Division of Resources and Environment
Department of Environmental Biology

Phytoplankton Ecology in the Upper Swan River Estuary, Western Australia: with Special Reference to Nitrogen Uptake and Microheterotroph Grazing

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Doctor of Philosophy
of
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S. M. Jane Horner Rosser

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DEDICATION

I dedicate this thesis to the memory of my beloved parents, Jim and Isa Horner.

The completion of this work fulfils a lifelong ambition and realises their early edict — you can be a doctor or a lawyer! This is especially for my Dad who has instilled in me the belief that you should always strive for the best and then some! I'm sorry it has taken so long.

The road to success is not always smooth. There are many and varied tests that one confronts in striving to reach personal goals. Completion of this dissertation has been difficult on a personal and scientific level. Most difficult has been the passing of both my parents. That I have reached the end has been due to the enormous support and sacrifice of my husband, Tony, and my two children, Lauren and Nicole. From times of elation to times of despair they have stood beside me and supported me to the nth degree. I thank them and hope that time will show it was all worth it in the end.

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ABSTRACT

Phytoplankton succession and abundance in estuaries is known to be influenced by the relative strengths of various seasonally changing physical and chemical factors. Previous studies of Swan River Estuary phytoplankton biomass and composition have identified salinity, temperature, rainfall and nutrients as the most important controlling factors. These conclusions are generally based on analysis of data from river length transects and depth integrated day-time sampling. They describe influences affecting whole system phytoplankton abundance and succession. Many of the typical seasonal blooms that develop are ephemeral and only extend over relatively small areas.

The focus of this study is a single site, Ron Courtney Island, considered typical of the upper estuary region. This region of the estuary was chosen as representative of the section of river most influenced by allochthonous nutrient input. It has been the region of most frequent and intense algal blooms over the past decade. The factors, physical, biological or physiological, that have the greatest influence on controlling phytoplankton biomass under various ambient conditions for this system are determined.

While previous studies have recognised the importance of nitrogen to phytoplankton growth in the Swan River Estuary, they have focused on NO₃⁻, with only anecdotal reference to the importance of the alternative nitrogen source, NH₄⁺. This is the first study to explore the influence of different nitrogen source fluxes on phytoplankton biomass in the upper Swan River Estuary. The roles of physiological adaptation to, and preferences for, 'new' (NO₃⁻), recycled (NH₄⁺) and organic (urea) nitrogen sources in relation to ambient nutrient levels are explored. Specific uptake rates (v), normalised to chlorophyll a, for NO₃⁻, NH₄⁺ and urea were 0.2 ± 0.04 – 1831.1 ± 779.19, 0.5 ± 0.26 – 1731.6 ± 346.67 and 3.0 ± 0.60 – 2241.2 ± 252.56 ng N µg Chla⁻¹ respectively. Urea concentration (14.8 – 117.7 µg urea-N l⁻¹) remained relatively constant over the12 month study period. Measured ambient specific uptake rates for urea represent between 27.5% and 40.4% of total N uptake over the annual period February 1998 – January 1999. Seasonal nitrate uptake over the same period constituted only 11.3% (± 10.77%, n=12) to 24.4% (± 13.02%, n=12) with the highest percentage during winter, when nitrate levels are elevated. It is suggested that urea provides a nutrient intermediary over
the spring - summer period during transition from autotrophic to heterotrophic dominated communities.

Grazing and nitrogen recycling are intricately connected by simultaneously providing top-down biomass control and bottom-up nutrient supply. Zooplankton (> 44 μm) grazing has been shown to reduce up to 40% of phytoplankton standing stock at times. Microheterotrophs (<300 μm) can reduce phytoplankton biomass production by up to 100% (potential production grazed, 11.1% day⁻¹ - 99.6 % day⁻¹) over an annual cycle. This correlated to mean seasonal day-time grazing loss of $80.47 \pm 3.5$ ngN μg Chla⁻¹ h⁻¹ in surface waters and $20.17 \pm 9.7$ ngN μg Chla⁻¹ h⁻¹ at depth (4.5m). Night time grazing for surface and bottom depths resulted in similar nitrogen loss rates ($13.03 \pm 4.84$ ngN μg Chla⁻¹ h⁻¹).

Uptake rates for nitrate ($r^2 0.501$) and urea ($r^2 0.512$), along with temperature ($r^2 0.605$) were shown to have the greatest influence on phytoplankton distribution over depth and time. This research emphasises the need for more detailed investigations into the physiology of nutrient uptake and the effects of nutrient fluxes on phytoplankton biomass and distribution. Further research into the roles of organic nitrogen and pico and nanoplankton in this system is recommended.
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CHAPTER 1: Phytoplankton Ecology and Eutrophication: An Introduction

1.1 Phytoplankton and algal blooms

Phytoplankton are microscopic autotrophic or mixotrophic organisms that occur in water ranging from fresh to hypersaline. These organisms often exist in a state of quasi-equilibrium, where biomass production and loss processes are in balance (Evans and Parslow, 1985) resulting in no net population growth and a relatively static biomass. Phytoplankton ecology and an understanding of the factors regulating bloom development and decline are a pivotal component in the overall health of any aquatic system. Phytoplankton biomass, growth rate and species composition can be influenced by a number of environmental factors including light, turbidity, nutrient availability, temperature and grazing. Periodically there may be transient departures from the status quo when population densities and biomass may increase markedly resulting in what has historically been classified as a bloom (Parel, 1988; Hallegraeff, 1993). This basic characterisation of what constitutes a bloom, historically based in terms of biomass, has been re-evaluated over recent decades in the light of the increasing scientific and public awareness of phytoplankton populations, or harmful algal blooms (HAB) that have deleterious effects, either directly or indirectly, on human socio-economic conditions (Smayda, 1997).

Algal blooms may cause non-toxic effects on humans, such as skin rashes and irritation or may cause the death of fish through induced anoxia in the water column in conditions of high biological oxygen demand overnight or, alternatively or additionally, they may cause species specific hepatotoxic or neurotoxic effects depending on the type of bloom. HABs and non-toxic blooms may have deleterious effects on the commercial and recreational use of rivers, requiring management or intervention to minimise economic or social impacts. Ability to predict their occurrence or positively influence or reduce their effects will be important in the maintenance of socially, recreationally and economically viable waterways.
1.2 The Issue of Eutrophication

The level of eutrophication of a system is assessed on the biomass and persistence of high concentrations of phytoplankton chlorophyll a (Chla). Globally there has been an increasing concern over anthropogenic eutrophic conditions that lead to higher primary production (Epstein et al., 1993) and consequently more productive heterotrophic communities. An increasing frequency of HABs has been linked to increasing levels of eutrophication (McComb, 1981; Smayda, 1990; Epstein et al., 1993; Hallegraeff, 1993; John, 1994; Justić et al., 1995; Paerl et al., 1999). There are three major anthropogenic influences affecting phytoplankton biomass and bloom development. Pollution from external sources of nutrients such as sewage and fertilisers, soil erosion and acid rain can add nitrogen and phosphorus to a system. Atmospheric deposition, land run-off, and river flow play an important role in regulating estuarine primary production and biomass accumulation (Malone et al., 1988; Paerl et al., 1990; Gallegos et al., 1992; Mallin et al., 1993). Indirect effects influencing phytoplankton biomass include changes in trophic interactions in populations. This may be a result of phytoplankton removal by pelagic zooplankton, benthic filter feeders and/or higher predators such as planktivorous fish species, with the consequent reduction of controlling effect that these higher orders have on the biomass of lower trophic levels (Shapiro and Wright, 1984; Post and McQueen, 1987; Buskey et al., 1997). Habitat loss, particularly of riparian vegetation and wetlands that sequester nutrients (Chambers, 1987; Epstein et al., 1993), results in an increase in the amounts of nitrogen and phosphorus entering aquatic systems. Wetlands are capable of removing up to 98% of the nitrogen and phosphorus from the water passing through them (Chambers, 1984 & 1987).

Nutrient loadings to waterways around the world have increased with increases in human activity (Heathwaite et al., 1996) but there have also been changes in nutrient quality and stoichiometry (Justić et al., 1995), with a concurrent increase in both organic and inorganic nitrogen concentrations (Butler et al., 1979; Smayda, 1989 & 1990). The paradoxical situation has been noted (Paasche et al., 1984), of algal blooms occurring in conditions of very low nutrient, especially nitrogen. Increased capacity for waterways to support algal growth has lead, in many cases, to blooms of such intensity as to cause catastrophic deoxygenation and death of higher consumers. These cultural or accelerated eutrophic conditions are primarily a result of changes in land use through
agricultural practices and industrial and population-based waste production (Reynolds, 1997). This global phenomenon has created the need for management strategies that enable predictive ability for phytoplankton succession (Roelke, 1998; Roelke et al., 1999). This will require an understanding of the complex interaction of environmental and biological factors that control phytoplankton growth and community composition.

1.3 Eutrophication in the Swan River - causes for concern - the need for action.

Extreme climatic variability coupled with clearing of remnant vegetation for agricultural purposes have led to modifications to the hydrology and ecology of many river and estuary systems in Australia (Harris, 1995). Relatively dense human settlement combined with changes in land-use of catchment areas, such as clearing of remnant vegetation for agricultural purposes, have had major impacts on water quality of estuarine ecosystems both in Australia and worldwide (Harris, 1995). Major changes in land-use of the Swan coastal plain, Western Australia, since early European settlement (Riggert, 1978) plus modifications to the hydrology of the Swan River have lead to a decline in the health of the Swan-Canning Estuary system. Warning signs have been periodic events symptomatic of eutrophication, such as red tides (Hamilton et al., 1999), fish kills, cyanobacterial blooms (Hamilton, 2000) and the accumulation of organic matter in the bottom sediments of deep holes (Douglas et al., 1996).

1.3.1 Location and description

The Swan River Estuary (31°57'S Latitude and 116°04'0'E Longitude), fed by a catchment area of approximately 121,000 km² (Peters and Donohue, 2001), is the second largest estuary in south-western Western Australia. It enters the Indian Ocean at the port of Fremantle and extends approximately 60km upstream from its mouth to Ellen Brook, one of its subsidiary streams. The Swan River Estuary is usually considered to include a lower estuary which has a large tidally driven marine influence, between Perth Water and Fremantle, and an upper estuary, the 40km of water between the causeway and Guildford (see Figure 1.1). This section of the river is narrow and shallow (generally < 4 m depth) with deeper pockets (6 m) and exhibits strong seasonal differences influenced by tidal and climatic conditions (Hodgkin, 1987). Its hydrodynamic properties are regulated primarily by climate. Cool wet winters
(maximum mean 17.4°C during July), with approx. 630 mm rain between April and
November, create seasonal river flow. This flow diminishes during the hot, dry summer
(maximum mean 29.9°C during February) to create a true tidally-driven estuary situation
A salt wedge penetrates some 50 km upstream as the winter rainfall run-off declines.
Average upper estuary depths are 2-3 m. A series of deeper pockets (6 m depth) occur
along the upper reaches of the river and these are reported to have higher
concentrations of NH$_4^+$ and PO$_4^{3-}$ (Douglas et al., 1996; Jack, 1987). A recognised
pattern of bloom succession (John, 1987; Thompson and Hosje, 1996; Twomey and
John, 2001) has been established within the system (see Figure 3.2). Chlorophyte-
dominated blooms occur in the upper estuary during early spring, giving way to
dinophyte- and cryptophyte-dominated blooms through the summer and autumn
periods. Seasonal variation of rainfall, and its subsequent effect on the spatial
distribution of salinity, coupled with nutrients, have been shown to influence the
distribution and succession of phytoplankton species (John, 1984 & 1987; Thompson,
2001; Twomey and John, 2001).

Hodgkin (1987) describes the Swan River as a seasonal estuary. The Swan River Estuary
dynamics were classified more recently by Stephens and Imberger (1996) as varying
between a winter rain-driven gravitational overflow and a salt wedge condition governed
by both discharge and topographic constraints, with the degree of flushing influenced
by tidal dynamics. This system alternates between three distinct phases. It is a poorly
flushed water course during the summer drought period, or a river during periods of
high rainfall where the subsequent high discharge flushes away the salt water. Between
these two phases are periods of high stratification where the salt-wedge regime is
established during periods of decreasing (late spring) or increasing (autumn-early winter)
river discharge (Douglas et al., 1996). This salt wedge penetrates some 50km upstream
as the winter rainfall run-off declines. Like other estuaries of south-western Australia,
the Swan is unusual for its highly variable biological and hydrological characteristics.
These are a reflection of the extreme seasonality of river flow, a direct consequence of
rainfall patterns (Spencer, 1956; Stephens and Imberger, 1996).

Eutrophication is a recognised and often severe problem in many rivers and estuaries
world wide, including rivers on the eastern seaboard and in the south-west of Australia.
Since European settlement there has been a 16 fold increase in total nutrient yields in the Swan-Avon river catchment (Viney and Sivapalan, 2001). The trend towards larger

Figure 1.1 Location map for the Swan-Canning Estuary, Perth, Western Australia showing lower, middle and upper sections of the Swan Estuary and the major landmarks of Perth city, the port of Fremantle and Guildford in the upper reaches. Inset: Location of Swan-Canning river systems in south-west of Western Australia.

and more persistent bloom events has been noted over the past few decades as being cause for concern (John, 1987 & 1994; Thompson and Hosja, 1996; Thompson et al.,
Several catastrophic events due to algal blooms in the Swan River have already occurred. Of note was the January 1992 deoxygenation of the entire length of the Swan River between the causeway and Midland (see Figure 1.1) following the collapse of a large dinoflagellate bloom. This resulted in mass mortality of fish and benthic invertebrates (Deeley et al., 1993). Although toxic cyanobacterial (blue-green algae) blooms have occurred sporadically in the Swan River, giving rise to increased management efforts (Thompson et al., 1997), concern that conditions conducive to development of harmful algal blooms in the Swan River may occur were realised early in 2000 (January 2000). A large-scale bloom of the fresh-water hepatotoxic blue-green alga Microcystis aeruginosa form flos-aquae (Wittrock) Kircher 1898 (Komarek and Anagnostidis, 1999) (Jacob John, pers.comm.; Hamilton, 2000) during late January and early February of this year followed unseasonal rainfall and caused thick green scums along shorelines. This HAB, the largest recorded for the Swan River to date, necessitated the closure of the whole length of the Swan River (approx 50 km) for a period of 12 days (John, 2000). Extensive fish kills (>250 000 mortalities recorded) occurred again in 2003 (April – June) caused by the ichthyotoxic dinoflagyte Karlodinium micrum (density >10^6 cells ml^-1) and were attributed to elevated nitrate levels from runoff in the Swan-Canning catchment area following high rainfall and a subsequent warm spell (http://www.wrc.wa.gov.au/srt/algalalert/FishKillAlgae.pdf, access date 29/12/2003). The extent of these HAB, combined with its drastic effect on the socioeconomic aspects of the river, reinforced the need for waterways health management and a predictive ability for the future reduction or elimination of nuisance and harmful algal blooms.

The main theme that emerged from a Scientific Committee on Oceanic Research (SCOR) working group on mathematical models in biological oceanography was the need “to have at least as much information on the fluxes as on the biomasses” (Platt et al., 1981) for an understanding of biological oceanographic systems. The most common fluxes considered are through, firstly, trophic transfers such as grazing, predation, egestion and, secondly, detritus formation and also elemental cycling which includes nutrient uptake, excretion and advective processes. Fluxes are a product of population density and organism physiology. Top-down and bottom-up controls operate simultaneously. The relationship between top-down and bottom-up control varies depending on the scale of interest, and has important consequences for how we
model phytoplankton biomass control in a natural food web. Grazing and nitrogen recycling are intricately connected; i.e. the presence of large zooplankton simultaneously provides top-down control of biomass and bottom-up nutrient supply (Glibert, 1998). It has become increasingly evident that an evaluation of the relative strength of each, predators and resources, is needed to determine how the development and sustainability of populations or communities is regulated.

The issue of "nuisance" blooms in the Swan River estuary has primarily focused on bottom-up control of phytoplankton dynamics relating to investigations into the physical and chemical conditions of the estuary (John, 1987; Gerritze, 1992; Hosja and Deely, 1994; John, 1994; Douglas et al., 1996; Thompson and Hosja, 1996; Hamilton et al., 1999; Horner Rosser, 1998; Horner Rosser and Thompson, 2001). However, little is known about the relationship between phytoplankton biomass and zooplankton grazers in the Swan-Canning Estuary (Rose, 1998). Studies have demonstrated that copepod grazing, by locally occurring Gladioferens imparepes and Sulcanus inflectus, can account for loss of up to 45% of standing stock (Griffin et al., 2001; Griffin and Rippingale, 2001), but little is known about the impact of other locally occurring zooplankton grazers. It has been recognised that grazing pressure can both enhance, through nutrient release and recycling, and reduce phytoplankton biomass (Stone, 1990; Svensson and Stenson, 1991; Ferrier-Pagès and Rassouzadegan, 1994; Koepfli and Lewitus, 1995). The importance of nano- and micro-zooplankton in foodwebs and planktonic community dynamics (Ferrier-Pagès and Rassouzadegan, 1994; Burkill et al., 1995), particularly those tending towards eutrophy, has been emphasised. No studies detailing the microheterotroph community or its role in top-down control of phytoplankton biomass and/or species composition have been undertaken for the Swan-Canning Estuary which, like other southern Western Australian estuaries, is subject to seasonally highly varying environmental conditions.

1.4 General Objectives of this Research

This study has been one component of a collaborative project with the aim of enabling predictive management strategies for the minimisation of eutrophication and control or prevention of harmful algal blooms (HAB) in the Swan and Canning Rivers. Based on a two dimensional model developed by the Centre for Water Research, University of
Western Australia under the guidance of Dr David Hamilton, this project was designed to provide ecological information on the phytoplankton assemblages of the upper Swan River.

Figure 1.2 Outline of the Estuarine Research Foundation of WA funded interdisciplinary project to model the Swan-Canning Estuary, showing the relationship of this phytoplankton project to the whole.  
(Modified from Hamilton, 1996)

The aim of this study was to determine the extent to which phytoplankton populations in this region are controlled by physico-chemical parameters (bottom-up) or biological (top-down) factors. That is, to determine which factors, physical, biological or physiological, have the greatest influence on controlling phytoplankton biomass under various ambient conditions for this system. It provides the first information on nitrogen fluxes influencing the phytoplankton biomass occurring in the upper Swan River Estuary. This region of the estuary was chosen as representative of the region
most influenced by allochthonous input and which historically has been the region of most frequent and intense algal bloom activity over the past decade. Flux rates and biomass information from this research have already been used to validate a model of the Swan River developed as a tool to aid predictive modelling and management of the Swan River environment (Hamilton et al., 1999).

The objectives of this thesis, presented in the form of separate chapters, are:

1. To establish short- (within bloom) and medium-term (annual) diurnal variation in the physico-chemical environment of the upper Swan River Estuary (Chapter 2).

2. To determine short-term (within bloom) and medium-term (annual) diurnal variation in phytoplankton species composition of the upper Swan River Estuary (Chapter 3).

3. Following previous studies that indicate nitrogen to be the limiting nutrient in this system, to determine flux rates of nitrogen species within the system. Seasonal uptake kinetics for the inorganic nitrogen sources ($\text{NO}_3^-$ and $\text{NH}_4^+$) and the organic nitrogen source (urea) were determined (Chapter 4). The nitrogen source(s) of preference and the rates of uptake of different nitrogen sources (nitrate, ammonium and urea) on a short-term (within bloom) and medium-term (annual) time scale (Chapters 5 and 6) are determined and compared with ambient levels during the study and related to long-term trends in ambient nutrient levels.

4. To determine the microheterotroph species composition and grazing pressure of micro-plankton ($<300\mu\text{m}$) and nano-plankton ($<20\mu\text{m}$) size classes in the upper Swan River Estuary (Chapter 7). The influence of mesotrophs, specifically Copepod grazing, on phytoplankton biomass was investigated by S. Griffin (2003) as part of a different sub-programme of the larger collaborative study.

5. To combine the results from the different research sections in this study to determine the balance between bottom-up and top-down control for the upper Swan River Estuary (Chapter 8). Ultimately the data collected will be used to validate a predictive model developed for the Swan River, in collaboration with The Centre for Water Research at the University of Western Australia (with Dr David Hamilton). Due to time constraints, this component of the analysis and
interpretation will be beyond the scope of this dissertation. The aim will be to
enhance or facilitate predictive management capabilities for this economically
and recreationally important river system

As each Chapter deals with a different aspect of phytoplankton ecology (i.e.
environmental or physiological conditions), each chapter is presented with its own
relevant literature review, techniques section and discussion. Therefore a degree of
repetition has been unavoidable. Published papers relevant to this research project, plus
seminars presented at National or International scientific meetings and based on the
results of this research, are listed in Appendix III.

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CHAPTER 2: Physico-Chemical Environment of
the Upper Swan River Estuary

2.1 Abstract

Phytoplankton succession and abundance in estuaries is known to be influenced by the
relative strengths of various seasonally changing physical and chemical factors. Previous
studies of Swan River Estuary phytoplankton biomass and composition have identified
salinity, temperature, rainfall and nutrients (N and P) as the most important controlling
factors (Hodgkin, 1987; John, 1987; Thompson and Hosje, 1996; Thompson et al., 1996;
Thompson, 2001). These conclusions are generally based on analysis of data from river
length transects and depth integrated daytime sampling. They describe influences
affecting whole system phytoplankton abundance and succession. Many of the typical
seasonal blooms that develop only extend over relatively small areas. This study focuses
on a single site, Ron Courtney Island, typical of the series of deeper pockets of the
upper estuary region (Douglas et al., 1996). It examines physico-chemical parameters
over medium time scales (weeks to months), on a depth and diurnal basis, to determine
which factor or combination of factors most influence typical localised blooms. Data
are compared with published recognised patterns to validate their general applicability
and has been used in later chapters as a basis for physiological studies.

Environmental factors in the period 1995 - 1998 were within previously reported
ranges, although it is recognised that inter-year variability is high. Environmental
variables most strongly related to chlorophyll levels at a single location were saturated
oxygen ($r^2 = 0.64$), orthoP ($r^2 = 0.58$) and ammonium ($r^2 = 0.52$). Diurnal distribution
indicated strong differentiation of surface and bottom phytoplankton during day time
that was not apparent at night. Depth related diurnal chlorophyll distribution patterns
indicated differences between seasonal blooms and changing patterns within blooms
that could be related to nutrient availability. Phytoplankton bloom development at
individual locales may not be influenced by the same factors identified from large area
or transect data sets. While broad scale knowledge gives insight into whole estuary
dynamics, localised depth-related studies may prove more insightful in managing
phytoplankton growth typical of succession bloom development in different regions of
the estuary.
2.2 Introduction

The processes and mechanisms governing spatial and temporal phytoplankton distribution patterns have been the focus of aquatic research for a number of decades (Riley, 1976; Turpin and Harrison, 1979; Dustan and Pinckney, 1989; Franks, 1992; Pinckney et al., 1998; Thompson, 1998; Hambright and Zohary, 2000). High phytoplankton diversity is fostered by both biotic (inter-specific competition and grazing) and abiotic (the influence of various physical and chemical parameters on the growth, productivity and competitive ability of phytoplankton species) mechanisms (Hutchinson, 1961; Pinckney and Dustan, 1990; Hambright and Zohary, 2000). In view of concerns over world-wide increasing occurrence of nuisance or harmful algal blooms (Epstein et al., 1993), the focus over the past two decades has turned to better understanding the roles of these factors in controlling species composition, abundance, distribution and succession, with the aim of developing predictive or preventative management strategies.

Phytoplankton abundance in estuaries is known to depend on factors such as light (Cloern, 1987), nutrients (D’Elia et al., 1986) and zooplankton grazing (Verity, 1987). It is regulated by the relative strength of structuring factors that change seasonally to form an interactive regulatory system (Paerl, 1988; Vanni and Temte, 1990; Levine et al., 1997). Environmental control of phytoplankton assemblages can be influenced by allogenic parameters, or those factors not under biological control, such as macronutrients, light availability, salinity and temperature (e.g. Levasseur et al., 1984; Cloern, 1987; Reynolds, 1988; Granéli et al., 1990; Reynolds, 1997). Factors which represent or are influenced by biological processes, such as life cycle, nutrients, water quality and predation, may also influence phytoplankton dynamics (Smayda, 1980). These autogenic parameters are those that organisms can regulate to a significant degree or that are regulated by other trophic levels. In some situations, regulation by single factors such as turbidity (Cloern, 1987) or nitrogen (Rudek et al., 1991; Mallin et al., 1993) may influence phytoplankton production year round in sections of some estuaries. In others, production is regulated by seasonal changes in limiting factors that may relate to changes in river flow (Fischer et al., 1995; Thompson 2001) or physical factors such as light and temperature (Pennock and Sharp, 1994). Evidence of changing community structure in response to progressive elevations in nutrient levels can be detected from
long-term monitoring of species composition and the physico-chemical environment (Harris, 1980).

Hydrological alterations to water courses may also impact on the system's ability to support algal growth through changes in flow rates and thus turbulence and light penetration, or through effects on salinity regimes as a result of the opening of channels or the imposition of restrictive barriers such as weirs and dams. All these changes have, at one time or another, altered the physico-chemical status quo of the Swan River since its settlement by Europeans since 1829 (Riggert, 1978).

2.3 Nitrogen

The concentrations of different nitrogenous nutrients in aquatic systems and the processes by which these nutrients are transformed, play an important role in the growth and physiology of phytoplankton. Nitrogen in groundwater, streams, rivers and estuaries may be present in several forms, most of which are soluble, highly mobile and readily available to plants. Both inorganic forms, (the oxidised nitrogen species, NO$_3^-$ and NO$_2^-$ and reduced species, NH$_4^+$), are utilised in phytoplankton growth. Processes associated with nitrogen cycling include fixation, dissimilatory reduction (respiratory reduction, denitrification), assimilatory reduction, nitrification, and ammonification (Webb, 1981).

Nitrate

Nitrate is the final oxidation product of nitrogen compounds in aquatic systems. Its concentration in these environments is influenced by microbial oxidation of ammonia, advective transport into euphotic surface waters, uptake by primary producers or denitrification in anoxic conditions (Grashoff et al., 1983). Nitrate concentrations in deep-sea and oceanic euphotic zones are well documented and are typically less than 70 µg-N L$^{-1}$ in the surface waters of the Pacific, Atlantic and Indian Oceans (Vaccaro, 1965). The concentration of nitrate in freshwater is variable and affected by land-use, groundwater and wastewater input (McCarthy, 1980).

Low summer NO$_3^-$ concentrations occur coincident with little or no groundwater discharge (especially in Mediterranean climates) and high biological uptake in catchment
or drainage networks. Atmospheric input of nitrogen, in the form of N₂ gas, into aquatic systems, or from overland and/or groundwater flow associated with catchment rain events, has been recognised in the past decade as a significant allochthonous nitrogen source (Caraco et al., 1992; Mallin et al., 1993; Paerl and Fogel, 1994; Paerl, 1997; Peterls and Pearl, 1997; Hu et al., 1998; Paerl et al., 1999).

Loss of nitrate occurs through denitrification, which is the successive reduction of fixed NO₃⁻ and NO₂⁻ to gaseous N₂O and to N₂. It occurs in sediments and is microbiologically mediated. Denitrification is one of the key processes in marine nitrogen cycles, although the relative importance of nitrogen fixation and denitrification in estuaries has been questioned (Howarth et al., 1988; Seitzinger, 1988). Denitrification processes within estuaries are influenced by nitrate availability, oxygen inhibition, organic matter supply and temperature and benthic infaunal activity (Seitzinger, 1988; Rysgaard et al., 1994; Kana et al., 1998; Nowicki et al., 1997). Denitrification in sediments is a major nitrogen sink in aquatic environments, converting useable inorganic nitrogen to a non-useable gaseous form and, in the process, altering the stoichiometric ratios of nutrients available to primary producers.

**Ammonium**

Of the usually monitored nitrogen compounds (NO₃⁻, NH₄⁺ and urea), ammonium is the most difficult to accurately measure. Concentrations of NH₄⁺ in most oceans, coastal areas and unpolluted waters are generally low (≤ 1.0 μM) and extremely variable over small spatial and temporal scales, due to the influence of biological activity (Glibert, 1982; La Roche, 1983; Goldman and Caron, 1985). Ambient NH₄⁺ levels may often be below the limits of detection of analytical techniques. Measurement of its cycling dynamics is usually made using ¹⁵N-tracer techniques (eg Glibert et al., 1988), although Neuer and Franks (1993) used a seawater dilution technique based on the Landry and Hassett (1982) dilution technique originally used for estimating grazing impact (see Chapter 7).

Ammonium (NH₄⁺) is generated (or regenerated) in the water column or sediments through the degradation of organic matter by bacterially mediated de-amination (Seitzinger, 1988; Rysgaard et al., 1994; Kana et al., 1998), or through animal excretion.
(McCarthy, 1980) and can be generated on short spatial and temporal scales (Goldman and Caron, 1985). While the concentration of NH$_4^+$ relative to other nutrients may be low, regeneration rates are variable and may be high relative to ambient concentrations (i.e., Glibert, 1982), providing a source of available nutrient. NH$_4^+$ may be lost to a system through nitrification to NO$_3^-$. This has been shown to be a rapid and irreversible loss process for this ion in the Delaware River (Lipschultz et al., 1986) since, due to high NH$_4^+$ availability, the resulting NO$_3^-$ was not utilized and was subsequently lost through advective processes.

The occurrence of diurnal variation in ammonium concentrations appears variable. Diurnal periodicity in NH$_4^+$ concentrations have been reported for the sub-arctic Pacific (Wheeler and Kokkinakis, 1990), while no such variation was found by Glibert (1982) for the Southern Ocean, by Collos et al., (1997) in an enclosed aquatic system or by Takahashi et al. (1995) in a lake environment. Diurnal changes reported by Priddle et al. (1997) in near-surface ammonium concentrations in the Southern Ocean (ie max 1.4 mM at night, min 0.1 mM at noon) were largely explained by the nocturnal release of ammonium by zooplankton and daytime utilization by phytoplankton, indicating a tight coupling over a diurnal period between grazers and phytoplankton.

**Organic Nitrogen and Urea**

Dissolved organic nitrogen (DON) constitutes a significant proportion of total available nitrogen in marine, estuarine and freshwater environments (Sharp, 1983; Thurman, 1985; Seitzinger and Sanders, 1997) and is present in the whole water column at significant concentrations (generally 2-10 μM), even in oceanic waters where dissolved inorganic nitrogen (DIN) species are undetectable (Sharp, 1983). Although its exact composition is variable and is not well understood, it includes nitrogen compounds such as urea, dissolved free amino acids (DFAA), purines and ureides (Antia et al., 1991). Tracer experiments using $^{15}$N suggest that the available DON pool can be rapidly produced from inorganic N additions and be available for subsequent utilization by the phytoplankton population (Bronk and Glibert, 1991 & 1993; Bronk et al., 1994).

Inputs of organic nitrogen to estuaries from rivers can account for between 14 - 90% (av. 37%, n=17) of total nitrogen loading (Seitzinger and Sanders, 1997 and references
there-in). Release of DON into the aquatic environment appears ubiquitous (Bronk et al., 1994; Slawyk and Raimbault, 1995).

Urca is just one component of DON and is often detected in higher concentrations in aquatic environments subjected to higher levels of human impact. In unpolluted oceanic, coastal and estuarine waters, urea levels are generally low and variable, ranging from 0 – 5.9 μg-atoms N l⁻¹ (0 – 165.2 μg urea N l⁻¹) for samples analysed immediately or filtered prior to freezing [Table 5.1, p196, in Morris, 1980]. Urea concentrations in sea surface waters have been detected in the range of 2.7 – 21% of DON levels (Carpenter et al., 1972). Morris and Lewis (1992) present riverine values for urea of 15.7 – 249.2 μg Nl⁻¹. A time series of lacustrine urea concentrations in Lake Kinneret, Israel, showed urea to be at greater concentrations than reported for marine environments, but always less abundant than ammonium (Berman, 1974). Concentrations reported for estuaries range between 0.84 – 249.2 μg urea-N l⁻¹ (Antia et al., 1991, and references there-in). Sources of urea in aquatic systems include excretion from certain phytoplankton and zooplankton species, bivalve molluscs, teleost fishes, various crustaceans and elasmobranchs that are known to accumulate urea as an osmoregulator. It may also be produced in the process of bacterial degradation of purines, pyrimidines and organic matter, including senescent phytoplankton (various references, in Antia et al., 1991). Significant urea sources have been demonstrated from benthic macrofauna (Lomstein et al., 1989) and copepod grazing on phytoplankton (Bidgarc, 1983), while urea excretion in the copepod Arcatia tonsa was reported to range between undetectable and 28 ng N copepod⁻¹ h⁻¹ (Miller and Glibert, 1998).

2.4 Phosphorus

Orthophosphate (filtered or soluble reactive phosphorus, FRP or SRP), the form of phosphate preferred by all organisms, is the major source of this element in natural waters available to phytoplankton for primary production. The primary separation of phosphorus into dissolved and particulate is based on separation using a 0.45 μm membrane filter. Total phosphorus (the sum of inorganic orthophosphate and organic phosphorus forms) seldom exceeds 2.0 μM. While uniformly low phosphorus levels are found in oceanic regions (Eppley et al., 1973; Dugdale, 1967) seasonal cycles are more pronounced in coastal and estuarine regions where concentrations are influenced by in
situ regeneration rather than allochthonous sources (Nalewajko and Lean, 1980). Phosphorus concentrations in marine and freshwater sediments are often higher than water column levels.

2.5 Eutrophication and changing stoichiometry

Phytoplankton growth is considered to be nutrient limited if the balance of carbon, nitrogen and phosphorus in the environment varies from the Redfield ratio for C:N:P of 106:16:1 (Redfield, 1958). In freshwater ecosystems the incorporation rate of nitrogen into plant tissue is usually controlled by P availability, since freshwaters are typically P limited (Correll, 1998). Estuaries are typically nitrogen limited, with some variation in nutrient limitation in brackish waters (Smith and Longmore, 1980; Nixon, 1995; Correll, 1998). An analysis of the stoichiometry of dissolved nutrients in 10 large world rivers and in two river-dominated coastal ecosystems prone to eutrophication, suggests that proportions of dissolved silica, nitrogen and phosphorus in rivers carrying nutrients of anthropogenic origin, as well as in the coastal waters strongly influenced by those rivers, have changed historically in a way that reflects the Redfield ratio (Si:Na:P = 16:16:1) (Justić, Rabalais and Turner, 1995; Justić et al., 1995). It is likely that coastal phytoplankton productivity has increased under these favourable nutrient conditions and was accompanied by an increasing incidence of noxious phytoplankton blooms and bottom water hypoxia (Justić et al., 1995).

2.6 The Swan River Estuary physical and chemical environment

Environmental awareness and the concern over the eutrophication of waterways has only been popular since the early 1970s. However, systematic monitoring of the physical and chemical environment of the Swan and Canning Rivers has been ongoing since 1958 (Riggert, 1978) and records of nutrient data for the Swan River date back to 1944 (Jack, 1987; Henderson et al., 1998). Studies of the ecology of the Swan-Canning Estuary and catchment areas, with a view to ecosystem management, include routine water quality monitoring and investigations into phytoplankton populations, (e.g. John, 1984; Jack, 1987; Thompson and Hosja, 1996; Deeley and Paling, 1998; Twomey and John, 2001).
The Swan River, which varies seasonally between fresh and salt conditions, has been shown to vary between P and N limitation throughout the year, dependant on rainfall and river flow (Thompson and Hosja, 1996). From a study of long-term trends in data sets in the estuary, for nitrogen and phosphorus concentrations, Gerritse et al., 1998, indicate a two- to three-fold increase in P concentration since the 1940s. Even so, it has been demonstrated that nitrogen is 20 times more limiting than phosphorus for this system (Thompson and Hosja, 1996). The Avon, a tributary of the Swan River and which drains 98.5% of the 121000 km$^2$ catchment area, contributes most of the N (0.03 kg ha$^{-1}$ y$^{-1}$ or 65%) and a high percentage of the P (< 0.01 kg ha$^{-1}$ y$^{-1}$ or 32%) to the estuary (Peters and Donohue, 2001). Nutrient supply to the estuary has been closely related to disposal of animal wastes and to the type and rate of fertilizer application on the Swan Coastal Plain (Peters and Donohue, 2001) while recent research suggests that nutrients supplied though groundwater input may be important during low-flow periods (Linderfelt and Turner, 2001).

Since previous studies indicate nitrogen to be the most limiting macronutrient for the Swan River estuary, a major aspect of this study has been to explore the significance of different nitrogen sources to the growth and maintenance of phytoplankton in this system. Reliance on recycled nitrogen during times of minimal inorganic nitrogen availability has been suggested, based on anecdotal evidence, but not yet verified (Douglas et al., 1996; Thompson, 2001). To date there has only been speculation about the importance of ammonium-N to phytoplankton growth and succession and no information about the availability or importance of organic nitrogen to phytoplankton growth in this system.

### 2.7 Objectives

This section of the study was designed to provide physical and chemical information that directly related to the times and depths from which species composition analysis (Chapter 3) and rate determination experiments (Chapters 4 – 7) were made, to provide an indication whether the environmental parameters encountered during this study were "typical" of the conditions previously reported (ie Thompson, 2001) and which of these were more influential in affecting phytoplankton distribution. The aims of this section of the research were:
Firstly, to measure diurnal temporal trends of allochthonous and autogenic environmental parameters in order to determine what role, if any, depth and diurnal variations in the physical and chemical environment play in phytoplankton biomass and distribution patterns.

Secondly, for a single site over an annual period, which factor or combination of factors had the greatest influence on distribution. Depth and/or diurnal differences in physical and chemical parameters were related to phytoplankton biomass distribution, using chlorophyll concentration as an indicator of phytoplankton biomass.

2.8 Materials and Methods

2.8.1 Sampling strategy

The upper estuary region was chosen for this study because it is this region that has shown the most obvious signs of nutrient stress and phytoplankton bloom increases. The Ron Courtney Island site (RCI, Figure 2.1) was of particular interest because it had been the site of a number of previous studies that may provide useful background or comparative data. Measurements were made on two temporal scales: one, to measure monthly variations related to depth and time of day to provide seasonal information; the other, a shorter time-scale looking at changes over the course of single bloom events typical of the upper Swan River Estuary environment. Data collected from the temporal study was related to annual long-term trends to determine the validity of ascribing this study’s findings to a more general temporal trend for the system.

Physical and chemical profiles for spring 1995 to summer 1999 were collected from the RCI site at midday, either during routine weekly or fortnightly (winter) monitoring runs with the Swan River Trust (temperature, salinity, oxygen and chlorophyll) during 1995 and 1996. Monthly sampling runs were made noon and night for physiological studies from February 1998 to January 1999. During these runs profiles for NO₃⁻, NH₄⁺, urea, temperature, salinity, oxygen and irradiance were made. Separate profiles were made at morning (approx. 0900 h), afternoon (1400 h) and night (2400 h) for specific bloom periods (spring chlorophyte, November 1995; summer mixed dinophyte, January 1996; and autumn dinophyte-dominated (Oxyrrhis marina) blooms).
Short-term (weekly, within specific blooms) and longer-term (monthly) annual surveys were undertaken of diurnal trends in allogenic and autogenic parameters using the RCI site (Figure 2.1) as representative of the upper Swan River estuary. Sampling stations for physical (temperature, salinity, oxygen and light) and chlorophyll profiles were established at the RCI site and at two sites either side of this (Figure 2.1, Inset C), encompassing a two kilometre stretch of the upper region of the Swan River Estuary. Nutrient profiles were conducted at the RCI site only.

In addition, during spring 1995 and summer 1996, *in situ* diurnal profiles of temperature, salinity and oxygen were made using duplicated water quality logging systems (Yellow Springs Instrument – Grant model 3800). Chlorophyll *a* (Chla) was monitored *in situ* using a Sea-Tech Inc. fluorometer. Two sets of instruments were deployed from an anchored barge and positioned at 0.5m and 3m depths. Each logged at 15 min intervals over 3 days (spring) and 5 days (summer) at the Ron Courtney Island site. Detailed information on diurnal changes over a period of 3 - 5 days for surface and bottom depths provided evidence of cyclical variations in physical parameters and phytoplankton biomass.

### Solar insolation and Irradiance

Trends in annual solar insolation were determined for Perth (Lat. 32°0' Long. 116°0') for the period June 1997 to March 1999. Data were collected by the Western Australian Meteorological Bureau (Mt Lawley weather station).

Vertical profiles of irradiance (air, surface (0.1m) and 1m-depth increments) were made monthly at midday for the RCI site and sites 1 - 4 using a LiCor Model LI-250 light meter with 4π collector. From these profiles monthly diffuse light attenuation coefficients (k) for photosynthetically active radiation (PAR) were calculated using values of irradiance measured at the surface (I₀) and at depth, z (I₀) at time, t, according to the Beer-Lambert Law for uniform liquids (Reynolds, 1997)

\[
I_z = I_0 e^{-kz}
\]

The compensation depth, Z₀ (taken as 10% of surface light in this case) was calculated as representative of the lower limit of the photosynthetic region. The ratio of mixing depth, z₀ (assumed to be equivalent to water depth) to euphotic depth, Z₀, was calculated as an indication of turbulence.
Rainfall
Rainfall averages were calculated from daily rainfall data provided by the Meteorological Bureau of WA and collected at the Perth Airport over a five year period (1995-1999). Monthly rainfall and the distribution trends are plotted as a box plot to show medians and identify outliers occurring over the period of this study. Weekly rainfall totals were calculated for the 13 month period January 1998 – January 1999 inclusive.

2.8.2 Sampling Profiles

Temperature, Salinity and Oxygen
For the individual bloom studies (November 1995, January 1996, May 1996), in situ depth profiles (0.5 m increments) of temperature, salinity and oxygen were made using a Hydrolab H20 Multiprobe Logger (Hydrolab Corporation, Austin Texas, USA) at each of the sampling times (morning, afternoon and night).

During 1998-1999 temporal study profiles (surface and 1m depth increments) were made for temperature, salinity, oxygen and pH using a TPS Model MC-81 Conductivity-Salinity-pH-Temperature meter (TPS Pty Ltd, Brisbane Australia) with resolution capabilities for salinity (0.1‰ ± 0.3%), pH (0.01 pH units ± 0.01) and temperature (0.1°C ± 0.2°C). Oxygen (mg l⁻¹) was measured using a YSI Model 51B Meter (Yellow Springs Instrument Co., Ohio USA; accuracy ±0.38 ppM).

Dissolved oxygen values (mg l⁻¹) were converted to % saturation values using the following formula: %saturation = [O₂] measured * 100/O₂ solubility at saturation together with Table 6.1 of O₂ saturation in relation to temperature in Wetzel and Likens (1991, p 74).

Nutrients and Chlorophyll a
Monthly nutrient concentration profiles for nitrogen and phosphorus species were made for the Ron Courtney Island (RCI) site only. Samples were collected at noon by positive displacement pump from surface (0.25 m) and 1m depth increments. Night-time samples were collected in a similar manner for surface and bottom (4.5 m) only as a comparison with day-time profiles and physiological data (Chapters 5-7). Diurnal profiles for algal biomass distribution (as estimated by Chla) were collected for all five
sampling sites (RCI and sites 1 - 4, see Figure 2.1). Samples were held on ice in the dark prior to processing at the laboratory.

Samples for Chla were filtered (Millipore GF/C) and stored frozen for later analysis according to the acetone extraction method and subsequent pigment analysis using the equations of Jeffrey and Humphrey (Parsons et al., 1984). After sonication and overnight acetone extraction, sample absorbance was read on a Varian spectrophotometer. Calculations of total chlorophyll a (Chla) were made according to the equations of Jeffrey and Humphrey (cited in Parsons et al., 1984). Samples for dissolved nutrient analysis were filtered through 0.45 μm cellulose nitrate membrane (Whatman). Unfiltered samples for particulate and total nutrient analysis plus all filtered samples were stored frozen (−4°C) until subsequent analysis by the Marine and Freshwater Research Laboratories, Institute for Marine Studies, Murdoch University, WA.

Diurnal depth averaged means and standard deviations for all nutrients were calculated from all data points gathered in daytime water column profiles, which included surface and bottom samples for night time samples.

### 2.8.3 Data analysis

Differences between particular depths and times of day and between seasonal nutrient profiles were tested using the non-parametric Wilcoxon's signed-ranks test for two groups, arranged as paired observations. Correlation analysis of physical and chemical parameters was performed on the means of variables for each sampling period and a correlation matrix constructed. These results, combined with data from subsequent chapters (3 - 6), are used in principal component analysis (PCA). PCA explored the relationship between environmental factors and the diurnal depth related distribution. PCA plots are presented in Appendix I. Graphical representation and curve fitting was achieved with Sigmaplot 4.0 (SPSS Inc., Chicago USA) or Microsoft EXCEL (Microsoft Corp., USA).
Figure 2.1 Location map for study sites in the upper Swan River Estuary.
A. Location of Swan River in the south-western Western Australia. B. Location of sampling Ron Courtney Island (RCI) sampling area in the upper Swan River estuary. C. Location of main study site at RCI and comparison sites in 2km stretch up and down stream. Site 1 Tonkin Overpass (at channel marker), site 2 Mid-stream below RCI, site 3 Channel marker, site 4 Mid-stream above RCI, site 5 RCI.
2.9 Results

2.9.1 Solar Insolation and Irradiance

The annual insolation levels for the Perth region are high, with maximum intensity of 33 x 10^6 J occurring during January (Figure 2.2), dropping to approximately 50% of this during mid-winter. Seasonal light attenuation curves (Figure 2.3) indicate the greatest variability at the surface during autumn and spring. The spring (increasing insolation) and autumn (declining) periods exhibit the greatest changes. Monthly attenuation (or extinction) coefficients \( k \) for the water column are presented in Figure 2.3 and indicate highest \( k \) values occurred during August, September and November of 1998, dipping slightly in October. Attenuation coefficients were fairly consistent \((1.7 \pm 0.23)\) over all other months. Calculated compensation depths, \( Z_c \) (depth at which light intensity is 10% that of surface light), show a compensation depth maximum of 2.69 (Table 2.1). Despite the shallow water (<6 m) at this site, compensation depths were between 26 and 50% of water depth over the 12 month period.

![Graph showing seasonal variation in solar radiation](image)

**Figure 2.2** Annual seasonal variation in solar radiation for Perth WA (Lat. 31°57' S, Long. 116°04' E) over a 5 year period (1995-1999). Values represent daily integrals. Data courtesy WA Bureau of Meteorology.
Figure 2.3 Seasonal irradiance and monthly attenuation coefficients for an upper Swan River site, RCI, for 1998.

A-D  Seasonal light attenuation curves for Ron Courtney Island. Calculations made according to Beers Law, $I_z = I_0 e^{-kz}$, where $I$ is irradiance at surface (0) or depth ($z$) metres. Error bars represent 3 monthly variations. E Monthly extinction coefficients for RCI, calculated using Beer-Lambert Law.

Light profiles for Ron Courtney Island indicated that the seasonal maximum mean intensity in surface waters (0.25 m) was in summer ($1402 \pm 333.4$ µEin sec$^{-1}$) with the
lowest seasonal mean surface irradiance of 558 ± 464.3 μEin sec⁻¹ recorded during winter. Attenuation coefficients, k, calculated from Beer’s Law, ranged between 1.41 and 3.58 m⁻¹ (Table 2.1). Maximum attenuation rates occurred during August to November. Minimum surface irradiances were recorded for August and September (see Figure 2.3) while minimum insolation (air reading) was measured during May and June. The compensation depth (Zwu, 10% light level) throughout the year occurred between 2 and 3 metre depth. The ratio of mixing depth (Zm, assumed to be equivalent to water depth) to euphotic depth (Zwu) was always < 6 (see Table 2.1).

Table 2.1 Seasonal light regime characteristics of Ron Courtney Island, an upper estuary site.

<table>
<thead>
<tr>
<th>Month</th>
<th>Julian day</th>
<th>z (m)</th>
<th>k (m⁻¹)</th>
<th>Zwu/Zm</th>
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<tr>
<td>February 98</td>
<td>49</td>
<td>5</td>
<td>1.62</td>
<td>2.5</td>
</tr>
<tr>
<td>March 98</td>
<td>77</td>
<td>5</td>
<td>1.56</td>
<td>2.4</td>
</tr>
<tr>
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<td>115</td>
<td>5</td>
<td>1.65</td>
<td>2.4</td>
</tr>
<tr>
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<td>145</td>
<td>5.25</td>
<td>1.41</td>
<td>2.0</td>
</tr>
<tr>
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<td>172</td>
<td>5</td>
<td>2.03</td>
<td>2.7</td>
</tr>
<tr>
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<td>204</td>
<td>5.5</td>
<td>1.92</td>
<td>3.1</td>
</tr>
<tr>
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<td>248</td>
<td>5</td>
<td>3.58</td>
<td>3.1</td>
</tr>
<tr>
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<td>5</td>
<td>3.22</td>
<td>3.2</td>
</tr>
<tr>
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<td>5</td>
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<td>3.9</td>
</tr>
<tr>
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<td>5</td>
<td>3.07</td>
<td>4.4</td>
</tr>
<tr>
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<td>362</td>
<td>5</td>
<td>2.00</td>
<td>2.9</td>
</tr>
<tr>
<td>January 99</td>
<td>5</td>
<td></td>
<td>1.65</td>
<td>2.2</td>
</tr>
</tbody>
</table>

2.9.2 Rainfall

Rainfall is seasonal with winter rain and summer drought conditions. Over the five year period from 1995 to 1999 summer (December – February) monthly rainfall means of 8 ±12.7 mm (range 0 – 45.6 mm month⁻¹) were recorded. Rainfall increased over autumn to peak in winter (June – August) with a mean monthly winter rainfall of 144 ± 24 mm (range 76 – 232 mm). Rainfall then declined gradually over spring. A boxplot (Figure
2.4) indicates that, during this 5-year period, February 1995, April 1997 and January 1999 had unusually high monthly rainfalls, whilst, in June 1999, rainfall was unusually low. During the 1998-99 seasonal study the onset of winter rains occurred just after the May sampling, with a significant rain event on May 26th (23.6 mm), following the hottest May day on record (26°C). Prior to this event, only sporadic falls of generally <10 mm were recorded. There was no rain recorded over the period January 10 - 29 (dinophyte bloom) 1996, with 1.8 mm recorded at the nearby Perth Airport on 30th January (data courtesy of Bureau of Meteorology of Western Australia).

![Rainfall Chart](image)

**Figure 2.4** Monthly rainfall averages over the five year period from 1995 to 1999 for Perth, Western Australia. Outliers marked indicate higher than usual rainfalls recorded for February 1995, March 1997 and January 1999. Data collected at Perth Airport, courtesy of Meteorological Bureau of WA.

### 2.9.3 Temperature

Water column temperatures reflect seasonal changes in air temperature (Figure 2.5). Profiles for surface (0.25 m) and 0.5 m depth increments to the bottom (4.5 – 6 m depending on tide and season) show maximum temperatures recorded during the
summer months (December, January & February, depth averaged mean 26.9 ± 0.6°C, n=33) and dropping to 14.5 ± 1.3°C (n=33) during the winter months (June, July & August). Spring and autumn average temperatures were similar (22.1 ± 3.3°C and 22.5 ± 2.4°C respectively), although they exhibited greater depth-related variation than summer and winter months.

**Figure 2.5** Maximum and minimum air temperature ranges and diurnal surface and bottom water temperatures.

A. Monthly mean and standard deviations for air temperature over a 2 year period (1996-97, Perth Airport. Data courtesy of Meteorological Bureau of WA.) Surface (B) and bottom (C) diurnal temperature differences for RCI over a 12 month period (1998 - 99). ■ day, □ night

### 2.9.4 Salinity

Salinity over an annual period (Figure 2.6) reflects the seasonal rainfall pattern with stratification evident following the onset of winter rains (May) and the establishment of stronger river flow from run-off. Monthly salinity profiles indicate strong stratification occurring from the onset of the rains in May. High mixing rates create a uniform salinity profile during July to September. By November the intrusion of the salt wedge increases surface and bottom salinities and by March the entire water column is considered totally marine.
Figure 2.6 Annual pattern of surface (0.25m) and bottom (5m) salinities at RCI, February 1998 to January 1999.
Salinities reflect recognised patterns established for the Swan River Estuary.

2.9.5 Oxygen
Temporal trends in oxygen concentration (Figure 2.7) indicate a strong discontinuity recorded in June when anoxic conditions occur below 2 m depth. By July oxygen levels had elevated to between 6 - 8 mg l\(^{-1}\). Bottom oxygen concentrations were also low (1.9 mg l\(^{-1}\)) during November and December. Peak oxygen concentrations were recorded in surface samples during April (10.8 mg l\(^{-1}\)) and July (10.8 mg l\(^{-1}\)).

2.9.6 Nutrients
Nitrate
Nitrate levels ranged between 2 μg N l\(^{-1}\) during summer and autumn (periods of low to no rainfall, see Figure 2.4) and 741 μg N l\(^{-1}\) during winter (Figure 2.8). Surface (0.25 m) and bottom (4.5 - 5 m) concentrations are similar throughout the year. The exception was June, when surface NO\(_3^-\) was 7 times bottom concentration (~700 μg NO\(_3^-\) -N l\(^{-1}\) vs ~100 μg NO\(_3^-\) -N l\(^{-1}\)). This corresponded with the onset of winter rains in May, which produced strong downstream flow resulting in lower surface salinity and a strong stratification in salinity, temperature and oxygen at about 2 m depth. The winter months of July and August had the most consistently high concentrations throughout the water column (depth averaged mean 310.2 ± 6.52 μg NO\(_3^-\) -N l\(^{-1}\), n=12), while
Figure 2.7 Seasonal day time oxygen profiles and diurnal surface and bottom comparisons at RCI site, 1998-1999.

A, B & C Annual diurnal trend in oxygen concentration and % saturation for surface (0.25m) and bottom (5m) depths at RCI, February 1998 to January 1999. Surface oxygen supersaturated during April, May, July and October. Night time surface saturation in October possibly due to wind mixing.
Figure 2.8 Annual midday nitrate profiles for RCI, February 1998 to January 1999.

Low values throughout the water column February to May. In May the onset of winter rains elevates surface levels, followed by uniform mixing throughout the water column during June – September. Levels return to minimum as fluvial flow diminishes.

summer months (January and February) were consistently low (depth averaged mean 8.6 μg ± 4.13 NO$_3$-N l$^{-1}$, n=18).

The high degree of variability in NO$_3$ concentration over the year is reflected in the large standard error value compared with the mean (114 ± 149.12 μg NO$_3$-N l$^{-1}$, n=75) across all day time depths and times. Wilcoxon’s rank sum analysis indicated no significant difference (P>0.05) in NO$_3$ concentration between surface and bottom day time or night time samples, day and night surface samples or day and night bottom samples.

**Ammonium**

Concentrations of NH$_4^+$ in surface waters (0.25 m) during the 1998-99 temporal study ranged between 4 and 95 μg NH$_4^+$-N l$^{-1}$, while bottom (5m) concentration was generally higher, ranging between 32 - 439 μg NH$_4$-N l$^{-1}$ (Figure 2.9). In general, when peaks of
NH$_4^+$ occur in bottom samples, the water column NH$_4^+$ levels are elevated between 3 m depth and the bottom (5 m), with lower concentrations in shallower (0.25 – 2 m) depths. The highest concentrations were in bottom samples during June, when

![NH$_4^+$ Concentration vs. Depth and Month](image)

**Figure 2.9** Annual midday ammonium profiles for RCI, February 1998 to January 1999. Highest values at mid to bottom depths coincided with onset of first rains in May.

stratification occurred and bottom salinity was high (24 ppt) and dissolved oxygen was low (0 mg l$^{-1}$). Wilcoxon’s signed-ranks test showed a significant difference (P<0.005) between NH$_4^+$ concentration in day time surface and bottom samples over the 12 month study period. Night time surface and bottom samples and night versus day surface and bottom samples were not significantly different (P>0.05). There was no significant difference (P<0.005, n=18) for ambient NH$_4^+$ concentration profiles between summer and winter during the 1998-99 period.

**Urea**

Urea concentration was used as an indicator of organic nitrogen levels in this study. Over the 12-month study period the diurnal urea concentration range throughout the
water column was 14.8 – 117.74 μg urea N l\(^{-1}\) (Figure 2.10). Diurnal depth-averaged mean for the year was 40.1 ± 14.9 μg urea N l\(^{-1}\) (n=62). Urea profiles indicate that concentration is uniform throughout the water column during the April – August period.

![Image](image-url)

**Figure 2.10** Annual midday urea profiles for RCI, February 1998 to January 1999.

(i.e. from mid-autumn through winter) with levels ranging between 14.8 μg urea-N l\(^{-1}\) and 42.8 μg urea-N l\(^{-1}\) (mean 28.8 ± 8.3 μg urea-N l\(^{-1}\), n=27). During September to November the surface levels of urea were lower than bottom, whilst highest levels were recorded in surface water during December and January (62 - 64 μg N-urea l\(^{-1}\)), although overall the summer levels are higher throughout the water column than during other seasons. Wilcoxon's signed-ranks tests showed day time surface and bottom urea concentrations were significantly different (P<0.05), with no significant difference (P>0.05) between depths at night or between night and day for the same depths.

Although urea concentrations showed little variation over the annual period 1998 - 1999, the relative proportion of urea to total available nitrogen indicated urea
constituted the major nitrogen source (104% - 287% total DIN) during summer for both surface and bottom depths (Figure 2.11), dropping to 4% during winter.

There was a significant difference between surface and bottom TKN values over a 12 month temporal period (P>0.05, n=22). A correlation analysis between TKN and Chla for both surface and bottom samples over the temporal study indicated no correlation (P>0.05, n=9). Organic nitrogen levels calculated as the difference between Kjeldahl-N and NH$_4^+$, ranged between 253.5 - 1367 μg N$_{org}$ l$^{-1}$, with an annual day time mean over all depths of 778.8 ± 229.27 μg N$_{org}$ l$^{-1}$.

Figure 2.11 Nitrogen sources as % of total ambient nitrogen (NO$_3^-$ + NH$_4^+$ + urea) for day (A) and night (B).
Figure 2.12 Monthly nitrogen species concentrations for February 1998 – January 1999 at RCI, upper Swan River Estuary. NO$_3^-$ (A), NH$_4^+$ (B), urea (C), organic N (D), Kjeldahl N (E) and Total N (F) day time surface and bottom concentrations.
Phosphorus

Total P
The range of concentration of total P (TP) over the twelve months February 1998 to January 1999 was 38 - 189 μg P l⁻¹ (n=83). Diurnal depth-averaged mean for TP for this period was 84.0 ± 27.4 μg P l⁻¹. Monthly profiles of day time TP concentrations are presented in Figure 2.13A). While, graphically, for 8 of the 12 monthly sampling period bottom (~5m) TP ≥ surface (0.25m) TP concentrations, statistically there is no significant difference (P>0.05) in daytime TP concentrations between these two depths. No significant difference was found between day and night surface or bottom TP levels (P>0.05) although surface and bottom TP concentrations were found to be significantly different (P<0.025).

![Graph illustrating annual variation in total P for February 1998 – January 1999 at RCI](image)

**Figure 2.13** Annual variation in total P for February 1998 – January 1999 at RCI
Figure 2.14 Annual variation in ortho-P, organic P and total P for February 1998 - January 1999 at RCI.
Ortho-P
The range of concentration of P as ortho-P over the twelve months February 1998 to January 1999 was 3 – 58 µg P l⁻¹ (n=94). Monthly profiles of day time ortho-P concentrations are presented in Figure 2.13B. Diurnal depth averaged mean for ortho-P for this period was 24.2 ± 15.20 µg P l⁻¹. Day time bottom concentrations were significantly greater (P<0.005) than surface concentrations of ortho-P. There was a significant difference (P<0.01) between day and night surface concentrations, while day and night bottom samples were not significantly different (P>0.05).

Organic P
This nutrient was calculated from subtracting the ortho-P value from the total P value determined in analyses above. Organic P levels form the larger component of the total phosphorus levels in the water column.

Chlorophyll a
In situ intensive monitoring of chlorophyll levels at surface and bottom during spring 1995 (November) and summer 1996 (January) show a clear diurnal pattern of rise and fall of chlorophyll concentration in surface waters, peaking during the day (Figure 2.7 D & H). Coinciding with this is an alternating cycle of peaks and troughs in bottom chlorophyll concentration during summer but not during spring, where bottom levels remain relatively constant.

2.9.7 Short-term (within bloom) studies
All short-term studies were conducted on blooms typical of recognised seasonal blooms. The environmental parameters (salinity, oxygen, temperature and nutrients) during these times were within previously reported ranges (John, 1987; Thompson, 2001).

The spring weekly monitoring captured the end of a chlorophyte bloom dominated (90 %) by *Chlamydomonas globosa*, with surface chlorophyll levels exceeding 50 µg Chla l⁻¹ on November 21 (day 325, Figure 2.2 D). Chla remained relatively constant at 3 m depth for the spring intensive monitoring period, while 0.3 m levels exhibited daily variations that may reflect small vertical movement of phytoplankton between surface
waters and 1 – 2 m depth. Weekly monitoring of a typical summer mixed dinophyte bloom over a four week period from January 10 to 24 showed Chla levels ranging between 4.86 ± 0.4 and 44 ± 2.4 over the 3 week period. Barge data from a similar mixed dinophyte bloom 2 weeks later, (February 22-28, 1996), showed Chla levels exhibited marked diurnal patterns, with evidence of decreases in surface Chla at night and an associated rise in bottom Chla. These corresponded with increases in cell dinophyte numbers for night time bottom samples (see Chapter 3).

Figure 2.15 1998-99 temporal diurnal Chla profiles for RCI
Figure 2.16 Annual diurnal chlorophyll for surface and bottom depths at RCI, February 1998 – January 1999.

Depth-averaged temperatures over the November 1995 study period (22.8 ± 1.04°C, n=17) fall within ranges previously reported (Hodgkin, 1987; Thompson, 2001). Temperature and oxygen levels during spring 1995 showed diurnal variation with daytime elevation of surface values and a return to uniform values at night (Figure 2.2 A & C). The temperatures recorded for surface samples during the January 1996 dinophyte bloom study were higher than average (29.1°C at 0.25 m on 10th January 1996) with a depth-averaged mean of 28.5 ± 0.40°C (n=12). The second week of this bloom study was particularly hot with a maximum air temperature recorded at Perth Airport of 40.2°C. Two weeks later surface temperatures had cooled to 25.1°C with depth-averaged values of 25.4 ± 0.52°C. The intensive 24 h sampling for summer shows evidence of a thermal inversion forming on two occasions during the night. In general, bottom temperatures remained steady, rising 1°C over the 5-day period, while surface temperatures varied diurnally, by up to 2°C, in response to diurnally fluctuating air temperatures.

November 1995 oxygen profiles for both weeks (Nov 14 & 21) showed supersaturation (10.35 mg l⁻¹ or 129% saturation) extending from surface to 2 m depth. Below this oxygen levels decreased gradually to approximately 80% saturation at 5m depth. Oxygen profiles taken during the January 1996 dinophyte bloom study indicated super-
saturating concentrations (103% and 107.5%) at 0 - 0.5 m depths, with 98% saturation at 1 m. A rapid drop over the next 0.5 m to 65.5% indicates a discontinuity layer. Below this the O₂ gradient dropped gradually to approximately 34% saturation near the bottom. Diurnal changes in oxygen levels (% saturation) are evident in both surface and bottom samples with greater ranges in surface than bottom. During the May 1996 study oxygen was supersaturated in the top 1m with a strong decreasing gradient to 3.4 mg l⁻¹ (44% saturation) at 5 m depth, a profile similar to that in 1998.

Surface and bottom salinity levels during the spring (November 1995) intensive monitoring were uniformly low (< 5 ppt). During this period the bottom salinity was greater than surface salinity, indicating the onset of the salt wedge intrusion into this region of the river. During intensive monitoring salinity levels were consistently low for surface (4.2 ± 0.69 ppt, n=264) and bottom (3.9 ± 0.39, n=264) depths over three diurnal periods (Julian day 325 - 328, 1995, Figure 2.16 B). While no rain fell during this study period, over the previous 2 weeks 25.4 mm was recorded. During the January 1996 bloom study, salinity levels ranged between 25 ppt at the surface to 32 ppt at 5 m. No rainfall was recorded in during this month. The 5 day intensive monitoring period during summer showed that a salinity gradient exists, with salinity at 3m up to 3 ppt higher than at 0.5 m (Figure 2.16F). Bottom salinities are stable due to strong development of a marine intrusion (salt wedge) at this depth by this time of the year. It is suggested that variability in surface salinity may be related to tidal fluctuations. May salinities were still high at 26.4 (0.1 m) – 32.9 (4.5 m). The 26.4 mm rainfall recorded in the 2 weeks prior to this sampling period could explain the drop in surface salinity.

2.10 Ordination analysis

Ordination analysis revealed a grouping based on depth, with those samples at the top right hand side of the ordination tending to be the day time bottom samples, while those on the bottom left were day time surface samples (Figure 2.17). All night time samples were positioned mid-way between these two groups, with surface and bottom samples overlapping. Based on Pearson and Kendall correlations with ordination axes (n=46) the environmental variables most strongly related to phytoplankton distribution data were temperature ($r^2 = 0.61$), saturated oxygen ($r^2 = 0.64$), orthoP ($r^2 = 0.58$)
Figure 2.17 Comparison of diurnal fluctuations of temperature, salinity oxygen and Chla at surface (0.3m) and bottom (5m) during spring and summer at RCI site. A – D spring Chlorophyte bloom (Nov 21-24, 1995). E – F summer dinophyte bloom (Feb 22 1996)
and ammonium ($r^2 = 0.52$) (see Appendix I). PCA plots and side scatter plots are presented in Appendix I.

### 2.11 Discussion

Whilst a long-term data set for physical and chemical parameters have been collected, there has been little or no information on diurnal-related changes that may enhance the growth of one or more phytoplankton species. This study concentrates on changes in local factors at an upper estuary site to investigate parameters influencing the local development and maintenance of algal biomass. The monitoring of physical and chemical parameters in this study serves to ascertain that conditions over the duration of this research fall within the recognised pattern of environmental conditions established for the Swan River Estuary. This provided justification to extend the findings of this study to other times and other areas of the estuary. It provides diurnal information over an annual cycle to enable a statistical investigation into the major factors influencing local phytoplankton distribution for an upper estuary site. This is deemed significant for the initiation of the localised ephemeral seasonal blooms typical of the succession described for this river.

A number of factors have been implicated in the control of phytoplankton biomass development. Jordan et al. (1991) showed that for the Rhode River, a sub-estuary of Chesapeake Bay, chlorophyll concentrations in the spring are controlled by riverine nitrate inputs, while summer concentrations are controlled by the regeneration of inorganic N from organic matter produced during spring. Gibbs and Vant (1997) working in Beatrix Bay, New Zealand, found phytoplankton growth to be light limited during winter and N, but not P, limited during summer. Atmospheric deposition of nitrogen and the increased nutrients entering estuaries following catchment rain events can significantly influence primary and biomass accumulation (Mallin et al., 1993; Paerl et al., 1990). Mallin et al. (1993) concluded that the magnitude of estuarine primary production and the periodicity of algal blooms can be related to variations in the upper watershed rainfall and its subsequent regulation of down-stream river flow.

Historically, nitrate levels in the Swan River have been reported to vary seasonally, with high values ($50 - 300 \mu g l^{-1}$) during winter and summer values generally negligible ($\leq 20$...
(Jack 1987). For 1993/4 Douglas reports summer concentrations of NO$_3$ of between 2.5 and 71 µg N l$^{-1}$ and bottom concentrations between 2.5 and 43 µg N l$^{-1}$ at Ron Courtney Island, an upper estuary site. Thompson and Hosja (1996), working at the same site in 1994, found a significant correlation ($r^2 = 0.69$) between surface nitrate levels and the total rainfall for the preceding week. The timing and magnitude of physical forcing events, particularly rainfall, appear to be the crucial factors influencing summer and autumn bloom events in this system (Thompson, 1998). The occurrence of the first major rain event for the year (May) was reflected by a peak in the surface NO$_3$ concentrations in the subsequent (June) sampling period. This study shows that, following the onset of winter rains in May 1998, there was a substantial rise in surface (0.25 m) NO$_3$ concentration from 4 to 741 µg NO$_3$-N l$^{-1}$ (i.e 53 µM compared with the reported rise to 70 µM in May-June 1994, Thompson and Hosja, 1996). There was a concurrent rise in bottom (4.5m) NH$_4$+ concentration from 65 to 439 µg NH$_4$+-N l$^{-1}$. No effect on urea concentration was evident. Thompson and Hosja (1996) and Douglas et al. (1996) identify a strong relationship between rainfall and phytoplankton biomass that is suggested to be due to a reduction in (potential) N-limitation.

While most studies recognise the importance of atmospheric input to nitrate levels, pelagic nitrification has been recognised as the major spring and summer source of NO$_3$ (55% annual input) in the Narragansett Bay and Providence River estuaries, resulting in increased oxygen demand near sediments (Berounsky and Nixon, 1993). High oxygen levels in surface waters during spring and summer are common and are the consequence of phytoplankton photosynthesis and wind-induced mixing (Salmon, 1996). Oxygen profiles in the upper estuary have indicated that, when the salt wedge is present, stratification induces anoxic conditions in the deeper water layers (Douglas et al., 1996; Chan and Hamilton, 2001), perhaps due to increased nitrification levels. Stratification in June, when anoxic conditions prevailed below 2 m depth, coincided with elevations in bottom NH$_4$+, organic P and total P concentrations, in keeping with reported anoxic release of these nutrients from sediments under anaerobic conditions (Douglas et al., 1996).

Ammonium concentrations in natural waters vary from <10 µg NH$_4$+-N l$^{-1}$ to >30 mg N l$^{-1}$ in some waste waters (Clesceri et al., 1989). Measurement can be particularly
sensitive to such facts as contamination by atmospheric ammonium or rapid changes in 
$\text{NH}_4^+$ due to chemical transition of organic N compounds such as urea to ammonium 
ions. $\text{NH}_4^+$ has been reported to occur throughout the year in the upper Swan River 
Estuary (Jack, 1987), although levels reported for summer ($40 - 150 \mu \text{g NH}_4^+-\text{N l}^{-1}$) are 
generally lower than those for winter ($100 - 500 \mu \text{g NH}_4^+-\text{N l}^{-1}$). During the 1993/4 
study at RCl, $\text{NH}_4^+$ ranges for summer surface were $2.5 - 19 \mu \text{g NH}_4^+-\text{N l}^{-1}$ and $2.5 - 
163 \mu \text{g NH}_4^+-\text{N l}^{-1}$ for bottom depths (4-5 m). During 1998 (this study) the range for 
summer was within previously reported ranges.

During the individual bloom studies (1995 & 1996) the concentrations of $\text{NH}_4^+$ were 
equal to or in excess of $\text{NO}_3^-$ concentration (ratio of $\text{NH}_4^+: \text{NO}_3^-$ ranged from 1 to 21). 
Nitrate levels throughout the water column were uniformly higher during the spring 
sampling times than the summer and autumn sampling times. During the summer 
bloom the highest nitrate levels were detected in day time bottom samples. By autumn, 
nitrate was depleted ($<5 \mu \text{g NO}_3^--\text{N l}^{-1}$) and ammonium was present in lower 
concentrations ($10 - 13 \mu \text{g NH}_4^+-\text{N l}^{-1}$) throughout the water column than for summer. 
As with previous studies (Douglas et al., 1996; Jack, 1987; John, 1984), the highest 
concentrations of ammonium occurred at depth. Shallow ground water has been a 
suggested source of $\text{NH}_4^+$ (Linderfelt and Turner, 2001). Douglas et al. (1996) and 
references cited therein report increased $\text{NH}_4^+$ concentrations in bottom water 
associated with anoxic conditions and the presence of the salt wedge. Anoxic release 
and density driven displacement of pore-water have been suggested as mechanisms for 
$\text{NH}_4^+$ accumulation in the deeper water. This would explain the high negative 
correlation between $\text{NH}_4^+$ and PAR ($r^2 = -0.51$) and oxygen ($r^2 = -0.64$). The higher 
nitrate concentrations that were measured at depth during week three of the dinophyte 
bloom may be caused by nitrification in the overlying aerobic bottom water (2.6 - 3.1 
mg DO l$^{-1}$) of the $\text{NH}_4^+$ released from the sediments under anaerobic conditions.

High ammonium releases from sediments, which may be enhanced by the activity of 
benthic infauna (Pennifold and Davis, 2001), have been reported for the upper Swan 
River Estuary (up to 137 mg $\text{NH}_4^+$ m$^{-2}$ d$^{-1}$, Douglas et al., 1997; Pennifold and Davis, 
2001) and Port Phillip Bay (up to 216 mg $\text{NH}_4^+$ m$^{-2}$ d$^{-1}$, Berelson et al., 1998). Their 
estimates, although based on extrapolation from daily to annual fluxes, indicate that
benthic nutrient release may provide a significant and important nutrient source in this ecosystem. Diurnal profiles for NH$_4^+$ indicated elevated concentrations at depth during summer and autumn.

While much of the DON released from phytoplankton results from cell death and lysis, or from grazing, active DON excretion from phytoplankton itself has been measured up to 13.3 nmol N l$^{-1}$ h$^{-1}$ (Pujo-Pay et al., 1997) and contributes towards the total DON pool. Bronk and Ward (1999) found that 32 ± 17% of nitrogen incorporated into particulate nitrogen (PN) as NH$_4^+$ or NO$_3^-$ was released as DON in a variety of environments, from relatively eutrophic estuaries to oligotrophic oceans. They suggest that variable DON production rates are caused by differences in grazing pressure and the state of physiological stress of the phytoplankton. Urea was monitored in this study as an indication of how much DON was available for phytoplankton growth. Annual profiles show urea levels to remain fairly uniform for depth and time with annual mean concentrations of 28.8 ± 8.3 μg urea-N l$^{-1}$ (n=27). Fluctuations in NO$_3^-$ and NH$_4^+$ levels over the seasons result in urea constituting 107 - 287% of total DIN during summer, and decreasing to only 4% total DIN during winter.

It is not unusual for lakes and rivers to be deficient in biologically available phosphorus (Reynolds, 1997). Where phosphorus in the form of orthophosphate ions (HPO$_4^{2-}$, H$_2$PO$_4^-$) and organic compounds is available, phytoplankton have evolved elaborate mechanisms for the sequestering of this from waters where the concentrations are often below the limits of analytical detection (<0.3 μg P l$^{-1}$). For this latter reason total phosphorus (TP) is often used to described the status of P in systems under investigation. Under oxic conditions sediments may act both as a sink (Bostrom and Petterson, 1982) or a source (Bortelson, 1970; Spear, 1970; Sridharan and Lee, 1974) for phosphorus. Under anoxic conditions sediments act as a source for phosphorus. For 1993/4 Douglas (1996) reports a summer PO$_4$ surface range of 1 - 22 μg N l$^{-1}$ and bottom range of 10 - 61 μg N l$^{-1}$. During winter PO$_4$ concentrations range between 3 and 50 μg N l$^{-1}$ and become quite variable (depth averaged mean 20 ± 15.0 μg N l$^{-1}$).

Regeneration rates for phosphorus ranging between 15 and 205 ng P l$^{-1}$ h$^{-1}$ have been reported in lake planktonic communities. Of this 77% was regeneration in the <40 μm
fraction, indicating that micro-organisms were a major factor in P regeneration (Hudson and Taylor, 1996). Working in lotic waters (rivers) Basu and Pick (1996) found a positive relationship between Chla and TP ($r^2 = 0.76$). No correlation was found between TKN and chlorophyll $a$ over an annual cycle ($P > 0.05, n=9$). This suggests that the nitrogen content of the particulate is not predominantly of living phytoplankton origin. Investigation into the levels of microbial biomass and its importance in trophic transfer should be made.

Light availability can limit phytoplankton growth in turbid, nutrient rich estuaries (Irigoin and Castel, 1997). When the ratio of mixing depth ($Z_m$) to eutrophic depth ($Z_e$) is $< 6$, then net primary production is possible and a bloom can be initiated (Kromkamp and Peene, 1995). This ratio was always below 6 during the 1998-99 temporal study. For values greater than 6 to be obtained at the RCI site, the euphotic zone would need to be limited to a depth of only 0.8 m, a situation which rarely, if ever occurs. Maximum attenuation rates during August to November are thought to be a result of increased turbidity due to a combination of rain-induced water flow and an increase in picoplankton density at this time of year. In terms of light available for photosynthesis, the ratio of mixing depth to euphotic depth is high during the winter period (June to September ranges between 2.7 and 3.1) and peaks during October and November (3.9 and 4.4 respectively), indicating reduced light penetration, possibly due to turbulence. Summer and autumn are characterised by lower values (2 – 2.5) which indicate a higher light penetration level.

Seasonal salinity shifts have been implicated as a master factor in determining the distribution of the diverse biota in the Swan River Estuary (Hodgkin and Vicker, 1987; John, 1994). Salinity profiles were relatively uniform with depth, with the exception of June, when a lowering of surface salinity following the onset of seasonal rains produced a distinct halocline. Coincidental with this drop in surface salinity was a peak in surface $\text{NO}_3^-$ concentration and the development of an anoxic layer below 2 m depth. By July river flow had been established and salinity had dropped to a uniform 7 ppt throughout the water depth.

When the abundance of nitrogen relative to other major nutrients, carbon and phosphorus, in the environment is less than the Redfield atomic ratio (C:N:P of 106 : 16
then nitrogen is considered to be potentially limiting to phytoplankton growth (Redfield, 1958). Changing stoichiometric ratios of nutrients, in their case Si:N ratio, in rivers is hypothesised by Officer and Ryder (1980) to potentially exacerbate eutrophication by reducing diatom growth in favour of noxious flagellate growth. This has been demonstrated for a number of large world rivers and for coastal regions under riverine influence (Justić, Rabalais and Turner, 1995; Justić, Rabalais, Turner and Dortch, 1995; Smayda, 1990).

Phosphorus limitation, based on N:P ratios, has previously been demonstrated for certain times of the year in the Swan River Estuary (Thompson and Hosja, 1996) Using bioassay techniques Thompson and Hosja (1996) have linked increased N input as NO$_3^-$ N from rainfall during spring and summer rain events with a transition from N to a marginal P-limitation. The 1998 period N : P ratio for the RCI site support this in terms of available N and P, with high winter Redfield rates (up to 30:1) indicating very high proportion of N to P available, thus making P to be the limiting nutrient. Monthly N : P ratios for the upper Swan during 1998 - 1999 indicated much lower than the Redfield ratio of 16 for N : P was evident for surface water in all but two months (mean N : P ratio 11.9 ± 2.81, n=10) and for bottom water (mean N : P ratio 11.1 ± 4.37, n=11) in all but one month over an annual cycle, indicating an environmental situation of nitrogen limitation. The months in which nitrogen was not limiting were June, July (surface) and August (bottom), times of high rainfall (see Figure 2.4) and non-stratification in the water column.

Supply (or loading) ratios of biologically available nitrogen and phosphorus, N : P, have often been suggested as the major determinants for the presence or absence of N$_2$ fixing cyanobacteria in aquatic environments. For example, the unprecedented bloom of *Aphanizomenon ovalisporum* that occurred in Lake Kinneret, Israel, from mid-September through October 1994 derived most of the N required for growth directly or indirectly from DON rather than from N$_2$ fixation (Berman, 2001). This would suggest that factors other than apparent low N : P ratios were important in causing the outgrowth of the cyanobacteria. Increasing evidence that some components of the dissolved organic nitrogen (DON) pool can play an active role in supplying N nutrition, either directly or indirectly to phytoplankton implies, that this source of N must be
considered in any attempt to apply the N : P ratio approach to predict or explain phytoplankton population composition.

2.11.1 What factors are important for localised ephemeral blooms?

Previous findings ascribe phytoplankton succession in the Swan River Estuary to be primarily controlled by salinity, rainfall and nutrient (NO₃) availability (Hodgkin, 1987; Thompson, 2001; Twomey and John, 2001). PCA analysis of data from this study implicates a different combination of physico-chemical factors regulating diurnal distribution of phytoplankton classes at one locale over an annual period. The principal influencing factors for depth and diurnal related phytoplankton distribution over an annual cycle, at one site, are temperature ($r^2 = 0.51$, axis 1), oxygen saturation ($r^2 = 0.64$, axis 2), orthoP ($r^2 = 0.58$, axis 2) and NH₄⁺ concentration ($r^2 = 0.52$, axis 2) (Appendix I, PCA analysis). These factors, along with physiological factors (see Chapter 5), control phytoplankton species composition and distribution for ephemeral blooms typical of those recognised in seasonal succession patterns for phytoplankton in the upper Swan River Estuary. While correlation analysis indicated that ambient urea concentration was not highly significant ($r^2<0.50$) in terms of its relationship with Chla distribution (Appendix I), it does represent a relatively constant nitrogen source throughout the year that may enable phytoplankton growth to continue when inorganic sources are depleted (see Chapter 5).

2.12 Conclusions

Identification of the factors limiting phytoplankton growth and productivity in estuaries is crucial to the development of effective management strategies for the protection of the health of these systems. The scale at which these factors are investigated influences the results obtained. This study shows that focussing on local blooms enables a finer resolution of environmental factors regulating diurnal phytoplankton biomass distribution. Of greatest influence to the local development of phytoplankton biomass in the upper Swan River Estuary are temperature, and the concentrations of orthoP, oxygen and ammonium. This is a region that has been recognised as having a higher eutrophic influence than lower regions. Important information is gained from analysing the physico-chemical regulatory factors in whole estuary or river systems. Knowledge
of the seasonal characteristics enable recognition of unseasonal changes to the regulatory factors that can, and have, led to extensive HABs affecting large tracts of the whole estuary.

Information on a more local scale enables finer resolutions of factors regulating the smaller and more common ephemeral blooms that occur as part of the natural seasonal cycle. Studies such as these may provide insight into management issues that can be tackled on a local scale.

2.13 References


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CHAPTER 3: Species composition and distribution patterns for phytoplankton in the upper Swan River Estuary

3.1 Abstract

This study correlated temporal and depth differences in phytoplankton species distribution with various physical and chemical parameters in the upper Swan River Estuary with the aim of identifying environmental or physiological factors that may enhance one species' competitive advantage over others. Chlorophyll fractionation analysis indicated a year-round dominance of nano-phytoplankton. Species diversity was highest during autumn, a time of transition between the marine tidally-dominated summer period and the high flow fresh period of the winter wet. A higher proportion of small species, such as Pyramimonas and Cryptophyte species, occurred during summer 1998 and 1999 at a time when previous studies indicate dominance by dinophytes. The principal environmental factors related to phytoplankton distribution at one site over an annual period were found to be saturated oxygen \((r^2 = 0.64)\), ortho phosphate \((r^2 = 0.58)\) and ammonium \((r^2 = 0.52)\). Species distribution analysis over the course of several blooms showed diurnal vertical migration occurred during a period of negligible allochthonous nitrogen input to the system. This behaviour was an apparent and varying response to depleting \(NH_4^+\) in upper water levels. It provides evidence of the importance that access to alternative nitrogen sources has for species success. Accessibility to partitioned resources also plays an important role in the successful growth of motile forms during periods of low nutrient input. An understanding of species depth distribution patterns related to partitioned environmental factors may provide better predictive ability in the management of aquatic environments in general.

3.2 Introduction

Phytoplankton communities comprise a dynamic, highly diverse multi-species mixture exhibiting rapid successional shifts in response to environmental changes (Gallegos, 1992; Gilbert et al., 1995; Marshall and Nesius, 1996). The diversity, dynamics, succession and distribution of phytoplankton populations are continually changing in response to a complex interplay of physical, chemical and biological factors in their
environment (Hallegraeff and Reid, 1986; Smayda, 1980) and the community composition is a result of a complex interaction of structuring factors exhibiting changing relative strengths (Vanni and Terme, 1990).

The influence that any specific factor will have on succession is dependent on the degree to which the population can adapt physiologically to changes in the factor. A phytoplankton bloom may start from a specific point (see Figure 3.1A) and spread to a wider area (Figure 3.1B). This bloom 'hot-spot' may be related to specific combinations of physical and chemical parameters and species composition at particular depths. However, single factor regulation is not usual in the natural environment except under

![Figure 3.1 A](image1)

![Figure 3.1 B](image2)

**Figure 3.1** Aerial imagery of *Akashiwo sanguinea* bloom at Ron Courtney Island (RCI) site, upper Swan River Estuary.
Photos taken approx 2hrs apart and show initial localised bloom development (A) and subsequent wider area dispersal (B). Arrows indicate regions of high surface chlorophyll indicative of bloom densities. The wake of a motorboat (w, see A) and the darker tracks of boats cutting through the bloom (t, see B) are evident. (Aerial imagery courtesy of P Jernakoff)
particular circumstances, since *in situ* all these factors exert a collective and simultaneous influence on phytoplankton species growth and abundance (Vanni and Temte, 1990).

### 3.3 Swan River Phytoplankton

From early in Perth’s history there have been reports of nuisance algal blooms occurring in the Swan River (Hodgkin and Vicker, 1987). Although these early reports were primarily of macroalgal accumulations, reference to eutrophication due to nutrient pollution has been made since as early as 1912, when sewage was first discharged into the Swan River from a treatment plant in the Burswood area, upstream of the causeway (see Figure 1.1). Despite the problems that algal blooms can cause, studies to identify the microalgal community and determine factors controlling its growth and distribution in the Swan River did not begin until the work of John in the 1980s (John, 1983 a, b, 1984).

Species information for the Swan River estuary has established a recognised pattern of bloom succession (John, 1987; Thompson, 1998) within the system that is influenced by hydrological factors. Chlorophyte-dominated blooms occur in the upper estuary during early spring, giving way to mixed dinoflagyte- and cryptophyte-dominated blooms through the summer and autumn periods (Figure 3.2). The influence of allogenic factors in the regulation of successional changes in the Swan River has indicated the strong influence of salinity, temperature and rainfall (Hodgkin, 1987; John, 1987). Seasonal variation in rainfall and its subsequent effect on the spatial distribution of salinity has been shown to influence the distribution and succession of phytoplankton species (John, 1984 & 1987; Chan and Hamilton, 2001; Twomey and John, 2001). Reports of shifts from diatom-dominated to dinoflagellate-dominated phytoplankton communities have not been substantiated by recent historical (1980-81) and present-day (1994-95) comparisons of the Swan River phytoplankton community (Twomey and John, 2001), although they do not dismiss anecdotal evidence for this.

The annual pattern of phytoplankton species succession has also been linked to nutrient availability (Thompson, 2001) although Twomey and John (2001) concluded that nutrient levels were not significantly different from those recorded in the 1980s since
within- and between-year variations were greater than long-term shifts in ambient concentrations. Even so, the largest recorded harmful algal bloom (HAB) to date in the Swan River caused by an hepatotoxic cyanobacterium necessitated closure of the river to all use for a length of 50km over a 12 day period (John, 2000). Increased public awareness of the issue of eutrophication and HABs, apparent increasing intensity of blooms, concern over anoxia in the bottom waters and the occurrence of toxic blooms have all lead to increased efforts to manage the Swan River (Thompson and Hosja 1996).

Phytoplankton species succession is of major significance to phytoplankton dynamics and the coupling of the phytoplankton community to higher trophic levels (Smayda, 1980). As phytoplankton community composition is the result of a complex interaction of structuring factors exhibiting changing relative strengths (Vanni and Temte, 1990) the triggers for bloom development may be a combination of very localised factors. Factors interacting to enable point source biomass development (see Figure 3.2), where certain species out-compete others, are not fully understood and may be different for each aquatic system.

Routine monitoring of the species composition and biomass of phytoplankton in the Swan River estuary is made by Government agencies from day-time depth-integrated samples taken at various points along the river on a weekly or biweekly basis. No studies to date have investigated depth and diurnal related differences in phytoplankton species distribution for this system. The relative contribution of different size classes of phytoplankton to the overall species composition has not been analysed. Species composition and distributional changes over the course of individual blooms have not been monitored nor related to concurrent changes in environmental factors to try to ascertain the factors influencing the maintenance and decline of such blooms.

Knowledge of species composition changes with depth, when related to other environmental factors measured over the same time and position framework, may help in better understanding these processes. This information, when linked with physical and chemical profiles, may provide insight into species transitions and successful algal growth under different environmental situations. It may provide an insight into smaller
time and spatial scale factors influencing algal growth in the Swan River Estuary that may be applicable to other aquatic systems.

3.4 Objectives

1. To investigate diurnal and depth related species distribution at Ron Courtney Island (RCI) site, as representative of the upper Swan River Estuary environment.

2. To determine species composition and distribution patterns over the course of specific bloom events. At shorter timescales compositional shifts and the occurrence of such factors as vertical migration may influence bloom development by favouring certain species to out-compete through their ability to access partitioned resources.

3. To correlate these data with the findings of Chapter 2 and explore the link between physico-chemical parameters and diurnal species distribution. This may help understand the development of bloom ‘hot-spots’.

3.5 Materials and Methods

3.5.1 Field work

Opportunistic sampling of specific blooms was made at various sites in the upper Swan River Estuary as they occurred. These included Success Hill, Sandringham and Ron Courtney Island as shown in Figure 2.1. A monthly monitoring programme was undertaken at the Ron Courtney Island site, a deeper site (6 m depth) typical of deeper pockets found in upper regions of the Swan River.

Chlorophyta- (spring, November 1995 & 1997), mixed dinophyta- (summer, January 1996) and cryptophyta- (autumn, May 1996) dominated bloom events were targeted at Ron Courtney Island (RCI, ~5 m), a site representative of the deeper pockets of the upper Swan River estuary. Three depths (surface - 0.25 m, mid - 2.5 m and bottom - 4.5 m) were sampled. During the spring and summer periods, samples were taken at three times (approximately 0900 h - am, 1400 h - pm and 2400 h - night) over a diurnal period. During autumn there were two sampling times (1200 h - noon and 0300 - 0400 h - night) over a diurnal period. Triplicate water samples were collected at each
sampling period using a purpose-built 6-litre horizontal water sampler designed to
collect from a narrow (15 cm) depth range (Rippingale, pers. comm.).

During 1998-1999 monthly surface and bottom samples were collected at noon and
night for species enumeration and identification using a peristaltic pump and wide-bore
tygon tubing as described in Chapter 2. Physical profiles (surface and 1 m - depth
increments) for temperature, salinity oxygen, light (noon) and chlorophyll a (Chla),
were also made at this time, at the RCI site and sites approximately 1 km above and
below this (see Figure 2.1). This was to verify that trends seen were consistent within
this region of the river and not merely artefacts of patchiness or advection. In addition,
samples for nutrient analyses were also collected in the same manner (see Chapter 2)
from the RCI site.

3.5.2 Laboratory work

Species identification and enumeration were made from Lugols-preserved samples. It is
generally desirable to have an uncertainty of ≤ 10%, which can be achieved by counting
a minimum of 400 cells. Uncertainty (error margin) is indirectly proportional to the
number of cells counted and is calculated by \( \frac{1}{\sqrt{n}} \times 100\% \) where \( n \) is the total number of
cells counted. Species abundance (no. cells ml\(^{-1}\)) was calculated. To minimise
uncertainty, 20 fields of view or 500 individuals (at 380x or 240x magnification) were
counted, using the Utermöhl (1958) technique and a Leitz Inverted microscope.
Photomicrographs were taken for identification purposes.

Size fractionation of chlorophyll was determined from the initial GF/C filtered
(nominal pore size 1.2 µm), 20 µm and 300 µm screened samples used in the grazing
experiments (see Chapter 7) with chlorophyll analyses performed as described (see
Chapter 2).

3.5.3 Analysis

Comparison of seasonal chlorophyll data was made using the Wilcoxon Signed-Ranks
test for two groups, arranged in pairs (Sokal & Rohlf, 1969).

Diversity indices (\( H' \)) were calculated according to Shannon-Weaver index:
\[ H' = - \sum_{i=1}^{k} p_i \log p_i \]

where \( k \) is the number of species, \( p_i \) is the proportion of the observations found in category \( i \). Maximum possible diversity (\( H'_{\text{max}} \)) for \( k \) species is

\[ H'_{\text{max}} = \log k \]

and Pielou's evenness (\( J' \)), or the relative diversity is

\[ J' = \frac{H'}{H'_{\text{max}}} \]

Species composition was analysed and related to physico-chemical parameters using multivariate analysis techniques (PC-Ord vers. 3.18). Canonical correspondence analysis was used to interpret species ordination with the following environmental variables presented in Chapter 2: Chla, temperature, salinity, dissolved oxygen, PAR, orthoP, organic P, total P (TP), NO\textsubscript{3}\textsuperscript{−}, NH\textsubscript{4}\textsuperscript{+}, urea, Kjeldahl N (KN), total N (TN) and NiP ratio. Results are presented in the form of PCA plots (see Appendix I).

### 3.6 Results

Results of species composition analysis has been sectioned into annual trends which can be compared with previously reported trends in species composition for this section of the estuary, and species compositional changes observed over the course of individual blooms. A list of most common species occurring over the 1998-1999 study period is presented in Table 3.1.

Fractionation of chlorophyll over the twelve month period February 1998 to January 1999 indicated that chlorophyll levels for the < 300 \( \mu \text{m} \) and < 20 \( \mu \text{m} \) fractions were similar (Figure 3.3). No significant difference between the chlorophyll content of the < 20 \( \mu \text{m} \) and < 300 \( \mu \text{m} \) fractions (\( P > 0.05 \)) was found over this twelve month period. Chlorophyll in the < GF/C filtered samples (nominally < 1.2 \( \mu \text{m} \)) had negligible amounts (< 1 \( \mu \text{g l}^{-1} \)) of Chla for February to July with August through to November having up to 9 \( \mu \text{g Chla l}^{-1} \).
Figure 3.2 Four year temporal species composition trends from 1995 to 1999 at an upper Swan River estuary site (Ron Courtney Island).

Following obliquely down the page will show the transition between major groups over annual periods. (Data courtesy Swan River Trust)
Table 3.1 Phytoplankton species occurring in the upper Swan River Estuary

This list contains those species encountered over the 12-month period from February 1998 to January 1999 that were dominant or obvious. It is not a comprehensive list. (Classification according to Tomas 1997; Horner, 2002)

Division Chromophyta
(Phylum Heterokontophyta, Round et al. 1990)

Class Bacillariophyceae (diatoms)

Order Bacillariales (pennate forms)
"Cocconelis sp." Ehrenberg

Sub-order Bacillariineae (raphid pennate)
Family Bacillariaceae
"Cylindrotheca closterium" (Ehrenberg) Lewin & Reimann

Family Naviculaceae
"Gyrosigma fasciola" Ehrenberg 1839
"Pleurosigma sp." W. Smith 1852
"Navicula sp."
"Nitzschia sp."

Sub-class Bacillariophycidae (Round et al., 1990)

Order Surirellales
Family Entomoeidaceae
"Entomoneis tenuistriata" John

Class Coscinodiscophyceae

"Cyclotella meneghiniana" Kutzi
"Skeletonema costatum" (Grev.) Cl.
"Stephanodiscus sp" Ehrenberg
"Thalassiosira weissflogii" (Grunow) G. Fryxell & Hasle

Sub-order Rhizosolenineae
"Rhizosolenia pungens" Cleve-Euler 1937

Class Chrysophyceae

Order Chrysosphaerales
Family Aurosphaeraceae
"Meringosphaera sp" Lohmann

Class Cryptophyceae

"Plagioselmis prolonga" Butcher 1967
"Cryptomonas sp (2 species)" Ehrenberg 1832

Class Dictyochophyceae
Order Pedinellales
  Family Pedinellaceae
    *Apedinella* sp. Thronsden 1971

Class Dinophyceae
Order Prorocentrales
  Family Prorocentraceae
    *Prorocentrum gracile* Schütt
    *P. micas* Ehrenberg
    *P. minimum* (Pavil.) Schil.

Order Gymnodiniales
  Family Gymnodiniaceae
    *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup
      (Synonym *Gymnodinium sanguineum* Hirasaka)
    *Amphidinium fusiforme* Martin
    *Cochlodinium sp.* Schütt 1896
    *Gymnodinium nelsonii* Martin 1929
    *G. simplex* (Lohmann) Kofoid and Swezy 1921
    *G. punctatum* Pouchet 1887
    *Gyrodinium uncatenum* Hulbur 1957
    *Gyrodinium sp.* Kofoid & Swezy 1921

  Family Polykrikaceae
    *Polykrikos schwartzii* Bütschli

Order Peridiniales
  Family Calcidinellaceae
    *Scripsiella sp.* Balech ex Loeblich III 1965

  Family Protoperidiniaceae
    *Protoperidinium sp.* Bergh

*Oxyrrhis marina* Dujardin 1841

Class Prymnesiophyceae
Order Prymnesiales
  Family Prymnesiaceae
    *Chrysochromulina sp.* Lackay 1939
    *Prymnesium sp.* Massart ex Conrad 1926

Division Chlorophyta
Class Chlorophyceae
  *Chlamydomonas globosa* Snow

Class Euglenophyceae
Order Euglenales
Family Eutreptiaceae

*Eutreptiella sp.* Da Chuna 1913

Class Prasinophyceae

Order Chlorodendrales

Family Halosphaeraceae

*Pyramimonas grossii* Parke

*P. cf parkeae* Norris and Pearson

*Micromonas sp.* Manton & Parke 1960

### 3.6.1 Annual trends

Species compositional trends over the study period followed established successional patterns (John, 1987; Thompson and Hosja, 1996; Twomey and John, 2001) (Figure 3.2) although small species such as *Pyramimonas* spp were sporadically abundant numerically throughout the 1998-1999 monthly study. In general, spring chlorophyte blooms, dominated by *Chlamydomonas globosa* (97% in November 1995 and 98% in November 1998), gave way to mixed phytoflagellate assemblages dominated by dinoflagellates and cryptophytes during summer and autumn with winter species composition dominated (98.6%) by diatoms.

During spring 1998 (September, October and November, n=8) seasonal species richness was 22. A suite of two classes, Chlorophyceae (71%) and Euglenophyceae (11.5%) constituted the bulk of the monthly diurnal cell count over the two depths (0.25 m and 4.5 m) and comprised only two species, *C. globosa* (Chlorophyte) and *Eutreptiella sp* (Euglenophyte). The next most abundant class was the Dinophyceae (10% of seasonal total with seven species), followed by the Bacillariophyceae (6%, six species), the Cryptophyceae (1.5%, 12 species) and the Prasinophyceae and Chrysophyceae each < 0.1% total cell count and each with only one species. During this study depth integrated counts (data courtesy Swan River Trust (SRT)) indicated that the spring 1995 and 1997 blooms were both dominated (>80% total cell count) by the chlorophyte *C. globosa.*
During summer 1998, 20 species were identified. Prasinophyceae (86% total cell count, n=12) were the dominant class with four species observed, Pyramimonas grossii, P. cf parkeae plus another unidentified Pyramimonas spp, and a very small unidentified prynnesiophyte. Cryptophyceae (three species) were next most numerous (5% total count). Chrysothryceae (three species) constituted 4% of total counts and were dominated by Apedinella sp. (3.4%). Next most abundant were the Bacillariophyceae (3%, three species), Dinophyceae (2%, five species) with Chlorophyceae (two species) and Euglenophyceae having a combined count of < 1%. By the end of summer 1998 Scrippsiella and G. simplex were again dominant (80% of total cells). Summer 1996 blooms comprised a mix of phytoflagellate species dominated by the dinoflagellates Scrippsiella sp., G. simplex and O. marina (depth integrated data courtesy SRT).

The early autumn phytoplankton population in 1998 (March) was dominated by a mixed dinophyte assemblage (Gyrodinium 73% and Scrippsiella sp. 7%). By April Pyramimonas grossii dominated the species composition (61%) with cryptophyte numbers increasing (12%) and peaking in May, corresponding to the high percentage of this group in 1996. During autumn 1998 (March, April and May, n=11) 33 species were
recorded, with 14 Dinophyceae species dominating (42% of total monthly count) the community. Euglenophyceae were the next numerically abundant (35% total monthly count) with only one species (*Eutreptiella* sp) being recorded. Prasinophyceae (five species) constituted 11% of monthly total counts followed by diatoms (4%, seven species) and Chrysophyceae (4%, one species), Chlorophyceae (3%, two species), with Cryptophyceae (two species) and Dictyochophyceae (*Apedinella* sp) each < 1% of monthly total cell count. In contrast, during the autumn 1996 period Cryptophyceae were most numerous (53.7% of total seasonal cell count, based on SRT integrated counts), with the next most numerous class being dinophytes (24.4% total seasonal cell count, dominated by *O. marina*) and chlorophytes/prasinophytes (12%). During the autumn specific bloom study (May 1996) *O. marina* was dominant (84% cell count).

During winter of 1998, 22 species were recorded for surface and bottom diurnal samples. Bacillariophyceae dominated (98.6 % total monthly count, n=10) with high numbers of pennate diatoms (> 80% of diatoms) occurring. These diatom species were dominated (80%) by *Cylindrotheca closterium*. The remaining classes included Prasinophyceae (five spp), and Cryptophyceae (three spp), with Chrysophyceae and Euglenophyceae each having one species each and combined constituted < 2% of total monthly cell counts. Winter 1996 (SRT integrated data) was dominated by diatoms (84% total count, n=9) with the centric diatom *Cyclotella meneghiniana* being conspicuous. Cryptophytes (11%) were the next most abundant group. Chlorophyte, prasinophyte, dinophyte, chrysophyte and raphidophyte numbers were all < 1% total seasonal count.

These seasonal patterns of phytoplankton community composition for 1998-99 can clearly be seen in Figure 3.4.

Seasonal diversity indices have been calculated for combined day and night samples on a monthly (Table 3.2) and seasonal (Table 3.3) basis. Species richness indicates that September and March had highest species numbers (22 and 25 respectively) with November lowest (two species). The difference between seasonal richness and the sum of monthly richness over a season represents the number of species present in more than one month.
Figure 3.4 Seasonal composition of phytoplankton community at RCI during the period February 1998 and January 1999.
Dominance of chlorophytes in spring, prasinophytes in summer, cryptophytes and euglenophytes in autumn and diatoms in winter is clearly shown.

When seasonal richness is compared to the sum of monthly richness values it is evident that certain species are common for more than one month. Summer, autumn and winter have 17, 19 and 15 species respectively that occur in more than one month, while in spring this number is only eight.

Highest potential diversity ($H'_\text{max}$) occurred in autumn (1.519, Table 3.3). Highest Shannon-Weaver diversity index ($H'$) was calculated for June (0.986, Table 3.2) and the season of winter (1.002, Table 3.3). Pielou's evenness ($J'$), calculated as ratio of $H'$: $H'_\text{max}$ indicates winter to be the most homogeneous period.

3.6.2 Individual bloom studies
The spring chlorophyte blooms were dominated (90%) by *C. globosa*. Diurnal distribution patterns for these bloom types, based on percentage of total cell numbers in
surface and bottom samples (Fig. 3.5a), indicated that the bulk of the chlorophyte biomass remained in the surface waters. This is supported by Chla profile data (see Figure 2.17).

The summer bloom (January 1996) was a mixed assemblage dominated (average 77%) by three dinoflagellate species, Gymnodinium simplex (average 51%), Scrippsiella sp (average 17%) and Oxyrrhis marina (average 9%). Plots of species compositional changes over the four week period of the bloom (January 10 – 31, 1996), based on integrated compositional analysis, showed an initial increase followed by a decline of both diatoms and dinoflagellates, with dinoflagellates the numerically dominant group (Figure 3.8).

The autumn mixed dinophyte bloom was dominated by O. marina (average 74% of total cell count), with the next most abundant species being Gyrodinium sp., Scrippsiella sp. and Entroplidella sp. Diurnal distribution patterns (Figure 3.5d) indicated a preference for the deeper (4.5 m) bottom water levels, rather than surface (0.25 m) waters, during both day and night.

Chla concentrations, indicated in Figure 3.5 a-d as mean (± SD), show that night time surface Chla values are approximately half daytime values for spring (~20 : ~45 µg Chla 1⁻¹, Figure 3.5a) and summer week1 (~30 : ~15 µg Chla 1⁻¹, Figure 3.5b). By week 3 of the summer bloom Chla levels had risen. Autumn Chla levels were highest in bottom night-time samples.
Table 3.2 Monthly species diversity and evenness for an upper estuary site (RCI) over an annual cycle, Feb 1998 - Jan 1999.

Maximum species richness (for combined day and night samples at surface and bottom) occurred in March, with maximum Shannon-Weaver diversity index ($H'$) in June. Highest potential diversities ($H'_{\text{max}}$) occurred in April and June. August had the highest Pielou evenness ($J'$) indicating greatest homogeneity.

<table>
<thead>
<tr>
<th></th>
<th>September</th>
<th>October</th>
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<td>6</td>
<td>2</td>
<td>16</td>
<td>9</td>
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<td>13</td>
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<td>$H'$</td>
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<td>0.324</td>
<td>0.055</td>
<td>0.300</td>
<td>0.618</td>
<td>0.696</td>
<td>0.518</td>
<td>0.942</td>
<td>0.619</td>
<td>0.986</td>
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<td>0.778</td>
<td>0.301</td>
<td>1.204</td>
<td>0.954</td>
<td>1.079</td>
<td>1.398</td>
<td>1.279</td>
<td>0.903</td>
<td>1.279</td>
<td>0.699</td>
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<tr>
<td>$J'$</td>
<td>0.252</td>
<td>0.417</td>
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<td>0.645</td>
<td>0.371</td>
<td>0.737</td>
<td>0.685</td>
<td>0.771</td>
<td>0.360</td>
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</tbody>
</table>

Table 3.3 Seasonal species diversity and evenness for an upper estuary site (RCI) over an annual cycle, Feb 1998 – Jan 1999.

Richness represents the number of species recorded per 3-month period. Shannon-Weaver diversity index ($H'$) and Pielou evenness ($J'$) show autumn to have the greatest number of species and the maximum potential diversity ($H'_{\text{max}}$). Winter is the most homogeneous season. Data used is combined monthly data for day and night samples from surface and bottom depths.

<table>
<thead>
<tr>
<th></th>
<th>SPRING</th>
<th>SUMMER</th>
<th>AUTUMN</th>
<th>WINTER</th>
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<tr>
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<td>20</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>$H'$</td>
<td>0.485</td>
<td>0.5052</td>
<td>0.775</td>
<td>0.270</td>
</tr>
<tr>
<td>$H'_{\text{max}}$</td>
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<td>1.3010</td>
<td>1.519</td>
<td>1.342</td>
</tr>
<tr>
<td>$J'$</td>
<td>0.361</td>
<td>0.3883</td>
<td>0.510</td>
<td>0.201</td>
</tr>
</tbody>
</table>
3.6.3 Diurnal Vertical Migration

Chlorophyll $a$ profiles above and below the study site showed that, although there is some variation in biomass, the profile pattern is fairly consistent over this portion of the river at these times, indicating that the trends seen at the study sites are real, persisting over a series of sites on either side of the study site. They do, therefore, truly represent the chlorophyll profile patterns (i.e. phytoplankton biomass distribution patterns) existing rather than patchiness or advective artefacts. It is evident from species composition and distribution analysis for the blooms studied that migratory behaviour of different species varies. Chlorophyte distribution patterns showed that $C. 	ext{ globosa}$ remain predominantly in the surface waters throughout the diurnal cycle (Figure 3.5).

In contrast, there was an apparent observed shift in behaviour from no diurnal vertical migration (DVM) during week 1 of the January dinophyte bloom to an established nocturnal vertical migration into deeper water by week 3 (Figure 3.5c & d). DVM was occurring for the dinophyte species that constituted the bloom. Pooled diurnal distribution patterns for these three species indicated no diurnal vertical migration during week 1 (Figure 3.5b) of the bloom, while marked diurnal vertical migration had been established by week 3 (Figure 3.5c). It appears that migration to midwater depths (week 2, Figure 3.6) precedes accessing the deepest water in week 3. DVM was further supported by in situ monitoring of chlorophyll profiles during the dinophyte bloom reported elsewhere (Hamilton et al., 1999) and from barge data in February (mixed dinophyte bloom, see Figure 2.17). The change from no DVM to DVM behaviour in January corresponded to a progressive drop in water column (surface and midwater) nutrient (NH$_4^+$) concentration over the three week bloom period (Figure 3.7).

3.6.4 Ordination analysis

Diurnal comparisons of temporal vertical distribution (Figure 3.9) show a high degree of variability in composition between day and night samples from the same 24hr period. This may be explained by advective influences due to tidal movement. However, ordination analysis (Figure 3.10) revealed a grouping based on depth, where those samples at the top right hand side of the ordination plot tended to be the daytime bottom samples, while those on the bottom left of the plot were daytime surface.
Figure 3.5 Diurnal species distribution patterns and chlorophyll concentrations for surface and bottom samples

a) Spring Chlorophyte (Chlamydomonas globosa - 90% of total cell count). b) & c) Summer mixed Dinophyte (pooled Gymnodinium simplex, Scrippsiella sp. & Oxyrrhis marina - av. 77% of total cell count). d) Autumn Dinophyte (O. marina - av. 74% of total cell count)

Chlorophyll concentration (SD) in units of μg Chl a l⁻¹ for each time period is given
Figure 3.6 Diurnal chlorophyll distribution over the course of a 3 week typical summer mixed dinophyte bloom, January 1995, RCI.

There is an apparent increasing shift in chlorophyll density from surface to bottom depths as the bloom progresses.

samples. All night time samples were positioned midway between these two groups with surface and bottom samples overlapping.

Based on Pearson and Kendall correlations with ordination axes (n=46) the environmental variables most strongly related to chlorophyll levels were saturated oxygen ($r^2 = 0.64$), ortho-Phosphate ($r^2 = 0.58$) and ammonium ($r^2 = 0.52$). These plots can be seen in Appendix I.
Figure 3.7 Changes in diurnal profiles of $\text{NO}_3^-$ and $\text{NH}_4^+$ during spring, summer and autumn blooms.

a & b Spring 1995 $\text{NO}_3^-$ and $\text{NH}_4^+$ profiles. c & d Summer 1996 $\text{NO}_3^-$ and $\text{NH}_4^+$ profiles, week 1 & 3. e & f Autumn 1996 $\text{NO}_3^-$ and $\text{NH}_4^+$ profiles, noon only. ● morning, ■ afternoon, △ night

---Week 1, -----Week 2, ---Week 3
Figure 3.8 Species composition changes over a mixed Dinophyte bloom (January 1996) at Ron Courtney Island.
Counts were made from integrated samples (depth approx. 5m) at RCI site. Data courtesy of Swan River Trust
Figure 3.9 Diurnal distribution for phytoplankton classes at Ron Courtney Island, February 1998 to January 1999.
Daytime (A) and Night-time (B) counts from Surface (0.25 m) and Bottom (4.5 - 5 m) depths.
b = bottom  s = surface
Figure 3.10 Principal component analysis relating diurnal phytoplankton species composition data with environmental factors.

Day (AM) surface and bottom samples are distinctly different. Surface and bottom night (PM) samples (open symbols) lie medially between these two extremes and overlap.
Figure 3.11 NMS analysis of phytoplankton classes with environmental parameters. Multidimensional scaling shows a strong linear relationship between axis 2 and dinoflagellate abundance. Dinophyte abundance is related to O₂ saturation ($r^2 = 0.64$), and concentrations of orthoP ($r^2 = 0.58$) and NH₄⁺ ($r^2 = 0.52$).

3.7 Discussion

Freshwater discharge in the Swan-Canning estuary has been demonstrated to influence the succession between marine, estuarine and freshwater phytoplankton taxa according to the extent to which it hinders the intrusion of marine water into the lower and upper estuary (Chan, 1990). Species composition determined during this research concur with previously reported species successional patterns for estuarine systems (ie Pinckney et al., 1998) and established patterns identified for the Swan River (John, 1987; Thompson & Hosja, 1996). Margalef (1978) predicted that under conditions of high turbulence centric and pennate diatoms should dominate. This was found to be the case in the upper Swan River Estuary during both 1996 and 1998 where winter species
composition was dominated (48 - 95% total cell counts) by diatom assemblages, with the dominant pennate diatom being *Cylindrotheca closterium*, with *Thalassiosira weissflogii* and *Cyclotella meneghiniana* the dominant centric diatoms. Over the twelve month period of this study (February 1998 to January 1999) all samples comprised a suite of, on average, three species that constituted ≥ 80% of total cell counts. This suite of three dominant species changed over the course of the year. Species richness ranged between 2 (November 1998) and 25 (March 1998) on a monthly basis with seasonal ranges between 20 and 33 (see Tables 3.2 & 3.3). Low species richness during summer was also reported by Deeley and Paling (1998) and related to the highly impacted nature of the upper Swan River estuary. Differences between 1996 seasonal bloom composition (January & May) and composition for 1998 indicated a higher number of small species in the latter year.

While species compositional trends have been established for this system, there has been no detailed data on diurnal depth distribution patterns for this system. It is evident that both within blooms and over a 12 month period, there are changing patterns of distribution that have ramifications, not only for productivity and nutrient uptake, but also for grazing control by higher order consumers. Species counts have shown that the Prasinophytes, such as *Pyramimonas grossii,* were numerous in surface and bottom depths for all seasons except winter. Cryptophytes were numerous for all seasons but there was a marked preference for bottom (av. 19.4%, n=8) over surface samples (av. 9.3%, n=8) during the day. Centric diatoms, species of which occurred throughout the year, were usually one of the minor contributors to the 80% count, while pennate diatoms only occurred in significant numbers in the early spring sample (August 1998).

Principal component analysis based on phytoplankton class groups shows a marked depth related separation over the annual period, with the greatest separation between daytime surface (0.25 m) and bottom (4.5 m) samples (Figure 3.10). Night surface and bottom samples lie midway between these two extremes and overlap, indicating that nocturnal distribution does not reflect any depth separation by light-related factors.

Ordination analysis of phytoplankton species composition and environmental parameters indicate that, for a single site over an annual period, saturated O₂, orthoP
and ammonium concentrations are most strongly related to phytoplankton distribution, in particular dinophyte distribution. At this finer level of resolution (one site, depth and time of day considered) the factors controlling phytoplankton distribution vary from those identified from integrated whole-system analyses.

This study indicates that most of the chlorophyll in the upper Swan River is contained in phytoplankton able to pass through a mesh. Where chlorophyll in the < 20 µm fraction exceeds levels in the <300 µm (October and January) it is suggested that a trophic cascade is operating (see Chapter 7). The dominance of nano- autotrophs (0.2 – 20 µm) in this system, as indicated by chlorophyll fractionation over the twelve month period (February 1998 - January 1999), supports evidence for the importance of nano-size phytoplankton in aquatic ecosystems. This has been demonstrated for lakes (Pick, 1991; Fahrensteil and Carrick, 1992; Chang and Petersen, 1995), rivers (Edwards et al., 1990), estuaries (Revelante and Gilmartin, 1978; Malone, 1980; Malone et al., 1991; Iriate and Purdie, 1994), coasts (Hallegraeff, 1981) and oceans (Ituriaga and Mitchell, 1986). Bernhard and Peele (1997) found that nanoplanckton contributed 79 -92% of total Chla in the Northern Puget Sound estuary. They are responsible for a significant fraction of total primary production and have been implicated as important in trophic transfer of carbon, nutrient and energy in microbial foodwebs in plankton communities (Stockner and Antia, 1986). Their small size and large surface to volume ratio makes them able to out-compete larger species for nutrients (van den Hoek et al., 1995). Thus, despite varying evidence (see Carrick and Schelske, 1997 and refs there in), an increasing compositional component of pico-plankton may be indicative of increasing levels of eutrophication.

As previous studies of the Swan River Estuary have not reported size fractionation data for chlorophyll, it is difficult to ascertain that any shift towards smaller species has occurred. Twomey and John (2001) compared species composition between 1980-81 and 1994-95 and concluded that no significant shift was evident. However, they do not dismiss circumstantial evidence for more frequent dinoflagellate blooms in the upper estuary during the summer and autumn of 1994 - 1995. Since the early studies focussed primarily on diatom composition (John, 1984) it may be that detailed information on phytoflagellate composition was not comprehensive. Since no research into the microbial foodweb of this system has been undertaken to date, this is an aspect of
phytoplankton species composition in the Swan River that requires further detailed investigation.

3.7.1 The role of diurnal vertical migration in different blooms

Vertical migration in phytoflagellates is a well established phenomenon (e.g. Watanabe and Kohata, 1991; Villarino and Figueiros, 1995; Kamykowski and Yamazaki, 1997; Kamykowski et al., 1998). Although many reasons have been postulated for this behaviour, in general it has been described as an endogenously controlled rhythmic diurnal migration. During the summer dinophyte bloom, diurnal vertical distribution patterns indicate that diurnal vertical migration, which was not evident in the first week of the study, was established by week 2 and week 3 of the bloom. A simultaneous study conducted from the Swan River Trust barge, the Seagull, moored at the RCI site used in situ fluorescence detectors (Sea Tech Inc. fluorometer) set at two depths (0.5 m & 3.0 m) to detect migrating phytoplankton. Results obtained showed daytime surface Chla levels approximately 20 μg l⁻¹ higher than bottom (3.0 m) levels and that this situation reversed correspondingly during the night (see Figure 2.17). This offers supporting evidence for vertical migration as an explanation for species composition changes by showing two peaks in chlorophyll at each depth that coincided with the migration down by midnight and then up again near dawn through the water column (Salmon, 1996). These changes in migration corresponded with depletion from surface to bottom of ambient nitrogen levels in the water column over the study period. Depletion in ambient nutrient concentration was also shown to relate to diurnal uptake of nitrogen species at various depths over the diurnal period (see Chapter 6). This suggests that vertical migration, rather than being a fixed endogenous diurnal response, is an alterable behavioural adaptation to access nutrient from deeper water in response to declining water column concentrations.

The role of DVM in optimising photosynthetic capability and nutrient acquisition (Kamykowski et al., 1998) is reinforced by observed changes in DVM behaviour over the course of the summer bloom. It indicates that DVM behaviour can alter under changing conditions of water-column nutrient availability (Fupperley et al., 1969). Cells undergo DVM in order to balance nutrient and photosynthetic requirement, thus enabling maximum growth under the existing environmental conditions. It also
suggests that DVM is regulated by factors other than circadian or endogenous rhythms. The importance of the role of DVM in maintaining a balance between near-bottom night-time nitrogen uptake and near-surface day-time photosynthesis, resulting in net population growth, has been demonstrated elsewhere (Hamilton et al., 1999) using the date from this study in conjunction with modelling techniques.

3.8 Conclusion

The high variability of species composition between years makes annual comparisons difficult. Previous studies based on nutrient data have indicated that the within and between year variability over the past 30+ years is too great to clearly indicate increases in eutrophic condition (Twomey and John, 2001).

For the RCI site the physico-chemical parameters most strongly related to Chla concentration distribution were O$_2$ ($r^2 = 0.64$) and orthoP. ($r^2 = 0.58$), in the first axis. It has been noted that motile species representative of several algal classes usually follow the initial dominance of non-motile species (Smayda, 1980; p496). Motile species in the upper Swan River Estuary are dominant during summer and autumn, a period typified by low ambient nitrate concentrations. It is suggested that this may be related to the ability for these forms to access partitioned resources through vertical migration. Ammonium, a product of decomposition (of non-motile species settling into deeper water), was found to be one of the environmental variables most strongly related to Chla levels.

This study demonstrates changing patterns of vertical migration of species which coincided with altering ambient nitrogen profiles. This phenomenon was particularly apparent during summer, a period when allochthonous nutrient supply from precipitation, run-off or groundwater discharge is minimal. These changing patterns of vertical migration and species distribution may influence and be influenced by nutrient distribution patterns. The effect that this has on bloom seeding and development may be a necessary consideration in understanding bloom establishment and perseverence.

There seems to be a dominance of nano-phytoplankton throughout this study period, with small Prasinophytes ($< 15$ $\mu$m) dominating during Autumn. While the relatively high proportion of small ($< 20$ $\mu$m) phytoplankton species, as evidenced by chlorophyll
fractionation, may indicate increasing eutrophication, since this group of phytoplankton has not been previously investigated for the Swan River system, this is speculation. A recommendation is made for further research emphasis on nanoplanckton (< 20 μm) in the Swan-Canning Estuary in terms of biomass, productivity and its contribution to the foodweb.

3.9 References


CHAPTER 4: Nitrogen uptake kinetics for inorganic and organic nitrogen sources

4.1 Abstract

Uptake kinetics for spring chlorophyte-dominated, summer and autumn dinophyte-dominated and winter diatom-dominated blooms provide an indication of the variability of physiological responses to different nitrogen source additions, both within and between specific seasonal bloom assemblages of phytoplankton. Summer and autumn phytoplankton assemblages exhibited typical Michaelis Menten saturated uptake kinetics for $\text{NO}_3^-$, $\text{NH}_4^+$ and urea. Diatom dominated assemblages exhibited linear, nonsaturating uptake for $\text{NO}_3^-$. *Chlamydomonas*-dominated spring blooms showed complex, mixed mode $\text{NO}_3^-$ uptake kinetics where both linear and saturating uptake modes occurred simultaneously, while the response to $\text{NH}_4^+$ was variable, with both linear and saturating uptake recorded at different times. The ramification of linear uptake response to nitrogen concentration, assuming uptake and assimilation occur at the same rate and in the absence of other controlling factors, is the potential for an increase in biomass that is proportional to the increase in eutrophication levels. It is suggested that mixed mode uptake may be indicative of an imminent successional change in the phytoplankton community.

4.2 Introduction

To understand the potential of any system to support phytoplankton growth, there is a need for knowledge of the physiological aptitude of the phytoplankton assemblage to utilise the ambient nutrient regime. Although monitoring gives an indication of changes in static ambient nutrient levels, it provides no indication of the uptake or turnover rates; that is, the rates at which nutrients are being used, or recycled for use, within the system. Monitoring of nutrient levels alone is too simple an approach for reaching an understanding of the processes influencing or controlling phytoplankton biomass and growth. It fails to take into account fluxes, as these occur at time or spatial scales which are not detected by such monitoring techniques, but which have important small-scale temporal and spatial ramifications for bloom development (Sterner, 1994). It also does not consider the physiological aspects of nutrient uptake that influence the ability of a species to grow or out-compete another under different ambient nutrient regimes.
Growth rates and nutrient uptake rates in natural systems are not in steady-state (Rhee et al., 1981) but will fluctuate according to large and small-scale spatial and temporal variations in nutrient availability. In a theoretical exploration of nutrient uptake and growth kinetics in phytoplankton, Morel (1987) recognised:

the dynamic behaviour of the uptake and growth processes, in relation to the frequency and amplitude of nutrient variations in nature which...govern what the optimum average nutrient concentration is for a particular organism.

Competition for nutrients and differences in nutrient uptake activity between species affects the dominance and species composition of phytoplankton in aquatic systems. The relationship between phytoplankton physiology and nutrients has been described by three different models. The Michaelis Menten model (Dugdale, 1967) describes the relationship between ambient nutrient concentration and the rate of uptake of that nutrient. The Droop model (Droop, 1973) looks at the relationship between the cell quota of nutrients and the phytoplankton growth rate. The Monod model (Tilman and Kilham, 1976) shows the relationship between ambient nutrient concentration and growth rate of the phytoplankton. Parameters in these models differ between phytoplankton species and these differences are used to explain mechanisms for interspecific competition and species succession.

### 4.2.1 Michaelis Menten uptake kinetics

Growth rates and nutrient uptake rates in natural systems are not in steady-state but will fluctuate according to large and small-scale spatial and temporal variations in nutrient availability. The ability of certain species to maximise growth through short-term rapid utilisation of such fluctuations may provide the mechanism for competitive success in a mixed population and thus the impetus for successional change (Rhee et al., 1981).

Physiological responses to nitrogen (N) supply are often used to characterise the nitrogen status of natural phytoplankton populations. The basic expression derived to describe the relationship between nutrient concentration and uptake rate is a Michaelis Menten rectangular hyperbola of the form $V = V_{\text{max}} S \cdot (k_s + S)^{-1}$, where $V$ is the uptake velocity of the limiting nutrient, $V_{\text{max}}$ is the maximum uptake velocity, $S$ is the substrate concentration of the limiting nutrient and $k_s$ is the value of $S$ at which $V =$
$V_{max}/2$ (the half-saturation constant). This has been shown to be valid for natural populations of marine phytoplankton (e.g. MacIsaac and Dugdale, 1969; Caperon and Meyer, 1972; Kanda et al., 1985) and heterotrophs (Parsons and Stricklands, 1962; Vaccaro and Jannasch, 1966), and for individual phytoplankton species (e.g. Eppley and Thomas, 1969; Bates, 1976; Conway et al., 1976; Parslow et al., 1984; Lieberman et al., 1994).

Maximum uptake rates ($V_{max}$) vary among phytoplankton species (e.g. Holm and Armstrong, 1981). Differences in maximum uptake rates can be used to explain mechanisms that lead to changes in community structure in response to altering modes of limiting nutrient supply (Turpin and Harrison, 1979; Sakshaug and Olsen, 1986; Suttle et al., 1987; Olsen, 1989; Watanabe and Miyazaki, 1996). Increasing $V_{max}$ has been shown to be a general response to the onset of nutrient limitation for phosphorus (Perry, 1976; Gotham and Rhee, 1981), inorganic carbon (Miller et al., 1984) and ammonium (Eppley and Renger, 1974; McCarthy and Goldman, 1979; Goldman and Glibert, 1982). This does not appear to be the case for nitrate (Horrigan and McCarthy, 1981; Dortch, 1982). It has been shown that in stable, nitrogen-limited environments, it is $\text{NH}_4^+$ which is readily regenerated, that supports the majority of primary production (Dugdale and Goering, 1967; Eppley et al., 1977; Eppley and Peterson, 1979). Thus, a strategy for maximising $\text{NH}_4^+$ uptake during times of $\text{NH}_4^+$ limitation, is through an increase in $V_{max}$, makes evolutionary sense. The explanation for the lack of this response to nitrate lies in the fact that nitrate provides only a minor component of nitrogen nutrition during times of N-limitation and so such uptake enhancing mechanisms for this nitrogen source have not evolved (Turpin, 1988).

An indication of the rate at which different populations respond to different nitrogen sources will provide an indication of the potential for population growth under various ambient nitrogen regimes. Any change in the kinetic parameters that lowers the substrate concentration necessary for a given transport rate represents an adaptation for enhancing the uptake of a limiting resource. Three adaptations are possible: an increase in substrate saturation rate, or maximum uptake rate ($p_{max}$ or $V_{max}$), a decrease in the half saturation constant $k$, indicating an increased affinity for the the limiting nutrient, and both an increase in $p_{max}$ and a decrease in $k$ (Turpin, 1988).
Various factors, such as masking of initial transport kinetics by prolonged incubation, dilution of isotopic tracer (i.e. changing atom % enrichment of $^{15}$N) over the course of the incubation through remineralisation processes (Glibert, 1982), dissolved organic production or decomposition (Axler et al., 1986), or changes in substrate concentration, may influence the results of uptake determinations. Other things being equal, a cell with lower $k_1$ and/or higher $V_{max}$ for nitrogen will be at a competitive advantage at low nitrogen concentration (Flynn, 1998) over cells without such a physiological adaptation.

### 4.2.2 Tracer methodology: Isotope techniques in flux measurements of aquatic systems

The use of a tracer method in determining the amount of a substance present (tracer statics) or the rate at which a substance is mixed, transported or exchanged (tracer kinetics) has long been recognised in many and diverse areas of science, from medicine to hydrology (Sheppard, 1962).

The use of tracer techniques originated in medical research in the early 1950s and extended into the area of marine science more than a decade later (Smith and Horner, 1981). These techniques present a mechanism for determining the amount of a substance present (tracer statics), or the rate of processes in which a substance is being mixed, transported or exchanged (tracer kinetics), without the need for disruptive or destructive sampling (Sheppard, 1962). Isotopic tracers have been widely used for measuring nutrient cycling in the marine environment (for review see Harrison, 1983), with the use of stable isotopes for the determination of flux rates in biological systems being popular since its early appearance in the aquatic literature in the late 1960s.

The use of tracer kinetics is of particular benefit when determining the rate of incorporation of material into compartments that are not readily accessible without disruption or perturbation of the system under investigation. The use of $^{14}$C to determine the rate of incorporation of dissolved inorganic carbon (DIC) into the particulate fraction (POC) in order to measure productivity is an example. The theoretical and mathematical considerations in calculating these rates from compartmental analysis techniques have been presented by a number of authors (i.e. Sheppard, 1962; Smith and Horner, 1981).
The use of the stable isotope $^{15}$N for the determination of nitrogen uptake and remineralisation rates in aquatic biological systems has been in use since the early 1960s. The majority of papers reporting N-uptake determinations use as their technique the model provided by Dugdale and Goering (1967) who present the uptake in terms of rate of change, as per the equation of Sheppard (1962),

$$V = \frac{da_t}{dt}(a_2-a_1)^{-1} \quad \text{(Eq 4.1)}$$

where $V$ is the uptake rate of $^{15}$N, $da_t/dt$ is the rate of accumulation of $^{15}$N into the particulate fraction over time, $t$; $a_2$ and $a_1$ represent the amount of tracer in the two compartments being monitored. Since in this case tracer is only added to one compartment the value ($a_2 - a_1$) represents the atom% enrichment of tracer added. This equation may be rewritten in the following form

$$V = \frac{a_t}{R \times t} \quad \text{(Eq 4.2)}$$

where $a_t$ is the atom % excess, $R$ is atom % enrichment of the source and $t$ is the incubation time (h). This number represents the percentage of N incorporated as $^{15}$N. By multiplying this value by the amount of particulate N present (PN), the absolute uptake rate of N ($\rho$), in terms of mass, can be calculated, ie

$$\rho = PN \times \frac{a_t}{R \times t} \quad \text{(Eq 4.3)}$$

This absolute uptake rate has units of mass volume$^{-1}$ time$^{-1}$.

Stable isotope techniques provide a sensitive measure of kinetic response that is particularly suited to low concentration determinations because they are able to detect increases in $^{15}$N content relative to the normally low natural abundance (0.37%) of $^{15}$N. An enrichment of only 0.01 atom% is usually considered analytically significant (Dugdale and Goering, 1967; McCarthy et al., 1977). The use of $^{15}$N labelled nitrogen compounds in the determination of Michaelis Menten uptake kinetics therefore provides a sensitive measure of nutrient uptake processes, particularly at low concentrations. The $^{15}$N technique is based on the assumption that there is no
isotopic discrimination, that uptake remains linear and that the atom % enrichment of the $^{15}$N source remains constant over the course of the incubation (Harrison, 1983). Limitations of the method include a sample requirement of typically 1 – 10 μM N (Holmes et al., 1998) for measurement on the mass spectrophotometer. One of the benefits of using this technique is that it does not require knowledge of the concentrations of substances under investigation. The uptake rate is calculated on the basis of rate of accumulation of the labelled source over the incubation period (Sheppard, 1962), represented by the atom % excess value (atom % - background).

It is at this point that there is some confusion in the literature over the use and interpretation of the results of isotope enrichment determinations of nitrogen uptake. The use of the term 'enrichment' may lead to confusion when using this technique. The effects of isotope enrichment and nutrient enrichment must be recognised as representing quite different attributes in this technique. The term 'enrichment experiment' has different connotations and consequences for isotope studies than for studies of nutrient dynamics. Isotope enrichment refers to the addition of even trace amounts above background of an isotope, while nutrient enrichment refers to substantially elevating the concentration of the nutrient in question. Dilution effects are greater in conditions where the concentration of added $^{15}$N is low relative to ambient $^{14}$N, as is the case where the recommended additions of ≈ 10% ambient are used (Dugdale and Goering, 1967). This recommended addition level is reported to avoid the effects of nutrient enrichment, eliciting enhanced or luxury nutrient uptake during the first 20 - 30 minutes of incubation (Goering et al., 1964; Glibert and Goldman, 1981; Harrison, 1983). However, maintaining low levels of nutrient enrichment in the experimental system results in creating maximum dilution of the atom % enrichment factor (R), when the dilution effect of added N, relative to ambient nutrient, is taken into consideration (see critique in Appendix III).

When stable isotope techniques are coupled with a standard method of analysing uptake kinetics, such as the Michaelis Menten technique, of analysing uptake kinetics, it enables accurate determination of small uptake rates from low concentration additions of a tracer to low ambient concentration incubation media. Inherent problems using stable isotopes that may influence uptake rate measurements include non-linear uptake and isotope dilution and over the course of the incubation (Glibert, 1982; Glibert et al.,
1982; Harrison, 1983). Those associated with kinetic parameter determination include length of incubations, non-constant substrate and/or isotope concentration, presence of interacting nutrients and cellular N status of phytoplankton under investigation (Flynn, 1998). Many factors may influence the results of uptake rate determinations. These include the masking of initial transport kinetics by prolonged incubation, the repression of transport by ammonium-nitrate interaction, changing substrate concentration or the dilution of isotopic tracer (i.e. $^{15}\text{N}$) caused by remineralisation processes over the course of incubations.

La Roche (1983) working in a eutrophic environment (the Bedford Basin, Nova Scotia) found that a 4h incubation time minimised the chances of substrate exhaustion and problems related to isotope dilution (Glibert et al., 1982). Incubation periods of 2 - 6 h appear to avoid problems related to isotope dilution and overcome the bias introduced in some cases by initial high rate or surge uptake (Dugdale and Wilkerson, 1986).

Despite these inherent problems associated with the determination of uptake rates and the associated rate parameters of maximum uptake rate ($V_{\text{max}}$) and half saturation constant ($k_{\text{m}}$), the use of stable isotope techniques provides the most sensitive method for measuring N uptake at low (less than detectable ($3 - 5 \mu\text{g N l}^{-1}$) by wet chemistry techniques) concentrations.

The uptake of nitrogen and its incorporation or synthesis into new cellular material may not coincide temporally, depending on the nutritional state of the cells (Morel, 1987; Wheeler et al., 1982). It cannot be assumed that uptake directly reflects assimilation, particularly where vertical migration is evident, such as is the case in the Swan River (Hamilton et al., 1999; Horner Rosser, 1998 & 1999; Horner Rosser and Thompson, 2001). Growth on $\text{NH}_4^+$, even with the added energy requirements of vertical migration, enables dinophyte species to out-compete other non-migratory species (Lieberman et al., 1994) during a time when $\text{NH}_4^+$ is the more prevalent inorganic nitrogen source (Douglas et al., 1996).

The utilisation of chemostat or cyclostat monospecific cultures, while providing a useful tool for 'elucidating general physiological, biological and genetic principles related to ecology' (Rhee et al., 1981) cultures, does not give information on these parameters as they relate to populations existing under quasi-steady-state conditions in the natural
environment. For this reason, studies which look at responses of natural assemblages to variations in environmental conditions, under in situ or as near natural conditions as possible, will provide a more realistic picture of population response to such variations. Knowledge of the kinetic parameters for a species or population will give an indication of the (preconditioned) preference for uptake of a particular nitrogen species over another and the ambient concentrations of the nutrient in question to support maximum growth rates. Knowledge of the rate at which different populations respond to different nitrogen sources will provide an indication of potential for growth under various ambient nitrogen regimes.

4.3 Objectives

Phytoplankton blooms in the upper Swan River Estuary develop at times when ambient nitrate concentration is low or often below detectable limits (summer and autumn). The ability or adaptability of phytoplankton populations to use alternative nitrogen sources was investigated. The potential alternative nitrogen sources for phytoplankton growth chosen were the inorganic source, \( \text{NH}_4^+ \), able to be derived from sediment or water column regenerative processes, and the organic nitrogen form, urea, not previously investigated in the Swan-Canning system. A comparison of kinetic parameters was made in an attempt to determine which of these nitrogen sources appeared to play a significant role in nitrogen nutrition during different seasons.

The main aim of this section was to determine seasonal uptake kinetics for the different nitrogen species; two inorganic sources (\( \text{NO}_3^- \) and \( \text{NH}_4^+ \)), and an organic source (urea). Uptake kinetics were related to species composition and ambient concentrations of these three nitrogen sources to determine whether physiological adaptations to the ambient N-species concentrations present are indicated in seasonal species assemblages.

4.4 Materials and methods

From the established annual successional pattern (see Figure 3.1) blooms typical of each season were sampled to establish N-source uptake kinetics. Uptake kinetics for three inorganic nitrogen sources (\( \text{NO}_3^- \), \( \text{NH}_4^+ \) and urea) were determined for spring chlorophyte (November 1997 & 1998), summer mixed dinophyte (February 1998), autumn dinophyte (May 1998) and winter diatom (July 1998) blooms in the upper Swan
River estuary, Western Australia. For the purposes of this study a particular bloom is designated according to the percentage of cells (>50%) belonging to one particular group of phytoplankton, i.e. diatoms or chlorophytes.

Kinetic experiments presented here were conducted on surface samples only at two sites (Sandringham and Success Hill, see Fig 1.1) during November 1997 and at the Ron Courtney Island site during 1998. Surface water samples were collected, using a 6-litre horizontal whole-water sampler, and gently pre-screened through a 300 μm Nitex screen before dispensing into 500 ml Schott bottles. Water samples for ambient nitrogen (NO₃ -, NH₄ + and urea) were filtered (0.45 μm, Nucleopore) and stored frozen for subsequent analysis. Samples for chlorophyll were filtered (Millipore glass-fibre, nominal pore size 1.2 μm) and the filters stored frozen for later analysis.

To 500 ml aliquots of pre-screened surface water, a range of nitrogen concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 μM) were added, in the form of ¹⁵N-NO₃ (99.9atom% N), ¹⁵N-NH₄ + (99.9atom% N₂) or ¹⁵N-urea (99.9atom%) (ISOTEC, USA).

All kinetic experiment samples in 1997 were incubated in situ for 4 hours at their collection depth to ensure ambient temperature and light conditions were maintained. All 1998 incubations (4 h) were performed in a constant temperature room under simulated ambient temperature and light conditions (as determined from field measurements at the time of sampling) using quartz halogen lights under variable rheostat control. Light measurements were made using a Licor model LI-250 light meter with 4π collector. All incubations were terminated by filtering (<250 mm Hg pressure) a known volume of sample onto GF/C filters that were then stored frozen in pre-combusted (550°C) foil packets until subsequent analysis for ¹⁵N content.

Filters for ¹⁴N:¹⁵N ratio, total N and total C measurement were dried at 60°C overnight and processed for combustion on a RoboPrep-CN Biological Sample Converter. Subsequent measurement of stable isotope ratios and PON were made on a VG Micromass Spectrophotometer. Standardisation was with N₂ gas and dual low enrichment standards (carbon and nitrogen) were run after every 8 - 10 samples.

Nitrogen uptake rates were determined by the ¹⁵N-enrichment method first described by Dugdale and Goering (1967). This study investigates nitrogen source uptake over
various seasons and various levels of ambient nitrogen concentration, as demonstrated in chapter 2. Realistic comparisons of uptake rates can only be made if the dilution effects on R are taken into consideration. Therefore the effect of isotope atom % dilution on the calculated ρ has been incorporated into all uptake rate calculations.

Absolute uptake rates (μg N l⁻¹ h⁻¹) were calculated according to the Dugdale and Goering (1967) equation \( \rho = N \alpha \left( \frac{R}{R_0} \right)^{-4} \) (Eq. 4.1), where \( \rho \) is absolute uptake expressed as μg N l⁻¹ h⁻¹, \( N \) is the total particulate nitrogen in μg N, \( \alpha \) is the atom % excess of \(^{15}\text{N} \) (= atom% - background), \( R \) represents atom % enrichment \( \left[ \alpha \ast \frac{S_t}{S_0} \right] \), \( \alpha \ast \) is the atom % enrichment of labeled \(^{15}\text{N} \) source, \( S_t \) is the concentration of labeled \(^{15}\text{N} \), \( S_0 \) is the concentration of unlabelled \(^{14}\text{N} \) and \( t \) is incubation time (h). Particulate nitrogen (PN) was determined at the end of incubations to reduce the effect of changing PN over the experiment (Harrison, 1983). Isotopic dilution due to regeneration was assumed negligible due to short incubation times (Dugdale and Wilkerson, 1986).

Specific uptake, \( \nu \) (ng N μg Chla⁻¹ h⁻¹), is the absolute uptake, \( \rho \), normalised to Chla and was calculated according to the equation \( \nu = \rho \cdot \text{Chla} \) (Eq. 4.2), where \( \rho \) is the absolute uptake (μg N l⁻¹ h⁻¹) and Chla is total chlorophyll \( a \) (μg Chla l⁻¹).

Chla concentrations were determined according to the spectrophotometric method following acetone extraction (Parsons et al., 1984). Specific uptake (\( \nu \) ng N μg Chla⁻¹ h⁻¹), was calculated using Chla as the normalising factor according to the equation \( \nu = \rho \ast \text{Chla}^{-1} \). Means and standard deviations (n=3) for absolute and specific uptake have been used where relevant.

Results of Michaelis Menten uptake kinetics were analysed by a linear transform of the data to an inverse reciprocal plot of concentration (S) versus concentration/specific uptake normalised to Chla (S/V), according to the recommendations of Dowd and Riggs (1965).

### 4.5 Results

Blooms which were typical of each season were sampled to establish N-source uptake kinetics. They showed that there were two seasons, spring and winter, where nitrogen
species uptake did not follow the usual pattern of saturating uptake, or Michaelis Menten kinetics. Uptake kinetic parameter determinations for the different seasons are presented graphically in Figures 4.1 to 4.4, with a summary of all parameter determinations given in Table 4.1.

4.5.1 Spring

\( V_{\text{max}} \) was much lower (11.8 compared with 72.9 ng NO\textsubscript{3}\textsuperscript{-}N \ \mu g \ Chl a\textsuperscript{-1} h\textsuperscript{-1}) and \( k_s \) values higher (79.8 compared with 3.1 \mu M NO\textsubscript{3}\textsuperscript{-}) for specific uptake during a period of higher ambient NO\textsubscript{3}\textsuperscript{-} concentration (4.7 - 7.1 \mu M NO\textsubscript{3}\textsuperscript{-} in spring 1997 and only 2.6 \mu M in spring 1998). At this time a linear uptake component was also evident.

There was consistent linear uptake of NH\textsubscript{4}\textsuperscript{+} when ambient NH\textsubscript{4}\textsuperscript{+} concentrations were in the range of 3.29 \mu M (1998) to 3.9 \mu M (1997), although the magnitude of uptake varied by two orders of magnitude. When ambient NH\textsubscript{4}\textsuperscript{+} was higher (7.9 \mu M), saturating uptake according to Michaelis Menten kinetics was evident, with \( V_{\text{max}} \) of 491.74 and \( k_s \) of 9.37 \mu M.

Uptake kinetics for urea followed the classic Michaelis Menten model with a high \( V_{\text{max}} \) of 611.3 ng urea-N \ \mu g \ Chl a\textsuperscript{-1} h\textsuperscript{-1} compared with other estimates made, and \( k_s \) of 18.5 \mu M in 1998 (Figure 4.2C). In 1997 the \( V_{\text{max}} \) was 1.17 ng urea-N \ \mu g \ Chl a\textsuperscript{-1} h\textsuperscript{-1} and \( k_s \) of 3.34 \mu M (Figure 4.3C). Spring chlorophyte urea uptake for the November 1997 Success Hill site (see Fig 1.1) also exhibited typical Michaelis Menten saturation kinetics, although \( V_{\text{max}} \) was very low (1.16 ng urea-N \ \mu g \ Chl a\textsuperscript{-1} h\textsuperscript{-1}) compared with all other \( V_{\text{max}} \) parameters measured.

During spring ambient concentration ranges of 4 – 50 \mu g N l\textsuperscript{-1} (0.2 \mu M - 3.6 \mu M) for NO\textsubscript{3}\textsuperscript{-} and 3 – 283 \mu g N l\textsuperscript{-1} (0.2 \mu M - 20.2 \mu M) for NH\textsubscript{4}\textsuperscript{+} were measured over the diurnal period. The response to NO\textsubscript{3}\textsuperscript{-} and NH\textsubscript{4}\textsuperscript{+} for the 1997 and 1998 chlorophyte blooms was variable over the three spring blooms studied (Figure 4.1 A & B, Figure 4.2 A & B). The Success Hill NO\textsubscript{3}\textsuperscript{-} uptake kinetics fitted well to a hyperbolic function (r\textsuperscript{2} = 0.99, see Figure 4.2 A). However, the very high \( k_s \) (79.8 \mu M) suggests that for the range of NO\textsubscript{3}\textsuperscript{-} concentrations used (0.1 – 20 \mu M), both 1997 blooms showed similar trends of linear uptake for high (>5 \mu M) nitrate concentrations (Figure 4.2 A & D). At low NO\textsubscript{3}
concentrations, there was evidence of non-linear saturating, or active, uptake (Figure 4 A - C) for the Sandringham bloom. By separating the linear component of the Sandringham kinetic curve for NO$_3^-$ (Figure 4.3 A & B), then comparing linear slopes for the two chlorophyte blooms (Figure 4.3 C), it can be shown that the linear component of the uptake rates for these two blooms are identical (slope $\approx 1$, $r^2 = 0.99$).

NH$_4^+$ uptake for chlorophyte blooms subject to ambient NH$_4^+$ concentrations of 3.9 and 3.3 $\mu$M exhibited linear uptake kinetics, while saturation uptake occurred in the conditions of higher (7.9 $\mu$M) ambient NH$_4^+$ at the Sandringham site (Figure 4.2 E).

4.5.2 Summer

Summer 1998 ambient NO$_3^-$ levels at the time of sampling were 1.14 $\mu$M at the surface and decreased with depth to 0.6 $\mu$M between 1 m and 3 m then increased again to 1.2 $\mu$M at the deepest point (4.5 m). The range of NH$_4^+$ concentrations was 1.14 $\mu$M at the surface decreasing to 1.79 $\mu$M at depth. Urea surface concentration was 4.57 $\mu$M.

Summer uptake kinetics followed Michaelis Menten saturating uptake kinetics for all nitrogen sources examined (Figure 4.4 A-C). Specific uptake ($V_{\text{max}}$) for NO$_3^-$, NH$_4^+$ and urea were 350.6, 44.2 and 597.1 ng N $\mu$g Chla$^{-1}$ h$^{-1}$ respectively. Lowest $k_s$ value was for NH$_4^+$ at 1.75 $\mu$M, with $k_s$ for NO$_3^-$ of 3.56 $\mu$M and for urea of 6.42 $\mu$M. The NH$_4^+$ $k_s$ value was very close to the ambient NH$_4^+$ concentration measured (1.75 $\mu$M).

4.5.3 Autumn

During autumn NO$_3^-$ and NH$_4^+$ levels were much lower at the surface (0.68 $\mu$M for NO$_3^-$ and 1.36 for NH$_4^+$) than throughout the rest of the water column (1.36 - 1.57 $\mu$M for NO$_3^-$ and 3.57 - 5.86 $\mu$M for NH$_4^+$). Urea surface concentrations were uniform throughout the water column (1.63 - 1.78 $\mu$M), with a surface concentration of 1.78 $\mu$M.

As for summer, the uptake kinetics during autumn followed Michaelis Menten saturating kinetics for all three nitrogen species examined (Figure 4.4 D-F). The autumn mixed cryptophyte and prymnesiophyte bloom exhibited highest $V_{\text{max}}$ for urea (242.31 ng N $\mu$g Chla$^{-1}$ h$^{-1}$) and lowest $k_s$ for NO$_3^-$ (2.89 $\mu$M, see Table 4.1).
Figure 4.1 1998 Spring chlorophyte and winter diatom specific uptake kinetics determined on naturally occurring phytoplankton assemblages from RCI. NO₃⁻ (A & D), NH₄⁺ (B & E) and urea (C & F). \( V_{\text{max}} \) (Numbers in bold on S (µM) axis represent ambient concentration. – o – Linear regression of transformed data (SIV).

Ambient nitrogen concentration indicated by ↑ (value in bold).
Figure 4.2 Chlorophyte specific uptake kinetics at Success Hill and Sandringham sites (November 1997).

NO$_3$ (A & D), NH$_4^+$ (B & E) and urea (C). — specific uptake, – o — linear regression of transformed data (SAV vs S), ambient nitrogen concentration indicated by † (value in bold). —— 95% confidence limits of linear regression.
Figure 4.3 Mixed-mode NO₃⁻ uptake by Sandringham site, chlorophyte bloom.
Specific uptake (A) has been separated into its component uptake modes (B; • linear, △ non-linear) and the linear portion of this is correlated against the linear portion of the hyperbolic function of uptake (concentrations < kₑ) for the Success Hill NO₃⁻ plot from Fig. 4.2 A (C).
Figure 4.4 1998 Summer dinophyte and Autumn dinophyte specific uptake kinetics determined on naturally occurring phytoplankton assemblages from RCI. NO₃⁻ (A & D), NH₄⁺ (B & E) and urea (C & F). —— specific uptake, – o – Linear regression of transformed data (SAV vs S), ambient nitrogen concentration indicated by ↑ (value in bold).
Table 4.1 A comparison of uptake kinetic parameters, $V_{\text{max}}$ (ng N μg Chl$^{-1}$ h$^{-1}$) and $k_s$ (μM), and ambient surface nitrogen concentration (μM) for NO$_3^-$, NH$_4^+$ and urea for Spring, Summer, Autumn and Winter phytoplankton assemblages in the upper Swan River Estuary. k$_s$ values in italics represent slopes of linear relationships. nd indicates no data available.

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<td>(Winter) RCI'98</td>
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4.5.4 Winter

Winter ambient NO$_3^-$ concentration range of 291 – 317 μg NO$_3^-$-N l$^{-1}$ (20.8 μM – 22.6 μM) while the NH$_4^+$ ambient concentration range measured was 22 – 80 μg NH$_4^+$-N l$^{-1}$ (1.6 μM - 5.7 μM). Ambient surface (0.25 m) concentrations of NH$_4^+$ and urea were 1.57 and 1.86 μM respectively during winter while NO$_3^-$ comprised a much greater portion (21.43 μM) of the inorganic nitrogen source at this time.

Winter NO$_3^-$ uptake kinetics at this time were linear (slope 1.14, $r^2 = 0.98$, see Figure 4.1 D). Winter uptake kinetics for both NH$_4^+$ and urea followed typical Michaelis Menten style uptake, with $V_{\text{max}}$ of 104.91 ng NH$_4^+$-N μg Chla$^{-1}$ hl$^{-1}$ and 89.13 ng urea-N μg
Chla$^1$ h$^{-1}$, and $k_v$ values of 5.79 $\mu$M and 1.95 $\mu$M respectively (Figure 4.1 E & F). Uptake kinetics for urea followed the classic Michaelis Menten model with $V_{\text{max}}$ of 611.3 ng N $\mu$g Chla$^{-1}$ h$^{-1}$ and $k_v$ of 18.5 $\mu$M in 1998 (Figure 4.2 C), while in 1997 the $V_{\text{max}}$ was 1.17 ng N $\mu$g Chla$^{-1}$ h$^{-1}$ and $k_v$ of 3.34 $\mu$M (Figure 4.3 C).

4.6 Discussion

It has been recognized for some time that species-specific nutrient uptake kinetic parameters, $V_{\text{max}}$ and $k_v$, can be used to explain species competition involving a limiting nutrient (Dugdale, 1967; Harrison et al., 1989). This study determined uptake kinetics for natural assemblages of phytoplankton. A seasonal comparison of $V_{\text{max}}$ and $k_v$ parameters for different nitrogen sources, when compared with ambient N-source availability, shows that the winter blooms may be physiologically acclimatised to take advantage of the relatively higher ambient levels of nitrogen in the form of nitrate that occur at this time of year. The linear relationship between uptake rate and ambient concentration implies that elevated levels of nitrate in the system would enable an increase in phytoplankton uptake and a corresponding increase in algal biomass.

With ambient urea concentrations (1.67 $\mu$M) greater than those for either NO$_3^-$ (0.68 $\mu$M) or NH$_4^+$ (1.36 $\mu$M) during autumn, it is possible that urea functions as an important N-source for these phytoplankton species. This supports the findings of Kudela and Cochlan (2000), working with the red tide organism Lingulodinium polyedrum, that urea could potentially provide a large proportion of the nitrogen requirement at ambient urea concentrations.

The preferences indicated for the spring bloom are not as clearly defined from these parameters. Spring blooms have acclimatised to take advantage of higher available NO$_3^-$ concentrations reported to occur during this time (Jack, 1987; John, 1987; Douglas et al., 1996). River flow had not stopped and so a re-supply of NO$_3^-$ from terrestrial run-off and the occasional rain shower that occurred during spring in this climate was available. In general Chlamydomonas-dominated spring blooms exhibited saturating uptake for NO$_3^-$ in both years, although complex mixed mode NO$_3^-$ uptake kinetics were recorded, indicating active uptake was occurring at lower concentrations ($<5$ $\mu$M) with a linear response dominating uptake at higher concentrations ($>5$ $\mu$M) (Figure 4).
Ambient NO$_3^-$ levels at this time were 7.1 μM and therefore either uptake mode may have been in operation, although active uptake would provide a more competitive edge. The lower k, and higher V$_{\text{max}}$ parameters during a period of relatively higher ambient NO$_3^-$ concentration (4.7-7.1 μM NO$_3^-$; spring 1997 cf. 2.6 μM NO$_3^-$; spring 1998) indicate an adaptive ability to suit environmental conditions. Since urea had the lowest k, value for spring (1.0 μM, Table 4.1) then this nitrogen source can be taken-up at a greater rate than NO$_3^-$ and NH$_4^+$ under conditions of reduced nitrogen availability.

In one case analysed, there was evidence of active uptake when NO$_3^-$ levels were less than 0.5 μM (Figures 4.2 D & 4.3) that may represent competition by another species for that nitrogen resource. The ramification of linear uptake response to nitrogen concentration, in the absence of other controlling factors, is the potential for an increase in biomass that is proportional to the increase in nitrogen loading.

Despite the inherent problems outlined, the Michaelis Menten uptake kinetics presented here provide an indication of the variability of physiological responses to nitrogen addition for uptake of different nitrogen sources, both within and between specific seasonal natural bloom assemblages of phytoplankton. While non-conformity to Michaelis Menten uptake is known to occur, mixed mode uptake is rarely reported. There was clear evidence of bimodal uptake of NO$_3^-$ at the Sandringham site (Figure 4.3) which, when separated out, revealed a remaining linear component of equal rate to the Success Hill site (linear correlation coefficient = 1.0; r$^2$ = 0.99, see Figure 4.3 C). Although V$_{\text{max}}$ for urea was the same as for the summer bloom, the k, value was an order of magnitude lower, giving the spring bloom the ability to grow faster on lower ambient levels of urea. It is suggested that the linear component of this uptake for both sites in 1997 may be attributed to *Chlamydomonas*, as this was, in both cases, the dominant (> 80%) phytoplankton present. Since ambient NO$_3^-$ concentration at this time was 7.1 μM, surge uptake as a result of nutrient depletion prior to nutrient addition is probably not the cause of this non-linear uptake at the Sandringham site. It may be a result of uptake by *Apedinella*, which was the second most dominant species at this site and which was not present in significant numbers at Success Hill. If this were the case, the much lower k, value would indicate that this species was possibly capable of out-
competing *Chlamydomonas globosa* for available nitrate and so might increase in numbers to dominate the assemblage.

Spring and summer blooms have also been shown to be sustained by nitrogen from regeneration processes, even though the ambient nutrient concentrations may give the impression of a nutrient-deplete situation (Horner Rosser and Thompson, 2001). The 1998 spring study indicated that NH₄⁺ was strongly preferred (linear uptake, slope 15.9, \( r^2 = 0.98 \)) (Table 1). However, the two 1997 sites exhibited linear response to both NO₃⁻ and NH₄⁺ but with a comparatively low uptake:concentration ratio (slope approx. 0.1). Summer dinoflagellate blooms appear to be adapted to use the higher levels of regenerated nitrogen that occurred at this time (Horner Rosser and Thompson, 2001). Rainfall and river flow had ceased and the system had shifted to a micro-tidal estuarine hydrology (Stephens and Imberger, 1996). There was a measurable nutricline, with bottom NH₄⁺ levels up to 43 µM (Douglas *et al.*, 1996), which was shown to be depleted over the course of a bloom (Horner Rosser and Thompson, 2001). Dinophytes have the advantage of being able to actively take-up nutrients in apparently low ambient nutrient conditions.

### 4.7 Conclusions

While the Swan is considered on the basis of the frequency and intensity of algal blooms as being meso- to eutrophic, the highest density of phytoplankton occurs at times and sites in the system when nutrients, in particular nitrogen, are at often less than detectable concentrations. During periods of minimal allochthonous nitrogen (NO₃⁻) input to the upper estuary (summer and autumn) physiological adaptation to the alternate N sources of NH₄⁺ and urea, as indicated by higher \( V_{\text{max}} \) or lower \( k_s \), is evident. As demonstrated (see Chapter 2), these two nitrogen sources were available throughout the year. Physiological adaptation to uptake of both NH₄⁺ (lowest \( k_s \)) and urea (highest \( V_{\text{max}} \)) coincided with periods of minimum nitrate and greater NH₄⁺ and urea concentration. If the \( k_s \) parameter reflects pre-conditioning to nutrient concentration then those determined at the RCI site for the uptake of various nitrogen sources, indicate that \( k_s \) values exceeded measured ambient nitrogen concentrations by 5 times \((n=12)\). It is clear from this study that nitrogen sources other than nitrate are important in the growth and maintenance of phytoplankton species throughout the year. In particular,
the role of organic sources needs to be further investigated as a significant potential source for nitrogen nutrition.

Knowledge of potential physiological responses and of nutrient flux rates, factors that cannot be elucidated from point-in-time monitoring practices, will enable a better predictive ability for phytoplankton growth and successional change. The use of $^{15}$N labelled nitrogen compounds in the determination of Michaelis Menten uptake kinetics provides a sensitive measure of nutrient uptake processes, particularly at low concentrations. The appearance of multi-mode uptake kinetics may provide an indication of a potential bloom event enabled by the ability of some species within the population being able to physiologically out-resource others. It may be possible to predict a potential bloom development based on a physiological potential for rapid growth. It is hypothesised that this mixed mode uptake may be indicative of an imminent successional change in the phytoplankton community and that this aspect of community physiology be further investigated.

Although techniques for flux measurements are well established, they have not been widely used in Australia for the monitoring or understanding of systems tending towards eutrophication. They provide a useful tool in the elucidation of nutrient processes that, when used in conjunction with current monitoring techniques, will provide a more realistic understanding of phytoplankton growth and succession.

### 4.8 References


CHAPTER 5: Seasonal nitrogen uptake rates and preferential uptake of inorganic and organic nitrogen and comparison of C:N ratios

5.1 Abstract

Nitrogen uptake estimates for the upper Swan River Estuary over an annual cycle are comparable with those reported from other estuarine systems. There is a strong preference for recycled and organic nitrogen sources such as NH$_4^+$ and urea over allochthonous or 'new' nitrogen, NO$_3^-$. A relatively constant level of urea was available in the water column over an annual cycle. Levels of NO$_3^-$ fluctuated between negligible during summer and autumn, to peaks following the onset of seasonal rains. NH$_4^+$ concentrations were consistently greater in deeper water and peaked at this depth following the onset of rains. Daytime specific uptake rates (normalised to Chla) were generally an order of magnitude greater than nighttime or bottom rates. Based on total ambient nitrogen uptake rates ($\Sigma v$) for all nitrogen species examined, NH$_4^+$ (41% - 55%) and urea (28% - 40%), formed the greatest proportion of available N. Seasonal comparisons of total uptake show night uptake at depth during summer and winter were > 100% of surface uptake. This is explained by diurnal vertical migration of dinoflagellates during summer. The winter result is suggested to be due to higher microbial uptake rates at depth.

Diurnal depth-related phytoplankton distribution was found by PCA analysis to be most significantly influenced by two physiological flux measurements, $v$NO$_3^-$ ($r^2 = 0.50$) and $v$NH$_4^+$ ($r^2 = 0.51$) rather than physico-chemical factors. This aspect should be considered in management strategies.

5.2 Introduction

Nitrogen sources and sinks, and the ability of phytoplankton to access and utilise these, are important factors in an aquatic system's ability to support algal productivity. Nitrogen present in groundwater, streams, rivers and estuaries may occur in several forms, most of which are soluble, highly mobile and readily available to plants. Nitrogen sources potentially available in aquatic systems are principally inorganic forms (nitrate, nitrite and ammonium) and, generally considered of less importance, dissolved
organic forms (urea and free amino acids). Both inorganic forms, the oxidised nitrogen species, NO$_3^-$ and NO$_2^-$ and reduced species, NH$_4^+$, are utilised in phytoplankton growth.

Primary production based on nitrogen assimilation is categorised as new or regenerated, depending on the nitrogen substrate used. Nitrate (NO$_3^-$) is generally considered to generate new production, while ammonium (NH$_4^+$) fuels production based on regenerated or recycled nitrogen (Dugdale and Goering, 1967; Eppley and Petersen, 1979; Harrison, 1980). Regeneration rates range for aquatic systems between 66 and 1512 ng N l$^{-1}$ h$^{-1}$. The contribution of microplankton excretion to regeneration is reported as 0.39 – 574 ng N l$^{-1}$ h$^{-1}$ (Table 5.1). Atmospheric nitrogen, in the form of inorganic or organic nitrogen, has also been recognised as a quantitatively important source of external or ‘new’ nitrogen for a range of marine and freshwater ecosystems (Paerl, 1993, 1995 and 1997; Paerl and Fogel, 1994; Paerl et al., 1999; Jassby et al., 1994; Peierls and Paerl, 1997; Hu et al., 1998). Studies of NH$_4^+$ uptake have shown a predominance of regenerated production (maximum f-ratio < 0.47, where f-ratio is the relative proportion of new production to total primary production) for Indian Ocean phytoplankton communities where NH$_4^+$ supplied 53 – 99% of the nitrogen requirement (Mengesha et al., 1999) and was the major nitrogen substrate used.

In temperate estuaries, the relative proportions of new and regenerated N often varies seasonally and changes are associated with changes in microbial community composition and foodweb structure. Relatively high NH$_4^+$ levels are associated with communities dominated by small flagellated phytoplankton and foodwebs regulated by bacteria-nanoplankton-protozoan consortia (in Lewitus et al., 1998). Shifts between dependence on different nitrogen sources can be related to seasonal conditions. Maguer et al. (1998) demonstrated that spring phytoplankton growth was dominantly fuelled by nitrate (75% total uptake) and, by late spring and summer, ammonium uptake was substantial, when ammonium regeneration by microheterotrophs can satisfy between 62 – 100% of the total phytoplankton nitrogen requirements.

The complex interaction between nitrogen sources has been investigated and indicates that, in many situations, a preference for ammonium is evident (Dortch, 1990; Flynn et al., 1997) although, at low ammonium concentrations, this may not always be the case.
(Dortch, 1990; Flynn et al., 1997). A review by Dortch (1990) showed the repression of transport by ammonium-nitrate interaction to be highly variable and less of a problem in uptake rate measurements for natural populations than previously thought. Yin et al. (1998) have demonstrated that depression of nitrate uptake by ammonium was not evident for the diatom Thalassiosira pseudonana grown under low light conditions. This would enable both nitrogen sources to be utilised under conditions of increased nitrogen requirement.

Table 5.1 Regeneration rates for ammonium for various environments and sources.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Regeneration rate (ng N l⁻¹ h⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligotrophic water</td>
<td>238</td>
<td>Ferrier-Pagès &amp; Rasoulzadege 1994</td>
</tr>
<tr>
<td>Sub-arctic ocean</td>
<td>224 (night)</td>
<td>Wheeler et al. 1989</td>
</tr>
<tr>
<td></td>
<td>&lt; 5.6 (day)</td>
<td></td>
</tr>
<tr>
<td>Coastal</td>
<td>308-1512</td>
<td>Selmer &amp; Sorensson 1986</td>
</tr>
<tr>
<td></td>
<td>117 - 525 †</td>
<td>Neuer &amp; Franks 1993</td>
</tr>
<tr>
<td>upwelling</td>
<td>274 - 900 †</td>
<td>Dickson &amp; Wheeler 1995</td>
</tr>
<tr>
<td>non-upwelling</td>
<td>239 - 408 †</td>
<td></td>
</tr>
<tr>
<td>Lake</td>
<td>66 - 272</td>
<td>Takahashi et al. 1995</td>
</tr>
</tbody>
</table>

From microplankton excretion

<table>
<thead>
<tr>
<th>Species</th>
<th>Regeneration rate (ng N l⁻¹ h⁻¹)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagellate</td>
<td>0.389 - 0.584</td>
<td>Ferrier-Pagès &amp; Rasoulzadege 1994</td>
</tr>
<tr>
<td>Ciliate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strombidium sulcatum</td>
<td>380 - 571</td>
<td>Ferrier-Pagès &amp; Rasoulzadege 1994</td>
</tr>
<tr>
<td>(oligotrophic water)</td>
<td>308 (fed algae)</td>
<td>Gast &amp; Horstman 1983</td>
</tr>
<tr>
<td>Euplotes vanus †† (oceanic)</td>
<td>574 (fed bacteria)</td>
<td></td>
</tr>
<tr>
<td>(&lt; 200μm fraction)</td>
<td>8.4 - 378</td>
<td>Maguer et al. 1999</td>
</tr>
</tbody>
</table>

† These values converted from reported d⁻¹ to h⁻¹ assuming constant remineralisation rate over 24h
† † Used minimum ciliate densities reported for S. sulcatum to calculate h⁻¹ rate

Uptake and Regeneration

Phytoplankton populations can achieve near maximum growth rates, indicating non-nutrient limited growth, in conditions of low ambient concentrations such as
oligotrophic ecosystems (Goldman et al., 1979; McCarthy and Goldman, 1979; Harris, 1986). Close coupling between the uptake and regeneration of ammonium has been demonstrated (Harrison, 1978; Axler et al., 1981; Glibert et al., 1982; La Roche, 1983). A 1:1 relationship between these two processes has been reported for an open ocean study (Paasche, 1988). Microplankton (<100 µm) has been suggested as the source of much of the regenerated nutrients required for primary production (Harrison, 1978; Caperon et al., 1979; Glibert, 1982; Goldman and Dennet, 1985; Ferrier-Pagès and Rasoulzadegan, 1994) with reports of an average of 36% of the annual NH$_4^+$ uptake requirement coming from rapid remineralisation (La Roche, 1983). Rates of ammonium excretion by the copepod Acartia tonsa were found to range between non-detectable and 28 ng N copepod$^{-1}$ h$^{-1}$. (Miller and Glibert, 1998)

**Diel periodicity**

Diel periodicity has important implications in uptake and regeneration rate comparisons or diurnal N budget calculations. Goering et al. (1964) first demonstrated marked diel cycles in the potential uptake of NO$_3^-$ and NH$_4^+$ by phytoplankton in the N-depleted Sargasso Sea. A number of studies have provided evidence of uptake periodicity, with maximum uptake occurring during the day and reduced uptake rates at night, particularly for NO$_3^-$. The influence of light on NO$_3^-$ and NH$_4^+$ uptake and regeneration must be considered. Dark uptake of NO$_3^-$ does not occur for certain phytoplankton species (eg Karenia mikimotoi, Paasche et al., 1984; Chlorella and Nannochloropsis, Hipkin et al., 1983) in N-replete water as there is an absolute light requirement for this to occur. Others, such as *Proorocentrum minimum* have been shown to assimilate NO$_3^-$ in the dark, even in N-replete conditions (Paasche et al., 1984). It was suggested that this species relies on continual N uptake to match its daytime carbon assimilation.

**Dissolved Organic Nitrogen (Urea)**

It has been hypothesised that organic nitrogen may control phytoplankton succession when inorganic nitrogen becomes exhausted (Butler et al., 1979), with those species able to utilise DON having a competitive advantage. Piedras et al. (1998) suggest that an understanding of the ability to utilise DON components will help in understanding nitrogen micro-cycles in aquatic ecosystems, especially the interaction between N-cycles
of heterotrophs and phytoplankton. Discrepancies between uptake of $^{15}$N and the appearance of $^{18}$N in the particulate nitrogen (PN) (Gilbert et al., 1982; Price et al., 1985) have been attributed to release of $^{15}$N into the dissolved organic nitrogen (DON) pool (Bronk and Ward, 1999). In order to develop a biologically available N budget for an aquatic ecosystem, it is necessary to quantify both the DIN inputs and the portion of organic N (both particulate and dissolved) that is biologically available (Seitzinger and Sanders, 1997).

Recent research (Berman, 2001) has emphasised the importance of including nitrogen made available to phytoplankton, either directly or indirectly through the DON pool, in determining N supply to aquatic planktonic microbiota. Berg et al. (1997), looking at the relative importance of DON uptake to a brown tide organism, the chrysophyte Aureococcus anophageferens, found that potential urea uptake was substantially greater than uptake of other N-sources. It contributed 58% - 64% of total N uptake compared with only 5% - 8% for nitrate.

Sloth et al. (1995) showed marked differences in the fate of organic nitrogen deposited on sediments and demonstrated the importance of bioturbation and other physical mixing. Their results indicated that moderate loading of sediments with organic material increased N removal through denitrification, while high loading decreased denitrification.

**N versus P limitation**

The relative proportion of carbon:nitrogen:phosphorus (C : N : P) in phytoplankton is considered a gauge of the physiological state of the cell. This ratio, the Redfield ratio, is in proportions of the order of 106 : 16 :1 (or 6 : 1 for C : N) in cells which are growing at maximal rates and therefore not under nutrient limitation. Variation from this may indicate nutrient limitation.

Limitation by nitrogen and phosphorus have been reported (review by Howarth et al., 1988; Fisher et al., 1992; Bernhard and Peele, 1997). Research implicates nitrogen (specifically nitrate) rather than phosphorus as the limiting nutrient for phytoplankton biomass and productivity in a variety of marine aquatic ecosystems (Ryther and Dunstan, 1971; Fong et al., 1993; Mallin, 1994; Chapelle et al., 1995; Jansson et al., 1996;
Thompson and Hosja, 1996). Historically, nitrogen has been considered the nutrient limiting phytoplankton growth in coastal (Ryther and Dunston, 1971) and oceanic environments (Eppley et al., 1973), while in freshwater ecosystems the incorporation rate of nitrogen into plant tissue is usually controlled by P availability, since freshwaters are typically P limited (Correll, 1998), although Levine et al. (1997) found seasonal variation N and P limitation in Lake Champlain, Canada, with P the principle limiting nutrient during summer. In other studies a combination of both N and P was needed to positively affect phytoplankton growth. The variable response to N and P may be a response to seasonal changes in freshwater run-off altering the nutrient loading and N:P ratio (D’Elia, 1987; Fisher et al., 1992). The situation in estuaries seems variable. While estuaries are typically considered nitrogen limited, some variation in nutrient limitation in brackish waters is apparent (Smith and Longmore, 1980; Nixon, 1995; Correll, 1998). Fleming et al. (1998), working with sub-temperate estuarine phytoplankton, found Redfield ratios of particulate organic nitrogen and particulate organic phosphorus (PON:POP) often indicated nitrogen limitation. Nitrogen fixing Cyanobacteria have a competitive advantage over other species in environments with low N:P ratios.

5.3 **Nitrogen research in the Swan River Estuary**

The Swan River, which varies seasonally between fresh and salt conditions, has been shown to vary between P and N limitation throughout the year, dependant on rainfall and river flow (John, 1987; Thompson and Hosja, 1996). Research by Thompson and Hosja (1996) has indicated, through the use of elimination bioassay techniques, that for the Swan River estuary nitrogen, rather than phosphorus, is the limiting nutrient. Based on strong correlation between rainfall and nitrate concentration for the Swan River Estuary during a 1993 - 1994 study (r² = 0.69) nitrate was suggested as the primary source of N for algal growth in the Swan River estuary (Thompson and Hosja, 1996). Their research into nutrient requirements for phytoplankton in the Swan River has demonstrated that nitrogen is up to 20 times more limiting to biomass development than phosphorous in this system.

Major differences in nitrogen metabolism and adaptations to environments with variable nitrogen supply have been demonstrated for various phytoplankton species (Dortch and Conway, 1984). An understanding of the nitrogen requirements and preferred nitrogen
sources for different blooms may provide an insight into the mechanisms of bloom development and maintenance. It could also provide an insight into determining the most appropriate remediation strategies aimed at maintaining bloom biomass below 'nuisance levels'.

5.4 Objectives

Despite many projections on the importance of nitrogen and, in particular, ammonium, on the success of phytoplankton growth in this system (Spencer, 1956; Jack, 1987; Douglas et al., 1996), there have been no studies measuring the uptake rates of nutrients for the Swan River phytoplankton communities. This study has attempted to clarify the nature of ambient population response to different N-sources, with a view to better understanding the potential for growth based on the forms of nitrogen available at various depths and times of year. This information is currently unavailable.

The major aim was to determine monthly and seasonal nitrogen uptake by phytoplankton communities of the three nitrogen species $\text{NO}_3^-$, $\text{NH}_4^+$ and urea representing "new" inorganic-based production, regenerated inorganic-based production and organic-based production respectively. Kinetic uptake parameters determined in Chapter 4 were used to adjust maximum uptake rates determined experimentally to ambient uptake rates. A measure of the potential phytoplankton ambient nitrogen uptake rates over an annual cycle was determined. This could then be related to annual trends in physico-chemical parameters (see Chapter 2) and to species compositional changes (see Chapter 3) in the upper Swan River estuary for the same period.

The role of dissolved organic nitrogen in phytoplankton nutrition was evaluated as a possible nitrogen source that has, to date, not been considered for the Swan River Estuary. Uptake rates for urea-N were determined over a twelve month period and used as an indication of the relative importance of organic nitrogen compared with inorganic forms (i.e. $\text{NO}_3^-$ and $\text{NH}_4^+$) in this system.

Uptake rates and the measured urea profiles over a 12-month period were compared with those for nitrate and ammonium to determine which nitrogen source was the
preferred source and therefore more likely to have an impact on the growth of phytoplankton in this system.

5.5 Materials and Methods

5.5.1 Field monitoring
During the 1998-99 monthly sampling regime, water was collected by positive displacement pump from surface (0.25 m) and bottom (4.5 m) depths at the Ron Courtney Island site (see Figure 2.1) at noon (between 1200 h and 1300 h) and at night 1 - 2 hours before sunrise (between 0300 h and 0600 h depending on season). All samples were gently pre-screened through a 300μm Nitex screen, kept cool and transported under low light conditions to the laboratory within one hour of collection.

Chlorophyll measurements were made from triplicate surface (0.25 m), mid-water (2.5 m) and bottom (4.5 m) water samples taken at the sample site. Chlorophyll determinations were made following standard methods (Strickland and Parsons, 1972). After sonication and overnight acetone extraction, sample absorbance was read on a Varian spectrophotometer. Calculations of total chlorophyll a (Chla) were made according to the equations of Jeffrey and Humphrey (1975, cited in Parsons et al., 1984).

Samples for species composition analysis were collected by pooling the remaining water from the three 6-litre water samples (collected for nitrogen uptake determinations, see below), gently mixing and then sub-sampling. Species identification and enumeration were made from Lugols-preserved samples as previously described (see Chapter 3).

Nutrient and chlorophyll samples were filtered under low (< 250 mm Hg) pressure through GF/C filters. Filters and filtrate were stored frozen for later analysis. Ambient nutrient profiles for NO$_3^-$ and NH$_4^+$ were obtained from the routine monitoring program by the Water and Rivers Commission or determined from samples sent to an analytical laboratory. Urea determinations using the diacetyl monoxime method (Price and Harrison, 1987), with a detection limit of 0.05 μM, were made on samples filtered under low (< 250 mm Hg) pressure.
5.5.2 Nitrogen uptake measurements

Nitrogen uptake rates were determined by the $^{15}$N dilution method of Dugdale and Goering (1967) as described in Chapter 4. In this case samples were inoculated with nitrogen at a concentration of 2 μM-$^{15}$N in the form of either $^{15}$N-NO$_3^-$ (99.9 atom% N), $^{15}$N-NH$_4^+$ (99.9 atom% N) or $^{15}$N-urea (99.9 atom% N) (ISOTEC, USA) and incubated in a controlled environment under ambient light and temperature conditions, as determined in the field, for four hours. Absolute uptake rates (μg N l$^{-1}$ h$^{-1}$) were calculated as previously described (Chapter 4) according to the equations of Dugdale and Goering (1967).

It is assumed that the calculated uptake rates presented here are maximal due to perturbation through addition of excess N-source (>10% ambient) above that being experienced in the environment (Goering et al., 1964; Dugdale and Goering, 1967; Glibert and Goldman, 1981; Harrison, 1983). It is possible to calculate ambient uptake rates by using a modification of the Monod equation for calculating nutrient limited growth from nutrient saturated growth-rate determinations. By substituting the uptake rate (ρ) for the nutrient saturated growth rate ($r_{max}$), then this equation becomes

$$\rho_{amb} = \rho S (k_s + S)^{-1} \quad \text{Eq. 5.1}$$

where $\rho_{amb}$ is the calculated uptake at ambient concentration, $\rho$ is the measured uptake under excess nitrogen (NO$_3^-$, NH$_4^+$ or urea) addition, $S$ is the substrate concentration and $k_s$ is the half-saturation constant (where $V = V_{max}/2$). Therefore monthly uptake rates (ρ), assumed to approximate $V_{max}$, were converted to ambient uptake rates ($\rho_{amb}$) using seasonal $k_s$ values determined from uptake kinetics experiments (see Chapter 4) and the above equation. All uptake rates are presented as ambient specific uptake rates (μg N ng Chla$^{-1}$ h$^{-1}$) based on the above and previously reported calculations (Eq. 4.1, 4.2 and 5.1).

Relative Preference Indices (RPI) were calculated according to the formulae:

$$\text{RPI} = \frac{\nu_{NH_4^+}}{(\nu_{NO_3^-} + \nu_{NH_4^+})^{-1}} \quad \text{Eq. 5.2}$$

$$\text{RPI}_{urea} = \frac{\nu_{urea}}{(\nu_{NO_3^-} + \nu_{urea})^{-1}} \quad \text{Eq. 5.3}$$
This is similar to the f-ratio, $v_{NO_3^-} (v_{NO_3^-} + v_{NH_4^+})^{-1}$ of Dugdale and Goering (1967) that provides a measure of the relative preference for new vs. regenerated nitrogen (Flynn 1998). As it is a direct function of the ratios of each uptake rate, a value of 0.5 indicates that each N source is taken-up equally. Values approaching one indicate that $NH_4^+$, or regenerated N, is preferred, while values approaching 0 show $NO_3^-$, or 'new' N, to be the preferred N-source (see Table 5.2).

Most studies investigating remineralisation, uptake, grazing and production (not reported in this document) use teams of 4+ people for field and laboratory work. For reasons of limited resources it was beyond the scope of this project to conduct remineralisation rate determinations. Where possible, these have been estimated from comparisons of changes in ambient $NH_4^+$ concentrations between consecutive sampling periods (see Chapter 6).

5.5.3 Data Analysis

Correlation coefficients between uptake rates and physico-chemical parameters were compared and presented as a correlation matrix. Principle component analyses of environmental and physiological data (PCA plots and side scatter plots) are presented in Appendix I. Graphical representations and curve fitting was achieved with SigmaPlot 4.0 (SPSS Inc., Chicago USA) or Microsoft EXCEL (Microsoft Corp., USA).

5.6 Results

Annual ranges of nitrogen specific uptake rates ($v$), normalised to units of chlorophyll $a$, for surface (0.25 m) $NO_3^-$, $NH_4^+$ and urea are compared with corresponding ambient N concentration in Figures 5.1 – 5.3. Absolute uptake rate ($p$) ranges obtained, over all times and depths, for the period February 1998 to January 1999 were 0.001 - 25.6 $\mu$g N l$^{-1}$ h$^{-1}$ for $NO_3^-$ and 0.003 – 36.4 $\mu$g N l$^{-1}$ h$^{-1}$ for $NH_4^+$. Specific uptake rates ($v$) were 0.2 ± 0.04 – 1831.1 ± 779.19, 0.5 ± 0.26 – 1731.6 ± 346.67 and 3.0 ± 0.60 – 2241.2 ± 252.56 ng N $\mu$g Chl$a^{-1}$ for $NO_3^-$, $NH_4^+$ and urea respectively (Figures 5.1, 5.2 & 5.3, A & C). Bottom (4.5 m) ambient uptake maximums were an order of magnitude lower during the day for all three nitrogen species (44.4 ± 15.39, 135.8 ± 34.24 and 81.0 ± 11.39 ng N $\mu$g Chl$a^{-1}$ for $NO_3^-$, $NH_4^+$ and urea respectively) (Figures 5.1, 5.2 & 5.3, B & D). The
Figure 5.1 Monthly ambient specific uptake rates and concentrations of NO$_3^-$ at RCI, February 1998 – January 1999.
Note scale change in (C)
Figure 5.2 Monthly ambient specific uptake rates and concentrations of NH₄⁺ at RCI, February 1998 – January 1999
Figure 5.3 Monthly ambient specific uptake rates and concentrations urea at RCI, February 1998 – January 1999
Figure 5.4 Diurnal comparison of specific uptake rates (v) for nitrogen sources for two depths over an annual cycle at RCI. 

$\text{NO}_3^-$ (A, B, C & D), $\text{NH}_4^+$ (E, F, G, & H) and urea (I, J, K & L) uptake, normalised to Chla, indicate Day surface (0.25m) rates of an order of magnitude greater than 4.5m and night both depths with least uptake for $\text{NO}_3^-$ uptake at night and at depth. February 1998 uptake rates were generally high for all species. Note day surface scale change. ■ day surface □ night surface ▬ day bottom ▪ night bottom (nd = no data)
large range in uptake rates produces a standard deviation of between 1.5 and 2 times the daily mean values for all nitrogen species investigated. For all nitrogen species studied the February 1998 ambient uptake rates are an order of magnitude greater than all other uptake rates determined for the remainder of 1998 and for those measured for the same month during 1996.

Corresponding ambient nutrient profiles (Figures 5.1 - 5.3, B & D) indicate very low (often < detection limits) levels of NO$_3^-$ throughout the water column during summer and autumn. A sudden spike in surface concentration follows immediately after the first rains. Uniform distribution through the water column is quickly established. The majority of total nitrogen during summer and autumn come from NH$_4^+$ and urea. Urea levels in surface and bottom depths are fairly uniform, while NH$_4^+$ is higher at depth during day time.

Diurnal comparison of specific uptake rates (v) for nitrogen sources for two depths over an annual cycle at RCI are presented in Figure 5.4. These indicate night-time uptake for NH$_4^+$ and urea for both depths (0.25 m and 4.5 m) to be greater than day time rates measured.

Total ambient specific uptake, $\Sigma \rho_{amb}$ (NO$_3^-$+NH$_4^+$+ urea) Table 5.2 and Figure 5.5A) indicated ranges of 4 ± 0.4 to 5533 ± 609.6 ng N µg Chla$^{-1}$ h$^{-1}$ over the period February 1998 to January 1999. February 1998 had much higher total uptake than the summer period (December and January) in early 1999. The relative proportions of the total each nitrogen species contributes are presented in bar graph form (Figure 5.5 B-E). Ammonium and urea tend to be taken up in similar proportions to total, with nitrate a smaller fraction of total nitrogen uptake over most of the year.

Diurnal comparisons of total specific uptake, $\Sigma \rho$, show deeper $\Sigma \rho$ (4.5 m) to be proportionally higher than surface $\Sigma \rho$ rates during night-time (Table 5.2). Day-time bottom (4.5 m) $\Sigma \rho$ was generally < 20% of surface rates. In contrast, night time $\Sigma \rho$ rates indicated bottom uptake to be 3 - 170 % of surface rates, the highest proportion being during summer and winter.
Seasonal ambient specific uptake rates uptake rates for NO$_3^-$, NH$_4^+$ and urea at RCI site, February 1998 - January 1999 (Figure 5.5) show highest surface uptake during summer and autumn for all nitrogen species, with bottom uptake rates showing little variation in specific uptake between seasons but large within-seasons variation.

**Table 5.2** Comparison of day and night total nitrogen specific uptake, $\Sigma$ (NO$_3^-$, NH$_4^+$, urea) (ng N $\mu$gChla$^{-1}$ h$^{-1}$) for surface (0.25m) and bottom (4.5m) depths over an annual cycle, February 1998 to January 1999, at Ron Courtney Island, an upper estuary site. Total ambient specific uptake rates (ngN $\mu$gChla$^{-1}$ h$^{-1}$) are means of sums of triplicate (n=9) measurements. (nd: no data)

<table>
<thead>
<tr>
<th>Season</th>
<th>Month</th>
<th>Surface Mean</th>
<th>sd</th>
<th>Bottom Mean</th>
<th>sd</th>
<th>Surface Mean</th>
<th>sd</th>
<th>Bottom Mean</th>
<th>sd</th>
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</thead>
<tbody>
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<td>134.0</td>
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<td>5075</td>
<td>202.6</td>
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<tr>
<td></td>
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<td>64</td>
<td>35.7</td>
<td>4</td>
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<td>1</td>
<td>0.1</td>
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<tr>
<td></td>
<td>M</td>
<td>693</td>
<td>737.4</td>
<td>135</td>
<td>36.5</td>
<td>113</td>
<td>10.6</td>
<td>59</td>
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<tr>
<td></td>
<td>J</td>
<td>773</td>
<td>71.8</td>
<td>nd</td>
<td></td>
<td>24</td>
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<td>75</td>
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<td>38.3</td>
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<td>9.9</td>
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<tr>
<td></td>
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<td>6.1</td>
<td>20</td>
<td>2.4</td>
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<tr>
<td></td>
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<td>48</td>
<td>83.7</td>
<td>152</td>
<td>75.7</td>
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<tr>
<td></td>
<td>D</td>
<td>677</td>
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<td>9.0</td>
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<td>200</td>
<td>35.5</td>
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<tr>
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<td>J</td>
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<td>191</td>
<td>4.0</td>
<td>nd</td>
<td></td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

For all seasons the uptake of NO$_3^-$ was a small fraction (11% - 25%) of total specific uptake ($\Sigma$V) while uptake of NH$_4^+$ and urea comprised equal portions of $\Sigma$V (Figure 5.5). Total ambient specific uptake, averaged over the three weeks during February 1996, compared with the value obtained for February 1998 (Table 5.4) shows considerable variability between years, with 1996 values only a fraction (13-28%) of February 1998 values. In contrast May values for 1996 are generally higher than those for 1998.
Figure 5.5 Seasonal comparison of N-source specific uptake relative to total specific uptake (Σv). NO₃⁻ always forms the least proportion of total uptake. (n=12)

Table 5.3 Seasonal comparison of diurnal total specific uptake rates of nitrogen (Σp) between bottom (4.5 m) and surface (0.25 m) depths.
Numbers are calculated from mean seasonal total uptakes (Table 5.2) and represent bottom as percentage of surface uptake (n=3).

<table>
<thead>
<tr>
<th></th>
<th>Day %</th>
<th>Night %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>sd</td>
</tr>
<tr>
<td>Autumn</td>
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</tr>
<tr>
<td>Winter</td>
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<td>13.01</td>
</tr>
<tr>
<td>Spring</td>
<td>39.7</td>
<td>49.78</td>
</tr>
<tr>
<td>Summer</td>
<td>18.2</td>
<td>9.35</td>
</tr>
</tbody>
</table>

RPI Values
Seasonal RPI values for NH₄⁺ and urea versus NO₃⁻ indicate preference for NH₄⁺ and urea throughout the year for both surface and bottom depths (Figure 5.5). An equal preference for NH₄⁺ and NO₃⁻ or urea and NO₃⁻ was found in 25% and 44% of the cases respectively. Eleven averages (69%) indicated a strong preference for ammonium
(RPI$_{H_4}$ + > 0.7) over nitrate. Urea was strongly preferred (RPI$_{urea}$ > 0.8) in five of 16 averages.

### 5.6.1 C:N ratios

Ratio of carbon to nitrogen in the particulate fraction over the twelve-month study period (Figure 5.6), exhibited a greater range for bottom samples (4.4 – 12.8) than for surface samples (5.3 – 11.9). Annual day surface mean was 9.1 ± 1.38, n= 33) and bottom mean was 8.9 ± 1.19 (n= 36). Night time surface mean was 7.6 ± 1.89 and bottom mean was 8.6 ± 2.58. There was no significant difference (P > 0.05) in particulate C:N ratios for replicate samples incubated for 4 hours with each of three different nitrogen sources, NO$_3^-$, NH$_4^+$ and urea. C:N ratios generally exceeded the Redfield ratio with an annual overall average of 8.4 ± 1.34. Average seasonal C:N ratio was below the predicted Redfield ratio only for night-time surface samples during summer (Figure 5.9). At all other times mean seasonal ratio values exceeded the Redfield ratio although standard error bars indicated variability which, at times, (particularly night bottom samples), dipped below this value. Daytime surface samples generally had higher C:N ratios than surface night time samples. Exceptions to this were May, where this trend was reversed, and the period August to October where samples from both times were similar.

Correlation analysis of uptake rates with environmental parameters and Chla distribution (Table 5.5), showed a significant relationship (P > 0.5) between vNO$_3^-$ and vNH$_4^+$ (P = 0.71), urea concentration (P = 0.83) and PAR (P = 0.53). There were also significant relationships between vNH$_4^+$ and vurea (P = 0.94), between orthoP and ambient NH$_4^+$ concentration, and between ambient NO$_3^-$ concentration and the N:P ratio of phytoplankton.
Figure 5.6 Total ambient specific uptake ($\Sigma (\nu NO_3^- + \nu NH_4^+ + \nu urea)$) (A) and proportional uptake of nitrogen species (B - E) over an annual period at RCI. • $NO_3^-$, • $NH_4^+$, • urea (nd = no data)
Figure 5.7 Seasonal specific ambient uptake rates for NO₃⁻, NH₄⁺ and urea at RCI site, February 1998 - January 1999.

Results indicate highest surface uptake during summer and autumn for all nitrogen species, with bottom uptake rates showing little variation between seasons. Note scale difference between surface and bottom plots and large within seasons variation in specific uptake. Means, 5th and 95th percentiles and error bars (n=9).
Figure 5.8 Relative Preference Indices (RPI) for ammonium and urea over nitrate.
RPI calculations based on ambient specific uptake rates and Elrifi and Turpin (1987) equation (Equ 5.3 and 5.4) n=9.
Table 5.4 Comparison of potential total ambient specific nitrogen uptake calculated for surface and bottom depths during 1996 and 1998. Values presented for February 1996 are means of triplicate samples taken over three consecutive weeks (n=9). November 1995 are means of triplicate samples from two consecutive weeks (n=6). All other values are means of triplicate samples from one sampling time (n=3). Inter-year variability is pronounced and variable between seasons.

<table>
<thead>
<tr>
<th>Month</th>
<th>Time</th>
<th>Depth (m)</th>
<th>Potential total ambient N-uptake (ngN·μg Chl·a·h⁻¹)</th>
<th>1996</th>
<th>1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>February</td>
<td>Day</td>
<td>0.25</td>
<td>2406.1 ± 561.80</td>
<td>5532.7 ± 609.57</td>
<td></td>
</tr>
<tr>
<td>(summer)</td>
<td></td>
<td>4.5</td>
<td>694.1 ± 60.34</td>
<td>822.4 ± 643.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Night</td>
<td>0.25</td>
<td>1644.3 ± 620.12</td>
<td>4096.7 ± 949.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5</td>
<td>2746.8 ± 618.12</td>
<td>5075.1 ± 202.58</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>Day</td>
<td>0.25</td>
<td>2891.2 ± 716.55</td>
<td>6928.2 ± 727.4</td>
<td></td>
</tr>
<tr>
<td>(autumn)</td>
<td></td>
<td>4.5</td>
<td>429.9 ± 29.41</td>
<td>134.9 ± 36.47</td>
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</tr>
<tr>
<td></td>
<td>Night</td>
<td>0.25</td>
<td>1915.3 ± 88.95</td>
<td>113.3 ± 10.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5</td>
<td>763.8 ± 134.35</td>
<td>59.2 ± 4.80</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td>Day</td>
<td>0.25</td>
<td>294.5 ± 113.87</td>
<td>533.8 ± 561.55</td>
<td></td>
</tr>
<tr>
<td>(spring)</td>
<td></td>
<td>4.5</td>
<td>198.4 ± 147.82</td>
<td>48.3 ± 83.74</td>
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</tr>
<tr>
<td></td>
<td>Night</td>
<td>0.25</td>
<td>654.7 ± 428.51</td>
<td>152.3 ± 75.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5</td>
<td>519.6 ± 374.67</td>
<td>69.3 ± 13.83</td>
<td></td>
</tr>
</tbody>
</table>

5.7 Discussion

The dynamic nature of nutrient cycling and nutrient fluxes enables natural assemblages of phytoplankton to grow at rates which permit biomass doubling in just a few days, even when nutrient concentrations are bordering on the limits of analytical detection (Eppley et al., 1977; McCarthy and Goldman, 1979). This occurs through adaptation of physiological processes that affect cell growth, such as adaptation of nutrient uptake and utilisation in response to environmental availability (Gibbs and Vant, 1997; Iriarte and de la Sota, 1997; Lomas and Glibert, 1999; Yin et al., 1998, Ullrich et al., 1998; Kudela et al., 1997), or productivity in response to altering light quality and availability (Gibbs et al., 1997; Irigoien and Castel, 1997; Kudela, 1997; Ullrich, 1998). Differences in the physiological state of phytoplankton appear more influenced by environmental factors such as light intensity and short-, medium- or long-term nitrogen depletion than by species differences (Morris, 1981).
Table 5.5 Correlation matrix of ambient nutrient concentrations and measured specific uptake rates for February 1998 to January 1999 at Ron Courtney Island. Values of $r^2 > 0.5$ (indicated in red) are considered significant.

<table>
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<th>$\nu\text{NH}_4^+$</th>
<th>$\nu\text{urea}$</th>
<th>PAR</th>
<th>orthoP</th>
<th>orgP</th>
<th>TP</th>
<th>KN</th>
<th>TN</th>
<th>[NO$_3^-$]</th>
<th>[NH$_4^+$]</th>
<th>[urea]</th>
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<tr>
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</tr>
<tr>
<td>$\nu\text{urea}$</td>
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<td>-0.10</td>
<td>0.03</td>
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<td></td>
</tr>
<tr>
<td>[urea]</td>
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<td>0.09</td>
<td>0.11</td>
<td>0.05</td>
<td>0.03</td>
<td>0.19</td>
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<td>0.57</td>
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<td>-0.19</td>
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</tbody>
</table>
Figure 5.9  Seasonal C:N ratio for day and night surface (0.25 m) and bottom (4.5 m) samples at Ron Courtney Island, upper Swan River Estuary for the period February 1998 to January 1999. Values above Redfield ratio of 6 (dotted line) indicate non-nitrogen limited growth (n=9).

**Monthly uptake rates**

Phytoplankton growth is usually measured as a whole community response with chlorophyll representing an index of abundance (Smayda, 1997). When determined for naturally occurring assemblages this represents the sum of the different growth rates of the component species and also must incorporate chlorophyll losses due to concurrent grazing pressure within the community being assessed. Thus, in this case, it represents net community growth rate.

Absolute uptake rate ($\rho$) ranges obtained, over all times and depths, for the period February 1998 to January 1999 were 0.001 - 25.6 $\mu g$ N l$^{-1}$ h$^{-1}$ for NO$_3^-$ and 0.003 – 36.4 $\mu g$ N l$^{-1}$ h$^{-1}$ for NH$_4^+$ and fall within the range of uptake rates for NO$_3^-$ and NH$_4^+$ reported elsewhere (MacIsaac and Dugdale, 1969; Paasche et al., 1984; Neuer and Franks, 1993; Lieberman et al., 1994). Upper rates for both NO$_3^-$ and NH$_4^+$ in this study (25.6 and 36.4 $\mu g$ N l$^{-1}$ h$^{-1}$) indicates that total absolute uptake rates (range 0.01 ± .003 and 81.4 ± 1.34 $\mu g$ N l$^{-1}$h$^{-1}$) for both day and night-time rates are similar to reported values, although both NO$_3^-$ and NH$_4^+$ upper ranges are higher. Day-time surface total absolute uptake ($\rho_{total}$) in the upper Swan River estuary exceeds night-time
surface uptake at all times of the year, with mean day surface uptake six times that of night surface and bottom rates.

The RCI site total specific uptake ranged between 0.004 and 5.53 μg N μg Chl a⁻¹ h⁻¹. Specific uptake rates are usually presented in units of time as the incorporation of ¹⁵N into the particulate fraction is normalised against total N in the sample. In this study specific uptake rates have been normalised against Chl a rather than N, as in Levasseur et al. (1990), and constant Chl a : N is assumed (Kokkinakis and Wheeler, 1987) in order to compare uptake per unit chlorophyll between months and to enable a direct comparison with grazing rates which are based on chlorophyll loss per day.

The greater proportion of the total specific uptake at all times and all depths throughout the 12 month period investigated was due to urea and pNH₄⁺. Nitrate uptake was dominant in surface waters during winter, when nitrate levels were elevated following seasonal rains. This supports the findings that in waters low in NO₃⁻ (< 5 mM) phytoplankton growth was fuelled primarily by regenerated N (71% total uptake) whilst, in NO₃⁻ rich waters (> 20mM), growth was supported by new N (83% total) (Kokkinakis and Wheeler, 1987). Seasonal diurnal comparisons of surface and bottom uptake rates indicate day time bottom (4.5 m) uptake to be < 20% of surface (0.25 m) rates. Night time bottom uptake is seasonally a much higher percentage of day-time uptakes (53 – 170%), exceeding surface uptake during summer and winter. The diurnal migration of dinophytes has been demonstrated for the January 1996 summer bloom (Rosser and Thompson, 2001; Hamilton et al., 1999). Higher bottom uptake rates during winter, which is dominated by diatoms, particularly pennate forms (see Chapter 3) may be due to enhanced periphytic diatom growth under favourable conditions of light and turbidity.

Winter typically has high levels of detrital material and associated bacterial biomass may be significant. Results from the Delaware Estuary suggest that NH₄⁺ uptake by bacteria is relatively low in eutrophic systems (Hoch and Kirchman, 1995). This has important implications for the role of heterotrophic bacteria in the N cycling of marine environments and also suggests phytoplankton are responsible for most of the uptake. It is suggested that the reverse situation may explain high uptake rates in the upper Swan
River Estuary. The seasonal role of bacteria in nitrogen cycling in the Swan needs further examination.

Temporal variability in physical and chemical factors has been shown to influence phytoplankton population growth and community structure (Harris, 1986; Thompson, 1998 and 2001). A statistical comparison of uptake rates, ambient nutrient concentrations and chlorophyll a distribution over an annual cycle indicated a significant positive correlation ($r^2 > 0.7$) between uptake rates for the three nitrogen sources investigated. The relationship between urea uptake and ammonium uptake was highly significant ($r^2 = 0.94$).

**Table 5.6** Comparison of absolute uptake rates for nitrate and ammonium from a variety of aquatic environments.

Literature values have been converted to $\mu$m N $l^{-1}$ $h^{-1}$ for comparison with rates determined in this study.

<table>
<thead>
<tr>
<th>Site</th>
<th>Locality</th>
<th>N uptake range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NO$_3^-$ ($\mu$gN $l^{-1}$ $h^{-1}$)</td>
<td>NH$_4^+$ ($\mu$gN $l^{-1}$ $h^{-1}$)</td>
</tr>
<tr>
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</table>

The significant correlation between NO$_3^-$ uptake and PAR is not unexpected since uptake of NO$_3^-$ is facilitated by energy from photosynthesis, although dark uptake by phytoplankton does occur (Conway and Whitledge, 1979, Paasche et al., 1984; Kanda et al., 1985). At the RCI site there is an apparent base dark nitrate uptake rate that is evident for surface and bottom phytoplankton communities at night and for bottom
communities during the day. Light enhanced nitrate uptake for surface samples during the day creates a 6-fold increase in nitrate uptake. In contrast Takahashi *et al.* (1995) found total uptake rates (NO$_3^-$ + NH$_4^+$ + urea) in the range 0.062 – 0.59 μg N l$^{-1}$ h$^{-1}$ with no clear difference between night and daytime uptake rates. They concluded that light was not a significant factor in nitrogen uptake regulation in Lake Biwa, Japan, but rather related to temperature and nitrogenous nutrient concentration.

**RPI**

The use of relative preference indices has indicated a hierarchy of preference for ammonium over urea and nitrate for a variety of aquatic systems (Glibert *et al.*, 1982; McCarthy *et al.*, 1982; Dortch *et al.*, 1991; Twomey *et al.*, 2002). Stolte and Riegman (1996) caution interpretation of relative preference indices (RPI) as reflecting ambient nutrient concentrations rather than nutrient preference under conditions of large discrepancies between ambient nitrogen source concentrations. For this reason the Elrifi and Turpin equation (1987) was used to eliminate the double inclusion of an ambient nutrient concentration correction factor. This factor is included in the calculation of ambient specific uptake rate (see Eq. 4.1 and 5.1). The upper Swan River Estuary phytoplankton communities also exhibit a strong preference for recycled and organic nitrogen forms over nitrate. It was found that for the 12 month period February 1998 to January 1999, urea and ammonium were preferred over nitrate as nitrogen sources, with the majority of cases (63%, n = 32) where RPI > 0.70.

Combining correlation analysis information with the data on RPI indices and proportion of total uptake contributed by each of these nitrogen sources, indicates that the phytoplankton community has a strong affinity for internally recycled nitrogen, whether of an organic or inorganic nature.

**C:N ratios and growth on N-sources**

Based on C:N ratios, it has been shown that there is variability in a species' ability to assimilate N from different nitrogen sources. Levasseur *et al.* (1993) found that phytoplankton grown on different N-sources have different responses in terms of the effect on C:N ratios. A diatom (*Chaetoceros gracilis*) and phytoflagellate (*Akashiwo sanguinea*) grown under culture conditions, had higher C:N ratios when grown in urea based media rather than NO$_3^-$ or NH$_4^+$. PC:PN ratios for all samples generally exceeded
the Redfield ratio, with an annual overall average of 8.4 ± 1.3. Samples incubated with different nitrogen sources showed no significant difference in PC:PN ratios. There was not a great deal of separation between the final C:N ratios of phytoplankton incubated for 4 h on different nitrogen sources (Figure 5.7) in this study. What can be seen are variations in C:N ratios between surface and bottom samples. March, April and June night-time bottom samples have higher C:N ratios than the corresponding surface samples. Since phytoplankton are not photosynthetically active at night and are unable to produce carbon skeletons, it is assumed that the high ratios are a result of lowered or limiting nitrogen levels in the particulate fraction.

The role of organic nitrogen in the upper Swan River Estuary

Tracer experiments using $^{15}$N suggest that the available DON pool can be rapidly produced from inorganic N additions and be available for subsequent utilisation by the phytoplankton population (Bronk and Glibert, 1991 &1993; Bronk et al., 1994). Urrea concentrations in sea surface waters have been detected in the range of 2.7 – 21% of dissolved organic nitrogen (DON) levels (Carpenter et al., 1972). Ranges for urrea concentration in unpolluted waters (0 - 165.2 µg urea-N l$^{-1}$), riverine (15.7 - 249.2 µg urea-N l$^{-1}$) and estuaries (0.84 - 249.2 µg urea-N l$^{-1}$) have been reported (Antia et al. 1991; Seitzinger and Sanders, 1997). A time series analysis of lacustrine urrea concentrations in Lake Kinneret, Israel, showed urrea to be at greater concentrations than reported for marine environments, but always less abundant than ammonium (Berman, 1974).

Levels of urrea have not previously been monitored or related to phytoplankton growth in the Swan River Estuary. Monitoring at an upper estuary site for the Swan River Estuary detected ranges of 14.8 – 117.7 µg urea-N l$^{-1}$, within the reported range of urrea concentrations in a variety of aquatic habitats (0.43 – 124.6 µg urea-N l$^{-1}$, Antia et al., 1991), with a diurnal depth-averaged mean of 40.1 ± 14.9 µg urea-N l$^{-1}$ over an annual period (February 1998 – January 1999). Urea concentration represented 104% - 287% of total DIN (NO$_3^-$ + NH$_4^+$) during summer (see Chapter 2). Inputs of organic nitrogen to estuaries from rivers can account for 14 - 90% (av. 37%, n = 17) of total nitrogen loading (Seitzinger and Sanders, 1997 and references therein).
The measured ambient specific uptake rates for urea (3.0 ± 0.6 – 2241.2 ± 252.56 ng urea-N μg Chl a⁻¹ h⁻¹) fell within the previously reported range for estuarine waters (0.43 – 124.6 μg urea-N l⁻¹; Antia et al., 1991). Based on seasonal averages, this represents 27.5% - 40% of total N uptake over the annual period. This is lower than the 70% - 80% of total uptake reported for the Chesapeake Bay estuary (Glibert, 1999). Both systems report the highest proportion of urea uptake occurring in summer.

It has been reported that ciliates are a particularly important source of purines, one of the components of DON, in aquatic environments and that there may be a nitrogen cycle involving ciliates and phytoplankton mediated by purines (Antia et al., 1991; Palenik and Hensen, 1997). The maximum measured uptake rates and highest RPI values for urea, another DON component, occurred during the winter months at a time when microheterotroph grazer species composition analysis showed strombolid ciliates to be the dominant grazer and also highest numerically during that season. During this season the pennate diatom Gyrodiniotheca closterium, an indicator of organic pollution (Jacob John, pers. comm.), was dominant. It is suggested that phytoplankton growing at this time are conditioned to make use of organic nitrogen sources that may be produced by heterotrophic community members.

5.8 Conclusions

Because N-uptake, unlike C-uptake, can continue at night, calculation of daily N-uptake requires diurnal studies. This study has found that night time bottom uptake is seasonally a much higher percentage of day time uptakes (53 – 170%), exceeding surface uptake during summer and winter. Seasonal diurnal comparisons of surface and bottom uptake rates indicate day-time bottom (0.4 m) uptake to be < 20% of surface (0.25 m) rates.

Differences in the magnitude of diurnal periodicity between NO₃⁻ and NH₄⁺ uptake may be a result of ‘buffering’ by heterotrophic bacteria (Cochlan et al., 1991). It has been suggested that these bacteria may be responsible for a significant fraction of the total NH₄⁺ uptake (Laws et al., 1985; Wheeler and Kirchman, 1986). For this reason there needs to be further work in the Swan River Estuary to determine the extent to which
bacteria influence uptake and regeneration rates and support directly or indirectly the growth of autotrophic, heterotrophic and mixotrophic phytoplankton species.

Urea and NH$_4^+$ species constitute the highest proportion of total ambient specific nitrogen uptake (Σp$_{amb}$) at most times and depths throughout the 12 month period in this system. Many studies have highlighted the importance of NH$_4^+$ to phytoplankton growth over a range of aquatic environments. While anecdotal evidence for the importance of NH$_4^+$ has been postulated, to date most studies have focussed on the influence of NO$_3^-$ on Swan River Estuary phytoplankton distribution and succession. This study confirms anecdotal evidence of the importance of deep NH$_4^+$ to the success of vertically migrating dinophyte species during summer.

Greater understanding of the role of DON should be an important focus for future work in N-cycling in the Swan and Canning Rivers and Estuary, as urea forms such a seasonally significant nitrogen source (up to 287% total DIN in summer). Further research into urea or DON cycles in this system is recommended.

5.9 References


Price, N. M., W. P. Cochlan and P. J. Harrison. 1985. Time course of uptake of inorganic and organic nitrogen by phytoplankton in the Strait of Georgia:


CHAPTER 6: Phytoplankton of the Swan-Canning Estuary: a comparison of nitrogen uptake by seasonal bloom assemblages

6.1 Abstract

Spring Chlorophyte- and summer and autumn mixed Dinophyte-dominated phytoplankton blooms occurring in the upper reaches of the Swan-Canning Estuary have been studied to compare species composition and diurnal distribution and to determine preferential uptake rates for different nitrogen sources. Species distribution patterns over diurnal cycles showed Dinophyte species (Gymnodinium simplex, Scripsiella sp. and Oxyrrhis marina) exhibiting diurnal vertical migration patterns which changed during a three-week bloom period. The Dinophyte behaviour appeared to be related to measured changes in ambient nitrogen profiles over the course of the bloom. Oxyrrhis marina dominated the autumn bloom and was not shown to be strongly migratory over diurnal periods at this time. Absolute uptake rates ($\rho$, approximating $V_{med}$) for nitrogen, in the form of $^{15}$NO$_3^-$, $^{15}$NH$_4^+$ or $^{15}$N-urea, were compared between seasonal bloom assemblages and ranged between 0.02 and 12 $\mu$g N l$^{-1}$ h$^{-1}$ ($\text{NO}_3^-$ 0.02 - 7; $\text{NH}_4^+$ 0.4 – 9; urea 0.1 – 12 $\mu$g N l$^{-1}$ h$^{-1}$). Specific uptake rates ($\nu$) ranged between 2 and 1770 ng N $\mu$g Chla$^{-1}$ h$^{-1}$ (NO$_3^-$ 2 - 290; NH$_4^+$ 19 – 1770; urea 6 – 1730 ng N $\mu$g Chla$^{-1}$ h$^{-1}$). Relative Preference Indices (RPI) for NO$_3^-$ (new N) vs. NH$_4^+$ (recycled N) were calculated to determine whether allochthonous or autochthonous nitrogen sources were implicated in supporting these blooms. Results indicated equal physiological preference (RPI $\sim$ 0.5) at all depths examined for NO$_3^-$ and NH$_4^+$ during spring and autumn, and for summer surface assemblages. There was a strong preference for NH$_4^+$ (RPI $\sim$ 0.95) at mid and bottom depths during summer and weaker preference at mid and bottom depths during autumn.

From flux measurements, it was found that apparent reappearance rates for NH$_4^+$ ranging between 1% and 1030% of absolute uptake rates occurred during summer, with decreases equivalent to 100% of ambient uptake rate occurring at depth at night. Nutrient profiling alone, in the absence of knowledge of nutrient fluxes, was insufficient in assessing possible nutrient control over phytoplankton growth and biomass.
maintenance. Within this system there is the potential for phytoplankton growth and bloom maintenance to occur supported solely by recycling of NH₄⁺.

6.2 Introduction

Research into the preferential uptake of different nitrogen sources has indicated that this can vary with phytoplankton class and seasonal environmental conditioning for a variety of aquatic systems (Balone et al., 1998; Mallin et al., 1999; Collos et al., 2003; Fan et al., 2003; Tungaraza et al., 2003).

Although much attention has been given to the importance of reducing phosphorus levels in the Swan-Canning Estuary, previous research into phytoplankton growth has indicated that nitrogen, rather than phosphorous, is the limiting nutrient for this estuarine system. In their research into nutrient requirements for phytoplankton in the Swan River, based on bioassay studies, Thompson and Hosja (1996) demonstrated that nitrogen is up to 20 times more limiting to biomass development than phosphorus in this system. Chapelle et al. (1995) report similar findings from using three-dimensional hydrodynamic and biological model simulations which indicated reduction of nitrogen inputs to be more effective in limiting phytoplankton growth than reductions to phosphorus loadings.

Despite many projections on the importance of nitrogen and, in particular, ammonium, on the success of phytoplankton growth in this system (Spencer, 1956; Jack, 1987; Douglas et al., 1996), there have been no studies measuring the uptake rates of nutrients for the different Swan River phytoplankton seasonal bloom communities. An understanding of the nitrogen requirements and preferred nitrogen sources for different blooms may provide an insight into the mechanisms of local bloom development and maintenance. This would help in determination of the most appropriate remediation strategies aimed at maintaining bloom biomass below 'nuisance levels'.

6.3 Objectives

The objectives of this study were as follows: First, to determine the physiological predisposition of bloom assemblages during spring, summer and autumn to take-up different nitrogen sources. Maximum nitrogen uptake rates (\(V_{\text{max}}\)) and preferential
nitrogen sources were determined for the different major bloom types occurring during spring to autumn in the upper Swan River Estuary.

Second, to relate these uptake rates and preferences, together with the seasonal nitrogen source availability, to the pattern of succession for phytoplankton blooms (see Chapter 2). The occurrence of diurnal vertical migration in certain bloom species was related to ambient nitrogen profiles.

Third was to compare calculated ambient uptake rates with diurnal changes in ambient nutrient concentrations for the preferred N source, NH₄⁺, for the summer bloom in order to determine the relationship between uptake rates and apparent ambient nutrient availability in bloom development and/or maintenance.

6.4 Materials and Methods

6.4.1 Field monitoring

Chlorophyte-dominated (spring, November 1995 & 1997), and mixed Dinophyte-dominated (summer, January 1996 and autumn, May 1996) bloom events were studied at Ron Courtney Island (RCI), an area representative of the deeper (−6 m) sites of the upper Swan River Estuary. Chlorophyll measurements were made from triplicate surface (0.25 m), mid-water (2.5 m) and bottom (4.5 m) water samples taken at the sample site, and from profiles (1 m increments) made at RCI and several sites above and below the RCI site. This sampling was to verify that trends in chlorophyll distribution patterns seen at the RCI experimental site were representative of trends occurring over a larger area of the river, and not attributable to local patchiness or advection. Samples for species composition analysis were collected by pooling the remaining water from the three 6-L water samples (collected for nitrogen uptake determinations, see below), gently mixing and then sub-sampling. Species identification and enumeration were made from Lugols-preserved samples as described in Chapter 3.

Ambient nutrient profiles for NO₃⁻ and NH₄⁺ were obtained from the routine monitoring program by the Water and Rivers Commission or determined from samples sent to an analytical laboratory. Nutrient and chlorophyll samples were filtered through GF/C filters and the filters stored frozen for later analysis. Nitrate was measured by the
cadmium reduction method and ammonium was measured by the phenate method (Clesceri et al., 1989). Chlorophyll determinations were made following standard methods (Strickland and Parsons, 1972).

6.4.2 Nitrogen uptake

Nitrogen uptake rates were determined by the $^{15}$N dilution method of Dugdale and Goering (1967) from water samples collected at the RCI site. For seasonal bloom studies three depths (surface, 0.25 m; midwater, 2.5 m and bottom, 4.5 m) were sampled. The spring (November) and summer (January) blooms were sampled at three times (approx. 0900 h-am, 1400 h-pm and 2400 h-night) over a diurnal period. The autumn bloom (May) was sampled twice (1200 h, noon and 0300 – 0400 h, night). Water samples were collected as described in Chapter 4 methods and processed as described in Chapter 5 for $^{15}$N uptake rate determinations. All specific bloom studies were incubated in situ at the depth of collection to maintain ambient light and temperature conditions. Bottles were suspended in purpose built racks in a staggered manner to ensure no shading occurred from those at shallower depths.

Absolute uptake rates ($\rho$, $\mu$g N l$^{-1}$ h$^{-1}$), specific uptake rates ($\nu$, ng N $\mu$g Chl$\alpha$ $^{-1}$ h$^{-1}$) and Relative Preference Indices (RPI) were calculated as outlined in Chapters 4 and 5. In order to determine whether observed changes in ambient ammonium concentration between sampling times reflected the uptake rates determined, a comparison of observed vs. expected NH$_4^+$ concentrations was made. Uptake rates ($\rho$, approximating $V_{\text{max}}$) determined for summer were converted to ambient uptake rates using a $k_s$ value (4.4 $\mu$M) for summer NH$_4^+$ uptake, determined by the authors (Chapter 4), and the Michaelis Menten model (Maclsaac and Dugdale 1969), $V_{\text{amb}} = (V_{\text{max}} - S)(k_s + S)^{-1}$, where $V_{\text{amb}}$ is the calculated uptake at ambient concentration, $V_{\text{max}}$ is the measured uptake under excess NH$_4^+$ addition, $S$ is the substrate concentration and $k_s$ is the half-saturation constant (where $V = V_{\text{max}}/2$).

Uptake of NH$_4^+$ was calculated between subsequent sampling periods (i.e. morning to afternoon, afternoon to night) and this was compared with the absolute change in ambient NH$_4^+$ concentration over this same period (see Table 6.4). Uptake rate was assumed to remain constant over this time period and ambient NH$_4^+$ concentration
differences relative to expected ambient nutrient concentration were based on calculated loss due to the measured absolute uptake rate (µg N l⁻¹ h⁻¹) corrected to ambient. The observed change in ambient NH₄⁺ concentration was calculated as follows:

\[ \delta [\text{NH}_4^+]_{\text{observed}} = [\text{NH}_4^+]_a - [\text{NH}_4^+]_a \]  \hspace{1cm} (Eq. 6.1)

where \([\text{NH}_4^+]_a\) is the ambient concentration at time \(t_1\), and \(t_2\) represents a consecutive time of day sampling period, ie morning and afternoon or afternoon and night.

To calculate the expected change in ambient concentration, the ambient absolute uptake rate \(V_{\text{amb}}\) for one sampling time, say morning, was multiplied by the number of hours between the first \((t_1)\) and subsequent \((t_2)\) sampling time.

\[ \delta [\text{NH}_4^+]_{\text{expected}} = V_{\text{amb}(t)} \times (t_2-t_1)h \]  \hspace{1cm} (Eq. 6.2)

The calculated total expected loss of NH₄⁺ over this time period was then compared to the observed change determined as above (see Table 6.4 later).

### 6.5 Results

#### 6.5.1 Uptake rates

Addition of 2 µM N as \(^{15}\text{N}-\text{NO}_3^-, \(^{15}\text{N}-\text{NH}_4^+\) or \(^{15}\text{N}-\text{urea}\) in these experiments represented an average nutrient enrichment of 540% ambient (range 16 – 1100%) and therefore uptake rates represent \(V_{\text{max}}\) as defined in Michaelis Menten uptake kinetics. Absolute uptake rate (µ) ranges for NO₃⁻, NH₄⁺ and urea respectively (Table 6.1) were 0.02 – 7, 0.4 – 8 and 0.1 – 12 µg N l⁻¹ h⁻¹. Absolute uptake rates translated to higher specific uptakes rates when biomass levels, represented by Chlₐ, were lower, as in morning bottom samples for November week 1. The results of statistical between-depth comparisons of mean uptakes at each time period for each week (see Table 6.2) and between-weeks comparison (Table 6.3) are included to show the significance of variability of uptake \(V_{\text{max}}\) over depth and time.
Spring

Spring ranges of absolute uptake rates were 0.1 - 6, 0.4 - 6 and 0.2 - 7 μg N l\(^{-1}\) h\(^{-1}\) for NO\(_3^-\), NH\(_4^+\) and urea respectively. Plots of specific uptake rates (\(v\)) for NO\(_3^-\), NH\(_4^+\) and urea (Figure 6.1 a - c) show a wide variation with both depth and time. The 1995 spring chlorophyte blooms (Figure 6.1 a - f) showed specific uptake in the ranges of 7 - 283, 19 - 488 and 6 - 552 ng N μg Chla\(^{-1}\) h\(^{-1}\) for NO\(_3^-\), NH\(_4^+\) and urea respectively. There was no significant difference (\(p > 0.05\)) between depth averaged mean uptake of NO\(_3^-\) or NH\(_4^+\) for week 1 (both 113 ± 1 ng N μg Chla\(^{-1}\) h\(^{-1}\)) and week 2 (94 ± 14 and 174 ± 31 ng N μg Chla\(^{-1}\) h\(^{-1}\) respectively), while urea uptake for week 2 (depth average mean 239 ± 48 ng N μg Chla\(^{-1}\) h\(^{-1}\)) was significantly higher than week 1 (average 9 ± 0.008 ng N μg Chla\(^{-1}\) h\(^{-1}\)). Day time uptake of NO\(_3^-\) and NH\(_4^+\) during both weeks of this bloom showed variation over depth (\(P < 0.05\), see Table 6.2), while urea uptake was not significantly different between depths during either week. There were no significant night-time differences between depths for specific uptake of NO\(_3^-\), NH\(_4^+\) or urea during either week of the spring bloom (\(P > 0.05\)). By week 2 of this bloom, bottom uptake favoured NH\(_4^+\) (RPI range 0.80 - 0.91), while there was a strong preference for NO\(_3^-\) at mid-depth in the morning (RPI = 0.24).

Summer

For the summer dinophyte bloom (January 1996), absolute uptake (\(p\), approximating \(V_{\text{max}}\)) ranges were 0.02 - 7, 0.8 - 8 and 0.1 - 12 μg N l\(^{-1}\) h\(^{-1}\) (Table 6.1) for NO\(_3^-\), NH\(_4^+\) and urea respectively. Specific uptake rates (\(v\)) for NO\(_3^-\), NH\(_4^+\) and urea were 2 - 289, 63 - 1766, and 21 - 569 ng N μg Chla\(^{-1}\) h\(^{-1}\) respectively (see Figure 6.1 g - o). NH\(_4^+\) and urea had the highest \(V_{\text{max}}\) values. Both NH\(_4^+\) and urea uptake rates generally exceeded NO\(_3^-\) uptake at mid and bottom depths. Within-week comparison of specific uptakes between depths (see Table 6.2) showed no significant difference (\(P > 0.05\)) during daytime week 1 for NH\(_4^+\), while differences were significant between depths for NO\(_3^-\) and urea. Morning week 2 and afternoon week 3 mid-water NH\(_4^+\) uptake rates were significantly greater than surface uptake (\(P < 0.02\), see Table 6.2). NO\(_3^-\) specific uptake rates were significantly higher (\(P < 0.05\)) in surface waters during the day for all three weeks of this bloom. Bottom NO\(_3^-\) uptake rates were significantly higher at night-time for weeks 2 and 3. Surface ranges of specific uptake for NO\(_3^-\) during the day were 87 -
289 ng N µg Chla⁻¹ h⁻¹ and for night time were 29 – 158 ng N µg Chla⁻¹ h⁻¹. Maximum uptake rates were obtained in surface samples (average 175 ± 97 ng N µg Chla⁻¹ h⁻¹), while average mid- and bottom-water NO₃⁻ uptake was approximately half this (average 74 ± 8 ng N µg Chla⁻¹ h⁻¹). The bottom NO₃⁻ uptake rate exceeded surface uptake on one occasion (night-time, third week of a dinoflagellate bloom; see Figure 6.1 o). Surface uptake of urea during the day (average 436 ± 190 ng N µg Chla⁻¹ h⁻¹) was generally significantly higher (P < 0.05) than mid and bottom uptake rates during week 1 and week 3 of the summer bloom. Night time uptake of urea for week 1 was lowest at mid-water depth, while week 2 and week 3 showed significant decreases (P < 0.02) with increasing depth.

**Autumn**

The autumn (May 1996) Dinophyte bloom ranges for absolute uptake for NO₃⁻, NH₄⁺ and urea were 0.08 – 2.9, 0.5 – 2.9 and 1.1 – 6.6 µg N l⁻¹ h⁻¹ respectively. Specific uptake rates recorded for NO₃⁻, NH₄⁺ and urea were 30.6 – 612, 217 – 932.3 and 193 – 1049 ng N µg Chla⁻¹ h⁻¹ respectively (see Figures 6.1 p & q). Daytime rate measurements for NH₄⁺ and urea were approximately twice those for NO₃⁻. Depth-averaged mean urea uptake (day: 1122 ± 704 ng N µg Chla⁻¹ h⁻¹, night: 706 ± 287 ng N µg Chla⁻¹ h⁻¹) was significantly higher (P < 0.05) than NH₄⁺ uptake for the same periods. Night-time uptake rates for NH₄⁺ and urea were similar throughout the water column, but were approximately half day time rates for surface uptake. Bottom uptake rates for NO₃⁻ at night were about five-fold larger than daytime rates.
Figure 6.1 Specific uptake rates for seasonal bloom assemblages.

6.5.2 Nutrient profiles

Nutrient profiles (Figure 6.2 a-f) taken over diurnal periods showed that, from spring through to autumn, ambient nitrate concentrations were very low (<5 – 16 μg l\(^{-1}\)) throughout the water column. In all but two cases (November 21 Figure 6.2 d-f, & January 24 Figure 2 m-o) surface NO\(_3^−\) was below detectable levels (<5 μg l\(^{-1}\)) in surface, mid and bottom depths over 24 hours for November 21, and during day time for January 17 (Figure 6.2 j and k) and May 28 (Figure 6.2 p). Ammonium levels ranged between <5 (denoted as 2.5 μg l\(^{-1}\) for graphical and analytical purposes) and 170 μg l\(^{-1}\). The highest concentration occurred during summer, and the lowest occurred during the third week of the summer bloom (January 24, range < 5 – 8 μg l\(^{-1}\)). The concentration of NH\(_4^+\) was equal to or in excess of NO\(_3^−\) concentration, with the ratio of NH\(_4^+\):NO\(_3^−\) ranging from 1 to 21. Highest NH\(_4^+\) concentrations were always associated with bottom samples. Night time decreases in NH\(_4^+\) concentration at 4.5 m (bottom) depths were apparent during the first week of the spring bloom (November 14; from 46 μg l\(^{-1}\) down to 5 μg l\(^{-1}\) NH\(_4^+\)-N) and for all summer bloom measurements (January 10, from 170 down to 130 μg l\(^{-1}\) NH\(_4^+\)-N; January 17, from 36 down to 19 μg l\(^{-1}\) NH\(_4^+\)-N and January 24, from 46 down to 5 μg l\(^{-1}\) NH\(_4^+\)-N.

Concentrations of inorganic nitrogen species for different blooms varied with depth and time of day. Spring NO\(_3^−\) (Figure 6.2 A) concentrations decreased from week 1 (November 14; depth averaged mean 6 g N l\(^{-1}\)) to week 2 (November 21; depth averaged mean < 5 μg N l\(^{-1}\)). Concentrations of NH\(_4^+\) over the duration of November 14 (week 1, Figure 6.2 B) decreased for each water column sample (November 14; depth averaged mean morning 39 μg N l\(^{-1}\), night 5 μg N l\(^{-1}\)). Depth averaged mean daytime NH\(_4^+\) concentrations decreased from week 1 (30 ± 17 μg N l\(^{-1}\)) to week 2 (21 ± 4 μg N l\(^{-1}\)) but were not significantly different (P > 0.05). Concentrations of NH\(_3^+\) were always higher at depth.

Summer NO\(_3^−\) concentration (Figure 6.2 C) ranged between < 5 – 15 μg N l\(^{-1}\) (average 5 ± 3 μg N l\(^{-1}\)). Although there is apparent variation between depths and times of day, there appears to be a general trend in NO\(_3^−\) reduction throughout the water column over
the bloom. There is a small increase in the concentration of NO₃⁻ in bottom samples between morning (11 µg N l⁻¹) and afternoon (15 µg N l⁻¹), with a decrease at night (6 µg N l⁻¹) for the January 10 nutrient profiles. Summer NH₄⁺ profiles (Figure 6.2 D) indicate higher concentrations occurring with increasing depth. The high bottom (4.5m) NH₄⁺ concentrations of the first week (153 ± 31 µg N l⁻¹, approximately four-fold increase over surface level were reduced to 5 µg N l⁻¹ by night time of the third week. Autumn noon NO₃⁻ and NH₄⁺ profiles only were obtained (Figure 6.2 E and F) and indicated a uniform nutrient concentration throughout the water column. NH₄⁺ levels (depth averaged mean 13 ± 3.5 µg N l⁻¹) were consistently more than an order of magnitude higher than NO₃⁻ levels (depth averaged mean <0.5 µg N l⁻¹).

**Preferential uptake related to ambient nutrient concentrations**

As NH₄⁺ was the preferred dissolved inorganic nitrogen source in most cases, calculated absolute uptake rates were compared with observed changes in ambient NH₄⁺ concentrations for the summer bloom, to determine whether static measurements of nutrient concentrations could indicate potential nitrogen limitation for phytoplankton. The ambient NH₄⁺ concentration exceeded that which would be expected (on the basis of loss of NH₄⁺) from the uptake rates measured, by between 1% and 1028% in the majority of periods presented. Consistent exceptions were bottom night time comparisons in both weeks, when the ambient NH₄⁺ concentration was found to be less than would be predicted from projections based on the measured afternoon uptake rates. There was an unaccounted loss at depth (4.5 m) at night of between 30% and 769% of the measured uptake. During week 3 surface ambient nutrients were two times lower than expected concentrations.
Table 6.1 Summary of absolute uptake rates, ambient nutrient concentrations and $R_{\text{NH}_4}$ values for the spring Chlorophyte, summer and autumn Dinophyte blooms.

Surface (0.25 m, s) Midwater (2.5 m, m) Bottom (4.5 m, b) depths. All values of 2.5 denote concentrations below detection limits (<5 $\mu$g N l$^{-1}$). * designates day time nutrient concentrations used to calculate night time uptake rates. Italicised nutrient values are Swan River Trust midday values (others unavailable), n/d no data.

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<td>m</td>
<td>b</td>
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<td>Urea</td>
<td>0.37</td>
<td>0.35</td>
<td>0.2</td>
</tr>
<tr>
<td>Ambient conc. (µg N l$^{-1}$)</td>
<td>[NO$_3$]</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>[NH$_4$]</td>
<td>38</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>R$_{\text{NH}_4}$</td>
<td>0.5</td>
<td>0.51</td>
<td>0.55</td>
</tr>
<tr>
<td>21 Nov 1995 Chlorophyte</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Absolute uptake (µg N l$^{-1}$ h$^{-1}$)</td>
<td>NO$_3^-$</td>
<td>1.47</td>
<td>1.15</td>
</tr>
<tr>
<td>Urea</td>
<td>1.35</td>
<td>0.36</td>
<td>0.47</td>
</tr>
<tr>
<td>Ambient conc. (µg N l$^{-1}$)</td>
<td>[NO$_3$]</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>[NH$_4$]</td>
<td>16</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>R$_{\text{NH}_4}$</td>
<td>0.48</td>
<td>0.24</td>
<td>0.8</td>
</tr>
<tr>
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<tr>
<td>Absolute uptake (µg N l$^{-1}$ h$^{-1}$)</td>
<td>NO$_3^-$</td>
<td>6.69</td>
<td>0.03</td>
</tr>
<tr>
<td>Urea</td>
<td>3.59</td>
<td>2.31</td>
<td>1.7</td>
</tr>
<tr>
<td>Ambient conc. (µg N l$^{-1}$)</td>
<td>[NO$_3$]</td>
<td>2.5</td>
<td>7</td>
</tr>
<tr>
<td>[NH$_4$]</td>
<td>2.5</td>
<td>100</td>
<td>160</td>
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<tr>
<td>R$_{\text{NH}_4}$</td>
<td>0.35</td>
<td>0.99</td>
<td>0.99</td>
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<tr>
<td>Absolute uptake (µg N l$^{-1}$ h$^{-1}$)</td>
<td>NO$_3^-$</td>
<td>3.9</td>
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<tr>
<td>Urea</td>
<td>3.46</td>
<td>1.94</td>
<td>0.82</td>
</tr>
<tr>
<td>Ambient conc. (µg N l$^{-1}$)</td>
<td>[NO$_3$]</td>
<td>2.5</td>
<td>2.5</td>
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<tr>
<td>[NH$_4$]</td>
<td>2.5</td>
<td>10</td>
<td>27</td>
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<tr>
<td>R$_{\text{NH}_4}$</td>
<td>0.47</td>
<td>0.84</td>
<td>0.92</td>
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<tr>
<td>Absolute uptake (µg N l$^{-1}$ h$^{-1}$)</td>
<td>NO$_3^-$</td>
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<td>Urea</td>
<td>8.41</td>
<td>2.41</td>
<td>1.04</td>
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<tr>
<td>Ambient conc. (µg N l$^{-1}$)</td>
<td>[NO$_3$]</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>[NH$_4$]</td>
<td>38</td>
<td>30</td>
<td>48</td>
</tr>
<tr>
<td>R$_{\text{NH}_4}$</td>
<td>0.68</td>
<td>0.97</td>
<td>0.96</td>
</tr>
<tr>
<td>28 May 1996 Dinophyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute uptake (µg N l$^{-1}$ h$^{-1}$)</td>
<td>NO$_3^-$</td>
<td>0.98</td>
<td>0.49</td>
</tr>
<tr>
<td>Urea</td>
<td>1.49</td>
<td>0.97</td>
<td>0.54</td>
</tr>
<tr>
<td>Ambient conc. (µg N l$^{-1}$)</td>
<td>[NO$_3$]</td>
<td>3.68</td>
<td>2.05</td>
</tr>
<tr>
<td>[NH$_4$]</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>R$_{\text{NH}_4}$</td>
<td>0.6</td>
<td>0.66</td>
<td>0.87</td>
</tr>
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</table>
Table 6.2 Within-weeks comparison of specific uptake rates using a one-tailed t-test assuming unequal variances

<table>
<thead>
<tr>
<th></th>
<th>Morning</th>
<th>Afternoon</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO$_3^-$</td>
<td>NH$_4^+$</td>
<td>Urea</td>
</tr>
<tr>
<td><strong>Spring Chlorophyte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surf-mid</td>
<td>–</td>
<td>–</td>
<td>**</td>
</tr>
<tr>
<td>mid-bott</td>
<td>–</td>
<td>**</td>
<td>–</td>
</tr>
<tr>
<td>surf-bott</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surf-mid</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mid-bott</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>surf-bott</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Summer Mixed Dinophyte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surf-mid</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>mid-bott</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>surf-bott</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>week 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surf-mid</td>
<td>*</td>
<td>**</td>
<td>–</td>
</tr>
<tr>
<td>mid-bott</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>surf-bott</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>week 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surf-mid</td>
<td>–</td>
<td>–</td>
<td>**</td>
</tr>
<tr>
<td>mid-bott</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>surf-bott</td>
<td>–</td>
<td>–</td>
<td>**</td>
</tr>
<tr>
<td>Noon</td>
<td>NO$_3^-$</td>
<td>NH$_4^+$</td>
<td>Urea</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Autumn Dinophyte</strong> (O. marina)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>surf-mid</td>
<td>–</td>
<td>–</td>
<td>*</td>
</tr>
<tr>
<td>mid-bott</td>
<td>–</td>
<td>–</td>
<td>**</td>
</tr>
<tr>
<td>surf-bott</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*p < 0.05; ** p < 0.02; *** p < 0.002

Table 6.3 Between-weeks comparison of specific uptake rates for surface (0.25m) and bottom (4.5m) samples of the summer Dinophyte bloom using a one-tailed t-test assuming unequal variances

<table>
<thead>
<tr>
<th>Summer Dinophyte</th>
<th>NO$_3^-$</th>
<th>NH$_4^+$</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>am</td>
<td>pm</td>
<td>night</td>
</tr>
<tr>
<td><strong>Surface</strong> (0.25m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk1-wk2</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>wk2-wk3</td>
<td>–</td>
<td>–</td>
<td>***</td>
</tr>
<tr>
<td>wk1-wk3</td>
<td>*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Bottom</strong> (4.5m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wk1-wk2</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>wk2-wk3</td>
<td>***</td>
<td>–</td>
<td>**</td>
</tr>
<tr>
<td>wk1-wk3</td>
<td>**</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*p < 0.05; ** p < 0.02; *** p < 0.002
Figure 6.2 Diurnal nutrient profiles at RCI.
A & B Spring 1995 NO$_3^-$ and NH$_4^+$ profiles. C & D Summer 1996 NO$_3^-$ and NH$_4^+$ profiles, week 1 & 3. E & F Autumn 1996 NO$_3^-$ and NH$_4^+$ profiles, noon only. ● morning, ■ afternoon, Δ night

--- Week 1, ····· Week 2, — — — Week 3
Table 6.4 Comparison of observed with expected [NH₄⁺] based on nutrient profiles and calculated ambient uptake rates from the preceding time period, based on measured \( V_{\text{max}} \), \( k_s \) and Michaelis Menten model for uptake.

\( \delta[\text{NH}_4^+] \) represents the change in NH₄⁺ concentration. (All values are in units of \( \mu \text{g N L}^{-1} \))

<table>
<thead>
<tr>
<th></th>
<th>Surface am - pm</th>
<th>pm - night</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta[\text{NH}_4^+] ) observed</td>
<td>+6.50</td>
<td>+2.00</td>
</tr>
<tr>
<td>( \delta[\text{NH}_4^+] ) expected</td>
<td>-0.70</td>
<td>-35.86</td>
</tr>
<tr>
<td>Difference</td>
<td>+7.20</td>
<td>+37.86</td>
</tr>
<tr>
<td>( V_{\text{ambient}} )</td>
<td>0.14/h</td>
<td>1.23/h</td>
</tr>
<tr>
<td>Reappearance rate</td>
<td>1.40/h</td>
<td>3.29/h</td>
</tr>
</tbody>
</table>

**Bottom**

<table>
<thead>
<tr>
<th></th>
<th>Surface am - pm</th>
<th>pm - night</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta[\text{NH}_4^+] ) observed</td>
<td>+10.00</td>
<td>-40.00</td>
</tr>
<tr>
<td>( \delta[\text{NH}_4^+] ) expected</td>
<td>-6.14</td>
<td>-30.58</td>
</tr>
<tr>
<td>Difference</td>
<td>+16.14</td>
<td>-9.42</td>
</tr>
<tr>
<td>( V_{\text{ambient}} )</td>
<td>1.70/h</td>
<td>2.78/h</td>
</tr>
<tr>
<td>Reappearance rate</td>
<td>3.20/h</td>
<td>-0.86/h</td>
</tr>
</tbody>
</table>

**DINOPHYTE: Jan 10, 1996 (week 3)**

<table>
<thead>
<tr>
<th></th>
<th>Surface am - pm</th>
<th>pm - night</th>
</tr>
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<tbody>
<tr>
<td>( \delta[\text{NH}_4^+] ) observed</td>
<td>-32.00</td>
<td>+2.00</td>
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<tr>
<td>( \delta[\text{NH}_4^+] ) expected</td>
<td>-16.04</td>
<td>-3.72</td>
</tr>
<tr>
<td>Difference</td>
<td>-15.96</td>
<td>+5.76</td>
</tr>
<tr>
<td>( V_{\text{ambient}} )</td>
<td>3.21/h</td>
<td>0.34/h</td>
</tr>
<tr>
<td>Reappearance rate</td>
<td>-3.20/h</td>
<td>0.52/h</td>
</tr>
</tbody>
</table>

**Bottom**

<table>
<thead>
<tr>
<th></th>
<th>Surface am - pm</th>
<th>pm - night</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta[\text{NH}_4^+] ) observed</td>
<td>-2.00</td>
<td>-41.00</td>
</tr>
<tr>
<td>( \delta[\text{NH}_4^+] ) expected</td>
<td>-2.30</td>
<td>-4.72</td>
</tr>
<tr>
<td>Difference</td>
<td>+0.03</td>
<td>-36.28</td>
</tr>
<tr>
<td>( V_{\text{ambient}} )</td>
<td>0.46/h</td>
<td>0.43/h</td>
</tr>
<tr>
<td>Reappearance rate</td>
<td>0.06/h</td>
<td>-3.30/h</td>
</tr>
</tbody>
</table>
6.6 Discussion

It is generally accepted that additions representing < 10% ambient nitrogen are tracer additions and enable rate determinations that are representative of ambient uptake (Dugdale and Goering, 1967; Cochlan et al., 1991; and others) and will not induce enrichment effects. The uptake rates presented here must therefore be considered as $V_{\text{max}}$ values since $^{15}$N addition in the form of NO$_3^-$, NH$_4^+$ or urea represented between 16 and 1000 times the ambient concentration. In the blooms studied, $V_{\text{max}}$ was found to have a highly significant variability both temporally and spatially within and between weeks. Based on the measured $V_{\text{max}}$ uptake rate, relative preference indices calculated for NH$_4^+$:NO$_3^-$ and urea:NO$_3^-$ uptake (RPI) indicated that, in most mid-water and bottom measurements, NH$_4^+$ and urea were the physiologically preferred N-sources. Preference for NH$_4^+$ has also been demonstrated for Prorocentrum minimum-dominated field assemblages in Chesapeake Bay (Fan et al., 2003). Urea was the dominant nitrogen source for a mixed dinoflagellate-dominated bloom in the Neuse River (Fan et al., 2003). However, no apparent preference for NH$_4^+$ over NO$_3^-$ for surface uptake, day or night, was determined for any of the blooms studied. The preferred inorganic nitrogen source at mid- and bottom-water depths during summer was NH$_4^+$ (RPI = 0.95).

The range of absolute uptake rates observed in this study (0.02 – 11.5 µg N L$^{-1}$ h$^{-1}$) falls within the range of uptake rates for NO$_3^-$ and NH$_4^+$ reported elsewhere (MacIsaac and Dugdale, 1969; Paasche et al., 1984; Berounskey and Nixon, 1993; Neuer and Franks, 1993; Lieberman et al., 1994; Collos et al., 2003). The amount of particulate material present varies considerably, both between depths and sampling times, as indicated by the Chla levels representing phytoplankton biomass shown on Figure 6.1 a-d. Absolute uptake is affected by particulate biomass. Most studies reporting uptake rates normalise to particulate organic nitrogen (PON), which results in a unitless rate and removes the effect of differing detrital levels between samples (Dugdale and Goering, 1996). In this study Chla, rather than detrital PON was used as the normalising factor to enable direct comparisons of uptake between sampling periods with varying phytoplankton loads and so provide a more realistic comparative basis for uptake. Similar trends in specific and absolute uptake rates suggested that no spatial or temporal separation uptake and growth was occurring. Normalising to Chla also enabled a direct comparison between
calculated specific uptake rates and grazing rates for phytoplankton, expressed in units of Chla (see Chapter 7).

The concentration of NH$_4^+$ was equal to or in excess of NO$_3^-$ concentration (ratio of NH$_4^+$/NO$_3^-$ ranging from 1 to 21). Nitrate levels throughout the water column were uniformly higher during the spring sampling times than the summer and autumn sampling times. The higher nitrate concentrations seen during the first week of the spring bloom are a result of external inputs from rain and terrestrial run-off from the winter rains. This had been reduced to below detection limits (< 5 µg l$^-1$) throughout the water column by the second week, during which time the uptake of NH$_4^+$ and urea had significantly increased at all depths. During the summer bloom the highest nitrate levels were detected in day time bottom samples. By autumn, nitrate was depleted (<5 µg N l$^-1$) and ammonium was present in lower concentrations (10 – 13 µg N l$^-1$) throughout the water column than for summer. As with previous studies (John, 1984; John, 1987; Jack, 1987; Douglas et al., 1996; Douglas et al., 1997), the highest concentrations of ammonium occurred at depth. Shallow groundwater has been a suggested source of NH$_4^+$ (Linderfelt and Turner, 2001). Douglas et al. (1996) and references cited therein, report increased NH$_4^+$ concentrations in bottom water associated with anoxic conditions and the presence of the salt wedge. Anoxic release and density driven displacement of pore-water have been suggested as mechanisms for NH$_4^+$ accumulation in the deeper water. The higher nitrate concentrations that were measured at depth during week three of the dinophyte bloom may be caused by nitrification in the overlying aerobic bottom water (2.6 - 3.1 mg DO l$^-1$) of the NH$_4^+$ released from the sediments under anaerobic conditions.

The general pattern of nitrogen uptake and preference for different nitrogen sources generally reflected the levels of available ambient nitrogen sources occurring at that time and depth. Results indicated that the spring Chlorophytes are adapted to utilise NO$_3^-$ and NH$_4^+$ while urea increased in importance as a nitrogen source as other sources were reduced. The summer and autumn blooms occur at times of very low rainfall, with negligible external nitrate input, and low ambient nitrate levels. Uptake rates indicate a physiological ability to take up nitrogen in the form of ammonium and urea. Both these reduced nitrogen sources can be made available through biological processes within the water column. By autumn, when ambient nitrogen concentrations have been depleted
and the winter rains have not yet commenced, the phytoplankton exhibit a high physiological capacity, as indicated by high \( V_{\text{max}} \) parameters, for the uptake of both inorganic and organic nitrogen forms.

Higher daytime uptake rates for nitrate in surface samples compared with mid- and bottom-water daytime samples are consistent with there being a photosynthetically-linked dependency on energy from ATP in the chloroplast. Nitrate uptake can also occur in the dark, with the energy requirement coming from cellular respiration (Bates, 1976). In this study diurnal uptake rates for the blooms studied indicate that nitrate uptake at the surface was occurring at similar rates during both day and night. Dark uptake of NO\(_3^-\) is evident in bottom night-time samples during week 3 of the summer dinophyte bloom (Figure 6.1 c). Elevated nitrate concentrations in bottom water samples during the day may have pre-conditioned this response. Dark uptake is also evident in the autumn dinophyte bloom, where there is a fivefold increase in dark NO\(_3^-\) uptake at depth between day and night-time measurements. There is no significant difference in surface uptake of NO\(_3^-\) and NH\(_4^+\) for the spring Chlorophyte bloom and the first week of the summer Dinophyte bloom, while uptake rates for NH\(_4^+\) generally exceed those of NO\(_3^-\) for the remainder of the times sampled.

The role of dissolved organic nitrogen, in the form of urea, is important in this system, although its significance to different bloom types appears variable. Urea \( V_{\text{max}} \) was low (6 - 12 ng N \( \mu \)g Chla\(^{-1}\) h\(^{-1}\)) during the first week of the spring bloom (see Figure 6.1 a-c), but significantly increased (P < 0.05) by the second week, with highest specific uptake rates measured at night (99 ± 30 ng N \( \mu \)g Chla\(^{-1}\) h\(^{-1}\)). Summer and, in particular, autumn urea \( V_{\text{max}} \) were comparable to or exceeded NH\(_4^+\) \( V_{\text{max}} \) uptake rates. By autumn the specific uptake rates for urea were the greatest measured (max surface noon 1727 ± 400 ng N \( \mu \)g Chla\(^{-1}\) h\(^{-1}\)) and were higher than NH\(_4^+\) uptake rates for that period (702 ± 176 ng N \( \mu \)g Chla\(^{-1}\) h\(^{-1}\)). At that time total inorganic nitrogen levels in the water column were uniformly low, with no external inputs from sources such as rainfall or terrestrial run-off occurring since the previous spring. Fan et al. (2003) also report urea as a significant contributor to the nitrogen nutrition of dinophyte-dominated blooms in the Neuse Estuary. They demonstrate that Phaeocystis is physiologically adapted to utilise NH\(_4^+\) as it becomes more available, while diatoms are not similarly adapted. High \( V_{\text{max}} \)
for urea and NH$_4^+$ in the upper Swan River Estuary during times significantly reduced nitrogen (NH$_4^+$ and urea) availability suggests that seasonal blooms occurring at these times (summer and autumn dinophyte-dominated blooms) are physiologically adapted to utilise nitrogen sources that are made available through biological processes. The increasing uptake of urea over the spring bloom also suggests that organic nitrogen may play an important role in the maintenance of bloom assemblages as they deplete ambient inorganic nitrogen sources.

### 6.6.1 The role of diurnal vertical migration in different blooms

It is evident from species composition and distribution analysis for the blooms studied that migratory behaviour of different species varies. Chlorophyte distribution patterns showed that *C. globosa* remain predominantly in the surface waters throughout the diurnal cycle (see Figure 3.5 a). In contrast, there was an apparent observed shift in behaviour from no diurnal vertical migration (DVM) during week 1 of the dinophyte bloom to an established nocturnal vertical migration into deeper water by week 3 (see Figure 3.5 b and c). DVM was occurring for the dinophyte species that constituted the bloom. DVM was further supported by *in situ* monitoring of chlorophyll profiles during the dinophyte bloom reported here (Figure 2.17) and elsewhere (Salmon, 1996; Hamilton *et al.*, 1999). The change from no DVM to DVM behaviour corresponded to a drop in water column (surface and mid-water) nutrient (NH$_4^+$) concentration.

Changes in DVM behaviour over the course of the summer bloom support the role of DVM in optimising photosynthetic capability and nutrient acquisition (Watanabe *et al.*, 1991; Kamykowski, 1995; Kamykowski *et al.*, 1998). It also suggests that DVM behaviour can alter under changing conditions of water-column nutrient availability (Eppley and Thomas, 1969). Cells that display DVM behaviour can balance their nutrient and photosynthetic requirements, thus enabling maximum growth under the existing environmental conditions. It also suggests that DVM may be regulated by factors other than circadian or endogenous rhythms. The importance of the role of DVM in maintaining a balance between near-bottom night-time nitrogen uptake and near-surface daytime photosynthesis, resulting in net population growth, has been demonstrated elsewhere (Hamilton *et al.*, 1999) with modelling techniques that utilised uptake data from this study.
6.6.2 Preferential uptake related to ambient nutrient profiles

Uptake rates have been shown to reflect the ambient nutrient concentrations from the preceding sampling period (see Table 6.1). However, ambient concentrations did not necessarily reflect potential uptake rates; where nutrient levels show a decline, there is a corresponding decline in absolute uptake rates at the subsequent sampling time. This is demonstrated by comparing observed and expected ambient NH$_4^+$ concentrations for week 1 and week 3 of the summer dinophyte bloom, where ambient nutrient profiles were measured at more than one time during a diurnal period. This mass balance approach has been used by Lipschultz et al. (1986) to validate measured uptake rates with changes in ambient nitrogen species concentrations. Comparing calculated ambient uptake, based on $V_{\text{max}}$ and the Michaelis Menten model, and changes in ambient NH$_4^+$ concentration suggests that uptake does not reflect the change in ambient NH$_4^+$ concentration between consecutive experimental times (i.e. morning to afternoon; afternoon to night). In five of the eight cases presented, there is a greater concentration of ammonium in the water column than could be expected based on measured uptake rates. The inferred rate of ‘reappearance’ of NH$_4^+$ was generally equal to or greater than the rate of uptake and represents NH$_4^+$ production or input rates of between 0.06 and 3.3 μg N l$^{-1}$ h$^{-1}$. Apparent rates of ‘reappearance’ of NH$_4^+$, where the observed ambient nutrient concentration exceeded that which could be expected from the measured uptake rates, ranged between 13% - 1000% of the uptake rates. The ‘reappearance’ of NH$_4^+$ is sufficient, in most cases, to support algal growth at the measured uptake rates, irrespective of absolute ambient nutrient levels. In contrast, seasonal variation in uptake and regeneration for ammonium in coastal communities were found insufficient to support phytoplankton nitrogen demand (Collos et al., 2003). Possible environmental sources of these higher than expected NH$_4^+$ concentrations may be from remineralisation and microbial ammonification of PON and DON, zooplankton excretory products, ground-water input, tidal advection, or a combination of these. Increases in ammonium at night have been attributed to higher night time excretion rates primarily attributable to organisms <35 μm in size (Caperon et al., 1979).

On three occasions, both night-time bottom and surface samples during week 3 of the summer bloom (see Table 6.4), the nutrient levels reflected a greater consumption than production of NH$_4^+$. On these occasions there was an apparent loss of NH$_4^+$ of
between 31% and 767% of measured uptake rates. Possible explanations for this loss are that $^{15}$NH$_4^+$ incorporated into microbial biomass was not retained on the GF/C filters and so not included in the measurement, or that increased levels of nitrification may be occurring.

It is important when determining potential nutrient limitation on phytoplankton growth that a measure of the nutrient flux is obtained, rather than absolute values of the nutrient in question. Community regeneration rates of 0 - 2.4 µg l$^{-1}$ h$^{-1}$ in the light and 0.28 – 4.2 µg l$^{-1}$ h$^{-1}$ in the dark (van Rijn et al., 1987) have been reported for the Mississippi River. Glibert et al. (1982) demonstrated that regeneration of NH$_4^+$ over relatively brief periods could supply the daily nitrogen requirements of phytoplankton when there were no losses to the system. It is clear that the present results, obtained from comparing actual changes in ambient nutrient concentrations with expected changes in nutrient concentrations, give an indication of community nutrient remineralisation rates that are in the order of those reported elsewhere.

The uptake of nitrogen and its incorporation or synthesis into new cellular material may not coincide temporally, depending on nutritional state of the cells (Wheeler et al., 1982; Morel, 1987). It cannot be assumed that uptake directly reflects assimilation, particularly where vertical migration is evident, such as is the case in the Swan River (Hamilton et al., 1999). However, growth on NH$_4^+$, even with the added energy requirements of vertical migration, enables dinophyte species to out-compete other non-migratory species during a time when NH$_4^+$ is the more prevalent inorganic nitrogen source (Douglas et al., 1996).

Tight coupling between uptake and regeneration of ammonium has been documented for a number of water bodies (Harrison, 1978; Glibert, 1982; Probyn and Licas, 1987). Regeneration of ammonium from organic compounds by microbial decomposition of organic matter and by animal excretion has long been recognised as providing potential nutrient requirements for phytoplankton growth (Postma et al., 1982; Scavia et al., 1984). Nutrients released by larger animals may represent a more concentrated ‘point source’ on the microscale (Lehman and Scavia, 1982). Copepod excretion has been indicated as a potentially significant NH$_4^+$ source during periods of low ambient nitrogen for Swan River phytoplankton communities (Griffin, 2003). The results of this study are
consistent with previous reports of regeneration rates equal to or greater than uptake or assimilation rates (Harrison, 1978; Axler et al., 1981; Glibert, 1982 & 1988, Glibert et al., 1991). This would indicate that there was a reappearance rate which can provide ammonium for uptake but which was not discernible by standard point-in-time ambient nutrient determinations. This hidden source of ammonium provides sufficient nitrogen to allow phytoplankton growth in an apparently nutrient deplete environment. For both bloom types, the bottom night-time observed uptake rates were lower than that expected from extrapolating uptake rates. This may indicate that NH₄⁺ was being lost due to processes other than phytoplankton uptake, e.g. conversion to other forms of nitrogen.

6.7 Conclusions

The successional pattern of phytoplankton species occurring in the upper reaches of the Swan River Estuary appears to be related to changes in available nitrogen sources. The degree of variability of V_max between depths, time of day and duration of bloom for all nitrogen sources indicates that the physiological response of phytoplankton to nutrient availability is extremely dynamic within a bloom event. A relationship between the preferred nitrogen source exhibited by different bloom types and ambient nitrogen concentrations at that particular time of year supports the premise that the phytoplankton species occurring are adapted to utilise the nitrogen species that prevail. During spring blooms, which occur towards the end of the wet season, when nitrate levels are higher throughout the water column, algae show an initial preference for NO₃⁻. During summer and autumn blooms ammonium and urea were preferentially selected. Ammonium can be generated within the water column through such processes such as nitrification, ammonification and excretion, and is available at higher concentrations than nitrate, particularly at times when no external nitrogen inputs are occurring through the catchment (Thompson and Hosja, 1996; Thompson, 2001). Preliminary population studies indicated that partitioning nitrogen uptake and primary production through nocturnal vertical migration provides an ecological advantage for those migratory species able to access these resources (Hamilton et al., 1999). This enables access to regenerated nitrogen sources that would otherwise be lost to the planktonic system through senescence and settlement out of the water column.
The role of dissolved organic nitrogen, for example urea, is important in this system, although its significance to different bloom types appears variable. Urea concentrations remain relatively stable throughout the year compared with other nitrogen sources, in particular nitrate which has highly seasonal availability. It is suggested that the importance of urea is related to the low ambient levels of inorganic nitrogen sources at certain times of the year and that it provides a reliable and relatively constant nitrogen resource throughout the year.

Knowledge of ambient nutrient concentrations alone does not give a realistic picture of the potential for nutrient regulation of phytoplankton growth. To be able to make predictions of the potential effects of nitrogen species in regulating bloom development and maintenance, information on flux rates as well as ambient nutrient concentrations is needed for all potential nitrogen sources, organic and inorganic, throughout the year. A better understanding of the role of organic nitrogen for the Swan-Canning Estuary is needed. Nitrate and ammonium (re)generation processes appear, at times, to be able to provide sufficient nitrogen for maintenance of algal biomass.

If workable strategies for reducing bloom intensity and frequency, through the manipulation of nitrogen availability, are to be successful for the Swan River Estuary, then the various flux processes influencing available nitrogen should be considered. These processes appear to play an important role, throughout the water column, in maintaining nutrient levels and in promoting and sustaining phytoplankton biomass, and thereby influencing the pattern of phytoplankton bloom succession.

### 6.8 References


CHAPTER 7: Microheterotroph grazers and their impact on phytoplankton biomass

7.1 Abstract

Microheterotroph species composition at a typical upper Swan River Estuary site was determined and its potential role in top-down control of phytoplankton populations investigated. Microheterotroph species composition over an annual cycle (1998 – 99) indicated a predominance of aloricate ciliates from four genera, with *Strombolidium* numbers peaking during winter. Tintinnid (loricate) ciliates and heterotrophic dinoflagellates were next most abundant. Rotifers (*Brachionus* sp and *Synchaeta*) occurred sporadically throughout the year. Pallial feeding, which is the capture and external digestion of prey using a haptomere and mucous veil, was evident in the dominant heterotrophic dinoflagellate grazers (ie *Gyrodinium aff. spirale*). This is shown for the first time for the dinoflagellate *Akashiwo sanguinea*. Prey selectivity, as evidenced by monospecific cell accumulations within pallial veils and mucous aggregations, may provide a mechanism for effecting changes in the species composition at the lower trophic level.

The potential for microheterotrophs to exert a top-down effect on the biomass of algae has been estimated. From within-bloom intensive studies and longer-term temporal investigations of grazing rates relative to the potential phytoplankton production, it was found that the microheterotroph grazing community could significantly decrease phytoplankton biomass and so control phytoplankton bloom events. Grazing during a mixed bloom event typical of summer showed classic grazer control of phytoplankton biomass with Chla levels peaking and declining as grazing pressure increased. The average annual grazing pressure exerted by microheterotrophs reduced potential phytoplankton growth by about 80%, slowing overall phytoplankton biomass increase. Peak grazing rates were observed to reduce phytoplankton by up to 50% of standing stock. Annual grazing rate determinations, based on a recognised dilution technique, indicated that 32 – 149% (mean 79.4 ± 32, n=10) of potential phytoplankton production is grazed by microheterotrophs in the < 300 μm size class. The isolated nano-heterotrophic (< 20 μm) component was shown to graze 30 – 355% (67.9 ± 27,
n=12) of potential production. Microheterotrophs are able to substantially reduce algal biomass and so influence the development and/or maintenance of algal blooms.

7.2 Introduction: Microheterotroph grazing in aquatic ecosystems

Microheterotroph grazing of phytoplankton and its contribution to nutrient cycling and the food-web has been the subject of increasing interest over the past two decades (Landry and Hassett, 1982; Burkhill et al., 1987; Weisse, 1991; Hall et al., 1993; Strom et al., 1993; Verity et al., 1993; Ferrier-Pages and Rassoulzadegan, 1994; Kamiyama, 1994; Landry et al., 1994; Burkhill et al., 1995; Dagg, 1995; Froneman et al., 1996; Froneman and McQuaid, 1997). Included in the microheterotroph community are mixotrophic and heterotrophic flagellates, aloricate (naked) ciliates ranging in size from about 10 μm to 4.5 mm and tintinnids (loricate ciliates) in the range 20 to 200 μm. These organisms constitute a major component of the microzooplankton in most marine environments (Alder, 1999; Petz, 1999 and refs there-in). In open ocean environments, it is estimated that over 50% of dinoflagellate species are heterotrophic or mixotrophic (Gaines and Elbrachter, 1987) and that their relative contribution to the microzooplankton may become progressively more important with increasing latitude (Burkhill et al., 1993).

The biomass of microzooplankton with phytoplankton may be tightly correlated in estuarine and coastal waters (Verity, 1987; Burkhill et al., 1993). Marked seasonal differences in the relative contribution of pico-, nano- and microplankton to community biomass have been reported (Lewitus et al., 1998). Research in the 1970s indicated that the dynamics of aquatic ecosystems is more dependant on limnological variations and size selective predation than taxonomic community structure (Paloheimo et al., 1984). Arndt et al. (1990) found changes in the functional grouping of ciliate communities (bactivores versus algivores) were influenced by temporal changes in bacterial and phytoplankton production. The role of pico- (0.2 - 2 μm), nano- (2 - 20 μm) and micro-sized (20 - 200 μm) grazers has been recognised as having significant impact on phytoplankton biomass in oceanic (Jackson, 1980; Burkhill et al., 1993; Froneman et al., 1996), coastal (Burkhill et al., 1987; Hall et al., 1993; Goosen et al., 1996) and estuarine environments (Sherr et al., 1991; Dagg, 1995; Koepfler and Lewitus, 1995).
Phytoplankton biomass and production were dominated in a eutrophic lake by pico- (<3 \( \mu \text{m}, \sim 5\% \)) and nano-phytoplankton (< 20 \( \mu \text{m} \)) (Hansen and Christoffersen, 1995). These small phytoplankton species provide a potential food source for nano- and micro-zooplankton.

The importance of microheterotrophic activity in pelagic foodwebs has been recognised as providing a significant route for the transfer of energy to higher trophic levels (Kuosa and Kivi, 1989; Dagg, 1995). Included in this route of the foodweb are many mixotrophic, bactivorous and omnivorous phytoflagellates. In recent years the technique of autofluorescence microscopy has enabled the distinction of autotrophic from heterotrophic microplankton by utilizing the natural autofluorescence of chlorophyll and phycobilin photosynthetic pigments (MacIsaac and Stockner, 1993). The method enables quantification of total number of autotrophs in individual size classes and enables carbon:chlorophyll \( a \) ratios (Booth et al., 1993). Carbon or nitrogen biomass estimates for autotrophic and heterotrophic components of the microplankton can be made based on calculations using measured cell dimensions and reported or measured carbon or nitrogen content values for species or groups (i.e. Parsons et al., 1961; Mullin et al., 1966; Verity et al., 1992; Booth, 1993).

### 7.2.1 Grazing rate determinations

Feeding experiments on aquatic meso- and macro-scale invertebrate grazers often involves the isolation of individuals, supply of specific foods and monitoring of changes in particle density, gut fluorescence and even gut content analysis. For pico-, nano- and micro-size organisms gut analyses are difficult. Grazing rate determinations have been made by calculating feeding rates of individual organisms on specific prey species, from differences in cell counts of prey cells before and after exposure to the grazers (Bockstahler and Coates, 1993; Strom and Buskey, 1993), or from counts of ingested cells after timed exposure of grazers to prey (Hansen, 1992; Pedrós-Alió et al., 1995). The use of \(^{14}\text{C}\) labelled algal cells has been used to track trophic transfers through different size classes in the foodweb (Smith et al., 1979), with incorporation rates representing grazing rate. The use of live or heat-killed labelled prey (Putt, 1991) or fluorescently labelled beads representing algal cells of specific sizes (Simek et al., 1990; Sherr et al., 1991; Hall et al., 1993; Thompson et al., 1993) provides an indication of
grazing rate and prey selectivity. However, it may not be appropriate for measuring in situ grazing rates of all phagotrophs (Pace and Bailiff, 1987; Head and Harris, 1994).

Although the techniques outlined above provide feeding rates for individual organisms and allow determination of prey selectivity or preference, many are difficult to conduct on the pico, nano and micro size class of planktonic grazers. They are not designed to provide information on community grazing rates where multiple consumers, each grazing at different rates, contributes to the net grazing rate. The dilution method, first used by Landry and Hassett (1982), provides a method of measuring net community grazing through an inverse log transformation of chlorophyll a (Chla) levels, measured for a serial dilution of natural waters. The method assumes that there is no prey selectivity and that grazing is the result of random encounters between predator and prey. Results are often presented in terms of feeding rates per individual grazer, following estimates of grazer abundance. The method has been further extended to determine feeding preferences through photosynthetic pigment partitioning (Burkhill et al., 1987). By incorporating HPLC techniques for the analysis of photosynthetic pigments, some indication of the occurrence of prey selectivity within the natural assemblage can be estimated.

7.2.2 Food preferences and feeding mechanisms in heterotrophic and mixotrophic flagellates.

The suitability of different organisms as food for heterotrophic microplankton may influence grazing rate control of phytoplankton biomass (Buskey and Cammie, 1995). Burkhill et al. (1987) demonstrated selectivity by microzooplankton for dinoflagellates, cryptophytes and prasinophytes and against diatoms. Factors influencing the suitability of prey include physical factors such as size, shape and concentration (Frost, 1972; Fenchel, 1980), and physiological or biochemical factors such as toxicity of prey species to all or some life-cycle stages of predators (Granéli et al., 1989; Smaey and Villareal, 1989; Buskey and Cammie, 1995). These latter factors are used as criteria for categorising blooms as harmful algal blooms (HABs), which can have deleterious effects on the ecology of aquatic environments.

It is estimated that approximately 50% of dinoflagellate species are heterotrophic or mixotrophic (Gaines and Elbrächter, 1986; Lessard and Swift, 1985). These can be
important for their role in grazing on smaller phytoplankton in both oceanic and coastal waters (Burkhill et al., 1993). Growth rate and abundance of the heterotrophic dinoflagellates *Gyrodinium dominans* and *G. spirale* were shown to rapidly increase after an outbreak of the red-tide organism *Karenia mikimotoi*, and played an important part in the bloom disappearance (Nakamura et al., 1995). Phagotrophy is widespread among photosynthetic dinoflagellates, although actual feeding mechanisms have only been observed in a relatively few species (Hansen, 1998). Phagotrophy has only been recorded for three photosynthetic genera of Prymnesiophytes (Haptophytes), *Chrysochromulina*, *Prymnesium* and *Coccolithus* (Green, 1991). Prymnesiophytes, such as species of *Chrysochromulina*, which are between 2 – 20 μm in size, have been reported to ingest a broad range of live and inert particles in the size range of 0.33 μm – 6 μm. *Chrysochromulina* species can live autotrophically or phagotrophically (Hansen, 1998). The haptomere, a filamentous appendage that acts like a tentacle, can function as a food-capturing device (Hansen, 1998). Captured prey is then ingested phagotrophically (van den Hoek et al., 1995; Hansen, 1998).

Oligotrichs and tintinnids have a feeding preference for dinoflagellates and cryptophytes (Burkhill et al., 1987) and do not graze diatoms. Photosynthetic pigment analysis, combined with the dilution technique (Landry and Hassett, 1982; Buskey et al., 1997) also provides evidence that microzooplankton feed preferentially on chlorophytes and prasinophytes.

In the Swan River Estuary there have been a number of studies of macrozooplankton species composition and grazing (ie Bhuiyan, 1966; Hodgkin and Rippingale, 1971; Rippingale and Hodgkin, 1974; Gaughan and Potter, 1994; Griffin and Rippingale, 2001; Griffin et al., 2001; Griffin, 2003). Microheterotroph species composition and its role in top-down control of phytoplankton populations in the Swan River have not been previously investigated.

### 7.3 Objectives

This section of the study aimed to assess the potential for microheterotrophs to exert biomass or compositional control of phytoplankton during bloom and non-bloom conditions. This was investigated through within bloom intensive studies and longer-
term investigations of composition changes and grazing rates relative to potential phytoplankton growth. Specifically the objectives addressed were

- to determine species composition trends for the microheterotrophic grazing community (< 300 μm) over an annual period for an upper estuary site.
- to measure differences in grazing rates, monthly over an annual cycle, to estimate seasonal impact of microheterotroph grazing on phytoplankton biomass.
- to measure the change in grazing pressure and grazer species composition over the course of a typical (summer dinophyte) bloom.

7.4 Materials and Methods

7.4.1 Microheterotroph species composition

Annual species composition trends for the microheterotroph community were determined from monthly surface and bottom water samples which were collected from the study site at Ron Courtney Island (February 1998 to January 1999), as described in Chapter 3 (Phytoplankton Species Composition), during noon and night-time sampling trips (designated day 1), using a positive displacement pump. Whole water samples collected the following day (day 2) at noon for the grazing experiments, as described below, were also analysed for species composition and a comparison between day 1 and day 2 was made. Temporal variations in species composition and vertical distribution were determined from the same replicate samples (designated day 1) that were collected, using a positive displacement pump, for diurnal phytoplankton species composition (see Chapter 3), physico-chemical (see Chapter 2) and physiological experimentation (see Chapters 4 and 5). This ensured valid comparison between phytoplankton and grazers as sampling effort and methods for both were the same. After removal of water needed for other experiments the remaining water was pooled, gently mixed and sub-sampled for phytoplankton and grazer species identification and enumeration.

During the summer Dinophyte seasonal bloom study (January 1996) weekly whole water samples were collected from three depths (0.25 m, 2.5 m and 4.5 m) over a four week period using a purpose-built 6-litre horizontal water sampler, designed to collect from a narrow (15cm) depth range (Rippingale, pers.comm). Samples were preserved for identification. Changes in grazer species composition and diurnal depth-related
distribution patterns were established for the course of the bloom. Sub-samples (120 ml) for species composition from all samples were preserved with acid Lugols and stored for later identification and enumeration. Patterns of species composition for the microheterotroph community were determined from counts (20 fields of view or 500 individuals) of known volumes, using the Utermöhl settling technique (Reid, 1983: Utermöhl, 1958) and a Leitz Inverted Microscope (see Methods, Chapter 3.5). Photomicrographs were made using a Wild Photoautomat MPS45. Species were identified using various keys including Wood (1954), Corliss (1979), Patterson and Hedley (1992), Foissier and Berger (1996), Hasle and Syvertsen (1996), Tomas (1997), and others. Where possible, identification to genus and species has been made. However, as this study is not intended to be a comprehensive taxonomic treatise, but rather an exploration of factors controlling phytoplankton biomass levels, identification of grazers to Class level has been used to compare with a similar classification level of phytoplankton (Chapter 3) in ordination analysis of species composition trends. Statistical analysis was made on the basis of class groupings to ensure that classification resolution for phytoplankton and grazer communities was made at the same level.

Species diversity indices were calculated from cell counts according to the formula \( d = (S-1)^*\ln N^\lambda \) (Margaleff, 1951, in Parsons et al., 1977), where \( d \) is the diversity index, \( S \) is the number of species present and \( N \) is the total number of individuals.

Multi-response permutation procedure (MRPP), a randomisation test that evaluates differences in species composition based on Euclidean distance measure (PC-ORD Vers. 3.18; John et al., 2002) was used to explore the relationship between species composition, at class level, of microheterotroph grazers and phytoplankton for each month over an annual cycle (February 1998 to January 1999). MRPP has the advantage of not requiring multivariate normality and homogeneity of variances, factors that are seldom met when dealing with ecological community data (http://www.okstate.edu/artsci/botany/ordinate/index.html).

Non-metric Multidimensional Scaling (NMS) was used to highlight the similarities and differences of sampling times and depths based on their species composition and physico-chemical parameters. Results are presented as two-dimensional biplots (Appendix I).
7.4.2 Determination of grazing rates

In all cases the grazing rates were determined according to the dilution method (Landry and Hassett, 1982; Campbell and Carpenter, 1986; Burkhill et al., 1987). The theoretical considerations for this technique are detailed further in Burkhill et al. (1987). Grazing rate was determined monthly for two size fractions (< 300 μm and < 20 μm) over a 12-month period at the Ron Courtney Island site to determine the relative importance of nanoplankton and microplankton on phytoplankton biomass control over an annual cycle. Weekly grazing rates and species composition were compared during the course of a typical summer dinoflagellate bloom. A comparison of the proportion of phaeophytin to Chla was used as an independent indicator of the level of grazing activity evident in the field (Hallegraeff, 1981).

All grazing rate determinations were made from whole water samples collected, as previously described, at the Ron Courtney Island (RCI) site in the upper Swan River Estuary (see Figs 1.1, 2.1). Whole water sampling is recommended to minimise damage to delicate ciliate organisms (Petz, 1999). During the summer Dinophyta bloom (January 1996) samples were collected from surface water at approximately 0800 hr. Monthly grazing rates (February 1998 to January 1999) were determined from samples collected at midday to coincide with samples taken for nitrogen uptake experiments and physico-chemical analyses. Due to resource limitation during the temporal study grazing experiments were performed on samples collected one day later (designated day 2) at the same time of day and location as all other physiological experiments (nitrogen uptake and productivity). To ensure grazing rates were made on comparable plankton assemblages, species composition was compared between consecutive collection days (day 1 and 2, as previously described).

In all cases, water was transported to the laboratory within an hour of collection. Half the sample volume was filtered through Millipore GF/C filters. The remaining water was gently screened through either 300 μm or 20 μm Nitex screen to provide pico+nano+micro (< 300 μm, subsequently referred to as micro-) or pico+nano (< 20 μm, subsequently referred to as nano-) size classes, as required, for conducting grazing rate determinations. Dilutions of 75%, 50% and 25% of whole community were made using GF/C filtered water as the diluant. Incubations of GF/C filtered (Millipore
GF/C filters, nominal pore size 1.2 μm) water samples alone were also made to provide an indication of the presence of autotrophic bacteria in this size fraction. Nitrate (0.01 μM final concentration of NO₃) was added to individual incubations in the temporal study to reduce possible nutrient limitation to phytoplankton over the course of the incubations. All samples were incubated in a constant temperature room at ambient noon surface temperature under a 12:12 L:D regime at an irradiance of 615 - 630 μmol photons m⁻² s⁻¹ using 'cool white' fluorescent tubes (GroLux®) positioned on either side of the incubation containers. This provided a consistent light intensity throughout the study that approximated the 0.5m depth light equivalent in the estuary. Incubations were terminated by filtering a known volume onto GF/C filters, that were then stored frozen for later spectrophotometric analysis for chlorophyll concentration according to the acetone extraction method (Strickland and Parsons, 1972). After sonication and overnight acetone extraction, sample absorbance was read on a Varian DMS 90 UV Visible spectrophotometer.

Calculations of chlorophyll pigments (Chla, b and c) were made according to the equations of Jeffrey and Humphrey (1975, in Parsons et al., 1984). Phaeopigments were measured (with a better than 10% precision at the 0.5 mg Chla m³ level) using a Turner Designs fluorometer following standard methods as described in Parsons et al. (1984). Chlorophyll concentrations for the two weeks prior to the January 1996 Dinophyte study were taken from routine monitoring data collected at RCI by the Swan River Trust.

Values for apparent algal growth coefficients (k; in units of day⁻¹) and grazing coefficients (g; in units of day⁻¹) were determined by least squares linear regression analysis of the relationship between rate of change in Chla concentration, (1/Ln (P/P₀)*t), and the relative concentration of grazers in the experimental chambers (dilution factor), as described in Landry and Hassett (1982) and based on the assumption that change in density of phytoplankton, P, over some time, t, can be represented by the exponential equation

\[ P_t = P_0 e^{k \cdot g \cdot t} \]  

Eq 7.1.
where \( k \) and \( g \) are instantaneous coefficients of population growth and grazing mortality, respectively. Single factor ANOVA analyses were performed on the results. Potential production \((P_p, \mu g \text{ l}^{-1} \text{ day}^{-1})\), realised production \((P_r, \mu g \text{ l}^{-1} \text{ day}^{-1})\) and potential production grazed \((P_g, \% \text{ day}^{-1})\) were calculated as follows, according to the equations of Verity et al. (1993), using the notation of Landry and Hassel (1982),

\[
g = k - (\ln P_r - \ln P_o) \cdot t \quad \text{Eq 7.2}
\]

\[
P_g = (P_r - P_p) \cdot 100 \cdot P_p \quad \text{Eq 7.3}
\]

\[
P_p = P_o e^{k \cdot t} - P_o \quad \text{Eq 7.4}
\]

\[
P_r = P_o e^{k \cdot \psi} - P_o \quad \text{Eq 7.5}
\]

\[
P_g = (P_r - P_p)(100)/P_p \quad \text{Eq 7.6}
\]

where \( g \) (day\(^{-1}\)) is the grazing coefficient (slope calculated by dilution technique), \( k \) (day\(^{-1}\)) is the apparent phytoplankton growth coefficient (x-intercept calculated by dilution technique), \( P_o \) (\( \mu g \text{ l}^{-1} \)) is the initial Chla and \( P_r \) is the final Chla after time, \( t \) (decimal day). The proportion of initial Chla standing stock \((P_i)\) turned over, as \% d\(^{-1}\), was calculated as

\[
P_i = 1 - e^{x \cdot 100} \quad \text{Eq 7.7}
\]

Growth rates for \(< 1.2 \mu m\) size class were determined by the formula

\[
r = \ln(t_o) - \ln(t_o)^{+}t^{-1} \quad \text{Eq 7.8}
\]

where \( r \) is the growth rate in units per day, \( t_o \) is the final Chla concentration, \( t_0 \) is initial Chla concentration and \( t \) is the time of incubation in decimal hours.
CHAPTER 7: MICRO-HETEROTROPH GRAZING

7.5 Results

7.5.1 Species composition

Within Bloom trends – Summer dinophyte bloom

Grazer species composition changed over the 3 weeks of the summer (January 1996) mixed dinophyte bloom. Day time (noon) pattern of distribution with depth, as a percentage of total grazers counted, remained relatively constant over this period, with approximately 50% of grazers occurring in surface samples, 40% in mid-water and only 10% in bottom samples (see Table 7.1). Heterotrophic dinoflagellates, loricate and aloricate ciliates dominated the grazer species composition over the January dinophyte bloom at Ron Courtney Island (RCI). The dinophyte Oxyrrhis marina dominated the grazer species composition (80% of total grazers) during week 1 of the bloom study. A change in species domination by week 3 was apparent, with different heterotrophic dinoflagytes dominant (63% of total grazers). Abundance of O. marina had reduced to 31% of total grazer composition, with only 1% in NH₄⁺ rich bottom waters. Ciliates (loricate and aloricate) were a minor component (only 3% of total grazers during week 1, doubling to 6% by week 3) of the grazing assemblage during this bloom.

Monthly Grazer Species Composition

Monthly species composition analysis (Figure 7.1), based on counts from day 2 whole water samples (to minimise damage to ciliate species (Petz, 1999)), indicated a seasonal pattern that showed ciliates (loricate and aloricate) being the dominant (up to 100% of total grazers, av. 51%) microheterotrophic grazers occurring in the upper Swan River Estuary over the 1998 - 99 period. Diurnal patterns for surface and bottom (day 1 counts) show Ciliates constituted 17% - 100% of total grazer counts. Aloricate ciliates (Oligotrichs) ranged from 20% to 89% of total grazer numbers and were dominant (89% grazers) during winter. Ciliates from five major groups (Monodinium, Didinium, Mesodinium, Enterodinium and Strombolidium) are represented (see Figure 7.3). Next most abundant groups were heterotrophic dinoflagellates (at times up to 73% grazers) and tintinnids (up to 49% grazers). Tintinnids typical of the upper Swan River Estuary, represent five different Genera, are presented in Figure 7.4. Rotifers and various larval bivalves, polychaetes and copepod species (generally comprising < 13%
Table 7.1 Trends in daytime grazer species composition and distribution over a summer (January 1996) dinophyte bloom for surface (0.25 m) mid-water (2.5 m) and bottom (4.5 m) depths showing heterotrophic dinophytes dominated the grazer community over the course of this bloom.

<table>
<thead>
<tr>
<th>Major groups</th>
<th>Cell Counts (no cells ml⁻¹)</th>
<th>Week 1</th>
<th>Week 3</th>
</tr>
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<td>2.5m</td>
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<tr>
<td>Ciliates</td>
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<td>13</td>
</tr>
<tr>
<td></td>
<td>Loricate (2 spp)</td>
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<td>16</td>
</tr>
<tr>
<td>Dinophytes</td>
<td>Heterotrophic (3 spp)</td>
<td>18</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td><em>Oxyrrhis marina</em></td>
<td>532</td>
<td>310</td>
</tr>
<tr>
<td>Others</td>
<td>Bivalve</td>
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<td>5</td>
</tr>
<tr>
<td></td>
<td>Rotifer</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Polychaete worm</td>
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<td>0</td>
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<tr>
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<tr>
<td></td>
<td>% Weekly Total</td>
<td>48.2</td>
<td>38.1</td>
</tr>
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Figure 7.1 Annual pattern of grazer species composition at an upper estuary site (February 1998 to January 1999).
A Monthly comparison of grazer and phytoplankton abundance. Note scale differences
B. Monthly ratios of grazer classes to total diurnal grazer composition over an annual cycle.
(Data from day 1)

Figure 7.2 Diurnal depth distribution of grazer groups as % of total grazers for RCI (February 1998 – January 1999).
(Key as for Figure 7.1, Data from day 1)
grazers) occur sporadically throughout the year and, because of their low counts and sporadic occurrences, are grouped into 'others' (Figure 7.5). A high number of the phagotrophic haptophyte, *Chrysochromulina* sp. were counted within the 'other' category (67% of grazers) during December 1998, but were not apparent at other times of the year. Average annual species diversity indices for phytoplankton (0.37 ± 0.226) and microheterotrophs (0.39 ± 0.166) were not significantly different. Seasonal comparison of grazer species diversity indices (Table 7.3) indicated lowest diversity during winter (0.17 ± 0.006), with no significant difference between the spring, summer and autumn values of 0.35 ± 0.074, 0.47 ± 0.155 and 0.47 ± 0.166 respectively with maximum grazer species numbers (10) occurring at these seasons.

![Figure 7.3 Ciliates of the upper Swan River Estuary](image)

*Figure 7.3 Ciliates of the upper Swan River Estuary*

A – *Mesodinium* sp.; B – *Strombolidium* sp; C – benthic ciliate; D – *Strombolidium* sp. *Endodinium*; F – *Mesodinium rubrum*; G – *Entodinium* H – *Strombolidium* sp.; I – *Entodinium* sp. (Scale = 10 or 20 µm as indicated)
Figure 7.4 Tintinnids of the upper Swan River Estuary
A – Poroecus sp.; B – Tintinnopsis sp.; C – Favella aff. taraikaensis; D – Eutintinnus lusus-undae; E – Tintinnopsis sp.; F – Tintinnidium aff. balcheri; G – Tintinnidium aff. semiciliatum. (Scale = 5, 10, 20 or 50 μm as indicated)
Figure 7.5 Various grazer groups of the upper Swan River Estuary. A – Polychaete larva; B – Rotifer; C – Brachionus sp.; D – Polychaete; E – Xenostrobus secures larva; F – Synchaeta; G – Spionid polychaete trophophore larva, H – bivalve larvae; I – Copepod nauplius larva. (Scale = 20 or 100 μm as indicated)
<table>
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<tr>
<th>Taxon</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliophora</td>
<td><em>Dididium</em> sp Stein 1859</td>
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<tr>
<td></td>
<td><em>Monodidium</em> sp Fabre-Domerque 1888</td>
</tr>
<tr>
<td></td>
<td><em>Mesodinium rubum</em> (Lohmann, 1908)</td>
</tr>
<tr>
<td></td>
<td><em>Entodinium</em> sp</td>
</tr>
<tr>
<td></td>
<td><strong>Strombiliidiidae</strong></td>
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<tr>
<td></td>
<td><em>Strombiliidium</em> sp Schewiakoff 1892</td>
</tr>
<tr>
<td>Tintinnidae</td>
<td>Sub family Salpingellinae</td>
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<tr>
<td></td>
<td><em>Eutintinnus lusus-undae</em> (Entz. Sr. 1885)</td>
</tr>
<tr>
<td></td>
<td><em>Favella aff. Taralkaensis</em> Hada, 1932</td>
</tr>
<tr>
<td>Family Tintinnidiidae</td>
<td><em>Tintinnidium aff. Semiciliatum</em> Sterkii, 1879</td>
</tr>
<tr>
<td></td>
<td><em>T. aff. Balechi</em> Barria de Cao, 1981</td>
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<tr>
<td></td>
<td><em>Tintinopsis</em> spp. X2</td>
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<tr>
<td></td>
<td><em>Poroecus</em> sp Cleve 1902</td>
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<tr>
<td>Dinophyceae</td>
<td><em>Akashiwo sanguinea</em> (Hirasaka) Hansen &amp; Moestrup</td>
</tr>
<tr>
<td></td>
<td><em>Gymnodinium punctatum</em> Pouchet 1887</td>
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<tr>
<td></td>
<td><em>Gyrodinium aff. simple</em> (Lohmann 1908)</td>
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<tr>
<td></td>
<td><em>Oxyrrhis marina</em> Dujardin, 1841</td>
</tr>
<tr>
<td>Prymnesiopyceae</td>
<td><em>Chrysochromulina</em> sp Lackey</td>
</tr>
<tr>
<td>Rotifer</td>
<td><em>Brachionus</em> sp Pallas</td>
</tr>
<tr>
<td></td>
<td><em>Synchaeta</em> sp Ehrenberg</td>
</tr>
<tr>
<td>Copepoda</td>
<td><em>Gladiorerens imparipes</em> Thomson (nauplii)</td>
</tr>
<tr>
<td></td>
<td><em>Sulcanus conflictus</em> Nicholls, 1945 (nauplii)</td>
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<tr>
<td>Mollusca</td>
<td><em>Xenostrobus seures</em> Mueller, 1786</td>
</tr>
</tbody>
</table>

MRPP analysis of species composition based on a point in time once a month sampling regime indicated no apparent relationship between grazer and phytoplankton species distribution in time or depth.
Comparison of day versus night grazer composition

A comparison of day versus night surface abundance, based on day 1 counts and months when both day and night counts were available, (March, April, June, August, November and December 1998), indicated that grazer numbers in surface waters during the night are greater than day time numbers (Figure 7.6). Night time surface abundance (annual mean 15.7 x 10^3 cells L^-1, n = 6) is significantly higher than day time (annual mean 9.1 x 10^3 cells L^-1, n=6) (P > 0.05, single factor ANOVA), possibly indicating diurnal migration of grazers into surface waters. This seems particularly apparent during winter when the night values as a percentage of day values were 245% for June and 350% for August.

Figure 7.6 Diurnal microheterotroph abundance in surface waters (0.25 m) for an upper estuary site (RCI) over the period February 1998 to January 1999. Night time abundance as a percentage of daytime counts (*) generally exceeded 100%. (Data collected on day 1 of monthly sampling regime)
A comparison of total species counts for successive days (designated day 1 and day 2) noon sampling periods over the February 1998 – January 1999 period was made to determine whether grazing rates for day 2 were similar to those of day 1. This would validate the use of results from consecutive days. Results indicated a positive correlation (slope 0.547, \( r^2 0.428, n = 19 \)) between total day 1 and day 2 counts. Of the major groups (aloricate ciliates, tintinnids and dinophytes), correlations between the two days for tintinnid abundance was 0.735 (\( r^2 0.41, n=7 \)), and 0.46 (\( r^2 0.45, n=6 \)) for dinophyte abundance. Aloricate ciliates exhibited a higher correlation coefficient of 1.7878 (\( r^2 0.91, n = 6 \)), indicating that ciliate numbers for day 2 were consistently more numerous (178%) than day 1 numbers. This may be attributable to the sampling methods. Whole water sampling is less damaging to delicate ciliate structures (Petz, 1999).

**Pallial feeding in dinophyte species**

During species composition analysis evidence of mucous feeding veils for certain species was apparent, with the retractable haptonere visible in some (Figure 7.7). Pallial veils were observed (generally < 10% individual species count) on *Oxyrrhis marina* (day time January 1996 and December 1998), *Gyrodinium aff. simple* (March 1996, see Figure 7.6 C & D) and *Akashiwo sanguinea* during various months (March 1996, March 1998, April 1998, see Figure 7.7 A & B). April 1998 had the highest occurrence of feeding veils for *A. sanguinea* with 57% day-time and 47% night-time individuals having veils.
Figure 7.7 Akashiwo sanguinea (A & E) and Gyrodinium aff. simple (C, F & G) exhibit pallial veils for prey and particle capture. G aff. simple exhibits plastic body form. B & D show outlines of mucous veil with captured particles. Haptomere is used to hold and draw prey in. (Scale bar = 10μm)
Table 7.3 Monthly and seasonal diversity indices for upper estuary phytoplankton and microheterotroph grazers.

Diversity Indices ($d$), calculated according to $d = (S-1) \times \ln N$ (Margaleff, 1951) from species numbers ($S$) and total individual counts ($N$, cells $l^{-1}$), show seasonal grazer diversity generally reflects seasonal phytoplankton diversity. Subscripts indicate grazer (g) or phytoplankton (p).

<table>
<thead>
<tr>
<th>Month</th>
<th>$S_p$</th>
<th>$N_p$</th>
<th>$d_p$</th>
<th>$d_p$</th>
<th>$S_g$</th>
<th>$N_g$</th>
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<td></td>
<td></td>
<td>monthly</td>
<td>seasonal</td>
<td></td>
<td></td>
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<td>seasonal</td>
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<tr>
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<td>n/d</td>
<td>Winter</td>
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<td>Winter</td>
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<tr>
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<td>7</td>
<td>2400000</td>
<td>0.408</td>
<td>0.47±0.155</td>
</tr>
</tbody>
</table>

mean 0.37±0.226

mean 0.39±0.166
7.5.2 Grazing rates

Within bloom trends in microheterotroph grazing

Linear regression plots of $1/\ln (P_1/P_0)^{t}$ versus dilution factor (Figure 7.8 a - d), from weekly grazing rate determinations during the summer (January 1996) mixed dinophyte bloom at RCI, showed an increase in potential grazing rate ($g$) from 0.34 day$^{-1}$ to 1.0 day$^{-1}$ over the four week period. There was a corresponding increase in potential phytoplankton growth rate ($P_e$) over this period (Table 7.4) while Chla levels, which peaked during the week prior to this study, initially declined from week 1 to week 3 (Figure 7.8 c), then showed an increase by the fourth week. Potential production grazed ($P_e$) was not able to keep the bloom development in check during the first two weeks ($P_e < 0$) but, by the third and fourth weeks, grazing was reducing potential production by 61% and 86% respectively (Figure 7.8 f). This corresponded to a reduction of phytoplankton standing stock, in terms of chlorophyll content, of 9.3 $\mu$g Chla day$^{-1}$ in week 3 and 31.2 $\mu$g Chla day$^{-1}$ in week 4. This pattern fits a classic oscillation pattern of a predator - prey cycle for interspecies competition such as the Lotka-Volterra-type model (Sharov, 1996; Kretzschmar et al., 1991).

Monthly trends in size fractionated grazing rates.

Monthly grazing rate measurements for the nano ($< 20 \mu$m) and micro ($< 300 \mu$m) size fractions, based on field samples collected from surface waters at noon, provide a conservative estimate of daily grazing since grazer abundance has been shown to be considerably greater during night time sampling. Apparent phytoplankton growth coefficient for the nano-plankton ($< 20 \mu$m) fraction between February 1998 and January 1999 ranged between 0.005 (July) and 5.474 (June) over the 12 month period (mean 2.18 ± 1.5), while the grazing coefficient ranged between 0.057 and 1.406 (mean 0.48 ± 0.6) (Table 7.5). Microplankton ($< 300 \mu$m) apparent growth coefficients ($k$) and grazing coefficients ($g$) over the 12 month period exhibited a narrower range of between 1.224 and 3.809 (mean 2.18 ± 0.8) and 0.062 and 1.214 (mean 0.46 ± 0.3) respectively. The February, April, May, July, September, November, December and January nanoplankton ($< 20 \mu$m) grazing coefficients were higher than those recorded for the microplankton ($< 300 \mu$m) fraction (compare Tables 7.5 and 7.6). As is evident from
Figures 7.9 and 7.10, confidence limits on the regressions indicate a large variability in the data. The sample with least variation for both fractions was December, with $r^2$ values of 0.8147 ($< 20 \mu$m) and 0.8798 ($< 300 \mu$m).

Potential production ($P_p$), realised production ($P_r$) and potential production grazed ($P_g$) are presented for $< 20 \mu$m in Table 7.5 and $< 300 \mu$m in Table 7.6. Mean $P_g$ over the 12 month period was higher for the $< 20 \mu$m fraction (av. 53.8 ± 36.45, $n=11$) than for the $< 300 \mu$m fraction (av. 40.9 ± 26.50, $n=12$). A comparison of the potential production grazed (% day$^{-1}$) for both fractions (Figure 7.11 B) showed the $< 20 \mu$m fraction to have higher effect on reducing phytoplankton production than the $< 300 \mu$m fraction for a number of months.

< 1.2 \mu m size class

Initial chlorophyll a levels detected in GF/C filtered water was variable, averaging approximately 1 $\mu$g Chla l$^{-1}$ (range 0.2 – 2.1 $\mu$g Chla l$^{-1}$, see Figure 7.12 A). Final Chla analysis showed (ie Chla concentration at the end of incubation) that this generally did not change. However, during December there was a noticeable increase in the bacterioplankton size chlorophyll levels over the course of the 24h incubation. A 9-fold increase in Chla was measured in this fraction over the course of some 24-hour incubation periods. Growth rates calculated from this chlorophyll increase represented doubling times of 6 – 12 hours.

Phaeopigments

A comparison of Chla and phaeopigment concentration between surface and bottom depth over diurnal periods for the 12 month period (see Fig. 7.12 A & B) reveals Chla ranges from 0.5 to 123 $\mu$g Chla l$^{-1}$ over the 12 month period (mean 14.4 ± 20.6 $\mu$g Chla l$^{-1}$, $n = 40$) and phaeopigment ranges between zero and 25 $\mu$g l$^{-1}$ (mean 4.82 ± 4.73 $\mu$g l$^{-1}$, $n = 40$) with maximum values for November night time surface samples. An analysis of the ratio of phaeopigment to Chla (Figure 7.12 C) shows this to be quite variable, with a mean phaeo:Chla of 0.76 ± 0.95 ($n=40$) and maximum ratios occurring for both surface and night time samples during September. This corresponds with daytime grazer densities of $10^6$ cells l$^{-1}$, dominated by the aloricate ciliate Strombidium.
Figure 7.8 Dinophyte bloom grazing rates.
Linear regression analysis to determine apparent growth (k, day⁻¹) and grazing (g, day⁻¹) A. week1, B. week2, C. week3, D. week4, E. Comparison of Chla (○), apparent grazing rate (■) and g:k ratio (●) over the four week bloom and the preceding 2 weeks. F. Comparison of potential production grazed (P₄, ●, % day⁻¹) and standing stock grazed (△) over the bloom.
Figure 7.9 Monthly Pico + Nano (< 20 μm) grazing rates for Ron Courtney Island. Dashed line represents 95% confidence limits on linear regression.
Figure 7.10 Monthly Pico + Nano + Meso (< 300 μm) grazing rates for Ron Courtney Island.
Dashed line represents 95% confidence limits on linear regression.
Table 7.4 Estimates of Chla production and consumption by microheterotroph grazers (< 300 μm size class) over a four week dinoflagellate bloom (January 1996) in the upper Swan River Estuary.

Growth and grazing in surface samples (0.25m) from Ron Courtney Island site were determined by dilution method. Calculated phytoplankton growth coefficient $k$ (day$^{-1}$) and calculated grazing coefficient $l_1$ (day$^{-1}$) were determined from initial Chla $P_o$ (μg l$^{-1}$) and final Chla, $P_i$ (μg l$^{-1}$) according to the Landry and Hassett equations (see Fig. 7.5 equations). Linear correlation coefficients ($r^2$) and p values indicate goodness of fit. Potential production grazed was calculated according to the equations of Verity et al. (1993) where Potential production $P_p$ (μg Chla l$^{-1}$.day$^{-1}$) = $P_o e^{k-P_o}$. Realised production $P_r$ (μg Chla l$^{-1}$.day$^{-1}$) = $P_o e^{k-P_o}$. and Potential production grazed $P_g$ (%.day$^{-1}$) = ($P_p-P_r$)/(100/$P_p$).

\[
\begin{array}{cccccccc}
|g| & k & r^2 & p & P_o & P_i & P_p & P_g \\
\text{(day$^{-1}$)} & \text{(day$^{-1}$)} & & & (μg.L$^{-1}$) & (μg.L$^{-1}$) & (μg.L$^{-1}$.day$^{-1}$) & (μg.L$^{-1}$.day$^{-1}$) & (μg.L$^{-1}$.day$^{-1}$) & (μg.L$^{-1}$.day$^{-1}$) \\
Week 1 & 0.342 & -1.270 & 0.7743 & 0.8797 & 39.5 ± 1.55 & 20.7 ± 4.38 & -28.428 & -31.643 & -11.31 \\
Week 2 & 0.522 & -0.519 & 0.7667 & 0.8841 & 17.6 ± 0.16 & 14.4 ± 0.76 & -7.125 & -11.393 & -59.90 \\
Week 3 & 0.482 & 0.980 & 0.4852 & 0.1977 & 15.2 ± 1.40 & 15.4 ± 1.37 & 25.334 & 9.819 & 61.24 \\
Week 4 & 1.002 & 1.336 & 0.5746 & 0.6645 & 36.4 ± 5.47 & 59.3 ± 1.30 & 102.022 & 14.427 & 85.86 \\
\end{array}
\]
Table 7.5 Estimates of chlorophyll a production and consumption by microheterotroph grazers < 20 μm size class during 1998 - 99 in the upper Swan River Estuary.

Growth and grazing in surface (0.25 m) samples from Ron Courtney Island site were determined by dilution method and potential production grazed calculated according to the equations given in the Methods section. \( k \text{ (day}^{-1}) \) = calculated phytoplankton growth coefficient, \( IgI \text{ (day}^{-1}) \) = calculated grazing coefficient; \( P_p \text{ (μg l}^{-1}) \) = Chl_{inhib}; \( P_p \text{ (μg l}^{-1} \text{ day}^{-1}) \) = potential production = \( P_p e^{k \cdot t} - P_r \); \( P_r \text{ (μg l}^{-1} \text{ day}^{-1}) \) = realised production = \( P_p e^{k \cdot t} - P_r \); \( P_g \text{ (μg l}^{-1} \text{ day}^{-1}) \) = potential production grazed = \( (P_p - P_r) \times (100/P_r) \).

| Month     | | \( |g| \) (day\(^{-1}\)) | \( k \) (day\(^{-1}\)) | \( r^2 \) | \( P_o \) (μg l\(^{-1}\)) | \( P_p \) (μg l\(^{-1}\) day\(^{-1}\)) | \( P_r \) (μg l\(^{-1}\) day\(^{-1}\)) | \( P_g \) (%) day\(^{-1}\) |
|-----------|----|----------------|--------------------|--------|----------------|----------------|----------------|----------------|
| February  | 0.87 | 2.899        | 0.2671             | 1.3    | 22.299         | 8.587           | 61.492         |
| March     | 0.143 | 3.755        | 0.2177             | 26.59  | 1109.991       | 956.546         | 13.644         |
| April     | 0.48  | 1.173        | 0.7446             | 18.82  | 41.995         | 18.811          | 55.206         |
| May       | 0.574 | 1.892        | 0.2996             | 6.28   | 35.372         | 17.181          | 51.427         |
| June      | 0.057 | 5.474        | 0.0064             | 13.89  | 3296.606       | 3113.185        | 5.564          |
| July      | 0.98  | 0.005        | 0.0629             | 6.79   | 0.035          | 11.394          |               |
| August    | 0.473 | 1.436        | 0.4852             | 6.83   | 21.868         | 11.053          | 49.458         |
| September | 1.169 | 0.981        | 0.7036             | 1.551  | 2.587          | -0.266          | 110.268        |
| October   | 0.287 | 2.899        | 0.5655             | 62.621 | 1074.273       | 790.631         | 26.403         |
| November  | 0.225 | 3.169        | 0.0905             | 14.211 | 323.741        | 255.65          | 21.033         |
| December  | 1.042 | 1.332        | 0.8147             | 15.412 | 42.981         | 5.186           | 87.934         |
| January   | 1.406 | 1.171        | 0.6964             | 17.979 | 40.031         | -3.76           | 109.392        |
Table 7.6 Estimates of Chla production and consumption by microheterotroph grazers <300 μm size class during 1998 - 99 in the upper Swan River Estuary. Growth and grazing in surface (0.25 m) samples from Ron Courtney Island site were determined by dilution method and potential production grazed calculated according to the equations given in the Methods section. Calculated apparent phytoplankton growth coefficient $k$ (day$^{-1}$) and calculated grazing coefficient $lgi$ (day$^{-1}$) were determined from dilution experiments and initial $P_o$ (μg l$^{-1}$) and final $P_f$ (μg l$^{-1}$) chlorophyll a (Landry and Hassett, 1982). Potential production $P_p$ (μg l$^{-1}$ day$^{-1}$) = $P_o$e$^{k-P_g}$, realised production $P_r$ (μg l$^{-1}$ day$^{-1}$) = $P_o$e$^{k-P_g}$; Potential production grazed, $P_g$ (% day$^{-1}$) = ($P_o$-$P_r$)(100/$P_o$).

| Month     | $|lgi|$ (day$^{-1}$) | $k$ (day$^{-1}$) | $r^2$ | $P_o$ (μg l$^{-1}$) | $P_p$ (μg l$^{-1}$ day$^{-1}$) | $P_r$ (μg l$^{-1}$ day$^{-1}$) | $P_g$ (%)的日增长 |
|-----------|-------------------|-----------------|-------|-------------------|-----------------------------|-----------------------------|----------------------|
| February  | 0.695             | 3.199           | 0.2670 | 1.31              | 30.634                      | 14.617                      | 52.287               |
| March     | 0.412             | 2.255           | 0.8865 | 27.6              | 235.536                     | 146.681                     | 37.724               |
| April     | 0.115             | 3.809           | 0.2705 | 19.09             | 841.708                     | 748.197                     | 11.110               |
| May       | 0.239             | 3.067           | 0.0709 | 6.25              | 127.976                     | 99.442                      | 22.297               |
| June      | 0.422             | 2.092           | 0.4709 | 16.11             | 114.453                     | 69.505                      | 39.272               |
| July      | 0.123             | 1.748           | 0.1096 | 12.47             | 59.144                      | 50.856                      | 14.0138              |
| August    | 0.596             | 1.208           | 0.6581 | 7.15              | 16.781                      | 6.036                       | 64.030               |
| September | 0.324             | 2.213           | 0.2947 | 1.76              | 14.331                      | 9.878                       | 31.074               |
| October   | 0.712             | 1.865           | 0.4760 | 24.3              | 132.606                     | 52.688                      | 60.267               |
| November  | 0.062             | 1.323           | 0.0008 | 16.93             | 46.610                      | 42.790                      | 8.195                |
| December  | 0.603             | 2.151           | 0.8798 | 16.86             | 128.013                     | 62.410                      | 51.247               |
| January   | 1.214             | 1.224           | 0.5988 | 14.67             | 35.222                      | 0.148                       | 99.579               |
Figure 7.11 A. Initial chlorophyll a levels from monthly sampling at Ron Courtney Island from February 1998 to January 1999.

B. Potential grazing rate expressed as % of potential production over an annual cycle for micro (< 300 μm) and nano (< 20 μm) heterotrophs.
Figure 7.12 Temporal diurnal trends in Chla and phaeopigment levels at RCI.
A & B. Surface and bottom Chla and phaeopigments levels (SD, n=3) over an annual cycle.
Mean Phaeo:Chla value (χ) marked as a solid line.
• Surface (0.25 m) day ▼ Bottom(4.5 m) day ○ Surface night ▲ Bottom Night
Seasonal highs in phaeopigment levels occurred for spring and summer, with autumn and winter levels relatively lower.

### 7.6 Discussion

#### 7.6.1 Microheterotroph species composition

Temporal species composition analysis has shown that the grazing community comprised a diverse group of protozoans during the spring and summer months. Ciliate species were dominant in winter (average 54%, with maximum 89% in August 1998), with the aloricate ciliate *Strombidium* (Oligotrich) being conspicuous. Results concur with similar findings for the Kariega Estuary, South Africa (Froneman and McQuaid, 1997), where ciliate grazers represented 40 – 65% of total protozoan numbers. A comparison of abundance for noon samples between consecutive days indicated a positive correlation for within groups and overall counts. Natural variations in population numbers and the problem of being able to sample the same water body between sampling times, will have an effect on the results of a comparison of this nature. Although the general composition of samples between days was similar, it was noted that counts for aloricate ciliates were consistently higher for day 2 samples. This difference is attributed primarily to differences in sampling techniques. Whole-water sampling techniques are recommended for the enumeration of aloricate ciliates because of the fragile nature of these organisms (Petz, 1999). Sampling by positive displacement pump (day 1), although recognised as being gentle on organisms (Petz, 1999), apparently caused sufficient damage to the delicate ciliate forms as to effectively reduce their numbers by approximately 59%.

Generally species diversity indices are lower for grazers than for phytoplankton. Grazer diversity was greatest during summer and autumn, also periods of maximum phytoplankton species diversity. MRRP analysis of species composition in this study found no relationship between grazer and phytoplankton species composition. Basu and Pick (1996) report no relationship between Chlα and zooplankton biomass when studying factors regulating phytoplankton and zooplankton biomass in temperate rivers. Since sampling for grazing was made on a monthly time basis, the grazing determinations may be made at any point during a bloom or non-bloom cycle. Correlation analysis over a twelve month period between phytoplankton and grazer
species, based on this sampling regime, would not be able to detect such a successional relationship due to time-scale and timing issues. A possible explanation for the February low phytoplankton and high grazer diversity indices is that it is a consequence of sampling at the end of a bloom cycle when grazer growth and diversity is maximal within the community and the phytoplankton diversity low following grazing pressure. It is suggested that the low diversity index for phytoplankton and the apparent absence of grazers during November indicates that sampling occurred during the initial stages of a monospecific bloom of *Chlamydomonas globosa*.

Comparison of grazer abundance between day and night sampling indicates a higher number of grazers present in surface waters at night. This is particularly noticeable during the winter months when aloricate ciliates are dominant in the microheterotroph community. This observation supports previous reports of nocturnal negative geotaxis vertical migration in grazing species (Tranter *et al.*, 1981; Pedrós-Alió *et al.*, 1995; Kamykowski, 1997).

From studies of a summer dinophyte bloom it is apparent that, although day time grazer distribution through the water column did not appear to change greatly, there was a shift in species composition from cryptophyte dominated to dinophyte dominated grazing. The majority (> 80%) of grazers were found in mid to surface waters at noon. This coincides with the peak phytoplankton distribution during the day (see Figure 3.3). There was also an apparent change in the vertical migration behaviour of the heterotrophic dinophyte *Oxyrrhis marina*, that coincided with phytoplankton species behaviour change over the course of the bloom (see Figure 3.3). MRRP correlation analysis between phytoplankton and grazer species distribution over an annual period found no significant relationship (see Appendix I). This may be due inconsistent sampling from different portions of bloom development and decline cycle, resulting in extremely large variations in data obtained.

### 7.6.2 Grazing rates

The Landry & Hasset (1982) dilution technique is frequently used for community grazing rate determinations. Theoretical considerations are outlined in the original manuscript. One of the basic assumptions for the dilution technique is that the predator-prey interactions are a result of random chance encounters. However, prey
selectivity has been demonstrated for a number of heterotrophic microplankton, such as the heterotrophic flagellates Paraphysomonas imperforata (Goldman and Dennett, 1990), Bodo saltans (Kinetoplastidae), Spumella sp. (Chrysomonadida) (Jurgens and DeMott, 1995) and Chrysochromulina (Prymnesiophyceae) (Jones et al., 1993). Prey selectivity has also been suggested for some aplastidic flagellates (Pace and Balliff, 1987). The fact that there is evidence of prey selectivity in this study, through the observation of single species concentrations collected on feeding veils and in mucous strands (see Figure 7.5), indicates that this basic precept is not always valid. While not all grazer activity in the community is a result of random encounter, the net grazing rate determinations should provide an indication of community grazing rates. As a result of prey selectivity operating in some but not all of the grazer community, rate determinations may have greater within or between concentration variations, or skewed linear transforms that may be a result of concentration-related detection for selective grazers within the community. Variability could also be influenced by presence and/or absence of voracious individuals within replicate samples. The information gained from these grazing rate determinations provides the first estimates for microheterotroph grazing in the Swan River Estuary. Despite inherent problems in conforming stringently to the basic assumptions of the technique, the rates determined provide an indication of net community grazing and therefore the technique has merit when used in comparing top-down versus bottom-up control of algal biomass based on whole community studies.

The results of many grazing studies are presented in terms of chlorophyll grazed per individual (see Frost, 1972 and refs there-in; Griffin, 2003). As this study investigates community grazing rates in assemblages comprising an average of 6 different grazing species, the grazing rates determined are presented as potential production grazed (%) based on the equations of Verity et al. (1993). Extrapolation of results obtained from a mixed community study to give a 'per individual' grazing rate does not seem appropriate, valid or useful. Grazing rates presented in terms of phytoplankton community Chla loss overcomes the problem of accommodating various feeding rates attributable to different organisms within the grazing community. It provides a net community grazing estimate from the parameters determined by the dilution experiments (Landry and Hassett, 1982).
Within bloom trends in microheterotroph grazing

Grazing rates determined for noon surface samples indicated not only an increase in total grazer cell counts from week 1 (23000 cells l\(^{-1}\)) to week 3 (182000 cells l\(^{-1}\)), but also a shift in species dominance from cryptophyte (80% total) to dinophyte (63% total) grazers. Over the course of the bloom the grazing coefficient increased and then declined. Although the apparent growth rate increased over this period, the ratio of gk peaked and declined in a pattern that reflected the peak and decline of the chlorophyll a concentrations present. Comparison of chlorophyll a concentration change and the ratio of gk shows the peak and decline of gk to follow the same pattern. The apparent recovery in Chla levels by week 4 may indicate that, while grazing rates are still increasing, phytoplankton growth rate is also increasing, possibly as a result of additional nutrient availability through DON released by grazers through faecal material and/or sloppy feeding.

Monthly trends in size fractionated grazing

Grazing rate determinations based on the dilution technique indicate that 32 – 149% (mean 79.4 ± 32, n=10) of potential phytoplankton production is grazed by the < 300μm size class. Within that range the nano-heterotrophic component grazes 30 – 355% (av. 67.9% ± 27, n = 12) of potential production. These rates are 24hr (daily) estimates for those grazers present in the noon sample. As grazer abundance during day time sampling was significantly lower than night time abundance, it is probable that grazing rates determined from night time samples would be higher, simply because of higher abundance per sample. Considering these rates in conjunction with grazing rates determined for mesoheterotrophs (copepods) in the same system, where up to 40% of standing stock can be reduced (Griffin et al., 2001), it is apparent that total grazing within the microplankton (organisms <300 μm) has the potential to control phytoplankton biomass. By comparison, grazing rates corresponding to daily losses of approximately 5% (day) and 2% (night) of initial standing stock and about 50% (day) and 65% (night) of potential production respectively have been reported for a South African estuary (Froneman and McQuaid, 1997). These grazing rates are determined over a 24h period and therefore represent a daily rate, incorporating any diurnal rate changes that may occur. It must be noted that the samples on which these rate
estimates were based were collected at noon and therefore had a reduced number of grazers present than if the samples had been collected at night.

Monthly linear regression plots of $1/\ln (P_e/P_{e0})^t$ vs. dilution factor over an annual cycle (February 1998 – January 1999) for nano-plankton ($< 20 \mu m$ size-class, Figure 7.7) and micro-plankton ($< 300 \mu m$ size-class, Figure 7.8) show much more variability and greater departures from linearity than for the within bloom study. This variability is consistent with rates determined from samples taken on a temporal basis rather than a bloom basis since rates may be determined from a community at any stage of the bloom development and decline cycle. From a monthly comparison of grazing rates it is apparent that there is no direct proportionality between the two size classes examined ($< 300 \mu m$ and $< 20 \mu m$, see Figure 7.5 B). It appears that there is a component within the larger size fraction ($20 - 300 \mu m$) that has a negative effect on the grazing rate of the smaller fraction so that, in isolation ($< 20 \mu m$ only), this size fraction produces a much larger grazing pressure on the phytoplankton biomass. Caution must therefore be used when interpreting this data on a size fraction basis. The higher level of grazing evident in the $< 20 \mu m$ fraction compared with the $< 300 \mu m$ fraction suggests that heterotrophic predation by organisms in the larger fraction may be limiting grazing in the smaller size fraction. When these smaller grazers of pico-phytoplankton in the $< 20 \mu m$ fraction are unchecked by the larger heterotrophs the grazing rate is higher. One or more heterotrophic organisms within the size range $20 - 300 \mu m$ may be feeding on grazers in the $< 20 \mu m$ fraction and so reducing the grazing pressure on phytoplankton biomass. Thus a trophic cascade is operating within this system at these times.

Variation between replicates is particularly evident in $< 300 \mu m$ fraction. Possible explanations for this may be the occurrence of accumulations of chlorophyll-containing cells in mucous aggregates formed during pallial feeding. Another possible source of variation is the presence or absence of low numbers of highly voracious individuals in one or more of the replicates. This would either decrease or increase the Chla levels within those samples.

One of the basic assumptions for the dilution technique is that prey capture follows random encounters of predator and prey. While this may be the case, prey selectivity is
suggested by mono-specific cell accumulations within the pallial veils (see Figure 7.4). The occurrence of mucous slimes containing aggregates of specific microplankton species (see Figure 7.4) indicates food selectivity by heterotrophic or mixotrophic dinoflagellates. This may influence the accuracy of grazing rates determined using the dilution method. The clumping of chlorophyll may artificially raise Chla determinations for individual samples, thus increasing variation between replicates and causing lower than expected r² values in linear estimations.

**Picoplankton fraction**
Photosynthetic picoplankton (< 2 µm) and their contribution to production is a recognised integral component of pelagic foodwebs (Stockner and Antia, 1986; Sherr and Sherr, 1988; Carrick and Schelske, 1997). It may be assumed that there are no grazing organisms in the <1.2 µm size fraction and that changes in the chlorophyll content in this fraction represents autotrophic processes since grazers have been eliminated from the experimental chamber. The chlorophyll present may be due to microflagellates, Chla-containing cyanobacteria and/or Chla fragments generated by grazing or cell breakage (Azam & Hodson, 1977; Krempin et al., 1981). In general, dilution experiments of this type rely on the diluant being devoid of chlorophyll and not in itself a source of potential grazing material. A substantial increase in the Chla content of the GH/C filtered water fraction was demonstrated for certain months (up to 9-fold, see Figure 7.8 A), which suggests that, at times autotrophic bacteria may be an important source of primary production in the microbial foodweb. This has previously been overlooked in the Swan River Estuary.

**Phaeopigments**
Ambient levels of Chla and Chla degradation products (phaeopigments), which may arise from phytoplankton decomposition or sequential degradation of Chla caused by grazing (Bidigare et al., 1986), were monitored throughout this study. A comparison of the ratio of phaeopigment:Chla can give an alternative indication of the level of heterotrophy (and phytoplankton health) in the system.

In a healthy exponentially growing phytoplankton population the ratio of phaeopigments:Chla would be high (>1). An increase in phaeopigments and subsequent decrease in phaeopigment: Chla ratio or relative percentage of phaeophytin
to Chla has been used to indicate grazing activity (Hallegraeff, 1981), but may also indicate senescence of the phytoplankton population or high levels of plant-based organic debris in the water. The peak phaeopigment level occurred in night-time surface samples, following a peak in Chla the previous month (Oct., 122 μg Chla l⁻¹). Elevated phaeopigment levels at depth (4.5 m) may be indicative of a higher proportion of senescent phytoplankton cells that have settled out of the water column and/or higher levels of grazing. Elevated phaeopigments occurring in night-time surface waters, as seen in April, June and November (Figure 7.8 B) provides strong evidence of nocturnal grazing at this depth and also indicates changes in grazing behaviour through the water column over a diurnal period. This supports previous evidence of nocturnal vertical migration of zooplankton grazers (Tranter et al., 1981; Pedró-Alió et al., 1995; Kamykowski and Yamazaki, 1997).

Pallial feeding in Dinophyte species

There is evidence that pallial feeding is used by a number of the species occurring within the Swan River Estuary heterotrophs. The two species most commonly observed to have pallium veils were Akashiwo sanguinea and Gyrodinium aff. simple. The dinoflagellate A. sanguinea has previously been reported as feeding by engulfing prey (Hansen 1998), but not using feeding veils.

7.7 Conclusions

Microheterotroph grazing in the upper Swan River Estuary occurs as a result of a diverse assemblage of species. Dominant species vary throughout the year in response to changes in phytoplankton composition and levels of particulate organic debris present in the water column. Aloricate species known to feed on organic debris and small phytoplankton species dominate during winter periods when these are in high concentrations. Larger grazers, such as rotifers and larger tintinnid species, as well as grazers able to phagocytose or externally ingest cellular material, are more prevalent when larger phytoplankton species are present.

Results of this study support size-fractionated Chla studies in the Kariega Estuary, South Africa that showed phytoplankton standing stock was dominated by nano- and pico-phytoplankton (Froneman and McQuaid, 1997). For all months studied the Chla
levels in the < 20 μm and < 300 μm fraction were not significantly different (P > 0.5), indicating that all Chlα present was in fact nano-phytoplankton chlorophyll.

Grazing by mesoheterotrophs, in particular copepod species such as the locally occurring *Suleanus conflictus* and *Gladioferens imparipes*, was shown to be highly important in attenuating a dinoflagellate bloom that occurred over a 3-week model simulation period (Griffin *et al.*, 2001) and generally reduced phytoplankton biomass by <40% standing stock in the upper Swan River Estuary (Griffin, 2003). It is evident from this study that the microheterotroph component of the grazing community has the potential to significantly influence algal biomass. On average the annual grazing pressure exerted by this suite of organisms reduces potential growth by about 80%, slowing overall phytoplankton biomass increase. Microheterotroph grazing alone can, at times, effect reductions of up to 50% of standing stock and may also effect compositional change through selective feeding and the operation of trophic cascades. Microheterotrophs are able to substantially reduce algal biomass and so influence the development of algal blooms.

A more comprehensive understanding of the trophic importance of bacterioplankton in the microheterotroph community and the related detrital foodweb acting in the upper Swan-Canning Estuary is needed to provide a fuller understanding of the trophic transfer pathways operating to control phytoplankton biomass in this system.

### 7.8 References


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CHAPTER 8: A Synthesis of Control Mechanisms Regulating Phytoplankton Communities for the Upper Swan River Estuary

8.1 Introduction

Numerous studies have attempted to allocate relative importance of allogenic and autogenic factors to phytoplankton biomass (Gilbert 1998; Griffin and Rippingale 2001; Koepfler and Lewitus 1995; Matveev 1995; Schütte, 1998; Weisse 1991). Studies considering bottom-up control of phytoplankton growth and distribution, often consider whole systems (Baird and Ulanowicz, 1989; Basu and Pick, 1996; Thompson and Hosja, 1996; Gallegos and Jordan, 1997) rather than localised sites or environment types within a system. Yet it is on a local scale that the majority of typical blooms occur. Blooms characteristic of certain areas of a system are usually transient and do not spread over extensive tracts of an estuary. Incidental HABs resulting from anomalies in the regular cycle of environmental conditions (i.e. unseasonal rain, elevations in nutrient), such as those causing closure to kilometers of the Swan River Estuary in 2001 and 2003, usually occur on larger spatial scales. These ephemeral seasonal blooms may be better predicted and managed more effectively with an understanding of the physiology and flux processes associated with their development.

While nitrate nitrogen has been shown to be limiting in the Swan River Estuary system (Thompson and Hosja, 1986) there is little information on availability. No studies have been made on the use of other nitrogen sources by phytoplankton, and there was no information on uptake rates. Few studies on the effect of grazing on phytoplankton biomass have been conducted (Griffin and Rippingale, 2001) and there is no information on the microheterotroph component of the plankton or its influence on phytoplankton biomass.

The principal aim of this study was to investigate which factors most influenced phytoplankton biomass and distribution for an upper estuary site. This study considered physical and chemical parameters in conjunction with nitrogen-based physiological studies. The availability of alternate nitrogen sources was determined. The parameters chosen were based on cycling of nitrogen as this has been demonstrated to be a limiting nutrient in this system (Thompson and Hosje, 1996; Thompson, 2001).
Using these flux rates in conjunction with measured physico-chemical environmental parameters, an attempt was made to determine which factors, or combination of factors, exerted most influence on species distribution and composition on a localised basis.

An outcome of this project was to make a comparative assessment between the effects of top-down control of phytoplankton biomass by microheterotroph grazers and bottom up control by nutrient availability, specifically nitrogen sources. For this the microheterotroph community was identified and enumerated and size fractionated grazing rates were determined over an annual cycle.

## 8.2 Methods

To determine the factors having the greatest influence on biomass and distribution of phytoplankton over an annual cycle in the upper Swan River Estuary required the combining the results from various component studies of this research (see Figure 8.1). Information on diurnal and depth related variation in physical and chemical parameters (Chapter 2), species composition and distribution (Chapter 3), phytoplankton physiological adaptation to different nitrogen sources (Chapter 4), specific nitrogen source preference and uptake (Chapters 5 & 6) and grazing pressure exerted by the microheterotroph community are linked (see Figure 8.3) to produce an integrated analysis of the phytoplankton ecology at this upper estuary site.

Phytoplankton species composition analysis results indicate much lower than expected diversity indices. This is most likely due to the sampling method. Whole water samples were sub-sampled and settled. This would capture only the most numerous phytoplankton. Phytoplankton species analysis are usually based on net tows (vertical or oblique) that sample a large volume of water and so capture a variety of less numerous species. However, whole water sampling was necessary for physiological experiments to be performed so that trauma to the organisms was minimised (Petz, 1999).

Resource limitation has necessitated a number of compromises in the way samples were collected (i.e. the necessity to run these over a two days instead of concurrently) and experiments omitted ($^15$N remineralisation measurements). Results have been analysed based on assumptions of negligible remineralisation occurring over the course of
incubations. Comparisons of realised ambient NH$_4^+$ change with predicted ambient change based on rate estimates indicate that this may not be the case.

**Figure 8.1** Relationship of process and ambient measurements used to determine the net effect of nitrogen supply and grazing on phytoplankton growth and the principal factors controlling phytoplankton biomass distribution.
Grazing rates were determined by the dilution technique (Landry and Hassett, 1982) where it is assumed that no chlorophyll growth occurs in the diluent fraction (GF/C filtered, nominal pore size 1.2µm). In systems supporting autotrophic bacterial populations this may not be the case. In such a situation the potential production calculated would include a confounding factor that would increase in effect as the dilution increased. While most of the incubations did not show any evidence of this there was elevated Chla in the diluent during October and December. This aspect of the Swan River phytoplankton population needs quantifying and the technique needs modification to account for this.

Canonical correspondence analysis was used to interpret species ordination (Chapter 3) with the following environmental variables presented in Chapter 2: Chla, temperature, salinity, dissolved oxygen, PAR, orthoP, organic P, total P (TP), NO₃, NH₄, urea, Kjeldahl N (KN), total N (TN) and N:P ratio. Results are presented in the form of PCA plots. Multi-response Permutation Procedure (MRPP), a randomisation test that evaluates differences in species composition based on some distance measure, was used to relate phytoplankton species composition with grazer species composition. (MRPP has the advantage of not requiring multivariate normality and homogeneity of variances, factors that are seldom met when dealing with ecological community data).

Grazing and uptake rates were compared in terms of nitrogen uptake or loss per unit of chlorophyll per unit of time. Grazing rate determinations provided a percent value of net potential production grazed (Pg %) per day (Chapter 7). Using this factor and the monthly specific ambient uptake rates (Chapter 5), mean seasonal uptake and loss values in mg N µg Chla⁻¹ h⁻¹ were calculated. For this, production was assumed to be equivalent to nitrogen uptake. Grazing rate was assumed to be constant over the 24 hour period. Nitrogen uptake data obtained from laboratory experiments in this study have been used to provide input to validate a two dimensional model, based on the laterally averaged estuary model TISAT developed to simulate the movement of temperature, salinity and dinoflagellate Chla and its associated internal nitrogen store, with specific emphasis on the response to physical factors and vertical migration (Hamilton et al., 1999).
8.3 Synthesis

A major aim of phytoplankton population monitoring and modelling studies is to try to predict what factor or set of factors is controlling the composition and biomass of the phytoplankton populations.

Increases in levels of anthropogenic nutrient inputs in nitrogen sensitive estuarine and coastal environments (Paerl, 1988; Nixon, 1995) have lead to phytoplankton communities that exhibit higher biomass, shifts in community composition and a growing frequency and magnitude of nuisance and harmful algal blooms (Hallegraeff, 1993; Richardson, 1997). This has been a concern for the Swan and Canning Rivers Estuaries in Western Australia.

Physical factors can have an effect on the physiological process affecting phytoplankton growth. Whilst numerous studies have shown a relationship between physical and/or chemical parameters and algal biomass (Je Anderssen et al., 1994; Mallin, 1994; Gallegos and Jordan, 1997), others have found no significant relationship exists (Rhudy et al., 1999).

This study provides the first physiologically derived flux measurements for phytoplankton community nitrogen utilisation in the Swan River Estuary. It investigates nitrogen source preferences and relates these to ambient nitrogen availability. Diurnal and depth-related phytoplankton distribution is related to both physico-chemical and physiological parameters to determine which factor, or combination of factors, exert most control on phytoplankton abundance and succession. The Ron Courtney Island site was chosen as representative of one of the deeper sites of the upper Swan River Estuary, an area exhibiting elevated eutrophic status and thus a greater risk potential for blooms. It also had the added advantage of being the location for a number of previous and concurrent studies, and was one of the Swan River Trust's routine monitoring sites.

**Phytoplankton composition**

Species composition studies for phytoplankton indicated conformation to recognised seasonal successional patterns (Thompson and Hosia, 1996) over the course of this study. Principal component analysis (PCA) of diurnal distribution patterns with physico-chemical and physiological parameters indicated strong depth-related
differences in day-time population distribution that were not apparent at night. Depth related abundance estimates for typical seasonal blooms indicated differences in species distribution patterns. Spring chlorophyte blooms, dominated by Chlamydomonas globosa, maintained distribution in surface waters throughout the bloom. During the summer mixed dinophyte bloom, dominated by Scripsiella sp., Gymnodinium simplex and Oxyrrhis marina, there was an apparent shift in vertical migration (positive geotaxis) at night over the course of the bloom. Using modelling techniques this diurnal vertical migration was shown to provide an important component of dinophyte nutrition over the bloom (Hamilton et al., 1999). Winter was dominated by pennate diatoms which may be dislodged benthic diatoms. Cylindrotheca closterium, a species of pennate diatom that is recognised as an indicator of elevated organic levels (Jacob John, pers. comm.) was the dominant diatom at this time. This supports evidence presented that indicates organic nitrogen as an important component of the phytoplankton nitrogen nutrition in this system.

No significant difference (P > 0.05) was found between the < 20 µm and the < 300 µm fractions over the 12 month period, February 1998 to January 1999. For this study the majority of photosynthetic cells were in the pico- and nano- plankton fractions (<20 µm). There has been speculation that a shift from larger to smaller phytoplankton species has occurred in this estuary. Based on species composition data from 1980 - 81 and 1994 – 95, Twomey and John (2001) concluded no significant shift in major groups of species had occurred. Unlike previous studies, the present study included nano and picoplankton. As previous studies of the Swan River Estuary have not reported information on chlorophyll size fractionation, it is difficult to draw any conclusions on possible shifts in phytoplankton community size structure from this study alone.

**Microheterotrophs species composition**

Microheterotroph species composition over an annual cycle (1998 - 99) indicated a predominance of alocate ciliates from four genera, with Strombidium numbers peaking during winter. Tintinnid (loricate) ciliates and heterotrophic dinoflagellates were next most abundant. Rotifers (Brachionus sp. and Synchaeta) occurred sporadically throughout the year.
Pallial feeding, which is the capture and external digestion of prey using a haptomere and mucous veil, was evident in the dominant heterotrophic dinoflagellate grazers (e.g., *Gyrodinium aff. spirale*). This is shown for the first time for the dinoflagellate *Akashiwo sanguinea*. Prey selectivity, as evidenced by monospecific cell accumulations within pallial veils and mucous aggregations, may provide a mechanism for effecting changes in the species composition at the lower trophic level.

**Environmental factors**

Routine monitoring of river health indicators by the Swan River Trust determines the state of nutrient loadings, temperature, salinity, oxygen and chlorophyll levels, plus identifies and counts phytoplankton to monitor presence and abundance of potentially toxic or nuisance algal species. The monitoring programme is filling critical gaps in the knowledge of the Swan River Estuary that may assist in determining the best strategies to use for remediation. Various remediation techniques are currently being trialed in an attempt to modify river conditions conducive to bloom development. Methods trialed to date, with varying degrees of success, include oxygenation and destratification techniques and the application of a modified clay (Phoslock™) to bind phosphorus in the sediment so it is not available for phytoplankton growth.

The Swan-Canning Cleanup Programme, implemented and run over the past two years through the Swan River Trust, with cooperation from a number of bodies such as the Water and Rivers Commission, Agriculture Western Australia, the Department of Environmental Protection and the Western Australia Department of Planning and Infrastructure, uses integrated catchment management and land-use management strategies in an attempt to reduce nutrient inputs to these river systems.

**Physiological Studies**

Physico-chemical parameters over the study period conformed to previously reported ranges, although large inter-annual variability has been recognised (Thompson, 2001, Twomey and John, 2001). This is most likely true of uptake rates as well, but would require further studies. A comparison of total ambient uptake averaged over the three weeks during February 1996 with the value obtained for February 1998 (Table 8.3), shows considerable variability between years, with 1996 values only a fraction (13 - 28%) of calculated February 1998 total ambient specific uptake rates.
Monitoring programmes that use only static point-in-time nutrient concentration measurements fail to assess nutrient flux. The physiological response of phytoplankton assemblages to nutrient availability in the upper Swan River Estuary is extremely dynamic. Total ambient specific uptake ($\Sigma V$) over an annual cycle ranged between 4 ng N $\mu g$Chl a$^{-1}$ h$^{-1}$ and 5333 ng N $\mu g$Chl a$^{-1}$ h$^{-1}$. Daytime $\Sigma V$ near the bottom (4.5m) was generally <20% of surface rates, while this increased to up to 170% of surface rates at night. Ambient specific uptake rates ($V$) for different nitrogen sources ($= V_{\text{max}}$) exhibit great variability between depths and time of day, both within and between blooms and between years. Physiological adaptations to utilise alternate nitrogen sources are evident during periods of negligible allochthonous NO$_3^-$ input. Higher maximum uptake ($V_{\text{max}}$), and lower half saturation constants ($k_{\text{c}}$) for NH$_4^+$ and urea coincide with periods when these two nutrients form the largest component of available N. The importance of atmospheric input to nitrate levels, pelagic nitrification, has been recognised as the major spring and summer source of NO$_3^-$ (55% annual input) in the Narragansett Bay and Providence River estuaries and results in increased oxygen demand near sediments (Berounsky and Nixon, 1993). While this has not been explored in this study, it may help to explain the higher than expected NO$_3^-$ uptake rates for NO$_3^-$ at depth.

The conclusions drawn from the dinophyte bloom 2-D mathematical simulation validated with nitrogen uptake data from this study (Hamilton et al., 1999) indicated that spatial and temporal separation of nitrogen access (night, bottom) and photosynthetic activity (day, surface) was an important factor in these organisms ability to survive.

This research has highlighted the importance of ammonium as a nitrogen source for phytoplankton growth during most seasons, particularly during summer when allochthonous nitrate sources are negligible. For more than the past two decades it has been recognized that tight coupling exists between nitrogen (NH$_4^+$) uptake and regeneration for various water bodies (Caperon et al., 1979; Glibert, 1982; Probyn, 1987; Probyn and Licas, 1987). Regeneration rates equal to or greater than uptake or assimilation rates have been reported by Harrison (1978), Axler et al. (1981), Glibert (1982, 1988) and Glibert et al. (1991). Despite this knowledge, techniques for determining nutrient uptake and regeneration rates are not generally included in routine environmental monitoring programmes. To date there has been little research into the relative influences of ammonium inputs from tributaries, sediments and water column
recycling on phytoplankton growth in the Swan River Estuary. Preliminary estimates based on observed changes in ambient ammonium concentration over time, related to expected changes calculated on the basis of measured uptake rates (Chapter 6), indicate that regeneration of ammonium within the water column may be sufficient to maintain phytoplankton biomass when ambient measurements indicate this nutrient to be at very low to negligible (<0.005 mg l\(^{-1}\), below detection limits). Further research into ammonium generation and regeneration are recommended; this understanding could result in a management strategy for decreasing excessive phytoplankton biomass.

The transition from predominantly NO\(_3^-\) preference in surface waters for Chlorophyte blooms to the high preference for NH\(_4^+\) in summer may reflect a transition from autotrophic to a progressively more heterotrophic system through summer and autumn. Urea (or DON) may provide the transitional link between allochthonous nitrogen-based autotrophic spring communities and the autochthonous nitrogen-based heterotrophic summer and autumn communities (Bronk et al., 1998).

One aspect of nitrogen uptake that has not been investigated for this system is the role of bacteria in nitrogen resource competition. Bacteria : phytoplankton uptake ratios of NH\(_4^+\) and PO\(_4^{3-}\) increase with decreasing nutrient concentration because bacteria assimilate nutrients more efficiently than phytoplankton. Bacteria can therefore out-compete phytoplankton in conditions of low nutrient, which may lead to a higher incidence of heterotrophic or mixotrophic species. High uptake rates measured at times of high detrital loadings may also incorporate a degree of microbial nitrogen uptake. One method for investigating this would be to use autotroph inhibiting substances.

**Ordination analysis**

Phytoplankton communities are complex and highly diverse multi-species assemblages characterised by rapid successional shifts in species composition in response to dynamic environmental changes (Gallegos et al., 1992; Glibert et al., 1995; Marshall and Nesius, 1996).

Most research into spatiotemporal patchiness of phytoplankton communities considers these as single uniform entities. Using Multi-Response Permutation Procedures (MRPP), a non-parametric procedure for testing the hypothesis of no difference
between two or more groups of entities, species data can be compared with physical, chemical and physiological data to provide an indication of which combination of factors most influences species composition and distribution. MRPP has the advantage of not requiring assumptions (such as multivariate normality and homogeneity of variances) that are seldom met with ecological community data. This enables phytoplankton communities to be considered on a species basis rather than as single uniform entities (Pinckney et al., 1998).

The development of trophic models to describe ecosystems relied on defining food webs in terms of transfer of biomass, organic matter or energy through broad trophic categories such as herbivores or first order carnivores in a manner that did not incorporate factors such as species composition or population age structure (Steele and Frost, 1977). Multivariate ordination methods are currently used in exploring the relationships between various environmental parameters and changes in species composition over time and space. These techniques ‘order’ or arrange sites or samples along axes on the basis of data on variables, for example environmental factors and species composition, to estimate which factors have the greatest influence on that species composition. They do not have a requirement for the statistical rigour with which environmental studies so often find difficult to comply. The outcome is a two-dimensional diagram (biplot) in which sites/samples are represented by ‘points’ in two-dimensional space. If the points are close together, the sites/samples are similar in variables (environmental conditions or species composition). If the points are far apart the sites/samples are dissimilar. The degree of similarity is represented by the distance within the space of the biplot. Correlation analyses and PCA results (biplots and side scatter plots) are presented in Appendix I.

Correlation analysis of uptake rates with environmental parameters and Chla (Appendix I) showed significant relationships (P > 0.05) between nitrate uptake and ammonium uptake (P = 0.71), urea concentration (P = 0.83) and PAR (P = 0.53). There were also significant relationships between ammonium uptake and urea uptake (P = 0.94), between ambient orthoP and ambient NH₄⁺ concentrations (P = 0.68) and between ambient nitrate concentration and the N:P ratio of phytoplankton (P = 0.57). Negative correlations between temperature and nitrate (p = -0.68) can be explained because most nitrate occurs during the winter months. The negative correlation between orthoP and
PAR (P = -0.51), orthoP and oxygen saturation (P = -0.51) and ammonium and saturated oxygen (p = -0.64) is a result of orthoP and ammonium being at greatest concentration near the sediment at times of low oxygen. Phosphorus release from sediments under anoxic conditions has been reported at the Ron Courtney Island site (Douglas et al., 1996).

Principal component analysis (PCA) of the species composition data, by class, combined with the physical, chemical and physiological data, show that 54.8% of the variance can be explained in 3 dimensions. Ideally this should be only 2 dimensions. Two of the three primary factors explaining 22% of the variance (axis 1) in phytoplankton species distribution in the upper Swan River Estuary over an annual cycle are nitrate uptake ($r^2 = 0.50$) and urea uptake ($r^2 = 0.51$). The third is a routinely monitored parameter, temperature ($r^2 = 0.61$). Axis 2 included saturated oxygen ($r^2 = 0.64$), orthoP concentration ($r^2 = 0.58$) and ammonium concentration ($r^2 = 0.52$) (Appendix I, Table 2 and Figures A2 – A7). This is in contrast to the study by Rhudy et al. (1999) who found no significant relationship between seasonal cell densities of the Texas brown tide organism (Aureoumbra lagunensis) and either physical or chemical parameters measured. Although salinity and nitrate (or rainfall) may be governing distribution and composition of biota in the Swan River Estuary (Hodgkin, 1987; Thompson, 2001), neither has been identified as a factor in determining diurnal and spatial distribution at a specific site.

**Redfield Ratio as an indicator of nutrient limitation.**

The ratio of total nitrogen to total phosphorus (N:P) in the water column is often used as an indication of the limiting nutrient affecting phytoplankton growth in accordance with the Redfield ratio. According to the Redfield ratio carbon, nitrogen and phosphorus occur in the ratio 106:16:1. Variations from this are deemed to indicate nutrient limitation. The Redfield ratio has also been used on particulate organic matter to indicate whether phytoplankton growth has been nutrient limited. In this case it is usual to compare C:N ratios. A discrepancy exists in between results of N:P ratios from the water column and C:N ratios in the particulate fraction taken from that water column. According to water column nutrient ratios taken over the 12 month period from February 1998 to January 1999 (see Chapter 2) the average N:P ratios of 12 ± 2.8 (n=10) for surface and 11 ± 4.4 (n=11) for bottom depths are well below the Redfield N:P ratio of 16 and indicate nitrogen is limiting in this system over this period. An
examination of the particulate C:N ratios for the same period (see Chapter 5) indicate values in excess of the Redfield C:N ratio of 6.6:1 for most of the year which would be interpreted as non-nitrogen limited growth. Perhaps the discrepancy can be explained by considering the rapid flux of nitrogen within the water column that can be utilised by phytoplankton as fast as it is made available. This 'unseen' nitrogen source has been implicated from some of the uptake rate measurements where changes in ambient concentrations over time do not reflect the actual measured uptake rates (see Chapter 6). Because of its rapid turnover rate it has not been detected by wet chemistry or static monitoring techniques. The presence of higher than detected nitrogen levels is also suggested by k_s values in excess of ambient nitrogen measured. This may indicate preconditioning of phytoplankton communities to higher than ambient nitrogen levels that could be explained by high flux rate nitrogen species such as urea and remineralised ammonium.

**Dissolved Organic Nitrogen (DON)**

The importance of dissolved organic nitrogen to the nitrogen nutrition of Swan River Estuary phytoplankton has not previously been considered for this system. This study looks at urea as one component representative of DON. Urea has proved to be as important as ammonium to phytoplankton nitrogen nutrition. Urea and NH_4^+ species constitute the highest proportion of total ambient specific nitrogen uptake (\(\Sigma P_{amb}\)) at most times and depths throughout the 12 month period in this system. Monitoring at an upper estuary site for the Swan River Estuary detected ranges of 15 – 117.7 \(\mu\)g urea-N L^1. This is within the reported range of urea concentrations in a variety of aquatic habitats (0.43 – 124.6 \(\mu\)g L^1, Antia *et al.*, 1991). The diurnal depth-averaged mean was 40 \(\pm\) 14.9 \(\mu\)g urea-N L^1 over an annual period (February 1998 – January 1999). Urea concentration represented 104% - 287% of total dissolved inorganic nitrogen (NO_3^- + NH_4^+) during summer (see Chapter 2). Measured ambient specific uptake rates for urea (3 \(\pm\) 0.6 - 2241 \(\pm\) 252.6 ng urea-N \(\mu\)g Chl a^1 h^1) represent between 28% (\(\pm\) 13.5, n=12) and 40.4% (\(\pm\) 19.2, n=12) of total N uptake over the annual period February 1998 – January 1999. This is lower than the 70% -80% of total uptake reported for Chesapeake Bay estuary (Gilbert, 1999). Both systems report highest proportion of urea uptake for summer. Seasonal nitrate uptake over the same period constituted only 11% (\(\pm\) 10.8,
n=12) to 24% (± 13.0, n=12) with the highest percentage during winter when nitrate levels are elevated.

**Diurnal Vertical Migration (DVM)**

Changing DVM behaviour over the course of the summer bloom supports the role of DVM in optimising photosynthetic capability and nutrient acquisition (Kamykowski et al., 1998). It indicates that DVM behaviour can alter under changing conditions of water-column nutrient availability. It also suggests that DVM is regulated by factors other than circadian or endogenous rhythms. The importance of the role of DVM in maintaining a balance between near-bottom night-time nitrogen uptake and near-surface day-time photosynthesis, resulting in net population growth, has been demonstrated elsewhere (Hamilton et al., 1999) using the date from this study in conjunction with modelling techniques. This shows that the nutrient input derived from nocturnal uptake at depth is an important factor in the growth of these migrating species. Resource partitioning between photosynthesis and nitrogen, specifically NH$_4^+$ uptake provides a competitive advantage.

**Microheterotroph grazing control of phytoplankton biomass**

Top-down and bottom-up controls operate simultaneously. Their relationship varies depending on the scale of interest, and has important consequences for how we model phytoplankton biomass control in a natural food web. The relative influence of grazing and nutrient limitation, top-down versus bottom-up control, on phytoplankton species composition and biomass has been recognised as an important factor in regulating algal biomass (Vanni and Terme 1990; Lewitus et al. 1998). Estuarine phytoplankton blooms are generally nutrient limited (Nixon et al. 1986). However, Lewitus et al. (1998) demonstrated that this was not a consistent trend throughout the year for North Inlet, a tidally driven salt-marsh estuary in South Carolina. Winter diatom-dominated assemblages were limited by nutrient availability rather than grazing, while summer bloom biomass, dominated by phototrophic nano- and pico-size class species such as flagellates and *Synechococcus* sp., was not nutrient limited but controlled by grazing pressure.
### Chapter 8: Synthesis of Phytoplankton Regulating Factors

#### Table 8.1 Comparison of seasonal uptake and loss estimates of phytoplankton communities using different nitrogen sources.

Loss rates are based on percent production grazed (Pp%) determined in Chapter 7 and have been applied to monthly uptake data. All values in units of ng N µg Chla⁻¹ h⁻¹.

<table>
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<th></th>
<th>Day 0.25 m Uptake</th>
<th>Night 0.25 m Uptake</th>
<th>Day 4.5 m Uptake</th>
<th>Night 4.5 m Uptake</th>
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In order to make a direct comparison between nitrogen uptake and grazing rates this investigation presented nitrogen uptake rates and net community grazing rates in terms of chlorophyll \(a\) (Chla). The Chla : biomass ratio is variable (20-100 \(\mu g\) C \(\mu g\) Chla\(^{-1}\)) because of variation in cell size amongst species (there is often only a weak correlation between Chla and phytoplankton biomass), measurement of photosynthetic pigments is the most practically effective method for estimating of phytoplankton biomass (Baker, 1987).

Since the grazing rate of natural assemblages comprising mixed populations was under investigation the results are presented in the form of grazing as a percent of apparent production according to the equations of Verity (1993) (see Chapter 7).

### 8.4 Recommendations

This research highlights the need for knowledge of the flux rates within a system to adequately understand or predict the systems potential for support of phytoplankton growth. An ability to develop a predictive model for any ecosystem requires that all component are included and that knowledge of the fluxes between component pools within the system is necessary in order to understand the limitations of the system as a whole. For this reason an ecological model that does not include relevant information on the physiological parameters of the component populations will fail to adequately describe the system.

While this study provides initial estimates on grazing for the upper estuary, no information is available for other regions of the Swan River Estuary. Most investigations into uptake and remineralisation and grazing effects on aquatic communities are undertaken by teams of researchers (ie Glibert et al., 1991; Glibert et al., 1992; Bronk et al., 1998). Resource limitation has necessitated a number of compromises in the way samples were collected (ie the necessity to run these over a two days instead of concurrently) and experiments omitted (\(^{15}\)N remineralisation measurements). Further research into this area will need the resources (man-power) to be able to overcome these issues.

The next stage in the development of a comprehensive understanding of the Swan-Canning ecosystem is to include the effect that the microbial population dynamics has
on the food-web. As outlined in Chapter one the impact that new methods have had in advances in plankton ecology emphasizes the fact that ecological research and its development is methods limited. The revolution over the past few decades in understanding the ecology of aquatic microbes has been made possible by the rapid improvements in methods combined with a change in emphasis from cultures and laboratory measurements to field measurements of ambient microbial community abundance, activity and growth. When methods improve in one area, then the shortcomings existing in that and other areas become evident. Future research focused on nanoplanckton in the Swan-Canning Estuary in terms of biomass, productivity and its contribution to the foodweb is required. Once we understand the interplay between physical and chemical environment and the physiological interplay between macro and micro-fauna and flora, then only by including the pico- and nano- plankton components (including viruses) will a complete understanding of the interactivities controlling aquatic systems be attainable.

It is evident that changing patterns of vertical migration and species distribution may influence and be influenced by nutrient distribution patterns. The effect that this has on bloom seeding and development may be a necessary consideration in management strategies for understanding bloom establishment and perseverance.

Additional research into the role of phytoplankton and grazers <20mm is needed.

8.5 References


APPENDIX I: PCA analysis

The following data was analysed using PC-ORD Version 3.18

For PCA analysis of phytoplankton distribution and composition with environmental factors only those samples with phytoplankton present were used. Where data was missing the average of all data was used to enable the ‘site’ to be included in analysis.

PCA

Pearson and Kendall Correlations with Ordination Axes  N = 46

PC-ORD Version 3.18

PCA

Pearson and Kendall Correlations with Ordination Axes  N= 46

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<th>2</th>
<th>3</th>
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<td>tau</td>
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<tr>
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Correlation of phytoplankton group distribution with diurnal depth related environmental factors over a 12 month period shows temperature (T), NO\textsubscript{3}\textsuperscript{-} uptake (vNO\textsubscript{3}) and urea uptake (v urea) in axis 1 and oxygen saturation (satox), orthoP and NH\textsubscript{4}\textsuperscript{+} in axis 2 to be the major factors influencing phytoplankton distribution at RCI over an annual period.

Values > 0.5, highlighted in red, are considered significant. (T, r\textsuperscript{2} = 0.605), NO\textsubscript{3}\textsuperscript{-} uptake (vNO\textsubscript{3}, r\textsuperscript{2} = 0.501)
********** PRINCIPAL COMPONENTS ANALYSIS -- Samples in Variable space *****
PC-ORD, Version 3.18
27 Nov 2001, 0:17
PCA

VARIANCE EXTRACTED, FIRST 5 AXES

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Most of variance (54.8%) can be explained within 3 axes.
APPENDIX I: PCA ANALYSIS

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Correlation coefficients for environmental parameters for RCI over 1998-99. Correlations > 0.5 are deemed significant.

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Correlation matrix for ambient nitrogen species concentrations and specific uptake rates over 1998-99 at RCI. Factors with significant correlations (>0.5) are highlighted.
Figure AI.1 Principal Component Analysis of environmental factors with samples times and depths.

Strong separation of day-time surface (lower left) and bottom (upper right) samples. Night samples from both depths show no marked separation. First 2 letters of sample code refers to month, first number represents time of day (1 = day; 2 = night), second number represents depth (1 = surface, 0.25m; 2 = bottom, 4.5m).
Figure A1.2 PCA plot of environmental factors showing influence of temperature on phytoplankton diurnal distribution over an annual period. Symbols increase in size with increasing temperature.

Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
Figure Al.3 PCA plot of environmental factors showing influence of nitrate uptake (vNO$_3$) on phytoplankton distribution.

Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
Figure AL.4 PCA plot of environmental factors showing influence of urea uptake (vurea) on phytoplankton distribution.

Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
Figure AI.5 PCA plot of environmental factors showing influence of oxygen saturation (satox) on phytoplankton distribution.

Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
Figure Al.6 PCA plot of environmental factors showing influence of orthoP concentration on phytoplankton distribution.

Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
Figure A1.7 PCA plot of environmental factors showing influence of ammonium concentration ($\text{NH}_4^+$) on phytoplankton distribution.

Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
**NMS Grazers**

Pearson and Kendall Correlations with Ordination Axes  \(N=32\)

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Pearson and Kendall Correlations detected no distinct relationship between phytoplankton and grazer classes for the 1998-1999 period \((P<0.5)\).

**Figure A1.8** NMS plot of grazers with phytoplankton distribution showing no distinct relationship.
Figure A1.9 Non-metric multidimensional scaling ordination of grazer groups and phytoplankton groups.

The NMS showed that there was a strong linear relationship between Axis 2 and dinoflagellate abundance, in other words as you go from top to bottom in the ordination diagram there is a linear reduction in abundance of dinoflagellates. Symbols increase in size with increasing grazer numbers. Side scatter plots and statistics are included. Sample codes are as follows: first two digits represent month (01 = February 1998), next two digits represent time of day (1 = day, 2 = night) and depth (1 = surface, 0.25m, 2 = bottom, 4.5m).
APPENDIX II - Phytoplankton counts, by class, for surface (0.25m) and bottom (4.5m) depths at Ron Courtney Island, upper Swan River Estuary, for the period February 1998 to January 1999.

A. Noon counts, B. Night counts. This data was used in conjunction with grazer data for PCA comparison of day vs. night and surface vs bottom distribution of phytoplankton and grazers.

A. Day counts of phytoplankton groups

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<th>Crypto</th>
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<th>Dictyo</th>
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nd: Not determined

### APPENDIX II - PHYTOPLANKTON COUNTS FOR THE PERIOD FEBRUARY 1998 TO JANUARY 1999

#### Cell counts per Class

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#### B Night counts of phytoplankton groups

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### Appendix II - Phytoplankton counts for the period February 1998 to January 1999

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APPENDIX III: Enrichment experiments – a confusion in terms leading to erroneous uptake rate measurements

Many papers refer to the equations of Dugdale and Goering as the method by which they calculated the uptake rate but few actually state the equations used. During the early 1970s another form of the equation became apparent. It is presented in Harrison (1983) with no reference to its origin. In this equation the atom % enrichment factor, \( R \), is given the form \( 100\% \times \frac{S_u}{S_u + S_0} \). On inspection it is apparent that the erroneous interpretation of the atom % enrichment of the \(^{15}\text{N}\) source has become the factor 100 that has been included in subsequent uses of the formula. While atom % enrichment may vary between 5\% and 99.999\% (Aldrich, ICN Chemical Suppliers), it is not clear from the literature whether the value 100, rather than the actual atom % enrichment factor, has been incorporated in the calculation. Where atom % enrichments of sources are close to 100\% this is not an issue. However, where this enrichment falls short of this (say 60\%) its assignment as 100\% will affect the uptake rates calculated (Eppley et al., 1977; McCarthy, 1980). The work of Gilbert et al. (1982) has partly addressed this problem with the incorporation of a correction factor for changing \( R \) over the course of the incubation. By using a modification of the earlier Blackburn-Caperon model (Blackburn, 1979; Caperon et al., 1979) for simultaneous measurement of uptake and remineralisation, the inclusion of a correction for the \(^{15}\text{N}\) released back into the water over the incubation period avoided the uncertainty in estimating atom % enrichment. Their calculation of \( P(\rho) \), rather than \( \rho \), incorporates the determination of change in \(^{15}\text{N}\) in the aqueous fraction from the start \((R_0)\) to the end \((R)\) of the incubation. It assumes that the remineralisation rate is constant over the course of the incubation.

The following formula is used to calculate \( R \) when the effect of ambient nutrient dilution on the atom % enrichment factor is considered:

\[
R = \text{atom}\% \text{ enrichment of source} \times \left( \frac{S_u}{S_u + S_0} \right)
\]

where \( S_u \) represents unlabelled substrate (\(^{14}\text{N}\)) concentration and \( S_k \) is labelled (\(^{15}\text{N}\)) substrate concentration. The second term in the expression for \( R \), \( \left( \frac{S_u}{S_u + S_0} \right) \), represents a dilution correction factor included to account for the dilution of the enriched source by ambient \(^{14}\text{N}\). When using field collected water samples for
incubations this dilution factor may have a significant impact on the uptake rates calculated. The measurement of ambient nitrogen concentration is in itself a possible source of significant error in the calculations, especially where ambient nitrogen is close to or below the detection limits of wet chemistry techniques. Ammonium concentrations in natural waters vary between <10μg NH₄⁺-N l⁻¹ to >30mg-N l⁻¹ in some waste waters (Clesceri et al., 1989) and measurement can be particularly sensitive to contamination by atmospheric ammonium or rapid changes following chemical transition of organic N compounds such as urca to ammonium ions. In a situation where labelled ¹⁵N is added to an incubation medium already containing a ¹⁴N source, then the enrichment will be affected by a dilution factor. Consider the following three scenarios:

Scenario 1: Tracer addition is much greater than ambient ¹⁴N concentration. In this enrichment situation the affect of dilution on the atom % enrichment (R) is small (approaches 0) and there is little effect on the measured uptake rates.

Scenario 2: Tracer addition represents a nitrogen concentration equal to ambient ¹⁴N. In this case the dilution factor becomes 0.5 and there is a reduction in R of 50%. This results in a 50% increase in calculated uptake rates.

Scenario 3: Tracer addition represents only 10% of the ambient nitrogen concentration. Note that this is the recommended addition level in order to reflect ambient uptake rates (Dugdale and Goering, 1967). Higher additions are thought to create artificially high uptake rates (surge uptake) through luxury uptake following perturbation by excess nutrient addition, or nutrient enrichment (Dugdale and Wilkerson, 1986). An addition of 10% of ambient will have the effect of reducing the dilution factor to approximately 0.1, and thus result in an approximately 90% reduction in R. The overall effect on equations 2 and 3 will be to greatly increase the calculated uptake rate.

In a situation where uptake rate is determined from laboratory incubations in solutions made using the enriched N as the sole source of N, then S₀ becomes zero and the dilution factor becomes 1. Where ¹⁵N additions (isotope enrichment) are much greater than ambient ¹⁴N concentrations the dilution effect on R is minimal (R approaches 0) and the dilution effect makes little difference to the calculated p value. If ¹⁵N addition
APPENDIX IV: List of Publications


