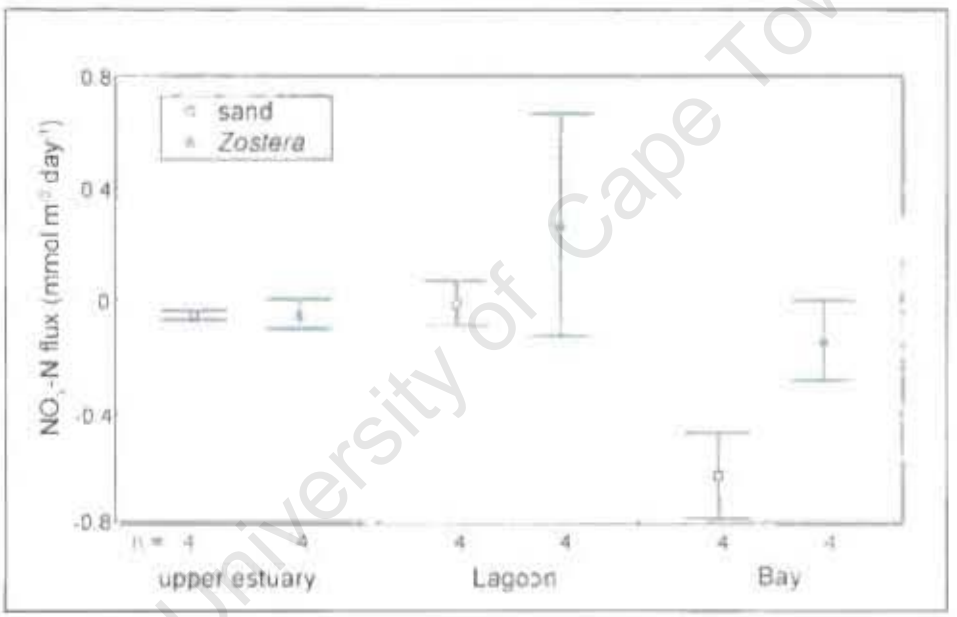


Figure 3.10. The NO_x-N flux in the upper estuary, Lagoon and Bay during the winter.

The fluxes of NH_4-N in the Ashmead during the winter increased from sites A3 to A7, indicating higher NH_4-N fluxes from the sediments at the sites closer to the mouth of the Estuary (Figure 3.11). A similar pattern of NH_4-N in the Ashmead was not observed during the summer (refer to Figure 3.8). The fluxes of DIP

during the winter in the Ashmead did not reflect an increase similar to the fluxes of NH_4-N at these sites (Figure 3.11).

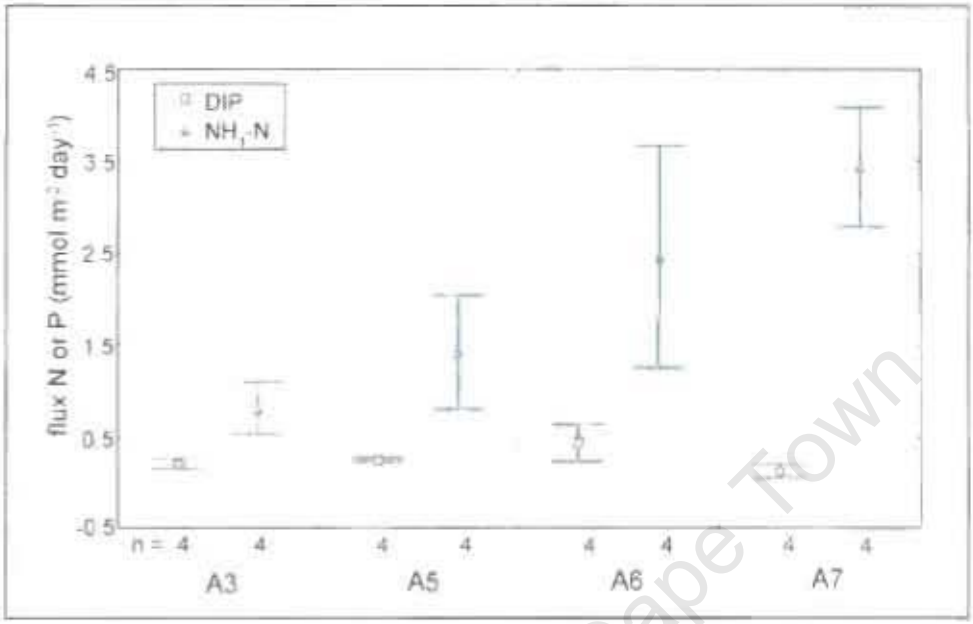


Figure 3.11. The fluxes of NH_4-N and DIP in the Ashmead during winter. There was a clear pattern of increasing NH_4-N fluxes at sites closer to the mouth of the Estuary in the Ashmead. However, this pattern was not observed in the fluxes of DIP at these same sites.

The fluxes for NH_4-N and DIP during winter at the sites of the upper estuary, Lagoon and Bay are shown in Figure 3.12. Overall, they presented a pattern similar to that observed in Ashmead, where the fluxes of these nutrients were higher at the downstream sites (i.e., upper estuary < Lagoon < Bay). The NH_4-N fluxes in the *Zostera* site in the Bay were significantly higher ($p<0.05$) than the NH_4-N fluxes in *Zostera* in either the upper estuary or the Lagoon. The NH_4-N fluxes at the sand site in the Bay were significantly higher ($p<0.05$) than the sand site in the upper estuary, but were not significantly higher ($p>0.05$) than those in the Lagoon.

The DIP fluxes at the *Zostera* site in the Bay were significantly higher ($P<0.05$) than the DIP fluxes at the sand site in the Bay. A similar pattern for NH_4-N flux was found, with significantly higher ($P<0.05$) flux at the Zostera site compared with the sand site (Figure 3.12). Taylor (1992), described a similar pattern for NH_4-N flux between a macrophyte bed and a tidal creek (sand) in the Kariega estuary with a NH_4-N flux from a bed of *Sarcocornia perennis* higher than it was from a nearby tidal creek. However, Taylor (1992) found the opposite pattern for DIP flux than was observed in the Bay (Figure 3.12), with the tidal creek with a sand bottom exporting more DIP than the adjacent bed of *Sarcocornia perennis*.

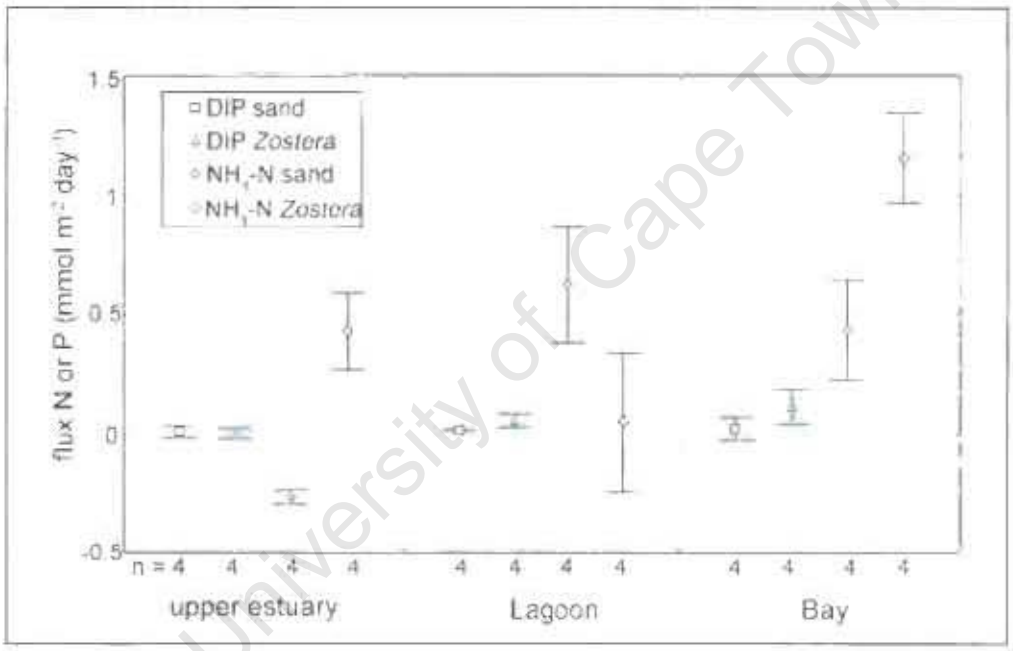


Figure 3.12. The fluxes of NH_4-N and DIP at the sand and *Zostera* sites in the regions of the main channel during winter. Note the sand site in the Lagoon region was intertidal.

Regional differences in fluxes

Another goal of these benthic flux experiments was to determine if there were differences in the fluxes of the measured nutrients between the four regions of the Estuary. In this section the spatial differences in fluxes are examined by pooling all fluxes of each nutrient in each region, with regard to season, regardless of the substrate (e.g., sand or *Zostera*) on which the chambers were deployed.

The NH_4-N fluxes for the Bay region were significantly higher ($p<0.05$) than all other regions during the summer, while the other regions showed no significant difference ($p>0.05$) from each other (Figure 3.13). The higher NH_4-N fluxes in the Bay were questionable, and were likely caused by the higher than expected initial nutrient concentrations of this deployment, which resulted from upwelling in the adjacent ocean in the days prior to this deployment. The DIP fluxes for the 4 regions during summer were remarkably consistent.

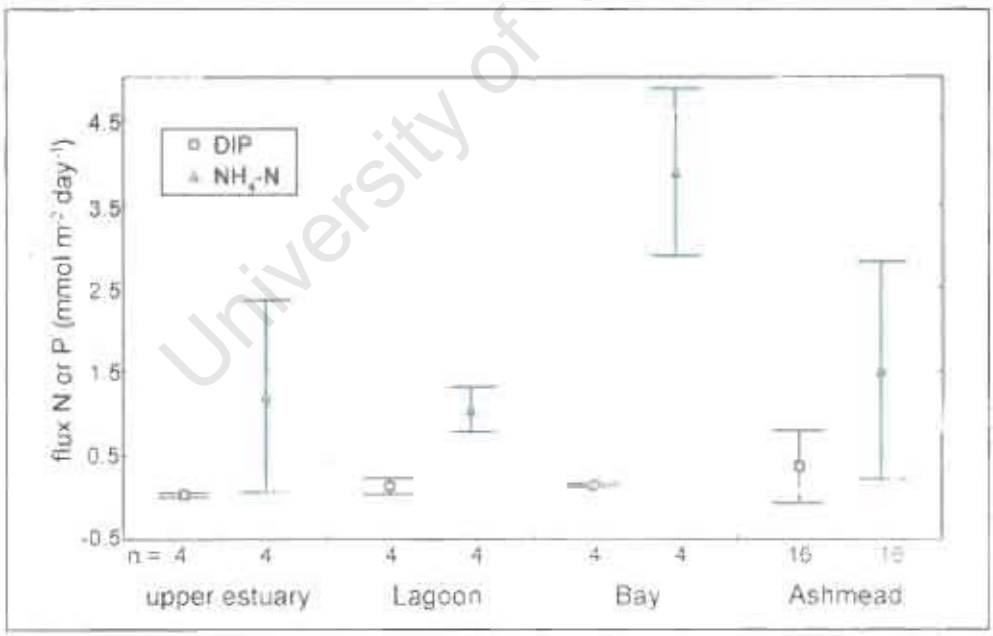


Figure 3.13. The fluxes of NH_4-N and DIP during the summer in each region of the Estuary.

The NH_4-N fluxes were significantly higher ($p<0.05$) in the Bay than in the Lagoon during the winter (Figure 3.14). Overall, the NH_4-N fluxes during the winter increased in the Bay and Ashmead when compared to the Lagoon and upper estuary. The DIP fluxes in the 4 regions of the Estuary during the winter reflected a similar pattern as the NH_4-N fluxes in these 4 regions. The Lagoon DIP fluxes were significantly higher ($p<0.05$) than the upper estuary, and DIP fluxes in the Bay were significantly higher ($p<0.05$) than in the Lagoon (Figure 3.14).

This is the same pattern that would be expected during the summer, when productivity would be expected to increase with a rise in temperature and hours of daylight, but this was not shown by data represented in Figure 3.13. Overall, on an estuary-wide average, the NH_4-N fluxes are higher during the winter (2.0 mmol m^{-2} day^{-1}) than during the summer (1.7 mmol m^{-2} day^{-1}). Further, there was no significant difference ($p<0.05$) in the NH_4-N or DIP fluxes between winter and summer at any of the Ashmead channel s tes. These results do not conform to the patterns in benthic fluxes of ammonium measured in other estuaries, where the NH_4-N fluxes were higher in the summer than in the winter (e.g., Dollar et al., 1991; An and Joye, 2001). As discussed previously, it is widely accepted, and demonstrated in many estuaries, that NH_4-N fluxes increase during the summer, due in part to a decrease in oxic layer depth. However, while the oxic layer measurements during winter and summer showed a general decrease in oxic layer depth during summer, the NH_4-N fluxes did not increase during summer. The reason for this may be the low concentration of NH_4-N in the overlying water that constantly drives the net vertical flux of NH_4-N out of the sediments. However further detailed studies are required to completely resolve this process.

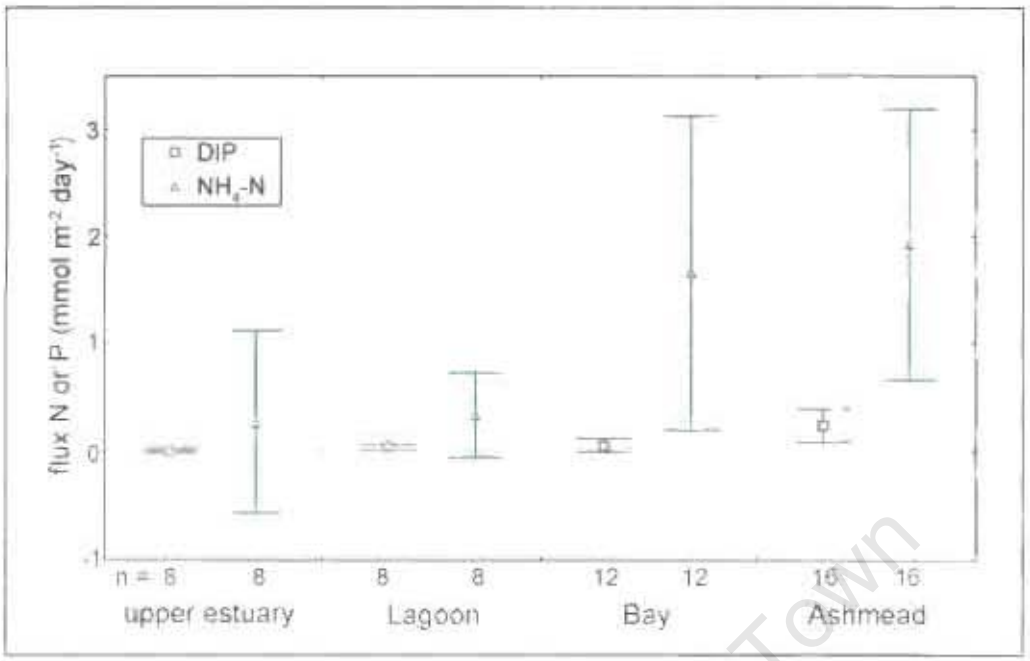


Figure 3.14. The fluxes of NH_4-N and DIP during the winter in each region of the Estuary.

Day to night differences in fluxes

A comparison between the fluxes of NH_4-N and DIP between the first day of the deployment and the first night of the deplcyment revealed that, regardless of season, these fluxes were always higher during the first night than the first day. During the 11 winter deployments throughout the 4 regions of the Estuary, 3 deployments showed significantly higher ($p<0.05$) fluxes of NH_4-N and 3 deployments showed significantly higher ($p<0.05$) fluxes of DIP. During the 7 summer deployments, 2 showed significantly higher ($p<0.05$) fluxes of NH_4-N and 3 showed significantly higher ($p<0.05$) fluxes of DIP. Interestingly, the upper estuary region was the only region that never displayed significantly higher ($p<0.05$) fluxes of NH_4-N or DIP between the day and night, during either the winter or the summer.

Nutrient fluxes during low oxygen conditions

As stated earlier one deployment in the upper estuary, Lagoon and Bay regions during the summer, demonstrated higher than expected fluxes of NH_4-N and DIP, when the concentration of oxygen fell below 2.5 mg L^{-1} in 2 or more of the 4 chambers. Oxygen concentrations of 2.4 and 2.1 mg L^{-1} were measured in 2 of the upper estuary, benthic flux chambers on January 29, 2002. In the Lagoon, oxygen concentration in all 4 chambers was below 2 mg L^{-1} on February 25, 2002, following 2 days of full sun and air temperatures in excess of 30 °C, coupled with limited tidal circulation (i.e. neap tides). The Bay deployment in the summer displayed oxygen concentration less than 2.5 mg L^{-1} in 3 out of 4 benthic flux chambers, as measured on January 24, 2002.

The low oxygen concentrations in the Bay and Lagoon were measured at the 07:00 sampling, 24 hours into the deployment. It was assumed this was due to relatively high oxygen use by respiration during the night as a similar variations in oxygen concentrations were observed by An and Joye (2001), and found to be due to net benthic respiration and photosynthesis. This consumption of oxygen during the night, and the resulting increase in the fluxes of NH_4-N are illustrated in Figure 3.16.

The fluxes of NH_4-N under these low oxygen conditions were higher in the regions closer to the mouth (i.e., upper estuary < Lagoon < Bay), as shown in Figure 3.15. This trend was similar to the trend shown in Figure 3.13, for these same regions during the summer, when oxygen levels did not fall below the threshold of 2.5 mg L^{-1} in any of the benthic flux chambers. However, the deployments in which the oxygen levels were at or below this threshold, displayed higher fluxes of NH_4-N and DIP as shown in Table 3.8.

Table 3.8. The average flux of NH_4-N and DIP (mmol m^{-2} day^{-1}) during low oxygen conditions (NH_4-N/low and DIP/low), and these fluxes when oxygen concentrations remained in expected ranges (NH_4-N/high and DIP/high). The significant increases ($p<0.05$) are underlined.

	NH_4-N/high	NH_4-N/low	DIP/high	DIP/low
upper estuary	1.2	2.2	0.0	0.2
Lagoon	1.0	4.6	0.1	0.5
Bay	3.8	6.0	0.2	0.4

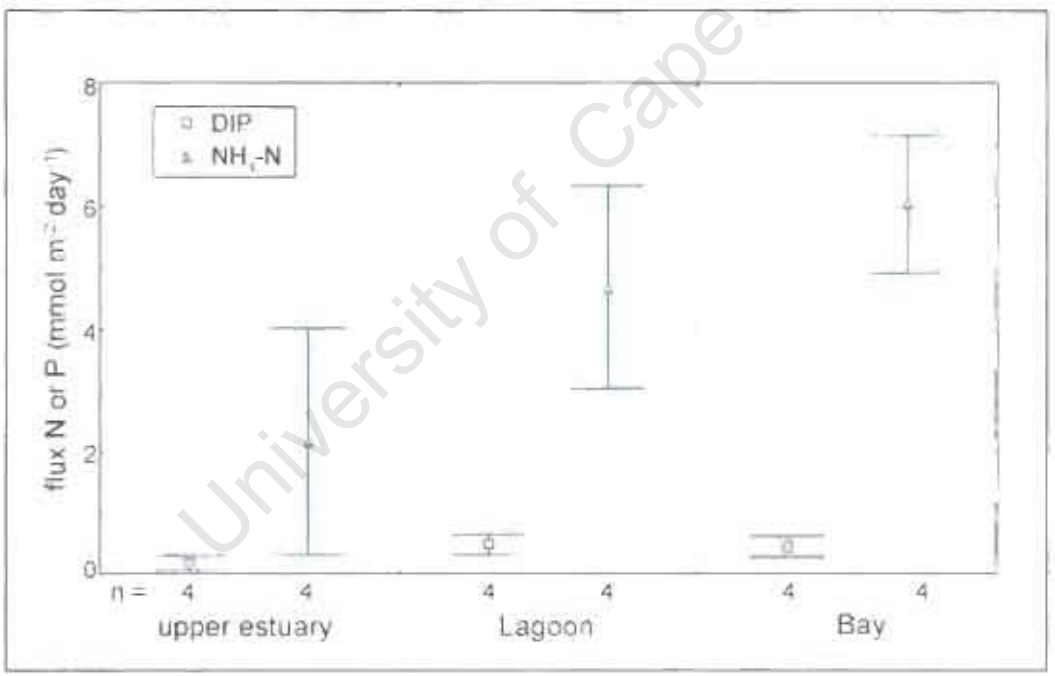


Figure 3.15. The benthic flux of NH_4-N and DIP in the upper estuary, Lagoon and Bay regions under low oxygen conditions during the summer.

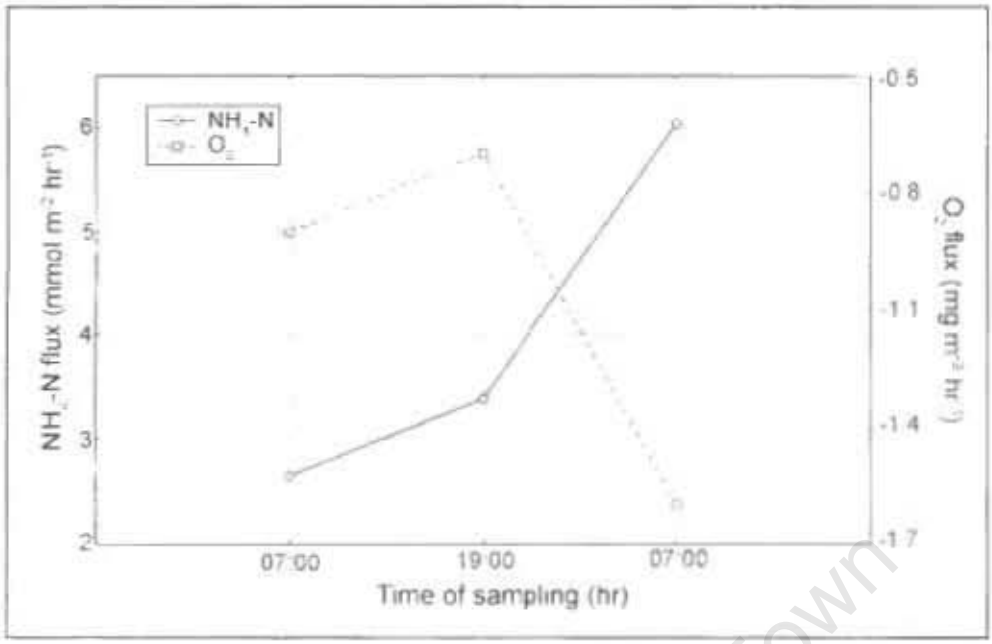


Figure 3.16. The average benthic fluxes of NH_4-N and oxygen in the Bay region during low oxygen conditions. Note the rapid incline in the production of NH_4-N and decline in oxygen during the night, when benthic respiration mops up the oxygen, and oxygen fluxes increase.

Summer benthic chamber deployments in the Ashmead channel did not display low oxygen concentrations below the 2.5 mg L^{-1} threshold during the summer deployment schedule. Allanson et al. (2000a) found that dissolved oxygen concentrations in the Ashmead were higher than in the main channel, and suggested that phytoplankton growth, and extensive eelgrass meadows in the lower intertidal areas as the reason for higher oxygen production in this region.

Many South African estuaries become isolated from the sea by the formation of a sand berm across the mouth (Allanson et al., 1999). When these estuaries become isolated from tidal ebb and flow, a decrease in oxygen concentrations may follow. Allanson et al. (2000a) found that the tidal inflow associated with upwelling events substantially increased cissolved oxygen concentrations throughout the Bay and Lagoon up to Belvidere. Additionally, measurements of

dissolved oxygen taken in conjunction with the deployment of the benthic flux chambers indicated that during periods of reduced tidal exchange (i.e. neap tides) oxygen concentrations were lower than during spring tides. It follows that an estuary mouth closure could result in a decrease in dissolved oxygen concentrations and therefore the fluxes of NH_4-N and DIP from the sediments would be expected to follow these changes.

3.5 DIP desorption from fluvial sediments

The results from the water column monitoring Part II, section 2.3 revealed the highest concentration of DIP occurred during the late summer in the upper estuary and Lagoon. Since these DIP concentrations were higher than DIP concentrations found in either of the end-member source waters, it was suspected that there was an internal source of DIP within the Knysna Estuary (refer to section 2.3). The 21% reduction in DIP concentration measured in filtered water samples as opposed to unfiltered water samples suggested that suspended solids were this source (refer to section 2.1).

Many studies have discussed the role of estuarine sediments in adsorption and desorption of P (Nixon et al., 1996; Howarth et al., 1995; Froelich, 1988). The review by Howarth et al. (1995) stated that desorption of P from particles in many estuaries tends to buffer the concentration of dissolved P at concentrations of 30 - 60 ug L^{-1}. The DIP concentrations measured in the Knysna Estuary falls in this range. The lowest concentrations of DIP were found during the dry season and the highest during the wet season. This suggested an association between the increases in DIP concentration and the increase in river flow during the wet season.

The characteristics of the soils found in the Knysna catchment basin assist the transport of runoff from the forest soils following substantial rainfall (Figure 3.17 and refer to section 1.6). It follows that this lateral movement of water from the forest soils transported organic particulate material including sediment into the rivers that drain into the Estuary. Therefore, much of the sediments transported by this method will be deposited in the Estuary with the runoff associated with the strongest storms of the wet season.

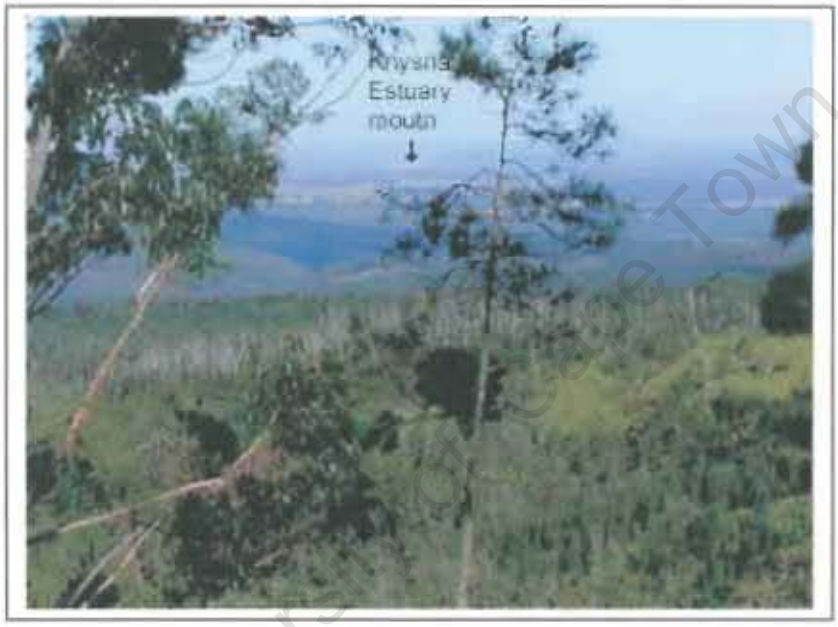


Figure 3.17. The mountainous and forested catchment area of the Knysna River Estuary. Approximately 1/3 of the catchment area was commercial forest plantation. Two commercially grown types of trees are shown in the foreground, the pine (center) and eucalyptus (left).

Froelich (1988) established that clay particles exhibit adsorption and desorption to phosphate (P) as a result of their natural oxide coatings. He described 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. For most natural clays and oxides, this fast reaction can account for 50% – 90% of the

total response. Froelich (1988) described experiments in which maximum sorption occurred at pH levels of 4.8 – 6. At this pH level, the $H_2PO_4^-$ ion is considered to be the dominant form of P adsorbed to the surface of the clay particle. When these particles were exposed to the higher salinity water, the $H_2PO_4^-$ ion was released.

The soils of the catchment area that drain into the Knysna Estuary are acidic, and the runoff from these soils had a pH near 5 (refer to section 1.4). The pH of the Estuary ranged from 7 to 8. Given this pH difference, the waters of the catchment area that drain into the Estuary were ideally suited to transport P on the clay particles that wash into the river during the wet season by the process described by Froelich (1988).

South African Forestry Company Limited (SAFCOL) began fertilization of pine tree *Pinus radiata* with P-based fertilizers in the forestry plantations in 1986. From the years 1986 to 1997, P-based fertilizer in granular form was spread manually on the surface of the newly planted plots. During the years 1998 to 2001 tabular forms of P-based fertilizer were buried in the soil at a depth of 35 cm with each new seedling planted. The granular form of fertilizers was distributed at ratios of 19.5 kg P hectare^{-1} to 8 kg N hectare^{-1}, while the introduction of tabular fertilizers reduced this P to N ratio to 19 to 8, (Korne Yonker, SAFCOL, personal communication). From GIS data provided by SAFCOL, it was calculated that 9% of the catchment area had been fertilized by these two methods, resulting in 70 tons of P introduced to the catchment basin soils from 1986 to 2001.

Methods

It was suspected that P introduced as fertilizers in the commercial plantations within the catchment basin, resulted in increased DIP concentrations in the Estuary. To test if sediments in river waters contained P bound to their surfaces, 1000 ml water samples were collected in acid-washed glass bottles from Jubilee Creek, the Knysna River at the Charlesford Weir (station K.R.) and at station S28 on the Salt River (refer to Figures 1.4, 1.8 and 1.9).

Although Jubilee Creek is in the adjacent Goukamma catchment basin it is representative of a high catchment river of the area, which was not affected by runoff containing fertilizers, as the plantations above this site were planted prior to 1986 and remain unfertilized. The collection of water samples from high in the Knysna catchment basin was not possible due to difficult access. The sites K.R. and S28 contain runoff from plantation forest fertilized with P-based fertilizer as described above.

The samples from K.R. and S28 were collected after 46 mm of rainfall on August 30, 2001. The Jubilee Creek sample was collected after 24 mm of rainfall on September 27, 2001. All samples represented high flow rates with visible suspended sediments in the collected samples.

One method widely used to determine the amount of bioavailable P introduced by sediments contained in river waters entering estuaries involves the resuspension of extracted sediments in low P seawater, and measuring the change in DIP concentration of this seawater after a period time (Froelich, 1988). However, low phosphate seawater for this method was unavailable.

As an alternative method, a buffer solution was made up with 0.19 g sodium hydroxide (NaOH) dissolved in 150 ml of distilled water hereafter referred to as

NaOH solution. This solution was used in conjunction with chemically pure sodium chloride (NaCl) to raise the pH and salinity of the sample to that of water found in the Knysna Estuary. The DIP concentration of the NaOH solution was equal to the distilled water.

The samples were shaken until all sediments were suspended, and three sets of 100 ml aliquots were decanted from each sample. The DIP concentrations were measured in the first aliquot without further processing, hereafter known as unfiltered. The second set of aliquots were filtered through Whatman GF/F removing suspended solids prior to measuring for DIP concentration, hereafter known as filtered. To the third set of 100 ml aliquots, 0.3 ml of NaOH solution and 3 g of chemically pure sodium chloride (NaCl) were added raising their pH to approximately 7.3 and salinity to 30 ppt, hereafter known as treated. These values were chosen as representative of pH and salinity that are found in the upper estuary and Lagoon regions during wet season. The treated aliquots were mixed by shaking for 2 minutes and allowed to stand for 15 minutes prior to analyzing each for DIP. The median value of these three aliquots was chosen to represent each condition.

Results and Discussion

The K.R. and S28 treated samples displayed higher DIP concentrations than the K.R and S28 unfiltered samples, and these unfiltered samples were higher than the K.R and S28 filtered samples. The Jubilee Creek sample did not display a decrease in DIP concentration when filtered, and only a small increase when this sample was treated (Table 3.9).

Table 3.9. The DIP concentration (μmol L^{-1}) in samples collected upstream (Jubilee Creek) and downstream (K.R. and S28) of commercial plantations fertilized with phosphate. High DIP concentrations of treated samples represent the release P bound to suspended sediment. Values given are the median value of three replicates.

	filtered	unfiltered	treated
Jubilee Creek	0.2	0.2	0.3
Knysna River (K.R.)	0.6	1.5	5.4
Salt River (S28)	0.6	2.8	4.9

In the K.R. and S28 treated samples, increasing the pH and salinity to values found in the Estuary, released the P bound to the surface of the sediments. When the K.R. and S28 samples were filtered prior to analysis for DIP, removing suspended sediments, the DIP concentrations were lower than in the unfiltered samples. The additions of reagents used to determine DIP concentration induced the partial desorption of DIP from the surface of sediments in the unfiltered samples. The samples from Jubilee Creek differ from the samples K.R. and S28 as they did not contain sediments downstream of forest plantations where P was used as fertilizer.

It was concluded that sediment, presumably clays, of fertilized forest contain P bound to their surface lattices. These sediments were transported downstream to the Knysna Estuary during periods of strong river flow. The exposure of these sediments to estuarine water (with higher salinity and pH than were found in the Knysna River) resulted in the release of the phosphate ion from the sediment surface, and increased the DIP concentrations in the estuarine water.

It was suspected that desorption of P from fluvial sediments increased the DIP concentrations in the Estuary in the days and weeks following the strongest river flows of the year. HWST conditions should maximize the release of DIP from sediments into the Estuary, by allowing the sediments in the upper inter-tidal zone to contact higher pH and saline water. In support of this point, the highest DIP concentrations, which were measured during the water column nutrient monitoring, were observed in the February 2001 HWST surveys, following strong river flow events in January 2001 (refer to section 2.3, *Dissolved Inorganic Phosphate*).

This mechanism is similar to the one described by Prastka and Jickells (1995) where tidal state played an important role in P release from sediments of the Great Ouse Estuary, England. Their data indicated that P flux from the sediment was enhanced when high salinity water with low dissolved P replaced the lower salinity water with high dissolved P during tidal exchange. They indicated that the rapid desorption rate for P of 10 minutes, and the longer adsorption time of several hours enhanced the removal of dissolved P from the estuary sediments to the overlying water.

Several authors describe how the diversity of processes that control the fate of P in estuaries complicates the classification of estuaries as either sources or sinks for P (Prastka et al., 1998; Nixon et al., 1996; Howarth et al., 1995). Knysna Estuary was no exception to this statement, showing behavior as source and sink for P during different times in a yearly cycle. The activities in the catchment basin affecting P inputs and sediment transport coupled with hydrology, and biological considerations of P flux, require that Knysna Estuary be considered individually with regard to these issues. While there was evidence that P was supplied to the Knysna Estuary from the 70 tons of P used as forestry fertilizers there was no evidence, thus far, that the resulting increases in DIP concentration are problematic to the Estuary. For example, the DIP fluxes in the upper estuary

and Lagoon regions during summer were not higher than expected. However, future research on the Knysna Estuary should consider the potential for DIP sorption from sediments entering from the Knysna and Salt rivers.

Part IV: Discussion

In a recent review on nutrients in estuaries, Nedwell et al. (1999) stated in their introduction, "Much of the research in the past 20 years has been directed towards furthering understanding of the effects of increased nutrient loads in estuaries, with consequent focus on high-nutrient, eutrophic estuaries; while there is a relative lack of information on low-nutrient, oligotrophic estuaries." This dissertation was the first body of work that measured the internal processes influencing the nutrient loads in a South African estuary. In accomplishing this goal, it contributes to the understanding of how a low-nutrient, oligotrophic estuary was affected by increased nutrient loads.

Currently, the majority of estuaries in South Africa do not seem to be experiencing problems associated with increased nutrient loads (e.g., eutrophication), which have 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 low-nutrient estuaries, than it has been with understanding problem-specific processes such as freshwater flow requirements (*vide* Adams et al., 2002). However, if South Africa estuaries remain unstudied with respect to the processes that govern N and P cycling, then it is 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 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 industrialization, limited use of fertilizers, and limited population densities living on the estuarine littoral and in the catchments.

While small in comparison to its Northern Hemisphere counterparts, the Knysna Estuary is the largest of the microtidal estuaries in South Africa. The early work of Day et al. (1952) revealed the diversity of habitats contained within the Knysna Estuary and the variety of flora and fauna that populated this unique ecosystem. Over the past 50 years, studies have continued to describe the rich variety of fauna (Day, 1967; Allanson et al., 2000b; Hodgson et al., 2000) and macrophytes (Day, 1967; Maree, 2000), which are found in the Estuary. The hydrological conditions of the Knysna Estuary have been well established (Schumann, 2000; Largier et al., 2000). The nutrient concentrations in the Estuary were investigated at a fundamental level by Grindley (1985) and further elaborated on by Allanson et al. (2000a). The rich base of knowledge associated with these studies established the Knysna Estuary as one of the best locations in South Africa to conduct a study of the processes internal to the Estuary, which govern nitrogenous and phosphatic cycling. Additionally, the development of Knysna Estuary's littoral by human intervention lags behind North American and European estuaries by decades, allowing it to serve as an example of a near-pristine estuary with respect to nutrient cycling.

The compartmentalization of the Knysna Estuary into four regions based on its hydrological characteristics had distinct benefits to this study. First, by dividing the Estuary into manageable regions it was possible to make comparisons between these regions to illustrate the localized impact from the anthropogenic influences. Second, there are estuarine management implications associated with understanding the processes studied in each region, as each region of the Knysna Estuary has characteristics found in other South African estuaries and low-nutrient estuaries found worldwide. The understanding of processes gained

during this study provides a background from which to begin investigations into intrinsic processes in other estuarine systems, both in South Africa and abroad.

The use of nutrient models in South African estuaries

Increasingly, models have been used to assess the biogeochemical functions of estuaries of South Africa (see Dupra et al., 2002). In some instances, models may represent the only published information available on the nutrients of these estuaries. Webster et al. (2001) have shown that models which are used to describe estuaries where the nutrient load varies through the year, or use time-averaged concentrations of nutrients may not accurately describe the biogeochemical functions of these estuaries. The danger for South African estuaries is that lacking more detailed studies, estuarine managers could rely solely on models to make long-term and far-reaching management decisions on these estuaries, especially with regard to making decisions of acceptable nutrient loads.

Nutrient models are based on considerable data from well-studied estuarine systems in the Northern Hemisphere. When applied to areas such as South Africa, they rely on the assumption that estuaries, which are largely unstudied with respect to N and P cycling, behave principally in a similar manner to those that have been exhaustively studied. However, as stated by Nedwell et al. (1999), although considerable progress has been made in understanding nutrient cycling processes in individual estuaries, we are still some time away from being able to generalize these results from other estuaries to predict nutrient cycling in unstudied systems. Therefore, while the trend toward estuarine modeling is likely to persist, a caveat should be issued that models should follow accurate, reliable, and detailed field data (as they often do in the Northern Hemisphere) and not be seen as a replacement of quality data.

The Knysna Estuary in a broader perspective

The main focus of this dissertation was to measure the processes that govern N and P uptake and production within the Knysna Estuary in an attempt to determine if the Estuary was successfully maintaining its oligotrophic status through the utilization of its nutrient load. In this section, I attempt to place these measurements in a broader perspective by illustrating how the processes in the Knysna Estuary function in relation to each other, and how the processes in the Knysna Estuary compare to other, comprehensively studied estuarine systems. I make these comparisons in an attempt to answer the question; was the Knysna Estuary functioning to its capacity to maintain its oligotrophic status, or can nutrient loads increase?

Knowledge of nutrient inputs to an estuary is essential for management of nutrient over-enrichment problems (Howarth et al., 2002). As a first-step in this study, the nutrient inputs to the Knysna Estuary were established through fieldwork. The number of samples from monitoring the numerous nutrient inputs did not parallel the large number of samples obtained from the high frequency water-column monitoring program. Consequently, I felt it unwise to extrapolate from a small number of samples, to system-wide annual estimates of nutrient input, and I have avoided doing this whenever possible in this dissertation.

The atmospheric deposition of DIN (ammonium and nitrates) was one instance where I have taken the liberty of extrapolating a small number of samples (n = 4) to provide a yearly estimate of DIN contributed by wet deposition from the atmosphere to the surface of the Estuary. I have done so to make the point that the AD-N was a significant source of N to the Knysna Estuary, and therefore was likely to be a considerable source of N inputs for other South African estuaries. This supports the findings of Paerl et al. (2002) that AD-N can account for a substantial portion of the N introduced to estuaries that lack substantial

terrigenous sources of N. Further, Howarth et al. (2002) noted that AD-N was largely ignored in many nutrient budgets. For example, in South African estuaries, AD-N has not been considered as an input of N in published nutrient budgets (*vide* Dupra et al., 2002). Future South African estuarine management decisions should, therefore, include AD-N as a potential source of N to these estuaries.

The nutrient loading from the parent-river of the Estuary, the Knysna River, was still low in comparison to the nutrient loading received through rivers to the estuaries in the Northern Hemisphere (e.g., Chesapeake Bay and River Colne estuary). This was largely due to the low development of the Knysna catchment, as opposed to the developed catchments that drained into the Chesapeake Bay and River Colne estuary (*vide* Dauer et al., 2000; Ogilvie et al., 1997). Data on loading from the sewage treatment works effluent that entered the Ashmead (refer to Table 2.1) indicated that this flow at times can exceed the loading from the Knysna River (refer to Table 2.3) to the Estuary. However, this dissertation revealed that loading from the Knysna River was not completely benign (e.g., urea loading post-storm, section 2.4 and DIP loading *via* fluvial sediments, section 3.5).

With regard to nutrient loading and increases in nutrient concentrations in the Estuary, the annual changes in urea concentration were the most unexpected results of this study. The increase in urea concentration following the spring storm (refer to section 2.4) was, in large part, explicable by the increases in urea loading from the rivers and storm drains of the Estuary. However, the sustained increases in urea concentrations during the summer were not easily explained by loading and indicated non-point sources of urea contributing to this increase. The utilization of urea by the phytoplankton showed that this increase in urea was not lost to the Estuary, as it made up approximately 50% of the measured nitrogenous nutrition required by the phytoplankton during the summer.

The loading of urea from pit toilets to the Estuary through the storm drains was considered in Section 2.3, *urea*. A source of urea that was not considered in this study was the possible leakage of urea from underground sewer pipes that serve the town of Knysna. The sewer pipes of Knysna are up to 5 meters below the town, which was below the water level of the Estuary (Bruce Berwick, operations manager of Knysna STW, personal communication). Evidence of pipe leakage was found when treated effluent from the sewage treatment works was used to irrigate the Sparrebosch golf course in 2000, and the high salinity of this treated effluent killed the grass almost overnight (Peet Joubert, South African National Parks Board, personal communication). It follows that if there is an exchange of raw sewage with estuarine water through the leaking sewer pipes under the town of Knysna, this exchange would be greatest during the summer holiday season when the town population peaks. This may be a source of the higher urea concentration in the Estuary observed during the February 2001 sampling (refer to Figure 2.12).

Anderson et al. (2002) warned that shifts in species composition have often been attributed to changes in nutrient supply ratios, and that microflagellates, primarily dinoflagellates, are most frequently associated with low nitrate concentrations, higher ammonium, urea or DON supply. Glibert et al. (2001) found that high urea concentrations preceded large blooms of the dinoflagellate, *Prorocentrum minimum* in the Chesapeake Bay. Although similar nutrient loads will not necessarily have the same effect on a different environment, in general, the relationship between nutrient delivery and the development of blooms of many harmful algal species remains poorly understood (Anderson et al., 2002). The Knysna Estuary may prove unique among estuaries that experience an increase in nutrient inputs through anthropogenic influences, in that its increased nutrient loads included a substantial proportion of N as urea. The finding that urea, a largely terrestrial source of N, fulfills half of the nitrogenous uptake requirement for the phytoplankton of the Knysna Estuary during summer, should serve as a

warning of the changes associated with over-enrichment. Such changes are on the horizon for the Knysna Estuary, unless current management practices concerning the Estuary change.

The chlorophyll-*a* concentrations in the Estuary on average remain typically less than 5 $\mu g\ L^{-1}$, and there is no discernable change between these average modern concentrations of chlorophyll-*a* and those made historically (Grindley, 1985; Allanson et al., 2000). However, chlorophyll-*a* concentrations in excess of 100 $\mu g\ L^{-1}$ have been measured in the canals of the Knysna quays during the fall of 2002 (refer to Figure 1.1) (Professor Allanson, unpublished data). Further, plankton blooms during late fall in the Knysna Estuary have been observed to reduce the visibility of a Secchi disc to less than 50 cm for periods up to a week (personal observation). Phytoplankton display species-specific uptake rates, and the availability of nutrients (e.g., nutrient supply ratios) have been shown to be crucial in determining the uptake and growth of a particular species (Glibert et al., 1982; Anderson et al., 2002). Therefore, the evidence that nutrient loads in the Knysna Estuary are affecting the Estuary may not be obvious throughout the year or in all locations of the Estuary. The signs of increasing eutrophication in the Knysna Estuary will more likely be manifested in small, localized problems that become more frequent over time, rather than a dramatic increase in the average algal biomass of the Estuary.

Benthic fluxes were, like the phytoplankton uptake rates, not substantially different to those found in other estuarine systems. Dollar et al. (1991) mentioned the benthic fluxes of ammonium from nine coastal systems that ranged from 0 to 5.6 mmol $m^{-2}\ d^{-1}$, with an average of 1.8 mmol $m^{-2}\ d^{-1}$, in these systems. For example, the average benthic flux of ammonium in Tomales Bay during summer was 3.2 mmol $m^{-2}\ d^{-1}$ and the average annual bay-wide benthic flux of this nutrient was 2.0 mmol $m^{-2}\ d^{-1}$. In another example, Galveston Bay,

Texas displayed average benthic fluxes of 0 mmol m^{-2} d^{-1} during winter and 0.8 mmol m^{-2} d^{-1} during summer (An and Joye, 2001).

The Knysna Estuary had an average benthic flux of ammonium during the summer of 1.7 mmol m^{-2} d^{-1}. During winter, this average increased to 2.0 mmol m^{-2} d^{-1}. It was not apparent why this flux should increase during the winter, when established understanding of estuarine biogeochemistry suggest that it should be higher during the summer. However, overall these data indicated that the average annual benthic flux of this nutrient was very similar to many other coastal systems. This adds further evidence that while the Estuary was operating at low-nutrient concentrations in the water column during the course of this study, its intrinsic processes of phytoplankton uptake and benthic flux of ammonium were characteristic of estuarine systems with higher nutrient loads.

In summary, based on the data that phytoplankton uptake and benthic fluxes were operating at a similar capacity to estuarine systems in the Northern Hemisphere, coupled with the considerable proportion of nitrogenous input and utilization that urea represents. A wise management strategy, in light of the potential for increased nutrient loads to the Knysna Estuary, would be to limit the present nutrient loading due to urea to the Estuary.

The value of healthy mudflats to the Knysna Estuary

The oligotrophic, low-nutrient status of the Knysna Estuary may change if its benthic processes do not retain their present state or improve. The benthic fluxes of nutrients measured during well-oxygenated conditions, and the increase in these fluxes measured under low-oxygen conditions, demonstrated the value of maintaining the dissolved oxygen concentrations in the Estuary (refer to Table

3.8). When oxygen concentrations decreased in the water column, the benthic fluxes of ammonium and DIP increased (refer to Figure 3.16). An increase in algal biomass in the Estuary would be expected to follow a sustained increase in the benthic flux of nutrients resulting from a decrease in dissolved oxygen concentration.

Liptrot (1978) documented the increase in growth of *Enteromorpha* mats associated with the mouth closure in the Swartvlei estuary, which is 20 kilometers west of Knysna. The rapid increase in the growth of these algal mats may be linked to increases in the benthic fluxes of ammonium and DIP after the mouth closed. However, a decrease in dissolved oxygen concentration immediately following the mouth closure (i.e., hours or days) was not shown by Liptrot (1978), as measurements of dissolved oxygen occurred on a weekly basis.

The threat of a mouth closure at the Knysna Estuary is inconceivable; the rocky heads at the mouth and large tidal prism ensure that this will not happen at shorter than geological time scales in the foreseeable future. However, there may be areas of the Estuary, which are cut off from tidal circulation in the future, if even for a period of days during low tidal exchange (e.g., neap tides), due to shifting sandbars and increased rates of sedimentation (*vide* Marker, 2000). The substantial tidal exchange of the Knysna Estuary assisted in increasing the dissolved oxygen concentrations (Allanson et al., 2000a; this dissertation). It follows that any area of the Estuary that is cutoff from tidal circulation can be expected to show a decrease in dissolved oxygen concentration, and an increase in the benthic fluxes of ammonium and DIP.

The Ashmead serves as an example of a region of the Knysna Estuary in which the benthic processes operated in an expected manner, even under the impact of higher nutrient loads entering this shallow channel from storm drains and the

sewage treatment works. I believe this was largely due to the increased dissolved oxygen concentrations in this region, as shown by Allanson et al. (2000a), in addition to the intensive tidal flushing it receives. If either of these two features should change, the water column nutrients (ammonium and DIP) would be expected to increase due to an increase in the flux of these nutrients from the sediments. Therefore, increases in signs of eutrophication (e.g., increased algal growth) would ensue.

The concentration of organic material in the sediments of the Estuary has changed as a result of sediments entering the Estuary. The increase in sedimentation as a result of the failed Simola golf estate venture serves as an example of this (refer to section 3.2, and Figure 1.2). It was not clear from the information available on the organic material in the Knysna Estuary that the portion of "new" organic material entering the Estuary was increasing the net benthic fluxes of nutrients. However, the benthic fluxes suggested that in the Bay and Ashmead, the regions closest to the mouth and therefore most influenced by the sea, a higher proportion of the organic material introduced to these sediments was utilised. This increase utilisation of organic material resulted in an increase in the benthic flux of ammonium and DIP relative to the Lagoon and upper estuary regions. This is not to say that sediments in the Lagoon and upper estuary region did not serve a valuable role to the Estuary by contributing to concentration of ammonium and DIP, as well as the uptake of nitrates from the water column when they were in excess of sediment concentrations. The sediment processes in the Lagoon and upper estuary regions reflected the lower net benthic metabolisms of these regions.

The importance of benthic processing of nitrogen and phosphorus may be greatest where extensive areas of saltmarsh or mudbanks increase the benthic area in an estuary (Nedwell et al., 1999). The investigations into the benthic processes controlling N and P in the Knysna Estuary support this statement, and

underscore the important role benthic processes play in the nutrient cycling in the Knysna Estuary. Therefore, it is crucial that the value of these benthic areas is realised by future estuarine management decisions, and that decisions are made to protect these areas from blanketing sedimentation, or other forms of degradation, which will reduce or destroy their present capacity for nutrient cycling.

Part V: Bibliography

Adams, J.B., Bate, G.C., Harrison, T.D., Huizinga, S., Taljaard, S., van Niekerk, L., Plumstead, E.E., Whitfield, A.K. and Wooldridge, T.H. 2002. A method to assess the freshwater inflow requirements of estuaries and application to the Mtata Estuary, South Africa. *Estuaries* 25:1382 - 1393.

Allanson, B.R., Baird, D. and Heydorn A.E.F. 1999. Perspectives. pp. 321 - 327. In: Allanson, B.R. and Baird, D. (eds.) Estuaries of South Africa. Cambridge University Press.

Allanson, B.R., Maree, B. and Grange, N. 2000a. An introduction to the chemistry of the water column of the Knysna Estuary with particular reference to nutrients and suspended solids. *Transactions of the Royal Society of South Africa* 55:141 - 162.

Allanson, B.R., Nettleton, J. and de Villiers, C.J. 2000b. Benthic macrofauna richness and diversity in the Knysna Estuary: a 50 year comparison. *Transactions of the Royal Society of South Africa* 55:188 - 197.

Amend, M. and Shanks, A. 1999. Timing of larval release in the mole crab *Emerita talpoida*. Marine Ecology Progress Series 183:295 – 300.

An, S. and Joye, S.B. 2001. Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnology and Oceanography* 46:62 - 74.

Anderson D.M., Glibert, P.M. and Burkholder, J.M. 2002. Harmful algal blooms and eutrophication: Nutrient sources, compositions and consequences. *Estuaries* 25:704 - 726.

Balls, P.W. 1994. Nutrient inputs to estuaries from nine Scottish east coast rivers; influence of estuarine processes on inputs to the North Sea. *Estuarine, Coastal and Shelf Science* 39:329 - 352.

Berg, G.M., Glibert, P.M., Jørgensen, N.O.G., Balode, M. and Purina, I. 2001. Variability in inorganic and organic nitrogen uptake associated with riverine nutrient input in the Gulf of Riga, Baltic Sea. *Estuaries* 24:204 - 214.

Billen, G. 1982. Modeling the processes of organic matter degradation and nutrients recycling in sedimentary systems. pp.15 - 52. In: Nedwell, D.B. and Brown, C.M. (eds.), Sediment Microbiology. Academic press, London.

Bohn, H.L. 1971. Redox potentials. *Soil Science* 112:39 - 45.

Boynton, W.R. and Kemp, W.M. 1985. Nutrient regeneration and oxygen consumption by sediments along an estuarine salinity gradient. *Marine Ecology Progress Series* 23:45 - 55.

Breitenbach, F.V. 1974. Southern Cape Forest and Trees. Government Printer, Pretoria.

Brock, D.A. 2001. Nitrogen budget for low and high freshwater inflows, Nueces Estuary, Texas. *Estuaries* 24:509 - 521.

Brown, A.C. and McLachlan, A. (eds.) 1990. Interstitial ecology. pp. 145 - 162. In: Ecology of Sandy Shores. Elsevier Science Publishers B.V., Amsterdam.

Christensen, P.B., Nielsen, P.B., SØrensen, J. and Revsbech, N.P. 1990. Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. *Limnology and Oceanography* 35:640 - 651.

Cloern, J.E. and Nichols, F.H. (eds.) 1985. Time scales and mechanisms of estuarine variability, a synthesis from studies of San Francisco Bay. pp. 229 - 237. In: Temporal Dynamics of an Estuary: San Francisco Bay. Dr W. Junk Publishers, Dordrecht.

Cowling, R. and Richardson, D. 1995. Fynbos South Africa's Unique Floral Kingdom. Fernwood Press. Pretoria.

Dauer D.M., Ranasinghe, J.A. and Weisberg, S.B. 2000. Relationship between benthic community condition, water quality, sediment quality, nutrient loads, and land use patterns in Chesapeake Bay. *Estuaries* 23:80 - 96.

Day, J.H. 1967. The biology of Knysna Estuary, South Africa. pp. 397 - 407. In: Lauff, G.H. (ed.) Estuaries. Washington, American Association for the Advancement of Science.

Day, J.H. (ed.) 1981. Estuarine ecology with particular reference to southern Africa. A.A. Balkema, Cape Town.

Day, J.H., Millard, N.A.H. and Harrison, A.D. 1952. The ecology of South African estuaries. Part III: Knysna, a clear open estuary. *Transactions of the Royal Society of South Africa* 33:367 - 413.

Dollar, S.J., Smith S.V., Vink S.M., Oberbski S. and Hollibaugh J.T. 1991. Annual cycle of benthic nutrient fluxes in Tomales Bay, California, and contribution of the benthos to total ecosystem metabolism. *Marine Ecology Progress Series* 79:115 - 125.

Dortch, Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. *Marine Ecology Progress Series* 61:83 - 201.

Dugdale, R. C. and Goering, J.J. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnology and Oceanography* 12:196 - 206.

Dupra, V., Smith, S.V., David, L.T., Waldron, H., Marshall Crossland, J.I. and Crossland, C.J. 2002. Estuarine systems of Africa (Regional Workshop II): carbon, nitrogen and phosphorus fluxes. LOICZ reports & studies no. 20. LOICZ International Project Office, Netherlands.

Dyer, K.R., 1997. Estuaries: A Physical Introduction (2nd edition). London, John Wiley and Sons Inc.

Eaton, A.D., Clesceri, L.S. and Greenberg, A.E. (eds.). 1995. 19th edition. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.

Froelich, P.N. 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: a primer on the phosphate buffer mechanism. *Limnology and Oceanography* 33:649 - 668.

Glibert, P.M., Goldmann, J.C. and Carpenter, E.J. 1982. Seasonal variation in the utilization of ammonium and nitrate by phytoplankton in the Vineyard Sound, Massachusetts, USA. *Marine Biology* 70:237 - 249.

Glibert, P.M., Magnien, R., Lomas, M.W., Alexander, J., Fan, C., Haramoto, E., Trice, M. and Kana, T.M. 2001. Harmful algal bloom in the Chesapeake and coastal bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. *Estuaries* 24:875 - 883.

Glud, R.N., Forster, S. and Huettel, M. 1996. Influence of radial pressure gradients on solute exchange in stirred benthic chambers. *Marine Ecology Progress Series* 141:303-311.

Grasshoff, K., Ehrhardt, M. and Kremling, K. (eds.) 1983. Determination of ammonium (9.4), determination of urea (9.5). pp. 150 - 162. In: Methods of Seawater Analysis, 2nd edition, Verlag Chemie, Weinheim.

Grindley, J.R. 1985. Estuaries of the Cape, Part 2. Synopses of available information on individual systems. Report No. 30: Knysna (CMS 13). In Heydorn, A.E.F. and Morant, P.D. (eds.) *CSIR Research Report* 429.

Grindley, J.R. and Snow, C.S. 1983. Environmental effects of the discharge of sewage effluent into Knysna lagoon. School of Environmental Studies, University of Cape Town.

Hansen, J.I., Henriksen, K., and Blackburn, T.H. 1981. Seasonal distribution of nitrifying bacteria and rates of nitrification in coastal marine sediments. *Microbial Ecology* 7:297 - 304.

Harcourt-Baldwin, J-L. 1996. Hydrography of the Knysna Estuary. Honours thesis, University of Cape Town.

Haw P.M. 1984. Freshwater requirements of the Knysna Estuary. MSc thesis, University of Cape Town, School of Environmental Studies. (Research Report No. 55).

Henriksen, K., Hansen J.I., and Blackburn, T.H. 1981. Rates of nitrification, distribution of nitrifying bacteria, and nitrate fluxes in different types of sediment from Danish waters. *Marine Biology* 61:299 - 304.

Head, P.C. 1985. pp. 178 - 181. Practical Estuarine Chemistry. Cambridge University Press.

Hodgson, A.N., Allanson, B.R. and Cretchley, R. 2000. An estimation of the standing stock and population structure of *Upogebia africana* (Crustacea: Thalassinidae) in the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55:187 - 196.

Howarth, R.W., Sharpley, A. and Walker, D. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25:656 - 676.

Howarth, R.W., Jensen, H.S., Marino, R. and Postma, H. 1995. Transport to and processing of P in near-shore and oceanic waters. pp. 323 - 345. In: Tiessen, H. (ed.) Phosphorus in the Global Environment. John Wiley, New York.

Hughes, D.A. 1983. Preliminary investigations into isolated flood event modelling with specific reference to the southern cape coastal lakes region. Rhodes University, Department of Geology, Hydrological Research Unit. Report no. 3/83.

Hughes, D.A., and Görgens, A.H.M. 1981. Hydrological Investigations in the Southern Cape Coastal Lakes Region. Rhodes University, Department of Geology, Hydrological Research Unit. Report no. 1/81.

Hunt, A.P., Parry, J.D., and Hamilton-Taylor, J. 2000. Further evidence of element composition as an indicator of the bioavailability of humic substances to bacteria. *Limnology and Oceanography* 45:237 - 241.

Huettel, M. and Gust, G. 1992. Solute release mechanisms from confined sediment cores in stirred benthic chambers and flume flows. *Marine Ecology Progress Series* 82:187-197.

Jenkins, M.C. and Kemp, W.M. 1984. The coupling of nitrification and denitrification in two estuarine sediments. *Limnology and Oceanography* 29:609 - 619.

Largier, J.L., Attwood, C. and Harcourt-Baldwin, J-L. 2000. The hydrographic character of the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55:107 - 122.

Liptrot, M.R.M. 1978. Community metabolism and phosphorus dynamics in a seasonally closed South African estuary. MSc thesis, Rhodes University, Grahamstown.

Lomas, M.W., Trice, M.T, Glibert, P.M., Bronk, D.A. and McCarthy, J.J. 2002. Temporal and spatial dynamics of urea uptake and regeneration rates and concentrations in Chesapeake Bay. *Estuaries* 25:469 - 482.

Louw, J.H. and Scholes, M. 2002. The influence of site factors on nitrogen mineralization in forest soils of the Mpumalanga escarpment area. *Southern African Forestry Journal* 193:47 - 63.

Marker, M.E. 2000. A descriptive account of sand movement in the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55:129 - 139.

Maree, B. 2000. Structure and status of the intertidal wetlands of the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55:163 - 176.

Morant, P. and Quinn, N. 1999. Influence of man and management of South African estuaries. pp. 289 - 320. In: Allanson, B.R. and Baird, D. (eds.) Estuaries of South Africa. Cambridge University Press.

Nedwell, D.B. and Trimmer, M. 1996. Nitrogen fluxes through the upper estuary of the Great Ouse, England: the role of the bottom sediments. *Marine Ecology Progress Series* 142:273 - 286.

Nedwell, D.B., Jickells, T.D., Trimmer, M. and Sanders, R. 1999. Nutrients in Estuaries. pp. 43 - 84. In: Nedwell D.B. and Raffaelli D.G. (eds.) Advances in Ecological Research, Estuaries. Academic Press.

Nedwell, D.B., Hall, S-E., Andersson, A., Hagstrøm, Å.F. and Lindstrøm,E.B. 1982. Seasonal changes in the distribution and exchange of inorganic nitrogen between sediment and water in the Northern Baltic (Gulf of Bothnia). *Estuarine, Coastal and Shelf Science* 17:169 - 179.

Nixon, S.W. 1981. Remineralization and nutrient cycling in coastal marine ecosystems. pp. 111 - 138. In: Neilson, B.J. and Cronin, L.E. (eds.) Estuaries and Nutrients. The Humana Press. New Jersey.

Nixon, S.W., Hunt, C.D. and Nowicki, B.L. 1986. The retention of nutrients (C,N,P), heavy metals (Mn, Cd, Pb, Cu), and petroleum hydrocarbons in Narragansett Bay. pp. 99 - 122: In: Lasserre, P. and Martin, J-M. (eds.) Biogeochemical Processes at the Land-Sea Boundary. Elsevier Science Publishers B.V., Amsterdam.

Nixon, S.W., Ammerman, J., Atkinson, L., Berounsky, V., Billen, G., Boicourt, W., Boynton, W., Church, T., DiToro, D., Elmgren, R., Garber, J., Giblin, A., Janke, R., Owens, N., Pilson, M.E.Q. and Seitzinger, S. 1996. The fate of nitrogen and phosphorus at the land-sea margin of the North Atlantic Ocean. *Biogeochemistry* 35:141 - 180.

Oliver, H., Lotter, A.F. and Lemcke, G. 2001. Loss of ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology 25:101 - 110.*

Ogilvie, B., Nedwell, D.B., Harrison, R.M., Robinson, A. and Sage, A. 1997. High nitrate, muddy estuaries as nitrogen sinks: the nitrogen budget of the River Colne estuary (United Kingdom). *Marine Ecology Progress Series* 150:217 - 228.

Paerl, H.W., Dennis, R.L. and Whitall, D.R. 2002. Atmospheric deposition of nitrogen: Implications for nutrient over-enrichment of coastal waters. *Estuaries* 25:677 - 693.

Parsons, T.R., Maita, Y. and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Prastka, K.E. and Jickells, T.D. 1995. Sediment geochemistry of phosphorous at two intertidal sites on the Great Ouse Estuary, S.E. England. *Netherlands Journal of Aquatic Ecology* 29:245 - 255.

Prastka, K., Sanders, R. and Jickells, T. 1998. Has the role of estuaries as sources of sinks of dissolved inorganic phosphorous changed over time? Results of a K_d study. *Marine Pollution Bulletin* 36:718 - 728.

Reddering, J.S.V. 1994. Supply of land-derived sediment and its dispersal in the Knysna Estuary: an environmental appraisal. Council for Geoscience. Pretoria. Report No.1994 - 0024.

Reddering, J.S.V. and Esterhuysen, K. 1984. Sedimentation in the Knysna Estuary. University of Port Elizabeth, Department of Geology. *Rosie Report No.9.*

Risgaard-Petersen, N., Rysgaard, S., Nielsen, L.P. and Revsbech, N.P. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnology and Oceanography* 39:573 - 579.

Rysgaard, S., Christensen, P.B. and Nielsen, L.P. 1995. Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. *Marine Ecology Progress Series* 126:111 - 121.

Schumann, E.H. 2000. Oceanic exchanges and temperature variability in the Knysna Estuary. *Transactions of the Royal Society of South Africa* 55:123 - 128.

Schumann, E.H. 1999. Wind-driven mixed layer and coastal upwelling processes off the south coast of South Africa. *Journal of Marine Research* 57:671 - 691.

Schumann, E.H., Largier, J.L. and Slinger, J.H. 1999. Estuarine hydrodynamics. pp. 27 - 52. In: Estuaries of South Africa. (eds.) Allanson, B.R. and Baird, D. Cambridge University Press.

Soil Classification Working Group. 1991. Soil Classification: A Taxonomic System for South Africa. Department of Agricultural Development, Pretoria.

Switzer, T. and Waldron, H. 2002. Knysna Lagoon, Western Cape. pp.24 - 31. In: Estuarine Systems of Africa (Regional Workshop II): Carbon, Nitrogen and Phosphorus Fluxes. LOICZ Reports & Studies no. 20. LOICZ International Project Office, Netherlands.

Taylor, D.I. 1992. The influence of upwelling and short-term changes in concentrations of nutrients in the water column on fluxes across the surface of a salt marsh. *Estuaries* 15:68 - 74.

Trimmer, M., Nedwell, D.B., Sivyer, D.B. and Malcolm, S.J. 1998. Nitrogen fluxes through the lower estuary of the river Great Ouse, England: the role of bottom sediments. *Marine Ecology Progress Series* 163:109 - 124.

Tyson, P.D. (ed.) 1971. Outeniqualand: The George-Knysna Area. The South African Landscape, No. 2. South African Geographical Society. Braamfontein.

Valiela, I. 1991. Ecology of water columns. pp. 29 - 56. In: Barnes, R.S.K. and Mann, K.H. (eds.) Fundamentals of Aquatic Ecology, second edition. Blackwell science, Malden, Massachusetts, USA.

Valiela, I. and Teal, J.M. 1979. The nitrogen budget of a salt marsh ecosystem. *Nature* 280:652 - 656.

Waldron, H.N. and Probyn, T.A. 1991. Short-term variability during an anchor station study in the southern Benguela upwelling system: Nitrogen supply to the euphotic zone during a quiescent phase in the upwelling cycle. *Progress in Oceanography* 28:153 - 166.

Webster, I.T., Parlow, J.S. and Smith, S.V. 2000. Implications of spatial and temporal variation for biogeochemical budgets of estuaries. *Estuaries* 23:341 - 350.

Wolaver, T.G., Zieman, J., Wetzel, R. and Webb, K.L. 1983. Tidal exchange of nitrogen and phosphorous between a mesohaline vegetated marsh and the surrounding estuary in the lower Chesapeake Bay. *Estuarine, Coastal and Shelf Science* 16:321 - 332.

Whitfield, A.K. 1992. A characterization of South African estuarine systems. *South Africa Journal of Aquatic Sciences* 18:89 - 103.

Part VI: Appendixes

APPENDIX A

Tidal prism

Main channel:	Estuary Width (m)	Channel Width (m)	Mudflat Area (m^2)	Estuary Area (m^2)	LWST Depth (m)	Block Length (m)
Ocean - X14	750	160	437500	629500	7.9	1200
X14 – M14	730	170	308000	401500	7.1	550
M14 – X13	580	200	159600	243600	10.6	420
X13 – M13	500	300	86000	215000	5.1	430
M13 – X12	730	260	204350	336950	4.1	510
X12 – M12	1140	170	514100	604200	4.4	530
M12 – X11	1340	170	713700	817400	3.9	610
X11 – M11	1830	300	673200	805200	3.2	440
M11 – X10	1900	230	734800	836000	2.7	440
X10 – M10	1270	290	372400	482600	2.4	380
M10 – X9	750	320	109650	272850	3.9	510
X9 – M9	680	200	112800	206800	5.4	470
M9 – X8	930	200	138700	214700	4.1	380
X8 – M8	1050	200	212500	262500	2.0	250
M8 – X7	1170	240	427800	538200	2.3	460
X7 – M7	1220	220	320000	390400	2.7	320
M7 – X6	1350	270	270000	337500	1.8	250
X6 – M6	1350	520	182600	297000	1.8	220
M6 – X5	1410	470	216200	324300	1.6	230
X5 – M5	1600	690	309400	544000	2.4	340
M5 – X4	1180	450	350400	566400	3.1	480
X4 – M4	850	360	313600	544000	3.6	640
M4 – X3	930	640	110200	353400	3.4	380
X3 – M3	1040	620	184800	457600	1.7	440
M3 – X2	880	530	87500	220000	1.9	250
X2 – M2	840	430	139400	285600	2.2	340
M2 – X1	660	260	144000	237600	2.4	360
X1 – M1	990	100	445000	495000	2.8	500
M1 – K1	690	40	455000	483000	2.0	700
K1 – K2	400	60	272000	320000	1.6	800
K2 – K3	130	60	53900	100100	1.0	770
K3 – K4	90	60	23100	69300	1.0	770
K4 – K5	70	50	16800	58800	1.8	840
K5 – K6	60	40	15000	45000	1.1	750
K6 – K7	40	40	0	20000	1.0	500
K7 – KR	30	30	0	19800	0.7	660
	Estuary Mean Width (m)	Channel Mean Width (m)	Mudflat Area (m^2)	Estuary Area (m^2)	LWST Mean Depth (m)	Channel Length (m)
	866	260	9114000	13035800	3.1	18120

Main channel Continued:	HWST Channel /olume(m^3)	LWST Channel Volume(m^3)	HWST Mudflat Volume(m^3)	Total HWST Volume(m^3)	Maximum Tidal Range(m)	Prism Per Block(m^3)
ocean – X14	1862400	1516800	393750	2256150	1.8	739350
X14 – M14	832150	663850	277200	1109350	1.8	445500
M14 – X13	1041600	890400	143640	1185240	1.8	294840
X13 – M13	890100	657900	77400	967500	1.8	309600
M13 – X12	782340	543660	183915	966255	1.8	422595
X12 – M12	558620	396440	462690	1021310	1.8	624870
M12 – X11	591090	404430	642330	1233420	1.8	828990
X11 – M11	660000	422400	605880	1265880	1.8	843480
M11 – X10	455400	273240	661320	1116720	1.8	843480
X10 – M10	462840	264480	335160	798000	1.8	533520
M10 – X9	930240	636480	98685	1028925	1.8	392445
X9 – M9	676800	507600	101520	778320	1.8	270720
M9 – X8	448400	311600	124830	573230	1.8	261630
X8 – M8	190000	100000	191250	381250	1.8	281250
M8 – X7	452640	253920	385020	837660	1.8	583740
X7 – M7	316800	190080	288000	604800	1.8	414720
M7 – X6	243000	121500	243000	486000	1.8	364500
X6 – M6	411840	205920	164340	576180	1.8	370260
M6 – X5	367540	172960	194580	562120	1.8	389160
X5 – M5	985320	563040	278460	1263780	1.8	700740
M5 – X4	1058400	669600	315360	1373760	1.8	704160
X4 – M4	1244160	829440	282240	1526400	1.8	696960
M4 – X3	1264640	826880	99180	1363820	1.8	536940
X3 – M3	954800	463760	166320	1121120	1.8	657360
M3 – X2	490250	251750	78750	569000	1.8	317250
X2 – M2	584800	321640	125460	710260	1.8	388620
M2 – X1	393120	224640	129600	522720	1.8	298080
X1 – M1	230000	140000	400500	630500	1.8	490500
M1 – K1	106400	56000	409500	515900	1.8	459900
K1 – K2	163200	76800	244800	408000	1.8	331200
K2 –K3	129360	46200	48510	177870	1.8	131670
K3 – K4	129360	46200	20790	150150	1.8	103950
K4 – K5	142800	75600	13440	156240	1.6	80640
K5 – K6	72000	33000	9750	81750	1.3	48750
K6 – K7	34000	20000	0	34000	0.7	14000
K7 – KR	21780	13860	0	21780	0.4	7920

	HWST Channel /olume(m^3)	LWST Channel Volume (m^3)	HWST mudflats Volume(m^3)	HWST Total Volume(m^3)	Exchange Volume (HW-LW)	Main channel Tidal prism Volume (m^3)
	20178190	13192070	8197170	28375360	53.5%	15,183,290

Tidal prism

Ashmead:	Estuary Width (m)	Channel Width (m)	Mudflat Area (m^2)	Estuary Area (m^2)	LWST Depth (m)	Block Length (m)
Main – A9	500	70	90000	107500	1.7	250
A9 – A8	1080	70	466000	503100	2.0	530
A8 – A7	1320	100	835000	893000	1.4	580
A7 – A6	1170	50	1018000	1045000	1.2	540
A6 – A5	510	60	258000	295800	0.9	630
A5 – A4	620	40	262000	287600	0.9	64
A4 – A3	530	40	166000	186400	1.0	510
A3 – A2	470	0	235000	235000	0.0	540
A2 – A1	490	0	303000	303000	0.0	620

Estuary mean width (m)	Channel mean width (m)	Mudflat Surface Area (m^2)	Estuary Surface Area (m^2)	LWST Mean Depth (m)	Ashmead Channel Length (m)
743	48	3633000	3856400	1.0	4840

	HWST Channel Volume(m^3)	LWST Channel Volume (m^3)	HWST Mudflat Volume (m^3)	Total HWST Volume (m^3)	Max Tidal Range (m)	Prism Per Block (m^3)
Main – A9	61250	29750	81000	142250	1.8	112500
A9 – A8	140980	74200	419400	560380	1.8	486180
A8 – A7	185600	81200	751500	937100	1.8	855900
A7 – A6	81000	32400	916200	997200	1.8	964800
A6 – A5	102060	34020	206400	308460	1.6	274440
A5 – A4	56320	23040	170300	226620	1.3	203580
A4 – A3	40800	20400	107900	148700	1.3	128300
A3 – A2	0	0	141000	141000	1.2	141000
A2 – A1	0	0	181800	181800	1.2	181800

HWST Channel /olume(m^3)	LWST Channel Volume(m^3)	HWST Mudflats Volume(m^3)	Total HWST Volume (m^3)	Exchange Volume (HW-LW)	Ashmead Tidal Prism Volume (m^3)
668010	295010	2975500	3643510	91.1%	3,348,500

Total HWST Channel /olume(m^3)	Total LWST Channel Volume(m^3)	Total HWST Mudflat Volume(m^3)	Total HWST Estuary Volume (m^3)	Knysna Estuary Tidal Prism Volume (m^3)
20846200	13487080	11172670	32018870	18,531,790

Total Mudflat Area (m^2)	Total Estuary Area (m^2)	Mean LWST Depth (m)
12747000	16892200	2.7

Appendix B,
Nutrients

Core stations: Upper estuary	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
Station M1							
5/6/2000	34.9	1.7	3.9	1.1	1.6	1.0	HWST
5/6/2000	30.1	0.8	3.7	0.7	1.2	2.7	LWST
10/6/2000	33.3	1.9	3.2	0.3	1.2	1.7	HWNT
10/6/2000	31.4	1.1	3.1	0.7	1.9	1.0	LWNT
8/8/2000	33.8	0.9	1.5	0.7	1.0	2.1	HWNT
8/8/2000	33.5	0.5	1.6	0.6	0.2	3.1	LWNT
16/8/2000	34.4	0.2	1.7	2.1	0.8	2.1	HWST
16/8/2000	32.1	0.2	0.7	3.5	0.9	*	LWST
27/10/2000	28.4	1.4	2.5	0.8	0.6	*	HWST
27/10/2000	16.2	1.6	5.4	0.4	1.6	*	LWST
3/11/2000	26.6	2.2	2.4	0.7	1.1	*	HWNT
3/11/2000	21.5	1.5	3.9	0.5	0.8	*	LWNT
9/2/2001	30.8	1.1	1.1	1.8	1.4	*	HWST
9/2/2001	27.1	2.1	2.1	0.6	1.1	*	LWST
16/2/2001	24.1	1.2	1.6	0.5	1.4	*	HWNT
16/2/2001	18.5	1.2	0.4	0.3	1.6	*	LWNT
Station M2							
5/6/2000	34.9	1.7	3.6	1.0	1.6	1.7	HWST
5/6/2000	31.8	1.0	4.2	0.9	1.5	2.4	LWST
10/6/2000	33.4	2.0	2.9	0.5	1.0	0.3	HWNT
10/6/2000	32.8	1.2	2.2	0.8	1.6	2.1	LWNT
8/8/2000	34.2	0.9	1.4	0.8	1.0	*	HWNT
8/8/2000	34.3	0.9	1.2	0.9	0.6	3.1	LWNT
16/8/2000	34.5	0.2	1.8	0.4	0.9	1.7	HWST
16/8/2000	33.3	0.5	1.1	0.8	0.8	0.7	LWST
27/10/2000	30.0	1.4	2.2	0.9	0.8	*	HWST
27/10/2000	20.9	2.4	4.0	0.5	2.0	*	LWST
3/11/2000	29.7	2.8	2.4	0.9	2.0	*	HWNT
3/11/2000	27.4	1.6	2.9	0.7	0.5	*	LWNT
9/2/2001	31.3	1.0	1.0	1.7	1.6	*	HWST
9/2/2001	28.7	2.1	1.7	0.7	1.8	*	LWST
16/2/2001	27.4	1.5	1.5	0.6	3.0	*	HWNT
16/2/2001	23.4	0.9	0.8	0.5	1.2	*	LWNT
Station M3							
5/6/2000	34.8	1.5	4.4	0.8	1.1	2.1	HWST
5/6/2000	32.3	1.3	4.3	0.9	0.8	3.8	LWST
10/6/2000	33.8	1.9	2.5	0.2	1.0	2.1	HWNT
10/6/2000	33.3	1.4	3.2	0.8	1.6	2.4	LWNT
8/8/2000	34.6	0.8	1.7	0.9	1.2	*	HWNT
8/8/2000	34.2	1.0	2.0	1.0	0.3	3.8	LWNT
16/8/2000	34.6	0.2	1.7	0.4	1.0	0.7	HWST
16/8/2000	*	0.7	1.3	0.8	0.9	*	LWST
27/10/2000	30.8	1.7	2.0	0.8	0.9	*	HWST
27/10/2000	23.1	2.2	3.6	0.6	1.0	*	LWST
3/11/2000	31.1	1.9	2.1	0.7	0.8	*	HWNT

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
3/11/2000	30.6	2.6	2.5	0.8	0.6	*	LWNT
9/2/2001	31.7	1.3	1.3	1.7	1.4	*	HWST
9/2/2001	29.2	1.9	1.9	0.8	1.0	*	LWST
16/2/2001	27.7	1.0	1.7	0.7	1.9	*	HWNT
16/2/2001	27.5	1.0	15.7	0.7	1.1	*	LWNT
Lagoon							
Station M4							
5/6/2000	34.7	1.7	4.7	1.0	1.6	1.7	HWST
5/6/2000	33.8	1.5	4.6	1.0	0.8	3.1	LWST
10/6/2000	33.8	1.7	2.5	0.3	0.9	2.7	HWNT
10/6/2000	33.7	1.4	3.4	0.9	0.5	2.4	LWNT
8/8/2000	34.4	1.1	1.8	0.9	1.0	1.0	HWNT
8/8/2000	34.4	0.9	1.4	0.8	0.2	3.4	LWNT
16/8/2000	34.7	0.2	2.7	0.4	0.8	2.1	HWST
16/8/2000	34.5	1.3	1.6	1.0	0.9	1.7	LWST
27/10/2000	32.0	1.7	1.7	0.8	0.6	*	HWST
27/10/2000	25.0	2.2	2.9	0.7	1.0	*	LWST
3/11/2000	30.9	1.8	2.2	0.7	0.5	*	HWNT
3/11/2000	31.0	1.6	2.6	0.9	0.7	*	LWNT
9/2/2001	31.9	1.3	1.1	1.6	1.9	*	HWST
9/2/2001	30.3	2.1	1.8	0.9	1.4	*	LWST
16/2/2001	30.8	1.5	1.6	0.8	2.1	*	HWNT
16/2/2001	28.9	1.1	0.9	0.6	0.7	*	LWNT
Station M5							
5/6/2000	34.8	1.7	5.1	1.1	1.3	2.1	HWST
5/6/2000	33.9	1.6	4.7	1.0	0.8	3.1	LWST
10/6/2000	34.1	1.9	2.6	0.3	1.0	3.4	HWNT
10/6/2000	33.7	1.5	3.0	0.9	0.5	3.4	LWNT
8/8/2000	34.7	1.3	1.4	0.8	1.2	2.7	HWNT
8/8/2000	34.6	1.6	1.5	1.0	0.4	2.1	LWNT
16/8/2000	35.0	0.3	2.9	0.5	0.5	1.4	HWST
16/8/2000	34.6	1.4	1.8	1.0	0.9	3.4	LWST
27/10/2000	33.5	1.4	2.0	0.7	0.8	*	HWST
27/10/2000	30.2	2.0	2.5	0.8	0.8	*	LWST
3/11/2000	33.0	1.6	2.2	0.7	0.7	*	HWNT
3/11/2000	31.7	1.9	2.3	0.9	0.7	*	LWNT
9/2/2001	33.8	1.5	0.9	1.4	2.7	*	HWST
9/2/2001	31.4	2.3	1.3	0.8	1.3	*	LWST
16/2/2001	32.9	2.2	2.0	0.9	1.5	*	HWNT
16/2/2001	30.8	1.4	1.3	0.9	1.1	*	LWNT
Station M6							
5/6/2000	34.6	1.7	5.6	1.0	1.3	1.4	HWST
5/6/2000	33.9	1.5	4.9	1.0	0.8	1.4	LWST
10/6/2000	34.3	1.9	3.4	0.8	1.2	1.0	HWNT
10/6/2000	33.7	1.6	2.8	0.9	0.4	1.4	LWNT
8/8/2000	34.7	1.4	1.7	0.8	1.1	*	HWNT
8/8/2000	34.5	2.2	1.4	1.0	8.1	2.1	LWNT

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
16/8/2000	35.2	0.8	3.4	0.7	0.6	2.1	HWST
16/8/2000	34.6	1.5	1.7	1.0	0.9	1.4	LWST
27/10/2000	33.9	1.3	2.1	0.6	1.0	*	HWST
27/10/2000	30.6	2.0	2.7	0.9	1.1	*	LWST
3/11/2000	33.3	1.6	2.3	0.8	0.9	*	HWNT
3/11/2000	32.2	2.2	2.7	0.8	0.8	*	LWNT
9/2/2001	34.0	1.5	0.9	1.3	2.2	*	HWST
9/2/2001	31.5	2.0	1.6	0.8	1.8	*	LWST
16/2/2001	33.1	2.2	2.9	0.9	1.6	*	HWNT
16/2/2001	31.8	1.6	2.4	1.1	1.3	*	LWNT
Station M7							
5/6/2000	34.5	1.7	5.3	0.9	1.0	3.1	HWST
5/6/2000	33.9	1.5	4.8	1.0	0.6	4.5	LWST
10/6/2000	34.4	2.1	3.3	0.3	0.9	1.0	HWNT
10/6/2000	33.7	1.7	3.7	1.0	0.5	2.4	LWNT
8/8/2000	34.7	1.5	1.8	0.9	1.0	*	HWNT
8/8/2000	34.5	1.3	1.5	0.9	0.3	3.4	LWNT
16/8/2000	35.2	0.4	4.1	0.6	0.6	1.0	HWST
16/8/2000	34.6	1.6	1.8	1.1	0.8	1.0	LWST
27/10/2000	33.9	1.4	1.4	0.6	0.5	*	HWST
27/10/2000	30.7	2.0	2.0	0.8	1.3	*	LWST
3/11/2000	33.5	1.6	2.5	0.7	0.9	*	HWNT
3/11/2000	32.5	1.6	1.5	0.7	0.5	*	LWNT
9/2/2001	34.3	1.4	1.0	1.1	1.8	*	HWST
9/2/2001	31.6	2.1	1.6	0.9	2.2	*	LWST
16/2/2001	33.4	2.3	2.5	0.8	1.4	*	HWNT
16/2/2001	31.7	1.3	2.0	0.9	1.2	*	LWNT
Station M8							
5/6/2000	34.5	1.8	5.4	0.8	1.0	3.4	HWST
5/6/2000	33.9	1.4	4.9	1.0	0.6	5.5	LWST
10/6/2000	34.5	2.0	3.1	0.4	1.2	2.7	HWNT
10/6/2000	33.7	1.6	2.9	1.2	0.5	2.7	LWNT
8/8/2000	35.2	1.6	2.3	0.9	0.9	0.3	HWNT
8/8/2000	34.6	1.4	1.6	0.9	0.4	4.8	LWNT
16/8/2000	35.3	0.6	5.1	0.6	0.4	1.0	HWST
16/8/2000	34.9	1.5	1.8	1.0	0.9	1.0	LWST
27/10/2000	34.3	1.0	2.0	0.5	0.5	*	HWST
27/10/2000	30.8	2.0	2.0	0.9	0.8	*	LWST
3/11/2000	33.8	1.5	2.9	0.8	0.7	*	HWNT
3/11/2000	32.1	1.8	2.5	0.8	0.8	*	LWNT
9/2/2001	34.4	1.5	0.9	0.9	4.4	*	HWST
9/2/2001	31.8	2.0	1.3	0.8	1.3	*	LWST
16/2/2001	33.6	2.4	2.0	0.8	1.5	*	HWNT
16/2/2001	32.1	1.6	2.6	1.0	1.2	*	LWNT
Station M9							
5/6/2000	34.5	1.9	5.5	0.9	1.1	3.4	HWST
5/6/2000	33.9	1.5	4.9	1.0	0.4	3.4	LWST
10/6/2000	34.7	2.2	2.9	1.3	0.6	2.4	HWNT
10/6/2000	33.9	1.6	2.9	1.2	0.8	1.7	LWNT

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
8/8/2000	34.8	1.5	2.6	0.9	1.1	*	HWNT
8/8/2000	34.7	1.7	1.4	0.9	0.4	2.1	LWNT
16/8/2000	35.2	0.5	4.9	0.7	0.5	1.7	HWST
16/8/2000	34.7	1.5	1.7	1.0	0.8	1.4	LWST
27/10/2000	34.4	1.2	1.7	0.6	0.6	*	HWST
27/10/2000	31.4	1.7	2.3	0.9	0.9	*	LWST
3/11/2000	34.0	1.5	3.0	0.8	0.6	*	HWNT
3/11/2000	31.9	1.4	2.1	0.8	0.4	*	LWNT
9/2/2001	34.9	1.5	0.8	1.1	1.4	*	HWST
9/2/2001	31.7	1.9	1.4	0.8	1.0	*	LWST
16/2/2001	33.7	2.6	1.8	0.8	1.6	*	HWNT
16/2/2001	32.1	1.3	2.1	0.9	1.1	*	LWNT
Station M10							
5/6/2000	34.3	1.8	6.1	0.9	0.9	7.2	HWST
5/6/2000	34.1	1.5	4.8	1.0	0.5	3.4	LWST
10/6/2000	34.8	2.0	3.1	1.4	0.6	2.1	HWNT
10/6/2000	34.1	1.6	2.4	1.0	0.9	2.7	LWNT
8/8/2000	34.9	1.6	3.5	0.9	1.0	0.3	HWNT
8/8/2000	34.8	1.8	1.6	0.9	0.3	1.4	LWNT
16/8/2000	35.3	1.1	4.0	0.9	0.4	*	HWST
16/8/2000	34.8	1.6	1.8	1.0	1.0	1.4	LWST
27/10/2000	34.6	0.6	1.9	0.5	0.4	*	HWST
27/10/2000	32.1	1.6	2.1	0.9	0.7	*	LWST
3/11/2000	34.0	1.3	3.4	0.7	1.0	*	HWNT
3/11/2000	32.7	1.7	2.0	0.8	0.3	*	LWNT
9/2/2001	34.8	1.2	0.8	0.9	1.8	*	HWST
9/2/2001	32.4	1.9	1.1	0.7	0.8	*	LWST
16/2/2001	33.8	2.0	1.6	0.7	1.6	*	HWNT
16/2/2001	32.9	1.3	2.4	1.1	1.3	*	LWNT
Bay							
Station M11							
5/6/2000	34.0	2.1	5.2	0.9	1.1	3.4	HWST
5/6/2000	34.0	1.5	4.7	1.0	0.6	4.1	LWST
10/6/2000	34.8	1.6	4.0	1.2	0.4	3.1	HWNT
10/6/2000	34.2	1.5	3.2	1.2	0.6	2.4	LWNT
8/8/2000	34.8	1.4	4.5	0.9	1.1	*	HWNT
8/8/2000	34.9	2.2	1.4	0.8	0.3	3.8	LWNT
16/8/2000	35.3	1.3	4.8	1.0	0.2	1.7	HWST
16/8/2000	34.7	1.4	1.8	1.0	0.8	1.0	LWST
27/10/2000	34.5	1.1	1.4	0.5	0.8	*	HWST
27/10/2000	32.4	1.6	2.0	0.9	0.8	*	LWST
3/11/2000	34.2	1.5	2.9	0.6	0.6	*	HWNT
3/11/2000	32.7	1.1	2.1	0.7	0.6	*	LWNT
9/2/2001	34.7	1.1	0.6	0.9	3.8	*	HWST
9/2/2001	32.0	2.8	1.2	0.7	1.4	*	LWST
16/2/2001	34.2	2.3	0.9	0.7	1.5	*	HWNT
16/2/2001	32.9	1.5	2.2	1.1	1.3	*	LWNT

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
Station M12							
5/6/2000	33.5	1.6	6.2	0.8	0.6	5.8	HWST
5/6/2000	34.5	1.5	4.8	1.0	0.5	4.1	LWST
10/6/2000	35.1	0.4	1.9	0.7	0.0	5.5	HWNT
10/6/2000	34.6	1.7	4.6	0.9	1.0	1.7	LWNT
8/8/2000	34.9	1.3	7.8	1.0	0.9	3.8	HWNT
8/8/2000	35.1	2.1	2.9	0.9	0.4	1.4	LWNT
16/8/2000	35.3	1.0	4.7	0.9	0.4	2.1	HWST
16/8/2000	34.8	1.5	2.5	1.0	0.8	0.3	LWST
27/10/2000	34.6	0.8	2.2	0.5	0.3	*	HWST
27/10/2000	32.7	1.8	2.2	0.8	0.8	*	LWST
3/11/2000	34.7	0.3	1.5	0.3	0.4	*	HWNT
3/11/2000	33.8	1.5	2.5	0.8	0.3	*	LWNT
9/2/2001	34.8	1.3	0.6	0.8	3.0	*	HWST
9/2/2001	32.6	2.3	1.1	0.7	1.9	*	LWST
16/2/2001	34.5	1.5	6.6	0.8	4.2	*	HWNT
16/2/2001	33.7	1.5	1.6	0.8	1.1	*	LWNT
Station M13							
5/6/2000	33.6	1.5	6.2	0.8	0.6	5.8	HWST
5/6/2000	34.2	1.5	4.9	1.0	0.6	3.8	LWST
10/6/2000	35.0	1.6	1.7	0.7	0.4	7.6	HWNT
10/6/2000	34.6	1.8	2.6	1.2	0.8	2.4	LWNT
8/8/2000	34.9	1.0	9.9	1.1	0.9	*	HWNT
8/8/2000	35.0	2.2	3.1	0.9	0.5	5.5	LWNT
16/8/2000	35.3	1.1	4.0	1.0	0.4	2.1	HWST
16/8/2000	34.8	1.5	2.2	1.0	0.8	1.4	LWST
27/10/2000	34.5	0.8	2.3	0.5	0.3	*	HWST
27/10/2000	32.7	1.6	2.2	0.8	0.4	*	LWST
3/11/2000	34.8	0.3	2.0	0.3	0.7	*	HWNT
3/11/2000	34.1	1.5	2.2	0.8	0.3	*	LWNT
9/2/2001	34.8	1.2	0.7	0.8	2.1	*	HWST
9/2/2001	32.6	2.4	1.4	0.7	1.3	*	LWST
16/2/2001	34.5	1.8	11.9	1.0	1.8	*	HWNT
16/2/2001	34.3	1.3	1.3	1.5	1.6	*	LWNT
Station M14							
5/6/2000	33.5	1.4	5.2	0.7	0.9	6.5	HWST
5/6/2000	34.4	1.3	4.1	0.9	0.5	6.9	LWST
10/6/2000	35.1	0.5	2.2	0.6	0.0	9.3	HWNT
10/6/2000	34.7	1.4	3.0	0.8	0.6	2.7	LWNT
8/8/2000	34.8	0.8	10.7	1.1	1.2	*	HWNT
8/8/2000	35.0	1.9	4.4	1.0	0.3	1.4	LWNT
16/8/2000	35.2	1.0	4.6	1.0	0.3	0.7	HWST
16/8/2000	35.1	1.3	3.5	1.0	0.8	2.4	LWST
27/10/2000	34.5	1.0	2.3	0.5	0.4	*	HWST
27/10/2000	33.3	1.7	1.9	0.8	0.5	*	LWST
3/11/2000	34.8	0.1	1.7	0.3	1.0	*	HWNT
3/11/2000	34.2	1.1	2.7	0.6	1.0	*	LWNT
9/2/2001	34.8	1.3	0.7	0.7	1.7	*	HWST

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
9/2/2001	32.9	2.2	1.6	0.7	0.9	*	LWST
16/2/2001	34.3	1.7	8.1	1.0	1.8	*	HWNT
16/2/2001	34.1	1.2	1.6	0.8	1.3	*	LWNT
Ashmead							
Station A3							
3/6/2000	34.6	1.2	8.4	1.1	0.6	11.0	HWST
3/6/2000	*	1.8	3.7	1.8	1.2	0.7	LWST
9/6/2000	34.6	3.6	2.1	3.5	2.3	*	HWNT
9/6/2000	*	1.9	0.7	2.6	0.8	*	LWNT
9/8/2000	33.7	5.8	6.3	7.7	1.0	10.0	HWNT
9/8/2000	*	2.2	0.9	6.5	1.5	4.8	LWNT
17/8/2000	35.1	1.8	5.0	0.8	0.5	2.1	HWST
17/8/2000	*	2.1	1.3	2.4	1.1	2.4	LWST
29/10/2000	34.0	1.9	3.4	0.7	0.2	*	HWST
29/10/2000	33.7	3.2	1.8	1.6	1.1	*	LWST
4/11/2000	32.6	8.4	1.7	5.5	1.5	*	HWNT
4/11/2000	32.7	7.8	1.1	4.2	1.5	*	LWNT
7/2/2001	34.5	2.6	1.4	3.1	1.8	*	HWST
7/2/2001	33.6	3.4	0.6	2.2	1.3	4.5	LWST
15/2/2001	32.6	7.0	1.7	4.7	1.7	3.4	HWNT
15/2/2001	31.8	5.4	3.2	3.7	1.8	*	LWNT
Station A4							
3/6/2000	35.1	0.4	8.2	0.9	0.5	9.6	HWST
3/6/2000	*	2.0	4.8	2.3	1.4	0.7	LWST
9/6/2000	35.1	3.0	2.2	1.8	1.6	*	HWNT
9/6/2000	34.2	2.5	1.1	3.7	1.0	*	LWNT
9/8/2000	34.7	5.0	2.7	4.5	1.9	0.3	HWNT
9/8/2000	*	4.3	3.7	9.4	1.9	*	LWNT
17/8/2000	35.2	1.1	5.1	0.7	0.5	*	HWST
17/8/2000	*	2.6	1.4	2.6	0.7	1.0	LWST
29/10/2000	34.6	0.8	2.5	0.5	0.0	*	HWST
29/10/2000	33.7	3.2	2.0	1.9	1.1	*	LWST
4/11/2000	33.2	5.5	1.2	3.1	1.2	*	HWNT
4/11/2000	31.9	3.2	2.8	7.9	1.6	*	LWNT
7/2/2001	34.7	2.0	1.2	1.9	1.8	*	HWST
7/2/2001	33.4	2.8	0.5	2.6	2.1	*	LWST
15/2/2001	33.6	4.9	1.6	1.9	3.1	*	HWNT
15/2/2001	31.6	6.7	2.4	6.0	2.0	*	LWNT
Station A5							
3/6/2000	*	1.9	4.2	2.0	1.0	1.4	LWST
9/6/2000	34.7	2.3	2.3	1.2	1.0	2.7	HWNT
9/6/2000	34.8	4.1	2.2	5.6	1.0	*	LWNT
9/8/2000	34.7	4.5	3.4	3.0	1.6	0.7	HWNT
9/8/2000	33.4	5.1	4.3	11.4	1.7	4.3	LWNT
17/8/2000	35.3	1.0	2.8	0.7	0.4	1.4	HWST
17/8/2000	34.6	2.9	2.4	3.7	1.0	1.4	LWST
29/10/2000	34.6	0.6	3.2	0.5	0.2	*	HWST
29/10/2000	33.0	5.3	3.4	5.8	1.3	*	LWST

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
4/11/2000	33.6	4.7	0.7	1.9	1.1	*	HWNT
4/11/2000	32.7	7.1	1.8	5.0	1.4	*	LWNT
7/2/2001	34.8	2.0	1.2	1.6	1.4	*	HWST
7/2/2001	33.1	3.6	3.4	5.8	0.8	*	LWST
15/2/2001	33.8	3.5	1.6	1.7	1.5	3.4	HWNT
15/2/2001	31.2	6.8	1.9	13.2	2.0	*	LWNT
Station A6							
3/6/2000	34.9	0.3	8.7	0.8	0.5	7.3	HWST
3/6/2000	*	2.8	5.1	2.6	1.0	*	LWST
9/6/2000	35.0	1.9	3.8	1.0	0.9	*	HWNT
9/6/2000	34.2	3.8	1.3	4.6	0.9	*	LWNT
9/8/2000	34.9	3.9	2.4	1.8	1.2	1.4	HWNT
9/8/2000	34.0	5.9	4.1	10.7	2.1	3.9	LWNT
17/8/2000	35.3	0.6	3.3	0.6	0.3	2.4	HWST
17/8/2000	34.6	3.3	2.5	3.9	0.8	*	LWST
29/10/2000	34.7	0.8	2.8	0.4	0.1	*	HWST
29/10/2000	33.4	4.3	2.4	3.8	1.3	*	LWST
4/11/2000	33.9	3.5	0.6	1.6	1.7	*	HWNT
4/11/2000	33.0	5.4	1.8	4.6	1.4	*	LWNT
7/2/2001	34.7	1.7	1.0	1.0	1.4	*	HWST
7/2/2001	33.2	2.1	2.7	5.2	4.5	*	LWST
15/2/2001	33.7	3.8	1.5	0.9	1.1	*	HWNT
15/2/2001	32.1	7.7	12.1	7.6	1.8	*	LWNT
Station A7							
3/6/2000	34.9	0.4	12.6	0.9	1.5	3.5	HWST
3/6/2000	34.0	2.2	4.1	1.6	1.2	1.4	LWST
9/6/2000	34.9	1.7	5.2	0.8	0.8	*	HWNT
9/6/2000	34.3	2.6	1.2	3.1	0.9	*	LWNT
9/8/2000	35.2	2.9	4.5	0.8	1.5	*	HWNT
9/8/2000	34.2	5.0	3.6	6.4	1.8	3.9	LWNT
17/8/2000	35.3	0.6	3.2	0.5	0.2	3.8	HWST
17/8/2000	34.7	3.5	2.3	3.2	0.8	0.3	LWST
29/10/2000	34.7	0.4	2.3	0.4	0.0	*	HWST
29/10/2000	33.7	3.5	2.1	2.4	1.1	*	LWST
4/11/2000	34.1	2.8	0.9	1.0	0.5	*	HWNT
4/11/2000	33.9	2.9	0.7	2.1	1.0	*	LWNT
7/2/2001	34.8	1.6	1.2	0.6	1.5	*	HWST
7/2/2001	33.3	1.5	2.2	4.3	1.3	3.8	LWST
15/2/2001	34.4	2.8	1.6	0.5	2.1	3.1	HWNT
15/2/2001	32.6	6.8	1.5	6.0	2.0	*	LWNT
Station A8							
3/6/2000	34.9	0.5	13.9	0.9	0.3	10.1	HWST
3/6/2000	34.2	1.8	4.6	1.8	0.9	0.7	LWST
9/6/2000	35.3	0.9	3.2	0.6	0.6	6.9	HWNT
9/6/2000	34.8	1.9	1.1	1.6	0.3	*	LWNT
9/8/2000	35.1	2.3	8.0	0.8	1.0	2.1	HWNT
9/8/2000	34.9	4.1	2.4	3.6	1.5	2.0	LWNT
17/8/2000	35.3	0.4	3.8	0.5	0.4	2.4	HWST

	Salinity (ppt)	NH_4-N (μmol L^{-1})	NO_X-N (μmol L^{-1})	DIP (μmol L^{-1})	Urea (μmol L^{-1})	Chlorophyll-*a* (μg L^{-1})	Tidal State
17/8/2000	35.2	3.4	2.1	2.6	1.0	*	LWST
29/10/2000	34.8	0.5	3.1	0.4	0.0	*	HWST
29/10/2000	34.0	3.1	1.4	1.7	1.0	*	LWST
4/11/2000	34.4	1.1	0.9	0.6	0.3	*	HWNT
4/11/2000	34.0	2.6	0.5	1.9	1.2	*	LWNT
7/2/2001	34.8	1.6	0.9	0.4	2.3	*	HWST
7/2/2001	33.7	1.5	0.7	2.1	2.3	*	LWST
15/2/2001	34.5	2.4	1.6	0.6	1.0	*	HWNT
15/2/2001	33.2	6.0	1.6	4.7	2.7	*	LWNT
Station A9							
3/6/2000	34.7	0.6	11.6	0.8	0.5	*	HWST
3/6/2000	34.6	2.0	4.0	1.9	1.0	1.4	LWST
9/6/2000	35.0	0.8	2.9	0.6	0.9	4.1	HWNT
9/6/2000	34.7	2.5	1.7	1.8	0.6	*	LWNT
9/8/2000	35.3	2.0	7.2	0.8	1.1	2.7	HWNT
9/8/2000	35.0	3.3	1.9	1.8	1.2	2.3	LWNT
17/8/2000	35.3	0.5	2.6	0.7	0.2	2.7	HWST
17/8/2000	35.1	3.1	2.0	1.9	0.9	0.3	LWST
29/10/2000	34.8	0.2	3.3	0.4	0.1	*	HWST
29/10/2000	34.1	2.3	1.2	1.5	1.0	*	LWST
4/11/2000	34.7	1.1	1.1	0.4	0.5	*	HWNT
4/11/2000	34.1	2.9	0.5	1.5	0.4	*	LWNT
7/2/2001	34.8	1.5	0.9	0.9	2.2	*	HWST
7/2/2001	33.8	1.3	0.7	1.9	2.0	*	LWST
15/2/2001	34.5	1.4	1.5	0.4	1.1	3.4	HWNT
15/2/2001	33.7	4.3	2.4	2.2	2.4	*	LWNT
Additional Stations:							
Station X15							
27/10/2000	34.8	0.7	1.9	0.5	0.3	*	HWST
27/10/2000	33.3	1.6	1.8	0.6	0.4	*	LWST
3/11/2000	34.3	1.1	2.8	0.7	1.1	*	HWNT
3/11/2000	33.3	1.3	2.4	0.9	0.4	*	LWNT
13/11/2000	34.6	0.8	1.1	0.2	4.3	*	HWST
13/11/2000	31.0	4.5	1.9	0.8	5.5	*	LWST
14/11/2000	25.1	5.5	2.2	0.8	9.0	*	LWST
16/11/2000	30.2	3.0	2.9	0.8	1.6	*	LWST
9/2/2001	34.8	1.2	0.6	0.8	1.6	*	HWST
9/2/2001	33.4	2.3	1.3	0.6	1.2	*	LWST
16/2/2001	34.4	1.6	1.8	0.5	1.6	*	HWNT
16/2/2001	33.9	1.8	1.1	0.8	1.0	*	LWNT
Station A1							
3/6/2000	*	1.7	9.3	1.3	0.6	*	HWST
3/6/2000	34.1	1.7	4.7	1.2	1.0	3.4	LWST
9/8/2000	*	2.9	1.6	1.4	1.1	2.7	HWNT
17/8/2000	*	2.7	3.7	2.6	0.9	0.7	HWST
17/8/2000	*	3.4	1.6	1.5	0.8	1.0	LWST
29/10/2000	34.8	2.8	2.2	1.6	0.6	*	HWST

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
29/10/2000	33.9	5.4	1.8	1.8	1.2	*	LWST
4/11/2000	33.5	3.1	1.5	0.9	0.5	*	HWNT
4/11/2000	33.2	5.5	0.4	1.2	1.4	*	LWNT
7/2/2001	33.6	2.2	1.6	5.5	2.1	*	HWST
7/2/2001	33.3	1.5	1.6	1.0	0.3	*	LWST
15/2/2001	32.2	4.6	*	1.1	9.6	*	HWNT
15/2/2001	32.9	3.6	1.5	1.7	1.7	*	LWNT
Station A2							
3/6/2000	*	2.5	5.8	1.2	0.9	1.0	HWST
Station A10							
17/8/2000	*	1.0	4.7	0.7	0.4	2.7	HWST
17/8/2000	*	2.2	1.1	1.5	1.0	*	LWST
29/10/2000	34.8	0.6	2.7	0.4	0.9	*	HWST
29/10/2000	34.7	1.1	0.4	1.1	0.4	*	LWST
4/11/2000	34.3	2.7	0.7	0.9	0.7	*	HWNT
4/11/2000	34.1	3.3	0.3	1.4	0.9	*	LWNT
7/2/2001	35.1	1.5	1.0	0.9	2.5	*	HWST
7/2/2001	34.9	0.8	0.3	1.4	0.5	*	LWST
15/2/2001	34.1	3.0	0.6	0.8	1.2	*	HWNT
15/2/2001	33.6	3.9	2.1	2.1	2.0	*	LWNT
Post Storm:							
Station M1							
13/11/2000	3.8	4.0	4.3	0.5	5.0	*	LWST
13/11/2000	0.8	2.4	10.5	3.5	2.2	*	HWST
14/11/2000	0.7	4.5	7.3	0.3	3.7	*	LWST
16/11/2000	5.8	6.9	14.8	0.2	5.2	*	LWST
Station M2							
13/11/2000	9.9	5.5	2.8	0.5	9.8	*	LWST
13/11/2000	2.2	3.4	11.3	0.4	1.9	*	HWST
14/11/2000	1.4	4.8	10.2	0.3	7.0	*	LWST
16/11/2000	7.6	4.9	14.0	0.2	2.1	*	LWST
Station M3							
13/11/2000	13.4	5.6	2.3	0.4	4.4	*	LWST
13/11/2000	19.8	4.1	5.5	0.4	1.7	*	HWST
14/11/2000	9	7.4	3.3	0.4	3.3	*	LWST
16/11/2000	9.8	5.7	13.9	0.4	2.0	*	LWST
Station M4							
13/11/2000	18.8	5.3	1.0	0.4	2.7	*	LWST
13/11/2000	30.2	4.5	2.6	0.9	3.2	*	HWST
14/11/2000	16.7	5.8	1.1	0.4	4.3	*	LWST
16/11/2000	17	8.0	10.5	0.5	4.3	*	LWST
Station M5							
13/11/2000	29.2	4.5	0.8	0.8	2.4	*	LWST
13/11/2000	31.9	3.5	1.7	0.8	3.3	*	HWST
14/11/2000	20.3	9.1	1.0	0.7	5.2	*	LWST
16/11/2000	20.7	8.4	9.1	0.6	6.1	*	LWST

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
Station M6							
13/11/2000	32.2	2.7	1.6	0.8	5.5	*	HWST
14/11/2000	19.2	8.3	1.3	0.6	9.7	*	LWST
16/11/2000	21.3	5.9	7.3	0.6	7.9	*	LWST
Station M7							
13/11/2000	29.6	5.2	0.8	0.8	3.1	*	LWST
13/11/2000	32.9	2.5	1.7	0.7	5.9	*	HWST
14/11/2000	19.1	4.7	1.8	0.7	2.0	*	LWST
16/11/2000	21.7	18.7	5.2	0.7	8.3	*	LWST
Station M8							
13/11/2000	29.8	4.7	0.8	0.9	2.8	*	LWST
13/11/2000	33.4	2.1	1.5	0.6	4.3	*	HWST
14/11/2000	20	5.9	2.3	0.6	5.7	*	LWST
16/11/2000	22.5	7.0	5.2	0.7	5.9	*	LWST
Station M9							
13/11/2000	29.1	5.9	2.3	0.7	7.1	*	LWST
13/11/2000	33.7	2.2	1.6	6.0	7.5	*	HWST
14/11/2000	20.1	4.9	1.7	0.6	1.8	*	LWST
16/11/2000	23.3	6.3	5.3	0.7	4.1	*	LWST
Station M10							
13/11/2000	30.8	4.5	1.2	0.8	4.3	*	LWST
13/11/2000	34.0	1.8	1.9	4.0	*	*	HWST
14/11/2000	21.6	5.9	1.8	0.6	3.7	*	LWST
16/11/2000	24.3	6.3	7.2	0.7	5.2	*	LWST
Station M11							
13/11/2000	30.8	5.5	1.4	0.8	4.0	*	LWST
13/11/2000	34.4	1.4	1.5	4.0	1.3	*	HWST
14/11/2000	23.4	6.4	1.6	0.6	5.4	*	LWST
16/11/2000	25.2	5.7	6.2	0.7	4.1	*	LWST
Station M12							
13/11/2000	31.3	4.4	1.7	0.8	7.6	*	LWST
13/11/2000	34.7	0.6	1.5	0.2	2.9	*	HWST
14/11/2000	25.2	5.5	1.4	0.7	5.5	*	LWST
16/11/2000	26.6	5.7	4.4	0.8	4.6	*	LWST
Station M13							
13/11/2000	31.5	4.5	1.6	0.8	5.3	*	LWST
13/11/2000	34.7	0.4	1.3	0.3	2.6	*	HWST
14/11/2000	26.4	4.0	2.1	0.8	6.3	*	LWST
16/11/2000	28.4	4.7	3.2	0.8	9.4	*	LWST
Station M14							
13/11/2000	31.6	4.9	1.7	0.8	4.7	*	LWST
13/11/2000	34.7	1.1	1.1	0.3	4.1	*	HWST
14/11/2000	28	4.1	1.3	0.7	3.7	*	LWST
16/11/2000	30.2	3.9	6.6	0.8	3.8	*	LWST

	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)	Urea ($\mu mol\ L^{-1}$)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Tidal State
upper estuary, K1 - K10:							
Station K10							
29/9/2000	8	1.5	0.9	0.1	4.2	*	HWST
Station K9							
29/9/2000	17.8	2.2	1.3	0.0	14.5	*	HWST
19/3/2001	0.1	1.2	4.4	1.8	0.0	*	HWNT
Station K8							
29/9/2000	18.8	1.7	1.5	0.1	2.2	*	HWST
19/3/2001	0.1	1.2	4.4	2.2	0.0	*	HWNT
Station K7							
29/9/2000	24.5	1.5	1.5	0.3	5.3	*	HWST
19/3/2001	0.1	1.6	4.9	3.0	0.0	*	HWNT
Station K6							
29/9/2000	26	0.7	0.6	0.2	2.4	*	HWST
19/3/2001	0.2	1.3	5.6	1.9	0.0	*	HWNT
Station K5							
29/9/2000	29.1	2.4	1.1	0.2	22.3	*	HWST
19/3/2001	0.4	1.6	5.5	2.8	0.1	*	HWNT
Station K4							
29/9/2000	31.1	0.5	0.9	0.2	0.1	*	HWST
19/3/2001	0.6	1.8	6.2	2.2	0.1	*	HWNT
Station K3							
29/9/2000	32.4	1.4	3.6	0.6	8.7	*	HWST
19/3/2001	1.8	2.2	6.9	1.7	0.1	*	HWNT
Station K2							
29/9/2000	33.8	2.3	1.7	0.7	21.1	*	HWST
19/3/2001	3.5	2.7	6.9	1.8	0.2	*	HWNT
Station K1							
29/9/2000	34.1	2.6	1.5	0.8	18.2	*	HWST
19/3/2001	7.9	3.5	5.2	2.5	0.3	*	HWNT

Storm Drains:

Station	Description	Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)
S5	Ashmead boat ramp				
	17/5/2000	3	5.3	27.7	1.7
	30/8/2001	0.3	31.3	124.9	2.7
S6	Ashmead east end of walkway				
	17/5/2000	2	5.3	2.4	3.1
	30/8/2001	0.2	29.8	106.0	4.3
S7	Strand street, Ashmead				
	30/8/2001	5.4	58.2	482.6	7.3
S8	Ashmead wharf pipe				
	30/8/2001	0.1	29.8	16.6	4.0
S10	George Rex dr., 60 cm pipe				
	30/8/2001	1.7	42.9	244.1	11.0
S12	George Rex dr., twin 80 cm pipes				
	30/8/2001	0.3	31.3	17.0	4.7
S15	Golf course, twin 50 cm pipes				
	30/8/2001	1.1	48.7	32.2	9.3
S17	North flow				
	3/8/2000	0.3	3.7	52.4	150.0
	3/11/2000	*	72.4	163.1	17.4
	20/2/2001	2.0	21.4	141.7	3.8
	30/8/2001	0.2	34.2	39.8	8.7
S18	South flow, STW				
	3/8/2000	1.3	51.8	60.0	57.7
	3/11/2000	*	9.5	75.0	150.0
	20/2/2001	2.5	26.9	53.6	252.3
	30/8/2001	1.7	37.8	296.4	163.3
S19	Woodbourne pan outflow				
	30/8/2001	0.8	32.0	107.0	7.3
S22	Thesen causeway, west of road				
	30/8/2001	0.5	50.9	199.2	7.7
S23	Kada stream				
	15/11/2000	2.8	56.5	7.9	1.7
	30/8/2001	0.4	53.8	78.6	6.3
S24	Railway bridge stream				
	15/8/2000	1	28.5	62.8	4.9
	15/11/2000	1	45.9	6.2	3.4
	30/8/2001	0.4	9.5	206.7	9.7
S28	Salt River at culvert				
	8/11/2000	0.3	2.2	0.6	0.1
	15/11/2000	0.3	5.5	2.9	0.6
	20/2/2001	0.3	10.5	2.6	0.8
	24/7/2001	0	3.5	20.8	0.9
	30/8/2001	0.2	2.5	68.7	1.0
S29	Oyster catcher drain				
	30/8/2001	0.4	40.0	80.8	7.7
S30	Ashmead pan				
	30/8/2001	2.1	53.8	193.4	4.7

		Salinity (ppt)	NH_4-N ($\mu mol\ L^{-1}$)	NO_X-N ($\mu mol\ L^{-1}$)	DIP ($\mu mol\ L^{-1}$)
KR	Knysna River at Charlesford weir				
	19/3/2000	0	1.4	4.3	0.1
	29/9/2000	0	1.3	0.2	0.1
	27/10/2000	0	0.6	7.6	0.2
	15/11/2000	0	2.5	10.4	0.3
	20/2/2001	0	1.9	1.0	0.2
	24/7/2001	0	1.1	4.3	0.1
	31/8/2001	0	1.0	8.6	1.2

Storm drains, continued Station	Date	Urea ($\mu mol\ L^{-1}$)	Oxygen ($\mu g\ L^{-1}$)	Rainfall(mm) previous 24h.(72h.)	Flow rate ($m^3\ hr^{-1}$)
S5	17/5/2000	3.7	12.2	0 (0)	0.01
	30/8/2001	7.8	*	46 (46)	23.10
S6	17/5/2000	3.5	5.3	0 (0)	0.03
	30/8/2001	2.5	*	46 (46)	132.00
S7	30/8/2001	10.4	*	46 (46)	5.59
S8	30/8/2001	1.9	*	46 (46)	0.96
S10	30/8/2001	5.6	*	46 (46)	3.75
S12	30/8/2001	5.8	*	46 (46)	2.25
S15	30/8/2001	4.5	*	46 (46)	471.24
S17	3/8/2000	2.2	8.69	0 (0)	61.72
	3/11/2000	8.3	*	0.5 (7)	116.64
	20/2/2001	6.7	7.01	0 (0)	21.60
	30/8/2001	8.1	*	46 (46)	555.43
S18	3/8/2000	5.9	8.55	0 (0)	216.00
	3/11/2000	16.7	*	0.5 (7)	41.97
	20/2/2001	8.1	8.55	0 (0)	24.69
	30/8/2001	2.4	*	46 (46)	1,080.00
S19	30/8/2001	2.1	*	46 (46)	3,600.00
S22	30/8/2001	7.0	*	46 (46)	85.71
S23	15/11/2000	18.1	*	0 (39)	0.72
	30/8/2001	3.0	*	46 (46)	86.90
S24	15/8/2000	6.2	9.1	0 (0)	0.15
	15/11/2000	17.0	*	0 (39)	0.36
	30/8/2001	8.0	*	46 (46)	38.88

		Urea (umol L^{-1})	Oxygen (ug L^{-1})	Rainfall(mm) Previous 24h.(72h.)	Flow Rate (m^3 hr^{-1})
S28	8/11/2000	3.3	*	11.5 (14)	17.40
	15/11/2000	14.4	*	0 (39)	17.40
	20/2/2001	5.2	*	0 (0)	5.70
	24/7/2001	*	7.7	10.5 (34)	228.00
	30/8/2001	4.1	*	46 (46)	598.50
S29	30/8/2001	3.7	*	46 (46)	23.14
S30	30/8/2001	3.0	*	46 (46)	100.55
KR	19/3/2000	2.2		0 (31)	1,123.60
	29/9/2000	2.0		0 (0)	274.00
	27/10/2000	2.3		0 (29)	2,331.00
	15/11/2000	5.8		0 (39)	11,281.00
	20/2/2001	2.0		0 (0)	952.50
	24/7/2001	*		10.5 (34)	10,740.00
	31/8/2001	3.4	*	5 (51)	5,812.00

Appendix C,
Stations

name	Site Description	Latitude, south	Longitude, east
M1	Buoy 1	34 01.597	22 59.287
X1	Buoy 2	34 01.814	22 59.406
M2	Buoy 3	34 01.942	22 59.661
X2	Buoy 4	34 02.018	22 59.873
M3	Buoy 5	34 02.016	23 00.095
X3	Buoy 6	34 02.104	23 00.282
M4	Buoy 7	34 02.214	23 00.493
X4	Buoy 8	34 02.546	23 00.400
M5	Buoy 9	34 02.830	23 00.373
X5	Buoy 10	34 02.945	23 00.538
M6	Buoy 11	34 02.963	23 00.674
X6	Buoy 12	34 02.899	23 00.855
M7	Buoy 13	34 02.847	23 00.934
X7	Buoy 14	34 02.695	23 01.018
M8	Buoy 15	34 02.512	23 01.079
X8	Buoy 16	34 02.371	23 01.198
M9	Buoy 17	34 02.295	23 01.367
X9	Buoy 18	34 02.265	23 01.604
M10	Buoy 19	34 02.359	23 01.843
X10	Buoy 20	34 02.493	23 02.075
M11	Buoy 21	34 02.653	23 02.309
X11	Buoy 22	34 02.828	23 02.533
M12	Buoy 23	34 03.214	23 02.665
X12	Buoy 24	34 03.499	23 02.630
M13	Buoy 25	34 03.682	23 02.656
X13	Buoy 26	34 03.870	23 02.770
M14	Buoy 27	34 04.159	23 03.028
X14	Buoy 28	34 04.349	23 03.148
M15	Buoy Y1	34 02.990	23 02.700
X15	mid channel at yacht club	34 02.573	23 02.622
A1	Ashmead 1	34 02.618	23 02.907
A2	Ashmead 2	34 02.542	23 03.225
A3	Ashmead 3	34 02.441	23 03.655
A4	Ashmead 4	34 02.590	23 03.979
A5	Ashmead 5	34 02.862	23 03.904
A6	Ashmead 6	34 03.108	23 03.700
A7	Ashmead 7	34 03.337	23 03.502
A8	Ashmead 8	34 03.363	23 03.167
A9	Ashmead 9	34 03.515	23 02.880
A10	Ashmead 10	34 03.603	23 03.426
KR	Knysna River at Charlesford weir	33 59.907	23 00.195
K1	upper estuary	34 01.287	22 59.601
K2	upper estuary	34 01.007	22 59.979
K3	upper estuary	34 00.792	23 00.413
K4	upper estuary	34 00.672	23 00.891
K5	upper estuary	34 00.462	23 01.047
K6	upper estuary	34 00.326	23 00.566
K7	upper estuary	34 00.073	23 00.45

name	Site Description	Latitude, south	Longitude, east
K8	upper estuary	33 59.883	23 00.370
K9	upper estuary	33 59.877	23 00.251
K10	upper estuary	33 59.907	23 00.195
upper estuary benthic	Benthic study site for the upper estuary	34 00.773	23 00.531
Lagoon benthic	Benthic study site for the Lagoon	34 02.372	23 01.123
Bay benthic	Benthic study site for the Bay	34 02.782	23 02.878
S5	Ashmead boat ramp	34 02.394	23 03.781
S6	Ashmead east end of walkway	34 02.409	23 04.000
S7	Ashmead, end of Strand street	34 02.463	23 03.226
S8	Ashmead wharf pipe	34 02.410	23 03.608
S10	George Rex dr., #2, 60 cm pipe	34 02.538	23 04.073
S12	George Rex dr., #4, twin 80 cm pipes	34 02.633	23 04.111
S15	George Rex dr., #7, golf course	34 03.340	23 04.171
S17	George Rex dr., #9, North flow	34 02.757	23 04.175
S18	George Rex dr., #10, STW, South flow	34 02.830	23 04.191
S19	George Rex dr., #11, Woodbourne pan	34 04.122	23 04.166
S22	Thesen Island causeway, west of road	34 02.557	23 02.891
S23	Kada stream	34 02.271	23 02.378
S24	Railway bridge, west of tracks at the N2	34 02.219	23 02.243
S28	Salt River at dirt road bridge	34 01.489	23 01.969
S29	Oyster catcher, drain to west of facility	34 02.404	23 02.591
S30	Ashmead pan	34 02.391	23 03.405

APPENDIX D,

Benthic Flux	substrate	DIP flux mmol P m-2 d-1	NH_4-N flux mmol N m-2 d-1	NO_X-N flux mmol N m-2 d-1	D.O. minimum mg L-1
Ashmead A3					
26/6/2001, 24 hr	Caulerpa, Zostera	0.6	2.5	0	*
26/6/2001, 33 hr	Caulerpa, Zostera	0.6	1.3	0	*
18/02/2002, 24 hr	Caulerpa, Zostera	0.4	5.1	0	*
18/02/2002, 36 hr	Caulerpa, Zostera	0.4	4.3	-0.1	5.4
Ashmead A5					
25/07/2001, 24 hr	Caulerpa, Zostera	0.8	4.4	0	*
25/07/2001, 33 hr	Caulerpa, Zostera	1.0	6.0	0	*
5/02/2002, 24 hr	Caulerpa, Zostera	0.7	2.0	0	5.5
Ashmead A6					
12/06/2001, 24 hr	Caulerpa, Zostera	3.2	7.6	0	*
12/06/2001, 33 hr	Caulerpa, Zostera	2.8	6.2	-0.1	*
20/02/2002, 24 hr	Caulerpa, Zostera	3.2	8.6	0	*
20/02/2002, 36 hr	Caulerpa, Zostera	2.4	2.4	0	3.0
Ashmead A7					
4/07/2001, 24 hr	Caulerpa, Zostera	0.4	10.7	0	*
4/07/2001, 48 hr	Caulerpa, Zostera	0.4	6.0	-0.1	*
4/03/2002, 24 hr	Caulerpa, Zostera	0.3	3.1	0	*
4/03/2002, 36 hr	Caulerpa, Zostera	0.4	3.2	-0.5	5.5
Bay					
6/06/2001, 24 hr	Zostera	0.4	9.6	0	*
6/06/2001, 33 hr	Zostera	0.8	8.4	-0.3	*
18/07/2001, 24 hr	sand	0.1	3.9	0	*
18/07/2001, 33 hr	sand	0.1	6.6	-0.3	*
25/09/2001, 24 hr	sand	0.2	3.0	0	*
25/09/2001, 33 hr	sand	0.0	2.1	-0.4	*
24/01/2002, 24 hr	Zostera	1.3	18.8	0	2.3
27/02/2002, 24 hr	sand	0.5	12.1	-0.1	*
27/02/2002, 36 hr	sand	0.6	12.3	-3.1	3.3
Lagoon					
6/22/2001, 24 hr	sand	0.3	1.2	0	*
6/22/2001, 33 hr	sand	0.2	0.7	0.2	*
7/22/2001, 24 hr	Zostera	0.0	2.6	0	*
7/22/2001, 33 hr	Zostera	-0.1	1.3	0	*
2/02/2002, 24 hr	sand	0.4	2.8	0	*
2/02/2002, 36 hr	sand	0.4	3.1	-0.5	2.9
25/02/2002, 24 hr	Zostera	1.5	12.4	0	*
25/02/2002, 36 hr	Zostera	1.6	14.4	-0.2	1.5
Estuary					
6/17/2001, 24 hr	sand	0.0	-1.0	0	*
6/17/2001, 33 hr	sand	0.0	-0.4	-0.1	*
7/01/2001, 24 hr	Zostera	0.0	2.4	0	*
7/01/2001, 33 hr	Zostera	0.0	1.9	0	*
28/01/2002, 24 hr	sand	0.4	5.0	0	*
28/01/2002, 36 hr	sand	0.3	4.0	-0.1	2.1
13/02/2002, 24 hr	Zostera	0.1	2.9	0	*
13/02/2002, 36 hr	Zostera	0.1	4.1	-0.2	3.4

Concentration of nutrients in chamber	DIP initial/final (*u*mol L-1)	NH_4-N initial/final (*u*mol L-1)	NO_X-N initial/final (*u*mol L-1)
Ashmead A3			
26/6/2001, 24 hr	1.4 / 1.7	3.3 / 8.4	0.7 / 0.4
26/6/2001, 33 hr	1.4 / 2.0	3.3 / 9.7	0.7 / 0.4
18/02/2002, 24 hr	1.1 / 1.7	3.8 / 6.5	2.3 / 2.3
18/02/2002, 36 hr	1.1 / 1.9	3.8 / 5.6	2.3 / 2.2
Ashmead A5			
25/07/2001, 24 hr	0.6 / 1.3	3.1 / 6.2	4.0 / 3.9
25/07/2001, 33 hr	0.6 / 1.6	3.1 / 7.1	4.0 / 3.8
5/02/2002, 24 hr	2.9 / 3.6	3.0 / 5.0	0.6 / 0.4
Ashmead A6			
12/06/2001, 24 hr	0.8 / 2.1	3.4 / 11.0	2.1 / 2.0
12/06/2001, 33 hr	0.8 / 2.3	3.4 / 12.6	2.1 / 1.6
20/02/2002, 24 hr	0.6 / 3.8	2.1 / 10.7	0.5 / 0.2
20/02/2002, 36 hr	0.6 / 4.3	2.1 / 11.8	0.5 / 0.2
Ashmead A7			
4/07/2001, 24 hr	3.2 / 2.9	3.6 / 14.4	3.2 / 2.9
4/07/2001, 48 hr	3.2 / 2.9	3.6 / 22.2	3.2 / 2.9
4/03/2002, 24 hr	0.6 / 0.9	2.5 / 5.6	3.0 / 1.0
4/03/2002, 36 hr	0.6 / 1.0	2.5 / 7.3	3.0 / 0.7
Bay			
6/06/2001, 24 hr	0.9 / 1.2	3.1 / 11.6	4.0 / 3.6
6/06/2001, 33 hr	0.9 / 2.0	3.1 / 15.5	4.0 / 3.2
18/07/2001, 24 hr	0.7 / 0.8	2.2 / 3.6	2.1 / 1.6
18/07/2001, 33 hr	0.7 / 0.8	2.2 / 12.3	2.1 / 1.2
25/09/2001, 24 hr	0.8 / 0.9	4.9 / 7.9	4.0 / 3.3
25/09/2001, 33 hr	0.8 / 0.8	4.9 / 8.0	4.0 / 2.6
24/01/2002, 24 hr	1.2 / 2.6	3.7 / 22.5	1.5 / 0.9
27/02/2002, 24 hr	1.0 / 1.5	2.9 / 14.9	17.5 / 11.5
27/02/2002, 36 hr	1.0 / 1.6	2.9 / 21.4	17.5 / 7.9
Lagoon			
6/22/2001, 24 hr	0.7 / 0.9	3.1 / 3.3	1.7 / 2.6
6/22/2001, 33 hr	0.7 / 0.9	3.1 / 3.9	1.7 / 2.2
7/22/2001, 24 hr	0.6 / 0.6	2.5 / 4.4	0.7 / 0.7
7/22/2001, 33 hr	0.6 / 0.5	2.5 / 13.8	0.7 / 0.8
2/02/2002, 24 hr	0.7 / 1.1	2.2 / 5.4	2.9 / 1.4
2/02/2002, 36 hr	0.7 / 1.1	2.2 / 6.9	2.9 / 1.2
25/02/2002, 24 hr	1.1 / 2.6	3.0 / 17.5	0.9 / 0.5
25/02/2002, 36 hr	1.1 / 2.7	3.0 / 29.6	0.9 / 0.4
Estuary			
6/17/2001, 24 hr	0.3 / 0.3	1.8 / 0.9	1.1 / 1.0
6/17/2001, 33 hr	0.3 / 0.3	1.8 / 1.4	1.1 / 1.0
7/01/2001, 24 hr	0.3 / 0.3	2.7 / 5.7	2.9 / 2.1
7/01/2001, 33 hr	0.3 / 0.3	2.7 / 5.4	2.9 / 2.3
28/01/2002, 24 hr	0.6 / 1.1	2.9 / 23.4	1.3 / 1.2
28/01/2002, 36 hr	0.6 / 1.3	2.9 / 27.0	1.3 / 1.0
13/02/2002, 24 hr	0.7 / 0.8	2.2 / 6.0	1.9 / 1.5
13/02/2002, 36 hr	0.7 / 0.8	2.2 / 8.8	1.9 / 1.2