Nitrogen uptake by phytoplankton in the Huon Estuary: with special reference to the physiology of the toxic dinoflagellate *Gymnodinium catenatum*

By

Paul B. Armstrong

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National Centre for Marine Conservation and Resource Sustainability

University of Tasmania

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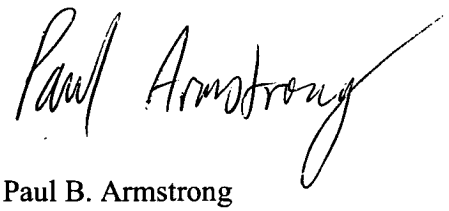

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Abstract

The Huon Estuary is a micro-tidal estuary in south-east Tasmania that is an important area for salmon aquaculture. In 2008 the salmon aquaculture industry in Australia is worth $260M (AUD) per year. Salmon aquaculture began in the Huon Estuary in the 1980's and production has since increased significantly. The Huon Estuary is nitrogen (N) limited and salmon farming is a significant input of N to this ecosystem. Both industry and government regulators are alert to the potential for eutrophication and increased harmful algal blooms if the assimilative capacity for N of the estuary is exceeded. As part of a larger project on the ecology of the Huon Estuary, this PhD research has two main objectives; firstly to determine whether phytoplankton in the Huon Estuary are using nitrogen that had, primarily, an oceanic source (e.g. nitrate) or was more locally supplied or regenerated (e.g. ammonium and urea) and secondly to examine the physiology of *G. catenatum* a toxic dinoflagellate that dominates the summer and autumn Huon Estuary phytoplankton biomass in many years.

Uptake rates of NO_3^-, NH_4^+ and urea were measured on four occasions (28-29 May 2003, 23-24 Sept 2003, 18-19 Nov 2003, and 24-25 Feb 2004) in the Huon Estuary using a ^{15}N tracer technique. Uptake rates were measured at Garden Island and Hideaway Bay in the lower estuary and at 5 and 20 m during the day and also at 5 and 20 m during the night. The mean uptake rates (mean across time of year, site, time of day and depth) for NH_4^+ (0.13 μg N μg chl *a* h^{-1}) and urea (0.09 μg N μg chl *a* h^{-1}) were 4.5 and 3.2 times higher than the uptake of NO_3^- (0.3 μg N μg ch l*a* h^{-1}). Overall NH_4^+, NO_3^- and urea were responsible for 52, 37.5 and 10.5% of N uptake respectively.

Gymnodinium catenatum is a toxic dinoflagellate that blooms periodically in the Huon Estuary and in years that it blooms it dominates the phytoplankton biomass and has caused closure of shellfish farms in the area. Laboratory experiments on effect of temperature and irradiance on growth rate, effect of different nitrogen species on growth rate and preferential uptake of different nitrogen species by *G. catenatum*

were undertaken to better understand the physiology of this species and to test a hypothesis that *G. catenatum* vertically migrates to access NH_4^+ at depth during summer. The effect of 12 different temperatures ranging from 11.9-25.2 °C and irradiances from 5-283 μmol photons m^{-2} s^{-1} on growth and biochemical composition of *G. catenatum*. The highest predicted growth rates (>0.2 d^{-1}) occurred during summer and autumn as might be expected based on observations of bloom dynamics of this species in the Huon Estuary which supports both a summer and autumn bloom in many years

G. catenatum was able to grow using NO_3^-, NH_4^+ or urea as its sole nitrogen source. There was no significant difference in growth on any of these nitrogen sources. Preferential uptake of NH_4^+, NO_3^- or urea was examined by growing *G. catenatum* on a mixture of NO_3^-, NH_4^+ and urea. The results clearly showed that NH_4^+ was taken up first, followed by NO_3^- and finally urea. Maximum mean uptake rates were 170, 98 and 30 pg $cell^{-1}$ $hour^{-1}$ respectively for NH_4^+, NO_3^- and urea.

In addition to the laboratory experiments the nitrogen uptake characteristics of a bloom of *G. catenatum* was examined at Pelican Island, Southport (30-31/03/2004) nearby the Huon Estuary. Mean urea uptake was greatest (0.045 μgN μg chl *a* h^{-1}) followed by NH_4^+ (0.029 μgN μg chl *a* h^{-1}) and the lowest uptake was of NO_3^- (0.025 μgN μg chl *a* h^{-1}). For *G. catenatum* growing in the Huon Estuary it seems increasingly apparent that it functions as a nitrogen scavenger. When N concentrations are exhausted, it is able to migrate through the water column seeking whatever form of nitrogen is available.

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1 INTRODUCTION

1.1 Eutrophication in coastal ecosystems

Eutrophication is widely considered to be one of the greatest threats to estuarine and coastal ecosystems around the world (Howarth *et al.*, 2002, Howarth *et al.*, 2005). Eutrophication as defined by Nixon (1995) is an increase in the rate of supply of organic matter to an ecosystem. The rate of organic matter supply to an aquatic ecosystem can increase through a number of different mechanisms. Clearing of catchments is widely associated with more runoff and a greater supply of organic matter and nutrients into downstream water bodies. Agriculture and sewage may be the source of more inorganic nutrients which can, in turn, support more photosynthesis and thereby contribute more organic matter into an ecosystem (Laws, 1993). The organic loading itself may result in anoxia thereby changing the cycling of N and P sometimes resulting in these nutrients becoming periodically more abundant in the water column. Thus there are a range of different mechanisms whereby an aquatic ecosystem may become more eutrophic (Smith *et al.*, 1999). The responses of ecosystems, however, to an increase in organic matter supply can be complex depending on a large number of physical and biological factors (Cloern, 2001). In most cases the organisms that can directly use the nutrients, typically photosynthetic autotrophs, are the first to respond to eutrophication (Philippart & Cadee, 2000). The magnitude of these responses, however, is determined by the nature of the inputs and by complex interactions between physics, chemistry and biology within the water body and its sediments.

In some coastal ecosystems moderate eutrophication is considered beneficial because it increases primary productivity and this in turn increases the biomass of fish and/or shellfish species for human consumption (Nixon, 1990, Jørgensen & Richardson, 1996). However in most coastal ecosystems eutrophication has deleterious effects such as:

- Shifts in phytoplankton community composition. Decoupling of base trophic levels with higher trophic levels that can result in a shift towards toxic species of phytoplankton.
- Low dissolved oxygen concentrations (hypoxia) and absence of oxygen (anoxia) can be the result of increased plant, animal and bacterial respiration caused by increased organic matter from primary production (autochthonous) or organic matter input from outside the ecosystem (allochthonus).
- Increased turbidity caused by greater phytoplankton biomass can degrade or destroy seagrass and macroalgae habitats for which light transmission down through the water column to the bottom is important.

In almost all ecosystems, sometime during the annual cycle one nutrient or a sequence of different nutrients can become limiting to primary production (Elser *et al.*, 1990). When a nutrient is limiting for primary productivity an increase in the rate of supply of that nutrient will stimulate primary productivity (Smayda, 1989). Typically more biomass is produced as a response to more nutrients (Clark, 1989, Vollenweider, 1992). Thus increased nutrient inputs also result in an increased organic matter supply to the ecosystem. For temperate coastal ecosystems primary productivity in these systems is usually limited by the availability of N (Ryther & Dunstan, 1971, Howarth & Marino, 2006). The reason that eutrophication is becoming such a widespread threat to coastal ecosystems is that there has been a rapid increase in the amount of nitrogen getting into our aquatic ecosystems from sewage and agriculture (Nixon, 1990) (Galloway *et al.*, 2004). In the 1950's fertiliser production using the Haber process began resulting in the steady increase in N fertilizer and subsequent runoff of relatively more N into the coastal zone. A classic example is the steady rise in N in the plume off the Mississippi River during the last 50 years (Turner *et al.*, 1998). In this case there has been a relative decline in the silicate concentrations producing a large scale example of selective resource limitation with an impact on the ecology ranging from phytoplankton to fish. The continental shelf off the Mississippi River has seen the ratio of silicate to dissolved in

organic nitrogen loading ratio has declined from around 3:1 to 1:1 during this century because of fertilizer application, agriculture and other land-use practices in the watershed. Diatoms require dissolved silicate and their growth can become Silicate limited when the atomic ratio of silicate to dissolved inorganic nitrogen (Silicate:DIN) approaches 1:1. Considerable research indicates this shift in N loading is the primary reason this coastal ecosystem now produces a large anoxic zone potentially supporting disruptive harmful algal blooms (Turner et al., 1998).

Harmful algal blooms (HAB) have been classified depending upon their type of impact into three categories (after Hallegraeff, 1993):

1. Those not directly toxic to humans or other organisms. This type of HAB is typically a large bloom that causes high biological oxygen demand (BOD) during decomposition. Particularly in situations with low rates of mixing the resulting hypoxia or anoxia can lead to the death of other organisms. Large fish kills have been associated with this type of bloom.
2. Toxic algal blooms caused by a range of species, most commonly dinoflagellates, that have a negative biological effect upon humans. For example a range of species produce toxins which effect people once ingested. These include:
    a. PSP - paralytic shellfish poisoning, same toxins are found in cyanobacteria
    b. DSP - diarrheic shellfish poisoning
    c. ASP - Amnesic shellfish poisoning
    d. Ciguatera poisoning
    e. NSP - neurotoxic shellfish poisoning
3. Blooms causing direct negative biological effects upon organisms other than humans. For example HAB species that kill fish via damaged or clogged gills and a range of other known and unknown mechanisms.

Four explanations for the apparent increase in algal blooms have been proposed: a greater scientific awareness of toxic species; the growing utilization of coastal waters for aquaculture; the stimulation of plankton blooms by domestic, industrial and agricultural wastes and/or unusual climate conditions; and the transportation of algal cysts either in ships' ballast water or associated with moving shellfish stocks from one area to another (Hallegraeff, 1993). Most of the species known to cause HABs are dinoflagellates.

The continuous plankton recorder transects of the North Atlantic (Edwards & Richardson, 2004, Edwards *et al.*, 2001) have shown clear evidence that whole regions of the ocean have increased abundances of dinoflagellates. The mechanism proposed to explain the growing dominance of dinoflagellates observed in the pelagic ocean is warming due to climate change. The proponents hypothesized that warming of the ocean surface due to climate change results in increased temperature stratification. An increase in stratification would reduce vertical mixing, the major process whereby nitrate is injected into the euphotic zone. It is not yet clear whether global warming is having a global impact on stratification through warming, or polar ice melting or increased precipitation. If large scale changes to stratification do occur the consequences could be significant in terms of increase HABs. The seasonal pattern of stratification and blooms may provide some insights.

Low stratification or high turbulence has a seasonal dynamic in the temperate zone. Conditions of high turbulence and high nutrients are typically found in winter. The diatom blooms that are associated with the transition from winter into spring suggest diatoms are more capable of coping with turbulence than species that occur later in a seasonal succession. This seasonal cycle of diatoms in spring often leading to dinoflagellate blooms in summer or autumn and the commonly associated reduction in turbulence was conceptualized by Margalef (1978). Margalef (1978) defined niches for diatoms and dinoflagellates along 2 axis, one the concentration of nutrients and the second the amount of turbulence (Fig. 1). Others have refined Margelef's seminal work (Margalef, 1978) by expanding the conceptual space and populating it

with species and their characteristic behaviours (e.g. Smayda & Reynolds, 2001). There can be no doubt that many ecosystems have a seasonal transition to lower turbulence and an increased proportion of dinoflagellates but the underlying causes of this succession can be complex (Glibert *et al.*, 2008). In contrast considerable experimental research on diatoms has successfully separated the multitude of factors associated with this seasonal dynamic and substantiated the strong association between enhanced growth and greater turbulence (Litchman, 1998, Litchman & Klausmeier, 2001, Litchman *et al.*, 2004).

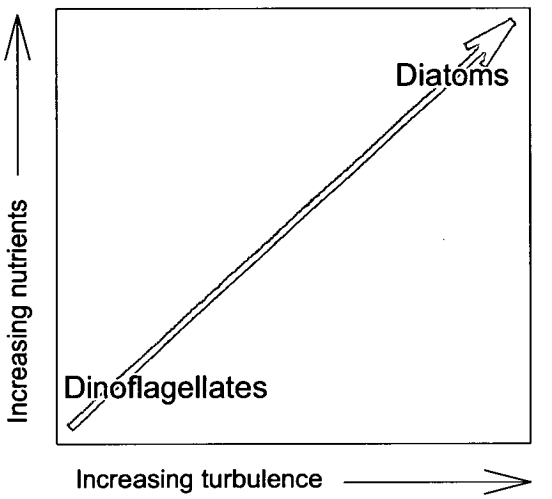

Figure 1.1 A simple conceptual map of the niche space associated with nutrient concentrations and turbulence. Adapted from Margalef (1978).

In the coastal zone there is growing evidence that HABs are linked with increasing eutrophication (Smayda, 2002) especially inputs of N (Paerl, 1997, Paerl, 1988). Other limiting nutrients have certainly been observed with Fe increasingly recognized for limiting growth in high nitrogen low chlorophyll *a* areas that are remote from atmospheric inputs of dust. Some blooms are stimulated by additional P, or other compounds including cobalt and vitamins (Segatto *et al.*, 1997). While there is insufficient evidence to be conclusive regarding the frequency, duration and extent of different types of nutrient limitation there are several lines of evidence that coastal

ecosystems are more likely to be periodically limited by the availability of N than other nutrients (Graneli *et al.*, 1990, Ryther & Dunstan, 1971, Bricker *et al.*, 1999).

One consequence of the importance of nitrogen to phytoplankton dynamics has been a great deal of research upon the nitrogen nutrition of phytoplankton. It has been commonly assumed that phytoplankton should grow better on NH_4^+ than NO_3^-, particularly under conditions of low irradiance, as growth on NO_3^- imposes a substantial extra cost in terms of reducing power. There is, however, little evidence of this factor being a significant determinant of growth rates even at very low irradiances (Thompson *et al.*, 1989). Many species preferentially take up NH_4^+ over NO_3^- when both are present, with some indication of a threshold effect (Dortch *et al.*, 1991, Dortch, 1990). A considerable amount of research has been focused on answering the question, if not determined by an energetic constraint then what would control the balance between NO_3^- and NH_4^+ assimilation?

Although there is substantial geographic and temporal variation one of the most persistent observations of phytoplankton ecology at temperate latitudes is a spring bloom that consumes most of the available NO_3^- in the euphotic zone. Frequently this bloom is composed largely of diatoms leading to a hypothesis that diatoms may have enhanced genetic capabilities and thus be physiologically more capable of using NO_3^-. Experiments to test this hypothesis have shown that some species do grow better on particular forms of NO_3^- (Levasseur *et al.*, 1993) but not necessarily diatoms. Experiments on diatoms in turbulent environments also have a long history examining aspects of their physiology that might be advantageous (Marra, 1978) relative to dinoflagellates (Chan, 1978). More recently there has been a refinement of the general hypothesis that turbulence favours diatoms to include NO_3^- uptake and reduction as a method to use the excess light energy that must periodically be acquired by a phytoplankton cell in a well mixed water column (Lomas & Glibert, 2000, Lomas & Gilbert, 1999) and a chlorophyll content adapted to the average irradiance. Indeed some fraction of the competitive advantage diatoms possess in turbulent environments seems to be associated with the reduction of NO_3^-.

Temporal variability in nutrient availability exists on many time scales such as seasonal fluctuations at mid to high latitudes (Parsons & Takahashi, 1973), estuaries which experience nutrient pulses associated variations in flow (Mallin *et al.*, 1993) and on shorter time scales especially in near shore environments (Fong *et al.*, 1993). If the nutrient pulses are rare relative to the life spans of the organisms then long lived species will dominate, while intermediate pulses should give a mixture of species and a rise in diversity (Floder & Sommer, 1999). Long periods of high nutrient availability with low N:P ratios are often associated with a loss of diversity and nuisance algal blooms (Birch *et al.*, 1981).

Harmful algal blooms, however, are rarely diatoms and frequently occur later in the season when NO_3^- is relatively less available. Many HAB have also been associated with greater availability of reduced N such as NH_4^+ or dissolved organic N (DON). Relatively little research has been conducted on the capacity of phytoplankton to use organic N for growth. Most of this has focused on urea as a proxy for all forms of organic N. Early research demonstrated the importance of urea in the natural environment, where it was frequently observed to be ≈50% of all N uptake (e.g. McCarthy, 1972). Similar results have been reported in a range of studies although there are relatively few studies and a generic perspective is not easily obtained. Other organic forms of dissolved N have received relatively little attention, although the pioneering work of Antia (e.g. Antia & Harrison, 1991 and references therein) did show considerable capacity of many species to use many forms of DON even though their growth rates were low. In the field it has been shown that phytoplankton will use a range of DON (Hellebust, 1970, Hollibaugh, 1976). Use of DON is still being actively investigated today (e.g. Stolte *et al.*, 2002, Bronk *et al.*, 2007) and some interesting recent work again showing taxonomic differences in DON use (Wawrik *et al.*, 2009). The use of DON and the use of specific forms of DON would seem to have a significant genetic component that may be important in defining the ecological niche occupied by some species, a subject that requires further investigation.

Perhaps the most intriguing differences in the use of NO_3^- versus NH_4^+ is the contrast between the two dominant picoplanktors, *Synechococcus* and *Prochlorococcus*. These two genera dominate the world's oceans yet *Prochlorococcus* has only very limited capacity to use NO_3^-. Although the lack of nitrate reductase is not universal among strains of *Prochlorococcus* it is important in determining its niche (Moore *et al.*, 1998, Rocap *et al.*, 2003). Thus the availability of different forms of nitrogen is an important factor contributing to the relative success and productivity of different phytoplankton (Berg *et al.*, 1997). Typically, the abundance of dinoflagellates can be correlated with low nitrate concentrations and high rates of NH_4^+ or dissolved organic nitrogen (DON) supply (Carlsson *et al.*, 1998). Many studies show that phytoplankton biomass may increase with overall nitrogen availability (Boynton *et al.*, 1982) and the DON component may shape the phytoplankton succession and lead to a harmful algal bloom (Paerl, 1988) . While molecular techniques are making it easier to assess whether a species has the potential to use a particular form of N they cannot tell us whether this capacity actually provides a competitive advantage. Field experiments that measure fluxes and laboratory experiments that assess outcomes with, and without, a specific nitrogen source are still the major tools to determine whether a specific nitrogen compound is important in ecology of a particular species. This sort of research seems likely to be required for all HAB species.

As discussed above the availability of particular forms of nitrogen often has a seasonal dynamic but it may also have a spatial component. For example, point sources can provide locally elevated concentrations of particular forms of nitrogen. Often the euphotic zone can be stripped on DIN during a spring bloom. The nitrate in deeper waters is not readily available to photosynthetic organisms. Some large scale dinoflagellate blooms have been shown to vertically migrate during the dark for this N returning to the euphotic zone for light energy during the day (Eppley *et al.*, 1968, Hasle, 1950, Cullen & Horrigan, 1981). Considerable work has been done on the physiology of vertical migration through temperature and nutrient gradients (Kamykowski, 1981, Kamykowski, 1995, Kamykowski & Yamazaki, 1997). Field

observations indicate that *G. catenatum* vertically migrates through temperature, salinity and nutrient gradients in the Huon Estuary (Doblin *et al.*, 2006). Testing the hypothesis that *G. catenatum* may vertically migrate to access NO_3^- or NH_4^+ was one of the major components of this research.

In the coastal zone and following the spring bloom the water column is often resupplied with DIN in forms other than NO_3^-. If the water column is stratified this can result in elevated NH_4^+ near the bottom. This is especially true if dissolved oxygen is low (Laws, 1993) due to inhibition of de-nitrification (Bonin & Raymond, 1990). In stratified ecosystems with low vertical exchange the NH_4^+ gradient can be considerable and again dinoflagellates may migrate vertically to access this N source.

1.2 The Huon Estuary

The Huon River estuary and its catchment is located in southeast Tasmania between latitude 42° 45′ S and 43° 45′ S (Figure 1.2 and Figure 1.3). Tasmania is the southernmost island state of Australia. Tasmania has a maritime climate that is dominated by zonal westerly's, resulting in a variable cool temperate climate. The Huon Estuary is a drowned river valley ~ 40km long and 4.5km wide at the mouth (at Huon Island) where it joins the southern end of the D'Entrecasteaux Channel, a semi-protected channel formed between the Tasmanian mainland and Bruny Island (Figure 1.3). The depth ranges from 40m at the mouth to 10 m at Port Huon, above which the depth decreases rapidly to between 2 and 4 m deep on the east and west sides of Egg Island (Figure 1.2). It is a salt wedge estuary, with the marine water extending from the mouth of the estuary up to Ranelagh, upstream of Huonville (Figure 1.2) where saltwater can penetrate under low river flows.

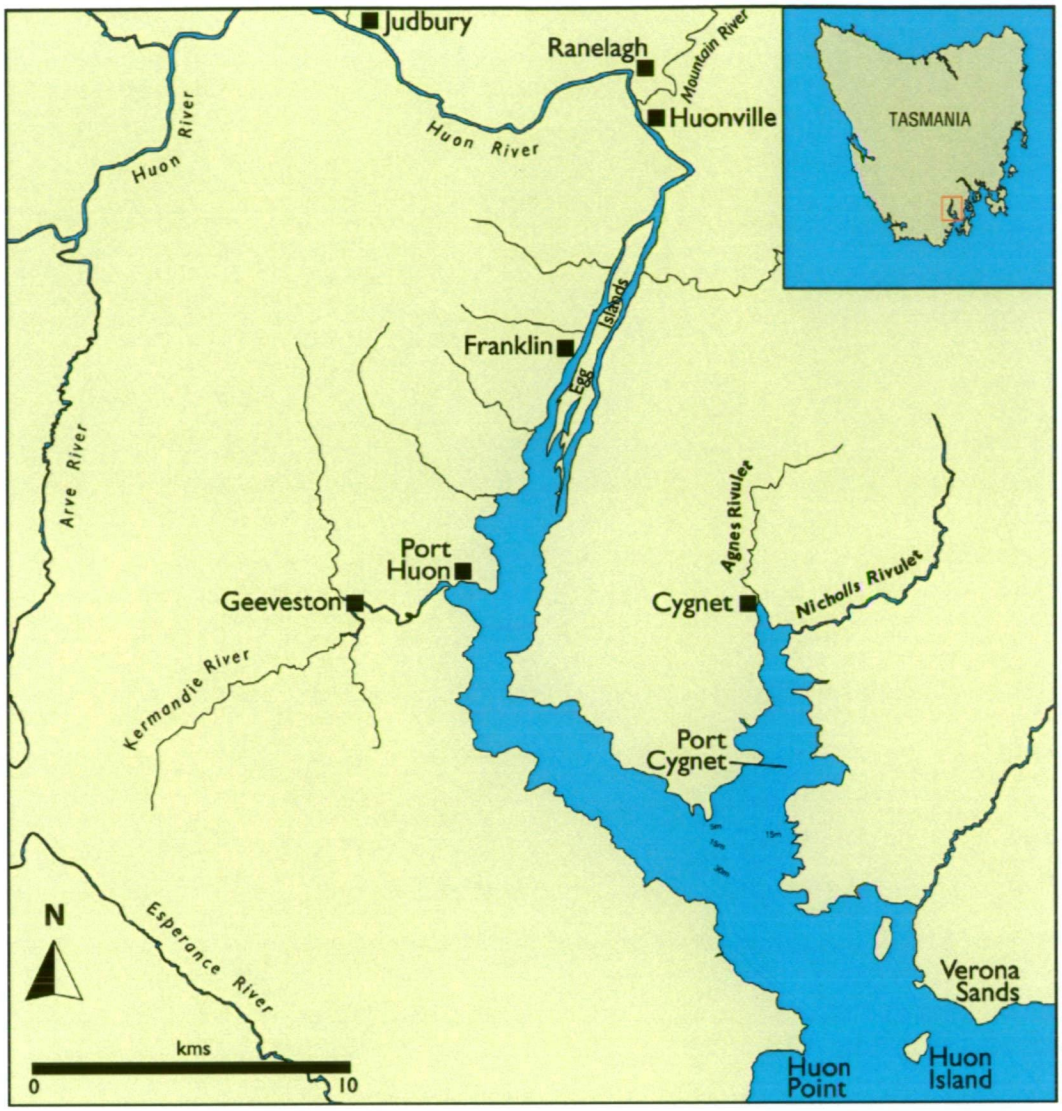

Figure 1.2 Location and features of the Huon Estuary in south-eastern Tasmania, adapted from CSIRO Huon Estuary Study Team (2000).

The catchment of the Huon Estuary is classified as largely natural as it has been subject to only moderate modification by human activity. Much of the upper catchment remains as undisturbed native forest, increasing areas of the mid- and lower-catchment are now been subjected to managed forestry activities. Approximately, 5.6 % of the total catchment has been cleared- for a patchwork of primary agriculture activity such as horticulture and livestock grazing. Human

settlement is sparse, approximately 15,000 spread across a series of small townships along the lower reaches of the River, and also bordering the estuary.

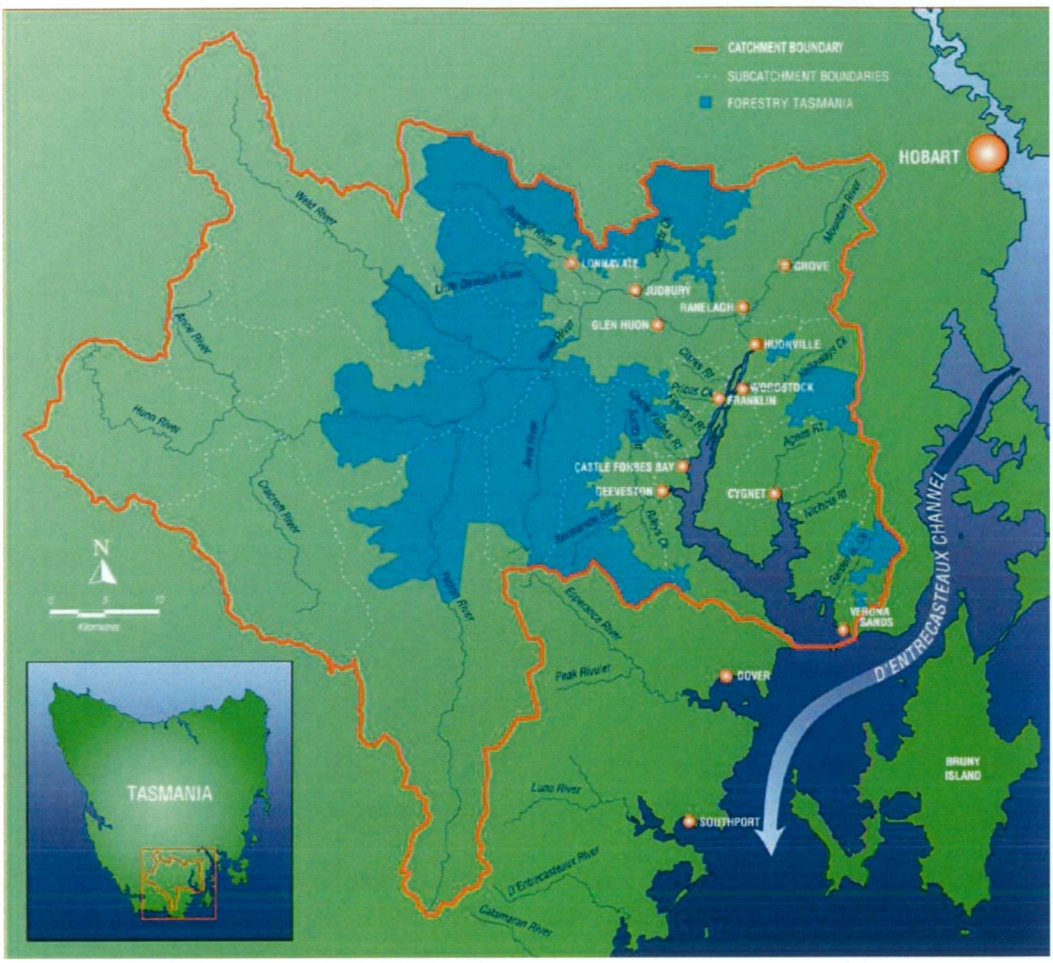

Figure 1.3 Location of the Huon Estuary catchment, adapted from CSIRO Huon Estuary Study Team (2000). Also in this map is the D'Entrecasteaux Channel, Port Esperance (Dover) and Southport.

Salmon aquaculture began in the 1980s and has grown significantly since then. Salmon farming is one of the largest aquaculture industries in Australia, estimated to be worth 260 million dollars in 2008 and it is expected to continue to grow. Almost all salmon produced in Australia is from Tasmania and the majority of salmon farms are situated in the south-east where there are plenty of sheltered sites and the water temperature is most suitable. When the salmon aquaculture industry began in the

early 1980's there was limited understanding and knowledge of environmental effects. As salmon farming continued to grow in Tasmania it became increasingly recognised that expansion needed to be underpinned by a sound scientific knowledge of the effects of salmon farming on the environment.

In 1996 a large study commenced in the Huon Estuary- the Huon Estuary Study (HES). The goal of the HES was to examine the physical and biological characteristics and environmental status of the estuary and to gain an integrated understanding of the system. One of the key drivers for the research was too examine potential impacts of salmon farming on the Estuary. The 3 year HES involved collection of physical, chemical and biological data throughout the estuary and the development of a relatively simple (2D box) coupled hydrodynamic and bio-geochemical model. Nutrient data from the Huon Estuary Study indicated that it is a N limited system.

Models based upon data from the HES predicted that a doubling in the production of salmon would result in only a small increase in the likelihood of algal blooms, while greater inputs could significantly increase the likelihood of phytoplankton blooms and potential eutrophication, but that further investigation was required to improve understanding of the links between nitrogen and phytoplankton growth and biomass in the Huon Estuary. A key gap identified from the HES was the lack of knowledge on how different forms of N may affect the phytoplankton biomass and its composition in the estuary.

The biogeochemical model from the more recent Aquafin CRC was used to calculate nitrogen budgets for the Huon Estuary and D'entrecasteaux Channel in 2002 (Volkman *et al.*, 2009). The largest input of N (60%) to the Huon Estuary and D'entrecasteaux Channel comes from the surrounding marine waters. This N is mostly delivered in winter and is primarily NO_3^-. The Huon River delivers about 23 % but this is mostly considered refactory N. Salmon aquaculture accounts for 17% of the N and while it is only a relatively small amount almost all of this N is labile NH_4^+.

For the D'entrecasteaux Channel and Huon Estuary in 2002, N from salmon aquaculture was estimated to be 843 tonnes: 313 tonnes of N input to the Huon and 543 input into the D'Entrecasteaux Channel. The salmon aquaculture industry has increased production significantly since 2002 and in 2009 it is estimated that there will be a 210% increase in N to 1747 tonnes across both the Huon Estuary and D'Entrecasteaux Channel. But industry and regulators have capped production in the Huon Estuary, so only 243 tonnes of N will be input to the Huon in 2009. However there will be a ~3 times increase in the D'Entrecasteaux from 530 to 1747 tonnes in 2009.

Dinoflagellates are important components of the phytoplankton community in the Huon Estuary, often forming the majority of the biomass. Periodic blooms of the toxic species, *G. catenatum,* result in closure of shellfish farms in the Huon Estuary and D'Entrecasteaux Channel (Hallegraeff *et al.*, 1989). This large dinoflagellate species (35um) intermittently forms dense and often mono-specific blooms in the Huon Estuary and is a major contributor to the phytoplankton biomass in the estuary during blooms (Thompson *et al.*, 2008, Hallegraeff *et al.*, 1995). Blooms occur only in some years and not in others and appear to be associated with particular climatic triggers (early summer rainfall followed by periods of low winds; Hallegraeff et al., 1995). However, these triggers have not proven to be universally required as significant blooms have occurred without these cues. During bloom years, the phytoplankton biomass is also much greater than would be predicted from the available NO_3^- in the estuary. As *G. catenatum* is capable of rapid diurnal vertical migration it has been hypothesised that these blooms may be using NH_4^+ derived from bottom waters (Doblin et al., 2006).

1.3 Research Objectives

This research will combine laboratory and field experiments to address the current lack of knowledge on the effects of nutrient input composition on phytoplankton in the Huon Estuary. The first objective of this research was to determine whether

phytoplankton in the Huon Estuary are using nitrogen that has an oceanic source (primarily NO_3^-) or more locally supplied or regenerated source (primarily NH_4^+ and urea). Given the large role *G. catenatum* plays in the Huon Estuary with high biomass blooms forming during summer and autumn it was also considered that better understanding the physiology of *G. catenatum* would be key to understanding phytoplankton dynamics in the Huon Estuary. The second objective of this research was to understand the physiological responses of *G. catenatum* to light, temperature and different nitrogen species. Specifically, this thesis sets out to:

- Determine the nitrogen uptake preference and dynamics of the seasonal phytoplankton assemblage in the Huon Estuary (Chapter 2)

- Investigate the effect of temperature and irradiance on growth rate and biochemical composition of *G. catenatum* (Chapter 3)

- Describe the physiology and nutrient uptake dynamics and preference of the dinoflagellate *Gymnodinium catenatum*, a significant seasonal contributor to the phytoplankton biomass in the Huon Estuary (Chapters 4).

- Determine whether diurnally migrating *G. catenatum* blooms are able to access and uptake nitrogen available at depth during the night in the field (Chapter 5).

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2 NITROGEN UPTAKE BY PHYTOPLANKTON IN THE HUON ESTUARY, SOUTH-EAST TASMANIA, AUSTRALIA

2.1 Introduction

The Huon Estuary has been the subject of intensive environmental studies since 1996 as part of a program to ensure the sustainability of aquaculture in the Estuary. One of the key topics being addressed is the link between nutrients and phytoplankton blooms in the estuary. The Huon Estuary Study (Team, 2000) suggested that phytoplankton growth in the Huon Estuary is limited primarily by the availability of nitrogen (N).

In coastal ecosystems the main sources of N used by phytoplankton are nitrate (NO_3^-), ammonium (NH_4^+) and urea (Twomey *et al.*, 2005). Some phytoplankton are able to use all these sources of N for growth (Antia *et al.*, 1975). However there is evidence that some species/groups of phytoplankton favour/prefer one form of nitrogen over another. For example diatoms have been shown to be associated with increased NO_3^- uptake (Heil *et al.*, 2007, Berg *et al.*, 2003, Bode & Dortch, 1996). In addition to some species groups having preferences for particular N substrates there are a number of environmental factors that also have an effect on N uptake, including: temperature, light, substrate concentration and inhibition (Varela & Harrison, 1999, Dortch, 1990).

Dinoflagellates are important components of the phytoplankton community in the Huon Estuary, often forming the majority of the biomass. Periodic blooms of the toxic species, *G. catenatum,* result in closure of shellfish farms in the Huon Estuary

and D'Entrecasteaux Channel (Hallegraeff *et al.*, 1989). This large dinoflagellate species (35um) intermittently forms dense and often mono-specific blooms in the Huon Estuary and is a major contributor to the phytoplankton biomass in the estuary during blooms (Thompson *et al.*, 2008, Hallegraeff *et al.*, 1995). Blooms occur only in some years and not in others and appear to be associated with particular climatic triggers (early summer rainfall followed by periods of low winds; Hallegraeff et al., 1995). However, these triggers have not proven to be universally required as significant blooms have occurred without these cues. During bloom years, the phytoplankton biomass is also much greater than would be predicted from the available NO_3^- in the estuary. As *G. catenatum* is capable of rapid diurnal vertical migration it has been hypothesised that these blooms may be using NH_4^+ derived from bottom waters (Doblin *et al.*, 2006). Field observations indicate that *G. catenatum* vertically migrates diurnally from 5m to 20m through temperature, salinity and nutrient gradients in the Huon Estuary (Doblin et al., 2006). Testing the hypothesis that *G. catenatum* may vertically migrate to access NO_3^- or NH_4^+ was one of the major components of this research. For this reason N uptake experiments were to be setup at 5m and 20m during the day when it was expected that *G. catenatum* would be concentrated at 5m. Experiments were also set up at 5m and 20m during the night when it was expected that *G. catenatum* would be concentrated at 20m. However, large blooms of *G. catenatum* were rare in the Huon Estuary in 2002, 2003 and 2004. So our 2003-2004 field trips, were unable to provide information about the N uptake strategies of *G. catenatum* during a vertically migrating bloom. For this reason we focused on determining which nitrogen sources: nitrate (NO_3^-), ammonium (NH_4^+) or urea are important for supporting phytoplankton growth in the Huon Estuary. These field experiments have focused upon whether the phytoplankton are using nutrients that have an oceanic source (primarily nitrate) or more locally supplied or regenerated nutrients (primarily ammonium and urea). Given that phytoplankton in Australian estuaries are nitrogen limited (Harris, 2001) the possibility exists that additional nitrogen inputs to the ecosystem may cause an increase in phytoplankton biomass or increase blooms of nuisance or toxic species.

The research in this chapter is designed to investigate which of these N sources supports the growth of phytoplankton in this region.

2.2 Methods

The research included 4 field trips in the Huon Estuary on the 28-29 May 2003, 23-24 September 2003, 18-19 November 2003, and 24-25 February 2004. It is recognized that 4 sampling trips per year, even when statistically different, may not fully represent seasonal affects. Regardless, for the sake of simplicity in presentation, these temporal periods are referred to as seasons. During these field trips a ^{15}N tracer technique was used to measure uptake of 3 different nitrogen (N) sources (NO_3^-, NH_4^+ and urea) by the natural phytoplankton assemblage. Two sites, Garden Island (latitude 43° 16' 3" S longitude 147° 6' 30" E) and Hideaway Bay (latitude 43° 16' 14" S longitude 147° 5' 2" E), were used for this field work (Figure 2.1). N uptake experiments were setup at 5 m and 20 m water depth during both the day and night.

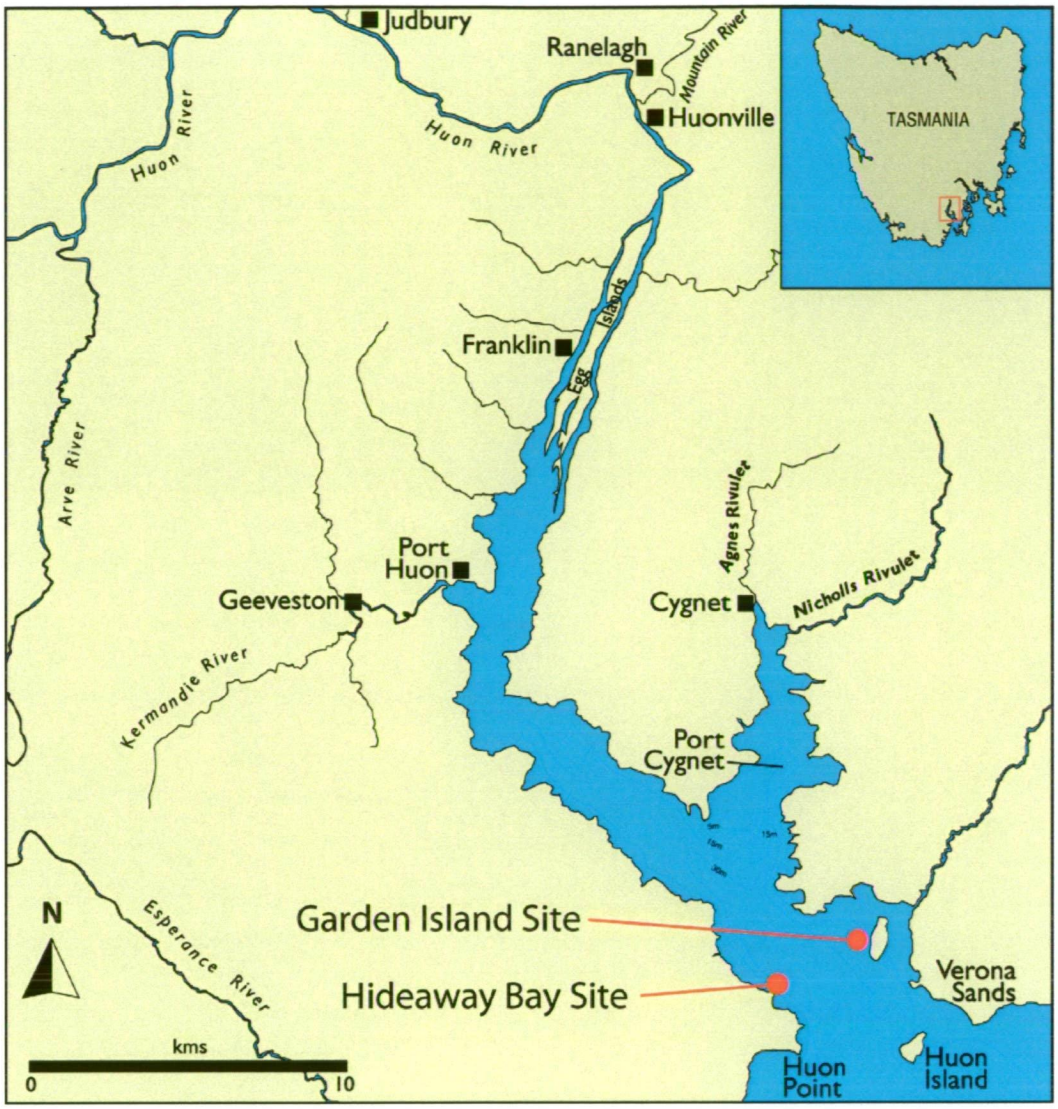

Figure 2.1 Location for nitrogen uptake experiments in the Huon Estuary during 2003-2004.

Four field trips (28-29 May 2003, 23-24 Sept 2003, 18-19 Nov 2003, and 24-25 Feb 2004) were undertaken to measure N uptake at Garden Island and Hideaway Bay: 2 sites near the mouth of the Huon Estuary. In this section of the thesis data collected on the September 23-24 is considered early spring, November 18-19 is referred to as late spring, February 24-25 is late summer and May 28-29 is late autumn. A ^{15}N Tracer technique (Dugdale & Goering, 1967) was used to measure the uptake of NO_3^-, NH_4^+ and urea at 5 m and 20 m depths during the day and night. Water was

collected from the 2 depths using a 10 l Niskin bottle and dispensed into 500ml glass Schott bottles made by Schott Duran™. The water collected for the incubations were not pre-filtered before dispensing into the 500 ml Schott bottles. There were two main reasons for not pre-filtering the water for the incubations. Firstly, ensuring that long chains of *G. catenatum* were not excluded from incubations. *G. catenatum* chains of 4 to 8 cells are common. A quick estimate of the size of these chains, 30 microns x 8 cells = 240 microns suggests that screening at 200 microns (a commonly used screen) would exclude a proportion of this species. Secondly, we wanted to estimate the real in situ N uptake rate including, losses due to grazing. For each depth three 500 ml schott bottles were spiked with 0.3 μM ^{15}N- NO_3^- (99.3 atom percent ^{15}N), three 500 ml schott bottles were spiked with 0.1 μM ^{15}N-NH_4^+ (99.6 atom percent ^{15}N) and three 500 ml schott bottles were spiked with 0.068 μM ^{15}N-urea (98.61 atom percent ^{15}N). In addition at each depth one 500ml schott bottle was filled with water but not spiked with any ^{15}N substrate, this unspiked bottle was used to determine the background ^{15}N (un-enriched atom % excess). These samples were incubated in 500 ml Schott bottles for 4 hours in-situ at the depths they were collected from. Four hour incubations were chosen because they are short enough to limit the chances of substrate exhaustion (La Roche, 1983) and also reduce the problems caused by substrate dilution(Glibert *et al.*, 1982), but an incubation period of 2-6 hours is also long enough to minimise the bias introduced by initial high uptake rates that sometimes occur in phytoplankton (Dugdale & Wilkerson, 1986). After the incubation the water samples were filtered onto pre-combusted (450°C for 4 hours) 25 mm Whatman™ glass fibre filters and stored frozen until analysis. The filters were dried in an oven at 60°C overnight before they were analysed using a Carlo Erba NA1500 CNS analyzer interfaced via a Conflo II to a Finnigan-MAT Delta S isotope ratio mass spectrometer to determine the N isotope ratios. Absolute uptake rates were calculated using the Dugdale and Goering (1967) equation:

$$\rho = Na_t(Rt)^{-1}$$

where ρ is the absolute uptake ($\mu g\ N\ l^{-1}\ h^{-1}$), N is the total particulate nitrogen (μg N), a_t is the atom % excess of ^{15}N (= atom % - background), R is the atom % enrichment $[a_e\ (S_L/(S_L + S_U))]$, a_e is the atom % enrichment of labelled ^{15}N source, S_L is the concentration of labelled ^{15}N, S_U is the concentration of unlabelled ^{14}N and t is the incubation time (h). Specific uptake v ($\mu gN\ \mu gChla^{-1}\ h^{-1}$) is the absolute uptake ρ normalised to chl*a* and is calculated using this equation:

$v = \rho/Chla$

Where, ρ is the absolute uptake ($\mu g\ N\ l^{-1}\ h^{-1}$) and Chl*a* is total chlorophyll a (μg Chl*a* l^{-1}).

It is common for researchers investigating N uptake to normalise N uptake to particulate N (PN). In this research N uptake has been normalised to chl *a* as an indicator of phytoplankton biomass. The fact is that chl *a* is the preferred method of measuring phytoplankton biomass worldwide. It is well known that measurements of particulate N or particulate C are not as reliable, primarily due to the potential contamination by detritial C or N. A number of highly respected researchers have proposed methods to improve the estimation of phytoplankton biomass from POC or PON including Karl Banse (Banse, 1977) or methods that rely on neither (Holm-Hansen & Booth, 1966) but these methods are complex or difficult to apply to individual samples. One result is that very few researchers report phytoplankton biomass in units of PN or PC. In spite of these difficulties a number of researchers have normalized their N uptake measurements to PN and reported them in this manner. This may have more to do with the fact that the analysis required to obtain the results from the uptake experiments also gives a value for PN, rather than any more strategic reasoning. The truth is that N per cell shows about the same amount of variability as chl *a* per cell (Thompson, 1999) in response to irradiance and temperature. For these reasons we have normalized N uptake to chl *a*.

When water was collected for the N uptake experiments, triplicate 10 ml samples were taken for measurement of ambient NO_3^-, NH_4^+ and urea concentrations and 2 L was filtered for pigment analyses using high performance liquid chromatography (HPLC).

Nutrient samples were stored at -20 °C prior to analysis. Analytical techniques for nitrate and/or nitrite (Wood *et al.*, 1967), silicate (Murphy & Riley, 1962) and phosphate (Armstrong, 1951) were adapted and performed using Quick-Chem™ methods on a flow injection LACHAT instrument as per the following protocols for nitrate and/or nitrite (Quik-Chem™ Method 31-107-04-1-A; detection limit 0.03~ mM), silicate (Quik-ChemTM Method 31-114-27-1-D; detection limit ~0.05 mM) and phosphate (Quik-ChemTM Method 31-115-01-1-G; detection limit ~0.02 mM). Ammonium was measured using the technique of Kerouel and Aminot (Kerouel & Aminot, 1997) adapted for flow injection (Watson *et al.*, 2004)with a detection limit of ~0.05 mM. Urea samples were analysed using a diacetyl monoxime technique according to the method of (Mulvenna & Savidge, 1992). To reduce the health risks associated with thio-semicarbazide, a reagent for this technique, we substituted semicarbazide (a less toxic but similar compound).

It was not possible to measure ambient concentrations of nutrients prior to commencing an experiment. Consequently fixed concentrations of NO_3^-, NH_4^+ and urea were added as listed above, with a target of ~ 10% of the ambient concentration. The majority (59%) of ^{15}N additions were <15% of ambient concentrations. There were, however, a number of instances where ambient concentration were less, at certain depths or times of the year, than anticipated. Adding less ^{15}N may have been more appropriate at these times of the year although it increases the likelihood of running out of labelled substrate and thus underestimating uptake rates. Under conditions of extreme N limitation where 0.3 μM ^{15}N- NO_3^-, 0.1 μM ^{15}N-NH_4^+ or 0.068 μM ^{15}N-urea represent substantially more than 15% of the ambient concentration the labelled N is no longer a tracer but becomes an increasingly important source. Under these conditions it is likely that real in situ uptake rates were

overestimated by the technique. The worst cases were for nitrate in late spring and late summer (Table 2.1) where uptake of nitrate is probably over estimated in the results presented here.

Table 2.1 Estimated proportions (% of ambient concentration) of ^{15}N added as a tracer for all field trips in the Huon Estuary.

Date	site	time	depth (m)	% ^{15}N NH_4^+	% ^{15}N NO_3^-	% ^{15}N urea
28/05/2003	Hideaway Bay	day	5	11	9	22
			20	18	9	19
		night	5	8	8	16
			20	19	7	13
	Garden Island	day	5	7	9	33
			20	12	7	20
		night	5	15	8	25
			20	12	8	13
23/09/2003	Hideaway Bay	day	5	13	9	14
			20	32	8	16
		night	5	25	8	20
			20	65	8	20
	Garden Island	day	5	60	8	11
			20	91	8	13
		night	5	43	8	19
			20	98	8	15
18/11/2003	Hideaway Bay	day	5	15	430	21
			20	16	54	17
		night	5	10	820	32
			20	13	40	14
	Garden Island	day	5	114	3000[a]	17
			20	13	44	15
		night	5	229	3000[a]	34
			20	20	52	16
24/02/2004	Hideaway Bay	day	5	36	295	15
			20	7	53	15
		night	5	23	485	11
			20	6	58	13
	Garden Island	day	5	32	663	10
			20	10	61	9
		night	5	7	51	8
			20	23	1785	9

[a] **% enrichment calculated by using the detection limit (0.03 mM) of the NO_3^- detection method used.**

2.2.1 CTD profiles

A Seabird™ SBE19$^+$ conductivity, temperature and depth (CTD) profiler was used to measure the salinity, temperature and fluorescence of the water column during the field experiments. The measurements throughout the water column presented are the

downwards cast with the initial period removed where the instrument was beginning to pump water through the sensors.

The CTD sensors: salinity, temperature and fluorescence are factory calibrated. The sensors were further checked prior to fieldwork by measurement of standard samples for salinity and temperature and field samples for fluorescence.

2.2.2 Phytoplankton Counts

Water collected for the surface N uptake experiment during the day was used to enumerate phytoplankton. One litre water samples were taken and preserved in the field using Lugol's iodine fixative solution (110 g potassium iodide, 50 g iodine, 1 litre distilled water, 100 ml glacial acetic acid) to approximately 2% final concentration. The samples were stored until they could be counted under the light microscope. Prior to counting the Lugol's preserved samples were transferred to 1 litre measuring cylinders (volume recorded – V_1) and allowed to settle for at least 24 hours. After this time, approximately 900 ml were siphoned off and the remaining sample was transferred to a 100-ml measuring cylinder and again allowed to settle for at least 24 hours. Then approximately 90 ml were siphoned off, the final volume recorded (V_2) and thoroughly mixed before a 1-ml aliquot was taken, placed in a Sedgwick Rafter counting chamber and examined using an Olympus IX71 microscope to identify and count the phytoplankton.

The Sedgwick Rafter counting chamber has a grid of 1000 squares, each of 1 µl. For microplankton, (cells generally larger than 20 µm diameter) at least 100 squares or 10% of the counting chamber was scanned (except in cases where there were dense blooms of one or more microplankton species, when at least one column of 20 squares was scanned) at 200x magnification. For nanoplankton, (2-20 µm in diameter) the chamber was examined under 400x magnification until at least 300 cells of the dominant nanoplankton "species" had been counted. Flagellates in the nanoplankton were grouped, as time constraints did not allow fuller identification.

Cells per litre =

cell "species" count * (1000 / number squares counted) * (V_2 *1000 / V_1)

2.2.3 CHEMTAX

Pigment data from HPLC analysis was further analysed to give the proportions of chlorophyll *a* present in the following algal taxonomic categories: Cyanophyta, Prochlorophyta, Chrysophyta, Cryptophyta, Bacillariophyta (diatoms), Haptophyta, Prasinophyceae, and Dinophyta (Mackey *et al.*, 1996). Initial input ratio matrices were adjusted from those proposed in Mackey, Mackey *et al.* (1996) by including four types of Haptophytes:

Haptophyte N – Type 1 = fucoxanthin e.g. *Isochrysis spp.*

Haptophyte S- Type 2 = Chl c_3 + fucoxanthin e.g. *Ochrosphaera neopolitana*

Haptophyte Type 3 = Chl c_3+ 19'Hexanoyloxyfucoxanthin + fucoxanthin + and sometimes 19'butanoyl derivative e.g. *Chyrsochromulina strobulis*

Haptophyte Type 4 = c_3 + 19'butanoyloxyfucoxanthin +19'hexanoyloxyfucoxanthin and fucoxanthin e.g. *Imantonia rotunda.*

The peridinin:chlorophyll *a* ratio was modified from 0.515:1 as in Mackey, Mackey *et al.* to 0.36:1 after Hallegraeff, Nichols *et al.* (1991). The latter was based on extensive culture studies for *Gymnodinium catenatum.* This has the effect of increasing the proportion of the chlorophyll *a* that CHEMTAX will allocate to dinoflagellates for a given amount of peridinin. Based on personal observations this results in CHEMTAX estimates of dinoflagellates that are more consistent with the estimated proportion of biomass calculated by cell counts and adjusted for cellular bio-volumes.

2.2.4 Statistical analysis

2.2.4.1 Phytoplankton Dynamics

In order to determine whether there were significant effects of season on the phytoplankton composition we first determined whether phytoplankton data from both sites could be pooled for subsequent analysis. The pigment data were percentage data, thus nonparametric tests were used. The effect of site was tested for using a signed Wilcoxin ranks test. There was no significant difference in the phytoplankton community associated with site and the data from the 2 sites were pooled together. The effect of season on abundance of four phytoplankton groups: diatoms, dinoflagellates, cryptophytes and prasinophytes (the main contributors to the phytoplankton assemblage throughout the year) was tested using a Kruskal-Wallis analysis of variance (ANOVA) on ranks for each phytoplankton group. A Dunn's *post hoc* test was used to make multiple comparisons following the Kruskal-Wallis ANOVA on ranks.

2.2.4.2 Nitrogen uptake data

The Hideaway Bay and Garden Island N uptake data sets comprised 96 independent observations of N uptake (Table 1.1). A few samples were lost and the plan for a balanced experimental design (time of day, depth, season) were only 91% completed with 3 (x 3 replicates) missing data points. In Hideaway Bay data there were no chl *a* results for the September field trip during the night at Depth (20m) for calculating specific uptake rates. A balanced dataset was required for multifactor ANOVA, so the corresponding chl *a* results from the Garden Island were used to replace these missing chl *a* results. The CTD plots from both Hideaway Bay and Garden Island both showed similar fluorescence profiles and based on fluorescence estimates of chl *a* were both ~0.25 $\mu g\ L^{-1}$ at depth (20m) during the night. The Garden Island data Set was missing nitrogen uptake measurements for September at the surface (5 m) during the day and February at depth (20m) during the night. To enable analysis using a

multifactor ANOVA a balanced data set was achieved by constructing the missing data from the mean and standard deviation across the 4 field trips (Table 2.1) for the corresponding depth and time (Little & Rubin, 1987).

Comparisons between the Hideaway Bay and Garden Island sites results for NH_4^+, NO_3^-, urea and absolute N (NH_4^++NO_3^-+urea) uptake using paired t tests. The tests for normality and equal variance were Kolmogorov-Smirnov test (with Lilliefors' correction) and Levene median test. Where data was not normally distributed (NH_4^+, Urea and absolute N (NH_4^++NO_3^-+urea) uptake) 0.05 was added to make all values positive and then they were log transformed. Following the transformation only urea did not conform to a normal distribution. Where there were no significant differences between sites data was pooled across sites for subsequent statistical analyses.

An overall analysis to determine whether there were differences between uptake of NH_4^+, NO_3^- and urea in the Huon Estuary (averaged across season, time of day and depth) was undertaken using a non-parametric Kruskal-Wallis ANOVA on ranks test ($P=<0.001$) and post hoc multiple comparisons made using Tukey's Test.

A subset of the data: nitrogen uptake during the day and at the surface (5m) was used to examine the effect of time of year on absolute and specific nitrogen (NH_4^++NO_3^-+urea) uptake. For absolute N uptake (NH_4^++NO_3^-+urea), data were log transformed and analysed by ANOVA. Post hoc multiple comparisons of this data were made using the Holm-Sidak test. Specific N uptake (NH_4^++NO_3^-+urea) data were analysed by ANOVA and post hoc multiple comparisons were made using the Holm-Sidak test.

Differences between specific uptake of NH_4^+, NO_3^- and urea at four times throughout the year (during the day and at surface (5m)) were also analysed using a two-way ANOVA, followed by post hoc multiple comparisons by the Holm-Sidak. Prior to analysis by ANOVA, data were transformed (log(x+0.05)), but they still failed the Levene Median test for equal variance.

Multi-factor ANOVA was used to examine the effect of season, time of day, and depth on specific uptake of NH_4^+, NO_3^- and urea independently. Many attempts were made to find a suitable way to transform NH_4^+, NO_3^- and urea datasets to meet the requirements of ANOVA for normality and equal variance, however, they were not successful. A log transform (log(x+0.05) visibly improved the shape of the distribution of the NH_4^+, NO_3^- and urea datasets, therefore were used, even though the data still failed the tests for normality and equal variance. Although ANOVA is reasonably robust to lack of homogeneity of variance and normality (Sokal & Rohlf, 1995) the reader is thus cautioned that the conclusions of these statistical analysis should be viewed with some caution.

Where data was transformed for analysis, the Multifactor ANOVA results (least squares means and standard errors) were back-transformed for reporting in the text and figures. As standard errors cannot be directly back-transformed upper and lower 95% confidence intervals were reported. The 95% confidence intervals were calculated as below:

Lower 95% confidence interval= 10^(X-SE*t)

Upper 95% confidence interval= 10^(X+SE*t)

Where X=mean, SE=standard error and t is the t statistic (α=0.05) for n-1 degrees of freedom. Both the mean and standard error are to be in the log form for this calculation. To improve the accuracy of the 95% confidence intervals the standard errors from the ANOVA were not used, because use of these standard errors is only accurate when the assumptions for equal variance in the dataset are met. As our NH_4^+, NO_3^- and urea data did not pass the requirements for equal variance, standard errors were calculated for each cell of the ANOVA using the relevant original data.

2.3 Results

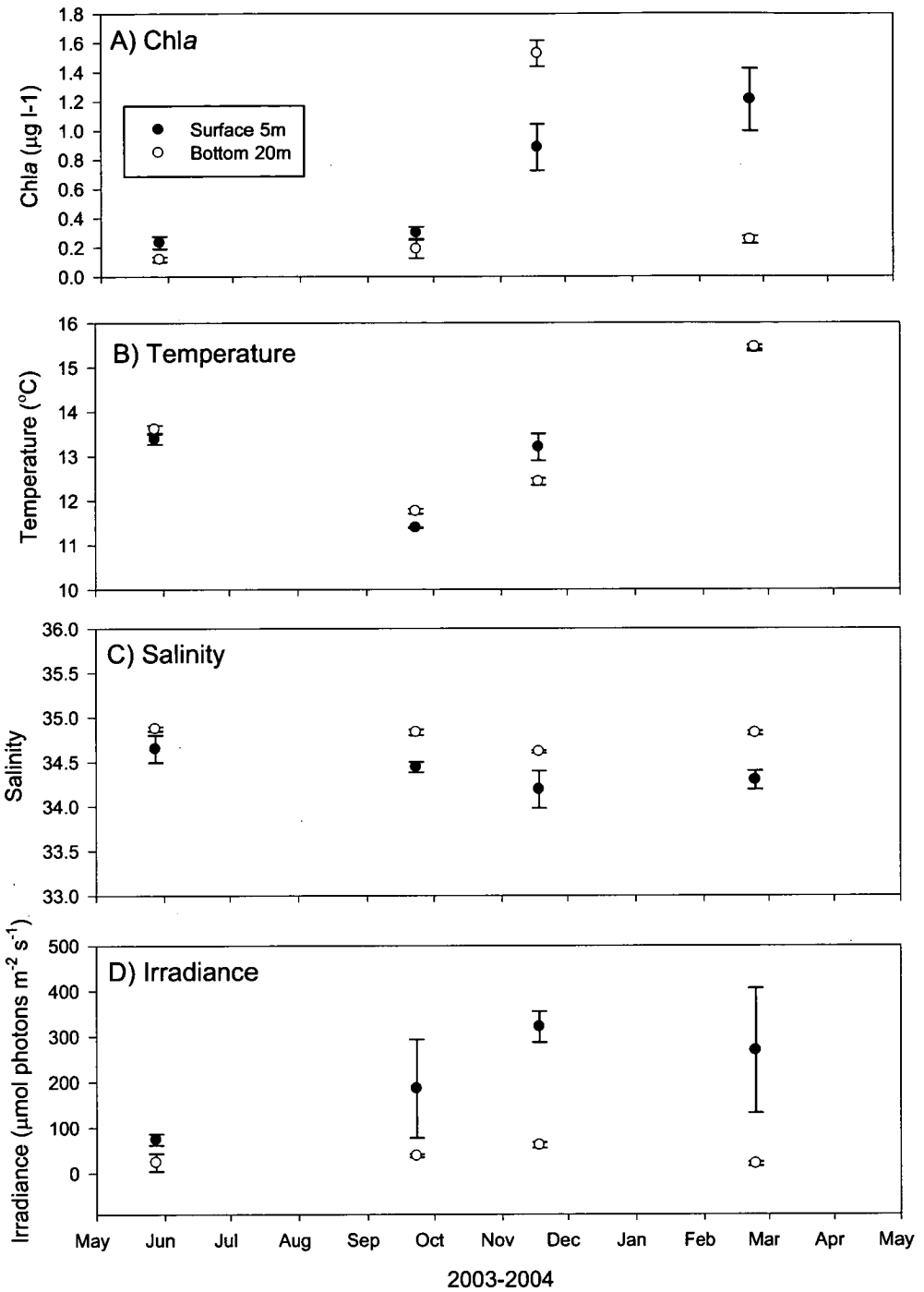

Figure 2.2 A) Chl*a*, B) temperature, C) salinity and D) irradiance at the surface (5m) and bottom (20m) during the four field trips in the Huon Estuary.

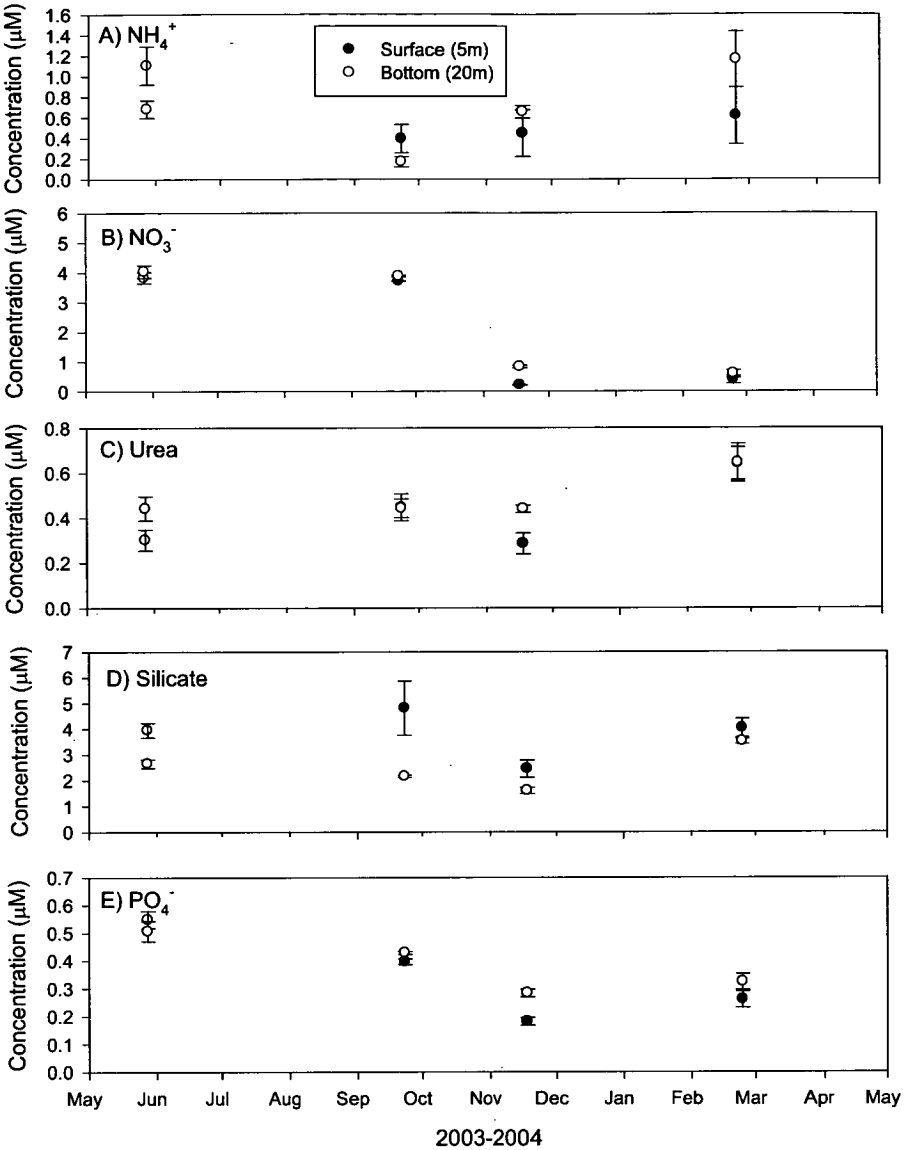

Figure 2.3 Mean ambient concentrations of nutrients (NH_4^+, NO_3^-, urea, Silicate and PO_4^{3-}) at surface (5m) and bottom (20m) depth during each of the field trips.

2.3.1 Late autumn

Physical conditions

During the late autumn field trip the temperature ranged from 13.5 °C at the surface and was 13.7 °C at a depth of 30 m (Figure 2.4). Salinity was also fresher (34.0) from the surface to 3m depth while at depths below the salinity was closer to a salinity expected of a full marine environment (>34.8). At the surface irradiance was 90 μmol photons m^{-2} s^{-1} and decreased quickly in the surface layers. At depths of 30 metres there was very little irradiance ~40 μmol photons m^{-2} s^{-1}.

Chemical conditions

In late Autumn [NO_3^-] and [Silicate] were high at ~4 μM in the surface (5m), at depth (20m), [NO_3^-] was similar at ~4 μM but the [Silicate] was 2.8 μM. In the surface (5m) [NH_4^+] and [urea] were 1.1 and 0.3 μM respectively. At depth (20m) [NH_4^+] was lower, 0.7 μM and [urea] was slightly increased at 0.4 μM. At the surface (5m) and depth (20m) [PO_4^{3-}] was 0.55 and 0.5 μM, respectively.

Biological conditions

During the late autumn field trip chlorphyll *a* (chl*a*) was lowest 0.23 and 0.1 ug l^{-1}, respectively in both the surface (5m) and at depth (20m). Fluorescence was at its maximum near the surface 0.7 units and decreased with depth to 0.3 units. The phytoplankton assemblage was dominated by dinoflagellates (49% of total chl *a*). The next two most dominant groups were prasinophytes and diatoms 23 and 19% of total chl *a*, respectively. The remainder of the phytoplankton consisted of cryptophytes and haptophytes type N (only contain fucoxanthin). Based upon CHEMTAX analysis of the HPLC pigment data there were no significant differences in phytoplankton community composition between site, day or night and surface or depth. There was no microscopic examination of samples in late autumn to substantiate the CHEMTAX phytoplankton community composition.

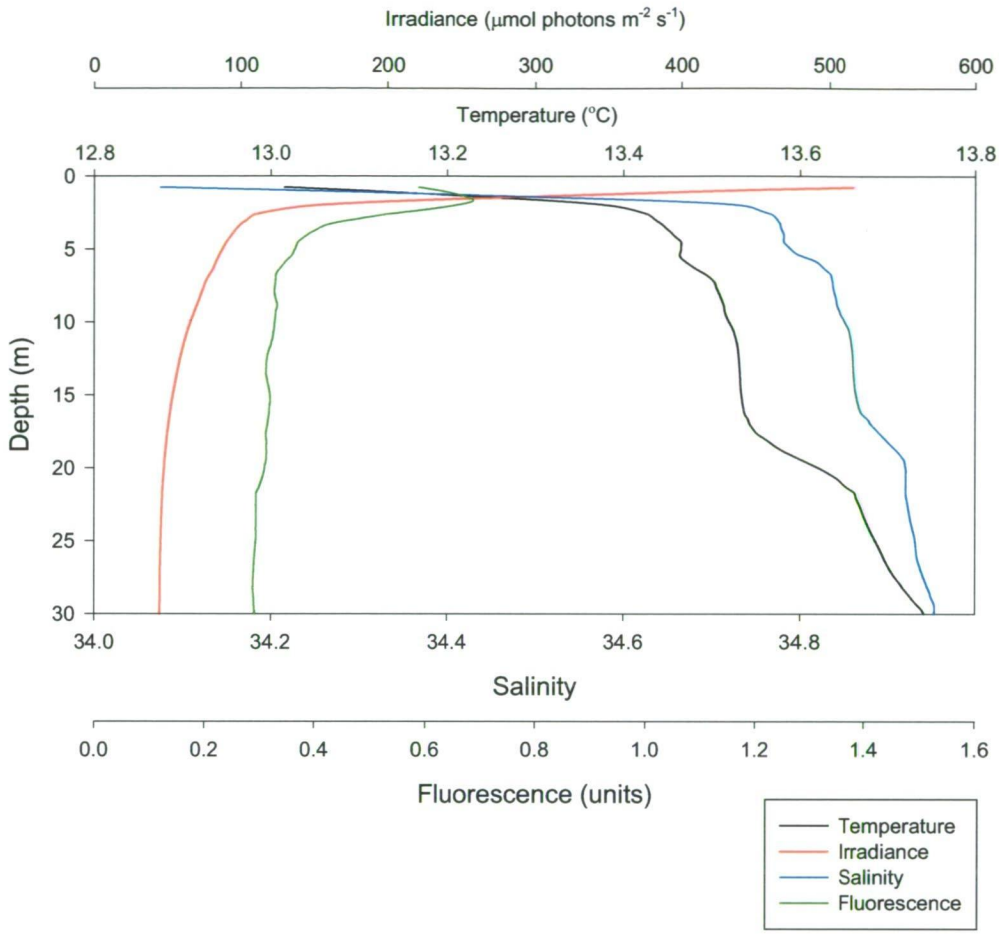

Figure 2.4 Conductivity, temperature and depth profile of Hideaway Bay site during the day for the late autumn (28/05/2003) N uptake experiment.

2.3.2 Early spring

Physical conditions

Temperature was at its lowest, 11.4 and 11.6 °C during early spring in the surface (5m) and at depth (20m) respectively. Irradiance had increased from late autumn to early spring in both the surface (~300 µmol photons m^{-2} s^{-1}) and depth (~50 µmol

photons m^{-2} s^{-1}). Relative to late autumn the salinity was lower (34.5 and 34.8) in early spring at surface and depth, respectively.

Chemical conditions

NO_3^- concentrations were still ~ 4 μM in both the surface (5m) and at depth (20m) in early spring. The concentration of NH_4^+ reached was at its lowest in both the surface and at depth, 0.4 and 0.2 μM respectively, during early spring compared with the other field trips. The concentrations of urea at both depths were ~0.5 μM. The concentrations of silicate were at their highest in the surface (5m) during the early spring ~5 μM. At depth the silicate concentration was less than half this concentration (2.3 μM). Concentrations of phosphate (PO_4^{3-}) were ~0.4 μM at surface (5m) and depth (20m).

Biological conditions

During early spring the chl*a* concentrations were low and similar in both the surface and at depth (0.3 and 0.2 μg l^{-1}, respectively). Fluorescence was greatest near the surface 0.3 units and decreased to ~ 0.27 units at depths below 17 m. Phytoplankton were dominated by diatoms at the surface (30-40% of total chlorophyll *a*) and even more dominated at depth (50-60%). Dinoflagellates represented 20-30% of the phytoplankton. There was one exception to these generalisations at Hideaway Bay during the night at the surface (5m). In this sample dinoflagellates accounted for > 60% of the phytoplankton. The remainder of the phytoplankton were composed of chryptophytes, prasinophytes, chlorophytes, haptophytes types N and S and cyanobacteria. The chlorophytes and cyanobacteria seemed to be restricted to the surface (5m) samples. The most numerous diatoms in this sample were *Pseudonitzschia pseudodelicatissima* (1248 cell L^{-1}), *Nitzscia closterium* (793), *Skeletonema costatum* (521 cell L^{-1}) and *Chaetoceross spp.* >15μm (401 cell l^{-1}) (Table 2.1). The dinoflagellates were represented by 15 μm *Gymnodiniod* dinoflagellates (273 cell l^{-1}), *Gymnodinium catenatum* (132 cell l^{-1}) Dinophysis *spp.*

(36 cell l^{-1}) and *Ceratium spp.* (27 cell l^{-1}). There were no cryptophytes observed in these samples. It very likely that they were identified in CHEMTAX because of the alloxanthin in the relatively large population of pigmented *Dinophysis* species with chloroplasts that originate from cryptophytes via the intermediate host of *Myrionecta rubra* (=*Mesodinium rubrum)* (Park *et al.*, 2006, Nagai *et al.*, 2008)

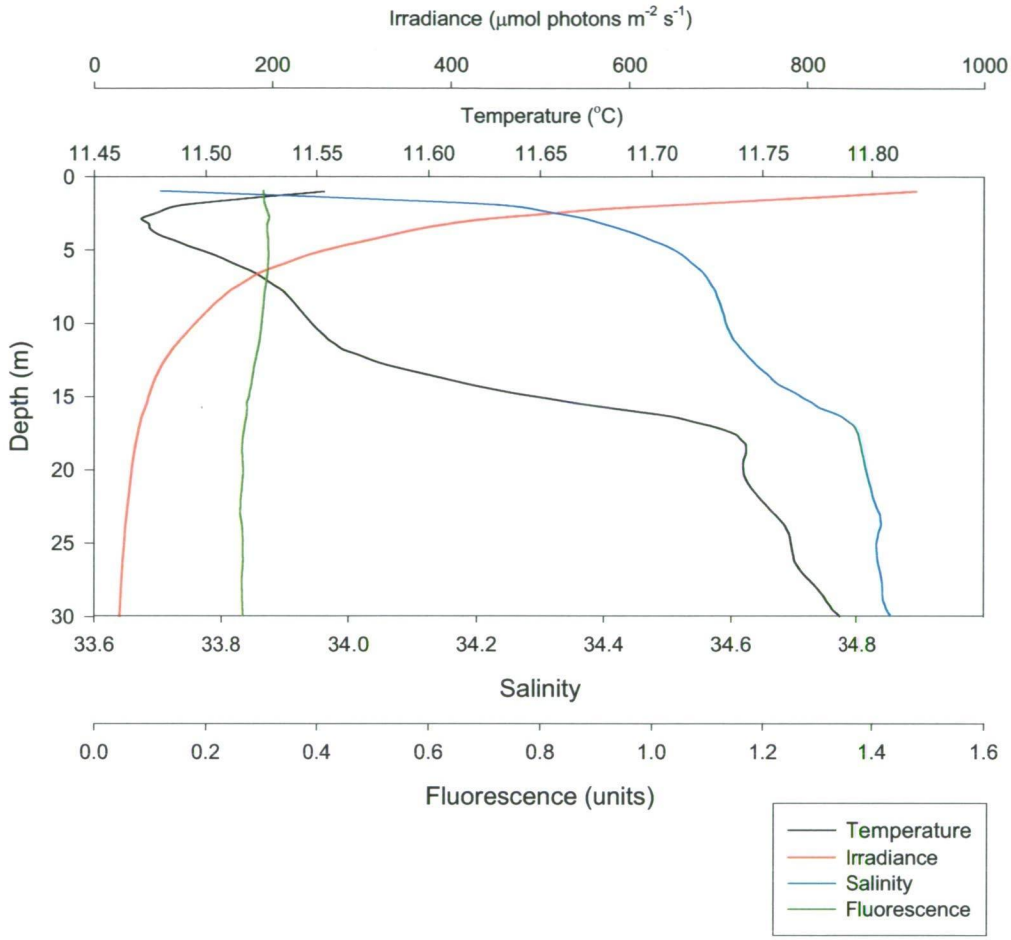

Figure 2.5 CTD profile of Hideaway Bay site during the day for the early spring (23/09/2003) N uptake experiment.

2.3.3 Late spring

Physical conditions

The temperature was increased at both depths, the temperature in the surface was slightly higher 13.2 °C, than at depth 12.8 °C. The irradiance was at it greatest during late spring experiment being 350 and 50 µmol photons m^{-2} s^{-1} in the surface and at

depth respectively. The salinity was lower (34.4 and 34.6) than in the previous early spring field trip at surface (5m) and depth (20m) respectively.

Chemical conditions

The concentration of NO_3^- in late spring decreased in the surface and at depth to 0.2 and 1 μM respectively from the NO_3^- concentrations ~ 4 μm in early spring. The NH_4^+ concentration increased from its lowest concentrations in early spring to 0.5 and 0.7 μM for the surface and at depth respectively. The urea concentration showed a slight decrease from early spring to late spring, from 0.5 to 0.38 μM in the surface. However at depth urea concentration remained the same as in early spring ~0.44. Silicate was at its lowest concentration in the surface and at depth, 2.5 and 2 μM respectively. PO_4^{3-} also reached its lowest concentration during late spring in the surface and at depth, 0.2 and 0.3 μM respectively.

Biological conditions

Chl*a* increases in the surface and at depth to 0.9 and 1.5 μg l^{-1}respectively during late spring. Fluorescence was at its lowest near the surface (0.4 units) and then reached its maximum (0.8 units) at ~ 7 m. The chl *a* then decreases again with depth to 0.6 μg l^{-1} at 25m. The phytoplankton community shifted to a cryptophyte (40-60% of total chlorophyll *a*) domination. Diatoms and prasinophytes were the next most dominant components of the community. There were also a small portion of dinoflagellates in all samples and some cyanobacteria present in the surface samples from Hideaway Bay. There was one sample that had a clearly different composition from all the others. This sample was dominated instead by diatoms and had a large % of dinoflagellates. There were no clear trends between sites, time of day or depth. The cryptophytes were comprised of small round flagellates (863 cell l^{-1}). The diatoms were dominated by a high numbers of *Guinardia flaccida* (41223 cell l^{-1}) and *Rhizosolenia fragillissima* (4607 cell l^{-1}). The majority of the dinoflagellates were

Ceratium spp.(1024 cell l^{-1}), *Dinophysis spp.*(228 cell l^{-1}) and *G. catenatum* (33 cell l^{-1}).

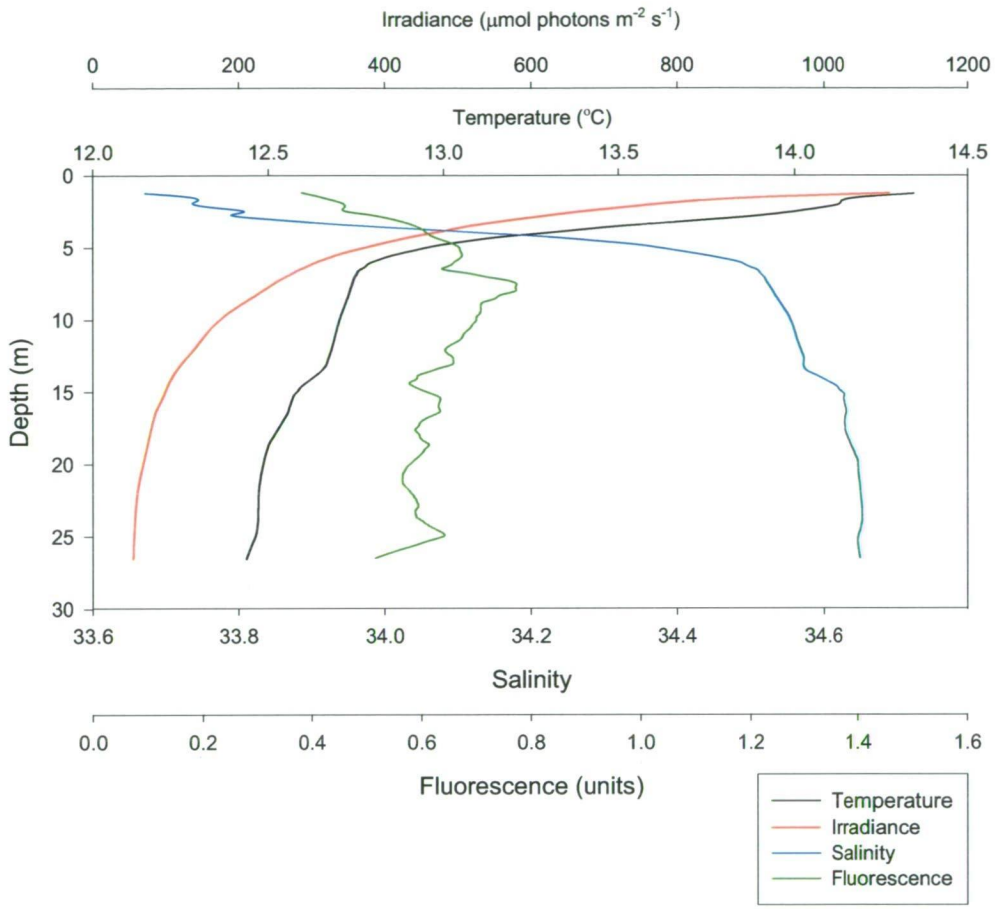

Figure 2.6 CTD profile of Hideaway Bay site during the day for the late spring (18/11/2003) N uptake experiment.

2.3.4 Late summer

Physical conditions

Temperatures were at their highest in late summer, 15.5 °C for both surface (5m) and at depth (20m). However the irradiance had decreased since the field trip in early spring and was now at 130 µmol photons m^{-2} s^{-1} in the surface (5m) and at ~20 µmol

photons m^{-2} s^{-1} at depth (20). In the surface (5m) the salinity was 34.4 the same as late spring. However at depth (20 m) the salinity was 34.8 slightly greater than in late spring.

Chemical conditions

NO_3^- was still at low concentrations in both the surface and at depth during late summer, 0.3 and 0.4 μM, respectively. Both NH_4^+ and urea showed increases from late spring to late summer. NH_4^+ concentrations increased to 0.6 μM in the surface and to 1.1 μM in at depth (20 m). Urea showed an increase to 0.6 μM in both the surface and at depth from concentrations below 0.4 μM in late spring. Silicate increased from its lowest concentrations that occurred during late spring to 4 μM and 3.7 μM in the surface and at depth, respectively. PO_4^{3-} showed a similar trend increasing from the lowest concentrations that occurred in late spring to ~0.3 μM in both the surface and at depth.

Biological conditions

Chl*a* concentration reached their maximum 1.2 ug l^{-1} in the surface (5 m) during late summer. However at depth (20 m) the chl*a* concentration decreased to 0.3 ug l^{-1} close to the lowest chl*a* concentrations recorded at depth during late autumn and early spring. Fluorescence showed that the max concentrations, >1.2 units occurred between 3 and 10 m depth and decreased to between 0.1 and 0.3 units. Composition of samples was not clearly dominated by any one taxonomic group. A mixture of diatoms, cryptophytes, prasinophytes and dinoflagellates were present. These samples had the most variable composition and there were no clear trends associated with time of day, location or depth. There were also small proportions of haptophyte N and S and cyanobacteria groups in some of these samples. The most numerous diatoms were *S. costatum* (938150 cell l^{-1}), *N. closterium* (49499 cell l^{-1}), *Chaetoceross spp.* (29695 cell l^{-1}). The dinoflagellates were represented by *G.*

catenatum (2567 cell l^{-1}), *Dinophysis spp.* (344 cell l^{-1}), *Ceratium spp.* (226 cell l^{-1}) and *Prorocentrum spp.* (15 cell l^{-1}). Small 5-10 um round cryptophtes (1355 cell l^{-1}).

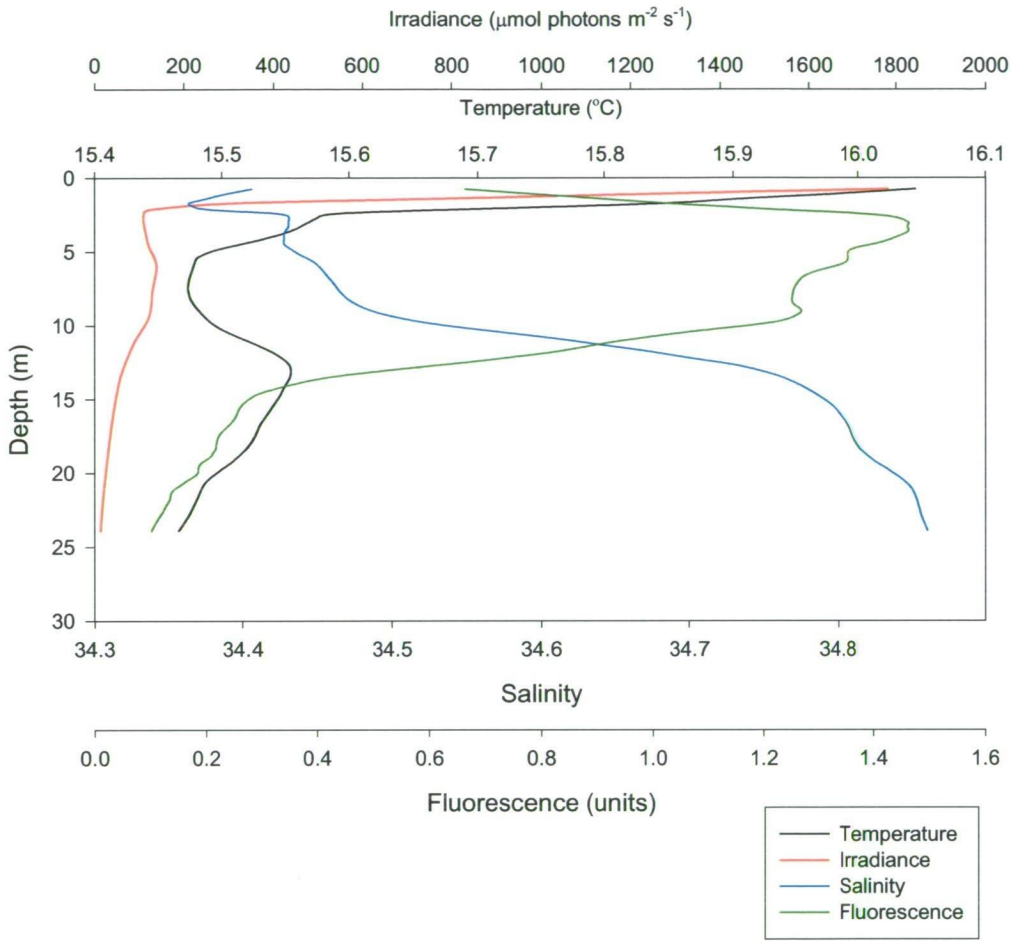

Figure 2.7 CTD profile of Hideaway Bay site during the day for the late summer (24/02/2004) N uptake experiment.

2.3.5 Effect of Season on phytoplankton composition

Season affected the four main phytoplankton groups: dinoflagellates, diatoms, cryptophytes and prasinophytes that were recorded during the four field trips in different ways (Figure 2.9). Dinoflagellates were responsible for 48 and 29% of the total chl *a* in late autumn and early spring respectively. This was reduced in late

spring and late summer where only 7 and 14% respectively, of the total chl *a* belonged to the dinoflagellates. The cryptophytes showed the opposite pattern where 9 and 10% of total chl*a* was attributed to them in late autumn and early spring respectively. Whilst in late spring and late summer the cryptophytes accounted for a much larger amount of the chl*a*, being 40 and 29% respectively. The high proportion of 'cryptophytes' during late spring and late summer probably represents Dinophysis species. During early spring and late summer the diatoms contributed the most to total chl *a*, 47 and 38% respectively. During late autumn and late spring the diatoms contributed less to the total chl*a*, 18 and 31% respectively. The prasinophytes were greatest during late autumn and late spring, 23 and 21% respectively. In early spring the prasinopytes were at their lowest, 9% of the total chl *a*. During late summer the prasinophytes accounted for 15% of the total chl *a*.

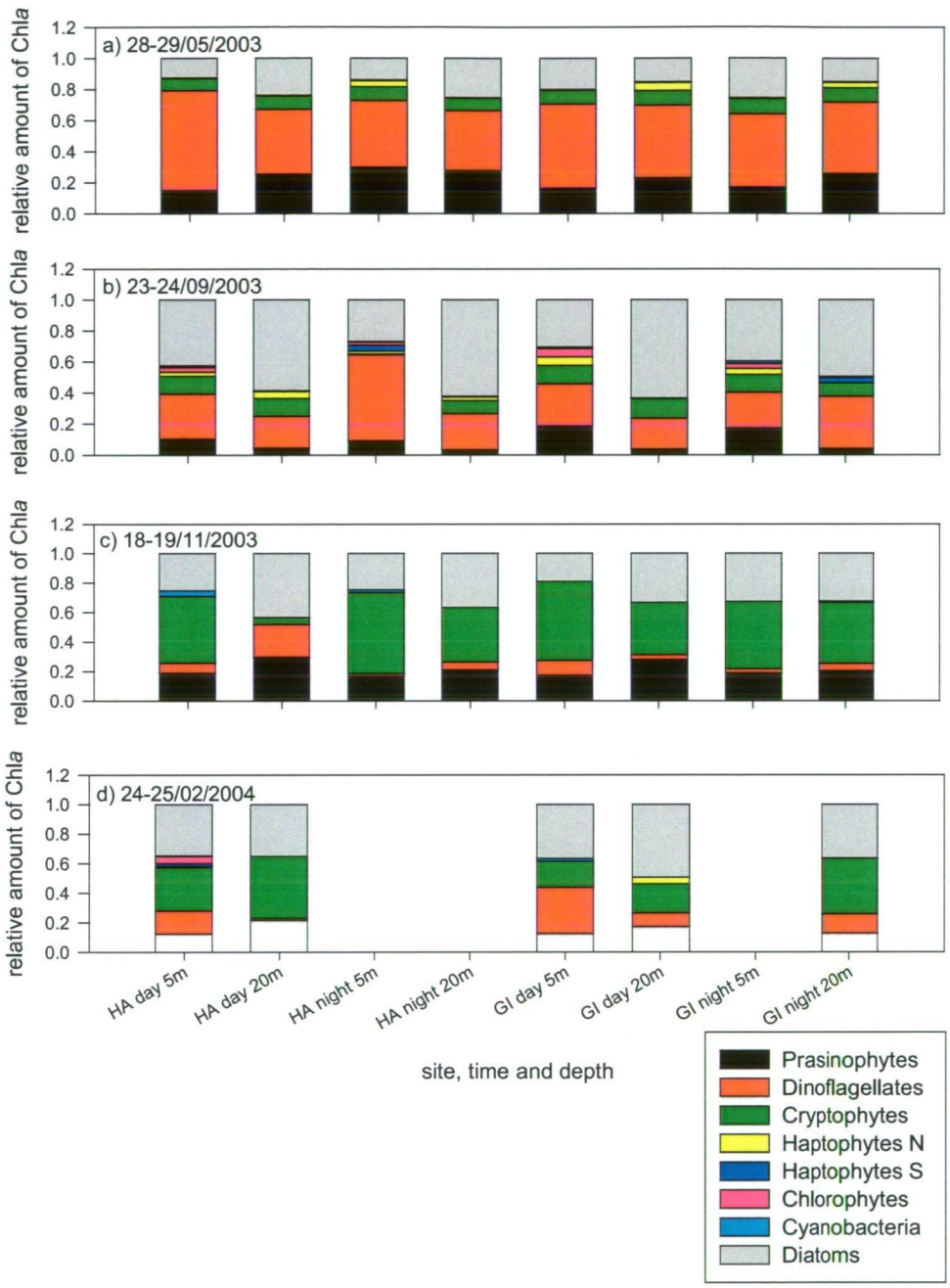

Figure 2.8 Phytoplankton composition derived from pigments from HPLC analysis of samples and subsequent analysis using Chemtax V1.1. Hideaway Bay and Garden Island in the Huon estuary during late autumn (28-29/05/2003), early spring (23-24/09/2003), late spring (18-19/11/2003) and late summer (24-25/02/2004are presented.

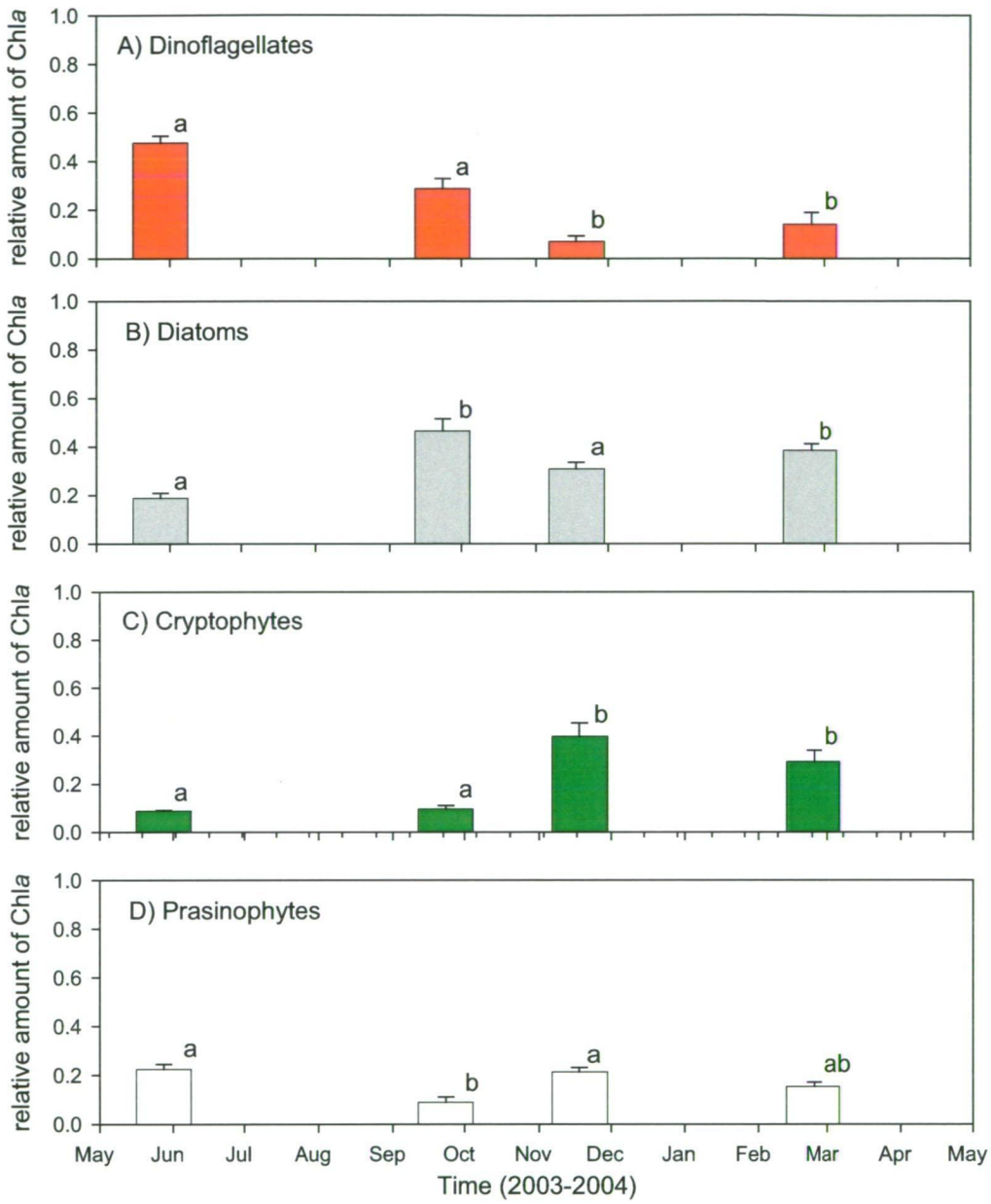

Figure 2.9 Mean phytoplankton abundance (relative amount of chl *a*) on four field trips A) dinoflagellate, B) diatom, C) cryptophyte and D) prasinophyte (across site, depth, time) phytoplankton in the Huon Estuary. Standard errors are displayed (n=4).

Table 2.2 Phytoplankton composition by cell count of field trips undertaken in the Huon Estuary. Cell counts for the first trip 28-29/05/2003 were not available.

TAXON	Huon 23/09/2003 (cells l^{-1})	Huon 18/11/2003 (cells l^{-1})	Huon 24/02/2004 (cells l^{-1})
Amphora	54		
Cerataulina pelagica		682	519
Coscinodiscus sp	21	319	871
Lauderia annulata	86	82	
Melosira	352		
Nitzschia closterium	793	2385	49499
Nitzschia sp	91		
Pleurosigma	18	171	
Pseudonitzschia pseudodelicatissima	1248	2136	19234
Guinardia striata = Rhizosolenia stolterfothii		569	56
Skeletonema costatum	521	1602	**938150**
Eucampia		360	
Grammatophora	311		
Paralia			280
Guinardia delicatula = Rhizosolenia delicatula	227	936	
Corethron criophilum	77		
Chaetoceros spp > 10um	401	1988	6630
Chaetoceros spp < 10um		1681	29695
Leptocylindrus danicus	91	341	104
Guinardia flaccida	9	**41223**	61
Dactyliosolen fragillissimus = Rhizosolenia fragillissima		4607	1608
Rhizosolenia fallax	58	557	787
Gymnodinioid dinoflagellate 15um	273		
Prorocentrum			15
Ceratium spp	27	1024	226
Dissodinium (Pyrocystis) lunula		13	
Dinophysis spp	36	228	344
Gymnodinium catenatum	132	33	2657
Mesodinium rubrum	**1450**		
flagellates 5-10 um round		863	1355

2.3.6 Comparison of Nitrate, Ammonium and Urea uptake: all Huon Estuary data.

In terms of understanding the nutrient dynamics of the Huon Estuary the comparison of nitrate, ammonium and urea uptake is the most basic, and potentially the most useful, for comparison with other studies. Paired t tests showed there was no

significant difference between sites for NH_4^+ (P=0.612), NO_3^- (P=0.565), urea (P=0.194) and combined N (NH_4^++NO_3^- +urea)(P=0.327). This enabled pooling of data across sites. For each measurement of NO_3^- uptake there was a corresponding measure of NH_4^+ and urea uptake. There were significant differences between NH_4^+, NO_3^- and urea uptake rates (P=<0.001). Pair-wise comparisons between them revealed that uptake of NH_4^+ was statistically greater than the uptake of NO_3^- and urea. And urea uptake was also statistically greater than nitrate uptake.

Table 2.3 An overall comparison of the uptake of nitrate, ammonium and urea by phytoplankton in the Huon Estuary sampled during four seasons, at two locations, two times of day and two depths (n=96).

	nitrogen source	median uptake rate	mean uptake rate	std. dev.	std. err.	% total uptake [c]
specific uptake [a]	NH_4^+	0.082	0.133	0.145	0.015	52.0
	NO_3^-	0.018	0.027	0.028	0.003	10.5
	Urea	0.042	0.096	0.126	0.013	37.5
uptake [b]	NH_4^+	0.031	0.090	0.13	0.014	50.3
	NO_3^-	0.009	0.019	0.03	0.003	10.6
	Urea	0.015	0.071	0.133	0.014	39.7

[a] ($\mu g\ N \cdot \mu g\ chl a^{-1} \cdot h^{-1}$)
[b] ($\mu g\ N \cdot L^{-1}\ hour^{-1}$)
[c] total uptake = $NH_4^+ + NO_3^- +$ Urea

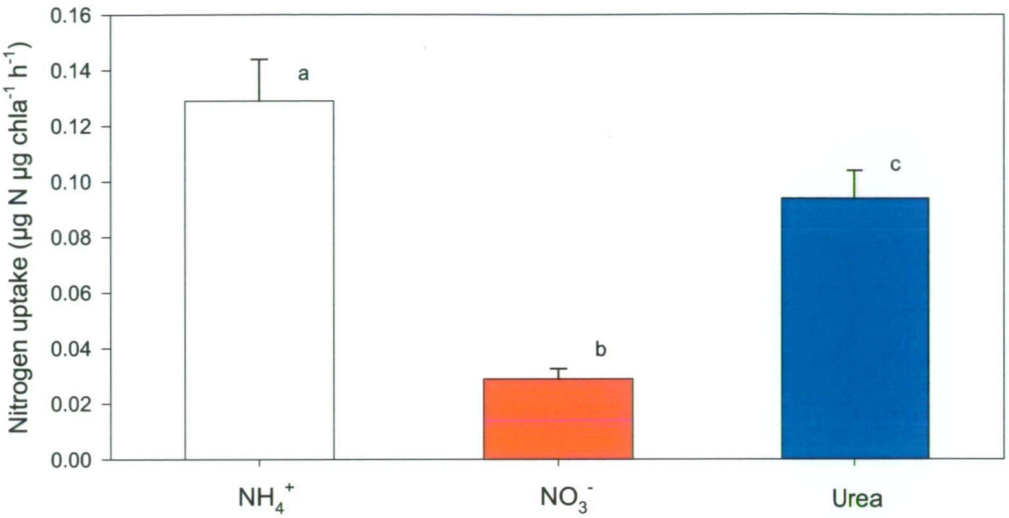

Figure 2.10 Mean specific uptake rates for ammonium, nitrate and urea averaged across all depths, times, and seasons). Standard error are displayed (n=96) and where there are different subscripts on the bars there were significant differences.

2.3.6.1 Effect of season on combined nitrogen uptake and specific uptake of different nitrogen species

The difference in combined N (NH_4^++NO_3^- + urea) uptake between the four field trips was examined (Figure 2.11). Combined N uptake was at its lowest (0.010 µg N l^{-1} h^{-1}) on the late autumn field trip (28-29/05/2003). The early and late spring field trips (23-24/09/2003 and 18-19/11/2003) were both increased (0.201 and 0.282 µg N l^{-1} h^{-1}, respectively) compared to the late autumn field trip (28-29/05/2003). However Combined N uptake was at its greatest (0.820 µg N l^{-1} h^{-1}) in late summer (24-25/02/2004).

When combined N (NH_4^++NO_3^- + urea) uptake was standardised to chl *a*, there was a slightly different pattern (Figure 2.12). Combined N uptake was lowest in late autumn and late spring. Combined N uptake in early spring and late summer were both significantly greater than in late autumn, however late spring was not significantly different to early spring. Only late summer combined N uptake was significantly greater than late spring.

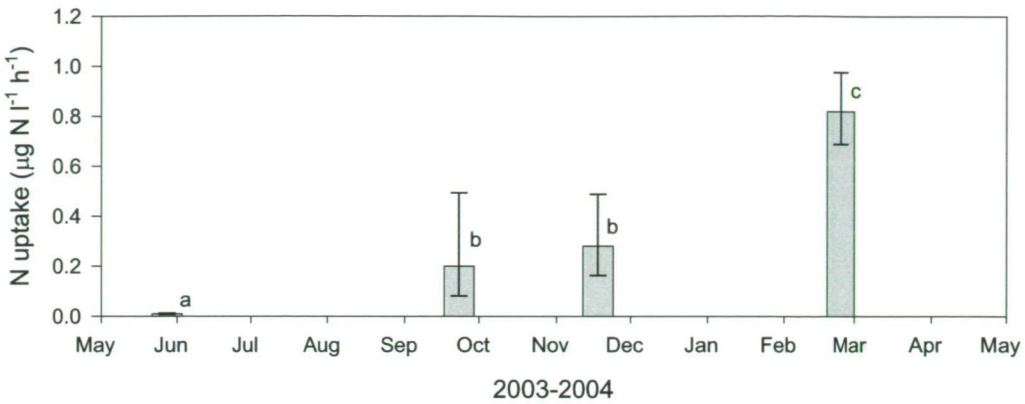

Figure 2.11 Mean combined nitrogen uptake (NH_4^++NO_3^-+urea pooled across both sites) in the surface (5m) and during the day for the four field trips in the Huon Estuary. 95% confidence intervals (n=6) are displayed and significant differences between field trips are indicated where subscripts on bars are different.

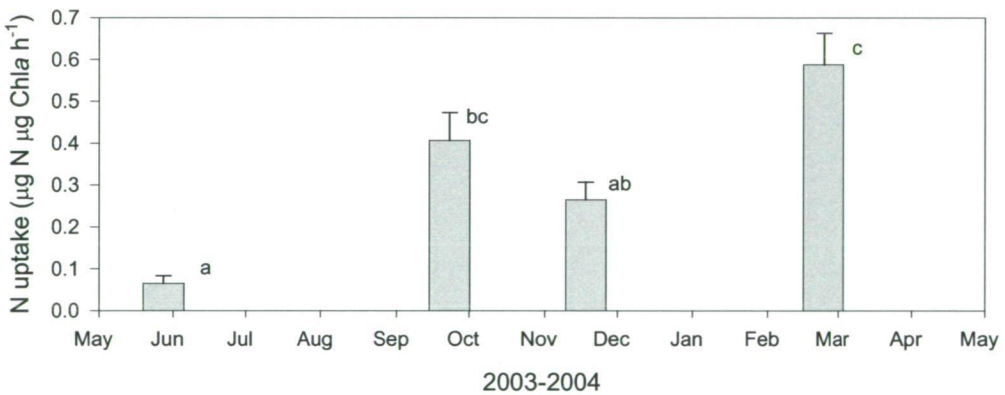

Figure 2.12 Mean specific combined nitrogen uptake (NH_4^++NO_3^-+urea pooled across both sites) in the surface (5m) and during the day for the four field trips in the Huon Estuary. Standard error bars (n=6) are displayed and significant differences between field trips are indicated where subscripts on bars are different.

The pattern of specific N uptake between NH_4^+, NO_3^- and urea was unique for each of the fieldtrips (Figure 2.13). On the late autumn field trip (28-29/05/2003) NH_4^+ uptake (0.040 µg N µg chl *a* h^{-1}) was ten times greater than NO_3^- uptake (0.003 µg N µg chl *a* h^{-1}), but urea uptake (0.017 µg N µg chl *a* h^{-1}) was not different from either NH_4^+ or NO_3^- uptake. The early spring field trip (23-24/09/2003) showed a similar

pattern in N uptake to late autumn. However uptake of all the N species were significantly different from each other and from greatest to lowest, they were: NH_4^+ (0.248 μg N μg chl *a* h^{-1}), urea (0.087 μg N μg chl *a* h^{-1}) and NO_3^- (0.046 μg N μg chl*a* h^{-1}). In late spring (18-19/11/2003) NH_4^+ uptake (0.186 μg N μg chl *a* h^{-1}) was significantly greater than both NO_3^- (0.028 μg N μg chl *a* h^{-1}) and urea uptake (0.036 μg N μg chl*a* h^{-1}). In late summer (24-25/02/2004) uptake of NH_4^+ (0.238 μg N μg chl *a* h^{-1}) and urea (0.242 μg N μg chl *a* h^{-1}) are both greater than NO_3^- (0.066 μg N μg chl *a* h^{-1}) uptake.

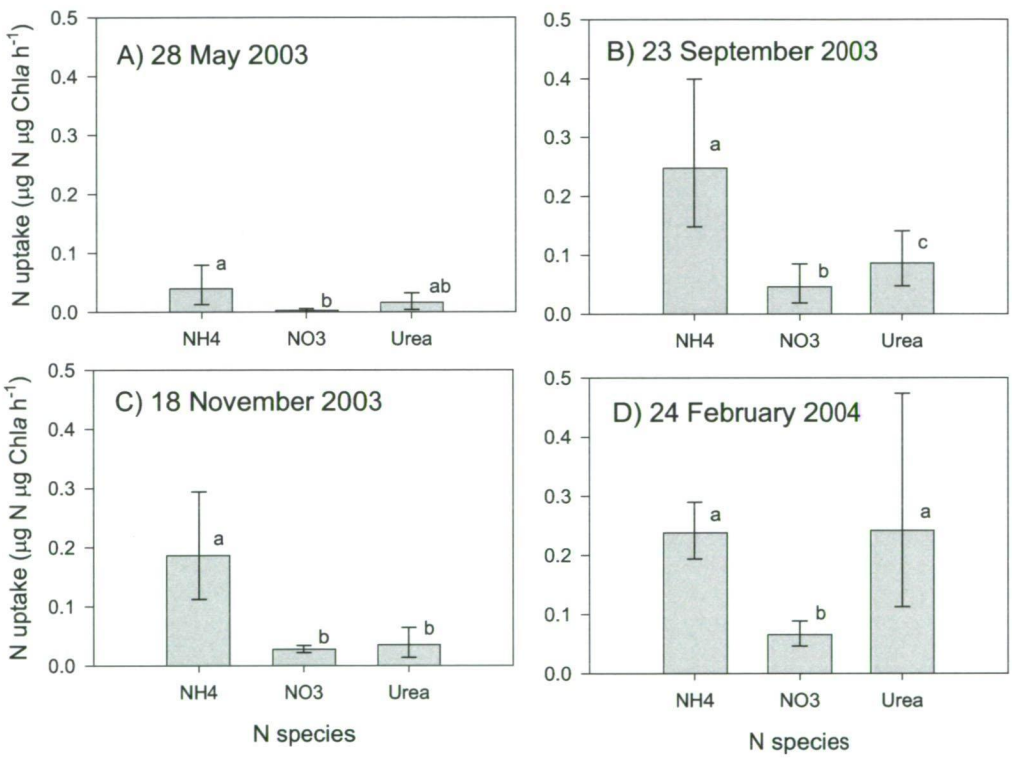

Figure 2.13 Uptake of NH_4^+, NO_3^- and urea in the surface (5m) during the day in A) 28 May 2003, B) 23 September 2003, C) 18 November 2003, D) 24 February 2004. 95 % confidence intervals (n=6) are displayed and different subscripts on bars indicate where there are Significant differences.

2.3.6.2 Effect of Season, time of day and depth on ammonium uptake

Main Factors: Season, time of day and depth were all significant for NH_4^+ uptake (Table 2.4). However, an unambiguous interpretation of the main effects for NH_4^+,

was not possible because there was an interaction between season, time of day and depth, such that the effect on one factor is not consistent at all combinations of the other factors.

There was an interaction between season and depth for NH_4^+, but it was further complicated by the fact that the interaction between season and depth in the surface (5m) is dependent on the level of time of day (Figure 2.14 A and B). NH_4^+ uptake during the day at surface (5m) was lowest in late autumn, 0.040 μgN μg chl a^{-1} h^{-1}. NH_4^+ uptake during the day at surface (5m) increased by 4 times or more in early spring, late spring and late summer, 0.248, 0.186 and 0.238 μgN μg chla^{-1} h^{-1}, respectively. In late spring at the surface (5m) NH_4^+ uptake decreased by ~4 times from 0.186 during the day to 0.044 μgN μg chl a^{-1} h^{-1}, during the night. At the surface (5m) during late autumn, early spring and late summer there was no effect of time of day on uptake of NH_4^+.At depth (20m) the interaction between season and depth is not dependant on the time of day (Figure 2.15). NH_4^+ uptake at bottom (20m) during late autumn and late spring respectively 0.012 and 0.020 μgN μgchla^{-1} h^{-1}, was between 5-10 times lower than in early spring or late summer respectively, 0.103 and 0.114 μgN μg chl a^{-1} h^{-1}. There were no significant differences in NH_4^+ uptake when time of day was evaluated at depth (20m) when the four field trips were averaged (Figure 2.16).

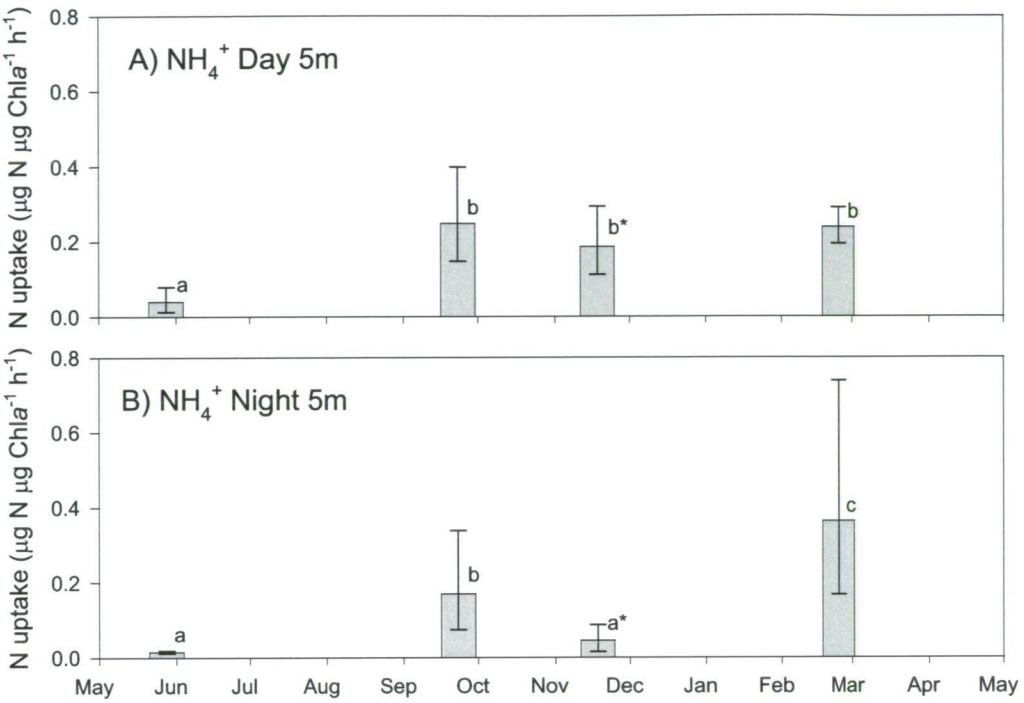

Figure 2.14 Mean NH_4^+ uptake from four field trips A) Day at surface (5m) and B) Night at surface (5m). 95% Confidence intervals (n=6) are displayed and different subscripts on bars indicate where there are significant differences between fieldtrips. Where there is an * on bars from the same field trip in both A) and B) subfigures this indicates a significant difference between them.

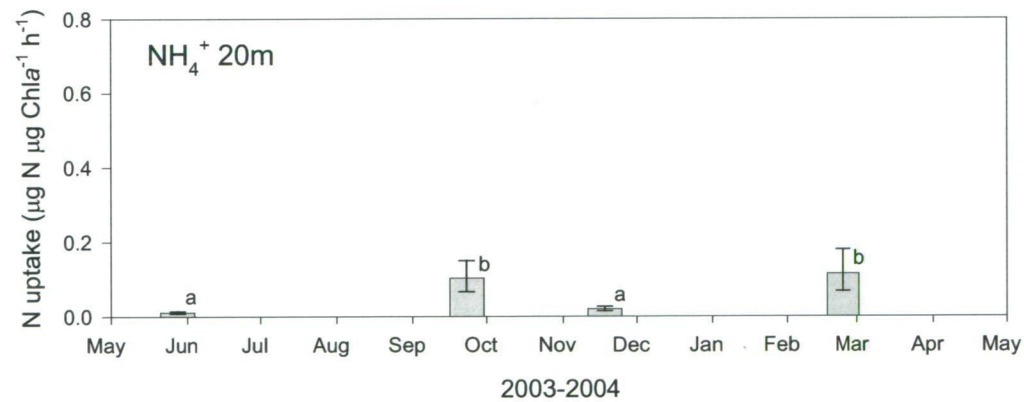

Figure 2.15 Mean NH_4^+ uptake from four field trips at depth (20m). 95% Confidence intervals (n=12) are displayed and different subscripts on bars indicate where there are significant differences.

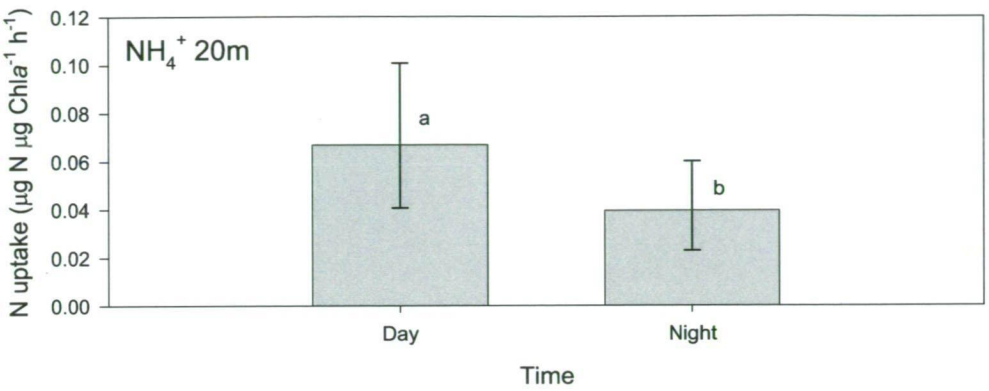

Figure 2.16 Mean NH_4^+ uptake during the day and night at depth (20m). 95% Confidence intervals (n=24) are displayed and different subscripts on bars indicate where there are significant differences.

2.3.6.3 Effect of Season, time of day and depth on nitrate uptake

The effects of the main factors, season and depth, had significant effects upon NO_3^- uptake (Table 2.4). The effect of season on NO_3^- was dependant on the depth. The time of day (day or night) did not have a significant effect on uptake of NO_3^-. There was no effect of time of day on uptake of NO_3^- during any of the field trips. There was an interaction between season and depth for NO_3^-. In the surface (5m) uptake of NO_3^- was lowest in late autumn, 0.002 µg N µg chl a^{-1} h^{-1}, when compared to all the other field trips (Figure 2.17). In the surface (5m) NO_3^- uptake was greater in both early spring and late summer, 0.046 and 0.053 µg N µg chla^{-1} h^{-1} in late autumn or late spring. The NO_3^- uptake in late spring in the surface 0.022 µg N µg chl a^{-1} h^{-1} was approximately 10 times greater than in late autumn but less than half of the NO_3^- uptake rates for early spring and late summer in the surface (5m). At depth (20m) NO_3^- uptake was low in late autumn, late spring and late summer, 0.0038, 0.0065 and 0.01 µg N µg chl a^{-1} h^{-1}, but during early spring there was an increase in NO_3^- uptake by ~5 times. In late spring at bottom (20m), NO_3^- uptake was 3.3 times lower than late spring in the surface (5m). In late summer at bottom (20m) NO_3^- uptake was 5.5 times lower than late summer in the surface (5m).

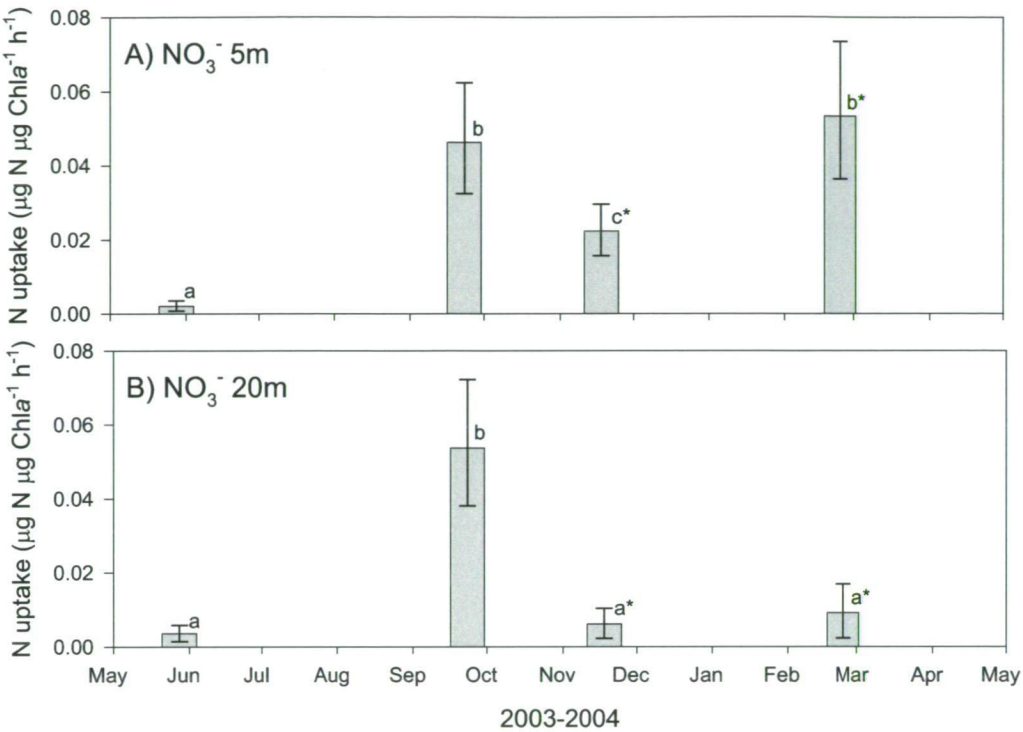

Figure 2.17 Mean NO_3^- uptake from four field trips A) surface (5m) and B) depth (20m). 95% Confidence intervals (n=12) are displayed and different subscripts on bars indicate where there are significant differences between fieldtrips. Where there is an * on bars from the same field trip in both A) and B) subfigures this indicates a significant difference between them.

2.3.6.4 Effect of Season, time of day and depth on urea uptake

The main factors of season, time of day and depth all had significant effect on urea uptake (Table 2.4). The effect of season on urea uptake was dependant on the depth (Figure 2.18) and time of day (Figure 2.19). There was also an interaction between time of day and depth for urea uptake (Figure 2.20).

Uptake of urea during the day was at its lowest in late autumn (0.011 µg N µg chl a^{-1} h^{-1}) and late spring (0.019 µg N µg chl a^{-1} h^{-1}). Early spring (0.075 µg N µg chl a^{-1} h^{-1}) and late summer (0.111 µg N µg chl a^{-1} h^{-1}) urea uptake during the day was at least 3 times greater than late autumn and late spring uptake. Urea uptake at night in early

spring (0.116 μg N μg chl a^{-1} h^{-1}) and late spring (0.061 μg N μg chl a^{-1} h^{-1}) was 1.5-3 times greater than uptake during the day in early spring and late spring.

Uptake of urea in the surface (5m) was least during late autumn, 0.014 μg N μg chl a^{-1} h^{-1} (Figure 2.19). Compared to late autumn urea uptake in the surface (5m) during early spring and late spring increased by almost 10 times to 0.123 and 0.087 μg N μg chl a^{-1} h^{-1} respectively. The greatest urea uptake in the surface (5m) was during late summer, 0.286 μg N μg chl a^{-1} h^{-1}. At depth (20m) Urea uptake was 10-20 times greater in early spring (0.07 μg N μg chl a^{-1} h^{-1}) and late summer (0.046 μgN μg N μg chl a^{-1} h^{-1}) than in late autumn (0.004 μg N μg chl a^{-1} h^{-1}) and late spring (0.006 μgN μg N μg chl a^{-1} h^{-1}).

Uptake of urea was ~ 2 times greater at night in the surface (5 m) (0.134 μg N μg chl a^{-1} h^{-1}) compared to the day in the surface (5m) (0.073 μg N μg chl a^{-1} h^{-1}) (Figure 2.20). During the day, urea uptake in the surface (5m) (0.073 μg N μg chl a^{-1} h^{-1}) was ~3 times greater than urea uptake in the bottom (20m) (0.025 μg N μg chl a^{-1} h^{-1}). During the night urea uptake in the surface (5m) (0.134 μg N μg chl a^{-1} h^{-1}) was 4.8 times greater than urea uptake in the bottom (20m) (0.028 μg N μg chl a^{-1} h^{-1}).

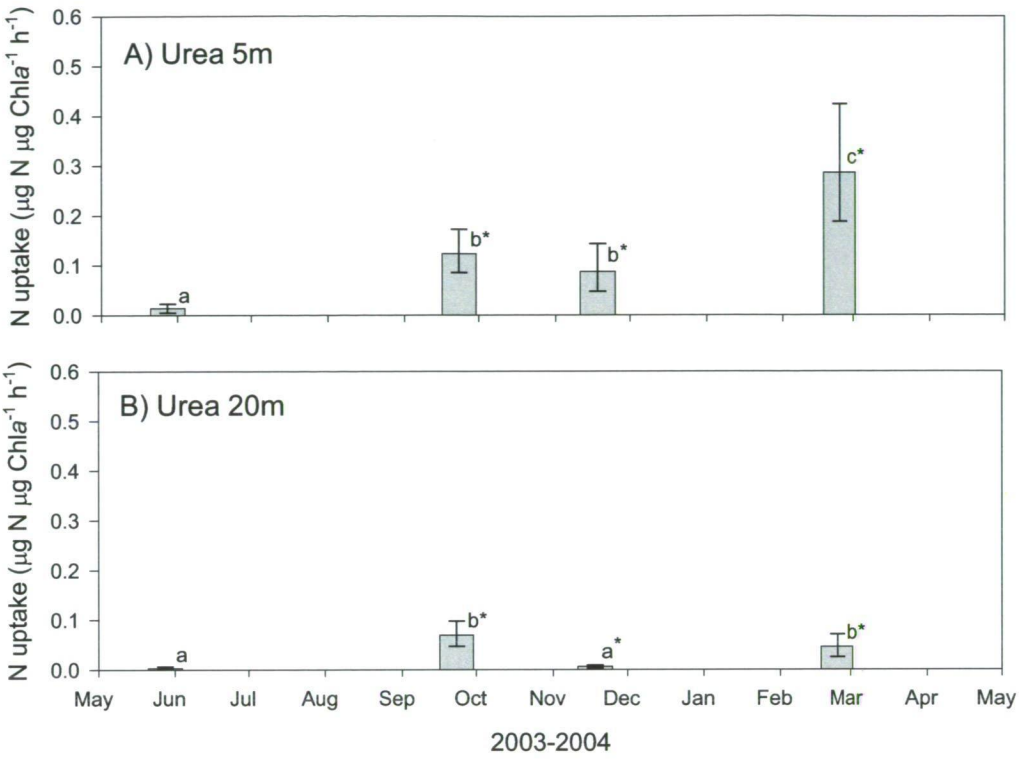

Figure 2.18 Mean Urea uptake from four field trips A) surface (5m) and B) depth (20m). 95% Confidence intervals (n=12) are displayed and different subscripts on bars indicate where there are significant differences between fieldtrips. Where there is an * on bars from the same field trip in both A) and B) subfigures this indicates a significant difference between them.

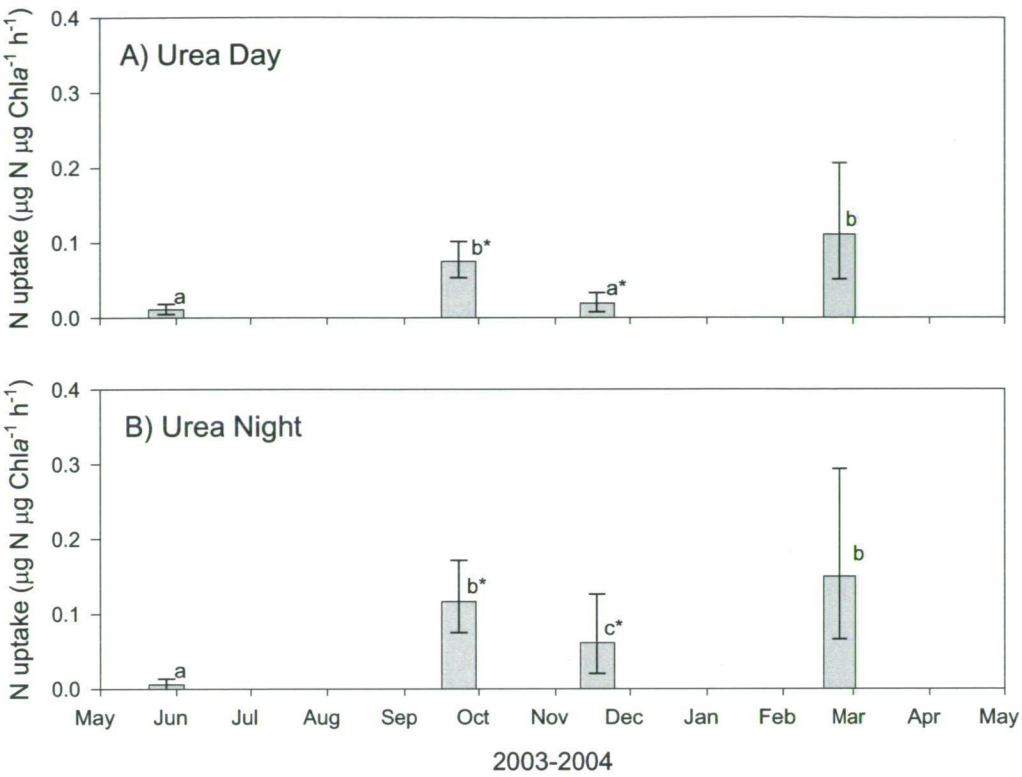

Figure 2.19 Mean Urea uptake from four field trips A) Day and B) Night. 95% Confidence intervals (n=12) are displayed and different subscripts on bars indicate where there are significant differences between fieldtrips. Where there is an * on bars from the same field trip in both A) and B) subfigures this indicates a significant difference between them.

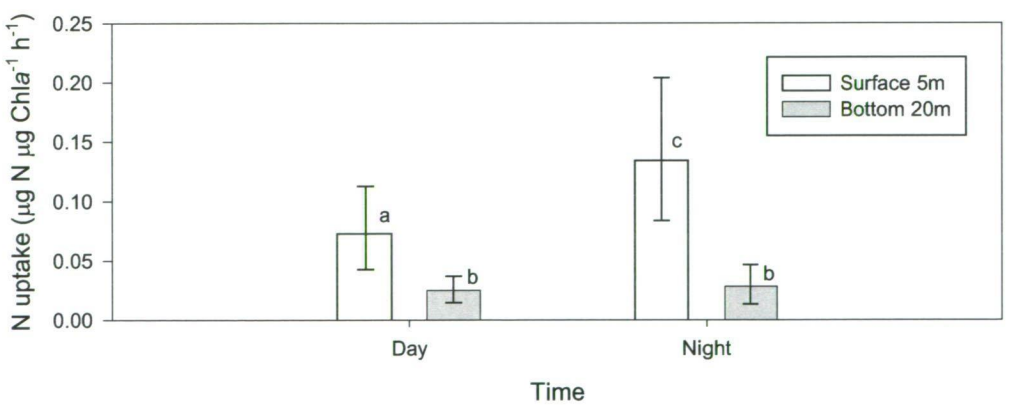

Figure 2.20 Mean Urea uptake during the day and night and at surface (5m) and depth (20m). 95% Confidence intervals (n=24) are displayed and different subscripts on bars indicate where there are significant differences.

Table 2.4 Summary of effects of season, depth, time of day and any interactions between these factors on the uptake of NH_4^+, NO_3^- and urea. See Materials and Methods for details of statistical analysis.

Factor	NH_4^+	NO_3^-	Urea
Season (S)	Significant effect P=<0.001*	Significant effect P=<0.001*	Significant effect P=<0.001*
Time of day (T)	Significant effect P=<0.001*	No effect	Significant effect. P=0.001*
Depth (D)	Significant effect P=<0.001*	Significant effect P=<0.001*	Significant effect P=<0.001*
S x T	Significant interaction P=0.039	No interaction	No interaction
S x D	Significant interaction. P=0.031	Significant interaction. P=<0.001.	Significant Interaction. P=<0.001.
D x T	No interaction	No interaction	Significant interaction. P=0.005.
S x D x T	Significant interaction. P=0.005	No interaction	No interaction

*** Main effects do not have a simple interpretation because the factor's effect depends upon the level of another factor. Need to look at interactions of this factor with other factors.**

2.3.6.5 % N uptake for NH_4^+, NO_3^- and urea

NH_4^+ made up for more than 50% of the N uptake for each of the four field trips (Table Table 2.6). During the late autumn field trip NH_4^+ was at its greatest percentage (62.7%) of the N uptake. Urea uptake was the next most important

component of N uptake, ranging from 28.5-41.6%. Whilst NO_3^- made up the smallest percentage (7.3-15.8%) of the N uptake for each of the four field trips. The percentage of N uptake made up by NO_3^- was greatest in the early and late spring field trips, 15.8 and 10.3% respectively.

Table 2.6 Percentage of N uptake (NH4+NO3+Urea) for each of the four fieldtrips (Combined Times and Depths)

Field trip	% NH_4^+	% NO_3^-	% Urea
28-29/05/2003	62.7	8.8	28.5
23-24/09/2003	52.4	15.8	31.8
18-19/11/2003	50.7	10.3	39.0
24-25/02/2004	51.1	7.3	41.6

2.4 Discussion

2.4.1 Comparison of N uptake characteristics of the Huon Estuary with other ecosystems

Nitrogen uptake rates in the Huon Estuary are at low end of the range found in many other estuaries and coastal ecosystems that have been studied (Bode *et al.*, 2005, Rosser & Thompson, 2001, Twomey et al., 2005, Veuger *et al.*, 2004, Tremblay *et al.*, 2000, Furnas, 1983, Furnas *et al.*, 1986, Bode & Dortch, 1996, Fernandez *et al.*, 1996) . The low absolute rates of nitrogen uptake reflect the generally low biomass of phytoplankton found in the Huon Estuary. Analysis of more than 1000 samples from more than 20 sites collected during 1996-1998 in the Huon Estuary gave a median chlorophyll *a* concentration of 0.60 ug L^{-1} (Team, 2000). The low median biomass indicates the Estuary is largely oligotrophic. In comparison many of the other estuaries, fjords and coastal regions that have been studied are subject to higher nitrogen concentrations and are categorized as eutrophic for example, Chesapeake Bay (Glibert *et al.*, 1991, McCarthy *et al.*, 1977), Swan-Canning Estuary (Rosser & Thompson, 2001), the Thames (Middelburg & Nieuwenhuize, 2000), Neuse River

Estuary (Boyer *et al.*, 1994, Twomey et al., 2005), and the Pearl river estuary (Yin *et al.*, 2000).

Mean specific uptake rates for the Huon Estuary were: NH_4^+ (0.133 μg N·μg chla^{-1}· h^{-1}), urea (0.096 μg N·μg chla^{-1}· h^{-1}) and NO_3^- (0.027 μg N·μg chla^{-1}· h^{-1}). Whilst these N uptake rates are low in comparison to other ecosystems as discussed above, the phytoplankton of the Huon Estuary show similarities in nitrogenous nutrition to the N uptake characteristics of phytoplankton from the majority of these other estuarine, coastal and oceanic ecosystems.

The overall composition of N uptake in the Huon Estuary for NH_4^+, NO_3^- and urea 52, 10.5 and 37.5 % respectively. Reduced forms of N (NH_4^+ and urea) made up 89.5% of the N taken up, a result consistent with overall N uptake in many estuarine, coastal and some oceanic ecosystems: NH_4^+ and urea were taken up preferentially in Chesapeake Bay (McCarthy et al., 1977), In the western English Channel Regenerated forms of N accounted for up 70% of the overall N uptake (L'Helguen *et al.*, 1993), in the southern Atlantic near Brazil, NH_4^+ and urea accounted for 74% and 96% of oceanic and coastal N uptake, respectively (Metzler *et al.*, 1997), in the Swan-Canning Estuary evidence showed that NH_4^+ was most important N source overall (Rosser & Thompson, 2001) and at Station P in the Pacific over a long time series NH_4^+ and urea were responsible for the majority of the primary production (Harrison, 2002).

The overall N uptake characteristics are useful in understanding an ecosystem and which types of N are most important for supporting primary productivity on a large scale. The magnitude and composition (NH_4^+, NO_3^- and urea) of N uptake by phytoplankton is, however, affected by physical conditions like temperature, irradiance, and substrate concentrations which change on a range of temporal and spatial scales, So to understand N uptake in the Huon Estuary the effect of depth (surface 5m or bottom 20m), time of day (day or night) and Seasons: late autumn, early spring, late spring and late summer on magnitude and the composition (NH_4^+,

NO_3^- and urea) of N uptake were examined. These result provide insight into phytoplankton strategies and dynamics of Huon Estuary phytoplankton assemblage as relevant to N uptake. Season and depth were responsible for most of the variation in NH_4^+, NO_3^- and urea uptake with less effect of time of day. For this reason the following discussion on uptake of individual N species (NH_4^+, NO_3^- and urea) focuses on the effect of season and depth. For NH_4^+ and NO_3^- season was responsible for the majority of the variation and depth was responsible for less variation but still important. For urea, however, depth was responsible for the majority of the uptake variation, followed by season and with time of day responsible for the least.

2.4.2 Late autumn

During late autumn the chlorophyll *a* concentrations at 5 and 20m were ~ 0.2 μg L^{-1}, the lowest observed. There was a slight rise in concentration to ~ 0.6 μg L^{-1} closer to the surface. Based on the presence of peridinin CHEMTAX (Mackey et al., 1996) the dominant taxa were dinoflagellates with 48% of the total chlorophyll *a*. The dominant species were *G. catenatum* and *Ceratium* spp. (Thompson et al., 2008). Late autumn dominance of the phytoplankton community by dinoflagellates is a common situation in temperate estuarine ecosystems (Marshall *et al.*, 2005, Smayda & Reynolds, 2001, Lopes *et al.*, 2007). In the Huon Estuary this high percentage of dinoflagellates probably represents a residual summer-autumn community which may be better adapted to growth at higher temperatures. The near zero growth rate of *G. catenatum* at 12°C (see Figure 3.22 Chapter 3) suggests it would be severely disadvantaged at 13.5°C relative to the growth rates of many other species at this temperature (Eppley, 1972). For example the diatom *Skeletonema costatum,* was also present in the Huon Estuary, and has been reported to grow at a rate of ~3 divisions per day at 10°C (Falkowski, 1977).

During the late autumn field trip combined N uptake was low 0.010 μg N l^{-1} h^{-1}, ~86 times less than the greatest absolute uptake from the late summer field trip. Nitrogen

uptake rates are typically low in late autumn/winter (L'Helguen *et al.*, 1996, Wilkerson *et al.*, 2006, Collos *et al.*, 2003, Wafar *et al.*, 2004) because of low irradiance and/or low temperature which limit phytoplankton growth. Specific uptake of NH_4^+, NO_3^- and urea made up for 62.7, 8.8 and 28.5% of the total specific N uptake respectively. NH_4^+ and urea made up 91.2 % of the total N uptake in autumn. That NO_3^- contributes only 8.8% of total N uptake in the Huon in late autumn is likely because the residual summer-autumn phytoplankton community is not well adapted to using NO_3^- (late autumn ambient NO_3^- ~4uM). The distribution of uptake between N forms in the Gulf of St Lawrence during autumn was very similar to that seen in the Huon Estuary, with NH_4^+ (62%) and urea (28%) being preferred while NO_3^- only made up 10% of the total N uptake (Tremblay et al., 2000). In contrast, however, to the Huon Estuary the low nitrate uptake during autumn in the Gulf of St Lawrence may be a result of the low ambient NO_3^- concentrations (<1 μM at 3 of 4 stations). In the Western English Channel during autumn there were some differences in N composition compared the Huon Estuary. NH_4^+ accounted for only 45% of the total N uptake and NO_3^- and urea almost equal amounts 23 and 21% respectively and NO_2^- made up the remaining 10% (Wafar et al., 2004). The relatively high ambient NO_3^- concentrations (4-5 μM) coupled with a phytoplankton assemblage adapted to exploit are responsible for the increase in NO_3^- uptake but clearly NH_4^+ and urea were still the preferred N sources in autumn as they were being taken up at higher rates than NO_3^- while ambient NH_4^+ concentrations (0.1 μM) are much lower in comparison to NO_3^-.

2.4.3 Early spring

Chl*a* increased from late autumn to early spring by ~1.3 and ~1.5 times in the surface and bottom respectively. CHEMTAX showed a significant shift in phytoplankton composition towards diatoms based on the increase in the pigment fucoxanthin. The dominant diatoms by abundance were *Pseudonitzschia pseudodelicatissima*, *Nitzschia closterium* and *Skeletonema costatum.* There were also low numbers of

Gymnodinium catenatum and a small *Gymnodiniod* dinoflagellate. A shift to diatom dominance is typical of spring phytoplankton assemblages in temperate locations (Smayda, 1980)

Uptake of NH_4^+, NO_3^- and urea increased by ~20 times from late autumn to early spring. This increase in N uptake coincided with the lowest temperatures of all the field trips (12°C) and a 4 -5 times increase in irradiance when compared with irradiance from the late autumn fieldtrip. Both NH_4^+ (51.4%) and urea (31.8%) were still the largest component of the total n uptake, But the greatest NO_3^- uptake rates were measured during this early spring field trip (16.9 %) in comparison to the other field trips. Peak NO_3^- uptake rates are associated with a diatom dominated spring bloom in other temperature marine ecosystems e.g., the Gulf of St Lawrence (Tremblay et al., 2000), Menai Straight (Rodrigues & Williams, 2002) and San Fransisco Bay (Wilkerson et al., 2006). The low temperatures and high irradiance typical of spring conditions in temperate locations can create a situation where phytoplankton cells capture more light energy than they are capable of converting to growth. It has been hypothesized that one component of the diatoms' ability to exploit these spring conditions could be an ability to take up NO_3^- and reduce it using excess photosynthetic energy, then releasing the more reduced N forms (NO_2-, NH_4^+ and DON), thus using up potentially harmful excess energy(Lomas & Gilbert, 1999).

2.4.4 Late Spring

Chlorophyll *a* increased from early spring to late spring, by ~3 and ~8 times in the surface and bottom depths respectively. The large increase in chl *a* at the bottom to a concentration ~ 2 times the surface chl *a* suggests the decline and subsequent sinking of the spring bloom as observed in other ecosystems with strong diatom communities (Waite *et al.*, 1992a, Waite *et al.*, 1992b). The absolute and specific N uptake rates were not significantly different relative to early spring, however, the late spring absolute uptake rate increased by ~1.4 times from the early spring. This increase was associated with a large increase in phytoplankton biomass. In addition the specific N

uptake rate decreased by ~1.5 times from the early spring suggesting that the phytoplankton assemblage may be N limited (Smith, 1982, Howarth *et al.*, 1988). Simultaneously ambient NO_3^- concentrations and N:P ratios all indicate the likelihood of N limitation in late spring (Figure 2.21). The molar N:P during late spring was 2.3, or about 2 times less than in late autumn and early spring and well below the Redfield ratio for N:P of 16 (Hecky & Kilham, 1988). The ambient concentrations of NO_3^- fell from ~4 μM in early spring to 0.21 μM in late spring at the surface. These concentrations are well below 1 μM, the half saturation constant for uptake NO_3^- by coastal phytoplankton assemblages (Dortch, 1990, Eppley *et al.*, 1969). It appears likely that it is N limiting growth and indirectly responsible for the sinking of the spring bloom as was the case in the sub arctic study (Waite et al., 1992a).

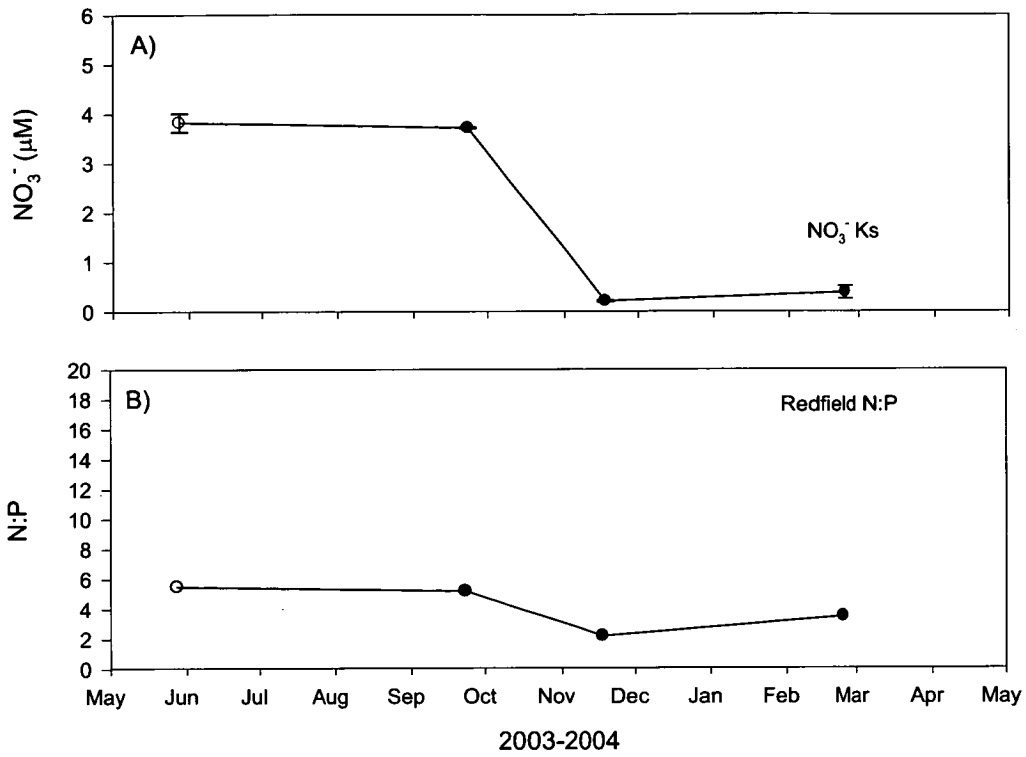

Figure 2.21 Change in surface (5m) A) NO_3^- (µM) and B) N:P on the four field trips in the Huon Estuary. Reference lines are included for half Saturation constant (Ks) for NO_3^- uptake by coastal phytoplankton (Eppley et al., 1969) and Redfield ratio for N:P (Redfield, 1958)

2.4.5 Late Summer

In contrast with late spring where phytoplankton biomass was greatest at the bottom (20m) due to the decline and sinking of the spring bloom, during late summer the greatest chl *a* concentration of all field trips, 1.2 µg l^{-1} was at the surface (5m). At the bottom (20m) chl *a* had fallen to a concentration, 0.25 µg l^{-1} comparable to the low concentrations in the bottom (20m) during late autumn and early spring field trips.. In late summer the water temperature was at 15.5 °C, the highest temperature of all the fieldtrips and irradiance in the surface (5m) was 267 µmol photons m^{-2} s^{-1} relatively high in comparison to the irradiance from the late autumn fieldtrip at the same depth. These favourable conditions for phytoplankton growth coupled with a

stratified water column result in a mixed phytoplankton assemblage. The most dominant phytoplankton groups represented in this phytoplankton assemblage according to CHEMTAX analysis of pigments, were in order of dominance: diatoms, cryptophytes, dinoflagellates. The most abundant diatoms by abundance were *Pseudonitzschia pseudodelicatissima*, *Nitzschia closterium* and *Skeletonema costatum*. The dinoflagellates were dominated by *Gymnodinium catenatum* but *Dinophysis* spp and *Ceratium* spp. were also present in reasonable abundance. The CHEMTAX identification of cryptophytes during late summer associated with relatively high concentration of alloxanthin is hypothesized to represent mostly pigmented *Dinophysis* species. Their chloroplasts that originate from cryptophytes via the intermediate host of *Myrionecta rubra* (=*Mesodinium rubrum)(Nagai et al., 2008, Park et al., 2006)*. There has not yet been any ecological studies of this complex multi trophic level interaction since the recent discovery that it was required for alloxanthin to be present in *Dinophysis* (Nagai et al., 2008, Park et al., 2006). A mixed phytoplankton assemblage, including diatoms and dinoflagellates in summer is characteristic of the Huon Estuary (Team, 2000) and other temperate estuaries and coastal areas including Chesapeake Bay (Adolf *et al.*, 2006, Marshall et al., 2005)

The specific N uptake in late summer was 9 times greater than during late autumn but was not significantly different to the specific uptake rates measured in early spring. Whilst the magnitudes of specific N uptake during late summer and early spring were not significantly different, there were notable differences in the importance of the various N substrates. Ammonium uptake was equally important during early spring and late summer being 52 and 51 % of total N uptake, respectively. The differences were in NO_3^- uptake which peaked in early spring at 16% but was only half that in late summer. During late spring and late summer ambient NO_3^- concentrations of ~ 0.2 μM were well below the half saturation constant for coastal phytoplankton limit the capability of phytoplankton to take up nitrate at the surface. In contrast urea reached its greatest concentration in late summer and made up the greatest proportion of N uptake for the late summer fieldtrip at 42%. The importance of urea and other

forms of DON in summer has been reported in many systems including: Neuse River Estuary (Twomey et al., 2005), Chesapeake Bay (Lomas *et al.*, 2002), and the Scheldt Estuary (Andersson *et al.*, 2006).

2.4.6 Summary

Ammonium uptake was most important throughout the year. Nitrate uptake, however, played an important role in the early and late spring. The results are consistent with the hypothesis that NO_3^- may be important as a method for diatoms to get rid of excess energy in the high irradiance conditions that spring produces. Urea was important as a N source in all seasons but, particularly during late spring and late summer when NO_3^- was in low supply.

Overall the uptake of N in the Huon Estuary was dominated by NH_4^+ (52%). Considering the availability of the various forms of N the ammonium preference shown by the phytoplankton community was 9 times greater than for nitrate. These results are consistent with those from other ecosystems. The strong dependence of phytoplankton in the estuary on NH_4^+ and urea for N highlights the need to know both the input of N into the ecosystem and the rates of nutrient cycling to manage this ecosystem.

2.5 Appendix

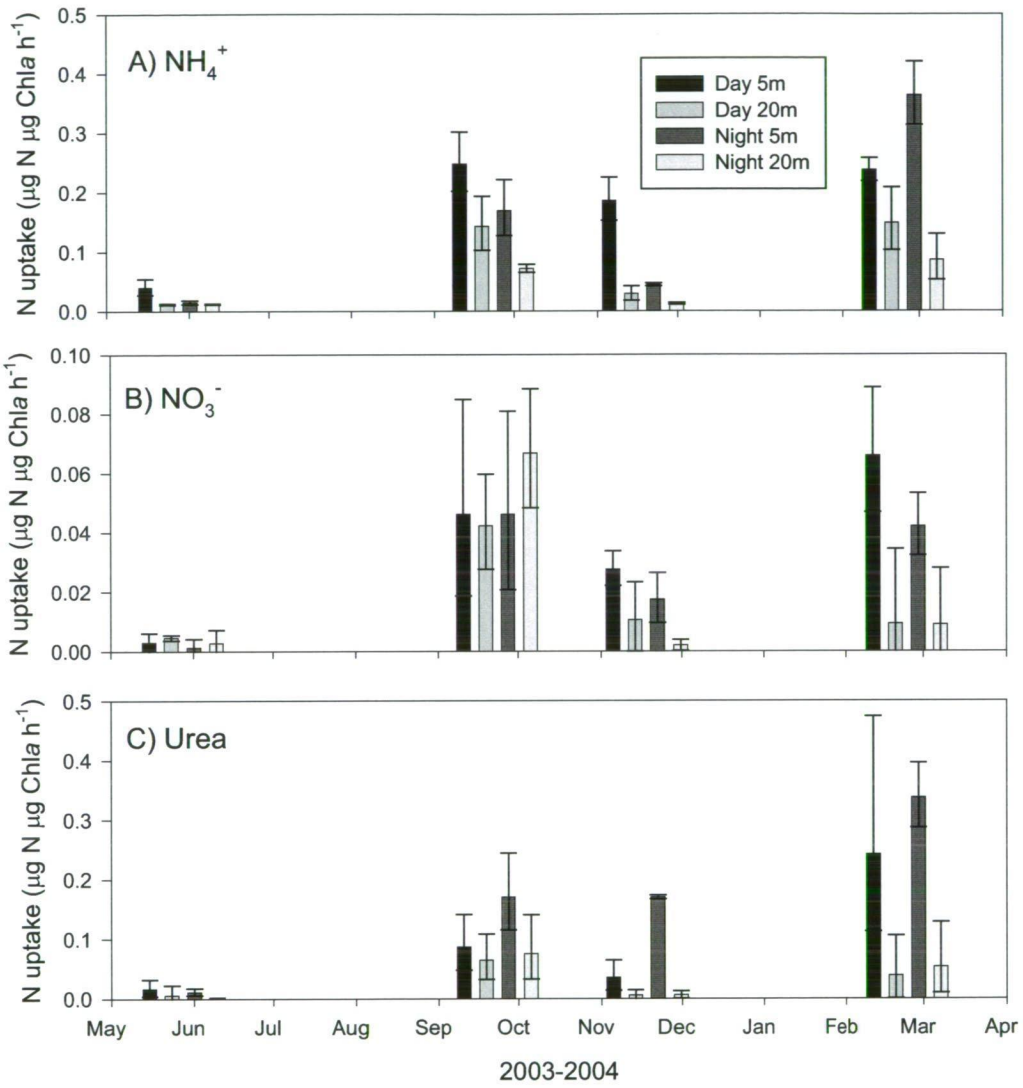

Figure 2.22 Mean uptake rates (from Hideaway Bay and Garden Island) during the day at 5m, night at 5m, day at 20m and night 20m for A) NH_4^+, B) NO_3^- and C) Urea. Error bars are 95% Confidence intervals. Note the different N uptake scale for NO_3^-.

Table 2.4 Results of ANOVA for effect of Season on absolute nitrogen uptake during the day and at surface (5m) in the Huon Estuary.

Source of Variation	DF	F	P
Season	3	79.195	<0.001
Residual	20		
Total	23		

Table 2.5 Results of ANOVA for effect of Season on specific nitrogen uptake during the day and at surface (5m) in the Huon Estuary.

Source of Variation	DF	F	P
Season	3	16.111	<0.001
Residual	20		
Total	23		

Table 2.6 Results of ANOVA for effect of Season on specific uptake of NH4, NO3 and Urea during the day and at surface (5m) in the Huon Estuary.

Source of Variation	DF	F	P
Season (S)	3	46.612	<0.001
Nitrogen Species (N)	2	55.956	<0.001
S x N	6	4.666	<0.001
Residual	60		
Total	71		

Table 2.7 Results of ANOVA for effect of Season, time of day and depth on NH_4^+ uptake in the Huon Estuary

Source of Variation	DF	F	P
Season (S)	3	62.908	<0.001
Time of day (T)	1	15.021	<0.001
Depth (D)	1	58.071	<0.001
S x T	3	2.930	0.039
S x D	3	3.099	0.031
T x D	1	0.045	0.833
S x T x D	3	4.549	0.005
Residual	80		
Total	95		

Table 2.8 Results of ANOVA for effect of season, time of day and depth on NO_3^- uptake in the Huon Estuary.

Source of Variation	DF	F	P
Season (S)	3	53.774	<0.001
Time of day (T)	1	1.472	0.229
Depth (D)	1	22.495	<0.001
S x T	3	2.515	0.064
S x D	3	15.374	<0.001
T x D	1	2.249	0.138
S x T x D	3	0.0214	0.478
Residual	80		
Total	95		

Table 2.9 Results of ANOVA for effect of season, time of day and depth on urea uptake in the Huon Estuary.

Source of Variation	DF	F	P
Season (S)	3	63.157	<0.001
Time of day (T)	1	12.492	<0.001
Depth (D)	1	113.643	<0.001
S x T	3	3.368	0.023
S x D	3	15.095	<0.001
T x D	1	8.293	0.005
S x T x D	3	2.702	0.051
Residual	80		
Total	95		

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3 EFFECT OF TEMPERATURE AND IRRADIANCE ON THE GROWTH AND BIOCHEMICAL COMPOSITION OF THE TOXIC DINOFLAGELLATE *GYMNODINIUM CATENATUM* FROM SOUTH-EAST TASMANIA, AUSTRALIA

3.1 Introduction

To increase our understanding of Huon Estuary bloom dynamics and their inter-annual variability it is necessary to characterise the physiological responses of the dominant species to different temperature and irradiance conditions. *Gymnodinium catenatum* (*G. catenatum*) has been identified as an important part of the phytoplankton community whose bloom dynamics are poorly understood in the Huon Estuary. Several studies have examined the effect of temperature on other strains of *G. Catenatum* (Bravo & Anderson, 1994, Ellegaard *et al.*, 1993, Band-Schmidt *et al.*, 2004) but these are limited in their scope and application in models as they only examine growth at one irradiance, more recently Yamamoto, *et al.*, (2002) has examined the effect of temperature on a Japanese strain and published the first growth versus irradiance curve for this species.

The effect of temperature and irradiance on growth in phytoplankton has been studied widely. The effect of temperature on growth rate of phytoplankton has been described by a variety of relationships including exponential (Eppley, 1972), Linear (Montagnes *et al.*, 2003), and even Sigmoid (Bouterfas *et al.*, 2002). In general, growth rate increases with temperature to a maximum. At temperatures greater than the temperature where growth is at its maximum there is a sharp decrease in growth

rate. Thompson (2006) has reviewed several models for the effect of growth rate on phytoplankton and concludes that "Logan's (1976) model provides the best parameterisation of the empirical relationship between temperature and micro-algal growth rate". While generalised models of growth rate as a function of temperature have been developed in the past increasingly models capable of predicting species succession and phytoplankton community composition are required to better manage our ecosystems. Hence, there is a growing need for species specific models for effect of temperature on growth rate.

The relationship between growth rate and irradiance has been described by a number of saturating curves (rectangular hyperbola). The advantages and disadvantages of more commonly used curves has been investigated by Jassby and Platt, (1976). These curves can all be used to estimate parameters such as μ_{max}, α, and E_k. Where μ_{max} is the maximum growth rate above which increases in irradiance do not increase growth rate, α is the initial slope of the light limited section of the curve and E_k is the irradiance coefficient (irradiance at the point where the initial slope intercepts the μ_{max}). Some of the models include a parameter for the compensation irradiance, E_c (irradiance where growth=respiration) and most can be easily modified to include a parameter for E_c.

The relationship between growth rate (μ_{max}) and temperature is well established and is discussed above, but the other parameters of growth versus irradiance curves (α, E_k and E_c) have also been shown to vary between classes of phytoplankton (Banse, 1976) and with temperature (Verity, 1982b, Yoder, 1979). Unfortunately there are only relatively few studies (Li & Morris, 1982, Thompson, 1999, Geider *et al.*, 1985, Verity, 1982b, Palmisano *et al.*, 1987) that report how the physiological parameters α, E_k and E_c vary in response to both light and temperature. Most of these use only a narrow, and sometimes inappropriate, range of temperatures and irradiances. These constraints limit both understandings of phytoplankton physiology and attempts to make the science more predictive.

More recently the mechanisms behind growth and physiological acclimation of phytoplankton to irradiance and temperature have begun to be elucidated. Of particular interest is the conclusion that seemingly opposite temperature and irradiance conditions result in the same responses: growth at high temperature and high irradiance is physiologically similar to growth at low temperature and a comparatively low irradiance (Maxwell *et al.*, 1994). Biochemical components like chlorophyll a (chl *a*) content (quota) also show a similar response in these seemingly opposite temperature and irradiance conditions (Wilson *et al.*, 2003). The reason for this is that the energy balance of the cell as determined by 'excitation pressure' on photosystem II (PSII) is what triggers the physiological response(s) of the cell. So at high temperatures reactions for growth are accelerated and the demand for energy by the cell is high. If the irradiance is sufficiently high to provide enough energy for the cell then chl *a* quota will be maintained at a low level. Because the rate of chemical reactions is rapidly reduced at lower temperatures, a similar response occurs at low temperature and at much lower irradiance where the cell cannot process very much light energy through the biochemical reactions of photosynthesis so that the excitation pressure on PSII is still high. Thus even at a low irradiance the chl*a* quota will be maintained at a low level. At high or low temperatures under reduced irradiance it would be expected that the chl *a* quota would be increased to increase energy captured per cell but that the absolute irradiance that stimulates chl *a* synthesis will vary with temperature. Consequently understanding phytoplankton physiology requires that the effects of temperature and light be considered together because they both play a role in determining the physiological status of the cell.

In this study the effect of temperature ranging from 12 to 28.5°C and irradiances from 5 to 458 μmoles of photons m^{-2} s^{-1} on the growth rate and biochemical composition of a Huon Estuary strain GCHU02 of *G. catenatum* were investigated, with the intention of determining:

- the growth rates of a Huon Estuary strain of *G. catenatum* under different combinations of temperature and light;

- the physiological responses of this species to variation in irradiance and temperature, and;
- the parameters necessary to model the gross growth rate of this species in the Huon Estuary.

To achieve this, growth versus irradiance curves were constructed from the growth rates of each of the twelve different temperatures examined. A comparison of these results to other strains that have been isolated from a number of other coastal areas around the world is presented and discussed. Relationships between parameters of the growth versus irradiance curves with temperature were used to construct a model for the growth response of this species to a range of temperature and irradiance conditions likely to be encountered in the Huon Estuary and other temperate ecosystems that this species currently inhabits or may inhabit in the future.

3.2 Methods

3.2.1 Strain and culture conditions

A toxic strain of *G. catenatum*, GCHU02 (Ellegaard & Oshima, 1998) was used in this experiment. The GCHU02 strain was isolated from the Huon Estuary on the 6/6/1986 by Susan Blackburn This strain is maintained at the CSIRO Collection of Living Microalgae in GSe growth media at 20 °C.

Stock cultures were maintained in 40 ml of a seawater medium (GSe) with GPM nutrients (Loeblich, 1975) and the addition of 10^{-8} M of Selenium (IV) in 50ml Erlenmeyer flasks at 18 °C, bottom illumination of 150 μmol photons m^{-2} s^{-1}(Philips deluxe cool white fluorescent tubes) and a 12:12 light:dark cycle. Irradiances were measured with a Biospherical Instruments® QSL-100 meter using a 4 π spherical sensor. Experimental cultures were grown in GPM (Loeblich, 1975) + 10^{-8} M of Selenium (IV) enriched medium prepared from seawater (collected from offshore eastern Tasmania, 42 36.00 S 148 14.00 E), treated with activated charcoal

(McLachlan, 1973), filtered (1μm, followed by 0.45 μm) autoclaved in a Teflon container, and adjusted to ~28 practical salinity units (PSU) seawater adding sterile Milli-Q® water (MQ). The nutrients were added via a sterile Millipore Sterivex™ 0.22 μm filter under the sterile conditions of a laminar flow hood (Clemco CF 435). Once sterile, all medium transfers and culture inoculations were undertaken in the sterile conditions of a laminar flow hood (Clemco CF 435).

3.2.2 Temperature and irradiance gradient incubator table

The experiment used a 45 mm thick aluminium block in which a temperature gradient could be established by heating one end and cooling the other using two different temperature water baths (Figure 3.1). The different irradiances were established at each temperature using different density computer generated black dots, laser-printed onto transparency film. The printed transparencies were placed between the fluorescent tubes and the cultures which were illuminated from underneath. At each temperature in the gradient table there were 6 different irradiances. The gradient table was designed to be used with 70 ml polycarbonate containers. A 50 ml borosilicate test tube (Pyrex™ or Kimax™) was attached to these containers via a polypropylene custom made adapter (Figure 3.2) so that the fluorescence could be easily measured by inverting the culture vessel and placing the test tube into a Turner Designs model 10 AU fluorometer (Mountainview, California, USA).

The irradiance inside an empty culture vessel was measured at each location in the gradient incubator table using a Biospherical Instruments® QSL-100 meter with a 4 π spherical sensor (California, USA). Six fluorescent tubes (Osram cool white) were used to illuminate the gradient incubator table from below.

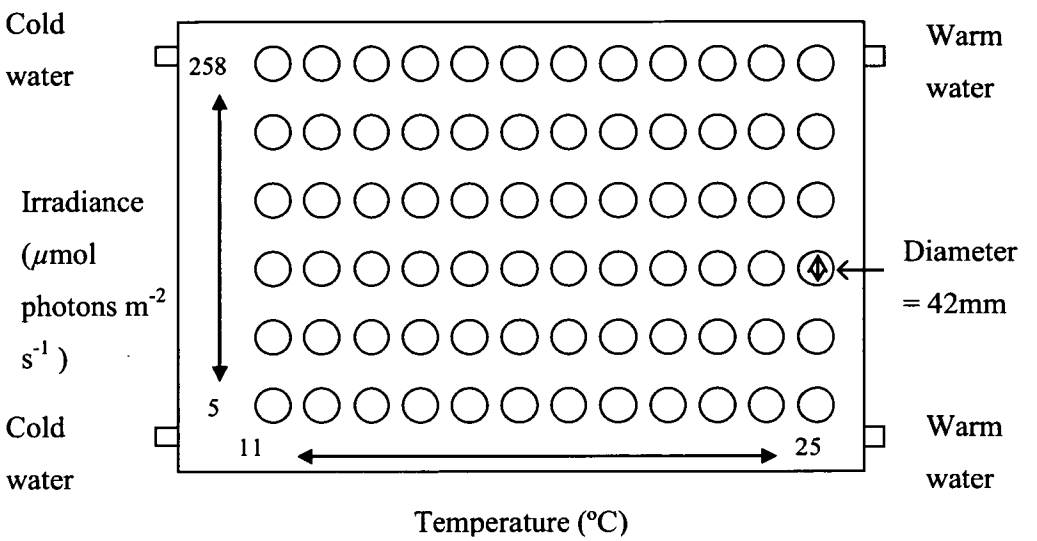

Figure 3.1 Layout of the temperature and irradiance gradient table.

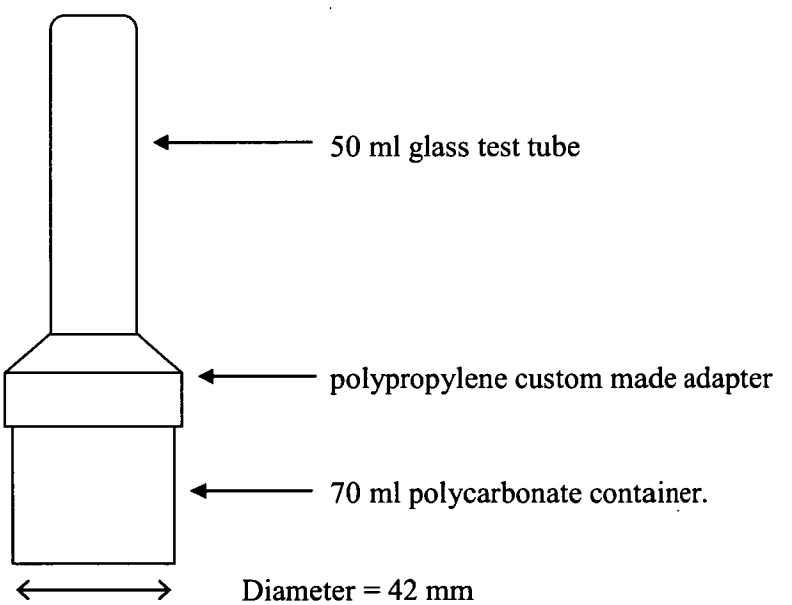

Figure 3.2 70 ml polycarbonate container with 50 ml glass test tube attached to the top via a polypropylene custom made adapter.

3.2.3 Effect of culture vessels

G. catenatum was grown in three different culture vessels to determine if the type of culture vessel affects growth rate in this species (after (Metaxas, 1989)). In particular, it was necessary to determine if the 70 ml polycarbonate container that had to be used with the temperature gradient table would have a negative effect on growth rate in comparison to other culture vessels commonly used in the laboratory. Qualitative effects of culture vessels have been observed on growth of *G. catenatum* before, however they have not been tested experimentally. Three replicate cultures were grown in 50ml Pyrex™ or Kimax™ test tubes with polypropylene tops, 70 ml polycarbonate containers with 50 ml test tube "tops" (Figure 3.2) and 250 ml Pyrex™ flasks with 50 ml test tube type side-arms and polypropylene tops. The cultures were inoculated so they would have a starting cell concentration of 1.12×10^{-5} L^{-1}. The cultures were grown at 18 °C, 130 µmol photons m^{-2} s^{-1} and a 12:12 L:D for nineteen days. Fluorescence of the cultures was measured every three to four days and initial and final cell counts were also taken so that the growth rate could be determined.

3.2.4 Preconditioning of µ versus I experimental cultures

Twelve cultures were grown at 18 °C and six irradiances ranging from 5 to 283 µmol photons m^{-2} s^{-1} for three weeks to acclimatise the cells to the irradiances that would be used in the experiment. Using these cultures as inocula, temperature acclimation was incremental as follows. Twenty-four 70 ml polycarbonate containers each containing 48 ml GSe medium were added to the centre of the temperature and irradiance gradient table (18.2,18.9, 20.7 and 21.1 °C). At each temperature in the gradient incubator table there were six different irradiances. These vessels were inoculated with 2 ml from the irradiance acclimated cultures that had the closest irradiance to that location in the gradient incubator table. After a week the cultures at 18.2 and 18.9 °C in the gradient incubator table were shifted to the next lowest

temperature and the cultures at 20.7 and 21.1 °C shifted to the next highest temperature. The shifted cultures were replaced by another twelve vessels inoculated from the appropriate irradiance acclimated cultures. Every four to five days this process was repeated until all seventy-two cultures had been added to the gradient incubator table. All the cultures were incubated at their experimental temperature for a further week prior to commencing the experiment described in section 3.2.5.

3.2.5 Temperature and irradiance experiment

From the seventy-two pre-experimental cultures, seventy-two new cultures were started in 70 ml polycarbonate containers with 50 ml test tubes attached via a custom-made adaptor (Figure 3.2). These new cultures were set up by visually assessing the density of the acclimatised individual pre-experimental cultures (see section 3.2.4). Depending on their density, 1,2,5 or 10 ml was transferred using a pipette under sterile conditions into the new 70 ml experimental polycarbonate containers, and GSe medium was added to make up a total culture volume of 50 ml. At some of the low irradiance extremes pre-experimental cultures had shown little or no growth and new cultures were inoculated from another culture at the same temperature but a higher irradiance. These seventy-two new cultures of *G. catenatum* were grown at 12 different temperatures: 11.9, 13.4, 15.5, 16.3, 18.2, 18.9, 20.7, 21.1, 22.8, 23.0, 24.9 and 25.2 °C (temperatures are those measured when lights were on)and at six different irradiances ranging from 5 to 283 µmol photons m^{-2} s^{-1} for forty-nine to seventy-two days. The temperature, irradiance and initial cell densities of the *G. catenatum* cultures in the gradient table experiment are listed in Table 3.1.The light:dark cycle used throughout this experiment was 14:10. One of the limitations of the gradient table experiment setup was that to achieve the irradiance conditions required for the experiment the fluorescent tubes providing the light had to be positioned almost immediately below the gradient table. Positioning the lights so close to the gradient table and cultures meant that when the lights were on it increased the temperature of some cultures. The cultures effected most by this

increase in temperature were those positioned in the lower temperature areas of the gradient table. When the lights were on some cultures increased in temperature by ~ 2 °C. To reduce this side effect of the lights increasing the temperature of some cultures a fan was added to dissipate the heat from under the gradient table.

Some of the cultures incubated at low irradiances across all temperature treatments did not exhibit any growth over the period of forty-nine days. For these "no growth" cultures, 40 ml was removed for chlorophyll analysis and new media added to replace the amount removed. The cultures were then shifted to the next highest irradiance every three to four days, until these 'no growth' cultures that were originally at irradiances below 25 μmol photons m^{-2} s^{-1} were at irradiances greater than 60 μmol photons m^{-2} s^{-1}. Throughout this twenty-three day period the fluorescence was measured four times.

At the conclusion of these experiments on the effects of light and temperature it was apparent that at the highest temperatures the irradiances were not saturating growth (see section 3.3.2.2). Consequently some additional experiments at the extremes of high temperatures and high irradiances were conducted (see section 3.2.6).

Table 3.1 Temperature, irradiance and initial starting cell densities for each culture of *G. catenatum* in the temperature gradient table.

Culture ID	Temperature (°C)	Irradiance (µMol photons m^{-2} s^{-1})	Initial Cells/l
A1	25.8	208	29000
A2	25.6	208	38000
A3	25.6	192	72000
A4	25.6	183	52000
A5	25.8	127	139000
A6	25.6	78	95000
A7	25.5	50	67000
A8	25.4	43	33000
A9	25	20	4000
A10	25	15	6000
A11	25	8	14000
A12	25	6	8000
B1	24	242	51000
B2	24	225	26000
B3	23.8	203	17000
B4	23.8	178	35000
B5	23.8	142	42000
B6	23.8	77	18000
B7	23.6	53	18000
B8	23.6	42	7000
B9	23.4	23	13000
B10	23.5	15	11000
B11	23	9	0
B12	23.2	8	1000
C1	22.4	238	3000
C2	22.2	233	28000
C3	22.2	197	10000
C4	22	180	15000
C5	22	125	12000
C6	22	67	14000
C7	22	50	13000
C8	22	40	13000
C9	22	27	20000
C10	21.8	16	7000
C11	21.8	8	4000
C12	21.6	6	0
D1	19.8	250	2000
D2	19.5	230	9000
D3	20.2	187	12000
D4	19.5	147	4000
D5	20.2	123	10000
D6	19.5	80	5000
D7	20.2	47	3000
D8	19.4	32	12000
D9	20	23	13000
D10	19.4	15	7000
D11	20	8	7000
D12	19	5	2000
E1	17.8	258	17000
E2	17	217	9000
E3	17.6	167	14000
E4	16.8	150	40000
E5	17.6	113	16000
E6	17	67	12000
E7	17.6	43	19000
E8	16.8	33	10000
E9	17.5	23	4000
E10	16.8	15	5000
E11	17.4	7	3000
E12	16.8	5	5000

Table 3.1 continued

Culture ID	Temperature (°C)	Irradiance (µMol photons m^{-2} s^{-1})	Initial Cells/l
F1	15	242	39000
F2	13.2	175	7000
F3	14.6	207	22000
F4	12.8	167	10000
F5	14.8	130	10000
F6	13	63	57000
F7	14.6	47	66000
F8	13.2	37	21000
F9	14.8	21	16000
F10	13.2	13	10000
F11	14.5	8	3000
F12	13	5	3000

3.2.6 Growth at high temperature and high light

3.2.6.1 Pre-acclimatisation

Two cultures were acclimatised in 50 ml Erlenmeyer flasks at 25.6 °C at two higher irradiances 291 and 458 µmol photons m^{-2} s^{-1} for eight days. The cell density of the acclimatised cultures was determined by cell counts at the end of the eight-day period.

3.2.6.2 Culture conditions

The cultures were illuminated from below with fluorescent tubes (Osram cool white). The light:dark period used was 14:10.

3.2.6.3 Growth at 28.5 °C

The 70 ml polycarbonate experimental vessels with 50 ml test tube attached via custom adapter (Figure 3.2) were filled with 50 ml of GSe medium and inoculated with 5 ml from the pre-acclimatised cultures (see section 3.2.6.1). The cultures were then grown at 291 and 458 µmol photons m^{-2} s^{-1} with the temperature increased to 28.5 °C and grown for fourteen days. Fluorescence was monitored and at the end of the experiment the cell density of the cultures was determined by cell counts.

3.2.6.4 Growth at 25.0 °C

Two experimental vessels with 50 ml GSe were inoculated with 5 ml from the pre-acclimatised cultures (see section 3.2.6.1). The new cultures were grown at 25.0 °C

and 291 and 458 μmol photons m^{-2} s^{-1} for seventeen days. In addition, another two culture vessels with 50 ml of GSe medium were inoculated with 5 ml from the experimental cultures grown at 28.5 °C (see section 3.2.6.2) and were also grown at 25 °C and 291 and 458 μmol photons m^{-2} s^{-1} for eighteen days. Fluorescence was measured every two to three days for the four cultures grown at 25 °C and growth rates were calculated according to the method outlined in section 3.2.7.

3.2.7 Growth rates

A 3 ml sample was taken from each of the seventy-two cultures and fixed with Lugol's iodine solution (Throndsen, 1978) for an initial cell count. Every three to four days throughout the experiment the fluorescence of the cultures was measured using a Turner Designs model 10 fluorometer (Mountainview, CA, USA). A strong linear relationship between cell density and fluorescence was observed under some conditions ($r^2 = 0.99$; n= 11; $P \leq 0.0001$) (Figure 3.3) although this may not have held for all treatments (see section 3.3.2.1). During fluorescence measurements the cultures were removed from the gradient table and kept insulated and in the dark until being mixed by two gentle inversions into the 50 ml test tubes. The tubes were wiped clean with a Kimwipe™ and inserted into the fluorometer. The fluorescence measurements were made at 10:00 each day. When the cultures had reached 120 fluorescence units (this was pre-determined as the fluorescence just prior to stationary phase) or the experiment was completed (whichever occurred first), 3 ml samples were taken for final cell counts. Both fluorescence measurements and cell counts were used to calculate the specific growth rate (μ) of each culture using the following equation (Guillard, 1973):

$$\mu = (1/\Delta t)\ln(N^t/N^0)$$

Equation 1

Where t is time, N^0 and N^t respectively are initial and final culture density measurements (fluorescence or cell counts).

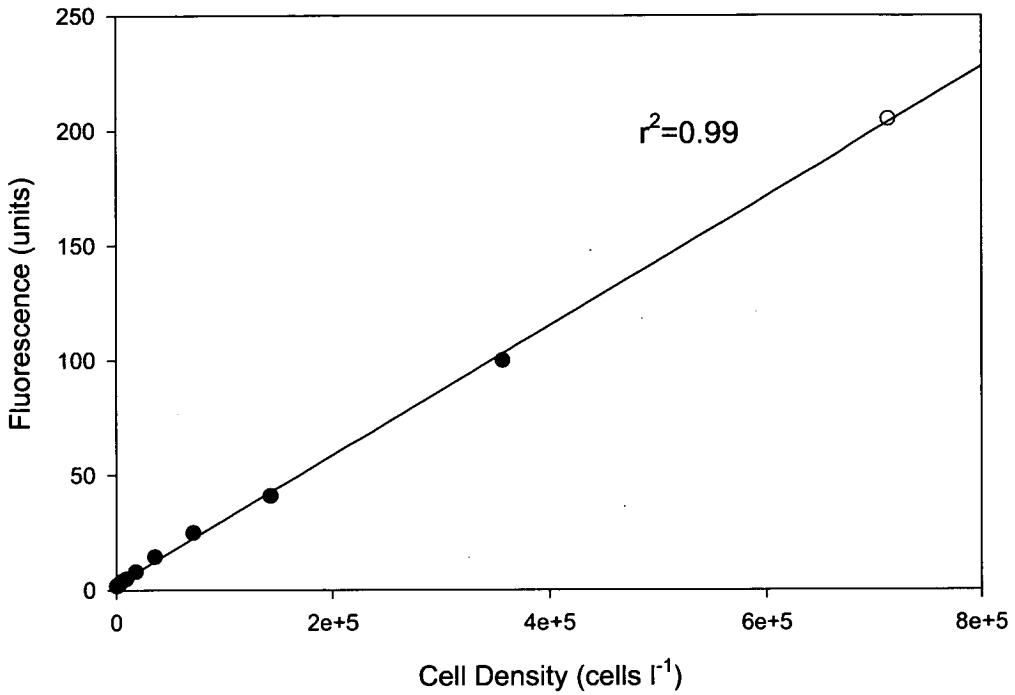

Figure 3.3 Cell density vs. fluorescence of *G. catenatum* fit with a linear model. Grown at 22.0 °C and ~300 µmol photons m^{-2} s^{-1}.

3.2.8 Growth vs. irradiance curves

To determine the physiological parameters of the growth rate versus irradiance curve for each of the temperature treatments, the data were fitted with the model Platt *et al.* (1975). This model was modified to include an x-intercept. The model is as follows:

$$\mu = \mu_{max} \tanh(\alpha E/\mu_{max}) - E_c$$

Equation 2

Where μ is growth rate at irradiance E, μ_{max} is the estimated maximum growth rate; α the initial slope of the curve and E_c the compensation irradiance. The growth rate data determined by fluorescence and cell counts were used to fit the model.

3.2.9 Biochemical analysis

When the cultures had reached 120 fluorescence units or the experiment was completed (see section 3.2.6), samples were taken for chemical analysis. For carbon and nitrogen analysis 20 ml of each culture was filtered under less than 5 mm Hg vacuum through a Pall™ A/E 13 mm glass fibre filter. These 13 mm filters were dried and analysed on a Leco™ CHN analyser. For Chlorophyll analysis 20 ml was filtered under less than 5 mm Hg vacuum through a Whatman™ GF/F filter. The filters were stored in cryogenic vials (Cryovial™) immersed in liquid nitrogen until extraction. All glassware used for extraction was cleaned in dilute Extran™ solution, rinsed three times with Milli-Q (MQ) water and once with acetone (AR grade). Frozen filters were cut into halves and placed in a clean 10 ml centrifuge tube. Three millilitres of 100% acetone was added to each tube. The tube was covered with Parafilm™ and vortexed for ≈ thirty seconds before placing the tube in an ice-water bath and sonicating (Unisonic™) the filter and acetone for fifteen minutes in the dark. The filters and acetone were then stored for at least eighteen hours at 4 °C. After this time, 0.2 ml MQ was added to each tube (to bring solvent to ≈ 90:10 acetone:water) and the filter and solvent sonicated for a further fifteen minutes in ice water and in the dark. Solvent and filter were then transferred to a Biorad™ column containing a small GF/F filter acting as a plug. The sample tubes were rinsed with 2 x 0.5 ml of acetone/water (90:10) which was quantitatively added to the Biorad™ column. Each Biorad™ column was fitted into a centrifuge tube and centrifuged for five minutes at 5000 rpm. The filtrate was stored overnight in the dark at 4 °C until just prior to analysis. Samples were analysed for chlorophyll *a* and *c*, using a GBC UV/VIS 916 spectrophotometer with 40 mm path length optical glass cells. Absorbance was read at wavelengths of 750, 664, 647 and 630 nm. The absorbance at 750 nm was subtracted from the absorbance at each of the other three wavelengths and substituted into the following equations (after (Jeffrey *et al.*, 1997)):

Chl*a* extract = $11.85A_{664}/l - 1.54A_{647}/l - 0.08A_{630}/l$

Equation 3

Chl*c* extract = $24.52A_{630}/l - 1.67A_{664}/l - 7.60A_{647}/l$

Equation 4

A = corrected absorbance at specified wavelength.

l = path length in cm.

The concentration of chlorophyll in the sample in $\mu g\ l^{-1}$ was obtained by the following equation:

Chl*x* sample = Chl*x* extract * (v/V)

Equation 5

v = volume of extract (ml).

V = volume of seawater filtered (l).

The total concentration of chlorophyll in the sample in $\mu g\ l^{-1}$ was obtained by the following equation:

Chl total = Chl*a* sample + Chl*c* sample

Equation 6

Some of the cultures at low irradiances across all temperature treatments did not exhibit any growth after forty-nine days. Consequently a decision was made to take a large volume (40 ml) for chlorophyll analysis, due to the cell density in these cultures being very low with insufficient culture available for carbon and nitrogen analysis. However a large amount of variation existed in the chl*a* values even after 40 ml was extracted for analysis from these very low density cultures. Therefore this chl*a* data was not included in any of the figures or analyses in this study.

3.2.10 *G. catenatum* energetics model

Using cellular energetics as a conceptual framework, an energetics model was developed for *G. catenatum*. The concepts and definitions used by Richardson et al. (1983) were adopted for the purposes of this study. The primary concept is that the amount of growth is determined by how much of the available light energy (potential growth) is used up by maintenance and capital energy costs. The excess energy can then be directed to growth processes:

μ = light energy – (capital costs + maintenance costs)

Equation 7

Where μ is growth, light energy is energy captured from light via photosynthesis; capital costs are energy costs associated with synthesising a new cell; and maintenance costs are associated with the survival of a cell (i.e. re-synthesis of cellular components and recovery of cellular solutes etc.).

Growth rate for *G. catenatum* was modelled by using the relationships between temperature and the parameters of the growth vs. irradiance curve, μ_{max}, α and E_c (see section 3.3.2.3, 3.3.2.4 and 3.3.2.6 respectively). These relationships were used to parameterise the modified growth vs. irradiance model of Platt *et al.* (1975). Growth rate was modelled from 11 to 35 °C, under light saturating conditions (1000 µmol photons m^{-2} s^{-1}) and light limiting conditions (30 µmol photons m^{-2} s^{-1}).

3.2.11 Statistical analysis

A one-way ANOVA was used to test if the type of culture vessel had a significant effect on growth rate. Sigmastat™ 2.03 was used for all statistical analyses and alpha was set at 0.05.

3.3 Results

3.3.1 Vessel effects on growth rate

Growth rates from *G. catenatum* cultures grown in the 70 ml polycarbonate container and the 250 ml sidearm flask were significantly greater (n= 12, P=0.019) than in the 50 ml Pyrex™ or Kimax tubes™ (Figure 3.4).

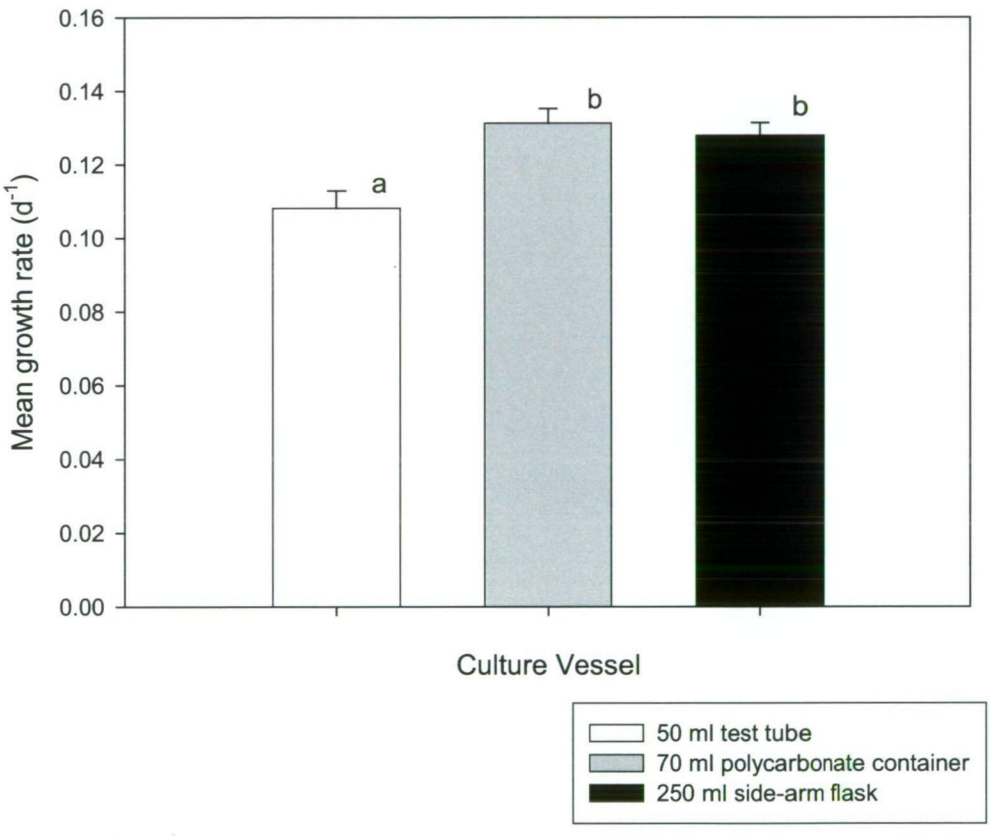

Figure 3.4 Mean growth rate and standard error (n=4) of *Gymnodinium catenatum* grown in three different culture vessels. Statistically significant differences are indicated by superscripts.

3.3.2 Effect of temperature and irradiance on growth

The *G. catenatum* culture at 20.8 °C and 67 μmol photons m^{-2} s^{-1} exhibited an eleven day lag phase before beginning to grow exponentially as indicated by the linear relationship of natural log fluorescence with time (Figure 3.5). There was a much longer lag phase of eighteen days at 16.3 °C and 23 μmol photons m^{-2} s^{-1} where the subsequent exponential increase in fluorescence was much slower. The initial lag phase was excluded from all the data for the seventy-two cultures, the data were then fit with a linear model and the growth rate determined according to the method of Guillard (1973). The examples given in Figure 3.5are representative of the seventy-two cultures for which growth was measured.

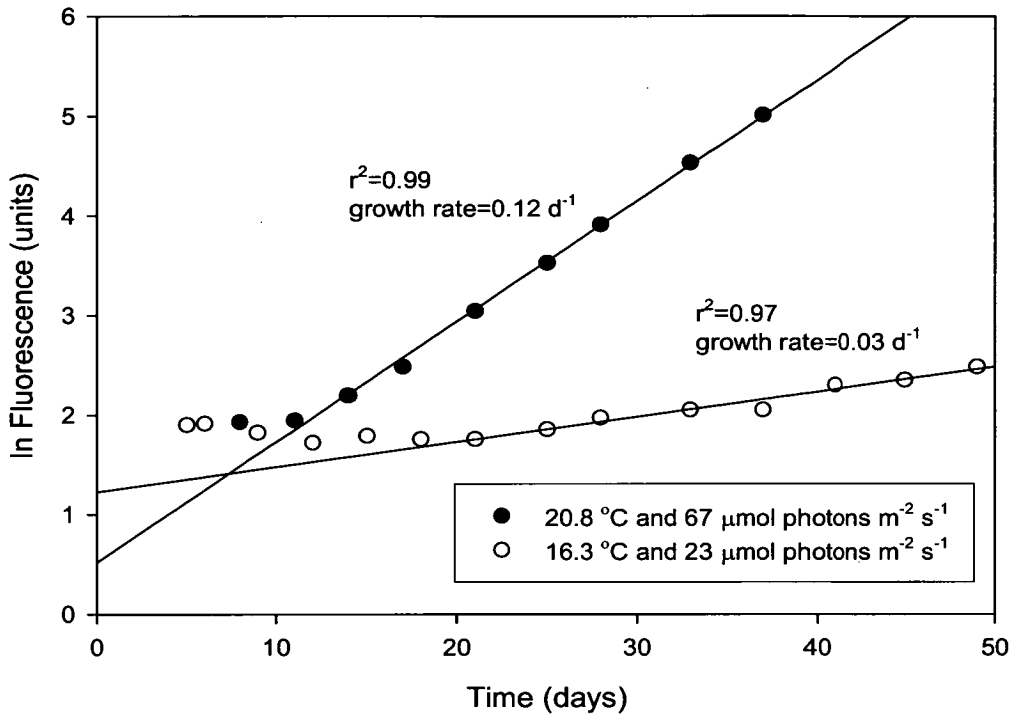

Figure 3.5 Fluorescence of two *Gymnodinium catenatum* cultures following inoculation on day zero and grown under different temperature and irradiance conditions.

3.3.2.1 Comparison of different methods for determining growth rate

The growth rates of *G. catenatum* cultures determined by in vivo fluorescence and cell counts were compared (Figure 3.6). Comparison of the growth rates from cell counts and fluorescence showed that low growth detected by cell counts was not well detected by fluorescence, for this reason it was decided that growth rates from cell counts would be used in preference to growth rates from fluorescence.

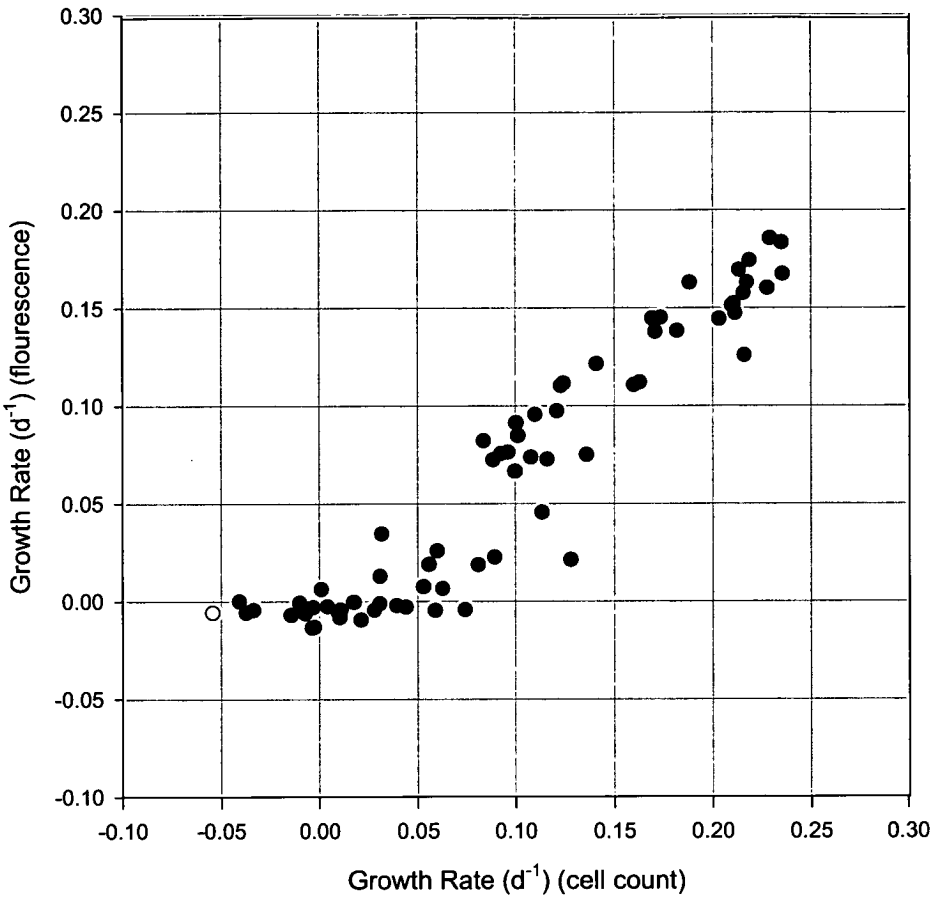

Figure 3.6 Comparison of growth rate by cell counts and growth rate by fluorescence of *Gymnodinium catenatum*.

3.3.2.2 Growth vs. irradiance curves

Growth versus irradiance curves were constructed using data from cultures grown at 11.9, 13.4, 15.5, 16.3, 18.2, 18.9, 20.7, 21.1, 22.8, 23.0, 24.9 and 25.2 °C. Within each temperature there were six different irradiances ranging from 4 to 283 µmol photons m^{-2} s^{-1}. Growth versus irradiance curves were constructed using growth rates calculated from cell counts. The modified model developed by Platt *et al.* (1975) (Equation 2) describes the majority of the data well (Figure 3.7). From the

growth versus irradiance curves several parameters with physiological significance can be determined: maximum growth rate (μ_{max}), initial slope (α), irradiance coefficient (E_k) and compensation irradiance (E_c). The parameters for each of the growth versus irradiance curves at each temperature are given in Table 3.2. The parameters of the growth versus irradiance curves ($_{\mu}$max, α, E_k and E_c) will be examined individually with respect to temperature in sections 3.3.2.3 to 3.3.2.6. There are several instances where parameters of the model at particular temperatures have not been included in figures. This is because there was not enough data to describe these parameters accurately, evidenced by large standard errors associated with these parameters in most cases. The parameters that have been excluded at particular temperatures are marked with an asterisk in Table 3.2.

The greatest observed growth rate in this experiment was 0.236 d^{-1} and occurred at 23 °C and 203 µmol photons m^{-2} s^{-1}(Figure 3.7). However it is important to note that at 24.9 and 25.2 °C growth rates showed little or no saturation even at irradiances greater than 200 µmol photons m^{-2} s^{-1}. Also the model (Equation 2) could not be fit to the 11.9 °C data because only one culture grew (0.021 d^{-1}) in this treatment at an irradiance of 175 µmol photons m^{-2} s^{-1}.

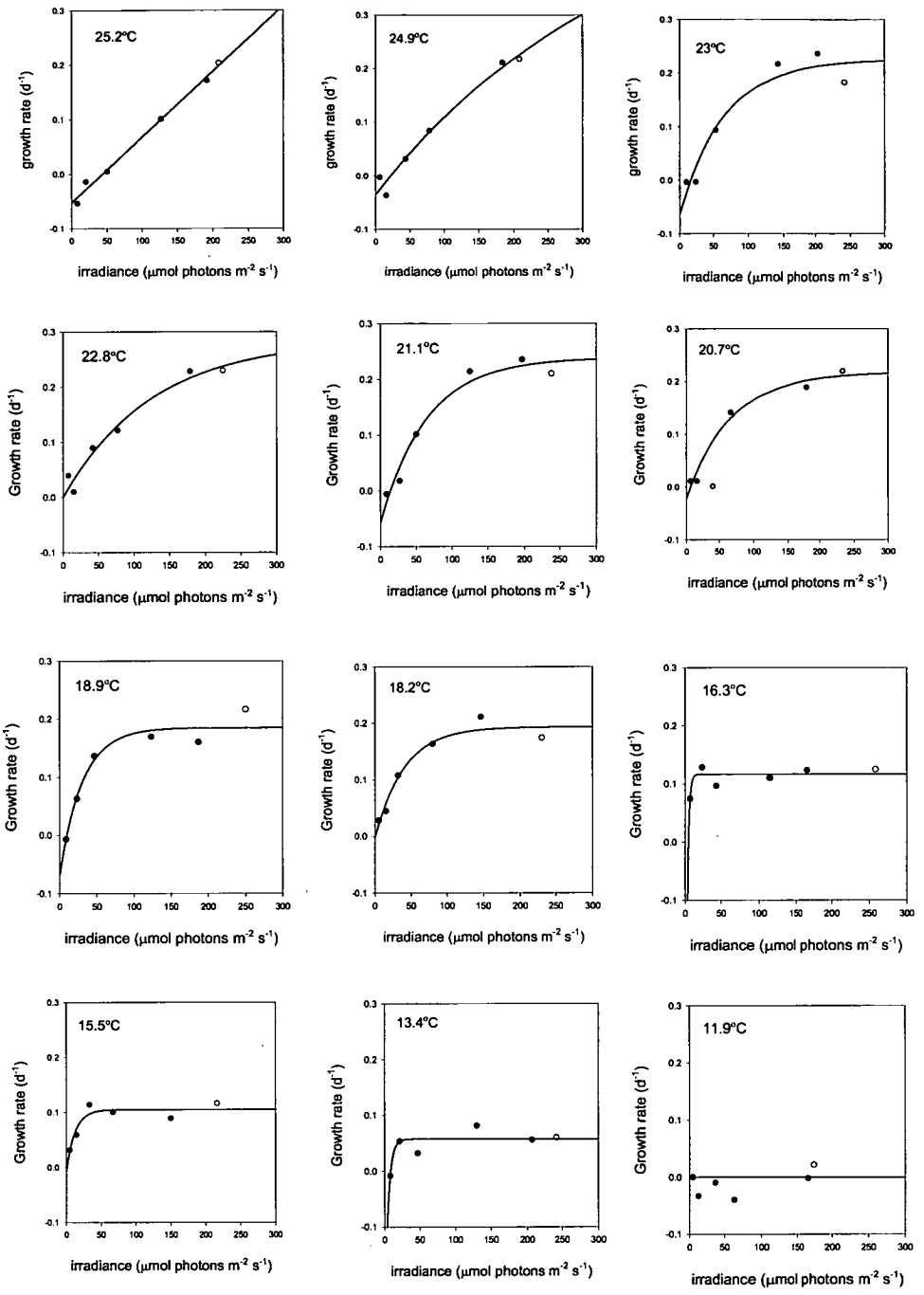

Figure 3.7 Growth vs. irradiance curves using the modified model of Platt *et al.* (1975) (Equation 2) for *Gymnodinium catenatum* at twelve different temperatures. A data point was excluded from the model fit at 20.7 °C (open circle).

Table 3.2 Parameters of the modified model of Platt *et al.* (1975) fit to the growth vs. irradiance data for *Gymnodinium catenatum* at twelve different temperatures. The standard error (SE) for these parameters are included. E_k was calculated by solving the modified model of Platt *et al.* (1975) (Equation 2) at the irradiance corresponding to half $_\mu$max.

Temperature (°C)	r^2	$_\mu$max	SE ($_\mu$max)	α	SE (α)	E_c	SE (E_c)	E_k
25.2	0.98	1.271E+03*	2.992E+06	0.0E+00*	2.20E-03	44	5.5	1.1E+11*
24.9	0.96	0.557*	0.615	2.8E-03	3.90E-03	22	7.4	230.8*
23	0.90	0.226	0.036	1.5E-02	8.20E-03	16	6.9	98.8
22.8	0.95	0.285	0.068	8.0E-03	4.40E-03	0	9.4	86.6
21.1	0.95	0.239	0.025	1.5E-02	5.30E-03	14	4.8	46.2
20.7	0.95	0.218	0.024	1.5E-02	5.90E-03	6	5.2	46.2
18.9	0.91	0.185	0.015	3.3E-02	1.15E-02	10	3.5	20.8
18.2	0.93	0.194	0.016	2.5E-02	8.90E-03	1	4.9	27.7
16.3	0.46	0.117	0.008	4.3E-01*	1.04E+01	4	79.0	1.6
15.5	0.75	0.105	0.010	7.9E-02*	4.81E-02	1	4.2	8.7
13.4	0.57	0.058	0.010	2.1E-01*	3.60E-01	8	1.8	3.3
11.9	0.00	1.891E+02	6.797E+10	0.0E+00*	4.68E+04	0*	1.6E+06	#N/A

*** indicate parameters not included in figures. The model could not determine these parameters accurately because of data limitations at some temperatures.**

3.3.2.3 The effect of temperature on maximum growth ($_\mu$max)

At those temperatures where μ_{max} could be estimated from the growth versus irradiance data (temperatures between 13.4 and 23 °C) there was a linear increase in μ_{max} as temperature increased (Figure 3.8). μ_{max} was smallest (0.064 d^{-1}) at 13.4 °C and greatest (0.285 d^{-1}) at 22.8 °C. The data were described well by Logan's (1976) model that was modified to include a y-intercept (r^2=0.97;n=12;P=<0.0001):

$$\mu_{max} = \{\alpha_1 e^{(\beta x)} - e\left[\beta T_m\left(\frac{T_m - x}{\Delta T}\right)\right]\} + y_0$$

Equation 8

Where μ_{max} is the maximum growth rate, α_1 is the rate of temperature-dependent processes at a basal temperature; β is equivalent to a composite Q_{10}; T_m is the upper temperature threshold; ΔT is the temperature range where the curve plateaus; x is the temperature; and Y_0 is the y-intercept of the curve.

Table 3.3 Parameters from the modified model of Logan (1976) for *Gymnodinium catenatum* GCHU02.

Parameter	Model Estimate	SE	P
α_1	0.32	1.32	0.82
β	0.07	0.76	0.93
T_m	48.78	429.42	0.91
ΔT	6.99	42.21	0.87
Y_0	-0.59	3.44	0.87

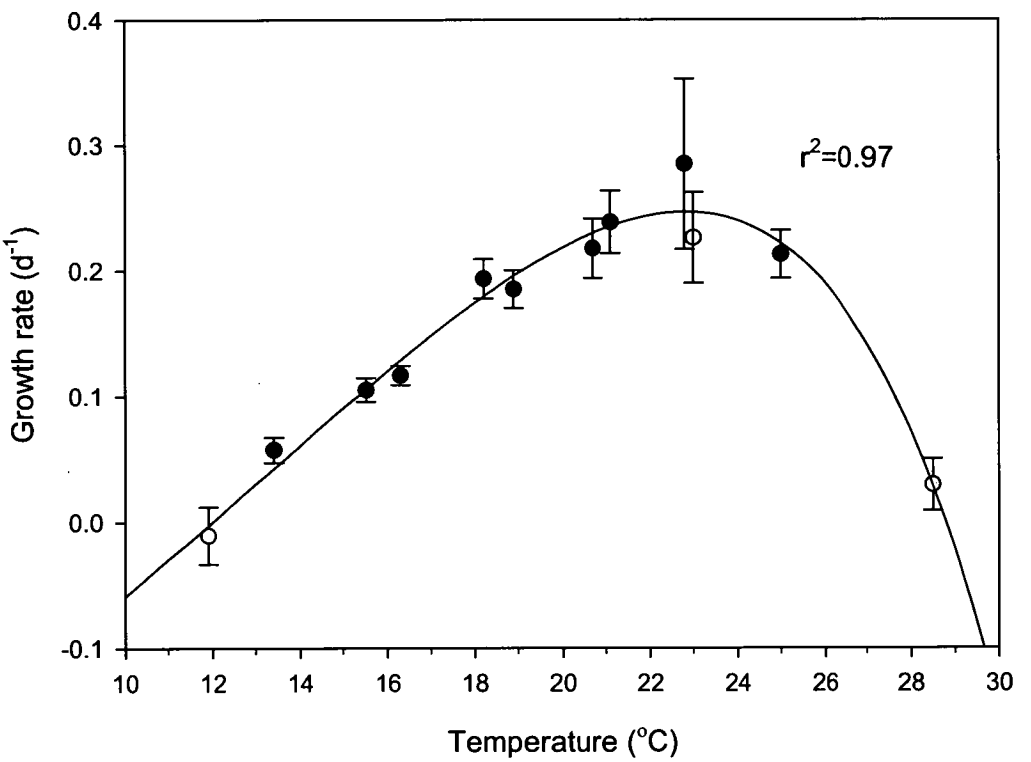

Figure 3.8 Effect of temperature on $_{\mu}$max of *Gymnodinium catenatum*. Each μ_{max} value is from the modified model of Platt *et al.* (1975). Standard errors were also estimated from the model. For details regarding the open circle see section 3.2.8 and for the grey circle see section 3.2.6.

3.3.2.4 The effect of temperature on the initial slope (α)

At temperatures <18 °C α was not included in Figure 3.9 for the reasons outlined in 3.3.2.2. At temperatures above 18 °C there was a decrease in α with increasing

temperature. The data had an r^2=0.81 when fitted with a linear model, y=0.096+-0.0038x (n = 7, P =0.0056).

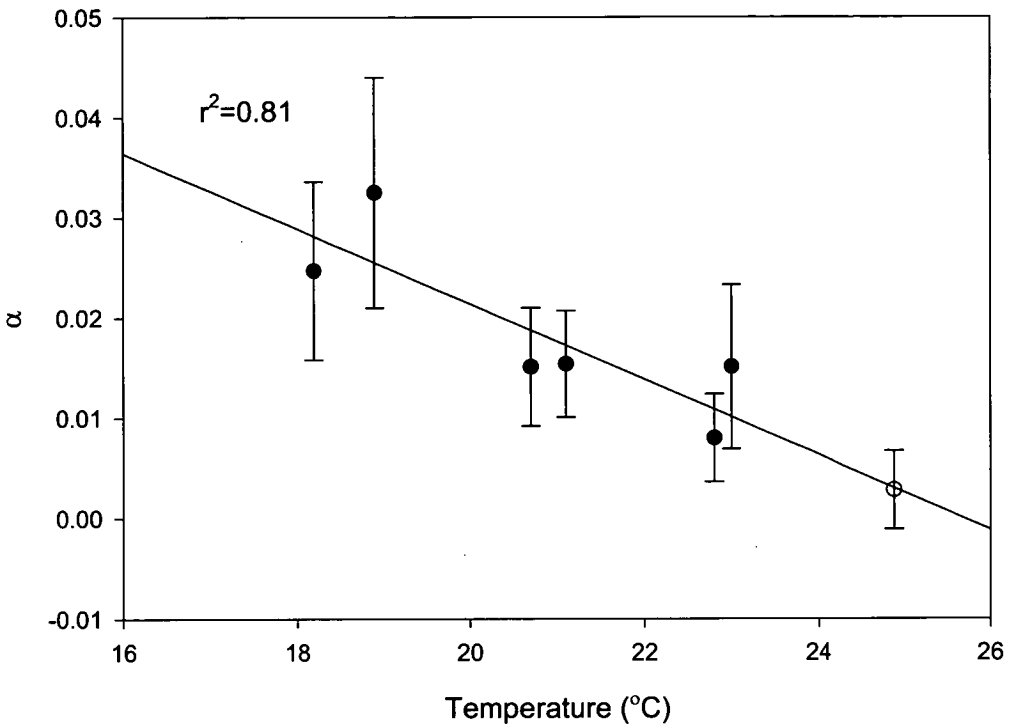

Figure 3.9 Effect of temperature on α of *Gymnodinium catenatum*. Each α value is from the modified model of Platt *et al.* ((1975)). The standard error for α was estimated from the model.

3.3.2.5 The effect of temperature on irradiance co-efficient (E_k)

The half-saturation co-efficient for growth as a function of irradiance (E_k) was estimated by solving (using the Solver add-in provided with Microsoft Excel) the modified model of Platt *et al.* (1975) (see Equation 2) for the irradiance at half the estimated μ_{max}. E_k increased with increasing temperature (Figure 3.10). The lowest E_k, 1.6 µmol photons m^{-2} s^{-1} was at 16.3 °C and the highest E_k, 98 µmol photons m^{-2} s^{-1} at 22.8 °C. The exponential model $y=0.035\exp^{0.34x}$ (r^2=0.98, n = 8, P≤0.0001) described the E_k data very well.

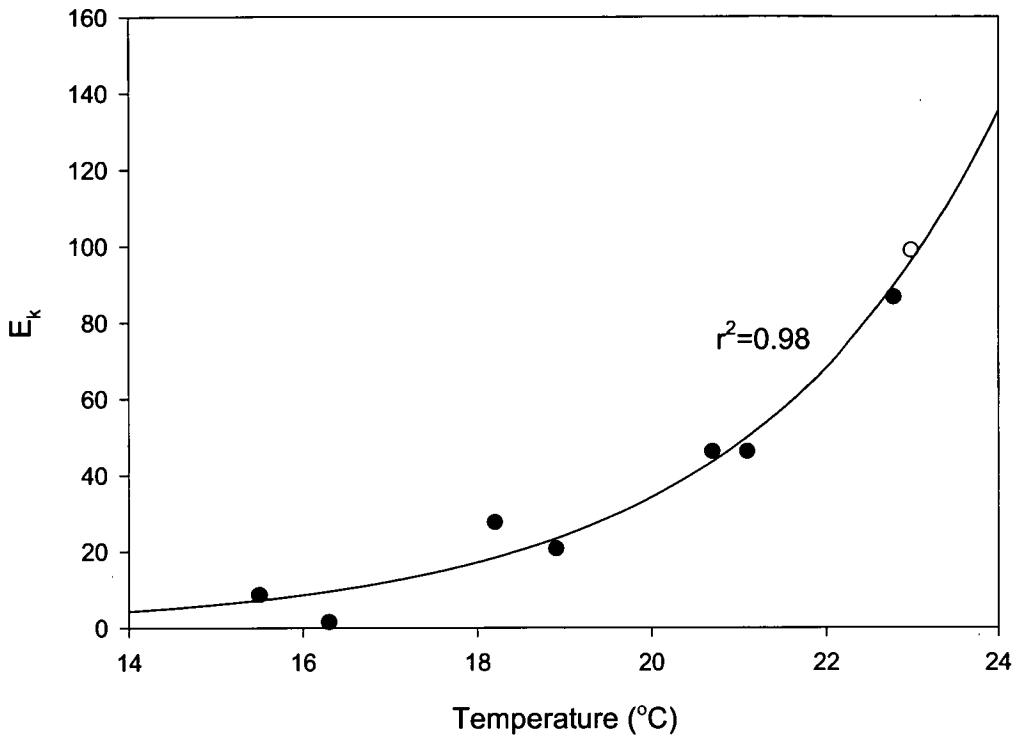

Figure 3.10 The effect of temperature on the half-saturation co-efficient for growth as a function of irradiance (E_k) for *Gymnodinium catenatum*. E_k was calculated from the model of Platt, Denman *et al.* (1975)

3.3.2.6 The effect of temperature on compensation irradiance (E_c)

The compensation irradiance (E_c) increased with increasing temperature (Figure 3.11). The highest compensation irradiance was 47 μmol photons m^{-2} s^{-1} at 25.2 °C. This trend was described well with the model $y=exp^{0.124x}$ (r^2=0.43, n = 11, P<0.001). The standard error at 16.3 °C is much larger than at any other temperature, because there is only one point on the initial slope of the curve, making it difficult for the model to estimate E_c without an associated large error.

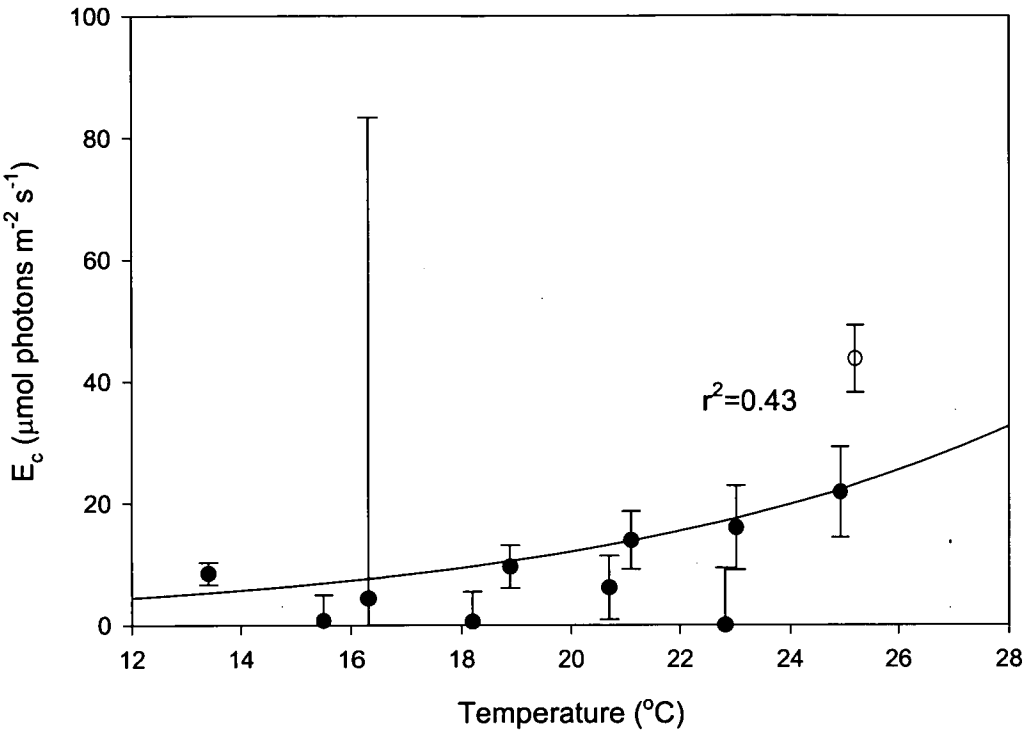

Figure 3.11 Effect of temperature on E_c of *Gymnodinium catenatum*. Each E_c value is from the modified model of Platt, Denman *et al.* (1975) (Equation 2). The standard error for E_c is also from the model.

3.3.3 Effect of high temperature and high irradiance on growth rate

Growth rates calculated from the change in fluorescence over time at 25 °C and 291 and 458 µmol of photons m^{-2} s^{-1} were not significantly different from each other ($P<0.05$). At 28.5 °C growth rates calculated from fluorescence and at 291 and 458 µmol photons m^{-2} s^{-1} were not significantly different from (Figure 3.12).

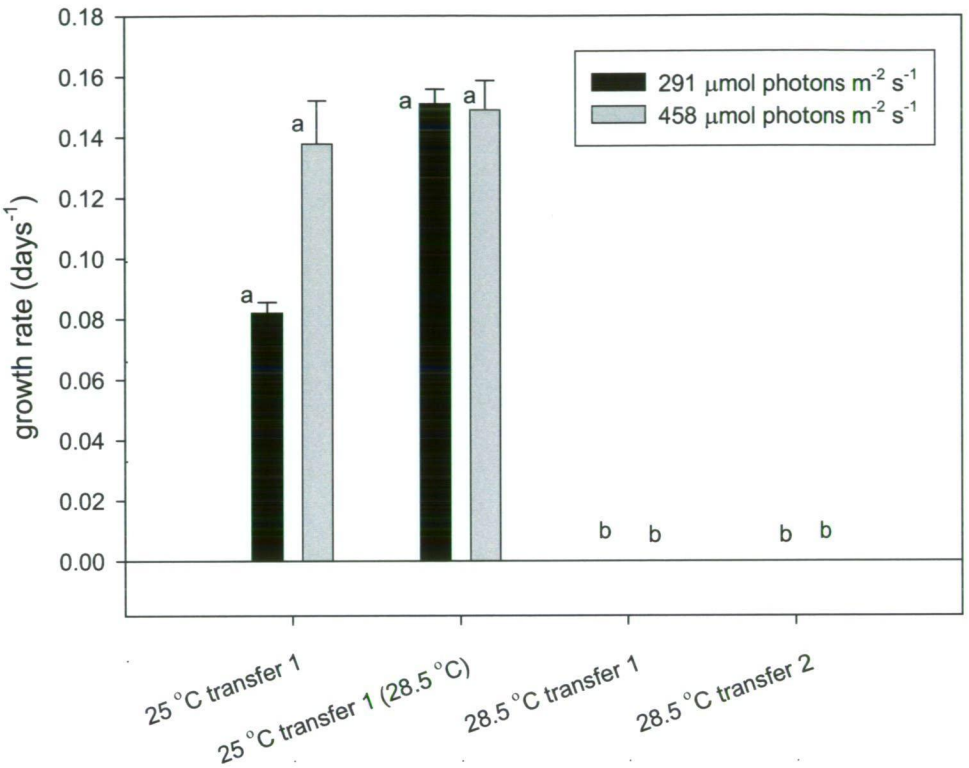

Figure 3.12 Growth rates of *Gymnodinium catenatum* at 25 and 28.5 °C and 291 and 458 µmol photons m^{-2} s^{-1}. Superscript text indicates where there were significant differences (P<0.05) between growth rates.

The growth rates were added to the growth versus irradiance curve with those from 24.9 to 25 °C. The combined data fit the model well (Figure 3.13). The parameter estimates of the modified model of Platt *et al.* (1975) are included in Table 3.4.

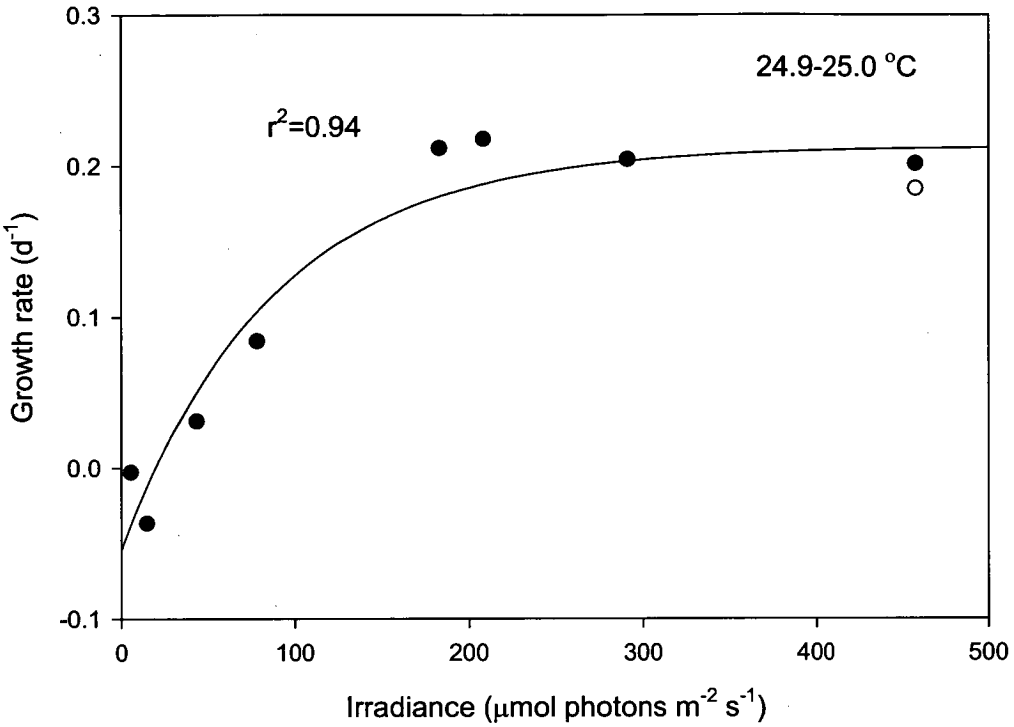

Figure 3.13 Growth vs. irradiance curve of *Gymnodinium catenatum* grown at 24.9 to 25.0 °C with growth rates for cultures grown at 291 and 458 µmol photons m^{-2} s^{-1} added. The modified model of Platt, Denman *et al.* (1975) was fitted to the data.

Table 3.4 Parameter estimates from the modified model of Platt, Denman *et al.* (1975) for growth at 24.9 to 25 °C with new growth rates added from cultures grown at 298 and 454 µmol m^{-2} s^{-1}.

Parameter	Model Estimate	SE	P
μ_{max}	0.21	0.019	<0.0001
α	0.01	0.004	0.021
E_c	20.0	6.66	0.024

3.3.4 Effect of light and temperature on biochemical composition

The mean for chlorophyll *a*, carbon, and nitrogen quotas were 72, 4445 and 587 pg^{-1} respectively (Table 3.5). The mean C:chl *a* and C:N ratios are 57 and 8.1 respectively. These means are from *G. catenatum* grown at temperatures ranging from 13.4 to 25.2 °C and irradiances between 25 and 258 µmol photons m^{-2} s^{-1} (note

cultures grown at <25 µmole of photons m^{-2} s^{-1} across all temperatures were not included in these biochemical quotas; see section 3.2.9).

Table 3.5 Mean biochemical quotas of *Gymnodinium catenatum* grown at temperatures ranging from 13.4 to 25.2 °C and irradiances between 5 and 280 µmol photons m^{-2} s^{-1}.

	Chl *a* quota (pg $cell^{-1}$) (n=42)	C quota (pg $cell^{-1}$) (n=41)	N quota (pg $cell^{-1}$) (n=41)	C:Chl*a* (n=40)	C:N (n=40)
Mean	72	4445	587	57	8.1
S.E.	3.1	551.5	85.7	4.2	0.42
CV (%)	28	80	95	47	33

Table 3.6 Temperature range and number of cultures in each binned temperature group. Binned temperature groups were used to examine the trends in biochemical composition with temperature and irradiance.

Binned temperature (°C)	Temperature range included (°C)	Number of cultures
25	24.7-25.5	7
22.9	22.8-23.3	8
20.9	20.8-21.3	7
18.5	18.2-18.9	8
15.9	15.4-16.4	7
13.4	13.3-13.5	4

To examine trends in carbon and chlorophyll *a*, similar (Δ temp. ≈ 1 °C) temperature groups were binned together (Table 3.6). This did not occur at the lowest temperatures 11.9 °C and 13.4 °C where there were only four cultures which were sampled for chl*a* and carbon and all grown at 13.4 °C. Both carbon $cell^{-1}$ (carbon quota) and chlorophyll a $cell^{-1}$ (chl*a* quota) show an exponential decrease as irradiance increased in both 25 and 22.9 °C binned temperature groups (Figure 3.14). The carbon and chl*a* quota range from 1979 to 11069 pg $cell^{-1}$ and 61 to 132 pg $cell^{-1}$ respectively at 25 °C and from 2050 to 8153 pg $cell^{-1}$ and 52 to 108 pg $cell^{-1}$ respectively at 22.9 °C. At 25 °C the 5.6 fold decrease in carbon with increasing temperature is much larger than the 2 fold decrease in chl*a*. Similarly at 22.9 °C the difference in magnitude decrease for carbon and chl*a* quotas were maintained: a larger 4 fold decrease in carbon quota and only a 2.1 fold decrease in chl*a* quota.

With the exception of a slight decrease in carbon quota with increasing irradiance at 15.9, 18.5 and 20.9 °C the carbon and chl *a* quotas did not exhibit a clear decrease with increasing irradiance as at the higher temperatures. At 15.9, 18.5 and 20.9 °C, 95.2 % of carbon quotas are all <5000 pg cell^{-1}, or considerably lower than at the higher temperatures and lower irradiances. At 15.9, 18.5 and 20.9 °C, 82.6 % chl*a* quotas ranged between 50 to 100 pg cell^{-1}. At 13.4 °C the carbon quotas were greatest, between 7000 and 9000 pg cell^{-1} at the two intermediate irradiances. At the lowest and highest irradiances the carbon quotas were reduced to values between 5000 and 7000 pg cell-^{1}. The chl *a* quota at 13.4 °C was approximately 50 pg cell^{-1} across the different irradiances with the exception of a slight increase in chl*a* at the lowest irradiance (50 μmol photons m^{-2} s^{-1}).

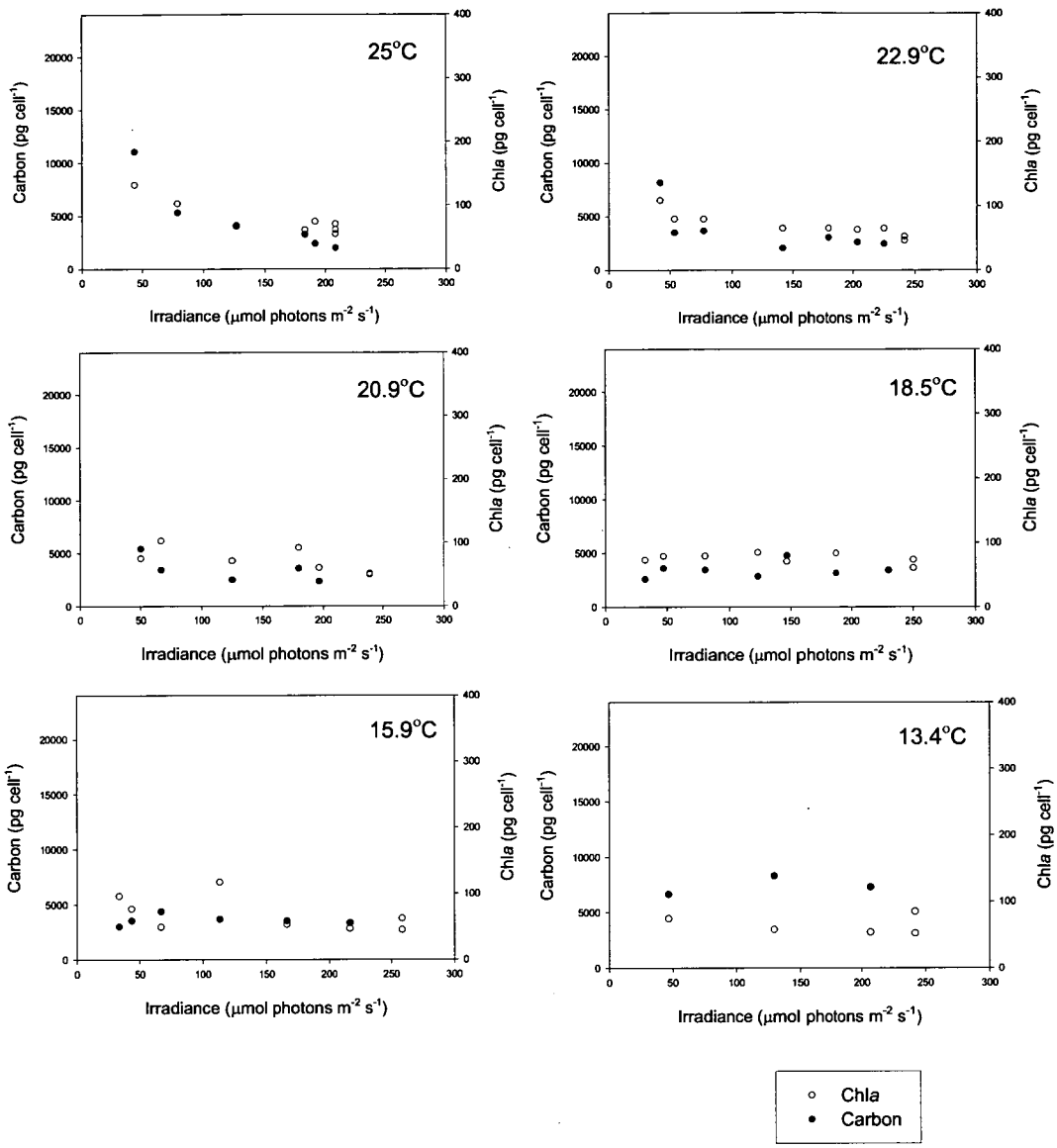

Figure 3.14 Effect of irradiance on C and chl*a* quotas for *Gymnodinium catenatum* are plotted on separate axes for six binned temperature groups.

There were not many data points available at any given temperature to examine the trends in C:chla (pg cell^{-1}:pg cell^{-1}) with irradiance. Given the variability in both carbon and chl*a* measurements, the analysis of trends was also statistically weak. To improve this situation the data sets were binned again as in Table 3.6. Out of the six

binned temperature groups, only the 25 °C binned temperature group had a slope (-0.212) significantly different from 0 (r^2=0.58; n=7; P=0.046). However, trends were apparent; the strongest trends were at temperatures of 25, 20.9 and 15.9 °C which had the highest r^2 values of 0.58, 0.41 and 0.29 respectively. The slopes of the regressions were all negative at the higher temperatures (25, 22.9 and 20.9 °C) and the negative slope decreased as temperature decreased until the slope of the C:chl*a* versus irradiance became positive for the lower temperature groups (18.5, 15.9 and 13.4 °C).

The complex interaction between irradiance and temperature on C:chl*a* (Figure 3.15) can be simplified by considering only the effects of the highest irradiances. At irradiances >200 μmol photons m^{-2} s^{-1} there was an exponential decrease in C:chl*a* as temperature increased (Figure 3.16). A linear model was fitted to the natural log of this data and the slope (-0.095 +/- 0.0157) and y-intercept (6.1 +/- 3.33) were both significantly different from 0 with respective P values of ≤0.0001 and ≤0.0001.

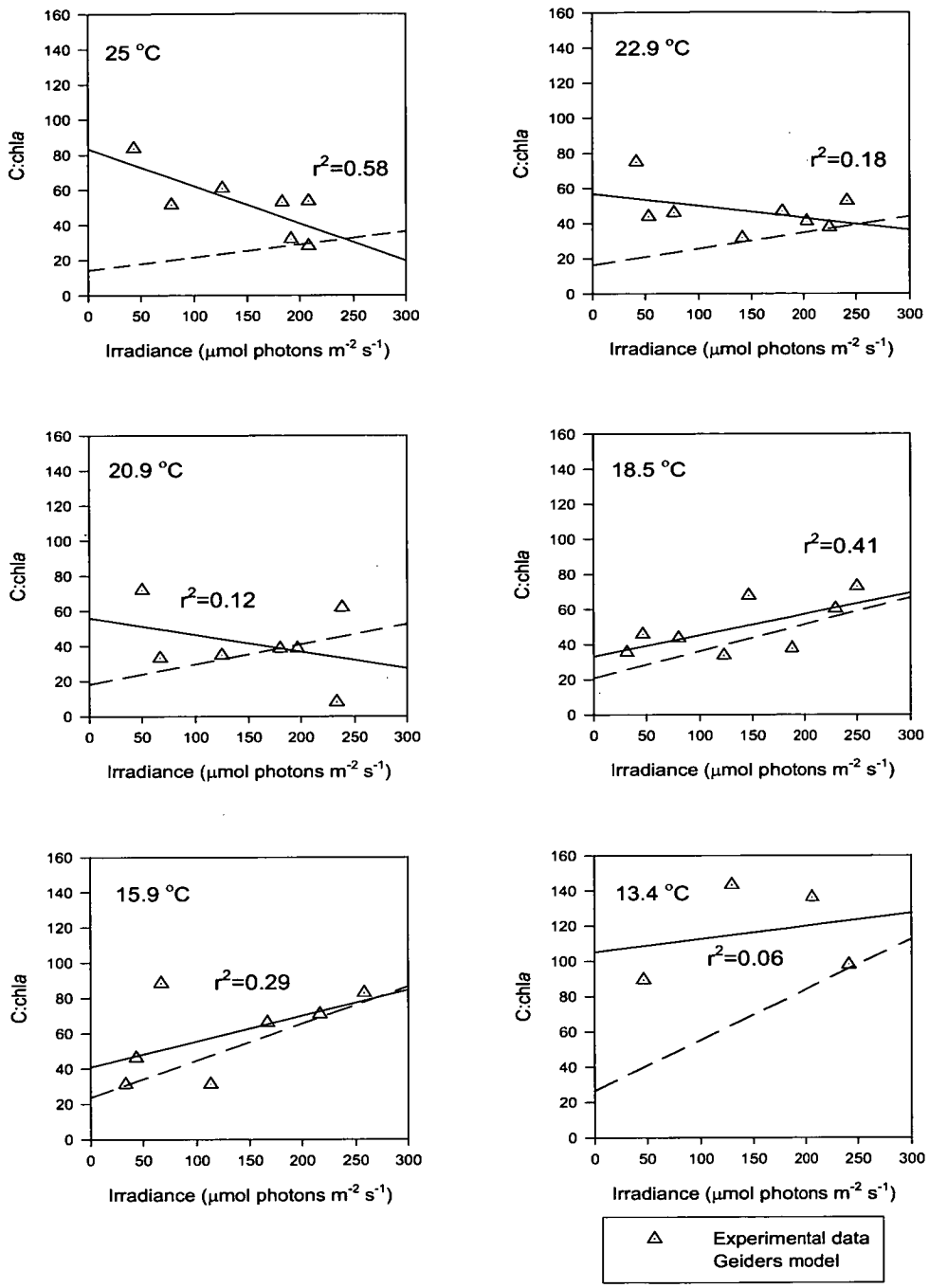

Figure 3.15 The effect of irradiance on C:chl*a* of *Gymnodinium catenatum* on cultures binned into six temperature groups. Straight lines are fitted to the C:chl*a* data for each binned temperature group. The dotted line represents the expected C:chl*a* ratio from Geider's model (Geider, 1987)

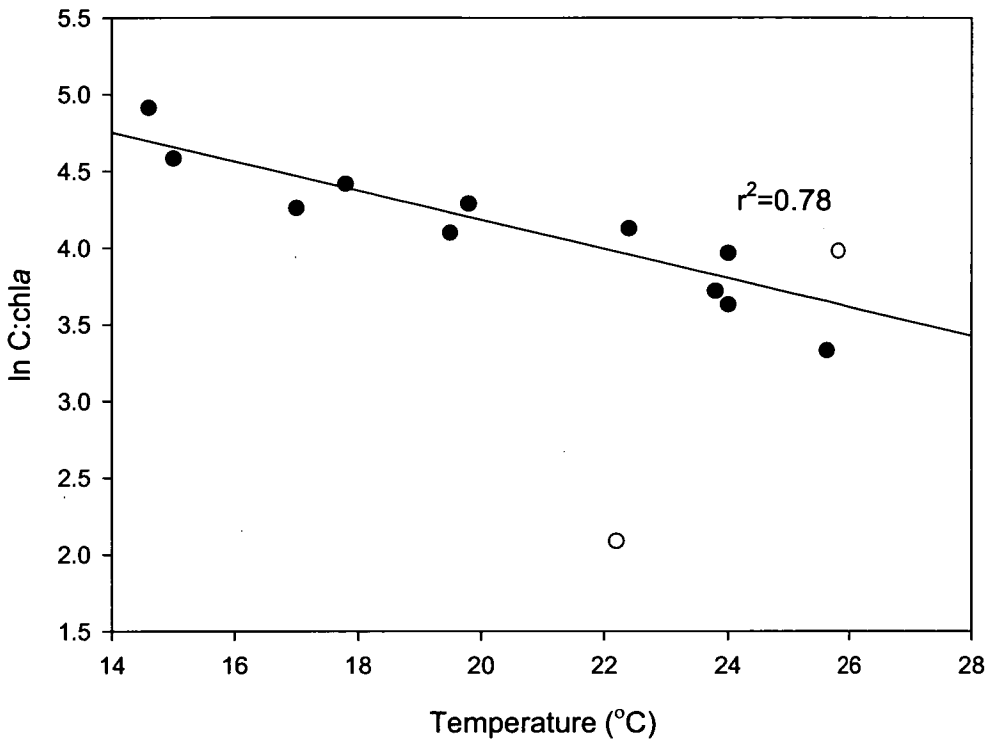

Figure 3.16 Effect of temperature on ln C:chla in *Gymnodinium catenatum* cultures grown at irradiances >200 µmol photons m^{-2} s^{-1}. The white circle was treated as an outlier and excluded from the regression.

3.3.5 A model for carbon: chlorophyll *a*

Plotting the slope of the regressions for C:chl*a* with irradiance for each of the temperature groups in Figure 3.15 showed that there was a clear trend for the slope to decrease as temperature decreased (Figure 3.17). The linear model fitted the slope data well (r^2=0.73; n = 6; P = 0.030):

slope=0.53+-0.028T

Equation 9

Where T= temperature and the slope is the slope of the C:chl*a* relationship with irradiance at that temperature.

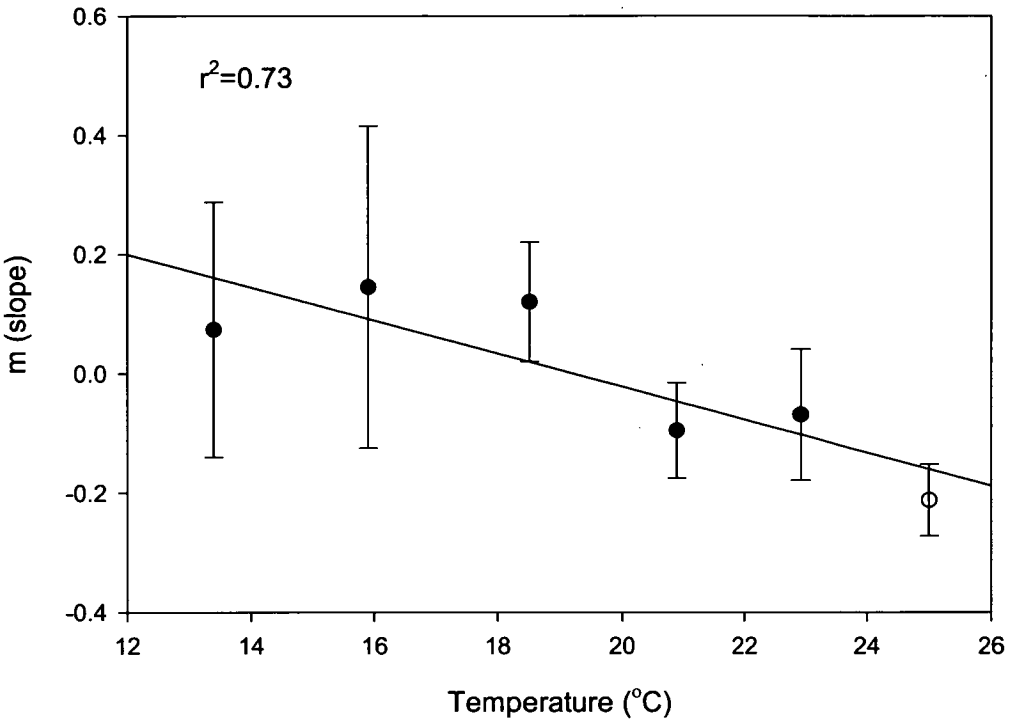

Figure 3.17 The effect of temperature on m(slope) of the line fit to C:chl*a* vs irradiance for *Gymnodinium catenatum* binned into six temperature groups (data from Figure 3.15).

The y-intercept for each of the lines fitted to the C:chl*a* versus irradiance in Figure 3.15 were plotted against temperature (Figure 3.18) and were described well with this equation (r^2 = 0.88; n = 6; P =0.041):

$$y\text{-intercept}=430+-42T+1.14T^2$$

Equation 10

where T = temperature and y intercept is the y-intercept of the C:chl*a* versus irradiance relationship at the temperature specified.

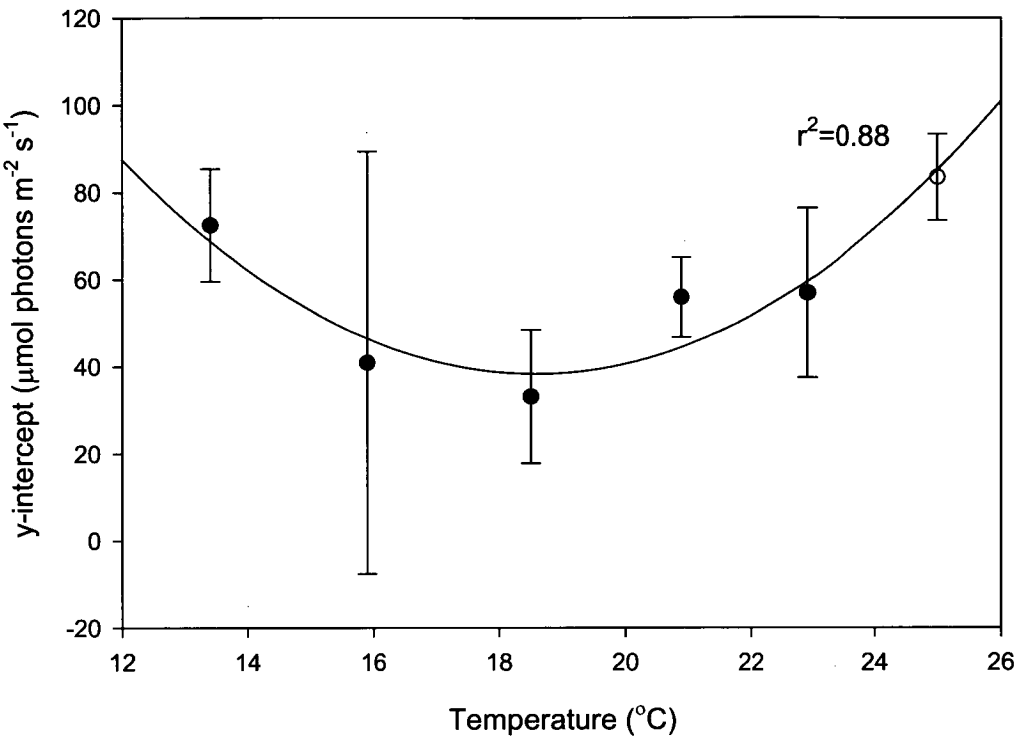

Figure 3.18 The effect of temperature on the y-intercept of the line fitted to C:chl*a* vs. irradiance for *Gymnodinium catenatum* binned into six temperature groups (data from Figure 3.15).

From these sub-models it is possible to create an overall model that describes the effect of temperature and irradiance on C:chl*a* by substituting Equation 9 and Equation 10 into a linear model equation and adding an irradiance term. The parameters of the model were refined using an algorithm for least-squares estimation of nonlinear parameters (Marquadt, 1963). The model is:

$$C:chla = (0.76 + (-0.036T)I) + (516 + (-48T) + 1.19T^2)$$

Equation 11

Where T= temperature and I =Irradiance.

The model is presented on three dimensions and the observed data are also included in Figure 3.19. C:chl*a* ratios were greatest (up to 143.3) at low temperature (≈ 14

°C) and high irradiances (>200 μmol photons m^{-2} s^{-1}). The C:chl*a* decreases as irradiance decreases and temperature increases to the lowest values 40 to 60 C:chl*a*. However as temperatures increase above 20 °C there was an increase in the C:chl*a* up to 75 to 85 with decreasing irradiance.

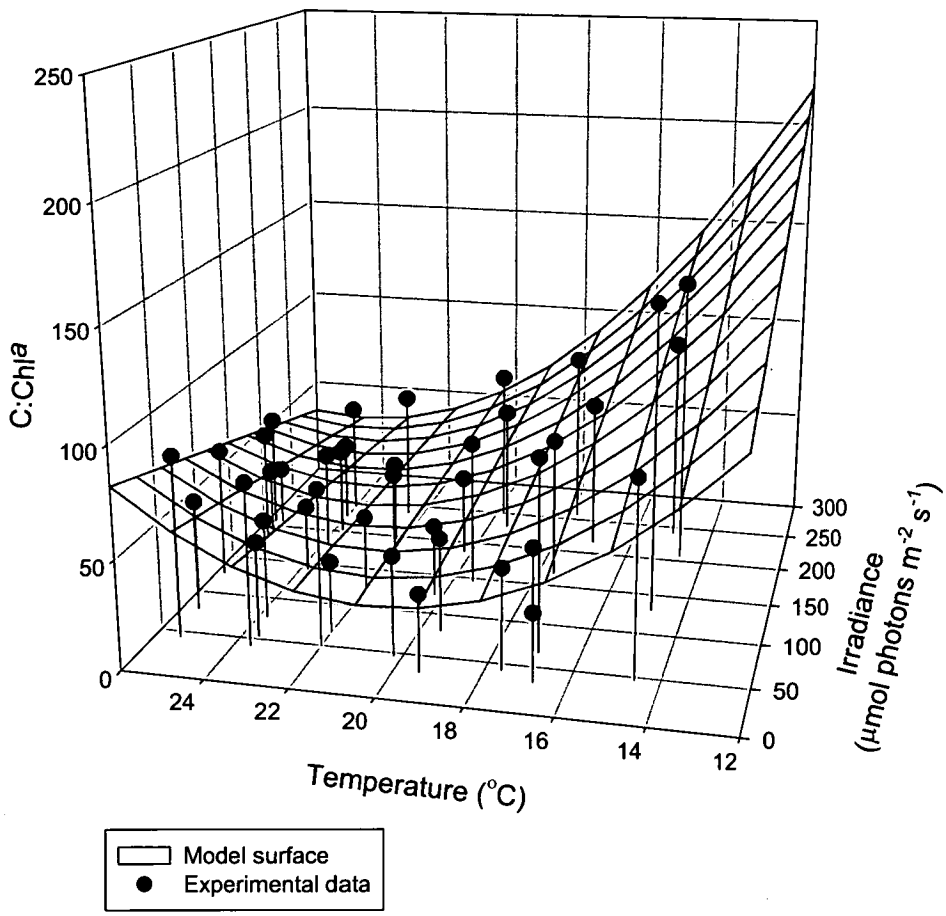

Figure 3.19 Effect of temperature and irradiance on C:chl*a* of *Gymnodinium catenatum*. A 3D surface from the model (Equation 11) is used to describe the data.

3.3.6 Energetics model

A simple conceptual model based on cellular energetics has been applied to light saturating (Figure 3.20) and light limiting (Figure 3.21) conditions for *G. catenatum*.

The results from the conceptual energetics model of *G. catenatum* describe the response of *G. catenatum* growth (modelled using *G. catenatum* growth vs. irradiance curves) well.

Input data for the maintenance costs used in the model comes from the estimates of E_c from each growth versus irradiance experiment at each temperature. These were shown to have an exponential relationship with temperature. Growth rates were simply μ_{max} values from each temperature. The relationship between capital costs and temperature were derived from the 'U' shaped carbon quota versus temperature data.

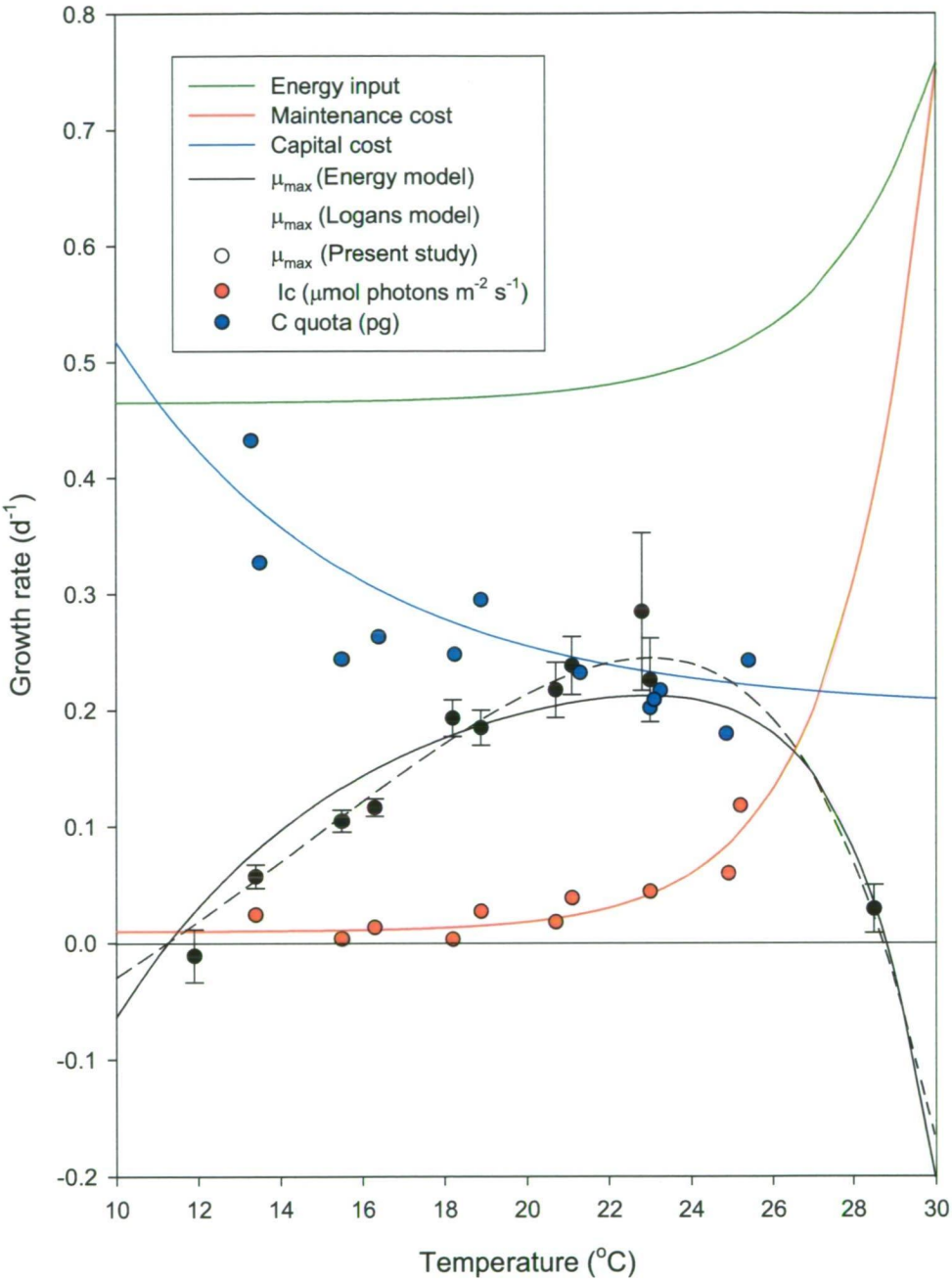

Figure 3.20 Model of temperature effect on energetic costs for *Gymnodinium catenatum* in light saturated (1000 μmol photons m^{-2} s^{-1}) conditions

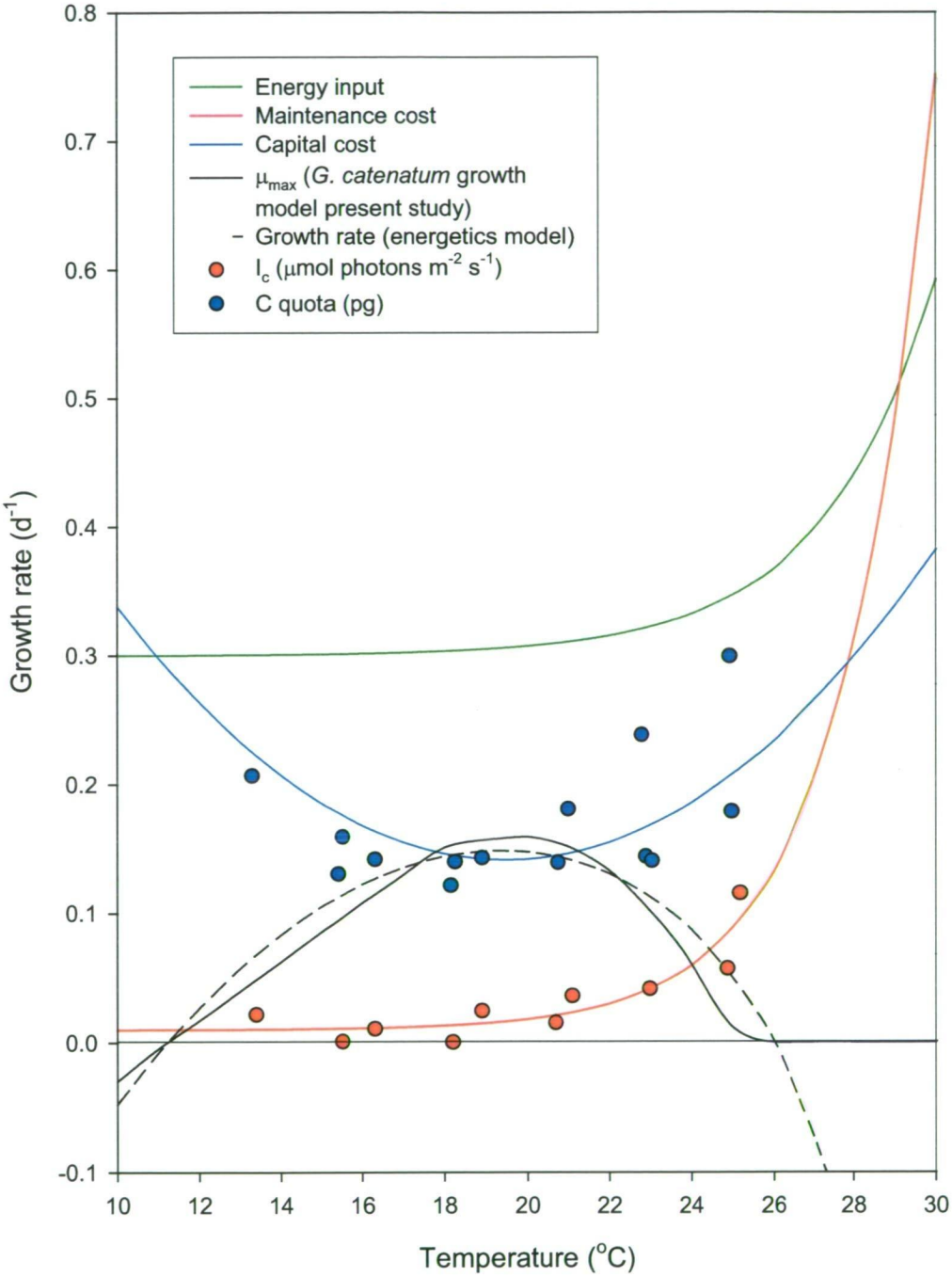

Figure 3.21 Model of temperature effect on energetic costs for *Gymnodinium catenatum* in light limited (<80 µmol photons m^{-2} s^{-1}) conditions.

3.4 Discussion

3.4.1 Effect of temperature and irradiance on *Gymnodinium catenatum* growth

Growth rates of *G. catenatum* grown in culture at temperatures ranging from 11.9-25.2 °C and irradiances ranging from 5 to 283 μmol photons m^{-2} s^{-1} are presented in a contour plot providing a visual summary of all the growth rate data collected in this experiment (Figure 3.22). The greatest growth rates of >0.2 d^{-1} occurred at combinations of temperatures between 20 to 25 °C and irradiances >140 μmol photons m^{-2} s^{-1}. There were also two areas where growth rates were ≤0 d^{-1}: where temperature was 11.9 °C and irradiance was <150 μmol photons m^{-2} s^{-1} and where temperature was >23 °C and irradiances were <35 μmol photons m^{-2} s^{-1}.

The physiology of *G. catenatum* will be discussed through examining the effect of temperature on the parameters of the growth vs. irradiance curve and effect of temperature and irradiance on the biochemical composition of this species.

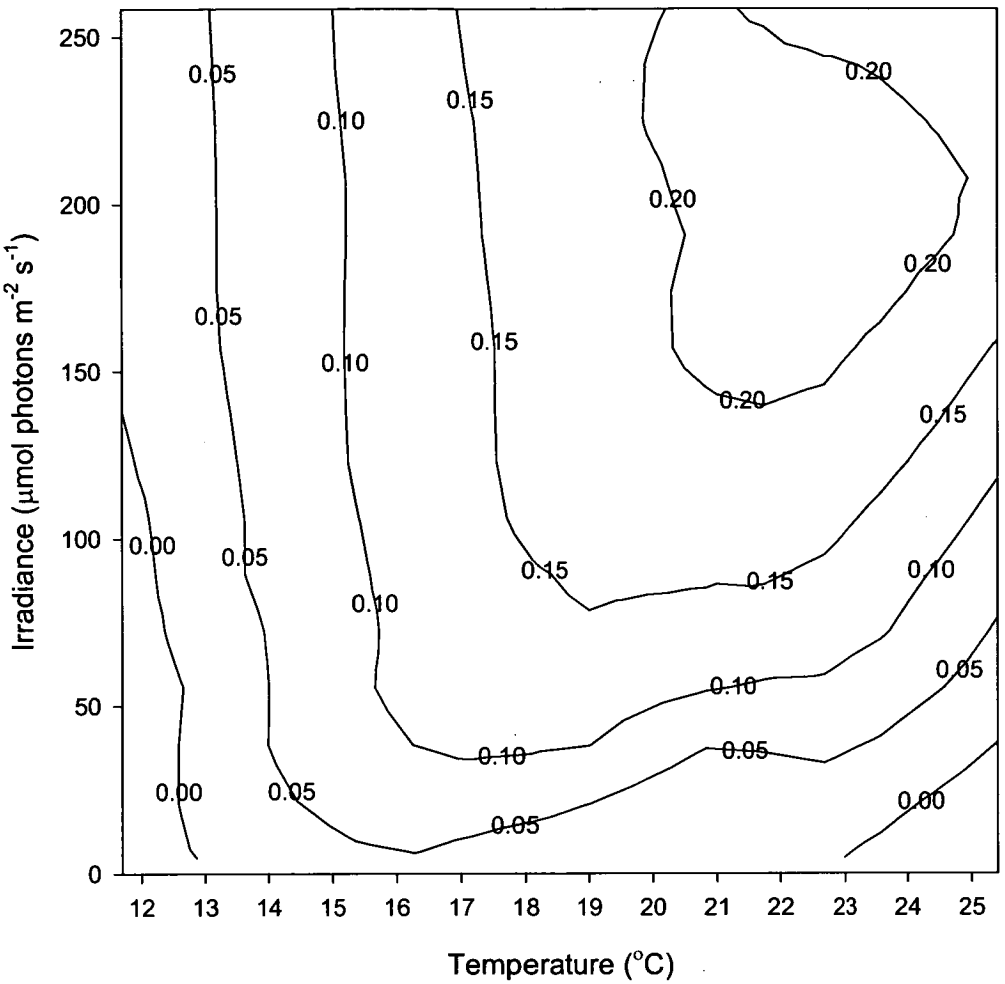

Figure 3.22 Contour plot of growth rate (d^{-1}) of *Gymnodinium catenatum* grown at different combinations of temperature and irradiance.

3.4.2 Effect of temperature on parameters of the growth vs. irradiance curve

3.4.2.1 Maximum growth rate (μ_{max})

Effect of temperature on μ_{max}

The μ_{max} increased linearly with temperature for *G. catenatum* in this study. The reason that Logan's (1976) model fit the data well (r^2=0.97) where none of the parameters are statistically significant is because Logan's model was made to fit exponential increases in μ_{max} with temperature. However, clearly the data increased linearly up to the greatest μ_{max} in this study. This result contrasts with several other studies that show that μ_{max} increases exponentially with temperature (Li & Morris, 1982, Ojala, 1993, Eppley, 1972). Montagnes *et al.*, (2003) provide some examples of linear increases in μ_{max} and argue that in the majority of cases μ_{max} increases linearly with temperature. But as pointed out by Thompson (Thompson, 2006) it is likely that what appears as linear relationships may in reality be exponential. This is because in many cases only a small temperature range has been examined, or experimental artefacts are caused by limiting light at high temperatures or the failure to measure growth at the temperature where μ_{max} is greatest (because of the abrupt transition from the greatest μ_{max} to temperatures that cause death for many phytoplankton).

Unfortunately the majority of alternative studies examining temperature and growth rate in *G. catenatum* did not use a sufficient range of temperatures to determine what kind of relationship there is between resource saturated growth rate and temperature (Blackburn *et al.*, 1989, Ellegaard et al., 1993, Yamamoto et al., 2002). Additionally, of the studies that have enough data to evaluate this relationship, the growth rates from Band-Schmidt et al. (2004) show a linear function of temperature while Bravo and Andersen (1994) provide results which appear exponential for the temperatures

up to 16°C. Therefore, existing studies show it is not possible to generalise about the exact nature of the relationship between resource saturated growth and temperature for this species. The GCHU02 strain examined in the present study showed a strong linear increase in μ_{max} with increasing temperature. A linear increase in μ_{max} is likely to be a disadvantage in competition with the many phytoplankton species that have exponential increases in μ_{max} (Li and Morris, 1982; Ojala, 1993)

Greatest μ_{max}

The greatest growth rates measured for *G. catenatum* in this study (>0.2 d^{-1}) occurred in cultures grown at temperatures from 20 to 25 °C and at irradiances ≈150 to 250 µmol photons m^{-2} s^{-1}. Earlier studies are similar in that the highest growth rates occur at temperatures >20 °C with the exception of Blackburn et al. (1989) where the growth rate of the Huon Estuary strain GCHU01 peaked at 17.5 °C and decreased quickly after 20 °C (Figure 3.23).

μ_{max} is the parameter of growth versus irradiance curves that indicates where growth is at a maximum, i.e. where growth rate no longer continues to increase with increasing irradiance. Because, of the scarcity of other μ_{max} values in the literature for *G. catenatum.* The results from the present study will be compared with growth rates from other studies (Figure 3.23), where irradiances are assumed to be excess to the requirements of *G. catenatum.* The conditions used in each of the other studies are included in Table 3.7. However, these assumptions based on other species may not always be accurate, particularly at extremes of temperature. At the highest temperature (25.2 °C) used in the initial study, growth was a linear function of irradiance up to 250 µmol photons m^{-2} s^{-1} with no indication of saturation. In a subsequent experiment, cultures were exposed to a temperature of 25 °C and higher irradiances (291 and 458 µmol photons m^{-2} s^{-1}). Growth rates at these higher irradiances were slightly lower than those at 208 µmol photons m^{-2} s^{-} indicating that growth was saturated at < 291 µmol photons m^{-2} s^{-1}.

These results indicate that in several studies light may have been limiting growth at higher temperatures and thus these may not be entirely accurate resource saturated growth rates. The fact that light requirements increase quickly at higher temperatures is evident in the exponential relationship between I_{sat} or E_k and temperature (Collins & Boylen, 1982). In the present study irradiances of≈25 μmol photons m^{-2} s^{-1} saturated growth at 13.4 °C, but in excess of 250 μmol photons m^{-2} s^{-1} was required to saturate growth at temperatures greater than 23 °C. Given the nonlinear nature of the increase in irradiance required to saturate growth with increasing temperature it is difficult to choose an irradiance that will saturate growth as temperature increases. One way of avoiding light limitation in experiments investigating resource saturated growth at a range of temperatures is to set the irradiance at $>2E_k$ at the highest temperature. In this case irradiance will be in excess of requirements at the lower temperatures. However by setting the irradiance at $>2E_k$ of the highest temperature, the risk of exposing the cells to levels of irradiance they cannot tolerate at lower temperatures appears. The cells will either have to employ strategies to ameliorate the effect of the excess irradiance, or are likely to suffer photo damage - both of which will impact on growth rate negatively.

Temperature limits for growth

The poor growth response at 11.9 °C indicates that this is the lower temperature limit for growth in this strain of *G. catenatum*. It is clear that temperature was limiting growth response because irradiance had little effect (only one culture grew at 0.021 d^{-1} in this treatment at irradiance of 175 μmol photons m^{-2} s^{-1}). Growth for GCHU02 was within the range of lower temperature limits reported by earlier studies (Ellegaard et al., 1993, Band-Schmidt et al., 2004, Bravo & Anderson, 1994, Yamamoto et al., 2002, Blackburn et al., 1989) in which growth ceased at temperatures ranging from 9 to 12.5 °C.

At 28.5 °C, *G. catenatum* growth rates calculated from fluorescence were not significantly different from zero. The Spanish strain (from water temperatures rarely

exceeding 20 °C) also showed growth rates of that decreased very quickly to near 0 d^{-1} growth rates at temperatures close to 28.5 °C (Blackburn et al., 1989, Bravo & Anderson, 1994), and the Australian GCHU01 strain examined by Blackburn, *et al.* (1989) had a growth rate close to 0 d^{-1} at 25 °C. Other strains isolated from Japan and Mexico (where water temperatures can reach 28 °C in Japan and in excess of 28 °C in Mexico) exhibited growth rates > 0.2 d^{-1} at 28.5 °C and still had relatively high growth rates at 30 °C (Band-Schmidt et al., 2004, Yamamoto et al., 2002). The ability of these two strains to grow better at higher temperatures may indicate there are ecotypes of *G. catenatum* better suited to sub-tropical water temperatures within the *G. catenatum* complex. The *G. catenatum* strain isolated from cysts in the sediments near Island Hven in Denmark showed growth rates similar to the other strains at temperatures from 10-15 °C but exhibited growth similar to the Mexican and Japanese strain at 28.5 °C. The Spanish strain (Bravo & Anderson, 1994) was similar to all other strains at temperatures from 10-15 °C, above 20 °C growth rates of this strain are greater than all the other strains.

While growth rates were negligible at 28.5 °C and 291 and 458 μmol photons m^{-2} s^{-1}, in the present study the majority of cells were still motile and appeared morphologically normal at the end of the experiment (characteristics expected from healthy cultures of *G. catenatum*). It appears the cultures were able to acclimatise sufficiently to the temperature extreme, but that under these conditions no growth (measured by the fluorescence method) was observed

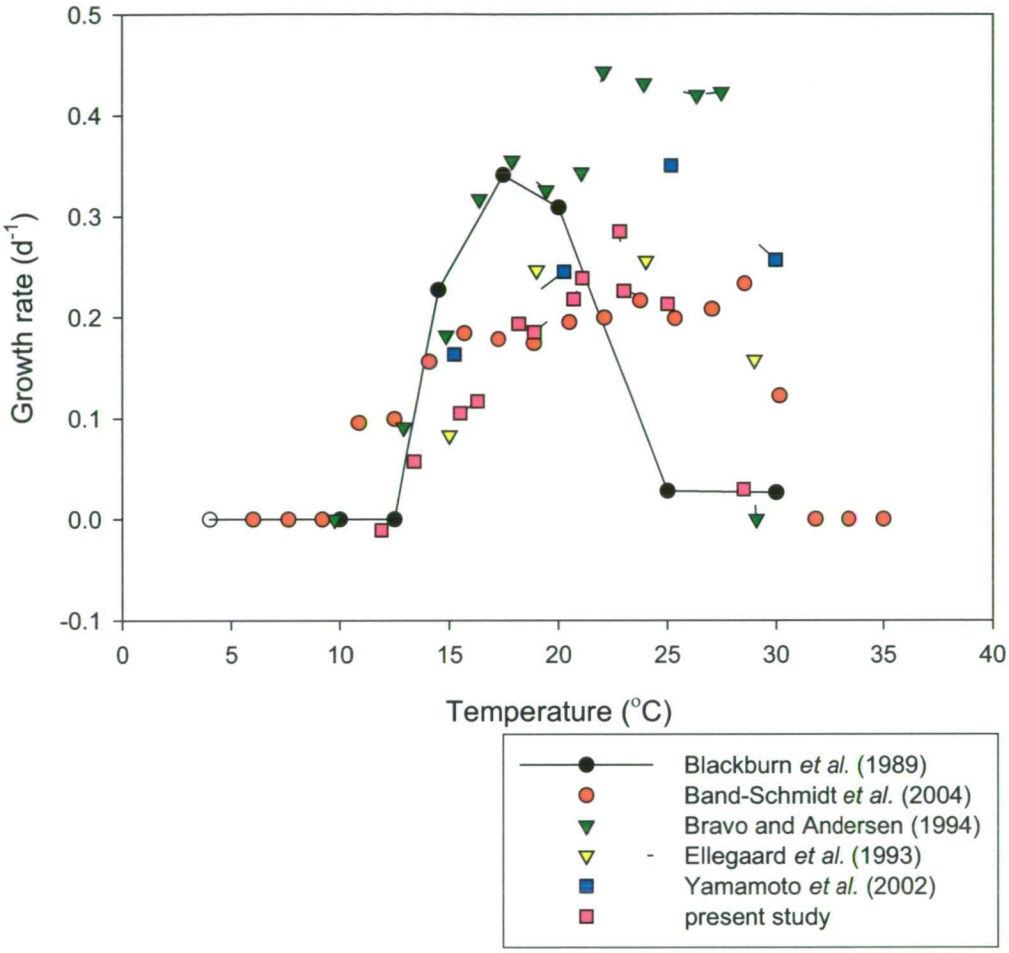

Figure 3.23 Effect of temperature on *Gymnodinium catenatum* growth rate in different studies. All growth rates were adjusted based on the daily irradiance of the present study (9.07 mol photons m^{-2} day^{-1}).

Table 3.7 Conditions for different experiments on effect of temperature on growth rate of *Gymnodinium catenatum*.

Study	Strain	Isolation location	Medium	Salinity	Irradiance (mol photons m^{-2} day^{-1})	Light: dark cycle
Blackburn et al. (1989)	GCHU01	Huon Estuary, Australia	GPM mod	26	6.48	12:12
Band-Schmidt et al. (2004)	GCCV10	Bahia Concepcion, Mexico	F2 mod	30	8.28	10:14
Bravo and Andersen (1994)	Not Specified	Galicia, Spain	K	N/A	7.56	14:10
Ellegaard et al. (2002)	Not Specified	Island Hven, Denmark	GPM mod	28	8.64*	16:8
Yamamoto et al. (2002)	Not specified	Hiroshima Bay, Japan	F2 mod	30	7.77	12:12
Present study	GCHU02	Huon Estuary, Australia	GSe	28	9.07	14:10

*** Irradiance N/A so used 150 µmol photons m^{-2} s^{-1} as this was the lowest irradiance used in the other studies.**

A comparison of growth vs. irradiance curves between the present study and (Yamamoto et al., 2002) showed that at lower temperatures (~15 °C) μ_{max} and α were not different between these strains. There was a difference between E_c, but it is likely that this may be an artefact of the small amount of data points on the initial slope in both studies.

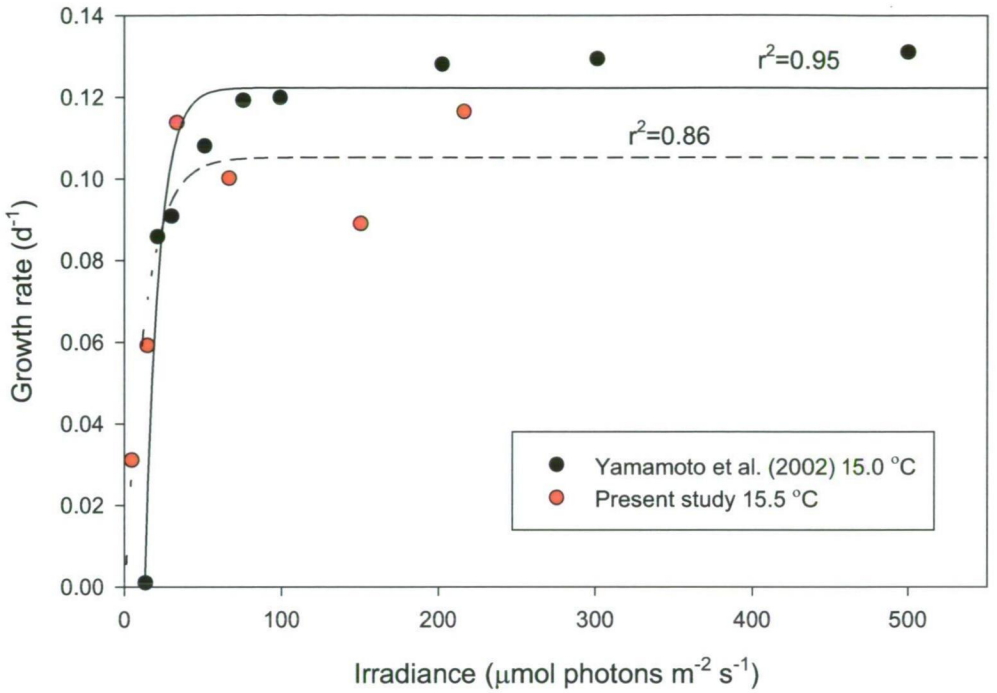

Figure 3.24 Gymnodinium catenatum growth vs. irradiance comparison: the modified model of Platt et al. (1975) (Equation 2) **is fitted to data from 15.0 °C** (Yamamoto et al., 2002) **and 15.5 °C from the present study.**

Based on mean temperature for the Huon Estuary (Figure 3.25) and results from the present study, some inferences can be made about growth of *G. catenatum* at different times of the year in the Huon Estuary. Between 150-250 days (winter approaching early spring) temperatures in the surface are ~11 °C or less and sub-surface temperatures are ~12 °C. Based on our experimental results *G. catenatum* will not be able to grow at temperatures this low. Temperatures at 50 days are ~16 °C and during summer in the Huon Estuary temperatures of 20 °C are not uncommon. But at temperatures ranging from 16-20 °C and saturating light *G. catenatum* will only be capable of growth ~0.1-0.2 d^{-1}.

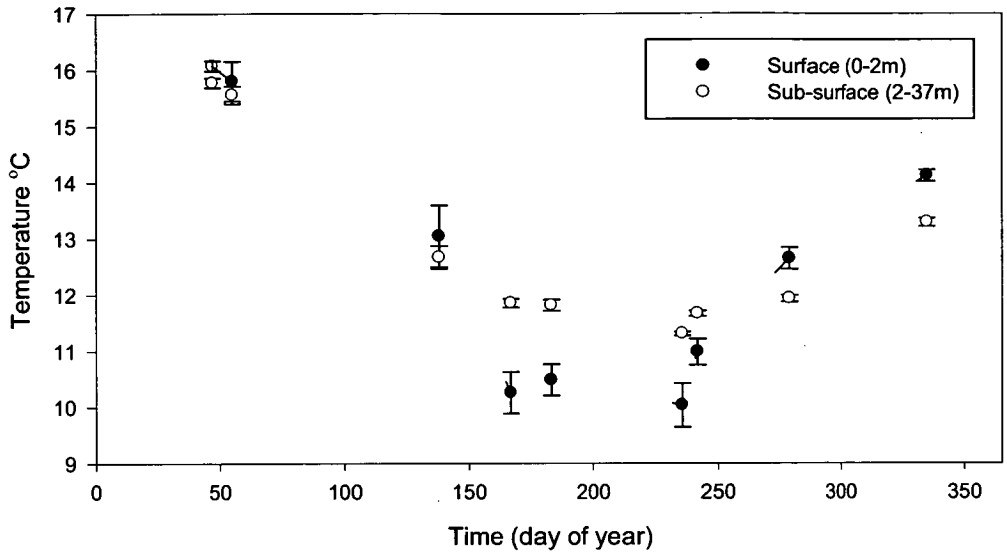

Figure 3.25 Mean (+/- 1 SE) temperature in the surface (0-2m) and sub-surface waters of the Huon Estuary (1996-1998). Data for the lower estuary (from Port Huon to Huon Island) from the Huon Estuary Study (CSIRO Huon Estuary Study Team, 2000).

3.4.2.2 Initial slope (α)

The initial slope (α) for *G. catenatum* was similar to that of other dinoflagellates (Prezelin & Sweeney, 1977, Garcia & Purdie, 1992). Dinoflagellates have been shown to be less efficient at converting light to growth and have lower initial slopes of the μ versus irradiance curve than diatoms (Langdon, 1987, Rivkin *et al.*, 1982). At temperatures greater than 18 °C, α decreases in order of magnitude from 0.03 to 0.002 and fits a line with a negative slope (r^2=0.81). This means that the growth efficiency of *G. catenatum* decreased as the temperature increased above 18 °C. Several studies have shown that respiration rate increases with temperature (Collins & Boylen, 1982, Vona *et al.*, 2004, Verity, 1982a, Verity, 1982b) It seems likely that the decreased growth efficiency of *G. catenatum* in this study is the result of increased respiration at these higher temperatures. However of the few studies that have looked at the effect of temperature on α, some show no effect of temperature (Thompson, 1999, Geider et al., 1985) while a few show an effect of temperature on α (Verity, 1982b, Palmisano et al., 1987). Most of the studies that have investigated

the effect of temperature on α were conducted on diatoms, chlorophytes and cyanophytes. There are no papers that examine the effect of temperature on α in dinoflagellates in any detail. The reason why *G. catenatum* exhibits an effect of temperature on α but isn't seen in other phytoplankton groups could be that diatoms and other groups have a much smaller respiration rate standardized to biomass, and that changes in α due to temperature may be difficult to elucidate. In comparison, dinoflagellates have a higher respiration rate standardized to biomass (Chan, 1978), also evidenced by their higher compensation irradiances (Langdon, 1988). Because of these differences in respiration/biomass the effect of temperature on α may be more easily observed in dinoflagellates and other species that have greater respiration/biomass.

3.4.2.3 Irradiance co-efficient (E_k)

E_k increased exponentially with temperature in *G. catenatum*. Our results were consistent with those of Collins and Boylen, (1982) whose study on the cyanobacteria *Anabeana variabilis* shows an exponential relationship between E_k and temperature. Few other studies have investigated the effect of temperature on E_k.

3.4.2.4 Compensation irradiance (E_c)

Dinoflagellates exhibit some of the highest compensation irradiances (Langdon, 1987), however they also exhibit a wide range of compensation irradiances in some cases comparable to diatoms which are considered to be one of the most efficient classes (Langdon, 1988). The compensation irradiance in our study ranged quite low (<1 μmol photons m^{-2} s^{-1}), comparable to some diatoms (Yoder, 1979, Langdon, 1987, Thompson, 1999), to relatively high (>20 μmol photons m^{-2} s-1) but not unusual for dinoflagellates (Langdon, 1987, Chan, 1978). There appeared to be a trend of increasing compensation irradiance with temperature in *G. catenatum* as might be expected if the respiration rate is increasing with temperature. It has been demonstrated (Langdon, 1988, Langdon, 1987) that E_c is positively correlated with the maintenance respiration (i.e. more energy derived from light by photosynthesis is

needed to match the higher respiration rate and hence the irradiance at which photosynthesis equals the respiration rate will be higher). Increases in E_c have also been observed when a culture is acclimated to a higher irradiance prior to determining an instantaneous photosynthesis or growth versus irradiance curve (Iriarte & Purdie, 1993). At high temperatures respiration is increased, because chemical processes are able to proceed at a higher rate. Under high light the base level respiration is also increased as the cell implements strategies to protect itself from the excess light. The present study and several others have shown that E_c can increase with temperature (Verity, 1982b, Palmisano et al., 1987) however this is not the case in all studies (Thompson, 1999, Li & Morris, 1982, Geider et al., 1985). Once again there is a lack of studies that examine the effect of temperature on E_c in dinoflagellates. However, it is possible that because of higher respiration rates in dinoflagellates there is no effect of temperature in E_c or that changes in E_c were significantly less and thus not detected in other more light efficient groups like the diatoms and chlorophytes.

3.4.3 Effect of temperature and irradiance on the bio-chemical composition of *G. catenatum*

Cellular energetics will be used as a framework for understanding the physiology of *G. catenatum* while discussing the effect of the temperature and irradiance on biochemical composition. Some terms and concepts described in Richardson, *et al.*, (1983), including "running costs", "capital costs" and "maintenance costs" will be used. Brief definitions of these terms are given below:

- Running costs: energy costs for running particular pathways, i.e. the AT P and reductant input per unit net product.

- Capital costs: energy costs associated with the synthesis of catalytic and structural cell material.

- Maintenance costs: energy costs associated with cell survival, i.e. re-synthesising protein or pigments that are continually breaking down.

Running costs are not considered important for responding to energy limitation, as biophysical and biochemical properties of organisms are very similar. However, reducing capital and maintenance costs are considered important for phytoplankton to adapt to energy limitation. For example, by reducing cell biomass, the cell requires less energy to divide, because a smaller amount of cell material needs to be synthesised, thus reducing capital costs. This also means that less cellular materials need to be repaired and resynthesised for the cell to survive, reducing maintenance costs.

The mean C, N and C:chl*a* averaged across cultures grown at all temperature and irradiances were highly variable as indicated by the co-efficient of variation of 33% for C:N and 47% for C:chl*a*. This indicates that there was an effect of temperature and irradiance on the biochemical composition, caused by *G. catenatum* adapting to growth over a wide range of conditions. The effect of temperature and irradiance on the biochemical composition will be discussed in detail in the following paragraphs.

The carbon and nitrogen quota of *G. catenatum* in this study displayed the 'u' shape that has been observed in a variety of species and different groups (Goldman & Mann, 1980, Goldman, 1977, Jorgensen, 1968, Thompson *et al.*, 1992) including a dinoflagellate (Nielsen, 1996) in response to temperature. Some studies have not found this 'u' shaped response, however these may be an artefact of the temperature range being too small or the irradiance being too high. Goldman (1977) explained this 'u' shaped response by asserting that it was "a temperature dependent uncoupling between growth and nutrient uptake, which is manifested as a change in cell size". The results from this study suggest that the accumulation of carbon and nitrogen are a response to unfavourable conditions of temperature and irradiance for growth and are described below.

Growth rate of *G. catenatum* is < 0.1 d^{-1} under two different scenarios in this experiment: when temperature drops below 16 °C, and at temperatures >20 °C and irradiance < 100 µmol photons m^{-2} s^{-1}. When these scenarios are examined energetically they are quite different. While C and N are accumulated under both scenarios, I suggest that their uses will be different because of the underlying differences in the energy status of the cell at these different conditions.

At low temperature the cells energy demand will be lower because of reduced respiration as indicated by the steeper initial slope and relatively low E_c measured in this study. Hence at low temperature, energy limitation is not the primary issue; but the impaired ability of the cell to process CO_2 because of the effect of lower temperature on the enzymes responsible for this process would be explain the increase in C and N (Morris & Glover, 1974, Raven & Geider, 1988). To counter this the cell produces more enzymes and hence this increases the C and N quotas.

At high temperature and low irradiance the cell is under severe energy limitation, because of higher energy demands at increased temperature and not enough light to meet those demands. In addition, the degradation of pigments and other cellular components speeds up at higher temperatures. *G. catenatum* responds by increasing quota of chl*a* so that it can harvest more energy from the available light. It is also suggested that the increase in carbon and nitrogen quota is related to the cell building more chloroplast and other structures required for increasing light harvesting capability. This would seem consistent with the low carbon and nitrogen quotas at the same temperature but higher irradiances. If increased carbon and nitrogen quotas were associated with thermal protection it would be expected that the quotas would be still high when irradiance increases.

Each of these coping mechanisms related to the effects of temperature directly on the cell or indirectly by causing energy limitation contribute to increasing both the capital and maintenance costs of the cell. It is a testament to the adaptability of phytoplankton to be able to maximise growth under a wide range of temperature and

irradiance conditions and also have strategies for protection and survival in extreme temperature and irradiance conditions.

3.4.4 Carbon:chlorophyll *a*

Eliminating irradiances that were not saturating, C:chl *a* was binned across irradiances > 200 umol photons m^{-2} s^{-1} and plotted against temperature, finding that C:chl*a* decreased exponentially (r^2=0.78) as temperature increased. This exponential decrease in C:chl*a* at saturating irradiance with temperature is consistent with the relationship predicted by the generalised model of Geider (1987) for effect of temperature and light on C:chl*a*. However, the C:chl *a* decreases as irradiance increases at 22.9 and 25.0 °C in contrast to the prediction of Geider's model. These results highlight the generalised nature of Geider's model and that *G. catenatum* is an example of a species which exhibits different responses in C: chl*a* to irradiance at high temperatures.

An empirical model for the effect of temperature and irradiance on the C: chl*a* of *G. catenatum* was developed using the C and chl *a* quotas obtained at different temperature and irradiances in this study. The model for *G. catenatum* predicts an increase in C:chl *a* at combinations of high temperature and low irradiances in spite of a two times increase in chl*a* which would drive the C:chl *a* down if it were not offset by a much larger increase in carbon. The much higher C:chl *a* values at low temperatures are the result of the large increase in C quota while the changes in chl*a* were relatively small in comparison.

An empirical model of C:chl *a* for a dinoflagellate that exhibits a carbon accumulation strategy under conditions unfavourable for growth may be useful for understanding primary production in systems that are dominated by dinoflagellates. However, we need to understand whether other dinoflagellates show similar responses to light and temperature before this a C:chl a model like this could be extrapolated to the dinoflagellates for predictive modelling.

3.4.4.1 Energetics model

Under light saturating conditions a combination of high but decreasing capital costs and a smaller increase in respiration with temperature result in an almost linear increase in μ_{max} from 11 °C up to the greatest μ_{max} at ≈23 °C . There is a sharp decline in μ_{max} as respiration begins to increase much faster at temperatures greater than 23 °C. These are consistent with our understanding of the variation in physiological processes as the cell's maintenance costs become greater than the capital costs and the energy input from the cell can no longer meet the cellular energy requirements as temperature approaches the physiological maximum. Therefore, μ_{max} is reduced and eventually the cell will no longer be able to survive at temperatures ≈28.5 °C, where the cell cannot resynthesise and repair its components faster than they are breaking down.

Under light limiting conditions, the energy input is reduced. This manifests itself as lower μ_{max} across the range of temperatures and also a lower maximum temperature where growth becomes zero. Under light limiting conditions capital costs play an important role in determining the rate of μ_{max} increase up to its greatest μ_{max} at ≈ 20 °C. In contrast to light saturating conditions, capital costs increase above the greatest μ_{max} presumably because the cell is building a greater light harvesting capacity. The increased capital costs at low light and high temperatures were the main reason for the rapid decrease in μ_{max} (in light saturating conditions increases in respiration were more important). It is because of the lower available energy (even after the cell increases its light harvesting capacity) that growth ceases at a lower maximum temperature (≈26 °C).

3.4.5 Summary

This is the most comprehensive study of the effect of irradiance and temperature on *G. catenatum* growth and physiology to date. Growth versus irradiance curves been determined for twelve temperatures from 11.9-25.0 °C and a comparison of the growth rates from the present study with five other studies on different *G. catenatum*

strains has revealed similarity between the growth responses of these strains particularly at temperatures between 10-20 °C. At temperatures >= 28.5 °C the strains from Japan and Mexico appeared to be able sustain growth rates approximately 0.2 d^{-1}, whilst the other strains did not grow. Indicating that there may be evidence for a tropical ecotype better suited to growth at higher temperatures.

The biochemical composition of *G. catenatum* has also been examined revealing that *G. catenatum* is able to adapt to low temperature conditions by increasing its Carbon quota. At higher temperatures and lower irradiances *G. catenatum* responds by increasing chl*a* but this is accompanied by an even greater increase in C quota. These results give us an insight into the strategies that this species uses for growth and survival in unfavourable conditions. An empirical model to describe the effects of light and temperature on C:chl *a* was constructed and may be useful information that helps advance modelling of this species or other similar species in the future.

Relationships between the parameters (μmax, α and E_c) from the growth versus irradiance curves and temperature were investigated and modelled. The models of the effect of temperature on μmax, α and E_c provide the basis for development of a *G. catenatum* growth model. For example this model could predict growth rates for *G. catenatum* in the Huon Estuary using modelled 3D high resolution temperature and irradiance data (Herzfeld *et al.*, 2005). A simple model of *G. catenatum* growth rate would enable comparison with historical bloom data and may helpgive to better understand the role of temperature and irradiance in the development of blooms of this species.

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4 NITROGEN PREFERENCE AND UPTAKE BY *GYMNODINIUM CATENATUM* IN CULTURE

4.1 Introduction

The toxic dinoflagellate *Gymnodinium catenatum* forms dense blooms in many coastal areas and estuaries around the world (Blackburn *et al.*, 1989, Carrada *et al.*, 1991, Fraga *et al.*, 1992, Holmes *et al.*, 2002, La Barbara-Sanchez & Gamboa-Maruez, 2001, Matsuoka & Fukuyo, 1994, Mee *et al.*, 1986, Nehring, 1995, Park *et al.*, 2004, Ramirez-Camarena *et al.*, 1999, Sordo *et al.*, 2001, Oh *et al.*, 2002). Phytoplankton productivity in the majority of these ecosystems are limited by nitrogen (N) (Boynton *et al.*, 1982), therefore, improving our understanding of the nitrogen uptake strategies that allow this relatively slow growing organism to dominate is critical for understanding bloom dynamics and improving predictive modelling for this species (McCarthy, 1972, McCarthy *et al.*, 1982).

There has been considerable research on the effect of physical conditions (e.g. temperature and/or salinity) on the physiology of *G. catenatum* (Band-Schmidt *et al.*, 2004, Blackburn et al., 1989, Bravo & Anderson, 1994, Ellegaard *et al.*, 1993, Yamamoto *et al.*, 2002) as well as nitrate (NO_3^-) and ammonium (NH_4^+) uptake kinetics (Flynn *et al.*, 1996, Yamamoto *et al.*, 2004) However, there is little information on the N preferences of this species or its ability use urea for growth, a N source whose importance is becoming increasingly recognised (Glibert *et al.*, 2004, Twomey *et al.*, 2005). With increased N-rich anthropogenic inputs to many of our estuaries and coastal ecosystems, there may be potential for HAB species to exploit changes in macronutrient ratios. Changes in these ratios (e.g. N:P or N:Si) can cause changes in phytoplankton composition. This has been demonstrated in cultures (Baek *et al.*, 2008), mesocosms (Escaravage & Prins, 2002), and also observed in the field

(Riegman *et al.*, 1992, Heil *et al.*, 2007). Furthermore, not only are macronutrient ratios important but also nutrient speciation has been shown to play a role in competitive interactions between phytoplankton (Riegman *et al.*, 1996, Heil et al., 2007, Baek et al., 2008)

The majority of coastal systems are N limited, and the ability for some species to use, or have enhanced growth rates on different N species can give a competitive advantage. Some species have increased growth on NH_4^+ when compared with NO_3^- or urea (Chang *et al.*, 1995, Leong & Taguchi, 2004, Chang & McClean, 1997). Other species are unable to grow using particular N species. For example, some species cannot use urea as a sole source (Lourenco *et al.*, 2002, Yamaguchi *et al.*, 2001) or growth is reduced when urea is utilised (Yamaguchi & Itakura, 1999, Tungaraza *et al.*, 2003). Some HAB species appear to prefer urea as a N-source, suggesting that urea may be important for bloom development (Kana *et al.*, 2004, Berg *et al.*, 1997). Riegman *et al.* (1996) has also demonstrated in laboratory cultures how the use and affinity for different N sources may cause changes in species composition of mixed phytoplankton communities.

G. catenatum bloom populations in the Huon Estuary, south east Tasmania are known to undergo diurnal vertical migration (DVM). It has been hypothesised that this allows access to NH_4^+ available at depth (Doblin *et al.*, 2006, Team, 2000). This could provide a distinct competitive advantage when N has been depleted in the upper layer of the estuary. To be able to test this hypothesis we sought to determine first if NH_4^+ or urea could be used as a sole N-source for growth, and whether NH_4^+ or urea afforded any advantage in growth rate. In addition we also wished to determine whether *G. catenatum* displayed a specific N-source preference when simultaneously exposed to similar concentrations of NH_4^+, NO_3^- or urea.

4.2 Methods

Three strains of *G. catenatum*; GCHU02, GCVS04 and GCLV01 were used in the following experiments. A pre experiment investigating the toxicity of ammonium used GCHU02, GCHU11 and GCDE08 strains. All the *G. catenatum* strains used in these experiments are non-axenic.These strains are maintained at the CSIRO Collection of Living Microalgae in GSe medium at 20 °C. The collection details for each of these strains are in Table 4.1. Individual experiments had different temperature and irradiance conditions because these experiments had to be carried out in temperature controlled rooms used primarily for maintaining the CSIRO collection of microalgae. So the temperature and irradiance in each experiment was determined by which temperature controlled room could be used for the experiment. Temperature and irradiance conditions are listed in the descriptions of each experiment.

Table 4.1 *Gymnodinium catenatum* strain details.

Collection Code	Source Locality	Isolation Date	Isolator
GCHU02	Huon Estuary	6/6/1986	S. Blackburn
GCHU11	Huon Estuary	20/6/1988	S. Blackburn
GCVS04	Huon Estuary (Verona Sands)	2002	M. de Salas
GCLV01	Louisville (Triabunna)	2002	M. de Salas
GCDE08	Derwent Estuary	15/6/1987	S. Blackburn

4.2.1 Determining tolerance to ammonium concentrations

Forty ml of GSe medium (Blackburn *et al.*, 2001) (without NO_3^-) was added to 15 culture vessels (70 ml polycarbonate containers (Labserv Biolab Ltd. Auckland New Zealand) with 50 ml test tube adapters (see Figure 3.2 in Chapter 3). These cultures were inoculated with different strains of *G. catenatum*: 5 with GCHU02, 5 with GCHU11 and 5 with GCDE08. NH_4^+ was added to all cultures so that each strain would have a culture growing at 10, 20, 40, 80 and 160 µM concentrations of NH_4^+. Culture conditions were: 20 °C, ≈300 µmol photons m^{-2} s^{-1} and 12:12 L;D cycle. Growth was calculated using measurement of *in-vivo* fluorescence as a proxy for

biomass, every 2-3 days over the 31 day s of the experiment. Growth rate was calculated according to (Guillard, 1973) (see 3.2.7 in Chapter 3).

Based on the results of the experiment above a second experiment was carried out to determine whether growth could be sustained using non-lethal concentrations of NH_4^+ as a sole source of nitrogen. For this experiment, duplicate cultures of HU02 and HU11 were grown with either 10 μM or 20 μM of NH_4^+ as a sole N source. Cultures grown on excess NO_3^- (initial concentration ~800 μM) were used as a control. Growth of all cultures was monitored using *in-vivo* fluorescence as previously described, and growth rates calculated using data from the exponential growth phase.

4.2.2 Growth on ammonium

Cultures of GCHU02 and GCVSO4 were preconditioned by N-starvation by growth in GSe medium without added NO_3^- for 14 days prior to the experiment, so that 'carry over' of NO_3^- into the treatments with different N sources would be minimized. Triplicate cultures of both strains were grown in 70 ml polycarbonate containers with 50 ml test tubes attached (see Figure 3.2 in Chapter 3) containing 47.5 ml of GSe medium without NO_3^- and the addition of 10 μM of NH_4^+. Triplicate control cultures contained 47.5 ml Gse medium containing NO_3^- in excess (~800 μM). All cultures were grown under optimal temperature and saturating light conditions (22 °C and 150 μmol photons m^{-2} s^{-1}) with a L:D cycle of 12:12. As NH_4^+ appeared to have negative effects on growth rate at concentrations of >40 μM, NH_4^+ was maintained at less than 20 μM with regular aseptic additions of sufficient $NH_4^+$to give an increase of 10μM when significant draw-down of NH_4^+ was estimated. Draw-down was monitored every 2-3 days using a spectrophotometric technique developed by Solorzano (1969), further details of this technique are given in Parsons, *et al.* (1984). Cultures were grown for 19 days and cell density monitored using *in-vivo* fluorescence ever 2-3 days and growth rates calculated as described in Chapter 2. Growth rates between days 3 and 6 (when NH_4^+ was not significantly depleted) were used to examine

significant difference between growth using NH_4^+ or NO_3^- as a sole N source and whether there were significant strain-specific responses between GCHU02 and GCVSO4. Statistical analyses used a 2 way ANOVA using the program Sigmastat 2.03 and α-value of 0.05. The data passed both the Kolmogorov-Smirnov test (with Lilliefors' correction) for normality and Levene's median test for equal variance.

4.2.3 Growth on Urea

Strains GCHU02 and GCVS04 were pre-conditioned by growing them in GSe/10 (GSe medium with 1/10 normal concentrations of nutrients) for at least 14 days. When cultures reached ~5 x 10^5 cells l^{-1}, one ml of culture was used as an inoculum for experimental cultures. Triplicate control and treatment cultures of both GCHU02 and GCVS04, were grown in 70 ml polycarbonate containers with 50 ml test tubes attached (see Figure 3.2 Chapter 3). Control cultures contained 50 ml GSe medium containing NO_3^- in excess (~800 μM). Treatment cultures contained 50 ml of GSe medium without NO_3^- and urea added to achieve an initial concentration of 10 μM. Cultures were grown at 23 °C and 160 μmol photons m^{-2} s^{-1} with a L:D cycle of 18:8. Cultures were grown for 14 days and cell density monitored using *in-vivo* fluorescence every 2-3 days and growth rates calculated as previously described (see 3.2.7 in Chapter 3). The concentration of urea was not monitored as the experiment progressed. However, assuming a maximum growth rate of ~0.3 d^{-1}, the N requirements for *G. catenatum* were calculated for the experimental period and urea was added periodically to make sure urea was in excess.

After the first 14 day experimental period each culture was used as an inoculum for a second set of experimental cultures. The second sets of cultures were inoculated to have the same starting cell densities. Growth rates of these cultures were monitored for a further 19 days and growth rates calculated as before. Growth rates from the first and second round of cultures were compared using a 2 way ANOVA using the program Sigmastat 2.03 and α-value of 0.05. The data passed both the Kolmogorov-

Smirnov test (with Lilliefors' correction) for normality and Levene's median test for equal variance.

4.2.4 Preferential uptake of different nitrogen species

Prior to the experiment, cultures of *G. catenatum* strains GCHU02, GCVS04 and GCLV01 were pre-conditioned by being grown for 14 days in GSe/10 (GSe at 1/10 concentration). After 14 days cell counts were undertaken to allow standardised (same number of cells ml^{-1}) inocula for experimental cultures.

Four 500 ml round bottom glass flasks capped with steri-stoppers manufactured by Heinz Herenzwere used because a larger volume of culture was required so that 2 ml samples could be removed from the culture every 3-5 days. The round bottom flasks provide better conditions than other culture vessels, presumably because of the large surface area that these vessels provide when 200-400 ml of media is added. When in culture *G. catenatum* has been observed to aggregate just below the surface and when provided with a larger surface areas grows better. These culture vessels had 420 ml of GSe with no added NO_3^-, and were inoculated with GCHU02, GCHU11, GCVSO4 or GCLV01 at an initial concentration of 2.9×10^3 cells l^{-1}. NH_4^+, NO_3^- and urea were all added aseptically at an equi-molar concentration of $\approx$4 µM. The cultures were then grown for 29 days under these conditions: 23.5 °C, 316 µmol photons m^{-2} s^{-1} and L:D 18:8. The GCHU11 culture displayed a much longer lag phase before it began to grow, for this reason it was excluded from the results for the purposes of this study.

Samples were taken at 3-5 day intervals throughout the experiment and stored at -20 °C for later analysis of NH_4^+, NO_3^- and urea concentration. Ammonium was measured using the technique of Kerouel and Aminot (Kerouel & Aminot, 1997) adapted for flow injection (Watson *et al.*, 2004)with a detection limit of ~0.05 mM. Nitrate and/or nitrite (Wood *et al.*, 1967), was measured using Quick-Chem™ methods on a flow injection LACHAT instrument as per the following protocols for

nitrate and/or nitrite (Quik-Chem™ Method 31-107-04-1-A; detection limit 0.03~ mM), Urea was analysed by a spectrophotometric technique to quantify the reaction of urea with diacetylmonoxime (Mulvenna & Savidge, 1992). To reduce the health risks associated with thio-semicarbazide, a reagent for this technique, we substituted semicarbazide (a less toxic but similar compound).

Samples for microscopic cell counts were also taken and counts carried out using 1ml samples in a Sedgewick-Rafter counting cell (ProsciTech Queensland, Australia). Phytoplankton in these samples were preserved using Lugol's iodine fixative solution (110 g potassium iodide, 50 g iodine, 1 litre distilled water, 100 ml glacial acetic acid) to approximately 2% final concentration. Cell counts were performed using an Olympus IX71 microscope at a magnification of 200x. After day 12, cell counts became unreliable due to an increasing proportion of the cells adhering to the bottom of the flasks. Some of these cells did not resuspend with gentle hand swirling, therefore specific uptake rates are only presented for the period prior to day 12.

An estimate of specific uptake was made by multiplying cell counts by the average N per cell. This estimate of N accumulation in the *G.catenatum* cells over time was then used to calculate the specific uptake. The average N per cell (587 pg N $cell^{-1}$) used in this calculation was determined in Chapter 3 of this thesis.

4.3 Results

4.3.1 Growth on different nitrogen species

Growth was observed for *G. catenatum* in GSe with addition of NH_4^+ at 10 µM and 20 µM, however, no growth was observed at concentrations above 40 µM, indicating that cells were dying. These results were confirmed by the growth of *G. catenatum* on NH_4^+ solely at both 10 and 20 µM. The growth rate achieved at 20 µM growth was not significantly different from that achieved on NO_3^- in the initial stages of the experiment. The growth rate at 10 µM NH_4^+ was significantly lower (by almost half

(P=0.02)) in comparison to the NO_3^- control, although there was no significant difference between growth on 10 or 20 μM of $NH4^+$.

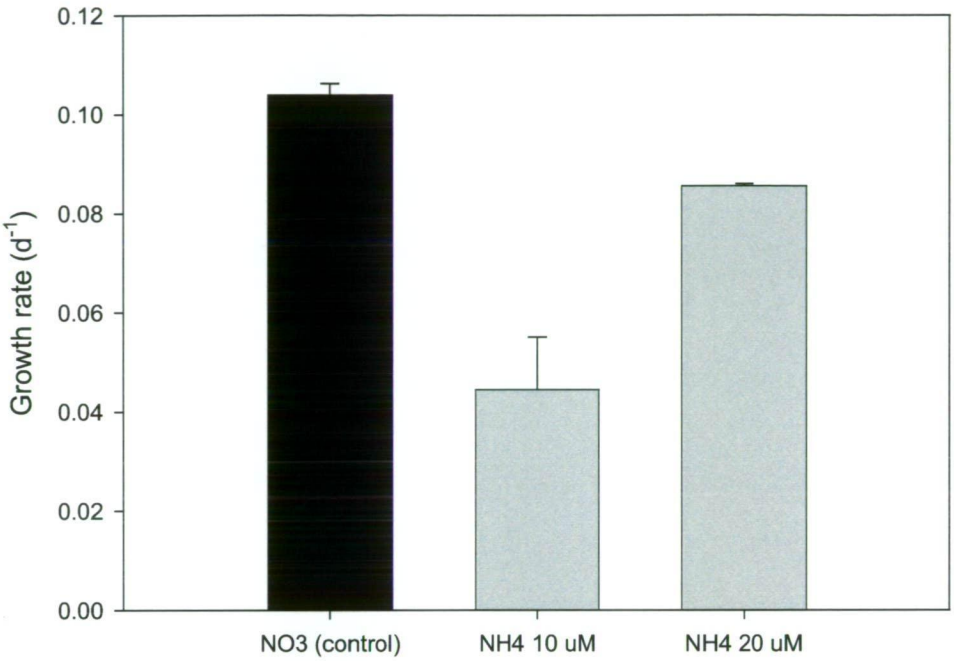

Figure 4.1 Mean growth of G. *catenatum* (GCHU02, GCHU11 and GCDE08) at different concentrations of NH_4^+. Subscripts indicate significant differences between treatments. Error bars are +1 standard error from the mean (n=3).

In the second set of experiments, where NH_4^+ concentrations were kept > 10 μM and less than 40 μM, there were no significant differences in *G. catenatum* growth rates when grown on NO_3^- or NH_4^+ (P=0.161) (Figure 4.2) and NO_3^- or urea (P=0.852) (Figure 4.3). Neither was there a significant difference between growth response of the GCHU02 and GCVSO4 strains when grown on NO_3^- or NH_4^+ (0.397) (Figure 4.2) and NO_3^- or urea (P=0.857) (Figure 4.3)

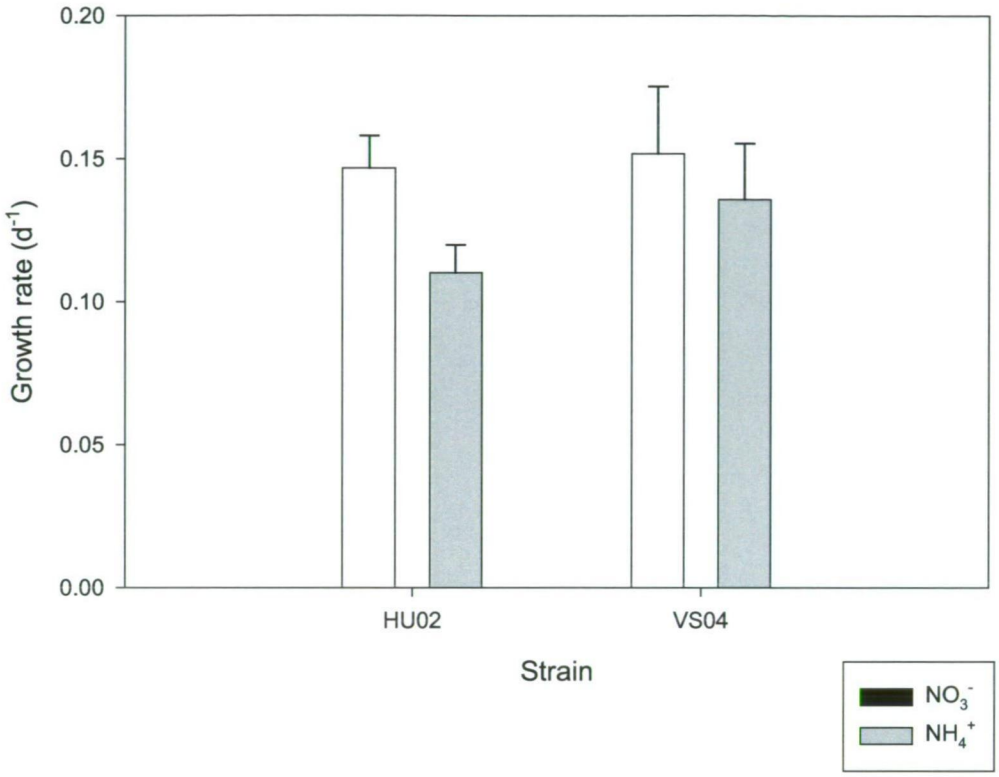

Figure 4.2 Growth of two strains of *Gymnodinium catenatum* (HU02 and VS04) on NO_3^- and NH_4^+. Error bars are +1 standard error from the mean (n=3).

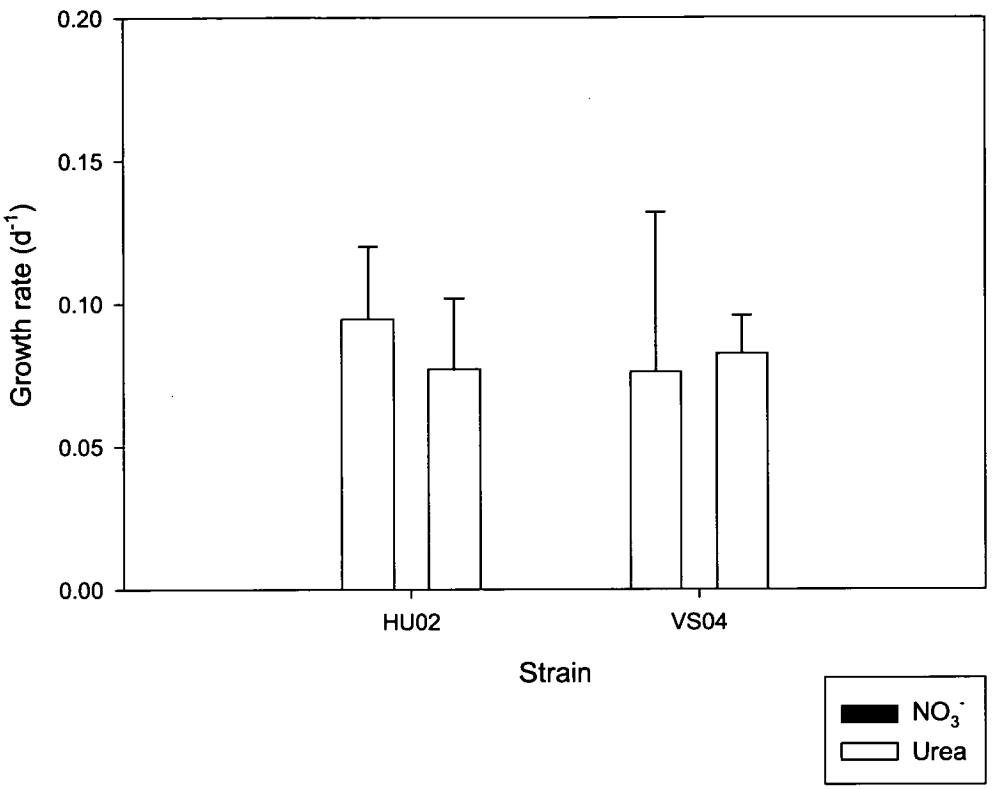

Figure 4.3 Growth of two strains of *Gymnodinium catenatum* (HU02 and VS04) on NO_3^- and urea. Error bars are +1 standard error from the mean (n=3).

4.3.2 Preferential uptake of different nitrogen species

Mean ambient NH_4^+ concentrations decreased sharply from 6.5 to 2.4 μM in the first 4 days (Figure 4.4). From 4 to 7 days mean ambient [NO_3^-] decreased in from 7.7 to 6.5 μM, with an increased rate of disappearance between days 7 and 13. This coincides with the lower availability of NH_4^+ and a decreased rate of disappearance in [NH_4^+] over this same time period. From day 13 to 18 the mean ambient concentration of urea started to be disappear at its fastest rate from 5.4 to 1.6 μM. By Day 18 the ambient [NH_4^+] and [NO_3^-] had reached their lowest. Between day 18 and 29 urea disappearance continues but at a reduced rate compared to the initial rate.

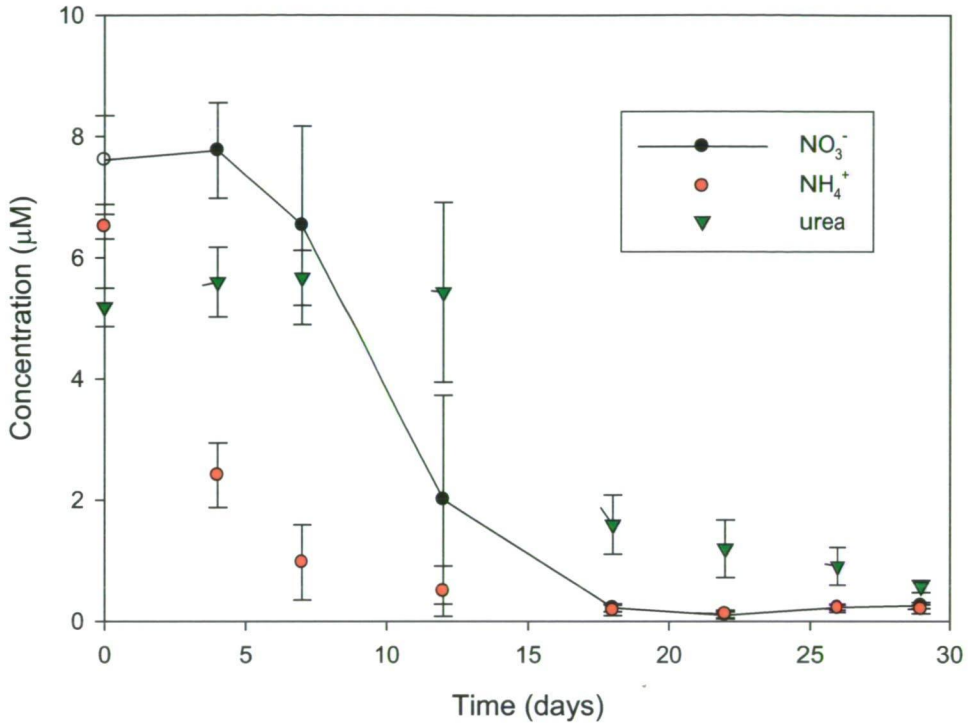

Figure 4.4 Preferential uptake of nitrogen species by *Gymnodinium catenatum*. Mean NO_3^-, NH_4^+ and urea concentrations for *G. catenatum* over the 29 day experiment period (data from HU02, VS04 and LVO1 strains). Error bars are +1 SEM (n=3).

The maximum specific uptake rate for NH_4^+ was 5.3 +/- 0.92 d^{-1} between the 0-4 day period (Figure 4.5). Uptake of NO_3^- was much lower by ≈6 times at 0.9 +/- 0.30 d^{-1} and occurring between days 7-12. Urea uptake peaked between days 12 and 18 at approximately 0.51 +/- 0.40 d^{-1}, about half the uptake rate of NO_3^-.

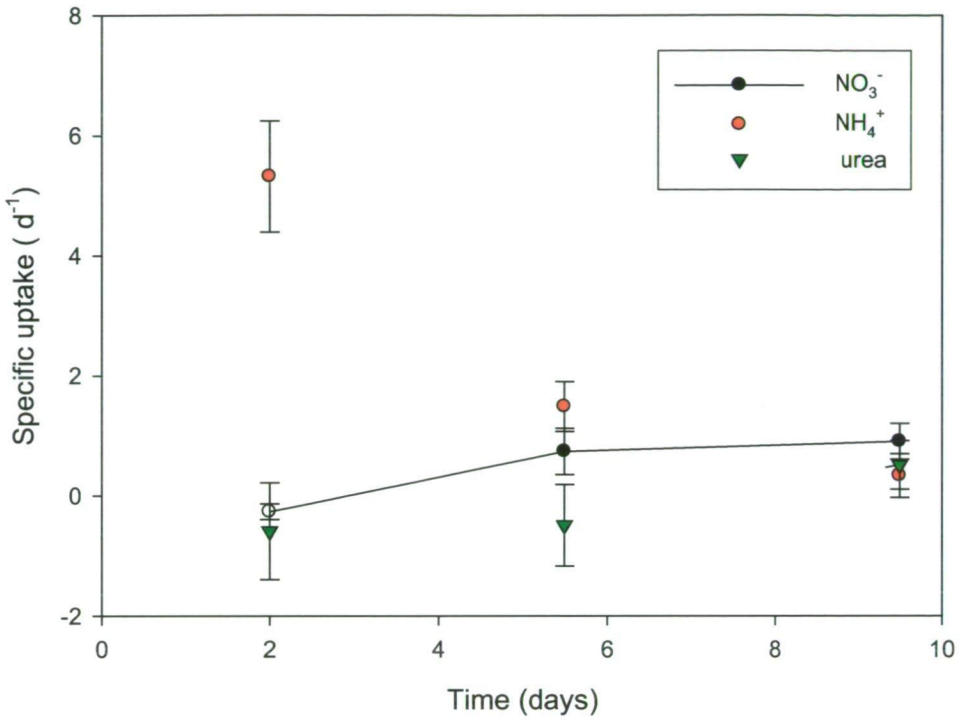

Figure 4.5 Mean uptake rates for NO_3^-, NH_4^+ and urea (data from GCHU02, GCVS04 and GCLVO1 strains) for *G. catenatum* over the 29 day experiment period. Error bars are +/-1 standard error from the mean (n=3).

4.4 Discussion

The strains of *Gymnodinium catenatum* from south east Tasmania are able to use NH_4^+, NO_3^- and urea equally well as sole N-sources for growth. This has been found in other studies, ten species of phytoplankton studied by Lourenco, Barbarino *et al.* (2002) were similar, in that the majority were able to grow soley on NH_4^+, NO_3^- or urea. The studied included 3 chlorophytes, 2 diatoms, a dinoflagellate, a cryptophyte, a cyanophyte, a prymnesiophte and a eustigmatophyte. There were two exceptions *Hillea* sp. (eustigmatophyte) and *Prorocentrum minimum* (dinophyte) which didn't grow on NH_4^+. However, this was probably because the concentrations used were very high (1.18 mM) and therefore likely to be toxic.

The effect of NH_4^+, NO_3^- and urea on growth rate of 4 phytoplankton species was also investigated by Levasseur, *et al.*, (1993). They showed *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *Dunalilella tertiolecta* and *Gymnodinium sanguineum* were all capable of growth using NH_4^+, NO_3^- and urea as sole N sources. However, growth rates decreased for these species investigated when grown on urea. The ability to grow equally well on urea may be an important advantage for *G. catenatum* over other species that cannot grow using urea as a sole N source or have substantially reduced growth rates on N in this form. The ability to grow equally well using all of these N species would be advantageous in an environment where N is the limiting nutrient and it is available in these different forms.

It is important to take into account the toxicity of NH_4^+ at higher concentrations to many phytoplankton. For example, our study showed NH_4^+ was toxic for *G. catenatum* at concentrations >40 μM and in other studies the concentrations may be very high (0.05-1.18 mM) as these concentrations are required for large scale batch algal culture (Lourenco et al., 2002, Flynn et al., 1996) In most oceanic and coastal environments, generally NH_4^+ concentrations experienced are much lower but NH_4^+ fluxes are relatively high, therefore providing a continuous supply of NH_4^+ at low concentrations (i.e. <2 μM) unlikely to be toxic to the majority of phytoplankton. So it is important to investigate the effect of NH_4^+ on growth of phytoplankton species at lower concentrations or non toxic concentrations if these results are going to be applied to understanding the physiology of phytoplankton in natural environments.

We have shown that *G. catenatum* cannot tolerate NH_4^+ concentrations in excess of 40 μM and that NH_4^+ at < 10 μM is limiting growth. This may indicate that *G. catenatum* has a relatively high half saturation constant for uptake of NH_4^+ and growth.

Table 4.2 gives half saturation constants (K_s) for phytoplankton from different groups including: Dinoflagellates, Diatoms, Raphidophytes, Prymnesiophytes, Prasinophytes and Chlorophtyes the majority of phytoplankton species have K_s values for NH_4^+ less

than 3 μM (Table 4.2). Hence, most phytoplankton are able to effectively take up NH_4^+ at low concentrations commonly found in the environment.

Table 4.2 Comparison of half saturation constants(K_s) for NO_3^- and NH_4^+ between phytoplankton species from different classes

Species	Group	NO_3^- Ks (uM)	NH_4^+ Ks (uM)	Reference
Gymnodinium catenatum	Dinophycae	0.42	1.02	Flynn 1996
Alexandrium catenella	Dinophycae	7.7	3.3	Matsuda
Alexandrium tamarense	Dinophycae	2.84	1.49	MacIsaac
Alexandrium tamarense	Dinophycae	1.31	0.12	Leong and Taguchi
Prorocentrum minimum	Dinophycae	5.0		Lomas and Glibert 2000
Gymnodinium splendens	Dinophycae	3.8	1.1	Eppley et al. 1969
Skeletonema costatum	Bacillariophyceae	0.4–0.5	0.8–3.6	Eppley
Phaeodactylum tricornutum	Bacillariophyceae	7	<1	Syrett
Thalassiosira Weissflogii	Bacillariophyceae	2.8		Lomas and Glibert 2000
Skeletonema costatum	Bacillariophyceae	0.4		Lomas and Glibert 2000
Chaetoceros sp.	Bacillariophyceae	3.1		Lomas and Glibert 2000
Chattonella antiqua	Raphidophyceae	2.81	2.19	Nakamura
Micromonas pusila	Prasinophyceae	~0.182	~0.182	Cochlan and Harrison
Pavlova lutheri	Prymnesiophycae	22.7		Lomas and Glibert 2000
Dunaliella tertiolecta	Chlorophyceae	11.1	–	Lomas and Glibert 2000
Dunaliella tertiolecta	Chlorophyceae	1.4	0.1	Eppley 1969

While growth rates of *G. catenatum* were not significantly different on NO_3^-, NH_4^+ or urea, when presented with a mixture of these N species (each at ~ 5-7 μM) preference for uptake is in this order: $NH_4^+ > NO_3^- >$ urea. Uptake rates from the present study of NH_4^+ were initially 5.3 +/- 0.92 d^{-1}, far greater, by ~6-8 times, than uptake rates of NO_3^- and urea respectively at 0.9 +/- 0.30 and 0.5 +/- 0.4 d^{-1}.These results indicate *G. catenatum* has a strong preference for NH_4^+. The uptake kinetic results for NO_3^- and NH_4^+ from Flynn, Flynn *et al.* (1996) would suggest this also. However, uptake kinetics cannot be used to infer uptake preference directly. Based on first principles for balanced growth it would be expected that the N uptake rates of *G. catenatum*

would equal its growth rate. However, it has been shown in several studies that when cells are starved of N, as in this study, that when they are resupplied with N they exhibit uptake greater than their growth rate. This is commonly referred to as "surge uptake" (Parslow *et al.*, 1984b, Parslow *et al.*, 1984a). Our results show that initial uptake of NH_4^+ is almost 20 times the maximum growth rate of *G. catenatum* (0.30 d^{-1}). This surge uptake is also reported by Flynn *et al.* (1996) for *G. catenatum* but the magnitude of increase was ~2.5 times less than in the present study. In the present study the uptake of NO_3^- and urea are also at rates greater than the growth rate but they have relatively large standard errors associated with them. It might be expected that the uptake of N would return to rates similar to the growth rate after an initial surge in uptake to recover after N starvation.

4.4.1 Summary

We have demonstrated that *G. catenatum* is physiologically diverse in its N uptake capabilities. It is able to grow equally well using NO_3^-, NH_4^+ and urea. Why is it then that *G. catenatum* has preferences for NH_4^+ and NO_3^- over urea? Perhaps while it poses no penalties for growth when energy from light is saturating, the preferences may be related to the preferences under energy limited circumstances. For example, *Alexandrium minutum* (dinophyceae) showed variations in growth rate when grown on NO_3^- that were highly dependent on the level of irradiance (Chang & McClean, 1997).

The present study and that of (Flynn et al., 1996) show that *G. catenatum* is capable of responding to pulses of N by 'surge uptake'(compared to max growth rate 0.3 d^{-1}) when it is in a N starved condition. In addition, it has been clearly demonstrated that *G. catenatum* can grow equally well on NH_4^+, NO_3^- or urea. Combined, these physiological capabilities of *G. catenatum* increase its ability to compete for N in the environment and would contribute to its ecological success.

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5 NITROGEN UPTAKE DURING A *GYMNODINIUM CATENATUM* BLOOM AT SOUTHPORT, SOUTH-EAST TASMANIA, AUSTRALIA

5.1 Introduction

It appears that there has been an increase in the occurrence of harmful algal blooms (HABs) in Australia (Hallegraeff, 1992) and around the world (Anderson *et al.*, 2002, Hallegraeff, 1993). The results of these HABs includes fish kills, human health issues including such as PSP, DSP and the loss of biological diversity and productivity. That eutrophication plays an important role directly or indirectly in the stimulation of many HABs is now widely accepted (Anderson et al., 2002, Heisler *et al.*, 2008). Coastal and estuarine systems phytoplankton are primarily nitrogen limited(Downing, 1997). Hence, the increased interest in nitrogen nutrition of HAB species such as *Alexandrium minutum* (Chang & McClean, 1997), *Heterosigma cartarae* (now Heterosigma akashiwo) (Chang & Page, 1995),*Gymnodinium sanguineum* (now *Akashiwo sanguinea*) (Doucette & Harrison, 1991). In addition mechanisms that allow dinoflagellates to bloom when nitrogen availability is low are increasingly under investigation. These include the use of organic N such as urea and behaviours such as the vertical migration to obtain nitrogen from deeper in the water column (Cullen and Horrigan 1981). In some dinoflagellate species this behaviour has been proven to be a strategy for maximising photosynthesis and nutrient uptake where nutrients and light are vertically separated (MacIntyre et al. 1997, Ault 2000). *G. catenatum* has been observed undertaking a strong vertical migration (Toshinori et al. 2001) and in the Huon Estuary, when it is the dominant phytoplankter, it has been observed to vertically migrate from ~ 5m to ~ 20 m on a daily basis (Team, 2000, Doblin *et al.*, 2006). From these observations it was hypothesized that the blooms of

this toxic species may be facilitated by its vertical migration and possible access to nitrogen from depth. Large blooms of *G. catenatum* were rare in the Huon Estuary in 2002, 2003 and 2004. So our 2003-2004 field trips, while providing information on the N uptake by the mixed phytoplankton community, were unable to provide information about the N uptake strategies of *G. catenatum* during a bloom. Discovery of a significant bloom of *G. catenatum* at Dover and Southport, some 15 km south of the Huon Estuary, allowed us to prepare a field trip to measure N uptake of *G. catenatum* in the field, and to specifically investigate the uptake of N during vertical migration of this species.

5.2 Methods

The N uptake experiments were set up in Southport adjacent to Pelican Island (latitude 43° 27' 00" S longitude 146° 58' 28" E) in autumn 2004 on the 30-31 March. During this field trip the same ^{15}N tracertechnique as used in the Huon Estuary (Chapter 4) was used to measure the uptake of three different nitrogen (N) sources (NH_4^+, NO_3^- and urea) by the natural phytoplankton assemblage. Based on the observations of vertical migration of *G. catenatum* in the Huon Estuary (Team, 2000) it was intended that suitable depths would be selected based on the depth of the chl*a* maximum as indicated by fluorescence profile of the water column. Because of technical limitations a vertical profile of fluorescence could not be obtained for the day sampling. Alternatively, we sampled near the surface (5m) and also near the bottom (15m) during the day. For the night sampling a fluorescence profile was obtained prior to sampling and sampling was undertaken near the surface (5m) and also at the depth of the fluorescence maximum (10m).

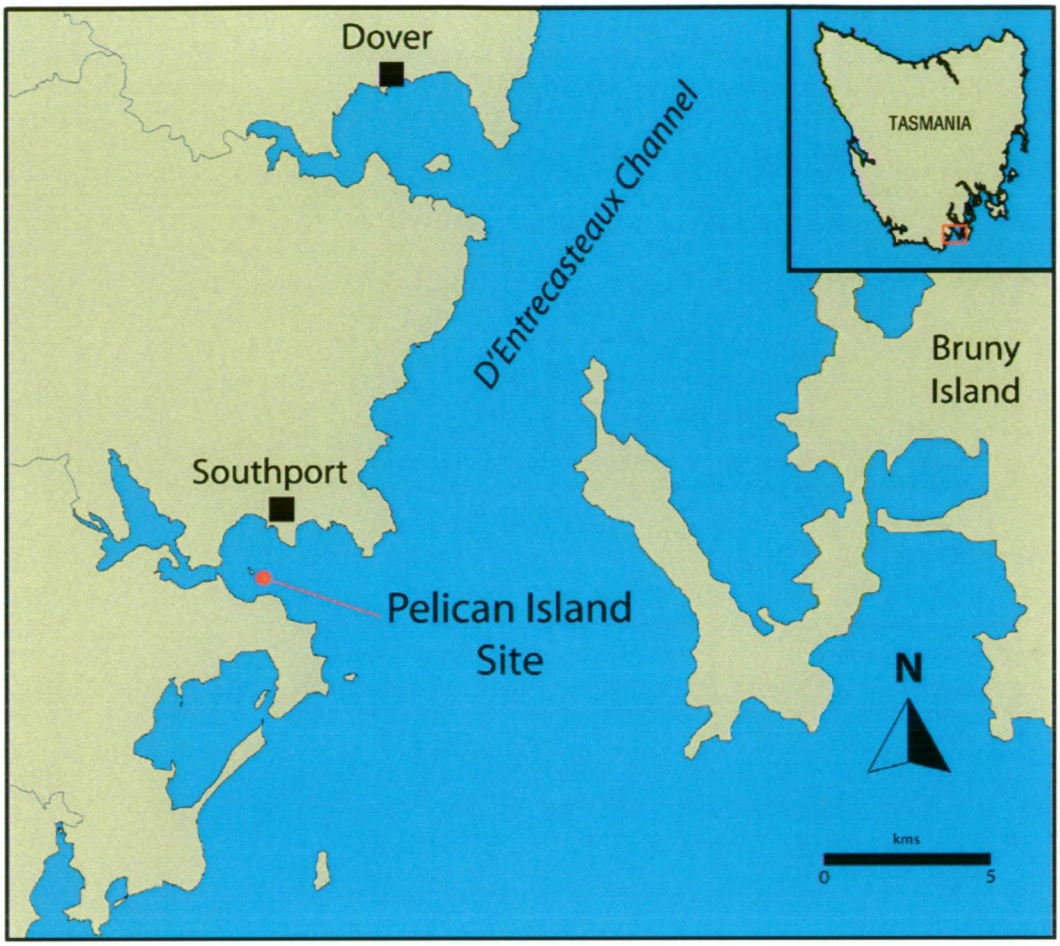

Figure 5.1 Location for nitrogen uptake experiments near Pelican Island at Southport, 30-31/3/2004.

5.2.1 Nitrogen uptake

The ^{15}N tracer technique (Dugdale & Goering, 1967) was used to measure the uptake of NH_4^+, NO_3^- and urea at surface (5m) and bottom (15m) during the day and surface (5m) and chl*a* maximum (10m) during the night . Water was collected from the selected depths using a 10 l Niskin bottle and dispensed into 500 ml glass Schott bottles manufactured by Schott Duran™. The water collected for the incubations was not pre-filtered before dispensing into the 500 ml Schott bottles. There were two main

reasons for not pre-filtering the water for the incubations. Firstly, ensuring that long chains of *G. catenatum* were not excluded from incubations. *G. catenatum* chains of 4 to 8 cells are common. A quick estimate of the size of these chains, 30 microns x 8 cells = 240 microns suggests that screening at 200 microns (a commonly used screen) would exclude a proportion of this species. Secondly, we wanted to estimate the real in situ N uptake rate including, losses due to grazing. For each depth three 500 ml Schott bottles were spiked with 0.3 μM ^{15}N- NO_3^- (99.3 atom percent ^{15}N), three 500 ml Schott bottles were spiked with 0.1 μM ^{15}N-NH_4^+ (99.6 atom percent ^{15}N) and three 500 ml schott bottles were spiked with 0.068 μM ^{15}N-urea (98.61 atom percent ^{15}N). In addition at each depth one 500ml schott bottle was filled with water but not spiked with any ^{15}N substrate, this unspiked bottle was used to determine the background ^{15}N (un-enriched atom % excess). These samples were incubated in 500 ml Schott bottles for 4 hours in-situ at the depths they were collected from. Four hour incubations were chosen because they are short enough to limit the chances of substrate exhaustion (La Roche, 1983)and also reduce the problems caused by substrate dilution(Glibert *et al.*, 1982) , but an incubation period of 2-6 hours is also long enough to minimise the bias introduced by initial high uptake rates that sometimes occur in phytoplankton (Dugdale & Wilkerson, 1986). After the incubation the water samples were filtered onto pre-combusted (450°C for 4 hours) 25 mm Whatman™ glass fibre filters and stored frozen until analysis. The filters were dried in an oven at 60°C overnight before they were analysed using a Carlo Erba NA1500 CNS analyzer interfaced via a Conflo II to a Finnigan-MAT Delta S isotope ratio mass spectrometer to determine the N isotope ratios. Absolute uptake rates were calculated using the Dugdale and Goering (1967) equation:

$$\rho = N a_t (Rt)^{-1}$$

where ρ is the absolute uptake (μg N l^{-1} h^{-1}), N is the total particulate nitrogen (μg N), a_t is the atom % excess of ^{15}N (= atom % - background), R is the atom % enrichment [a_e (S_L/(S_L + S_U))], a_e is the atom % enrichment of labelled ^{15}N source, S_L is the concentration of labelled ^{15}N, S_U is the concentration of unlabelled ^{14}N and t is the

incubation time (h). Specific uptake v (μgN μgChla^{-1} h^{-1}) is the absolute uptake ρ normalised to chl*a* and was calculated using this equation:

$v = \rho/\text{Chl}a$

Where, ρ is the absolute uptake (μg N l^{-1} h^{-1}) and Chl*a* is total chlorophyll a (μg Chl*a* l^{-1}).

It is common for researchers investigating N uptake to normalise N uptake to particulate N (PN). In this research N uptake has been normalised to chl *a* as an indicator of phytoplankton biomass. The fact is that chl *a* is the preferred method of measuring phytoplankton biomass worldwide. It is well known that measurements of particulate N or particulate C are not as reliable, primarily due to the potential contamination by detritial C or N. A number of highly respected researchers have proposed methods to improve the estimation of phytoplankton biomass from POC or PON including Karl Banse (Banse, 1977) or methods that rely on neither (Holm-Hansen & Booth, 1966) but these methods are complex or difficult to apply to individual samples. One result is that very few researchers report phytoplankton biomass in units of PN or PC. In spite of these difficulties a number of researchers have normalized their N uptake measurements to PN and reported them in this manner. This may have more to do with the fact that the analysis required to obtain the results from the uptake experiments also gives a value for PN, rather than any more strategic reasoning. The truth is that N per cell shows about the same amount of variability as chl *a* per cell (Thompson, 1999) in response to irradiance and temperature. For these reasons we have normalized N uptake to chl *a*.

Samples were collected for the determination of ambient nutrient concentrations but not analyzed until after the experimental determination of uptake rates. For the purpose of the experiment ambient concentrations were assumed to be 1 μM NH_4^+, 3 μM NO_3^- and 0.68 μM urea and high purity ^{15}N NH_4^+, NO_3^- and urea tracers were added to produce a 10% rise in estimated ambient concentration. In this experiment

ambient nutrient concentrations were lower than expected resulting in enrichments significantly greater than 10% for NH_4^+ +, NO_3^- (Table 1.1), however, the actual concentration of ^{15}N NH_4^+ and NO_3^- were still low relative to other measurements in the Huon Estuary (Thompson *et al.*, 2008) and other coastal and estuarine ecosystems. Hence, the N uptake rates derived from these incubations were indicative of potential ambient uptake rates for NH_4^+ and NO_3^-.

Table 5.1 Percentage (% of ambient concentration) of ^{15}N added as a tracer for the Southport fieldtrip.

Date	Site	Time	depth (m)	%^{15}N NH_4^+	%^{15}N NO_3^-	% ^{15}N urea
30/03/2004	Pelican Island, Southport	Day	5	250	3000	9
			15	16	21	13
		Night	5	250	3000	21
			20	89	85	16

*** % enrichment calculated by using the detection limit (< 0.03 mM) of the NO_3^- detection method used.**

5.2.2 Nutrient Analysis

Water samples were taken from the water collected for N uptake experiments. The water samples were not filtered prior to storage. These water samples were stored at -20 °C prior to nutrient analysis. Analytical techniques for nitrate and/or nitrite (Wood *et al.*, 1967), silicate (Murphy & Riley, 1962) and phosphate (Armstrong, 1951)were adapted and performed using Quick-Chem™ methods on a flow injection LACHAT instrument as per the following protocols for nitrate and/or nitrite (Quik-Chem™ Method 31-107-04-1-A; detection limit 0.03~ mM), silicate (Quik-ChemTM Method 31-114-27-1-D; detection limit ~0.05 mM) and phosphate (Quik-ChemTM Method 31-115-01-1-G; detection limit ~0.02 mM). Ammonium was measured using the technique of Kerouel and Aminot (Kerouel & Aminot, 1997) adapted for flow injection (Watson *et al.*, 2004)with a detection limit of ~0.05 mM. Urea samples were analysed using a diacetyl monoxime technique according to the method of (Mulvenna & Savidge, 1992). To reduce the health risks associated with thio-semicarbazide, a reagent for this technique, we substituted semicarbazide (a less toxic but similar compound).

5.2.3 High performance liquid chromatography

Two L of water was filtered for pigment analyses by high performance liquid chromatography (HPLC). Most pigment samples were analyzed by high performance liquid chromatography (HPLC) using methods developed by Wright et al. (1991) for extraction and gradient elution. The separated pigments were detected at 436 nm and identified against standard spectra using Waters EmpowerTM software. Concentrations of all pigments were determined from standards (SigmaTM or DHI Denmark). Primary pigments identified by HPLC included: Chl c_1 and c_2, peridinin, 9'-cis-neoxanthin, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, prasinoxanthin, violaxanthin, diadinoxanthin, alloxanthin, diatoxanthin, lutein, zeaxanthin, Chl *b*, Chl *a*, β, ε-carotene and β,β-carotene.

5.2.4 Phytoplankton Counts

Water collected for the surface N uptake experiment during the day was used to enumerate phytoplankton. One litre water samples were taken and preserved in the field using Lugol's iodine fixative solution (110 g potassium iodide, 50 g iodine, 1 litre distilled water, 100 ml glacial acetic acid) to approximately 2% final concentration. The samples were stored in plastic containers in the dark until they could be counted under the light microscope. Prior to counting the Lugol's preserved samples were transferred to 1 litre measuring cylinders (volume recorded – V_1) and allowed to settle for at least 24 hours. After this time, approximately 900 ml were siphoned off and the remaining sample was transferred to a 100-ml measuring cylinder and again allowed to settle for at least 24 hours. Then approximately 90 ml were siphoned off, the final volume recorded (V_2) and thoroughly mixed before a 1-ml aliquot was taken, placed in a Sedgwick Rafter counting chamber and examined using an Olympus IX71 microscope to identify and count the phytoplankton.

The Sedgwick Rafter counting chamber has a grid of 1000 squares, each of 1 μl. For microplankton, (cells generally larger than 20 μm diameter) at least 100 squares or 10% of the counting chamber was scanned (except in cases where there were dense blooms of one or more microplankton species, when at least one column of 20 squares was scanned) at 200x magnification. For nanoplankton, (2-20 μm in diameter) the chamber was examined under 400x magnification until at least 300 cells of the dominant nanoplankton "species" had been counted. Flagellates in the nanoplankton were grouped, as time constraints did not allow fuller identification.

Cells per litre =

cell "species" count * (1000 / number squares counted) * (V_2 *1000 / V_1)

5.2.5 CTD profiles

A Seabird™ SBE19$^+$ conductivity, temperature and depth (CTD) profiler was used to measure the salinity, temperature and fluorescence of the water column during the field experiments. The measurements obtained on the downward cast through the water column are presented with the initial period removed when the instrument was held at surface until flow past the sensors was established.

The CTD sensors: salinity, temperature and fluorescence were factory calibrated. The factory calibrations were confirmed by comparison with independent instrumentation for salinity, temperature and fluorescence.

5.2.6 CHEMTAX

Pigment data from HPLC analysis was further analysed to give the proportions of chlorophyll *a* present in the following algal taxonomic categories: Cyanophyta, Prochlorophyta, Chrysophyta, Cryptophyta, Bacillariophyta (diatoms), Haptophyta, Prasinophyceae, and Dinophyta (Mackey *et al.*, 1996). Initial input ratio matrices

were adjusted from those proposed in Mackey, Mackey *et al.* (1996) by including four types of Haptophytes:

Haptophyte N – Type 1 = fucoxanthin e.g. *Isochrysis spp.*

Haptophyte S- Type 2 = Chl c_3 + fucoxanthin e.g. *Ochrosphaera neopolitana*

Haptophyte Type 3 = Chl c_3+ 19'Hexanoyloxyfucoxanthin + fucoxanthin + and sometimes 19'butanoyl derivative e.g. *Chyrsochromulina strobulis*

Haptophyte Type 4 = c_3 + 19'butanoyloxyfucoxanthin +19'hexanoyloxyfucoxanthin and fucoxanthin e.g. *Imantonia rotunda.*

The peridinin:chlorophyll *a* ratio was modified from 0.515:1 as in Mackey, Mackey *et al.* to 0.36:1 after Hallegraeff, Nichols *et al.* (1991). The latter was based on extensive culture studies for *Gymnodinium catenatum.* This has the effect of increasing the proportion of the chlorophyll *a* that CHEMTAX will allocate to dinoflagellates for a given amount of peridinin. Based on personal observations this results in CHEMTAX estimates of dinoflagellates that are more consistent with the estimated proportion of biomass calculated by cell counts and adjusted for cellular bio-volumes.

5.2.7 Statistical analysis

A three-way ANOVA was used to examine the effect of N species (NH_4^+, NO_3^- and urea), time of day, and depth on N specific uptake. The data failed the Kolmogorov-Smirnov test (with Lilliefors' correction) for normality but passed Levene's median test for equal variance. A log transform (log(x+0.05)) visibly improved the shape of the distribution of the N uptake data however following this transformation the data still failed the test for normality. However, because of the robustness of ANOVA to non normal data (Sokal & Rohlf, 1995), the three way ANOVA was still used to analyse the data.

Where data was transformed for analysis, the three-way ANOVA results (least squares means) were back-transformed for reporting in the text and figures. As standard errors cannot be directly back-transformed upper and lower 95% confidence intervals were reported. The 95% confidence intervals were calculated as below:

Lower 95% confidence interval= $10^{(X-SE*t)}$

Upper 95% confidence interval= $10^{(X+SE*t)}$

Where X=mean, SE=standard error and t is the t statistic (α=0.05) for n-1 degrees of freedom. Both the mean and standard error were first transformed using $\log_{10}$ for these calculations.

5.3 Results

5.3.1 Phytoplankton composition

CHEMTAX analysis of pigments showed that the phytoplankton composition was dominated by dinoflagellates at Southport at all depths sampled during the day and the night (Figure 5.3). During the day at 5m dinoflagellates accounted for over 96 % of the phytoplankton, the remainder being 2.2, 1.2 and 0.6% of prasinophytes, diatoms and haptophytes N, respectively (Figure 5.2). During the day at 15m and during the night at 5m and 10m at least 75% of the phytoplankton were dinoflagellates. Diatoms were more abundant than during the day at 5m, accounting for between 6 and 8 %. Cryptophytes, or possibly Dinophysis, were 2-4 % of total chlorophyll *a*, while the remainder of the phytoplankton was haptophytes type N (containing only fucoxanthin). There were was large numbers of cryptophytes 26812 cells l^{-1} observed in these samples(Table 5.2). The CHEMTAX results were confirmed by cell counts of the surface (5m) sample collected during the day (Figure 5.3). The cell counts showed that there were high numbers of *G. catenatum* (86 229 cell l^{-1}) and also a number of diatom species and some cryptophytes. The diatoms

with the greatest abundances were *Leptocylindrus danicus* and *Skeletonema costatum* with 10031 and 9375 cells l^{-1} respectively.

During the bloom of *G. catenatum* sampled at Pelican Island Southport, there was a narrow fluorescence maximum in excess of 7 fluorescence units at ~ 8m during the day (Figure 5.4 A). At night the maximum fluorescence occurred at ~11m and the peak of fluorescence was much wider and spread over a greater depth range (6-13m) (Figure 5.4 B).

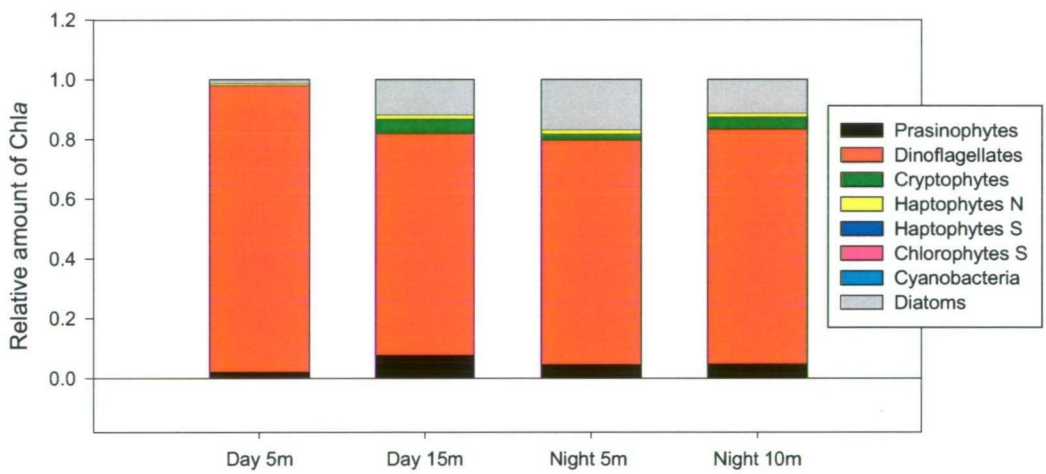

Figure 5.2 Phytoplankton composition (based on HPLC determined pigments and subsequent CHEMTAX analysis from samples during bloom of *G. catenatum* at Pelican Island, Southport during Day and night and Surface (5m) and depths (10 and 15m).

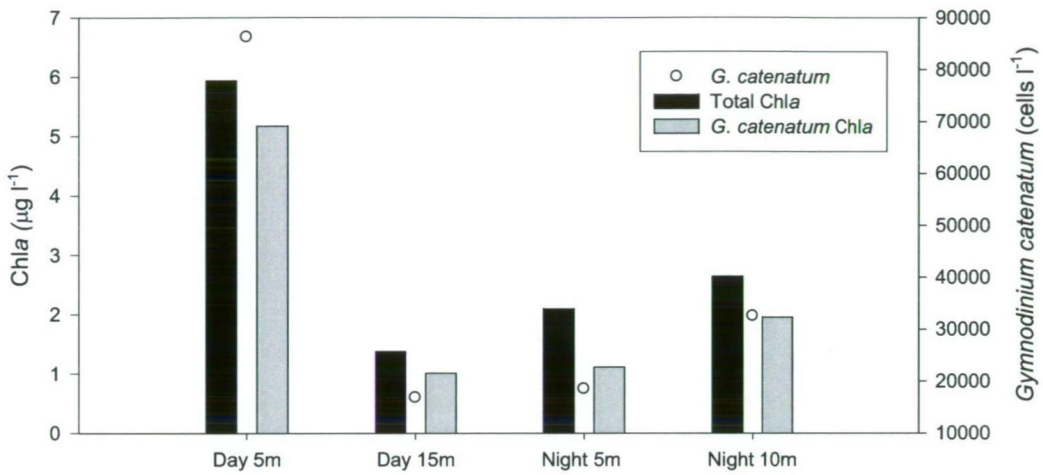

Figure 5.3 Total chl *a*, chl *a* attributed to *G. catenatum* and G. catenatum cell counts at Pelican Island, Southport during Day and night and Surface (5m) and depths (10 and 15m). G. catenatum attributed chl *a* calculated based on 60 pg chl *a* cell^{-1} (Hallegraeff et al., 1991).

Table 5.2 Representative cell count of lugols fixed phytoplankton collected from 5m during the day at Southport 30/03/2004.

TAXON	Cell count (cells l^{-1})
Melosira	562
pennate diatom 15um	375
Skeletonema costatum	9375
Thalassiosira spp	187
Guinardia delicatula = Rhizosolenia delicatula	6094
Chaetoceros spp > 10um	1875
Chaetoceros spp < 10um	6375
Leptocylindrus danicus	10031
Dactyliosolen fragillissimus = Rhizosolenia fragillissima	187
Rhizosolenia fallax	469
dinoflagellate 6-8um	3656
Ceratium spp	1856
Dinophysis spp	56
Protoperidinium sp	562
Gymnodinium catenatum	86229
cryptophytes small 7 -10um	26812
flagellates 5-10 um round	6187

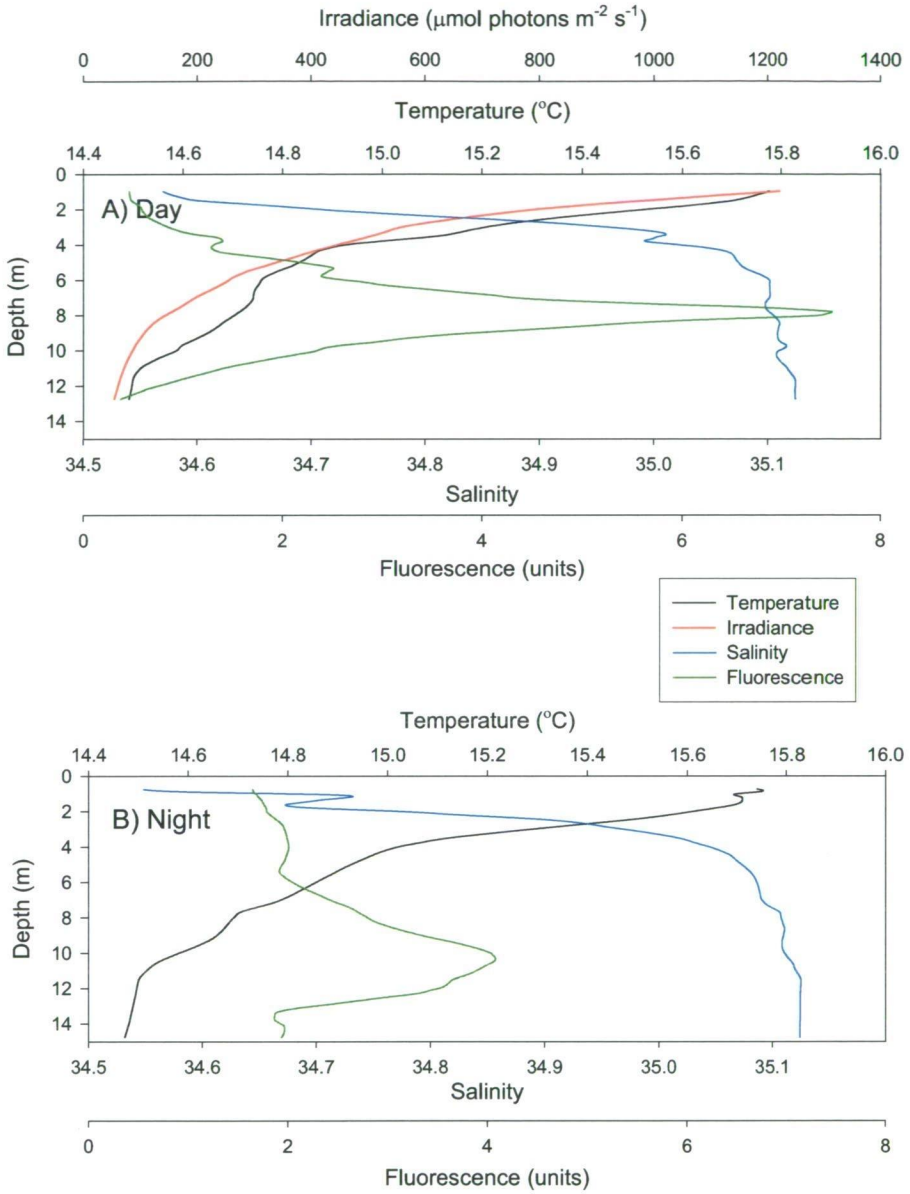

Figure 5.4 Profiles of temperature, irradiance, salinity and fluorescence at Pelican Island, Southport (30-31/03/2004) at A) day and B) night.

5.3.2 N uptake

Ambient, tracer and total NH_4^+, NO_3^- and urea concentrations are shown in Table 5.3.

Table 5.3 Ambient, tracer and total NH_4^+, NO_3^- and urea concentrations at different times and depths for Southport N uptake experiment.

	Day 5m	Day 15m	Night 5m	Night 10m
Ambient NH_4^+	0.036	0.645	0.036	0.113
Tracer NH_4^+	0.100	0.100	0.100	0.100
Total NH_4^+	0.136	0.745	0.136	0.213
Ambient NO_3^-	0.000	1.606	0.000	0.533
Tracer NO_3^-	0.300	0.300	0.300	0.300
Total NO_3^-	0.300	1.906	0.300	0.833
Ambient Urea	0.793	0.522	0.330	0.435
Tracer Urea	0.068	0.068	0.068	0.068
Total Urea	0.861	0.590	0.398	0.503

Specific uptake rates for NH_4^+, NO_3^- and urea ranged from 0.012-0.063, 0.011-0.040 and 0.012-0.081 μgN μgChla^{-1} h^{-1}, respectively (Figure 5.5) During the day at the surface, night at surface and night at depth urea uptake was 58.1, 43.9, 47.0 %, respectively of total N uptake (Table 5.4). There was only one instance (day at 15m) where NO_3^- uptake was greater than urea; when it was 51.7 % and urea only accounted for 24.5% of the total N uptake. NO_3^- uptake showed the greatest variability ranging from 12.8 % during the day at 5m to 51.7 % day at Depth (15m). Uptake of NH_4^+ ranged from 22.4 to 34.3 % during the day at surface (5m) and night at surface (5m) respectively.

Table 5.4 Percentage uptake of NH_4^+, NO_3^-, and urea during the day at 5 and 15m and during the night at 5 and 10m for the Southport fieldtrip.

Date	Site	Time of day	Depth (m)	%NH_4^+	% NO_3^-	% urea
30/03/2004	Pelican Island, Southport	Day	5	29.1	12.8	58.1
			15	23.8	51.7	24.5
		Night	5	34.3	21.8	43.9
			10	22.4	30.7	47.0

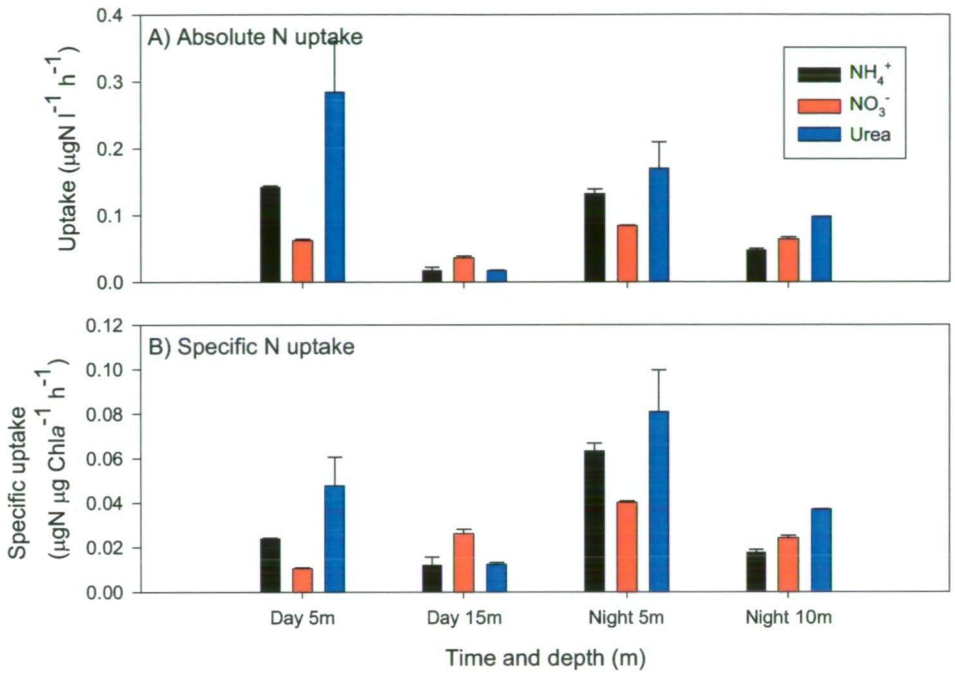

Figure 5.5 Absolute and specific uptake rates of NH_4^+, NO_3^- and urea at Pelican Island, Southport during the day and night and at the surface (5m) and depth (10 and 15m).

When averaged across time of day and depths NH_4^+, NO_3^- and urea specific uptake rates were 0.0292, 0.0253 and 0.0446 µg N µg chl a^{-1} h^{-1} respectively or expressed as percentage of total N uptake (NH_4^++ NO_3^- + urea) 29.5, 25.5 and 45% respectively (Figure 5.6)

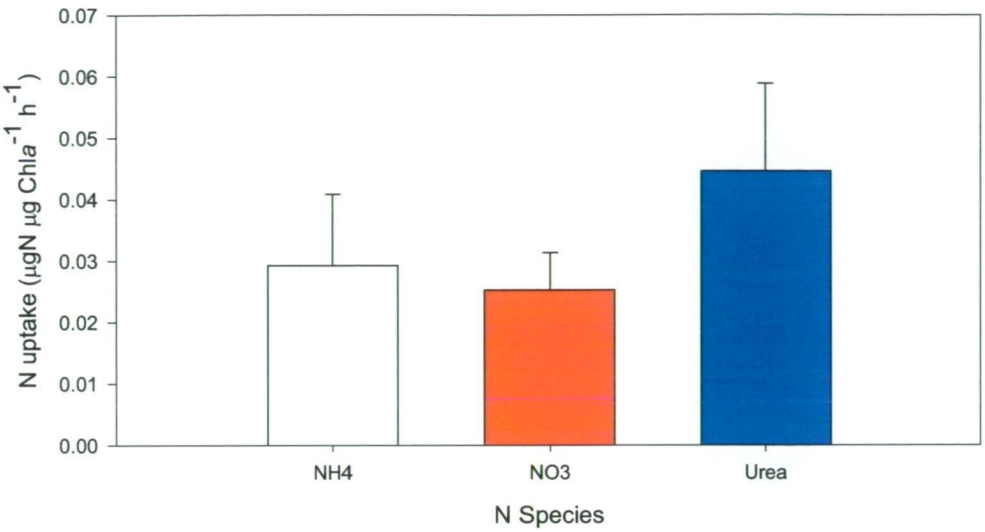

Figure 5.6 Mean uptake for NH_4^+, NO_3^- and urea when both times and depths were combined from Southport (see Fig 1.1 for location). Standard errors are included (n=12)

The main effects of N species, time of day and depth on N uptake were all significant but interpretation of their effects was complicated because of the significant interactions between N species and depth and between time of day and depth (Table 5.5).

Table 5.5 Three way ANOVA for the effect of Nitrogen species (N), Time of day (T) and Depth (D) on the specific uptake of nitrogen at Southport during a *Gymondium catenatum* bloom.

Factor	Nitrogen uptake
N species (N)	Significant effect P=0.048
Time of day (T)	Significant effect P=<0.001
Depth (D)	Significant effect P=<0.001
N x T	No Interaction
N x D	Significant Interaction P=<0.001
T x D	Significant Interaction P=0.005
N x T x D	No Interaction

While acknowledging the complications caused by the significant interaction some general trends in N uptake were evident. There was an ~ 2 times increase in the

specific uptake of NH_4^+, NO_3^-, and urea during the night when compared to uptake during the day (Figure 5.7). NH_4^+ and urea uptake at the surface was ~ 3 times greater than near the bottom but there was no effect of depth on NO_3^- uptake (Figure 5.8). At the surface urea uptake was ~ 3 times greater than NO_3^- uptake and 1.5 times greater than NH_4^+ uptake. In addition NH_4^+ uptake was ~ 2 times greater than NO_3^- uptake in the surface. Total specific nitrogen uptake was 2.3 times greater in the surface at night than in the surface during the day (Figure 5.9).

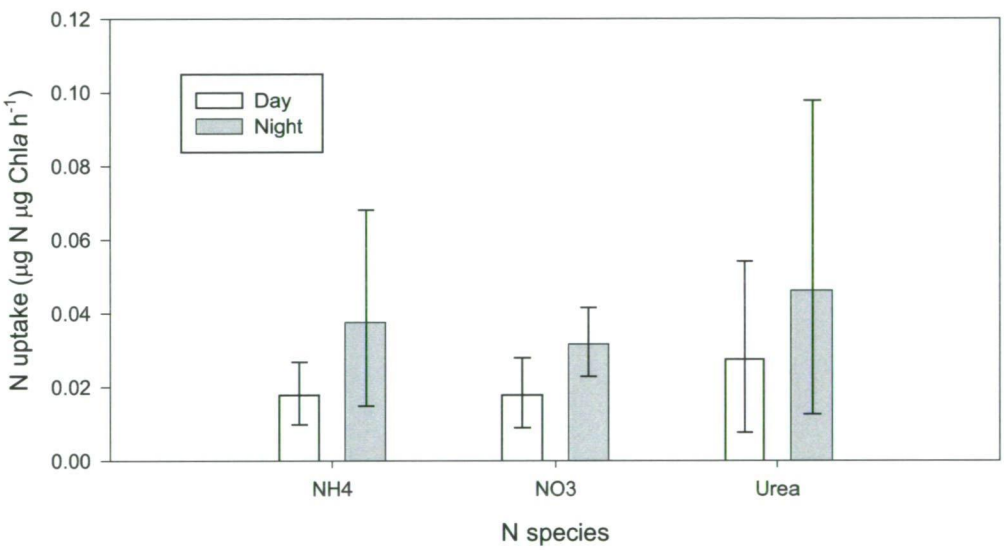

Figure 5.7 Uptake of NH_4^+, NO_3^-, and urea during the day and night at Southport. Least square means and upper and lower 95% confidence intervals.

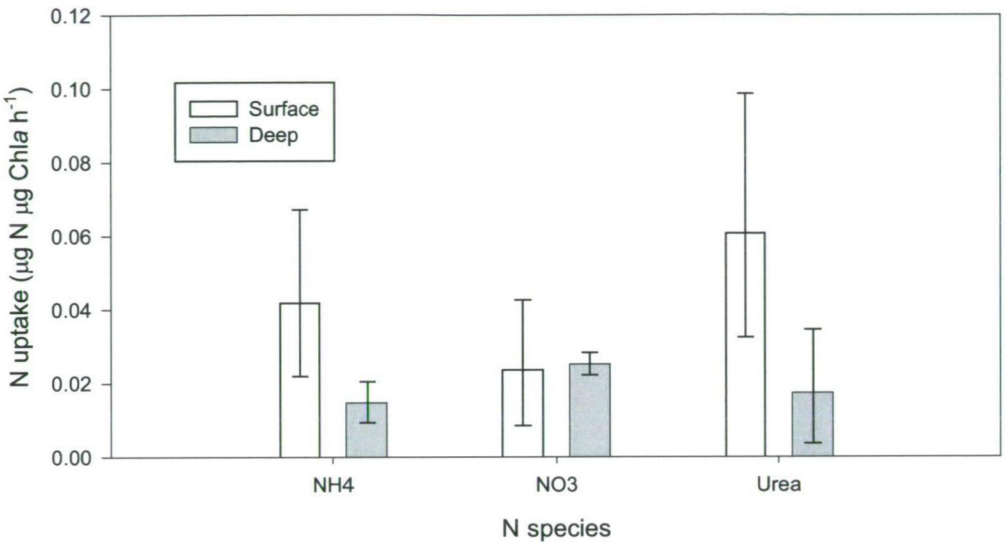

Figure 5.8 Uptake of NH_4^+, NO_3^-, and urea at surface and bottom depths in Southport. Least square means and upper and lower 95% confidence intervals are shown.

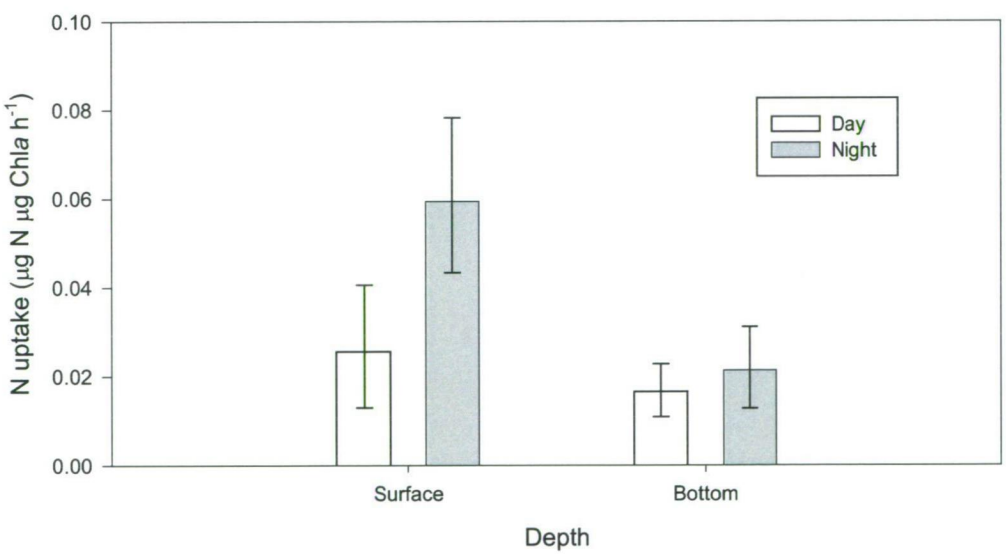

Figure 5.9 Uptake of the three different N species at surface and bottom during the day and night at Southport. Least square means and upper and lower 95% confidence intervals are shown.

5.4 Discussion

Gymnodinium catenatum dominated the phytoplankton assemblage sampled near Pelican Island, Southport. *G. catenatum* was responsible for 87 and 73% of the Chl*a*, during the day at 5 and 15 m respectively and 53 and 74% of the Chl*a* during the night at 5 and 10m respectively. CHEMTAX results based on HPLC pigments indicated that the other phytoplankton groups responsible for the remaining Chl*a* were diatoms, cryptophytes, prasinophytes and haptophytes N, listed in order of their contribution. The Chl *a* biomass and species composition of this bloom at Southport was similar to the summer and autumn blooms previously observed in the Huon Estuary with mean Chl *a* values of ~3 $\mu g\ l^{-1}$ and *G. catenatum* responsible for greater than 50% of the phytoplankton assemblage (Thompson et al., 2008). It is suggested that the N uptake results from this *G. catenatum* dominated bloom at Southport will be useful in gaining insight into the behaviour and N uptake capabilities that enables *G. catenatum* to bloom so successfully in the South East of Tasmania and other parts of the world.

Specific uptake rates for NH_4^+, NO_3^- and urea ranged from 0.012-0.063, 0.011-0.040 and 0.012-0.081 μg N μg chl $a^{-1}\ h^{-1}$. The specific uptake rates from Southport were at the lower end of the range reported by Rosser and Thompson (Rosser & Thompson, 2001) in the much more eutrophic Swan River.

When averaged across time and depth urea uptake was greatest at 45% and NH_4^+ and NO_3^- are both roughly equal in their contributions at 29.5 and 25.5 %, respectively. In comparison to the late autumn and late summer fieldtrips in the Huon estuary urea was responsible for a greater proportion of the phytoplankton uptake. It seems likely that the reason for this is that in southport both the preferred N sources (chapter 4) $NH_4^+$and NO_3^- have been exhausted in the surface and hence *G. catenatum* is forced to use urea and exploit deep NO_3^- via vertical migration. While in the Huon Estuary its hypothesised that NH_4^+ uptake is more important during these times of year because of the added input of NH_4^+ from salmon farming. These results are also in

good agreement with many studies which have shown that urea uptake is important for many phytoplankton species (Berman & Bronk, 2003), especially autumn dinoflagellate blooms (Glibert and Terlizzi 1999).

Results from a series of experiments on *G. catenatum* show that this species grows equally well on NH_4^+, NO_3^- and urea as sole N sources (see Figure 4.2 and Figure 4.3 in Chapter 4), but when presented with a mixture of NH_4^+, NO_3^- and urea they are taken up in the order NH_4^+>NO_3^-> urea. This preference for NH_4^+ and NO_3^- indicates that there must be low supply of both of these substrates for urea uptake to be the most important N source for this *G. catenatum* dominated bloom at Southport. The ability of *G. catenatum* to vertically migrate (Doblin et al., 2006) means that to understand more clearly the behaviour and N uptake capabilities of this species in the field it is important to examine N uptake during the day and night and at depths related to its position in the water column.

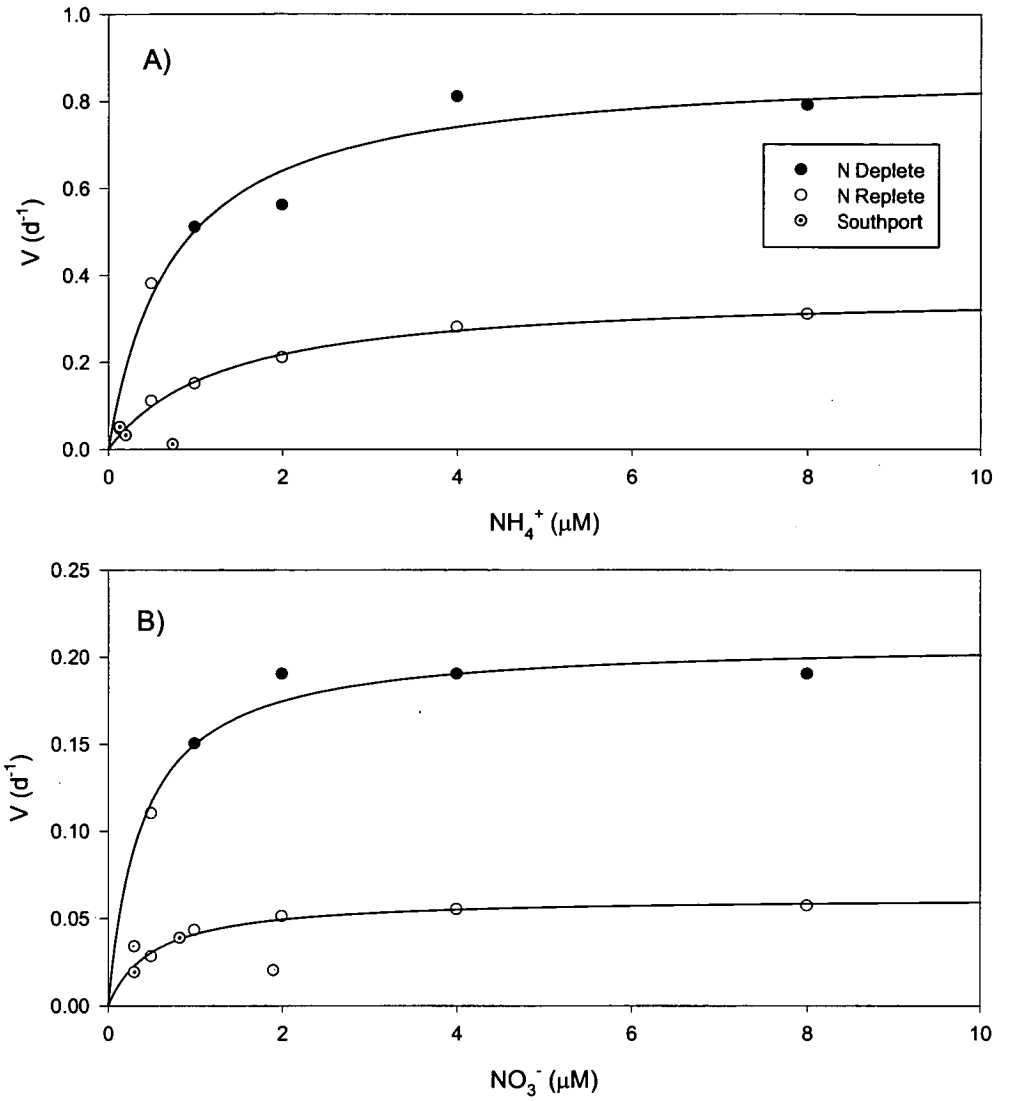

Figure 5.10 Comparison with A) NH_4^+ and B) NO_3^- *G. catenatum* uptake kinetics data from Flynn, Flynn *et al.* (1996).

5.4.1 Day

During the day at 5m urea uptake accounted for 58.1% of the N uptake, while NH_4^+ and NO_3^- only accounted for 29.1 and 12.8 % respectively. Urea uptake was greater during the day at 5m because ambient concentrations of NH_4^+ and NO_3^- were very

low 0.04 and <0.01 μM (below detection level) respectively. At such low concentrations the rate at which *G. catenatum* can take up any N substrates is substantially reduced (Flynn et al., 1996). However while urea is not the preferred source of N it was present at a much greater concentration, 0.79 μM hence the phytoplankton were taking up urea at a greater rate. While urea was being taken up at the greatest rate NH_4^+ and NO_3^- were also being taken up at lesser but significant rates. A considerable fraction of the NH_4^+ and NO_3^- uptake was associated with the ^{15}N tracer which was added at 2.5 and 30 times (respectively) greater concentrations than ambient. If the NH_4^+ and NO_3^- could have been added at truly tracer levels (10% of ambient concentrations) NH_4^+ and NO_3^- uptake would have been much less and urea would have made an even greater % of the uptake under these conditions.

During the day at 15m NO_3^- uptake was greatest, responsible for 51.7% of N uptake and NH_4^+ and urea were responsible for almost equal amounts 23.8 and 24.5% respectively. The N uptake rates at depth appear to be related to the ambient substrate concentrations with NO_3^- uptake being greatest because it is available at concentrations of 1.6 μM, greater than double the concentration of NH_4^+ or urea during the day at this depth.

The fluorescence profile is in the shape of a sharp peak with a maximum fluorescence at ~8m indicating that during the day the greatest concentration of phytoplankton are at 8m and based on the *G. catenatum* dominated samples from the 5 and 15m samples and what we know about vertical migration of *G. catenatum* (Team, 2000, Doblin et al., 2006) it seems likely that *G. catenatum* is positioning itself at 8m during the day where it can access light for growth but also N in the form of elevated NO_3^- concentrations like those observed at 15m. At 8m Irradiance is approximately 160 μmol photons m^{-2} s^{-1} and temperature 15.8°C. E_k calculated from the model in Chapter 3 for 15.8 °C was 7.1 μmol photons m^{-2} s^{-1}. These laboratory results indicate that the irradiance of 160 μmol photons m^{-2} s^{-1} would be saturating growth and well in excess of the requirements for *G. catenatum* at this temperature. This type of

strategy has been reported by other dinoflagellates including (e.g. Ault, 2000)(Cullen and Horrigan 1981; Eppley, Holmhans *et al.* 1968; Hasle 1950.

5.4.2 Night

During the night at 5m urea uptake was 43.9% of N uptake, while NH_4^+ and NO_3^- were responsible for 34.3 and 21.8%, respectively. Uptake during the night at 5m was similar in many ways to what was seen during the day at 5m. Urea uptake was most important and ambient concentrations of urea (0.32 μM at surface) were greater than either NH_4^+ or NO_3^- uptake. Also, in-situ uptake rates of NH_4^+ and NO_3^- were probably overestimated because of relatively high concentrations of the ^{15}N tracer, as was also the case for the day at 5m. It might be expected that uptake rates of these N species would be lower at night because absence of light usually reduces the uptake of N substrates (MacIsaac & Dugdale, 1972) particularly NO_3^- which requires more energy for uptake and assimilation (Syrett, 1981). Clearly uptake rates are not reduced at night, in fact they are greater. It can be hypothesized that high noctural uptake rates resulted from cells that were N depleted. Under these conditions a range of cultures and field experiments have shown uptake of N, including NO_3 in the dark at rates usually only seen during the day (Harrison, 1976, Cochlan *et al.*, 1991). During the night at 10m uptake of urea was 47% ot total N uptake, NO_3^- 30.7% and NH_4^+ 22.4%. Uptake rates of both urea and NO_3^- were greater than the usually preferred NH_4^+, possibly because they were available at greater concentrations, 0.43 μM and 0.53 μM respectively compared to NH_4^+ at 0.11 μM.

At night the shape of the fluorescence peak broadened and the maximum in fluorescence was deeper and less concentrated than the shallower the daytime fluorescence maximum (Figure 5.4 B). Within the broad night time fluorescence maximum the distribution of biomass was skewed towards the bottom of the maximum. This suggests that the majority *G. catenatum* dominated phytoplankton community found concentrated at 8m during the day probably migrated deeper to 10m at night. In addition to this modest migration the phytoplankton spread out more

ranging from 5-13 m. Going deeper may be important for accessing NO_3^- and urea at greater concentrations but spreading out may also be important in maximising the substrate concentration per cell and scavenging any available N in the water column. This strategy may act to maximising uptake under conditions of very low ambient concentrations of NO_3^- in these and other similar coastal and estuarine systems worldwide.

5.4.3 Summary

This field study confirmed that *G. catenatum* uses NH_4^+, NO_3^- and urea in the field as was also observed in the laboratory (Chapter 4). The results from this field study were consistent with the preferences for N substrates found in the laboratory, in this order NH_4^+> NO_3^- >urea. The low ambient concentrations of $NH_4^+$and NO_3^- in the surface layer clearly suggest the bloom was in a relatively late phase. Clearly at this stage of the bloom recycled N or organic N such as urea became a very important source of N. The ability of this species to vertically migrate means that during the day *G. catenatum* is able to position itself in the water column where it receives light in excess of its requirements for growth while migrating to depth also allows it to access greater concentrations of NO_3^-. During the night when light is not available *G. catenatum* vertically migrated deeper in the water column and was able to access the greater concentrations of NO_3^- at these depths. While the vertical migration observed in this field study was not as dramatic as seen during the Huon Estuary study, the water column was not as deep and the N relatively closer to the surface. There is sufficient evidence from this study to validate the hypotheses from Doblin et al, (2006) that *G. catenatum* vertically migrates to access nitrogen at depth.

5.5 Appendix

Table 5.6 Results of ANOVA for effect of nitrogen species, time of day and depth on nitrogen uptake at Southport. Data were log transformed (log(x+0.05)) failed Kolmogorov-Smirnov test (with Lilliefors' correction) for normality but passed levene's median test for equal variance.

Source of Variation	DF	F	P
N Species (N)	2	3.455	0.048
Time of Day (T)	1	20.482	<0.001
Depth (D)	1	32.518	<0.001
N x T	2	0.178	0.838
N x D	2	10.081	<0.001
T x D	1	9.77	0.005
N x T x D	2	0.733	0.491
Residual	24		

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6 SUMMARY

6.1 Background

The Huon Estuary receives a significant input of oceanic nutrients during winter and surface nutrients rise to > 3 μM nitrate, 0.4 μM phosphate, ~ 15 μM silicate (Fig. 1). . The Huon River is the major source of fresh water to the Estuary, it has a seasonal flow with a long-term average of ~ 30 m s^{-1} in summer (Dec-Feb) rising to ~ 130 ms^{-1} during late winter (July-Aug). The Huon Estuary is a salt wedge with seasonally variable surface outflow and inflow at depth. The Huon River is a major source of refractory nitrogen (~ 600 to 1100 tonnes annually) and dissolved silica to the Huon Estuary and D'Entrecasteaux Channel but a source of very little DIN. The Huon River dissolved organic N input is poorly quantified and the nitrogen forms are very poorly characterized. During the transition to spring nitrate and phosphate concentrations decline and by summer the nitrate concentrations are near zero while phosphate and silicate concentrations remain relatively high, suggesting a nitrogen limited ecosystem (Figure 6.1). It is only during summer that surface nitrate and ammonium concentrations are relatively similar. The finfish aquaculture industry contributes a significant amount of nitrogen to the Huon Estuary, ~ 313 tonnes of mostly ammonium in 2002. Both industry and government regulators are alert to the potential for eutrophication and increased harmful algal blooms if the assimilative capacity for N of the estuary is exceeded. As part of a larger project on the ecology of the Huon Estuary, this PhD research has two main objectives; firstly to determine whether phytoplankton in the Huon Estuary were using nitrogen that had, primarily, an oceanic source (e.g. nitrate) or was more locally supplied or regenerated (e.g. ammonium and urea) and secondly to examine the physiology of *G. catenatum*.

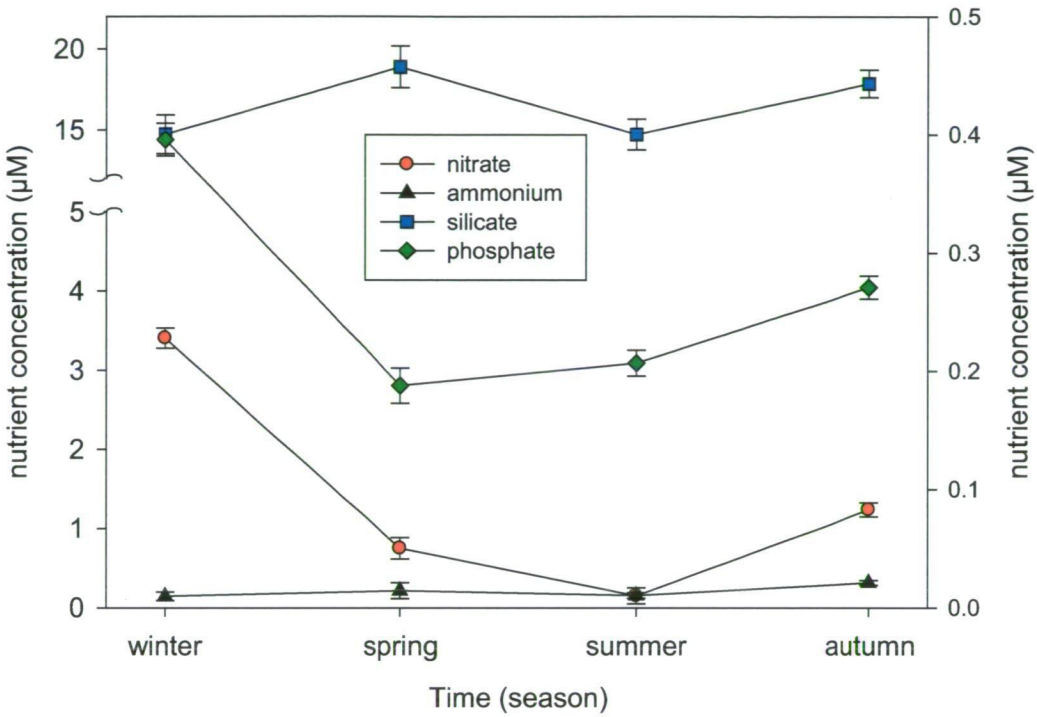

Figure 6.1 Surface nutrient concentrations from weekly samples at 5 sites during 1996-1998 (adapted from Thompson et al. 2008). The y-axes on the left is for nitrate and silicate and the y-axes on the right is for ammonium and phosphate. Mean +/- 1 SE.

6.2 Nitrogen uptake dynamics in the Huon Estuary

The availability of different forms of nitrogen in the Huon Estuary follows the pattern of seasonal abundance seen elsewhere in temperate ecosystems. During winter nitrate concentrations are high while during summer nitrate concentrations are low and primary production relies heavily upon urea and ammonium (Twomey *et al.*, 2005). The availability of ammonium depends upon inputs and the recycling of particulate organic matter. The former is dominated by the ~ 800 tonnes supplied by the aquaculture industry. In addition the aquaculture industry supplies a considerable portion of its N load during summer when other inputs of DIN are estimated to be

low. Based upon what we know about the species that typically form HABs this input of DIN into a N limited system during summer could be expected to exacerbate HAB in this ecosystem.

During this research, the uptake of NH_4^+, NO_3^- and urea at 2 locations, 2 depths, during the day and night and at four times throughout the year provided the basis to assess the nitrogen uptake dynamics by phytoplankton in the Huon Estuary (Chapter 2). Reduced forms of N (NH_4^+ and urea) accounted for the majority of the overall N uptake, 52% and 37.5% respectively, while NO_3^- uptake was responsible for only 10.5% of the overall N uptake. Studies of N uptake in other coastal and estuarine ecosystems have also shown that reduced N forms account for a large component of the overall N uptake (e.g. McCarthy *et al.*, 1977, L'Helguen *et al.*, 1993, Metzler *et al.*, 1997, Harrison, 2002, Rosser & Thompson, 2001) and that these forms of N are actively preferred in many cases (e.g. O'Donohue *et al.*, 2000, Dauchez *et al.*, 1991, Keene *et al.*, 1991, Middelburg & Nieuwenhuize, 2000).

Nitrate uptake accounted for the smallest component on all field trips but peaked at ~15% of the N uptake during early spring. During the winter to spring transition [NO_3] was drawn down and only low concentrations were measured on late spring and late summer field trips. This also coincided with the spring diatom bloom in the Huon Estuary sinking out of the water column in late spring when the chlorophyll *a* concentrations were greater at 20 m than at 5m.

The NH_4^+ accounted for >50% of N uptake by phytoplankton on all 4 field trips (late autumn, early spring, late spring and late summer). Urea was the next largest component of the N uptake on all 4 field trips but became a larger component, up to ~40% of the N uptake during the late spring and late summer field trips. There is very little work on the seasonality of N uptake although this shift from nitrate to more ammonium and urea uptake has been observed by other researchers following a spring bloom (e.g. Bury *et al.*, 2001, Kudela & Dugdale, 2000, Bronk *et al.*, 1998, Torres-Valdes & Purdie, 2006).

Hydrodynamic and biogeochemical modelling of the Huon Estuary and D'Entrecasteaux Channel indicated a significant rise in expected chlorophyll *a* concentrations in response to the DIN loading from aquaculture (Volkman *et al.*, 2009). The modelling also suggests the magnitude of this impact will not be uniform in time or space. Some locations are predicted to experience no change and other will have a substantial rise in chlorophyll *a* for most of the year. One of the challenges identified in the modelling was that the DIN sources and phytoplankton responses will be displaced in time and space making attribution difficult. A number of locations were identified as potentially susceptible to phytoplankton blooms associate with the N loading from aquaculture. In response to the modelling and the identified environmental risks a monitoring program has been put in place. This program spans the region and will regularly assess the environmental status of key parameters such as phytoplankton composition and biomass, dissolved oxygen and ammonium concentrations.

6.3 Physiology and ecology of *G. catenatum*

This thesis represents the most comprehensive study of the effects of irradiance and temperature on *G. catenatum* growth and physiology to date. Growth versus irradiance curves were determined for twelve temperatures from 11.9-25.0 °C and a comparison of the growth rates from the present study with five other studies on different *G. catenatum* strains has revealed considerable similarity between the growth responses of these strains particularly at temperatures between 10-20 °C. At temperatures >= 28.5 °C the strains from Japan and Mexico appeared to be able sustain growth rates approximately 0.2 d^{-1}, while the other strains did not grow, indicating that Japanese and Mexican populations may constitute an warm-water ecotype. Relationships between the parameters (μ_{max}, α and E_c) from the growth versus irradiance curves and temperature were investigated and modelled. The models of the effect of temperature on μ_{max}, α and E_c provide the basis for development of a *G. catenatum* growth model useful to predictive ecological

responses in natural populations. This model can be applied by scientists or natural resource managers to determine when growth of this species will be fastest and whether the ecosystem they are investigating can support growth year round. For example the Huon Estuary winter temperatures are at the lower limit of growth of the strain examined in this research. In addition to this at temperatures from 16-20 °C, typical of summer in the Huon Estuary *G. catenatum* would only be capable of growth rates ranging from 0.1-0.2 d^{-1}.

The biochemical composition of *G. catenatum* was also examined revealing that *G. catenatum* adapts to low temperature conditions by increasing its carbon (C) quota. At higher temperatures and lower irradiances *G. catenatum* responded by increasing chl*a* but this was accompanied by an even greater increase in C quota. These results give us insights into the strategies that this species uses for growth and survival in unfavourable conditions. At low temperatures *G. catenatum* increases C quota as observed in other species (Sakshaug & Andresen, 1986) possibly as a mechanism to allow survival through cold periods. In the case of *G. catenatum* it might be a carbon acquisition strategy that will lead to sexual reproduction and cyst formation. At high temperatures and low irradiances the increase in carbon quota was accompanied by an increase in chlorophyll *a* per cell. Increased carbon and chlorophyll *a* quotas are a common occurrence under these conditions (Geider, 1987) where cells require more structures (e. g. chloroplasts) for light capture and the enzymes are capable of high rates of operation. An empirical model to describe the effects of light and temperature on C:chl *a* was constructed and may be useful information that increases our understanding of physiological responses in this species or other similar species in the future.

This work also demonstrated that *G. catenatum* has diverse N uptake capabilities. It grows equally well using NO_3^-, NH_4^+ or urea in the laboratory (Chapter 4) and this is also evident from the field studies (Chapters 4, 5). The preference for NH_4^+ and NO_3^- over urea may reflect the energetic and biochemical costs associated with uptake and assimilation of the various sources. Perhaps, while it poses no penalties

for growth when energy from light is saturating, the preferences may be related to the preferences under energy limited circumstances. For example, variation in the growth rate of *Alexandrium minutum* (Dinophyceae) on NO_3^- were highly dependent on the level of irradiance (Chang & McClean, 1997).

The ability of *G. catenatum* to vertically migrate means that during the day *G. catenatum* is able to position itself in the water column where it receives light in excess of its requirements, and migrates to depth at night to access greater concentrations of NO_3^-, probably also NH_4^+ and urea. While the vertical migration observed in this field study was not as dramatic as seen during the Huon Estuary study (CSIRO Huon Estuary Study Team, 2000, Doblin *et al.*, 2006), the bloom did not take place in as deep a water column and the N was relatively closer to the surface. There is sufficient evidence from this study to validate the hypotheses from Doblin, Thompson *et al.* (2006) that *G. catenatum* vertically migrates to access nitrogen at depth.

For *G. catentatum* growing in the Huon Estuary it seems increasingly apparent that it functions as a nitrogen scavenger. When N concentrations are exhausted, it is able to migrate rapidly through the water column seeking whatever form of nitrogen is available. Thus the dominance of this species is perhaps not due to it occupying a specialist niche associated with NH_4^+, but rather due to its greater capacity to access uptake N from different parts of the water column when it may be in short supply nearer the surface. This was clearly demonstrated by the experiments conducted at Southport during a *G. catenatum* bloom (Chapter 5). Urea was the most important N source (~ 40%) at the late stage of the bloom. The low ambient concentrations of NH_4^+ and NO_3^- in the surface layer were clearly insufficient to support more growth. At the bottom of the euphotic zone the vertically migrating bloom was able to find NO_3^- and was fully capable to exploiting this N resource with NO_3^- uptake dominating (~52%) over NH_4^+ and urea uptake. Although nitrate reductase was not measured it has been shown for other dinoflagellates that high nitrate reductase

activity is present during blooms, even when ammonium is abundant relative to nitrate concentrations (Harrison, 1973).

The present study and that of (Flynn *et al.*, 1996) shows that *G. catenatum* is capable of responding to pulses of N by 'surge uptake'(relative to a maximum growth rate 0.3 d^{-1}) when it is in a N starved condition. Surge uptake is another mechanism that a species may use to exploit patches of elevated nutrients potentially giving them a competitive advantage relative to species without this capability (Cochlan & Harrison, 1991). Our results show that 'surge' uptake rates of NH_4^+ were almost 20 times the maximum growth rate of *G. catenatum* (0.30 d^{-1}).

Based on this combined new understanding of the physiology and ecology of *G. catenatum* from lab and field studies, *G. catenatum* has a competitive advantage over non migratory species in environments where available nutrients (nitrogen) are vertically separated from sufficient irradiance for growth. The chain-forming nature and the tendency to form progressively longer chains during blooms can increase swimming speed (Fraga *et al.*, 1987) allowing *G. catenatum* to vertically migrate over substantial depths, and this capacity appears to be more important than which N species is available in the water column.

6.4 Future Research

While this study has made significant progress in understanding the nitrogen uptake dynamics of phytoplankton in the Huon Estuary, there are significant gaps in our current knowledge of the sources of nitrogen and the bloom dynamics of *Gynmodinium catenatum* in the Huon Estuary system. Two areas for future research are:

- **Terrestrial sources of nitrogen:** The Huon River remains a potentially major source of dissolved organic N (DON) input to the Estuary that is poorly quantified and very poorly characterized. Recent hydrodynamic and biogeochemical modelling (Volkman et al., 2009) suggests that this N is

largely refractory and exported out to sea, but it is currently unknown what proportion is retained and recycled in Huon Estuary, or may be used by phytoplankton in the estuary.

- **Development of a species specific model for *Gymnodinium catenatum*:** A model that enables the prediction of growth rate based on temperature and irradiance was developed in Chapter 3. Combining this growth model with the increased understanding of the physiology of *G.catenatum*: N uptake preferences, capability for 'surge' uptake (Chapter 4) and vertical migration strategy to scavenge N (NH_4^+, NO_3^- and urea) (Chapter 5) and other important parameters like uptake kinetics for *G. catenatum* (NO_3^- and NH_4^+) from Flynn, Flynn *et al.* (Flynn et al., 1996), *G. catenatum* mortality rates (Volkman et al., 2009) and grazing in Huon Estuary (Thompson *et al.*, 2008). A species specific model could then be coupled to a physical model of the Huon Estuary and D'Entrecasteaux Channel and used to compare with field data in trying to elucidate the drivers of the development of *G. catenatum* blooms. There may also be potential to apply a species specific model to other temperate ecosystems where *G. catenatum* is a problem.

6.5 References

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