The eco-physiology of macroalgae from a temperate marine embayment in southern Australia

A thesis submitted for the degree of Doctor of Philosophy

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lighthouse to the sea

plunge deep
there is a place
as fluid as silence
as free as air
as colored as light
and there is space
to spin
to breathe
to forget
to neglect
and dream
with the childlike
quiet of the weeds

s.j.c. '98

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Declaration

This thesis is submitted in accordance with the regulations of Victoria University of Technology in fulfilment of the requirements for the degree of Doctor of Philosophy. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and no material previously published or written by another person except where duly acknowledged or referenced.

Stuart J. Campbell

[redacted]

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List of Abbreviations

α	Photosynthetic efficiency
ANOVA	Analysis of variance
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
Chl *c*	Chlorophyll *c*
B	Biomass
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorus
DMSO	Dimethylsulfoxide
I	Irradiance
I_c	Compensation irradiance
I_{max}	Saturating irradiance
K_m	Half saturation constant
K^s_m	Half saturation constant for surge uptake
K^{ass}_m	Half saturation constant for assimilation uptake
n.s.	Not significant
n.a.	Not available
N_c	Critical nitrogen content
N_{max}	Maximum tissue N
N_q	N subsistence quota
N_{req}	Nitrogen required to support maximum growth
N_{red}	N pools that allow reduced growth
N_s	N pools in excess of those necessary for maximum growth
P_{max}	Gross photosynthetic capacity

P-I	Photosynthesis versus Irradiance
PPB	Port Phillip Bay
PPFD	Photosynthetic Photon Flux Density
PSI	Photosystem I
PSII	Photosystem II
PSU	Photosynthetic Unit
PSS	Practical Salinity Scale
PQ	Photosynthetic Quotient
RuBPCase	Ribulose Carboxylase
S	Substrate concentration
SCUBA	Self-contained underwater breathing apparatus
T_{max}	Storage capacity at non-limited growth
T_{red}	Possible duration of reduced growth
μ	Specific growth rate
μ_{max}	Maximum specific growth rate
V^{ass}	Assimilation uptake
V^{s}	Surge uptake
V_{max}	Maximum uptake capacity
V^{s}_{max}	Maximum surge uptake
V^{ass}_{max}	Maximum assimilation uptake

All other symbols represent SI units

Glossary of algal authorities

* indicates no authorities provided by author

Chlorophyta

Caulerpa remotifolia Sonder (Chlorophyta)

Chaetomorpha linum (Müller) Kützing

Cladophora vagabunda (Linnaeus) van den Hoek

Cladophora sericea (Hudson) Kützing

Cladophora aff. *albida* (Hudson) Kützing

Cladophora prolifera (Roth) Kützing

Codium fragile subsp. *tomentosoides* (Suringar) Hariot

Codium decoratium (Woodward) Howe

*Codium dwarkense**

Enteromorpha intestinalis (Linnaeus) Link

Enteromorpha prolifera (Muller) J. Agardh Bliding

Ulva curvata (Kützing) DeToni

Ulva fasciata Delie

Ulva fenestrata Postels et Ruprecht

Ulva lactuca Linnaeus

Ulva lobata (Kützing) Setchel and Gardner

Ulva rigida C. Agardh

Ulva rotunda (Kützing) DeToni

Rhodophyta

Acrosorium uncinatum (Turner) Kylin

Ceramium rubrum (Hudson) C. Agardh

Chondrus crispus Stackhouse

Gracilaria pacifica Abbott

Gracilaria edulis (Gmelin) Silva

Gracilaria foliifera (Forsskål) Børgesen

Gracilaria tenuistipitata Zhang et Xia

Gracilaria tikvahiae McLachlan

Gracilaria verrucossa (Hudson) Papenfuss

Hypnea musciformis(Wulfen) Lamouroux

Neogardhiella baileyi Harvey ex Kützing

Polysiphonia decipiens Montagne

Polysiphonia lanosa (Linnaeus) Tandy

Polysiphonia nigrescens Smith (Greville)

Porphyra perforata J. Agardh

Porphyridium purpureum Bory, Drew and Ross

Spyridea hypnoides *

Phaeophyta

Chordaria flagelliformis (O.F. Mueller) C. Agardh

Ecklonia radiata (C. Agardh) J. Agardh

Ectocarpus confervoides (Roth) Le Jolis

Ectocarpus siliculosus (Dillwyn) Lyngbye

Dictyota dichotoma (Hudson) Lamouroux

Fucus distichus Linnaeus subsp. *edentatus* (de la Pylaie)

Fucus evanescens (C. Agardh)

Fucus spiralis Linnaeus

Fucus vesiculosus Linnaeus

Hincksia sordida (Harvey) Clayton

Laminaria digitata and (King and Schramm 1976)

Laminaria saccharina (Linnaeus) Lamouroux

Laminaria groenlandica Rosenvinge

Macrocystis pyrifera (Linneaus) C. Agardh

Phyllariopsis purpurascens (C. Agardh) Henry et South

Pilayella littoralis (Linnaeus) Kjellman

Ptelonia fascia (Müller) Kuntze

Sargassum thunbergii (Mertens ex Roth) O. Kuntze

*Sargassum baccularia**

Scytosiphon lomentaria (Lyngbye) Link

Undaria pinnatifida (Harvey) Suringar

List of publications and conference presentations

Campbell, S.J. and Burridge, T.R. (1998). Occurrence of *Undaria pinnatifida* (Phaeophyta Laminariales) in Port Phillip Bay, Victoria, Australia. *Marine and Freshwater Research*, **49**: 379-381.

Campbell, S.J., Bité, J.S. and Burridge T.R. (1999). Seasonal patterns in the photosynthetic capacity, tissue pigment and nutrient content of different developmental stages of the introduced kelp *Undaria pinnatifida* (Phaeophyta : Laminariales) in Port Phillip Bay, south-eastern Australia. *Botanica Marina*, in press.

Campbell, S.J. (1999). The uptake of ammonium by 4 species of macroalgae in Port Phillip Bay, Victoria, Australia. *Marine and Freshwater Research*, in press.

The photosynthetic response of macroalgae to sewage enrichment. Poster Presentation. *Australasian Society for Ecotoxicology, Inaugural Conference*, Sydney, Australia, June 1994.

The seasonal photosynthetic capacity of macroalgae from Port Phillip Bay. Paper presentation. *Australasian Society for Phycology and Aquatic Botany*, Stradbroke Is., Queensland, Australia, July, 1995.

Productivity and nutrient uptake by the Japanese kelp *Undaria pinnatifida* in Port Phillip Bay. Paper Presentation. *Australasian Society for Phycology and Aquatic Botany*, Hobart, Australia, January 1997.

Ecology and physiology of introduced macroalgae in two marine embayments in Victoria, Australia. Paper Presentation. *Australasian Society for Phycology and Aquatic Botany*, Dunedin, New Zealand, July, 1998.

Abstract

This study investigated the effects of nitrogen and phosphorus on the growth and eco-physiology of a number of dominant species of macroalgae at a site in Port Phillip Bay (PPB), a large shallow water marine embayment located on the central southern coast of Victoria, Australia (Fig. 1.1). Port Phillip Bay is an extensive marine embayment which receives high loads of nutrients from wastewater outlets, drains and diffuse sources. These nutrients promote the growth of fast growing macroalgae which lead to eutrophication and decline of other marine plant and animal species. To provide a functional understanding factors that promote the proliferation of fast growing macroalgae it is necessary to understand the physiological mechanisms that respond to changing environmental conditions. This thesis investigated the physiological processes (i.e. photosynthesis, growth, nutrient uptake) of three species of macroalgae, *Hincksia sordida* (Harvey) Clayton (Phaeophyta), *Polysiphonia decipiens* Montagne (Rhodophyta) and *Ulva* sp. (Chlorophyta) in response to a range of environmental regimes. All species were collected from a site 500 m offshore Werribee, Port Phillip Bay, in south-eastern Australia from a depth of 3 m. The uptake of nutrients was also examined for an additional species, *Undaria pinnatifida* (Harvey) Suringar, which invaded the site under examination during the study.

Photosynthetic rates (measured via an oxygen electrode) of each species were measured over an annual cycle and were found to be similar between *Hincksia sordida* and *Ulva* sp., which were 2- to 3-fold higher than *Polysiphonia decipiens*. The photosynthetic capacities of all species were influenced by light and temperature although the maintenance of high photosynthetic rates at low temperatures by *Hincksia sordida* coincided with increased N availability. The photosynthetic and growth response of each species during summer and winter was measured in relation to N and P concentrations using augmented seawater in laboratory culture. The positive relationship between N availability, and growth, photosynthetic performance, tissue N and pigment content recorded for all species suggests that photosynthesis in these macroalgae may be limited by N. The optimisation of pigment concentration

with N availability appears to be directed towards maximising photosynthetic capacity and growth. An increase in accessory pigment concentration with N limitation was postulated as an adaptive mechanism necessary to maintain productivity during N limitation. The relationship between growth and tissue nutrients reflected seasonal differences in tissue nutrients and provides a functional explanation for observed growth strategies of species. Information for each species on the storage capacity and critical tissue N limits for growth was also determined. There was no relationship between growth and P concentration.

Kinetic data were collected for ammonium uptake for each of the four species using batch culture techniques. The decline in assimilation uptake after a period of rapid surge uptake was indicative of saturated intracelluar pools of N controlling uptake across the cell membrane. By combining uptake and previously established growth kinetics it was shown that the relative importance of surge uptake to growth varied amongst species. Saturated uptake also varied amongst species over the range of ammonium concentrations tested. Maximum uptake rates (V_{max}) were relatively high in *Hincksia sordida*, mature *Undaria pinnatifida* and *Ulva* sp. suggesting that these species can exploit high concentrations of DIN, are inclined to be N limited, and may dominate N enriched environments in Port Phillip Bay. These taxa also showed evidence of bi- or multi-phasic uptake which allows for ammonium uptake at both low and high concentrations. *Polysiphonia decipiens* showed a relatively high capacity for uptake at low concentrations which is consistent with a high storage capacity for N found for this species. The higher affinity for N by immature *Undaria pinnatifida* than mature *Undaria pinnatifida* may partly explain high productivity rates of mature populations in the field.

Long-term (14 d) ammonium and phosphate-P uptake were found to be dependent on the seasonal nutritional history of the algae. Higher rates were found in the fast growing ephemeral species *Hincksia sordida* and *Ulva* sp. compared with *Polysiphonia decipiens*. An inverse relationship between ammonium uptake and macroalgal N status for *Hincksia sordida* and *Ulva* sp. was consistent with higher

summer ammonium uptake rates than winter rates. This implies tissue nutrient saturation that may impose feedback controls on rapid uptake mechanisms. For *Hincksia sordida* and *Polysiphonia decipiens* the dependence of long-term N uptake on P availability was found in summer cultures only, but the dependence of phosphate-P uptake on N availability was found for *Hincksia sordida* and *Ulva* sp. in summer and winter. This implies a potential disposition to N limitation, as demonstrated by their fluctuating biomass over the annual cycle. In contrast, phosphate uptake by *Polysiphonia decipiens* was found to be independent of N availability which may increase its P uptake efficiency.

The data generated in this thesis may be used as inputs to existing coastal models to forecast the effects of nutrients on macroalgae and episodes of eutrophication in Port Phillip Bay.

Chapter 1

1.1 General Introduction

Estuarine and near-shore marine environments are particularly vulnerable to pollution from industrial, agricultural and domestic waste-water effluents. These effluents often contain high concentrations of nutrients which can stimulate aquatic macrophyte productivity (Kautsky 1982; Lee and Olsen 1985; Nixon et al. 1986; Sfriso et al. 1992; Valiela et al. 1992; Peckol et al. 1994; Duarte 1995; Jeffrey et al. 1995). Macroalgae are an important component of coastal marine environments and a common source of primary production for food webs in these environments. They are often used as indicators of the intensity of pollution impact (Munda 1982; Lavery and McComb 1991a; Horrocks et al. 1995; Pederson and Borum 1996). The study of macroalgal communities, their biological relationships and their interactions with the changing physical environment can contribute to our understanding of the impacts of pollution.

Two commonly used terms that describe the consequences of nutrient enrichment of aquatic environments are:

> **Eutrophication:** which is an environment change towards a higher concentration of nutrients, leading to an increase in primary productivity with positive effects on the whole trophic chain and,
>
> **Hypereutrophication:** which is the enrichment of a particular environment with nutrients to a stage when the oxygen consumed can no longer be renewed leading to negative environmental impacts (Fonselius 1978).

One of the consequences of coastal eutrophication on macrophytes is a reduction in species richness and diversity (Borowitzka 1972; Edwards 1972; Littler and Murray 1975; May 1985; Tewari and Joshi 1988). This is often caused by a proliferation of

opportunistic taxa better suited to high nutrient conditions. These taxa are able to outcompete existing macroalgae previously suited to lower nutrient concentrations and may dominate coastal environments to the degree that mono-specific stands occur. Such changes in macroalgal abundance often represent a shift in dominance from long lived late successionals (perennials) to faster growing opportunistic algae, that culminates in a reduction of vertical stratification and structural complexity of the algal community (Murray and Littler 1975; Munda 1980; Tewari and Joshi 1988; Brown et al. 1990).

Morand and Briand (1996) and Valiela et al. (1997) provide reviews of eutrophication caused by excessive macroalgal growth. Species that have proliferated worldwide include *Chaetomorpha* spp., (Lavery et al. 1991a; McGlathery et al. 1997), *Cladophora* spp., (Gordon et al. 1981; Schramm and Booth 1981; Lapointe and O'Connell 1989; Thybo-Christesen et al. 1993), *Enteromorpha* spp. (Tewari 1972; Pregnall and Rudy 1985; McComb and Lukatelich 1990; Wheeler and Björnsäter 1990; Lavery et al. 1991a), *Ulva* spp. (Borowitzka 1972; Thom and Albright 1990; Lavery et al. 1991b; Sfriso 1995; Viaroli et al. 1996; Pederson 1997), *Pilayella* spp. (Wallentinus 1978; Wilce et al. 1982; Thybo-Christesen et al. 1993), *Gracilaria* spp. (Lapointe 1985; Valiela et al. 1992; Pickering et al. 1993) and *Ectocarpus* sp. (Jeffrey et al 1993).

The proliferation of these species are thought to represent dynamic shifts in coastal ecosystems from those primarily limited by light (i.e. seagrass and perennial macrolagal communities) to systems dominated by fast growing algae, limited essentially by nutrient availability (Valiela et al. 1997). Such shifts are direct indications of eutrophication. The physiological traits (e.g. P_{max}, alpha, pigment and nutrient contents and nutrient uptake rates) of these common macroalgae are commonly elevated by nutrient enrichment and therefore can be used as indicators of nutrient enrichment in coastal waters. The study of these indicators may provide process based explanations for changes in species composition arising from eutrophication. Such characteristics are early warning indicators of the condition and

metabolic efficiency of macroalgae and the nearshore marine ecosystems which they inhabit.

Few studies have examined relationships between nutrient availability and macroalgal productivity in nutrient enriched coastal ecosystems in southern Australia. Detailed studies have been restricted to the Peel Harvey Estuary in Western Australia (Gordon et al. 1981; Gordon and McComb 1989; Lavery et al. 1991; Lavery and McComb 1991a). Port Phillip Bay (PPB) is a large shallow water marine embayment located on the central southern coast of Victoria, Australia (Fig. 1.1). It is subject to semi-diurnal tides with a maximum amplitude of almost 1 m. Approximately 50% of PPB's total volume is at a depth less than 10 m. Much of this area provides habitat for macroalgal and seagrass communities. In the central northern region of PPB these communities are exposed to annual inputs of approximately 4000 tonnes of nitrogen and 1800 tonnes of phosphorus from the Western Sewage Treatment Plant, Werribee.

The physiological responses of these communities to nutrient exposure in PPB is unknown but nutrient inputs have been causally linked to excessive growth of the unattached Chlorophyte *Cladophora fascicularis* from 1976 to 1979 (Brown et al. 1980). Since 1992, the filamentous unattached Phaeophyte *Hincksia sordida*, has exhibited prolific growth on an annual basis from February to August (pers. obs.). The species periodically inhabits depths of 2 to 8 m, for about 1 to 3 weeks at a time, and covers extensive areas of the seabed (> 2 km^2) occurring as epibenthic mats up to 0.5 m thick. Other species which dominate nutrient enriched areas of Port Phillip Bay include *Ulva* spp. and common Rhodophyte taxa such as *Polysiphonia decipiens* which possesses a filamentous morphology. The two most common forms of *Ulva* in Port Phillip Bay are *Ulva lactuca* and *Ulva rigida.* Together with *Hincksia sordida* and other macroalgae these species form expansive, intertwined epibenthic mats that are capable of smothering and/or displacing other species. The introduced Laminarian Phaeophyte *Undaria pinnatifida* invaded the site during the study and appeared capable of displacing local species. It was included in part of the study to provide direct comparisons between its physiology and those of the other species examined.

Fig. 1.1 Study site location in proximity to the Western Treatment Plant, Werribee, Port Phillip Bay and location of sewage outlets.

The general aim of this study was to examine the physiology of macroalgae from a site offshore the Western Sewage Treatment Plant in relation to seasonal environmental factors. Chapter 2 aims to quantify the effects of changing light, temperature and nutrient availability over an annual cycle on photosynthetic responses. The data obtained was used to develop nutrient budgets for each species. It was important to examine the direct effects of nutrient enrichment on these macroalgae by controlling light and temperature in the laboratory and quantifying the limiting effects of nutrients on their physiology and growth (Chapter 3). Data from these experiments on the relationships between growth and nitrogen utilisation were examined and interpreted in terms of the functional advantages that may be conferred to particular species in changing nutrient environments (Chapter 4).

It is possible that in near-shore waters where nutrient input is highly seasonal, species with the fastest rates of uptake may have an advantage in utilising periodic nutrient pulses. Alternatively uptake rates may be of little importance for macroalgae which have developed different nutrient requirements or strategies of nutrient utilisation such as luxury consumption. Experiments undertaken in Chapter 5 examined the short-term ammonium uptake requirements of macroalgae to assess any competitive advantage this may confer. Such mechanisms may be influence by the long-term nutrient uptake strategies of macroalgae in relation to previous exposure to nutrient concentrations. Experiments in Chapter 6 aimed to establish whether long term uptake strategies differed between species and were dependent on prior nutrient exposure. Such strategies are useful in predicting the dynamics of macrolagal growth in relation to seasonal changes in nutrient inputs. Chapter 7 contains the main conclusions of the study.

Chapter 2

The seasonal eco-physiology of macroalgae from Port Phillip Bay.

2.1 Introduction

Light and temperature are considered to be the primary factors regulating the *in situ* metabolism and growth of macroalgae (Davison 1991). Nutrient availability has also been shown to influence productivity (DeBoer 1981; Dawes et al. 1984) and both nitrogen (N) and phosphorus (P) are often discharged in pulses to coastal waters from industrial, agricultural and sewage sources. Nitrogen has frequently been implicated as the limiting nutrient in marine temperate waters (Lobban et al. 1985), but P has also been shown to limit growth of seaweeds in temperate marine and estuarine waters (Gordon et al. 1981; Manley and North 1984; Connolly and Drew 1985; Lavery and McComb 1991a; Lavery et al. 1991; Wheeler and Björnsäter 1992). These nutrients have directly caused excessive growth and dominance of ephemeral and opportunistic macroalgae that effect shifts in macroalgal community composition (Sfriso et al. 1987; Lavery et. al. 1991; Fong et al. 1993a, 1993b; Valiela et al. 1997).

The tissue nutrient composition of macroalgae varies as a function of taxonomic affinity (Neill 1976) and often reflects the seasonality of N and P concentrations in coastal waters (Björnsäter and Wheeler 1990; Horrocks et al. 1995). The Redfield (1958) water column N:P ratio of 16 is used as an indicator of balanced phytoplankton growth, however this may be inappropriate for macroalgae due to differing optimal ratios of N:P between species (Atkinson and Smith 1983). Moreover, the discharge of N to coastal waters in pulses often results in variable tissue N:P ratios. Nutrient enrichment experiments have also shown that biomass and growth are often proportional to N enrichment and P concentrations remain constant (Fong et al. 1993a). In addition, the high nutrient demands of large standing stocks of macroalgae occurring in coastal waters may influence the chemical composition of seawater

(Wheeler and Björnsäter 1992). For example, in the Lagoon of Venice DIN depletion of the water column in spring was correlated with the rapid growth of *Ulva rigida* (Chlorophyta) (Sfriso et al. 1992; Viaroli et al. 1992; Sfriso and Marcomini 1997). In Waquoit Bay, U.S.A., *Cladophora vagabunda* (Chlorophyta) and *Gracilaria tikvahiae* (Rhodophyta) depleted nutrients from the water column in summer (Peckol et al. 1994).

Measures of tissue nutrients in relation to water column nutrient availability over an annual cycle are necessary to determine the seasonality of nutrient limitation in macroalgae (Wheeler and Björnsäter 1992). Seasonally high N:P and low carbon (C) to N ratios in macroalgae may reflect seasonal P limitation and/or N sufficiency. Low N:P ratios and high C:N ratios may reflect P sufficiency and N limitation respectively (Wheeler and Björnsäter 1992). Given their capacity to integrate pulses of nutrients over time, macroalgae may provide a tool for detecting long term trends in water column nutrients and be useful bioindicators of eutrophication in coastal waters (Fujita 1985; Hwang et al. 1987; Lyngby 1990; Fong et al. 1994).

The requirement of macroalgae for nutrients and the limiting effect of nutrients on their growth can be reflected in physiological indicators of macroalgal productivity. These have included measures of photosynthesis (Lapointe et al. 1984a; Levy and Gannt 1990; Fillit 1995; Rivers and Peckol 1995; Vergara et al. 1997), and tissue N and pigment concentrations (Lapointe et al. 1984b; Fujita et al. 1988; Chopin and Gallant 1995; Sfriso 1995). Measures of photosynthetic performance include maximum photosynthetic rate or $P_{max,}$ and photosynthetic efficiency or α. Photosynthetic efficiency is derived from the initial slope of the photosynthesis-irradiance (P-I) curve. Under light limitation, α is controlled primarily by pigment levels and N availability whilst P_{max} is controlled by enzymatic (RuBPCase) activity often directly influenced by temperature (Lapointe and Duke 1984; Levavasseur et al. 1991).

Other parameters may be derived from the P-I curve. The onset of saturating photon irradiance or I_k provides an estimate of the efficiency of photosynthesis at low light. The compensation irradiance or I_c provides a measure of photon irradiance at which net photosynthesis is zero or equal to respiration (Arnold and Murray 1980). A general trend to low I_c and low I_k reflects lower respiratory quotients in winter months and lower light requirements of winter algae compared with summer algae (Dawes et al. 1978; Hoffman and Dawes 1980).

Seasonal changes in temperature, light and nutrient availability can all influence pigment concentrations in macroalgae. Tissue pigment concentrations often reach maxima during conditions of low light and high nutrient availability (Rosenberg and Ramus 1982a, b; Geertz-Hansen and Sand-Jensen 1992). Seasonal changes in accessory pigment to chlorophyll *a* ratios have also been recorded during seasonal N enrichment, especially in the Rhodophyta (DeBoer and Ryther 1978; Lapointe and Ryther 1979; Rosenberg and Ramus 1982a, b). Such changes have illustrated the structural adaptability of the chloroplasts or photosynthetic unit (PSU) to optimise its photosynthetic performance in response to changing ambient light and nutrient concentrations (Ramus 1981).

Little is known about the photosynthetic characteristics of macroalgae from PPB on a seasonal cycle and their physiological responses to changes in light, temperature and nutrient availability. Some studies have documented the seasonal photosynthetic rates of similar types of macroalgae to those found in Port Phillip Bay such as, *Ulva lactuca* (Brinkhuis 1977; Fillit 1995; Rivers and Peckol 1995), *Ulva curvata* and *Ulva rotunda* (Vergara et al. 1997), *Polysiphonia nigrescens* (Rhodophyta) (King and Schramm 1976), and filamentous ephemeral Phaeophyta (King and Schramm 1976, Wallentinus 1978). These studies have generally shown that maximum photosynthetic rates occur during periods of maximum growth in spring and summer. Translating these values to Port Phillip Bay macroalgae is difficult because of the seasonally changing nutrient environment in Port Phillip Bay waters which may influence macroalgal productivity.

2.1.1 Aims

The aim of this study was to examine the temporal variability in the photosynthetic performance and tissue nutrient and pigment concentrations of three species of macroalgae from Port Phillip Bay, *Hincksia sordida* (Harvey) Clayton (Phaeophyta), *Polysiphonia decipiens* Montagne (Rhodophyta) and *Ulva* sp. (Chlorophyta). As it was not possible to distinguish between the two common species of *Ulva* (i.e. *Ulva lactuca* and *Ulva rigida*) in the field, no effort was made in this study to distinguish between the two. Therefore data presented are for *Ulva* sp. in this chapter and for studies in all other chapters. Little is known about the photosynthetic characteristics of these taxa over an annual cycle in PPB, or the effects of nutrient availability and temperature on their physiological responses. Such information may determine the utility of a particular species as a potential bioindicator of the nutrient status of inshore PPB waters and may be used to indirectly infer nutrient limitation. Measures of standing biomass were also made to examine the relationship between physiology and *in situ* productivity. Annual productivity and nutrient budgets for each taxa were derived from this information.

2.2 Methods

2.2.1 Study site and sample collection

Plants were collected from a single site (3 m depth), 500 m offshore, at an area 2 km north-east of Point Wilson in Port Phillip Bay, Victoria (38°4.05'S, 144°31.4'E.). The site is located about 1 km from the Murtcaim Drain which is one of four sewage outlets into Port Phillip Bay from the Western Treatment Plant, Werribee (Fig. 1.1). The benthic habitat consists of low lying broken rocky/rubble reef interspersed with soft sediments. Whole thalli of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. were collected from the reef from April 1995 to April 1997. In total 21 sampling events took place on a 4-6 week sampling basis. Plants were collected using SCUBA and kept at ambient temperature during transport to the laboratory.

2.2.2 Biomass measures

Quadrat size determination

Biomass of individual species of macroalgae were made by harvesting and weighing macroalgae from five haphazardly placed 0.0625 m^2 quadrats placed in 80-100% vegetated areas. The size of the quadrats was determined by recording the biomass of the 3 species from 3 sizes of quadrats (0.0625, 0.25, 0.5 m^2), repeated five times (Müeller-Dombois and Ellenberg 1974). The quadrat size at which the cumulative number of species was not exceeded by more than 10% by the next highest quadrat size was chosen for field sampling (i.e. 0.0625 m^2). The approximate area of the site examined was 150 m^2.

Sample size determination

The optimum number of quadrats for measuring the biomass of each of the species was determined by initially measuring biomass of macroalgae from 15 0.0625 m^2 quadrats. Mean biomass and 95% confidence intervals for each of the 3 species from

each quadrat were determined for increasing numbers of quadrats from n = 3 onwards (Fig. 2.1). The variability in the data was lowest at n = 15. Given the small difference in variability from n = 5 onwards, 5 was chosen as the sample number of monthly biomass measures for each species. Post-hoc power calculations indicated that at this level of replication the power achieved to detect a 30% change between mean species biomass was relatively low at 0.55, and the power to detect a 40% change was 0.81.

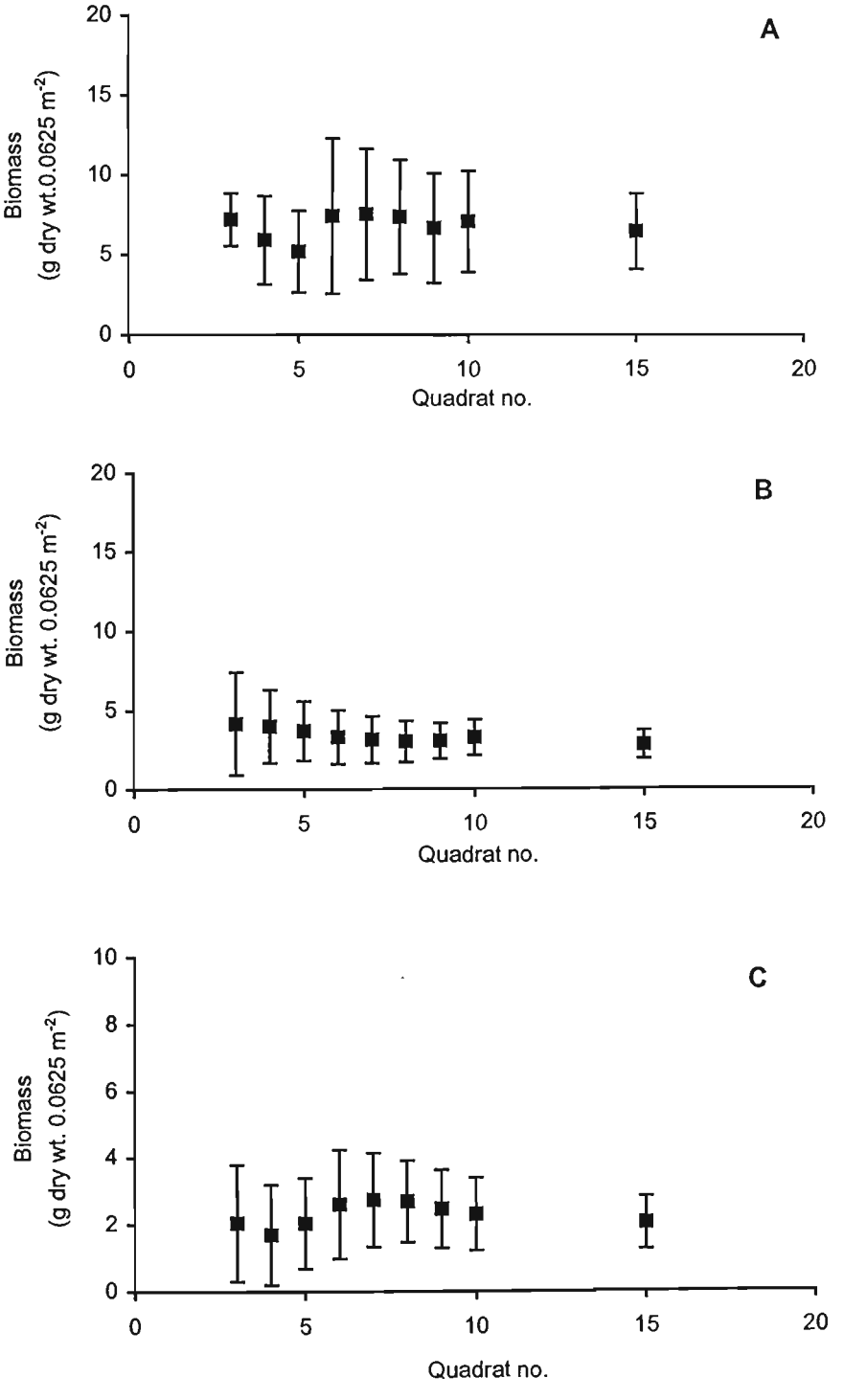

Fig. 2.1 Biomass of (A) *Hincksia sordida*, (B) *Polysiphonia decipiens* and (C) *Ulva* sp. for various numbers of sample quadrats from n = 3 to 15. Values represent means ± 95% confidence intervals.

2.2.3 Field measurements

Midday light, temperature and salinity measures were recorded in the field each month. All measurements were taken at a depth of 3 m. Photon flux density was measured using a quantum meter (Li-892 with fitted UWQ cosine sensor, Licor); salinity and temperature were recorded using a temperature/salinity meter (Model 33, Yellow Springs Incorporated, Ohio, U.S.A).

2.2.4 Nutrient analyses

Analysis of water samples (n = 3) for inorganic nitrogen (NH_4-N and NO_3-N) and inorganic phosphorus (PO_4-P) were undertaken using a U.V. spectrophotometer (Shimadzu UV1201, LKB Biochrom, England) and the methods of Strickland and Parsons (1972).

Plant samples for tissue N and P were dried, homogenised and 130 mg sub-samples were digested in concentrated sulphuric acid (H_2SO_4) and perchloric acid (H_2O_2). For detailed methods refer to Appendix 1. Tissue N and P concentrations were determined using a Novaspec II nutrient Autoanalyser (Pharmacia) using methods GDWB 26 and 34 of the American Public Health Association (1990). Organic C was determined using a modification of the method described by Allen (1989) (Appendix 2). This involved digestion of 10 mg of dried, homogenised plant tissue in sulphuric acid and perchloric acid.

2.2.5 Photosynthesis

Healthy portions of adult plants were shaken in 0.2 μm filtered seawater to remove any grit or sand and cleaned of any epiphytic growth. Pieces of thalli (~0.1 g fresh weight) were cut from the middle of mature healthy plants on the day of collection and kept overnight in 20 L aquaria containing aerated natural seawater at 15°C, under a PFD of 150 μmol $m^{-2} \cdot s^{-1}$ (36 W 'cool white' fluorescent tubes) and a 12:12 light:dark cycle. Seawater was enriched to concentrations of 50 μg NH_4-N L^{-1}

(NH_4Cl) and 50 μg PO_4-P L^{-1} (NaH_2PO_4). The preparation of plant pieces on the day of collection reduced possible effects of wound respiration on the photosynthetic measures (Bidwell and McLachlan 1985). All pieces were preconditioned with 1% iodine (Betadine, w/v 0.75% iodine, Faulding Health Care) for 5 min to inhibit bacterial contamination.

P-I curves were obtained via the measurement of oxygen production/consumption using 4 replicate plant pieces for each sampling period. Measurements were dependent upon the availability of plants and were made throughout the 2 days following collection. The transient bloom macroalga *Hinckisa sordida* was sampled each time it was found in 1995 and 1996. The more common taxa *Ulva* sp. and *Polysiphonia decipiens* were sampled monthly for photosynthetic measures during 1996.

Oxygen exchange of the plants was measured in a circular Plexiglas chamber (750 ml volume) fitted with an oxygen electrode (StrathKelvin model 781, Scotland). The oxygen electrode was calibrated daily both in air saturated water and a 1% Na_2SO_3 solution at constant experimental temperature. A resolution of 0.01 mg O_2 L^{-1} could be attained with the apparatus. The seawater in the chamber was stirred by a magnetic bar to reduce boundary layer effects on the diffusion of inorganic C into the alga (Koch 1993). A constant *in situ* temperature (± 0.1°C) was maintained by a water bath with the aid of a refrigeration unit and thermo-circulator. For each replicate measurement the chamber was replenished with filtered seawater (0.2 μm) which was autoclaved to O_2 concentrations < 30% of saturation to reduce photosynthesis inhibition resulting from high oxygen tensions (Littler 1979). To provide sufficient inorganic C in the seawater medium, $NaHCO_3$ was added to a concentration of 3 mM. For each species the algal dry weight to chamber volume ratio was less than 0.03 g dry wt. L^{-1}. This ratio provided optimal photosynthetic rates as determined by preliminary studies (data not shown).

A slide projector (Kodak Carousel, model S-AV 2050) with a neutral density filter was used to provide photosynthetically active radiation (400-700 nm). Each algal piece was subject to 10 different PPFD's (0, 10, 25, 50, 100, 150, 200, 400, 800 1600 μmol m^{-2} s^{-1}) adjusted by varying distance between light source and plant chamber and measured using a quantum meter (Li-892 with UWQ sensor, Licor). The single plant pieces were incubated initially for 3 minutes to acclimatise the alga and then measurements of O_2 exchange were taken over 10 minute intervals. Each alga was weighed after being oven dried at 70°C for 24 h until constant dry weight was achieved and photosynthetic rates were expressed as mg O_2 evolved g dry $wt.^{-1}$ h^{-1}.

2.2.6 Pigment analysis

Tissue pigment analyses were made with fresh pieces of healthy tissue (0.05 g fresh weight) located adjacent to the tissue used for photosynthesis experiments. Photosynthetic rates (mg O_2 evolved) were also normalised to g Chl a^{-1} h^{-1}.

Chlorophyll (Chl) *a*, *b*, *c* and fucoxanthin were extracted for 20 minutes in dimethyl sulfoxide (DMSO) and Chl *a* and *c* were further extracted for 30 minutes in methanol (Duncan and Harrison 1982). The concentration of pigments extracted in DMSO were determined from measurements of absorbance at a wavelength of 665 nm (Chl *a*), 631nm and 582 nm (Chl *c*) and 665 nm, 631 nm, 582 nm, and 480 nm (fucoxanthin). Pigment concentrations were calculated according to equations of Seeley et al. (1972). The extractions in methanol of Chl *a* were determined at 668 nm and 665 nm, and Chl *c* was determined at 668 nm and 635 nm. Concentrations were calculated according to the equations of Jeffrey and Haxo (1968). Chl *b* was determined at 652 nm and 665 nm according to the equation outlined in Porra et al. (1989).

Phycorytherin content was measured by homogenising 0.1 g (fresh weight) tissue with 0.1 M phosphate buffer (pH 6.5). The homogenate was centrifuged at 426 *g* for 20 minutes and phycorytherin content was determined at 565 nm and calculated according to the equations of Rowan (1989). All extracts were corrected for turbidity

by measuring absorbance at 750 nm. This was then subtracted from pigment concentrations which were expressed as mg g dry wt.$^{-1}$. The optical density of all supernatant samples was analysed with a spectrophotometer (Shimadzu, UV1201) .

2.2.7 Photosynthetic parameters

Calculation of the photosynthetic parameters P_{max}, α and I_c were made by fitting P/I data to a rectangular hyperbolic model (Jassby and Platt 1976) using a non-linear curve fitting program. The equation takes the form:

$$P = P_{max} \cdot (1 - \exp(-\alpha \cdot (I - I_c) / P_{max})) \qquad \text{(Eq. 2.1)}$$

where P is the gross photosynthetic rate, P_{max} is the maximum gross rate of photosynthesis, α is the slope of the curve or photosynthetic efficiency, I is the irradiance and I_c is the compensation irradiance. The theoretical onset of light saturation (I_k) was subsequently calculated from P_{max}/α (Talling 1957). P_{max} was normalised to both a gram dry weight and gram chlorophyll *a*.

2.2.8 Annual productivity and N budgets

Monthly midday photon flux densities (PFD's) were measured *in situ* at a depth of 3 m (Campbell and Burridge 1998; Fig 2.4B), and were fitted to the model;

$$I_t = I_{sat} * \sin^2(t*\pi/P) \qquad \text{(Eq. 2.2)}$$

where I_t is the PFD (μmol m^{-2} s^{-1}) at any time t since dawn, I_{sat} is PFD at solar noon, P is daylength (h). This did not account for modification by atmospheric conditions (e.g. cloud cover).

This model gave a prediction of the duration of incident saturating (I_k) and non-saturating PFD's. Productivity budgets (mg C g dry wt.$^{-1}$ d^{-1}) for each of the species and the total algal biomass were calculated according to the following model:

$$\text{Productivity} = [(P \times I) + \Sigma (p_i \times I_i)] \times 2.67 \times B \qquad \text{(Eq. 2.3)}$$

where P = monthly mean P_{max}, I = duration of saturating light (I_k), p_i = mean photosynthetic rate for a given duration (h) of non-saturated light (I_i), 2.67 = atomic conversion factor from O_2 output to C assimilation (assuming PQ = 1:1) and B = mean biomass m^{-2}.

The biomass of macroalgae per unit sample area was multiplied by either mean photosynthetic rate or mean tissue N of individual species to calculate productivity (g C m^{-2} d^{-1}) and N requirements (g N m^{-2}) respectively. "All algae" estimates were based on the total biomass of macrolagae sampled, including the 3 species examined, and the mean photosynthetic rates and tissue N contents of the 3 species. Monthly estimates (Appendix 4-6) were derived from daily estimates. Annual productivity rates were then calculated. Productivity expressed as dry weight (g dw m^2 y^{-1}) was calculated by dividing carbon productivity rates by 0.3 (assuming a mean 30% C content from lab. measures). Annual N requirements were derived by productivity (g dw m^2 y^{-1}) multiplied by mean tissue N content. Biomass turnover per year was calculated as productivity (mg C m^{-2} y^{-1}) / biomass (mg C m^{-2}).

2.2.9 Statistical analyses

Data were tested for assumptions of normality by examining heterogeneity of variance (Cochrans test) and skewness of data (residuals and outliers). Non-normal data were subject to the log transformation, $\log_e(x+1)$. ANOVA was used to test for differences in monthly photosynthetic rates ($p < 0.05$). Least squares linear regression was employed to test for the significance of the relationships between tissue nutrient, pigment and seawater nutrient data. SYSTAT (vs. 5.03, Systat Inc., USA) was used for all linear and non-linear statistical analyses.

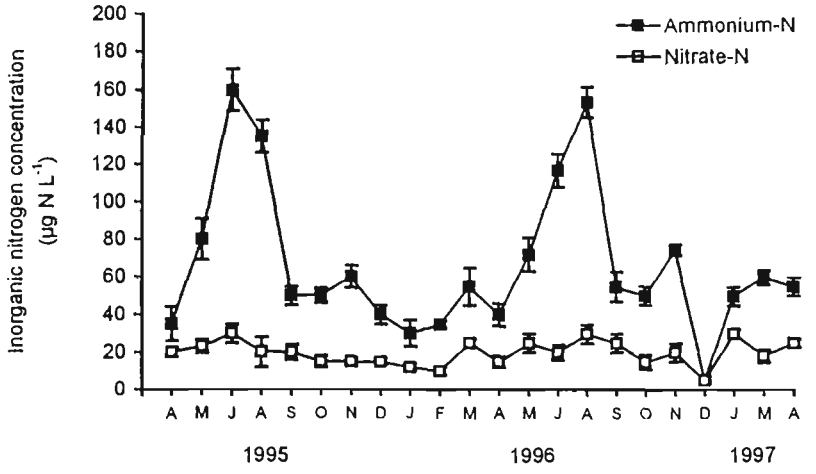

Fig. 2.2 Monthly dissolved inorganic nitrogen concentrations at the site offshore Murtcaim Drain, Werribee, Port Phillip Bay, from April 1995 to April 1997. Values represent means ± 1 s.e, $n = 3$.

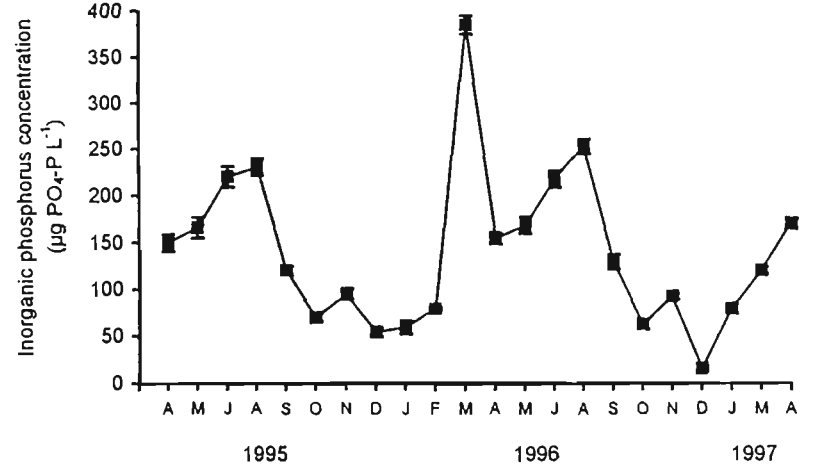

Fig. 2.3 Monthly dissolved inorganic phosphorus concentrations at the site offshore Murtcaim Drain, Werribee, Port Phillip Bay, from April 1995 to April 1997. Values represent means ± 1 s.e, n = 3.

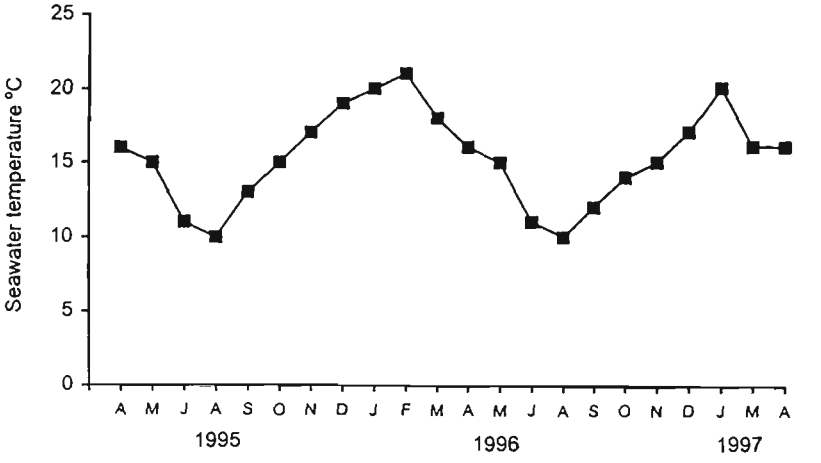

Fig. 2.4 Monthly bottom (3 m depth) seawater temperature at the site offshore Murtcaim Drain, Werribee, Port Phillip Bay, over a two year period.

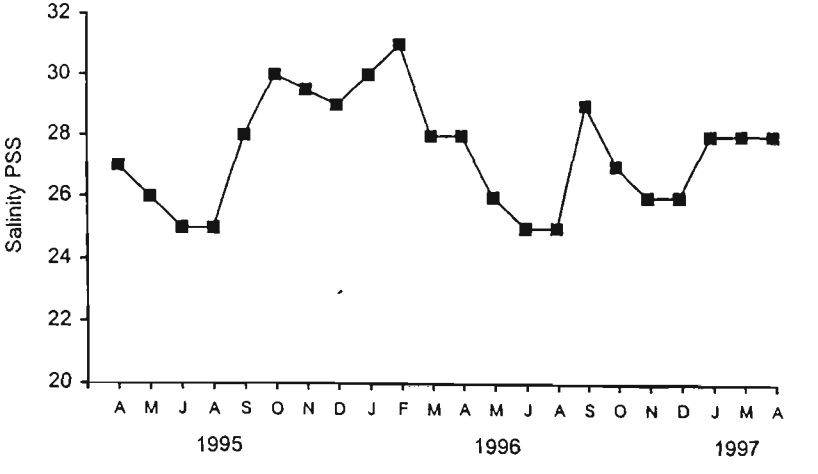

Fig. 2.5 Monthly bottom (3 m depth) seawater salinity at the site offshore Murtcaim Drain, Werribee, Port Phillip Bay, from April 1995 to April 1997.

2.3 Results

2.3.1 Field physico-chemical parameters

Field measurements of DIN, DIP, temperature and salinity exhibited seasonal variation over a two year period from April 1995 to April 1997 (Figs. 2.2 to 2.5). Concentrations of ammonium ranged from 35 to 160 μg NH_4-N L^{-1}, nitrate ranged from 5 to 30 μg NO_3-N L^{-1}, and phosphate ranged from 16 to 384 μg PO_4-P L^{-1}. Minima were generally recorded in summer months (December to February) and maximum concentrations were recorded in winter months (July to August) (Figs. 2.2 and 2.3). Bottom sea-water temperatures ranged from 10-11°C in July-August to 20-21°C in January-February (Fig. 2.4). Salinity ranged from 25 to 29 PSS but showed no clear seasonal trend (Fig. 2.5).

During winter, the lower angles of the sun and cloud cover resulted in considerably lower midday light intensities at 3 m depth (90-295 μmol m^{-2} s^{-1}), compared to autumn spring and summer days (300-865 μmol m^{-2} s^{-1}), irrespective of cloud cover.

2.3.2 Seasonal biomass

The variation in macroalgal biomass over the two year period of study is shown for the three species in Fig. 2.6. *Hincksia sordida* tends to bloom under calm conditions during sustained periods of low wind and rainfall. The biomass of this species shows a pronounced seasonality from April 1995 to April 1997, commonly increasing between autumn and winter for both years but also during late summer in 1995. *Ulva* sp. exhibits a bimodal trend with peak biomass in summer and spring and *Polysiphonia decipiens* showed some variation in biomass but no marked seasonal response.

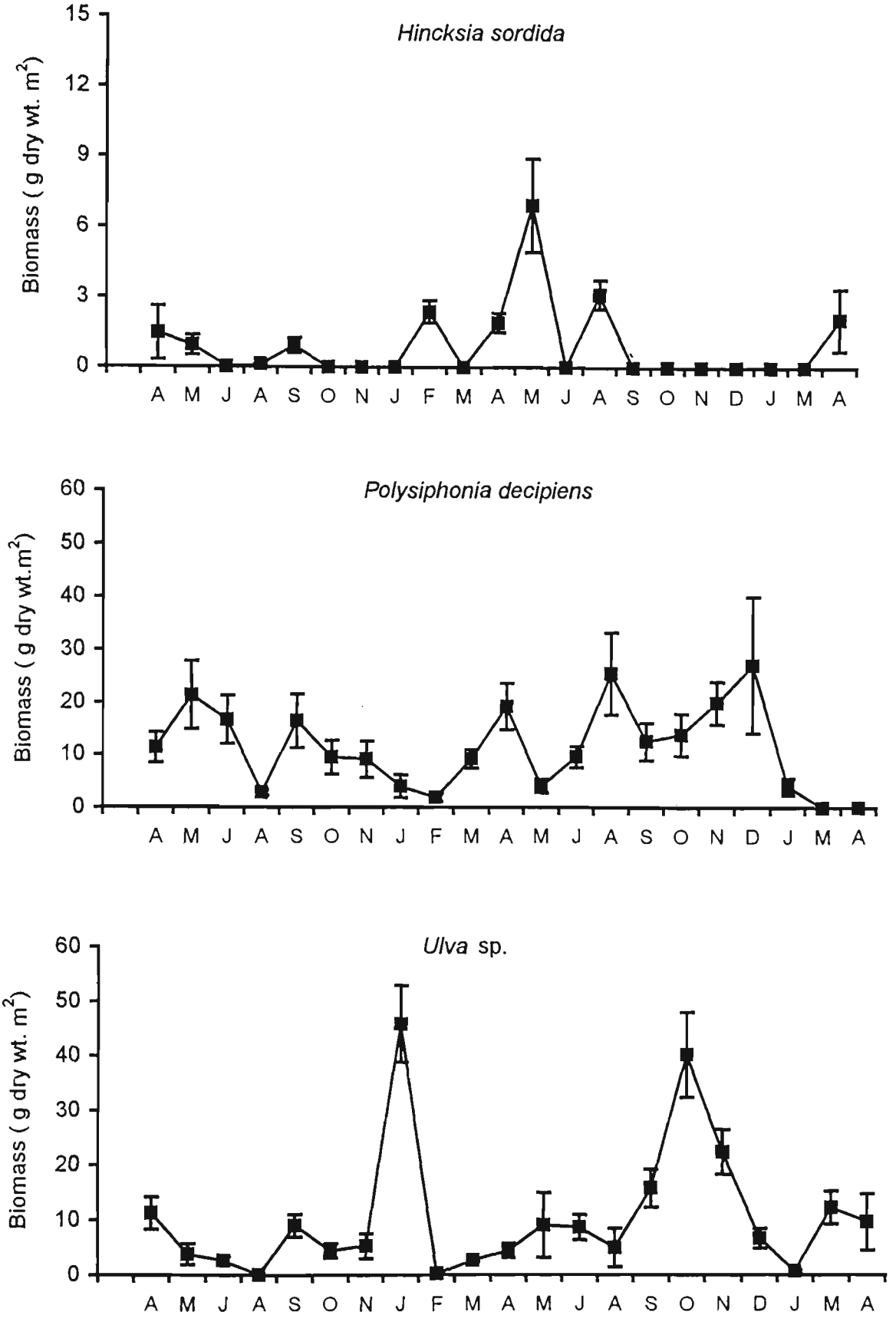

Fig. 2.6 Monthly biomass of selected species at a site (3 m depth) offshore Murtcaim Drain, Werribee, Port Phillip Bay from April 1995 to April 1997. Values represent means ± 1 s.e., n = 5.

2.3.3 Seasonal photosynthetic parameters

P_{max} (normalised to dry weight and chlorophyll *a*) of the 3 macroalgae exhibited marked seasonality. The P_{max} of *Hincksia sordida* was lowest in autumn, with values throughout the year ranging from 18.6 to 31.0 mg O_2 g dry wt.$^{-1}$ h^{-1} (Fig. 2.7). The P_{max} of *Polysiphonia decipiens* (range: 9.55 to 14.39 mg O_2 g dry wt.$^{-1}$ h^{-1}) and *Ulva* sp. (range: 11.99 mg O_2 g dry wt.$^{-1}$ h^{-1} to 31.36 mg O_2 g dry wt.$^{-1}$ h^{-1}) were lowest in winter and highest in summer. P_{max} therefore varied significantly ($p < 0.05$) across all months for each species (Appendix 3A and B) and a cubic polynomial, attesting unimodal seasonality, provides a significant fit for *Polysiphonia decipiens* ($r^2 = 0.66$) and *Ulva* sp. ($r^2 = 0.85$). Photosynthetic efficiencies (α) normalised to dry weight exhibit similar trends to P_{max} for each species (Fig. 2.8).

Compensation irradiance (I_c) for all species was generally higher in summer-autumn months compared to winter months (Fig. 2.9). No obvious seasonal trend was found for saturating irradiance (I_k) in *Hincksia sordida*, however *Polysiphonia decipiens* and *Ulva* sp. exhibited lower I_k values in winter suggesting a shift in the ratio of P_{max} to α (Fig. 2.10).

2.3.4 Seasonal tissue nitrogen, phosphorus and carbon

Tissue N and P generally exhibited peaks in winter months and were lowest in summer-autumn months for all species (Figs. 2.11 and 2.12). For *Hincksia sordida* mean tissue N ranged from 14.9 to 52.3 mg N g dry wt.$^{-1}$ and mean tissue P ranged from 1.5 to 5.2 mg P g dry wt.$^{-1}$. *Polysiphonia decipiens* showed least variation of all species in mean tissue N and P, ranging from 30.1 to 44.5 mg N g dry wt.$^{-1}$ and from 1.2 to 2.6 mg P g dry wt.$^{-1}$. In *Ulva* sp. tissue N ranged from 7.7 to 49.8 mg N g dry wt.$^{-1}$ and tissue P from 1.6 to 3.4 mg P g dry wt.$^{-1}$.

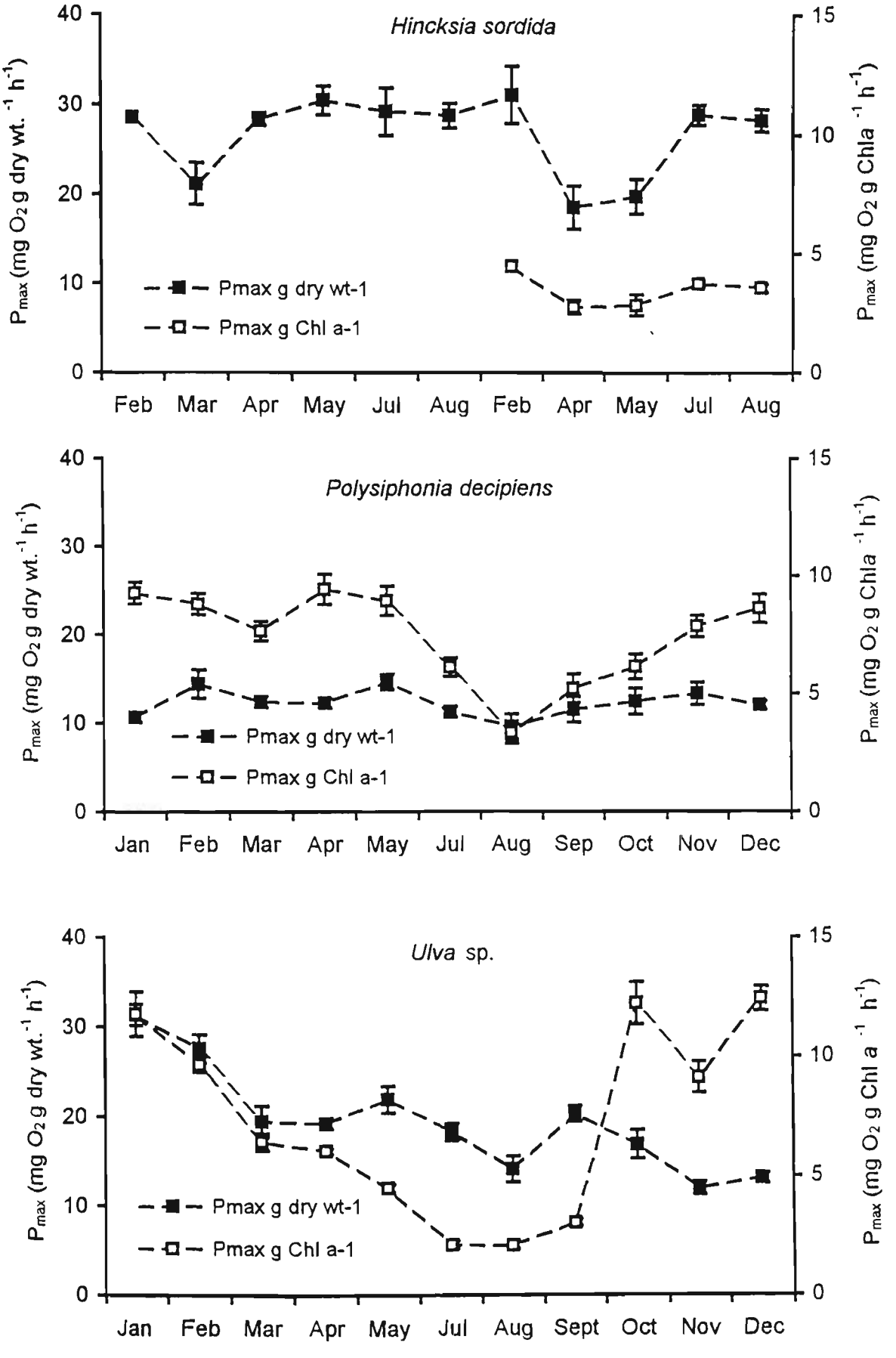

Fig. 2.7 Monthly variation in P_{max} for *H. sordida* in 1995 and for all three species in 1996. Values are expressed on a g dry wt.$^{-1}$ and a g Chl *a*$^{-1}$ basis and represent means ± 1 s.e., n = 4.

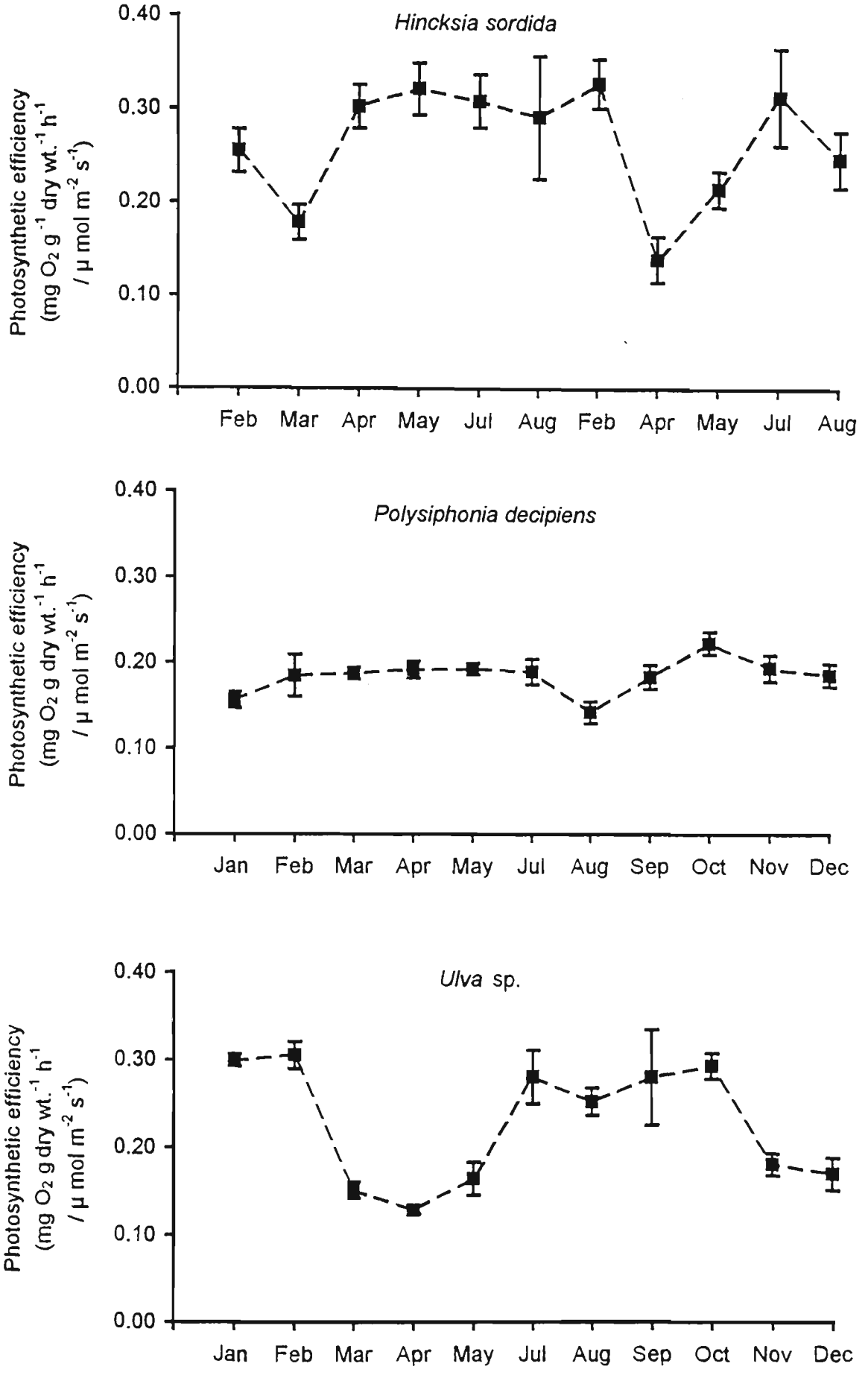

Fig. 2.8 Monthly variation in photosynthetic efficiency (α) for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

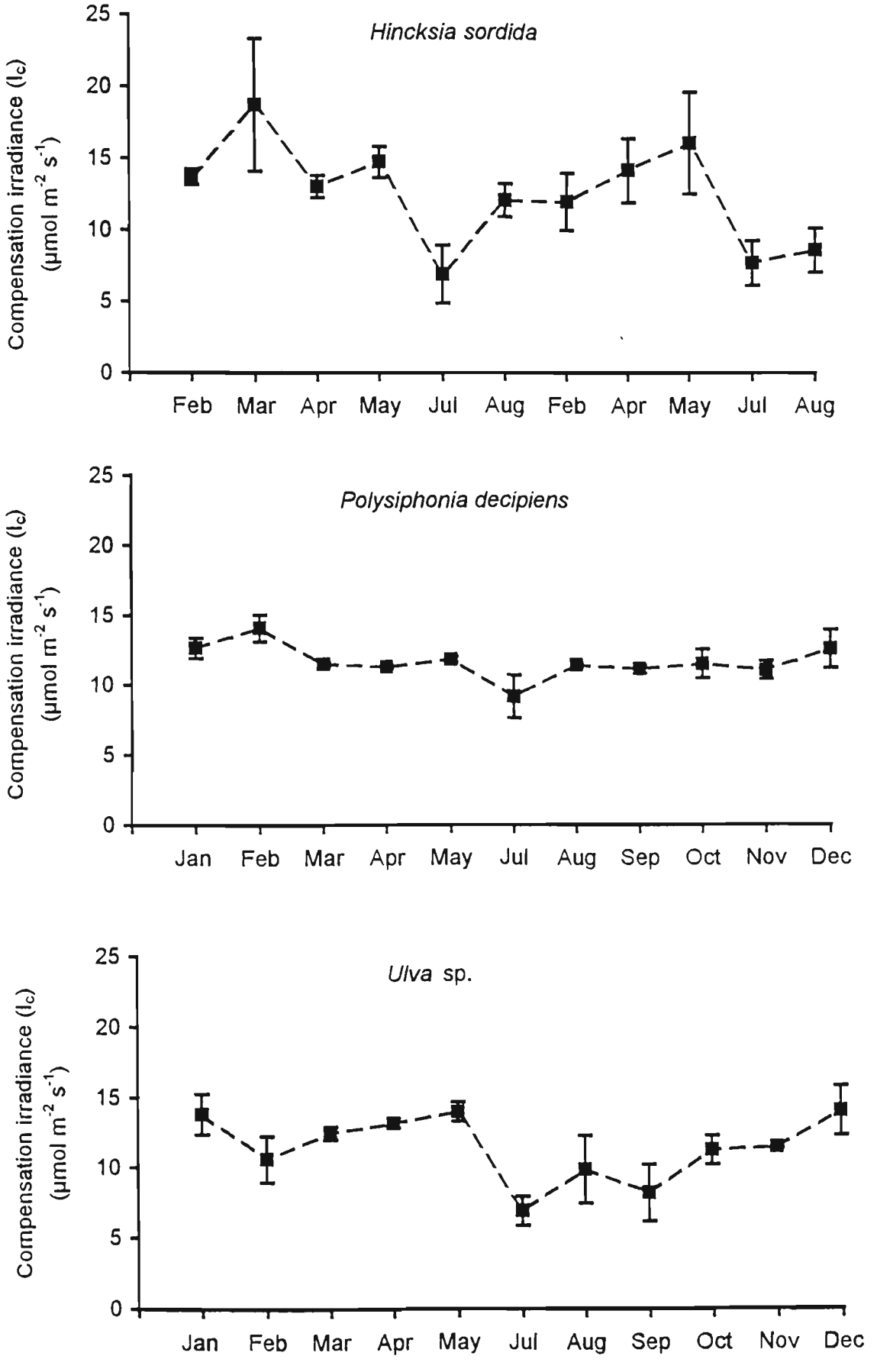

Fig. 2.9 Monthly variation in compensation irradiance (I_c) for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

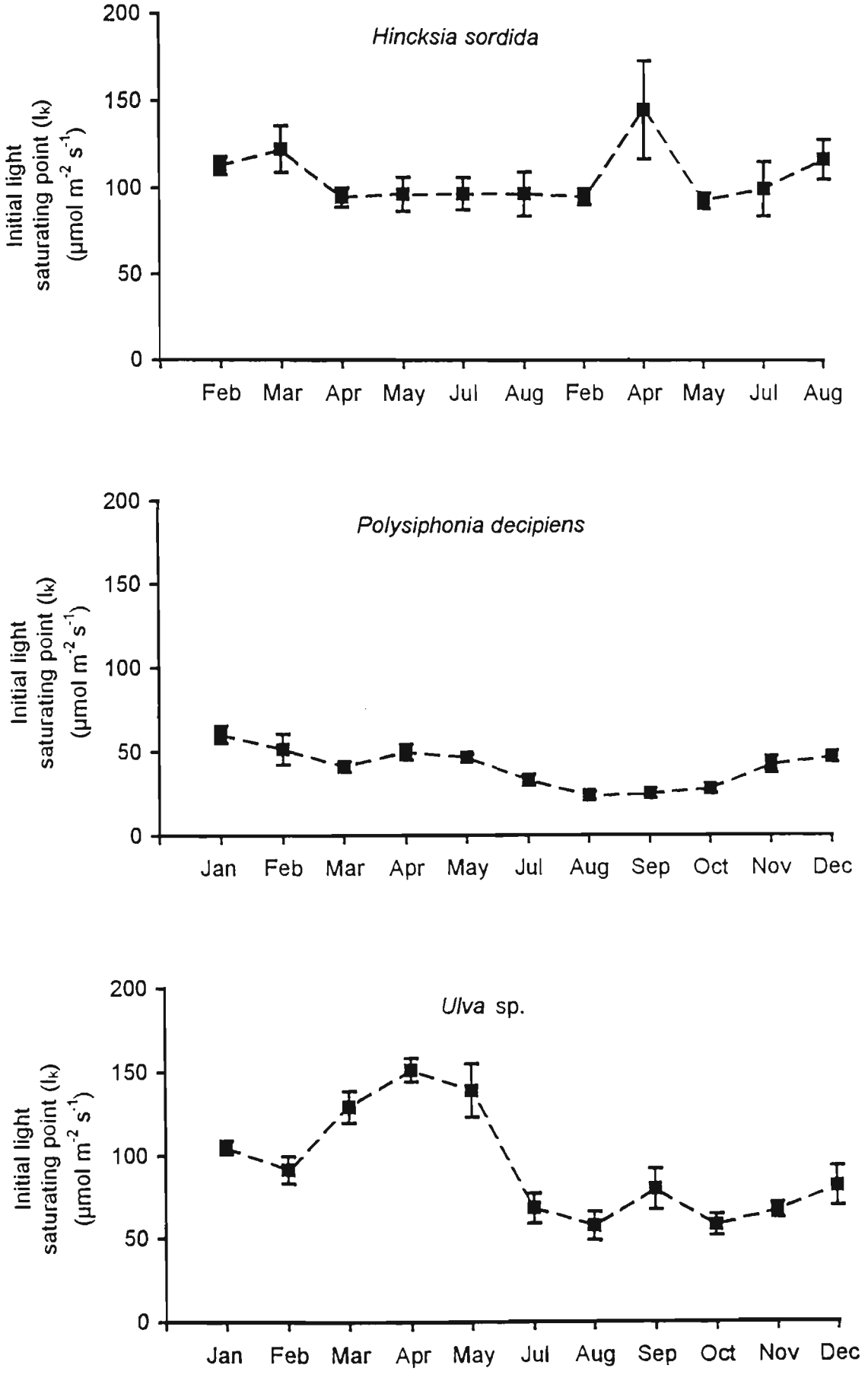

Fig. 2.10 Monthly variation in light saturation irradiance (I_k) for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

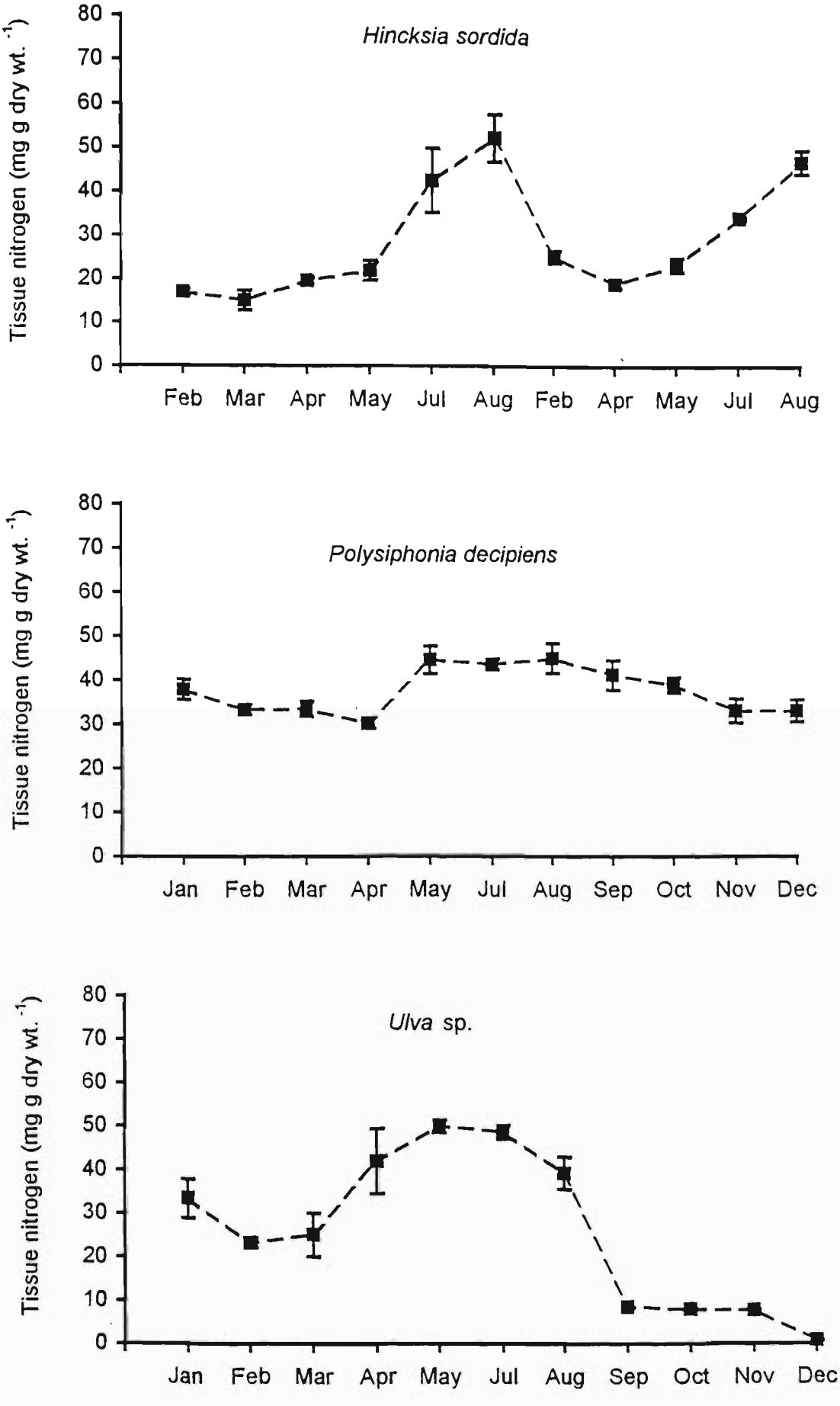

Fig. 2.11 Monthly variation in tissue nitrogen concentration for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

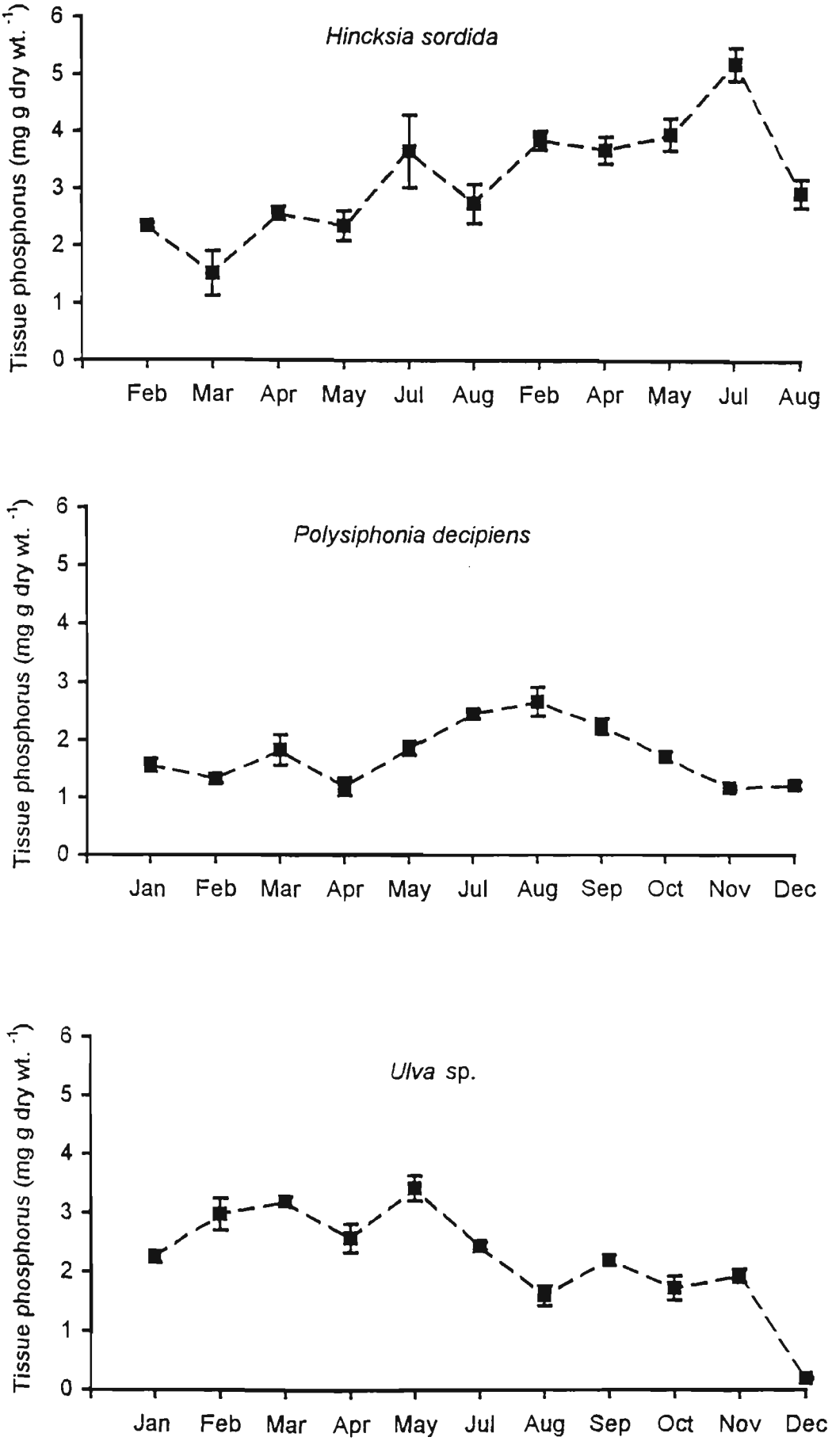

Fig. 2.12 Monthly variation in tissue phosphorus concentration for for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

Molar N:P ratios were also higher in winter months than summer months with mean values ranging from 11.9 to 45.8 (*Hincksia sordida*), 9.1 to 71.1 (*Ulva* sp.) and from 30.2 to 64.9 (*Polysiphonia decipiens*) (Fig. 2.13). Conversely, molar C:N ratios were lowest in winter and highest in summer-autumn months, ranging from 6.4 to 23.6 (*Hincksia sordida*), 8.2 to 47.8 (*Ulva* sp.) and 7.1 to 12.5 (*Polysiphonia decipiens*) (Fig. 2.14).

2.3.5 Tissue nutrients and seawater nutrients

There was a significant positive relationship ($p < 0.05$) between tissue N and ambient seawater DIN for each species (Fig. 2.15). The relationship between tissue P and ambient DIP was not significant for *Hincksia sordida*, *Polysiphonia decipiens* or *Ulva* sp.

2.3.6 Chlorophyll *a*

In *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. minimum mean chlorophyll *a* contents were recorded in summer and autumn months and highest values were generally found in winter months (Fig. 2.16). The annual range of chlorophyll *a* in *Hincksia sordida* was from 6.89 to 8.02 mg g dry wt.$^{-1}$. In *Polysiphonia decipiens* chlorophyll *a* ranged from 1.32 to 2.85 mg Chl *a* g dry wt.$^{-1}$. In *Ulva* sp. chlorophyll *a* ranged from a minimum of 1.06 mg g dry wt.$^{-1}$ to a maxima of 8.95 mg g dry wt.$^{-1}$.

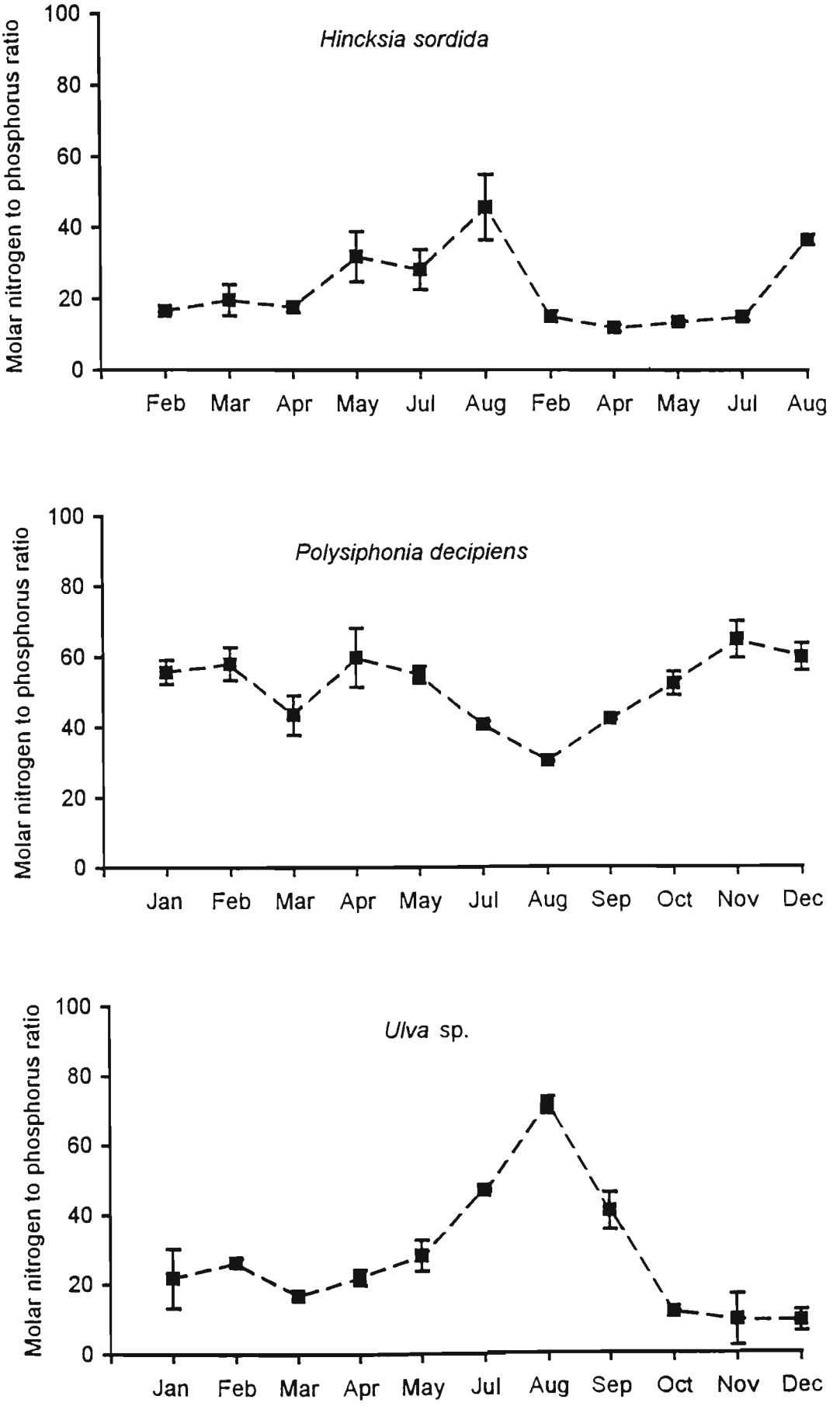

Fig. 2.13 Monthly variation in tissue nitrogen to phosphorus ratio for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

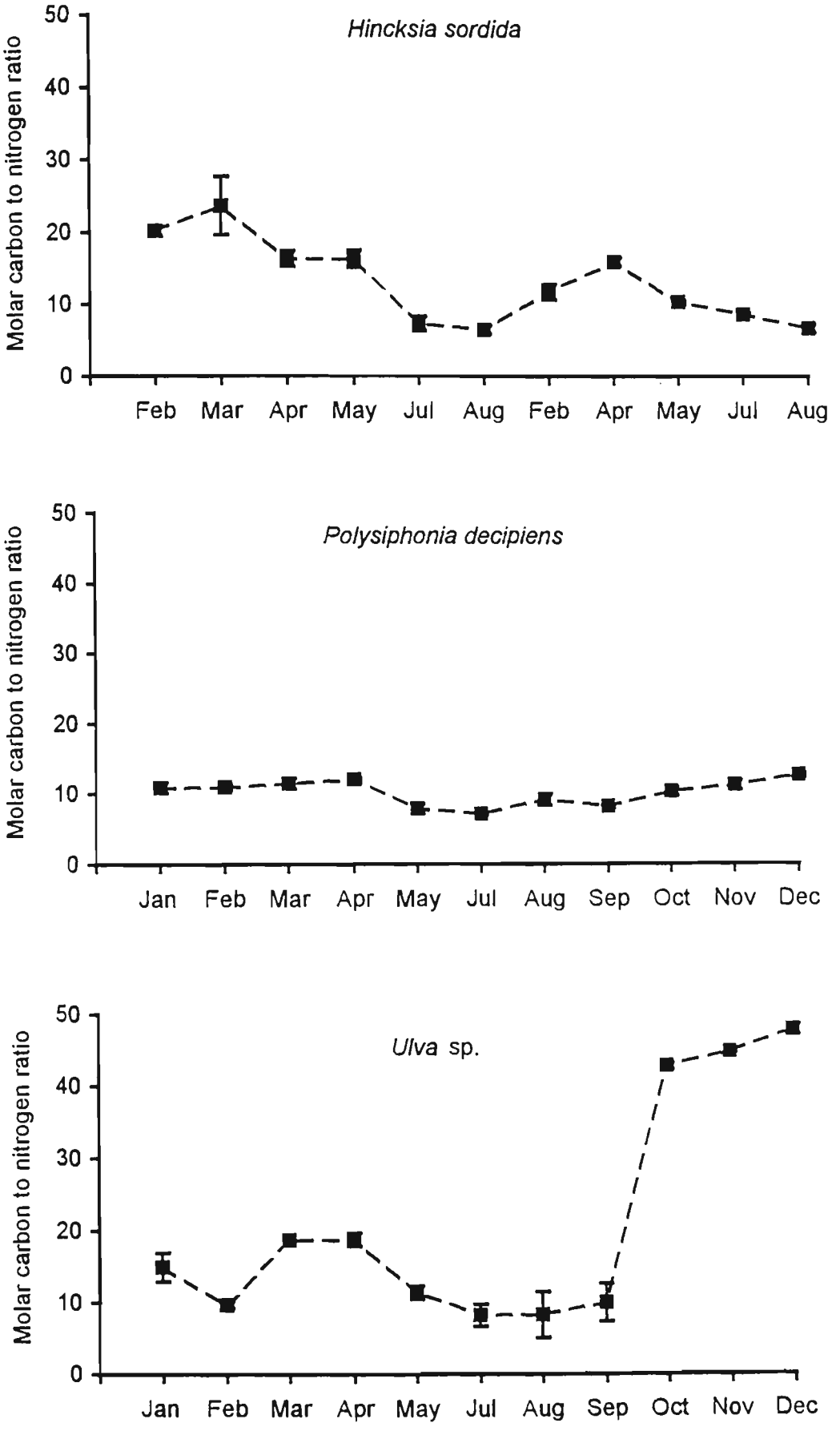

Fig. 2.14 Monthly variation in tissue carbon to nitrogen ratio for *H. sordida* in 1995 and for all three species in 1996. Values represent means ± 1 s.e., n = 4.

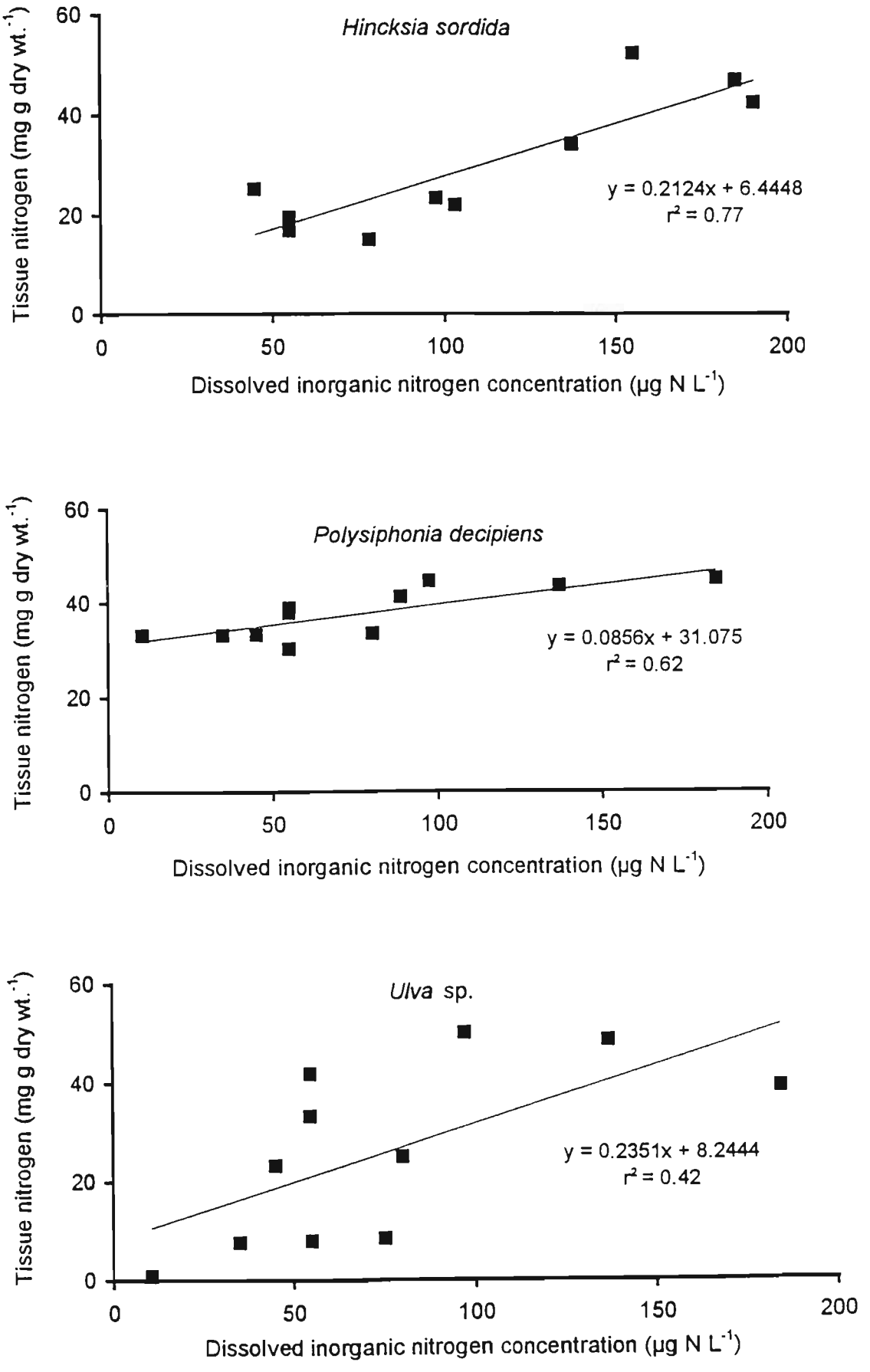

Fig. 2.15 Relationship between tissue nitrogen concentration and ambient dissolved inorganic nitrogen concentration in three species of macroalgae.

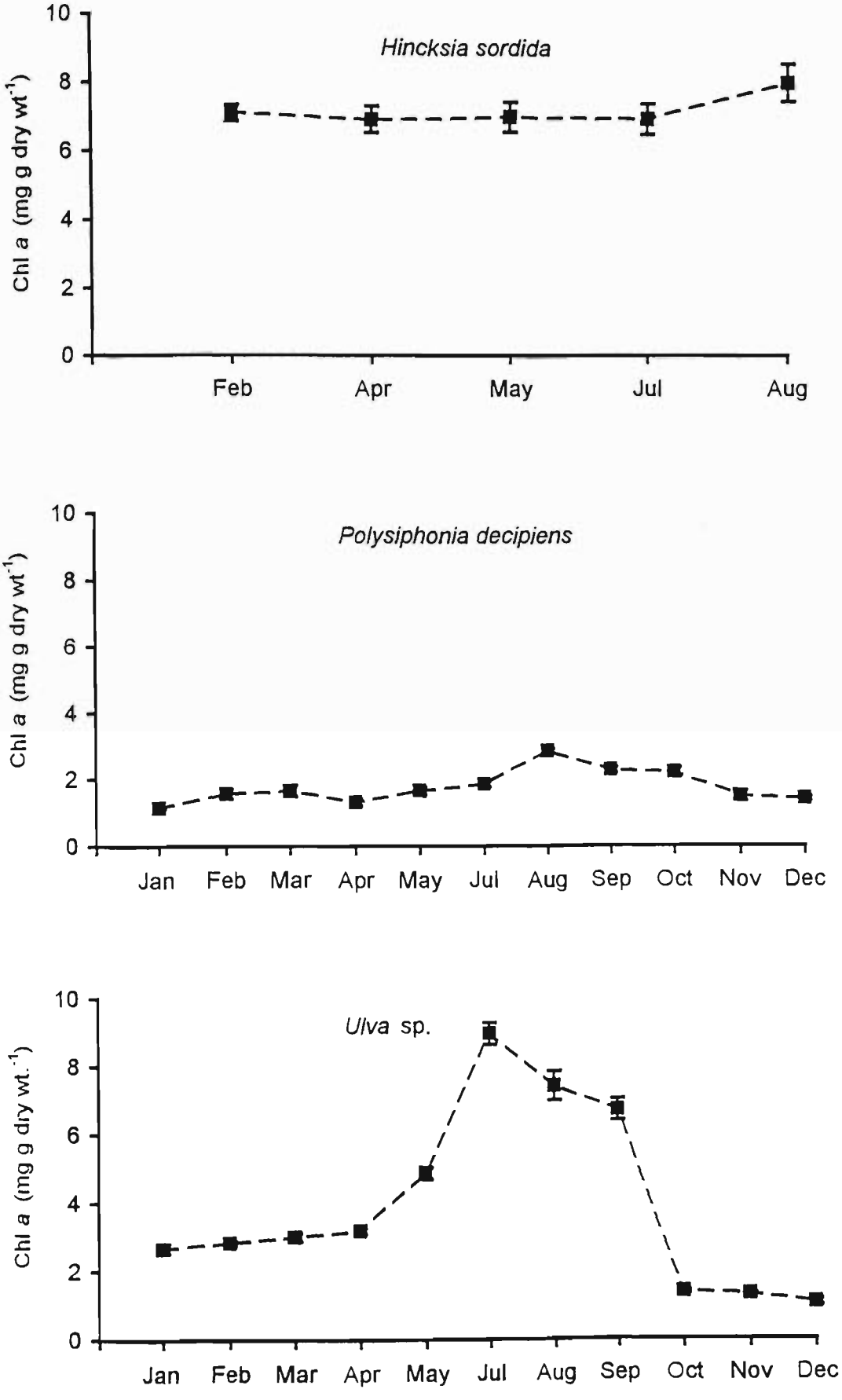

Fig. 2.16 Monthly variation in Chl *a* concentration for three species of macroalgae in 1996. Values represent means ± 1 s.e., n = 4.

2.3.7 Accessory pigments

Fucoxanthin, phycorytherin and Chl *b* content in *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. respectively were lowest in summer and autumn months and highest in winter months (Fig. 2.17). Mean values of fucoxanthin ranged from 0.711 to 1.29 mg g dry wt^{-1}; phycorytherin ranged from 0.14 to 0.77 mg g dry wt^{-1} and Chl *b* ranged from 0.04 mg g dry wt^{-1} to 1.27 mg g dry wt^{-1}. No seasonal trend was obvious for Chl *c* in *Hincksia sordida* which ranged from 1.80 to 4.16 mg g dry wt^{-1}.

2.3.8 Pigment ratios

Ratios of fucoxanthin : Chl *a*, phycorytherin : Chl *a* and Chl *b* : *a* in all species exhibited minima in summer and maxima in winter (Fig. 2.18). Mean values ranged from 0.10 to 0.19 (*Hincksia sordida*), 0.10 to 0.32 (*Polysiphonia decipiens*) and 0.029 to 0.166 (*Ulva* sp.). Chlorophyll *c* to *a* ratios in *Hincksia sordida* exhibited no seasonal trend and ranged from 0.26 in July to 0.60 in May.

2.3.9 Pigments and tissue nitrogen

There was no relationship ($p > 0.05$) between chlorophyll *a* and tissue N in *Hincksia sordida*. A significant linear relationship between fucoxanthin and tissue N was evident ($df = 14$, $F = 17.08$, $r^2 = 0.58$, $p < 0.001$). No significant relationships were evident between chlorophyll *a* and tissue N or between phycorytherin and tissue N in *Polysiphonia decipiens*. By contrast, for *Ulva* sp. there was a strong significant linear relationship between chlorophyll *a* and tissue N ($df = 32$, $F = 291.03$, $r^2 = 0.90$, $p < 0.001$) and between chlorophyll *b* and tissue N ($df = 32$, $F = 55.68$, $r^2 = 0.69$, $p < 0.001$).

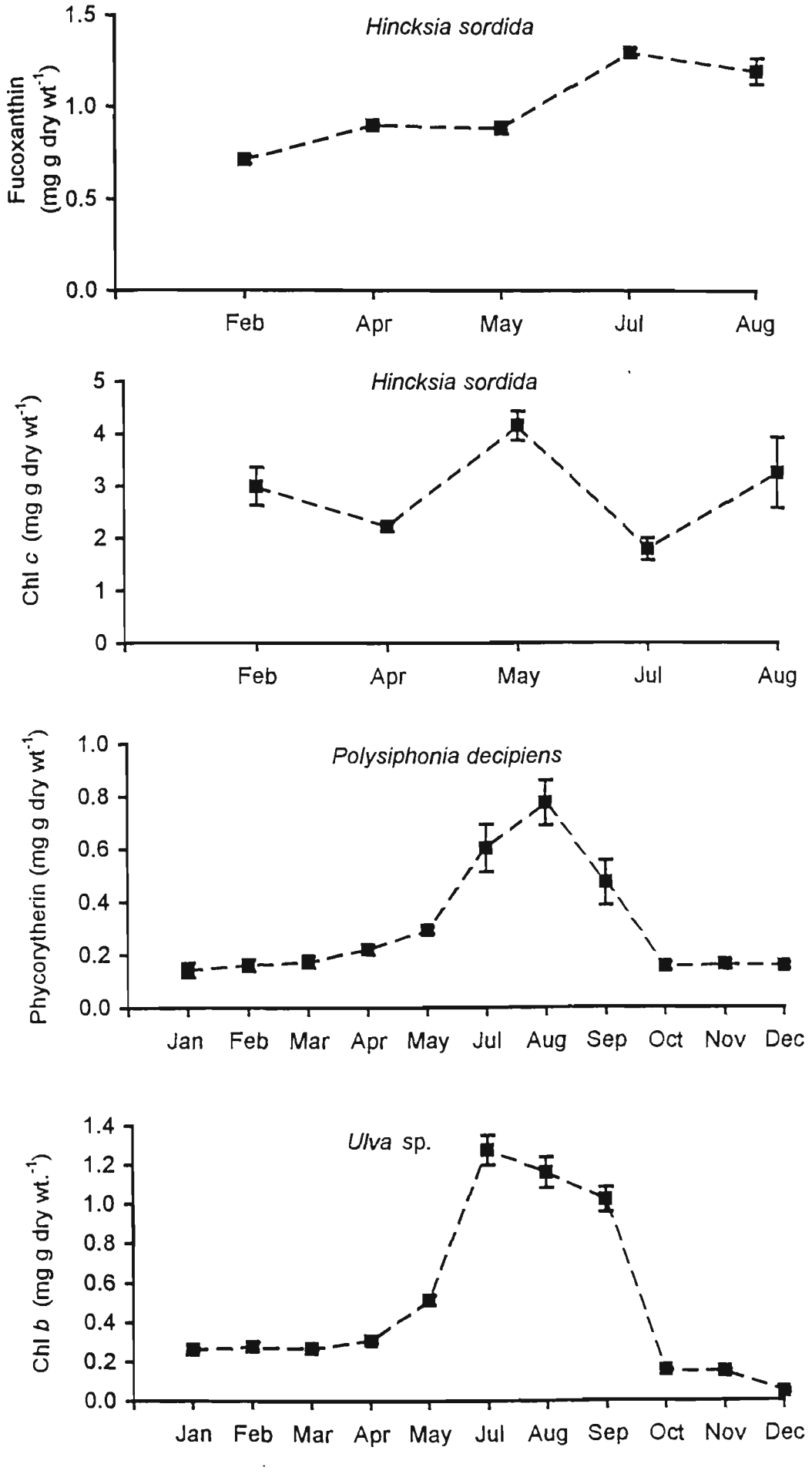

Fig. 2.17 Monthly variation in accessory pigment concentration for three species of macroalgae in 1996. Values represent means ± 1 s.e., n = 4.

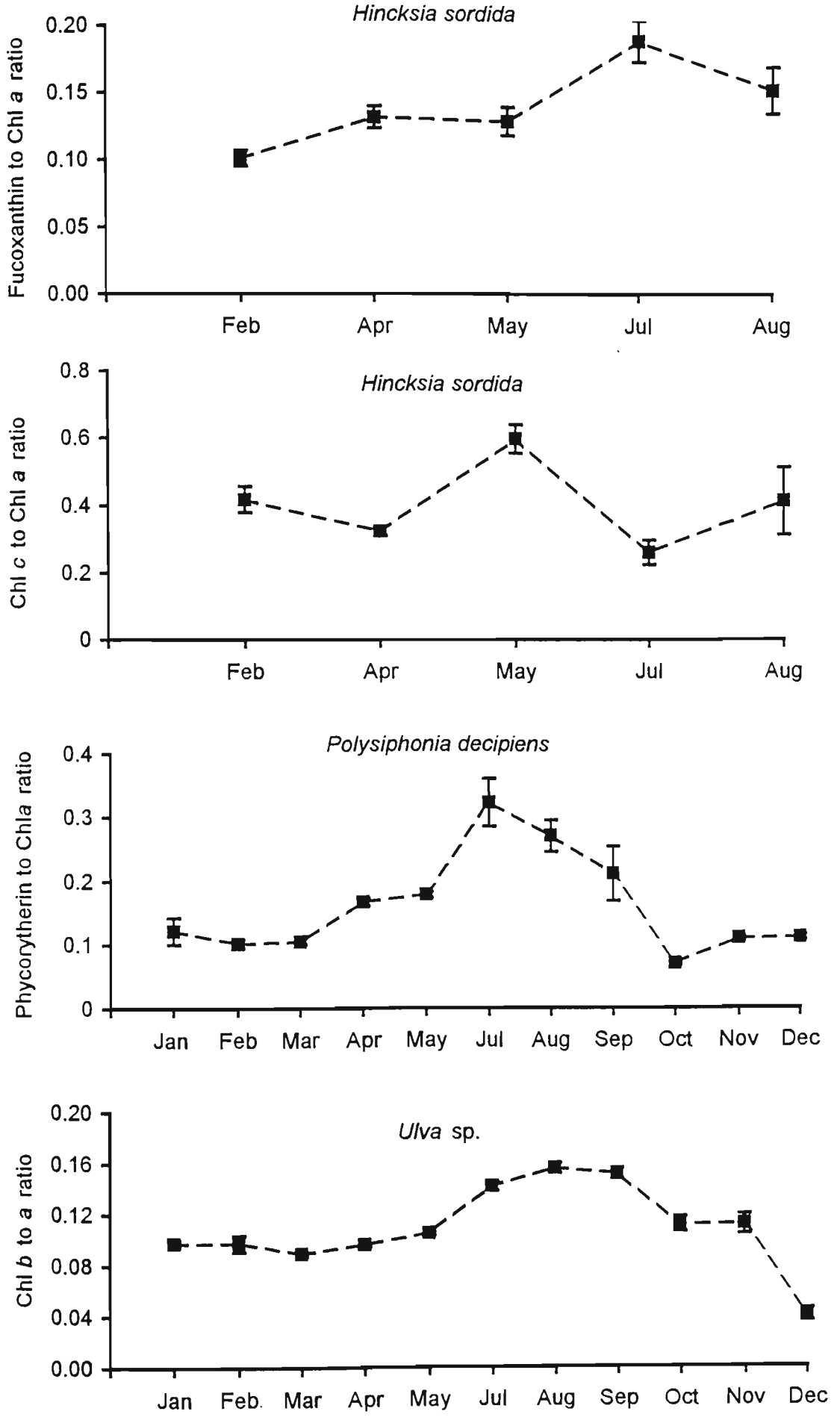

Fig. 2.18 Monthly variation in accessory pigment to Chl *a* ratios for three species of macroalgae in 1996. Values represent means ± 1 s.e., n = 4.

2.3.10 Pigments and tissue phosphorus

There was no significant relationship ($p > 0.05$) between chlorophyll *a* and tissue P for *Hincksia sordida* or *Ulva* sp. Significant but weak linear relationships between chlorophyll *a* and tissue P ($df = 29$, $F = 17.85$, $r^2 = 0.39$, $p < 0.001$) and between phycorytherin and tissue P ($df = 29$, $F = 21.26$, $r^2 = 0.43$, $p < 0.001$) were found for *Polysiphonia decipiens*.

2.3.11 P_{max} (normalised to dry weight) and pigments

There was no relationship ($p > 0.05$) between P_{max} and chlorophyll *a* content in *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. Similarly there was no relationship ($p > 0.05$) between P_{max} and fucoxanthin, phycorytherin and chlorophyll *b* in *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. respectively.

A summary of the above linear regressions between tissue nutrients (N and P) and physiological parameters (P_{max} and pigments) outlined in sections 2.3.9 to 2.3.11 are provided in Table 2.1.

Table 2.1 Relationships between tissue nutrients (N and P) (independent variable) and physiological parameters (dependent variable) for each species determined by linear regression of log transformed data. Significance level; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. = not significant.

Species	**Tissue N** vs. Chl *a*	Accessory pigments	P_{max}	**Tissue P** vs. Chl *a*	Accessory pigments	P_{max}
Hincksia sordida	n.s.	**	n.s.	n.s.	n.s.	n.s.
Polysiphonia decipiens	n.s.	n.s.	n.s.	**	***	n.s.
Ulva sp.	***	*	n.s.	n.s.	n.s.	*

2.3.12 Annual productivity and nutrient budgets

Annual estimates of productivity, N requirements and turnover (Table 2.2) were calculated from photosynthetic and standing biomass measurements. Carbon production was based upon the assumption that saturating light irradiance (I_k) for each species was reached for at least 8 hours a day, for each month throughout the year, as shown by modelling of *in situ* light data. Productivity at non-saturating light intensities varied amongst species and was highest for *Hincksia sordida* and lowest for *Polysiphonia decipiens*. A PQ ratio of C assimilation to O_2 evolution of 1:1 was assumed (Kindig and Littler 1980). Values are presented on a monthly basis in Appendices 4 to 6.

Ulva sp. exhibited the highest annual productivity, N requirement and turnover of the three species examined in 1996. These rates were about 2 to 3 times higher than those recorded for *Hincksia sordida* and *Polysiphonia decipiens*. *Ulva* sp. contributed 21.2% of the total N requirement of the whole community. *Polysiphonia decipiens* and *Hincksia sordida* contributed 11.6% and 7.8% respectively of the total N requirement of the entire macroalgal community. In total, the three species contributed to 51% of the estimated productivity of all species and 41% of the total N requirement for all taxa.

Table 2.2 Annual (1996) estimates of gross productivity, N requirements and turnover time for 3 species of macroalgae offshore the Western Treatment Plant, Werribee, Port Phillip Bay. Productivity (g C m^{-2} y^{-1}) estimates are based on mean oxygen production rates converted to carbon assimilation (assuming PQ = 1:1, gross saturating photosynthesis of 8 h d^{-1} plus non-saturating gross photosynthesis and respiration for 12 h d^{-1}). Dry weight productivity (g dw m^{-2} y^{-1}) assumes a C content of 30% of dry weight.

Species	Productivity (g C m^{-2} y^{-1})	Tissue nitrogen (mg N g dw^{-1})	Biomass (g dw m^{-2})	Biomass (g C m^{-2})	Nitrogen requirement (g N $m^{-2}y^{-1}$)	Productivity (g dw $m^{-2}y^{-1}$)	Biomass Turnover (no. times y^{-1})
Hincksia sordida	80	0.029	50.0	15.1	7.9	267	5.3
Ulva sp.	223	0.029	49.7	21.3	21.5	742	10.4
Polysiphonia decipiens	96	0.037	11.7	26.3	11.8	320	3.6
All algae	779	0.032	576	173	101.3	3197	4.5

2.4 Discussion

The study showed significant differences between photosynthetic performance, tissue nutrient and pigment contents of the species examined. The temporal variability in these parameters also differed between species and may be used to explain divergent growth strategies over the annual cycle. Photosynthetic capacity in 2 of the 3 species examined coincided with high summer temperatures and low nutrient availability, suggesting that light and or temperature were the primary factors limiting their productivity. Low saturating light irradiances (I_k) and high tissue N and pigment contents in winter generally served to optimise photosynthetic performance at low irradiances and maintain standing biomass in the third species examined. Species of macroalgae with such physiological traits are likely to be limited by nutrient availability. Further the relationships between tissue N and pigment content of the macrolagae examined and water column DIN concentration suggests that macroalgae are useful for detecting trends in exposure of marine communities to N inputs into Port Phillip Bay.

2.4.1 Seasonal standing biomass

The standing biomass of *Ulva* sp. reflects changes in P_{max}. This suggests that the optimisation of photosynthetic output by *Ulva* sp. during conditions of high light directly contributes to growth. No such relationship could be determined for *Hincksia sordida* or *Polysiphonia decipiens*. The peaks in biomass of *Hincksia sordida* from late summer through to winter are comparable with the seasonality of the free floating filamentous Phaeophyte *Pilayella littoralis* from Nahant Bay, USA (Wilce et al. 1982). This population exhibited maximum biomass in late summer and the absence of reproductive spores was insufficient for biomass maintenance from late spring to early winter.

The persistent standing biomass of *Polysiphonia decipiens* over the annual cycle suggests a growth strategy favorable to the storage of nutrient reserves, possibly at the expense of photosynthesis and growth (see Chapter 4). The transient growth of *Hincksia sordida* may preclude long-term nutrient storage. Its relatively high photosynthetic output in winter, when water column DIN concentration is high, may be utilised for pigment production. The importance of pigments for physiological maintenance during periods of relatively low growth has been reported for a number of algae (Rosenberg and Ramus 1982a; Geertz Hansen and Sand Jensen 1992; Horrocks et al. 1995; Pederson 1995; Fillit 1995) and is examined further in Chapter 3.

The proliferation of *Hincksia sordida* and *Ulva* sp. in summer and autumn respectively suggests that light and temperature may be primary factors influencing *in situ* productivity. *Hincksia sordida* blooms were sustained over periods of 2-3 weeks during conditions of low rainfall and/or calm winds (< 10 knots) such that water movement was reduced, turbidity decreased and light penetration increased. Whilst the sustained growth and photosynthetic efficiency of *Hincksia sordida* in winter may be facilitated by high DIN in the water column, it is possible that fluxes of porewater DIN facilitated growth of *Hincksia sordida* during periods of low water column DIN in summer and autumn.

Pregnall and Miller (1988) reported that large aggregations of the free living filamentous alga *Pilayella littoralis* (Phaeophyta) in Nahant Bay contributed to the greater flux of ammonium from sediment porewater. Ammonifying bacterial respiration increases during algal decay (Owens and Stewart 1982), and the subsequent reduction in available oxygen results in much of the N (as nitrate) being converted to ammonium by bacterial nitrate reduction ($NO_3^- \Rightarrow NH_4^+$). The period of time between decomposition of algal biomass and efflux of remineralised nutrients from sediments varies according to seasonal changes in temperature, oxygen availability, light and water movement (Pregnall and Miller 1988), and such factors

may influence the frequency of macroalgal blooms, such as *Hincksia sordida*, in Port Phillip Bay.

2.4.2 Seasonal photosynthetic characteristics

The seasonal trends in P_{max} and α for each of the species examined are generally consistent with published seasonal photosynthetic rates of related taxa. In these studies maximum rates of photosynthesis are achieved at spring and/or summer temperatures during observed growth periods. P_{max} values reported for *Ulva* sp. and *Polysiphonia* in this study are similar in magnitude to the range in rates recorded for *Polysiphonia* spp. (Fralick and Mathieson 1975; King and Schramm 1976) and *Ulva* sp. (Brinkhuis 1977; Brown et al. 1980; Rosenberg and Ramus 1982a; Lavery et al. 1991; Vergara et al. 1997). P_{max} rates obtained for *Hincksia sordida* (18.6 to 31.0 mg O_2 g dry wt.$^{-1}$ h^{-1}) were higher than other filamentous Phaeophyta such as *Ectocarpus confervoides* (P_{max} = 15 mg O_2 g dry wt.$^{-1}$ h^{-1}; King and Schramm 1976) and *Pilayella littoralis* (P_{max} = 16.3 mg O_2 g dry wt.$^{-1}$ h^{-1}; Wallentinus 1978).

The ability of *Polysiphonia decipiens* and *Ulva* sp. to exhibit optimal P_{max} and α at summer temperatures of 20°C suggests that temperature and/or light may limit photosynthesis during winter. This is consistent with the findings of Fralick and Mathieson (1975) who showed that temperature influenced photosynthesis in four species of *Polysiphonia*. Optimal photosynthetic rates were recorded at 27 to 30°C and lowest photosynthetic rates were found at 10°C. Light and/or temperature have also been shown to be the primary factors influencing photosynthesis of *Ulva* spp. (Waite et al. 1972; Brinkhuis 1977; Hernandez et al. 1997), although the importance of N in initiating *Ulva* growth has also been demonstrated (Zavodnik 1987; Sfriso et al. 1987; Hernandez et al. 1997). By contrast, the ability of *Hincksia sordida* to maintain a similar P_{max} and α in winter and summer is consistent with the generally accepted hypothesis that photosynthesis is largely independent of temperature at relatively low PFD (Kirk 1983; Lapointe and Tenore 1981; Peckol et al. 1994; Gayol et al. 1995). A similar trend was observed for the filamentous *Pilayella littoralis* (Phaeophyta) from the Baltic Sea, which exhibited equivalent P_{max} and α irrespective

of temperature in autumn (4°C) and spring (9.7°C) (Wallentinus 1980). Growth of *Pilayella littoralis* in the USA was found to be optimal at low temperatures (10°C) and low PFD's (Wilce et al. 1982).

This absence of a relationship in *Hincksia sordida* between either P_{max} or α and temperature (when measured at field temperatures) is consistent with some studies on macroalgae which suggest that photosynthetic enzyme activity is not influenced by temperature at low temperatures (Davison et al. 1991). These authors concluded that the independence of photosynthetic efficiency and temperature reflects the ability of photosynthetic Calvin cycle enzymes (e.g. RuBPCase) to acclimatise to changing temperatures and thus remain efficient during low temperatures. This is also a common feature of deep sublittoral macroalgae which are commonly exposed to sub-saturating light intensities, allowing light limited photosynthesis to be sustained independent of temperature. Such a strategy may enable *Hincksia sordida* to achieve optimal P_{max} at winter temperatures. The maintenance of photosynthesis by *Hincksia sordida* at elevated light and temperatures, despite low N availability, is almost certainly due to enhanced RuBPCase activity. This has been observed in a number of studies on other macroalgae (Lapointe and Duke 1984; Lobban et al. 1985). In contrast, the low P_{max} and *in situ* growth of *Ulva* sp. and *Polysiphonia decipiens* in winter suggests that photosynthetic enzyme activity in these species is directly influenced by temperature (Davison et al. 1991; Keubler 1991; Gomez et al. 1995).

Compensation irradiances (I_c) of the three species examined were within the range (ca. 3 to 20 μmol m^{-2} s^{-1}) of re-calculated values in *Ectocarpus confervoides* (Phaeophyta) and *Polysiphonia nigrescens* (Rhodophyta) (King and Schramm 1976) and *Ulva curvata* and *Ulva rotunda* (Chlorophyta) (Vergara et al. 1997). Re-calculated I_k values from P-I curves, of 30 to 65 μmol m^{-2} s^{-1} for *Polysiphonia nigrescens* (Rhodophyta) from autumn to spring (King and Schramm 1976), and of 140 μmol m^{-2} s^{-1} for *Pilayella littoralis* (Phaeophyta) during autumn (Wallentinus 1978), are comparable to *Polysiphonia decipiens* and *Hincksia sordida* respectively. I_k values recorded for *Ulva* sp. were lower than those recorded for *Ulva rigida*

(Chlorophyta) (200 to 300 μmol m^{-2} s^{-1}) from a shallow (0.5 to 2.0 m deep) estuarine embayment in the Peel Harvey Inlet, Western Australia (Lavery et al. 1991) but were within the range recorded for *Ulva lactuca* (100 to 200 μmol m^{-2} s^{-1}) during summer in Port Phillip Bay (Brown et al. 1980).

As all species were from the same depth and habitat the seasonal differences in the photosynthetic saturating irradiance (I_k) most likely reflect divergent adaptive strategies of these algae to changing light and temperatures rather than to morphology (Lüning 1985; Gayol et al. 1995). The low compensation irradiance (I_c) of all species, and low I_k for *Ulva* sp. and *Polysiphonia decipiens* during winter months, is indicative of adaptation to lower light and temperature conditions. Shade adaptation allows optimal photosynthesis during low photon flux availability to be sustained. Low temperatures also reduce I_k values by reducing enzyme activity and pigment concentration which influence α and photosynthetic maxima respectively (Gordon et al. 1981; Lapointe and Duke 1984).

The increase in I_k and I_c during summer coincides with maximum rates of photosynthesis and respiration as temperatures increase (Kuebler et al. 1991). Algae showing these characteristics would require a higher level of irradiance at summer temperatures than at winter temperatures to maintain positive carbon balance, and may be more vulnerable to low light levels in summer than in winter. The generally higher I_k for *Hincksia sordida* and *Ulva* sp. compared with *Polysiphonia decipiens* also suggests that during low light conditions in winter, the former two taxa are more likely to photosynthesise below light saturation than *Polysiphonia decipiens*. Photosynthesis in such algae may be more susceptible to decreases in irradiance although the ability of *Hincksia sordida* to maintain P_{max} irrespective of temperature may compensate for this apparent susceptibility. By contrast, P_{max} of *Polysiphonia decipiens* and *Ulva* sp. in winter were low compared to rates in summer, but this may be balanced by a reduced winter I_k yielding optimal α at low irradiances (Flores-Moya et al. 1995).

2.4.3 Seasonal tissue nutrients

Low summer and autumn tissue N:P ratios in *Hincksia sordida* and *Ulva* sp. fell below the Redfield (1958) ratio of 16, indicative of potential N limitation during these periods. By contrast, consistently high N:P ratios (greater than 16) throughout the year in *Polysiphonia decipiens* indicates N sufficiency. This is consistent with other studies which have found low tissue N and P during summer when external supplies of inorganic N and P are at their annual minima (Asare and Harlin 1983; Hanisak 1983; Fujita et al. 1989; Björnsäter and Wheeler 1990; Lyngby 1990; Pederson and Borum 1996). In the present study the tissues of *Hincksia sordida* and *Polysiphonia decipiens* reflected the ambient DIN, suggesting that these taxa may be used as bioindicators of N. Similar responses have been shown for other fast growing macroalgae such as *Spyridea hypnoides* (Rhodophyta) (McGlathery 1992), *Gracilaria* spp. (Rhodophyta) (Horrocks et al. 1995), *Enteromorpha* spp. (Chlorophyta) (Jeffrey et al. 1995) and *Ulva* spp. (Chlorophyta) (Piriou and Menesguen 1992; Viaroli et al. 1992; Hernandez et al. 1997). The relatively high tissue P in *Ulva* sp. in summer and autumn suggests rapid uptake and dependence on P by *Ulva* sp. In contrast Wheeler and Björnsäter (1992) found that tissue P in *Ulva fenestrata* showed little seasonal variability despite low DIP availability in summer.

2.4.4 Seasonal carbon assimilation

Carbon assimilation is directly controlled by light (i.e. as part of process of photosynthesis) and provides a surrogate measure for growth. The ratios between C and nutrients or C and pigments are affected by factors such as nutrient availability and plant physiology. These ratios may be used to characterise the physiological state and growth potential of macroalgae (Lapointe 1981). In the present study summer peaks in C:N and C:pigment ratios in both *Polysiphonia decipiens* and *Ulva* sp. reflect high C assimilation during photosynthesis relative to consumption. They may represent an accumulation of reserve carbohydrates in excess of growth requirements and often occur when N availability is low (Bird et al. 1982; Rosenberg and Ramus 1982b; Lapointe and Duke 1984; McGlathery 1992).

Therefore high C:N ratios would be expected to correspond to maximum photosynthetic rates, α and *in situ* biomass, as found in this study. This is consistent with studies conducted at relatively constant temperatures, where carbon assimilation and growth follow the patterns of P_{max} and α respectively (Flores-Moya et al. 1995). It is also concordant with studies that have found maximum growth rates and high C assimilation relative to N uptake and pigment synthesis (Lapointe and Tenore 1981; Lapointe 1987).

The possible accumulation of reserve carbohydrates in *Polysiphonia decipiens* and *Ulva* sp. indicates that photosynthesis and possibly growth of these taxa was not N limited. Reserve carbohydrates are often utilised in light independent C fixation to minimise energy losses associated with dark respiration during low temperatures and low light availability (Henley and Dunton 1995). Sand-Jensen (1988) found a strong relationship between growth and dark respiration in *Ulva lactuca* during extreme low temperature and light conditions. In such cases there is no relationship between C:N and P_{max} or growth and α. There was no relationship between C:N ratios and photosynthetic rates in *Hincksia sordida*. This may be attributed to the maintenance of relatively high P_{max} in winter due to elevated N availability and possible temperature adaptation, as there was no evidence of enhanced dark respiration in *Hincksia sordida* at low winter temperatures.

2.4.5 Seasonal pigment concentration

Seasonal changes in pigments, pigment ratios and tissue N and P concentrations were generally more pronounced in the opportunistic taxa *Ulva* sp. and *Hincksia sordida* than in *Polysiphonia decipiens*. When coupled with a high biomass turnover this suggests an enhanced ability of *Ulva* sp. and *Hincksia sordida* to assimilate nutrients when supply is greatest between autumn and winter. Rosenberg and Ramus (1982a) noted similar responses in pigments and tissue N in *Ulva* sp. compared to the Rhodophyte *Gracilaria foliifera*. Chopin et al. (1995) also found higher tissue P concentration in the filamentous Phaeophyte *Pilayella littoralis* (3.0 to 5.4 mg g dry $wt.^{-1}$) compared to the Rhodophyte *Polysiphonia lanosa* (2.2 to 3.8 mg g dry $wt.^{-1}$).

In *Ulva* sp the pronounced seasonal differences in P_{max}, when normalised to chlorophyll *a*, suggests that this pigment strongly influences photosynthetic performance. Further, the maintenance of α during winter in *Ulva* sp. and *Polysiphonia decipiens* suggests a reliance on DIN or DIP availability during winter. The positive linear relationships between pigments and tissue N was species dependent and supported the notion that N was the primary factor influencing pigment changes in the macroalgae studied. Significant positive correlations between chlorophyll *a* and tissue N in *Ulva* sp. suggests that chlorophyll *a* functions as a N storage pool, consistent with other studies of *Ulva* spp. (Lapointe and Tenore 1981; Rosenberg and Ramus 1982a, b; Duke et al. 1987; Geertz-Hansen and Sand-Jensen 1992). Rosenberg and Ramus (1982b) contended that N loading is the primary determinant of pigmentation in fast growing seaweeds. This was shown experimentally by the scant change in chlorophyll *a* in *Ulva* sp. and chlorophyll *a* and phycorytherin contents in *Gracilaria foliifera* in response to changes in photon flux density during peaks in N loading.

There was no relationship between chlorophyll *a* and tissue N in *Hincksia sordida* but the relationship between fucoxanthin and tissue N suggests that N is used to synthesise fucoxanthin in this species. By contrast, N is utilised primarily by chlorophyll *a* in other Phaeophyta, including *Fucus vesiculosus* (Brinkhuis 1977), *Laminaria saccharina* (Chapman et al. 1978), *Ptelonia fascia* (Williams and Herbert 1989) and *Sargassum thunbergii* (Gao 1990).

The absence of a relationship between pigment and N content in *Polysiphonia decipiens* is in contrast to the demonstrated relationships between pigments (chlorophyll *a* and/or phycorytherin) and N availability in other Rhodophyta (Lapointe 1981; Lapointe and Duke 1984; Levy and Gannt 1990; Chopin et al. 1995; Horrocks et al. 1995). The relatively low tissue N in summer and maintenance of high pigment concentrations in *Polysiphonia decipiens* suggests that accrued nutrient stores are being utilised for pigment synthesis and summer growth during periods of low N availability. It may be possible that *Polysiphonia decipiens* has a relatively

higher storage capacity for nutrients and/or a low requirement of N for growth (see Chapter 4). Higher concentrations of accessory pigments (and ratios of accessory pigments to chlorophyll *a*) in winter algae, compared to summer, also imply that accessory pigments may be a major storage pool for excess N. These relationships are examined experimentally in Chapter 3.

The weak relationship between both chlorophyll *a* and phycorytherin content and tissue P indirectly suggests that that N is required for growth of *Polysiphonia decipiens*. In studies of the Rhodophyta P has been found to influence protein synthesis, N uptake and pigment content by stimulation of Rubisco activity (Lapointe 1985; Parry et al 1985; Garcia-Sanchez et al. 1996). Consequently, *Polysiphonia decipiens* may have a dependence on DIP availability to enhance N assimilation and metabolism necessary for pigment development.

2.4.6 Annual budgets

The comparison of annual energy budgets between macroalgal communities worldwide is made difficult by the different methodologies employed and the measures they describe. The use of ^{14}C methods provide estimates somewhere between gross and net production (net carbon fixation) during daylight hours and provides no estimate of respiration. Derivation of P-I characteristics from oxygen exchange methods allows estimates of gross production and associated carbon and nutrient budgets based on a number of assumptions.

As in any extrapolation the scales of temporal (day to day) and spatial (if available) variability should be considered when interpreting results. In the present study there was little examination of spatial variability across the wider macroalgal community at the site of study. Therefore, the temporal trends in production rates and standing biomass pertain to a small (30 m x 30 m) ephemeral macroalgal community offshore Werribee in PPB, and provide inter-species comparisons which are useful for interpreting eco-physiological characteristics measured in the laboratory.

The maximum standing biomass of individual species in this study was low compared to the biomass reported for other fast growing macroalgae worldwide, but values for all algae combined are comparable with other studies (Table 2.3). Daily C assimilation of 0.867 to 3.203 g C m^{-2} d^{-1} (or 80 to 223 g C m^{-2} y^{-1}) for *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. are lower than those reported for *Enteromorpha* spp. (10 g C m^{-2} d^{-1} or 1100 C m^{-2} y^{-1}) (Pregnall and Rudy 1985) and *Chaetomorpha linum* (11 mmol C m^{-2} d^{-1} or 132 g C m^{-2} d^{-1}) (Krause Jensen et. al. 1996).

On a daily basis the opportunistic *Hincksia sordida* has a comparable productivity and N requirement with that of the sheet-like *Ulva* sp. and is likely to exhibit rapid growth and become a dominant component of the local benthic flora in N enriched waters of PPB. The relatively low annual productivity and turnover time of *Polysiphonia decipiens* is reflected by its comparably low fluctuation in biomass, low productivity and annual turnover over the annual cycle. Its ability to accumulate and maintain high tissue N reserves may enhance its ability to survive N limitation.

2.4.6 Summary

The physiological parameters measured for each species reflected the changing nutrient status of their environment and may explain observed shifts in species dominance throughout the annual growth cycle. The relationships between tissue N concentration and water column DIN concentration suggests that tissue N may be a useful tool for detecting trends in exposure of marine communities to N loads into Port Phillip Bay. Tissue C:N ratios also characterised the physiological state and possibly growth potential of the macroalgae examined. There was evidence that pigments were storage compounds for N in *Hincksia sordida* and *Ulva* sp. Inorganic P may also be important for physiological maintenance in *Polysiphonia decipiens* by enhancing N assimilation and metabolism necessary for pigment development. This is investigated in studies described in Chapters 3 and 6. Inter-species differences in physiology were reflected by differences in annual turnover times of carbon and N

budgets. The extent to which these budgets vary spatially throughout Port Phillip Bay requires further investigation.

Table 2.3 Standing biomass of fast growing annual macroalgae.

Species	Range, maximum or mean* biomass (g dry wt. m^{-2})	Location	Reference
Chaetomorpha linum	3600	Peel Inlet, Western Australia	McComb and Lukatelich (1990)
Enteromorpha intestinales	1900	Peel Inlet, Western Australia	McComb and Lukatelich (1990)
Enteromorpha spp.	38.5*	Langstone Harbour, England	Lowthion et al. (1985)
Enteromorpha spp.	35-375	Coos Bay Estuary, Oregon, USA	Pregnall and Rudy (1985)
Enteromorpha and *Ulva* spp.	100	Dublin Bay, Ireland	Jeffrey et al. (1995)
Gracilaria verrucossa.	200*	Sacca di Goro, Italy	Viaroli et al. (1994)
Gracilaria spp.	100-400	Indian River Lagoon, Florida, USA	Virnstein and Carbonara (1985)
Hincksia sordida	15	Port Phillip Bay, Australia	This study (1998)
Polysiphonia decipiens	14	Port Phillip Bay, Australia	This study (1998)
Undaria pinnatifida	498	Port Phillip Bay, Australia	This study (1998)
Ulva sp.	27	Port Phillip Bay, Australia	This study (1998)
Ulva spp.	370	Rhode island, USA	Thorne-Miller et al. (1983)
Ulva rigida	1500	Peel Inlet, Western Australia	Lavery et al. (1991)
All algae	123	Port Phillip Bay, Australia	This study (1998)

Chapter 3

The effects of nutrients on the eco-physiology of three species of macroalgae.

3.1 Introduction

The dominance of opportunistic macroalgae in near-shore coastal waters receiving nutrient inputs from urban sources has led to considerable research into the factors that control their growth (Howarth 1988; Fong et al 1993a; Pederson and Borum 1996) and photosynthesis (Wallentinus 1978; Chopin et al. 1995; Pederson 1995; Rivers and Peckol 1995). These factors (e.g. light, temperature and nutrient supply) interact in a complex manner (Sand-Jensen and Borum 1991). In Chapter 2 the role of N availability in controlling the variability in photosynthetic performance of *Hincksia sordida* over the annual cycle was discussed. It was also suggested that optimal light and temperature conditions in summer enable *Ulva* sp. and *Polysiphonia decipiens* to maintain maximum rates of photosynthesis during summer, and that N is important in maintaining photosynthetic performance during winter when temperature and light may be limiting.

Other studies have employed nutrient enrichment to directly quantify the effects of N and/or P on macroalgal photosynthesis (Topinka and Robbins 1976; Dawes et al. 1984; Chopin et al. 1995; Pederson 1995). Reductions in photosynthesis have coincided with reduced tissue nutrient and pigment concentrations and provided evidence for the dependence of photosynthesis on N availability (Ramus et al. 1976; Lapointe and Duke 1984; Pederson 1995). Nitrogen is an essential nutrient for protein and lipid synthesis and the synthesis of photosynthetic pigments and associated enzymes (Chapman and Cragie 1977). Nitrogen limitation also causes a reduction in thykaloid stacking (Rhiel et al. 1985, 1986) and this may increase the apparent chlorophyll specific efficiency of light harvesting (α) (Turpin 1991). Phosphorus also plays an important role in energy transfer through ATP and other high energy

enzymes and pigments involved in photosynthesis and respiration (e.g. ribulose-1,5,-biphosphate carboxylase or RuBPCase) (Prezelin and Nelson 1990). The interaction between pigment concentration and photosynthesis in macroalgae is also contingent on incident light availability and its effect on pigment levels. Under conditions of saturating light decreased pigment levels may coincide with increased photosynthesis whilst the opposite is true under light limitation (Lapointe and Duke 1984).

The photosynthetic unit (PSU) in macroalgae is composed of a large number of antenna pigments (e.g. chlorophyll *a*). These capture light energy and funnel it to traps (i.e. P700 in PSI and P680 in PSII) which contain no more than a few active chlorophyll *a* molecules (PSI) or accessory pigments (PSII) which convert the light energy to chemical energy. Nitrogen limitation has been shown to affect the light absorption cross section of the photosynthetic unit (PSII) and thus N limitation may increase, decrease or maintain α, depending on the species (Osborne and Geider 1986; Kolber et al. 1988). Structural changes in the photosynthetic unit (PSI and PSII) may change with ambient light and nutrient concentrations according to models proposed by Ramus (1981). Changes in the structure of PSU's may be inferred by altered ratios of accessory pigment to chlorophyll *a* in macroalgae associated with changes in spectral composition of light (Moon and Dawes 1976; Ramus et al. 1976; Ramus et al. 1977; Ramus 1981), nutrients (Levy and Gannt 1990) and maturation (Gomez and Wiencke 1996).

The primary aim of this chapter was to quantify the direct effects of N and P enrichment on the photosynthesis, tissue nutrient and pigment contents of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. The effects of nutrient enrichment may depend on the previous exposure to *in situ* dissolved inorganic nutrients and differences may be found both within and between species (Lapointe and Duke 1984; Lapointe 1987; Lapointe 1989). Macroalgae collected in summer and winter, after periods of low and high nutrient availability respectively, were acclimatised under identical laboratory conditions for 48 and physiological experiments conducted over a range of nutrient treatments. This methodology will provide needed information on

the seasonal changes in tissue nutrients and pigments in relation to nutrient history and supply which has never before been undertaken on south-eastern Australian marine macroalgae. It will also provide an empirical indication of seasonal nutrient limitation on photosynthesis. Finally, the adaptability of pigments to optimise photosynthetic efficiency when they are nutrient limited will be examined.

3.2 Methods

3.2.1 Sample collection and preparation

Whole thalli were collected during winter (June 1995-August 1995) and summer (December 1996-February 1996) from a site in Port Phillip Bay at 3 m depth approximately 500 m from shore (section 2.2.1). Plants were collected using SCUBA and kept at ambient temperature during transport to the laboratory.

Approximately 5 g (fresh weight) of plant material was cleaned of epiphytic material and washed in filtered (0.2 μm) seawater in the laboratory. Pieces of tissue were excised into portions on the day of collection and maintained for 48h in aerated natural seawater in 20 L aquaria at 15°C, under saturating PPFD of 150-200 μmol m^{-2} s^{-1} (36 W 'cool white' fluorescent tubes) and a 12:12 light:dark cycle. Acclimation of algae to constant photon flux and temperature regimes was undertaken to control for the effect of these factors on internal physiological characteristics. Seawater was enriched to ambient field concentrations of N (NH_4Cl) and P (NaH_2PO_4) typical of winter and summer concentrations. Initial (day 1) analyses of photosynthesis, pigments and tissue nutrients were carried out as described in sections 2.2.5 to 2.2.7.

3.2.2 Nutrient enrichment experiment

Tissue pieces (1 g) from the same plants used above were also used for the following nutrient enrichment experiment. Three replicate pieces (each 1 g fresh weight) of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. were placed in separate 4 L aquaria on the day after collection. This achieved initial dry weight to volume ratios of 0.04 to 0.06 to maintain optimal productivity rates (Littler 1979) and to provide

sufficient material for tissue nutrient analysis. Plants were subject to one of 3 nutrient treatments or 1 sewage effluent treatment and those plants that received seawater alone were used as controls (n = 3 for each of 5 treatments) (Table 3.1). The seawater used was obtained from open coastal waters, filtered (0.2 μm) and then air bubbled in aquaria for 1 h until DIN and DIP concentrations were undetectable. Each effluent/nutrient treatment was prepared and changed every 2 days for the duration of the experiment (14 days). Nitrogen was added to the cultures as NH_4Cl and P was added as NaH_2PO_4 from stock solutions. Secondary treated sewage effluent was collected every second day from the Western Treatment Plant, Werribee and analysed for DIN and DIP. In winter the effluent contained relatively high concentrations of DIN and DIP, whereas in summer the effluent remained relatively high in DIP but DIN concentrations were negligible. A volume of sewage was added to aquaria to produce required concentrations (Table 3.1). Winter sewage treatments were prepared to mimic concentrations in N and P enriched cultures, whilst in summer sewage enriched cultures were prepared at the same concentrations of P enriched cultures.

To prevent C limitation $NaHCO_3$ was added to a concentration of 3mM. The pH of all cultures was monitored daily and kept at 8.1-8.3. The cultures were maintained under constant saturating PPFD (200 μmol m^{-2} s^{-1} and a 12:12 h light:dark cycle) and temperature (15°C). The aquaria were bubbled with air to ensure water movement and O_2 and CO_2 equilibrium. Experiments were performed on separate occasions for each species during July-August 1995 (high DIN and DIP exposure) and February-March 1996 (low DIN and high DIP exposure) when ambient bottom water temperatures were 12°C and 19°C respectively.

3.2.3 Photosynthetic, tissue nutrient and pigment analyses

After each experiment plants were removed from treatments and measurements of photosynthesis, tissue N and P and pigment concentrations were made as described in sections 2.2.5 to 2.2.7. For photosynthetic measurements replicate plants (n = 3) from each of the 5 treatments were randomly selected over a 2 day period for measurement of photosynthesis.

Table 3.1 Nutrient conditions for experiments testing the effects of ammonium, phosphate and sewage on the photosynthesis, growth and nutrient uptake of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp.

	Treatments (concentration)					
Nutrient	N+P	+N	+P	SEW (sewage-winter)	SEW (sewage-summer)	no NP (control)
Ammonium ($\mu g\ NH_4\text{-}N\ L^{-1}$)	400	400	<1	400	< 1	< 1
Phosphate ($\mu g\ PO_4\text{-}P\ L^{-1}$)	500	< 1	500	500	500	< 1
Carbon ($mg\ L^{-1}$)	36	36	36	36	36	36
No. of replicates	3	3	3	3	3	3

3.2.4 Statistical analyses

Data were tested for assumptions of normality by examining heterogeneity of variance (Cochrans test) and skewness of data (residuals and outliers). Non-normal data was subject to the log transformation, $\log_e(x)$. For each treatment and experiment *t*-tests were used to compare physiological parameters measured on day 1 and day 14. A two-way ANOVA was employed to examine for effects of season (fixed factor) and nutrient enrichment (fixed factor) on physiological parameters measured at the end of the experiment. The significance level used was $p < 0.05$. Tukeys test was used for post hoc analyses of multiple comparisons among treatment means from significant ANOVA tests. The computer software SYSTAT (vs. 5.03, Systat Inc., USA) was used for all analyses.

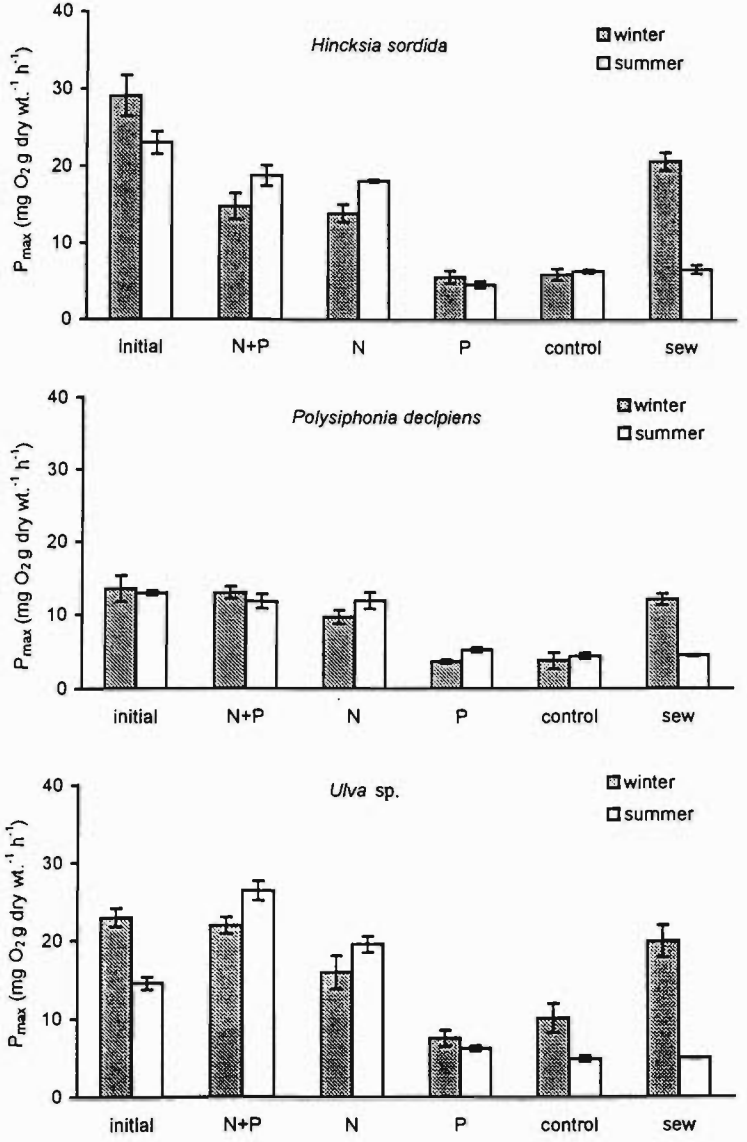

Fig. 3.1 Initial field P_{max} and P_{max} after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Nutrient enrichment includes nitrogen and phosphorus (N+P), nitrogen only (N), phosphorus only (P), no nitrogen or phosphorus (control) and sewage enriched (sew). Values are means ± 1 s.e., n = 3. Details of nutrient treatments are found in Table 3.1.

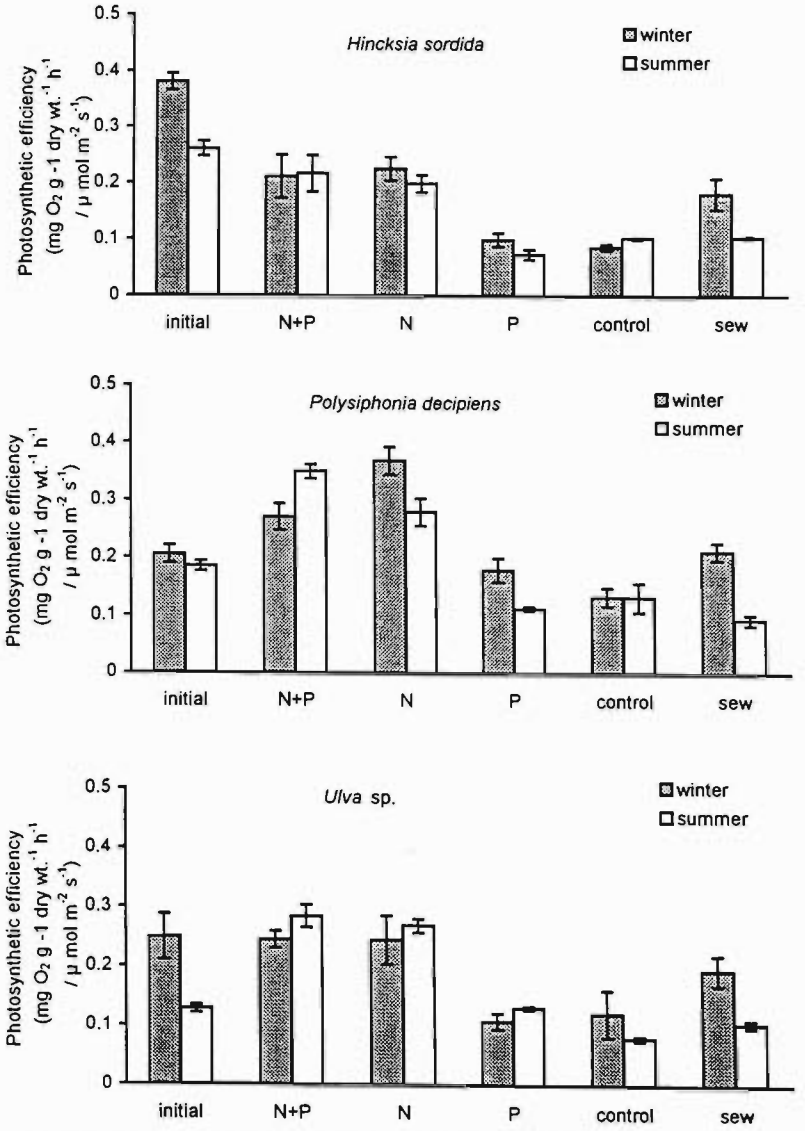

Fig. 3.2 Initial field photosynthetic efficiency(α)and α after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

3.3 Results

3.3.1 Photosynthetic performance: initial vs treatment responses

Winter and summer P_{max}

Winter P_{max} significantly decreased after 14 days of +N, +P and control treatments for *Hincksia sordida* and *Ulva sp.*, and in +P treatments of *Polysiphonia decipiens*. For each species P_{max} did not significantly change after 14 days of N+P and +sewage treatments (Fig. 3.1).

Summer P_{max} significantly decreased in *Hincksia sordida*, *Ulva* sp. and *Polysiphonia decipiens* for +P, control, and + sewage treatments after 14 days compared to values at day 1 (Fig. 3.1). In N+P and +N treatments the P_{max} of *Hincksia sordida* and *Polysiphonia decipiens* did not change significantly from day 1 to 14 but significant increases were evident for *Ulva* sp.

Winter and summer photosynthetic efficiency (α)

In cultures without added N, α significantly decreased after 14 days for *Hincksia sordida* (+P, control, +sewage), *Ulva* sp. (control, +sewage) and *Polysiphonia decipiens*, (+P, +sewage) (Fig. 3.2). In N enriched treatments of *Ulva* sp. (N+P, +N) and *Polysiphonia decipiens* (N+P) α significantly increased after 14 days.

In winter α decreased significantly in N enriched (N+P, +N, +sewage) *Hincksia sordida*. A significant decrease was found in treatments without added N (control, +P) in *Hincksia sordida* and in *Ulva* sp. (+P only) (Fig. 3.2). In +N enriched *Polysiphonia decipiens* significant increases in α occurred in +N enriched treatments.

3.3.2 P_{max} and α : seasonal and nutrient enrichment responses

After 14 d N enriched treatments had significantly higher P_{max} and α than controls in all algae, whilst P enrichment did not (Figs. 3.1 and 3.2; Tables 3.2 and 3.3). The significant interaction between season and nutrient treatment on P_{max} (Appendix 7) and α (Appendix 8) for each species reflected the significantly higher winter values than summer for particular treatments. Post hoc analyses found significantly higher P_{max} in winter sewage treatments for *Hincksia sordida*, higher P_{max} in winter control treatments for *Ulva* sp. and higher winter P_{max} and α in winter sewage treatments in *Ulva* sp. and *Polysiphonia decipiens* (Tables 3.2 and 3.3).

Table 3.2 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on maximum photosynthesis (P_{max}) for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.1. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	0.001	n.s.	0.001	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.001	n.s.

Table 3.2 cont.

Polysiphonia decipiens	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	0.001	n.s.	0.001	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.001	n.s.

Ulva sp.	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	n.s.	n.s.	0.004	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.001	0.005

Table 3.3 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on maximum photosynthetic efficiency (α) for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.2. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.027	0.001	n.s.	0.001	n.a.
Summer	0.028	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	n.s.	n.s.

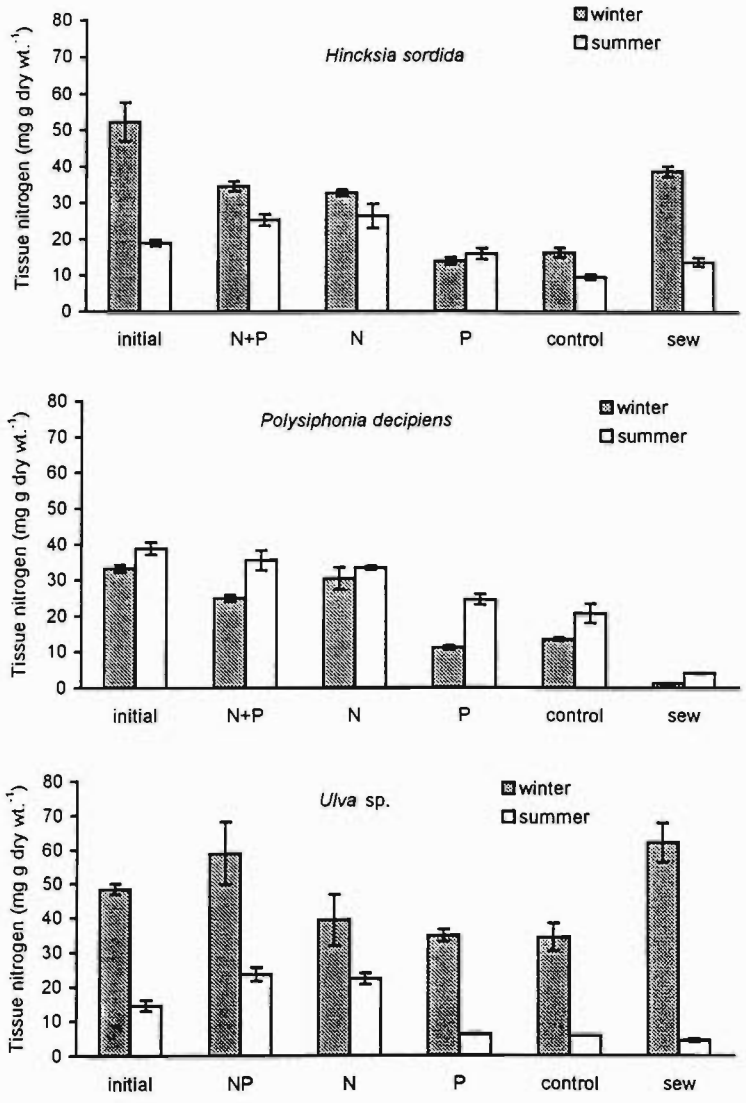

Fig. 3.3 Initial field tissue nitrogen concentrations and values after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

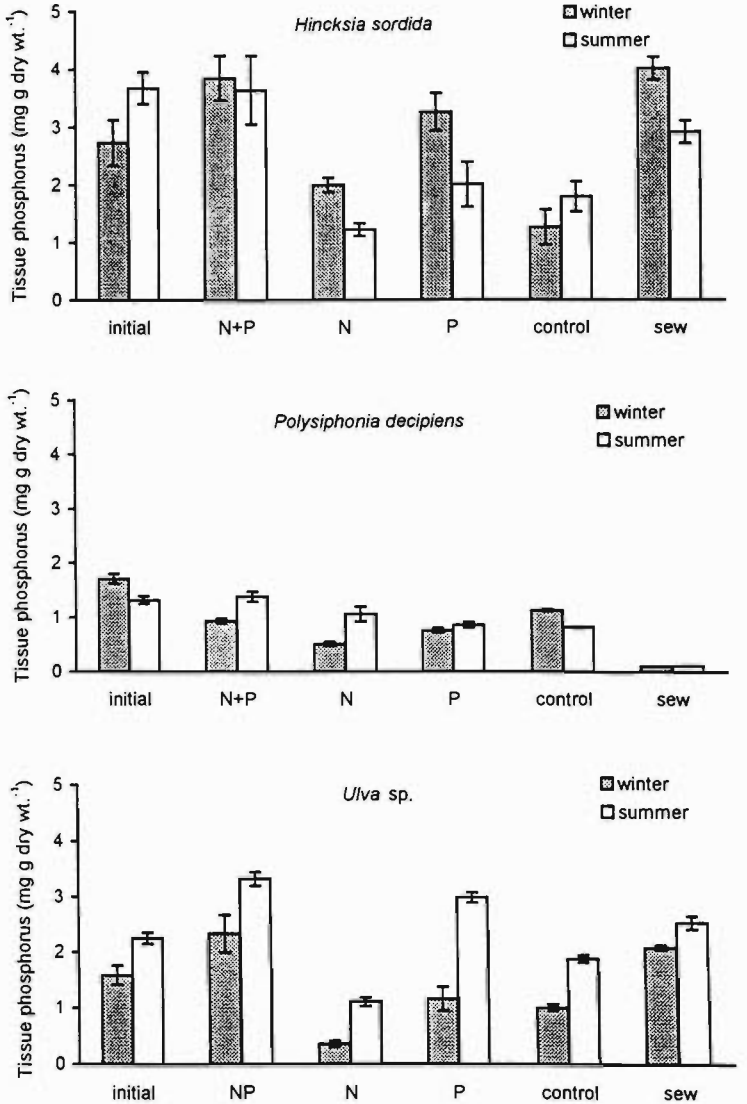

Fig. 3.4 Initial field tissue phosphorus concentrations and values after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

Table 3.3 cont.

Polysiphonia decipiens	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	0.001	n.s.	n.s.	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.004	n.s.

Ulva sp.	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.017	0.019	n.s.	n.s.	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	n.s.	n.s.

3.3.3 Tissue N and P: initial field vs. treatment responses

Effects of no nutrient additions (controls)

Tissue N in control treatments of winter and summer *Hincksia sordida* declined significantly over the 14 d period (Fig. 3.3). Tissue N concentrations of *Polysiphonia decipiens* also declined significantly over 14 d from initial field values. In *Ulva* sp. significant reductions in tissue N over the experimental period were found in summer (Fig. 3.3).

The tissue P of *Hincksia sordida* controls did not decline over the 14 d period (Fig. 3.4). In *Polysiphonia decipiens* tissue P declined in controls (Fig. 3.4). The decline in tissue P in *Ulva* sp. over the 14 d period was significant in summer only. Control tissue N:P ratios did not change significantly in all species from initial values (Fig. 3.5).

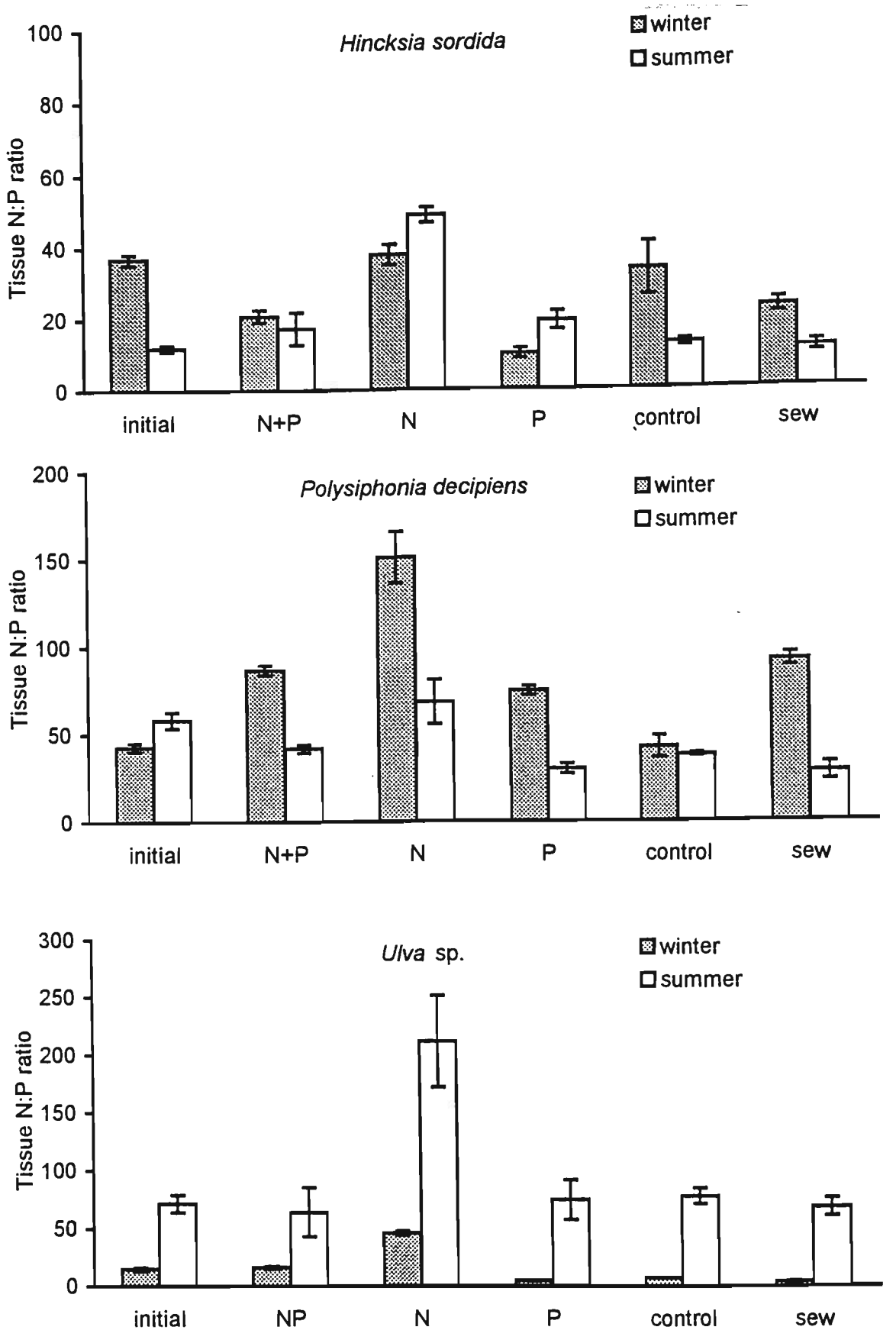

Fig. 3.5 Initial field nitrogen to phosphorus ratios and ratios after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

Nitrogen and phosphorus enrichment

Hincksia sordida enriched with N+P in summer were found to have a significant increase in tissue N over a period of 14 d from mean concentrations of 18.97 ± 0.89 to 25.26 ± 1.61 mg N g dry wt.$^{-1}$ (Fig. 3.3). No significant change in tissue N was apparent for winter *Hincksia sordida*, *Ulva* sp. or *Polysiphonia decipiens* during either season. Tissue P showed a significant decrease during winter in *Polysiphonia decipiens* and a significant increase during summer in *Ulva* sp. (Fig. 3.4).

In N+P enriched cultures the N:P ratios for *Hincksia sordida* did not change significantly from initial values after 14 d, but there was a significant increase for winter *Polysiphonia decipiens* from a mean of 51.6 to 74.1 and a significant decrease in summer *Ulva* sp. from a mean of 26.1 to 7.2 (Fig. 3.5).

Single nutrient enrichment

There was no effect of N enrichment on tissue N in cultures enriched with N only after 14 d (Fig. 3.3). Tissue P decreased significantly over the 14 d period in summer *Hincksia sordida*, in winter *Polysiphonia decipiens*, and in winter and summer *Ulva* sp. (Fig. 3.4) In summer the N:P ratios of all species declined significantly after 14 days. In winter only *Polysiphonia decipiens* was found to have reduced N:P ratios after 14 d (Fig. 3.5).

Winter and summer *Hincksia sordida*, enriched with phosphate, were found to have a significantly reduced tissue N from initial concentrations (Fig. 3.3). Significant reductions were apparent for *Polysiphonia decipiens* and *Ulva* sp. Tissue P decreased significantly from initial values over the 14 d in summer *Hincksia sordida*, winter *Polysiphonia decipiens* and in winter *Ulva* sp. (Fig. 3.4). There was no significant change in tissue P for winter *Hincksia sordida* or summer *Polysiphonia decipiens*. Tissue P in summer *Ulva* sp. significantly increased after exposure to a P enriched culture.

N:P ratios declined significantly after 14 d from initial field values in all species in summer exposed to P enriched cultures. This did not occur in winter plants (Fig. 3.5).

3.3.4 Tissue N and P: seasonal and nutrient enrichment responses

Tissue N

Nitrogen enrichment (including winter sewage treatments) caused a significant increase in tissue N compared to controls in both winter and summer experiments for all species (except for *Ulva* sp. in winter) (Fig. 3.3; Table 3.4).

Significant interactive effects of season and nutrient enrichment on tissue N were evident across all algae (Appendix 9). Post hoc analyses found significantly higher tissue N for *Ulva* sp. in winter compared to summer for each treatment (Table 3.4; Fig. 3.3). In *Hincksia sordida* significantly higher tissue N was found in winter compared to summer in sewage enriched and control treatments. Significantly lower tissue N was found in winter compared to summer in P enriched and sewage treatments for *Polysiphonia decipiens*.

Table 3.4 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on tissue N for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.3. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	0.001	n.s.	0.001	n.a.
Summer	0.001	0.001	0.006	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.001	0.003

Polysiphonia decipiens	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.007	0.019	n.s.	0.001	n.a.
Summer	0.002	0.001	0.030	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	0.001	0.001	n.s.

Ulva sp.	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	n.s.	0.028	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	0.001	0.036	0.001	0.001	0.001

Tissue P

N+P enrichment led to significant increases in tissue P concentration compared to controls in all species except for *Polysiphonia decipiens* in winter (Table 3.5; Fig. 3.4). Phosphate enrichment (including summer sewage treatments) increased tissue P in winter plants only (Table 3.5; Fig. 3.4).

Significant interactive effects of season and nutrient enrichment on tissue P were evident across all algae (Appendix 10). Post hoc analyses found significantly lower tissue P for *Ulva* sp. in winter compared to summer in N, P and control treatments (Table 3.5). Tissue P was also significantly lower in N and P and N treated *Polysiphonia decipiens* in winter compared to summer but no seasonal differences were found for *Hincksia sordida* (Table 3.5; Fig. 3.4).

Table 3.5 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on tissue P concentration for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.4. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	n.s.	0.001	n.s.	n.a.
Summer	0.001	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3.5 cont.

Polysiphonia decipiens	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	0.001	0.018	n.s.	n.a.
Summer	0.001	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	0.004	0.001	n.s.	n.s.	n.s.

Ulva sp.	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	0.001	n.s.	0.003	n.a.
Summer	0.029	0.050	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	0.001	0.001	n.s.	0.013

Tissue N:P ratios

In winter N:P ratios significantly increased in N+P, +N and sewage enriched cultures of *Polysiphonia decipiens*, and in all +N cultures (except for winter *Hincksia sordida*) relative to controls. N:P ratios decreased in P enriched cultures of *Polysiphonia decipiens* and *Hincksia sordida* in winter (Table 3.6; Fig. 3.5). In summer significant increases in N:P ratios were found for N+P enriched *Ulva* sp. and all +N enriched cultures relative to controls.

Significant interactive effects of season and nutrient enrichment on tissue N:P ratios were mostly evident across all algae (Appendix 11). There was no obvious seasonal trend in N:P ratios in *Hincksia sordida*, except for control plants which had higher N:P ratios in winter than summer (Table 3.6; Fig. 3.5). N:P ratios were significantly higher in most winter cultures than summer cultures in *Polysiphonia decipiens*. N:P

ratios in summer cultures of *Ulva* sp. were significantly higher than in winter cultures (Table 3.6; Fig. 3.5).

Table 3.6 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on tissue N:P ratios for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.5. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	0.001	n.s.	n.a.
Summer	n.s.	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	n.s.	0.012

Polysiphonia decipiens	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.004	0.001	0.034	0.002	n.a.
Summer	n.s.	0.049	n.s.	n.s.	n.a.
Winter vs Summer	0.005	0.002	0.001	0.001	n.s.

Ulva sp.	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	0.008	n.s.	n.s.	n.a.
Summer	0.037	0.001	n.s.	n.s.	n.a.
Winter vs Summer	0.001	0.001	0.001	0.001	0.001

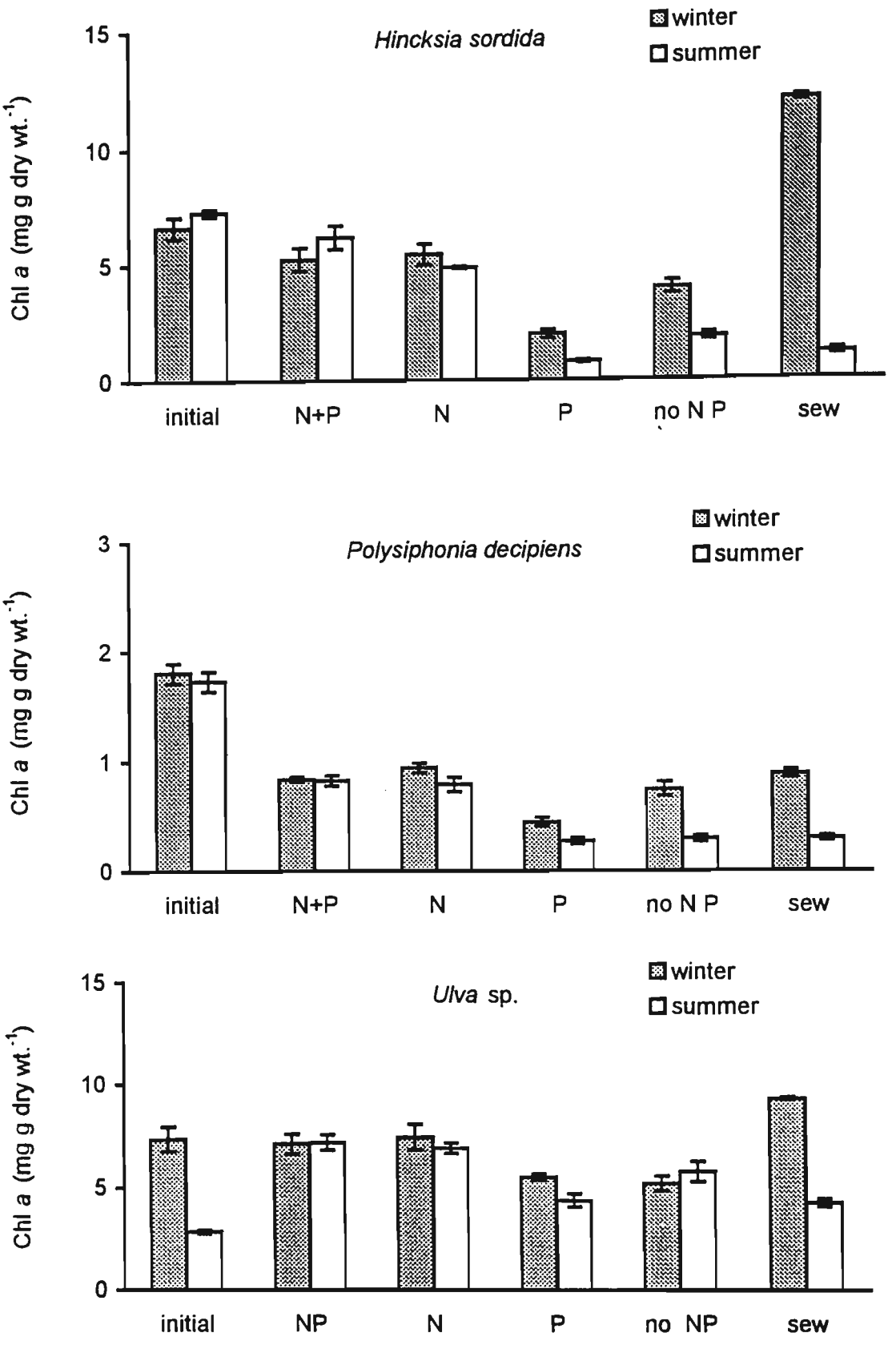

Fig. 3.6 Initial field chlorophyll *a* concentrations and values after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

3.3.5 Pigments: seasonal and nutrient enrichment responses

Chlorophyll a

Chlorophyll *a* was significantly increased compared to controls by N (N+P and +N) enrichment in *Hincksia sordida* and *Polysiphonia decipiens* in summer, by N (N+P and +N) enrichment in *Ulva* sp. in winter and by sewage enrichment of *Hincksia sordida* in winter (Table 3.7; Fig. 3.6). In P enriched treatments chlorophyll *a* was significantly lower than controls in *Hincksia sordida* and *Polysiphonia decipiens.* Sewage enrichment caused significant increases in chlorophyll *a* relative to controls in *Hincksia sordida* and *Ulva* sp. in winter but significant decreases in both these taxa in summer. Significant interactive effects of season and nutrient enrichment were found on chlorophyll *a* (Appendix 12). These were due to significantly higher concentrations of chlorophyll *a* in winter P enriched, sewage and control treatments compared to corresponding summer treatments (Table 3.7; Fig. 3.6).

Table 3.7 Table of post hoc significance values (Tukeys) of the effects of nutrient (N+P, +N, +P) and sewage enrichment on chlorophyll *a* concentration for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.6. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	0.001	0.001	n.a.
Summer	0.001	0.001	0.001	0.010	n.a.
Winter vs Summer	n.s.	n.s.	0.001	0.001	0.001

Table 3.7 cont.

Polysiphonia decipiens	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	0.002	n.s.	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	0.049	0.001	0.001

Ulva sp.	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.035	0.013	n.s.	0.001	n.a.
Summer	n.s.	n.s.	n.s.	0.049	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.001	n.s.

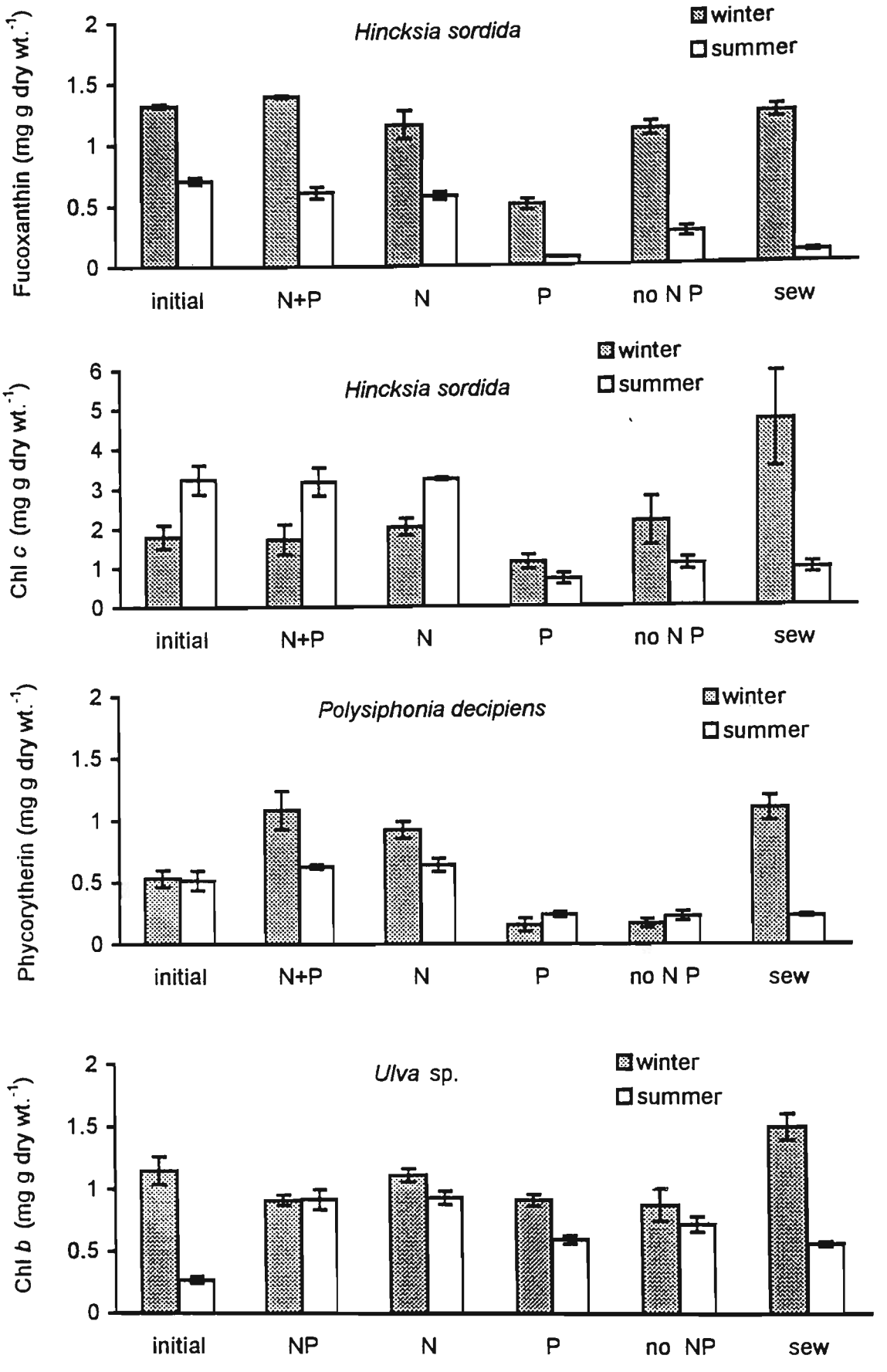

Fig. 3.7 Initial field accessory pigment concentrations and values after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

Accessory pigments

Nitrogen enrichment significantly increased accessory pigment concentrations for *Polysiphonia decipiens* and *Ulva* sp. (sewage only) in winter and for all accessory pigments for all species in summer. In *Hincksia sordida* fucoxanthin concentration decreased significantly in P enriched winter and summer cultures relative to controls (Table 3.8; Fig. 3.7).

The significant interactive effect of season and nutrient enrichment on accessory pigments shown for all species was due to significantly higher winter fucoxanthin and phycorytherin concentrations in N enriched *Hincksia sordida* and *Polysiphonia decipiens* respectively and significantly higher chlorophyll *b* in sewage enriched *Ulva* sp. in winter. Chlorophyll *b* was also higher in winter than in summer in +P treatments (Fig. 3.7).

Table 3.8 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on accessory pigments for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Data is based on values presented in Fig. 3.7. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida fucoxanthin	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	0.001	n.s.	n.a.
Summer	0.001	0.001	0.009	0.037	n.a.
Winter vs Summer	0.001	0.001	0.001	0.001	0.001

Table 3.8 cont.

Hincksia sordida chlorophyll *c*	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	n.s.	n.s.	n.a.
Summer	0.001	0.001	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	0.001	n.s.	n.s.	n.s.

Polysiphonia decipiens (phycorytherin)	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.001	0.001	n.s.	0.001	n.a.
Summer	0.002	0.001	n.s.	n.s.	n.a.
Winter vs Summer	0.007	n.s.	n.s.	0.001	n.s.

Ulva sp. (chlorophyll *b*)	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	n.s.	n.s.	n.s.	0.001	n.a.
Summer	n.s.	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	0.016	0.001	n.s.

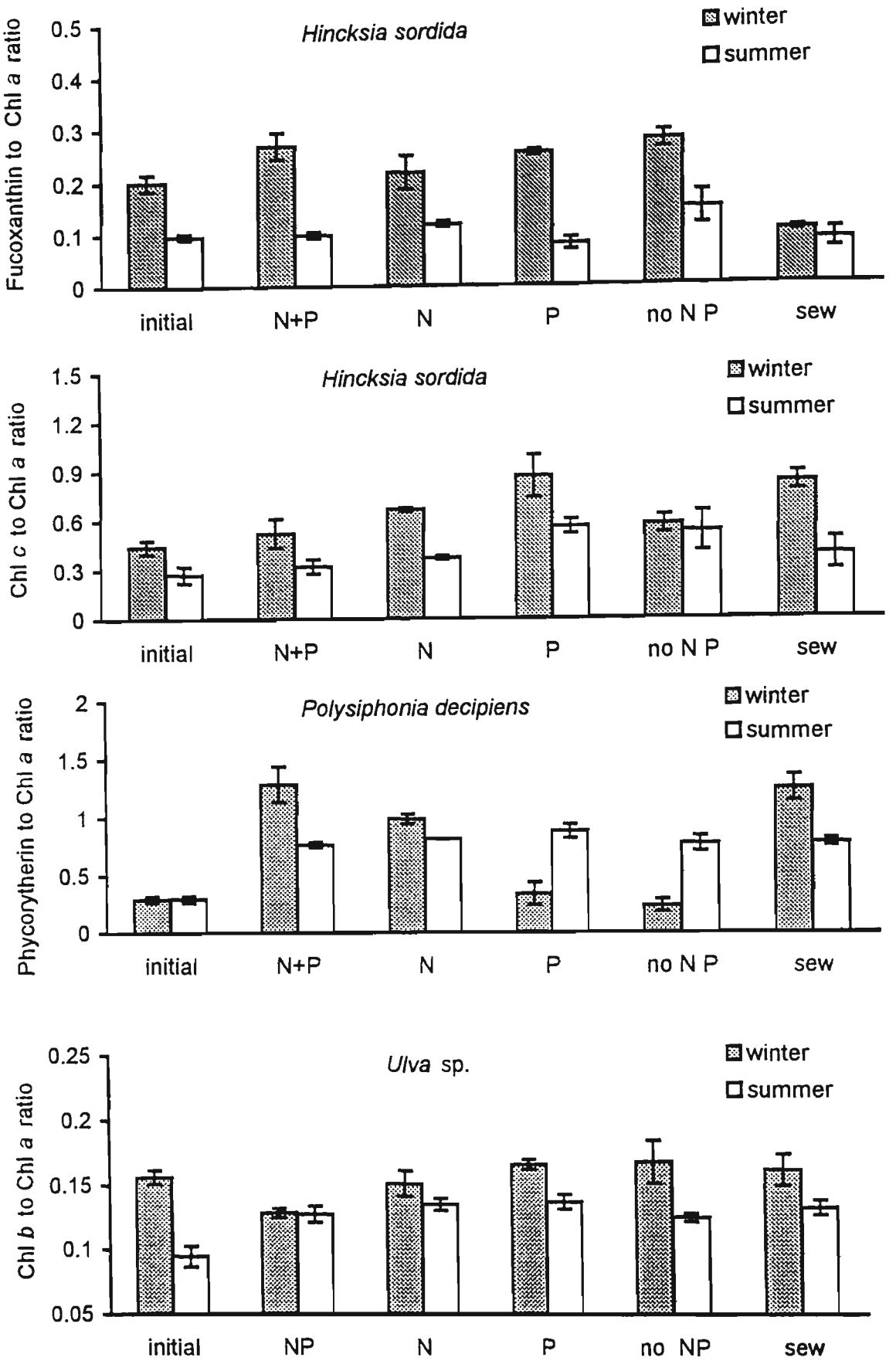

Fig. 3.8 Initial field accessory pigment to chlorophyll *a* ratios and ratios after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Details of nutrient treatments are shown in Fig. 3.1. Values are means ± 1 s.e., n = 3.

Accessory pigments to chlorophyll a ratios

Nitrogen enrichment contributed to significantly higher chlorophyll *c* : chlorophyll *a* and phycorytherin : chlorophyll *a* ratios during winter than treatments without added N in *Hincksia sordida* (sewage only) and *Polysiphonia decipiens* respectively (Table 3.9; Fig. 3.8). There was no significant effect of N or P enrichment on chlorophyll *b*: *a* ratios compared to controls for *Ulva* sp. (Fig. 3.8)

The interactive effects of season and nutrients on the ratios of accessory pigment to chlorophyll *a* were determined for each species (Appendix 13). In *Hincksia sordida* fucoxanthin : chlorophyll *a* ratios were two to three times higher in winter treatments than summer treatments except for sewage treatments. In *Hincksia sordida* the interactive effect on fucoxanthin : chlorophyll *a* ratios was due to elevated values in the winter sewage treatment as opposed to low values in summer (Fig. 3.8). For *Polysiphonia decipiens* this interaction was due to significantly higher phycorytherin : chlorophyll *a* ratios in winter N enriched plants than in summer but N depleted plants had higher phycorytherin : chlorophyll *a* ratios in summer than winter. In *Ulva* sp. there was a significant seasonal stimulation of chlorophyll *b*: *a* ratios in P enriched winter plants compared to P enriched summer plants but no interactive effect was evident (Appendix 14).

Table 3.9 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on accessory pigment to chlorophyll *a* ratios for 3 species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 3.8. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida (fucoxanthin)	N and P	N	P	Sewage	Control
Winter	n.s.	n.s.	n.s.	0.001	n.a.
Summer	n.s.	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	0.001	n.s.	0.001	n.s.	0.043

Hincksia sordida (chlorophyll *c*)	N and P	N	P	Sewage	Control
Winter	n.s.	n.s.	n.s.	n.s.	n.a.
Summer	n.s.	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	0.014	n.s.

Polysiphonia decipiens (phycorytherin)	N and P	N	P	Sewage	Control
Winter	0.001	0.001	n.s.	0.001	n.a.
Summer	n.s.	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	0.010	n.s.	0.001	0.025	0.001

Ulva sp. (chlorophyll *b*)	N and P	N	P	Sewage	Control
Winter	n.s.	n.s.	n.s.	n.s.	n.a.
Summer	n.s.	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	n.s.	n.s.	0.032.

3.4 Discussion

The findings of this study clearly demonstrate the importance of N in the maintenance of physiological processes in fast growing macroalgae in PPB and also suggest that P may play a role for N uptake in *Ulva* sp. Physiological processes within these opportunistic macroalgae vary with season and this difference may be attributed to nutritional history.

3.4.1 Photosynthesis

The seasonal photosynthetic responses of field collected plants and those enriched with ammonium suggests that summer field plants may be limited by N availability. As a result summer N enriched plants maintained or increased their photosynthetic performance (P_{max} and α) from initial field values. In contrast, N enriched winter plants showed slight decreases in photosynthetic capacity compared with initial values, indicative of N sufficiency. The significant interactive effect of season and nutrient enrichment on photosynthetic response (except for *Hincksia sordida*) mainly reflected the composition of sewage effluent which was N rich in winter and low in N in summer. It also showed that nutrient depleted *Ulva* sp. was able to maintain a higher photosynthetic capacity in winter than in summer, presumably due to an ability to utilise N rich tissue for photosynthesis. This may not be ecologically relevant given the ability of *Ulva* sp. to maintain high productivity at optimal light and temperature conditions despite low N availability (section 2.3.3).

Indicators of nutrient limitation such as P_{max} and photosynthetic efficiency (α), as measured in this study, suggested that N is the primary limiting nutrient in waters of PPB. Other studies have also recorded 2- to 4-fold increases in P_{max} and tissue N with additions of DIN. These include studies of the Rhodophytes *Gracilaria foliifera*, (Lapointe and Ryther 1979; Lapointe and Duke 1984) and *Gracilaria verrucossa* (Dawes et al. 1984), the Chlorophytes *Ulva lactuca* (Pederson 1995) and *Ulva fasciata* (Rosenberg et al. 1995), and the Phaeophytes *Ptelonia fascia* and *Laminaria saccharina* (Williams and Herbert 1989).

The absence of significant differences between plants treated with phosphate and untreated plants suggests that P alone does not limit plant performance. However, the enhancement of P_{max} in *Ulva* sp. when exposed to a culture enriched with both ammonium and phosphate may indicate an important role for P in the photosynthetic capacity of this plant. A similar trend in winter *Polysiphonia decipiens* also indicates a role for P in photosynthesis and is consistent with the apparent relationship between pigment content and tissue P identified for this species (section 2.3.10). Phosphorus plays an important role in energy transfer through ATP and other high energy enzymes and pigments involved in photosynthesis and respiration (e.g. ribulose-1,5,-biphosphate carboxylase or RuBPCase) (Prezelin and Nelson 1990).

ATP also drives transport mechanisms for NO_2, NO_3 and NH_4 by increasing activity of the enzyme glutamine synthetase (Layzell 1990). Therefore the availability of P may contribute to photosynthetic performance by facilitating N uptake. Waite and Mitchell (1972) found that additions of phosphate (at 500 μg P L^{-1}) contributed to an increase in carbon assimilation of ammonium enriched *Ulva lactuca* compared to plants grown in lower concentrations of phosphate (i.e. 100 μg PO_4-P L^{-1}). Phosphorus limited photosynthesis has also been documented in a number of macroalgae including *Gracilaria tenuistipitata* (Rhodophyta) (Garcia-Sanchez et al. 1996) and the tropical alga *Gracilaria tikvahiae* (Rhodophyta) (Lapointe 1987). In these algae phosphorus enrichment increased P_{max} in winter and summer but N did not affect the P_{max} of summer plants. In *Cladophora prolifera* (Chlorophyta) both N and P enrichment have been shown to increase photosynthetic output (Lapointe and O'Connell 1989) or have no influence on photosynthetic output (Bach and Josselyn 1979). Chopin et al. (1991) suggested that in *Agardhiella subulata* (Rhodophyta), phosphate may be an important regulator of carbon flow toward either glucose and starch or galactose and carrageenans.

The increase in photosynthetic efficiency (α) with ammonium enrichment in each of the species examined is consistent with the general view that α, under light limitation, is controlled primarily by pigment concentration and N availability (Levavasseur et al.

1991). The reduced α in plants that did not receive N enrichment may also be a function of lower RuBPCase activity. RuBPCase is a phosphorylated enzyme in algae which catalyzes the attachment of CO_2 to the five carbon sugar ribulose-1,5,-biphosphate (RuBP) during photosynthesis (Parry et al. 1985; Garcia Sanchez et al. 1996) and enables the initial production of 3-phosphoglycerate (PGA). RuBPCase is directly dependent on N availability as it utilises and stores N in excess quantities when N uptake exceeds growth requirements (Duke et al. 1987; Ekman et al. 1989). Photosynthetic efficiency reflects the ability of algae to utilise low light and a reduction in this ability was first attributed to N limited RuBPCase activity in *Laminaria longicruris* (Phaeophyta) (Chapman and Cragie 1977). Subsequently, reduced RuBPCase activity has been associated with reduced tissue N and pigment concentrations in *Ulva* spp. (Duke et al. 1986) and *Ulva curvata* and *Codium decoratium* (Duke et al. 1987), *Gracilaria tikvahiae* (Lapointe and Duke 1984), *Laminaria saccharina* (Phaeophyta) (Wheeler and Weidner 1983) and *Laminaria hyperborea* (Phaeophyta) (Kuppers and Weidner 1980). Correlations between N limitation and reduced α have also been recorded for *Porphyridium purpureum* (Rhodophyta) (Levy and Gannt 1990) and the filamentous alga *Cladophora vagabunda* (Chlorophyta) (Rivers and Peckol 1995).

In N limited phytoplankton an increase in chlorophyll specific α has been related to a reduction in thykaloid stacking and absorptivity leading to greater chlorophyll efficiencies in low light (Turpin 1991). McGlathery (1992) also reported increases in the chlorophyll specific α for the macroalga *Spyridea hypnoides* (Rhodophyta) with N limitation and associated this with a decrease in the concentration of chlorophyll. Measures of α in the present study have been reported per unit dry weight but the commensurate decreases in both α and chlorophyll *a* for N limited plants indicates that α per unit chlorophyll does not increase with N limitation. Hence N limited algae in this study appear to be no more efficient at harvesting low light than N sufficient algae. This suggests that these plants receive sufficient light in summer when N is limiting or that temperature ensures high productivity by promoting RuBPCase activity, despite N limitation.

3.4.2 Tissue nutrients

The decrease in tissue N concentration during experiments in this study , despite the high levels of nutrient enrichment, may be explained by the increased tissue growth resulting in a reduced N concentration per unit weight. This has been also found in *Ecklonia radiata* (Phaeophyta) (Paling 1991), *Sargassum muticum* (Phaeophyta) (Lewey and Gorham 1984) and *Phyllariopsis purpurascens* (Phaeophyta) (Flores-Moya et al. 1995). Rosenberg et al. (1984) attributed depletions in tissue nutrients at ambient seawater concentrations to the lack of water movement providing sufficient loading though this is unlikely to have occurred in this study as vigorous aeration and high nutrient concentrations were provided to relatively small (4L) culture chambers.

The significant effects of both N history (season) and N enrichment are probably suggestive of rapid uptake and luxury accumulation of N beyond the alga's growth requirements. This is consistent with the high tissue N maxima of all laboratory enriched plants (range 3.73-7.69%), in comparison to field collected plants. Comparable tissue N have been found previously in nutrient enrichment studies on similar types of algae. These include 3.3% for *Ulva rigida* (Chlorophyta) (Lavery and McComb 1991b), 5.5 % for *Ulva fenestrata* (Wheeler and Björnsäter 1992), 4.2 % for *Ulva rigida* (Fujita et al. 1989), 6.5 % for *Ulva lactuca* (Pederson and Borum 1996) and 5.2 % for *Ceramium rubrum* (Rhodophyta) (Pederson and Borum 1996).

Of all species examined *Ulva* sp. exhibited a capacity for luxury tissue P saturation as final tissue concentrations exceeded initial concentrations (section 3.3.3). In winter this was most prominent in N and P enriched cultures as opposed to P enriched cultures, but in summer P saturation was found in all P enriched cultures irrespective of N enrichment. Luxury phosphate uptake of up to 0.35 % in *Ulva* sp. compares favourably with studies of Western Australian estuarine Chlorophyta; including saturated tissue P of up to 0.88% and growth saturated tissue P of 0.33% in *Cladophora* aff. *albida* (Gordon and McComb 1989); saturated tissue P and growth saturated tissue P in *Ulva rigida* of 0.062% and 0.025% respectively and of 0.23% and 0.05% respectively in *Chaetomorpha linum* (Lavery and McComb 1991b). In

studies of Chlorophyta from the Pacific Northwest coast of the U.S.A. maximum saturated tissue P of 0.60% and 0.73% were recorded in *Ulva fenestrata* and *Enteromorpha intestinales* respectively (Björnsäter and Wheeler 1990).

Maximum tissue P in *Hincksia sordida* (0.42%) was higher than maximal concentrations in the Phaeophytes *Dictyota dichotoma* (0.082%; Aisha et al. 1995) and *Sargassum polyceratium* (0.13 %; Lapointe 1989) but comparable to *Ecklonia radiata* (0.5 %; Paling 1991) and *Macrocystis pyrifera* (0.5%; Manley and North (1984). The highest tissue P recorded of up to 0.15 % in *Polysiphonia decipiens* is in the mid range of that reported for other Rhodophyta; i.e. 0.5% in *Ceramium rubrum* (Lyngby 1990), 0.5% in *Porphyra limitata* (Wheeler and Björnsäter 1992) and up to 0.04% in *Gracilaria tikvahiae* (Lapointe 1989).

The absence of any significant difference in tissue N concentrations between N+P enriched and plants enriched with N alone is consistent with the studies by Pederson (1995) on *Ulva lactuca* and *Chaetomorpha linum*. In contrast Aisha et al. (1995) found increases in tissue N produced by the addition of N at low P concentrations were transient, in *Codium dwarkense* (Chlorophyta) and *Dictyota dichotoma* (Phaeophyta), compared to more persistent increases caused by N enrichment under non-limiting P conditions. Lapointe (1985) also found decreased tissue N in P limited plants of the Rhodophyte *Gracilaria tikvahiae*. These findings are consistent with photosynthetic responses of *Ulva* sp. in the present study and suggest that P limited plants may have a lower capacity for N utilisation. This supports the concept that P is important for the processes of N metabolism as previously discussed for both *Ulva* sp. and *Polysiphonia decipiens*.

The seasonal response of 1- to 3-fold higher winter tissue N and P compared to summer tissue N and P in *Ulva* sp. probably reflected initially high field tissue nutrient concentrations in winter, rather than greater nutrient uptake capacities by winter plants (for further discussion see Chapter 6). By contrast, the relatively high tissue P concentrations in field *Hincksia sordida* and *Ulva* sp. in summer, and in

phosphate enriched summer *Ulva* sp., and *Polysiphonia decipiens* (N+P only) suggests an increased uptake of phosphate in summer compared to winter (see Chapter 6) and implies that P may potentially limit growth in summer. Waite and Mitchell (1972) speculated that higher productivity of ammonium enriched *Ulva lactuca* at low phosphate concentrations compared with cultures at high P concentrations was due to increased synthesis of the enzyme alkaline phosphatase (APA). Alkaline phosphatase is primarily involved in dissolved inorganic and organic phosphate uptake across the cell membrane and contributes to enhanced P uptake and photosynthesis (Lobban et al. 1985). Low phosphate availability can induce the activity of the APA located on the outer surface of the cell either on the cell wall or in the cell membrane (Weich and Graneli 1989; Hernandez et al. 1992). It has been studied in a number of macroalgae including *Ulva lactuca* (Chlorophyta) (Weich and Graneli 1989) and *Cladophora prolifera* (Chlorophyta) (Lapointe and O'Connell 1989) and has been used as an indicator of P limitation (Hernandez et al. 1997). The seasonality of APA synthesis and activity warrants further investigation for species in PPB.

In contrast to the demonstrated relationship between N:P ratios and productivity for other macroalgae (Wheeler and Björnsäter 1992), N:P ratios in the present study may not be useful in delineating photosynthetic responses (or growth responses). This is because maximum productivity rates can be achieved at low (i.e. enriched with N and P) and high (i.e. enriched with N only) N:P ratios. The seasonal trend in *Polysiphonia decipiens* and *Ulva* sp. of higher tissue N:P ratios in winter appeared to reflect elevated or luxury amounts of tissue N in winter field plants compared to summer plants and higher summer tissue P, rather than P limitation.

Approximately equivalent N:P ratios in N+P enriched winter and summer plants of *Hincksia sordida* may reflect a high capacity for P uptake when enriched with N (see Chapter 6). In addition, the higher tissue P content of summer *Hincksia sordida* and *Polysiphonia decipiens* and winter *Ulva* sp. in N+P enriched cultures compared to P only enriched cultures also suggests that P uptake is dependent on N availability. This

is consistent with the trends in tissue N:P ratios in all N deficient plants, as both tissue N and P appear to decrease simultaneously such that N:P ratios remained essentially unchanged despite N limitation. Björnsäter and Wheeler (1990) also found a decline in the P content in N limited *Enteromorpha intestinales* and *Ulva fenestrata* (Chlorophyta) and similar observations were made for *Spyridea hypnoides* (Rhodophyta) (McGlathery 1992). The balance between tissue N and P during N limitation may indicate a coupling of N and P metabolism and is examined further in Chapter 6.

3.4.3 Pigments

The effects of N history (season) and N and P enrichment on the pigments chlorophyll *a*, fucoxanthin and phycorytherin across all 3 species is consistent with the theory that N is required for synthesis of proteins and lipids necessary for pigment development and photosynthetic performance (Chapman and Cragie 1977). Changes in the pigment concentrations of all species were dependent on N availability rather than adaptation to changing light intensity as laboratory cultures were maintained at saturating light intensities constant between treatments. The optimisation of pigment concentration is a biochemical acclimation to N availability that is directed towards maximising photosynthetic capacity and growth. It is a pattern that has been found in many species of macroalgae (Flores-Moya et al. 1995; Pederson 1995) and is also directly influenced by light adaptation (Lapointe and Duke 1984).

The increase in chlorophyll *a* in N enriched summer plants of *Hincksia sordida* and *Polysiphonia decipiens*, and not in winter plants, suggests that N is more limiting to pigment content in summer than in winter. By contrast the absence of an increase in chlorophyll *a* (compared to control plants) in summer *Ulva* sp. with N enrichment implies that this species may utilise N to produce other protein complexes or that growth may dilute chlorophyll *a* concentrations per unit weight. As there was little growth in *Ulva* sp. cultures during summer, the former explanation is most plausible. It should be noted that chlorophyll *a* concentrations increased in N enriched plants compared to field plants, and it is likely that with additions of higher concentrations of

N chlorophyll production may have exceeded control plants. Despite the apparent lack of utilisation of N by chlorophyll *a* in summer, the observed chlorophyll *a* production with N enrichment in winter *Ulva* sp. indicates that chlorophyll *a* is a major storage compound for N when ambient concentrations of DIN are relatively high. This finding is consistent with many studies on temperate macroalgae (Brinkhuis 1977; Chapman and Cragie 1977; Chapman et al. 1978; Lapointe and Tenore 1981; Duke et al. 1987; Williams and Herbert 1989; Gao 1990; Geertz Hansen and Sand Jensen 1992; Horrocks et al. 1995; Pederson 1995).

The observed reduction in pigment concentration with N limitation for all species suggests a decrease in either the number of PSU's (PSI plus PSII reaction centre and pigment protein complex) and/or a reduction in the size of antenna pigments (Ramus 1981). In red, green and brown algae the primary antenna for PSI is chlorophyll *a*, whilst PSII receives energy from chlorophyll *a* and *b* in the green algae and mostly from accessory pigments such as fucoxanthin and chlorophyll *c* in brown algae and phycobiliproteins in red algae (Dawes 1981). The observed redistribution of the antennae pigments in *Hincksia sordida* and *Polysiphonia decipiens* may serve to relieve N limitation and/or reduce the effects of light limitation in winter. Higher chlorophyll *c*: chlorophyll *a* ratios in N deficient (+P) *Hincksia sordida* compared to N sufficient plants in summer indicates that chlorophyll *c* may enable maintenance of PSII during N limitation. Similar differences were also observed for juvenile plants of *Sargassum thunbergii* (Phaeophyta) from nutrient poor and enriched waters (Gao and Nakahara 1990) and higher chlorophyll *c*: chlorophyll *a* ratios in nutrient limited apical tips of *Sargassum thunbergii* were also found (Gao and Nakahara 1990).

Nitrogen limitation appeared to have little effect on fucoxanthin: chlorophyll *a* ratios suggesting that fucoxanthin does not serve to relieve N limitation in *Hincksia sordida*. This may in part explain the reduced amounts of fucoxanthin in N limited summer plants compared to winter plants. Alternatively, the low concentrations of fucoxanthin may be due to sustained growth rates in summer plants, at least in the first week, which would cause decreases in pigment concentrations as algal biomass

increases (Lewey and Gorham 1984). The lower fucoxanthin: chlorophyll *a* ratio in sewage enriched cultures compared with controls in winter may also be associated high growth rates, however this was not evident in N enriched plants.

The increased phycorytherin: chlorophyll *a* ratios with N enrichment in *Polysiphonia decipiens* suggests that the antenna size of PSII is responsive to increases in N availability. Similar increases in phycorytherin: chlorophyll *a* ratios in the Rhodophytes *Gracilaria foliifera* (DeBoer and Ryther 1978; Lapointe and Ryther 1979; Lapointe 1981) and *Neoagardhiella baileyi* (DeBoer and Ryther 1978; Lapointe and Ryther 1979) have been recorded with increasing N availability. In addition, the higher phycorytherin: chlorophyll *a* ratios in N limited summer plants of *Polysiphonia decipiens* compared to winter plants suggests that PSII is more susceptible to N limitation in winter than summer. This is consistent with other studies on the Rhodophyta (Lapointe and Duke 1984; Levy and Gannt 1990; Chopin et al. 1995). By maintaining summer phycorytherin concentrations relative to chlorophyll *a* the alga is better able to maintain photosynthetic activity during N limitation (Lapointe 1981). This may provide an explanation for the possible uncoupling of phycorytherin and tissue N (Chapter 2). In contrast, there was little change in chlorophyll *b*: *a* ratios in *Ulva* sp. with N limitation which suggests that N limitation affects both PSI and PSII in *Ulva*. Comparable responses were found in *Ulva fasciata* with nitrate enrichment (Lapointe and Tenore 1981), in *Ulva lactuca* with ammonium enrichment (Pederson 1995) and in *Ulva* sp. with inorganic N enrichment (Rosenberg and Ramus 1982a).

3.4.4 Summary

The study also provides a quantitative explanation of the effects of nutrients on various physiological parameters in PPB macroalgae which can be used to explain enhanced primary production observed in this marine embayment. This study further supports the proposition that accessory pigments (excluding fucoxanthin) store excess N which may be utilised during rapid growth to synthesise new photosynthetic pigments. The maintenance of PSII in N limited summer plants, relative to N limited

winter plants, is of adaptive significance in that it would help to relieve N limitation during summer.

Chapter 4

The relationship between growth and nitrogen for three species of macroalgae.

4.1 Introduction

4.1.1 Factors affecting seaweed growth rate

Increased nutrient availability often leads to an increase in production of fast growing ephemeral macroalgae that are highly competitive for available nutrients and are capable of smothering and shading slower growing benthic macrophytes (Cambridge and McComb 1984; Cambridge et al. 1984; Silberstein et al. 1986). The growth of these algae is often seasonal, with higher growth rates generally occurring during spring and summer than in autumn and winter (Rosenberg and Ramus 1982b; Geertz-Hansen and Sand-Jensen 1992). Growth may also be subject to nutrient availability such that populations of algae show both rapid bursts and reductions in growth rates. Experimental evidence for nutrient limitation has been shown by the increased growth of macroalgae following N enrichment (Chapman and Cragie 1977; Pederson and Borum 1996) and P enrichment (Steffensen 1976; Manley and North 1984; Connolly and Drew 1985; Björnsäter and Wheeler 1990; Chopin et al. 1995).

Rapid uptake and luxury accumulation of N beyond the alga's growth requirements have been reported for a number of macroalgae including *Ulva fasciata* (Chlorophyta) (Lapointe and Tenore 1981), *Gracilaria* sp. (Rhodophyta) (Ryther et al. 1981) and in the Phaeophyta (Chapman and Cragie 1977; Gerard 1982; Paling 1991). Macroalgal nutrient limitation may also be dependent on seasonal *in situ* nutrient exposure and internal nutrient reserves (DeBoer 1981; Hanisak 1983; Lapointe 1987; Wheeler and Björnsäter 1992). Lapointe (1985) also found that severe P limitation can lead to N limited growth in *Gracilaria tikvahiae* (Rhodophyta). Interspecies differences in

nutrient requirements may be explained by their relative differences in growth rates. Fast growing species have higher nutrient requirements per unit biomass due to higher growth rates and doubling times of days to weeks compared to the slower growing algae (doubling times of weeks to months). Nutrient demands are also reflected by critical nutrient contents (Hanisak 1979) that are required to sustain maximum growth (Littler and Littler 1980; Pederson and Borum 1996). Slower growing algae with low critical nutrient contents are able to accumulate and store larger amounts of nutrient reserves than fast growing species with high critical nutrient contents. Stored nutrients may support maximum growth for longer periods of time and may confer a competitive advantage in areas of nutrient fluctuation (Pederson and Borum 1996). These nutrient requirements may vary significantly with light, temperature and relative growth rates (Sfriso 1995).

4.1.2 Growth kinetics and nutrient utilisation

The original theories of growth kinetics in algae were derived from studies on phytoplankton, where growth was limited by a single external substrate and new growth was assumed to be related to the uptake of the substrate. Several studies, however, failed to obtain data consistent with the hyperbolic relationship between growth and substrate concentration (Goldman 1977, 1979). Droop (1973) provided a model that equated growth to a limiting internal cellular nutrient concentration. This internal nutrient pool is used during growth and is constantly replenished by the uptake of external nutrients. The rate of external nutrient uptake is dependent on the concentration of nutrients in the external medium. Such assumptions of growth and external/internal nutrients depend on steady state growth where net nutrient uptake is in equilibrium with the internal pool, and growth rate is related to the total intracellular concentration (Lobban *et al.* 1985). This can be best achieved experimentally with a continuous but limiting nutrient supply and by maintaining relatively constant thallus weight to water volume ratios.

The quantifiable relationships between nutrient content and growth in laboratory cultures may be of limited value to field populations of algae, as seasonal

environmental factors (e.g. temperature, grazing pressure) that may change and influence growth rates in the field are difficult to simulate in the laboratory. It is logistically difficult, however, to experimentally examine nutrient limitation in the field and the variation in environmental factors may make determination of nutrient limitation difficult. The ease of which many environmental factors can be controlled in the laboratory means that specific questions about relationships between growth and nutrients can be effectively examined. Such studies are useful in determining whether an alga is limited by a particular nutrient over relatively long periods (e.g. weeks to months). Information on growth and tissue nutrient contents gathered during these types of experiments may also be useful to determine the ability of macroalgae to assimilate and store nutrients beyond their requirements for growth and utilise stored nutrients.

4.1.3 Aims

The aim of this study was to determine whether N and/or P were limiting to growth of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. The study also aimed to determine any differences in the growth of each species and their capacities to store and survive on internal nutrient reserves. The algae were either starved or subjected to a range of ammonium concentrations found in the field. Experiments were conducted in winter and summer to determine the effects of tissue nutrient status on growth responses.

4.2 Methods

4.2.1 Sample collection and preparation

Collection of algal material and conditions of preparation was carried out as described in section 3.2.1.

4.2.2 Nutrient enrichment experiments

Experiment 1

Nutrient enrichment experiments were carried out as described in section 3.2.2 to initially determine whether ammonium-N or phosphate-P was limiting to the growth of each taxa. Three replicate plants (n=3) were subject to one of 3 nutrient treatments or 1 sewage effluent treatment and those plants that received sea water alone were used as controls (Table 3.1). The seawater used for controls was obtained from open coastal waters, filtered (0.2 μm) and then air bubbled in aquaria for 1 h until DIN and DIP concentrations were undetectable.

Experiment 2

An additional set of experiments were set up under the same conditions as above and were designed to investigate the relationship between macroalgal growth and tissue N over a range of ammonium-N concentrations. Four replicate plants (n=4) (each 1 g fresh weight) of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. were placed in separate aquaria (i.e. 1 replicate per tank) filled to a volume of 4 L on the day after collection. Plants were subjected to one of 4 nutrient treatments. Those plants that received seawater (0.2 μm filtered) alone were used as controls (Table 4.1). Nitrogen was added to the cultures as NH_4Cl and P was added as NaH_2PO_4 from stock solutions. To prevent C limitation carbon was added by additions of $NaHCO_3$ to concentrations of 3mM. Each ammonium treatment was made up and changed every

2 days for the duration of the experiment (14 d). Experiments were performed on separate occasions for each species during June-August 1995 (high DIN and DIP exposure) and December-February 1996 (low DIN and high DIP exposure) when ambient bottom water temperatures were 10-12°C and 19-21°C respectively. After each experiment plants were removed from treatments and measurements of wet weight (blot dried on paper towel for 30 s) and tissue N and P were made. Each alga was also weighed for dry weight after being oven dried at 70°C for 24 h.

Table 4.1 Nutrient conditions for experiments testing the effects of ammonium-N, on the growth of *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp.

Nutrient	Treatments (concentration)				
	1	2	3	4	5
Ammonium ($\mu g\ NH_4\text{-}N\ L^{-1}$)	400	200	100	50	0
Phosphate ($\mu g\ PO_4\text{-}P\ L^{-1}$)	500	500	500	500	500
Carbon (mM)	3	3	3	3	3
No. of replicates	4	4	4	4	4

4.2.3 Growth rate

Growth rate was calculated from changes in fresh weight over a 14 d period. Plant material was trimmed at day 7 in order to maintain approximate biomass to water volume ratios during the experiment of 1g:4L. There was no significant difference between growth rates after 7 and 14 days. Therefore, the growth rates presented are means of growth rate after 7 and 14 days. Growth rates were calculated according to

equations outlined in Pederson and Borum (1996). The first method calculated growth as the actual percentage increase over time according to the formula :

$$\% g = (B_t - B_0)\, t^{-1} \qquad \text{(Eq. 4.1)}$$

The specific net growth rate (μ) was also calculated from changes in fresh weight biomass over time :

$$\mu = (\log_e B_t - \log_e B_0)\, t^{-1} \qquad \text{(Eq. 4.2)}$$

For both equations B_0 represents the initial and B_t the final biomass after t days incubation.

4.2.4 Fresh and dry weights

Fresh weights of all plant material were determined after blotting with tissues. Dry weights were determined after drying to constant weight at 70°C for 24 h.

4.2.5 Tissue nutrient analyses

Tissue N contents were determined on sub-samples of all plants after the growth experiments as detailed in section 2.2.4. Contents are expressed either as mg N or P g dry wt.$^{-1}$ or as % of dry weight.

4.2.6 Critical tissue nitrogen contentss

The N contents needed to support maximum growth of the three species were calculated according to the equations of Pederson and Borum (1996) and are described below.

The growth rates (μ) were plotted against the tissue N content for each sample, and data were fitted to the Droop equation (Droop 1973) using non-linear least-square regression (SYSTAT vs. 5.03, Systat Inc., USA).

$$\mu = \mu_{max} (1-N_q / N) \qquad \text{(Eq. 4.3)}$$

where μ_{max} is the maximum growth rate, N_q is the minimum tissue N content needed to sustain growth (the subsistence quota), and N is the actual tissue content in the alga. The critical N content, or N_c, was calculated from the intercept of the two lines represented by the maximum growth rate and the initial slope of the curve. The initial slope was approximated as the line going through the points where $\mu = 0.5\ \mu_{max}$.

4.2.7 Nitrogen storage capacity

The ability of different algal species to accumulate N was examined by comparing interspecific differences in the maximum tissue N content measured during the laboratory experiments. The N pool stored in excess of the requirements for maximum growth (N storage = N_s) was estimated as the difference between maximum (N_{max}) and critical N contents (N_c). The N storage capacity (T_{max}), defined as the duration (in days) this storage can sustain maximum algal growth without any compensatory N uptake from the external media, was calculated as:

$$T_{max} = \log_e(N_{max} / N_c)\ \mu_{max}^{-1} \qquad \text{(Eq. 4.4)}$$

When maximum growth has consumed internal stores of N below the critical level growth will continue at declining rates until the subsistence quota (N_q) is reached. The period of reduced growth (T_{red}), over which internal N pools are below the critical level ($N_{red} = N_c - N_q$), (i.e. when the tissue N content decreased below the critical limit (N_c) until the subsistence quota (N_q) was reached, was calculated as:

$$T_{red} = \log_e(N_c/N_q)\ (0.5\ \mu_{max})^{-1} \qquad \text{(Eq. 4.5)}$$

This equation assumes a linear decline in growth rate between N_c and N_q, providing an average growth rate of $0.5\ \mu_{max}$ during this period. Values of N_q were obtained from laboratory experiments.

4.2.8 Statistical analyses

Data were tested for assumptions of normality by examining heterogeneity of variance (Cochrans test) and skewness of data (residuals and outliers). Non-normal data was subject to log transformation, $\log_e(x+1)$. *t*-tests were used to detect for differences between growth rates measured after 7 and 14 d. Two-way ANOVA was employed to examine for effects of season and nutrient enrichment on the growth rates calculated at day 14. The significance level used was $p < 0.05$. Tukeys test was used to make multiple comparisons among treatment means from significant ANOVA tests. Pearson correlation coefficients were calculated to examine relationships between P_{max} and growth rates. The computer software SYSTAT (vs. 5.03, Systat Inc., USA) was used for all analyses.

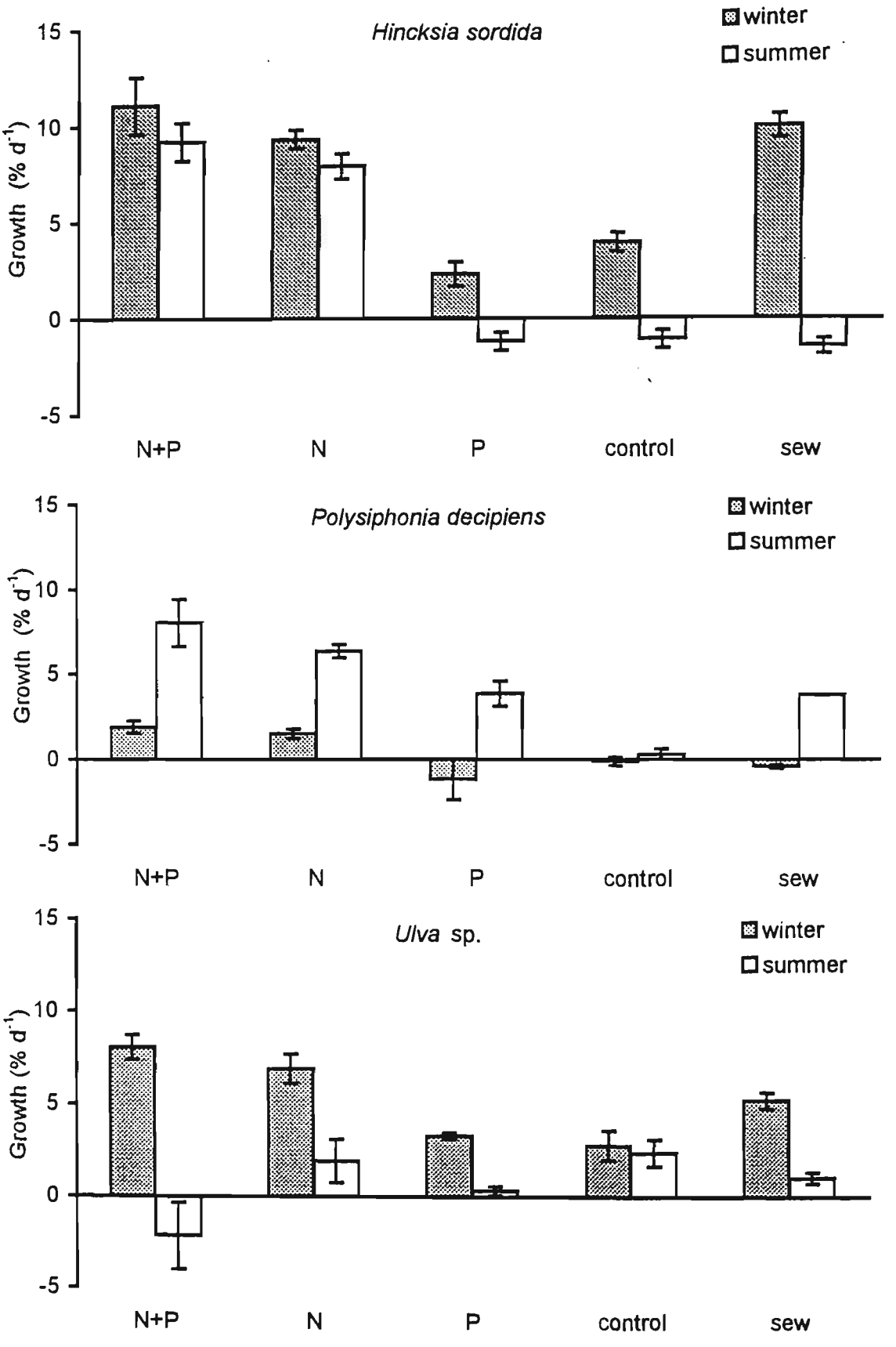

Fig. 4.1 Growth rates (% d^{-1}) after 14 days exposure to various nutrient treatments during winter and summer for three species of macroalgae. Nutrient enrichment includes nitrogen and phosphorus (N+P), nitrogen only (N), phosphorus only (P), no nitrogen or phosphorus (control) and sewage enriched (sew). Values are means ± 1 s.e., n = 3. Details of nutrient treatments are found in Table 3.1.

4.3 Results

4.3.1 Growth rates, nutrient responses

Experiment 1

Growth rates presented are the means of rates recorded after 14 d. Of the three species examined the maximum growth rate (% d^{-1}) was highest for *Hincksia sordida* (mean = 11.1 % d^{-1}), intermediate for *Ulva* sp. (mean = 10.2 % d^{-1}) and lowest for *Polysiphonia decipiens* (mean = 8.0 % d^{-1}) (Fig. 4.1). There was significant correlation between growth and P_{max} (section 3.3.1) for all of the 3 species examined (Table 4.1).

Table 4.2 Pearson correlation statistic, r, for the relationship between growth and P_{max} for 3 species of macroalgae in winter and summer. * represents significance at $p < 0.05$.

Species	r Winter	r Summer
Hincksia sordida	0.81*	0.89*
Polysiphonia decipiens	0.87*	0.83*
Ulva sp.	0.82*	-0.04

Growth (% increase d^{-1}) in N enriched treatments (N+P, +N, +sewage) (except for +sewage enriched *Polysiphonia decipiens* in winter) was significantly higher than growth in treatments without additional N (+P, control). Phosphorus enriched (+P, +sewage) growth did not significantly differ from sea water controls except for *Polysiphonia decipiens* in summer (Table 4.2; Fig. 4.1).

Growth rates for *Ulva* sp. and *Hincksia sordida* were significantly higher in winter than summer. The reverse was true for *Polysiphonia decipiens* (Table 4.2; Fig. 4.1). *Hincksia sordida* plants that did not receive additional N (+P, controls) were found to have significantly higher rates of growth in winter than summer, whilst N enriched (+N, N+P) plants did not differ seasonally. In contrast, growth of *Polysiphonia decipiens* was 3 to 4 fold higher in N and P enriched summer plants than equivalent winter plants. In *Ulva* sp. significantly higher growth rates were found in winter N enriched (N+P, +N) plants than summer plants. In winter, growth rates of sewage enriched *Hincksia sordida* and *Ulva* sp. were significantly higher than in summer, however, the trend was opposite in *Polysiphonia decipiens.*

The significant season-nutrient interaction (Appendix 15) reflected higher growth rates for *Hincksia sordida* and *Ulva* sp. in sewage treatments than in treatments without N addition during winter but not during summer. For *Polysiphonia decipiens* this interaction was due to significantly higher summer growth rates in N and P treatments than in controls during summer but not in winter (Table 4.2).

Table 4.3 Table of post hoc significance values (Tukeys) of the effects of nutrient (N and P, N, P) and sewage enrichment on growth for three species of macroalgae. Winter and summer values are compared to controls after 14 days of treatment exposure. Comparisons between winter and summer treatments are also presented. Analyses are based on values presented in Fig. 4.1. n.s. = not significant ($p > 0.05$), n.a. = not applicable.

Hincksia sordida	**N and P**	**N**	**P**	**Sewage**	**Control**
Winter	0.000	0.002	n.s.	0.001	n.a.
Summer	0.000	0.000	n.s.	n.s.	n.a.
Winter vs Summer	n.s.	n.s.	0.001	0.000	0.000

Table 4.3 cont.

Polysiphonia decipiens	N and P	N	P	Sewage	Control
Winter	n.s.	n.s.	n.s.	0.001	n.a.
Summer	0.001	0.001	0.030	0.012	n.a.
Winter vs Summer	0.001	0.001	0.001	0.001	n.s.

Ulva sp.	N and P	N	P	Sewage	Control
Winter	0.017	n.s.	n.s.	n.s.	n.a.
Summer	0.000	n.s.	n.s.	n.s.	n.a.
Winter vs Summer	0.000	0.008	n.s.	0.033	n.s.

Experiment 2

The log transformed mean algal growth rates (μ d^{-1}) obtained over the 14 d period increased asymptotically towards μ_{max} with increasing tissue N content in all algae during winter (Figs. 4.2 to 4.4). Of all species this relationship was strongest in *Hincksia sordida* during winter (r^2 = 0.84) and summer (r^2 = 0.83). Linear relationships were also significant for *Hincksia sordida* during winter (r^2 = 0.86) and summer (r^2 = 0.81). The non-linear relationship was weak for *Polysiphonia decipiens* in winter (r^2 = 0.52) but stronger in summer (r^2 = 0.78). Weak relationships were found for *Ulva* sp. in winter (r^2 = 0.25) and in summer. No significant relationships between μ_{max} and tissue P were found in winter or summer plants except for a significant linear relationship in summer *Polysiphonia decipiens* (df = 15, F = 99.22, p < 0.001) (Fig. 4.5).

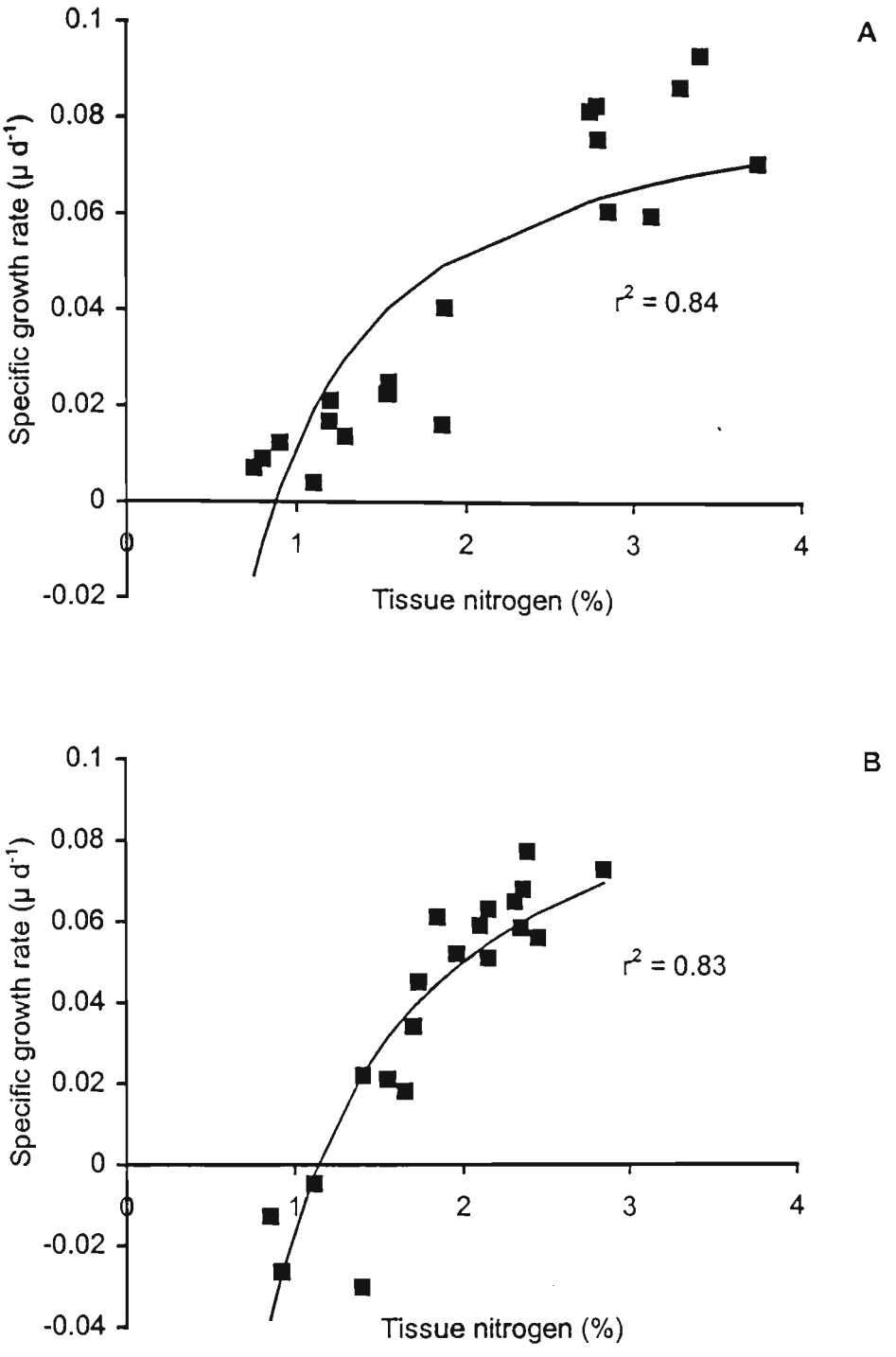

Fig. 4.2 Specific growth rates (μ) of *Hincksia sordida* versus tissue nitrogen concentration (%) in (A) winter and (B) summer, n = 20.

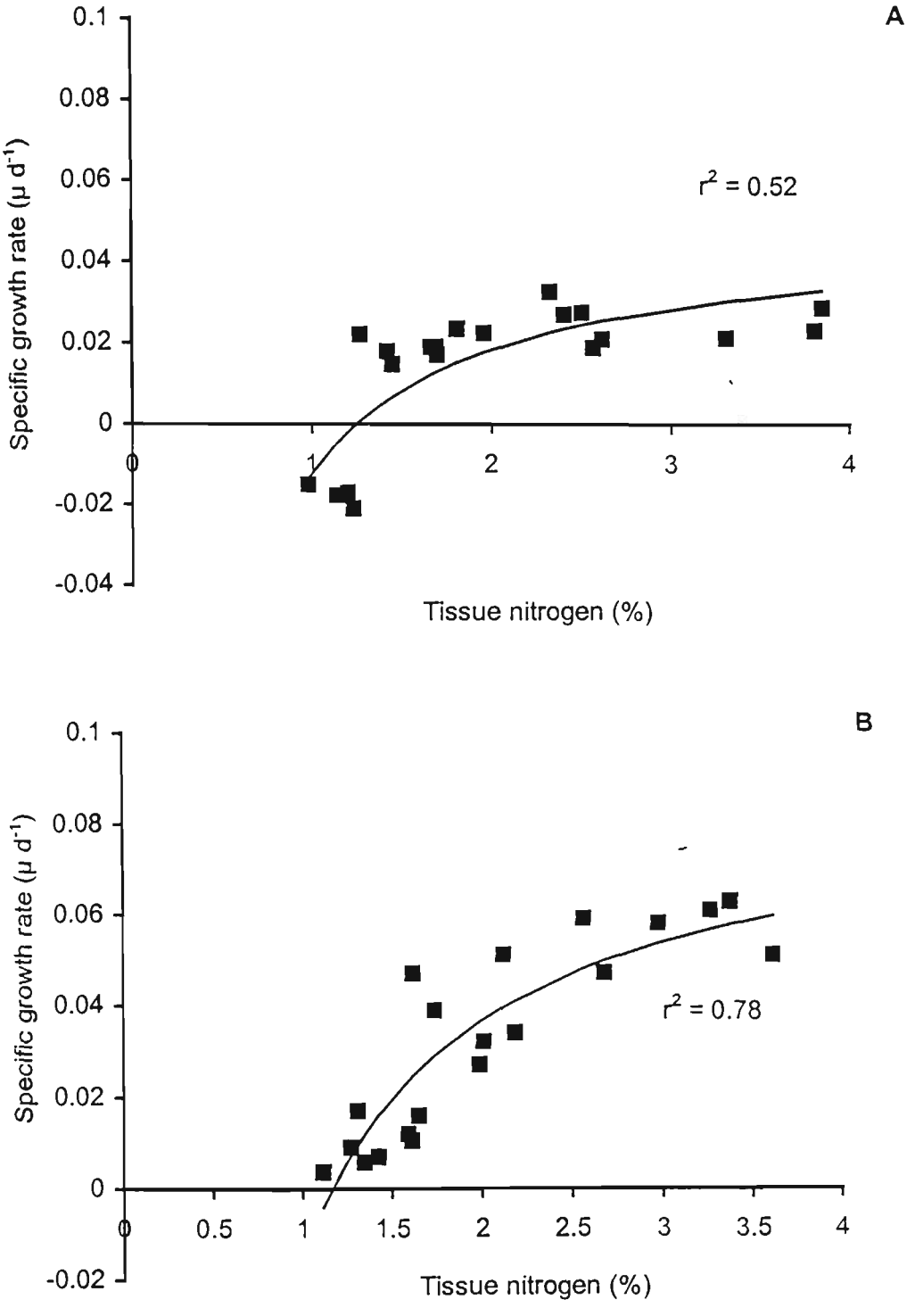

Fig. 4.3 Specific growth rates (μ) of *Polysiphonia decipiens* versus tissue nitrogen concentration (%) in (A) winter and (B) summer, n = 20.

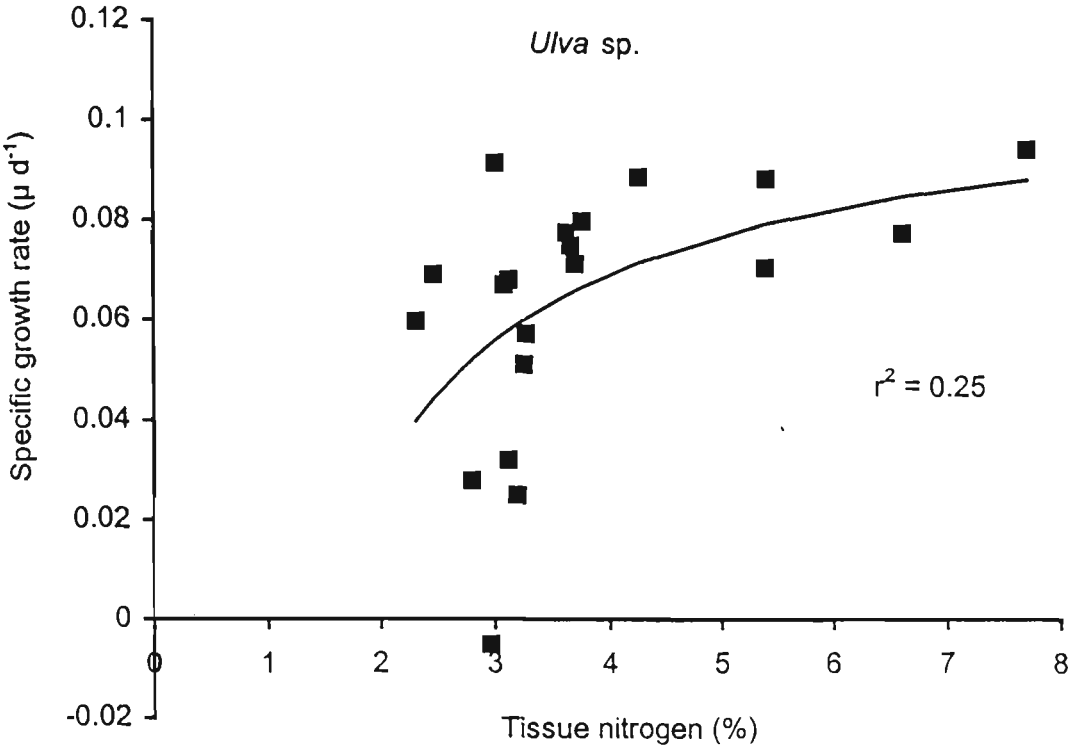

Fig. 4.4 Specific growth rates (μ d^{-1}) of *Ulva* sp. versus tissue nitrogen concentration (%) in winter, n = 20.

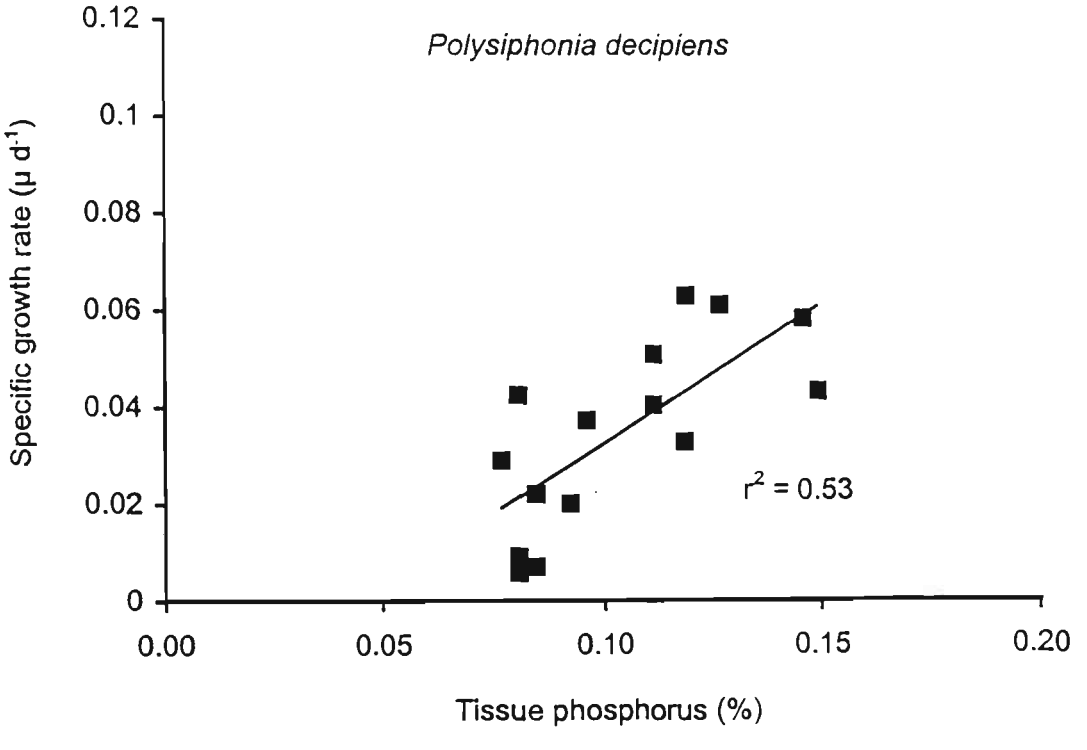

Fig. 4.5 Specific growth rates (μ d^{-1}) of *Polysiphonia* decipiens versus tissue phosphorus concentration (%) in summer, n = 20.

The maximum growth rates (μ_{max}) varied seasonally for all three species (Table 4.4). There was a 2- to 3-fold difference in maximum growth rate among species with *Ulva* sp. having the highest rate. During winter μ_{max} was lowest in *Polysiphonia decipiens* (0.049 d^{-1}) and highest in *Ulva* sp. (0.110 d^{-1}), whilst in summer lowest growth occurred in *Ulva* sp. (0.028 d^{-1}; derived from raw data) and highest in *Hincksia sordida* (0.116 d^{-1}).

Table 4.4 Maximum growth rates (μ_{max}), maximum tissue N (N_{max}), critical N (N_c) and subsistence quota (N_q) for three species of macroalgae from Port Phillip Bay. All values were obtained from laboratory experiments. n.c. = not computable. *derived from raw data

Species	μ_{max} (d^{-1})		N_{max} (mg N g dry wt.$^{-1}$)		N_c (mg N g dry wt.$^{-1}$)		N_q (mg N g dry wt.$^{-1}$)	
	winter	summer	winter	summer	winter	summer	winter	summer
Hincksia sordida	0.093	0.116	37.3	28.5	20.8	21.2	8.8	11.4
Polysiphonia decipiens	0.049	0.088	38.5	36.2	28.8	23.5	22.8	7.7
Ulva sp.	0.110	0.028*	76.9	27.5	27.5	n.c.	14.7	n.c.

4.3.2 Critical tissue N contents

Maximum tissue N (N_{max}) was highest in *Ulva* sp., and in winter taxa compared with summer taxa. Critical N contents (N_c) were highest during winter for *Polysiphonia decipiens* (28.8 mg N g dry wt.$^{-1}$) but similar between winter and summer for *Hincksia sordida* (20.8 mg N g dry wt.$^{-1}$ and 21.2 mg N g dry wt.$^{-1}$ respectively). Only winter values of N_c could be calculated for *Ulva* sp. due to low summer growth (Table 4.4). The subsistence quota (N_q) also varied seasonally for particular species, being lower during winter for *Hincksia sordida* and lower during summer for *Polysiphonia decipiens* (Table 4.4). The N_q for *Ulva* sp. in winter was intermediate

between the other two species. N_q could not be calculated for *Ulva* sp. in summer because of the absence of a relationship between growth and tissue N.

4.3.3 Nitrogen requirements and storage

The amount of N required to sustain maximum algal growth ($N_{req} = \mu_{max} \times N_c$) varied seasonally for *Polysiphonia decipiens* and *Hincksia sordida* with higher values in summer (Table 4.5). N_{req} also varied among species with lowest values found in summer *Polysiphonia decipiens* (14.1 mg N g dry wt.$^{-1}$ d^{-1}) and highest in winter *Ulva* sp. (30.3 mg N g dry wt.$^{-1}$ d^{-1}). The amount of N stored in excess of the critical limit ($N_s = N_{max} - N_c$) that supported maximum algal growth also varied among species, with lowest values in summer *Hincksia sordida* (7.3 mg N g dry wt.$^{-1}$ d^{-1}) and highest in winter *Ulva* sp. (49.4 mg N g dry wt.$^{-1}$ d^{-1}) (Table 4.5). Seasonal responses of N_s differed for *Hincksia sordida* and *Polysiphonia decipiens* with higher values for *Hincksia sordida* and *Polysiphonia decipiens* during winter and summer, respectively.

The tissue N content at which algal growth could proceed at N limited rates (T_{red}) was lowest in *Hincksia sordida* and *Polysiphonia decipiens* during summer (Table 4.5). During winter reduced growth rates could be supported for the longest time (34.06 d) in *Polysiphonia decipiens* and for the shortest time in summer *Hincksia sordida* and *Polysiphonia decipiens* (10.71 and 15.93 d respectively). The storage capacity (T_{max}), defined as the number of days that excess N (N_s) could support maximum growth was lowest in winter *Ulva* sp. (1.35 d). For *Hincksia sordida* and *Polysiphonia decipiens* similar values were evident in winter (5.92 and 6.28 d respectively) and in summer (2.55 and 4.91 d respectively) (Table 4.5).

Table 4.5 Nitrogen requirements at maximum growth rates ($N_{req} = \mu_{max} \times N_c$), N pools in excess of that necessary for maximum growth (N_s), N pools that allow reduced growth (N_{red}), storage capacity at non-limited growth (T_{max}), and the possible duration of reduced growth (T_{red}) based on N_{red} for three species of macroalgae from Port Phillip Bay. All values were obtained from laboratory experiments. n.c. = not computable.

Species	N_{req} (mg N g dry wt.$^{-1}$ d^{-1})		N_s ($N_{max} - N_c$) (mg N g dry wt.$^{-1}$)		N_{red} ($N_c - N_q$) (mg N g dry wt.$^{-1}$)		T_{max} (d)		T_{red} (d)	
	winter	summer	winter	summer	winter	summer	winter	summer	winter	summer
Hincksia sordida	1.93	2.46	16.5	9.4	10.3	9.8	6.28	2.55	18.62	10.71
Polysiphonia decipiens	1.41	2.06	9.7	12.8	9.2	11.8	5.92	4.91	34.06	15.93
Ulva sp.	3.03	n.c.	49.4	n.c.	12.8	n.c.	1.35	n.c.	11.39	n.c.

4.4 Discussion

4.4.1 Growth and nutrient limitation

The high growth rates shown by *Hincksia sordida* and *Ulva* sp. in response to N enrichment may reflect the short lifespan, relatively high photosynthetic rates (see section 3.3.2) and high reproductive capacities that are well suited to environments with a fluctuating nutrient supply (Clayton 1990). The specific maximum growth rates (μ_{max}) species were generally within the range of growth rates obtained in similar studies using *Ectocarpus* sp. (Phaeophyta) (Boalch 1968), *Ulva* spp. (Lapointe and Tenore 1981; Ramus and Venable 1987; Duke et al. 1989; Björnsäter and Wheeler 1990; Pederson 1995; Pederson and Borum 1996) and Rhodophyta (DeBoer et al. 1978; Lignell and Pederson 1987; Duke et al 1989; Laing *et al.* 1989; Pickering et al. 1993; Pederson and Borum 1996). Such rates do not account for tissue losses associated with grazing and erosion in the field and therefore represent maximum gross rates of growth.

The higher winter growth rate compared to summer for both *Hincksia sordida* and *Ulva* sp. illustrates a dependence of growth on previous field N exposure, hence, the growth of these species appears well suited to high DIN avalability. These trends contrast with other studies which have noted higher growth of *Ulva* sp. (Rosenberg and Ramus 1982b; Geertz-Hansen and Sand-Jensen 1992) and *Ceramium rubrum* (Rhodophyta) (Pederson and Borum 1996) in summer compared to winter. In contrast to the present study, these experiments were conducted in the field, and temperature, which influences photosynthesis (Davison 1991) may have contributed to the higher growth rates in summer. Relatively high growth by *Polysiphonia decipiens* in summer suggests a lesser reliance of this species on previous N exposure, possibly due to a utilisation of stored N reserves. The positive relationship between growth and maximum photosynthetic output for all species examined indicates that growth is coupled to metabolism under laboratory conditions. The commensurate increase in pigmentation and tissue N (section 3.3.4) suggests that these parameters may be useful

indicators of growth during N sufficiency in the field. However, differences between laboratory and field relationships may be expected because of factors that limit growth in the field (e.g. grazing pressure, light limitation, fragmentation).

The absence of a stimulatory effect of P on N enriched growth in any of the species studied suggests that growth of these algae was not P limited. This contrasts with the stimulated photosynthetic responses of N and P enriched *Ulva* sp. compared to N enriched plants in summer (section 3.2.2; Fig. 3.1). The significant linear relationship between tissue P and summer growth for *Polysiphonia decipiens* also indicates that P is utilised for growth, possibly at the expense of P storage. Phosphorus limitation of macroalgae has generally been reported in tropical species such as *Gracilaria tikvahiae* (Rhodophyta) (Lapointe 1985) and *Cladophora prolifera* (Chlorophyta) (Lapointe and O'Connell 1989). Lapointe (1985) showed that severe P limitation can lead to N limited growth in *Gracilaria tikvahiae.* This was attributed to the requirement of P containing molecules to cellular energetics (ATP, ADP) and membrane structure (phospholipids), two critical components of the N uptake system in algae. Lapointe (1985) suggested that a minimum P content may be required to maintain optimal N uptake. The growth of *Ulva lactuca* and *Cladophora albida* from temperate estuarine waters have also been shown to be P limited (Steffensen 1976; Gordon et al. 1981). The absence of P limited growth in summer and winter macroalgae in the present study is in agreement with findings of a recent study of *Ulva* sp. from Roskilde Estuary, Denmark (Pederson and Borum 1996), and supports the view that temperate marine macroalgae are not prone to P limitation.

During summer, the lack of growth in N replete *Ulva* sp. suggests that much of the N assimilated is initially stored as reserves to be used for physiological processes in the form of protein/pigment synthesis, as shown by the increased P_{max} and pigment contents in these algae (sections 3.3.2 and 3.3.5). The increase in physiological parameters, apparently at the expense of growth, have previously been documented for *Ulva* sp. (Rosenberg and Ramus 1982b; Duke et al. 1986). Such a strategy allows rapid N accumulation and is ecologically significant as excess storage of N may allow

growth when nutrient supply fluctuates. In contrast, Pederson and Borum (1996) found that N (as ammonium) enrichment significantly increased growth rates of *Ulva lactuca* and the red alga *Ceramium rubrum* (Rhodophyta) in spring and early summer (but not in winter) during non-limiting light and temperatures. It may be possible that the relatively short duration of the experiments in the present study (2 weeks) did not allow enough time for growth of *Ulva* sp. to be optimised following N uptake. An alternative explanation is that summer *Ulva* sp. was reproductive and undergoing spore formation, a factor that could lead to a decline in growth rates (Geertz-Hansen and Sand Jensen 1992). It is unlikely that this would explain the lack of N enriched growth by *Ulva* sp. in this study because ammonium enriched growth has been reported in reproductive *Ulva lactuca* (Geertz-Hansen and Sand Jensen 1992).

4.4.2 Growth and tissue nutrients

The saturated growth responses shown and *Polysiphonia decipiens* in relation to tissue N are indicative of luxury N uptake beyond immediate growth requirements, and suggest an uncoupling between growth and N uptake. By contrast, the weak relationship for winter *Ulva* sp. suggests that growth by this species is not saturated over the range of contents used. Significant linear relationships between growth and tissue N in *Hincksia sordida* also suggest that growth may not have been saturated. Hence, the saturated growth and tissue N parameters derived by fitting the Droop model may provide an under-estimation of the true saturating growth concentrations. Whether growth would increase linearly at higher concentrations than used in this study requires further examination. The poor relationship between growth and tissue N for summer *Polysiphonia decipiens* also suggests a utilisation of N for growth at the highest ammonium concentrations. The relationship between ammonium uptake and concentration for all species is examined in Chapter 5.

From the hyperbolic relationships between growth and tissue N critical threshold limits of N defined the minimum tissue N that was necessary to support maximum growth rates. All species exhibited similar ranges of N_c, ranges that are also comparable to those recorded for other macroalgae (Fujita et al. 1989; Pederson and

Borum 1996). In winter field contents of N (section 2.3.4) rarely fall below N_c therefore these algae are unlikely to be N limited in winter. In contrast the tissue N contents of *Hincksia sordida* and *Ulva* sp. in spring and summer (section 2.3.4) fall below the reported N_c values. Accordingly the growth of both species may be N limited during these periods.

The converse was true for *Polysiphonia decipiens* and the seasonal variation in N_c and N_q recorded for this species suggests an adaptive capacity of this species to tolerate low N availability by reducing its summer N threshold limit and allowing for maintenance of growth in summer. This is possibly achieved by utilising stored nutrients. At thallus N quotas below N_c it is advantageous for a species to maintain low N_q in environments subject to seasonal N fluctuations, as this defines the lower limit of survival (Probyn and Chapman 1982). Thus the relatively high N_q in summer compared to winter for *Hincksia sordida* implies an inability to store N for use when N supply is low and may in part explain the periodic decline of this taxa during spring and summer (section 2.3.2). The low winter N_q of *Hincksia sordida* would serve to enhance its ability to maintain a basal growth rate at a lower internal tissue N when external N availability is high. This implies that *Hincksia sordida* is predisposed to N limitation and more dependent on the rapid sequestering of N, rather than the utilisation of stored N reserves.

The relatively high N_c and N_q for winter *Ulva* sp. implies an inability to survive in low N environments. Tissue N in field collected *Ulva* sp. rarely falls below this N_q value and as such the significance of these values in *Ulva* sp. may be of little ecological importance. Probyn and Chapman (1982) also concluded that N_q had little ecological significance for the fast growing annual *Chordaria flagelliformis* (Phaeophyta), as high tissue N contents may simply reflect relatively high winter N availability and/or high N requirements. In summer the growth of *Ulva* sp. was unaffected by N enrichment, supporting previous findings that light and temperature were more important towards limiting productivity (section 2.4.1). In studies on *Ulva* spp. from N enriched estuarine waters, Lavery and McComb (1991b) and Pederson

(1996) found considerably lower critical N contentss (respectively 2.0% and 2.17 %) than that calculated for *Ulva* sp. (2.75%) during winter in this study and for *Ulva fenestrata* (3.2%) in non enriched coastal waters (Björnsäter and Wheeler 1990). Critical threshold limits for N in species of *Ulva* may therefore depend on the extent to which the water body is enriched with N, with low critical N values indicative of N sufficiency.

The greater demand for N by *Hincksia sordida* and *Ulva* sp. compared to *Polysiphonia decipiens* is also reflected by the higher N_{req} (N required to support maximum growth). For *Hincksia sordida* the higher N_{req} in winter and summer infers a greater dependence on N availability, supporting observations of rapid growth and bloom events. In summer, lower concentrations of DIN in ambient waters combined with relatively high demands for N by *Hincksia sordida* does not allow it to store as much excess N (N_s) in summer as in winter. The high demand for N by summer *Hincksia sordida* is reflected by a low physiological pool of N that inhibits growth (N_{red}), compared with other taxa. This is indicative of a susceptibility to N availability and may explain transiently low biomass recorded for *Hincksia sordida* during summer.

The ability of *Hincksia sordida* to use stored N reserves to support non-limited growth for only short periods (T_{max}) in summer (2.55 d) compared to winter (6.28 d) indicates that it is prone to N limitation in a shorter time in summer. By contrast *Polysiphonia decipiens* has a lower demand for N in summer which is reflected by the higher summer N pool in excess of that required for maximum growth (N_s = 12.7 mg N g dry wt.$^{-1}$) compared to its winter N_s (9.7 mg N g dry wt.$^{-1}$). The approximately equivalent T_{max} values for winter (5.92 d) compared to summer (4.91 d) therefore suggests that stored N reserves are available to support non-limited growth. High winter N requirements of *Ulva* sp. were reflected by relatively high N_c, N_{req}, N_s and N_{red} and a low T_{max} (1.35 d). This suggests that during winter conditions of light and temperature limitation most of the N pool in this species is used rapidly for growth, rather than being stored. The opposite was true in summer. These results are

comparable to those of Fujita (1985) who found that stored N could support growth of *Gracilaria tikvahiae* (Rhodophyta) for longer periods (14 d) than the faster growing Chlorophytes *Ulva* sp. (6 d) and *Enteromorpha intestinalis* (8 d), after which N limited growth occurred. Pederson and Borum (1996) also reported similar time periods during which stores of N could support growth for *Ceramium rubrum* (2.3 d) and the Chlorophytes *Ulva lactuca* (3.1 d) and *Cladophora sereica* (3.4 d).

This high requirement for N by *Hincksia sordida* and *Ulva* sp. means that internal N reserves could support reduced growth (T_{red}) for a shorter time (10.71 to 18.62 d) than for *Polysiphonia decipiens* (15.93 to 34.07 d). This infers a predilection of the two opportunistic species to N limitation. Pederson and Borum (1996) found that *Ulva lactuca* could survive on stored nutrients for shorter periods (6.5 d) compared to other fast growing algae such as *Ceramium rubrum*, *Cladophora sereica* and *Chaetomorpha linum* (8.7 to 10.1 d), suggesting that *Ulva lactuca* was most likely to experience N limitation. By contrast the perennial macrolaga *Fucus vesiculosus* (Phaeophyta) was able to survive on stored N for extended periods of up to 54 d (Pederson and Borum 1996), which is consistent studies that have focused on nutrient requirements of perennial macroalgae (Chapman and Cragie 1977; Zimmerman and Kremer 1986; Paling 1991).

4.4.3 Summary

The present study provides a functional explanation for the importance of N limitation in determining seasonal growth responses of fast growing macroalgae in PPB. *Polysiphonia decipiens* exhibited the lowest growth rates of all taxa and was best able to store and survive on reserves of N. Much of the acquired N in *Hincksia sordida* and *Ulva* sp. was allocated to growth and both species showed a relatively low capacity for N storage compared with *Polysiphonia decipiens*. The high requirement for N by *Hincksia sordida* and *Ulva* sp. makes these taxa susceptible to N limitation in summer when inputs of N to coastal waters are low. As a response to N limitation *Ulva* sp. was able to store N and use it for physiological requirements at the expense of growth. On the other hand *Hincksia sordida* was shown to exploit available N for

growth. Such mechanisms may allow these macroalgae to buffer the effects of fluctuations in external nutrient concentrations and sustain optimal growth rates over periods of days to weeks during N limitation.

Chapter 5

The uptake of ammonium nitrogen by macroalgae

5.1 Introduction

5.1.1 Nutrient uptake

Macroalgae acquire phosphorus (P) as orthophosphate (PO_4^{3-}) and nitrogen (N) as nitrate (NO_3^-) and/or ammonium (NH_4^+) (Paling 1991) although nitrite (NO_2^-) is utilised by some macroalgae (Hanisak 1983). The rates of uptake of these nutrient forms varies, dependent on availability and competitive interactions with other taxa, but ammonium is generally taken up at the highest rate (Hanisak 1983; Paling 1991). Nutrient ions enter macroalgal cells by one of three mechanisms: passive diffusion, facilitated diffusion and active transport. Passive diffusion is directly proportional to the difference between external and internal nutrient concentrations while facilitated and active transport are dependent on transport mechanisms (e.g. enzyme activated) that saturate membrane carriers as the external concentration of the ion increases (Lobban et al. 1985).

Ammonium and nitrate are assimilated into macroalgae by different metabolic pathways. Both are used in the formation of compounds of metabolic importance, such as amino acids, purines, pyrimidines, amino sugars and amines. Ammonium is already reduced and can therefore be directly incorporated into compounds such as amino acids. Oxidized ions such as nitrate must, however, first be reduced intracellularly to ammonium. The reduction of nitrate to ammonium occurs in the cytoplasm of the cell in two main steps: firstly nitrate is reduced to nitrite, catalyzed by the enzyme nitrate reductase (NR). The electron donor in macroalgae is usually NADH. Secondly, nitrite is transported to the chloroplasts for reduction to ammonium. This reduction is catalyzed by NR located in chloroplasts (Lobban et al. 1985).

The incorporation of ammonium into amino acids may occur with the formation of glutamic acid which is catalyzed by the enzyme glutamate dehydrogenase (GDH). Alternatively glutamine may be formed as the first product of ammonium assimilation, catalyzed by the enzyme glutamine synthetase (GS). Glutamic acid can then be formed by a second reaction where the amide group of glutamine is transferred to α-oxoglutaric acid from the Krebs cycle. The enzyme catalyzing this second reaction is glutamine-oxoglutarate aminotransferase (GOGAT) (Lobban et al. 1985). These two major pathways for ammonium assimilation have been respectively termed the GDH and GS-GOGAT pathways and both may be present simultaneously (McKenzie et al. 1979).

An alternative pathway by which ammonium can be assimilated by macroalgae is the carbamoyl phosphate pathway in which ammonium is incorporated into the amino acids citrulline and arginine and thence into thiamine and biotin (Lobban et al. 1985). In this process freely available ammonium combines with carbon dioxide to form carbamoyl phosphate which donates its carbomyl group to ornithine to directly produce citrulline (Lehninger 1973). Asparate facilitates the conversion of citrulline to argininosuccinate which is then converted to arginine. Ammonium for carbomyl phosphate synthesis may also be derived from the amide group of asparagine or glutamine (Lobban et al. 1985).

5.1.2 Ammonium uptake and growth kinetics: Michaelis-Menten equation

The relationship between the uptake rate of the nutrient ion and its external concentration is often documented in terms of the hyperbolic curve of the Michaelis-Menten equation developed for interpreting enzyme substrate kinetics (Mercer and Goodwin 1972) and is described as:

$$V = V_{max} \times [S / K_m + S],$$

where V is the rate of reaction (uptake), V_{max} is the maximum uptake rate, S is the substrate (nutrient ion) concentration and K_m is the half saturation constant (also

referred to as K_m), which is the concentration at which uptake rate is half of the maximum (V_{max}). The equation is based on a single substrate binding to one enzyme. The use of this equation to describe uptake and growth kinetics allows theoretical comparisons to be made between algae from different genera or locations (Lobban et al. 1985). Algae with high V_{max} are often interpreted to be well equipped to exploit pulses of high nutrients (Turpin and Harrison 1979; Hurd and Dring 1990). An alternative but not mutually exclusive interpretation is that high V_{max} may indicate the degree of nutrient limitation in an alga (D'Elia and D'Boer 1978).

Uptake (V) and growth (μ) versus substrate concentration (S) data may be fitted to this equation using non-linear regression techniques. Alternatively these parameters may be estimated from one of three linear transformation methods, some of which are more reliable for determining V_{max} or K_m values than others (Dowd and Riggs 1965). The various linearization methods available may result in considerable differences in the generated values of V_{max} and K_m if the experimental data do not fit the hyperbolic curve function of the Michaelis-Menten equation. Where data do fit this equation the general method of linearization used is S/V as a function of S (DeBoer 1981). This method is thought to show least variation in uptake rates of the same species. Where data does not fit this equation, rates are often derived from non-linear uptake (V) versus concentration (S) curves (Pederson and Borum 1997).

Deviation from saturation kinetics of the hyperbolic function may occur when internal nutrient pools are not fully depleted or when uptake involves both passive and active mechanisms (i.e. dual or multiphasic) (Lobban et al. 1985). The latter has been reported for *Gracilaria tikvahiae* (D'Elia and DeBoer 1978) and *Ulva rigida* (MacFarlane and Smith 1982; Lavery and McComb 1991b). Uptake corresponding to these models exhibits high affinity (low K_m and active uptake) at low concentrations and a low affinity (high K_m and high diffusion) at higher concentrations. Active uptake at low concentrations (e.g. via an amine cation porter) is often rate limited by diffusion across the unstirred boundary layer (MacFarlane and Smith 1982).

The ratio of V_{max}:K_m is an indication of the competitive ability of macroalgae for nutrient uptake at low concentrations. It is generally lower for late successional, long lived, coarsely branched species and appears to be less intraspecifically variable than other kinetic parameters (Wallentinus 1984a). K_m and V_{max} values have been found to be inconsistent between uptake studies of the same species, but provide useful comparisons of between species from particular habitats.

5.1.3 Factors affecting nutrient uptake

Nutrient uptake may correspond empirically to the hyperbolic function of Michaelis-Menten kinetics (Lobban et al. 1985), but uptake is actually controlled by a number of factors including biological characteristics such as morphology and age and physico-chemical characteristics such as light, temperature, nutrient availability and water motion. To overcome the potential for nutrient limitation in the short term ephemeral macroalgae with thin thalli and simple morphology exhibit high uptake rates as these depend on the amount of surface area exposed relative to volume or biomass (Wallentinus 1984a; Hurd and Dring 1990; Hein et al. 1995). Fast nutrient uptake counters losses of N rich tissue by actions such as grazing and wave action (Rosenberg and Ramus 1982b). These morphological and physiological attributes have been summarised in a functional form model (Littler and Littler 1980) which distinguishes between opportunistic and persistent forms of algae, representing the extremes along an ecological continuum. Uptake rates have also generally been shown to be higher in apical fronds, metabolically active portions and young whole plants than in slower growing older portions of plants (Topinka 1978; Harrison et al. 1986; Paling 1991).

Light can indirectly affect N uptake through its influence on photosynthesis which regulates ATP turnover via photophosphorylation (Falkowski 1983). The regulation and turnover of ATP has important ramifications for N uptake because ATP provides energy required for active transport in the GS-GOGAT pathways of ammonium assimilation. Light also promotes growth via photosynthesis and carbon skeleton formation which is necessary for protein synthesis and the incorporation of nutrient

ions into larger molecules, all of which require N. Temperature affects active nutrient uptake mechanisms moreso than passive mechanisms by altering enzyme reactivity and increasing ATP production through photosynthesis (Lobban et al. 1985). In general, optimum nutrient uptake rates occur at intermediate temperatures, being lower at higher temperatures and lowest at low temperatures. Increases in water motion can also enhance photosynthesis and nutrient uptake as transport boundary layers surrounding plants become less obstructive to nutrient diffusion (Parker 1981; Paling 1991; Koch 1993; Hurd et al. 1996).

The inorganic form of N (NH_4^+, NO_3^-, NO_2^{2-} or urea) and the concentration that is assimilated also affect the synthesis and concentration of the different amino acids in macroalgae (Dawes et al. 1974; Bird et al. 1982; Horrocks et al. 1995; Jones et al. 1996). Jones et al. (1996) reported that ammonium was primarily converted to the amino acid, citrulline, by *Gracilaria edulis* (Rhodophyta). The concentrations of amino acids phenylanaline and serine also increased with additions of ammonium. These results suggested that ammonium assimilation was facilitated by the alternative carbamoyl phosphate pathway which utilises citrulline and arginine. By contrast, nitrate enrichment produced increases in glutamic acid which is an important component of the GDH and GS-GOGAT pathways (Lobban et al. 1985). For *Gracilaria verrucosa* (Horrocks et al. 1995) and *Gracilaria edulis* (Jones et al. 1996) N limitation reduced the transfer of citrulline to arginine by inhibiting N metabolism and causing citrulline to function as a storage product in the chloroplast. This study conclusively showed that amino acids are sensitive indicators to different forms of bioavailable N. Hence, the experimental enrichment of macroalgae with different forms of DIN and the measurement of amino acid concentration may provide an indication of the source of N (e.g. point source N, remineralised N) in these algae and of the pathways that are used in N assimilation.

Nutrient uptake may also be dependent on the concentration of nutrients in algal tissue. Ammonium uptake has been found to decrease with increases in tissue N content (Rosenberg et al. 1984), and sustained or long term uptake (i.e. from days to

weeks) of ammonium and nitrate is generally higher in N starved or depleted plants than in N enriched plants (D'Elia and DeBoer 1978; Fujita 1985; Duke et al. 1986; Cohen and Neori 1991). The concentrations of nutrients in the water column can also affect uptake rates with increased rates commonly reported during high nutrient concentrations (DeBoer 1981; Hanisak 1983; Paling 1991; Lavery and McComb 1991a). Nutrient uptake rates, however, may decrease once tissue nutrients are saturated by nutrient enriched waters (Kautsky 1982).

5.1.4 Measurement of nutrient uptake

The measurement of ammonium uptake may be separated into a number of processes: diffusion across the boundary layer adjacent to the plant surface; transport from the external medium across the cell membrane; assimilation into dissolved inorganic nutrient pools; assimilation into insoluble organic macromolecules such as amino acids; and protein synthesis. This uptake process has been described in terms of three distinct phases in macroalgae (Lobban et al. 1985; Pederson 1994; Rees et al. 1998). First there is a phase of surge uptake that lasts for minutes to hours and exceeds the growth requirements for N (Fujita 1985; Pederson 1994). The length and magnitude of initial rates of ammonium uptake are concentration dependent and are regulated by the plant surface and boundary layers. In *Ulva rigida* (Fujita et al. 1988) and *Chaetomorpha linum* (McGlathery et al. 1996) initial rates of ammonium uptake often increase up to a critical intracellular pool of N

The second 'assimilation' phase is a lower but fairly constant rate that is internally controlled by the incorporation of intracellular N pools into amino acids, proteins and other macromolecules (Fujita et al. 1988). Such controls may be relaxed as the rate of N is incorporated in response to growth demands (Dortch 1982; Fujita et al. 1988). Both surge and assimilation uptake have been shown to change with the total N content of algae (D'Elia and DeBoer 1978; Harrison et al. 1989), but more recent studies suggest that internal controls primarily regulate assimilation uptake (Pederson 1994; McGlathery et al. 1996). The third and final phase of uptake is externally

controlled by the depletion of ammonium in the medium and represents concentration dependent transport of nutrients across the boundary layer.

Pederson and Borum (1997) demonstrated that the magnitude of surge uptake differed significantly between fast and slow growing macroalgae and that slow growing taxa are better suited to grow under low N availability than fast growing algae. The reduced reliance on external N by slow growing macroalgae means that V_{max} is correspondingly low and K_m is high, but these parameters depend on the storage and growth capabilities of particular algae. By combining the kinetics of growth and N uptake the abilities of different species to sustain maximum growth as a function of external N can be determined (Pederson and Borum 1997). The relative measures of V_{max} and N required to sustain non-limited growth provides an index to evaluate the capacity for taking up N in excess of immediate growth demands.

In order to relate the significance of nutrient uptake kinetics to growth it is important to define the relationship between steady state nutrient assimilation or uptake and growth. In phytoplankton, Turpin (1988) concluded that when a limiting nutrient complement per cell (cell quota) is constant the relative kinetics of nutrient assimilation are equivalent to those of growth. When nutrients such as N and P are limiting the cell quota can adapt or exhibit flexibility and in these cases the kinetic growth constant (K_μ) is lower relative to the kinetic uptake constant (K_m). Therefore the ratio of K_μ to K_m can be used as indicator of sensitivity to nutrient stress. This may also be applied to macroalgae, and for a given species the lower the K_μ to K_m ratio the higher is its capacity to grow under low external N or adapt to N limitation (Pederson and Borum 1997).

Most studies on nutrient uptake in macroalgae use batch or closed system techniques (Harlin and Wheeler 1985; Lobban et al. 1985; Paling 1991). These techniques involve incubating plant material in a sealed container (*in situ* or in the laboratory) with a known concentration of nutrient, and sampling after a specified time. Utilising a closed system may result in inhibition of photosynthesis due to decreases in

dissolved inorganic carbon and increases in oxygen concentration over the duration of the experiment. One way to minimise these effects is to reduce the amounts of algal material per unit volume but retain a sufficient quantity to measure uptake. Wallentinus (1984a) used plant material ranging from 0.02 to 0.5 g dry wt L^{-1}. To avoid oxygen and inorganic carbon fluctuations incubation times were minimised to less than 4 hours (D'Elia and DeBoer 1978; Paling 1991; Lavery and McComb 1991b; Wallentinus 1984a). Studies of nutrient uptake in closed systems must also ensure that adequate mixing occurs so that diffusion across the unstirred boundary layer is not inhibited, and light limitation by self shading is reduced.

For closed systems N uptake in macroalgae is often measured as the depletion of inorganic N from the medium according to one of two techniques. The multiple flask technique measures nutrient uptake over time of separately incubated algal specimens at different substrate concentrations. The multiple flask method is best used when examining surge uptake (Pederson 1994). Problems associated with the use of this technique to measure the second and third phases of uptake are associated with falsely including the surge component of uptake in calculations, leading to overestimation of uptake. Underestimation of the second and third phases of uptake may occur at low substrate concentrations that may be depleted totally over the time period.

The perturbation technique, whereby depletion of N is continuously recorded over short time intervals using the same tissue at different substrate concentrations, has been recommended as the preferred technique to examine assimilation and externally controlled uptake (Pederson 1994). However, multiple flask methods may be used to examine assimilation and externally controlled uptake provided that sufficient algal to volume ratios are used so that the medium is not depleted in nutrients over the course of the experiment. Surge uptake is then derived by subtraction from subsequent uptake rates. This would exclude problems associated with surge uptake continuing directly into externally controlled uptake, and with the potential to mistake high uptake at high concentration (i.e. surge uptake) for bi-phasic uptake (Pederson 1994).

5.1.5 Aims

The aim of this study was to determine the kinetics of surge and assimilation ammonium uptake using the multiple flask technique and compare Michaelis-Menten kinetics of four abundant species of macroalgae from nutrient enriched waters of Port Phillip Bay (PPB). The kinetics of N uptake were also evaluated with respect to N dependent growth kinetics determined in Chapter 4. The study will also evaluate species specific sensitivities to nutrient stress. The species studied were *Hincksia sordida*, *Ulva* sp., *Polysiphonia decipiens* and *Undaria pinnatifida*. A comparison of ammonium uptake between immature and mature stages of *Undaria pinnatifida* was also made. The study focused on the uptake of ammonium, as this is the primary nutrient limiting macroalgal growth and the most concentrated form of inorganic N in PPB.

5.2 Methods

5.2.1 Sample collection and preparation

Whole thalli were collected from a single site at 3 m depth approximately 500 m from shore in waters offshore of Werribee, Port Phillip Bay (38°4.05'S, 144°31.4'E.). Plants were collected by SCUBA, kept at ambient temperature and transported to the laboratory. Healthy portions of adult plants were shaken in 0.2 μm filtered seawater to remove any grit or sand, cleaned of any epiphytic growth and preconditioned with 1% iodine (Betadine, w/v 0.75% iodine, Faulding Health Care) for 5 min to inhibit bacterial contamination. Pieces or discs of algae (0.1 g fresh wt.) were cut from healthy mature thalli from the central regions of plant tissue and cleaned of any epiphytic material. For *Undaria pinnatifida* discs from blades of both mature plants (> 35 cm in length) with reproductive sporophylls and immature plants (< 10 cm in length) without reproductive sporophylls were used. For the three native taxa, experiments were conducted during June to August 1996 when ambient ammonium availability was high. For *Undaria*, experiments were conducted in October 1996.

To deplete internal pools of N pieces of thalli (~0.1 g fresh weight) were cut from the middle of healthy thalli on the day of collection and kept overnight in 20 L aquaria containing aerated natural seawater at 15°C. To mimic natural light exposure tissue pieces were kept under a 12:12 light:dark cycle (i.e. overnight in the dark and during the day under saturating light of 150 μmol m^{-2} s^{-1}; 36 W 'cool white' fluorescent tubes).

5.2.2 Ammonium nitrogen uptake experiments

Experiments were conducted in 1 L Ehrlenmeyer conical flasks aerated with compressed oil-free air to reduce boundary layer effects. Dye studies indicated that total mixing occurred within 5 to 10 s. Based on preliminary experiments in which the ammonium uptake of different ratios of plant weight to seawater volume were tested (data not shown), an initial weight to volume ratio of approximately 0.1 g L^{-1}

(fresh weight) was chosen for each species. This ensured that uptake could be recorded for the duration of the 6 h experiment and that self shading was reduced (Littler 1979).

5.2.3 Experimental design

Filtered seawater (0.2 μm) (1 L) was placed in 1 L glass flasks and a known amount of ammonium and phosphate were added to each flask. There were four replicates (except for *Undaria pinnatifida*; 3 replicates) and a control (no seaweed added) for each of four treatment concentrations of 50, 100, 200 and 400 μg NH_4-N L^{-1}, with 500 μg PO_4-P L^{-1} in each. Before the seaweeds were added, the flasks were aerated for 5 min at 15°C under a photon flux density of 150-200 μmol m^{-2} s^{-1} and a water sample was taken from each flask to determine initial ammonium concentrations. The plant material was then placed in the flasks, and the rate of ammonium uptake was determined for each flask from the depletion in ammonium at 15, 30, 60, 90, 120, 150, 180, 240 and 360 min. The ammonium uptake rate in the control flasks was minimal (range: 4.0-8.0 μg NH_4-N L^{-1}) and was subtracted from the uptake rates for each treatment. Fresh weights of all plant material were determined after blotting with tissues, and dry weights were determined after drying to constant weight at 70°C for 24 h.

5.2.4 Nutrient analysis

Water samples were collected from each flask using prewashed (deionized water) syringes and siphon tube (4 mm diam.) that had been flushed with 50 ml of the water sample. Water samples were filtered (0.45 μm GF/C Whatman) (10 ml) and then placed into sterile 15 ml plastic centrifuge tubes which were used for nutrient analysis. Nutrient analyses of water and tissue samples were carried out as described in section 2.2.4.

5.2.5 Calculation of uptake rates and kinetic constants

Surge uptake rates (V^s) were calculated from the linear portion of the ammonium depletion curves (Fig. 5.1). For *Hincksia sordida* and *Ulva* sp. V^s was calculated from

uptake between 0 and 15 min, whilst uptake between 0 and 60 min was used to calculate V^s for *Polysiphonia decipiens* and *Undaria pinnatifida*. Assimilation uptake (V^{ass}) was calculated between 90 and 360 min after initial exposure. Both uptake rates were calculated according to the equation of Hurd and Dring (1990):

$$\text{Uptake rate } (\mu g\ NH_4\text{-N g dry wt.}^{-1}\ h^{-1}) = (I - F \times L) / (DW \times T) \qquad \text{(Eq. 5.1)}$$

where I = initial concentration ($\mu g\ NH_4$-N L^{-1}), F = final concentration ($\mu g\ NH_4$-N L^{-1}), L = volume before sample removal, DW = dry weight (g) and T = length of experiment (h). Uptake rates (V) were plotted against the mean substrate concentration (S) for each time interval and the Michaelis-Menten function (Mercer and Goodwin 1972) was fitted to uptake data that showed evidence of saturation kinetics using non-linear, least squares regression (SYSTAT):

$$V = (V_{max} \times S)/(K_m + S) \qquad \text{(Eq. 5.2)}$$

where V_{max} is the maximum uptake rate and K_m is the half-saturation constant for uptake. Estimates of uptake at the concentrations used were made by fitting the estimated V_{max} and K_m constants to the Michaelis-Menten equation.

The ratio between V_{max} and the amount of N required to sustain maximum growth (N_{req}) (section 4.3.3) was calculated for each species. The potential gain of N of each algae during surge uptake was calculated by subtracting the rate of N that was accumulated during N assimilation from the rate during surge uptake:

$$\text{N gain} = (\text{mean } V^s - \text{mean } V^{ass}) \qquad \text{(Eq. 5.3)}$$

The period of growth (h) that could be sustained by surge uptake at low (50 $\mu g\ NH_4$-N L^{-1}) and high concentrations (400 $\mu g\ NH_4$-N L^{-1}) of ammonium was calculated according to the following equation of Pederson and Borum (1997):

$$\text{N gain} / N_{req}\ h^{-1} \qquad \text{(Eq. 5.4)}$$

where N gain equals the net gain from surge uptake (mean V^s - mean V^{ass}) and N_{req} $h^{-1} = N_{req}$ $d^{-1}/24$.

The relationship between growth rate (μ) and the concentration of ammonium (S) in the water can be estimated for algae by fitting growth and substrate concentration data (Figs. 4.2-4.5) to the Monod type equation (Pederson and Borum 1997):

$$\mu = (\mu^*_{max} \times S^*)/(K_\mu + S^*) \qquad \text{(Eq. 5.5)}$$

where μ^*_{max} is the maximum growth rate under steady-state conditions, and K_μ the half-saturation constant for growth and S^* is the steady-state substrate concentration. The constants μ^*_{max} and K_μ, however, were derived according to the equations of Turpin (1988) assuming no effects of stored N in the algae:

$$\mu^*_{max} = (\mu_{max} \times V_{max})/ [(\mu_{max} \times N_q) + V_{max}] \qquad \text{(Eq. 5.6)}$$

$$K_{\mu\cdot} = (K_m \times \mu_{max} \times N_q) / [(\mu_{max} \times N_q) + V_{max}] \qquad \text{(Eq. 5.7)}$$

where V_{max} and K_m are defined as described in equation 5.2 and μ_{max} and N_q are derived from growth versus tissue N plots (Figs. 4.2-4.5) calculated in section 4.3.1.

5.2.6 Statistical analyses

Uptake data were tested for assumptions of normality by examining heterogeneity of variance (Cochrans test) and skewness of data (residuals and outliers). Non-normal data were subject to the log transformation, $\log_e(x)$. ANOVA was employed to examine for differences in uptake between species. The significance level used was $p < 0.05$. Tukeys test was used for post hoc analyses of multiple comparisons among treatment means from significant ANOVA tests. The computer software SYSTAT (vs. 5.03, Systat Inc., USA) was used for all analyses.

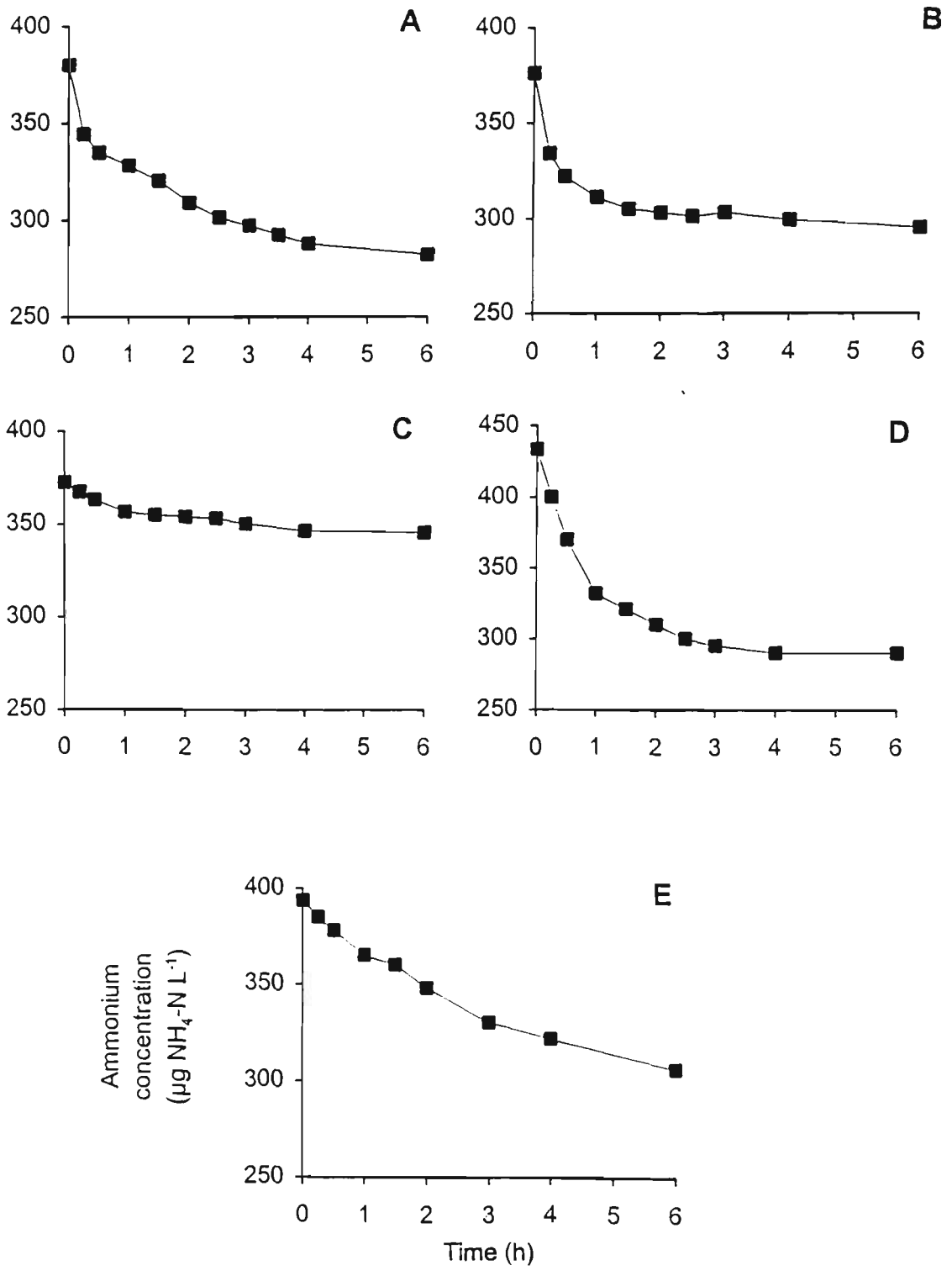

Fig. 5.1 Representative ammonium-N depletion curves: concentration (Y axis) versus time (X axis) for (A) *Hincksia sordida*, (B) *Ulva* sp., (C) *Polysiphonia* decipiens, (D) mature *Undaria pinnatifida* and (E) immature *Undaria pinnatifida* at nominal concentrations of 400 µg NH_4-N L^{-1}.

5.3 Results

5.3.1 Ammonium nitrogen uptake rates

Control solutions (without algae) showed minimal reductions in ammonium concentration when monitored over the 6 h period. Hence, the depletions in ammonium concentrations of the treatments could be attributed to biological uptake. The ammonium concentration in the medium decreased throughout the experiments for all species but was never completely exhausted. Representative ammonium uptake curves for each species are shown in Fig. 5.1. The ammonium depletion was found to be linear over the first 0-15 min for *Hincksia sordida* and *Ulva* sp. and between 0 and 60 min for *Polysiphonia decipiens* and *Undaria pinnatifida* ($r^2 > 0.90$ and $p < 0.05$). Over the duration of the experiments (360 min) the rate of ammonium depletion decreased and was best represented by a hyperbolic curve.

There were significant interspecies differences in surge uptake ($df = 4$, $F = 22.06$, $p < 0.05$) and assimilation uptake ($df = 4$, $F = 17.16$, $p < 0.05$) across the range of ammonium concentrations examined. Multiple range tests showed that surge ammonium uptake rates for *Hincksia sordida* were significantly higher than all other species and surge uptake of mature *Undaria pinnatifida* was significantly higher than immature *Undaria pinnatifida*. Assimilation uptake rates of all species were significantly higher than *Polysiphonia decipiens*. Assimilation uptake rates of immature *Undaria pinnatifida* were also significantly higher than *Hincksia sordida* and mature *Undaria pinnatifida*.

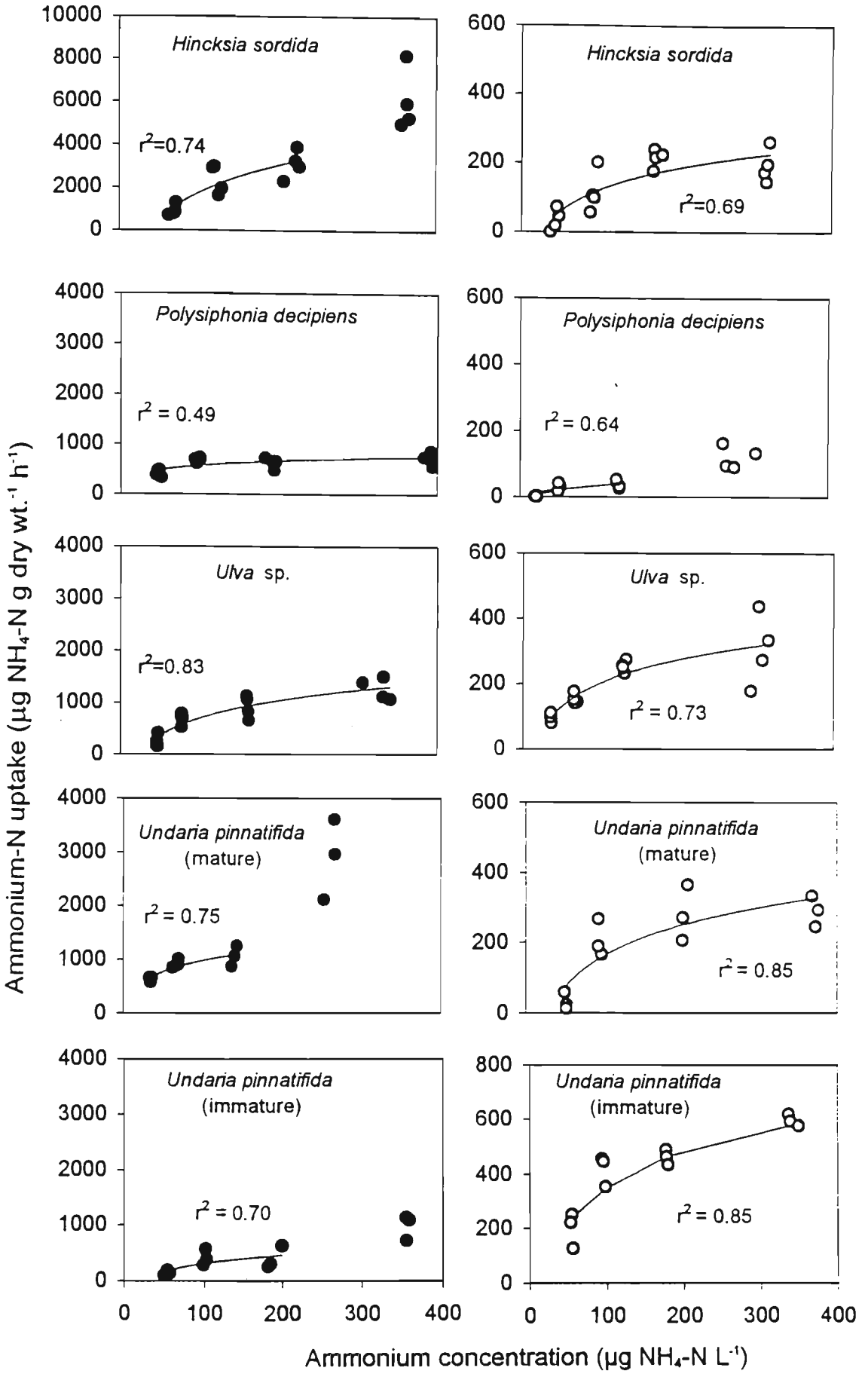

Fig. 5.2 Uptake rates of ammonium as a function of substrate concentration. Surge uptake (•) was measured over the initial 15 min to 60 min after exposure to ammonium. Assimilation rates (o) were measured later than 60 min after the experiments were initiated.

5.3.2 Uptake kinetics

The uptake of ammonium was highest when algae were first exposed to ammonium. For *Polysiphonia decipiens* and *Ulva* sp. this transient or surge phase of uptake plateaued as substrate concentration increased from 50-400 μg NH_4-N L^{-1}, and conformed to Michaelis-Menten saturation kinetics (Fig. 5.2). For *Hincksia sordida* and *Undaria pinnatifida* (immature and mature) uptake conformed to Michaelis-Menten saturation kinetics ($r^2 > 0.90$) up to 200 μg NH_4-N L^{-1}. All species exhibited saturation kinetics for the assimilation phase of uptake over the range of concentrations used, except for *Polysiphonia decipiens* which conformed to Michaelis-Menten saturation kinetics up to 200 μg NH_4-N L^{-1} (Fig. 5.2).

Kinetic constants exhibited large species specific variations for both surge and assimilation uptake (Table 5.1). The maximum rate of surge uptake (V^s_{max}) varied 14-fold, from 11231 μg NH_4-N g dry wt.$^{-1}$ h^{-1} in *Hincksia sordida* to 804 μg NH_4-N g dry wt.$^{-1}$ h^{-1} in *Polysiphonia decipiens*. The half saturation constant (K_m) varied 15-fold from 556 μg NH_4-N L^{-1} in *Hincksia sordida* to 36 μg NH_4-N L^{-1} in *Polysiphonia decipiens*. V_{max}:K_m ratios were highest in mature *Undaria pinnatifida*, *Polysiphonia decipiens* and *Hincksia sordida*, and lower in *Ulva* sp. and immature *Undaria pinnatifida*. The maximum assimilation rate of ammonium (V^{ass}_{max}) also increased with increasing substrate concentration but rates were 2- to 33-fold lower than surge uptake rates. The maximum rate of assimilation uptake (V^{ass}_{max}) varied 12-fold, from 794 μg NH_4-N g dry wt.$^{-1}$ h^{-1} in immature *Undaria pinnatifida* to 64 μg NH_4-N g dry wt.$^{-1}$ h^{-1} in *Polysiphonia decipiens*.

The half saturation constants (K_m) for assimilation uptake were lower than those for surge uptake in *Hincksia sordida*, *Ulva* sp. and immature *Undaria pinnatifida* but the opposite was true for *Polysiphonia decipiens* and mature *Undaria pinnatifida* (Table 5.1). K^{ass}_m values ranged from 174 μg NH_4-N L^{-1} in *Hincksia sordida* to 80 μg NH_4-N L^{-1} in *Polysiphonia decipiens*. The lower V^{ass}_{max} for assimilation uptake compared to surge uptake were reflected by lower V_{max}:K_m ratios for assimilation

uptake than surge uptake. In contrast to surge uptake V_{max}:K_m ratios for assimilation uptake were highest in immature *Undaria pinnatifida*.

Table 5.1 Kinetic parameters V_{max} (μg NH_4-N g dry wt.$^{-1}$ h^{-1}) and K_m (μg NH_4-N L^{-1}) of surge and assimilation ammonium uptake for 4 species of macroalgae. Parameters were derived from non-linear regressions of raw data using the Michaelis-Menten function and coefficients of determination (r^2) of this function are shown.

Species	Surge (V^s)				Assimilation (V^{ass})			
	V^s_{max}	K_m	V^s_{max}/K_m	r^2	V^{ass}_{max}	K_m	V^{ass}_{max}/K_m	r^2
Hincksia sordida	11231	556	20.2	0.94	340	74	1.9	0.91
Ulva sp.	2049	202	10.1	0.96	432	114.5	3.8	0.95
Polysiphonia decipiens	804	36	22.3	0.97	64	80	0.8	0.90
Undaria pinnatifida (mature)	1356	56	24.2	0.99	459	173	2.7	0.92
Undaria pinnatifida (immature)	1989	497	4.0	0.90	794	128	6.2	0.97

The calculated uptake rates (V^s), derived by applying V_{max} and K_m to the Michaelis-Menten equation at concentrations of 100 to 400 μg NH_4-N L^{-1}, were generally highest to lowest in the following order; *Hincksia sordida* > *Ulva* sp. ~ *Undaria pinnatifida* (mature) > *Polysiphonia decipiens* > *Undaria pinnatifida* (immature) (Table 5.2). The species specific capacity for surge uptake at 50 μg NH_4-N L^{-1} followed a similar trend except that *Polysiphonia decipiens* showed higher uptake than *Ulva* sp. The hyperbolic equations that describe surge uptake by *Hincksia sordida, Ulva* sp. and *Undaria pinnatifida* yielded the greatest coefficients of determination ($r^2 > 0.70$) than those found in *Polysiphonia decipiens* ($r^2 = 0.49$) (Table 5.2).

Table 5.2 Surge uptake rates (V^s) of NH_4^+ (μg N g dry $wt.^{-1}$ h^{-1}) at various nutrient concentrations (μg NH_4-N L^{-1}) determined from the kinetic parameters (Table 5.1) applied to the Michaelis-Menten equation. Hyperbolic functions, and their coefficients of determination (r^2), and uptake rate estimates were derived from saturated uptake kinetic parameters (i.e. ≤ 400 μg NH_4-N L^{-1}) (Fig. 5.1). V= nutrient uptake rate, S= substrate concentration.

Species	Surge uptake rates (V^s) at various concentrations (μg NH_4-N L^{-1})				Hyperbolic parameters
	50	100	200	400	
Hincksia sordida	926	1711	2970	4697	V = 1847 ln(S) - 6755 $r^2 = 0.74$
Polysiphonia decipiens	468	592	682	738	V = 133.6 ln(S) + 42.9 $r^2 = 0.49$
Ulva sp.	407	678	1019	1361	V = 490 ln(S) -1562 $r^2 = 0.83$
Undaria pinnatifida (mature)	640	869	1059	1189	V = 303.5 ln(S) - 544 $r^2 = 0.75$
Undaria pinnatifida (immature)	182	333	571	887	V = 212.2 ln(S) - 666.1 $r^2 = 0.70$

At low (50 μg NH_4-N L^{-1}) and high (400 μg NH_4-N L^{-1}) concentrations of N the net amount of N accumulated by surge uptake (N gain) far exceeded that taken up during assimilation (Table 5.3). The period of growth that could be sustained by surge uptake at low and high concentrations of ammonium (N gain / N_{req} h^{-1}) was highest for *Hincksia sordida* (10.8 and 73.2 h) and lowest for *Ulva* sp. (1.2 and 7.6 h) (Table 5.3).

Predicted values of maximum growth (μ^*_{max}), half saturation constants for growth (K_μ) and N gain were highest in *Hincksia sordida.* at 50 and 400 μg NH_4-N L^{-1} and lowest for *Ulva* sp. at 50 μg NH_4-N L^{-1} (Table 5.4). The ratio of V_{max}:N_{req} in *Hincksia sordida* was 8.5 times higher than in *Ulva* sp. and 10.2 fold higher than *Polysiphonia decipiens* (Table 5.4).

Table 5.3 The rate of ammonium taken up by surge and assimilation uptake at low (50 μg NH_4-N L^{-1}) and high (400 μg NH_4-N L^{-1}) ammonium concentrations. Parameters shown are the N gain (V^s-V^{ass}), the N requirement (N_{req}), and the period of growth at maximum rates that can be supported by the ammonium taken up during the surge uptake for 3 different species of macroalgae.

Species and uptake phase	Uptake rates (V) mean ± s.e. N gain (μg NH_4-N g dw h^{-1}) at 50 μg NH_4-N L^{-1}	400 μg NH_4-N L^{-1}	N_{req} d^{-1} (μg NH_4-N)	N_{req} h^{-1}	Growth (h) (N_{gain}/N_{req} h^{-1}) at 50 μg NH_4-N L^{-1}	400 μg NH_4-N L^{-1}
Hincksia sordida						
V^s	900 ± 127	6090 ± 721	1930	80.4	10.8	73.2
V^{ass}	34 ± 16	201 ± 26				
N gain	866	5889				
P. decipiens						
V^s	409 ± 37	741 ± 64	1410	58.8	6.8	10.6
V^{ass}	8 ± 3	119 ± 6				
N gain	401	622				

Table 5.3 cont.

Species	Uptake (V) mean ± s.e. and N gain (μg NH_4-N)		N_{req} d^{-1}	N_{req} h^{-1}	Growth (h)	
			(μg NH_4-N)		(N gain/N_{req} h^{-1})	
and uptake phase	50	400			50	400
	μg NH_4-N L^{-1}				μg NH_4-N L^{-1}	
Ulva sp.						
V^s	242 ± 59	1515 ± 165				
V^{ass}	92 ± 7	213 ± 54	3030	126.3	1.2	10.3
N gain	150	1302				
Undaria pinnatifida (mature)						
V^s	626 ± 29	2883 ± 434	n.a.	n.a.	n.a	n.a.
V^{ass}	31 ± 14	289 ± 25				
N gain	595	2594				
Undaria pinnatifida (immature)						
V^s	140 ± 27	971 ± 132	n.a.	n.a.	n.a.	n.a.
V^{ass}	198 ± 38	596 ± 12				
N gain	0	375				

n.a. = not available

Table 5.4 Growth and uptake kinetics. Predicted values of maximum growth (μ^*_{max}) and half saturation constants for growth (K_μ), under steady state conditions during the assimilation phase of uptake in 3 different species of macroalgae. Ratios of K_μ to half saturation constants for ammonium uptake (K^{ass}_m) and ratios of maximum surge ammonium uptake rates (V^s_{max}) to the N required to sustain maximum growth rates (N_{req} d^{-1}) are also shown.

Species	μ^*_{max} (d^{-1})	K_μ (μg NH_4-N L^{-1})	$K_\mu : K_m$	$V^s_{max} : N_{req}$ (d^{-1})
Hincksia sordida	0.027	122.9	0.706	5.81
P. decipiens	0.003	75.7	0.946	0.57
Ulva sp.	0.016	90.3	0.789	0.68

5.3.3 Tissue N

Tissue C:N ratios (range: 6.17-8.28) and tissue N concentrations (40.4 to 48.5 mg N g dry wt.$^{-1}$) were indicative of tissue N saturation and exhibited little interspecies variation (Table 5.5). *Hincksia sordida* took up about 13% of its total tissue N content at high substrate concentrations and this was 2-5 times higher than the other 3 species.

Table 5.5 Tissue C:N ratios, tissue N and the % of tissue N taken up by surge ammonium uptake for 4 species of macroalgae. Values represent means (s.e.), n = 4.

Species	Molar C:N ratios	Tissue N (mg N g dry wt.$^{-1}$)	% of total tissue N taken up by surge uptake
Hincksia sordida	6.37 (0.66)	46.9 (5.37)	13.0
Polysiphonia. Decipiens	7.09 (0.18)	44.5 (3.15)	1.7
Ulva sp.	8.21 (0.45)	48.5 (1.60)	2.6
Undaria pinnatifida (mature)	6.17 (0.30)	42.3 (0.15)	6.8
Undaria pinnatifida (immature)	8.28 (0.31)	40.4 (0.20)	2.4

5.4 Discussion

5.4.1 Ammonium nitrogen uptake rates

The transiently enhanced rates of ammonium uptake by all 4 algal species, after a period of N depletion, indicates a strong initial diffusive or surge component to uptake. Surge uptake is often characteristic of rapid diffusion into algal cells that are depleted in N (McGlathery et al. 1996). A comparison of surge uptake rates found in this study with rates determined for other macroalgae is presented in Table 5.5. At equivalent concentrations ammonium uptake rates of *Hincksia sordida* and *Undaria pinnatifida* were higher than those determined for other fast growing Phaeophyta, such as *Pilayella littoralis*, *Ectocarpus siliculosus* and *Scytosiphon lomentaria* from colder waters (Wallentinus 1984a). Lower rates of ammonium uptake recorded for these taxa may be due to a decrease in ATP production via the reduction of enzyme reactivity associated with low temperature acclimation (Lobban et al. 1985). The ammonium uptake rates of *Undaria pinnatifida* are comparable to rates determined for 8 week old thalli of the annual Phaeophyte *Sargassum baccularia* (Schaffelke and Klumpp 1997).

The ammonium uptake rates and kinetic parameters (V_{max} and K_m) exhibited by *Polysiphonia decipiens* and *Ulva* sp. were within the ranges of laboratory determined rates recorded for other species of Rhodophyta (D'Elia and DeBoer 1978; Wallentinus 1984a; Thomas et al. 1987) and Chlorophyta (Gordon et al. 1981; Rosenberg and Ramus 1984; Fujita 1985; Lavery and McComb 1991b; Peckol et al. 1994; Pederson 1994, Pederson and Borum 1997). The ammonium uptake rates of *Undaria pinnatifida* were considerably higher than those recorded for other structurally complex Phaeophyta at similar temperatures, such as *Ecklonia radiata*, (Paling 1991), *Fucus distichus* (Rosenberg et al. 1984), *Laminaria groenlandica* (Harrison et al. 1986) and *Sargassum* sp. (Paling 1991). This possibly reflects the relatively high photosynthetic rates reported for *Undaria pinnatifida* (Campbell et al. 1998). Comparisons between species have to be viewed with caution because the methods

Table 5.6 Uptake rates of ammonium (NH_4-N) expressed as μg N g dry wt.$^{-1}$ h^{-1} by temperate marine macroalgae at some ecologically relevant ambient nutrient concentrations (μg NH_4-N L^{-1}). See below for further details, calculation of uptake rates and explanation of symbols.

Species	Morphology#	V_{max}	K_m	V_{max}/K_m	μg NH_4-N L^{-1}				Temp. °C	Reference and habitat
					50	100	200	400		
Chlorophyta										
Ulva curvata	Ia	2576	280	9.2	390	678	1073	1515	20	Rosenberg and Ramus (1984) North Carolina, U.S.A. (estuary)
*Ulva lactuca**	Ia				360	720	1440	2880	20	Fujita (1985) Massachusetts, U.S.A. (estuary)
Ulva lactuca	Ia	2954	280	10.6	448	777	1231	1738	15	Pederson (1994) Roskilde Fjord, Denmark (estuary)
Ulva sp.	**Ia**	**2049**	**202**	**10.1**	**407**	**678**	**1019**	**1361**	**15**	**This study (1998) Victoria, Australia (shallow bay)**
*Ulva rigida**	Ia				92	178	352	698	25	Lavery and McComb (1991b) Peel Harvey Inlet, Australia (estuary)
Chaetomopha linum	IIa	1848	182	10.2	398	655	968	1270	15	Pederson and Borum (1997) Roskilde Fjord, Denmark (estuary)
Cladophora aff. *albida*	IIa	1820	289	6.3	268	468	744	1057	23	Gordon et al. (1981) Peel Harvey Inlet, Australia (estuary)
Cladophora sericea	IIa	1848	182	9.8	398	655	968	1270	15	Pederson and Borum (1997) Roskilde Fjord, Denmark (estuary)
Cladophora vagabunda	IIa	595	175	3.4	132	216	317	414	26	Peckol et al. (1994) Massachusetts, U.S.A. (estuary)

Table 5.6 cont.

Species	Morphology#	V_{max}	K_m	Vmax/ K_m	µg NH_4-N L^{-1} 50	100	200	400	Temp. °C	Reference and habitat
Phaeophyta										
Scytosiphon lomentaria	Ib	967	55	17.6	462	625	760	851	6	Wallentinus (1984a) Baltic Sea, Sweden
***Undaria pinnatifida* (mature)**	**Ib**	**1356**	**56**	**24.2**	**640**	**869**	**1059**	**1189**	**15**	**This study (1998) Australia (shallow bay)**
***Undaria pinnatifida* (immature)**	**Ib**	**1989**	**497**	**4.0**	**182**	**333**	**571**	**887**	**15**	**This study (1998) Victoria, Australia (shallow bay)**
Ectocarpus siliculosus	Ia	557	48.4	11.5	283	375	448	497	9	Wallentinus (1984a) Baltic Sea, Sweden
Hincksia sordida	**IIa**	**11231**	**556**	**20.2**	**926**	**1711**	**2970**	**4697***	**15**	**This study (1998) Victoria, Australia (shallow bay)**
Pilayella littoralis	IIa	454	31.5	14.4	278	345	392	421	8	Wallentinus (1984a) Baltic Sea, Sweden
Pilayella littoralis	IIa	550	68.3	8.1	232	327	410	470	1	Wallentinus (1984a) Baltic Sea, Sweden

Table 5.6 cont.

Species	Morphology[#]	V_{max}	K_m	V_{max}/K_m	µg NH_4-N L^{-1} 50	100	200	400	Temp. °C	Reference and habitat
Phaeophyta										
Chorda filum	III	333	48.2	6.91	170	225	268	297	9	Wallentinus (1984a) Baltic Sea, Sweden
Chodaria flagelliformis	III	2031	148	13.7	512	818	1166	1481	11	Probyn and Chapman (1982) Nova Scotia, Canada (open coast)
Chodaria flagelliformis	III	325	26	12.5	214	258	288	305	16	Rosenberg et al. (1984) Nova Scotia, Canada (open coast)
Sargassum sp.	III	139	231	0.6	25	42	64	88	20	Paling (1991) Perth, Australia (open coast)
Sargassum baccularia	III	1301	174	7.5	1042	1157	1225	1261	28	Schaffelke and Klumpp (1997) Queensland, Australia (open coast)
Ecklonia radiata	IV	228	733	0.31	15	27	49	81	20	Paling (1991) Perth, Australia (open coast)
Fucus distichus	IV	195	51	3.8	97	129	155	173	15	Rosenberg et al. 1984 Nova Scotia, Canada (open coast)
Fucus vesiculosus	IV	574	294	2.0	83	146	232	331	15	Pederson and Borum (1997) Roskilde Fjord, Denmark (estuary)
*Laminaria groenlandica**	IV				16.5	3366	66	132	13	Harrison et al. (1986) British Columbia, Canada (open coast)
Macrocystis pyrifera	IV	333	74	4.5	134	191	243	281	16	Haines and Wheeler (1978) St. Croix, Virgin Islands (open coast)

Table 5.6 cont.

Species	Morphology#	V_{max}	K_m	V_{max}/K_m	μg NH_4-N L^{-1} 50	100	200	400	Temp. °C	Reference and habitat
Rhodophyta										
Ceramium tenuicorne	IIa	2690	126	21.3	764	1190	1650	2046	15	Wallentinus (1984a) Baltic Sea, Sweden
Ceramium rubrum	IIa	3794	406	9.4	416	750	1252	1883	15	Pederson and Borum (1997) Roskilde ford, Denmark (estuary)
Polysiphonia decipiens	**IIb**	**804**	**36**	**22.3**	**468**	**592**	**682**	**738**	**15**	**This study (1998) Victoria, Australia (shallow bay)**
Gracilaria pacifica	III	422	141	3.0	111	175	248	312	16	Thomas et al. (1987) Britsh Columbia, Canada (open coast)
Gracilaria tikvahiae	III	334	22	15.2	230	272	300	316	20	D'Elia and De Boer (1978) U.S.A. (open coast)
Neogardhiella baileyi	III	420	63	6.7	186	258	319	363	20	D'Elia and De Boer (1978) U.S.A. (open coast)

Rates were determined from the kinetic parameters given in these studies, (V_{max} and K_m). * linear relationship so no calculated V_{max} and K_m. In these cases uptake rates were determined directly from linear equations at the concentrations shown.

after Littler and Littler (1980), 1a Thin tubular and sheet-like macroalgae; 1b Thin tubular and sheet-like macroalgae (several cell layers); II Filamentous, delicately branched (IIa Uniseriate, IIb Multiseriate); III Coarsely branched macroalgae; IV Macroalgae with thick leathery blades.

and conditions under which uptake is measured may differ between studies (Harrison et al. 1989).

The uptake rates reported in the present study are also higher than those reported for mats of filamentous algae such as *Cladophora vagabunda* (Peckol et al. 1994) and *Chaetomorpha linum* (McGlathery et al. 1997). In the latter study the spatial heterogeneity of the algal mat contributed to 10-fold variable rates and were dependent on variable productivity rates and nutrient availability. Assuming that such spatial heterogeneity occurs within mats of *Hincksia sordida* in the field, uptake rates derived for *Hincksia sordida* and other algae in the laboratory, are likely to overestimate those that would occur in the field. In extrapolating rates described in this study to those in the field account of the variability in nutrient flux (or loading) (i.e. where low concentrations may be sufficient for algal growth when the flow rate is high) must be made. In the absence of this type of data, or any information on uptake rates from continuous flow experiments, the uptake rates presented in this study are useful to examine interspecies differences in N uptake that may be used to explain growth strategies in the field.

5.4.2 Ammonium nitrogen uptake kinetics: morphology and life history

Differences in surface to volume ratios arising from cell thickness may contribute to the variation in ammonium uptake. Higher maximum ammonium uptake rates (V_{max}) were exhibited by the single celled thick (uniseriate) *Hincksia sordida* compared with the multi-celled sheet-like *Ulva* sp. and *Undaria pinnatifida*, and the multiseriate filamentous *Polysiphonia decipiens*. This may be a reasonable explanation for the higher uptake rates of filamentous *Hincksia sordida* as it would presumably have a higher surface to volume ratio than all the other species examined. Thomas and Harrison (1987) found 2-fold higher ammonium uptake rates for the thin, tubular *Enteromorpha intestinales* than for the coarsely branched *Gracilaria pacifica*. Lavery and McComb (1991b) reported a 1.5 to 6 times higher surface to dry weight ratio for

the uniseriate *Chaetomorpha linum* compared with *Ulva rigida*, and found correspondingly higher N uptake rates in *Chaetomorpha linum*.

The high ammonium uptake of *Undaria pinnatifida* compared with other large Phaeophyta (e.g. *Macrocystis pyrifera*, *Laminaria* spp., *Ecklonia radiata*: Topinka 1978; Harrison et al. 1986; Paling 1991) may also be a function of high surface to volume ratios. Blade tissue of *Undaria pinnatifida* is thin compared with these other species which possess multi-celled and relatively thick blades with a lower surface to volume ratio. High uptake of *Undaria pinnatifida* may be influenced by high growth rates as N is rapidly synthesised into amino acids. Such mechanisms may contribute to reduced feedback controls and result in higher ammonium uptake rates for fast growing compared with slower growing taxa (Ramus and Venable 1987; Duke et al. 1989; McGlathery et al. 1997; Pederson and Borum 1997). This is also consistent with the findings of Rosenberg et al. (1984) who showed that V_{max} in the annual *Chordaria flagelliformis* was 40% higher than in the perennial *Fucus distichus*. Paling (1991) found a similar trend between the annual *Sargassum* sp. and the perennial *Ecklonia radiata*.

The differences in uptake between immature and mature stages of *Undaria* indicate that the ecological relevance of surge uptake may be dependent on the life history stage of a particular alga. Uptake rates and V_{max} were slightly higher in mature plants of *Undaria pinnatifida* than immature plants but mature plants exhibited both saturated and non-saturated uptake kinetics. Adult plants therefore appear to compete successfully for the nutrient resource with younger plants. This is also consistent with the 10-fold higher V_{max}:K_m ratio for mature compared with immature *Undaria pinnatifida*.

The trends are consistent with methylamine (an ammonium analogue) uptake in *Macrocystis pyrifera* where mature blades had approximately double the rate of uptake than apical blades (Wheeler 1979). Wallentinus (1984a) reported that in *Fucus vesiculosus*, actively metabolising tissue had ammonium uptake rates 20% of that in

older portions of tissue. In other studies on the Phaeophyta nutrient uptake has generally decreased with plant age and/or tissue age (Topinka 1978; Harrison et al. 1986). In *Fucus spiralis* ammonium uptake per unit area or weight in older fronds and stipes were approximately 45% of those of apical fronds or whole young plants (Topinka 1978). Topinka (1978) suggested that this reflected the lower N demands of slower growing plant material. Harrison et al. (1986) found higher ammonium uptake rates in younger first year plants than slower growing second and third year classes of *Laminaria groenlandica* (Phaeophyta). Most of the plants used in the above studies were perennials. *Undaria pinnatifida* is an annual alga which lives for only 6-8 months of the year. In PPB it exhibits rapid growth rates in the field (Campbell and Burridge 1998) and can develop into mature reproductive plants in about two months. The equivalent or even higher ammonium uptake rates in adult plants compared to younger plants would presumably enable rapid growth and development throughout its life cycle so that optimal environmental conditions can be exploited.

5.4.3 Mechanisms of ammonium nitrogen uptake

Ammonium uptake by *Hincksia sordida* and *Undaria pinnatifida* may not soley be hyperbolic in character as elevated surge uptake at the highest N concentration is suggestive of bi-phasic uptake. That is, ammonium uptake has both a non-linear and linear component which is dependent on concentration. These results are comparable to a number of similar studies. Haines and Wheeler (1978) showed that ammonium uptake in *Macrocystis pyrifera* (Phaeophyta) was saturated up to concentrations of 22 μmol NH_4-N (or 308 μg NH_4-N L^{-1}) but above this concentration uptake increased linearly with concentration. In other Phaeophyta such as *Fucus evanescens* (Rosenberg and Ramus 1984) and *Fucus spiralis* (Topinka 1978) saturated hyperbolic kinetics were evident up to 40μmol NH_4-N (or 560 μg NH_4-N L^{-1}) and Wallentinus (1984a) found that the filamentous Phaeophyte *Pilayella littoralis* exhibited saturated uptake up to 70 μg NH_4-N L^{-1}. Other studies of Phaeophyta have recorded non-saturation or linear uptake kinetics up to 60 μmol NH_4-N L^{-1} (or 840 μg NH_4-N L^{-1}) in *Laminaria groenlandica* (Harrison et al. 1986) and ≤ 700 μg NH_4-N L^{-1} for *Ecklonia radiata* (Paling 1991). Bi-phasic models of uptake, as found for *Hincksia*

sordida, allows the plants to scavenge N at low concentrations and assimilate N at high concentrations, thereby buffering the effects of N fluctuation on a seasonal basis.

The saturated surge ammonium uptake exhibited by *Ulva* sp. and *Polysiphonia decipiens* at concentrations of 400 μg NH_4-N L^{-1} indicates that both algae are limited in their ability to sequester N. For the fast growing *Ulva* sp. limited uptake may be caused by feedback controls as intracellular pools accumulate because of low N storage capacities (see Chapter 4). For *Polysiphonia decipiens* N limitation may be associated with relatively low uptake rates and a subsequent inability to take full advantage of high DIN concentrations. Similar findings have been reported for other Rhodophyta. Haines and Wheeler (1978) studied nitrate and ammonium uptake in *Hypnea musciformis* and reported that saturation kinetics were reached for nitrate uptake at 308 μg NO_3-N L^{-1}. Saturated uptake was approached but not reached up to ammonium concentrations of 238 μg NH_4-N L^{-1}. Dual or bi-phasic systems of ammonium uptake have been recorded for some Rhodophyta including *Gracilaria tikvahiae* and *Neogardhiella baileyi* (D'Elia and DeBoer 1978) and *Gracilaria pacifica* (Thomas et al. 1987).

The substrate concentrations at which uptake rates of ammonium were half-maximal (K_m) for *Hincksia sordida*, *Ulva* sp. and immature *Undaria pinnatifida* were equivalent if not higher than field concentrations during winter (150-200 μg NH_4-N L^{-1}), suggesting that uptake is reasonably efficient at low field concentrations. The lower K_m for *Polysiphonia decipiens* and mature *Undaria pinnatifida* (K_m = 36 and 56 μg NH_4-N L^{-1} respectively) suggests a high uptake efficiency for these taxa at low ammonium concentrations. Differences between V_{max}:K_m ratios for surge and assimilation phases of uptake further highlight species specific divergence with respect to ammonium uptake at low concentrations. The higher surge V_{max}:K_m ratios compared to those for assimilation uptake in *Hincksia sordida*, *Polysiphonia decipiens* and mature *Undaria pinnatifida* infers a propensity to utilise ammonium at low concentrations and suggests that surge uptake is ecologically important for these taxa. For *Hincksia sordida* and mature *Undaria pinnatifida* this may be attributed to

high growth rates that require rapid acquisition and assimilation of N. For *Polysiphonia decipiens* this initial surge in ammonium uptake may be required to offset relatively low rates of N uptake and growth. Its capacity for ammonium uptake at low concentrations may partly be due to its ability to use stored N, causing relaxation of feedback controls (McGlathery et al. 1996). This is also consistent with the capacity of slow growing taxa to offset low uptake capacities by utilising internal N stores to cover their requirements for growth (Pederson and Borum 1997). The slower growth rate of *Polysiphonia decipiens* (section 4.3.1) may also enable it to expend energy on active uptake at lower concentrations.

The possible importance of surge uptake at low field concentrations differs to other studies where K_m was significantly higher than field DIN concentrations (Pederson and Borum 1997; Schaffelke and Klumpp 1998), and in studies where surge and assimilation V_{max}:K_m ratios have not differed from each other (Pederson 1994; Pederson and Borum 1997). Schaffelke and Klumpp's (1998) caveat was that ammonium uptake at low concentrations would be inefficient only if DIN concentrations remained low, which was unlikely in summer when spikes in nutrient concentrations result from high river inputs. An examination of Pederson's (1994) data shows that V_{max} to K_m ratios of *Ulva lactuca* declined, from 10.6 after 15 min to 4.1 after 300 min during multiple flask experiments. This reduction may reflect the importance of surge uptake for fast growing macroalgae such as *Ulva lactuca* at low and more natural concentrations of DIN. Alternatively this may illustrate the influence of externally controlled rates of uptake as substrate concentration was depleted. There was little evidence of externally mediated uptake over the 360 min period in the present study. Therefore surge uptake may be important at low concentrations for these macroalgae and contribute to high K_m relative to V_{max}. The smaller difference in V_{max}:K_m ratios between surge and assimilation uptake for *Ulva* sp. and immature stages of *Undaria pinnatifida* than for the 3 other species suggests that surge uptake at low concentrations is least important for these species or developmental stages of algae.

5.4.4 Uptake and N growth requirements

The similarity in tissue C:N and tissue N concentrations amongst species meant that interspecies variation in ammonium uptake rates could not be attributed to differences in tissue N status, as has been demonstrated for the Chlorophytes *Ulva rigida* (Fujita 1985) and *Enteromorpha prolifera* (OBrien and Wheeler 1985). Assimilation rates of ammonium uptake may, however, correspond to the incorporation of amino acids into macromolecules that in turn are dependent on high growth rates (Pederson 1994). Therefore assimilation uptake capacities for *Hincksia sordida* and *Ulva* sp. may be a function of high growth rates and the synthesis of amino acids. Relatively high assimilation capacities found for *Undaria pinnatifida* are consistent with observed rapid growth in the field (Campbell and Burridge 1998).

The high tissue N contents were above calculated critical N values and therefore were presumably non-limiting. This appears to contradict the contention that the presence of surge uptake is an adaptive response to N limitation (Rosenberg et al. 1984; Fujita 1985). Pederson (1994) proposed that the short-lived nature of surge uptake in *Ulva lactuca* meant that internal intracellular N pools were likely to be controlling surge uptake which contributed about 10% of the total tissue N content. In the present study *Hincksia sordida* took up a similar proportion (ca. 13%) of its total tissue N content at high substrate concentrations and this was about double that of mature *Undaria pinnatifida* and 5-fold higher than the other algae examined. As intracellular pools of DIN and amino acids represent about 5-20% of the total N content in fast growing macroalgae (Bird et al. 1982; Thomas and Harrison 1985; Horrocks et al. 1995) it is possible that these pools limit surge uptake in *Hincksia sordida* and *Undaria pinnatifida*. Such findings are consistent with studies on *Ulva rigida* (Fujita et al. 1988) and *Chaetomorpha linum* (McGlathery et al. 1996).

The relatively high V_{max}:N_{req} ratio for *Hincksia sordida* indicates that at enriched concentrations of ammonium this species could best take up N in excess of immediate growth demands. This uncoupling of N uptake and N requirements for growth commonly occurs in N limited algae where N uptake and assimilation are not limited

by internal carbon reserves (Rosenberg et al. 1984; Fujita 1985). McGlathery et al. (1996) found that the filling of internal storage pools of N in *Chaetomorpha linum* (Chlorophyta) required that N uptake and its incorporation into intracellular N pools exceeded metabolic N demand for growth. Under favourable light and temperature the filling of N pools was more than twice as fast as their depletion when N was limiting. This suggests that maximum pool sizes of N were reached such that growth depended upon the frequency of N supply rather than the duration of N supply. Given *Hincksia*'s relatively low propensity for N storage compared to other algae (section 4.3.3) its growth may also be highly dependent on the rapid accumulation of intracellular N pools to maximum levels. Consistent with its bi-phasic uptake strategy these pools would be facilitated by diffusion under relatively high concentrations of N. Such a strategy may explain the apparent uncoupling of its growth and surge uptake, as N is incorporated into amino acids, presumably via the GDH pathway (i.e. independent of ATP derived from photosynthesis), independent of its metabolic N demand for growth.

The 8- to 10-fold higher V_{max}:N_{req} ratio of *Hincksia* sordida compared to *Ulva* sp. and *Polysiphonia decipiens* parallels findings of Pederson and Borum (1997) who showed that ratios for *Ceramim rubrum* and *Chaetomorpha linum* were 2-fold higher than that recorded for *Ulva lactuca*. This suggests that *Ulva* sp. is a species predisposed to N limitation and is consistent with its low V_{max}:K_m ratio. The range of half saturation constants for growth under surge and assimilation uptake (20.9 to 122.9 μg NH_4-N L^{-1}) in the present study are comparable to values reported for species of similar morphological form (Gordon et al. 1981; Pederson and Borum 1997). These relatively low values suggest that all species examined are able to sustain high growth rates at low ammonium concentrations. This was also consistent with the comparatively similar K_μ:K_m ratios of the 3 macroalgae examined. This ratio can be used as an index of sensitivity to nutrient stress and the lower the ratio the higher is the capacity of a given species to grow under low external nutrient concentrations (Turpin 1988; Pederson and Borum 1997). The K_μ:K_m ratios in the present study (range: 0.706 to 0.946) are lower than those reported for *Ulva lactuca*

and *Ceramium rubrum* (range: 0.125-126) from Roskilde Fjord, Denmark (Pederson and Borum 1997). This suggests a comparatively superior capacity for PPB macroalgae to sustain growth at low N concentrations.

The role of surge uptake in supporting the growth of 3 of the algae examined (N gain/N_{req} h^{-1}) varied widely among species. Ammonium uptake occurred more rapidly than could be utilised for growth in all species but the relationship varied between concentrations of 50 and 400 μg NH_4-N L^{-1}. At the lowest concentration growth of *Hincksia sordida* and *Polysiphonia decipiens* could be supported for 6-9 times longer than *Ulva* sp. whilst at high concentrations *Hinckisa* growth could be supported for at least 6 times longer than either of the two other species. This reinforces the importance of surge uptake for *Hincksia sordida* and demonstrates its enormous capacity for N utilisation at both low and high concentrations. Growth of *Hincksia sordida* could be supported by surge N uptake at high DIN concentrations for 12 times as long than for N enriched macroalgae from waters low in nutrients (3-6 h) (Fujita 1985; Pederson and Borum 1997). This suggests that macroalgae from N replete waters such as PPB are able to take advantage of high concentrations of ammonium. The longer time that surge uptake could support growth of *Hincksia sordida* and *Polysiphonia decipiens* (7-11 h) supports the contention that these two taxa are conferred a competitive advantage at low DIN concentrations compared with *Ulva* sp. Therefore, according to this analysis, the growth of each species would likely become N limited as ammonium decreases in the following order: *Ulva* sp., *Polysiphonia decipiens*, *Hincksia sordida*.

5.4.5 Summary

The uptake and growth kinetics presented here has provided a functional account of the potential affinity for ammonium uptake by 4 species of macroalgae. Those species with relatively high N storage and slower growth capacities (e.g. *Polysiphonia decipiens*) appear reliant on active uptake mechanisms that utilise N at low concentrations. Those species with high surge uptake rates at high concentrations (e.g. *Hincksia sordida*, *Undaria pinnatifida*) showed bi-phasic uptake strategies that are well suited to exploit N independent of growth requirements and are indicative of

N limitation. However, surge uptake may not only be a function of N limitation but appears to be dependent on controls exerted by intracellular pools of N.

Chapter 6

The long-term uptake of nitrogen and phosphorus by three species of macroalgae.

6.1 Introduction

In previous chapters the relative capacities of macroalgae for short term ammonium uptake were described. Extrapolating these rates to long term nutrient uptake rates is problematic since there are many factors that may affect nutrient uptake. These include light, temperature, nutrient supply, internal nutrient contents and growth rates. Other studies suggest that nutrient uptake rates determined from depletion of nutrients over a full day are considerably less than those calculated from uptake over periods of minutes to hours, because short term nutrient depletion often consists mainly of elevated 'surge' uptake which remains constant over the first hour or more (Lavery and McComb 1991b; Paling 1991; McGlathery 1996; Pederson 1997).

In marine environments macroalgae are commonly observed to exhibit a seasonal pattern in tissue N and P that is reduced during summer when external nutrient supplies are at their annual minimum and growth rates are high (Chapman et al. 1978; Asare and Harlin 1983; Hanisak 1983). These patterns are also common in nutrient enriched near-shore environments (Birch et al. 1981; Lyngby 1990; Pederson and Borum 1996; this study: Chapter 2). Although total *in situ* N uptake is often high in winter because of elevated N supply and luxury uptake, uptake rates as a function of algal biomass may often be higher in summer when internal tissue N pools are reduced and growth is rapid (Rosenberg et al. 1984; Wheeler and Srivastata 1984; Duke et al. 1989; Fujita et al. 1989; Lohman and Priscu 1992; Peckol et al. 1994). In a number of experiments short term N uptake of algae starved of N has been found to be higher than in algae subject to N enrichment (Fujita 1985; Cohen and Neori 1991; Peckol et al. 1994). Few studies have examined long term nutrient uptake by macroalgae in relation to their nutritional history (Paling 1991; Pederson 1994). It

appears likely that a dynamic regulatory system of uptake may occur that is responsive to changes in tissue N and ambient DIN concentrations (Kopczak 1994). Other factors such as temperature and light may also regulate nutrient uptake (Ventura and Harlin 1976; Duke et al. 1989).

The limited information available on the uptake of phosphate by macroalgae indicates that, as with N, P uptake is highest when external availability is high (Gordon et al. 1981; Wallentinus 1984a; Hurd and Dring 1990). Tissue P concentrations have been shown to exhibit seasonal variation in relation to DIP supply (Lyngby 1990; Wheeler and Björnsäter 1992; Pederson and Borum 1996). There is also limited data on measured seasonal phosphate uptake rates of macroalgae although Hurd et al. (1993) found that in pilose forms of *Fucus spiralis* phosphate uptake over a range of laboratory concentrations was highest in winter than in other seasons. *In situ* nitrate and phosphate concentrations in the water column were at a maximum during winter.

The ratio of N:P can also regulate the relative uptake capacities of N or P (Frielander and Dawes 1985; Lapointe 1985, 1987; Aisha et al. 1995). There is evidence to suggest that N uptake can increase in the presence of P in some species (Aisha et al. 1995) whilst P uptake may be highest when N is non-limiting (Björnsäter and Wheeler 1990). Gordon et al. (1981) reported optimal growth rates for *Cladophora* aff. *albida* (Chlorophyta) in cultures enriched with both N and P. Lapointe (1985) also indicated that severe P limitation can lead to N limited growth in *Gracilaria tikvahiae* (Rhodophyta).

In assessing the importance of macroalgae in the nutrient cycling of coastal ecosystems it is necessary to establish whether there are seasonal differences in the nutrient uptake capacity of various taxa. The seasonal factors which govern N and P uptake over time must also be considered when estimating nutrient budgets for macroalgal communities. While several studies have found seasonal variation in nutrient uptake by macroalgae (Wallentinus 1978; Rosenberg et al. 1984; Lavery and

McComb 1991b; Lohman and Priscu 1992; Hurd et al. 1993; Peckol et al. 1994) it is uncertain whether Port Phillip Bay macroalgae exhibit this difference.

6.1.1 Aim

The aim of this study was to determine the long-term N and P uptake rates of macroalgae from Port Phillip Bay, specifically in relation to previous N exposure (high in winter c.f. low in summer) and seasonal differences in tissue nutrient reserves. In addition, the influence of N and P on the uptake capacity of each respective nutrient was examined. Comparisons were made with short-term uptake rates determined for the same species in previous experiments.

6.2 Methods

6.2.1 Sample collection and preparation

Whole thalli were collected during winter (June 1995-August 1995) and summer (December 1996-February 1996) and maintained in the laboratory as described in section 3.2.1.

6.2.2 Nutrient enrichment: ammonium and phosphate uptake

The data for this experiment was obtained from the same experiment outlined in section 3.2.2. Long term uptake (14 d) of ammonium and phosphate was measured for the three species of nutrient enriched macroalgae collected above. Experiments were conducted on separate occasions for each taxa. Nutrient uptake was measured from 3 replicates of 3 treatments (N+P, +N and +P) (n = 3 for each experiment and all treatments).

Algae were initially enriched with nutrients and then every second day for 14 d. Filtered (0.45 μm GF/C Whatman) water samples were intially collected from each aquaria and then 24 h after nutrient enrichment for the duration of the 14 d experiment. The collection of water samples is described in section 5.2.4.

Uptake rates were calculated from the net ammonium and phosphate depletion (in μg) over 24 h for each replicate alga every 2 days. Monitoring of aerated ammonium and phosphate solutions (controls: without algae) over 48 h periods showed no significant variation ($p > 0.05$) in concentration (data not shown), but slight changes in concentration of controls were accounted for. Increases in phosphate concentration that arose from possible efflux from tissues and resulted in no net uptake was reported as zero uptake. Rates were expressed as ammonium or phosphate uptake on a gram dry weight basis per hour (μg NH_4-N g dry $wt.^{-1}$ h^{-1}), assuming constant uptake over the 24 h period. Dry weight values were estimated from daily measures of fresh

weight and the significant linear relationship between fresh and dry weight for each species (data not shown).

6.2.3 Nutrient uptake parameters

Two nutrient uptake parameters were calculated for each replicate alga:

1. The total amount of nutrient removed from solution (μg N or P) over 14 d and;
2. The total of all nutrient uptake rates every 2 d (μg N or P g dry wt.$^{-1}$ h^{-1}) over the experimental period (14 d).

6.2.4 Nutrient analyses and units of measure

Water column and tissue nutrient analyses were carried out as outlined in section 2.2.4. Tissue nutrients were expressed on a unit biomass basis (mg g dry wt.$^{-1}$). In addition tissue nutrients were expressed on a per plant (or tissue piece) basis (mg tissue N g dry wt.$^{-1}$ x plant biomass (g) = mg tissue N plant^{-1}). This was calculated because of the variation in the weight of algae at the end of the experimental period. At high tissue weights dilution of tissue nutrient concentration may occur. Tissue concentration expressed on a per plant basis provides a measure of the total amount of nutrient within each replicate alga or tissue piece.

6.2.5 Statistical analyses

Uptake data were tested for assumptions of normality by examining heterogeneity of variance (Cochrans test) and skewness of data (residuals and outliers). Non-normal data was subject to the log transformation, $\log_e(x+1)$. Least squares linear regression was employed to test for the significance ($p < 0.05$) of the linear relationships between nutrient withdrawn from solution and change in tissue nutrients. For each treatment and experiment *t* tests were used to test for significance ($p < 0.05$) between uptake rates measured on day 1 and day 14. Two way ANOVA was employed to test for the effects of nutrient treatment (fixed factor) and season (fixed factor) on calculated uptake parameters. Post hoc analysis (Tukeys) was used to assess significant ($p < 0.05$) effects of treatment factors on the calculated uptake parameters (section 6.2.4).

6.3 Results

6.3.1 Tissue nutrients and uptake

It was found that ammonium and phosphate were not lost by physical mechanisms at the level of aeration used in the growth experiments (data not shown). Any depletion of ammonium or phosphate from solution can therefore be attributed to biological uptake.

Tissue nitrogen

Initial tissue N concentrations were described in Chapter 3 (Fig. 3.3). Both *Hincksia sordida* and *Ulva* sp. had significantly higher tissue N in winter than summer. There was no significant difference between winter and summer tissue N in *Polysiphonia decipiens*.

After 14 days of nutrient enrichment final tissue N contents (mg N $plant^{-1}$) were higher than initial tissue N contents (mg N $plant^{-1}$) in *Hincksia sordida* and *Ulva* sp. Final tissue N (mg N $plant^{-1}$) was less than or closely approximated initial tissue N (mg N $plant^{-1}$) in *Polysiphonia decipiens* (Fig. 6.1). For *Hincksia sordida* the increases in tissue N (mg N $plant^{-1}$) were generally greater in summer than in winter plants, but the opposite was true for *Ulva* sp.

The change in tissue N (mg N $plant^{-1}$) was not significantly related (all tests; df =11, $r^2 < 0.3$, $p > 0.05$) to the ammonium taken up from solution for any of the species examined (Fig. 6.2). This suggests that not all ammonium withdrawn from solution was stored as N in the plant tissues.

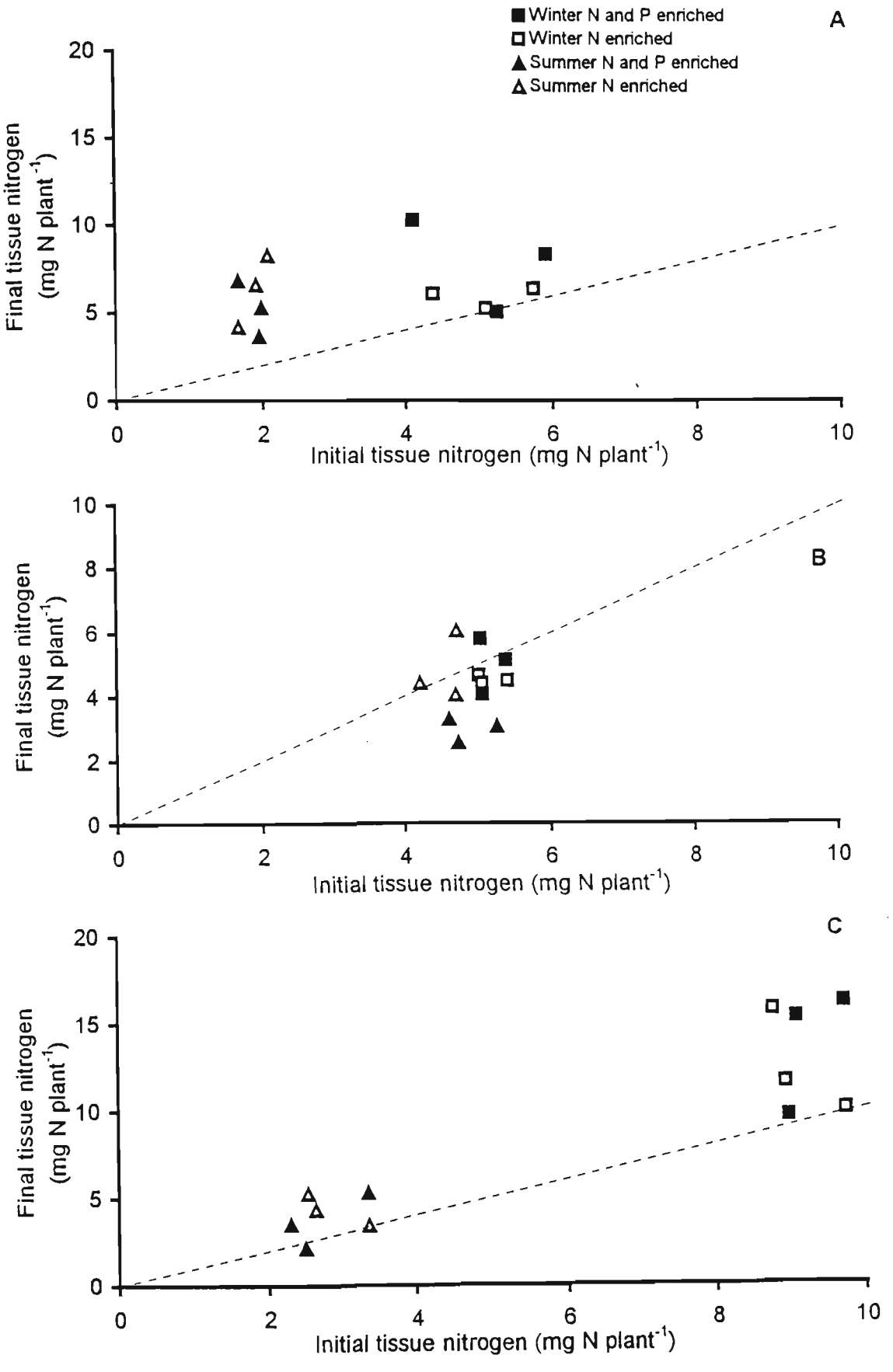

Fig. 6.1 The initial plant nitrogen (mg N plant^{-1}) compared to the final total plant nitrogen in (A) *Hincksia sordida*, (B) *Polysiphonia decipiens* and (C) *Ulva* sp. after 14 d enrichment with N and P or N in winter and summer. The legend in graph A applies to all graphs. The dashed line on the graphs has a gradient that equals 1. Data points above this line represent plants with a net uptake over the experimental period.

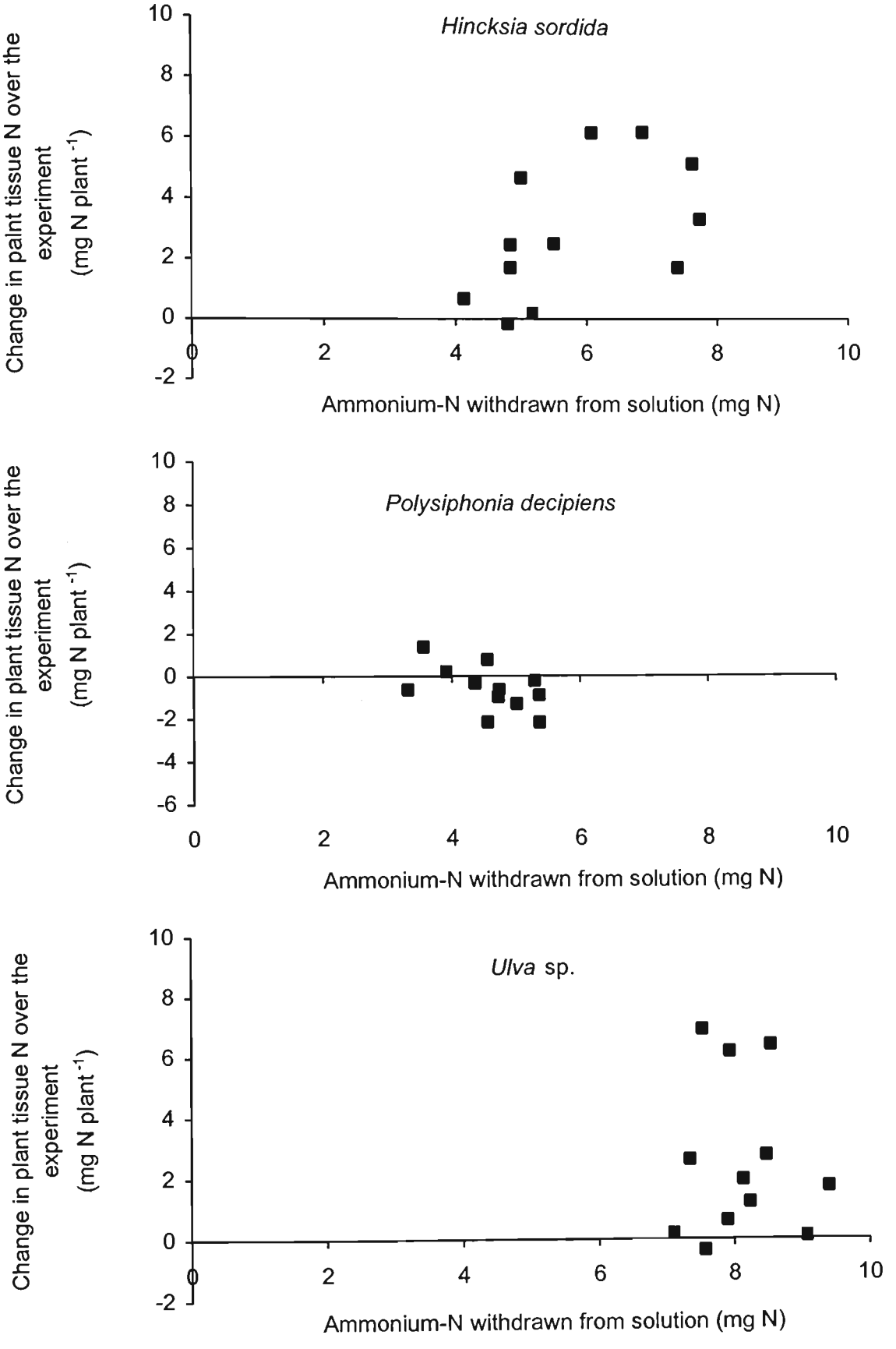

Fig. 6.2 The change in total plant nitrogen (mg N plant^{-1}) in three species of macroalgae compared to the amount of nitrogen taken up from the water column after growth in solutions containing 400 µg NH_4-N L^{-1} for 14 d; n = 12 for each species.

Tissue phosphorus

Initial tissue P concentrations were described in Chapter 3 (Fig. 3.4). There was no significant difference between winter and summer tissue P in any of the 3 species examined.

After 14 days of nutrient enrichment final tissue P contents (mg P $plant^{-1}$) were generally higher than initial tissue P contents (mg $plant^{-1}$) in *Hincksia sordida* (except for summer P enriched) and *Ulva* sp. (Fig. 6.3). Final tissue P contents (mg P $plant^{-1}$) were generally less than or closely approximated initial tissue P (mg P $plant^{-1}$) in *Polysiphonia decipiens*

For *Hincksia sordida* a significant positive relationship was found between the amount of phosphate withdrawn from solution (excluding P only enriched summer plants) and the change in tissue P (mg $plant^{-1}$) ($F = 7.09$, $df = 8$, $r^2 = 0.60$, $p < 0.05$). The gradient of this relationship was 0.5, therefore the ratio of phosphate removed from solution to P stored in the tissues was approximately 2:1 (Fig. 6.4A). For *Ulva* sp. the amount of phosphate removed from solution over the experiment also showed a significant positive relationship ($F = 13.54$, $df = 5$, $r^2 = 0.77$, $p < 0.05$) with the change in tissue P (mg P $plant^{-1}$) in winter plants (Fig 6.4C). The ratio of phosphate removed from solution to tissue P was approximately 1:1. No such relationship was evident for *Polysiphonia decipiens* (Fig 6.4B).

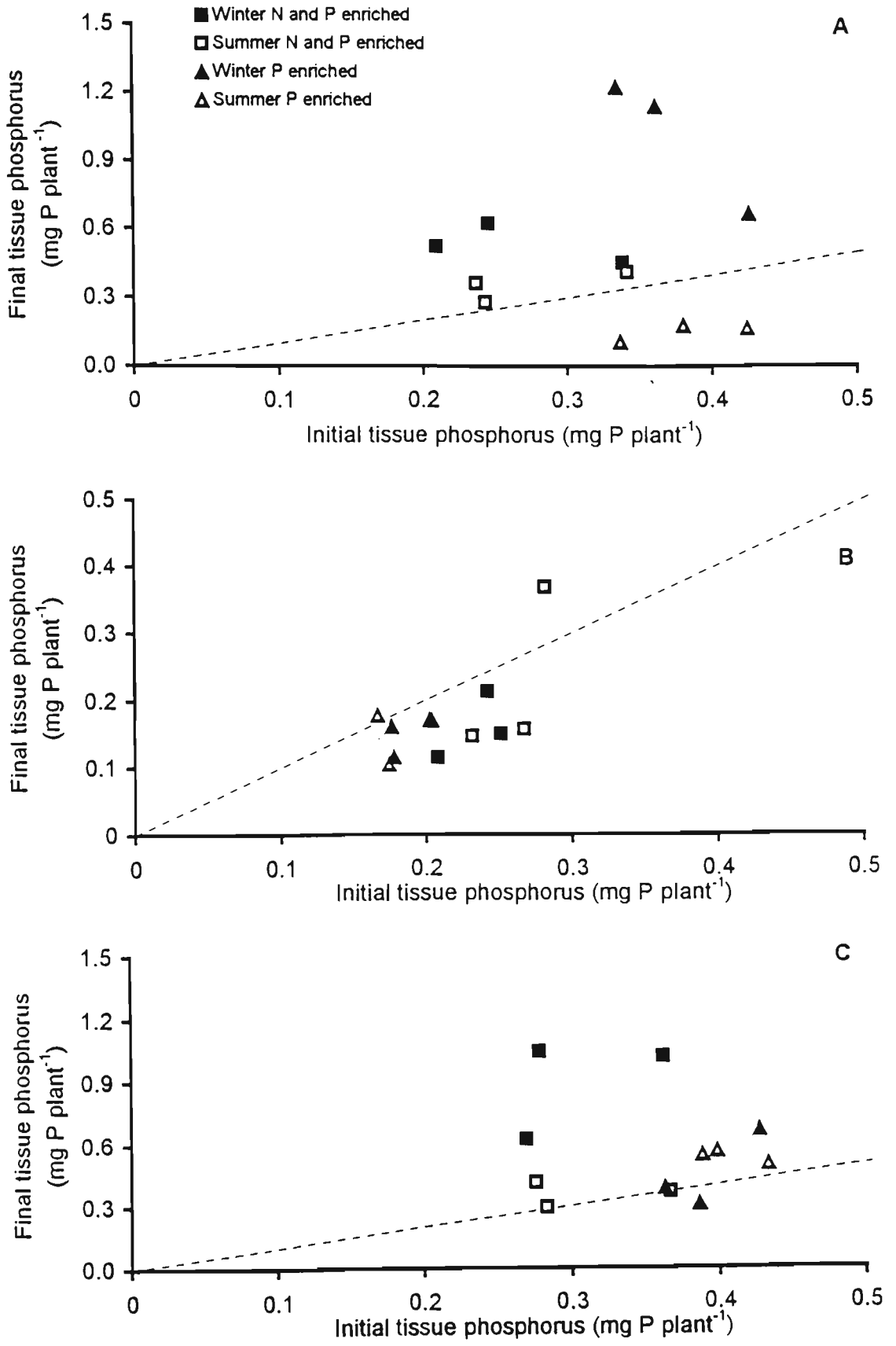

Fig. 6.3 The initial plant phosphorus (mg P plant^{-1}) compared to the final total plant phosphorus in (A) *Hincksia sordida*, (B) *Polysiphonia decipiens* and (C) *Ulva* sp. after 14 d enrichment with N and P or P in winter and summer. The legend in graph A applies to all graphs. The dashed line on the graphs has a gradient that equals 1. Data points above this line represent plants with net uptake over the experimental period.

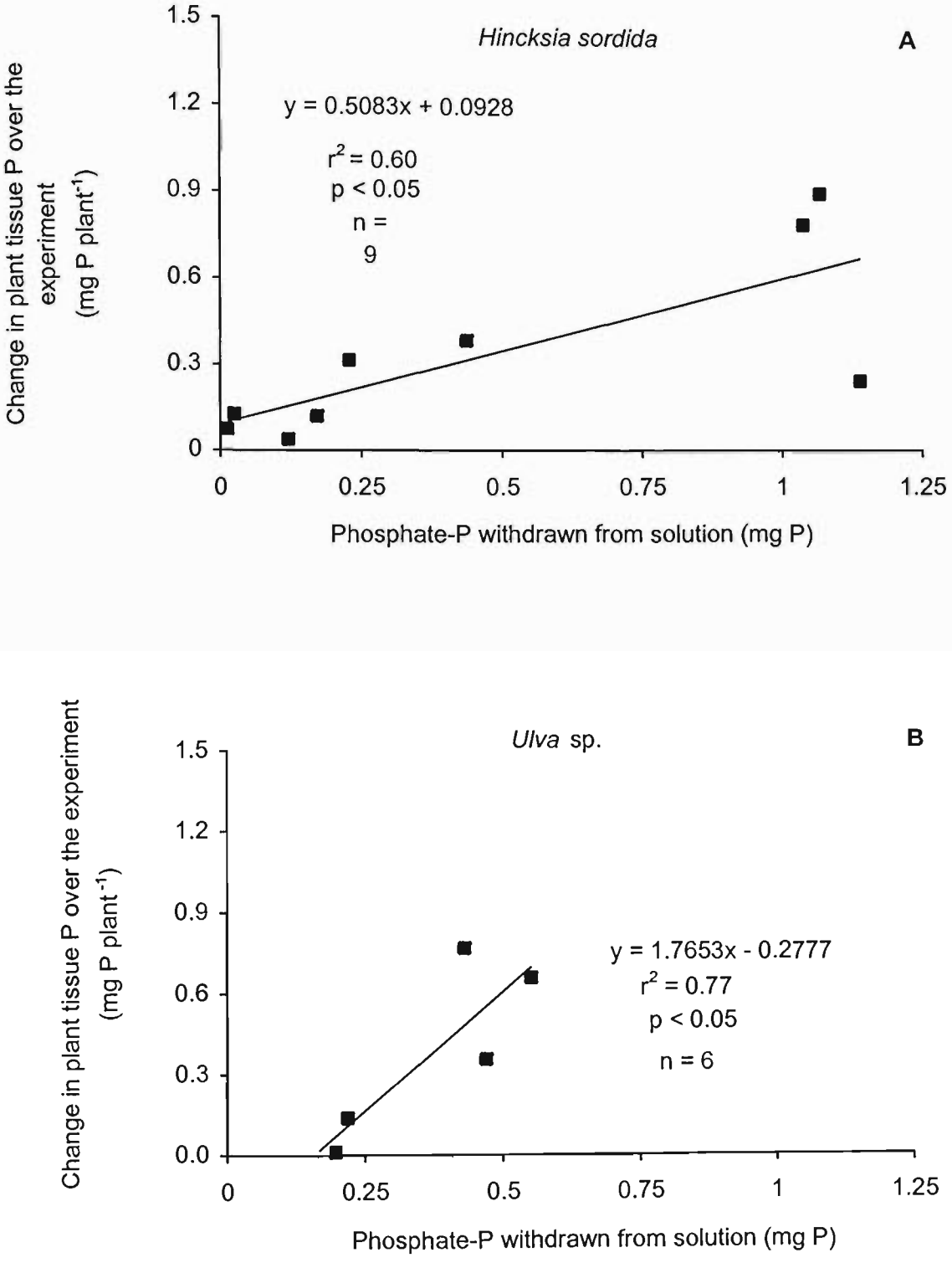

Fig. 6.4 The change in total plant phosphorus (mg P plant^{-1}) in two species of macroalgae compared to the amount of phosphate-P taken up from the water column after growth in solutions containing 500 μg PO_4-P L^{-1} after 14 d.

Table 6.1 Ammonium nitrogen uptake parameters for N+P and N enriched *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. over 14 d. Algae were collected from Port Phillip Bay in winter and summer. Values shown are means (s.e.), n = 3.

Nutrient enrichment and season	*Hincksia sordida*		*Polysiphonia decipiens*		*Ulva* sp.	
	NH_4-N removed (μg N)	**Uptake rate over time period** (μg NH_4-N g dry wt.$^{-1}$)	**NH_4-N removed** (μg N)	**Uptake rate over time period** (μg NH_4-N g dry wt.$^{-1}$)	**NH_4-N removed** (μg N)	**Uptake rate over time period** (μg NH_4-N g dry wt.$^{-1}$)
N + P enriched						
Winter	1203.1^{Aa} (2.99)	569.4^{Aa} (0.92)	1213.7^{Aa} (55.5)	281.5^{Aa} (24.8)	2034.2^{Aa} (52.4)	361.7^{Aa} (10.0)
Summer	1910.3^{Ab} (37.9)	530.6^{Aa} (13.1)	1244.2^{Aa} (8.9)	300.0^{Aa} (22.5)	1995.2^{Aa} (52.2)	567.2^{Ab} (38.1)
N enriched						
Winter	1161.6^{Aa} (75.0)	594.7^{Aa} (37.6)	1204.9^{Aa} (72.9)	307.4^{Aa} (32.3)	1832.8^{Ab} (32.1)	334.9^{Aa} (6.9)
Summer	1340.6^{Bb} (32.4)	605.4^{Aa} (19.2)	899.1^{Bb} (43.0)	221.0^{Ba} (5.2)	2246.1^{Bb} (67.4)	487.4^{Ab} (17.4)

Post hoc (Tukeys test) significance of two-way ANOVA is represented by superscript letters for each species. Within each column significant ($p < 0.05$) differences between nutrient treatments (for the same season) are indicated by different upper case superscript letters. Significant differences between seasons (for the same nutrient treatment) are indicated by different lower case superscript letters.

6.3.2 Nutrient uptake over time

Ammonium uptake

The seasonal and nutrient treatment differences in mean total ammonium uptake and total uptake rate are shown for each species in Table 6.1. The uptake of ammonium by *Hincksia sordida* in winter and summer experiments decreased significantly over the 14 d of the experiment and similar trends were evident for N+P enriched and N enriched experiments (Fig. 6.5). A significant effect of season on total ammonium uptake (Appendix 16A) indicates higher total ammonium uptake by N+P enriched plants in winter than in summer. Summer N alone enriched plants also had significantly higher total ammonium uptake than winter plants. A significant effect of nutrient treatment and a significant interaction between nutrient treatment and season indicates higher uptake by N+P enriched plants than N enriched plants in summer but not in winter (Table 6.1). Conversely, there was no effect of nutrient treatment, season or their interaction on ammonium uptake rate (Appendix 16B).

There was no significant difference between initial and final ammonium uptake rates of N+P enriched *Polysiphonia decipiens* in either winter or summer experiments. Initial ammonium uptake rates in N enriched *Polysiphonia decipiens* were significantly higher than final uptake rates (Fig. 6.6.). A significant effect of nutrient treatment, season and their interaction on total ammonium uptake (Appendix 16A) indicates higher total ammonium uptake by N+P enriched plants than N enriched plants in summer but not in winter, and higher ammonium uptake by N enriched plants in winter than in summer (Table 6.1). Conversely, a non-significant effect of nutrient treatment, season or their interaction was found for ammonium uptake rate (Appendix 16B).

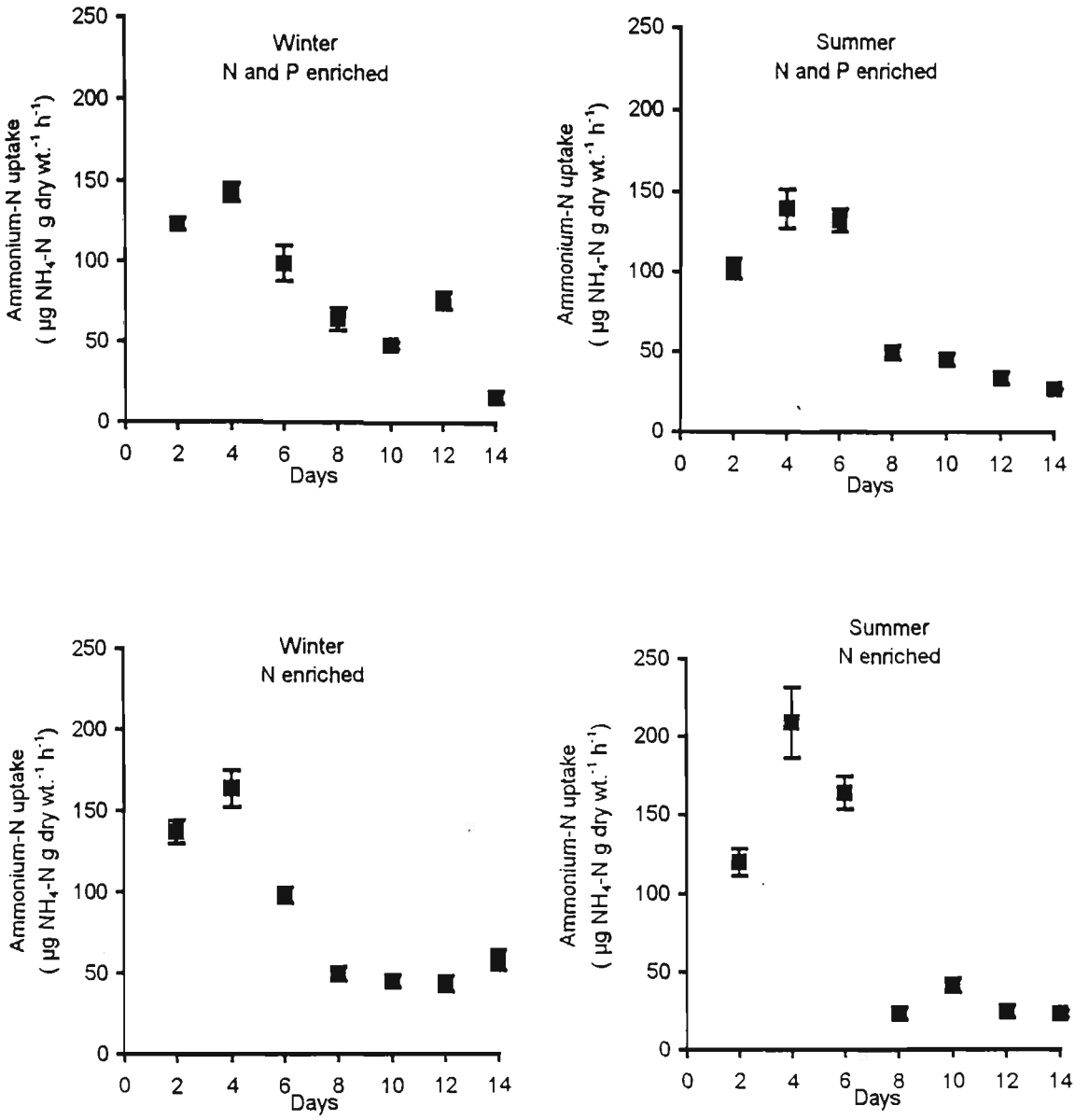

Fig. 6.5 Ammonium-N uptake by winter and summer plants of *Hincksia sordida* over the duration of the experiment grown in either N and P enriched (400 µg NH_4-N L^{-1} and 500 µg PO_4-P L^{-1}) or N enriched (400 µg NH_4-N L^{-1}) media. Values represent means ± 1 s.e., n = 3.

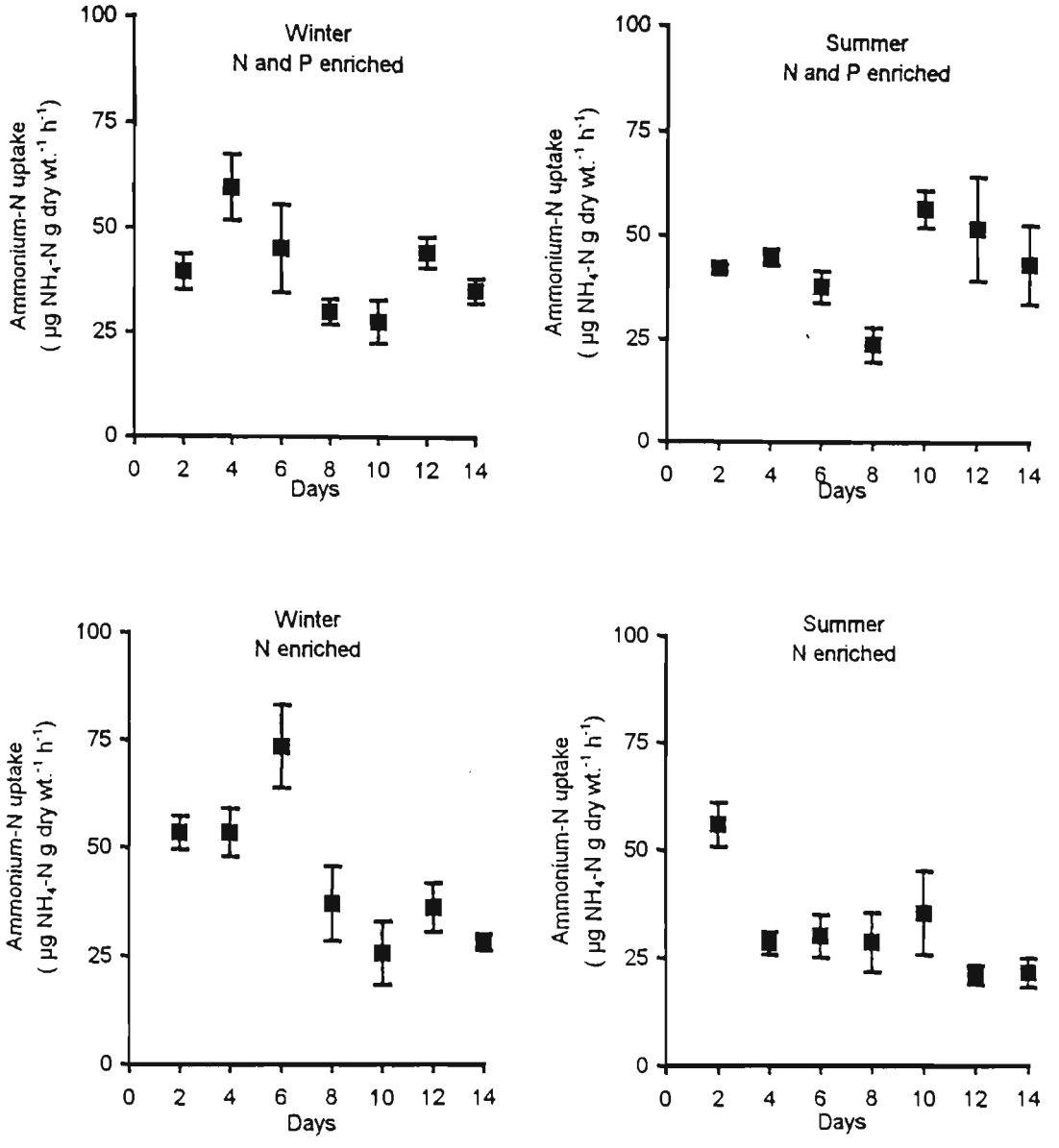

Fig. 6.6 Ammonium-N uptake by winter and summer plants of *Polysiphonia decipiens* over the duration of the experiment grown in either N and P enriched (400 µg NH_4-N L^{-1} and 500 µg PO_4-P L^{-1}) or N enriched (400 µg NH_4-N L^{-1}) media. Values represent means ± 1 s.e., n = 3.

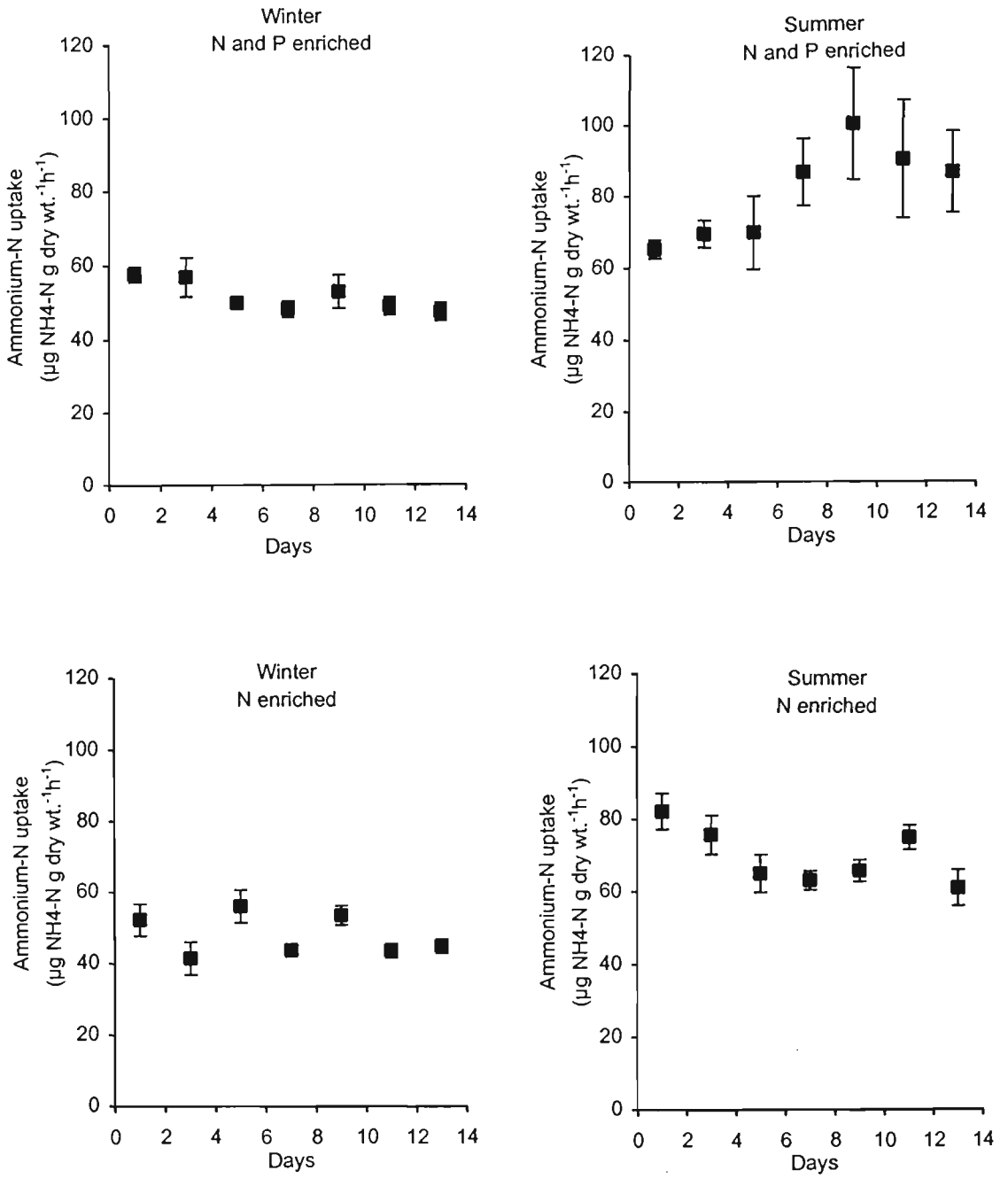

Fig. 6.7 Ammonium-N uptake in winter and summer plants of *Ulva* sp. over the duration of the of the experiment grown in either N and P enriched (400 µg NH_4-N L-1 and 500 µg PO_4-P L^{-1}) or N enriched (400 µg NH_4-N L^{-1}) media. Values represent means ± 1 s.e., n = 3.

For *Ulva* sp. ammonium uptake was relatively constant over the duration of winter and summer experiments in both N+P and N enriched plants (Fig. 6.7). A significant effect of season on ammonium uptake (Appendix 16A) indicates higher ammonium uptake by N enriched summer plants than N enriched winter plants (Table 6.1). A significant interaction between nutrient treatment and season on total ammonium uptake (Appendix 16A) indicates higher total uptake by N enriched plants than N+P enriched plants in summer and the opposite in winter (Table 6.1). A significant effect of season on ammonium uptake rate (Appendix 16B) was explained by significantly higher summer uptake rates than winter rates in N+P and N enriched plants. No significant differences were observed between N+P and N alone enriched plants (Table 6.1).

N:P ratios in plants enriched with N and P

Mean N:P ratios removed from solution were generally higher for all plants than the difference between initial and final tissue N:P ratios at day 14, except for winter *Polysiphonia decipiens* (Table 6.2).

Table 6.2 N:P ratios for 3 species of macroalgae before and after experimental enrichment with N+ P in winter and summer. The total N:P removed from solution is also shown. Values shown are means (s.e.).

N:P ratio status	*Hincksia sordida*		*Polysiphonia decipiens*		*Ulva* sp.	
	Winter	Summer	Winter	Summer	Winter	Summer
Initial	13.5 (0.95)	46.1 (11.48)	15.2 (1.95)	33.0 (8.57)	9.0 (1.09)	38.7 (1.94)
Final	11.6 (1.07)	44.3 (2.53)	58.0 (4.63)	52.3 (3.31)	15.7 (1.09)	70.7 (4.73)
Mean final - mean initial	-	-	42.8	19.3	8.7	32.0
Total removed from solution	16.1 (0.62)	21.0 (2.19)	40.4 (1.31)	91.0 (1.93)	18.3 (1.36)	52.8 (5.51)

Table 6.3 Phosphate-P uptake parameters for N and P and P enriched *Hincksia sordida*, *Polysiphonia decipiens* and *Ulva* sp. over 14 d. Algae were collected from Port Phillip Bay in winter and summer. Values shown are means (1 s.e.), n = 3.

	Hincksia sordida		*Polysiphonia decipiens*		*Ulva* sp.	
	PO_4-P removed (μg P)	**Uptake rate over time period** (μg PO_4-P g dry wt.$^{-1}$)	**PO_4-P removed** (μg P)	**Uptake rate over time period** (μg PO_4-P g dry wt.$^{-1}$)	**PO_4-P removed** (μg P)	**Uptake rate time period** (μg PO_4-P g dry wt.$^{-1}$)
N + P enriched						
Winter	70.8^{Aa} (19.7)	37.1^{Aa} (10.2)	94.6^{Aa} (20.3)	23.6^{Aa} (5.6)	121.0^{Aa} (8.9)	20.1^{Aa} (2.4)
Summer	270.2^{Ab} (76.4)	78.8^{Ab} (2.9)	195.7^{Aa} (36.6)	54.2^{Ab} (5.5)	525.3^{Ab} (69.1)	170.2^{Ab} (13.4)
P enriched						
Winter	13.2^{Ba} (8.5)	5.7^{Ba} (3.6)	270.1^{Ba} (21.2)	70.7^{Ba} (6.6)	48.5^{Ba} (3.8)	9.8^{Ba} (0.9)
Summer	102.2^{Bb} (4.3)	39.4^{Bb} (1.31)	557.1^{Bb} (61.7)	123.5^{Ba} (17.1)	349.0^{Bb} (29.6)	72.6^{Bb} (5.5)

Post hoc (Tukeys test) significance of two-way ANOVA is represented by superscript letters for each species. Within each column significant ($p < 0.05$) differences between nutrient treatments (for the same season) are indicated by different upper case superscript letters. Significant differences between seasons (for the same nutrient treatment) are indicated by different lower case superscript letters.

Phosphate uptake

The seasonal and nutrient treatment differences in mean total phosphate uptake and total uptake rate are shown for each species in Table 6.3. The uptake of phosphate by *Hincksia sordida* during winter and summer decreased over time (Fig. 6.8) with a significant difference between initial and final uptake rates in N+P enriched winter and summer plants, and P alone enriched summer plants (Table 6.3). A significant effect of nutrient treatment on total ammonium uptake (Appendix 17A) and phosphate uptake rate (Appendix 17B) indicates higher phosphate uptake by N+P enriched plants than P enriched plants in summer and winter. A significant effect of season on both uptake parameters indicates higher phosphate uptake by N+P enriched plants than P enriched plants.

For *Polysiphonia decipiens* phosphate uptake exhibited wide variation over time (Fig. 6.9). A significant effect of nutrient treatment on both uptake parameters (Appendix 17A and B) indicates higher uptake by P enriched plants than N+P enriched plants in winter and summer. A significant effect of season (Appendix 17A and B) on both uptake parameters indicates higher summer values than winter values (Table 6.3).

For N+P and P enriched *Ulva* sp. there was a significant decrease in phosphate uptake over 14 d in winter and summer (Fig. 6.10). A significant effect of nutrient treatment on total phosphate uptake and uptake rate (Appendix 17A and B) indicates higher uptake by N+P enriched plants than P enriched plants in winter and summer. A significant interaction between nutrient treatment and season (Appendix 17A and B) indicates that seasonality ($F = 295.69$) exerts most influence on phosphate uptake parameters irrespective of nutrient treatment ($F = 43.35$), with higher summer values than winter values (Table 6.3).

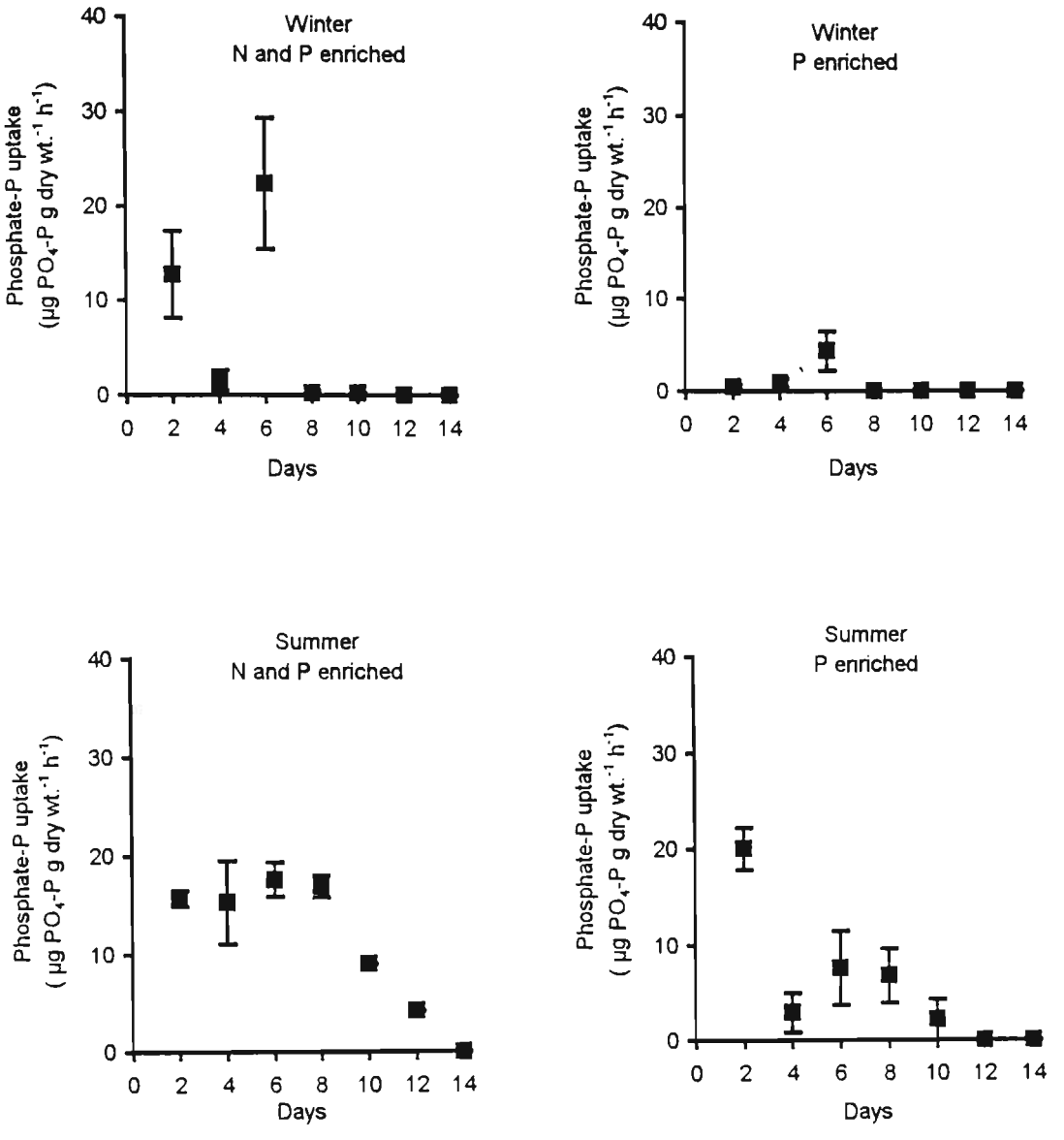

Fig. 6.8 Phosphate-P uptake by winter and summer plants of *Hincksia sordida* over the duration of the experiment grown in either N and P enriched (400 µg NH_4-N L^{-1} and 500 µg PO_4-P L^{-1}) or P enriched (500 µg PO_4-P L^{-1}) media. Values represent means ± 1 s.e., n = 3.

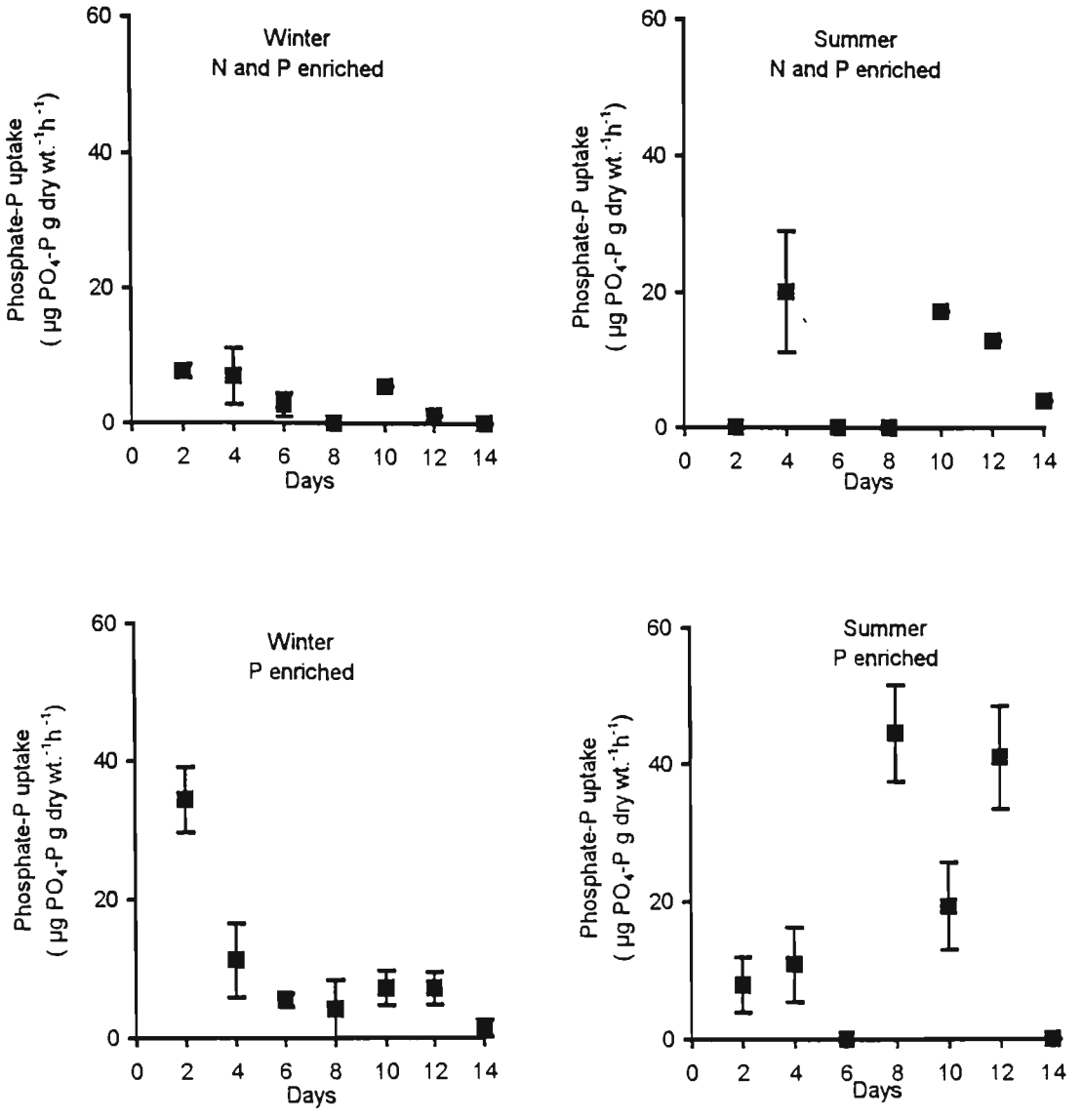

Fig. 6.9 Phosphate-P uptake by winter and summer plants of *Polysiphonia decipiens* over the duration of the experiment grown in either N and P enriched (400 µg NH_4-N L^{-1} and 500 µg PO_4-P L^{-1}) or P enriched (500 µg PO_4-P L^{-1}) media. Values represent means ± 1 s.e., n = 3.

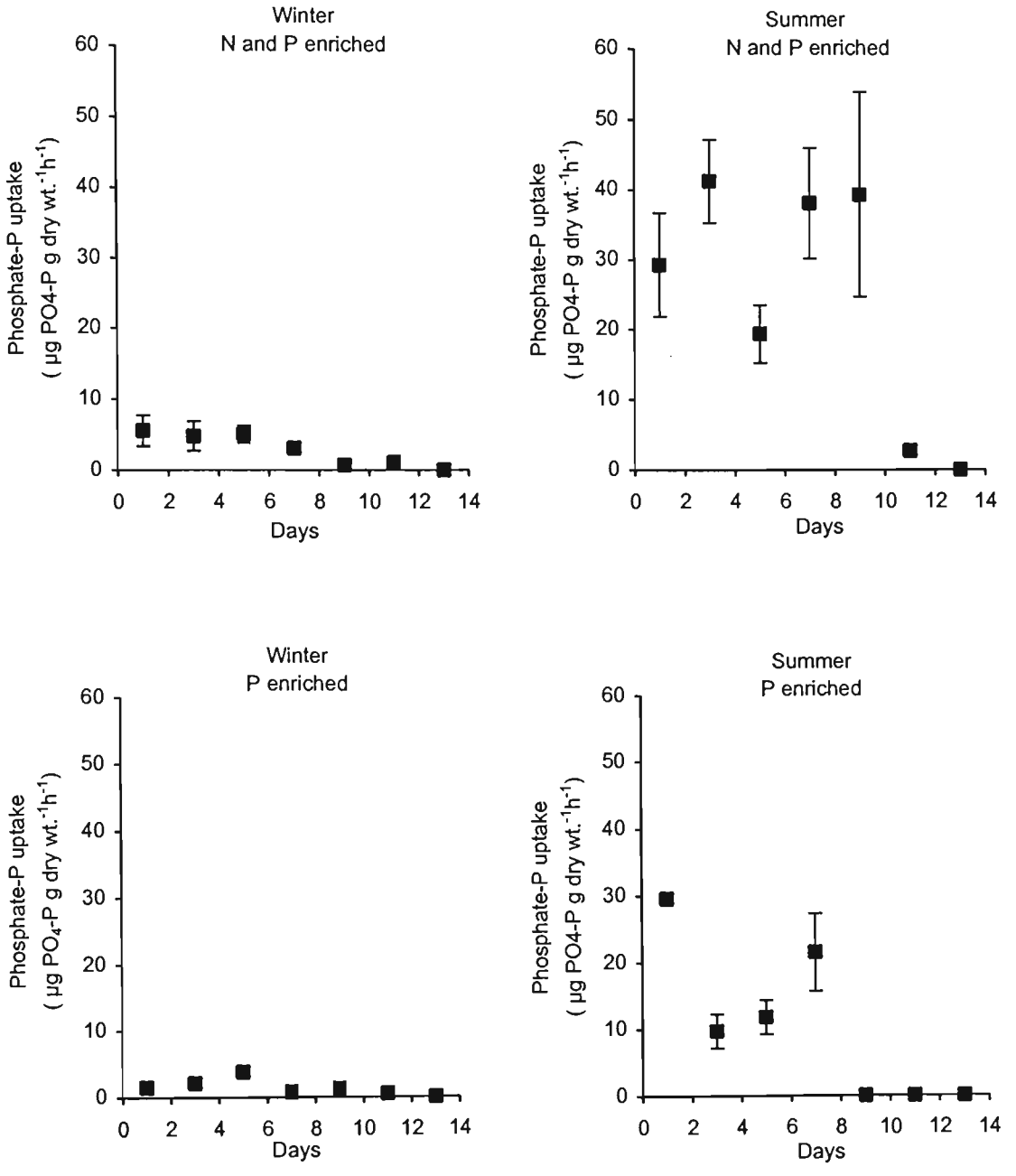

Fig. 6.10 Phosphate-P uptake in winter and summer plants of *Ulva* sp. over the duration of the of the experiment grown in either N and P enriched (400 µg NH_4-N L^{-1} and 500 µg PO_4-P L^{-1}) or P enriched (500 µg PO_4-P L^{-1}) media. Values represent means ± 1 s.e., n = 3.

6.4 Discussion

6.4.1 Ammonium uptake

There is limited information available on the daily uptake of N by macroalgae (Paling 1991; Pederson 1994; McGlathery et al. 1996). Most studies calculate N uptake over shorter periods of time (i.e. 0-4 h). The range in daily rates (expressed on an hourly basis) from this study are about 5 times lower than the short-term uptake rates reported by studies using similar morphological forms of macroalgae (Wallentinus 1984a). This could be attributed to the inability of macroalgae to sustain the rapid surge in N uptake that takes place when algae are initially exposed to elevated nutrient concentrations (Fujita et al. 1988). Daily uptake rates may provide a more accurate estimate of nutrient uptake during episodic pulses of nutrient exposure over periods of days to weeks. They can also provide useful data for input into nutrient budgets which can be used to determine long term nutrient requirements for macroalgal communities (Paling 1991).

The long-term ammonium uptake rates for the 3 species of macroalgae are generally lower than published values for similar morphological forms of algae from other studies. The highest mean daily rates reported for *Hincksia sordida* (25.85 to 183.08 μg NH_4-N g dry wt.$^{-1}$ h^{-1}), *Polysiphonia decipiens* of (15.4 to 89.4 μg NH_4-N g dry wt.$^{-1}$ h^{-1}) and *Ulva* sp. (33.52 to 119.73 μg NH_4-N g dry wt.$^{-1}$ h^{-1}) were lower than rates reported for *Gracilaria tikvahiae* (Rhodophyta) (9.81 to 18.90 μmol NH_4-N g fresh wt.$^{-1}$ h^{-1}; recalculated as 366 to 1455 μg NH_4-N g dry wt.$^{-1}$ h^{-1}), pulse fed (2 mM NH_4-N wk^{-1}) over a 28 day period (Frielander and Dawes 1985). McGlathery et al. (1996) reported high assimilation rates by *Chaetomorpha linum* of between 25 and 125 μmol NH_4-N g dry wt.$^{-1}$ h^{-1} (or 350-1750 μg NH_4-N) over a 30 d period. Pederson (1994) reported assimilation uptake rates by *Ulva lactuca* of 30 μmol NH_4-N g dry wt.$^{-1}$ h^{-1} (or 420 μg NH_4-N). The higher rates in these studies are possibly a function of starvation whereby unfilled intracellular N pools exert little control over the assimilation phase of uptake.

6.4.2 Ammonium uptake: seasonal and nutrient enrichment responses

The capacity of summer *Hincksia sordida* and *Ulva* sp. to assimilate greater amounts of ammonium than winter algae may have been due to greater storage capacity of summer algae because of a history of N depletion and low internal N reserves (section 4.3.2). The seasonally high ammonium assimilation in summer did not translate into increased photosynthetic performance (Chapter 3; Fig. 3.1) or growth (Chapter 4; Fig. 4.1), suggestive of an uncoupling of ammonium uptake and photosythesis. Such an uncoupling supports previous conclusions that surge uptake may comprise an important component of the uptake in these species (Chapter 5). The comparable ammonium uptake rates shown by winter and summer plants of *Polysiphonia decipiens* is not surprising given relatively similar tissue N between summer and winter plants. It also points to an uncoupling of uptake and growth as N is assimilated and stored at low growth rates (Chapter 4; Fig. 4.1) or even taken up at the expense of growth.

The relatively large differences between the increase in tissue N:P relative to the amount of N:P withdrawn from solution indicates a low storage capacity for all species in summer. A relatively high storage capacity for winter plants of *Polysiphonia decipiens* is inferred by increases in tissue N:P approximating N:P ratios withdrawn from solution and an absence of a seasonal difference in long-term uptake rates. These trends are consistent with differences in the N storage capacities of *Ulva lactuca* and *Chaetomorpha linum* (Pederson and Borum 1996). They are also depicted by reductions in ammonium uptake rates by *Hincksia sordida* and *Ulva* sp. and the sustained ammonium uptake by *Polysiphonia decipiens* over the course of the experiment. A decline in ammonium uptake with time has also been reported for *Gracilaria tikvahiae* (Frielander and Dawes 1985), *Ulva lactuca* (Pederson 1994), *Chaetomorpha linum* (McGlathery et al. 1996) over 28, 25 and 14 day periods respectively. The decline in N uptake by *Gracilaria tikvahiae* coincided with a reduced growth rate and an increased investment in photosynthetic pigment synthesis which in turn supported further N assimilation and storage (Frielander and Dawes

1985). These processes may explain the absence of growth in summer *Ulva* sp. and the lower growth of *Hincksia sordida* in summer (as discussed later).

Increasing uptake capacity when N availability is low or episodic is likely to be advantageous to fast growing macroalgae such as *Hincksia sordida* and *Ulva* sp. which are responsive to changes in N availability and dependent on N for immediate growth demands (Kopczak 1994). An association between high summer ammonium uptake rates and low tissue N has previously been demonstrated for short term ammonium uptake in *Ulva curvata* (Duke et al. 1989), *Chaetomorpha linum* (McGlathery et al. 1996) and *Enteromorpha* spp., *Ulva* sp. and *Gracilaria tikvahiae* (Fujita 1985). Such effects may be controlled by intracellular N pools and provide a temporary mechanism to buffer against the asynchrony of N supply and demand (McGlathery et al. 1996).

In each of the species examined the poor relationship between the amount of N removed from solution and changes in tissue N concentration may be explained by increases in tissue weight diluting the tissue N concentration. It seems an appropriate explanation for the present study because total amounts of tissue N per plant were often close to or exceeded those present initially. Similar findings for *Sargassum muticum* (Phaeophyta) (Lewey and Gorham 1984) and *Ecklonia radiata* (Phaeophyta) (Paling 1991) have been attributed to tissue N dilution. Paling (1991) found that for every 5 mg of ammonium removed from solution 1 mg was stored in the tissues whilst 0.8 mg of nitrate was stored for every 1 mg removed from solution. For *Hincksia sordida* a ratio of 1 mg removed to 0.9 mg taken up was evident across all experimental treatments. This implies that the filamentous *Hincksia sordida* has a highly competitive ability to sequester ammonium which was evident in short-term uptake studies (sections 5.3.1 and 5.3.2; Table 5.1). The disparity between the amount of N removed from solution and that stored in the algae may also imply that there was loss of dissolved or particulate N (DON and PON respectively) from the algal thallus. This may be due to low storage capacity associated with nutrient saturation. Alternatively bacteria could also take up inorganic N and lose it as DON

or PON (Lobban et al. 1985). The concentrations of DON or PON were not measured in these experiments.

6.4.3 Effect of phosphorus on ammonium uptake

The absence of any effect of P on ammonium uptake in winter cultures suggests that N uptake was unaffected by P availability. This is consistent with the similarity in photosynthetic and growth rates of N enriched algae irrespective of P enrichment (section 4.3.1). Lapointe (1985) concluded that the uncoupling or independent uptake of nutrients can be advantageous to algae, allowing particular nutrients to be exploited in the absence of other nutrients. Aisha et al. (1995) demonstrated that the presence of P had no affect ammonium uptake rate over a 24 h period in the Phaeophyte *Dictyota dichotoma*. Such uncoupling may allow transient 'bloom' taxa such as *Hincksia sordida* and *Ulva* sp. to sustain growth, irrespective of low P and light availability, by rapidly sequestering N in winter.

In contrast, the higher total amount of ammonium taken up in summer by P enriched *Hincksia sordida* and *Polysiphonia decipiens*, compared with P limited cultures, suggests that the process of N uptake by these taxa may be coupled to P availability. The higher ammonium assimilation rates translated into slightly higher growth rates for both species (Fig. 4.1) but not to higher photosynthetic performance (Figs. 3.1-3.2). For *Hincksia sordida* this it is unlikely to be of ecological importance given its low or absent biomass during low N availability. Phosphorus enrichment of N replete *Ulva* sp. resulted in higher photosynthetic rates (winter) than those shown for P limited cultures, suggestive of the direct nutritional effects of P on the photosynthetic capacity of *Ulva* sp.

The coupling of N and P uptake may allow N to be continually sequestered during periods of low N availability (e.g. summer) and relatively high P concentrations. Conversely, when N availability is high in winter it would be advantageous for *Polysiphonia decipiens* to maximise its uptake independent of other nutrients and increase storage of N. Such a strategy would allow it to remain competitive for the N

resource. It would be less reliant on an independent uptake strategy in summer due to its a capacity for utilisation of N reserves when N is limiting. The N uptake strategy of *Ulva* sp. in summer appears to differ from that observed for *Polysiphonia decipiens*. Its capacity to take up N in summer independent of P may allow it to take advantage of relatively low concentrations of N, irrespective of other nutrient forms. Such a strategy could be advantageous to annual species such as *Ulva* sp. which depend on rapid growth rates during spring and summer when light and temperature conditions are favourable.

The importance of P availability on N uptake and photosynthetic pigment synthesis has been demonstrated in studies on a number of taxa including the Rhodophyte *Gracilaria tenuistipitata* (Garcia-Sanchez et al. 1996) and the Chlorophytes *Ulva fenestrata* and *Enteromorpha intestinales* (Björnsäter and Wheeler 1990). Ammonium assimilation has been found to increase oxygen consumption and resulted in enhanced ATP production (Weger et al. 1988). Subsequent increases in dark respiration and the breakdown of complex carbohydrates in the mitochondrion may provide further energy for ATP production and N uptake in an ongoing cycle (Prezelin and Nelson 1990). It is possible that the seasonal and species specific responses of these biochemical pathways to changes in nutrient availability may explain the coupling or otherwise of N and P uptake in PPB macroalgae.

6.4.4 Phosphate uptake rates

Phosphate uptake exhibited disparity between removal from solution and change in tissue P concentration, especially in *Polysiphonia decipiens* and summer *Ulva* sp. As found with tissue N, final tissue P per plant approximated and often exceeded initial values. This suggests that P was being retained by the alga although there was evidence for leakage of P into the nutrient medium. The efflux of phosphate found in all species immersed in phosphate enriched seawater may partly explain the highly variable phosphate uptake rates recorded. This is consistent with studies that have shown that phosphate uptake is not a one-way movement into algal cells (Lapointe 1985; Hurd and Dring 1990; Aisha et al. 1995). Uptake is accompanied by an efflux

so that long term net phosphate uptake can be very low or even zero at times (Hurd and Dring 1990).

The apparent efflux of phosphate was most prominent in *Hincksia sordida* in which high uptake rates were followed by a period of zero uptake or a lag phase over periods of days. Lag phases has been reported in short term (0-6 h) studies on *Pelvetia canaliculata* and *Fucus* spp. (Phaeophyta) (Hurd and Dring 1990). These previous authors suggested that a lag phase may represent a period during which transmembrane carriers are stimulated or synthesised and P is bound to carrier sites so that uptake cannot occur. It is also possible that the algae were leaking organic material but it would presumably be energetically unfavourable to assimilate inorganic P and lose it as an organic form (Paling 1991). There is also the possibility that bacteria were taking up P. Either way, the observed rates of uptake probably represent the balance between uptake and loss of the nutrient rather than a single uptake process.

The range of mean daily net uptake rates for *Hincksia sordida* (0.23-22.4 μg PO_4-P g dry wt.$^{-1}$ h^{-1}), *Polysiphonia decipiens* (1.09-44.6 μg PO_4-P g dry wt.$^{-1}$ h^{-1}) and *Ulva* sp. (0.48-41.26 μg PO_4-P g dry wt.$^{-1}$ h^{-1}) are lower than short term rates reported for *Pilayella littoralis* (Wallentinus 1984a) and *Ulva rigida* (Lavery and McComb 1991). They are comparable to short term uptake rates reported for *Ectocarpus siliculosus* and *Ceramium teniucorne* (Wallentinus 1984a), and to the mean short-term (1 h) uptake rates (0.138 to 0.275 μmol g fresh wt.$^{-1}$ h^{-1} or re-calculated as 11.8-67.7 μg PO_4-P g dry wt.$^{-1}$ h^{-1}) reported for *Gracilaria tikvahiae* over a 28 d period (Frielander and Dawes 1985).

6.4.5 Phosphate uptake: seasonal and nutrient enrichment responses

The significantly higher phosphate uptake by summer plants compared with winter plants in all species could not be attributed to initial tissue P concentrations as there was little difference between winter and summer tissue concentrations. Higher P uptake by summer plants may indicate a dependence on DIP during summer when

field concentrations of DIN are relatively low. The decrease in P uptake rates over time in *Ulva* sp. was indicative of a low affinity for P, possibly due to tissue P saturation in both winter and summer plants. The maintenance of P uptake rates over the experimental period by *Hincksia sordida* and *Polysiphonia decipiens* suggests that tissue P saturation was not reached in these taxa implying utilisation or possibly leakage of P.

For *Hincksia sordida* and *Ulva* sp. the coupling of P uptake to N availability was in contrast to *Polysiphonia decipiens* which showed the highest P uptake in N limited cultures. Aisha et al. (1995) demonstrated the ability of the Chlorophyte *Codium dwarkense* to assimilate more P in the presence of N than in N limited cultures and Björnsäter and Wheeler (1990) also found that phosphate uptake in *Ulva fenestrata* was coupled to N availability. The reduction in P uptake with N limitation may be a function of a reduced requirement for P by biochemical processes involved in N uptake. This was demonstrated by the low photosynthetic and growth responses of N limited cultures (see Chapters 3 and 4). Alternatively the depressed physiological state that follows exposure to zero or low N availability may contribute to decreased energy available for assimilation of P or energetically unfavourable responses such as leakage of organic P into solution (Paling 1991). Stores of polyphosphates in macroalgae can also be depleted by enzymatic hydrolysis at times of nutrient stress (Lin 1977).

By contrast, the absence of any reliance of P uptake by *Polysiphonia decipiens* on N availability suggests an uncoupling of P and N uptake (as demonstrated for winter N uptake). It reinforces the notion previously put forward that, of the three taxa examined, this species is least affected by low N availability. Lapointe (1985) also showed an enhanced capacity for P uptake by *Gracilaria tikvahiae* under nutrient limited conditions and attributed this to P limitation and the importance of P towards regulating growth. The importance of P in *Polysiphonia decipiens* has been previously discussed in sections 3.4.1-2.

On the other hand, the low P uptake by N enriched *Polysiphonia decipiens* may be due to increased cellular energy requirements being expended on active N uptake kinetics (section 5.3.2). In the absence of N this taxa is able to use its energy to acquire P at optimal rates. Such a strategy is consistent with the hypothesis that this species has a high requirement for P independent of N availability and exhibits a unique nutrient uptake strategy compared with the other two taxa examined. As previously mentioned, the independent uptake of nutrients can be advantageous to algae, allowing particular nutrients to be exploited in the absence of other nutrients (Lapointe 1985). It may also be important for relatively slow growing taxa such as *Polysiphonia decipiens* which require P for metabolic purposes and are unable to rapidly exploit high concentrations of nutrients.

6.4.6 Summary

The long-term (over days) ammonium and phosphate uptake by macroalgae was found to be dependent on seasonal nutritional history and ambient nutrient availability. Such relationships are consistent with an inverse relationship between nutrient affinity and storage capacity (Pederson and Borum 1996). The higher uptake of ammonium by summer *Hincksia sordida* and *Ulva* sp. than winter cultures was in contrast with *Polysiphonia decipiens*. The latter species was able to assimalate ammonium irrespective of tissue N status, indicative of a high storage capacity. For *Hincksia sordida* and *Ulva* sp. the coupling of P uptake to N availability was also in contrast to *Polysiphonia decipiens* which showed the highest P uptake in N limited cultures.

The differences in these relationships between species offer insights into the relationship between the seasonal growth dynamics of individual taxa within macroalgal communities and their long-term nutrient requirements. Potential N limitation is implied by higher N uptake rates by algae previously starved of N. The contrasting seasonal responses of species with respect to the influence of P on N

uptake may reflect their differing seasonal requirements for N to maximise growth and/or increase reserves of N. In addition, the uncoupling of uptake and growth may sustain nutrient uptake and buffer against fluctuating nutrient availability and potential nutrient limitation. The coupling of long-term N and P uptake is also suggestive of the potential for P limitation in temperate marine environs. Conversely, the independence of N and P uptake (e.g. high P uptake in the absence of N) indicates that in some taxa (e.g. *Polysiphonia decipiens*) the requirement for external N may be offset by internal reserves of N.

Chapter 7

7.1 Conclusions

The work presented in this thesis was designed to meet three primary objectives;

1. To describe the changes in photosynthetic characteristics of dominant macroalgal species from Port Phillip Bay in relation to environmental factors;

2. To determine the direct effects of nutrient enrichment on physiology, nutrient requirements and growth of dominant macroalgae from this system; and

3. To describe short and long-term nutrient uptake characteristics of these algae.

The physiological responses (i.e. P_{max} , α and I_k, growth) of the species to changing nutrient supply were examined and these experiments provided explanations for the physiological mechanisms that contributed to seasonal shifts in species dominance. The importance of N towards pigment synthesis conclusively showed that N limits photosynthesis and growth in Port Phillip Bay macroalgae when other factors are non limiting. The monthly variation in maximum photosynthetic rate (P_{max}) and efficiency (α) for each of the species examined in this study reflected changing tissue nutrient status, pigmentation and external nutrient supply and light availability. Nitrogen to phosphorus and carbon to nitrogen ratios reflected storage capacities of nutrients. Elevated tissue N:P ratios implied N sufficiency and low N:P and C:N ratios implied N limitation. Hence, these macroalgae may be useful bio-indicators for detecting trends in N loads from nearby wastewater inputs.

The kinetics of short term ammonium uptake revealed that short-term uptake was either bi- or multiphasic. This means that while active ammonium uptake is saturated

at low concentrations total uptake can be rapidly increased by diffusive processes at high concentrations. Nutrient uptake mechanisms also varied seasonally and between species and provided clear insight into the different strategies that macroalgae may employ to take advantage of environmental conditions and buffer against limiting conditions. Such factors contributed to the wide temporal variation in primary production exhibited by each species over their annual growth cycle.

The following is a summary of the physiological attributes of each species, the environmental conditions that best suit these attributes and an assessment of whether these attributes are consistent with field observations.

Hincksia sordida

P_{max} of the opportunistic taxa, *Hincksia sordida*, showed high fluctuation and a unimodal seasonality consistent with changes in *in situ* biomass. Optimal P_{max} of *Hinckisa sordida* was maintained at low temperatures in winter when N supply was high, suggesting that this species would be more prone to N limitation in the field than the other species examined. Blooms of *Hincksia* increased in frequency between autumn and winter when temperatures decreased and N supply increased from the Western Treatment Plant. Therefore DIN appeared to be a major factor contributing to *Hincksia sordida* blooms. The ability of *Hincksia* to grow during periods of high N availability was also consistent with its high short-term N uptake capacity, allowing it to take advantage of high N inputs and maintain metabolic activity when temperature and light are limiting. The propensity for this species to be N limited was demonstrated by a low N storage capacity and a reduced capacity of internal reserves of N to sustain growth. The observed collapse in standing biomass of this species may therefore be partly due to a low capacity for N storage or survival at reduced growth rates, especially in late spring and early summer when N availability is low.
By late summer high growth rates of *Hincksia sordida* were again observed. The development of these blooms coincided with settled weather conditions, saturating

light conditions and elevated temperatures. This suggests that this species may also take advantage of elevated light and temperature conditions, irrespective of N supply. It is possible that the flux of remineralised nutrients from sediments may provide a N source when water column nutrients are scarce.

Further, characteristics of its uptake kinetics indicate that it is conferred a competitive advantage at low DIN concentrations and this may in part also explain its ability to bloom in summer. Surge uptake may be ecologically important to *Hincksia sordida* for utilisation at low DIN concentrations and could be dependent on controls exerted by intracellular pools of N. At high DIN concentrations the immediate growth demands of *Hincksia sordida* was suggestive of an uncoupling of N uptake and metabolic demand, as shown by higher ammonium uptake in summer than in winter, without commensurate photosynthetic or growth enhancement.

The decline in its ammonium-N and phosphate-P uptake over time is confirmation that this species has a weak capacity for N and P storage and are prone to nutrient limitation. An increase in PSII (accessory pigments) relative to PSI (Chl *a*) in *Hincksia sordida* may serve to optimise photosynthetic efficiency by synthesising new photosynthetic units during rapid growth and high N availability. Moreover, the maintenance of PSII in N limited summer plants, relative to N limited winter plants may be of adaptive significance by providing relief for N limitation during summer.

Polysiphonia decipiens

The photosynthetic rates and N requirement of *Polysiphonia decipiens* were relatively stable over the annual cycle suggesting that this species is limited less by N supply than the other opportunistic taxa. *Polysiphonia decipiens* also showed the lowest light requirement of all taxa which is typical of the Rhodophyta. Compared to the other species examined *Polysiphonia decipiens* has a larger capacity for N storage that enables survival for relatively long periods when N is limiting. This was

demonstrated to be an important characteristic that allowed *Polysiphonia decipiens* to buffer against fluctuations in N supply and therefore have a competitive advantage during periods of low N supply. The steady long-term ammonium-N uptake throughout both summer and winter for *Polysiphonia decipiens* was also consistent with its high storage capacity for N. *Polysiphonia decipiens* appears to offset relatively low uptake rates by assimilating equal amounts of N in winter and summer. The relatively consistent growth and standing biomass of this species may also be due to a high capacity for N storage which allows relatively long term survival at reduced growth rates (16 – 34 d) compared to other species examined.

The correlation between pigment concentrations and tissue P for *Polysiphonia decipiens* suggested that P is important for the maintenance of N assimilation for pigment synthesis. This was reinforced by the capacity of P enriched winter cultures to take up higher amounts of N in the long-term than P limited cultures. In addition, an increase in PSII (accessory pigments) relative to PSI (Chl *a*) P*olysiphonia decipiens* may serve to optimise photosynthetic efficiency during periods of high N availability. This supports the proposition that accessory pigments use and/or store excess N which then may be utilised during rapid growth to synthesise new photosynthetic units during N limiting conditions in summer. Moreover, the maintenance of PSII in N limited summer plants, relative to N limited winter plants may be of adaptive significance by providing relief for N limitation during summer.
The coupling of N to P availability may also serve to optimise N uptake at low concentrations (when P availability is high) and increase internal reserves of N. Conversely, a high P uptake capacity by *Polysiphonia decipiens* in the absence of N in winter and summer experiments implied a particular importance for P in this species. The independent uptake of P when N is limiting may be important for relatively slow growing taxa such as *Polysiphonia decipiens* which require P for metabolic purposes but are unable to rapidly exploit high concentrations of nutrients. This may enable

more efficient nutrient uptake that is less dependent on the availability of other nutrient forms.

***Ulva* sp.**

Light and temperature appear to be the primary determinants of *Ulva* sp. growth in Port Phillip Bay. In summer and autumn, when water temperatures are high and photon flux and daylength are intermediate to high, maximum photosynthetic rates coincided with maximum growth of *Ulva* sp. Enhanced P_{max} in N and P enriched *Ulva* sp. cultures revealed the importance of N and P to photosynthetic processes.

The requirement of N for P uptake by *Ulva* sp. inferred a propensity for N limitation. As a response to N limitation *Ulva* sp. was able to store N and use it for physiological requirements at the expense of growth. The decline in ammonium-N and phosphate-P uptake over time in *Ulva* sp. is confirmation that this species has a weak capacity for N and P storage and is prone to nutrient limitation, Difference in V_{max}:K_s ratios between surge and assimilation uptake indicated that surge uptake at low concentrations was not important for *Ulva* sp. and that this species is most likely to be N limited of all taxa examined. The uncoupling of N and P uptake inferred a capacity of *Ulva* sp. to maximise its nutrient uptake. This independent N uptake strategy of in summer may serve to optimise N uptake, irrespective of P availability, allowing plants to take advantage of favourable light and temperature conditions. Such a strategy may be of ecological importance for fast growing spring-summer annuals such as *Ulva* sp.

Undaria pinnatifida

For *Undaria pinnatifida* differences in short-term ammonium uptake kinetics were dependent on the maturity of the algae examined. Mature tissue was found to take up ammonium at rates significantly higher than immature tissue. Difference in V_{max}:K_s

ratios between surge and assimilation uptake indicated that surge uptake at low concentrations was less important for immature *Undaria pinnatifida* than mature *Undaria pinnatifida*. These findings suggest that mature plants are able to exploit DIN at low and high concentrations. As such *Undaria pinnatifida* is an important component of the macrolagal community and a dominant contributor to nutrient cycling in near-shore waters in PPB.

7.2 Concluding remarks

The study has yielded data on production rates and nutrient requirements of some subtidal macroalgae in Port Phillip Bay. The examination of the critical factors that control macroalgal physiology has provided a functional and conceptual understanding of the processes that govern productivity and growth of macroalgae in response to light, temperature and nutrients. The capacity of macroalgae to integrate nutrients over time, and their ability to exhibit physiological signals in response to temporal variation in nutrient availability, allows them to be used as indicators of nutrient enrichment in nearshore areas of Port Phillip Bay.

The significance of this study is that these assessments of macroalgal physiology provide an understanding of the environmental conditions and processes that dictate shifts in species composition and dominance. Such information is important if we are to predict the environmental triggers leading to eutrophication and implement controls to ameliorate its impacts on near-shore marine biological communities.

References

American Public Health Association (1990). *Standard methods for analysis of waters and wastewaters*. 19th edition. Washington, D.C.

Allen, S.E. (1989). *Chemical analysis of ecological materials*. Blackwell Scientific Publications, Oxford. 368 pp.

Aisha, K.A., Shabana, E.F., El-Abyad, M.S., Kobbia, I.A. and Schanz, F. (1995). Pulse feeding with nitrate and phosphate in relation to tissue composition and nutrient uptake by some macroalgae from the Red Sea at Ghardaqa (Egypt). *Journal of Basic Microbiology*, **35**: 135-145.

Arnold, K.E. and Murray, S.N. (1980). Relationships between irradiance and photosynthesis for marine benthic green algae (Chlorophyta) of differing morphologies. *Journal of Experimental Marine Biology and Ecology*, **43**: 183-192.

Asare, S.O. and Harlin, M.M. (1983). Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Rhode Island Sound. *Journal of Phycology*, **19**: 254-257.

Atkinson, M.J. and Smith, S.V. (1983). C:N:P ratios of benthic marine plants. *Limnology and Oceanography*, **9**: 568-574.

Axelrad, D.M., Poore, G.C.B., Arnott, G.H., Bauld, J., Edwards, R.R.C., Brown, V.B. and Hickman, N.J. (1981). The effects of treated sewage discharge on the biota of Port Phillip Bay, Victoria, Australia. In: *Proceedings of the first International Symposium on Nutrient Enrichment of Estuaries, Virginia*, USA. 29-31 May, 1979. (Neilson B.J. and Cronin L.E. eds.), Humana Press, New Jersey. pp. 279-306.

Bidwell, R.G.S. and McLachlan, J. (1985). Carbon nutrition of seaweeds: photosynthesis, photorespiration and respiration. *Journal of Experimental Marine Biology and Ecology*, **86**: 15-46.

Birch, P.B., Gordon, D.M. and McComb, A.J. (1981). Nitrogen and phosphorus nutrition of *Cladophora* in the Peel-Harvey estuarine system, Western Australia. *Botanica Marina*, **24**: 381-387.

Bird, K.T., Habig, C. and DeBusk, T. (1982). Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *Journal of Phycology*, **18**: 344-348.

Björnsäter, B.R. and Wheeler, P.A. (1990). Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). *Journal of Phycology*, **26**: 603-611.

Boalch, G.T. (1961). Studies on *Ectocarpus* in culture. II. Growth and nutrition of a bacteria-free culture. *Journal of the Marine Biological Association of the United Kingdom*, **41**: 287-304.

Borowitska, M.A. (1972). Intertidal algal species diversity and the effect of pollution. *Australian Journal of Marine and Freshwater Research*, **23**: 73-84.

Brinkhuis, B.H. (1977). Seasonal variations in salt-marsh macroalgae photosynthesis. II. *Fucus vesiculosus* and *Ulva lactuca*. *Marine Biology* **44**: 165-175.

Brown, V.B., Rowan, K.S. and Ducker, S.C. (1980). The effects of sewage effluent on the macrophytes off Werribee, Port Phillip Bay. Environmental Studies Program Task Report No. 273. Ministry for Conservation, Victoria.

Brown, V.B., Davies, S.A. and Synnot, R.N. (1990). Long term monitoring of the effects of treated sewage effluent on the intertidal macroalgal commnity near Cape Schanck, Victoria, Australia. *Botanica Marina*, **33**: 85-98.

Cambridge, M.L. and McComb, A.J. (1984). The loss of seagrass in Cockburn Sound, Western Australia. I. The time course and magnitude of seagrass decline in relation to industrial development. *Aquatic Botany*, **20**, 229-243.

Cambridge, M.L. Chiffings, A.W., Brittan, C. Moore, L. and McComb, A.J. (1984). The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. *Aquatic Botany*, **24**: 269-285.

Campbell, S.J. and Burridge, T.R. (1998). *The effects of sewage discharges on the ecological processes involving marine flora in southern Australian waters.* Urban Water Research Association of Australia, Research Report No. 140.

Campbell, S.J. and Burridge, T.R. (1998). Occurrence of *Undaria pinnatifida* (Phaeophyta Laminariales) in Port Phillip Bay, Victoria, Australia. *Marine and Freshwater Research*, **49**: 379-381.

Campbell, S.J., Bité, J.S. and Burridge T.R. (1999). Patterns in the photosynthetic capacity, tissue pigment and nutrient content of the introduced kelp *Undaria pinnatifida* (Phaeophyta : Laminariales) in Port Phillip Bay, Victoria, Australia *Botanica Marina*, **42**: in press.

Chapman, A.R.O. and Craigie, J.S. (1977). Seasonal growth in *Laminaria longicruris*: relation with dissolved inorganic nutrients and internal reserves of nitrogen. *Marine Biology*, **40**: 197-205.

Chapman, A.R.O., Markham, J.W. and Lüning, K. (1978). Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *Journal of Phycology*, **14**: 195-198.

Chopin, T., Gallant, T. and Davison, I. (1995). Phosphorus and nitrogen nutrition in *Chondrus crispus* (Rhodophyta): effects on total phosphorus and nitrogen content, carrageenan production, and photosynthetic pigments and metabolism. *Journal of Phycology*, **31**: 283-293.

Chopin, T., Hanisak, M. D. and Koehn, F.E. (1991). Effects of seawater phosphorus concentration on floridean starch content in *Agardhiella subulata* (C. Agardh) Kraft and Wynne (Rhodophyceae, Solieriaceae). *Journal of Applied Phycology*, **2**: 3-16.

Christie, N.D. (1981). Primary production in Langebaan Lagoon. In: *Estuarine Ecology with Particular reference to Southern Africa.* (Day, J.H. ed.), A.A. Balkema, Rotterdam. pp. 101-115.

Clayton, M.N. (1990). The adaptive significance of life history characters in selected orders of marine brown macroalgae. *Australian Journal of Ecology*, **15**: 439-452.

Cohen, I. and Neori, A. (1991). *Ulva lactuca* biofilters for marine fishpond effluents. i. ammonia uptake kinetics and nitrogen content. *Botanica Marina*, **34**: 475-482.

Connolly, N.J. and Drew, E.A. (1985). Physiology of *Laminaria*. IV. Nutrient supply and daylength, major factors affecting growth of *L. digitata* and *L. saccharina*. *Marine Ecology*, **6**: 299-320.

Davison, I. R. 1991. Environmental effects of algal photosynthesis: temperature. *Journal of Phycology*, **27**: 2-8.

Davison, I.R., Greene, R.M. and Podolak, E.J. (1991). Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Marine Biology*, **110**: 449-454.

Dawes, C.J., Lawrence, J.M., Cheney, D.P. and Mathieson, A.C. (1974). Ecological studies of Floridian *Eucheuma* (Rhodophyta Gigartinales). III. Seasonal variation of carrageenan, total carbohydrate, protein and lipid. *Bulletin of Marine Science*, **24**: 286-299.

Dawes, C.J., Moon, R.E. and Davis, M.A. (1978). The photosynthetic and respiratory rates and tolerances of benthic algae from a mangrove and salt marsh estuary: a comparative study. *Estuarine and Coastal Marine Science*, **6**: 175-185.

Dawes, C.J., Chen, C.-P., Jewett-Smith, J., Marsh, A. and Watts, S.A. (1984). Effect of phosphate and ammonium levels on photosynthetic and respiratory responses of the red alga *Gracilaria verrucosa*. *Marine Biology*, **78**: 325-328.

DeBoer, J.A. (1981). Nutrients. In: *The biology of seaweeds.* (Lobban, C.S. and Wynne, M.J. eds.), Blackwell Scientific Publications, Oxford. pp. 356-392.

DeBoer, J.A. and Ryther, J.H. (1978). Potential yields from a waste recycling algal mariculture system. In: *The marine plant biomass of the Pacific Northwest coast.* (Krauss, R. ed.), Oregon State University Press, Corvallis. pp. 231-249.

DeBoer, J.A., Guigli, H.J., Israel T.L., and D'Elia, C.F. (1978). Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. *Journal of Phycology*, **14**: 261-266.

D'Elia, C.F. and DeBoer, J.A. (1978). Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. *Journal of Phycology*, **14**: 266-272.

Dortch, Q. (1982). Effect of growth conditions on accumulation of internal nitrate, ammonium amino acids, and protein in three marine diatoms. *Journal of Experimental Marine Biology and Ecology*, **61**: 243-64.

Dowd, J.E. and Riggs, D.S. (1965). A comparison of estimates of Michaelis Menten kinetic constants from various linear transformations. *Journal of Biology and Chemistry*, **240**: 863-869.

Droop, M.R. (1973). Some thoughts on nutrient limitation in algae. *Journal of Phycology*, **9**: 264-272.

Duarte, C.M. (1995). Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia*, **41**: 87-112.

Duke, C.S., Lapointe, B.E. and Ramus, J. (1986). Effects of light on growth, RuBPCase activity and chemical composition of *Ulva* species (Chlorophyta). *Journal of Phycology*, **22**: 362-370.

Duke, C.S., Litaker, W. and Ramus, J. (1987). Seasonal variation in RuBPCase activity and N allocation in the chlorophyte seaweeds *Ulva curvata* (Kütz.) de Toni and *Codium decoratium* (Woodw.) Howe. *Journal of Experimental Marine Biology and Ecology*, **112**: 145-164.

Duke, C.S., Litaker, W. and Ramus, J. (1989). Effects of temperature, nitrogen supply and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. *Journal of Phycology*, **25**: 113-120.

Duncan, M.J. and Harrison, P.J. (1982). Comparison of solvents for extracting chlorophylls from marine macrophytes. *Botanica Marina*, **25**: 445-447.

Edwards, P. (1972). Benthic algae in polluted estuaries. *Marine Pollution Bulletin*, **3**: 55-60.

Ekman, P., Lignell, A. and Pederson, M. (1989). Localization of ribulose-1,5-biphosphate carboxylase/oxygenase in *Gracilaria secundata* (Rhodophyta) and its role as a nitrogen storage pool. *Botanica Marina*, **32**: 527-534.

Falkowski, P.G. (1983). Enzymology of nitrogen assimilation. In: *Nitrogen in the marine environment.* (Carpenter, E.J. and Capone, D.G. eds.), Academic Press, New York. pp. 839-868.

Fillit, M. (1995). Seasonal changes in the photosynthetic capacities and pigment content of *Ulva rigida* in a Meditteranean coastal lagoon. *Botanica Marina*, **38**: 271-280.

Flores-Moya, A., Fernandez, J.A. and Neill, F.X. (1995). Seasonal variations of photosynthetic pigments, total C, N, and P content, and photosynthesis in *Phyllariopsis purpurascens* (Phaeophyta) from the Strait of Gibraltar. *Journal of Phycology*, **31**: 867-874.

Fong, P., Zedler, J.B. and Donohue, R.M. (1993a). Nitrogen vs. phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnology and Oceanography*, **38**: 906-923.

Fong, P., Zedler, J.B. and Donohue, R.M. (1993b). Competition with macroalgae and benthic cyanobacterial mats limits phytoplankton abundance in experimental microcosms. *Marine Ecology Progress Series*, **100**: 97-102.

Fong, P., Donohue, R.M. and Zedler, J.B. (1994). Nutrient concentration in tissue of the macroalga *Enteromorpha* as a function of nutrient history: an experimental evaluation using field microcosms. *Marine Ecology Progress Series*, **106**: 273-281.

Fonselius, St. H. (1978). On nutrients and their role as production limiting factors in the Baltic. *Acta Hydrochimica et Hydrobiologia*, **6**: 329-339.

Fralick, R.A. and Mathieson, A.C. (1975). Physiological ecology of four *Polysiphonia* species (Rhodophyta, Ceramiales). *Marine Biology*, **29**: 29-36.

Frielander, M.D., and Dawes C.J. (1985). *In situ* kinetics of ammonium and phosphate and chemical composition of the red seaweed *Gracilaria tikvahiae*. *Journal of Phycology*, **21**: 448-453.

Fujita, R.M. (1985). The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. *Journal of Experimental Marine Biology and Ecology*, **92**: 283-301.

Fujita, R.M. and Goldman, J.C. (1985). Nutrient flux and growth of the red alga *Gracilaria tikvahiae* McLachlan (Rhodophyta). *Botanica Marina*, **28**: 265-268.

Fujita, R.M., Wheeler, P.A. and Edwards, R.L. (1988). Metabolic regulation of ammonium uptake by *Ulva rigida* (Chlorophyta): a compartmental analysis of the rate limiting step for uptake. *Journal of Phycology*, **23**: 560-566.

Fujita, R.M., Wheeler, P.A. and Edwards, R.L. (1989). Assessment of macroalgal nitrogen limitation in a seasonal upwelling region. *Marine Ecology Progress Series*, **53**: 293-303.

Gao, K. and Nakahara, H. (1990). Effects of nutrients on the photosynthesis of *Sargassum thunbergii*. *Botanica Marina*, **33**: 375-383.

Garcia-Sanchez, M.J., Fernandez, J.A. and Niell, F.X. (1996). Photosynthetic response of P-deficient *Gracilaria tenuistipitata* under two different phosphate treatments. *Physiologia Plantrum*, **96**: 601-606.

Gattuso, J.P. and Jaubert, J. (1985). Photosynthesis and respiration of *Caulerpa racemosa* (Chlorophyceae, Caulerpales) grown in aquaria: effects of light and temperature. *Botanica Marina*, **28**: 327-332.

Gayol, P., Falconetti, C., Chisolm, J.R.M. and Jaubert, J.M. (1995). Metabolic responses of low temperature-acclimated *Caulerpa taxifolia* (Chlorophyta) to rapidly elevated temperature. *Botanica Marina*, **38**: 61-67.

Geertz-Hansen, O. and Sand-Jensen, K. (1992). Growth rates and photon yield of growth in natural populations of a marine macroalga *Ulva lactuca*. *Marine Ecology Progress Series*, **81**: 179-183.

Gerard, V.A. (1982). *In situ* rates of nitrate uptake by the giant kelp, *Macrocystis pyrifera* (L.) C. Agardh: Tissue differences, environmental effects, and predictions of nitrogen limited growth. *Journal of Experimental Marine Biology and Ecology*, **62**: 211-224.

Goldman, (1977). Steady state growth of phytoplankton in continuous culture: comparison of internal and external nutrient equations. *Journal of Phycology*, **13**: 251-258.

Goldman, (1979). Outdoor mass cultures - II. photosynthetic yield limitations. *Water Research*, **13**: 119-136.

Gomez, I. and Wiencke, C. (1996). Photosynthesis, dark respiration and pigment contents of gametophytes and sporophytes of the Antarctic brown alga *Desmarestia menziesii*. *Botanica Marina*, **39**: 149-157.

Gomez, I., Wiencke, C. and Weykam, G. (1995). Seasonal photosynthetic characteristics of *Ascoseira mirabilis* (Ascoseirales, Phaeophyceae) from King George Island, Antarctica. *Marine Biology*, **123**: 167-172.

Gordon, D.M. and McComb, A.J. (1989). Growth and production of the green alga *Cladophora montagneana* in a eutrophic Australian estuary and its interpretation using a computer program. *Water Research*, **23** (5): 633-645.

Gordon, D.M., Birch, P.B. and McComb, A.J. (1980). The effects of light, temperature and salinity on photosynthetic rates of an estuarine *Cladophora*. *Botanica Marina*, **23**: 749-755.

Gordon, D.M., Birch, P.B. and McComb, A.J. (1981). Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Botanica Marina*, **24**: 93-106.

Haines, A.C. and Wheeler, P.A. (1978). Ammonium and nitrate uptake by the marine macrophytes *Hypnea musciformis* (Rhodophyta), and *Macrocystis pyrifera* (Phaeophyta). *Journal of Phycology*, **14**: 319-324.

Hanisak, M.D. (1979). Nitrogen limitation of *Codium fragile* spp. *tomentosoides* as determined by tissue analysis. *Marine Biology*, **50**: 333-337.

Hanisak, M.D. (1983). The nitrogen relationships of marine macroalgae. In: *Nitrogen in the marine environment*. (Carpenter, E.J. and Capone, D.G. eds.), Academic Press, New York. pp. 699-730.

Hanisak, M.D. (1990). The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand nitrogen nutrition in cultured seaweeds. *Hydrobiologia*, **204/205**: 79-87.

Harlin, M.M. and Wheeler, P.A. (1985). Nutrient uptake. In: *Handbook of phycological methods. Ecological field methods: macroalgae*. (Littler, M.M. and Littler, D.S. eds.), Cambridge University Press, London. pp. 493-508.

Harrison, P.J., Druehl, L.D., Lloyd, K.E. and Thompson, P.A. (1986). Nitrogen uptake kinetics in three year classes of *Laminaria groelandica* (Laminariales: Phaeophyta). *Marine Biology*, **93**: 29-35.

Harrison, P.J., Parslow, J.S. and Conway, H.L. (1989). Determination of nutrient uptake parameters: a comparison of methods. *Marine Ecology Progress Series*, **52**: 301-312.

Hein, M., Pederson, M.F. and Sand-Jensen, K. (1995). Size-dependent nitrogen uptake in micro- and macroalgae. *Marine Ecology Progress Series*, **118**: 247-253

Henley, W.J. and Dunton, K.H. (1995). A seasonal comparison of carbon, nitrogen, and pigment content in *Laminaria solidungula* and *L. saccharina* (Phaeophyta) in the Alaskan Arctic. *Journal of Phycology*, **31**: 325-331.

Hernandez, I., Niell, F.X. and Fernandez, J.A. (1992). Alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. *Journal of Experimental Marine Biology and Ecology*, **159**: 1-13.

Hernandez, I., Fernandez, J.A. and Niell, F.X. (1993). Influence of phosphorus status on the seasonal variation of alkaline phosphatase activity in *Porphyra umbilicalis* (L.) Kützing. *Journal of Experimental Marine Biology and Ecology*, **173**: 181-196.

Hernandez, I., Peralta, G., Perez-Llorens, L.,Vergara, J.J. and Niell, X.F. (1997). Biomass and dynamics of growth of *Ulva* species in Palmones River Estuary. *Journal of Phycology*, 33: 764-772.

Hoffman, W.E. and Dawes, C.J. (1980). Photosynthetic rates and primary production by two Florida benthic red algal species from a salt marsh and mangrove community. *Bulletin of Marine Science*, **30**: 358-364.

Horrocks, J.L., Stewart, G.R. and Dennison, W.C. (1995). Tissue nutrient content of *Gracilaria* spp. (Rhodophyta) and water quality along an estuarine gradient. *Marine and Freshwater Research*, **46**: 975-983.

Howarth, R.W. (1988). Nutrient limitation of net primary production in marine ecosystems. *Annual Revue of Ecology*, **19**: 89-110.

Hurd, C.L. and Dring, M.J. (1990). Phosphate uptake by intertidal algae in relation to zonation and season. *Marine Biology*, **107**: 281-289.

Hurd, C. L., Galvin, R.S., Norton, T.A. and Dring, M.J. (1993). Production of hyaline hairs by intertidal species of *Fucus* (Fucales) and their role in phosphate uptake. *Journal of Phycology*, **29**: 160-165.

Hurd, C.L., Harrison, P.J. and Druehl, L.D. (1996). Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of *Macrocystis integrifolia* from wave sheltered and exposed sites. *Marine Biology*, **126**: 205-214.

Hwang, S-P. L., Williams, S.L. and Brinkhuis, B.H. (1987). Changes in internal dissolved nitrogen pools as related to nitrate uptake and assimilation in *Gracilaria tikvahiae* McLachlan (Rhodophyta). *Botanica Marina*, **30**: 11-19.

Jassby, A. D. and Platt T. (1976). Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnology and Oceanography*, **212**: 215-222.

Jeffrey, S.W. and Haxo F.T. (1968). Photosynthetic pigments of symbiotic dinoflagellates (zooxanthellae) from corals and clams. *Biological Bulletin*, **135**: 149-165.

Jeffrey, D.W., Madden, B. and Rafferty, B. (1993). Beach fouling by *Ectocarpus siliculosus* in Dublin Bay. *Marine Pollution Bulletin*, **26** (1): 51-54.

Jeffrey, S.W., Jennings, M.T., Jennings, E., Madden, B. and Wilson, J.G. (1995). Nutrient sources for in-shore nuisance macroalgae - The Dublin Bay Case. *Ophelia*, **42**: 147-161.

Jones, A.B., Dennison, W.C. and Stewart, G.R. (1996). Macroalgal responses to nitrogen source and availability: amino acid metabolic profiling as a bioindicator using *Gracilaria edulis* (Rhodophyta). *Journal of Phycology*, **32**: 757-766.

Kautsky, L. (1982). Primary production and uptake kinetics of ammonium and phosphate uptake by *Enteromorpha compressa* in an ammonium sulfate industry outlet area. *Aquatic Botany*, **12**: 23-40.

Kindig, A.C. and Littler, M.M. (1980). Growth and primary production of marine macrophytes exposed to domestic sewage effluent. *Marine Environmental Research*, **3**: 81-100.

King, R. J. and Schramm, W. (1976). Photosynthetic rates of benthic marine algae in relation to light intensity and seasonal variations. *Marine Biology*, **37**: 215-222.

Kirk, J.T.O. (1983). *Light and photosynthesis in aquatic ecosystems*. Cambridge University Press, Cambridge. 401 pp.

Koch, E.W. (1993). The effect of water flow on photosynthetic processes of the alga *Ulva lactuca* L. *Hydrobiologia*, **260/261**, 457-462.

Kolber, A., Zehr, J. and Falkowski, P. (1988). Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. *Plant Physiology*, **88**: 923-929.

Kopczak, C.D. (1994). Variability of nitrate uptake capacity in *Macrocystis pyrifera* (Laminariales, Phaeophyta) with nitrate uptake and light availability. *Journal of Phycology*, **30**: 573-580.

Krause-Jensen, D., McGlathery, K. Rysgaard, S. and Christensen P.B. (1996). Production within dense mats of the filamentous macroalga *Chaetomorpha linum* in relation to light and nutrient availability. *Marine Ecology Progress Series*, **134**: 207-216.

Kuebler, J. E., Davison, I.R. and Yarish, C. (1991). Photosynthetic temperature adaptation in the red algae *Lomentaria baileyana* and *Lomentaria orcadensis*. *British Phycology Journal*, **26**: 9-19.

Kuppers, U. and Weidner, M. (1980). Seasonal variation of enzyme activities in *Laminaria hyperborea*. *Planta*, **148**: 222-230.

Laing, W.A., Christeller, J.T. and Terzaghi, B.E. (1989). The effect of temperature, photon flux density and nitrogen on growth of *Gracilaria sordida* Nelson (Rhodophyta). *Botanica Marina*, **32**: 439-445.

Lapointe, B. E. (1981). The effects of light and nitrogen on growth, pigment content, and biochemical composition of *Gracilaria foliifera* v. *angustissima* (Gigartinales, Rhodophyta). *Journal of Phycology*, **17**: 90-95.

Lapointe, B.E. (1985). Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: interactions between concentration and frequency of nutrient pulses. *Journal of Experimental Marine Biology and Ecology*, **93**: 211-222.

Lapointe, B.E. (1987). Phosphorus and nitrogen limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental study. *Marine Biology*, **93**: 561-568.

Lapointe, B.E. and Duke, C.S. (1984). Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *Journal of Phycology*, **20**: 488-495.

Lapointe, B.E. and O'Connell, J. (1989). Nutrient enhanced growth of *Cladophora prolifera* in Harrington Sound, Bermuda: eutrophication of a confined, phosphorus limited marine ecosystem. *Estuarine, Coastal and Shelf Science*, **28**: 347-360.

Lapointe, B.E., and Ryther, J.H. (1979). The effects of nitrogen and seawater flow on the growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor cultures. *Botanica Marina*, **22**: 529-537.

Lapointe, B.E. and Tenore, K.R. (1981). Experimental outdoor studies with *Ulva fasciata* Delile. I. Interaction of light and nitrogen on nutrient uptake, growth and biochemical composition. *Journal of Experimental Marine Biology and Ecology*, **53**: 135-152.

Lapointe, B.E., Tenore, K.R. and Dawes, C.J. (1984a). Interactions between light and temperature on the physiological ecology of *Gracilaria tikvahiae* (Gigartinales: Rhodophyta). I. Growth, photosynthesis and respiration. *Marine Biology*, **80**: 161-170.

Lapointe, B.E., Dawes, C.J. and Tenore, K.R. (1984b). Interactions between light and temperature on the physiological ecology of *Gracilaria tikvahiae* (Gigartinales: Rhodophyta). II. Nitrate uptake and levels of pigments and chemical constituents. *Marine Biology*, **80**: 171-178.

Lavery, P.S. and McComb, A.J. (1991a). Macroalgal-sediment nutrient interactions and their importance to macroalgal nutrition in a eutrophic estuary. *Estuarine, Coastal and Shelf Science*, **32**: 281-295.

Lavery, P.S. and McComb, A.J. (1991b). The nutritional eco-physiology of *Chaetomorpha linum* and *Ulva rigida* in Peel Inlet, Western Australia, *Botanica Marina*, **34**: 251-260.

Lavery, P.S., Lukatelich, R.J. and McComb, A.J. (1991). Changes in the biomass and species composition of macroalgae in a eutrophic estuary. *Estuarine,Coastal and Shelf Science*, **33**: 1-22.

Layzell, D.B. (1990). N_2 fixation, NO_3^- reduction and NH_4^+ assimilation. In: *Plant physiology, biochemistry and molecular biology.* (Dennis, D.T. and Turpin, D.H. eds.), Longman Scientific and Technical Publications, Singapore. pp. 212-223.

Lee,V. and Olsen, S. (1985). Eutrophication and management initiatives for the control of nutrient inputs to Rhode Island coastal lagoons. *Estuaries*, **8**: 191-202.

Lehninger, A.L. (1973). *Short course in Biochemistry*. Worth Publishers, New York. 452 pp.

Levavasseur, G., Edwards, G.E., Osmond, C.B. and Ramus, J. (1991). Inorganic carbon limitation of photosynthesis in *Ulva rotundata* (Chlorophyta). *Journal of Phycology*, **27**: 667-672.

Levy, I. and Gannt, E. (1990). Development of photosynthetic activity in *Porphyridium purpureum* (Rhodophyta) following nitrogen starvation. *Journal of Phycology*, **26**: 62-68.

Lewey, S.A. and Gorham, J. (1984). Pigment composition and photosynthesis in *Sargassum muticum*. *Marine Biology*, **80**: 109-115.

Lignell, A. and Pederson, M. (1987). Nitrogen metabolism in *Gracilaria secundata* Harv. *Hydrobiologia*, **151/152**: 431-441.

Lin,C. K. (1977). Accumulation of water soluble phosphorus and hydrolysis of polyphosphates by *Cladophora glomerata* (Chlorophyta). *Journal of Phycology*, **13**: 46-51.

Littler, M.M. (1979). The effect of bottle volume, thallus weight, oxygen saturation levels and water movement on apparent photosynthetic rates in marine algae. *Aquatic Botany*, **7**: 21-34.

Littler, M.M. (1980). Morphological form and photosynthetic performances of marine macroalgae: tests of a functional form hypothesis. *Botanica Marina*, **22**: 161-165.

Littler, M.M. and Littler, D.S. (1980). The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional/form model. *American Naturalist*, **116**: 25-44.

Littler, M.M. and Murray, S.N. (1974). The primary productivity of marine macrophytes from a rocky intertidal community. *Marine Biology*, **27**: 131-135.

Littler, M.M. and Murray, S.N. (1975). Impact of sewage on the distribution, abundance and community structure of rocky intertidal macro-organisms. *Marine Biology*, **30**: 277-291.

Lobban, C.S., Harrison, P.J. and Duncan, M.J. (1985). *The physiological ecology of seaweeds*. Cambridge University Press, Cambridge.

Lohman, K. and Priscu, J.C. (1992). Physiological indicators of nutrient deficiency in *Cladophora* (Chlorophyta) in the Clark Fork of the Columbia River, Montana. *Journal of Phycology*, **28**: 443-448.

Lowthion, D., Soulsby, G.P. and Houston, M.C.M. (1985). Investigation of a eutrophic tidal basin: part 1. Factors affecting the distribution and biomass of macroalgae. *Marine Environmental Research*, **15**: 263-284.

Lüning, K. (1981). Light. In: *The biology of seaweeds.* (Lobban, C.S. and Wynne, M.J. eds.), Blackwell Scientific Publications, Oxford. pp. 326-355.

Lyngby, J.E. (1990). Monitoring of nutrient availability and limitation using the marine macroalga *Ceramium rubrum* (Huds.) C. Ag. *Aquatic Botany*, **38**: 153-161.

MacFarlane, J.J. and Smith, F.A. (1982). Uptake of methylamine by *Ulva rigida*: transport of cations and diffusion of free base. *Journal of Experimental Botany*, **33**: 195-207.

Manley, S.L. and North, W.J. (1984). Phosphorus and the growth of juvenile *Macrocystis pyrifera* (Phaeophyta) sporophytes. *Journal of Phycology*, **20**: 389-393.

May, V. (1985). Observations on algal floras close to sewerage outlets. *Cunninghamia*, **1**: 385-394.

McComb, A.J. and Lukatelich, R.J. (1990). Inter-relations between biological and physicochemical factors in a database for a shallow estuarine system. *Environmental Monitoring and Assessment*, **14**: 223-238.

McGlathery, K.J. (1992). Physiological controls on the distribution of the macroalga *Spyridea hypnoides*: patterns along a eutrophication gradient in Bermuda. *Marine Ecology Progress Series*, **87**: 173-182.

McGlathery, K.J., Pederson, M. F. and Borum, J. (1996). Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta). *Journal of Phycology*, **32**: 393-401.

McGlathery, K.J., Krause-Jensen, D. Rysgaard, S. and Christensen, P.B. (1997). Patterns of ammonium uptake within dense mats of the filamentous macroalga *Chaetomorpha linum*. *Aquatic Botany*, **59**: 99-115.

McKenzie, G.H., Ch'ng, A.L. and Gayler, K.R. (1978). Glutamine synthetase/glutamine: α-ketoglutarate amino transferase in chloroplasts from the marine alga *Caulerpa simpliciuscula*. *Plant Physiology*, **63**: 578-582.

Moon, R.E. and Dawes, C.J. (1976). Pigment changes and photosynthetic rates under selected wavelengths in the growing tips of *Eucheuma isiforme* (C.Agardh) J. Agardh var. *denudatum* Cheney during vegetative growth. *British Phycological Journal*, **11**: 165-174.

Munda, I.M. (1982). The effects of organic pollution on the distribution of fucoid algae from the Istrian coast (vicinity of Rovinj). *Acta Adriatic*, **23**: 329-337.

Müeller-Dombois, D. and Ellenberg, H. (1974). *Aims and Methods of Vegetation Ecology*. John Wiley and Sons, New York.

Murray, S.N. and Littler, M.M. (1978). Patterns of algal succession in a perturbed marine intertidal community. *Journal of Phycology*, **14**: 506-512.

Neill, F.X. (1976). C:N ratio in some marine macrophytes and its possible ecological significance. *Botanica Marina*, **19**: 347-350.

Nixon, S.W., Oviatt, C.A., Frithsen, J. and Sullivan, B. (1986). Nutrients and the productivity of estuarine and coastal marine ecosystems. *Journal of Limnological Society of South Africa*, **12**: 43-71.

O'Brien, M.C. and Wheeler, P.A. (1987). Short term uptake of nutrients by *Enteromorpha prolifera* (Chlorophyceae). *Journal of Phycology*, **23**: 547-556.

Osborne, B.A. and Geider, R.J. (1986). Effect of nitrate-nitrogen limitation on photosynthesis of the diatom *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae). *Plant Cell Environment*, **9**: 617-625.

Paling, E.I. (1991). The relationship between nitrogen cycling and productivity in macroalgal stands and seagrass meadows. Ph.D thesis, University of Western Australia.

Parker, H.S. (1981). Influence of relative water motion on the growth, ammonium uptake and carbon and nitrogen composition of *Ulva lactuca* (Chlorophyta). *Marine Biology*, **63**: 309-318.

Parry, M.A., Schmidt, C.N.G., Cornelius, M.J., Keys, A.J. Millard, B.N. and Gutteridge, S. (1985). Stimulation of ribulose biphosphate carboxylase activity by inorganic orthophosphate without an increase in bound activating CO_2: cooperativity between the subunits of the enzyme. *Journal of Experimental Botany*, **36**: 1396-1404.

Peckol, P., DeMeo-Anderson, B., Rivers, J., Valiela, I., Maldonado, M. and Yates, J. (1994). Growth, nutrient uptake capacities and tissue constituents of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae* related to site-specific nitrogen loading rates. *Marine Biology*, **121**:175-185.

Pederson, M.F. (1994). Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): nature, regulation, and the consequences for choice of measuring technique. *Journal of Phycology*, **30**: 980-986.

Pederson, M.F. (1995). Nitrogen limitation of photosynthesis and growth: comparison across aquatic plant communities in a Danish estuary (Roskilde Fjord). *Ophelia*, **41**: 261-272.

Pederson, M. F. and Borum, J. (1996). Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. *Marine Ecology Progress Series*, **142**: 261-272.

Pederson, M. F. and Borum, J. (1997). Nutrient control of estuarine algae: growth strategy and the balance between nitrogen requirements and uptake. *Marine Ecology Progress Series*, **161**: 155-163.

Pickering, T.D., Gordon, M.E. and Tong, L.J. (1993). Effect of nutrient pulse concentration and frequency on growth of *Gracilaria chilensis* plants and levels of epiphytic algae. *Journal of Applied Phycology*, **5**: 525-533.

Piriou, J-Y. and Menesguen, A. (1992). Environmental factors controlling the *Ulva* sp. bloom in Brittany, France. In: *Marine Eutrophication and Polpulation Dynamics*. (Colombo, G., Ferrari, I., Cecchereli, U. and Rossi, R. eds.), Olsen and Olsen, Fredensborg, Denmark. pp. 111-116.

Porra, R. J., Thompson, W.A. and Kriedmann, P.E. (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta*, **975**: 384-394.

Pregnall, A.M. and Miller, S.L. (1988). Flux of ammonium from surf-zone and nearshore sediments in Nahant Bay, Massachusetts, USA in relation to free-living *Pilayella littoralis*. *Marine Ecology Progress Series*, **50**: 161-167.

Pregnall, A.M. and Rudy, P.P. (1985). Contribution of green macroalgal mats (*Enteromorpha* sp.) to seasonal production in an estuary. *Marine Ecology Progress Series*, **24**: 167-176.

Prezelin, B.B. and Nelson, N.B. (1990). The formation of ATP and reducing power in the light. In: *Plant physiology, biochemistry and molecular biology.* (Dennis, D.T. and Turpin, D.H. eds.), Longman Scientific and Technical Publications, Singapore. pp. 212-223.

Probyn, T.A. and Chapman, A.R.O. (1982). Nitrogen uptake characteristics of *Chordaria flagelliformis* (Phaeophyta) in batch mode and continuous mode experiments. *Marine Biology*, **71**: 129-133.

Ramus, J. (1978). Seaweed anatomy and photosynthetic performance: The ecological significance of light guides, heterogeneous absorption and multiple scatter. *Journal Phycology,* **14**: 352-362.

Ramus, J. (1981). The capture and transduction of light energy. In: *The biology of seaweeds.* (Lobban, C.S. and Wynne, M.J. eds.), Blackwell Scientific Publications, Oxford. pp. 458-492.

Ramus, J. and Rosenberg, G. (1980). Diurnal photosynthetic performance of seaweeds measured under natural conditions. *Marine Biology*, **56**: 21-28.

Ramus, J. and Venable, M. (1987). Temporal ammonium patchiness and growth rate in *Codium* and *Ulva* (Ulvophyceae). *Journal of Phycology*, **23**: 518-523.

Ramus, J., Beale, S.I., Mauzerall, D. and Howard, K.L. (1976). Changes in photosynthetic pigment concentration in seaweeds as a function of water depth. *Marine Biology*, **37**: 223-229.

Ramus, J., F. Lemons and C. Zimmerman. (1977). Adaptation of light-harvesting pigments to downwelling light and consequent photosynthetic performance of the eulittoral rockweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. *Mar. Biol.* **42**: 293-303.

Redfield, A.C. (1958). The biological control of chemical factors in the environment. *American Scientist*, **46**: 205-222.

Rees, A.V., Grant, C. M., Harmens, H. E. and Taylor, R. B. (1998). Measuring uptake rates of ammonium assimilation in marine algae: use of the protonophore carbonyl cyanide *m*-chlorophenylhydrazone to distinguish between uptake and assimilation. *Journal of Phycology*, 34: 264-272.

Rhiel, E. Morschel, E. and Wehrmeyer, W. (1985). Correlation of pigment deprivation and ultrastructural organisation of thykaloid membranes in *Cryptomonas maculata* following nutrient deficiency. *Protoplasma*, **129**: 62-73.

Rhiel, E. Krupinskak, K. and Wehrmeyer, W. (1986). Effect of nitrogen starvation on the function and organisation of thykaloid membranes in *Cryptomonas maculata* (Cryptophyceae). *Planta*, **129**: 62-73.

Rivers, J.S. and Peckol, P. (1995). Interactive effects of nitrogen and dissolved inorganic carbon on photosynthesis, growth, and ammonium uptake of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae*. *Marine Biology*, **121**: 747-753.

Rosenberg, G. and Ramus, J. (1982a). Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): photosynthesis and antenna composition. *Marine Ecology Progress Series*, **8**: 233-241.

Rosenberg, G. and Ramus, J. (1982b). Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates. *Marine Biology*, **66**: 251-259.

Rosenberg, G. and Ramus, J. (1984). Uptake of inorganic nitrogen and seaweed surface area:volume ratios. *Aquatic Botany*, **19**: 65-72.

Rosenberg, G., Probyn, T.A. and Mann, K.H. (1984). Nutrient uptake and growth kinetics in brown seaweeds: response to continuous and single additions of ammonium. *Journal of Experimental Marine Biology and Ecology*, **80**: 125-146.

Rosenberg, G., D.S. Littler, M. M. Littler, and Oliveira, E.C. (1995). Primary production and photosynthetic quotients of seaweeds from Sao Paulo State, Brazil. *Botanica Marina*, **38**: 369-377.

Rowan, K.S. (1989). *Photosynthetic Pigments of Algae*. Cambridge University Press, London.

Ryther, J.H., Corwin, N., DeBusk, T.A. and Williams, L.D. (1981). Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan 1979). *Aquaculture*, **26**: 107-115.

Sand-Jensen, K. (1988). Minimum light requirements for growth in *Ulva lactuca*. *Marine Ecology Progress Series*, **50**: 187-193.

Sand-Jensen, K. and Borum, J. (1991). Interactions among phytoplankton, periphyton and macrophytes in temperate freshwaters and estuaries. *Aquatic Botany*, **41**: 137-175.

Schaffelke, B. and Klumpp, D.W. (1997). Growth of germlings of the macroalga *Sargassum baccularia* (Phaeophyta) is stimulated by enhanced nutrients. *Proceedings of the 8th International Coral Reef Symposium*, **2**: 1839-1842.

Schaffelke, B. and Klumpp, D.W. (1998). Nutrient limited growth of the coral reef macroalga *Sargassum baccularia* and experimental growth enhancement by nutrient addition in continuous flow culture. *Marine Ecology Progress Series*, **164**: 199-211.

Schramm, W. and Booth, W. (1981). Mass bloom of the alga *Cladophora prolifera* in Bermuda: productivity and phosphorus accumulation. *Botanica Marina*, **24**: 419-426.

Seeley, G. R., Duncan M.J. and Vidaver W.E. (1972). Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. *Marine Biology*, **12**: 184-188.

Silberstein, K., Chiffings, A.W. and McComb, A.J. (1986). The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* Hook. F. *Aquatic Botany*, **24**: 355-371.

Sfriso, A. (1995). Temporal and spatial responses of growth of *Ulva rigida* C. Ag. to environmental and tissue concentrations in the Lagoon of Venice. *Botanica Marina*, **38**: 557-573.

Sfriso, A. and Marcomini, A. (1997). Macrophyte production in a shallow coastal lagoon. Part I: Coupling with chemico-physical paramaters and nutrient concentrations in waters. *Marine Environmental Research*, **44** (4): 351-377.

Sfriso, A., Marcomini, A. and Pavoni, B. (1987). Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice Lagoon. *Marine Environmental Research*, **22**: 297-312.

Sfriso, A., Pavoni, B. Marcomini, A. and Orio, B. (1992). Macroalgae, nutrient cycles and pollutants in the lagoon of Venice. *Estuaries*, **15**: 517-528.

Steffensen, D.A. (1976). The effect of nutrient enrichment and temperature on the growth in culture of *Ulva lactuca* L.. *Aquatic Botany*, **2**: 337-351.

Strickland, J.D.H. and Parsons, T.R. (1972). A practical handbook of seawater analysis. *Bulletin of the Fisheries Research Board of Canada*, **167**: 2nd Ed.

Tewari, A. (1972). The effect of sewage pollution on *Enteromorpha prolifera* var. *tubulosa* growing under natural habitat. *Botanica Marina*, **15**: 167.

Tewari, A. and Joshi, H.V. (1988). Effect of domestic sewage and industrial effluents on biomass and species diversity of seaweeds. *Botanica Marina*, **31**: 389-397.

Thom, R.M. (1984). Composition, habitats, seasonal changes and productivity of macroalgae in Grays Harbour estuary, Washington. *Estuaries*, **7**: 51-60.

Thom, R. M. and Albright, R.G. (1990). Dynamics of benthic vegetation standing stock, irradiance, and water properties in central Puget Sound. *Marine Biology*, **104**: 129-141.

Thomas, T.E. and Harrison, P.J. (1985). Effect of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata* (Rhodophyta). *Marine Biology*, **85**: 269-278.

Thomas, T.E., Harrison, P.J. and Turpin, D.H. (1987). Adaptations of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. *Marine Biology*, **93**: 569-580.

Thorne-Miller, B., Harlin, M.M., Thursby, G.B., Brady-Campbell, M.M. and Dworetzky, B.A. (1983). Variations in the distribution and biomass of submerged macrophytes in five lagoons in Rhode Island, U.S.A. *Botanica Marina*, **26**: 231-242.

Thomas, T.E. and Harrison, P.J. (1987). Rapid ammonium uptake and nitrogen interactions in five intertidal seaweeds grown under field conditions, *Journal of Experimental Marine Biology and Ecology*, **107**: 1-8.

Thybo-Christesen, M., Rasmussen, M.B. and Blackburn, T.H. (1993). Nutrient fluxes and growth of *Cladophora sericea* in a shallow Danish bay. *Marine Ecology Progress Series*, **100**: 273-281.

Topinka, J.A. (1978). Nitrogen uptake by *Fucus spiralis* (Phaeophyceae). *Journal of Phycology*, **14**: 241-247.

Topinka, J.A. and Robbins, J.V. (1976). Effects of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis*. *Limnology and Oceanography*, **21**: 659-664.

Turpin, D.H. and Harrison, P.J. (1979). Limiting nutrient patchiness and its role in phytoplankton ecology. *Journal of Experimental Marine Biology and Ecology*, **39**: 151-166.

Turpin, D. H. (1988). Physiological mechanisms in phytoplankton resource competition. In: *Growth and reproductive strategies of freshwater phytoplankton*. (Sandgren, C.E. ed.), Cambridge University Press, Cambridge, pp. 369-387.

Turpin, D.H. (1991). Effects of inorganic N availability on algal photosynthesis and carbon metabolism. *Journal of Phycology*, **27**: 14-20.

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P. DeMeo-Anderson, B., d'Avanzo, C., Babione, M., Sham, C.H., Brawley, J. and Lajtha, K. (1992). Coupling of watersheds and coastal waters: sources and consequences of nutrient enrichment in waquoit Bay, Massechusetts. *Estuaries*, **15**: 443-457.

Valiela, I. McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D. and Foreman, K. (1997). Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, **42(2)**: 1105-1118.

Viaroli, P. Pugnetti, A. and Ferrari, I. (1992). *Ulva rigida* growth and decomposition processes and related effects on nitrogen and phosphorus cycles in a coastal lagoon (Sacca di Goro, Po River Delta). In: *Marine Eutrophication and Polpulation Dynamics*. (Colombo, G., Ferrari, I., Cecchereli, U. and Rossi, R. eds.), Olsen and Olsen, Fredensborg, Denmark. pp. 77-84.

Viaroli, P., Bartoli, M. Bondavalli, C., Christian, R.R., Giordani, G. and Naldi, M. (1996). Macrophyte communities and their impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments. *Hydrobiologia*, **329**: 105-119.

Ventura, L. A. and Harlin, M.M. (1976). Ammonium uptake characteristics in *Gracilaria verrucosa* with respect to light intensity, temperature and ammonium pre-treatment. *Journal of Phycology*, **12** (Suppl.): 12.

Vergara, J.J., Perez-Llorens, L., Peralta, G., Hernandez, I. and Niell, F.X. (1997). Seasonal variation of photosynthetic performance and light attenuation in *Ulva* canopies from Palmones river estuary. *Journal of Phycology*, **33**: 773-779.

Virnstein, R.W. and Carbonara, P.A. (1985). Seasonal abundance and distribution of drift algae and seagrasses in the mid-Indian River Lagoon, Florida. *Aquatic Botany*, **23**: 67-82.

Waite, T. and Mitchell, R. (1972). The effect of nutrient fertilization on the benthic alga *Ulva lactuca*. *Botanica Marina*, **15**: 151-156.

Wallentinus, I. (1978). Productivity studies on Baltic macroalgae. *Botanica Marina*, **21**: 365-380.

Wallentinus, I. (1984a). Comparisons of nutrient uptake for Baltic macroalgae with different thallus morphologies. *Marine Biology*, **80**: 215-225.

Wallentinus, I. (1984b). Partitioning of nutrient uptake between annual and perennial seaweeds in a Baltic archipelago area. *Hydrobiologia*, **116/117**: 363-370.

Weger, H.G., Birch, D.G., Elrifi, I.R. and Turpin, D.H. (1988). Ammonium assimilation requires mitochondrial respiration in the light: a study with the green alga *Selenastrum minutum*. *Plant Physiology*, **86**: 688-692.

Weich, R.G. and Graneli, E. (1989). Extracellular alkaline phosphatase activity in *Ulva lactuca* L. *Journal of Experimental Marine Biology and Ecology*, **129**: 33-44.

Wheeler, P.A. (1979). Uptake of methylamine (an ammonium analogue) by *Macrocystis pyrifera* (Phaeophyta). *Journal of Phycology*, **15**: 12-17.

Wheeler, W. N. (1980). Pigment content and photosynthetic rate of fronds of *Macrocystis pyrifera*. *Marine Biology*, **56**: 97-102.

Wheeler, P.A. and Björnsäter, B.R. (1992). Seasonal fluctuations in tissue nitrogen, phosphorus, and N:P for five macroalgal species common to the Pacific northwest coast. *Journal of Phycology*, **28**: 1-6.

Wheeler, P.A. and Srivastata, L.M. (1984). Seasonal nitrate physiology of *Macrocystis integrifolia* Bory. *Journal of Experimental Marine Biology and Ecology*, **76**: 35-50.

Wheeler, P.A. and Weidner, M. (1983). Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. *Journal of Phycology*, **19**: 92-96.

Williams, S.L. (1984). Decomposition of the tropical macroalga *Caulerpa cupressoides* (West) C. Agardh: field and laboratory studies. *Journal of Experimental Marine Biology and Ecology*, **80**: 109-124.

Wilce, R. T., Schneider, C. W., Quinlan, A. V. and van den Bosch, K. (1982). The life history of free-living *Pilayella littoralis* (L.) Kjellm. (Ectocarpaceae, Ectocarpales) in Nahant Bay, Massachusetts. *Phycologia*, **21**: 336-354.

Williams, S.L. and Herbert, S.K. (1989). Transient photosynthetic responses of nitrogen-deprived *Petalonia fascia* and *Laminaria saccharina* (Phaeophyta) to ammonium resupply. *Journal of Phycology*, **25**: 515-522.

Zavodnik, N. (1987). Seasonal variations in the rate of photosynthetic activity and chemical composition of the littoral seaweeds *Ulva rigida* and *Porphyra leucosticta* from the North Adriatic. *Botanica Marina*, **30**: 71-82.

Zimmerman, R.C. and Kremer, J.N. (1986). *In situ* growth and chemical composition of the giant kelp, *Macrocystis pyrifera*: response to temporal changes in ambient nutrient availability. *Marine EcologyProgress Series*, **27**: 277-285.

Appendix 1

Tissue nitrogen and phosphorus digestion

Seaweeds were dried at 70°C for 24 h and 130 mg sub-samples were weighed and placed in 75 ml digestion test tubes, and held in preheated blocks at 320°C. To each tube 1.3 ml of sulphuric acid (H_2SO_4) was added and the tubes remained in the blocks for 30 seconds and then allowed to cool for 10-15 min. To each tube 0.5 ml of perchloric acid (H_2O_2) was added and tubes were kept in heat blocks for 10 to 15 min and then allowed to cool for 10-15 min. The process of adding H_2O_2, followed by heating and cooling, was repeated until the liquid was clear. Tubes were then placed in blocks for 45 min to burn off excess H_2O_2 and the tubes were allowed to cool for a further 10-15 min. A small amount of distilled water was added to the test tube and the liquid was shaken and poured into a sterilised plastic 50 ml sample tube. This liquid was then made up to 50 ml with distilled water. Samples were stored for 1 week in a dry, dark area prior to nutrient analysis.

Appendix 2

Tissue carbon digestion method

A wet digestion method (dichromate titration) was used to measure organic carbon content (Allen 1989). Measurement of organic carbon content by this method was checked by analysing 50 mg glucose. The result indicated a recovery of 94% of the organic carbon present in the glucose sample.

For each sample period five replicate dried plants were analysed for organic carbon content as follows: 10 mg dried algal tissue was weighed into 100 ml conical flasks to which was added exactly 10 ml $K_2Cr_2O_7$ (0.5N or 0.083 M) and 15 ml of an acid mixture (5:1 v/v of H_2SO_4 and H_2PO_4). The sample was boiled for 30 min at 300°C, then cooled for 20 min and the sides were rinsed with milli-Q pore water. 0.1 ml of the indicator reagent (*N*- phenylanthranilic acid dissolved in Na_2CO_3 solution) was added, and the sample was titrated with ferrous ammonium sulphate reagent (0.5 M). The ferrous ammonium sulphate reacted with the remaining dichromate with an endpoint (colour change) of brown/green to aqua/emerald green. Blanks (no algae) were run with each analysis. The calculation of percentage organic carbon content is as follows, if 1 ml of 0.5N dichromate is equivalent to 1.5 mg organic carbon then:

$$\% \text{ Organic C} = \frac{(\text{blank - sample}) \text{ ml} \times 0.15}{\text{sample weight (g)}}$$

Appendix 3

ANOVA of the effects of time (month) on (A) P_{max} g dry wt.$^{-1}$ h^{-1} and (B) P_{max} g chl *a*$^{-1}$ h^{-1} for 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 2.7.

(A)

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	10	80.45	4.72	0.001
	Error	33	17.03		
Polysiphonia decipiens	Season	10	0.066	2.36	0.031
	Error	33	0.028		
Ulva sp.	Season	10	0.351	18.95	0.001
	Error	33	0.019		

(B)

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	4	1.791	7.05	0.002
	Error	15	0.254		
Polysiphonia decipiens	Season	4	0.466	20.24	0.001
	Error	15	0.023		
Ulva sp.	Season	4	1.879	141.70	0.001
	Error	15	0.013		

Appendix 4

Estimates of productivity and nitrogen requirement of *Hincksia sordida* and *Polysiphonia decipiens* based on monthly photosynthetic and standing biomass values. Estimates are based on gross photosynthetic rates converted to carbon assimilated (assuming PQ= 1:1, based on daily gross saturated plus non-saturated photosynthesis and respiration for 12 h per day) and assume a C content 30% of dry weight.

Hincksia sordida	Productivity ($g\ C\ m^{-2}\ d^{-1}$)	Tissue nitrogen ($mg\ N\ g\ dw^{-1}$)	Biomass ($g\ dw\ m^{-2}$)	Biomass ($g\ C\ m^{-2}$)	Nitrogen req. ($g\ N\ m^{-2}$)	Biomass productivity ($g\ dw\ m^{-2}\ d^{-1}$)
Jan-96						
Feb-96	0.63	25.13	9.51	2.85	0.24	2.09
Mar-96						
Apr-96	0.51	18.97	12.70	3.81	0.24	1.70
May-96	0.64	23.33	15.27	4.58	0.36	2.13
Jun-96						
Jul-96						
Aug-96	0.87	46.92	12.61	3.78	0.59	2.89
Sep-96						
Oct-96						
Nov-96						
Dec-96						

Polysiphonia decipiens	Productivity ($g\ C\ m^{-2}\ d^{-1}$)	Tissue nitrogen ($mg\ N\ g\ dw^{-1}$)	Biomass ($g\ dw\ m^{-2}$)	Biomass ($g\ C\ m^{-2}$)	Nitrogen req. ($g\ N\ m^{-2}$)	Biomass productivity ($g\ dw\ m^{-2}\ d^{-1}$)
Jan-96	0.09	37.82	4.02	1.21	0.07	0.29
Feb-96	0.05	33.21	1.86	0.56	0.03	0.15
Mar-96	0.23	33.46	9.20	2.76	0.12	0.77
Apr-96	0.47	30.13	19.27	5.78	0.24	1.56
May-96	0.13	44.54	4.07	1.22	0.06	0.43
Jun-96	0.09	44.54	4.07	1.22	0.08	0.31
Jul-96	0.21	43.59	9.59	2.88	0.18	0.70
Aug-96	0.49	35.11	25.50	7.65	0.45	1.64
Sep-96	0.32	35.11	16.41	4.92	0.30	1.05
Oct-96	0.23	38.85	9.52	2.86	0.15	0.75
Nov-96	0.25	33.08	9.16	2.75	0.11	0.84
Dec-96	0.59	33.10	27.13	8.14	0.40	1.98

Appendix 5

Estimates of productivity and nitrogen requirement of *Ulva* sp. based on monthly photosynthetic and standing biomass values. Estimates are based on gross photosynthetic rates converted to carbon assimilated (assuming PQ= 1:1, based on daily gross saturated plus non-saturated photosynthesis and respiration for 12 h per day) and assume a C content 30% of dry weight.

Ulva sp.	Productivity ($g\ C\ m^{-2}\ d^{-1}$)	Tissue nitrogen ($mg\ N\ g\ dw^{-1}$)	Biomass ($g\ dw\ m^{-2}$)	Biomass ($g\ C\ m^{-2}$)	Nitrogen req. ($g\ N\ m^{-2}$)	Biomass productivity ($g\ dw\ m^{-2}\ d^{-1}$)
Jan-96	3.20	21.28	13.75	8.77	0.65	10.68
Feb-96	0.09	33.33	1.68	0.50	0.05	0.32
Mar-96	0.47	23.21	3.70	4.90	0.16	1.57
Apr-96	0.21	25.05	1.32	5.86	0.05	0.72
May-96	0.42	41.79	2.71	5.82	0.06	1.41
Jun-96	0.40	42.00	2.64	5.54	0.06	1.33
Jul-96	0.38	49.87	2.58	5.63	0.05	1.28
Aug-96	0.16	48.46	1.48	4.30	0.03	0.52
Sep-96	0.62	39.10	4.20	5.47	0.11	2.07
Oct-96	1.02	8.46	12.08	3.54	1.43	3.41
Nov-96	0.09	7.95	1.58	2.42	0.20	0.31
Dec-96	0.15	7.69	1.98	3.24	0.26	0.51

Appendix 6

Estimates of productivity and nitrogen requirement of all algae, based on monthly photosynthetic and standing biomass values. Estimates are based on gross photosynthetic rates converted to carbon assimilated (assuming PQ= 1:1, based on daily gross saturated plus non-saturated photosynthesis and respiration for 12 h per day) and assume a C content 30% of dry weight.

All algae	Productivity ($g\ C\ m^{-2}\ d^{-1}$)	Tissue nitrogen ($mg\ N\ g\ dw^{-1}$)	Biomass ($g\ dw\ m^{-2}$)	Biomass ($g\ C\ m^{-2}$)	Nitrogen req. ($g\ N\ m^{2}$)	Biomass productivity ($g\ dw\ m^{-2}\ d^{-1}$)
Jan-96	3.44	21.28	68.80	20.64	3.23	11.46
Feb-96	2.94	33.33	41.72	12.52	1.25	9.81
Mar-96	1.26	23.21	32.80	9.84	1.41	4.22
Apr-96	2.04	25.05	41.24	12.37	1.65	6.79
May-96	2.08	41.79	41.64	12.49	1.00	6.94
Jun-96	1.71	42.00	40.00	12.00	0.95	5.70
Jul-96	1.34	49.87	26.68	8.00	0.53	4.48
Aug-96	2.08	48.46	43.32	13.00	0.89	6.95
Sep-96	1.95	39.10	47.04	14.11	1.20	6.51
Oct-96	4.05	8.46	122.64	36.79	14.49	13.50
Nov-96	0.82	7.95	26.24	7.87	3.30	2.73
Dec-96	1.91	7.69	43.48	13.04	5.65	6.38

Appendix 7

Two-way ANOVA of the effects of season, nutrient enrichment and their interaction (SxE) on log transformed P_{max} of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.1.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.134	4.83	0.040
	Enrichment	4	1.670	60.32	0.001
	S x E	4	0.532	19.23	0.001
	Error		0.028		
Polysiphonia decipiens	Season	1	0.025	0.57	0.460
	Enrichment	4	1.596	36.63	0.001
	S x E	4	0.450	10.32	0.001
	Error		0.044		
Ulva sp.	Season	1	1.015	30.69	0.001
	Enrichment	4	1.932	58.43	0.001
	S x E	4	0.681	20.61	0.001
	Error		0.033		

Appendix 8

Two-way ANOVA on the effects of season, nutrient enrichment and their interaction (SxE) on log transformed photosynthetic efficiency (α) of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.2.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.004	2.79	0.111
	Enrichment	4	0.024	17.68	0.000
	S x E	4	0.002	1.49	0.244
	Error		0.001		
Polysiphonia decipiens	Season	1	0.009	12.25	0.002
	Enrichment	4	0.036	52.13	0.001
	S x E	4	0.006	8.94	0.001
	Error		0.001		
Ulva sp.	Season	1	0.001	0.50	0.487
	Enrichment	4	0.026	25.04	0.001
	S x E	4	0.003	3.19	0.035
	Error		0.001		

Appendix 9

Two-way ANOVA on the effect of season, nutrient enrichment and their interaction (SxE) on log transformed tissue nitrogen concentration of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.3.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	1.188	65.15	0.001
	Enrichment	4	0.928	50.88	0.001
	S x E	4	0.280	15.37	0.001
	Error		0.018		
Polysiphonia decipiens	Season	1	2.421	106.93	0.001
	Enrichment	4	0.582	25.72	0.001
	S x E	4	0.269	11.86	0.001
	Error		0.023		
Ulva sp.	Season	1	17.603	483.85	0.001
	Enrichment	4	1.074	29.53	0.001
	S x E	4	0.959	26.36	0.001
	Error		0.036		

Appendix 10

Two-way ANOVA of the effects of the effects season, nutrient enrichment and their interaction (SxE) on log transformed tissue phosphorus concentration of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.4.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.014	7.52	0.013
	Enrichment	4	0.040	21.01	0.001
	S x E	4	0.005	2.76	0.056
	Error	20	0.002		
Polysiphonia decipiens	Season	1	0.002	16.05	0.001
	Enrichment	4	0.001	11.56	0.001
	S x E	4	0.001	13.54	0.001
	Error	20	0.000		
Ulva sp.	Season	1	3.321	99.49	0.001
	Enrichment	4	2.027	60.73	0.001
	S x E	4	0.243	7.27	0.001
	Error	20	0.033		

Appendix 11

Two-way ANOVA on the effect of season, nutrient enrichment and their interaction (SxE) on log transformed tissue nitrogen to phosphorus ratio of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.5.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.293	4.01	0.059
	Enrichment	4	1.204	16.50	0.001
	S x E	4	0.657	9.01	0.001
	Error	20	0.073		
Polysiphonia decipiens	Season	1	4.319	115.59	0.001
	Enrichment	4	0.758	20.29	0.001
	S x E	4	0.260	6.95	0.001
	Error	20	0.037		
Ulva sp.	Season	1	34.25	469.50	0.001
	Enrichment	4	3.033	41.58	0.001
	S x E	4	0.762	10.44	0.001
	Error	20	0.073		

Appendix 12

Two-way ANOVA of the effect of season, nutrient enrichment and their interaction (SxE) on log transformed chlorophyll *a* concentration of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.6.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	4.778	243.24	0.001
	Enrichment	4	2.231	113.59	0.001
	S x E	4	1.466	74.63	0.001
	Error	20	0.020		
Polysiphonia decipiens	Season	1	0.234	121.09	0.001
	Enrichment	4	0.108	55.69	0.001
	S x E	4	0.034	17.83	0.001
	Error	20	0.002		
Ulva sp.	Season	1	0.284	26.36	0.001
	Enrichment	4	0.167	15.48	0.001
	S x E	4	0.187	17.36	0.001
	Error	20	0.011		

Appendix 13

Two-way ANOVA of the effect of season, nutrient enrichment and their interaction (SxE) on log transformed accessory pigment concentration of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.7.

Species and accessory pigment	Factor	df	MS	F ratio	P
Hincksia sordida (fucoxanthin)	Season	1	1.558	665.77	0.001
	Enrichment	4	0.175	74.90	0.001
	S x E	4	0.040	16.90	0.001
	Error	20	0.002		
Hincksia sordida (chlorphyll c)	Season	1	0.227	5.88	0.025
	Enrichment	4	0.420	10.86	0.001
	S x E	4	0.540	13.96	0.001
	Error	20	0.039		
Polysiphonia decipiens (phycorytherin)	Season	1	0.196	44.04	0.001
	Enrichment	4	0.264	59.35	0.001
	S x E	4	0.090	20.31	0.001
	Error	20	0.004		
Ulva sp. (chlorophyll *b*)	Season	1	0.936	57.35	0.001
	Enrichment	4	0.100	6.14	0.002
	S x E	4	0.220	13.47	0.001
	Error	20	0.016		

Appendix 14

Two-way ANOVA of the effect of season, nutrient enrichment and their interaction (SxE) on log transformed accessory pigment to chlorophyll *a* ratios of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 3.8.

Species	Factor	df	MS	F ratio	P
Hincksia sordida (fucoxanthin)	Season	1	4.105	77.78	0.001
	Enrichment	4	0.506	9.59	0.001
	S x E	4	0.200	3.78	0.019
	Error	20	0.053		
Hincksia sordida (chlorophyll *c*)	Season	1	0.203	29.88	0.001
	Enrichment	4	0.027	4.01	0.015
	S x E	4	0.012	1.76	0.177
	Error	20			
Polysiphonia decipiens (phycorytherin)	Season	1	0.005	0.99	0.330
	Enrichment	4	0.121	22.77	0.001
	S x E	4	0.141	26.54	0.001
	Error	20	0.005		
Ulva sp. (chlorphyll *b*)	Season	1	0.189	21.70	0.001
	Enrichment	4	0.023	2.70	0.060
	S x E	4	0.016	1.85	0.158
	Error	20	0.009		

Appendix 15

Two-way ANOVA of the effect of season, nutrient enrichment and their interaction (SxE) on log transformed growth rates of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Fig. 4.1.

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.012	152.35	0.001
	Enrichment	4	0.007	86.41	0.001
	S x E	4	0.002	20.81	0.001
	Error	20	0.000		
Polysiphonia decipiens	Season	1	0.010	139.99	0.001
	Enrichment	4	0.002	21.72	0.001
	S x E	4	0.000	5.69	0.003
	Error	20	0.000		
Ulva sp.	Season	1	0.014	100.96	0.001
	Enrichment	4	0.001	4.14	0.013
	S x E	4	0.002	15.83	0.001
	Error	20	0.000		

Appendix 16

Two-way ANOVA of the effect of season, nutrient enrichment and their interaction (SxE) on log transformed (a) long-term total ammonium-N uptake and, (b) long-term ammonium-N uptake rate of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Table 6.1.

(A)

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.278	71.46	0.001
	Enrichment	1	0.116	29.86	0.001
	S x E	1	0.074	19.14	0.002
	Error	8	0.004		
Polysiphonia decipiens	Season	1	0.053	6.98	0.030
	Enrichment	1	0.084	10.90	0.011
	S x E	1	0.075	9.80	0.014
	Error	8	0.008		
Ulva sp.	Season	1	0.025	13.07	0.007
	Enrichment	1	0.000	0.08	0.784
	S x E	1	0.037	19.19	0.002
	Error	8	0.002		

(B)

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	0.002	5.13	0.523
	Enrichment	1	0.022	0.45	0.053
	S x E	1	0.006	1.48	0.258
	Error	8	0.004		
Polysiphonia decipiens	Season	1	0.048	2.33	0.165
	Enrichment	1	0.035	1.70	0.228.
	S x E	1	0.111	5.40	0.056
	Error	8	0.021		
Ulva sp.	Season	1	0.505	94.36	0.001
	Enrichment	1	0.038	7.09	0.029
	S x E	1	0.004	0.73	0.419
	Error	8	0.005		

Appendix 17

Two-way ANOVA of the effect of season, nutrient enrichment and their interaction (SxE) on log transformed (a) long- term total phosphate-P uptake and, (b) long-term phosphate-P uptake rate of 3 species of macroalgae after 14 days of treatment exposure, based on data presented in Table 6.3.

(A)

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	11.39	29.58	0.001
	Enrichment	1	6.879	17.86	0.003
	S x E	1	0.873	2.27	0.171
	Error	8			
Polysiphonia decipiens	Season	1	1.617	18.95	0.030
	Enrichment	1	3.518	41.24	0.011
	S x E	1	0.001	9.80	0.014
	Error	8	0.085		
Ulva sp.	Season	1	8.801	295.69	0.001
	Enrichment	1	1.290	43.35	0.001
	S x E	1	0.200	6.74	0.032
	Error	8	0.030		

(B)

Species	Factor	df	MS	F ratio	P
Hincksia sordida	Season	1	7.727	19.89	0.002
	Enrichment	1	6.494	16.72	0.003
	S x E	1	1.825	4.70	0.062
	Error	8	0.388		
Polysiphonia decipiens	Season	1	1.514	22.76	0.001
	Enrichment	1	2.860	43.00	0.001.
	S x E	1	0.078	1.12	0.309
	Error	8	0.066		
Ulva sp.	Season	1	12.887	485.52	0.001
	Enrichment	1	1.830	68.96	0.001
	S x E	1	0.015	0.56	0.475
	Error	8	0.027		