Studies on the biomass, diversity and nutrient relationships of macroalgae and seagrasses in Lake Illawarra, New South Wales, Australia

Karin Rutten
University of Wollongong

Studies on the biomass, diversity and nutrient relationships of macroalgae and seagrasses in Lake Illawarra, New South Wales, Australia

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KARIN RUTTEN

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Thesis Declaration

I, Karin Rutten, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Earth and Environmental Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other institution.

Karin Rutten

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Abstract

Lake Illawarra is a shallow barrier lagoon, located on the south-eastern coast of Australia. Eutrophication, referring to the enrichment of water by inorganic plant nutrients (primarily nitrogen and phosphorus), is one of the key environmental problems in Lake Illawarra. Management of macroalgae in Lake Illawarra is a major issue; excessive blooms of macroalgae, resulting in odours, access problems and community concern over Lake health, have led to many management strategies, including direct harvesting of algal biomass. Little information is available on the factors responsible for excessive growth of macroalgae in Lake Illawarra, although over supply of nutrients has often been cited as the primary cause. The aim of this study was to investigate the distribution, diversity, biomass and nutrient relationships of seagrasses and macroalgae in Lake Illawarra, and to determine what contribution, if any, macrophytes make to the Lake's nutrient budget.

Firstly, detailed species lists and taxonomic descriptions were prepared for macrophytes occurring in Lake Illawarra, between June 2000 and July 2003. This study focused primarily on shallow (< 1 m depth), inshore areas of Lake Illawarra, where problematic macroalgal blooms frequently occur. Seagrasses found in Lake Illawarra are *Zostera capricorni*, *Ruppia megacarpa*, *Halophila ovalis* and *Halophila decipiens.* In addition, 35 species of macroalgae were recorded and described; these included: 14 species from 7 genera of green macroalgae; 9 species from 9 different genera of brown macroalgae; and, 8 species from 8 genera of red macroalgae.

The biomass of seagrasses and macroalgae in Lake Illawarra were documented seasonally (winter and summer) at four key Lake Illawarra sites; these included two *R. megacarpa* sites and two *Z. capricorni* sites. Average *R. megacarpa* and *Z. capricorni* dry weight (DW) biomasses (above and below-ground material) ranged from 54.8 - 440 g DW m^{-2} and 58.1 - 230 g DW m^{-2}, respectively. Significant die-back, particularly of *Z. capricorni*, occurred in winter; summer biomasses were up to 1.5 - 3.9 times higher than winter biomasses. Below-ground material (roots and rhizomes) comprised 20 - 45 % and 40 - 67 % of total plant biomass for *R. megacarpa* and *Z. capricorni*, respectively. Macroalgal biomass in 2000-03 was notably lower than in previous decades; this may be due to drought, as well as improvements in water quality. Maximum biomasses of macroalgae recorded in the present study were 150 - 370 g DW m^{-2}. Algal blooms were composed primarily of the filamentous chlorophytes, *Chaetomorpha linum* and *Chaetomorpha billardierii*. The highest seagrass (*R. megacarpa*) and macroalgal biomasses usually occurred at the Oasis Caravan Park site, located along the eastern Lake Illawarra peninsula.

Tissue nutrient analyses were conducted on the most abundant seagrasses (*Z. capricorni* and *R. megacarpa*) and macroalgae occurring at four sites in Lake Illawarra, between spring 2000 and

winter 2002. Total C contents of macrophytes varied from 23.3 - 42.0 % C for seagrasses, and 28.0 - 39.7 % C for macroalgae. The $\delta^{13}C$ and $\delta^{15}N$ contents of seagrasses ranged from -7.7 to -15.9 ‰ and 0.7 - 9.0 ‰, respectively. The most significant seasonal variations in seagrass $\delta^{13}C$ contents and, to a lesser extent $\delta^{15}N$ contents, occurred in *Z. capricorni* located at the source of fresh water input, Mullet Creek. Macroalgae showed a greater variation in isotopic signatures than the seagrasses, ranging from -4.9 to -19.8 ‰ ($\delta^{13}C$) and 1.8 - 14.6 ‰ ($\delta^{15}N$). Differences between species at the same site were often more significant than differences between the same species at different sites. Seagrass leaf N and P contents ranged from 1.74 - 4.13 % (mean ± s.e.: 2.62 ± 0.05 % N) and 0.12 - 0.59 % P (mean ± s.e.: 0.31 ± 0.01 % P); leaf N and P contents were typically double those of roots/rhizomes. N contents varied between species and sites, but P contents of *Z. capricorni* were usually significantly higher than *R. megacarpa. Z. capricorni* C and N contents increased in winter, corresponding to lower winter biomasses. Seagrass leaf biomass and tissue P contents peaked in summer 2002, which may be related to higher water column P concentrations in summer. Tissue N and P contents of macroalgae were more variable than those of the seagrasses, and ranged from 0.85 - 3.95 % N and 0.03 - 0.58 % P. The average C/P (808 ± 65) and N/P (47.9 ± 3.47) molar ratios of macroalgae were typically double those of the seagrasses. Low concentrations of tissue P, with respect to N, in *R. megacarpa* and macroalgae implied P limitation on several occasions, particularly when macrophyte biomasses were low. High tissue N contents in Lake Illawarra macrophytes suggested N limitation of biomass formation rarely occurred. Evidence of P, rather than N, limitation in macrophytes is surprising considering most data suggests N limitation of phytoplankton production in Lake Illawarra. The estimated pools of N and P contained in Lake Illawarra macrophyte biomass were similar to those present in the water column, but appeared minute when compared to the N and P stored within Lake Illawarra sediment.

Laboratory culture experiments were conducted to evaluate the response of the most problematic alga, *Chaetomorpha linum*, to nutrient enrichment. Water temperatures of 20 - 25°C were found to promote the highest growth rates (up to 27 % WW d^{-1}) of *C. linum*, but high growth rates (13 % WW d^{-1}) were also recorded at 10°C, the lowest winter water temperature recorded in Lake Illawarra. Enrichment with N, rather than P, had the greatest effect on *C. linum*; growth rates were significantly reduced in treatments without added N, but treatments with N-alone were statistically similar to N+P treatments. It was concluded that in Lake Illawarra, *C. linum* was strongly nitrogen limited. The ability of *C. linum* to grow successfully in culture, under a range of nutrient treatments, and without added phosphorus, in particular, correlates with the excessive growth of this alga in Lake Illawarra.

This study has made a significant contribution to the understanding of seagrass and macroalgal growth, biomass and distribution in Lake Illawarra. This information will assist with the long-term management of macroalgal problems in Lake Illawarra.

Publications arising to date from this study

Rutten, K., Morrison, R.J. and West, R.J. (2004) Macroalgae in Lake Illawarra, New South Wales, Australia. *Wetlands (Australia),* **21**(2), 103-114.

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CHAPTER 1 Introduction

1.1 General Introduction

An estuary has been defined as "…a semi-enclosed coastal body of water which has a free connection with the open sea and within which seawater is measurably diluted with freshwater derived from land drainage" (Pritchard, 1967). However, there are many coastal water bodies which do not strictly fit this definition as they may be permanently or intermittently closed to the sea; these water bodies are typically referred to as Intermittently Open and Closed Lakes and Lagoons (ICOLLs). Along the NSW coastline there are over 133 estuaries and embayments, 57 of which are classified as ICOLLs (West *et al.*, 1985). As 70 of these estuaries, 35 of which are ICOLLs, are located in the Illawarra and South Coast regions (West *et al.*, 1985), work conducted in the Illawarra region is particularly important and of regional significance.

Estuaries and their associated wetlands support a diverse array of aquatic and terrestrial life, from viruses and bacteria, through to plants and animals. They are important ecological regions as they are utilised by a wide variety of birds and other wildlife for migratory corridors, habitat, shelter, a source of food and breeding areas. Fish, including many commercially important species, and other marine organisms, also depend on estuaries for food, shelter, spawning and juvenile nurseries (Bell and Pollard, 1989).

Aquatic plants, such as seagrasses and macroalgae, play a crucial role in estuarine ecology. Seagrasses are marine flowering plants (angiosperms) which complete their life cycle completely submerged under water (Edgar, 2000). In Australia, there are about 30 species of seagrasses from 12 genera, with *Halophila ovalis, Halophila decipiens, Zostera capricorni, Zostera muelleri, Heterozostera tasmanica* and *Posidonia australis* represented along the New South Wales coastline (West, 1983; Kirkman, 1997). Seagrass beds are utilised by a wide variety of fish and invertebrates as habitats, breeding areas, nurseries, and as a source of food, both directly and indirectly by acting as a substrate for epiphytes (Dawes, 1998). Seagrasses are also important primary producers and improve water quality by stabilising bottom sediments, thus reducing turbidity and inhibiting the release of sediment-bound nutrients to the water column (Kirkman, 1997). Seagrass beds are often used as an indicator of the health of estuaries. Over the decades, many seagrass beds have been damaged or lost as a result of anthropogenic activities, such as eutrophication, the introduction of exotic pests, thermal pollution or mechanical damage by dredging and boating (Dawes, 1998). The distribution and abundance of seagrass beds has also been known to change dramatically over time due to natural causes, such as storms, wave action, or over grazing by animals (West *et al.*, 1985).

The term 'macroalgae' commonly refers to multicellular eukaryotic algae which may be easily observed with the naked eye. Macroalgal beds often perform much of the same ecologically

beneficial functions as seagrass beds, such as a habitat and food source for fish and invertebrates. However, an over supply of nutrients in many estuaries commonly results in excessive algal growth and the formation of nuisance algal blooms. These blooms reduce the aesthetic appeal of an estuary as they often accumulate along foreshore regions, produce foul odours during decomposition, cause anoxia and inhibit the growth of native macrophytes, such as seagrasses (Dawes, 1998). Vast quantities of macroalgal biomass are often harvested from estuaries in an effort to improve water quality and the aesthetic value. However, little is known of the immediate or long-term impacts of macroalgal harvesting on estuarine ecology, such as damage to fish and invertebrate populations, or the effect on estuarine nutrient budgets.

1.2 Statement of the Problem

Estuaries are considered to be valuable natural resources that require careful management. However, sustainable estuarine management is often hindered by substantial gaps in data and poor understanding of natural estuarine processes (National Land and Water Resources Audit, 2002). Without comprehensive and long term scientific data on estuaries, it is difficult to accurately assess the ecological condition, identify long-term trends and develop effective management strategies for estuaries. Optimal management of estuaries requires detailed scientific knowledge, long-term monitoring and research plans, and a strategic, integrated approach to catchment management (DLWC, 2003).

Management of macroalgae in Lake Illawarra is a major issue and the problem has been documented by several authors (e.g., Harris, 1977; Yassini, 1985; King *et al.,* 1997). Excessive blooms of macroalgae, resulting in odours, access problems and community concern over Lake health, have led to many management strategies, including direct harvesting of algal biomass (West, 1998; WBM, 2006). Although seagrass surveys have been conducted regularly (see WBM 1993, 1996, 1998, 2000), past assessments of macroalgal growth in Lake Illawarra have generally been restricted to short-term, infrequent studies, which often only include general observations and field-based biomass estimates. Macroalgae may be misidentified, or are often listed by genera only, as identification to species level is very difficult to achieve in the field. Little information is available on the factors responsible for excessive growth of macroalgae in Lake Illawarra, although over supply of nutrients has often been cited as the primary cause. It is not yet known what, if any, contribution macroalgal beds make to the Lake's nutrient budget. Nor is it understood what impact harvesting large quantities of macroalgal biomass from Lake Illawarra has on the Lake's nutrient budget or overall ecology.

Ecologically sustainable management of macroalgal problems in estuaries requires knowledge of the environmental factors determining macroalgal abundance and diversity, and the key factors limiting the growth of those species (Chambers *et al.*, 1999). The present study has examined the distribution, biomass and nutrient relationships of seagrasses and macroalgae in

Lake Illawarra, NSW, and some of the key factors relating to the excessive growth of macroalgae in the Lake. This information will assist with the long-term management of macroalgal problems in Lake Illawarra.

1.3 Thesis Aims and Objectives

The original aim of this study was to investigate the ecological impacts of macroalgal harvesting in Lake Illawarra, commissioned by the Lake Illawarra Authority (LIA) and funded through an Australian Research Council (ARC) Industry Linkage Grant. However, unusually dry weather conditions during 2000-3 (with a severe drought in 2002) meant there were few algal blooms in Lake Illawarra during this period, and little use of harvesting by the LIA. Consequently, the impacts of harvesting could not be studied directly and the research objectives were progressively altered to investigate related issues, namely:

- to develop a detailed description of macroalgal and seagrass species present in Lake Illawarra, including their seasonal and spatial distribution, biomass and taxonomy;
- to investigate the nutrient relationships of Lake Illawarra seagrasses and macroalgae, using tissue analysis of nitrogen, phosphorus, carbon and the stable isotopes of nitrogen and carbon;
- to investigate the key environmental parameters, namely temperature and nutrients, contributing to excessive growth of *Chaetomorpha linum* in Lake Illawarra; and,
- to determine the contribution that macrophytes make to the Lake Illawarra nutrient budget.

1.4 Thesis Outline

The remainder of Chapter One includes a description of the Lake Illawarra study area. Chapter Two includes a literature review on the ecology, growth and carbon, nitrogen and phosphorus nutrition of seagrasses and macroalgae. The materials and methodologies used are listed in Chapter Three. Chapter Four describes the distribution, taxonomy and identification of seagrasses and macroalgae in Lake Illawarra. A study on the biomass and nutrient relationships of Lake Illawarra macrophytes is presented in Chapter Five. Chapter Six describes factors relating to excessive growth of macroalgae, including the growth in culture of *Chaetomorpha linum*. Chapters Four to Six each contain separate introductions, results and discussion sections for the particular study area. The conclusions drawn from the study and recommendations for future work are given in Chapter Seven, and the references and appendices are listed at the end.

1.5 Study Site - Lake Illawarra

1.5.1 Site Description

Lake Illawarra is a shallow coastal lagoon, located 8 km south of Wollongong and 90 km south of Sydney, New South Wales (Figure 1-1; 34° 33'S, 150° 52'E). The Lake has a surface area of approximately 35 km^2 and a catchment of 235 km^2. Lake Illawarra is formed in a shallow depression with a coastal sand barrier on the eastern side of the Lake and a catchment extending from the Illawarra escarpment to the west of the Lake. The major tributaries draining into Lake Illawarra are Macquarie Rivulet, Mullet Creek and Duck Creek. The maximum depth of the Lake is approximately 3.8 m, with an average depth of 1.9 m, but about 25 % of the Lake is typically less than 1.2 m deep (LIA, 1995). Generally, the water level of the Lake is 25 - 30 cm above average sea level (Yassini and Clarke, 1986).

The shallowest areas of Lake Illawarra are densely populated with aquatic macrophytes, including seagrasses and macroalgae, which generally do not extend beyond 2 m depth. In 2000, seagrasses covered 7.86 km^2, which accounts for 22 % of the total Lake surface area (WBM, 2000). *Zostera capricorni* is the dominant seagrass in Lake Illawarra, occupying around 70 % of the total area of seagrass beds, followed by *Ruppia megacarpa* and small patches of *Halophila ovalis* and *Halophila decipiens*. The distribution of macrophytes in Lake Illawarra is described in further detail in Chapter 4.

1.5.2 Catchment Land Use

Extensive land clearing and agricultural, residential and industrial development of the Lake Illawarra catchment since European settlement in 1817 have led to a substantial deterioration in the ecological condition of the Lake (LIA, 1995; WBM, 2006). Urban development has resulted in increased sedimentation, trace metal contamination and nutrient enrichment of the sediment and water column, and excessive growth of macroalgae (Depers *et al.*, 1994).

Land use patterns in the Lake Illawarra catchment are presented in Table 1-1 and Figure 1-2. The upper western reaches of the Lake Illawarra catchment remain largely undeveloped due to the steep nature and inaccessibility of the Illawarra escarpment (Figure 1-1). However, there are a number of active and disused collieries and related facilities located on the escarpment (Figure 1-2). The lower foothills of the catchment have been extensively cleared of vegetation and have historically been utilised for agricultural purposes, such as dairy farming, horse breeding and training (LIA, 1995). Fertiliser runoff from rural areas is a common contributor to high nutrient loads in the catchment waterways.

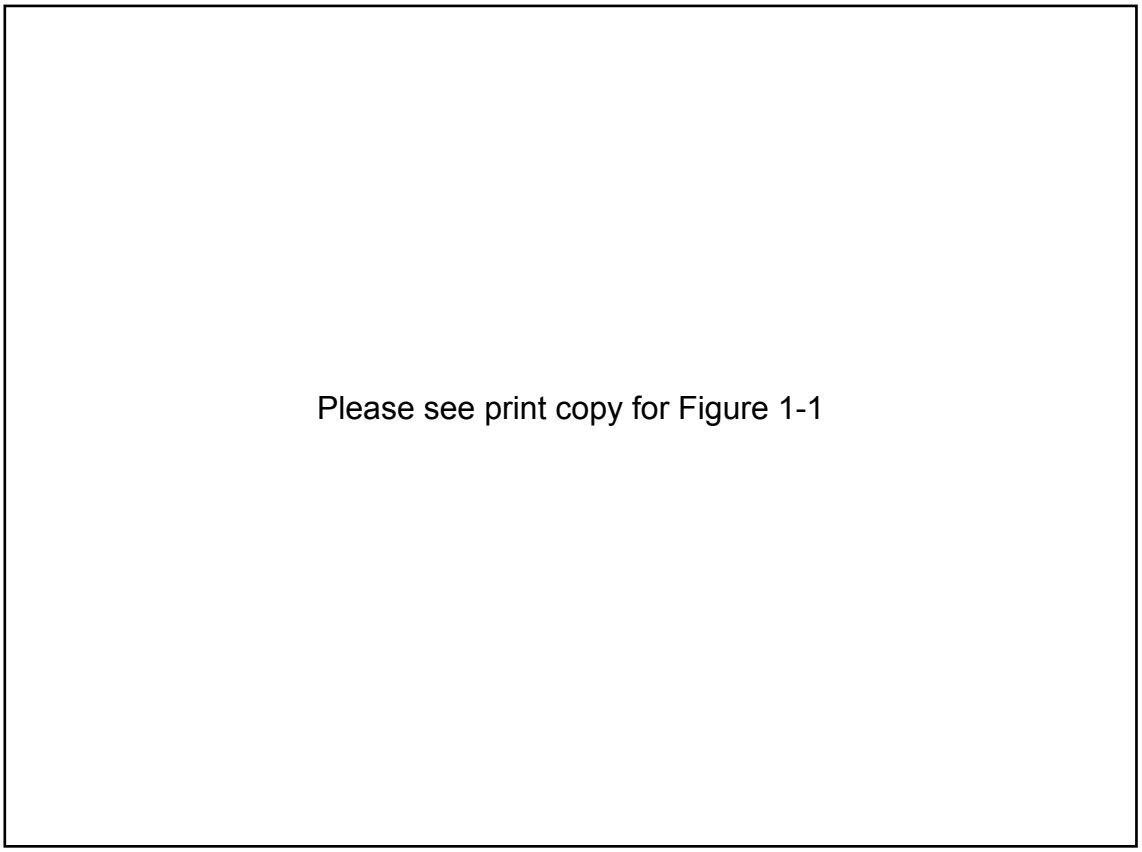


Figure 1-1: Location of Lake Illawarra, showing contour lines, major watercourses and catchment boundary (GIS data obtained from Geoscience Australia, 2003).

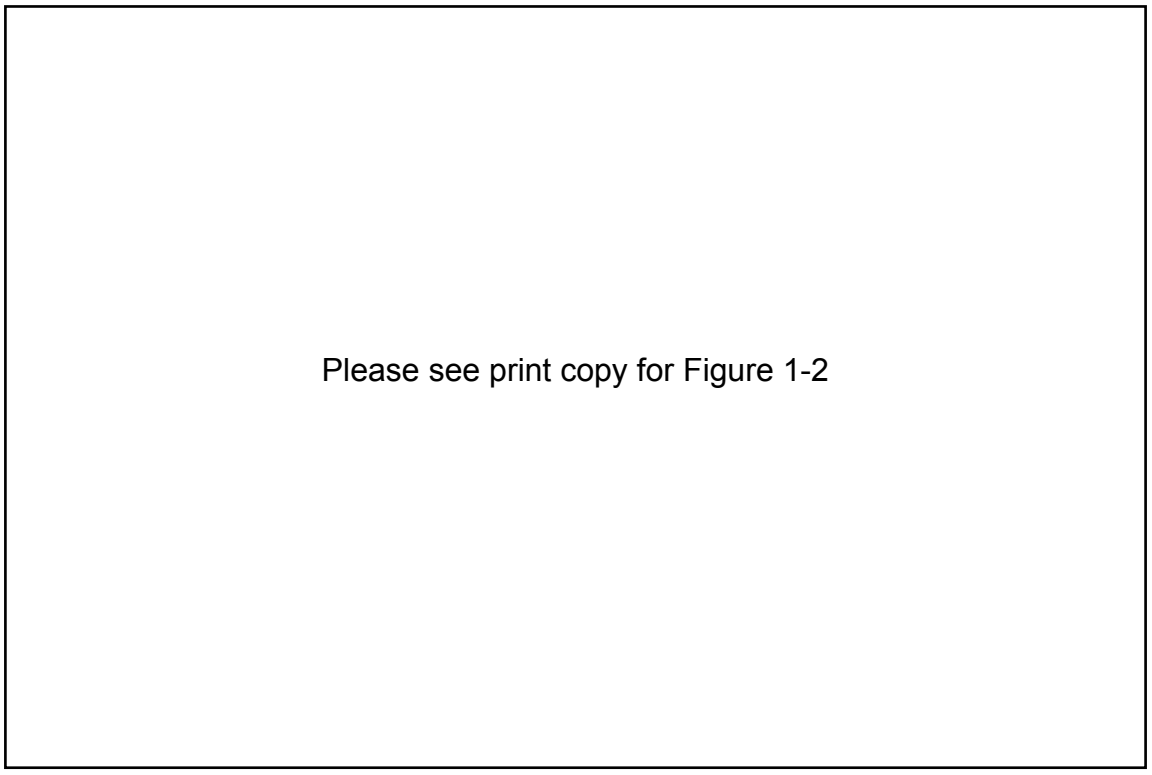


Figure 1-2: Land use in the Lake Illawarra catchment (GIS data obtained from Geoscience Australia, 2003).

Table 1-1: Land use in the Lake Illawarra catchment (after Depers *et al.*, 1994).

Please see print copy for Table 1-1

Much of the Lake Illawarra foreshore region is surrounded by residential development (Figure 1-2), which is continually expanding. The Lake Illawarra catchment is situated within two local government areas (LGAs): Wollongong City and Shellharbour City Councils. Population has been increasing rapidly in both of these LGAs and is placing increasing pressure on Lake Illawarra and its catchment. The population of the Lake Illawarra catchment was estimated to be about 90,000 people in 2000, but this figure is likely to increase rapidly with the development of the West Dapto housing estate (Sherman *et al.*, 2000). Past population growth in the Lake Illawarra catchment had been positively correlated with increasing nutrient enrichment of the Lake (Yassini and Clarke, 1986). Industrial activities, both within and surrounding the Lake Illawarra catchment, have potentially contributed to water quality problems and sediment contamination through direct discharges, stormwater runoff or air-borne particulate deposits. Industrial sources of pollution include emissions from the Port Kembla industrial complex and the former Kanahooka Smelter, and thermal pollution from the now disused Tallawarra Power Station (Depers *et al.*, 1994).

1.5.3 Climate

Rainfall across the Lake Illawarra catchment is strongly influenced by the escarpment. Average annual rainfall in the catchment ranges from 1600 mm on the crest of escarpment, decreasing to 1100 mm across the surface of the Lake (UOW and WCC, 1976). Annual rainfall recorded between 1893 and 2003 at Albion Park ranged from 469 - 2,640 mm, with an annual mean of 1,095 mm (Figure 1-3). Similarly, at Dapto, annual rainfall (1906 - 2003) ranged from 500 - 2324 mm annually (mean: 1,092 mm). To the north-east of the catchment, annual rainfall recorded at Port Kembla (1960 - 2003) ranged from 406 - 1853 mm, with an annual average of 1,152 mm (Bureau of Meteorology data). The region's climate is characterised by peaks and troughs in annual rainfall (Figure 1-3). During wetter periods, the Lake Illawarra catchment is prone to flooding, often resulting in extensive damage (Nanson and Hean, 1984), while drought conditions can occur in drier periods. The wettest and warmest months of the year are typically January through to March, while the winter months are the driest and coolest (Figure 1-4). Average daily evaporation is approximately 1 mm in winter and 4 mm in summer; when the Lake entrance is closed and rainfall is low, the Lake's water level can drop considerably (Standing Committee on Public Works, 1996).

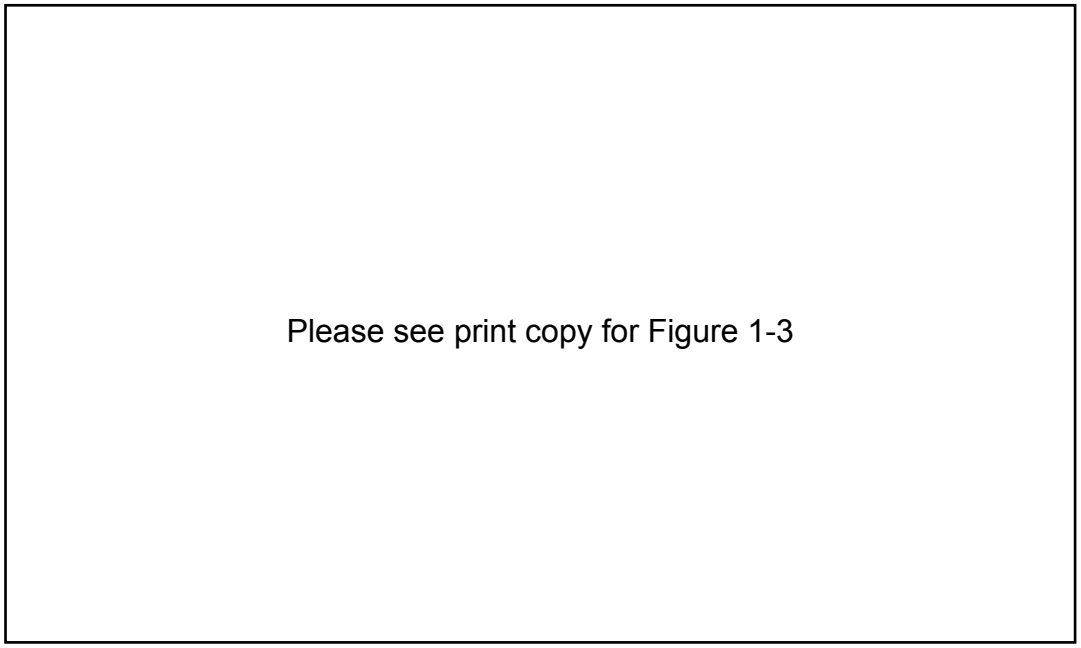


Figure 1-3: Annual rainfall recorded at Albion Park (data from Bureau of Meteorology, 2007).

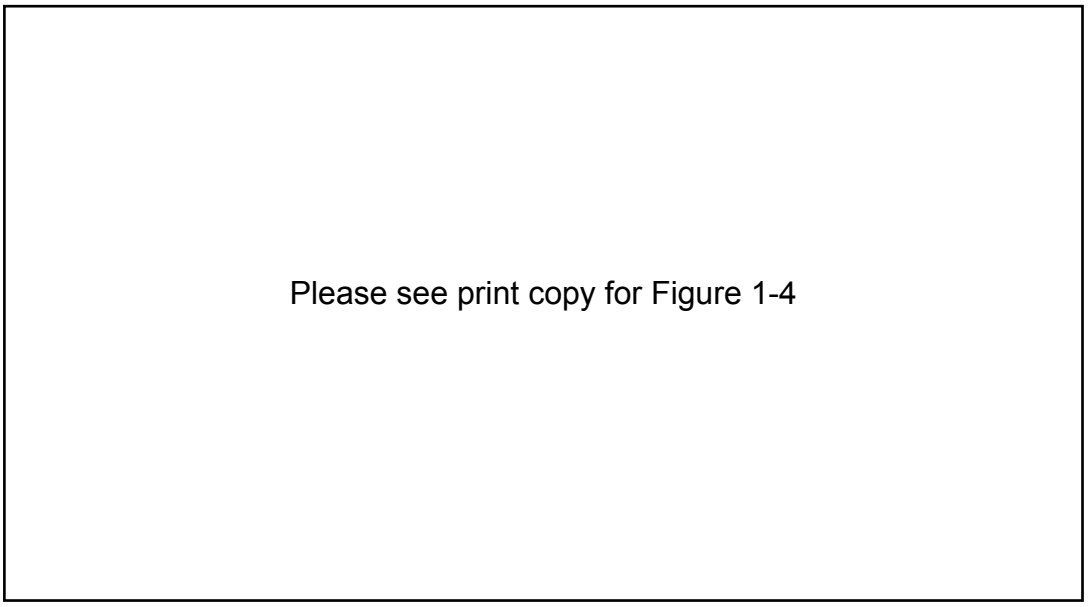


Figure 1-4: Average monthly rainfall and temperatures recorded at Port Kembla, 1960 - 2003 (data from Bureau of Meteorology, 2004).

1.5.4 Lake Entrance Conditions

Lake Illawarra is connected to the sea by a 3.7 km long entrance channel. As Lake Illawarra is a barrier lagoon, the entrance to the Lake naturally varies between open, closed, or moderately or heavily shoaled. The status of the entrance is largely determined by natural processes such as tidal exchange, wind and wave action, flooding and littoral drift (LIA, 1995). The main function of the Lake entrance, when open to the sea, is to allow tidal exchange, the flushing of flood waters, sediments, nutrients and pollutants out of the Lake, and to allow the migration of fish and other aquatic organisms between the Lake and the ocean (LIA, 1995). The entrance area is also

widely utilised by tourists and local residents for its beaches, boat ramps and other amenities, and for recreational activities such as fishing, swimming or windsurfing.

The tidal range in Lake Illawarra is minimal, varying from about 0.03 m when the entrance is shoaled, to 0.1 m when the entrance is well scoured (LIA, 1995). Heavy shoaling at the mouth of the Lake limits tidal exchange and occasionally results in complete closure (Depers *et al.*, 1994), which exacerbates water quality and flooding problems and limits the passage of marine species to and from the sea (LIA, 1995). To address these problems, substantial works were undertaken in 2000 - 2007 to try and ensure that the Lake's entrance remained permanently open, including dredging of the entrance channel and construction of training walls. However, the entrance area has still been prone to heavy shoaling, especially during extended periods of dry weather; the entrance subsequently closed over during the drought of 2002-3 and had to be mechanically reopened several months later.

1.5.5 Nutrient Enrichment

Substantial development and urbanisation of the Lake Illawarra catchment since European settlement has resulted in a significant reduction in water quality and enhanced sedimentation rates (LIA, 1995). Catchment runoff empties into the Lake from the major tributaries, including Mullet Creek, Macquarie Rivulet and Duck Creek, and a number of small streams and stormwater drains. Thirteen sewerage pumping stations in the catchment may also contribute to water pollution during wet weather sewerage overflows (Depers *et al.*, 1994). Runoff from the catchment typically carries heavy loads of sediment, nutrients, trace metals and other pollutants into the Lake. Additionally, the Lake is often highly turbid as a result of sediment inflow from the catchment, growth of phytoplankton and the re-suspension of fine particulate material by wind and wave action (Depers *et al.*, 1994; LIA, 1995).

While sediment infilling of coastal lakes is a natural evolutionary process, many estuaries are infilling more rapidly due to anthropogenically induced changes in catchment runoff, soil erosion and coastal processes (Roy *et al.*, 2001). Sediment washed in from the catchment often carries heavy loads of nutrients and pollutants into an estuary; large-scale declines of seagrasses in estuarine and coastal waters have been attributed to a reduction in the availability of light due to increased suspended sediments, turbidity and nutrient enrichment, which leads to macroalgal or phytoplankton blooms (Walker *et al.*, 1999). Infilling has been accelerated in Lake Illawarra by urban development of the catchment and has resulted in the formation and expansion of fluvial deltas at the mouths of creeks, expanding mudflats along the Lake's foreshore, and leads to increased shallowing of bays, therefore reducing the water depth available for boating and other recreational activities (LIA, 1995; Sherman *et al.*, 2000). Sources of sediment infilling in Lake Illawarra include bank erosion from numerous creek ands tributaries and land clearing for agricultural, residential and industrial purposes (LIA, 1995). Coastal processes are another

significant source of infilling, particularly in the Lake entrance channel; it has been estimated that tidal currents and flood flows transport, on average, 110,000 m^3 of sand into and out of the entrance channel annually (PWD, 1982, cited in LIA, 1995). Since European settlement, the Lake's sediment has become enriched to the point where release of nutrients from the sediment has been cited as the most significant process affecting Lake nutrient concentrations (LIA, 1995; Qu, 2004a).

Table 1-2 lists water quality parameters recorded in Lake Illawarra over the last three decades, including the high nutrient concentrations that have been documented in the Lake after wet weather events. Of particular interest are the concentrations of plant available nutrients, such as orthophosphate, ammonia and nitrate, which contribute to excessive growth of macroalgae. LIA (1995) reported that ammonia-N concentrations in Lake Illawarra had been steadily increasing between the 1970s and 1990s, possibly due to increasing amounts of organic matter and the subsequent fluxes of ammonia from the sediment. Ammonia concentrations in the middle of Lake Illawarra average 14 ± 4.5 $\mu g\ L^{-1}$ (Dongyan Liu, PhD Student, University of Wollongong, pers. comm., 2007), but in sheltered bays with limited circulation (e.g., Griffins Bay), water column ammonia concentrations may be up to 300 $\mu g\ L^{-1}$ (Lake Illawarra Authority data, pers. comm., 2007). Similarly, nitrate levels have also increased since the 1970s, which may be due to nitrification of the increasing ammonia concentrations (LIA, 1995). While anecdotal evidence suggests that water quality has deteriorated since European settlement, it is difficult to conclude that water quality is continuing to decline as the sparsity and incompleteness of the data set generally makes long-term comparisons difficult. Additionally, nutrient and water quality data often vary according to the weather conditions and status of the entrance prior to sampling, as well as the sampling and analytical techniques used. For example, nutrient concentrations are typically higher after heavy rain washes nutrients into the Lake, or high winds disturb sediment-bound nutrients.

The seasonal variations in dissolved inorganic phosphorus (DIP) and nitrogen (DIN) in Lake Illawarra (1996 - 2007) are shown in Figure 1-5. Concentrations of DIP were typically 2 - 3 times higher in summer and autumn, than in winter and spring. The lower DIN concentrations in 1999 - 2001 are probably related to lower than average rainfall and, therefore, catchment runoff, during that period (see, e.g., Morrison and West, 2004). The ratios of dissolved inorganic nitrogen to phosphorus in the water column suggest that Lake Illawarra is strongly nitrogen limited for primary production (Qu, 2004a).

Table 1-2: Average water quality parameters **(A)** and nutrient concentrations **(B)** recorded in Lake Illawarra, between 1976 and 2007 (values are mean, with range given in parentheses).

(A)

Year	pH	Secchi depth (m)	Dissolved Oxygen (mg L^{-1})	Dissolved Oxygen (%)	Chlorophyll-*a* ($\mu g\ L^{-1}$)	Salinity (ppt)	Reference
1972 - 76	-	1.7 (0.9 - 2.2)	7.7*	92.7*	-	-	UOW and WCC (1976)
1981 - 84	7.6 (5.9 - 8.3)	1 (0.1 - 2) *	7.9 (5 - 9.4) *	104*	4.9 *	33.0 (10 - 40)	Pacific Power data (cited in LIA, 1995)
1987 - 91	7.9 (7.3 - 8.4)	1.1 (0.4 - 2)	7.7 (4 - 10.5)	-	6.95	" "	Pacific Power data (cited in LIA, 1995)
1991	7.6		6.8	93.4	6.90	-	Scientific Services (1992a) [i]
1992	7.6		6.76	87.3	3.03	-	Scientific Services (1992b) [ii]
1996 - 00	7.9 (7.4 - 8.4)	1.1 (0.1 - 2.6)	8.09 (3.85 - 11.52)	101.3 (54.0 - 120.1)	5.07 (0.01 - 29)	30.5 (1.6 - 39.2)	Pacific Power data (pers. comm., 2004)
2000 - 01	8.0 (7.5 - 8.9)	1.0 (0.2 - 2.2)	8.06 (4.86 - 13.93)	105.5 (65.8 - 190.7)	0.17 (0 - 2.3)	31.9 (5.9 - 39.2)	LIA data (pers. comm., 2001)
2000 - 02	8.1 (7.3 - 8.8)	-	7.4 (4.4 - 10.5)	102.6 (62.7 - 150.4)	-	30.5 (27.9 - 37.8)	Present Study
2005 - 07	-	-	-	-	4.56 (0.1 - 28.0)	-	LIA data (pers. comm., 2007)

(B)

Year	Total-P ($\mu g\ L^{-1}$)	PO_4^{3-}-P ($\mu g\ L^{-1}$)	Total-N ($\mu g\ L^{-1}$)	NH_3-N ($\mu g\ L^{-1}$)	NO_3^--N + NO_2^--N ($\mu g\ L^{-1}$)	Reference
1972 - 76	47 (4 - 145)	14 (2.1 - 68.5) *	-	12 *	3.4 (0 - 200)	UOW and WCC (1976)
1981 - 84	85 *	39 (5 - 125) *	460 *	28 *	3.8 (5 - 50) *	Pacific Power data (cited in LIA, 1995)
1987 - 91	91 (40 - 270)	44.7 (10 - 155)	423 (150 - 950)	34.5 (5 - 400)	23.2 (5 - 100)	Pacific Power data (cited in LIA, 1995)
1991	78	62	393	<10	<10	Scientific Services (1992a) [i]
1992	116	50	690	40	130	Scientific Services (1992b) [ii]
1993 - 94	88 (40 - 180)	59.8 (25-120)	395 (0 - 650)	20.1 (0 - 60)	16.1 (0 - 180)	Water Board (LIA, 1995)
1994	180 (90 - 560)	100 (6 - 16)	500 (<150 - 13000)	(<150 - 12000)	24 (<20 - 60)	AWACS (1994)
1996 - 00	79 (4 - 190)	48 (2 - 1050)	610 (250 - 3760)	45 (10 - 2100)	20 (1 - 1000)	Pacific Power data (pers. comm., 2004)
2000 - 01	67 (6 - 140)	46 (3 - 130)	440 (27 - 1200)	20 (10 - 170)	6 (1 - 120)	LIA data (pers. comm., 2001)
2005 - 07	100 (4 - 820)	50 (4 - 330)	590 (170 - 1500)	130 (30 - 350)	-	LIA data (pers. comm., 2007)

[i] Based on 1 day of intensive sampling during dry weather; [ii] Based on 1 day of intensive sampling during wet weather.
* After SPCC (1986).

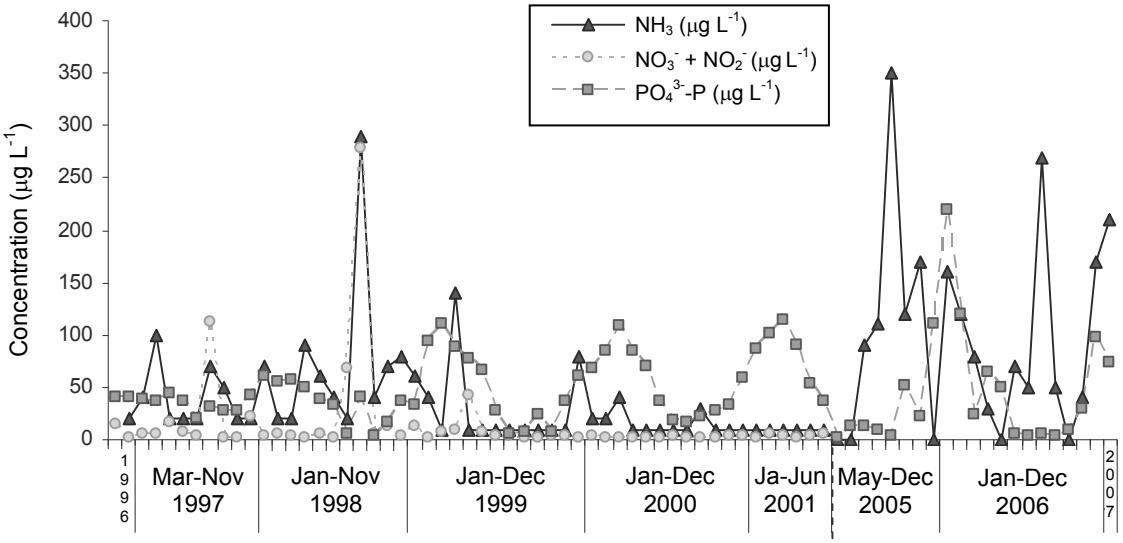


Figure 1-5: Concentrations of dissolved inorganic nitrogen and phosphorus in the water column, Lake Illawarra, 12/96 - 9/00 (Site LI5A: Pacific Power, pers. comm., 2004), 10/00 - 6/01 (Site 4: LIA, pers. comm., 2001) and 05/05 - 01/07 (Bevans Island: LIA, pers. comm., 2007).

1.5.6 Macroalgal Blooms

The main impact of nutrient enrichment in Lake Illawarra has been the excessive growth of macroalgae, which first became a significant problem in the early 1970s (LIA, 1995). Yassini (1985) reported that blooms composed largely of the filamentous green alga *Chaetomorpha* were particularly problematic in the 1980s, forming thick mats from the sediment to the water surface. For example, Yassini (1985) estimated that macroalgal blooms in winter 1985 covered 1.74 km^2, with an estimated total biomass of 71,000 tonnes (wet weight). Macroalgal blooms in Lake Illawarra are mostly confined to shallow areas of less than 0.6 m depth, largely due to a reduction in light attenuation with depth. Blooms are most likely to occur after periods of heavy rainfall, and extensive blooms have been recorded in winter, spring and summer (Yassini, 1985; Depers *et al.*, 1994; this study). Over the past few decades, problem areas for macroalgal blooms have been Griffins Bay, Koona Bay, Koong-Burry Bay, the Windang Peninsula and around Bevans Island (Yassini, 1985; King *et al.*, 1997).

Macrophyte beds, including macroalgae, can have many beneficial effects in an estuary. For example, macrophyte beds provide a structure for colonization by algal epiphytes and a subsequent habitat for organisms that feed on epiphytes (Chambers *et al.*, 1999). Many species of green algae, such as *Lamprothamnium* spp., are an important food source for water birds, especially swans (Noordhuis *et al.*, 2002). Large macrophyte beds also provide spawning grounds, shelter and refuge from predators for juvenile fish species and benthic invertebrates, including commercially important species (West, 1983; Sogard and Able, 1991). Additionally, macrophyte beds can improve water quality by taking up nutrients released from the sediment (McGlathery *et al.*, 1997), and by enhancing sedimentation and reducing the re-suspension of

sediment particles (Kufel and Kufel, 2001), thus restricting the release of sediment nutrients to the water column and reducing the availability of nutrients for phytoplankton.

Macrophyte beds also have many detrimental effects on an estuary. For example, the high oxygen consumption rates of dense mats of algae, such as *Chaetomorpha linum*, can substantially reduce dissolved oxygen concentrations within the mat, resulting in anoxia at the sediment-water interface (Lavery and McComb, 1991a). These anoxic conditions can enhance the release of nutrients from the sediment and decomposing bottom-layers of the algal mat, thus supporting further algal growth (Krause-Jensen *et al.*, 1996; Lavery and McComb, 1991a). The decomposition of decaying plant matter by aerobic bacteria can further reduce dissolved oxygen, resulting in DO stress and the subsequent death of fish and other aquatic organisms (Chambers *et al.*, 1999; Kamer and Fong, 2001). In Lake Illawarra, decomposition of the algal mats also produces an offensive odour and leads to the formulation of "black ooze" covering the sediment (Yassini, 1985; LIA, 1995). *Chaetomorpha* sp., and other filamentous algae which have detached from seagrass leaves, often form floating masses of entangled filaments which may sink to the Lake floor, or are transported by water movements and wind and accumulate on the Lake's foreshore (Yassini, 1985). These accumulations of large masses of macroalgae, as well as dead seagrass leaves, along the Lake's foreshore reduce the amenity of the Lake and restrict access to the Lake for wading, boating and various other recreational activities (see, e.g., Appendix 1A).

1.5.7 Macroalgal Harvesting

Since 1988, the Lake Illawarra Authority (LIA) has been manually removing substantial quantities of algal biomass from the Lake, at considerable expense to the authority (West, 1998). Past methods of harvesting macroalgae were to utilise a floating harvester in water deeper than 0.5 metres, and front-end loaders were used for foreshore removal of algal mats, decaying seagrass leaves and black ooze. It was estimated that approximately 3,000 tonnes of drained organic material was removed with the floating harvester each year prior to 2000, and about 5,000 tonnes of drained material was removed annually during foreshore cleanups (G. Clarke, pers. comm., 2003). Due to reduced macroalgal biomass in recent years, however, no harvesting of floating macroalgal biomass was conducted between 2000 and 2006 (WBM, 2006).

Macrophyte biomass is an important contributor to primary production and is also a significant sink for nutrients in estuaries. Seagrass and algal beds may be a significant contributor to anoxia during summer nights, especially in calm, shallow and stagnant estuarine waters, where stratification of the water column is likely to occur (Calado and Duarte, 2000). Eutrophication problems usually originate from the formation and subsequent decomposition of biomass, rather than the presence of nutrients (von Sperling, 1997). Therefore, harvesting of macrophytes may assist in preventing or lessening the impact of eutrophication by removing organic matter and

nutrients (Calado and Duarte, 2000). However, past studies (e.g., LIA, 1995) have shown that the amount of nutrients removed from Lake Illawarra during algal harvesting is insignificant when compared to other potential sources and sinks of nutrients in the Lake, such as sediment nutrient release and catchment runoff. Macroalgal harvesting also has the obvious benefits of preventing the accumulation of organically rich sediment (black ooze) and improving amenity and access to foreshore regions for boating and other recreational uses. However, the machinery, such as bulldozers, that is used to remove algae can have a negative effect on the ecology of the Lake by damaging the foreshore seagrass beds and compacting sediment, thus preventing re-establishment by seagrasses.

The main impacts of macroalgal harvesting relate to physical or biological disturbance, such as the removal of habitat and entire populations of small fish and invertebrates which inhabit the algal mats (Lavery *et al.*, 1999; Chambers *et al.*, 1999). During the present study, substantial quantities of fish eggs were found attached to the Lake Illawarra algal mats which are also inhabited by large populations of juvenile fish and crustaceans. Removal of vast quantities of these organisms through algal harvesting could potentially have an adverse effect on fish populations in Lake Illawarra and requires further investigation. According to Calado and Duarte (2000), macrophyte harvesting may also result in a further input of nutrients to the water column by disturbing sediment and resuspending organic matter which could, in turn, result in a reduction in dissolved oxygen. These authors suggest that these impacts of harvesting could be reduced by using a cutting device to only remove macrophytes above the sediment-water interface and avoiding harvesting during periods of water column stratification. Removal of algal mats can also provide space or other resources, such as light, which may initiate succession (Foster and Barilotti, 1990). Nevertheless, ecological populations often recover quickly following disturbance from harvesting. For example, while macroalgal harvesting on beaches in the Peel-Harvey Estuary, Western Australia, resulted in an immediate decline in macroalgal biomass and associated epifaunal and fish communities, most of these variables had recovered to values similar to non-harvested beaches within two months (Lavery *et al.*, 1999).

1.6 Summary

While Lake Illawarra has been the focus of many scientific studies and reports, there are still substantial gaps in knowledge, particularly regarding the factors responsible for excessive growth of macroalgae in Lake Illawarra. Although oversupply of nutrients (N and P) has often been cited as the cause of excessive algal growth, little information is available concerning the relationship between nutrients and algal biomass in Lake Illawarra. Additionally, it is not yet known what, if any, contribution macroalgal beds make to the Lake's nutrient budget. The present study has examined the distribution, biomass and nutrient relationships of seagrasses and macroalgae in Lake Illawarra. The following chapter includes a literature review on the ecology, growth, and nutrient (C, N and P) relationships of seagrasses and macroalgae.

CHAPTER 2 Literature Review

2.1 Introduction

Urban and industrial centres in Australia and Worldwide have historically been developed along coastlines or in the vicinity of available water supplies. As a result, coastal ecosystems, such as estuaries and lagoons, are commonly the receiving water bodies for much of society's urban, industrial and agricultural waste (Young, 1996). This often leads to water and sediment contamination in coastal ecosystems and alterations in the natural sedimentological, hydrological and biological regimes, such as invasion of non-indigenous species (Lavoie *et al.*, 1999) and a reduction in marine biodiversity and an increase in nuisance algal blooms (Paerl, 1988). Estuarine habitats are an important source of food, shelter and breeding areas for many birds, mammals, fish and other aquatic organisms, including many commercially important species. However, many estuaries and coastal ecosystems are considered to be under threat of degradation due to eutrophication of waterways. Eutrophication refers to the enrichment of plant waters by inorganic plant nutrients, usually nitrogen and phosphorus, and results in an increase in primary productivity (Kiely, 1997). Eutrophication is a natural process, although anthropogenic influences may cause excessive nutrient levels, leading to artificial enrichment of waters. The main consequences of increased nutrient inputs are an increase in plant biomass production, often leading to algal blooms (especially of species responsive to nutrient inputs), the loss of submerged macrophytes, and increased oxygen consumption in bottom waters (Painting *et al.*, 2007).

Nutrient enrichment can dramatically alter plant biomass in estuaries and shallow lagoons, as macroalgae that take up nutrients from the water column tend to replace macrophytes that take up nutrients from the sediment via root systems (Valiela *et al.*, 1997). Nutrient loading often leads to a proliferation of short-lived, fast-growing, bloom-forming macroalgae, and an eventual decline in seagrasses (McComb and Lukatelich, 1986; Chisholm *et al.*, 1997). For example, Hauxwell *et al.* (2001) determined that a macroalgal canopy of approximately 9 - 12 cm height over a *Zostera marina* bed was the critical point at which the growth and overall health of the seagrass declined. In eutrophic waters, light, rather than nutrients, tends to limit biomass formation of seagrasses and slow-growing attached macroalgae, as phytoplankton and fast-growing, free-floating macroalgae are superior competitors for available light and other resources (Brennan *et al.*, 1998). For example, in Waquoit Bay, Massachusetts, increasing nitrogen loads (above 30 kg N ha^{-1} yr^{-1}) resulted in an increased biomass of macroalgae and phytoplankton, and large-scale loss of *Zostera marina* beds due to light limitation (Bowen and Valiela, 2001). Eutrophication may also negatively affect seagrass beds through changes in sediment redox conditions and sulfide concentrations (Azzoni *et al.*, 2001). In addition, increased sedimentation restricts the ability of beneficial macroalgae to attach to the substrate, as well as reducing water quality, thereby reducing survival rates of some algae (Sanderson,

1997). A recent study by Krause-Jensen *et al.* (2007) showed that cover of opportunistic algae also declined with eutrophication (namely, increasing total nitrogen) and reduced water clarity.

The problematic, bloom-forming macroalgae are mostly green (Chlorophytes) and typically filamentous (e.g., *Chaetomorpha, Cladophora*), tubular or sheet-like (e.g., *Ulva*). These algae accumulate in extensive masses, entangled amongst seagrass beds or occasionally attached as epiphytes or to stable substrata. They often detach from the substrate and form free-floating masses, or drift inshore to form thick mats over the sediment surface. In eutrophic waters, thick mats of macroalgae often reach heights of 0.5 m and biomasses exceed 500 g DW m^{-2} (McGlathery, 2001). Algal blooms often increase turbidity, leading to a reduction in light transparency and death of attached macrophytes due to shading (Kiely, 1997). As algae die and sink to deeper waters, an increased load of organic detritus accumulates, requiring oxygen for decomposition. If the rate of oxygen consumption exceeds the rate of oxygen input then rapid deoxygenation of bottom waters may occur, ultimately leading to the development of anoxic conditions (Grant and Jickells, 1995), defined as waters with 0 mg O_2 L^{-1} (Kennish, 1997). This process is often exacerbated by thermal stratification, which occurs when surface water is continually heated and floats on top of the deeper, colder water; this prevents the transport of oxygen into the deeper water and results in nutrients from decomposing organic material not being recycled into the upper layers. Thus, oxygen and plant nutrients become limiting, resulting in low productivity (Grant and Jickells, 1995; Kiely, 1997).

This chapter reviews the ecology of seagrasses and macroalgae in shallow coastal environments, with particular reference to those genera found in Lake Illawarra (refer to Chapter 4). Environmental processes influencing the growth of macrophytes are discussed, followed by a review of the nutrient relationships (carbon, nitrogen and phosphorus) of macrophytes.

2.2 Ecology of Aquatic Macrophytes

The term “macrophyte” is used here to describe aquatic plants that are visible to the naked eye; this refers to the seagrasses, which are attached to the sediment via a root-rhizoidal system, as well as the larger algae or seaweeds, which may be attached to a stable substrate or free-floating. Aquatic macrophyte beds are extremely important components of coastal ecosystems; they provide habitat, shelter and a food source for various organisms and are thus inhabited by various fish and planktonic communities (van Donk and van de Bund, 2002). Submerged macrophyte beds serve as both sinks and sources of organic matter and nutrients; they can contain significant pools of organic carbon and are thus an important component of carbon and nitrogen fluxes in shallow lakes (Rooney and Kalff, 2000). Additionally, macrophyte beds have a stabilizing effect and are essential for maintaining clear water conditions as they limit mixing and inhibit erosion and resuspension of sediment and particulates (van Donk and van de Bund, 2002; Steinman *et al.*, 2002).

The presence of macrophyte beds can also have an important influence on phytoplankton dynamics. Macrophytes successfully compete with phytoplankton and periphyton for nutrients in the water column and can significantly affect the amount of light available for phytoplankton (van Donk and van de Bund, 2002). Kufel and Kufel (2001) determined that beds of *Chara* (Chlorophyta) act as important nutrient sinks in shallow lakes, through direct uptake of nutrients and by restricting the resuspension of sediment to the water column, thus limiting the availability of nutrients to phytoplankton. In addition, macrophytes release organic carbon compounds which enhance production and nutrient uptake by bacteria (Vähätalo and Søndergaard, 2002), thus reducing the availability of nutrients for phytoplankton (van Donk and van de Bund, 2002). However, after blooms of ephemeral macroalgae collapse and decay, nutrients released into the water column may stimulate phytoplankton production (Sfriso *et al.*, 1992; McGlathery, 2001).

Aquatic macrophytes which persist in estuaries must survive wide fluctuations in temperature, light, salinity, water level and ephemerality; these are often the most important variables determining the distribution and abundance of estuarine flora. The ability to tolerate these wide fluctuations, rather than extreme levels of each variable, may determine which macrophytes will grow in an estuary (Brock, 1986). The key factors affecting growth and photosynthesis of aquatic macrophytes are environmental variables, such as light availability, water temperature, water velocity and salinity, and concentrations of nitrogen, phosphorus and dissolved inorganic carbon (McComb and Lukatelich, 1986; Carr *et al.*, 1997; Lougheed *et al.*, 2001). These issues will be discussed further in the following sections.

2.2.1 Seagrasses

Seagrasses are angiosperms ("flowering plants") that complete their entire life cycle submerged below the water (Robertson, 1984). They typically colonise soft substrates, from sand to mud, in shallow, sheltered areas of estuaries, bays, lagoons, and lakes (West, 1983). At least 52 species of seagrass occur worldwide (West, 1989), with about 30 species found in Australian waters (Kirkman, 1997). Six seagrass species have been documented along the New South Wales coastline: *Posidonia australis, Zostera capricorni, Zostera muelleri, Heterozostera tasmanica, Halophila ovalis* and *Halophila decipiens* (West, 1989). However, a recent assessment by Les *et al.* (2002) concluded that *H. tasmanica, Z. capricorni* and *Z. muelleri* in Australia and New Zealand should be merged into a single species (*Zostera capricorni*), due to a lack of morphological and genetic differentiation between the species. Another aquatic genus, *Ruppia*, which commonly grows in association with the above seagrasses, is also considered here, but by definition, it is not a true seagrass; *Ruppia* is tolerant of a broad range of salinities, including fresh, brackish and marine, and pollination may occur above or below the water surface (Robertson, 1984). Three species of *Ruppia* occur in New South Wales: *Ruppia maritima, Ruppia megacarpa* and *Ruppia polycarpa* (West, 1989). In Lake Illawarra, there are four species of seagrass: *Zostera capricorni, Ruppia megacarpa, Halophila decipiens* and

Halophila ovalis (Harris, 1977; Yassini, 1985). The morphology and taxonomy of seagrasses found in Lake Illawarra is described further in Section 4.4.

Seagrass beds are important habitats and nursery areas for fish and provide a source of food, structure and shelter from predators (Bell and Pollard, 1989). Some marine animals (e.g., dugongs and green sea turtles) and fish (e.g., garfish and leatherjackets) graze directly on seagrasses, such as *Halophila* and *Halodule* (Walker *et al.*, 1999). Seagrasses are also an indirect food source for fish and other aquatic organisms, either through detrital production, or by providing a structural substrate for colonization by invertebrates and algal epiphytes (Walker *et al.*, 1999). Thus, the diversity and abundance of fish and other animals is usually greater in seagrass beds than in nearby unvegetated areas (Bell and Pollard, 1989; West and King, 1996; Jones and West, 2005). In addition, different seagrass beds (e.g., *Posidonia* versus *Zostera*) support different fish communities (Rotherham and West, 2002).

Seagrass beds slow water movement, thus facilitating deposition of particulate matter, such as sediment, organic material, eggs and larval invertebrates. Sediment and organic matter is subsequently trapped by the seagrass roots and rhizomes and bound into the sediment (Walker *et al.*, 1999). Thus seagrass beds play an important role in stabilising sediment, trapping nutrients from the water column, reducing turbidity and ultimately improving water quality (McComb and Lukatelich, 1986). Additionally, seagrasses influence nutrient fluxes through nutrient uptake and through the excretion and leakage of cell products (Walker *et al.*, 1999). Epiphytes attached to seagrasses and larger macroalgae can also contribute to primary production through nutrient uptake and release (Walker *et al.*, 1999).

Seagrasses, such as *Zostera* and *Ruppia*, typically exhibit a seasonal distribution, with biomass peaking in the warmer months and die-back of leaf material occurring during late autumn and winter (DeBoer, 2000; Menéndez, 2002; White, 2003). *Ruppia megacarpa* also exhibits a general seasonal trend in percent cover, above and below ground biomass, shoot density and leaf length; Carruthers *et al.* (1999) concluded that the abundance of *R. megacarpa* within Wilson Inlet, Western Australia, was related to the seasonal variation of hydrological variables (e.g., conductivity, turbidity and depth), which were controlled by climate and the annual opening of the estuary to the sea. Seagrasses have high growth rates and produce large quantities of biomass, especially leaf material (West, 1983), which is shed periodically and often forms dense wracks along the shoreline of coastal embayments (Jędrzejczak, 2002; Mateo *et al.*, 2003) and estuaries, such as Lake Illawarra (Ganassin, 1994). Most species of seagrasses use the soluble carbohydrate and proteins stored in rhizomes for regrowth when leaves are removed, with the below-ground portion (roots, rhizomes, small shoots) of the plant incorporating up to 90 % of the total biomass (Dawes, 1998). In seagrasses, the dominant storage carbohydrate is sucrose, which typically constitutes more than 90 % of the total soluble carbohydrate pool, and is primarily stored in the rhizomes (Touchette and Burkholder, 2002).

Having carbohydrates stored predominantly in the below-ground material would minimise loss of carbon (e.g., due to herbivory, or shedding of older leaves), and ensure maintenance of rhizomatous structures during periods of relative dormancy, such as winter (Touchette and Burkholder, 2002).

2.2.2 Macroalgae

The vast majority of marine and estuarine plants, other than seagrasses, belong to the group referred to as "algae". Algae are photosynthetic (pigmented), mainly aquatic, plants characterised by a simple structure, which is never differentiated into roots, stems and leaves, non-vascular, and without a sterile layer of cells surrounding the reproductive organs (Womersley, 1984; Allaby, 1998). The algae encompass several phyla and a vast array of forms, ranging from benthic or free-floating varieties readily seen with the naked-eye, to microscopic varieties, such as phytoplankton and minute epiphytes on seagrasses and larger macroalgae (Womersley, 1984). The major divisions of benthic algae are: Chlorophyta (green algae), Phaeophyta (brown algae), Rhodophyta (red algae), and to a lesser extent Cyanophyta (blue-green algae). Over 4400 species of marine algae have been recorded worldwide (Dawes, 1998) and the marine benthic algae occurring in NSW alone include 131 species of green, 140 species of brown and 449 species of red macroalgae (Millar, 2004). Of interest to this study are the macroalgae, those readily seen un-aided, and ranging in size from small epiphytes growing on seagrass leaves, to the larger bloom and mat-forming varieties.

Macroalgae occur in a wide range of environments, including fresh, brackish, marine, and hypersaline water. Macroalgal biomass can be an important shelter to juvenile fish populations and other animals (McComb and Lukatelich, 1986), as well as a food source; for example, *Ulva intestinalis* (formerly *Enteromorpha intestinalis*) is reported to be an important food source for lined shore crabs and topsmelt in southern Californian estuaries (Kamer and Fong, 2001). Charophytes (e.g., *Chara*, *Lamprothamnium*) are an important food source for water birds, especially swans, in shallow coastal lakes (Søndergaard *et al.*, 1996; Noordhuis *et al.*, 2002), including Lake Illawarra (Harris, 1977). Noordhuis *et al.* (2002) found that water birds absented Lake Veluwemeer, The Netherlands, when macrophyte biomass was reduced by eutrophication and subsequent increase in microalgal biomass. Restoration works were subsequently undertaken to reduce the nutrient supply, and hence eutrophication, and to recolonise *Chara* spp. in the lake. The study found that water bird numbers increased significantly with respect to increasing charophyte biomass in the lake, and that more birds ate charophytes than any other macrophyte species in the area.

Macroalgal biomasses are important contributors to primary production in estuaries and can also act as significant sinks for nutrients therein, by taking up nutrients directly from the water column, as well as intercepting the flux of nutrients from the sediment to the water column

(Krause-Jensen *et al.*, 1996). However, large masses of macroalgae can have a detrimental effect on aquatic ecosystems by depleting oxygen from the water column and sediment, which can lead to mortality of fish and other aquatic organisms (Kamer and Fong, 2001). In addition, macroalgal mats, as well as excessive epiphytic algal growth, can significantly shade seagrass beds and reduce their photosynthesis, leading to a decline in seagrass areas (McComb and Lukatelich, 1986; Bach *et al.*, 1992; McGlathery, 2001).

Growth and productivity of macroalgae are largely determined by light, temperature, nutrient concentrations and water movement (DeBoer, 1981; Fong and Zedler, 1993). The biodiversity of macroalgae tends to increase with increasing water clarity, salinity and hard substratum, but generally declines with increasing nutrient concentration, with a shift towards simple-structured, opportunistic algae with high growth rates (Krause-Jensen *et al.*, 2007). Macroalgae tend to be highly successful in a wide range of environments, due predominantly to a high tolerance to large variations in environmental factors, such as salinity, temperature and nutrient fluxes (Lotze and Schramm, 2000). Many species of green macroalgae have high nutrient uptake rates and large internal storage capacities, enabling them to take up nutrients quickly and proliferate in shallow estuaries where nutrient influxes are often episodic; for example, *Enteromorpha prolifera* (O'Brian and Wheeler, 1987), *E. intestinalis* (Kamer and Fong, 2001), *Chaetomorpha linum* (Lavery and McComb, 1991b; Krause-Jensen *et al.*, 1996, 1999), *Cladophora* aff. *albida* (Gordon *et al.*, 1981), *C. vagabunda* (Peckol *et al.*, 1994), *C. montagneana* (Gordon and McComb, 1989), *Ulva fasciata* (Lapointe and Tenore, 1981) and *U. lactuca* (Ho, 1987). Most species of nuisance green algae, such as *Ulva* or *Cladophora*, show a seasonal distribution, proliferating in one season and declining in the next (Womersley, 1984; Menéndez *et al.*, 2001). In temperate regions, macroalgae mats tend to reach a maximum biomass from spring through to summer, disappearing towards late summer or autumn (Fujita, 1985; Bolam *et al.*, 2000). *Chaetomorpha linum*, however, has the ability to persist over winter, taking up and storing nutrients when rainfall and nutrient fluxes may be higher, and thus supporting growth in summer when fluxes may be lower (Lavery and McComb, 1991b).

Both seagrass and algal beds may be significant contributors to anoxia during summer nights, especially in calm, shallow and stagnant estuarine waters, where stratification of the water column is likely to occur (Calado and Duarte, 2000). The density of macroalgal mats can have a significant effect on dissolved oxygen concentrations; Lavery and McComb (1991a) found that in low density *Chaetomorpha linum* mats ($\leq$ 312 g m^{-2}) dissolved oxygen concentrations were 15.2 % higher inside the mat than in the external water. The opposite effect was observed in dense mats ($\geq$ 668 g m^{-2}), where dissolved oxygen concentrations were 96 % lower inside the mat than outside.

Green macroalgal mats (e.g., *Ulva*, *Chaetomorpha*) tend to have a reducing effect on the underlying sediments, often resulting in anoxia and accumulation of toxic hydrogen sulfide

(Bolam *et al.*, 2000). These mats often have significant effects on the macrobenthos, such as, a decline in species richness; Bolam *et al.* (2000) found that *Enteromorpha prolifera* mats that were artificially implanted on intertidal sandflats caused a distinct shift in macrofaunal population from species found in natural conditions (e.g., *Pygospio elegans*) to those commonly found in organically polluted sediments (e.g., *Capitella capitata*).

2.2.3 Macrophyte Biomass

The biomass of macrophytes is usually measured through field-based sampling, such as assessments of percent coverage, physical removal and weighing of above- and/or below-ground material, or measuring of leaf length, shoot density and other characteristics. A number of studies have used aerial or digital photographs to estimate biomass of seagrass and macroalgal beds, usually in combination with *in situ* harvesting methods (e.g., Mellors, 1991; Long *et al.*, 1994; Robbins, 1997; Smith *et al.*, 2000; Håkanson and Boulion, 2002). Production (i.e., growth) of macrophytes can be assessed through experimental techniques, such as leaf marking, harvesting, or incubation experiments (Menéndez, 2002).

Macrophyte biomass in temperate waters generally increases in spring and reaches peak biomass in summer. Biomass tends to decline towards the end of summer or autumn, reaching a minimum in winter (Fernández-Aláez *et al.*, 2002; Menéndez, 2002). Macrophyte species that are ephemeral in growth, or those that die-back over winter, usually show greater fluctuations in biomass than those species that maintain growth throughout the year. Thus macroalgal growth can be highly variable, with maximum biomasses of green macroalgae (e.g., *Chaetomorpha* and *Ulva*) often in the order of 300 - 600 g DW m^{-2} (Hernández *et al.*, 1997; Malta and Verschuure, 1997; Fernández-Aláez *et al.*, 2002). Lavery *et al.* (1991) documented maximum macrophyte biomasses of 4,400 g DW m^{-2} in the Peel Inlet, Western Australia, in 1979; this value was obtained for a mixed seagrass-algal bed, of which 89 % of the total biomass was *Cladophora.* These authors also reported maximum biomass records for *Chaetomorpha linum* (3,600 g DW m^{-2}), *Enteromorpha intestinalis* (1,900 g DW m^{-2}) and *Ulva rigida* (1,500 g DW m^{-2}). The average biomass and production estimates of several seagrass species occurring Worldwide are listed in Table 2-1. Duarte and Chiscano (1999) estimated the average annual biomass production of 30 seagrass species worldwide to be 1012 g DW m^{-2} per year. The mean above- and below-ground biomass of all seagrass species reviewed was 223.9 ± 17.5 and 237.4 ± 28 g DW m^{-2}, respectively.

Table 2-1: Dry weight biomass and daily production of seagrasses (values are mean ± s.e., where given).

Species	Biomass (g DW m^{-2})	Production (g DW m^{-2} d^{-1})	Location	Reference
Cymodocea serrulata [t]	62.5	0.20 - 0.62	Mozambique	de Boer (2000)
Halophila ovalis [t]	0.3 - 11	n/a	Qld, Australia	Long *et al.* (1994)
Halophila ovalis [a]	54.8*	0.03*	Worldwide	Duarte and Chiscano (1999)
Halophila ovalis [b]	21.1*	0.01*	Worldwide	Duarte and Chiscano (1999)
Posidonia australis [t]	1111	6.4	W. Australia	Paling and McComb (2000)
Posidonia sinuosa [a]	140 - 190	1.6 - 2.5	W. Australia	Cambridge and Hocking (1997)
Ruppia cirrhosa	100 - 1,375	n/a	Mediterranean	Menéndez and Comin (2000)
Zostera capricorni [t]	45 - 364	n/a	Qld, Australia	Long *et al.* (1994)
Zostera capricorni [a]	191.4*	1.9*	Worldwide	Duarte and Chiscano (1999)
Zostera capricorni [b]	176.0*	0.44*	Worldwide	Duarte and Chiscano (1999)
Zostera capensis [t]	205	0.18	Mozambique	de Boer (2000)
Zostera marina [a]	26.6 - 136.3	n/a	France	Plus *et al.* (2001)
Zostera marina [b]	19.2 - 125.0	n/a	France	Plus *et al.* (2001)
Zostera marina [a]	150 ± 25	n/a	Norway	Duarte *et al.* (2002)

* average maximum biomass
[a] above-ground material
[b] below-ground material
[t] total biomass (above- and below-ground material)

2.2.4 Environmental Limitations on Macrophyte Growth

Seagrass and macroalgal growth is fundamentally complex, responding to a variety of environmental conditions such as light, temperature and nutrient concentrations, and can not be explained by any single parameter. Growth can vary spatially, seasonally and annually and having multiple species present in a single macrophyte community makes it difficult to treat the whole community as a single homogeneous unit (Sterner and Grover, 1998). Many authors (e.g., Peckol and Rivers, 1996) have suggested that despite a wide variation in nutrient loading rates in coastal waters, primary productivity estimates do not vary greatly. This suggests that the limits on primary productivity in estuaries are often determined by environmental factors other than nutrient loading (Peckol & Rivers, 1996). Meteorological and hydrological regimes, and associated fluctuations in temperature, light availability and salinity, have a significant influence on the seasonal and annual fluctuations of macrophyte biomass (Fernández-Aláez *et al.*, 2002). Other factors that influence macrophyte distribution and biomass include lake shape and form, latitude, hydrology, sediment slope and physical characteristics and water clarity (Rooney and Kalff, 2000). Seagrass and macroalgal growth can also be significantly affected by grazing pressure (e.g., by snails, isopods, amphipods) (Worm *et al.,* 2000), plant density (Israel *et al.*, 1995) and competition for resources between co-existing macrophyte species and epiphytes (Friedlander *et al.*, 1991; Anderson *et al.*, 1996; Fong *et al.*, 1996; Lin *et al.*, 1996; Coffaro and Bocci, 1997; Gacia *et al.*, 1999; Reyes and Sansón, 2001).

The availability of light is probably the critical factor determining macrophyte distribution, but seasonal fluctuations in water temperature and interactions between light and temperature are also significant factors (Rooney and Kalff, 2000). Productivity in aquatic macrophytes is

significantly affected by water temperature, which influences the rate at which chemical reactions take place (Carr *et al.*, 1997, and references therein). In addition, temperature can influence physiological processes in algae, such as the rate of diffusion or uptake of nutrients, thus regulating nutrient availability (Tsai *et al.*, 2005). Masini *et al.* (1995) reported the optimal temperature for photosynthesis of *Posidonia sinuosa* was 18 - 23°C and respiration rates at 23°C were 80 % higher than at 13°C. Touchette and Burkholder (2000b) noted that respiration rates in seagrasses tend to increase with increasing temperatures above 40°C. Both temperature and irradiance have been shown to have a significant effect on the growth of seagrasses, such as *Zostera capricorni, Syringodium isoetifolium, Halodule uninervis, Cymodocea serrulata, Halophila spinulosa* (Grice *et al.*, 1996) and *Posidonia oceanica* (Zupo *et al.*, 1997) and macroalgae, such as *Ulva* sp. (Lapointe and Tenore, 1981), *Ulva lactuca* (Steffensen, 1976), *Cladophora* sp. (Gordon *et al.*, 1981), *Cladophora montagneana* (Lavery *et al.*, 1991), *Chaetomorpha linum* (Arnold and Murray, 1980; King *et al.*, 1990), *Codium fragile*, *Enteromorpha intestinalis* (Arnold and Murray, 1980) and *Colpomenia peregrina* (Matta and Chapman, 1995). Taylor *et al.* (2001) noted that tolerance of temperature and irradiance may often be related to habitat; intertidal and shallow-water algae, for example, would tolerate higher temperatures and irradiances than deep-water algae. The effect of light availability on macrophyte growth is discussed further in Section 2.3.1.

Salinity in estuaries can be highly variable, depending on freshwater and saltwater influxes and evaporation. Many species of macroalgae and some seagrasses (e.g., *Ruppia maritima*: Lazar and Dawes, 1991) have been shown to be tolerant of a broad range of salinities. For example, Imai *et al.* (1997) found that *Rhizoclonium riparium* was tolerant of a wide range of salinities, from 0.1 - 34.0 ppt, although the rate of photosynthesis was significantly lower at the lowest salinities. Algae can adapt to fluctuating salinity regimes by regulating their internal solute concentration, but this process requires energy, which may result in a reduction in algal growth and productivity (Kamer and Fong, 2001). Taylor *et al.* (2001) found that the highest growth rates of nuisance green algae occurred in salinities similar to estuarine water, rather than sea water; growth increased with increasing salinity up to 70 - 80 % seawater (23.8 - 27.2 psu). *Chaetomorpha linum* also grew at a rate of about 4 % WW d^{-1} in 0 psu. Additionally, these authors showed that *Enteromorpha compressa* tolerated a broad range of salinities, but the highest growth rates of about 7 % WW d^{-1} were recorded at 6.8 PSU. Similarly, Martins *et al.* (1999) found the highest growth rates (up to 19.7 % d^{-1}) of *Enteromorpha intestinalis* occurred at 15 - 20 psu and the lowest growth rates (< 0.9 % d^{-1}) at salinities ≤ 5 psu and ≥ 25 psu. Kamer and Fong (2001) reported that *Enteromorpha intestinalis* in southern Californian estuaries proliferated despite significant fluctuations in nitrogen supply and salinity. These authors found that *E. intestinalis* responded very well to N addition, while salinity reduction reduced growth. However, high N additions (~18 to 152 μM NO_3^-) counteracted the negative effects of reduced salinity on algal growth rates and P uptake ability. Additionally, tissue N content of *E. intestinalis* decreased as salinity increased (from 15 to 35 psu), but was not

significantly affected by N enrichment, indicating that the alga was taking up and storing N even when reduced salinity hindered growth rates. Kamer and Fong (2001) also found that wet to dry biomass ratios of *E.* intestinalis increased as salinity decreased, but N enrichment ameliorated the negative effect of reduced salinity; under high N enrichment, wet to dry ratios at 15 psu were similar to those at 25 and 35 psu.

Increasing water depth and exposure (e.g., to wind, waves) are negatively associated with macrophyte biomass (Rooney and Kalff, 2000). Wave-exposed shorelines, for example, will often have a different macrophyte community structure than sheltered locations and will be dominated by robust, wave-tolerant species, such as the large kelps (Sanderson, 1997). Previous studies have demonstrated that increased water movement can significantly enhance macroalgal growth rates as well as the rate of nutrient uptake and photosynthesis (Wheeler, 1982; Hurd, 2000; Smit, 2002), and sporeling development (Ryder *et al.*, 2004). To enter a cell, ions move across the boundary layer of water adjacent to the outer cell of the thallus; low water movement can affect nutrient uptake rates because the boundary layer of water surrounding the cell will be thicker, so that the rate of diffusion of ions across this layer may limit uptake (Lobban and Harrison, 1997). For example, Smit (2002) found that at low concentrations of nitrate-N (4 μm), NO_3^--N uptake rates of *Gracilaria gracilis* were 2.5 times higher under high water movement (500 oscillations min^{-1}) than under still conditions. Site-related differences in growth of macroalgae may also be related to differences in water movement (Smit, 2002); for example, Glenn and Doty (1992) suggested that water motion accounted for 81 - 98 % of the variation in growth rates of *Kappaphycus alvarezii* (Rhodophyta) amongst different experimental pens.

2.3 Photosynthesis in Aquatic Macrophytes

Photosynthesis is the oxidation-reduction reaction by which plants and other organisms (e.g., certain protists and bacteria) convert light energy to chemical energy (Purves *et al.*, 1995). In green plants, energy absorbed by chlorophyll from sunlight is used to reduce carbon dioxide (CO_2), and subsequently produce glucose ($C_6H_{12}O_6$) and other organic compounds used for energy (Allaby, 1998), as summarised by the following equation:

$$6CO_2 + 12H_2O \xrightarrow{\text{light energy}} C_6H_{12}O_6 + 6H_2O + 6O_{2\,(gas)}$$

Water acts as a reactant in the process, and both water and oxygen (as gas) are released as products. The oxygen produced photosynthetically by green plants, particularly algae, is an extremely important source of atmospheric oxygen (Purves *et al.*, 1995). Photosynthesis is the result of two pathways; firstly, the “light reactions” used to produce adenosine triphosphate (ATP), and secondly, the “dark reactions” (or Calvin-Benson cycle) which use ATP to produce glucose. The first pathway utilises light energy to oxidise water. Hydrogen ions released during the oxidation of water are used to reduce nicotinamide adenine dinucleotide phosphate (NADP) to NADPH + H^+: Associated with these reactions, is the process of phosphorylation; the

conversion of adenosine diphosphate (ADP) to a highly energetic compound, adenosine triphosphate (ATP). The second pathway of photosynthesis ("dark reactions" or carbon fixation) is not light dependent, and uses the energy stored in the NADPH + H^+ and ATP obtained from phosphorylation to reduce carbon dioxide to glucose and other compounds required for plant growth (Purves *et al.*, 1995; Allaby, 1998; Fyfe, 2004).

Associated closely with photosynthesis, is respiration, referring to the oxidation and mobilization of stored carbohydrates (Touchette and Burkholder, 2000b). Where CO_2 is consumed and O_2 is released for photosynthesis, respiration uses O_2 and releases CO_2 (Lobban and Harrison, 1997). Both photosynthesis and respiration in aquatic macrophytes are significantly affected by light, water temperature, water motion and dissolved oxygen (Touchette and Burkholder, 2000b; Carr *et al.*, 1997; Hurd, 2000). In addition, many studies have shown that photosynthesis, and subsequently growth, of macroalgae increases significantly under increased nutrient enrichment (Peckol and Rivers, 1996).

2.3.1 Availability of Light

In shallow lakes, light availability is the key factor controlling photosynthesis (van Nes *et al.*, 2002) and can often be a limiting factor for seagrass and macroalgal growth as photosynthesis provides the energy required for nutrient uptake and assimilation (McGlathery *et al.*, 1997). The amount of light absorbed by macrophytes depends on the concentration of photosynthetic pigments; in seagrasses and algae, these pigments include chlorophyll *a* and a range of other chlorophylls, carotenes, xanthophylls, and phycobilins (in red and blue-green algae). The chlorophylls and other light-harvesting pigments absorb across a wide range of wavelengths, referred to as photosynthetically active radiation (PAR); PAR is defined as wavelengths of 350 - 700 nm (Lobban and Harrison, 1997).

The availability of light decreases with increasing water depth and distance from the shoreline, and is often a limiting factor for primary producers, such as phytoplankton (Cabeçadas, 1999), macroalgae (Bonis and Grillas, 2002; Kufel and Kufel, 2001; Steinman *et al.*, 2002) and seagrasses (Bach *et al.*, 1992). Light availability is also related to water turbidity and meteorological conditions (Fernández-Aláez *et al.*, 2002); for example, in the Peel Inlet, Western Australia, low light levels often limit macrophyte growth during winter and early spring when nutrient availability is high, whereas low nutrient concentrations tend to limit production during summer when light levels are higher (Lavery *et al.*, 1991). Eutrophication of coastal waters leads to increased turbidity and growth of macroalgae and phytoplankton, and a subsequent reduction in the availability of light to seagrasses; this reduction in light can significantly affect the depth range of seagrass growth, from a few centimetres to 1 - 2 m (Bach *et al.*, 1992). For example, *Ruppia megacarpa* in Wilson Inlet, Western Australia, showed a rapid response to increased turbidity and a subsequent reduction in light, with abundance decreasing in a matter of weeks (Carruthers *et al.*, 1999). Grice *et al.* (1996) found that

seagrasses (e.g., *Zostera capricorni*, *Halophila spinulosa* and *Cymodocea serrulata*) grown in outdoor culture experiments in Moreton Bay, Queensland, were significantly affected by changing light intensity; for all seagrass species, the productivity was higher in full sunlight (100 % incident light) than in low light treatments (5 to 50 % incident light). Grice *et al.* (1996) also found that in full sunlight, $\delta^{13}C$ values of seagrasses became 3 to 4 ‰ less negative, while productivity, lacunal areas and root biomass increased. In addition, the lowest concentrations of tissue nitrogen and the highest C/N ratios were generally recorded under full sunlight (100 % light intensity), suggesting that the seagrasses were becoming nutrient limited.

The availability of light, and associated reduction in productivity, becomes a limiting factor within dense mats of macroalgae, such as *Chaetomorpha* or *Cladophora* (e.g., Gordon *et al.*, 1981). When algal densities are low, sunlight is not fully exploited, whereas in medium algal densities, growth rates tend to increase to a maximum during the initial growth period when biomass is at a sufficient level to fully exploit incoming irradiance (Lapointe and Tenore, 1981). Dense algal mats, however, promote self-shading within the mat, reducing the amount of light reaching the bottom layers (Krause-Jensen *et al.*, 1996). Low irradiance levels are likely to limit primary production and, therefore, the uptake and assimilation of available nutrients by macroalgae, within dense algal mats (Krause-Jensen *et al.*, 1996; McGlathery *et al.*, 1997). Therefore, growth rates of macroalgae tend to decrease with increasing density due to self-shading and subsequent irradiance reductions (Lapointe and Tenore, 1981). For example, Peckol and Rivers (1996) found that while biomass of *Cladophora vagabunda* (220 g DW m^{-2}) was twice that of *Gracilaria tikvahiae* (109 g DW m^{-2}) in a dense algal bloom, *Cladophora* accounted for only half of the total mat productivity due to decreasing irradiance within the mat. They determined that light attenuation within the dense mats of *C. vagabunda* decreased sharply with depth and light, and was only 10 % of the surface irradiance at a depth of 2 cm. Furthermore, these authors suggested that in dense algal mats, such as *Cladophora*, decreasing light attenuation results in large sections of the mat becoming photosynthetically inactive, with respiratory processes dominating, resulting in a net release of carbon from the system, estimated at 0.14 g C m^{-2} day^{-1} for *C. vagabunda*, and 0.05 g C m^{-2} day^{-1} for *G. tikvahiae*. Lapointe and Tenore (1981) also found that in dense *Ulva* cultures grown under full sunlight and with sufficient nutrient concentrations, growth rates diminished as self-shading reduced light penetration, resulting in less light being absorbed and thus respiratory losses exceeded photosynthetic gains.

In addition, Peckol and Rivers (1996) determined that oxygen profiles within dense mats of *Cladophora vagabunda* declined considerably with depth, becoming anoxic within a few centimetres of the surface. Krause-Jensen *et al.* (1996) reported distinct diurnal variations and steep vertical gradients in oxygen and ammonium concentrations within mats of *Chaetomorpha linum* grown in incubation experiments (using low and high surface irradiance and enriched by a sediment nutrient flux). In the light treatments, oxygen production caused supersaturation in the mat's surface layers and nutrient uptake by *Chaetomorpha* substantially lowered the nutrient

flux to the water column. In addition, *Chaetomorpha* tissue nitrogen concentrations increased with mat depth, reflecting increasing nutrient availability towards the bottom layers and nitrogen limitation towards the upper layers of the mat. Productivity (measured as ^{13}C incorporation) and C/N ratios gradually declined towards the bottom of the mat, reflecting increasing light attenuation due to self-shading, and suggesting that the bottom layers of the mat were light limited. Light availability within the *Chaetomorpha* mat declined exponentially with depth, with irradiance reduced to 10 % of surface irradiance at 4 cm depth. Algal chlorophyll concentrations also increased significantly with depth under high surface irradiance, reflecting a decrease in irradiance and increase in the availability of inorganic nitrogen with increasing depth. *Chaetomorpha* chlorophyll concentrations varied over depth, ranging from 2.4 - 6.6 mg chl g^{-1} (dry weight), and were positively related to tissue nitrogen concentrations ranging from 11.3 to 24.8 mg N g^{-1} (dry weight); this indicated that the algae can adapt to decreasing light availability by increasing their chlorophyll content. In addition, Krause-Jensen *et al.* (1996) found that oxygen production rates within *Chaetomorpha linum* mats declined during the daylight hours and reached a minimum at the end of the light cycle. Respiration and decomposition processes in the lower layers of the *Chaetomorpha* mats resulted in high rates of oxygen consumption and created permanent anoxic conditions at the bottom of the mat and at the sediment surface. These reducing conditions at the sediment surface tend to enhance the release of chemicals such as PO_4^{3-}, NH_4^+ and H_2S, accelerating oxygen consumption and finally killing the algae.

High irradiances (e.g., during summer or at low tide), have a deleterious effect on productivity and growth of both seagrasses and macroalgae. Many studies have indicated that macroalgae tend to have a threshold of irradiance, above and below which growth rates will be detrimentally affected (e.g., *Grateloupia filicina*: Wong and Chang, 2000). Very high irradiance may result in photoinhibition, a mechanism that reduces photosynthetic rates, damages electron-transport proteins, impairs photophosphorylation and ultimately allows excess light energy to be dispersed as heat (Lobban and Harrison, 1997; Touchette and Burkholder, 2000b). In seagrasses, photoinhibition has been reported at light intensities greater than 1000 $\mu E\ m^{-2}\ s^{-1}$ (Touchette and Burkholder, 2000b, and references therein). Menéndez *et al.* (2001) suggested that high irradiance resulted in photodestruction of pigments, as evidenced by the whitish colour of fronds exposed at the surface of algal mats, and may inhibit production of *Gracilaria* in summer; incubation experiments showed that photosynthesis by *Gracilaria* was inhibited at high irradiances despite other variables, such as dissolved inorganic carbon concentrations and pH, being at optimal levels. Krause-Jensen *et al.* (1996) reported that *Chaetomorpha* mats grown under high light conditions were net oxygen productive, whereas oxygen consumption exceeded production in mats grown under low light conditions. These authors concluded that as algal density and self-shading increases, the proportion of shaded and consumptive biomass in the mat may become greater than the proportion of productive biomass and the mats may switch from net production to net consumption. Additionally, high oxygen consumption rates

could result in anoxia within the algal mat and water column, enhancing the release of nutrients from the sediment and decomposing algal biomass.

Although the rate of photosynthesis is highly dependent on irradiance, macroalgae tend to acclimate to variability in irradiance and nutrient regimes (Lobban and Harrison, 1997). For example, tissue nutrient concentrations often increase with increasing nutrient availability and decreasing irradiance (Krause-Jensen *et al.*, 1996). Lapointe and Tenore (1981) reported that growth of *Ulva fasciata* grown in outdoor cultures was not affected by nitrogen (nitrate-N) enrichment under low light conditions (62 ly day^{-1}), whereas under high light conditions (324 ly day^{-1}), growth rates increased significantly in association with increasing nitrogen enrichment. Nitrate uptake by *Ulva fasciata* was determined largely by daily nitrate additions and was also negatively correlated with irradiance levels. They concluded that algae growing quickly under high light required increasing amounts of nitrogen to sustain the increasing growth rates, whereas algae grown under low-light conditions had slower growth rates, requiring less nitrogen and therefore nitrogen enrichment had a lesser effect in the low-light treatments. Lapointe and Tenore (1981) also noted that algae grown in low light environments increases its chlorophyll content; *Ulva* grown under high light conditions contained half the Chlorophyll *a* concentrations of those plants grown under low light conditions. High irradiance is often associated with increased carbohydrate production as well as reduced chlorophyll levels, which leads to increased C/N and C:Chl *a* ratios. Increasing nitrogen enrichment, however, leads to increased growth rates, but decreased C/N and C:Chl *a* ratios in *Ulva fasciata*. It was suggested that C/N ratios and C:Chl *a* ratios usually only correlate with algal growth rates under nitrogen limited conditions.

2.3.2 Carbon

Photosynthesis is the basis of primary productivity, referring to the net incorporation of carbon into organic compounds. This includes C retained in the plant tissue, as well as organic carbon released back into the water column (as exudates or plant tissue), but does not include release of carbon as CO_2. Macroalgae use inorganic carbon as their primary source of C for photosynthesis, with CO_2 considered to be the preferred form of dissolved inorganic carbon (DIC) taken up, as it is readily diffused across cell and chloroplast membranes (Lobban and Harrison, 1997). Intertidal marine macroalgae, such as *Enteromorpha compressa*, can also take up CO_2 for photosynthesis from the air, as well as the water-column (Beer and Shragge, 1987), but the diffusion rate of CO_2 through water is 10,000 times slower than air (Lobban and Harrison, 1997). Inorganic carbon in seawater occurs as carbon dioxide, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions (DeBoer, 1981), with HCO_3^- comprising approximately 90 % of the DIC in standard seawater (Bidwell and McLachlan, 1985). Therefore, the photosynthetic capacity of submerged macrophytes may be limited by inorganic carbon when ambient CO_2 concentrations are low, due to the extremely low rate of CO_2 diffusion in water and, for example, in dense algal

mats, where CO_2 concentrations decrease rapidly with increased pH at the algal surface (Levavasseur *et al.*, 1991; Larsson and Axelsson, 1999; Menéndez *et al.*, 2001). However, some species of macroalgae may use HCO_3^- as a major source of DIC for their photosynthetic requirements, particularly when CO_2 concentrations are limiting; HCO_3^- utilization, and possibly direct uptake, has been documented for *Cladophora glomerata* (Choo *et al.*, 2002), *Enteromorpha compressa* (Beer and Shragge, 1987), *E. intestinalis* (Larsson *et al.*, 1997; Andría *et al.,* 2001) and a number of green, brown and red algae (c.f., Bidwell and McLachlan, 1985; Maberly, 1990; Larsson and Axelsson, 1999).

Water column CO_2 concentrations decrease with increasing pH, so that photosynthesis becomes more dependent on HCO_3^- as a source of dissolved inorganic carbon (Invers *et al.*, 1997). Beer and Rehnburg (1997) reported that some seagrasses (e.g., *Zostera marina*) can utilise HCO_3^-, but were still limited by inorganic carbon at the ambient inorganic carbon concentration of seawater where other parameters, such as light and other nutrients, were at sufficient levels to sustain photosynthetic growth. These authors suggested that this was because seagrasses, unlike macroalgae, did not have an efficient bicarbonate acquisition system. Therefore, changes in pH may also be a limiting factor for seagrass growth. Invers *et al.* (1997) found that *Posidonia oceanica* and *Cymodocea nodosa* exhibited a linear decrease in photosynthetic rates with increasing pH; photosynthetic rates at pH 8.8 were only 25 - 80 % of those at pH 8.2. *Zostera noltii*, however, maintained high photosynthetic rates up to pH 8.8, but showed a significant reduction at pH 9. These authors suggested that *Z. noltii* may be more efficient at low CO_2 concentrations, or better at utilising bicarbonate, than *C. nodosa* and *P. oceanica.* Menéndez *et al.* (2001) determined that the rate of DIC uptake, and hence photosynthesis by macroalgae which can only assimilate free CO_2, may also be indirectly affected by changes in pH; during the warmer summer months the combined increase in evaporation, pH levels, conductivity and water temperatures decreases the solubility of carbon dioxide, resulting in a reduction in CO_2 uptake by macroalgae. These authors found that the optimum rate of photosynthesis for *Ulva* sp. and *Chaetomorpha linum* was achieved between pH 6 and 7.5 in both spring and summer. For *Gracilaria verrucosa*, the optimal photosynthetic rate was between pH 6 and 7.5 in spring and between pH 6 and 8.5 in summer. The photosynthetic rate declined rapidly above the optimal pH for each alga. They suggested that the change in optimal pH range for photosynthesis of *Gracilaria* indicates that this species can adapt to changes in water pH, possibly providing it with a competitive advantage over other species.

Positive relationships have been found between macroalgal biomass and dissolved inorganic carbon concentrations in shallow lakes. For example, in Lake Veluwemeer, The Netherlands, Van den Berg *et al.* (2002) found that HCO_3^- concentrations dropped from 2.5 mM to <0.5 mM and 0.75 mM with increasing summer biomass of *Chara aspera* and *Potamogeton pectinatus*, respectively. Outside the macrophyte beds, however, HCO_3^- concentrations remained at 2.5 mM.

Similarly, Menéndez *et al.* (2001) found that water column CO_2 concentrations within a mixed mat of *Chaetomorpha linum* and *Gracilaria verrucosa* fluctuated diurnally, reaching a maximum of 20 μM in the morning and a minimum of 0.7 μM during the late afternoon. Bicarbonate concentrations followed a similar pattern, corresponding with decreasing surface irradiance. These authors concluded that this daily variation was related to uptake of carbon dioxide by the macroalgal mat. Matta and Chapman (1995) further suggested that the brown alga, *Colpomenia peregrina*, has a threshold level for the amount of water that can be lost during intertidal emersion, after which net carbon gain is affected by temperature and desiccation (i.e., the drying of the alga when exposed to air). Optimal rates of net carbon gain are achieved when the thallus is submersed, although net carbon gain can occur when the thallus is emersed. In winter, primary production and growth rates of *C. peregrina* were reduced as a result of reduced irradiance, lower water temperatures and desiccation stress. Matta and Chapman (1995) found that photosynthesis in *C. peregrina* was enhanced during the initial emersion phase, possibly due to evaporation of seawater around the thallus which would have served as a barrier for CO_2 diffusion into the thallus; once this seawater has evaporated, CO_2 is able to enter the thallus quicker, thus enhancing photosynthetic rates.

Macroalgal mats and seagrass meadows play an important role in both primary production, and secondary production, largely through the detrital food chain (West, 1983). Pregnall and Rudy (1985) estimated that annual production of dense intertidal mats of *Enteromorpha* sp. in the Coos Bay estuary (Oregon, USA) was 1,100 g C m^{-2} yr^{-1}. Similarly, Peckol and Rivers (1996) estimated that 1 - 5 cm thick mats of *Cladophora vagabunda* and *Gracilaria tikvahiae* in Waquoit Bay (Massachusetts, U.S.A) produced 443 g C m^{-2} yr^{-1} and 1,094 g C m^{-2} yr^{-1} at low and high nitrogen-loaded sites, respectively. Short-lived, simple-structured and sheet-like or filamentous algae usually have higher productivity rates than perennial species with complex structures and coarse thalli (King and Schramm, 1976). Arnold and Murray (1980) determined that benthic green algae (*Enteromorpha intestinalis, Codium fragile, Chaetomorpha linum, Ulva lobata and Ulva rigida*) showed a linear increase in photosynthetic rate with increasing irradiance in Californian field studies. Simple algae such as the sheet-forming *Ulva*, which have a thin-fronded morphology with high surface to volume ratio had higher photosynthetic production rates (9.2 mg C · g dry wt^{-1} hr^{-1}) than alga with more complex structures, such as the thick-stemmed, multi-branched and optically dense *Codium* (0.9 mg C · g dry wt^{-1} hr^{-1}).

Annual carbon production estimates for seagrass beds range from 292 g C m^{-2} yr^{-1} for *Halophila ovalis* (leaves: Hillman *et al.,* 1995), 361.1 g C m^{-2} yr^{-1} for *Ruppia cirrhosa* (leaves: Menéndez, 2002) and 1,093 g C m^{-2} yr^{-1} for *Zostera marina* (above and below-ground material: Sfriso and Ghetti, 1998) (Table 2-2).

Table 2-2: Average biomass (dry weight) and calculated rates of daily carbon production for seagrasses and macroalgae.

Species	Biomass (g DW m^{-2})	Production (g C m^{-2} d^{-1})	Location	Reference
Seagrass				
Halophila ovalis [a]	60 - 120	0.8 - 2.0	W. Australia	Hillman *et al.* (1995)
Halophila ovalis [t]	10.93 *	0.83 - 1.38	Indonesia	Erftemeijer and Stapel (1999)
Ruppia cirrhosa [a]	30 - 532 *	0.99	Spain	Menéndez (2002)
Posidonia oceanica [a]	220 - 622	0.19 - 0.40	Mediterranean	Pergent *et al.* (1997)
Zostera marina [a]	459	2.29	Venice	Sfriso and Ghetti (1998)
Macroalgae				
Cladophora montagneana	260	-0.23 - 1.52	W. Australia	Gordon and McComb (1989)
Cladophora vagabunda	37 - 220	0.6 - 1.0	U.S.A	Peckol and Rivers (1996)
Enteromorpha sp.	310	3.0	U.S.A	Pregnall and Rudy (1985)
Gracilaria tikvahiae	37 - 109	1.1 - 2.3	U.S.A	Peckol and Rivers (1996)

* ash-free dry weight

[t] total biomass (above- and below-ground material)

[a] above-ground material (leaves)

The decomposition and leaching of seagrass and macroalgae detritus is a significant source of carbon in estuaries (Rice and Tenore, 1981; Vähätalo and Søndergaard, 2002). Pregnall (1983) noted that highly photosynthetic green algae, such as *Enteromorpha*, contribute significant quantities of dissolved inorganic carbon into an estuary; much of the DOC released during photosynthesis is in the form of small organic compounds, such as amino acids, sugars, organic acids and sugar phosphates. Pregnall (1983) suggested that the release of dissolved inorganic carbon by intertidal *Enteromorpha prolifera* was enhanced during periods of reduced salinity and increased desiccation. *Ruppia megacarpa* has also been cited as a significant source of dietary carbon in estuaries, supporting secondary production via detrital release of C or direct grazing (Boyce *et al.*, 2001). Macroalgal biomass typically decomposes at a faster rate than that of seagrass, due to the chemical composition of the plant material; algal material containing a higher proportion of soluble ash, organic N and other components capable of undergoing hydrolysis, decays more rapidly than plants containing cross-linked celluloses and lignins (Rice and Tenore, 1981; Bourguès *et al.*, 1996). Bourguès *et al.* (1996) determined that biomass of *Monostroma obscurum* (Chlorophyta) decayed twice as fast as that of *Zostera noltii*; 76 % of the dry weight biomass of *M. obscurum* was lost after 25 days, compared to a 77 % loss after 66 days for *Z. noltii*. Anaerobic decomposition of both species resulted in a rapid release of inorganic N and P, and subsequent increase in C/N and C/P ratios of the decaying biomass, but *Z. noltii* appeared to be more efficient at recycling nutrients than the macroalgae.

2.4 Nutrients

Nutrient availability is one of the most important factors regulating the growth and reproduction of seagrasses and macroalgae, with nitrogen and phosphorus usually being the most important nutrients governing macrophyte growth. Nitrogen and phosphorus are typically described as limiting nutrients; this means that when all other environmental factors, such as light and

temperature, are suitable for macrophyte growth, the nutrient present at the lowest level necessary will limit growth (DeBoer, 1981). Phosphorus tends to be the key nutrient limiting macrophyte growth in fresh water and carbonate environments, such as coral reefs (McClanahan *et al.*, 2002). Nitrogen has often been cited as the most important nutrient limiting growth of macrophytes in temperate marine and shallow estuarine waters (Hanisak, 1983; Chambers *et al.*, 1999; Lotze and Schramm, 2000; Menéndez, *et al.*, 2002a).

Nutrients are introduced to coastal waterways by a number of natural processes. These include: terrestrial weathering and leaching of nitrogen or phosphorus bearing rocks and soils, direct land run-off, groundwater seepage, plant decomposition, oceanic mixing processes (e.g., upwelling), regeneration from decomposing marine primary and secondary producers (in the water column and sediments), dry deposition and atmospheric precipitation (GESAMP, 1990; Misztal, 1992; Knoppers, 1994). Nutrients are also derived from land-based anthropogenic activities, such as increased erosion and weathering, urban, industrial and agricultural runoff (e.g., fertilisers), domestic wastes (e.g., sewage and detergents), industrial effluent discharges and atmospheric emissions from fuel combustion and agriculture (GESAMP, 1990; Gerritse *et al.*, 1992). Animal wastes (e.g., excreta, dead bodies) contain considerable amounts of phosphorus (Cook and Williams, 1973) and organic nitrogen (unassimilated protein), which is converted to ammonia by heterotrophic bacteria (Sawyer *et al.*, 1994). Internal sources include possible nitrogen fixation by cyanobacteria, as well as pelagic and benthic nutrient regeneration. Nutrients are exported from coastal systems through tidal exchange, sediment accumulation and denitrification (Knoppers, 1994).

2.4.1 Nutrients Required by Macrophytes

The essential macronutrients required by macroalgae include C, H, O, P, K, N, S, Ca and Mg, all of which are present in some quantity in macrophytes and seawater (DeBoer, 1981; Table 2-3). Macroalgae also require inorganic micronutrients, including Fe, Cu, Zn, Mn, Si, Co, Mo, V, B, Cl, I, Br and Na. These trace elements are also essential for growth as catalysts in metabolic reactions, in osmotic regulation and several other functions (DeBoer, 1981; Table 2-4). Nutrients other than nitrogen and phosphorus are essential for growth and productivity of macroalgae and seagrasses, but are generally not considered limiting in aquatic systems. For example, sulfur is an important component of proteins; in seawater, the most common form of sulfur is sulfate, which typically occurs at a concentration of 0.025 M, and is therefore not likely to be a limiting nutrient for macroalgae growing in seawater (DeBoer, 1981). Calcium is important for the maintenance of cellular membranes, as well as being an integral component of the cellular structure of many species of macroalgae. Magnesium is an important component of chlorophyll (DeBoer, 1981), and is involved in many enzyme reactions, such as nitrate reduction, sulfate reduction, and phosphate transfers (Lobban and Harrison, 1997). Calcium and magnesium are

generally not considered to be limiting nutrients for macroalgal growth as both elements are typically present in seawater at concentrations sufficient to support algal growth (DeBoer, 1981).

Table 2-3: Average concentrations of some essential elements in seawater (after Brown *et al.*, 1989).

Please see print copy for Table 2-3

2.4.2 The Phosphorus Cycle

Phosphorus in the ocean occurs as dissolved inorganic phosphorus (DIP), dissolved organic phosphorus (DOP) and particulate phosphorus (Levinton, 1995; Figure 2-1). In seawater, DIP occurs primarily as orthophosphate species: HPO_4^{2-} (87 %), PO_4^{3-} (12 %) and $H_2PO_4^-$(1 %), which are ionised products of phosphoric acid ($H_3PO_4^-$) (Sawyer *et al.*, 1994; Millero, 1996; DeBoer, 1981). Macroalgae and marine phytoplankton take up phosphorus primarily as orthophosphate ions (PO_4^{3-}) (Levinton, 1995; Lobban and Harrison, 1997); this is converted to organic-P and reconverted to PO_4^{3-} following death (Millero, 1996). As a result, oceanic surface waters tend to be depleted in DIP due to biological uptake, whereas deeper waters tend to be enriched in DIP derived from decomposition of organic matter (Parker and Corbitt, 1992; Pilson, 1998).

Table 2-4: Functions and compounds of essential elements in macroalgae (Lobban and Harrison, 1997).

Please see print copy for Table 2-4

Please see print copy for Figure 2-1

Figure 2-1: The phosphorus cycle in ocean waters (after Kennish, 1997).

2.4.3 The Nitrogen Cycle

Nitrogen in the marine environment cycles between its particulate and dissolved organic and inorganic forms (Figure 2-2). Dissolved inorganic nitrogen (DIN) occurs in many forms, with the most abundant being: dimolecular nitrogen (N_2), nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3) and ammonium (NH_4^+). Organic nitrogen occurs in both particulate (PON) and dissolved (DON) forms, such as urea, nucleic acids and peptides (Libes, 1992; Levinton, 1995). The most important processes in the marine nitrogen cycle are briefly outlined below.

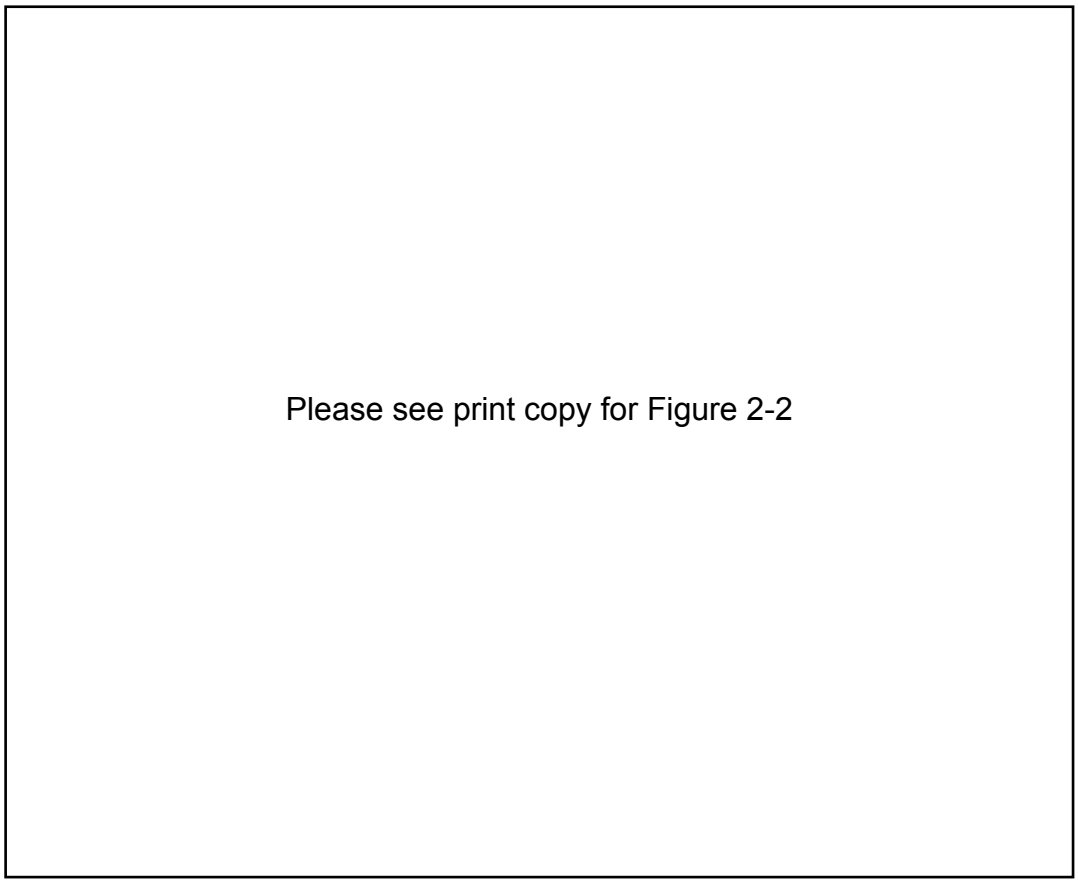


Figure 2-2: The nitrogen cycle in ocean waters (after Kennish, 1997).

Nitrogen fixation

Nitrogen fixation is the conversion of atmospheric nitrogen (N_2) to organic nitrogen, ammonia, nitrate or nitrite (Pilson, 1998), and is a source of bioavailable nitrogen in estuaries and coastal waters (Knoppers, 1994). Biological nitrogen fixation is the reduction of N_2 to ammonia (NH_3) by a few types of organisms; these include a limited number of species of heterotrophic or phototrophic bacteria, such as blue-green algae (Libes, 1992; Pilson, 1998). Most biological nitrogen fixation occurs in shallow benthic environments, such as saltmarsh, mangrove or

seagrass areas (Libes, 1992). As most plants can not utilise N_2 directly, biological nitrogen fixation supplies a significant proportion of the nitrogen required by primary producers (Libes, 1992; Vance, 2001). Seagrasses have high growth rates and require considerable amounts of nitrogen: up to 10 - 450 mg N m^{-2} d^{-1} (Dawes, 1998). Welsh *et al.* (1996) estimated that nitrogen fixation in *Zostera noltii* beds averaged 0.1 - 7.3 mg N m^{-2} d^{-1}, compared to 0.02 - 3.7 mg N m^{-2} d^{-1} in unvegetated sediments. Nitrogen fixation in the rhizosphere was calculated at 0.4 - 1.1 mg N m^{-2} y^{-1}, accounting for between 6.3 and 12 % of the annual fixed nitrogen requirement of the seagrasses.

Remineralization of Organic Matter

Remineralization refers to the decomposition of particulate organic nitrogen (PON), where solid nitrogen is converted to dissolved organic nitrogen (DON). The DON is rapidly degraded by heterotrophic bacteria, with ammonia released in the process, which subsequently reacts with H+ or H_2O to form ammonium (Libes, 1992). Nitrogen returned to the water column as NH_4 and DON may stimulate primary production, leading to increased production and decomposition of organic matter, and increased oxygen consumption. This excess mineralisation of organic matter may result in anoxia, thus inhibiting nitrification-denitrification, and resulting in further recycling of ammonium-N to the water column, subsequently enhancing primary productivity (Eyre and Ferguson, 2002).

Nitrification

Nitrification refers to the oxidation, by aerobic bacteria, of the ammonium ion (NH_4^+) into the nitrite (NO_2^-) or nitrate (NO_3^-) ion, as follows (Sawyer *et al.*, 1994):

$$2NH_4^+ + 3O_2 \xrightarrow{\text{Nitrosomonas bacteria}} 2NO_2^- + 2H_2O + 4H^+$$

$$2NO_2^- + O_2 \xrightarrow{\text{Nitrobacter bacteria}} 2NO_3^-$$

In estuaries and coastal waters, nitrification may be an important factor limiting the growth of phytoplankton and macroalgae, due to preferential uptake of ammonium over nitrate (Levinton, 1995; Section 2.5.2).

Denitrification

Denitrification is an important sink for nitrogen in estuaries and coastal waters, and may limit the degree of eutrophication in waters receiving substantial inputs of nutrients (Knoppers, 1994; Seitzinger, 1988). It is performed by heterotrophic bacteria and refers to the chemical reduction

of nitrate and nitrite to gaseous forms: nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2), as follows (Kiely, 1997):

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

Denitrification requires suboxic or anoxic conditions and the presence of large amounts of organic matter. It occurs in coastal sediments and upwelling zones, regions of relative water stagnancy and polluted estuarine waters (Libes, 1992). The nitrate required for sediment denitrification is sourced from: the diffusion of nitrate from the water column into the sediments; the production of nitrate in the sediments via nitrification of ammonia released from oxidation of organic matter (e.g., macrophytes, animals); and the transport of nitrate through the sediments via groundwater (Seitzinger, 1988). The loss of nitrogen via denitrification in freshwater and marine systems is considered a major contributor to nitrogen limitation in estuaries and coastal systems (Seitzinger, 1988; Knoppers, 1994).

Denitrification in estuaries is influenced by water column nitrate concentrations, water temperature, oxygen concentration, the depth of oxygen penetration, rates of sediment carbon metabolism, sediment trace metal concentrations, bioturbation and the abundance of primary producers (e.g., seagrass, macroalgae and macrofauna) (Seitzinger, 1988; Flemer *et al.,* 1998; Cornwell *et al.,* 1999; Tuominen *et al.*, 1999; Qu, 2004a). Eriksson and Weisner (1999) suggested that submerged macrophytes (e.g., seagrasses) can have significant effects on nitrification and denitrification processes, such as: providing a large surface area for the attachment of nitrifying and/or denitrifying bacteria; inducing changes in concentrations of DIC and pH; and altering sediment processes via rhizomous growth. In addition, the photosynthetic release of O_2 from submerged macrophytes, in daylight, may stimulate the transition of NH_4^+ to NO_3^- by nitrifying bacteria occurring in the sediment and as epiphytes. Night-time consumption of O_2 via macrophyte respiratory processes may, however, induce a shift from aerobic (i.e., nitrifying) to anaerobic (i.e., denitrifying) bacterial respiration in epiphytic populations, as well as inducing sediment denitrification by reducing O_2 concentrations in the water column (Eriksson and Weisner, 1999). Krause-Jensen *et al.* (1999) noted that dense algal mats can significantly suppress sediment denitrification; within dense mats of filamentous algae (e.g., *Chaetomorpha linum*), water movement over the sediment surface is dramatically reduced, so that the bottom layer of water becomes stagnant, resulting in anoxia at the sediment surface. In addition, the algae can effectively assimilate all nitrate from the water column, thereby limiting denitrification, and sediment nitrification is subsequently inhibited by the anoxic conditions below the algal mat.

2.5 Nutrient Uptake by Macrophytes

Both macroalgae and seagrasses take up nutrients from the water column, but seagrasses also take up nutrients from the sediment via roots and rhizomes (McComb and Lukatelich, 1986). For macroalgae, nutrients derived from recycling of nutrients from the underlying sediment and

decomposing algal mats is also an important source (Gordon *et al.,* 1981; Lavery *et al.*, 1991). These factors are discussed further in the following sections.

2.5.1 Phosphorus Uptake

Macrophytes take up phosphorus primarily as orthophosphate. Other sources of P include inorganic polyphosphates and organic-phosphorus compounds (Lobban and Harrison, 1997). The primary role of phosphorus is in photosynthesis and respiration, as energy transfer, through ATP and other compounds, as well being a component of many biomolecules (e.g., nucleic acid, proteins and phospholipids) (Lobban and Harrison, 1997). Photosynthesis in seagrasses can be depressed by up to 50 % under environmental P limitation (Touchette and Burkholder, 2000a). When deficient in phosphorus, algae can rapidly incorporate phosphorus at levels exceeding immediate growth requirements, with the excess P being incorporated into polyphosphates via polyphosphate kinase (Lobban and Harrison, 1997). The formulation of polyphosphates by many species of macroalgae is an important difference in phosphorus metabolism between algae and vascular plants (DeBoer, 1981). Macroalgae reported to accumulate polyphosphates include *Ceramium*, *Ulothrix* and *Ulva* (Lobban and Harrison, 1997; Lundberg *et al.*, 1989).

2.5.2 Nitrogen Uptake

Although nitrogen occurs in abundance in the air and in seawater, most species of macroalgae and seagrasses can not utilize it directly (DeBoer, 1981). Instead, nitrogen uptake by macrophytes requires inorganic sources of nitrogen (nitrate, nitrite and ammonium) to be incorporated into amino acids and proteins (Lobban and Harrison, 1997). Ammonium-N is the preferred form of dissolved inorganic nitrogen for algae and seagrasses; as ammonium is already present in a reduced form, it requires less biochemical energy for assimilation than other forms of dissolved nitrogen and can be directly incorporated into amino acids (Lobban and Harrison, 1997; Dawes, 1998; Shaw *et al.*, 1998). Touchette and Burkholder (2000a) noted that seagrasses take up nitrogen from pore-water, predominantly as ammonium-N, as well as the water-column, predominantly as nitrate-N. They found that 30 - 90 % of N required by *Zostera marina* was taken up from the water column.

After nitrate is taken up by macroalgae, it is either stored in the vacuole or reduced to nitrite via nitrate reductase (Crawford, 1995):

$$NO_3^- + NAD(P)H + H^+ \rightarrow NO_2^- + NAD(P)^+ \; H_2O$$

Nitrite is then transported to the chloroplast and reduced to ammonium, catalysed by nitrite reductase (Lobban and Harrison, 1997):

$$NO_2^- + 6Fe_{red} + 8H^+ \rightarrow NH_4^+ + 6Fe_{ox} + 2H_2O$$

where Fe_{red} and Fe_{ox} are ferredoxin in its reduced and oxidised forms, respectively (Figure 2-3).

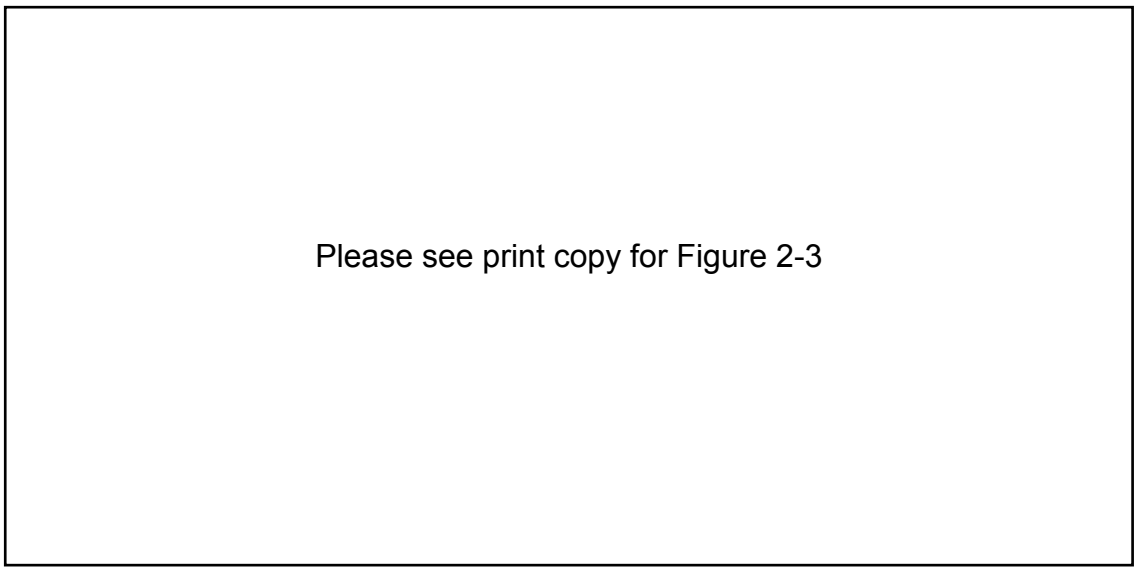


Figure 2-3: Summary of nitrogen uptake and assimilation within a eukaryotic algal cell (after Lobban and Harrison, 1997).

2.5.3 Growth of Macroalgae in Culture

Macroalgal growth in nutrient culture experiments generally tends to follow a distinct pattern (Figure 2-4). Firstly, an initial lag phase, where nutrients are rapidly taken up after deprivation and growth is minimal. A short burst of fast growth follows; this growth phase is usually exponential, so that plotting on a logarithmic graph produces a linear growth rate. The exponential growth phase is followed by a lengthy period of slow, steady growth, where internal and external nutrient pools are exhausted. Finally, growth rates decline and the alga eventually begins to decay (Lobban and Harrison, 1997).

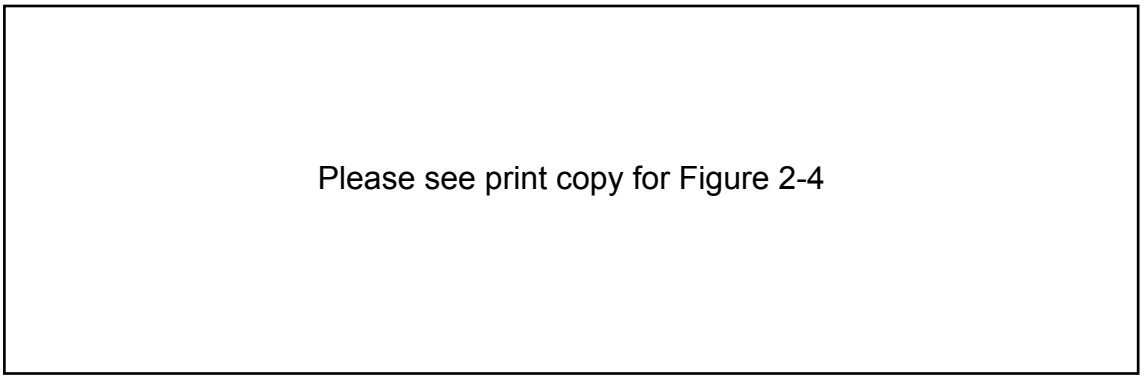


Figure 2-4: Typical pattern of growth in macroalgae (after Lobban and Harrison, 1997).

A simplistic approach to modelling macroalgal growth is to graph the change in mass over time; however, this type of graph will typically produce a curved fit. The most commonly used measure of growth is the specific growth rate in which the natural log is used to produce a linear

fit. The specific growth rate, μ, can be estimated using the change in mass from the initial biomass, B_0, to the final biomass, B_t, over time, t:

$$\mu = \frac{100[\ln(B_t / B_0)]}{t}$$

(after Lobban and Harrison, 1997). In batch culture experiments, nutrients are taken up rapidly during the exponential growth phase, and the alga often grows at the theoretical maximum rate (μ_{max}) when nutrients and other factors (e.g., temperature and light) are saturating. Algal growth rates would generally not be considered limited (e.g., by nutrients or other experimental manipulations) until growth slows after the exponential phase (Figure 2-4), and the algae are growing at a rate less than μ_{max}. As initial growth rates are high during the exponential growth phase of culture experiments, it is considered more appropriate to calculate growth rates after a "steady state" of growth and nutrient uptake has been achieved. To achieve a steady state, a fairly constant biomass must be maintained, for example through regular harvesting or increasing the nutrient concentration proportionally with the increasing biomass (DeBoer, 1981). Additionally, a steady state may be achieved using continuous-flow cultures or by regularly changing the growth medium so that the nutrient concentration remains fairly uniform. Attempts are made to keep the dilution rate high (i.e., a high water volume to plant biomass ratio) to maintain a fairly steady nutrient concentration (e.g., 1 g L^{-1} is typical for many studies). The time required to reach a steady state condition can be lengthy and is primarily dependent on growth rates and culture conditions (Lobban and Harrison, 1997). Growth rates of macroalgae determined in field-based studies usually average less than 3 % d^{-1} (e.g., *Cladophora montagneana*: Gordon and McComb, 1989), but growth rates up to 15 - 20 % d^{-1} may occur in laboratory culture studies (e.g., *Enteromorpha intestinalis:* Martins and Marques, 2002).

2.5.4 Nutrient Uptake Kinetics

Nutrient uptake kinetics in macroalgae and seagrasses have been reviewed by several authors (e.g., Dowd and Riggs, 1965; Lobban and Harrison, 1997; Touchette and Burkholder, 2000a), and will only be outlined briefly here. Assuming a steady-state enzymatic reaction, the Michaelis-Menten equation is used to determine the relationship between the initial velocity of the reaction or rate of nutrient uptake, v, and the external substrate concentration, a:

$$v = \frac{V_{max} a}{K_m + a}$$

V (often referred to as V_{max}) is the limiting rate theoretically obtained when the alga has been saturated with an infinite concentration of substrate. K_m (equivalent to K_s) refers to the Michaelis constant: the concentration of substrate at half of the maximal initial velocity (Dowd and Riggs, 1965; Cornish-Brown, 1995). When the initial velocity of reaction is measured in relation to

various substrate concentrations, it is customary to present the experimental data graphically to obtain estimates of V and K_m (Cornish-Bowden, 1995). A plot of v against a gives a rectangular hyperbola (Figure 2-5A), making estimations of V and K_m very difficult. Therefore, the experimental data is often plotted using one of the following linear transformations of the Michaelis-Menten equation (Dowd and Riggs, 1965; Cornish-Bowden, 1995):

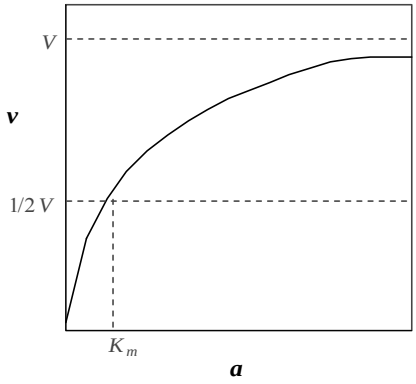
$$v = V - K_m\left(\frac{v}{a}\right) \qquad \text{(c.f., Figure 2-5B)}$$

$$\frac{a}{v} = \frac{K_m}{V} + \left(\frac{1}{V}\right)\cdot a \qquad \text{(c.f., Figure 2-5C)}$$

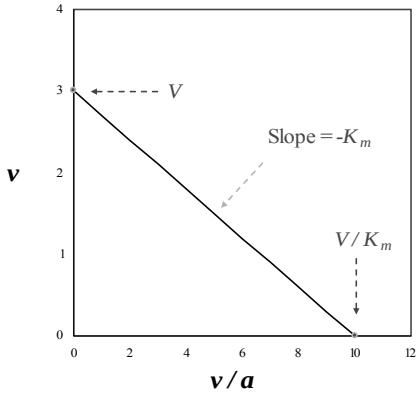
$$\frac{1}{v} = \frac{1}{V} + \left(\frac{K_m}{V}\cdot\frac{1}{a}\right) \qquad \text{(c.f., Figure 2-5D)}$$

The linear transformations described above are represented graphically in Figure 2-5B-D.

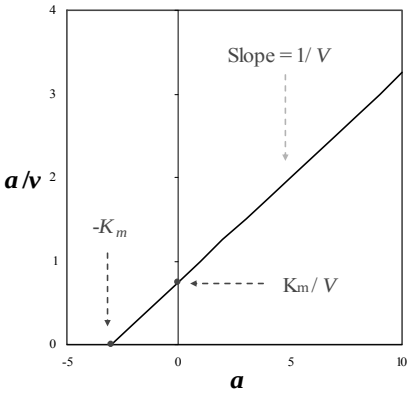
(A) v against a



(**B)** v against v/a



(C) a/v against a



(D) $1/v$ against $1/a$

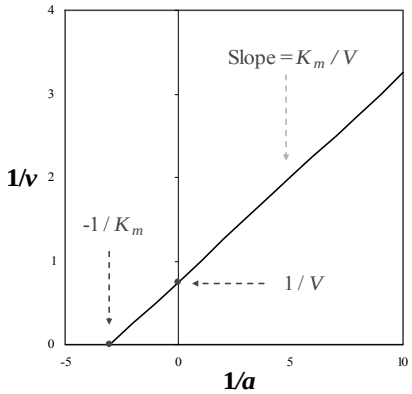


Figure 2-5: (A) Graphical representation of a reaction obeying the Michaelis-Menten equation. **(B-D)** Linear transformations of the Michaelis-Menten equation: **(B)** v against v/a, often referred to as the Eadie-Hofstee plot; **(C)** a/v against a, or the Hanes plot; **(D)** $1/v$ against $1/a$, commonly referred to as the Lineweaver-Burk plot (adapted from Cornish-Bowden, 1995).

In Figure 2-5C, for example, a/v is plotted against a to give a straight line with slope $1/V$ and intercepts of K_m/V and $-K_m$ on the a/v and a axes, respectively. Dowd and Riggs (1965) concluded that estimates of K_m and V obtained by plotting v against v/a (Figure 2-5b) or a/v against a (Figure 2-5c) were far better than those obtained by plotting $1/v$ against $1/a$ (Figure 2-5d). For experimental datasets in which the error of v was small, a/v against a gave more accurate estimates, whereas if the error of v was large and constant, or large and variable, better results were obtained by plotting v against v/a. Lavery and McComb (1991b) also found that the linear transform of a/v against a most accurately predicted V and K_m for their data of nutrient uptake rates in *Chaetomorpha linum.*

The kinetic parameters determined for a range of macroalgae are listed in Table 2-5. V and K_m are rarely constant, but are influenced by environmental and experimental conditions (e.g., light, temperature) as well as the nutritional status and physiological condition of the algal tissue (Hanisak, 1983). It is therefore difficult to relate V and K_m values to ecological conditions or compare values for different species obtained by different authors. It must also be noted that V often varies with the incubation period and it is therefore difficult to compare values obtained from different studies with varying incubation periods (Lobban and Harrison, 1997). For example, ammonium uptake rates measured over the first 10 minutes will often be higher than those measured over the first hour of incubation as uptake rates tend to decrease over time as the external substrate concentrations decline and internal pools are filled (Thomas and Harrison, 1987; Lobban and Harrison, 1997). In addition, water movement can have a significant effect on K_m values, but V is typically unaffected; thus some degree of caution must be exercised when comparing K_m values from different studies where water movement has not been taken into consideration (Smit, 2002).

Nitrate, ammonium and phosphate uptake kinetics in seagrasses have been reviewed by Touchette and Burkholder (2000a). They found that seagrasses leaves tend to have higher NH_4^+ uptake rates (V_{max} = 5 - 270 μmol g^{-1} dry weight hr^{-1}) than NO_3^- (V_{max} = 3.7 - 75 μmol g^{-1} dry weight hr^{-1}). Maximum inorganic P uptake rates ranged from 0.014 - 43 μmol g^{-1} dry weight hr^{-1}. In addition, uptake rates of both inorganic nitrogen and phosphorus were typically higher in seagrass leaves, than roots.

Table 2-5: Range of kinetic parameters, V_{max} (μmol (N or P) g^{-1} dry weight hr^{-1}) and K_m (μmol N or P) for phosphate, ammonium and nitrate uptake for selected species of macroalgae (values are mean ± s.e.).

Species	Temp	PO_4^{3-}-P		NO_3^--N		NH_4^+-N		Reference
	(°C)	V_{max} ± s.e.	K_m ± s.e.	V_{max} ± s.e.	K_m ± s.e.	V_{max} ± s.e.	K_m ± s.e.	
Chlorophyta								
Chaetomorpha linum	25	21.5	10.4	-	-	-	-	Lavery and McComb (1991b)
Cladophora aff. *albida*	23	4.1 ± 0.1	0.06 ± 0.1	42.1 ± 5.7	1.4 ± 0.7	130 ± 22	20.7 ± 11	Gordon *et al.* (1981)
Cladophora glomerata	15	2.42	0.28	161.9	9.42	299.3	52.1	Wallentinus (1984)
Enteromorpha spp.	15	-	-	129.4	16.6	-	-	Harlin (1978)
Enteromorpha spp.	20	-	-	-	-	996 ± 510	24.6 ± 8.6	Fujita (1985)
Enteromorpha ahlneriana	9-12	8.20	2.87	27.77	1.73	409.1	16.6	Wallentinus (1984)
Enteromorpha prolifera	12-14	~2 (max)	-	75.4 - 169	2.3 - 13.3	39.2 - 188	2.9 - 13.4	O'Brien and Wheeler (1987)
Enteromorpha intestinalis	15	46.9 [c]	17.3	237.3 [b]	43.7	439.1 [a]	66.4	Lotze and Schramm (2000)
Enteromorpha intestinalis	15	35.9 [d]	17.1	135.7 [d]	20.4	60.7 [d]	12.8	Lotze and Schramm (2000)
Ulva lactuca	20	-	-	-	-	138 ± 78	40.7 ± 8.5	Fujita (1985)
Ulva lactuca	15	-	-	-	-	244 ± 27 [a]	24 ± 7	Pedersen (1994)
Ulva rigida	25	8.8	3.6	59 - 85	18 - 33	-	-	Lavery and McComb (1991b)
Ulva rigida [e]	-	-	-	68.16 [b]	87.03	-	-	Naldi and Viaroli (2002)
Ulva sp.	15	-	-	-	-	146 [a]	14.4	Campbell (1999b)
Rhodophyta								
Gracilaria tikvahiae	20	-	-	-	-	144 ± 66	16.9 ± 3.5	Fujita (1985)
Gracilaria gracilis	15	-	-	34.6 ± 2.8	6.9 ± 2.3	160.5 ± 17.2	55.1 ± 8.9	Smit (2002)
Gracilaria gracilis	20	-	-	35.0 ± 1.8	5.6 ± 1.3	272.9 ± 122.8	97.8 ± 57.5	Smit (2002)
Polysiphonia decipiens	15	-	-	-	-	57.4 [c]	2.6	Campbell (1999b)
Phaeophyta								
Fucus vesiculosus (tip)	13-14	0.75	1.84	29.7	32.2	4.32	2.62	Wallentinus (1984)
Fucus vesiculosus (tip)	2-3	2.85	45.9	67.5	270.7	157.8	507.6	Wallentinus (1984)
Laminaria groenlandica	13	-	-	-	-	-	-	Harrison *et al.* (1986)
Hincksia sordida	15	-	-	-	-	802 [a]	39.7	Campbell (1999b)
Undaria pinnatifida (mature)	15	-	-	-	-	96.9 [c]	4.0	Campbell (1999b)
Pilayella littoralis	15	44.2 [c]	15.4	300.1 [b]	116.4	466.7 [a]	66.6	Lotze and Schramm (2000)

Incubation times: [a] 0 - 15 mins [b] 0 - 30 mins [c] 0 - 60 mins [d] 60 - 120 mins [e] N+P treatments

2.5.5 Nutrient Limitation

Nutrient limitation is a critical factor influencing the growth rates and ultimate biomass production of macroalgae. The two prevailing theories of nutrient limitation are the multiple limitation hypothesis and single factor (Liebig-type) limitation (Figure 2-6). Liebig's "law of the minimum" states that the growth rate of a plant, biomass formation and overall plant health are dependent on the availability of the scarcest essential nutrient that has a concentration below the growth requirements of the plant (Allaby, 1998). Plants can exhibit both single-factor responses (i.e., limitation by a single nutrient at a time) and multiple-limitation responses (e.g., limitation by nitrogen in combination with potassium or magnesium), but the two types of limitation should not occur at the same time and one should dominate over the other (Rubio *et al.*, 2003).

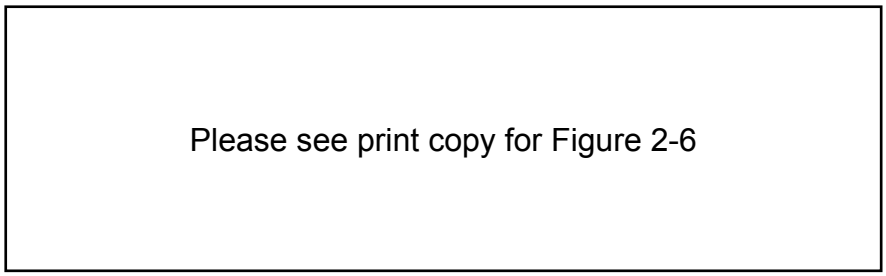


Figure 2-6: Comparison between the theoretical limitation models of plant growth response to increasing nutrient supply (after Rubio *et al*., 2003).

According to Kokum *et al.* (2002), studies of potential nutrient limitation due to an imbalance in the *in situ* N:P ratios relative to the growth requirements of the alga examine the effect of Liebig-type limitation of biomass production, rather than the effect of *in situ* nutrients on growth rates. Low N:P ratios which may imply limitation of biomass production (e.g., < 10:1) may not necessarily be limiting to algal growth rates if concentrations of both nutrients are saturating to growth rates in terms of Michaelis-Menten kinetics (Section 2.5.4). Limitation due to Michaelis-Menten kinetics usually applies to the exponential phase of an algal growth cycle (Figure 2-5A), when the concentration of a nutrient is less than that required to achieve maximum relative growth rates (μ_{max}). The faster growth rates lead to higher biomass production during the initial stages of growth. On the other hand, Liebig-type (single-factor) limitation of biomass production typically occurs after growth rates become limited by low nutrient concentrations at the end of a growth cycle (Kokum *et al.*, 2002).

It is important to note that nutrient limitation is not exclusively dependent on external conditions; internal (i.e., cellular) stores of nutrients and the allocation of cellular resources must also be considered when examining the potential response of an alga to nutrient limitation (van den Berg, 1998). In addition, it is equally important to consider the complex nature of ecosystem dynamics; algal growth and ultimate biomass production, for example, can be equally

dependent on the interactions between limiting factors (e.g., temperature and other mineral nutrients), rather than the addition of a single nutrient, such as nitrogen (Cullen, 1991).

2.5.6 Nutrient Uptake from Sediment

The recycling of nitrogen and phosphorus from the sediment is considered a significant source of nutrients for primary producers (McComb and Lukatelich, 1986; Seitzinger, 1988). Nutrients can be released from the sediment to the water column by resuspension of sediment particulate matter (e.g., by wind, dredging or biological disturbance), or via the transport of dissolved nutrients through turbulent mixing and diffusion (McComb and Lukatelich, 1986; Lohrer *et al.*, 2003). In shallow coastal lakes, such as Lake Illawarra, microphytobenthos play an important role in intercepting nutrients regenerated from the sediment, thus limiting availability to macrophytes and phytoplankton (Webster *et al.*, 2002; Qu, 2004a). Several studies have indicated that release of phosphorus from bottom sediments is one of the main sources of nutrients for macrophytes, whereas nitrogen is often acquired from both sediments and the water column (Kufel and Kufel, 2001). Nutrients associated with sediments are found in three forms: dissolved in the porewater of the sediment, adsorbed to the sediment grains, or fixed within the structure of the grains. The forms available to seagrasses include dissolved orthophosphate and ammonium in the sediment porewater and, to a lesser extent, the nutrients adsorbed to the sediment particles (Udy and Dennison, 1996).

Macroalgal blooms can significantly uncouple sediment-water biogeochemical cycles, such as the interception of nutrients regenerated from the sediment (Valiela *et al.*, 1997). Loose or floating macroalgal mats tend to show a preference for uptake of water column nutrients, as indicated by seasonal changes in tissue concentration (Lavery and McComb, 1991a). Dense macrophyte beds, however, tend to enhance sedimentation and reduce the resuspension of sediment particles; thus restricting the release of sediment nutrients to the water column (Kufel and Kufel, 2001). The rate of nutrient release from sediment tends to be temperature dependent and may contribute to higher growth rates of algae in warmer weather (Stimson *et al.*, 1996). Macrophyte beds may also promote the release of nutrients from sediments (van Donk and van de Bund, 2002). Sediment nutrients are released if anoxic conditions develop at the sediment surface; under dense macroalgal mats, anoxic conditions tend to develop at the sediment-water interface, promoting nutrient release from the sediment and supporting further algal growth (Lavery and McComb, 1991a; Section 2.3.1). Lavery and McComb (1991a) suggested that the mat-forming *Chaetomorpha linum* in the Peel Inlet may be less reliant on water column nutrients than *Cladophora* and *Ulva*, and may be more effective at generating reducing conditions at the sediment surface through tissue decomposition, leading to release of nutrients from the sediment.

In dense algal mats, in particular, decomposition of algal tissue and release of nutrients from the older, bottom layers of the mat may resupply nutrients to the upper layers (Krause-Jensen *et al.*, 1996). Owens and Stewart (1983) further noted that decomposition of macroalgal tissue at the sediment-water interface served as an input of nitrogen to the sediment, thus supporting remineralization and recycling of nitrogen back into the system. Nutrients may be released back into the overlying water column when macroalgal productivity is reduced by light limitation due to factors such as cloudy days or self-shading in the lower layers of dense algal mats (McGlathery *et al.*, 1997). In *Cladophora* cultures, Gordon *et al.* (1981) found high concentrations of organic nitrogen in the substrate of the highest N treatments; they suggested that the excess nitrogen that was taken up by the algae, but not incorporated into the tissue, may have been released back into the water as organic nitrogen. In addition, Kufel and Kufel (2001) noted that charophytes (e.g., *Chara, Lamprothamnium*) are capable of delivering oxygen to the underlying sediment which may positively affect nitrification - denitrification processes and limit the release of sediment phosphorus to the water column.

2.5.7 Factors Affecting Nutrient Uptake

Nutrient uptake by seagrasses and macroalgae may be influenced by a range of factors, such as surface area to volume ratio, plant part, the age and nutritional history of the plant, habitat and light attenuation (Lobban and Harrison, 1997). Lavery and McComb (1991b) suggested that in estuaries that have a seasonal nutrient input, such as the Peel Inlet, W.A., algal species that have faster nutrient uptake rates and can take advantage of periodic nutrient pulses would have a competitive advantage over species with slower uptake rates. For example, these authors reported that phosphate and ammonium uptake rates of *Chaetomorpha linum* were twice those of *Ulva rigida*. *U. rigida*, however, had higher nitrate uptake rates at low nitrate concentrations (below 750 μg NO_3^--N L^{-1}) but *C. linum* had higher uptake rates at higher concentrations.

Algae with high surface to volume ratios (e.g., sheet-like or finely branched species) are reported to have higher photosynthetic rates than algae of a thicker and more structurally complex morphology (Arnold and Murray, 1980). Wallentinus (1984) showed that short-lived, opportunistic, filamentous macroalgae (e.g., *Cladophora glomerata, Enteromorpha ahlneriana, Scytosiphon lomentaria, Dictyosiphon foeniculaceus* and *Ceramium tenuicorne*) typically had higher nutrient uptake rates than successional, long-lived species with low surface area to volume ratios (e.g., *Fucus vesiculosus, Furcellaria lumbricalis* and *Phyllophora truncata*). Duke *et al.* (1996) noted that the nutrient uptake capacity of macroalgae is a function of the surface area to volume ratio (SA/V), whilst the storage capacity of N is generally inversely correlated with the surface area to volume ratio. They suggested that as nitrogen is one of the primary factors limiting growth and biomass production of macroalgae in estuaries, variation in algal growth rates should correlate with variation in nitrogen supply. Furthermore, the relationship between macroalgal growth rates and nitrogen supply may also be a function of the surface are

to volume ratio. Species such as *Ulva*, with a high SA/V and high N uptake capacity, would be expected to have growth rates highly correlated with N supply. Whereas species such as *Codium*, which have a low SA/V and low N uptake capacity, would be expected to store N on a long-term basis and have growth rates which are not dependent on nitrogen supply. Duke *et al.* (1989) noted that although *Ulva* exhibited higher N uptake rates than *Codium*, it had a lower N storage capacity; the low SA/V ratio and therefore limited uptake capacity of *Codium* prevents it from exploiting sporadic supplies of nitrogen, but it has a high N storage capacity and is therefore able to continue growth even when water N concentrations are low. Additionally, Lavery and McComb (1991b) reported that *Chaetomorpha linum* took up ammonium and phosphate at twice the rate of *Ulva rigida*, possibly due to *C. linum* having a greater surface area to volume ratio than *U. rigida*. Conversely, Lotze and Schramm (2000) found that there was generally no significant difference in nutrient uptake or growth rates of the tubular *Enteromorpha intestinalis* (Chlorophyta) and filamentous *Pilayella littoralis* (Phaeophyta), indicating that substantial differences in surface area to volume ratio did not affect uptake or growth rates.

The age of the plant has been shown to significantly affect nutrient uptake rates in seagrasses (e.g., Borum *et al.*, 1989) and macroalgae. Harrison (1986) found that nitrate and ammonium uptake rates for first-year plants of *Laminaria* were 3 times higher than second- and third-year plants. Additionally, for first-year plants, there were no differences in uptake rates when nitrate or ammonium were added individually or together; thus, first-year plants could take up twice as much N when both nitrate and ammonium were present. The nitrate uptake capacity of macroalgae is also partly dependent on the nutritional history of the plant and subsequent tissue N status; nitrate uptake rates are often higher when the alga is N-limited, and lower when the alga is N-replete (Wheeler, 1982; Smit, 2002). For example, ammonium-N uptake by *Cladophora vagabunda, Gracilaria tikvahiae* (Peckol *et al.*, 1994) and *Gracilaria gracilis* (Smit, 2002) was faster under N-limited conditions. Similarly, Gordon *et al.* (1981) reported that while P uptake rates of *Cladophora* increased in proportion to increasing substrate concentrations, the highest uptake rates occurred after preconditioning in a P-free medium (Gordon *et al.*, 1981). McGlathery *et al.* (1997) found that ammonium uptake within dense mats of *Chaetomorpha linum* varied according to light and dark conditions and varied diurnally according to tissue N status. *Chaetomorpha* mats acclimated to low light conditions were N-saturated and showed a diel (24 hour) variation in ammonium uptake whereby uptake in the light exceeded that in the dark. However, mats acclimated to high light conditions were generally N-saturated and ammonium uptake was equally high in the light and dark.

2.6 Macrophyte Tissue Nutrient Concentrations

The monitoring of concentrations of nutrients in the water column has traditionally been used to assess the nutrient status of an estuary and the availability of those nutrients to macrophyte

productivity (Fong *et al.*, 1994). However, some studies (e.g., Fong *et al.*, 1993, 1994) have demonstrated that in warm temperate and subtropical coastal environments, there is little correlation between water column nutrient (N and P) concentrations and the productivity or abundance of primary producers. Water quality analyses can be difficult to interpret as nutrient influxes tend to be rapidly taken up by macrophytes, and thus nutrient concentrations in the water column are frequently below the detection limit of instruments. In addition, due to the shifting and movement of water and organisms, water samples often do not accurately represent the substrate in which the plants took up nutrients (Gerloff and Krombholz, 1966). The concentrations of nutrients in plant tissue, however, are commonly used to evaluate the nutritional status of the plant and are considered to be more representative of the availability of that nutrient in the plant's ambient environment. Nutrient concentrations in macroalgal tissue are a function of nutrients assimilated from the water column from a certain length of time prior to collection, thus tissue nutrient contents and C:N:P ratios can be a useful indicator of *in situ* nutrient status (Wheeler and Björnsäter, 1992; Villares and Carballiera, 2003). For example, Fong *et al.* (1994) found that tissue P concentrations of *Enteromorpha* sp. were a good indicator of water column P concentrations as well as the rate of P supply. Additionally, tissue N concentrations could also be used to indicate supply rate and water column nitrogen concentrations, but only when N was not limiting growth.

Gerloff and Krombholz (1966) noted that for aquatic plants, tissue nutrient analysis must be conducted on healthy and metabolically active plants; otherwise, low nutrient concentrations measured on dying or decaying plants may be mistakenly interpreted as being below the critical nutrient level. For example, Pérez-Lloréns *et al.* (1991) found that younger seagrass leaves typically contained more N than older portions; the N contents of *Zostera noltii* leaves from The Netherlands varied from 4.0 % N in the youngest leaves, to 2.9 % N in older leaves. Gerloff and Krombholz (1966) defined the critical nutrient concentration as the minimum tissue concentration required to achieve maximal growth. When all other parameters are at adequate levels to support growth, tissue nutrient concentrations below the critical level indicate a deficiency of that element, resulting in yields less than the maximum possible. Tissue nutrient contents above the critical level do not affect plant yields and are therefore considered to be luxury consumption. These authors suggested that the critical nutrient concentrations for aquatic angiosperms were 1.3 % N and 0.3 % P (dry weight). Critical tissue nitrogen and phosphorus concentrations for macroalgae range from: 1.2 - 2.1 % N, and 0.025 - 0.33 % P, respectively (Table 2-6).

Tissue nutrient content varies according to the species, type of tissue, age of the plant, season and other environmental conditions (Touchette and Burkholder, 2000a). Seagrasses have high growth rates and periodically shed older leaves, which results in a significant loss of nutrients that must be replenished to support growth. While nutrient availability can limit the growth of seagrasses, they can also take up excess nutrients in the water column or sediment, via leaves

and/or rhizomes, and store them for later use (Borum *et al.*, 1989). The tissue C, N and P values of some seagrass species (including *Ruppia*) occurring under natural conditions are listed in Table 2-7. Tissue C contents of seagrasses typically range from 29 - 40 % C (dry weight). The nitrogen and phosphorus concentrations of seagrass leaf tissue tends to vary markedly, ranging from 0.4 - 4.0 % N and 0.04 - 0.4 % P, respectively.

Table 2-6: Critical tissue nitrogen and phosphorus concentrations (% dry weight) for the growth of aquatic macrophytes.

Species	N (%)	P (%)	Locality	References
Chaetomorpha linum	1.2	0.05	W. Australia	Lavery and McComb (1991b)
Cladophora aff. *albida*	2.1	0.33	W. Australia	Gordon *et al.* (1981)
Codium fragile	1.90	-	Rhode Is., USA	Hanisak (1979)
Enteromorpha intestinalis	2.5	-	NW USA	Björnsäter and Wheeler (1990)
Ulva sp.	2.45	0.081	NW Spain	Villares and Carballiera (2003)
Ulva fenestrata	3.2	-	NW USA	Björnsäter and Wheeler (1990)
Ulva rigida	2.0	0.025	W. Australia	Lavery and McComb (1991b)
Gracilaria coronopifolia	3.20	0.27	Taiwan	Tsai *et al.* (2005)
Laurencia papillosa	2.06	0.078	Taiwan	Tsai *et al.* (2005)
Vallisneria americana	1.3	0.13	NW USA	Gerloff and Krombholz (1966)
Ruppia maritima	2.5 - 3.5	0.25 -0.35	Rhode Is., USA	Thursby (1984)

The luxury consumption of nutrients is a common characteristic of many macroalgae, such as *Ulva rigida* (Naldi and Viaroli, 2002), *Gracilaria edulis* (Costanzo *et al.*, 2000) and *Gracilaria gracilis* (Smit, 2002). These algae have the ability to take up N and/or P at levels far exceeding immediate growth requirements, and store it for later use when ambient nutrient concentrations are lower. For example, Gordon *et al.* (1981) found that *Cladophora* sp. cultured with high N and P substrate concentrations (> 0.4 mg L^{-1} N and 0.2 mg L^{-1} P), had very high tissue nutrient concentrations (up to 7.6 % N and 1.3 % P) which were well above levels critical for growth (2.1 % N and 0.33 % P). These authors suggested that *Cladophora* has a high internal storage capacity and undertakes “luxury” storage of nutrients under non-limiting conditions. Tissue nutrient concentrations of macrophytes fluctuate seasonally and are typically higher in winter and spring, probably due to luxury uptake of nutrients in winter when rainfall and therefore runoff may be higher; nutrients stored in the tissue may be used in the summer growing season when light and temperature are higher and ambient water column nutrient concentrations are lower (McComb and Lukatelich, 1986). Therefore, tissue nitrogen and phosphorus concentrations of macroalgae collected *in situ* often exceed the known critical nutrient concentrations for algal growth. Tissue nitrogen and phosphorus concentrations of macroalgae sampled in natural conditions range from 0.07 - 6 % N, and 0.01 - 0.7 % P, respectively (Table 2-8).

Table 2-7: Concentrations of carbon, nitrogen and phosphorus (% dry weight) in seagrasses growing under natural conditions (values are mean ± s.e., where given, with range in parentheses).

Species	Total C (%)	Total N (%)	Total P (%)	Locality	Reference
Cymodocea serrulata - leaves	-	1.48	0.17	NE Qld	Birch (1975)
Cymodocea serrulata - rhizomes		0.46	0.08	NE Qld	Birch (1975)
Halophila ovalis - whole plant	-	0.59	0.15	NE Qld	Birch (1975)
Halophila ovalis - whole plant	29	1.1	0.19	W. Australia	Atkinson and Smith (1983)
Halophila decipiens - whole plant	-	1.29	0.26	NE Qld	Birch (1975)
Posidonia australis - leaves	-	1.63	-	W. Australia	Paling & McComb (2000)
Posidonia australis - rhizomes	-	0.55	-	W. Australia	Paling & McComb (2000)
Phyllospadix scouleri - leaves	-	2.3 ± 0.22	0.7 ± 0.02	Mexico	Ramírez-García *et al.* (2002)
Ruppia maritima - leaves	29	2.1	0.16	Virginia	Atkinson and Smith (1983)
Ruppia megacarpa - leaves	35.2 (27.4 - 41.0)	2.40 (1.75 - 3.09)	0.28 (0.12 - 0.59)	NSW, Australia	Present study
Ruppia megacarpa - rhizomes	34.3 (26.7 - 39.7)	1.75 (1.22 - 2.24)	0.24 (0.10 - 0.57)	NSW, Australia	Present study
Thalassia testudinum - leaves	34.6 (29.4 - 39.5)	2.20 (1.71 - 1.67)	0.095 (0.053 - 0.201)	Florida, USA	Fourqurean and Zieman (1992)
Thalassia testudinum - leaves	-	2.27 ± 0.22	0.23 ± 0.15	Florida, USA	Fourqurean and Cai (2001)
Zostera capricorni - leaves	-	1.58	0.20	NE Qld	Birch (1975)
Zostera capricorni - leaves	32	1.1	0.27	NE Qld	Atkinson and Smith (1983)
Zostera capricorni - leaves	-	(1.36 - 1.68)	-	SE Qld	Boon (1986)
Zostera capricorni - leaves	39	1.6	0.20	SE Qld	Udy and Dennison (1997a)
Zostera capricorni - leaves	36.1 (24.2 - 42.0)	2.86 (1.89 - 4.13)	0.34 (0.21 - 0.56)	NSW, Australia	Present study
Zostera capricorni - rhizomes	-	0.53	0.15	NE Qld	Birch (1975)
Zostera capricorni - rhizomes	-	(0.71 - 0.74)	-	SE Qld	Boon (1986)
Zostera capricorni - rhizomes	31.4 (23.3 - 38.3)	1.27 (0.85 - 1.80)	0.23 (0.11 - 0.38)	NSW, Australia	Present study
Zostera marina - whole plant	36.3 (29.0 - 40.9)	2.33 (1.13 - 3.79)	0.38 (0.11 - 0.90)	California, USA	Fourqurean *et al.* (1997)
Zostera marina - leaves	-	1.8 (1.3 - 2.4)	-	Virginia, USA	Borum *et al.* (1989)
Zostera marina - leaves	37.6 (35.4 - 40.8)	2.3 (1.7 - 2.9)	0.23 (0.13 - 0.38)	Venice, Italy	Sfriso and Marcomini (1999)
Zostera marina - rhizomes	32.0 (20.7 - 37.0)	1.2 (0.9 - 1.7)	0.10 (0.06 - 0.15)	Venice, Italy	Sfriso and Marcomini (1999)
Zostera noltii - leaves	44.2 (41.1 - 46.9)	3.8 (3.6 - 3.9)	-	Portugal	Machás *et al.* (2006)

Table 2-8: Concentrations of carbon, nitrogen and phosphorus (% dry weight) in macroalgae growing under natural conditions (values are mean ± s.e., where given, with range in parentheses).

Species	Total C (%)	Total N (%)	Total P (%)	Locality	Reference
Chlorophyta					
Chaetomorpha linum	-	1.97 (1.10 - 2.19)	0.20 (0.04 - 0.24)	W. Australia	Lavery & McComb (1991)
Chaetomorpha linum	(18 - 23)	(1.2 - 1.45)	-	Mediterranean	Menéndez and Comin (2000)
Chaetomorpha spp.	36.0 (28.0 - 39.7)	3.06 (2.05 - 3.95)	0.16 (0.03 - 0.30)	NSW, Australia	Present Study
Cladophora aff. *albida*	-	3.2 ± 0.1	0.23 ± 0.008	WA	Birch *et al.* (1981)
Codium fragile	-	(1.64 - 2.60)	(0.28 - 0.49)	NW USA	Wheeler and Björnsäter (1992)
Codium isthmocladum	12.6 (6.0 - 21.2)	0.93 (0.52 - 1.44)	0.05 (0.02 - 0.15)	Caribbean & Florida	Lapointe *et al.* (2005)
Enteromorpha spp.	17.65	1.59	-	NW USA	Fujita (1985)
Enteromorpha sp.		(0.7 - 3.5)	(0.08 - 0.15)	SE USA	Fong *et al.* (1994)
Enteromorpha sp.	38.5 (25.2 - 44.8)	3.04 (0.07 - 5.9)	0.096 (0.025 - 0.26)	NW Spain	Villares and Carballiera (2003)
Enteromorpha intestinalis	-	(2.02 - 5.11)	(0.372 - 0.733)	NW USA	Wheeler and Björnsäter (1992)
Ulva sp.	39.7 (35.5 - 42.6)	2.88 (0.69 - 5.13)	0.097 (0.025 - 0.23)	NW Spain	Villares and Carballiera (2003)
Ulva fasciata	-	2.69 ± 0.29	0.12 ± 0.01	Hawaii	Larned (1998)
Ulva fenestrata	-	(2.44 - 5.48)	(0.32 - 0.60)	NW USA	Wheeler and Björnsäter (1992)
Ulva lactuca	-	(2.17 - 5.03)	(0.11 - 0.33)	Hong Kong	Ho (1981)
Ulva lactuca	25.56	1.02	-	NW USA	Fujita (1985)
Ulva lactuca	-	3.76 (2.22 - 5.27)	0.21 (0.086 - 0.31)	Hong Kong	Ho (1987)
Ulva lactuca	-	(0.7 - 4.1)	(0.05 - 0.58)	Denmark	Lyngby *et al.* (1999)
Ulva rigida	(21.1 - 32.7)	(0.99 - 3.5)	(0.027 - 0.27)	SE France	de Casabianca and Posado (1998)
Ulva rigida	28.5 (24.5 - 32.2)	2.7 (1.9 - 3.3)	0.09 (0.05 - 0.15)	Venice, Italy	Sfriso and Marcomini (1999)
Rhodophyta					
Gracilaria coronopifolia	(16.8 - 31.6)	(0.86 - 4.73)	(0.04 - 0.18)	Taiwan	Tsai *et al.* (2005)
Gracilaria edulis	-	(1.3 - 1.5)	-	NE QLD	Costanzo *et al.* (2000)
Gracilaria salicornia	-	0.93 ± 0.13	0.07 ± 0.03	Hawaii	Larned (1998)
Gracilaria verrucosa	(23 - 30)	(2.3 - 3.0)	-	Mediterranean	Menéndez and Comin (2000)
Laurencia papillosa	(16.6 - 26.1)	(0.74 - 3.87)	(0.03 - 0.14)	Taiwan	Tsai *et al.* (2005)
Phaeophyta					
Cystoseira myrica	(17 - 24)	(0.99 - 2.24)	(0.04 - 0.10)	Egypt	Abou-Aisha *et al.* (1997)
Sargassum echinocarpum	-	1.32 ± 0.08	0.08 ± 0.01	Hawaii	Larned (1998)

2.6.1 Effect of Nutrient Enrichment

Nutrient enrichment has been shown to significantly enhance the tissue N and P contents of macroalgae (Lotze and Schramm, 2000). Menéndez (2005) found that enrichment with N only, or a combination of N and P enrichment, had a significant effect on the tissue N content of *Chaetomorpha linum* grown in culture, but enrichment with P only did not significantly affect tissue N contents. Similarly, enrichment with N only had no effect on tissue P contents, but P only and N + P treatments resulted in significantly higher tissue P contents. Gordon *et al.* (1981) found that the tissue N and P content of *Cladophora* increased in proportion to substrate concentration. The amount of P taken up from the water column corresponded perfectly with increasing tissue P concentrations, and tissue P concentrations of 0.2 % were maintained for phosphorus substrate concentrations greater than 0.01 mg P L^{-1}. Similarly, in the lowest N treatments ($\leq$ 3 mg N L^{-1}), there was a linear relationship between the amount of N taken up and increasing tissue N concentrations, but at concentrations above 3 mg N L^{-1}, tissue N content did not increase with further N additions, showing a ceiling for N addition at about 7.6 % N (dry weight). Menéndez *et al.* (2002b) reported that N enrichment (with 120 μM NH_4^+ or 68 μM NO_3^-) resulted in a significant increase in *Chaetomorpha linum* tissue N contents, but had no effect on tissue P contents. Likewise, P-enrichment (18 μM PO_4^{3-}) resulted in a significant increase in tissue P, but did not affect tissue N. The increase in tissue P content was greater when the algae were enriched with P alone, compared to combined P and N (nitrate or ammonium) enrichment. In the P-only treatment, the N/P ratio was reduced from 80 to 6 after 10 days, indicating an N-deficiency. Enrichment by P + NH_4^+ or P + NO_3^- resulted in a reduction of the N/P ratio from 80 to 21 after 10 days, whilst enrichment by NO_3^--only and NH_4^+-only substantially increased the N/P ratio to about 200 after 10 days.

Peckol *et al.* (1994) concluded that tissue N content was a poor indicator of growth rates for *Gracilaria tikvahiae* and *Cladophora vagabunda* cultured *in situ*. Tissue N content and algal growth are often not linearly related as high tissue N levels can occur when N supply is high and the alga is growing successfully, or when excess N has been taken up and stored, but other environmental conditions (e.g., temperature, light, salinity) are limiting growth (Morgan and Simpson, 1981a, 1981b; Kamer and Fong, 2001). Morgan and Simpson (1981a) reported an inverse relationship between total tissue N and growth rates of *Palmaria palmata* (Rhodophyta) at temperatures of 6 - 18°C; plants grown slowly under low irradiance had higher N than plants grown quickly under high irradiance. Total plant nitrogen content of *P. palmata* was highest at the lowest temperature (6°C) and in all temperature treatments, the plant total N was also lowest at the highest irradiance and appeared to be dependent on growth rates; the highest N contents (3.1 - 4.2 % N) were associated with the slowest growth which occurred under low irradiances (19 ly·day^{-1} PAR), whereas the lowest N contents (2.4 - 3.0 % N) correlated with rapid growth rates under high irradiances (86 ly·day^{-1} PAR). Similarly, Menéndez *et al.* (2002b) documented a 10-fold increase in *Chaetomorpha linum* tissue P content (from 0.02 % to 0.2 %

P) 4 days after fertilisation with P (18 μmol PO_4^{3-} L^{-1}) alone, reducing the N/P ratio from 80 to 6. These authors suggested this substantial increase in tissue P would indicate luxury uptake of P by *C. linum,* although biomass did not increase significantly in P-only treatments, suggesting the lack of N had limited growth. Thus, high nutrient levels in macroalgae may be an indication of high growth rates occurring under high nutrient loading, or slow growth rates as a result of limitation by low temperatures and/or irradiance (Morgan and Simpson, 1981b).

2.6.2 Stoichiometric Ratios of C : N : P

Stoichiometric ratios of C:N:P can be used to indicate limitation by nitrogen or phosphorus during growth. The 'Redfield Ratio' is commonly used to describe the average molar ratio of carbon, nitrogen and phosphorus in marine phytoplankton, and averages 106:16:1 (Atkinson and Smith, 1983; Geider and La Roche, 2002). In phytoplankton, the average N/P ratio is 16:1 when nutrients are not limiting; but N/P ratios greater than 30:1 indicate deprivation of P during growth, whereas N/P ratios of < 10:1 indicate deprivation of N during growth (Atkinson and Smith, 1983). Hillebrand and Sommer (1999) suggested a C:N:P atomic ratio of 119:17:1 was optimal for growth of benthic microalgae, and that N/P ratios less than 13, and greater than 22, indicated N and P limitation, respectively. They also documented that C/N ratios of benthic microalgae increased with decreasing growth rates, regardless of N or P limitation, whereas C/P and N/P ratios only increased significantly with P limitation. The average C:N:P ratios of benthic macrophytes (seagrasses and macroalgae), however, are substantially different to the Redfield ratio (Table 2-9). Atkinson and Smith (1983) documented that C:N:P ratios of marine macroalgae and seagrasses worldwide ranged from 143:16:1 to 3550:61:1, with a mean of 700:35:1 and median of 550:30:1. They determined that macrophytes collected from low nutrient regimes had significantly higher C/N and C/P ratios than those from high nutrient environments, and that high C:N:P molar ratios in macroalgae may be indicative of low water nutrient concentrations limiting growth. Wheeler and Björnsäter (1992) concluded that for macroalgae (e.g., *Ulva fenestrata*, *Enteromorpha prolifera* and *Pelvetiopsis limitata*), an N/P ratio exceeding 11 - 24 suggested P limitation, whilst N/P ratios less than 8 - 16 suggested N limitation.

The growth of a macrophyte will be largely dependent on the availability of light (to fix carbon) and N. Macrophytes in different environments are expected to have differing C/N ratios, depending on the individual requirements of the plant (Baird and Middleton, 2002). Krause-Jensen *et al.* (1996) noted that the C/N ratio in algal tissue represents the relationship between assimilation and uptake of carbon and nitrogen, respectively, and is often used to determine possible nutrient limitation within algal mats. These authors found that in *Chaetomorpha linum* mats growing in low light, the low C/N ratios (12 - 15) indicated that assimilation of C was low compared to uptake of N and the entire mat was N saturated and light limited. In high light, however, C/N ratios decreased from the 33 at the surface to 22 at the bottom of the mat,

indicating a reduction in C assimilation compared to N uptake. They concluded that the bottom layers of the algal mat were light limited and N limitation increased towards the mat surface. Previous studies have indicated that the critical C/N ratio for many macroalgal species is between 10 and 15; values exceeding this ratio may indicate nitrogen limitation, whereas values below suggest nitrogen storage (Hanisak, 1983). However, as C/N ratios are related to both carbon and nitrogen metabolism, changes in the C/N ratio does not always indicate nitrogen limitation (Hanisak, 1979; 1983), but may often be or related to other environmental factors such as light intensity (Hanisak, 1983; Lapointe and Tenore, 1981).

Pérez-Lloréns *et al.* (1991) found that tissue nutrient (C, N, P) contents in *Zostera noltii* were dependent on the age of the leaves and the plant part (leaves, roots and rhizomes), with the highest nutrient contents occurring in the above-ground components, and the lowest C/N and N/P molar ratios occurring in the youngest leaves. Similarly, Borum *et al.* (1989) found that *Zostera marina* leaf N contents averaged 1.5 - 3.0 % (dry weight) for actively growing leaves, but declined with age, with N contents in the oldest leaves being only about half of that in the youngest leaves. Seagrass C:N:P ratios increase with age because older leaves have a greater proportion of structural carbon, compared to N and P (Pérez-Lloréns *et al.*, 1991), and have also been shown to decline significantly in winter. For example, seagrasses (*Halodule wrightii* and *Thalassia testudinum*) in the Gulf of Mexico exhibited a significant decline in C/N and N/P ratios between summer and winter; C/N ratios declined by 10 - 30 %, C/P ratios declined by 42 - 52 %, and N/P ratios declined by 30 - 35 % (Johnson *et al.*, 2006). Declines in C/N and N/P ratios during winter may be related to a reduction in plant growth due to reduced light and low temperatures (Fourqurean *et al.*, 1997; Johnson *et al.*, 2006).

Table 2-9: C:N:P atomic ratios of selected macroalgae and seagrasses growing under natural conditions (values are mean, with range given in parentheses).

Order	Species	C / N	C / P	N / P	Locality	Reference
Chlorophyta	*Chaetomorpha linum*	32.9 ± 2.15	3141 ± 32	63 ± 0.2	NE Spain	Menéndez (2005)
	Chaetomorpha spp.	15.5 (10.7 - 29.5)	781 (171 - 2910)	52.0 (8.1 - 151)	NSW, Australia	Present study
	Codium fragile	-	-	12.4 (8.5 - 16.8)	NW USA	Wheeler and Björnsäter (1992)
	Codium isthmocladum	16.7 (7 - 34)	745 (184 - 1638)	43.7 (15 - 78)	Caribbean & Florida	Lapointe *et al.* (2005)
	Enteromorpha sp.	17.6 (8.82 - 58.7)	1,239 (377 - 3594)	76.2 (32.4 - 209)	NW Spain	Villares and Carballiera (2003)
	Enteromorpha prolifera	-	-	15.4 (10.1 - 29.3)	NW USA	Wheeler and Björnsäter (1992)
	Ulva sp.	19.47 (9.61 - 65.1)	1,309 (463 - 3904)	69.9 (27.4 - 178)	NW Spain	Villares and Carballiera (2003)
	Ulva fenestrata	-	-	22.3 (11.2 - 30.1)	NW USA	Wheeler and Björnsäter (1992)
	Ulva reticulata	13.1	1,051	80	Hawaii	Atkinson and Smith (1983)
Rhodophyta	*Gracilaria* sp.	28.2	819	29	Virginia, USA	Atkinson and Smith (1983)
	Gracilaria mammalaris	10	321	33	Florida, USA	Lapointe *et al.* (2005)
Seagrasses	*Halodule wrightii* - leaves	14.6 - 17.4	556.7 - 614	45.2 - 31.5	Alabama, USA	Johnson *et al.* (2006)
	Ruppia maritima - leaves	15.8	457	29	Virginia, USA	Atkinson and Smith (1983)
	Ruppia megacarpa - leaves	17.5 (13.9 - 24.1)	415 (120 - 879)	23.9 (8.2 - 54.1)	NSW, Australia	Present study
	Ruppia megacarpa - rhizomes	23.3 (19.3 - 30.7)	459 (135 - 1023)	19.8 (6.5 - 43.0)	NSW, Australia	Present study
	Thalassia testudinum - leaves	18.5 (15.7 - 22.8)	1070 (448 - 1721)	58.6 (20.3 - 89.4)	Florida, USA	Fourqurean and Zieman (1992)
	Thalassia testudinum - leaves	20.3 - 23.4	1265 - 1183	53.56 - 62.2	Florida, USA	Johnson *et al.* (2006)
	Zostera capricorni - leaves	33.6	302	9	NE Qld	Atkinson and Smith (1983)
	Zostera capricorni - leaves	14.7 (11.4 - 17.9)	291 (142 - 445)	20.0 (10.5 - 30.4)	NSW, Australia	Present study
	Zostera capricorni - rhizomes	30.3 (18.0 - 50.2)	400 (207 - 779)	12.9 (8.0 - 20.8)	NSW, Australia	Present study
	Zostera marina - whole plant	19.7 (11.5 - 38.0)	273 (106 - 455)	14.6 (5.8 - 28.5)	California, USA	Fourqurean *et al.* (1997)
	Zostera marina - leaves	17.8	481	27	Rhode Is., USA	Atkinson and Smith (1983)
	Zostera marina - leaves	(15 - 20)	254 (184 - 440)	17 (14 - 32)	Oregon, USA	Kaldy (2006)
	Zostera marina - rhizomes	34 (20 - 60)	511 (130 - 784)	15 (7 - 22)	Oregon, USA	Kaldy (2006)
	Zostera noltii - leaves	12	104	9	The Netherlands	Pérez-Lloréns *et al.* (1991)
	Zostera noltii - rhizomes	21	127	6	The Netherlands	Pérez-Lloréns *et al.* (1991)

2.6.3 Stable Isotopes of C and N

Stable isotopes of C and N have been used extensively in environmental studies, to study trophic relationships, sources of primary productivity and utilization of organic matter or detritus (Cifuentes *et al.*, 1996; Camusso *et al.*, 1999). The abundance of an isotope is described in terms of *delta* (δ), which indicates the degree to which a sample's isotopic composition deviates from an international reference material; this variation is measured in parts per thousand (‰) (Lepoint *et al.*, 2004). Camusso *et al.* (1999) noted that negative (-) δ values indicate depletion and positive (+) δ values indicate enrichment of the heavier isotope (^{13}C and ^{15}N) compared to the lighter isotope (^{12}C and ^{14}N), relative to the standard materials of Pee Dee Belemnite for C and atmospheric $N_{2(gas)}$ for N, as follows:

$$\delta X = \left(\frac{\delta X_{sample}}{\delta X_{standard}} - 1 \right) \times 1000$$

where X is ^{13}C or ^{15}N, X_{sample} is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and $X_{standard}$ is the isotope ratio of the standard. An increase in the δ value indicates an increase in the amount of heavy isotope, and subsequent decrease in the light isotope. In contrast, a decrease in the δ value indicates a decrease in the heavy isotope components, and a subsequent increase in the lighter isotope content (Peterson and Fry, 1987).

Many studies have recorded significant spatial and temporal variations in the isotopic ratios of aquatic macrophytes (Cifuentes *et al.*, 1996). Boon and Bunn (1994) noted that significant variability (> 10 delta units) can occur in carbon and nitrogen isotopic values of the same species sampled at different sites at the same time, or at the same site at a different time of year. This high variability suggests that there are many environmental and other factors influencing the isotopic signatures of aquatic macrophytes; these include temperature, light availability, water movement, and the proximity to fresh-water and marine inflow and other sources of nutrients, such as groundwater, waste water or runoff (Smith *et al.*, 1976; McMillan and Smith, 1982; Hemminga and Mateo, 1996; France and Cattaneo, 1998; Boyce *et al.*, 2001). The $\delta^{13}C$ values of seagrasses are usually around -10 ‰, but values ranging from -23 to -3 ‰ have been reported in the literature (Lepoint *et al.*, 2004; McMillan *et al.*, 1980). Macroalgae have similar $\delta^{13}C$ values to seagrasses, and are usually in the range of -12 to -23 ‰ (Smith and Epstein, 1971), but extreme values of -8.8 to -32.4 ‰ have been documented (Fry *et al.*, 1982). The $\delta^{15}N$ values of seagrasses typically lie between 0 - 8 ‰, but can vary from -2 to 12.3 ‰ (Lepoint *et al.*, 2004). The $\delta^{13}C$ and $\delta^{15}N$ values of a range of seagrasses and macroalgae are listed in Table 2-10.

Table 2-10: Isotopic composition of macroalgae and seagrasses (values are mean, with range given in parentheses).

Order	Species	$\delta^{15}N$	$\delta^{13}C$	Locality	Reference
Chlorophyta	*Chaetomorpha* sp.	5.6	-17.9	Stagnone di Marsala, Sicily	Vizzini *et al.* (2002)
	Chaetomorpha spp.			NSW, Australia	Present Study
	Cladophora vagabunda	(3.2 - 5.4)	n/a	Waquoit Bay, USA	McClelland *et al.* (1997)
	Ulva lactuca	n/a	(-17.8 to -8.86)	Scotland	Maberly *et al.* (1992)
	Enteromorpha sp.	7.3	n/a	The Netherlands	Riera *et al.* (2000)
	Ulva lactuca	(2.3 - 7.8)	(-17.1 to -13.3)	Moa Point, New Zealand	Rogers (2003)
	Ulva rigida	11.1	-8.8	River Po, Italy	Camusso *et al.* (1999)
Rhodophyta	*Chondria* sp.	5.4	-17.5	Stagnone di Marsala, Sicily	Vizzini *et al.* (2002)
	Gracilaria tikvahiae	(5.4 - 7.9)	n/a	Waquoit Bay, USA	McClelland *et al.* (1997)
Phaeophyta	*Sargassum duplicatum*	2.7	-15.8	Ishigaki Island, Japan	Yamamuro *et al.* (1995)
Seagrasses	*Cymodocea serrulata* - leaves	4.5	-11.6	Moreton Bay, Qld, Australia	Grice *et al.* (1996)
	Halodule uninervis - leaves	2.6	-11.3	Moreton Bay, Qld, Australia	Grice *et al.* (1996)
	Halodule uninervis - leaves	2.4	-9.2	Moreton Bay, Qld, Australia	Udy and Dennison (1997a)
	Halophila decipiens - leaves	n/a	-10.2 (-12.3 to -8.5)	Virgin Islands, USA	McMillan *et al.* (1980)
	Halophila ovalis - leaves	-1.41	n/a	Dravuni Island, Fiji	Yamamuro *et al.* (2003)
	Halophila spinulosa - leaves	8.6	-15.4	Moreton Bay, Qld, Australia	Grice *et al.* (1996)
	Posidonia australis - leaves	n/a	-7.2	Corner Inlet, Vic., Australia	Nichols *et al.* (1985)
	Posidonia oceanica - leaves	2.8	-11.3	Stagnone di Marsala, Sicily	Vizzini *et al.* (2002)
	Ruppia megacarpa - whole plant	n/a	-11.15 (-17.8 to -6.6)	Peel Inlet, WA	Boyce *et al.* (2001)
	Ruppia megacarpa - leaves	3.9 (1.0 - 9.0)	-10.9 (-13.0 to -8.9)	NSW, Australia	Present Study
	Ruppia megacarpa - rhizomes	2.9 (0.9 - 7.5)	-10.6 (-12.2 to -9.1)	NSW, Australia	Present Study
	Syringodium isoetifolium - leaves	2.9	-6.6	Moreton Bay, Qld, Australia	Grice *et al.* (1996)
	Thalassia testudinum - leaves	n/a	(-13.2 to -9.9)	Virgin Islands, USA	Fry *et al.* (1982)
	Zostera capricorni - leaves	8.8	-10.2	Moreton Bay, Qld, Australia	Grice *et al.* (1996)
	Zostera capricorni - leaves	n/a	-10.8 (-12.4 to -9.2)	Not specified	Hemminga and Mateo (1996)
	Zostera capricorni - leaves	2.2	-7.4	Moreton Bay, Qld, Australia	Udy and Dennison (1997a)
	Zostera capricorni - leaves	4.4 (1.0 - 6.8)	-11.9 (-15.9 to -7.7)	NSW, Australia	Present Study
	Zostera capricorni - rhizomes	3.9 (0.2 - 6.3)	-11.2 (-13.8 to -7.7)	NSW, Australia	Present Study
	Zostera marina - leaves	9.7 (6.3-12.5)	-9.6 (-15.0 to -7.5)	California, USA	Fourqurean *et al.* (1997)
	Zostera noltii - leaves	5.7 (4.9 - 6.6)	-10.2 (-11.3 to -9.7)	Ria Formosa, Portugal	Machás *et al.* (2006)

Stable isotopes of carbon and nitrogen have often been used in food web analysis, by analysing the ratio of stable isotopes in animal tissue to determine the dominant plant groups at the bottom of the food chain (Fry and Parker, 1978; Fry *et al.*, 1982; Nichols *et al.*, 1985; Walker *et al.*, 1999). The isotopic composition of an animal is strongly determined by that of its food (Lepoint *et al.*, 2004), and as $\delta^{13}C$ values do not change greatly between different trophic levels in the food web, they can therefore be used to trace sources of primary productivity (Camusso *et al.*, 1999). Different plant groups use different photochemical pathways; this results in a consistent variation in the ratio of stable isotopes of C and N, which is also transferred conservatively to consumer species (Walker *et al.*, 1999). Stable isotopes have been used extensively as environmental tracers, such as mapping the source of sewage discharges or groundwater flow (Lepoint *et al.,* 2004). In estuaries, stable isotope studies have been used to identify organic matter derived from native as opposed to non-native sources, such as marine and terrestrial inputs (e.g., runoff and anthropogenic discharges) (Camusso *et al.*, 1999). ^{15}N levels in waste water are typically higher than those derived from atmospheric sources, so that primary producers incorporating ^{15}N from polluted waters will have higher $\delta^{15}N$ levels those from pristine environments (Bowen and Valiela, 2001). As inorganic nitrogen in waste water is typically of animal or human origin, it has a higher ^{15}N isotopic signature than marine water (Lepoint *et al.*, 2004). Additionally, Hansson *et al.* (1997) noted that the $^{15}N/^{14}N$ ratio was usually higher in samples (e.g., of particulate organic matter or marine organisms) collected from areas receiving waste waters, because nitrification and denitrification at the waste treatment facility resulted in isotopic fraction and thus enrichment of ^{15}N in waste water discharges.

Macrophyte $\delta^{15}N$ values fluctuate according to variations in the metabolic pathways used for nitrogen assimilation and the type of nitrogen assimilated, as well as the presence of nitrogen-fixing or denitrifying bacteria (Boon and Bunn, 1994). The variations in $\delta^{15}N$ values of seagrasses are related to incorporation of inorganic N, as well as sediment and water column geochemistry (Lepoint *et al.*, 2004). $\delta^{15}N$ values close to 0 ‰ may be related to nitrogen fixation by organisms within the seagrass community (Yamamuro *et al.*, 2003). Elevated $\delta^{15}N$ values in seagrasses may also be related to denitrification processes in marine and estuarine waters; denitrification leads to the loss of ^{14}N, and an enrichment of ^{15}N, from the inorganic N pool in the water column, and a subsequent enrichment of ^{15}N in the plants taking up that inorganic N from the water (Fourqurean *et al.*, 1997). Grice *et al.* (1996) reported that $\delta^{15}N$ values of seagrasses from Moreton Bay, Qld, appeared to be influenced by the species and collection site; $\delta^{15}N$ values were higher at eutrophic sites than those furthest from anthropogenic inputs, suggesting that the study of $\delta^{15}N$ values would be a useful tool for tracing sources and transfer of N in estuaries.

The $\delta^{13}C$ values of seagrasses and macroalgae are often highly variable, differing within and between species as well as spatially, seasonally and annually. Variability occurs in $\delta^{13}C$ values due to fluctuations in the isotopic signature of carbon fixed during photosynthesis, the metabolic

pathway used (e.g., C_3 or C_4) and differences in transport of carbon across the cell membrane (Smith and Epstein, 1971; Boon and Bunn, 1994; Coffin and Cifuentes, 1999). Lepoint *et al.* (2004) noted that $\delta^{13}C$ values of plants are directly related to photosynthesis; variations in the rate of photosynthesis and irradiance levels (e.g., due to depth, location, community structure, season) lead to variations in isotopic discrimination between ^{13}C and ^{12}C. High variability in $\delta^{13}C$ values may also be due to differing demands for carbon in carbon-limited systems (Grice *et al.*, 1996) or isotopic fractionation occurring during algal decomposition and fixation (Cifuentes *et al.*, 1996). Carbon isotope signatures are also affected by the form and source of inorganic carbon; inorganic carbon may be sourced from the water column and the sediment, where mineralization processes result in the release of CO_2, which may be taken up by seagrass roots (Hemminga and Mateo, 1996; Boyce *et al.*, 2001). $\delta^{13}C$ values can also indicate which plant sources of carbon (i.e., HCO_3 or CO_2) are utilised by macrophytes. Less negative values in aquatic macrophytes indicate the use of bicarbonate as the predominant C source; use of HCO_3 can occur when photosynthetic demand depletes CO_2 (Camusso *et al.*, 1999). In seagrasses, for example, high $\delta^{13}C$ values may indicate utilisation of bicarbonate ($\delta^{13}C$ = 0 ‰), as opposed to CO_2 ($\delta^{13}C$ = -9 ‰). Maberly *et al.* (1992) found that macroalgae thought to be capable of utilising HCO_3^- as a source of inorganic carbon had $\delta^{13}C$ values in the range -8.8 to -22.6 ‰. Those species that were thought to use CO_2 only had $\delta^{13}C$ values in the range -29.9 to -34.5 ‰. Additionally, some macrophytes growing in the intertidal zone, or partially emersed in shallow water, may have significantly lower $^{13}C/^{12}C$ ratios than completely submerged macrophytes, due to uptake of isotopically light atmospheric carbon dioxide (Cooper and McRoy, 1988). The ability to absorb atmospheric CO_2 ($\delta^{13}C$ = -7.8 ‰) and maintain photosynthesis during exposure to air has been demonstrated for some seagrasses, such as *Zostera muelleri, Z. marina* and *Z. noltii* (Hemminga and Mateo, 1996).

Changes in light and temperature result in changes in $^{13}C/^{12}C$ ratios in aquatic macrophytes, with $\delta^{13}C$ values becoming less negative with increasing light (Smith *et al.*, 1976). Grice *et al.* (1996) suggested that increases in light, temperature or numerous other factors may stimulate photosynthesis and productivity by seagrasses, thus increasing the demand for carbon; this may result in reduced discrimination against the heavier ^{13}C isotope as the lighter isotope, ^{12}C, is depleted. Therefore, more of the heavier isotope would be assimilated, causing $\delta^{13}C$ values of the seagrass to become less negative and resembling those of the ambient carbon source in the water. Grice *et al.* (1996) suggested that less negative $\delta^{13}C$ values in seagrasses exposed to high light intensities could be a result of physiological and morphological reactions. High light intensities (resulting in increased productivity) lead to declining tissue N contents. In response to lower nitrogen levels, seagrasses expand their root biomass to exploit a larger proportion of nutrients in the rhizosphere. To accommodate for the increased root biomass, lacunal areas in the shoots also increase in order to increase gaseous exchange. These expanded lacunal areas may provide a larger area for storage and recycling of CO_2, thus trapping the heavier ^{13}C isotope in the lacunae, increasing the amount of ^{13}C fixed for photosynthesis and resulting in a

less negative $\delta^{13}C$ signature. These authors predict that lower light intensities and thus lower productivities would result in the opposite of this process and thus more negative $\delta^{13}C$ values. In addition, Cooper and De Niro (1989) found more negative $\delta^{13}C$ values along a 38 m depth gradient in shallow *Posidonia oceanica* beds; the average $\delta^{13}C$ value of *P. oceanica* leaves was -11.0 ‰ at 5 m depth, but declined to -16.4 ‰ at 35 m. These authors attributed the decline in $\delta^{13}C$ values to reducing light levels with increasing depth and a subsequent decline in photosynthetic rates.

2.7 Local Macrophyte Studies

Several studies have been conducted regarding the biomass and distribution of seagrasses in Lake Illawarra (refer to Chapter 4), but limited information is available regarding the ecology of macroalgae in the Lake. Furthermore, information relating to the carbon, nitrogen and phosphorus contents of macrophytes in Lake Illawarra is particularly sparse. Localised studies include investigations by University of Wollongong research students, including a study of macroalgal biomass and distribution in Lake Illawarra (McConville, 2000) and a preliminary examination of carbon and nitrogen contents and $\delta^{13}C$ and $\delta^{15}N$ values of Lake Illawarra seagrass (Kuster, 2000). These studies are discussed further in Chapters 4 and 5, respectively.

CHAPTER 3 Materials and Methods

3.1 Introduction

This chapter describes the sampling sites, fieldwork and laboratory procedures used for the studies of macrophyte biomass, distribution and diversity in Lake Illawarra (Chapter 4 and 5). The methodologies used to examine the effect of temperature and variable nutrient regimes on the growth in culture of *Chaetomorpha linum* (Chapter 6) are discussed separately below.

3.2 Methodology used for the Studies of Macrophyte Biomass, Distribution and Diversity in Lake Illawarra

3.2.1 Site Selection

The sampling sites chosen for the biomass and nutrient studies (Chapter 5) and general macrophyte surveys (Chapter 4) were considered to be of particular interest and representative of macrophyte habitats in the whole Lake, easily accessible, and in similar locations to sites used in previous studies (e.g., King *et al.*, 1997). The sampling sites chosen for intensive biomass surveys and subsequent tissue nutrient analyses included two sites dominated by *Ruppia megacarpa* beds on the eastern Lake shore, Nicolle Road (NIC) and the Oasis Caravan Park (OCP) (Figure 3-1). These two sites were previously known to be subject to extensive macroalgal blooms and periodic macroalgal harvesting. Also studied were two sites dominated by *Zostera capricorni* beds, Purry Burry Point (PBP) on the eastern Lake shore, and Mullet Creek (MC) on the western shore of the Lake (Figure 3-1). In spring 2000, macrophyte biomass surveys were also conducted at Primbee Bay (PRIM), in the north-east, and the Lake Illawarra Village (LIV), on the eastern shore of the Lake. These two sites were excluded from future surveys due to the lack of suitable seagrass and/or macroalgal habitat. While macrophyte beds were sampled at similar locations within each site whenever possible, exact sampling locations varied according to seasonal fluctuations in seagrass and macroalgal coverage.

To further evaluate macrophyte diversity in Lake Illawarra, visual inspections and surveys were undertaken at a number of key sites, chosen to reflect the different habitats around the Lake. These sites included rocky substrate sites (Tuggerah Bay, Yallah Bay, Koonawarra Bay, Whyjuck Bay and Karoo Point) and mainly sand or silty substrate sites, including the Lake entrance channel, Koona Bay and Cudgeree Bay (Figure 3-1).

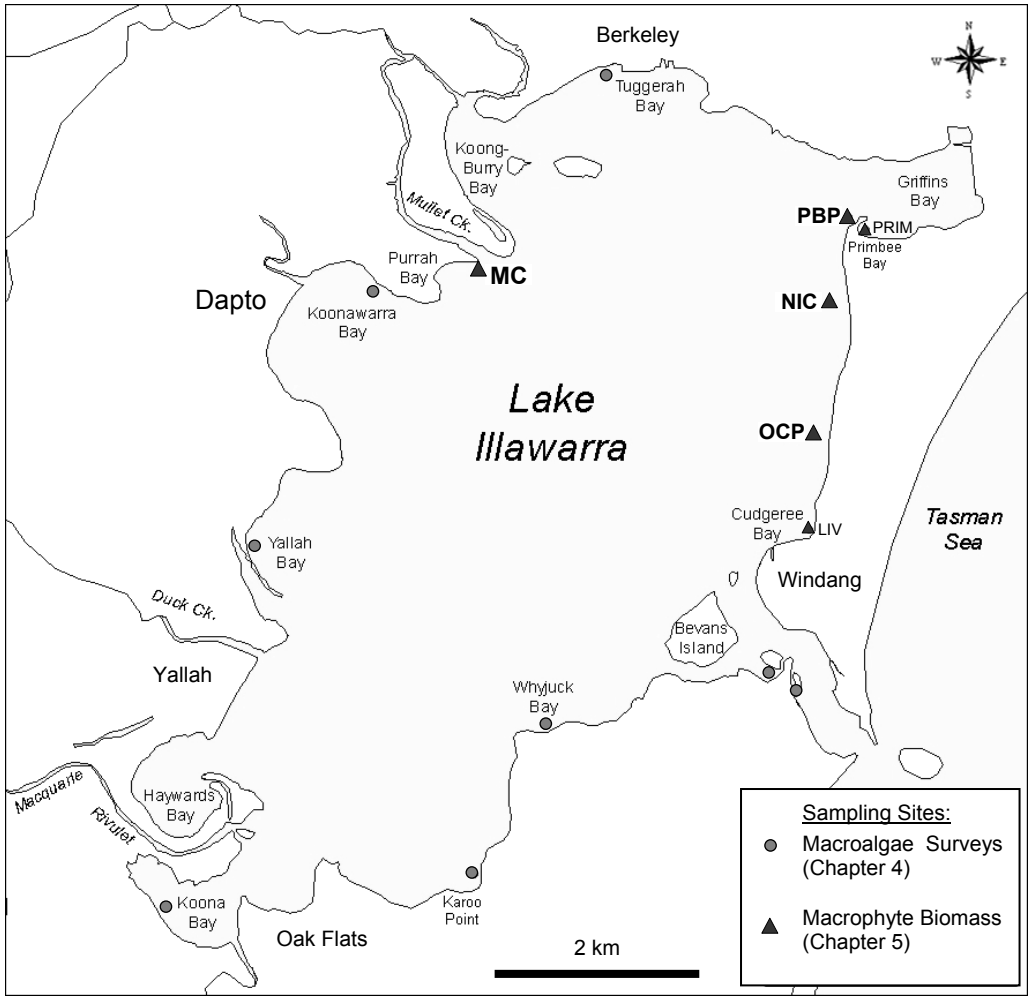


Figure 3-1: Sites used for seagrass and macroalgae biomass sampling (Chapter 5), and additional sites used for general ecological surveys of macroalgae (Chapter 4), Lake Illawarra, 2000 - 2003.

3.2.2 Sample Collection and Preservation

Macrophyte biomass surveys were conducted at four primary sites: the Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP) and Mullet Creek (MC), between spring 2000 and winter 2002. The surveys were conducted in mid-summer and mid-winter, to incorporate data from the two extremes in climate and assess seasonal variations in macrophyte biomass and nutrient contents.

At each site, four sub-sites of 15 m x 15 m were selected for biomass surveys; these included two sub-sites over seagrass beds considered representative of the site, and two sub-sites over inshore sand flats, typically with macroalgal coverage. These sub-sites were not permanent throughout the study due to fluctuating coverage of seagrass beds and algal mats, as well as fluctuating water level due to climatic variations and the intermittent opening and closing of the Lake's entrance to the sea. The seagrass sampling sites were typically 100 - 200 m from the shore, although occasionally it was necessary to sample closer to, or further from, the shore to

find suitable seagrass coverage. The macroalgae sub-sites were located approximately 10 - 50 m from the shore, depending on the abundance of macroalgal biomass. Sub-sites within the seagrass or macroalgae area were separated by a distance of 20 - 25 m. Within each sub-site, visual assessments were made of percentage seagrass and macroalgal cover to determine the average biomass per site.

Macrophyte biomass was sampled at 3 random areas within each sub-site; it was decided to collect biomass samples from 12 locations in total at each site due partly to time-constraints involved with collecting and analysing the material, as well as to limit the destruction of seagrass beds, particularly where coverage was sparse. Sampling was conducted by placing a small metal quadrat (0.25 m^2) in a random area and removing all vegetation (above and below ground) within the quadrat by hand. Vegetation was briefly rinsed free of sediment and debris using a plastic sieve *in situ*, and placed into polyethylene bags. Sediment samples were also obtained from each seagrass sampling location prior to removal of vegetation; samples were collected from the top 0 - 10 cm of the rhizoidal zone beneath the seagrass, using a pre-cleaned PVC pipe. All samples were kept on ice and transported back to the laboratory, after which they were either frozen immediately, or kept refrigerated for a short period (less than 72 hours) prior to sample preparation.

Additional surveys to assess the distribution, abundance and diversity of Lake Illawarra macroalgae were conducted bi-monthly, between June 2000 and June 2003. Sample collections were made whilst wading around the various sites, with several specimens of every distinguishable type collected initially, placed in plastic bags filled with seawater from the sites, and returned to the laboratory on ice for further examination. It was not considered appropriate to remove large amounts of organic material from the sites as this may have altered the macrophyte habitats and affected the results of later studies. Thus, in later surveys, collections were restricted to macroalgae which could not be identified in the field, were of particular interest to the study, under-represented in earlier collections, or for which fertile material had not yet been obtained. Where possible, macroalgae were collected *in situ*. However, as a large proportion of the macroalgal biomass in Lake Illawarra is free floating and regularly shifted by strong winds, a significant proportion of the collections included drift plants (i.e., detached and floating or entangled amongst other plants).

At each site, A *Yeo-Kal Intelligent Water Quality Analyser (Model 611)* was used to document water temperature, salinity, pH, conductivity, turbidity and dissolved oxygen (data provided in Appendix 3). Water depths at each site varied from 0.5 - 0.75 metres.

3.2.3 Macrophyte Sample Preparation

In the laboratory, the macrophyte samples were rinsed very briefly with tap water to remove sediment, shells and other debris, shaken and patted dry, then weighed to obtain a measure of the total wet weight biomass. A small portion (approximately 30 g) was removed to assess species content and biomass; of this sub-sample, the entire portion was separated according to macroalgal genera, living seagrass leaves and roots-rhizomes, and "leaf litter" (i.e., dead and decomposing seagrass leaves). Macroalgae were identified to species-level where possible, and all material was weighed wet, oven-dried to constant weight at 60°C, and re-weighed to determine dry weight and wet to dry ratios. A larger portion of each sample bag was separated into living seagrass leaves, rhizomes and macroalgae, rinsed briefly, weighed and dried in an oven at 60°C for 1 - 3 days, if necessary.

From each site, a total of 6 samples of seagrass, and 3 - 6 samples of the most abundant macroalgal genera (when present), were reserved for nutrient analysis. Samples prepared for nutrient analysis were ground to a fine powder using a clean mortar and pestle and passed through a 250 µm sieve; approximately 3 - 5 grams of dried material was prepared for each sample.

Selected samples of macroalgae were also preserved for later identification. Larger specimens were pressed onto high-grade art paper, using a plant press, and kept for future reference. Smaller samples not suited for pressing were preserved in sterile specimen jars, in a solution of 70 % Ethanol and 30 % seawater. Specimens preserved in this ethanol solution, however, often lost their colour over time. Specimens containing reproductive features were sectioned under a microscope and mounted onto slides using an aniline blue solution (Dr. A.J.K. Millar, pers. comm., 2001).

3.2.4 Identification of Macrophytes

Macroalgae were identified to species level wherever possible, using morphological features described in the literature, including Womersley (1984) and Kraft (2000) for green algae, Womersley (1987) for brown algae, and Millar (1990) and Womersley (1994, 1996, 1998, 2003) for red algae. For the rhodophytes, specimens with all reproductive features present were identified by Dr. A.J.K. Millar of the National Herbarium of Australia. Additionally, specimens collected and preserved by McConville (2000), and identified by staff of the National Herbarium of NSW, were used to verify the identifications of Lake Illawarra macroalgae collected during the present study. Recent classifications and changes in taxonomic status of the macrophytes described during the present study were verified using Algaebase (http://www.algaebase.org/, accessed 2004 - 2007).

Photographs were taken with a digital camera, with smaller specimens photographed through the eyepiece of an Olympus biological microscope, containing a 1 mm graticule with 0.01 mm divisions. The digital photographs were then transferred to a computer imaging program (Adobe Photoshop) for editing. Cell dimensions were determined using the program's measuring tool, with the result compared to the scale in each photograph. These measurements were averaged to determine an average cell length, width and length by breadth ratio for each species.

3.2.5 Sediment Sample Preparation

At each site, sediment samples were collected from the top 0 - 10 cm of the rhizoidal zone beneath the seagrass beds at the same time and location as the macrophyte sampling (see Section 3.2.2). This sediment sampling depth was intended to provide an average representation of the potential carbon, nitrogen and phosphorus loads available for uptake by seagrasses and was similar to that used in a number of similar studies comparing sediment-seagrass interactions (see, e.g., Udy and Dennison, 1997; Azzoni *et al.*, 2001). Grain size analysis of sediments was conducted using wet-sieving with deionized water. Sieves with mesh of 2000, 1000, 500, 250 and 63 µm were used, with all size groups oven-dried at 60°C until constant weight was achieved. Percentage content was calculated using dry weights. A portion of each sediment sample collected was wet-sieved with a 500 - 1000 µm sieve to remove debris such as shells and organic material. Where there was a high proportion of fine sediment (usually at Mullet Creek), an additional portion of the original sample was separated into a <63 µm fraction. Samples were dried at 60°C for 1 - 3 days, then ground with a mortar and pestle and stored for later analysis by external laboratories.

3.2.6 External Laboratory Analysis and Quality Control Samples

It was necessary to have samples analysed by different laboratories over the course of this study due to economic and practical reasons, such as equipment malfunctions and subsequent delays in analyses at particular laboratories. The total P contents of macrophyte tissue were analysed by Incitec Ltd (Queensland) in 2000 and 2001, and by Waite Analytical Services (South Australia), for the 2002 and 2003 batches. Sediment total P was analysed by Incitec Ltd. Macrophyte total organic C and δ^{13}C contents were analysed in 2000 by the UOW Geosciences laboratory. Macrophyte tissue and sediment were also analysed for total N, C, δ^{13}C and δ^{15}N contents by CSIRO Land and Water (South Australia) in 2001. The total N, C, δ^{13}C and δ^{15}N contents of macrophyte tissue and sediments (2002 - 2003 batches) were analysed at ISO-Analytical (United Kingdom). Several samples analysed with the earlier 2000-01 batches, including those with dubious results, were also re-analysed by ISO-Analytical. This laboratory included duplicate results for 20 % of the plant tissue and sediment samples submitted for analysis. Where necessary, all samples sent to ISO-Analytical were treated with an HCl solution to remove carbonate carbon prior to analysis (S. Brookes, pers. comm., 2003).

Standard Reference Material (1515 - Apple Leaves, National Institute of Standards and Technology, U.S. Commerce Department) was included with each batch of samples sent to external laboratories. The analytical results obtained for the standard reference material are outlined in Table 3-1. Total N concentrations were within the acceptable range of 2.25 ± 0.19 % N during each batch of analyses. Recovery of total P was slightly below the certified range of 0.159 ± 0.011 % P during the first two sample batches. Sample batches 3 and 4 were analysed by a different laboratory to Batch 1 and 2, and were within the acceptable range for total P. Variances between total C and δ^{13}C concentrations of the Apple Leaves was 4.1 % and 3.6 %, respectively. The δ^{15}N contents of the Apple Leaves analysed during batch 2 (-0.57 ‰) were markedly lower than Batch 4 (0.40 ‰); this may be due to equipment malfunctions affecting δ^{15}N measurements at the first laboratory (CSIRO Land and Water Laboratory, pers. comm., 2001).

Table 3-1: Analytical results for the standard reference material (NIST 1515, Apple Leaves), macrophyte tissue (*Ulva* sp.) and sediment quality control samples included with each external laboratory analysis (values are mean ± s.e.).

Reference Sample	Sample Batch #	Concentration of elements recovered (mean ± s.e.)				
		Total P (%)	Total C (%)	δ^{13}C (‰)	Total N (%)	δ^{15}N (‰)
Apple Leaves	Certified Conc.	0.159 ± 0.011	N/A	N/A	2.25 ± 0.19	N/A
	B1 (2000)	0.14	46.78 ± 1.04	-26.70 ± 0.12	N/A	N/A
	B2 (2001)	0.14	48.30	-25.88	2.19	-0.57 *
	B3 (2002)	0.15	N/A	N/A	N/A	N/A
	B4 (2003)	0.161	46.32	-26.82	2.33	0.40
Plant Tissue *(Ulva*)	B1 (2000)	0.10 ± 0	22.55 ± 1.13	-7.01 ± 0.11	0.85 ± 0.04	5.06 ± 0.07
	B2 (2001)	0.10 ± 0	22.70 ± 0.80	-7.05 ± 0.05	0.81 ± 0.04	4.87 ± 0.30 *
	B3 (2002)	0.08	N/A	N/A	N/A	N/A
	B4 (2003)	0.08 ± 0.002	28.47 ± 0.31	-6.63 ± 0.05	1.22 ± 0.01	7.49 ± 0.06
Sediment #1 (< 63 μm)	B2 (2001)	0.06	2.90	-21.15	0.26	2.64 *
	B4 (2003)	0.06	3.03 ± 0.05	-21.27 ± 0.11	0.30 ± 0	4.07 ± 0.08
Sediment #2 (< 500 μm)	B2 (2001)	0.03	3.30	-24.34	0.20	-0.98 *
	B4 (2003)	0.03	3.07	-24.38	0.21	1.19

* δ^{15}N results from "Batch 2" may be unreliable due to equipment malfunctions at that laboratory.

Internal quality control (QC) samples included with each laboratory analysis included a sample of *Ulva* sp., collected from Primbee Bay, August 2000, and a sediment sample of <63 μm, collected from Mullet Creek, November 2000. Approximately 40 grams of dried material was prepared for the macrophyte and sediment QC samples, with 2 - 4 replicate samples included with each laboratory analysis. Total P concentrations of the *Ulva* sp. QC sample varied from 0.10 % P (Batch 1 & 2) to 0.08 ± 0.002 % P (Batch 3 & 4); this lower result may be due to better accuracy in the later analyses. Total C, total N, δ^{13}C and δ^{15}N contents of the *Ulva* QC sample were all significantly higher ($p < 0.05$) in the Batch 4 analyses (Table 3-1); these results suggest that an incorrect QC sample may have been included with the Batch 4 samples. In addition, paired t-tests conducted on twelve *Zostera* (six leaf and six rhizome) samples analysed by the

two different laboratories (Batch 2 and 4) showed no significant differences ($p > 0.05$) between total C and N contents determined by CSIRO (Batch 2) and ISO-Analytical (Batch 4).

The $\delta^{13}C$ contents of the six *Zostera* rhizome samples were not significantly different ($p > 0.05$) between Batch 2 and 4, but the replicate *Zostera* leaf $\delta^{13}C$ contents analysed in Batch 4 varied significantly from Batch 2 ($T = 6.82$; $p < 0.01$). Likewise, the $\delta^{15}N$ contents of the replicate *Zostera* leaf and rhizome samples measured in Batch 4 were significantly higher than those in Batch 2 ($T = 7.58$; $p < 10^{-4}$). Due to significant differences between analytical results, particularly $\delta^{13}C$ and $\delta^{15}N$ contents, obtained from the different laboratories, the earliest seagrass tissue analyses were disregarded and only relevant C, N, $\delta^{13}C$ and $\delta^{15}N$ data analysed by ISO-Analytical (Batch 4) is discussed in the Results (Section 5.2). However, some C, N, $\delta^{13}C$ and $\delta^{15}N$ data from the Batch 2 analyses is presented in the Results; this data includes about nine macroalgal samples (*Ulva* spp. only, not *Chaetomorpha*) collected during the spring 2000 sampling round at Primbee Bay. For macroalgae species other than *Chaetomorpha,* temporal variations in tissue nutrient data were not discussed due to a lack of macroalgal biomass in later sampling rounds; therefore differences between laboratories is unlikely to impact those results.

Replicate sediment samples (Mullet Creek: < 63 μm and < 500 μm fractions) analysed with Batch 2 and 4 samples are provided in Table 3-1. Total P concentrations, total organic C and $\delta^{13}C$ concentrations showed little variation between the two sampling batches. However, $\delta^{15}N$ contents of sediment samples analysed with Batch 4 were significantly higher ($p < 0.05$) than Batch 2 levels. The Batch 2 analyses included all sediment samples from spring 2000, of which all relevant samples were re-analysed for C, N, $\delta^{13}C$ and $\delta^{15}N$ during the Batch 4 analyses. To maintain consistency in $\delta^{15}N$ contents across sampling rounds, only replicate sediment data obtained during the Batch 4 analyses were used in statistical analyses.

3.2.7 Statistical Analyses

Statistical analyses were conducted using the NCSS (Number Cruncher Statistical Systems) 2004 software. Spatial, temporal and interspecies variations in nutrient concentrations of macrophytes were evaluated using Analysis of Variance (ANOVA) with a 95 % probability level. Where differences between nutrient contents were significant, Tukey-Kramer multiple comparison tests ($\alpha = 0.05$) were used to determine significant differences. Differences between sub-sites (A and B) at each site were assessed with one-way ANOVA; as there were generally no significant differences ($p > 0.05$) between sub-sites, the A and B sub-site data were combined at each site. Comparisons between nutrient concentrations of seagrass leaves and rhizomes (of the same plant) were conducted using paired t-tests with 95 % probability. All sample groups were tested for normality and equality of variance prior to analysis; groups that failed the tests were log-transformed or analysed with non-parametric tests, such as the Kruskal-Wallis ANOVA or the Wilcoxon Signed-Rank test for difference in medians.

3.3 Methodology used for the Experimental Growth in Culture of *Chaetomorpha linum*

The following section describes the methods used for the *C. linum* growth experiments. Some methods were specific to individual experiments and will be described in more detail within those sections. Growth of *C. linum* was determined through changes in biomass; other methods of determining growth, such as measuring surface area or frond length, were considered too time-consuming and impractical for the masses of *C. linum* filaments.

3.3.1 Sample Collection

As various growth experiments were conducted over several months, it was not possible to use the same batch of *C. linum* for all experiments, thus fresh *C. linum* was collected when required from various sites around Lake Illawarra. Using *C. linum* from different locations also helped to encompass the natural variation and genetic diversity of *C. linum* populations found in the lake. The material collected was in a healthy condition and generally free of sediment and epiphytes. Clumps of filaments were rinsed in seawater then stored in aquaria and preconditioned for later use in experiments. Prevailing drought conditions in the summer of 2002-3 resulted in the absence of visible *C. linum* blooms in Lake Illawarra during 2003. Hence, healthy *C. linum* was unobtainable in early 2003 and subsequently the *C. linum* cultured during the pilot study (Dec 2002) was pre-conditioned and used in further experiments between February and May 2003. During mid-2003 small quantities of *C. linum* were collected from any possible site, such as the Windang Peninsula, Primbee Bay, and Gooseberry Island.

Seawater used in the experiments was collected every few days from a nearby beach. Although cleaner water may have been obtained offshore by boat, large quantities were required on a frequent basis, so collection by boat was not a viable option for these experiments. The seawater was passed through a 63 μm sieve to remove particulates on site before being transported to the laboratory in clean 20 L plastic bottles. Attempts were made to 'strip' the seawater of nutrients by leaving 1 - 2 kg of *C. linum* (separate to that used in experiments) in the seawater overnight, prior to filtering through Whatman filter papers.

3.3.2 Preconditioning and Culture Media

Knowledge of the nutritional history of the alga is important when attempting to relate growth rates to substrate concentrations, as algae will use internal storage reserves to grow when external nutrient concentrations are limiting (Harrison, 1986). When studying relationships between nutrients and growth of algae, such as *C. linum*, which have large storage capacities, a lengthy preconditioning period ensures that internal stores are relatively depleted and growth

can be better related to substrate concentration. Prior to use in experiments, *C. linum* was pre-conditioned for several weeks in 6 - 12 L aquaria filled with low-nutrient enriched seawater media (ESM), replaced weekly; it was assumed that internal nutrient stores would be substantially depleted after 4 - 6 weeks (Lavery and McComb, 1991b). Tissue nutrient analyses conducted on 6 *C. linum* samples randomly collected from the pre-conditioning tank returned nitrogen and phosphorus concentrations of 0.90 ± 0.07 % N and 0.04 ± 0.01 % P (dry weight), which were generally below the range of nutrient concentrations in field-collected *C. linum* (see Chapter 5).

ESM, rather than a synthetic seawater medium, was used in all experiments due to ease of preparation and because *Chaetomorpha linum* was reported to grow poorly in artificial media (Lavery and McComb, 1991). The ESM used for pre-conditioning and in all experiments contained trace metals and vitamins (Table 3-3) and was an adaptation of those described in McLachlan (1973) and other authors (Table 3-2). Nitrogen and phosphorus were added separately for each experiment and details are provided in the relevant experimental sections. No nitrogen or phosphorus was added to the ESM during the pre-conditioning phase (approximately 6 weeks), although the seawater would have contained background levels of nutrients (see, e.g., Table 2-3).

Table 3-2: Algal culture media described by previous authors ([a]Lavery and McComb (1991b); [b]Von Stosch (1963), cited in McLachlan 1973; [c]Von Stosch medium without N or P was used by Taylor *et al.* (2001) for *Chaetomorpha linum* culture; [d]Provasoli (1968), cited in McLachlan (1973); [e]Gordon *et al.,* (1981), adapted from Provasoli (1964)).

Medium	***SWM*** [a]	***Grund*** [b]	***ES*** [d]	***ASP$_{12}$*** [e]
Type of media	Enriched seawater	Enriched seawater	Enriched seawater	Artificial seawater
Species studied	*C. linum, Ulva rigida*	Rhodophyceae, *C. linum* [c]	Benthic & unicellular algae	*Cladophora*
Nitrogen	24 μmol N L^{-1} (as $NaNO_3$)	500 μmol N L^{-1} (as $NaNO_3$)	660 μmol N L^{-1} (as $NaNO_3$)	360 μmol N L^{-1} (as NH_4NO_3)
Phosphorus	8 μmol P L^{-1} (as KH_2PO_4)	30 μmol P L^{-1} (as Na_2HPO_4)	25 μmol P L^{-1} (as Na_2.glycero-phosphate)	7 μmol P L^{-1} (as K_2HPO_4)
Thiamine.HCl (μg L^{-1})	100	-	20	500
Biotin (μg L^{-1})	0.5	-	0.8	1.0
Cyanocobalamin (μg L^{-1})	0.5	1.0	1.6	0.2
Sodium silicate (μmol L^{-1})	200	-	-	53
Boron (μmol L^{-1})	43	-	185	185
Zinc (μmol L^{-1})	23	-	0.8	0.8
Manganese (μmol L^{-1})	5.5	0.1	7.3	7.3
Molybdenum (μmol L^{-1})	4.8	-	-	5.3
Cobalt (μmol L^{-1})	0.18	-	0.17	0.2
Copper (μmol L^{-1})	0.19	-	-	-
Iron (μmol L^{-1})	-	2	1.8	2
EDTA (μmol L^{-1})	-	10	26.9	34
Fe.EDTA (μmol L^{-1})	2.0	-	7.2	-

Table 3-3: Enriched seawater medium and preparation of working stock solutions used in preconditioning and culture experiments. Note that nitrogen and phosphorus were added separately for each experiment.

Supplement added to filtered seawater	Concen -tration ($\mu g\ L^{-1}$)	Primary Stock Solution (g added per 50 mL deionised water)	Working Stock Solution (added per 1000 mL deionised water)
Thiamine.HCl (Vitamin B_1)	100	0.5000 g	10 mL
Biotin (Vitamin H)	0.5	0.0025 g	10 mL
Cyanocobalamin (Vitamin B_{12})	0.5	0.0025 g	10 mL
Zn (as $ZnCl_2$)	52	0.5452 g	10 mL
Mn (as $MnCl_2.4H_2O$)	385	6.9267 g	10 mL
Mo (as $Na_2Mo_4.2H_2O$)	480	6.0487 g	10 mL
Co (as $CoCl_2.6H_2O$) (omitted)	12	0.2379 g	10 mL
Cu as ($CuSO_4.5H_2O$)	13	0.2497 g	10 mL
EDTA as ($Na_2EDTA.2H_20$)	584		0.749 g
Fe (as $FeCl_3.6H_2O$)	118		0.547 g

3.3.3 Media Preparation

The ESM was prepared according to the methods of McLachlan (1973) and NIES (2001). As the concentrations of vitamins, trace metals and macronutrients required for the ESM were very low, separate primary stock solutions were first prepared for each element, combined into a single working stock solution and diluted to volume (Table 3-3). The vitamin primary stock solutions were prepared by dissolving small amounts in deionised water, sterilized by filtering through 0.45 μm filter papers and frozen for later use. Separate primary stock solutions of Zn, Mn, Mo, Co and Cu were also prepared by dissolving the salts in the required volume of deionised water (Table 3-3). The working stock solution was prepared by filling a beaker with about 800 mL of deionised water, dissolving the required amount of $Na_2EDTA.2H_20$ while stirring, followed by $FeCl_3.6H_2O$ and 10 mL of each trace metal and vitamin from the primary stock solutions. The solution was then diluted to 1000 mL with de-ionised water, filtered (0.45 μm) and refrigerated. Enriched seawater medium was prepared prior to a water change by adding 1 mL of working stock per litre of filtered seawater and nitrogen and phosphorus at varying levels, depending on the individual experiment.

Although boron (as H_3BO_3) is often added to ESM and synthetic culture media at approximately 0.2 mmol L^{-1} (Table 3-2) it was omitted from these experiments as seawater generally contains about 0.4 mmol B L^{-1} (Table 2-3) and it is considered unnecessary and undesirable to add further boron (McLachlan, 1973). Sodium silicate (Na_2SiO_3) was also omitted from the ESM due to unavailability and whilst silicon is essential for diatom culture, it is probably not necessary for short-term culture of benthic macroalgae (McLachlan, 1973).

3.3.4 Experimental Apparatus

For the small-scale pilot studies, experiments were conducted in ten 850 mL glass jars (filled with 800 mL of ESM), topped with plastic petri-dish lids and placed on an east-facing windowsill that received the morning to midday sun. Jars were positioned randomly and rotated daily to ensure each jar received uniform irradiance. A small aquarium pump (maximum flow 8500 cc s^{-1}) was used to bubble air into the jars and aid water circulation. Plastic airlines were fed through a hole in the lid of each jar and connected to the pump via a gang valve system, adjusted to distribute air evenly through each tube. For all other experiments described below, *C. linum* was cultured in a growth cabinet (Thermoline Illuminated Refrigerated Incubator, model TLMRIL320-1-SD). *C. linum* filaments were placed in 600 mL beakers topped with plastic petri-dish lids and aerated via airline connected to two small aquarium pumps (Appendix 1F). Periodic tests showed that the water within the beakers remained at the designated growth cabinet temperature despite the aquarium bubblers pumping warmer air into the beakers. The beakers were randomly repositioned in the cabinet on a daily basis to ensure uniform exposure to any variation in environmental conditions within the cabinet. Early investigations showed that the position within the growth cabinet did not significantly affect growth rates.

3.3.5 Preparation of Plant Material

To determine changes in growth over extended time periods, it is preferable to use non-destructive sampling techniques, such as measurements of fresh weight, surface area or frond length. Sampling methods which require drying are destructive to the alga and not always suitable for ongoing experiments (Lobban and Harrison, 1997). However, the dry weight may be estimated throughout the experiment by taking subsamples, determining the wet to dry biomass ratio, and extrapolating this to the entire sample. Change in weight (both wet and dry) of *C. linum* was considered the most appropriate measure of growth. Other methods of determining growth, such as measuring the length of filament, or number and lengths of cell, were time-consuming and labour intensive, and thus abandoned.

At the start of each experiment, fresh tissue samples were taken from the pre-conditioning tank, rinsed in seawater, blotted dry with paper towel and weighed (fresh weight) on an analytical balance (4 decimal places); it is not believed that this method damaged the algae or inhibited growth. An initial biomass to volume ratio of 1 g L^{-1} (wet weight) was used in the experiments, following the methods of Lavery and McComb (1991) and based on the results of the pilot study (Section 6.2.1). Sub-samples of the algal tissue were taken to determine dry weights and wet to dry biomass ratios at the start and at the end of each experiment. Dry weights were determined after drying at 60°C to constant weight (usually 24 hours). After one week, the entire algal biomass was removed from the beakers, blotted dry and quickly wet weighed. The algal tissue was then cut-back to the original biomass (~1 g L^{-1}) after weekly weighing, to maintain a reasonably high water to algal biomass ratio. The harvested portion of algal tissue was wet

weighed and dried to constant weight, then the calculated wet to dry biomass ratios of the harvested portion were used to estimate the wet and dry weights of each algal sample.

3.3.6 Experimental Design

Pilot Studies

The aim of the first pilot study was to determine whether *C. linum* could be grown successfully under controlled laboratory conditions. Two different mass to volume ratios were used in the pilot study to determine how the ratio affected growth rates and which starting mass should be used in future experiments. One gram of algae (fresh weight) per litre was used in 5/10 jars and a larger mass of 2 g L^{-1} was used for the remaining 5 jars to compare growth rates resulting from different initial biomasses. Based on the findings of Taylor *et al.* (2001) and Lavery and McComb (1991), the seawater used in the pilot study was supplemented with non-limiting concentrations of 30 μmol L^{-1} PO_4^3-P and 70 μmol L^{-1} of NH_4NO_3, as both nitrate and ammonia are present in Lake Illawarra. The culture medium was replaced every three days after the algae had been removed from the jars, blotted dry, weighed and returned to the jars with fresh ESM. The plants were kept in enriched media for the first 21 days of the experiment and then placed in unenriched seawater and allowed to grow undisturbed for a further 28 days.

A second windowsill pilot study was conducted to examine the effects of different nitrogen species on *C. linum* growth. Culture media described in previous studies have contained $NaNO_3$, NH_4Cl or NH_4NO_3 as the source of nitrogen (Table 3-2), but *C. linum* grown in laboratory culture has been shown to have significantly higher growth rates under NH_4^+ than NO_3^- (Taylor *et al.*, 2001). In order to simplify the experiments and decide which nitrogen species (NO_3^-, NH_4^+ or both) would be used in further culture studies a small-scale experiment was conducted to determine whether growth rates of Lake Illawarra *C. linum* would differ under ammonium-N or nitrate-N. The nitrate v ammonium pilot study was conducted for 28 days using the methods described above and the ESM described in Section 3.3.2, replaced daily. Phosphorus was added at non-limiting concentrations of 30 μmol L^{-1} (3.8 mg L^{-1} of KH_2PO_4). Nitrogen was added at 100 μmol N L^{-1} d^{-1} (1.4 mg N L^{-1} d^{-1}) in both treatments; 5 out of the 10 treatments received 2.7 mg L^{-1} d^{-1} of NH_4Cl and the remaining 5 treatments received 8.5 mg L^{-1} d^{-1} of $NaNO_3$. The experiment began with an initial *C. linum* mass to volume ratio of 1 g L^{-1} and growth was allowed to continue uninterrupted throughout the experiment. The algae was removed from the jars daily, blotted dry, weighed and returned to the jars with fresh ESM. Dry weights were determined at the end of the experiment.

Nitrogen, Phosphorus and Temperature Experiments

A range of experiments were conducted with *Chaetomorpha linum* cultured under various temperatures and concentrations of orthophosphate, nitrate or ammonium, but with all other culture parameters at non-limiting levels. The aim of these experiments was to determine the response of *C. linum* to varying temperatures, P and N concentrations, and to compare Lake Illawarra *C. linum* culture experiments to those published by other authors (e.g., Lavery and McComb, 1991b; Taylor *et al.*, 2001). The experimental parameters were chosen to allow the generation of growth curves and to encompass average water temperatures and nutrient conditions in Lake Illawarra, particularly the high nitrogen and phosphorus concentrations that may occur after wet weather events (see, e.g., Table 1-2), thus triggering algal blooms.

Previous algal culture studies have demonstrated that less than 3 weeks incubation is often insufficient to establish significant differences in growth rates between nutrient treatments (e.g., Lavery & McComb, 1991b; Morgan and Simpson, 1981a). However, space restrictions and time constraints during the present study meant that it was not possible to run individual experiments for more than 3 weeks so shorter incubation periods were chosen. Each experiment was conducted within the growth cabinet for 1 - 2 weeks with light at 100 % saturation and 12 hour day-night periods. Six different treatments were used with 3 replicates per treatment and each experiment was replicated twice at different times. Although the inclusion of more replicates was desirable, lack of space in the culture chamber also meant that only a limited number of culture vessels could be used in the chamber during any one experiment. The ESM was changed daily in early experiments, although this had the undesirable affect of adding further trace levels of nutrients to the solutions and promoting excessive growth in the control and low nutrient treatments. Hence it was decided to replace the ESM weekly in later experiments and to add the required nutrients daily. Each 500 mL beaker contained approximately 0.5 g of *C. linum* (pre-conditioned, as described in Section 3.3.2) in 500 mL of ESM. Fresh weights were determined on the first, seventh and fourteenth day of each experiment and sub-samples were taken to estimate dry weights. To achieve steady state growth conditions in culture experiments, a relatively constant biomass should be maintained through periodic harvesting or by increasing the nutrient concentrations in proportion to the increasing biomass (DeBoer, 1981). Therefore, after one week, the algae were removed from the jars, blotted dry, weighed, harvested back to 0.5 g per 500 mL and returned to the jars in fresh ESM for a further week in the incubation cabinet.

Phosphorus experiments were conducted at 15, 20 and 25°C with ESM containing 50 μM NH_4NO_3 and phosphate-P at concentrations of 0, 10, 20, 30, 40, 80 and 100 μM. The ESM used in the nitrogen experiments contained 20 μmol KH_2PO_4 and nitrogen at concentrations of 0, 10, 30, 50, 80 and 100 μmol N L^{-1} as either NO_3^- or NH_4^+.

Temperature experiments were conducted for 2 weeks each at 10, 15, 20, 25 and 30°C, using a range of nutrient treatments:

- No added nutrients
- 10 μM P without added N
- 50 μM NH_4NO_3 without added P
- 50 μM NH_4NO_3 and 10 μM P
- 100 μM NH_4Cl and 10 μM P
- 100 μM $NaNO_3$ and 10 μM P

Using temperatures above 30°C was not considered necessary as previous studies (e.g., King, 1990) have determined that survival rates of *C. linum* cultured at 35°C were poor.

3.3.7 Calculations and Statistics

The relative growth rate, RGR (% increase per day), of C*. linum* was determined from wet and dry weight measurements at the start and end of each 7-day experimental period:

$$\mathrm{RGR} = \frac{100 \ln\left(B_2 / B_1\right)}{t_2 - t_1}$$

where B_2 and B_1 are the biomass (wet or dry weight) at times t_2 and t_1, respectively. Comparisons were made between each 7 day period (days 0-7 and days 7-14), when experiments were continued for 2 weeks. A cumulative growth rate (cRGR) was then calculated for days 0-14:

$$\mathrm{cRGR} = \frac{\ln\left(B_m / \mathrm{B_i}\right) + \ln\left(B_f / B_{mr}\right)}{t_f - t_i}$$

where B_i is the initial biomass, B_m is the biomass at the middle of the experiment (usually day 7), B_{mr} is the biomass returned to the beakers after harvesting on day 7, B_f is the final biomass (usually day 14) and t_i and t_f are the final and initial times, respectively. The yield, y (g L^{-1} d^{-1}), was also determined by dividing the daily wet and dry weight biomass change by the volume, V, of culture media used:

$$y = \frac{\left(B_2 - B_1\right)/V}{t_2 - t_1}$$

When the growth of an alga is exponential, the time taken for the algal biomass to double, t_D, can be determined by dividing the natural log of the number of generations (2, in this case) by the growth rate, μ (Valiela, 1984):

$$t_D = \frac{\ln(2)}{\mu} = \frac{t_2 - t_1}{\ln(2) \cdot \ln\left(B_2 / B_1\right)}$$

The doubling time assumes a constant growth rate and non-limiting conditions. It was used in this context to demonstrate the time taken for the algal biomass to double, given the growth rates calculated for each treatment over a 14 day period.

Statistical analyses were conducted using the NCSS (Number Cruncher Statistical Systems) 2004 and JMP 4.0 software packages. Temporal variations in growth rates (RGR) of the macroalgae were evaluated using Analysis of Variance (ANOVA), with a 95 % probability level. Repeated Measures (RM) ANOVAs were conducted when comparing measurements taken on the same plants, such as growth rates in Week 1 (0 - 7 days) compared to Week 2 (7 - 14 days) of treatment. However, significant ($p < 0.05$) interactions often occurred between growth rates in Week 1 and Week 2, making interpretation of the results difficult. For example, individual plants which had high growth rates in the first week of treatment, often had low growth rates in the second week of treatment, and vice versa. In addition, growth rates in the first week of the experiments were usually high in all treatments, regardless of enrichment; growth rates in the low nutrient or control treatments, however, usually declined significantly by the second week, presumably due to low nutrient concentrations restricting the formation of new biomass. Thus, few significant differences in growth rates occurred between treatments in Week 1, whereas Week 2 showed a greater differentiation between treatments. Therefore, one-way ANOVAs were conducted to compare growth rates between treatments in the second week of culture (7 - 14 days), as well as the cumulative growth rate (0 - 14 days). Where differences between growth rates were significant, Tukey-Kramer multiple comparison tests ($\alpha = 0.05$) were used to determine significant differences. All sample groups were tested for normality and equality of variance prior to analysis; groups that failed the tests were log-transformed or analysed with non-parametric tests, such as the Kruskal-Wallis ANOVA.

CHAPTER 4 Description and Ecology of Shallow Benthic Macrophytes in Lake Illawarra, New South Wales

4.1 Introduction

This chapter describes the distribution, ecology and identification of macrophytes (seagrasses and macroalgae) found in Lake Illawarra, between winter 2000 and winter 2003. Past research regarding the distribution and abundance of macrophytes in Lake Illawarra is also reviewed here. In Lake Illawarra, the shallowest areas of less than 2 m water depth are densely populated with aquatic macrophytes. These include the seagrasses (*Zostera capricorni*, *Ruppia megacarpa*, *Halophila ovalis* and *Halophila decipiens*) and a variety of green, brown and red macroalgae. This study deals only with seagrasses and the macroscopic forms of algae (i.e., those which can be readily observed with the naked eye); no attempts were made to examine the planktonic forms of algae, of which more than 100 species have been recorded from Lake Illawarra (King *et al.*, 1997). This study presents the only known compilation of all relevant material for macrophytes currently found in Lake Illawarra and is a key resource for future researchers and Lake managers.

4.2 Literature Review of Macrophyte Distribution and Biomass in Lake Illawarra

The following section reviews the historical records of seagrass and macroalgal distribution in Lake Illawarra; previous studies on Lake Illawarra macrophytes are compared to the distribution of macrophytes found in the present study, conducted between winter 2000 and winter 2003. Refer to Chapter 5 for a detailed analysis of the biomass of seagrasses and macroalgae found in Lake Illawarra during the present study.

4.2.1 Seagrass Distribution

Seagrasses cover extensive areas of Lake Illawarra, from the water's edge to a depth of about 2.2 m. In Lake Illawarra, there are four species of seagrass: *Zostera capricorni*, *Halophila ovalis, Halophila decipiens* and *Ruppia megacarpa*. *Zostera capricorni* is the dominant seagrass, occupying 72 % of the total area of seagrass beds in 2000 (WBM, 2000). Although *Ruppia* is not technically considered a seagrass (see Section 4.4), *Ruppia* is included with the seagrasses for the purposes of this study. *Ruppia megacarpa* usually grows inshore of the *Zostera* beds, predominately in shallow water (< 0.6 m) along the Windang Peninsula (Figure 4-1). *Halophila ovalis* and *H. decipiens* tend to grow in the deepest water (> 1.8 m) beyond the *Zostera* beds. In

addition, small *Halophila* beds were occasionally observed in very shallow water (< 0.5 m) along the northern and eastern shores of Lake Illawarra during the present study. Being the dominant macrophytes in Lake Illawarra, seagrasses have been studied in much greater detail than macroalgae. Studies on macroalgae in Lake Illawarra are limited in frequency and scope, and have occasionally been included as a component of the seagrass studies. The following sections provide an overview of previous studies relating to macrophytes in Lake Illawarra.

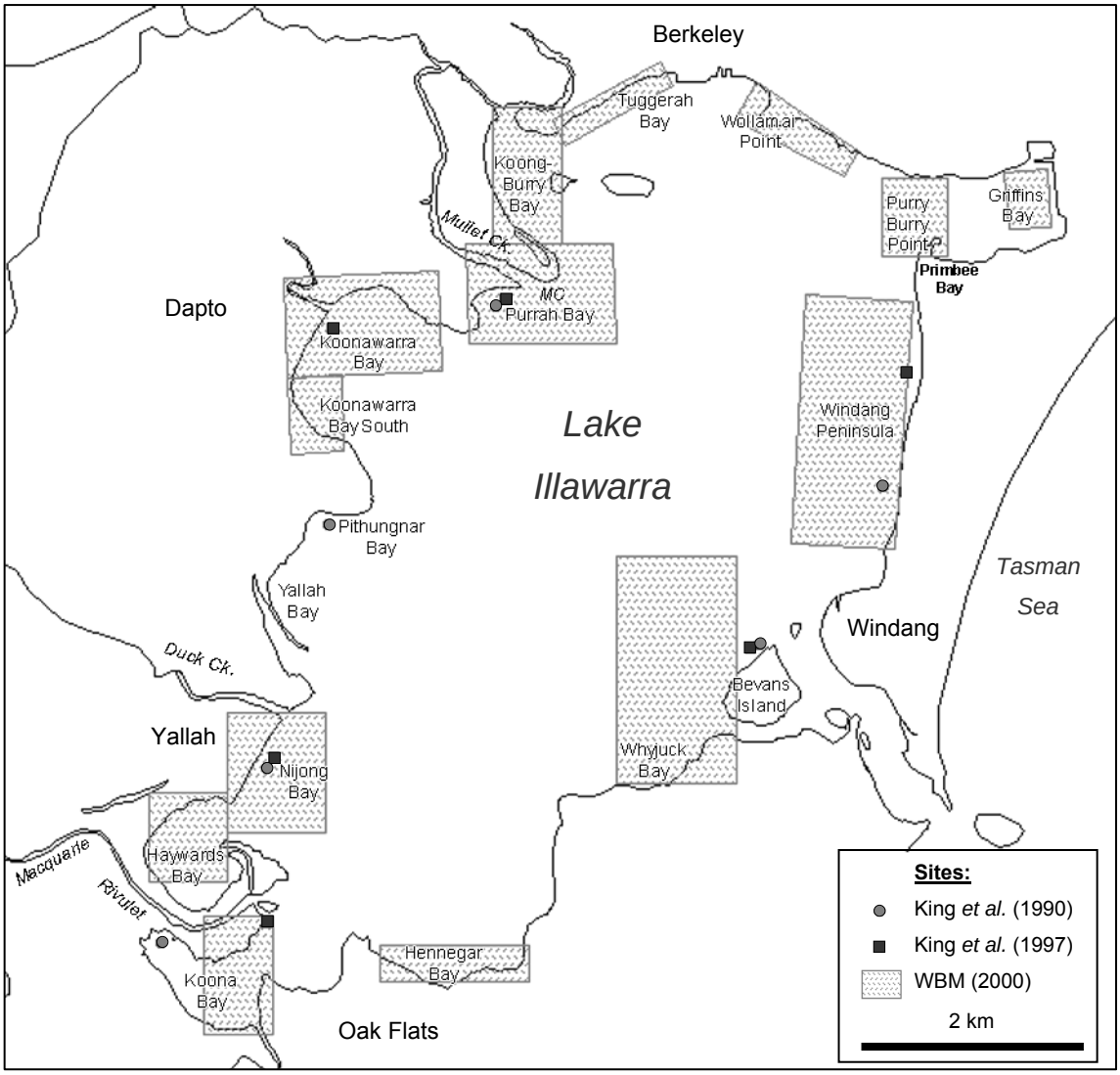


Figure 4-1: Sites used by King *et al.* (1990), King *et al.* (1997) and WBM (2000) to determine seagrass distribution and biomass.

Surveys of the Lake Illawarra seagrass beds have been conducted periodically by Harris (1977), Yassini (1985), West *et al.* (1985), King (1988), King *et al.* (1990), King *et al.* (1997), WBM (1993, 1996b, 1998, 2000), White (2003) and Tadkaew (2007). These surveys have differed greatly in assessment technique, as well as the scale of the studies and the individual sites surveyed by different authors (Figure 4-1). Also, improvements in technology (e.g., Global Positioning and Geographic Information Systems) are likely to have resulted in improved accuracy. Thus, the area of seagrass beds reported from Lake Illawarra is subject to variation,

due to differing methodologies between the studies, as well as variations due to short- and long-term natural fluctuations (WBM, 1993). Additionally, the results of seagrass surveys are strongly influenced by the season in which they are conducted as the *Zostera* beds typically decline during winter (King, 1988). However, past surveys have been conducted during different seasons; for example, the surveys by WBM (1993, 1995) were conducted in spring, King *et al.* (1990) and WBM (1998) in autumn, WBM (2000) in summer, and White (2003) during winter. Thus seasonal differences must be taken into account when comparing results between surveys. There have also been differences in reporting the species of seagrass mapped (WBM, 2000), as some past studies neglected to include the coverage of *Ruppia* and *Halophila.* Table 4-1 lists the estimated areas of seagrass beds in Lake Illawarra, between 1976 and 2003.

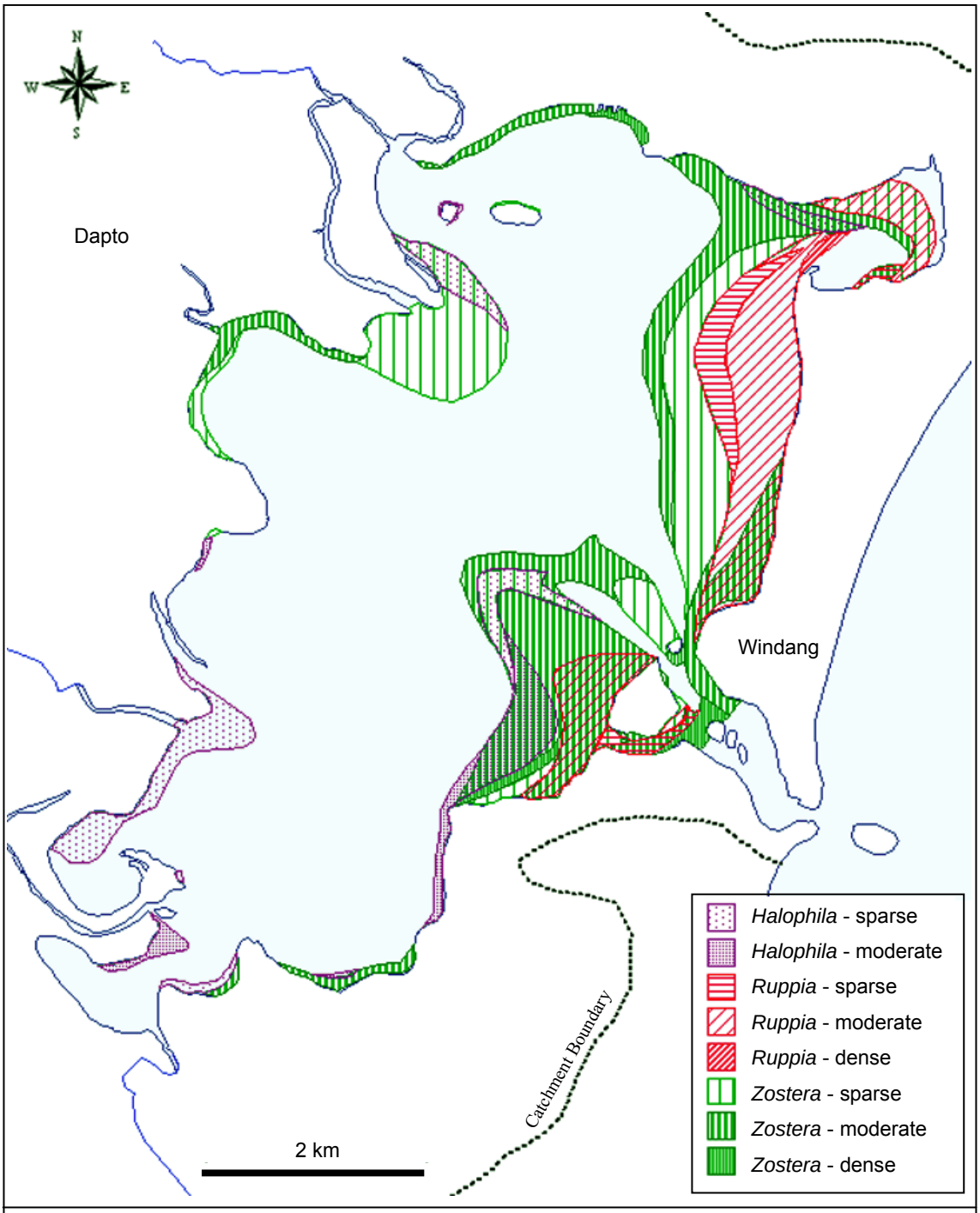
Table 4-1: Total area of seagrass beds in Lake Illawarra, 1976 - 2003.

Year of	Area of seagrass beds in Lake Illawarra (km^2)				Reference
Survey	*Zostera*	*Ruppia*	*Halophila*	Total	
1976	-	-	-	7.96	Harris (1976)*
1981	-	-	-	8.96	Evans & Gibb (1981)*
1985	-	-	-	5.12	West *et al.* (1985)
1987	8.11	3.29	1.89	10.70	King (1988)
1997	6.3	2.8 $	0.1 $	~10.0 $	King *et al.* (1997)
2000	5.65	1.67	0.54	7.86	WBM (2000)
2003	1.41	0.48	-	1.89	White (2003)

* Data cited in King (1988); $ Estimated using maps provided in King *et al.* (1997).

The distribution of seagrasses mapped by King (1988), WBM (2000) and White (2003) are shown in Figure 4-2, Figure 4-3 and Figure 4-4, respectively. The most extensive seagrass beds are found along the eastern shoreline of the Lake, from Griffins Bay to west of Bevans Island, and extend up to 1.8 km from the shoreline (King *et al.*, 1997). These eastern seagrass beds are typically composed of *Ruppia* in water shallower than 0.6 m, with a transition zone of *Ruppia* growing in association with *Zostera*, followed by *Zostera* growing beyond the *Ruppia* beds to a depth of approximately 2 m. *Zostera* also grows in narrower beds around much of the Lake shoreline, often in association with macroalgae, such as *Gracilaria* sp. *Ruppia* is largely confined to the Windang Peninsula and Purrah Bay (Figure 4-3), possibly because other areas of the Lake are unsuitable for colonisation (see Section 4.2.2). Coverage of *Halophila* in Lake Illawarra appears to be highly variable, and it is usually not found in winter. King (1988) estimated the area of *Halophila* beds at 1.89 km^2, whereas *Halophila* was barely present in 1997, and had increased to 0.54 km^2 in 2000 (Table 4-1). WBM (2000) mapped beds of *Halophila* growing in deep water beyond the *Zostera* beds in Whyjuck Bay, Koona Bay, Nijong Bay and Yallah Bay (Figure 4-3). During the present study, *Halophila* was also found growing in water less than 0.5 m deep, in association with *Zostera*, and in isolated patches near Purry Burry Point, Tuggerah Bay and Yallah Bay.

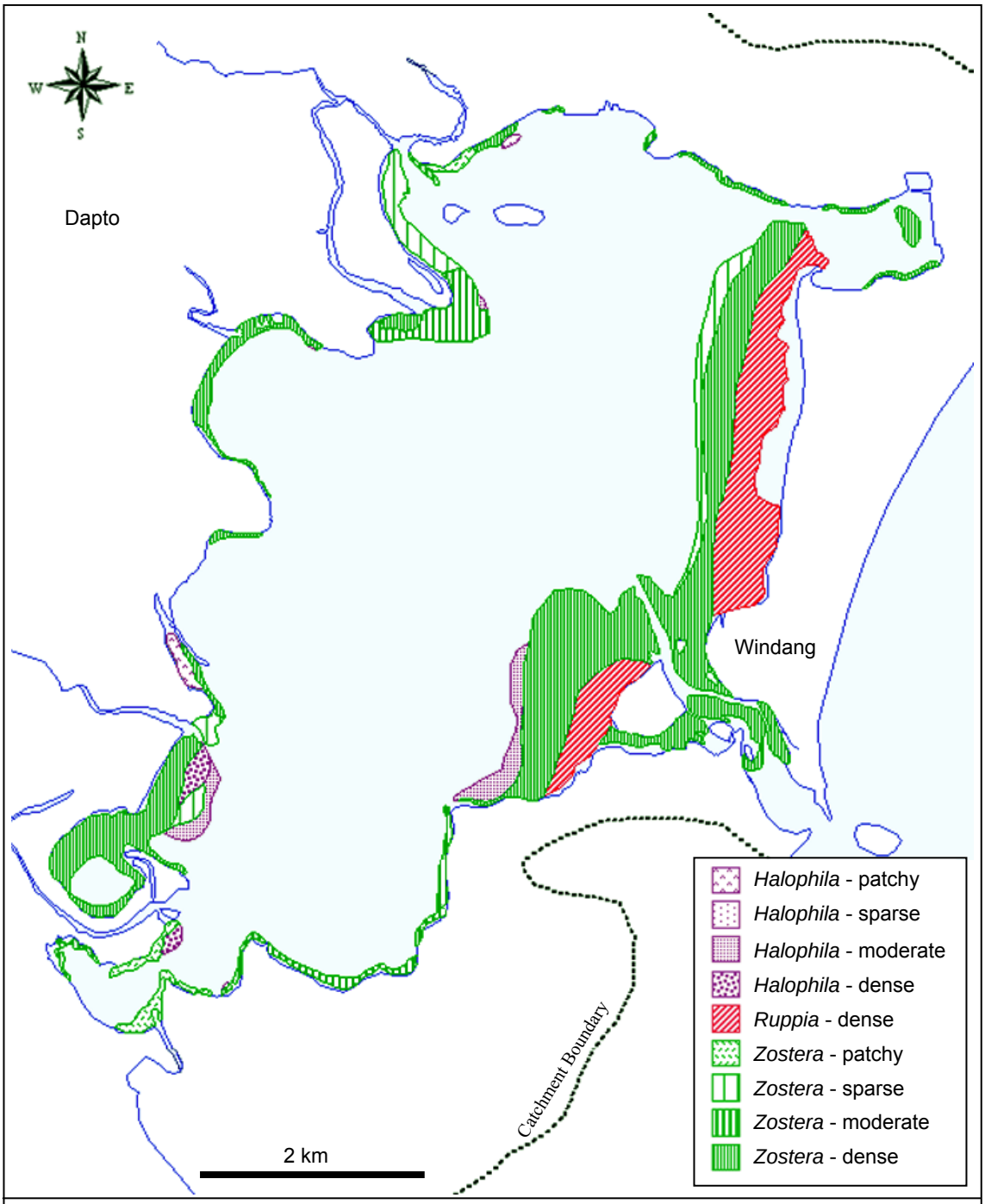
Seagrass areas in Lake Illawarra appear to have declined slightly since 1987, when King (1988) estimated coverage to be 10.7 km^2 of the Lake surface area (Figure 4-2; Table 4-1). Additionally, Figure 4-2 shows significant areas of *Zostera* and *Ruppia* in Griffins Bay, which were later lost due to dredging between 1989 to 1991 (WBM, 1993). In January 2000, seagrasses covered 7.86 km^2, which accounted for 22 % of the total Lake surface area (WBM, 2000; Figure 4-3), but the total area of seagrass beds is subject to variation (Table 4-1). The most notable difference in the area of Lake Illawarra seagrass beds was an approximate decline in area of more than 75 % between 2000 and 2003 (Table 4-1). White (2003) mapped the distribution of seagrass (mainly *Zostera*) in Lake Illawarra during winter and spring 2003, under combined conditions of drought and winter die-back of *Zostera*. She noted extensive die-back of *Zostera* and *Ruppia* occurred when the Lake water level dropped by approximately 0.5 m during the drought, exposing much of the *Zostera* flats along the Windang Peninsula, the Entrance channel, around Bevans Island and in Whyjuck Bay. Furthermore, White (2003) estimated that during winter 2003, dead, bleached or exposed seagrass areas amounted to 255.8 ha of the Lake surface area (Figure 4-4). By early 2004, however, many of the exposed areas of seagrass had regrown (Dr. R. West, University of Wollongong, pers. comm., 2004).



Legend: seagrass distribution categories used by King (1988):

- *sparse* growth: up to 15 % cover;
- *moderate* growth: 15 - 50 % cover; and,
- *dense* growth: greater than 50 % cover.

Figure 4-2: Seagrass distribution in Lake Illawarra, March 1987 (adapted from King, 1988).



Legend: Seagrass distribution categories used by WBM (2000):

- *patchy* growth: small areas of seagrass occurring within large areas of bare substrate;
- *sparse* growth: less than 1000 shoots m^{-2};
- *moderate* growth: 1000 - 1700 shoots m^{-2}; and,
- *dense* growth: more than 1700 shoots m^{-2}.

Figure 4-3: Seagrass distribution in Lake Illawarra, January 2000 (adapted from WBM, 2000).

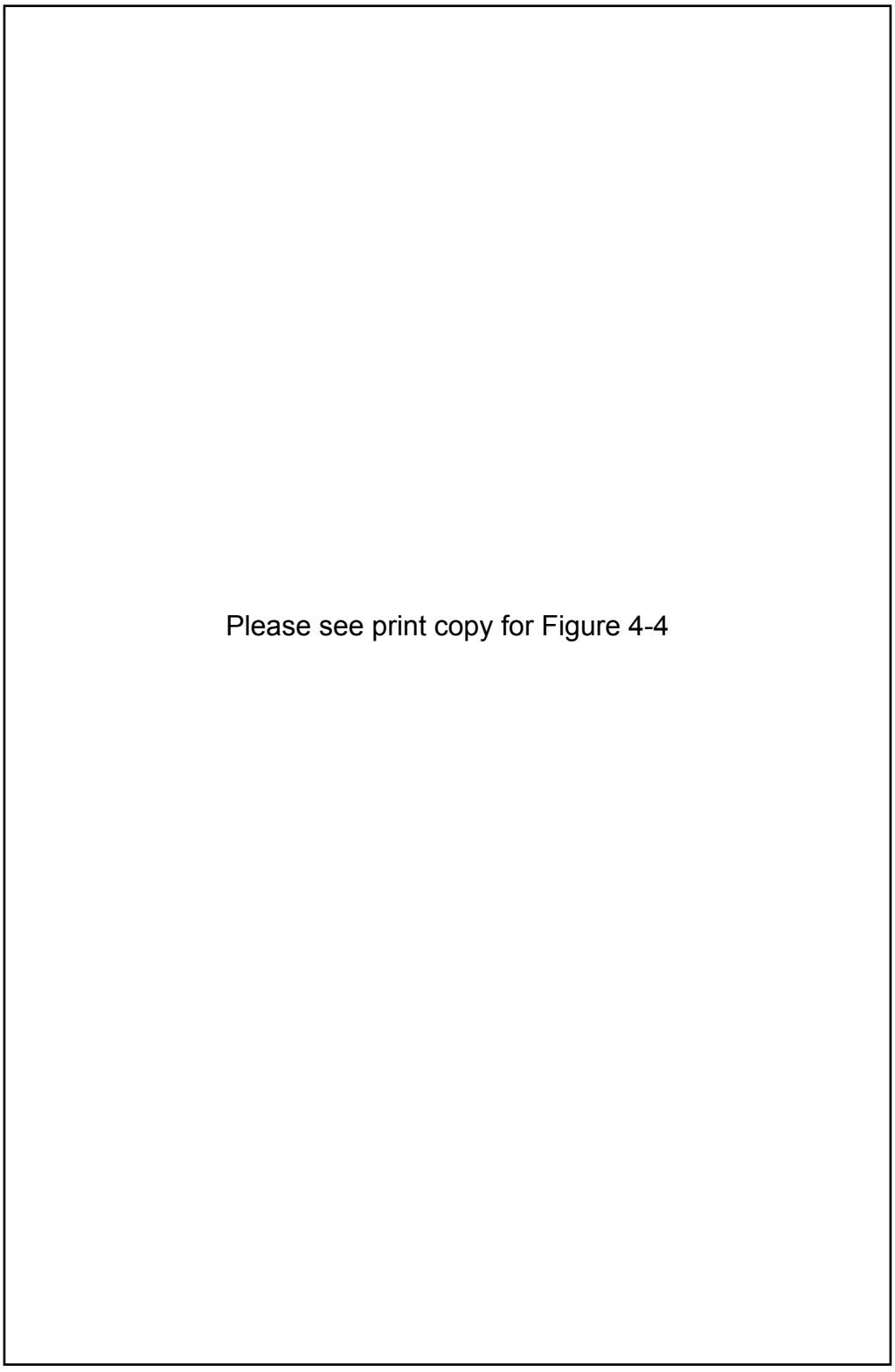


Figure 4-4: Seagrass distribution in Lake Illawarra, May - June 2003 (after White, 2003).

4.2.2 Factors Limiting Seagrass Distribution in Lake Illawarra

Harris (1977) concluded that the distribution of *Zostera* in Lake Illawarra was not limited by sediment-type, salinity, water temperature, or phosphorus deficiency. For example, *Zostera* in Lake Illawarra grew on a wide range of sediment types, with sand content ranging from 14 - 98 %, organic carbon from 0.5 - 12 %, and total phosphorus from 35 - 120 μg P g^{-1}. Likewise, *Zostera* persisted in salinities ranging from 3 ppt in Hooka Creek, to 35 ppt in the Lake's entrance channel, and water temperatures ranging from 11 - 35°C. While average nitrate concentrations were typically lower near dense *Zostera* beds than in the Lake proper, Harris (1977) concluded that nitrate or phosphate concentrations did not limit the distribution of *Zostera*. However, seagrass growth may have been inhibited in some areas close to urban drains where high nutrient concentrations resulted in the excessive growth of macroalgae, which shaded the *Zostera* (Harris *et al.*, 1980). The most important environmental factors limiting the growth of *Zostera* in Lake Illawarra appeared to be water depth and turbidity and, therefore, light availability (Harris, 1977). For example, in the less turbid areas of the Lake's entrance channel, Harris (1977) reported that the depth limit of *Zostera* growth was 2 m, whereas in more turbid areas, such as Griffins Bay and the mouth of Mullet Creek, *Zostera* was not found beyond 1 m depth. WBM (2000) also reported that *Zostera* did not extend beyond 1.9 m depth, whereas *Halophila ovalis* was recorded in depths of up to 2.2 m.

Dredging can also have significant short- and long-term impacts on seagrass distribution, through direct removal and alteration of seagrass habitat. Seagrass beds are likely to recover from dredging in the long-term, provided conditions, such as light penetration and substrate type, are suitable for re-colonisation (WBM, 1996a). For example, between 1988 and 1989, 5.7 ha of seagrasses were removed during dredging in Koonawarra Bay, mostly in shallow subtidal areas. In WBM's Koonawarra Bay study area (Figure 4-1), the area recolonised by *Zostera* covered 7.5 ha prior to dredging, but had increased to 14.5 ha five years after dredging, probably because the water depth (0.6 m) and the sandy substrate which remained after dredging were suitable for *Zostera* re-colonisation (WBM, 1993). However, dredging was also undertaken to a depth of 1.5 m in Griffins Bay between 1989 and 1991 (WBM, 1993), but *Zostera* beds had still not recovered in those areas by 1998, probably due to insufficient light availability (WBM, 1998).

Swans can cause significant, and often permanent, damage to *Zostera* beds by digging up and eating the young shoots and rhizomes (Wood, 1959). Additionally, removing the seagrass rhizomes destabilizes the sediment, so that it may be disturbed by water motion and winds, thus becoming oxidised and unsuitable for re-colonisation by *Zostera* (Wood, 1959). Harris *et al.* (1980) found that, along the eastern shore of Lake Illawarra, *Zostera* growth was sparse in water shallower than 40 cm deep due to intensive grazing on *Zostera* shoots and rhizomes by ducks and swans.

4.2.3 Seagrass Biomass

Previous studies have estimated the total biomass of *Zostera* in Lake Illawarra according to the total area of seagrass beds mapped, and the average biomass at particular sampling sites. WBM (2000) estimated the above-ground dry weight biomass of *Zostera* in Lake Illawarra to be approximately 439 tonnes, whereas King *et al.* (1997) estimated 794 tonnes of total *Zostera* dry weight biomass, including 324 tonnes of living leaf biomass. The average biomasses of *Zostera* documented by previous authors between 1988 and 2003 are listed in Table 4-2 (total biomass) and Table 4-3 (living leaf biomass only). Differences between the areas mapped, as well as sampling techniques (e.g., measurement of wet vs. dry weight biomass or living leaf vs. total biomass) make direct comparisons between the seagrass surveys somewhat difficult. Note that the sites used by WBM (1996, 1998, 2000) were somewhat different to those of King *et al.* (1990) and King *et al.* (1997) (Figure 4-1), making direct comparisons between sites difficult. Seagrass growth rates are typically higher in summer than in winter, when die-back often occurs (WBM, 2000), thus comparisons between sampling years is complicated by the surveys being conducted during different seasons.

4.2.4 Seagrass Condition

Previous seagrass surveys, such as those of WBM (1998, 2000), have found highly significant variability in the condition of *Zostera* (determined by leaf density, numbers and lengths) across Lake Illawarra (Table 4-4). The number of leaves per shoot appears to be fairly consistent across sites and between various years, whereas leaf lengths vary significantly (Table 4-4). Leaf length is expected to vary due to the continual cycle of shedding and regrowth, although some variation may be due to differences in water quality between sites (WBM, 2000). Much of the variability in seagrass condition across Lake Illawarra may be explained, in part, by environmental factors, such as substrate type, water currents, wave and wind exposure, nutrient concentrations and water temperature (WBM, 1993). For example, seagrasses are likely to have shorter leaf lengths in areas of high wave action than in sheltered environments (WBM, 2000). Likewise, higher seagrass densities at Purry Burry Point and Griffins Bay may be due to these sites having a higher sand content than sites on the western side of the Lake (WBM, 1993). Similarly, White (2003) observed that significant wind events (up to 64 km hr^{-1}) in September 2003 resulted in the total loss of above-ground biomass of *Zostera* from her Griffins Bay sampling site.

Table 4-2: Total biomass of seagrass (leaves, roots, rhizomes and detritus) recorded by King *et al.* (1990: wet weight) and King *et al.* (1997: dry weight).

Site	Wet weight (g WW m^{-2})			Dry weight (g DW m^{-2}) *	
	1988-89 (summer)	1989 (autumn - winter)	1989-90 (spring - summer)	1996-97 (spring - summer)	1997 (autumn)
Windang Peninsula	1426	1365	3076	146.18	192.10
Purrah Bay / Mullet Ck.	3276	1613	3354	131.08	65.67
Koonawarra Bay	-	-	-	127.10	99.04
Pithungnar Bay	1144	951	1421	-	-
Nijong Bay / Duck Ck.	1566	842	1398	98.91	185.82
Koona Bay	16	4	55	155.52	151.19
Bevans Island	2357	1270	2315	121.05	88.68
Reference	King *et al.* (1990)			King *et al.* (1997)	

***** The average wet to dry ratio of *Zostera* (total biomass) in King *et al.* (1997) was 7.3:1.

Table 4-3: Biomass of *Zostera* leaves recorded by WBM (1996b, 1998, 2000), White (2003) and the present study (refer to Chapter 5). Values are mean ± s.e., where given.

Site	Biomass of *Zostera* leaves (g DW m^{-2})				
	1995 (spring)	1998 (autumn)	2000 (summer)	2000 - 2002 (winter - summer)	2003 (winter)
Entrance	-	-	134.30	-	-
Windang Peninsula	-	-	115.70	-	16.87
Purry Burry Point	15.65	44.83	91.72	83.48 ± 9.7	-
Griffins Bay	17.48	55.52	77.44	-	-
Wollamai Point	-	-	99.38	-	-
Tuggerah Bay	12.83	4.37	39.14	-	-
Koong Burry Bay	-	-	6.03	-	-
Mullet Creek	16.01	53.70	20.38	99.25 ± 9.8	-
Koonawarra Bay	25.97	59.85	54.19	-	1.85
Koonawarra Bay South	25.24	15.54	165.95	-	-
Duck Creek	37.02	120.44	96.58	-	-
Haywards Bay	-	-	51.68	-	0
Koona Bay	-	-	38.80	-	-
Hennegar Bay	-	-	104.34	-	-
Whyjuck Bay	-	-	70.52	-	-
Reference	WBM (1996b)	WBM (1998)	WBM (2000)	Present Study	White (2003)

Table 4-4: Summary of *Zostera* condition data at selected sites in Lake Illawarra (after WBM, 1993, 1996b, 1998, 2000; White, 2003). Values are mean (standard error not provided).

Please see print copy for Table 4-4

4.2.5 Distribution of Macroalgae in Lake Illawarra

Macroalgal blooms in Lake Illawarra typically occur in shallow water of less than 0.6 m depth. Over the past few decades, problem areas for macroalgal blooms have been Griffins Bay, Koona Bay, Koong-Burry Bay, the Windang Peninsula and around Bevans Island (Yassini, 1985; King *et al.*, 1997; Figure 4-5). Macroalgae tend to have a strong association with seagrass beds, as the leaves provide an anchoring point for epiphytes, as well as free-floating plants which become tangled within the beds. King *et al.* (1990) observed that green macroalgae appeared to have a more permanent association with *Ruppia* than *Zostera*, as the green algae often bloomed over *Zostera* then disappeared, possibly because *Zostera* sheds its leaves periodically.

Previous studies on macroalgae in Lake Illawarra determined that the dominant genera were *Chaetomorpha*, *Enteromorpha*, *Ulva*, *Rhizoclonium*, *Gracilaria* and *Hypnea* (Yassini, 1985; King *et al.*, 1990; King *et al.*, 1997). During the present study, the dominant algal genus in Lake Illawarra was *Chaetomorpha* (identified as *C. linum* and *C. billardierii)*, which was widely

distributed in shallow, sheltered bays, with large masses often found entangled amongst seagrass beds. Other green algae, such as *Ulva* spp., *Cladophora* sp., *Rhizoclonium riparium* and *Codium* spp., were found frequently in the Lake, particularly along rocky shorelines (e.g., Yallah Bay and Tuggerah Bay), but biomasses were low compared to those of *Chaetomorpha* spp. The red algae, *Gracilaria* sp. and *Hypnea* sp., were also very common in Lake Illawarra, typically found growing attached to rocks along most of the Lake shoreline, and also growing amongst *Zostera* and *Ruppia* beds. The rhodophytes, *Polysiphonia* sp. and *Chondria* sp., were the most abundant algal epiphytes during the present study. The ecological characteristics of each alga are discussed further in Sections 4.3 - 4.7.

4.2.6 Macroalgal Biomass - Historical Overview

Macroalgal biomasses in Lake Illawarra appear to have peaked during the mid 1980s and early 1990s. Blooms typically occurred in winter, peaking in spring to early summer, and declining during autumn. Yassini (1985) estimated that macroalgal blooms, occurring mostly along the Windang Peninsula, covered 1.07 km^2 in spring 1984, with an estimated total biomass of 38,000 tonnes (wet weight), whilst the winter 1985 bloom covered 1.74 km^2, with an estimated total biomass of 71,000 tonnes (wet weight). The highest average biomasses documented by Yassini and Clarke (1986) were just over 700 g DW m^{-2} (Figure 4-6), with an average wet to dry ratio of 9:1. The maximum average macroalgal biomasses recorded by King *et al.* (1990), between 1988 - 1990, were less than 2 kg WW m^{-2} (approximately 220 g DW m^{-2}), with the highest biomasses occurring in Koona Bay, Pithungnar Bay, and along the Windang Peninsula (Table 4-5; Figure 4-5). Macroalgal biomasses were considerably lower in the 1990s, with King *et al.* (1997) recording a maximum biomass of 207 g DW m^{-2} in Koona Bay, but with biomasses at most other sites during that period being less than 70 g DW m^{-2}.

The excessive macroalgal blooms which occurred during the 1980s were attributed primarily to stormwater runoff, as well as leakages and wet weather overflow from the sewerage system (Yassini and Clarke, 1986). Since that period, the Lake Illawarra Authority, Sydney Water and the two local councils have undertaken substantial works to improve water quality and the overall amenity of the Lake. With the Lake management practices currently in place, it is unlikely that the levels of macroalgal biomass recorded during the 1980s and early 1990s will occur in the Lake again. Nuisance macroalgal blooms still occur in Lake Illawarra, however, particularly along the Windang Peninsula. White (2003) mapped macroalgal blooms covering 88.1 ha in winter 2003, predominantly in Haywards Bay, Koonawarra Bay, Koona Bay and the mouth of Mullet Creek. The maximum biomasses of macroalgae recorded in the present study (2000 - 2003) were at the Oasis Caravan Park and Primbee Bay sites (Figure 3-1), with 150 and 370 g DW m^{-2}, respectively. Macroalgal biomass recorded during the present study is discussed further in Chapter 5.

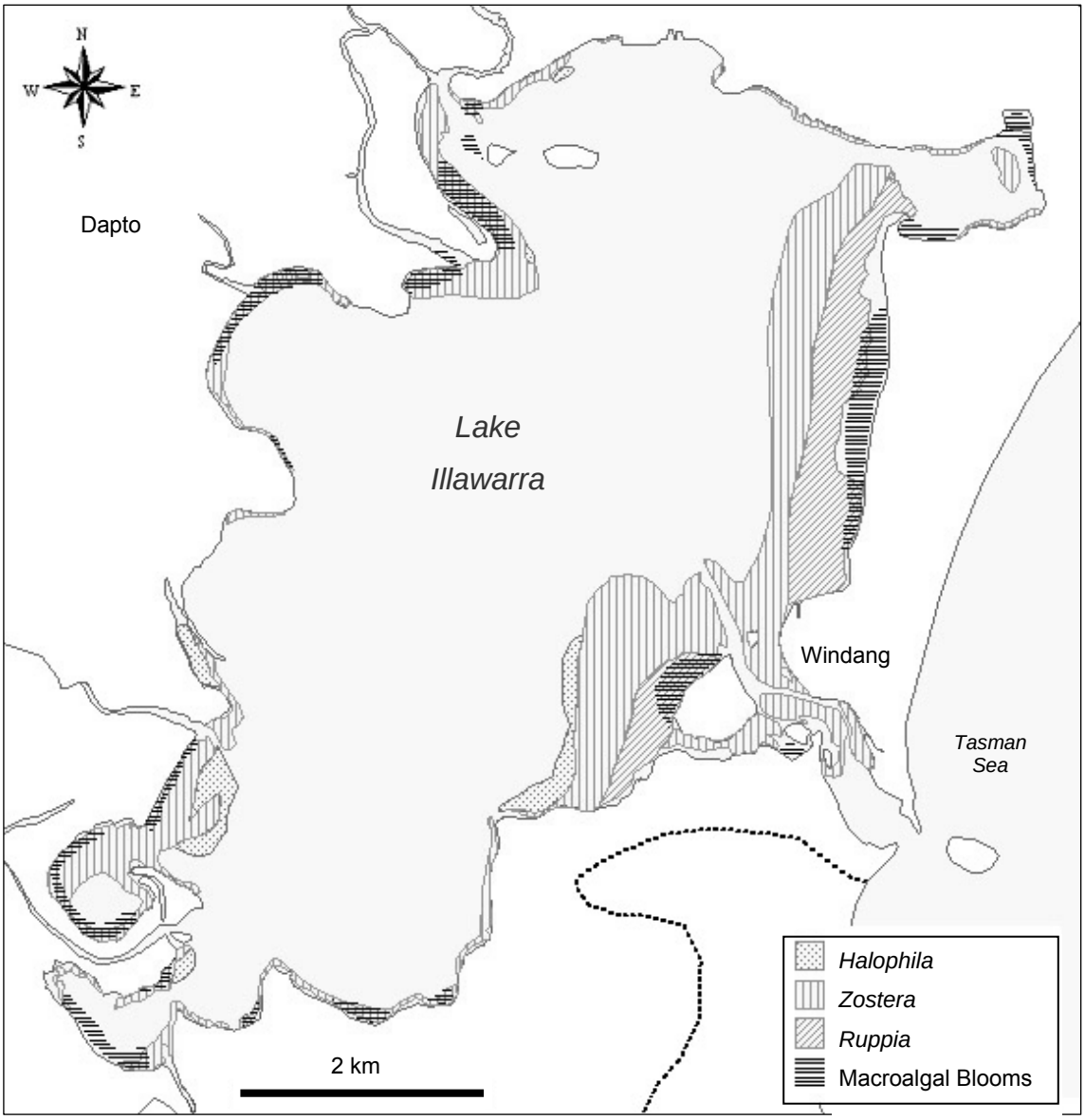


Figure 4-5: Lake Illawarra, showing areas where nuisance macroalgal blooms are likely to occur. Seagrass distribution is adapted from WBM Oceanics (2000); macroalgae distribution is adapted from Harris (1977), King *et al.* (1990) and the present study.

Table 4-5: Average biomass of macroalgae recorded by King *et al.* (1990, wet weight) and King *et al.* (1997, dry weight).

	Total biomass of macroalgae						
	Wet weight (g WW m^{-2})				Dry weight (g DW m^{-2})		
Site	**1988/89 (summer)**	**1989 (autumn)**	**1989 (winter)**	**1989 (spring)**	**1996 (spring)**	**1997 (summer)**	**1997 (autumn)**
Windang Peninsula	1121	697	980	1118	19.2	11.5	16.6
Purrah Bay / Mullet Ck.	82	29	661	546	16.5	14.3	26.5
Pithungnar Bay	657	471	430	1705	-	-	-
Yallah Bay	-	-	-	-	81.7	48.5	47.2
Koonawarra Bay	-	-	-	-	26.7	1.3	54.7
Nijong Bay / Duck Ck.	129	299	506	232	27.7	15.1	18.8
Koona Bay	868	144	342	1967	207.5	56.9	68.9
Bevans Island	584	38	53	336	19.9	4.4	57.2

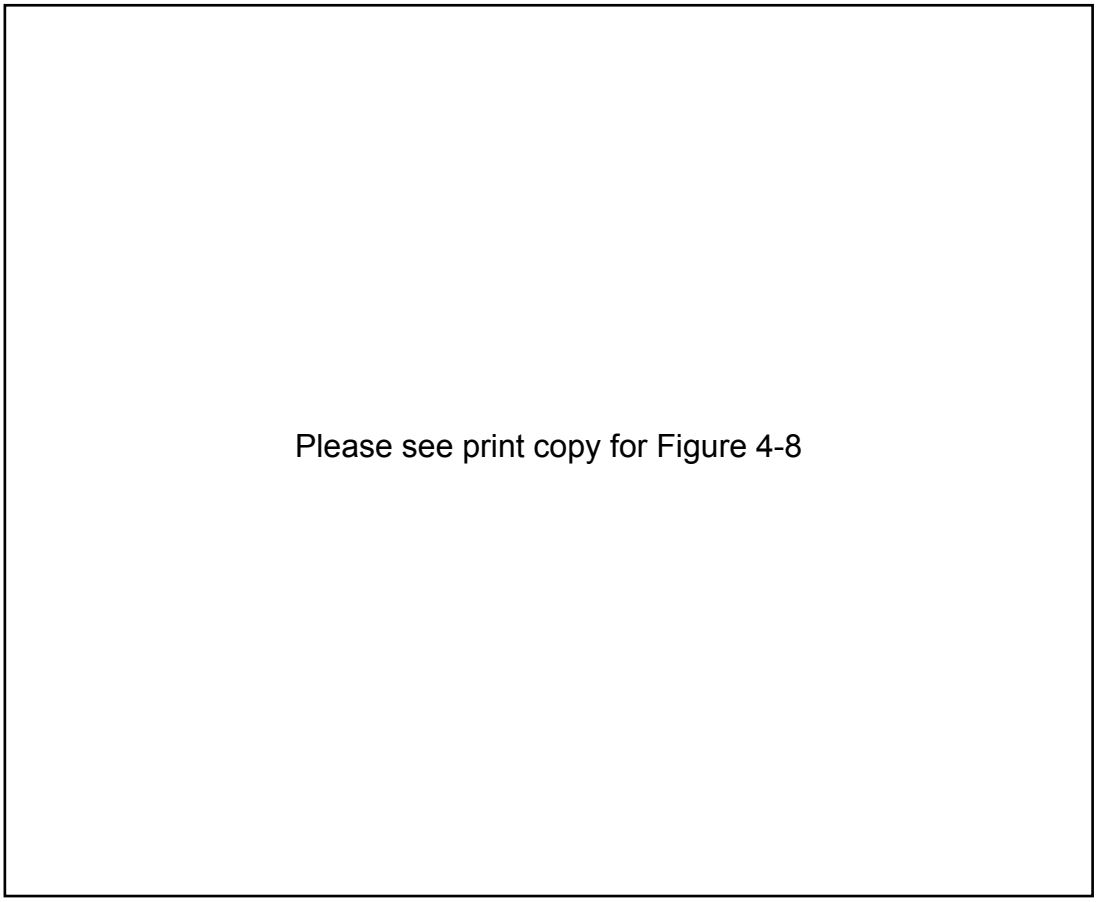


Figure 4-6: Seasonal variations in macroalgal biomass recorded at three Lake Illawarra sites by Yassini and Clarke (1986).

4.3 Results and Discussion: Description, Taxonomy and Ecology of Lake Illawarra Macrophytes

4.3.1 Introduction

Past assessments of macroalgal growth in Lake Illawarra have generally been restricted to short-term, infrequent studies, which often only included general observations, lists of the species present, and field-based identifications. Macroalgae may be misidentified, or are often listed by genera only, as identification to species level is very difficult to achieve in the field. This study is the first known attempt to provide a complete taxonomic and diagrammatic treatment of macrophytes found in Lake Illawarra. This section describes the macrophytes found in shallow areas of the Lake, between winter 2000 and winter 2003. Unless otherwise stated, all descriptions and measurements presented here are the work of the author. In total, 35 species are described, including 4 seagrasses, 14 species of green, 9 brown and 8 red macroalgae. A synopsis of the orders, families and genera of macrophytes found in Lake Illawarra during the present study is provided in Table 4-6. A comprehensive species list of those macroalgae

recorded by past authors and the present study on Lake Illawarra macrophytes is provided in Table 4-7.

Table 4-6: Synopsis of orders, families and genera of macrophytes found in Lake Illawarra, between winter 2000 and winter 2003 (current classification verified via Guiry, 2007).

Group	Order	Family	Genus
"SEAGRASSES"	ALISMATALES*	Hydrocharitaceae	*Halophila*
		Ruppiaceae	*Ruppia*
		Zosteraceae	*Zostera*
CHLOROPHYTA	ULVALES	Ulvaceae	*Ulva*
(Green Macroalgae)		Gayraliaceae	*Gayralia*
	CLADOPHORALES	Cladophoraceae	*Rhizoclonium*
			Chaetomorpha
			Cladophora
	CODIALES	Codiaceae	*Codium*
	DERBESIALES	Bryopsidaceae	*Bryopsis*
	CHARALES	Charophyceae	*Lamprothamnium*
PHAEOPHYTA	ECTOCARPALES	Ectocarpaceae	*Ectocarpus*
(Brown Macroalgae)			*Hincksia*
	SCYTOSIPHONALES	Scytosiphonaceae	*Petalonia*
			Scytosiphon
			Colpomenia
	FUCALES	Cystoseiraceae	*Cystoseira*
		Sargassaceae	*Sargassum*
		Hormosiraceae	*Hormosira*
		Seirococcaceae	*Phyllospora*
	LAMINARIALES	Alariaceae	*Ecklonia*
RHODOPHYTA	GIGARTINALES	Hypneaceae	*Hypnea*
(Red Macroalgae)	GRACILARIALES	Gracilariaceae	*Gracilaria*
	CERAMIALES	Ceramiaceae	*Spyridia*
			Ceramium
			Centroceras
		Rhodomelaceae	*Polysiphonia*
			Chondria
	HALYMENIALES	Halymeniaceae	*Grateloupia*

***** Reference: The Angiosperm Phylogeny Group (2003).

The Results and Discussion section is divided into four sub-sections concerning the four taxonomic groups of aquatic macrophytes found in Lake Illawarra:

(I) Seagrasses (including *Ruppia*)
(II) Green Macroalgae (Phylum Chlorophyta)
(III) Brown Macroalgae (Phylum Phaeophyta)
(IV) Red Macroalgae (Phylum Rhodophyta)

Note that plates (photographs) are listed at the end of each sub-section. Definitions of the taxonomic terms used are listed in footnotes. An alphabetical list of these taxonomic terms is also provided in Appendix 2.

Table 4-7: Macroalgae recorded in Lake Illawarra, 1975 - 2003.

Year / Reference	Chlorophyta	Phaeophyta	Rhodophyta
1975 - 1977 (Harris, 1977)	*Chaetomorpha linum* *Cladophora* spp. *Enteromorpha intestinalis* [1] *Percursaria* sp. *Rhizoclonium* sp. *Ulothrix* sp. *Lamprothamnium papulosum*	*Ectocarpus* sp.	*Ceramium* sp. *Chondria* sp. *Gracilaria confervoides* *Polysiphonia* sp.
1984 (Yassini, 1985)	*Chaetomorpha aerea* *Chaetomorpha linum* *Cladophora* spp. *Enteromorpha intestinalis* [1] *Ulva lactuca* *Ulvaria oxysperma*	*Colpomenia peregrina* *Cystophyllum onustum* [2] *Dictyota furcellata* *Ectocarpus siliculosus* *Endarachne binghamiae* *Petalonia fascia* *Sargassum* sp. *Scytosiphon lomentaria* *Ectocarpus siliculosus*	*Ceramium* sp. *Gracilaria verrucosa* *Gracilaria* sp. *Polysiphonia* sp.
1988 - 1990 (King *et al.*, 1990)	*Chaetomorpha linum* *Cladophora* sp. *Codium fragile* *Enteromorpha intestinalis* [1] *Rhizoclonium implexum* *Ulva lactuca* *Ulvaria* sp. *Lamprothamnium papulosum*	*Asperococcus fistulosus* *Colpomenia* sp. *Cystophora* sp. *Cystophyllum onustum* [2] *Ecklonia radiata* *Ectocarpus siliculosus* *Giffordia* sp. [3] *Phyllospora* sp. *Sargassum* sp.	*Gelidium* sp. *Gracilaria verrucosa* *Hypnea* sp. *Polysiphonia* sp.
1996 - 1997 (King *et al.*, 1997)	*Chaetomorpha linum* *Codium fragile* *Enteromorpha intestinalis* [1] *Rhizoclonium riparium* *Lamprothamnium papulosum*	*Cystophyllum onustum* [2] *Dictyota* sp. *Ecklonia radiata* *Hincksia* sp. *Sargassum* sp.	*Gracilaria verrucosa* *Hypnea valentiae* *Polysiphonia* sp. *Pterocladia* sp. *Spyridia filamentosa*
1998 (WBM, 1998)		*Sargassum* sp.	*Gelidiopsis variabilis* *Gracilaria verrucosa* *Hypnea valentiae*
2000 (McConville, 2000)	*Chaetomorpha billardierii* *Chaetomorpha indica* *Chaetomorpha linum* *Chaetomorpha* sp. *Cladophora chartacea* *Codium harveyi* *Enteromorpha compressa* [4] *Enteromorpha intestinalis* [1] *Enteromorpha ralfsii* [5] *Enteromorpha* sp. *Ulva* sp. *Ulva fasciata* *Ulva lactuca* *Gayralia oxysperma* *Rhizoclonium riparium* *Lamprothamnium papulosum*	*Colpomenia* sp. *Cystoseira trinodis* *Hincksia* sp. *Sargassum* sp.	*Chondria lanceolata* *Chondria* sp. *Gracilaria* sp. *Hypnea boergesenii* *Hypnea spinella* *Polysiphonia* sp.
2000 - 2003 (Present study)	*Bryopsis* sp.*** *Chaetomorpha aerea* *Chaetomorpha billardierii* *Chaetomorpha linum* *Cladophora* spp. *Codium fragile* *Codium harveyi* *Ulva compressa* *Ulva intestinalis* *Ulva fasciata* *Ulva lactuca* *Ulva ralfsii* *Ulva* sp. *Rhizoclonium riparium* *Lamprothamnium papulosum*	*Colpomenia* sp. *Cystoseira trinodis* *Ecklonia radiata* *Ectocarpus siliculosus* *Hincksia* sp. *Petalonia fascia* *Phyllospora comosa* *Sargassum* sp. *Scytosiphon lomentaria*	*Gracilaria edulis**** *Hypnea boergesenii**** *Spyridia filamentosa* *Ceramium* sp. *Centroceras clavulatum**** *Polysiphonia sphaerocarpa**** *Chondria angustissima**** *Grateloupia filicina****

*** New records for Lake Illawarra (identified by Dr. A.J.K Millar, National Herbarium of Australia).

[1] Currently referred to *Ulva intestinalis*

[2] Currently referred to *Cystoseira trinodis*

[3] Possibly *Hincksia sp*.

[4] Currently referred to *Ulva compressa*

[5] Currently referred to *Ulva ralfsii*

4.4 Results and Discussion (I): Description of Seagrasses in Lake Illawarra

The division Magnoliophyta includes the flowering plants, which usually have stems, leaves, roots and rhizomes (Edgar, 2000; Robertson, 1984). This division encompasses the marine plants commonly referred to as seagrasses - flowering plants that complete their life cycle completely submerged under water (Edgar, 2000). Seagrasses, other than *Halophila*, are characterised by a well-developed rhizome system and upright stems with linear, ribbon-like leaves (West, 1983). Seagrasses may occur from the midintertidal region to 60 m depth and form extensive beds which can vary in size from small, isolated patches to dense meadows covering many square kilometres (Nybakken, 1997). They typically colonise soft substrates, from sand to mud, in shallow, sheltered areas of estuaries, bays, lagoons, and lakes (West, 1983). There are less than 70 species of seagrasses globally (Walker *et al.*, 1999), with about 30 species from 12 genera represented in Australia (Kirkman, 1997). Six seagrass species are found along the New South Wales coastline: *Posidonia australis, Zostera capricorni, Zostera muelleri, Heterozostera tasmanica, Halophila ovalis* and *Halophila decipiens* (West, 1983). Another aquatic macrophyte, *Ruppia*, is also considered here, although *Ruppia* is not technically considered a seagrass as pollination in *Ruppia* may occur on or below the water surface, whereas pollination in seagrasses usually occurs below the water surface (West, 1983; Robertson, 1984).

4.4.1 Genus *Halophila* Thouars 1806

Halophila is one of the most distinctive seagrass species, characterised by small, ovate leaves, usually less than 7 cm in length, and which may be differentiated into a distinct petiole[1] and blade. It is distinguished from other seagrass species by the absence of a sheath at the base of the leaves and the structure of the flowers, which have bracts[2], whereas the flowers of other seagrass species are mostly ebracteate[3] (Robertson, 1984).

Three species of *Halophila* have been recorded in temperate southern Australian waters: *Halophila australis, Halophila ovalis* and *Halophila decipiens* (Edgar, 2000). *H. australis* is widely distributed in southern Australia, extending as far north as Dongara, Western Australia, and the central coast of New South Wales (Robertson, 1984). *H. ovalis* is widely spread in tropical and warm temperate waters, occurring along the eastern Australian coast as far south as the Victorian border. Along the western coast, *H. ovalis* extends to Cowaramup Bay on the south-western tip of Western Australia (Robertson, 1984). *H. decipiens* typically occurs north of the Tropic of Capricorn, although its south-eastern distribution extends to Mallacoota Inlet,

[1] **Petiole**: the stalk of a leaf.

[2] **Bracts**: leaf-like structures, unlike the normal foliage.

[3] **Ebracteate**: without bracts.

Victoria, and in Western Australia it occurs south to Cockburn Sound (Robertson, 1984). Additionally, two tropical species, *Halophila tricostata* and *Halophila capricorni*, have been recorded in deep water of the Great Barrier Reef (Larkum, 1995). Based on morphological features, both *Halophila ovalis* and *Halophila decipiens* were identified as occurring in Lake Illawarra during the present study.

Halophila ovalis (Brown) Hooker 1858

Halophila ovalis consists of much-branched stolons partly buried under sediment and bearing several pairs of small, ovate leaves, which are 10 - 40 mm in length and 5 - 20 mm in width (Robertson, 1984; Plate 4-1A). The leaves are glabrous[4], the leaf margins are entire[5], and have 10 - 14 pairs of crossveins per leaf (Plate 4-1B). There is usually only 1 root at each node (Robertson, 1984; Plate 4-1A). *H. ovalis* exhibits great variability in morphology, such as plant size and leaf-shape, which can be attributed to both environmental conditions and genetic differences (den Hartog, 1970, cited in Aston, 1973). The leaves, in particular, show a wide range of morphological variation, even from a single plant or bed; leaves may be oblong[6], obovate[7], or ovate[8] and slightly pointed at both ends (Robertson, 1984; Plate 4-1C).

H. ovalis flowers from December to February (West, 1983), and is distinguished from other species of *Halophila* mainly by the position of the flowers. *H. ovalis* is dioecious[9], meaning that the male and female flowers are borne on separate plants, and the female flowers have 3 styles and are positioned on the horizontal stolons (Robertson, 1984; Plate 4-1A). *H. ovalis* is relatively rare in Lake Illawarra, probably with a seasonal distribution, and generally growing in deeper water (0.8 - 2.2 m) in suitable habitat beyond the *Zostera* beds (WBM, 2000). During the present study, small beds of less than a few square metres in area were occasionally observed on mud flats in shallow waters (< 0.5 m depth) of Tuggerah Bay and near Purry Burry Point.

Halophila decipiens Ostenfeld 1902

Halophila decipiens occurring in southern Australian estuaries has been described as an annual plant, with the seeds germinating in summer, and the beds typically disappearing during winter (Kuo and Kirkman, 1995). It commonly grows on muddy substrates (West, 1983), and has been recorded in water depths of up to 85 m (den Hartog, 1970, cited in Larkum, 1995). In the present study, *H. decipiens* was found growing in small patches of less than 1 m diameter, on

[4] **Glabrous**: without hairs.

[5] **Entire**: not serrated.

[6] **Oblong**: with a rounded apex and base.

[7] **Obovate**: where the top of the leaf may be broader than the base (opposite of ovate).

[8] **Ovate**: egg-shaped, with a broader base (Robertson, 1984).

[9] **Dioecious**: the male and female flowers are borne on separate plants.

sandy sediments in shallow water along the Windang Peninsula, and in association with *H. ovalis*. It has also been recorded in deeper water (0.8 - 2 m) at Koonawarra Bay and Purrah Bay (Yassini, 1985).

Halophila decipiens is characterised by small, ovate-elliptical[10] leaves, which are 10 - 25 mm in length and 2.5 - 6 mm in width (Robertson, 1984; Plate 4-2A). It features a thin rhizome which grows on, or partially buried just below, the sediment surface (West, 1983). *H. decipiens* differs from *H. ovalis* by having occasionally hairy leaves with fine serrations on the leaf margins and only 5 - 9 pairs of crossveins per leaf (Robertson, 1984; Plate 4-2B, C). It is also monoecious[11], meaning that male and female flowers may be borne on the same plant, whereas *H. ovalis* is dioecious (Robertson, 1984).

4.4.2 Genus *Ruppia* Linnaeus 1753

Ruppia typically occurs in water less than 2 m deep, under conditions of good illumination, low water motion (Wood, 1959), and low salinities (West *et al.*, 1989). However, *Ruppia* is tolerant of a wide range of salinities, occurring in fresh, brackish, estuarine, marine and hypersaline waters (Robertson, 1984). In estuaries, *Ruppia* is commonly found growing on muddy sediments deposited from catchment runoff, or in areas where siltation or nutrient enrichment has occurred, such as Lake Illawarra (West, 1983). There are three species of *Ruppia* in New South Wales: *Ruppia megacarpa, Ruppia maritima,* and *Ruppia polycarpa* (West, 1983). The species of *Ruppia* are typically separated by the appearance of the fruits (refer to Robertson (1984) for full descriptions). Fertile material of *Ruppia*, however, is often difficult to find in Lake Illawarra; thus, while other species of *Ruppia* may be present, the *Ruppia* beds in Lake Illawarra are commonly referred to as *Ruppia megacarpa*.

Ruppia megacarpa Mason 1967

Ruppia megacarpa is widely distributed around southern Australia, from WA to NSW, occurring in perennial, enclosed water bodies such as estuaries, coastal salt-lakes and inland lakes (Jacobs and Brock, 1982; Robertson, 1984). *Ruppia* in Lake Illawarra typically grows in sheltered locations inshore of, or in association with, the *Zostera* beds, possibly because it is intolerant of high turbulence in less sheltered areas of the Lake (Harris *et al.,* 1980; King, 1990). *Ruppia* is mostly confined to sand or mud flats along the eastern side of the Lake, within a shallow depth range of 5 - 60 cm, as it requires shallow water for pollination (West, 1983). In Lake Illawarra, *Ruppia* occurs on sediments ranging from 68 - 97 % sand (Harris, 1977).

[10] **Elliptical**: in the shape of an ellipse (e.g., egg-shaped).

[11] **Monoecious**: the male and female flowers may be borne on the same plant (Robertson, 1984).

Ruppia megacarpa is characterised by thin, upright stems which are usually 20 - 30 cm in length (Plate 4-3A), although lengths of up to 2 m can occur (West, 1983). The leaves are blade-like, with a slightly indented tip, 2 - 30 cm in length, and usually less than 1 - 2 mm in width (Robertson, 1983; West, 1983; Plate 4-3B). Rhizomes are 1 - 2 mm in diameter (Plate 4-3C). Localised spreading occurs by seed and rhizomes (Jacobs and Brock, 1982). *Ruppia* flowers during the summer months, from November to February (Wood, 1959; Plate 4-3D), and pollination may occur above or below the water surface (Robertson, 1984).

4.4.3 Genus *Zostera* Linnaeus 1753

Zostera is the most abundant seagrass in south-eastern Australian estuaries (Wood, 1959) and is commonly referred to as “eelgrass” or ‘ribbonweed” (West, 1983). It is usually found growing on soft substrates (mud or sand), below the high tide mark in many coastal lagoons (Jacobs and Williams, 1980). *Zostera capricorni* extends along the NSW coastline, northwards into Queensland, and southwards into Mallacoota, Victoria (Jacobs and Williams, 1980). A species of similar morphology, *Zostera muelleri*, generally can not be separated from *Z. capricorni* in the field (West, 1983), but appears to have a more southerly distribution. The *Zostera* occurring in the Sydney Basin has traditionally been referred to as *Zostera capricorni* (West, 1983), whereas *Z. muelleri* occured in South Australia, Tasmania, Victoria, and as far north as Sussex Inlet in New South Wales (Jacobs and Williams, 1980). A recent assessment by Les *et al.* (2002), however, concluded that *H. tasmanica, Z. capricorni* and *Z. muelleri* in Australia and New Zealand should be merged into a single species (*Zostera capricorni*), due to a lack of morphological and genetic differentiation between the species. Jacobs *et al.* (2006) later suggested that these four species names be revised to subspecies of *Zostera muelleri* according to geographical distribution, with *Zostera capricorni* now referred to *Zostera muelleri* subsp. *capricorni* (Ascherson) Jacobs. The name *Zostera capricorni*, however, has been retained in this thesis to maintain consistency with previous research on Lake Illawarra seagrasses.

Zostera capricorni Ascherson 1876

Zostera capricorni is the dominant seagrass in Lake Illawarra, with WBM (2000) reporting that *Zostera* occupied 72 % of the total area of seagrass beds and covered a depth range of 0.1 - 1.9 m in 2000. Although *Zostera* in Lake Illawarra exhibits wide variations in morphology, previous studies (e.g., Harris, 1977) have concluded that Zostera in the Lake may all be encompassed within the single species *Zostera capricorni*. This species is characterised by linear leaves, with an average width of 2 - 5 mm and leaf length to 60 cm (Plate 4-4A). Leaves may have straight or notched leaf tips and generally have 4 - 5 longitudinal veins per leaf, with the outer veins merging with the inner veins near the leaf apex (Robertson, 1984; Plate 4-4B). The leaf apexes of *Zostera capricorni* vary in shape from rounded, truncate, or deeply notched

(Plate 4-4B); these variations can occur within a single bed or on individual plants (Wood, 1959). *Zostera* rhizomes are much-branched, with the roots clustered in 2 groups of 4 - 8 roots at each node (Plate 4-4C). Fertile shoots bear several spadices, with a short, pointed tip, and 6 - 10 male and female flowers per spadix[12] (Robertson, 1984; Plate 4-4D).

Zostera undergoes fast leaf growth during spring and summer (West, 1983). In Lake Illawarra, it typically attains maximum shoot length in summer and the minimum in winter (Harris *et al.*, 1980). Maximum flowering of *Zostera* typically occurs in summer, although flowering and seed production can occur from September up to August of the following year (Harris *et al.*, 1980). However, *Zostera* does not appear to colonize bare areas via seedlings, rather the most common form of spreading is by rhizome invasion into adjacent areas (Wood, 1959; Harris *et al.,* 1980). After flowering, most of the leaves die-off and only the rhizomes and some small shoots remain, so that, during autumn, the Zostera bed may appear devoid of any vegetative growth (Wood, 1959). The new growth cycle typically occurs between August and September, following the shedding of leaves from the previous season's growth (Harris *et al.*, 1980). In Lake Illawarra, the dead seagrass leaves either wash out into deeper water and sink, or accumulate on the foreshore in large, rotting masses (Yassini, 1985). Rapid leaf shedding also occurs following heavy rainfall, possibly due to increased water depth and wave action during flooding, as well as increased turbidity and reduced light availability (Harris *et al.*, 1980).

[12] **Spadix**: (spadices) a spike-like structure, usually enclosed in a spathe (i.e., the large bracts surrounding an inflorescence) (Robertson, 1984).

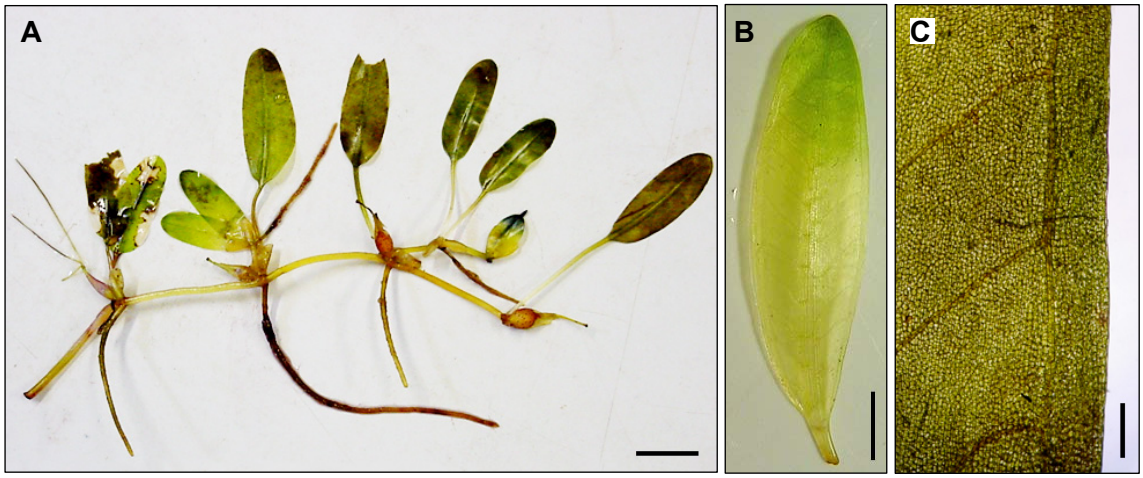


Plate 4-1: *Halophila ovalis* (PBP, 26/8/01). **(A)** Typical habit[13] of fertile plant, showing flower with three stigmas. Scale = 1 cm. **(B)** *A*verage leaf. Scale = 5 mm. **(C)** Leaf showing glabrous surface and entire margin. Scale = 200 μm.

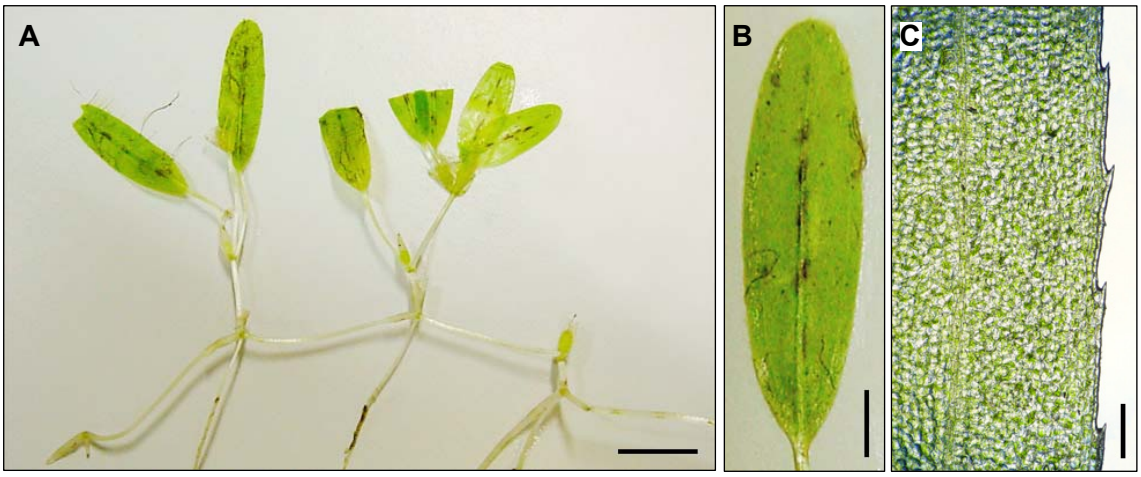


Plate 4-2: *Halophila decipiens* (NIC, 3/3/02). **(A)** Habit of fertile plant. Scale = 1 cm. **(B)** Typical leaf. Scale = 3 mm. **(C)** Serrulate leaf margin. Scale = 200 μm.

[13] **Habit**: refers to the characteristic or morphological form of a plant.

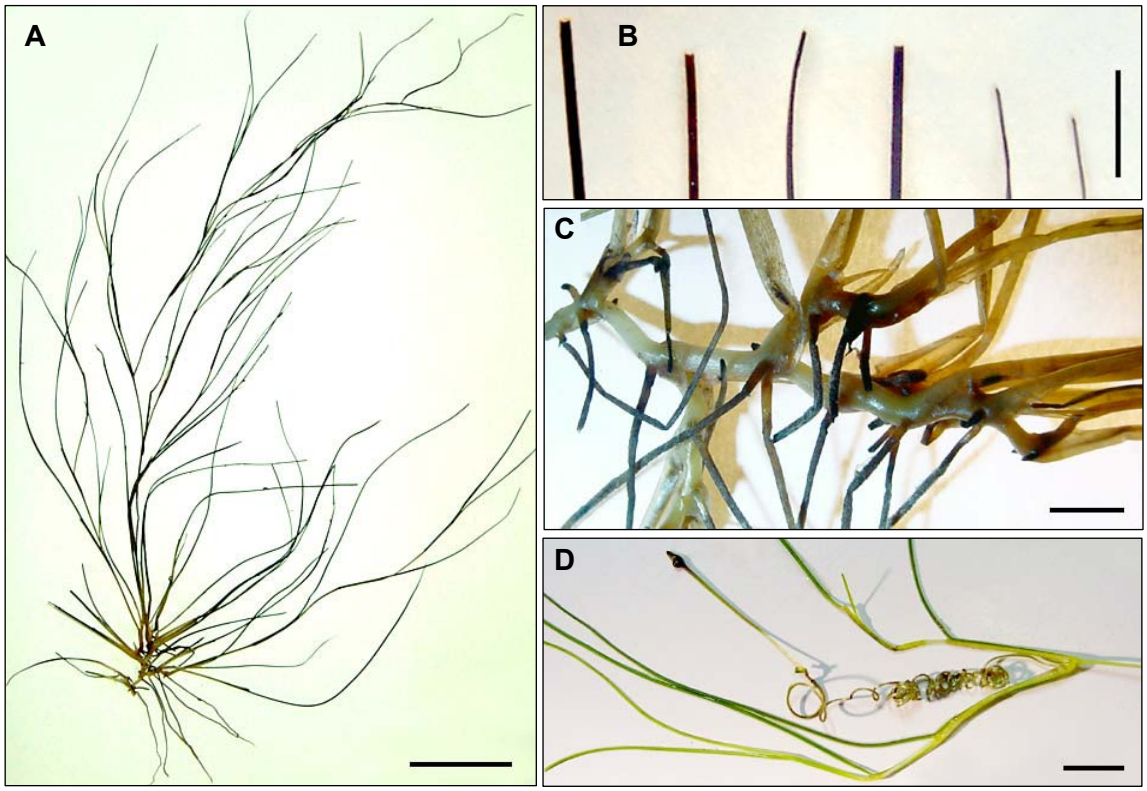


Plate 4-3: *Ruppia megacarpa* (OCP, 3/4/01). **(A)** Habit of plant. Scale = 5 cm. **(B)** Leaf tips. Scale = 5 mm. **(C)** Rhizome. Scale = 5 mm. **(D)** Flower. Scale = 1 cm.

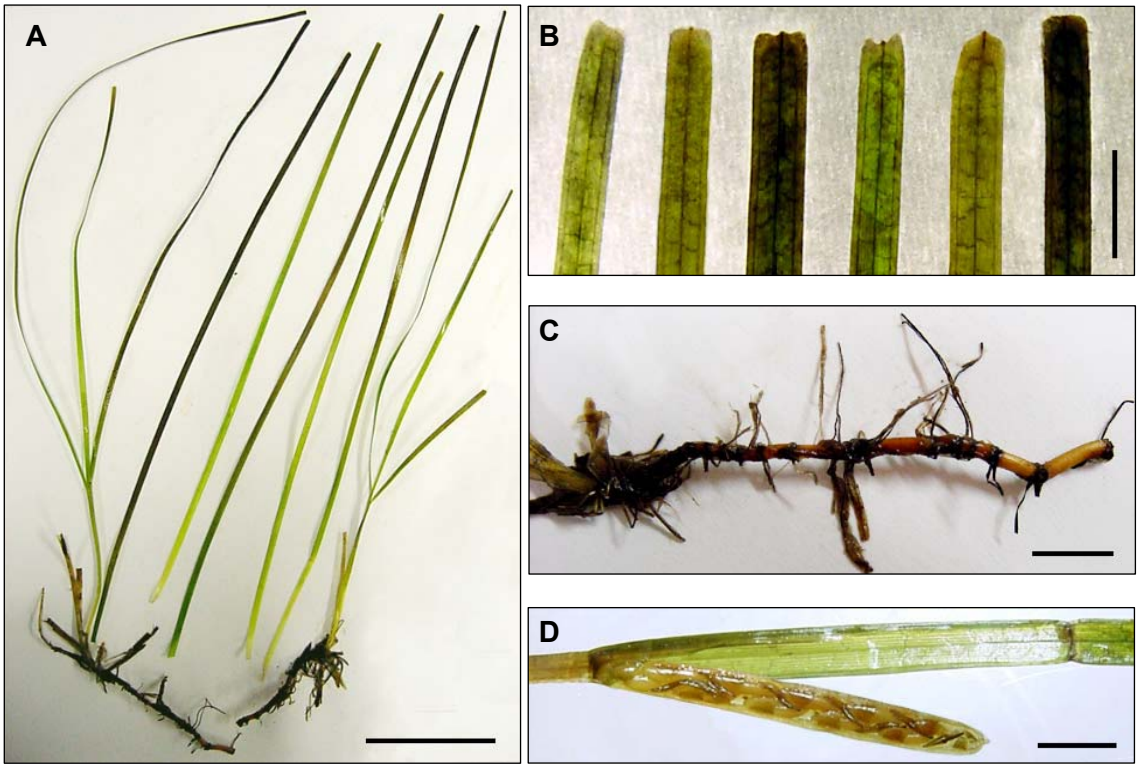


Plate 4-4: *Zostera capricorni*. **(A)** Habit of plant (Yallah Bay, 8/7/01). Scale = 5 cm. **(B)** Range of leaf tips exhibited on plants from a single area (PBP, 3/2/01). Scale = 5 mm. **(C)** Rhizome (Yallah Bay, 8/7/01). Scale = 1 cm. **(D)** Spadix (Purrah Bay, 25/11/00). Scale = 5 mm.

4.5 Results and Discussion (II): Description of Green Macroalgae (Chlorophyta) in Lake Illawarra

The division Chlorophyta encompasses the green algae, which are characterised by a green to yellowish-coloured thallus, and vary in size from phytoplankton, to the small epiphytic and larger benthic forms, as well as the loose-lying, mat-forming varieties (Womersley, 1984). The green macroalgae have been described as the most diverse of all the phyla, in terms of their morphological variability and the complexity of their life histories (Tanner, 1981). Thus, identification of the species of green macroalgae is often difficult to determine as the thalli show wide morphological variations as well as similarities within and between species and genera.

Of the three macroalgae divisions, the green algae have the smallest number of species, yet they are often the most abundant group, particular in disturbed or eutrophic habitats (Millar and Kraft, 1994b). In Lake Illawarra, green macroalgae account for a significant proportion of the total algal biomass as well as the total number of species present (refer to Chapter 5). The most common algae are the filamentous, bloom-forming genera, *Chaetomorpha* and *Ulva*, which often form extensive mats over sand flats and seagrass beds, particularly along the Windang Peninsula. Green macroalgae that have been recorded in Lake Illawarra from previous studies and the present study are listed in Table 4-7. The following sections describe and illustrate 14 species, from 8 genera, of benthic green macroalgae from Lake Illawarra. Note that all plates are listed at the end of this sub-section.

4.5.1 Genus *Ulva* Linnaeus 1753

Species of the order Ulvales (e.g., *Ulva*, *Gayralia* and *Ulvaria*) are very similar in appearance, thus making identification and classification difficult. Generally, *Ulva* is separated from *Gayralia* and *Ulvaria* by being distromatic[14] throughout the thallus, whereas *Gayralia* is monostromatic[15]. Recent molecular studies have suggested that the genera *Ulva* and *Enteromorpha* are not separated as distinct monophyletic groups and should be moved into the single genera *Ulva* (e.g., Hayden and Waaland, 2002; Hayden *et al.*, 2003; Shimada *et al.*, 2003). *Ulva* and *Enteromorpha* were previously separated by the appearance of the thallus; *Ulva* has a membranous thallus, whereas the thallus of plants previously referred to *Enteromorpha* are usually basally tubular and hollow. For the purposes of this study, macroalgae previously identified as *Enteromorpha* spp. in Lake Illawarra are now described under *Ulva*.

There are about 122 species of *Ulva* listed worldwide (Guiry, 2004), with 13 species recorded in New South Wales (Millar and Kraft, 1994b). The plants typically have a bright green appearance, varying from leaf-like to filamentous and tubular, and simple to much-branched. *Ulva* grows

[14] **Distromatic**: a cross-section shows two cell layers.

[15] **Monostromatic**: single layered - a cross-section shows only one cell layer (Womersley, 1984).

abundantly in the intertidal to subtidal zones of marine to brackish waters, and peaks in abundance between late winter and early spring in south-eastern Australia (Phillips, 1998; Kraft, 2000). Some species of *Ulva* may be attached to the substrate with a holdfast (e.g., on rock platforms), but it is often found free-floating and known to form extensive mats in eutrophic estuaries and sheltered bays (e.g., Pregnall and Rudy, 1985; Fong *et al.*, 1998; Martins and Marques, 2002).

The exact number of species belonging to *Ulva* is difficult to estimate due to taxonomic difficulties and past inconsistencies in the criteria used to apply species names (Kraft, 2000). Additionally, species of *Ulva* often show substantial morphological differences within species, but morphological variations between species are often slight and difficult to detect (Bliding, 1963, cited in Blomster *et al.*, 1998). Many of the morphological and anatomical characteristics used to identify *Ulva* can be extremely variable, and some populations of *Ulva* in southern Australia (Phillips, 1988), and the Netherlands (Malta *et al.*, 1999), have exhibited seasonal or habitat-induced variations in morphology, such that they appear to be separate taxa. Morphological variability in *U. lactuca* also appears to be related to plant density, water movement, temperature and photosynthetic photon flux (Phillips and Clayton, 1983; Israel *et al.*, 1995). Accurate classification of the species of *Ulva* requires morphological examination of populations sampled regularly from a range of habitats and seasons (Phillips, 1988), as well as molecular analysis and culture studies (Malta *et al.*, 1999). Thus, species of *Ulva* are very difficult to identify based on morphological features alone and may require molecular analysis to accurately differentiate between species (Innes, 1988; Blomster *et al.*, 1998).

Prior to Womersley (1984), most records of sheet-like *Ulva* in Australia had been referred to *Ulva lactuca* (commonly known as “sea lettuce”), but several more species have since been recognised on Australian coasts (see, e.g., Phillips, 1988; Millar and Kraft, 1994b; Kraft 2000). Past records of *Ulva* in Lake Illawarra (e.g., Yassini, 1985; King *et al.*, 1997) were classified as *Ulva lactuca* (sheet-like forms) and *Enteromorpha intestinalis* (filamentous forms), but it is likely that other species of *Ulva* also occur in the Lake. At least 5 - 6 species of *Ulva* were recognised in Lake Illawarra during the present study, although the exact classification of these species requires further investigation. In the present study, filamentous *Ulva* spp. (previously documented as *Enteromorpha* spp.) was relatively common in Lake Illawarra, forming extensive blooms over sand and mud flats, in sheltered bays and near areas of high nutrient loading (e.g., creeks, drains), and appeared to be most abundant between July and December. McConville (2000) recorded five species of *Ulva* in Lake Illawarra: *U. lactuca*, *U. fasciata*, *U. intestinalis, U. compressa* and *U. ralfsii*, with these new records having been verified by the National Herbarium of New South Wales. Each of these species was also recognized during the present study.

Ulva lactuca Linnaeus 1753

Ulva lactuca, a sheet-forming alga, has often been recorded from Lake Illawarra (Table 4-7) and classified as a nuisance macroalgal species by Yassini (1985), but it was relatively rare in Lake Illawarra during the present study. *U. lactuca* has a grass-green colour, usually has a single, entire or irregularly divided frond arising from the holdfast[16], and varies in size from 2 - 30 cm across and 2 - 20 cm high (Womersley, 1984; Plate 4-5A). The thallus of *U. lactuca* has a smooth surface and smooth margin (Womersley, 1984), but Lake Illawarra plants designated as *U. lactuca* occasionally had marginal microscopic teeth (Plate 4-5C). Phillips (1988) found that macroscopic, marginal teeth on *Ulva laetevirens* were related to water movement; marginal teeth occurred on thalli cultured in still water, as well as in field plants growing under conditions of extremely low water movement. The cells in surface view of *U. lactuca* tend to be polygonal to quadrangular, 13 - 23 μm in length and 8 - 25 μm in width (Phillips, 1988), with 1 - 3 pyrenoids[17] per cell (Womersley, 1984; Plate 4-5B). The fronds of *U. lactuca* are two cell layers thick, thickening towards the base. In cross-section, the cells are squarish to slightly rounded, with a length to breadth ratio of 1 - 1.4 (Womersley, 1984; Plate 4-5D); 17 - 25 μm high by 10 - 29 μm wide in the marginal region, and 18 - 32 μm high by 10 - 25 μm wide in the mid and basal regions of the thallus (Phillips, 1988).

Ulva fasciata Delile 1813

McConville (2000) recorded one specimen of *Ulva fasciata*, collected from the Windang Peninsula. *U. fasciata* differs from *U. lactuca* by having several long, smooth branches divided near the base, an average thallus height of 20 - 45 cm, and a grass-green to brownish-green colour on drying (Womersley, 1984; Plate 4-5E). The thallus margin may be smooth to irregular, or sometimes with marginal teeth (Phillips, 1988). The cells in surface view are polygonal to quadrangular in shape, 10 - 26 μm in length and 7 - 25 μm across (Phillips, 1988), arranged irregularly or in slight, often curved rows (Womersley, 1984), and with 1 - 4 pyrenoids per cell (Phillips, 1988; Plate 4-5F). The cells in cross-section are rectangular throughout the thallus; 16 - 36 μm high by 8 - 18 μm wide in the marginal region, 19 - 47 μm high by 10 - 24 μm wide in the mid region and 26 - 51 μm high by 8 - 26 μm wide in the basal region (Phillips, 1988). While the cell structure of the *Ulva* sp. collected from Tuggerah Bay (Lake Illawarra) (Plate 4-5E-F) appeared to match descriptions of *Ulva fasciata* given in Womersley (1984) and Phillips (1988), the general appearance of the thallus did not match these descriptions, as the Tuggerah Bay plants were not divided into numerous narrow branchlets.

[16] **Holdfast**: an attaching cell or organ, typically at the base of the plant (Womersley, 1984).

[17] **Pyrenoid**: a distinguishable region of the chloroplast, associated with carbohydrate synthesis.

Ulva intestinalis Linnaeus 1753

U. intestinalis (previously referred to *Enteromorpha intestinalis*) has been recorded by several authors as a nuisance species in Lake Illawarra (Harris, 1977; Yassini, 1985; King *et al.*, 1997). The thallus is light green to grass green in colour, unbranched, tubular and often tapering towards the base, loose-lying to mat-forming, and often gas-filled. Individual filaments in Lake Illawarra are up to 50 cm in length, 0.2 - 3 cm in width and of fairly uniform width throughout (Plate 4-6A). *U. intestinalis* is very similar in appearance to *U. compressa*, especially when detached and free-floating, but the main distinctions appear to be the presence of patches of cell rows and branches in *U. compressa* (Womersley, 1984). Although *U. intestinalis* is generally considered to be unbranched, previous workers have shown that branches could be induced by salinity changes or increases in irradiance (refer to Blomster *et al.*, 1998, and references therein). Cells of *U. intestinalis* identified in Lake Illawarra remain unordered throughout the thallus, are polygonal to rounded, 10 - 18 μm in length by 8 - 14 μm in width, and have 1 - 2 pyrenoids per cell (Womersley, 1984; Plate 4-6B, C).

Ulva compressa Linnaeus 1753

U. compressa was first recorded in Lake Illawarra by McConville (2000, as *Enteromorpha compressa*) and has also been recorded on numerous occasions during the present study. It is very common along rocky shorelines and often found attached to small rocks or shells on sand flats in the upper intertidal to subtidal zones. It is also a common epiphyte on *Zostera*, often detaching and forming small, free-floating masses over *Zostera* beds. The thallus is a light to medium green colour, erect and basally attached, with proliferous branching at the base of the thalli and occasional branches in the upper thalli (Plate 4-7A). Filament length varies from 0.5 - 30 cm, and filament diameter ranges from 1 mm to several cm across. The thallus is often compressed, with ruffled margins, and is narrow at the base and broadening considerably towards the tip. Some forms of *U. compressa*, particularly epiphytic forms, remain terete[18], are of relatively uniform diameter throughout and have more branches above than below. The cell structure is a combination of unordered areas and patches where the cells are in longitudinal and transverse rows. Cells tend to be polygonal to slightly rounded when unordered, and squarish when ordered, 10 - 16 μm in width, with the chloroplast filling most of the cell, and 1 (occasionally 2) pyrenoids per cell (Womersley, 1984; Plate 4-7B, C).

Ulva ralfsii (Harvey) Le Jolis 1863

The first record of *U. ralfsii* (as *Enteromorpha ralfsii*) in Lake Illawarra was by McConville (2000). During the present study *U. ralfsii* was occasionally found along the Windang Peninsula sand flats (e.g., Purry Burry Point). The thallus is a light grass-green colour and forms relatively small,

[18] **Terete**: cylindrical, but usually slightly tapering towards both ends.

loose-lying masses over shallow, inshore sand or mud flats in close proximity to *Zostera* or *Ruppia* beds. The filaments of Lake Illawarra specimens are 5 - 15 cm in length, unbranched, and are of a fairly uniform diameter of less than 250 μm throughout the length of the filament (Plate 4-8A). The cells of *U. ralfsii* are squarish to rectangular, in distinct longitudinal rows and often in transverse rows, with the chloroplast filling most of the cell, and 2 - 5 pyrenoids per cell (Womersley, 1984; Plate 4-8B, C).

4.5.2 Genus *Gayralia* K.L. Vinogradova 1969

Gayralia oxysperma (Kützing) Vinogradova *ex* Scagel *et al.* 1989

Gayralia oxysperma is the currently accepted name for *Ulvaria oxysperma* (Guiry, 2004). This species is sheet-like, very similar in appearance to *Ulva*, and common in marine to euryhaline waters, in habitats ranging from rocky coasts to estuaries (Woolcott and King, 1998). It is found predominantly during winter in southern Australian waters (Womersley, 1984). *G. oxysperma* (as *Ulvaria oxysperma*) has been described as translucent, a light to medium green colour, 2 - 12 cm high, relatively delicate, with multiple and irregularly shaped fronds, often with ruffled margins (Womersley, 1984; Woolcott and King, 1998). The thallus is monostromatic throughout, usually epilithic[19], and attached by a small holdfast (Womersley, 1984). *G. oxysperma* was not found in Lake Illawarra during the present study, although it has been recorded from Lake Illawarra by McConville (2000) and Yassini (1985, as *Ulvaria oxysperma*).

4.5.3 Genus *Rhizoclonium* Kützing 1843

The genus *Rhizoclonium* consists of about 20 species, distinguished from other genera of the Cladophoraceae by its unbranched filaments which form occasional lateral rhizoids[20] (Kraft, 2000). Millar and Kraft (1994b) recorded two species of *Rhizoclonium* from New South Wales: *Rhizoclonium implexum* and *Rhizoclonium riparium*, and McConville (2000) also listed *Rhizoclonium tortuosum* from Lake Wollumboola. Only *Rhizoclonium riparium* is known to occur in Lake Illawarra.

Rhizoclonium riparium (Roth) Harvey 1849

Rhizoclonium riparium commonly occurs as entangled masses of filaments in the intertidal zone of estuaries and generally shaded, calm water habitats (Womersley, 1984). In the present study, *Rhizoclonium riparium* was occasionally found over sand flats along the Windang Peninsula (e.g., near the Oasis Caravan Park) in late summer, forming loose-lying masses, or entangled with other algae, such as *Chaetomorpha*. The thallus of Lake Illawarra *R. riparium* is a light to

[19] **Epilithic**: attached to a hard substrate (e.g., rocks).

[20] **Rhizoid**: a single-celled or few-celled filament, used for attachment or absorption (Womersley, 1984).

medium green colour, composed of uniseriate[21], unbranched filaments, up to 15 cm in length (Plate 4-9A) and with occasional lateral rhizoids of 1 - 3 cells long (Plate 4-9B). The cells are 10 - 18 μm in width, 20 - 50 μm in length and with a length to breadth ratio of 1.5 - 4 (Plate 4-9C).

4.5.4 Genus *Chaetomorpha* Kützing 1845

Chaetomorpha is a widely distributed genus of about 50 species listed worldwide (Guiry, 2004), 8 of which have been reported in New South Wales (Millar and Kraft, 1994b). Species of *Chaetomorpha* occur in a range of habitats, from rough-water coasts to estuaries, where they frequently form nuisance algal blooms (e.g., Lavery and McComb, 1991b; Krause-Jensen *et al.*, 1999). The thallus of *Chaetomorpha* is unbranched, occurring as erect tufts, sometimes attached to the substrate, or as masses of loose-lying filaments (Womersley, 1984).

Chaetomorpha linum has traditionally been recorded as a nuisance species in Lake Illawarra (e.g., Yassini, 1985; King *et al.*, 1997). Two other, occasionally problematic, species of *Chaetomorpha* were recorded in the Lake during the present study: *Chaetomorpha billardierii,* and to a lesser extent, *Chaetomorpha aerea*. McConville (2000) also recorded the presence of *Chaetomorpha valida* and *Chaetomorpha indica* in Lake Illawarra, but these species were not found during the present study. *C. linum* and *C. billardierii* were the most abundant algae found in Lake Illawarra during the present study, forming extensive loose-lying, entangled masses, often overlying beds of seagrass and other macroalgae (see, e.g., Appendix 1A). *C. linum* and *C. billardierii* generally formed separate masses, although both species were occasionally found entangled together in blooms, and often with a mat of *C. linum* overlying a mat of *C. billardierii*. The blooms were most abundant during summer, although smaller masses persisted throughout the year. It should be noted that while the Lake Illawarra plants identified as either "*C. linum*" or "*C. billardierii*" appeared to be separate species, based largely on the length to breadth ratio of the cells, this morphological characteristic may vary according to the age of the plant or during cell division, and may not be a reliable indicator of species differentiation for the Lake Illawarra plants. As the cell lengths and other morphological characteristics of the "*C. linum*" and "*C. billardierii*" plants overlapped somewhat, it is unclear whether these specimens are separate taxa or environmental adaptations of the same species.

Chaetomorpha aerea (Dillwyn) Kützing 1849

Chaetomorpha aerea is found throughout southern Australia and extends north into Queensland and Western Australia, occurring in a range of habitats, from the intertidal to subtidal zones, in rock pools or as epiphytes on seagrass (Womersley, 1984). *Chaetomorpha aerea* was recorded only occasionally during the present study at Cudgeree Bay (near the Lake Illawarra Village), at

[21] **Uniseriate**: with the cells are arranged in a single row, and no more than one-cell wide.

the entrance to Mullet Creek, and at Yallah Bay, mainly during the summer months. *C. aerea* was found in relatively small masses of less than 1 m diameter, attached to sand or rocks, or growing epiphytically on *Zostera*. The thallus of *C. aerea* in Lake Illawarra is light green in colour, with a soft texture, and each filament is attached by an elongate basal cell (Plate 4-10A). The filament width is narrow at the base (about 100 μm), increasing in diameter to about 360 μm at the tip (Plate 4-10B-D). The length to breadth ratio of the cells varies from 1.5 - 2 towards the base, and is approximately 1 throughout the upper filament.

Chaetomorpha linum (Müller) Kützing 1849

Chaetomorpha linum is typically found in sheltered, calm water habitats, such as bays and lagoons (Womersley, 1984), and is particularly problematic in estuaries and other waterways that receive high nutrient loading (Lavery and McComb, 1991a; Peckol and Rivers, 1996). *Chaetomorpha linum* forms extensive blooms in sheltered bays and over sand flats and seagrass beds and has a depth range of 0 - 60 cm in Lake Illawarra (Yassini, 1985; present study). The thallus of *C. linum* in Lake Illawarra is a grass-green to dark-green colour and generally has a course, hair-like texture, occurring as masses of curved, unbranched filaments (Plate 4-11A). Filaments are up to 50 cm in length, are of a fairly uniform diameter throughout the length of the filament, and do not have basal attachment cells. In *C. linum* specimens from Lake Illawarra, cells were between 220 - 270 μm in width and 150 - 380 μm in length (Plate 4-11B). The average length to breadth ratio of the cells is 0.6 - 1, although several cells on the same filament may have a L/B closer to 1.5. Cells may also be very slightly incised at the cross walls (Womersley, 1984; Plate 4-11B). Reproduction is with both sporophytic[22] (Plate 4-11C) and gametophytic[23] phases (Plate 4-11D, E). In the latter stage, gametangial[24] branches grow from a part of an older filament, later detaching and becoming a separate filament.

Chaetomorpha billardierii Kützing 1847

Chaetomorpha billardierii is typically found in sheltered waters of the lower intertidal to upper subtidal zone, throughout southern Australia (Womersley, 1984). The distribution of *C. billardierii* in Lake Illawarra during the present study appeared to be confined to the Windang Peninsula, particularly around the Oasis Caravan Park and in Primbee Bay. The thallus has a soft, flaccid texture, and is dark green in colour, becoming yellow-green with age (Plate 4-12A). Filaments of *C. billardierii* from Lake Illawarra are between 5 - 30 cm in length, are of fairly uniform diameter throughout the length of the filament, and do not have basal attachment cells. In Lake Illawarra specimens, cell widths ranged from 170 - 250 μm (mean: 180 μm), cell lengths were between 140 - 510 μm (mean: 280 μm) and the cells had a length to breadth ratio of 0.8 -

[22] **Sporophytic**: the spore-producing phase in the life history of a plant.

[23] **Gametophytic**: the gamete-producing phase of a life history (Womersley, 1984).

[24] **Gametangial branches**: the sex organs which contain the gametes (Womersley, 1984).

3 (Plate 4-12B). Young specimens appeared to have longer cells than mature specimens due to cell division over time. Cells may also be slightly incised at the cross walls, and cell walls often collapse on drying.

4.5.5 Genus *Cladophora* Kützing 1843

Cladophora is a common and widely distributed genus, with 169 species listed worldwide (Guiry, 2004), including 22 species recorded in New South Wales (Millar and Kraft, 1994b). *Cladophora* is typically light green in colour, the thallus is filamentous, uniseriate, sparsely to profusely branched, and basally attached or loose-lying (van den Hoek and Womersley, 1984; Kraft, 2000). Species of *Cladophora* have traditionally been difficult to identify due to the morphological variability of the plant, depending on its age and exposure to various environmental parameters, such as light intensity or wave action (van den Hoek, 1963, cited in Kraft, 2000).

A number of past studies on Lake Illawarra macrophytes (e.g., Harris, 1977; King *et al.*, 1997) have recorded the presence of *Cladophora* in Lake Illawarra, but due to taxonomic difficulties these species are generally only identified to a generic level and, therefore, the number of species of *Cladophora* present in the Lake is unknown. Although efforts were made to identify the Lake Illawarra *Cladophora* to species level during the present study, this was not possible. McConville (2000) listed *Cladophora chartacea* and *Cladophora rhizoclonioidea* in Lake Illawarra, but as her sampling sites were confined to the eastern sand flats of the Lake, it is likely that other species of *Cladophora* inhabit the remainder of the Lake, particularly the rocky shorelines. Epiphytic forms of *Cladophora* often became problematic in Lake Illawarra (present study) when the seagrass leaves detached and accumulated inshore or become tangled amongst macrophyte beds; *Cladophora* blooms occasionally formed floating masses over the seagrass beds in summer (see, e.g., Plate 4-13E).

Cladophora spp.

During the present study, at least two species of *Cladophora* were found in Lake Illawarra, often attached to rocks, or as epiphytes on *Zostera* and *Ruppia.* The thallus of *Cladophora* sp. found during the present study is 2 - 10 cm high, a light to medium green colour, much branched, and forming dense tufts on rocks or growing as epiphytes on seagrasses. The epiphytic form of *Cladophora* sp. was typically less than 5 cm high (Plate 4-13A), with upper branches of 10 - 25 μm in diameter and a cell length by breadth of 4.5 - 7 (Plate 4-13B). A seemingly different *Cladophora* sp. was found growing on rocks on the western side of Lake Illawarra (Plate 4-13C). It was similar in appearance to that shown in Plate 4-13A, but featured significantly larger filaments and 2 - 3 branches per node (Plate 4-13D). Cells in the lower thallus are 100 - 150 μm

in diameter, with a cell L/B of 1.5 - 3, while cells in upper branches are 70 - 110 μm in diameter, with cell L/B of 2 - 5 (Plate 4-13D).

4.5.6 Genus *Codium* Stackhouse 1797

Species of *Codium* are epilithic, medium to dark green in colour, and vary greatly in shape, from the flattened or globular and lobed forms, to erect and much-branched varieties (Womersley, 1984). Guiry (2004) listed 100 species of *Codium* worldwide, and Millar and Kraft (1994b) recorded 12 species of *Codium* in New South Wales. In the present study, *Codium* was widely distributed around Lake Illawarra, particularly along rocky shorelines and attached to infrastructure, such as boat ramps. Identification of the species of *Codium* is determined by examining the habit of the plant and the shape of the utricles[25]; these are the swollen ends of cortical branches that form the outer layer of tissue in *Codium* (Womersley, 1984). Based on these morphological features, and previous studies (e.g., McConville, 2000), two species of *Codium* were found in Lake Illawarra during the present study: *Codium fragile* and *Codium harveyi.*

Codium fragile (Suringar) Hariot 1889

Codium fragile is found in cool temperate waters throughout south-eastern Australia, from Victor Harbor, SA, to Ballina, NSW (Womersley, 1984). *C. fragile* was fairly widely distributed around Lake Illawarra during the present study, and it was frequently found in the lower intertidal to subtidal zones, attached to hard substrate (e.g., rocks and shells). The thallus of *C. fragile* in Lake Illawarra is dark green in colour, erect, 5 - 30 cm high, with numerous branches and attached by a round basal disc (Plate 4-14A). *C. fragile* tends to be comparatively robust plants with thicker branches (greater than 3 mm in diameter) than *C. harveyi.* The utricles have mucronate[26] (i.e., pointed) tips, vary in size from 700 - 1450 μm in length and 130 - 400 μm in diameter, and have occasional to frequent hairs (Womersley, 1984; Plate 4-14C).

A number of subspecies of *C. fragile* are currently recognised (Guiry, 2004), including *Codium fragile* subsp. *tomentosoides*, which is considered an invasive pest in Australia, such as Port Phillip Bay and Western Port, Victoria (Campbell, 1999a), and other countries, including New Zealand (Trowbridge, 1995), Canada (Begin and Scheibling, 2003) and the United States (Mathieson *et al.*, 2003). Samples of *C. fragile* collected from Lake Illawarra during the present study appeared to resemble the subspecies *nova-zelandiae*, described in Womersley (1984).

[25] **Utricles**: the swollen, terminal ends of cortical branches that form the outer layer of tissue in *Codium.*

[26] **Mucronate**: (mucro) a short, sharp, pointed structure (Womersley, 1984).

Codium harveyi Silva 1956

Codium harveyi is also found in cool temperate waters of southern Australia, from Shark Bay, WA, to Lake Macquarie, NSW (Womersley, 1984). *C. harveyi* was first recorded in Lake Illawarra by McConville (2000), and was frequently found in Lake Illawarra during the present study, but appeared to be far less abundant than *C. fragile.* It was found in a similar habitat and often in the same location as *C. fragile* during the present study. *C. harveyi* in Lake Illawarra is a medium to dark green colour, erect, 4 - 25 cm high, with numerous branches (less than 4 mm in diameter) and basally attached (Plate 4-14B). The utricles are short, irregularly swollen, with rounded tips, 210 - 260 μm in length, 100 -180 μm in diameter and have frequent hairs (Womersley 1984; Plate 4-14D).

4.5.7 Genus *Bryopsis* Lamouroux 1809

A small filamentous green macrophyte, identified as *Bryopsis* sp. (Dr. A.J.K. Millar, pers. comm., 2001), was found occasionally in this study during winter, covering the underside of rocks, on the western shore of Lake Illawarra (e.g., near the Tallawarra Power Station, Yallah Bay). The species of *Bryopsis* was uncertain, but there are 7 species known from southern Australia (Womersley, 1984) and 3 species from NSW (Millar and Kraft, 1994b). The thallus of *Bryopsis* sp. in Lake Illawarra is 2 - 6 cm in height, epilithic and basally attached, with numerous axes (Plate 4-15A). The axes are 80 - 110 μm in diameter, with frequent lateral branchlets, which are more numerous higher on the axis than below, 20 - 50 μm in diameter, 0.3 - 3.4 mm in length, and elongating progressively towards the base (Plate 4-15B).

4.5.8 Genus *Lamprothamnium* Groves 1916

Lamprothamnium papulosum (Wallroth) Groves 1916

Lamprothamnium is a green macrophyte previously included by some authors (e.g., Womersley, 1984) under the phylum Charophyta, but it is currently accepted as a member of the phylum Chlorophyta (Dr. A.J.K. Millar, pers. comm., 2001). *Lamprothamnium* is generally not found in marine situations, but often occurs in brackish to saline ICOLLs of southern Australia (Womersley, 1984). *L. papulosum* was recorded extensively in Lake Illawarra by Harris (1977), particularly along the Windang Peninsula. During the present study, however, *L. papulosum* was found only occasionally, with the most significant biomass occurring amongst the dense *Ruppia* beds along the Windang Peninsula and Purrah Bay. *L. papulosum* also occurs in a number of South Coast Lakes, and is a preferred food item for black swans (Harris, 1977). The thallus of *L. papulosum* in Lake Illawarra is a light to medium green colour, up to 40 cm in height, erect and basally attached, but often becoming detached and loose-lying (Plate 4-15C). The axes are 2 - 8 mm in diameter, with branchlets 0.5 - 2 cm in length, clustered in a whorl around the axis, at internodes of 1 - 4 cm (Womersley, 1984; Plate 4-15D).

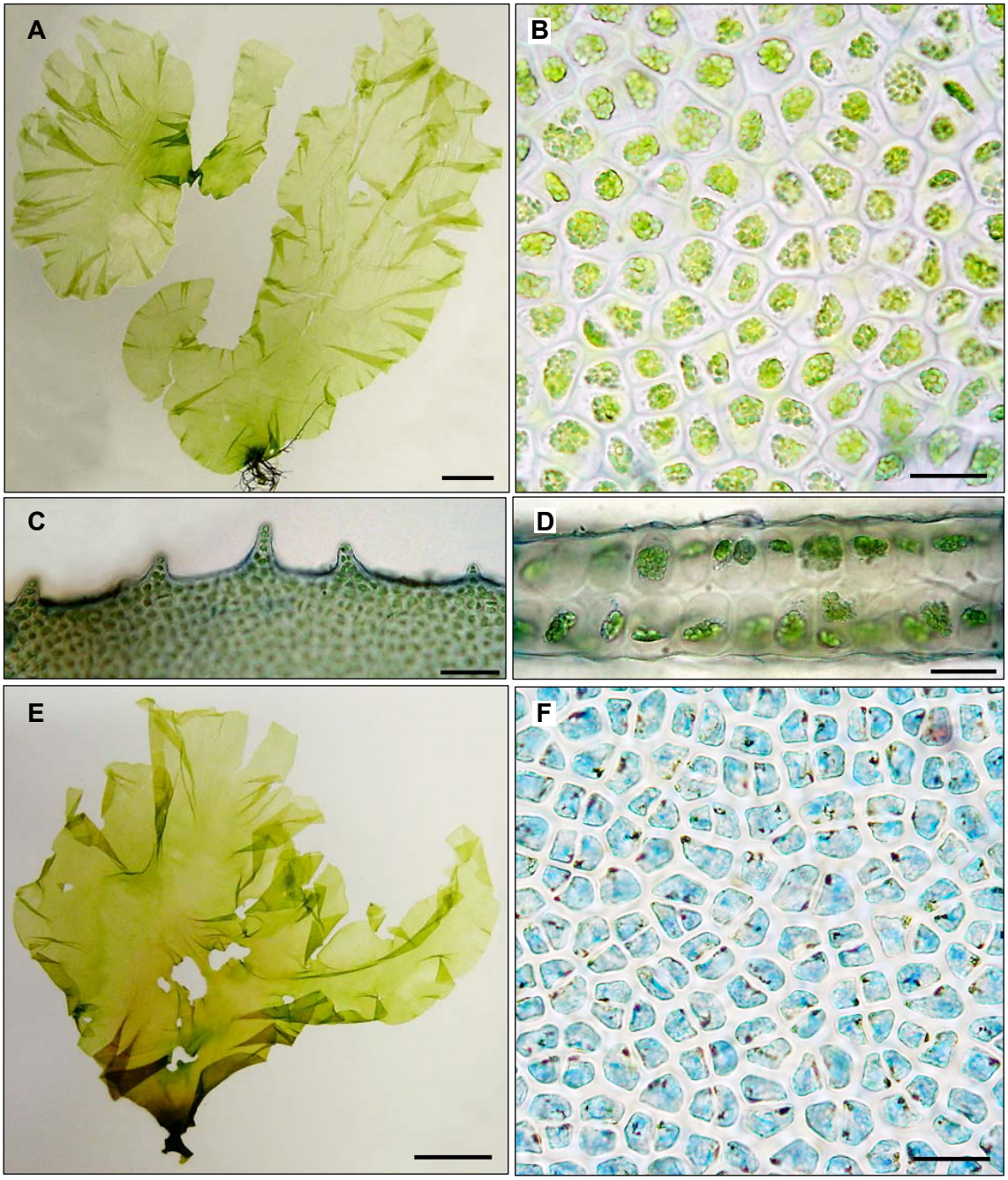


Plate 4-5: (A-D) *Ulva* sp. (Yallah Bay, 23/1/01), tentatively referred to *U. lactuca*. **(A)** Habit. Scale = 2 cm. **(B)** Surface view of cells. Scale = 20 μm. **(C)** Thallus margin with spines. Scale = 200 μm. **(D)** Cross-section through mid-thallus (aniline blue-stained). Scale = 50 μm. **(E-F)** *Ulva* sp. (Tuggerah Bay, 3/9/01), tentatively referred to *U. fasciata*. **(E)** Habit of dried plant. Scale = 1 cm. **(F)** Surface view of cells (aniline blue-stained). Scale = 20 μm.

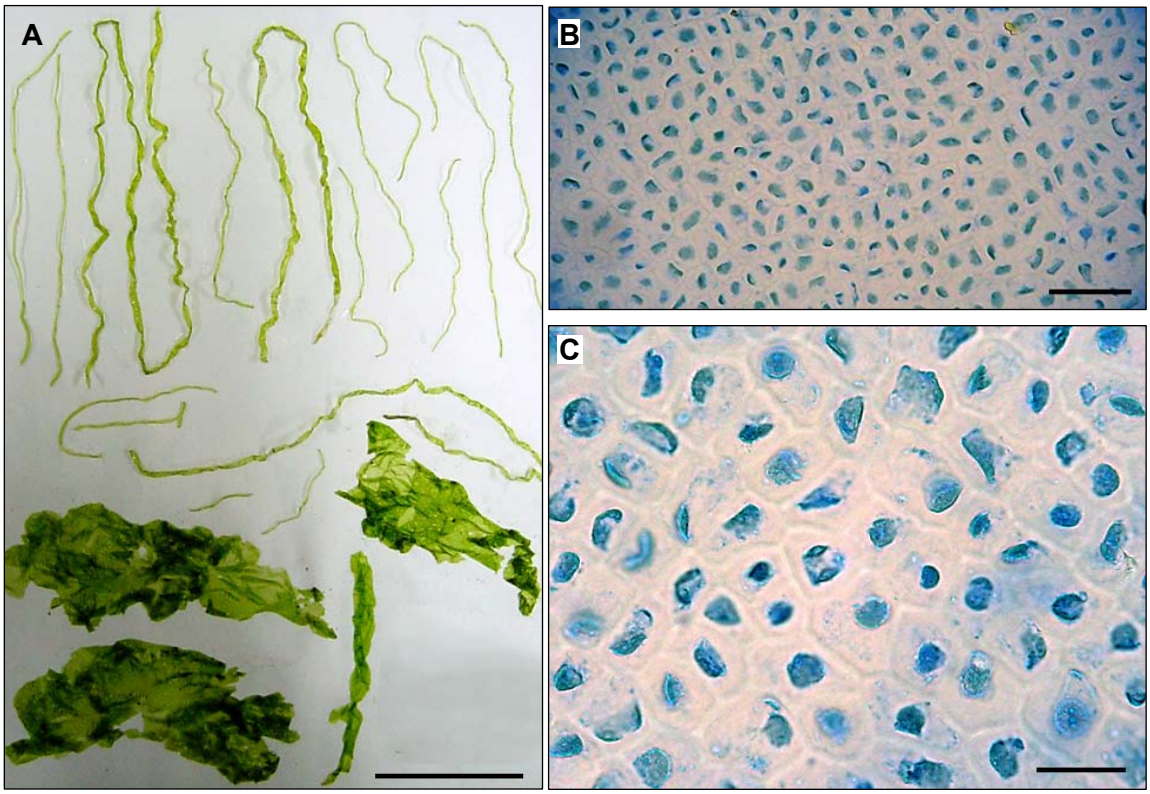


Plate 4-6: *Ulva intestinalis* (Yallah Bay, 8/7/01). **(A)** Habit. Scale = 5 cm. **(B-C)** Surface view of cells (aniline blue-stained). **(B)** Scale = 50 μm. **(C)** Scale = 20 μm.

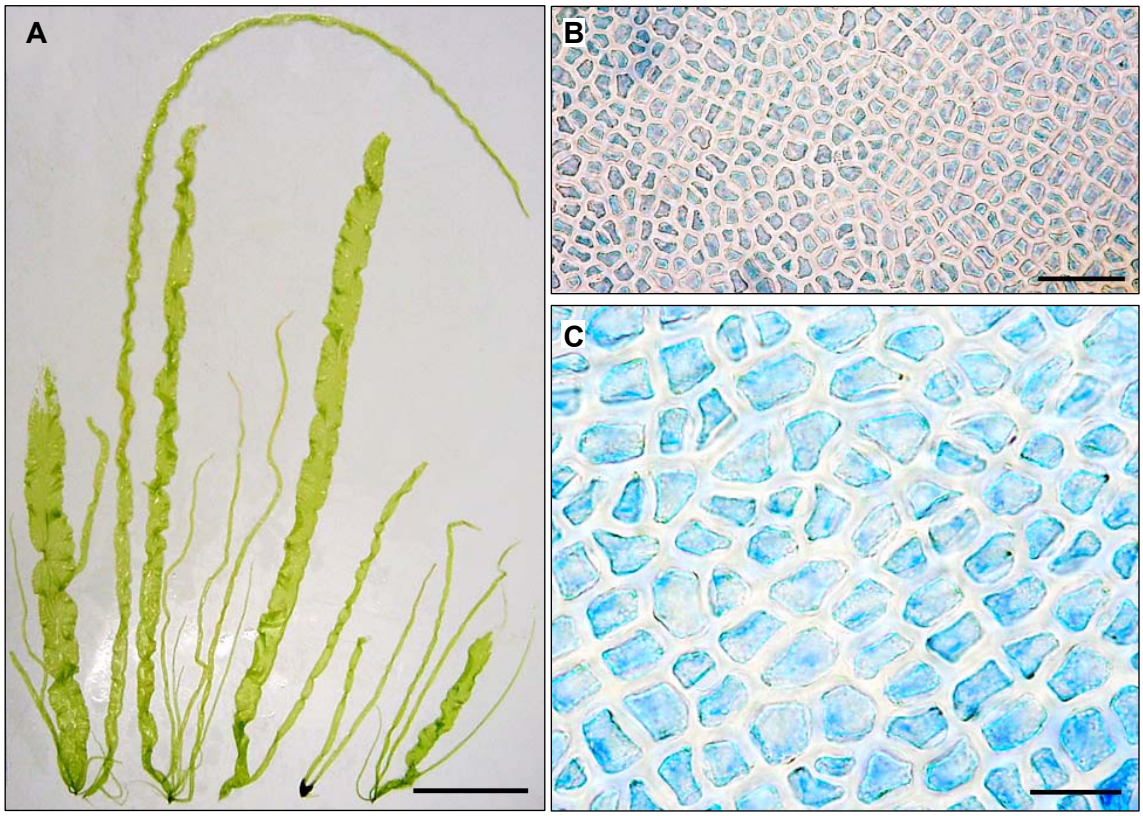


Plate 4-7: *Ulva compressa.* **(A)** Habit (Koonawarra Bay, 2/1/01). Scale = 5 cm. **(B-C)** Surface view of cells (aniline blue-stained) (NIC, 22/6/01). **(B)** Scale = 50 μm. **(C)** Scale = 20 μm.

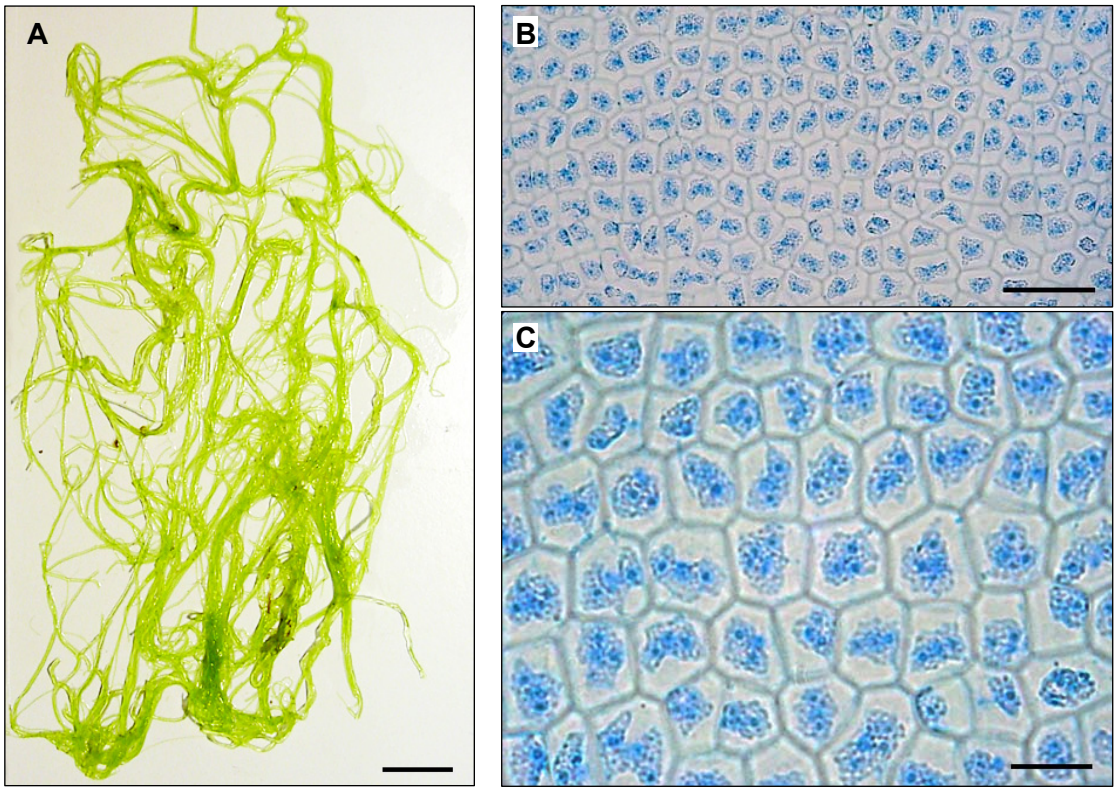


Plate 4-8: *Ulva ralfsii* (PBP, 6/6/01). **(A)** Habit. Scale = 1 cm. **(B-C)** Surface view of cells (aniline blue-stained). **(B)** Scale = 50 μm. **(C)** Scale = 20 μm.

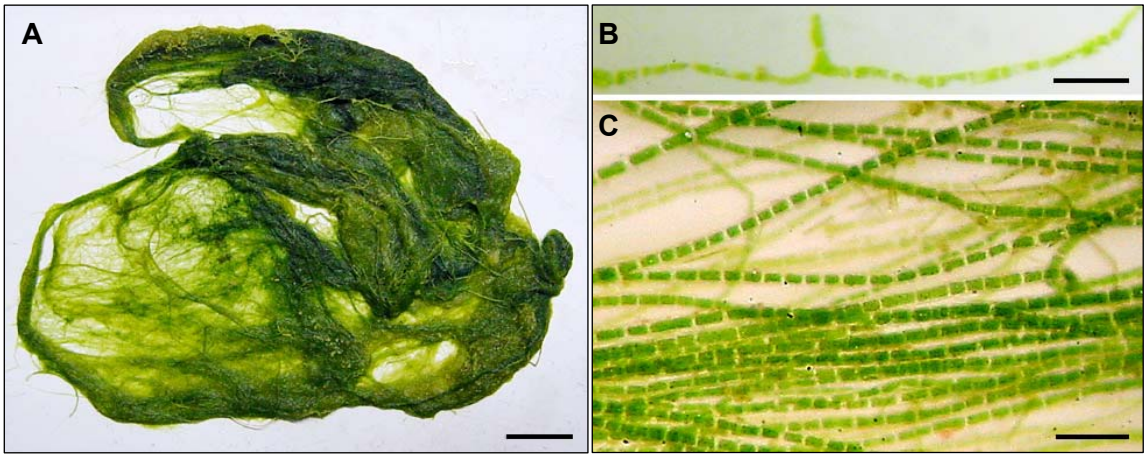


Plate 4-9: *Rhizoclonium riparium* (OCP, 3/2/01). **(A)** Habit. Scale = 1 cm. **(B)** Filament with lateral rhizoid. Scale = 100 μm. **(C)** Unbranched filaments. Scale = 100 μm.

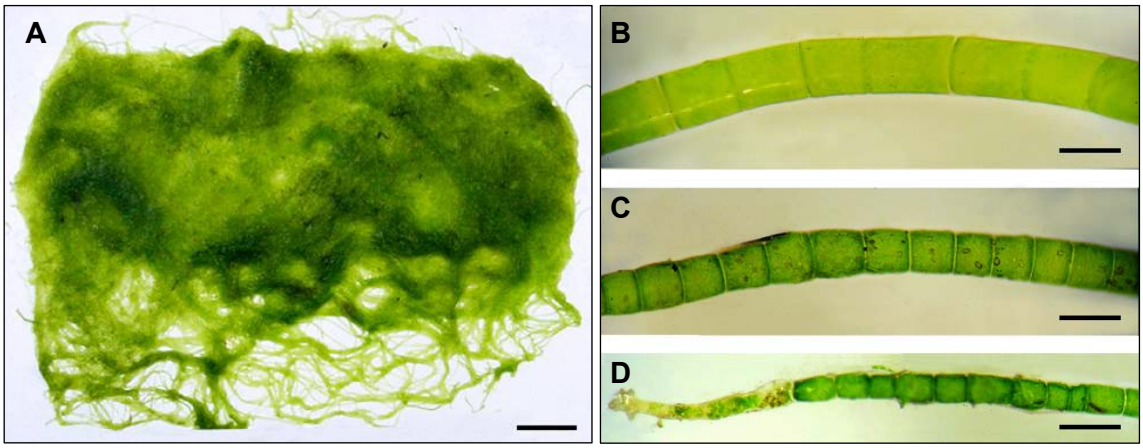


Plate 4-10: *Chaetomorpha aerea.* **(A)** Habit (LIV, 25/11/00). Scale = 2 cm. **(B-D)** Parts of filament (Yallah Bay, 8/7/01). Scale = 200 μm. **(B)** Upper filament. **(C)** Middle of filament. **(D)** Base of filament.

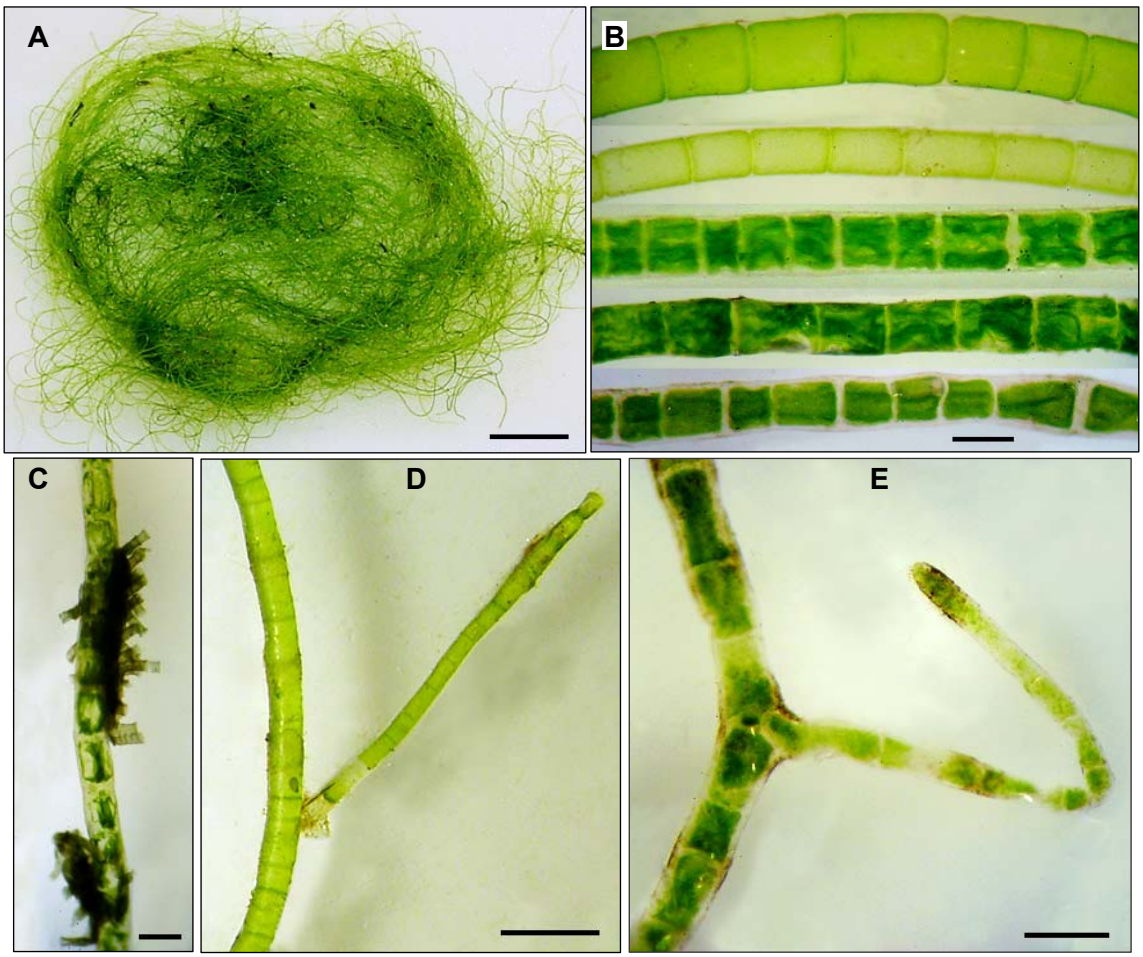


Plate 4-11: *Chaetomorpha linum*. **(A)** Habit (OCP, 23/1/01). Scale = 2 cm. **(B)** Filaments (Windang Peninsula, 20/1/01). Scale = 200 μm. **(C)** Filament with sporing structures (OCP, 25/11/00). Scale = 300 μm. **(D-E)** Filaments with gametangial branches (PBP, 20/5/01). **(D)** Scale = 1 cm. **(E)** Scale = 300 μm.

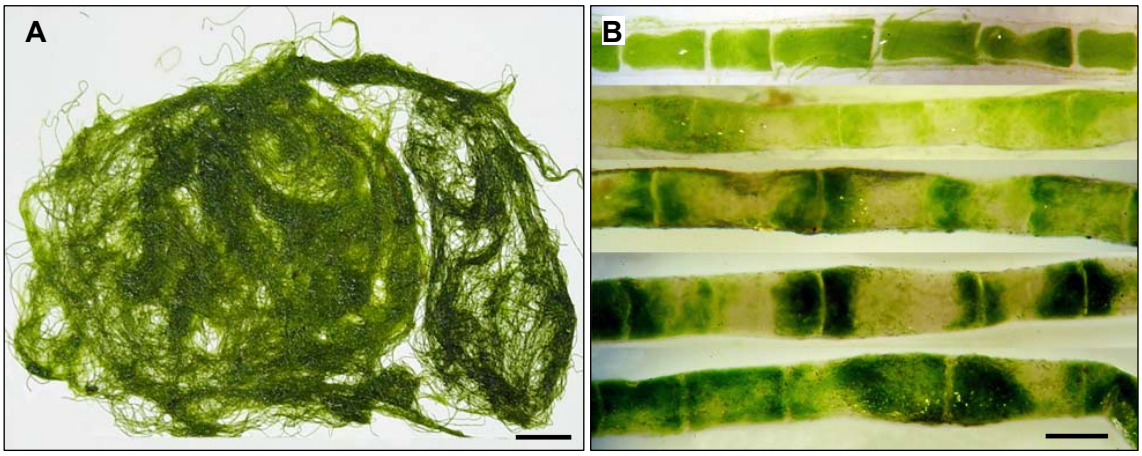


Plate 4-12: *Chaetomorpha billardierii* (OCP, 25/11/00). **(A)** Habit. Scale = 2 cm. **(B)** Parts of filaments. Scale = 200 μm.

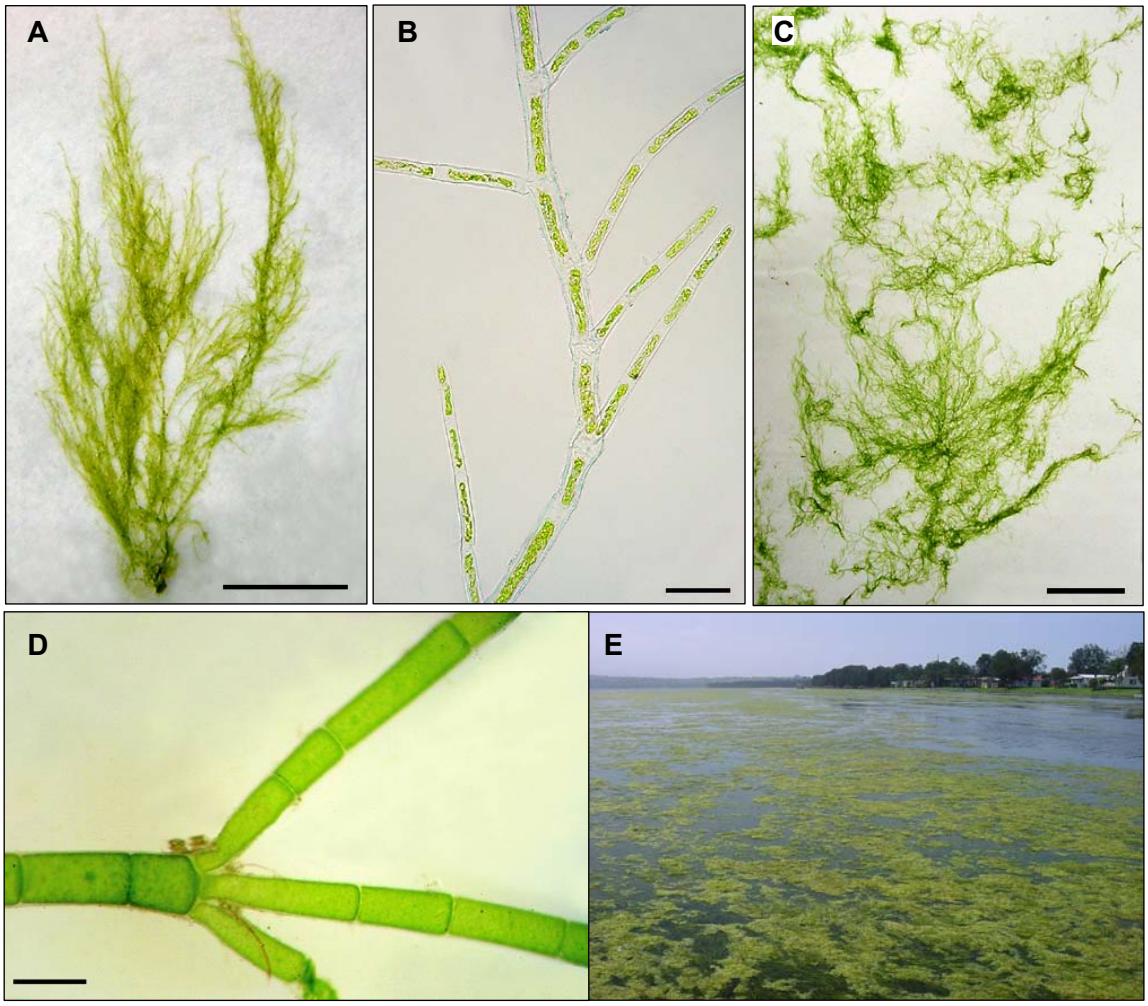


Plate 4-13: (A-B) *Cladophora* sp. (PBP, 25/11/00). **(A)** Habit as an epiphyte. Scale = 5 mm. **(B)** Upper branches. Scale = 50 μm. **(C-D)** *Cladophora* sp. (Yallah Bay, 13/6/01). **(C)** Habit. Scale = 2 cm. **(D)** Upper branches. Scale = 200 μm. **(E)** Bloom of *Cladophora* over *Ruppia* beds near the Oasis Caravan Park, Windang Peninsula (19/3/02).

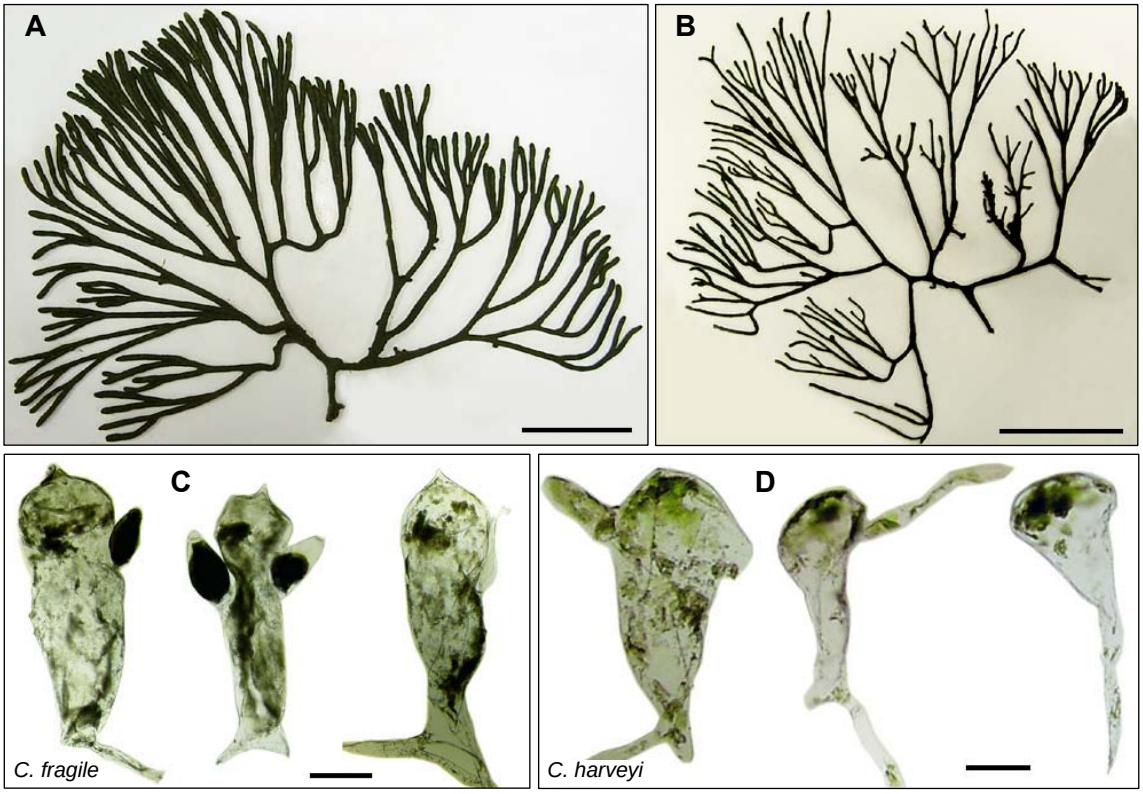


Plate 4-14: (A) *Codium fragile* (Tuggerah Bay, 6/6/01). Scale = 5 cm. **(B)** *Codium harveyi* (Tuggerah Bay, 6/6/01). **(C-D)** Utricles with gametangia. **(C)** *Codium fragile.* Scale = 200 μm. **(D)** *Codium harveyi.* Scale = 100 μm.

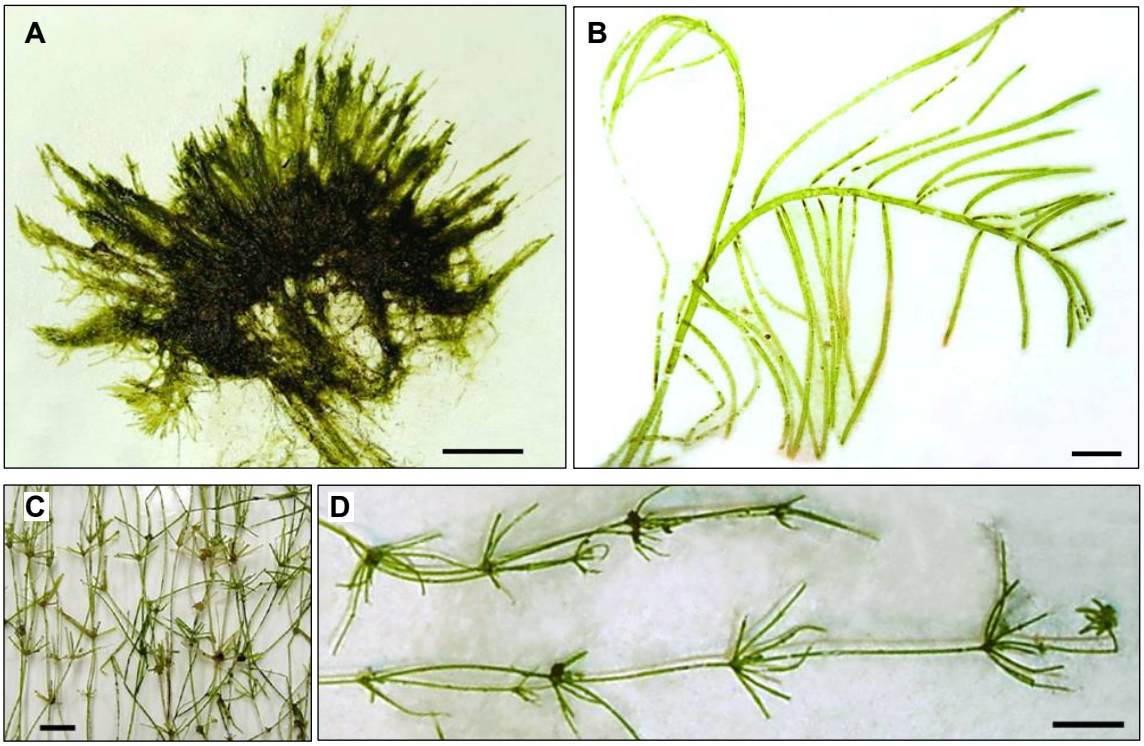


Plate 4-15: (A-B) *Bryopsis* sp. (Yallah Bay, Lake Illawarra, 8/7/01). **(A)** Habit. Scale = 1 cm. **(B)** Scale = 500 μm. **(C-D)** *Lamprothamnium papulosum* (OCP, 3/9/01). Scale = 1 cm. **(C)** Habit. **(D)** Part of branch.

4.6 Results and Discussion (III): Description of Brown Macroalgae (Phaeophyta) in Lake Illawarra

Like the green algae, the brown algae are highly variable in morphology, from tufted and filamentous to solid or hollow, globose[27], crustose[28], or vesicular[29]. The thallus can range in size from microscopic to several metres in length, with the giant kelps, such as *Macrocystis*, attaining lengths of up to 60 metres (Womersley, 1987). The Phaeophyta are a common group of approximately 265 genera and 1,500 species (Womersley, 1987), with 139 species listed in New South Wales alone (Millar and Kraft, 1994a). The brown seaweeds are a valuable natural resource; for example, kelps, such as *Laminaria*, *Durvillaea potatorum* and *Macrocystis pyrifera* have been harvested as a source of alginates, which were used as stabilisers and thickeners in many dairy products and cosmetics (Womersley, 1984). Brown macroalgae that have been recorded in Lake Illawarra from previous studies and the present study are listed in Table 4-7. The most abundant brown macroalgae in Lake Illawarra are of the order Fucales: the large seaweeds *Sargassum* and *Cystoseira*. Many of the brown algae appeared to have a seasonal distribution in Lake Illawarra, with the Ectocarpales (*Hincksia* and *Ectocarpus*) and Scytosiphonales (*Petalonia, Scytosiphon* and *Colpomenia*) found predominately during winter and autumn. The following sections describe and illustrate 9 species, from 9 genera, of benthic brown macroalgae from Lake Illawarra. Note that all plates are listed at the end of this sub-section.

4.6.1 Genus *Ectocarpus* Lyngbye 1819

Ectocarpus siliculosus (Dillwyn) Lyngbye 1819

E. siliculosus is found throughout Lake Illawarra, and was particularly common during autumn and winter in the present study, often forming small blooms over seagrass beds. The thallus of *E. siliculosus* in Lake Illawarra is a medium to yellowish-brown colour and 2 - 12 cm high (Plate 4-16A). *E. siliculosus* was found attached to rocks, epiphytic on seagrasses and macroalgae, or occasionally found detached and free-floating during the present study. The base is attached by rhizoids and branching is irregular and prolific from near the base. Lower filaments are 25 - 50 μm wide, with branchlets 8 - 16 μm wide. The cells have a length by breadth ratio of 0.8 - 2.2, several phaeoplasts[30] that are elongate, ribbon-like and simple or branched, with several pyrenoids (Womersley, 1987). The sporangia are plurilocular[31] and pedicellate[32], narrow to

[27] **Globose**: spherical or globular in shape.

[28] **Crustose**: forming a thin crust over the substrate.

[29] **Vesicular**: with vesicles - sac-like structures, partly gas-filled, used for flotation, especially in kelps.

[30] **Phaeoplast**: the photosynthetic plastid of brown algae (Womersley, 1987).

[31] **Plurilocular**: with many cells, each cell containing a single spore.

[32] **Pedicellate**: growing on a cellular stalk.

elongate in shape, 60 - 130 μm in length and 13 - 17 μm in diameter, tapering towards the apices (Plate 4-16B).

4.6.2 Genus *Hincksia* Gray 1864

Hincksia sp.

Hincksia sp. was reasonably common in Lake Illawarra during the present study, predominantly found during winter, growing as epiphytes on macroalgae, seagrass, or on rocks. *Hincksia* is very similar in appearance to *Ectocarpus* and microscopic examination of both species is generally required to differentiate the two. The thallus of *Hincksia* sp. in Lake Illawarra is a medium to dark brown colour, 1 - 3 cm high, and much branched (Plate 4-17A). Filaments are of fairly uniform diameter throughout, 7 - 20 μm in diameter, with a cell length by breadth ratio of 0.7 - 1.4 (Plate 4-17A). The cells contain numerous discoid pyrenoids, with one pyrenoid per phaeoplast (Womersley, 1987; Plate 4-17B). *Hincksia* sp. has plurilocular sporangia that are typically sessile[33] (Plate 4-17C), 60 - 125 μm long and 10 - 30 μm in diameter.

4.6.3 Genus *Scytosiphon* Agardh 1820

Scytosiphon lomentaria (Lyngbye) Link 1833

In the present study, *Scytosiphon lomentaria* was often found in Lake Illawarra during the cooler seasons, growing attached to rocks, and very occasionally as epiphytes on macroalgae (e.g., *Sargassum* and *Cystoseira*). The thallus is a medium to dark brown colour, 5 - 40 cm high, with one to several erect fronds extending from the small discoid holdfast. The fronds are 1 - 3 cm in diameter, hollow to terete (i.e., cylindrical and tapering), and often constricted at intervals, broadening from the discoid holdfast and tapering towards the apices (Womersley, 1987; Plate 4-18A).

4.6.4 Genus *Petalonia* Derbès *et* Solier 1850

Petalonia fascia (Müller) Kuntze 1898

Petalonia fascia was also found very occasionally in Lake Illawarra during autumn and winter in the present study, growing as an epiphyte on *Sargassum* and *Zostera*. The thallus is a light to medium brown colour, 2 - 10 cm high and 0.5 - 2.5 cm wide, with one or more fronds arising from a small discoid holdfast (Plate 4-18B). A transverse section through the thallus (not shown) of Lake Illawarra specimens fits the description of *P. fascia* in Womersley (1987). Yassini (1985) also recorded the presence of *Endarachne binghamiae*, which is very similar in morphological

[33] **Sessile**: without a stalk.

appearance to *Petalonia fascia*. In the present study, however, *E. binghamiae* was either not present, or could not be distinguished from *P. fascia* in the field.

4.6.5 Genus *Colpomenia* (Endlicher) Derbès *et* Solier 1851

Colpomenia peregrina (Sauvageau) Hamel 1937

In the present study, *Colpomenia peregrina* was found predominantly during the cooler seasons. It was a relatively common epiphyte of *Zostera* and larger macroalgae in Lake Illawarra, particularly at locations close to the Lake's entrance to the sea (e.g., the Windang Peninsula). The thallus of *C. peregrina* in Lake Illawarra is a light brown colour, membranous, globular or lobed and 0.5 - 6 cm in width (Plate 4-18C). The sori[34] spread across the thallus surface, with scattered hair groups, as per the description of Womersley (1987).

4.6.6 Genus *Cystoseira* Agardh 1820

Cystoseira trinodis (Forsskål) Agardh 1820

Cystoseira trinodis, formerly referred to *Cystophyllum onustum* (Womersley, 1987), was very common throughout Lake Illawarra during the present study, particularly around rocky foreshores. The thallus of *C. trinodis* in Lake Illawarra is a medium brown colour, 20 - 50 cm high, much branched and typically attached to hard substrate, such as rocks or shells. The thallus is divided into one to several stipes[35] from the discoid-conical holdfast, with primary branches up to 50 cm in length. It has numerous lesser branchlets, with basal flattened and leaf-like ramelli[36], 2 - 5 mm long and 0.3 - 1 mm in diameter (Plate 4-18D). Ramelli bear small ovoid vesicles, 0.8 - 1.5 mm in width and 1.5 - 3.5 mm in length, formed within the ramelli, usually in chains of 2 - 3. Receptacles[37] form at the ends of the ramelli, 0.5 - 1 mm in width and 1.5 - 5 mm in length, simple or occasionally once branched (Womersley, 1987; Plate 4-18E).

4.6.7 Genus *Sargassum* Agardh 1820

Sargassum sp.

Sargassum is a common genus, with over 300 species currently listed worldwide (Guiry, 2004), including approximately 25 species recorded in New South Wales (Millar and Kraft, 1994a). Taxonomic classification of *Sargassum* is difficult and requires mature, fertile specimens, with

[34] **Sori**: (singular = sorus) cluster of reproductive organs, occurring as a surface patch or raised group.

[35] **Stipe**: the stalk, between the holdfast and the fronds, or bearing primary branches.

[36] **Ramelli**: lesser or ultimate branchlets.

[37] **Receptacle**: the branch which holds the reproductive organs (Womersley, 1987).

basal parts present (Womersley, 1987). *Sargassum* occurred frequently throughout Lake Illawarra during the present study, typically along rocky shorelines, and often in association with *Cystoseira trinodis*. *Sargassum* found in the Lake during the present study could not be identified to species level, and was thus referred to *Sargassum* sp. The thallus of *Sargassum* sp. in Lake Illawarra is a medium to dark brown colour, much branched and 10 - 40 cm in length (Plate 4-19A). The stipe is simple or branched, 1 - 3 cm long and 1 - 2 mm in diameter, with 1 - 3 primary branches of up to 35 cm in length. The lateral "leaves" are lanceolate[38], serrate[39] and costate[40], 1 - 2.5 cm long and 1.5 - 6 mm wide. The vesicles are axillary[41], petiolate[42] and subspherical, 2 - 4 mm in diameter and mutic[43] (Plate 4-19B).

4.6.8 Genus *Hormosira* (Endlicher) Meneghini 1838

Hormosira banksii (Turner) Decaisne 1842

Hormosira banksii is a distinctive alga found occasionally in drift[44] during the present study, usually within 2 km of the Lake's entrance to the sea. *H. banksii* therefore may not be native to Lake Illawarra, but its presence is worth noting. The thallus of *H. banksii* is typically yellowish-brown to almost black in colour and 5 - 30 cm high, with one to several fronds from a small discoid holdfast. It is composed of branched chains of vesicular segments, subspherical to ovoid in shape and 0.4 - 1 cm in diameter, connected by narrow internodes (Womersley, 1987; Plate 4-19C).

4.6.9 Genus *Ecklonia* Hornemann 1828

Ecklonia radiata (Agardh) Agardh 1848

Ecklonia radiata was occasionally found in drift along the eastern Lake Illawarra foreshores during the present study, but these large plants typically inhabit wave-exposed coastlines (Edgar, 2000), rather than estuaries; therefore, specimens of *E. radiata* found during the present study are likely to have originated from outside the Lake. *E. radiata* plants found in Lake Illawarra were 0.5 - 1 m long, a medium to dark brown colour, with a single long, simple stipe, bearing a flattened blade and several laterals (Plate 4-19D). The lateral branches are narrower

[38] **Lanceolate**: tapering from a rounded base to an apex.

[39] **Serrate**: marginally toothed.

[40] **Costate**: with a midrib or central thickening of the branch.

[41] **Axillary**: situated at an angle from the axis.

[42] **Petiolate**: stalked leaves or vesicles.

[43] **Mutic**: without a terminal point (Womersley, 1987).

[44] **Drift**: refers to plants found free-floating or entangled amongst macrophyte beds, moved by wind or currents.

towards their base, simple or lobed, often with marginal spines and a smooth or corrugate (i.e., rippled) surface (Womersley, 1987).

4.6.10 Genus *Phyllospora* Agardh 1839

Phyllospora comosa (Labillardière) Agardh 1839

During the present study, large, detached sections of *Phyllospora comosa* were found periodically washed up on the Lake's foreshores, from the entrance channel to approximately 4 km from the Lake's entrance. It has also been recorded from Lake Illawarra by King *et al.* (1990), but these large plants may have been washed into the Lake through the entrance, rather than growing in the Lake itself. Plants of *P. comosa* found in Lake Illawarra are 0.6 - 1.5 m in length, yellowish-brown to medium brown in colour, with a single stipe bearing several long, flattened branches (Plate 4-19E). The primary branches bear numerous long, simple, linear laterals, often with marginal spines. Vesicles are scattered along the primary branches, forming at the base of some laterals, and are elongate-ovoid in shape, often with a short apical leaflet (Womersley; Plate 4-19F).

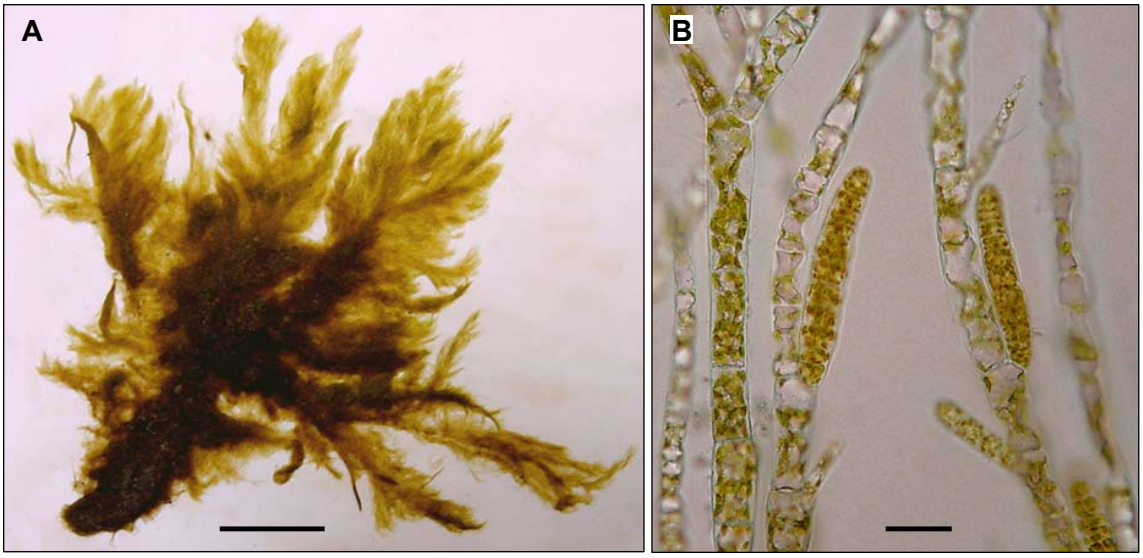


Plate 4-16: *Ectocarpus siliculosus.* **(A)** Habit (Purrah Bay, 20/5/01). Scale = 2 cm. **(B)** Narrow plurilocular sporangia. Scale = 50 μm.

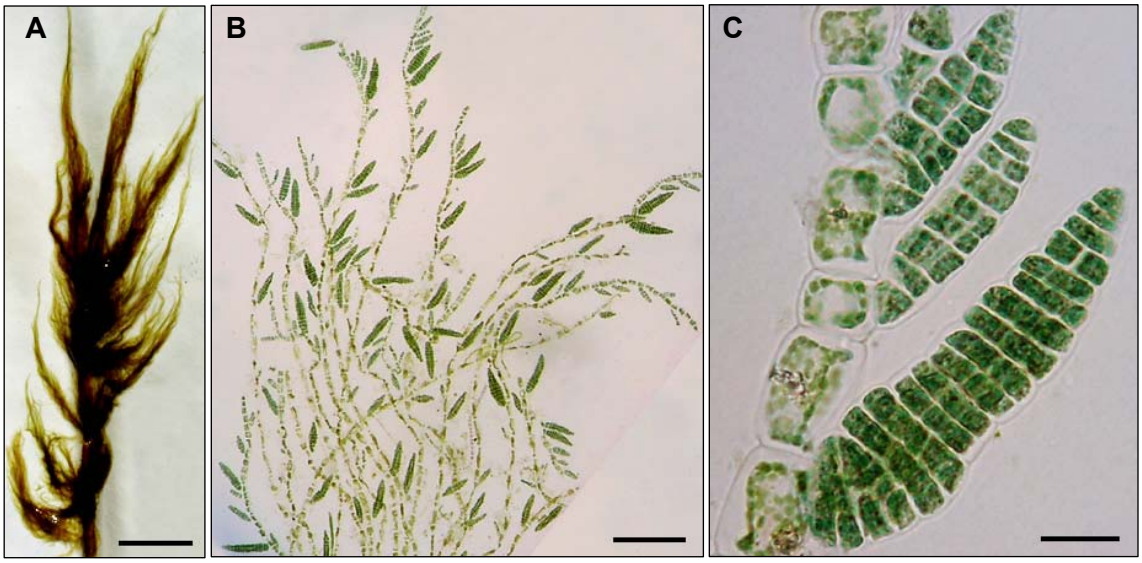


Plate 4-17: *Hincksia* sp. (PBP, 6/6/01). **(A)** Habit, epiphytic on *Zostera*. Scale = 2 cm. **(B)** Branches with sporangia. Scale = 200 μm. **(C)** Plurilocular sporangia. Scale = 20 μm.

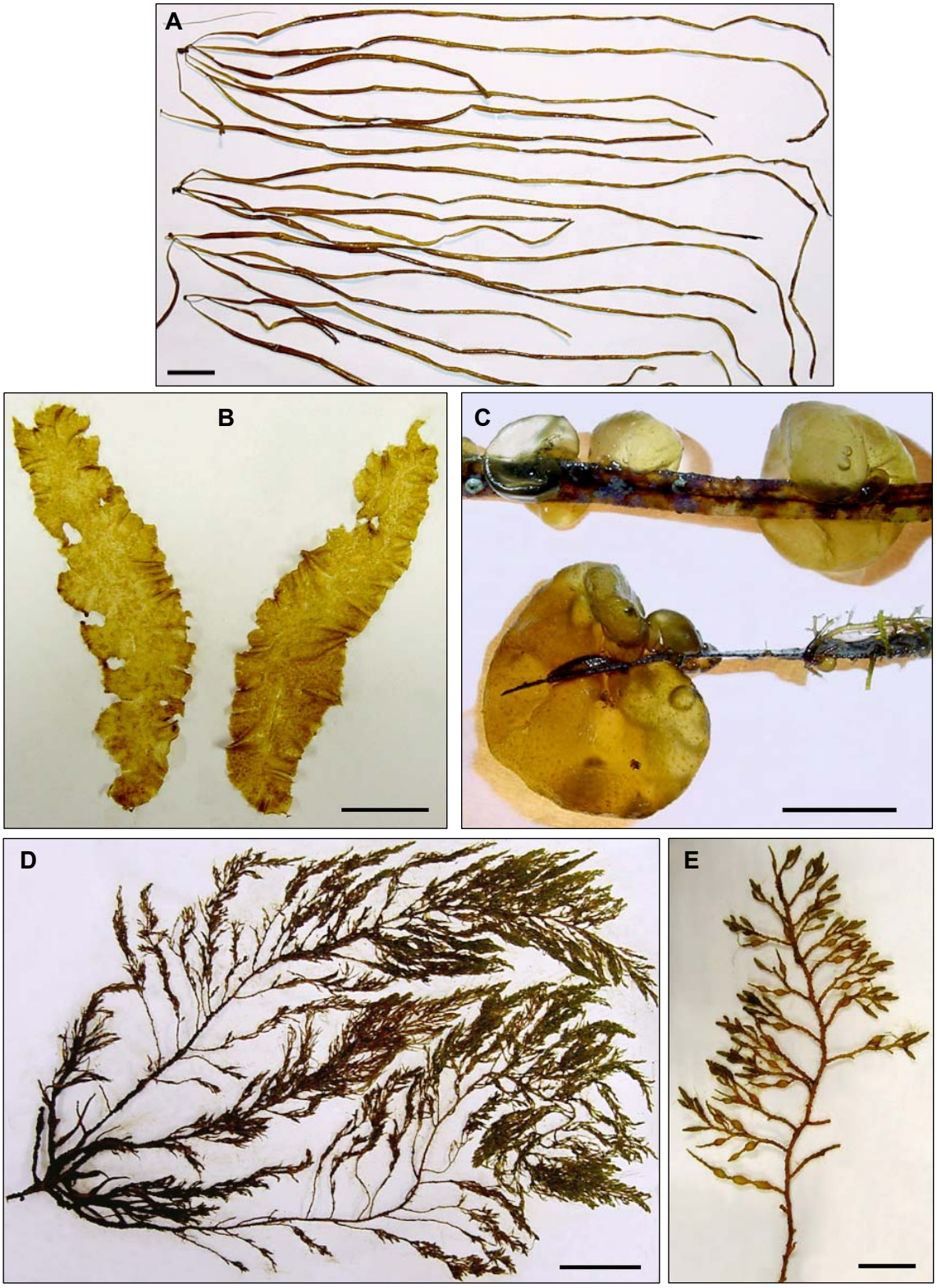


Plate 4-18: (A) *Scytosiphon lomentaria* (Yallah Bay, 4/8/01). Scale = 2 cm. **(B)** *Petalonia fascia* (Yallah Bay, 8/7/01). Scale = 2 cm. **(C)** *Colpomenia peregrina* (NIC, 4/8/01). Scale = 1 cm. **(D-E)** *Cystoseira trinodis* (Yallah Bay, 8/7/01). **(D)** Habit. Scale = 5 cm. **(E)** Upper branch with vesicles and receptacles. Scale = 1 cm.

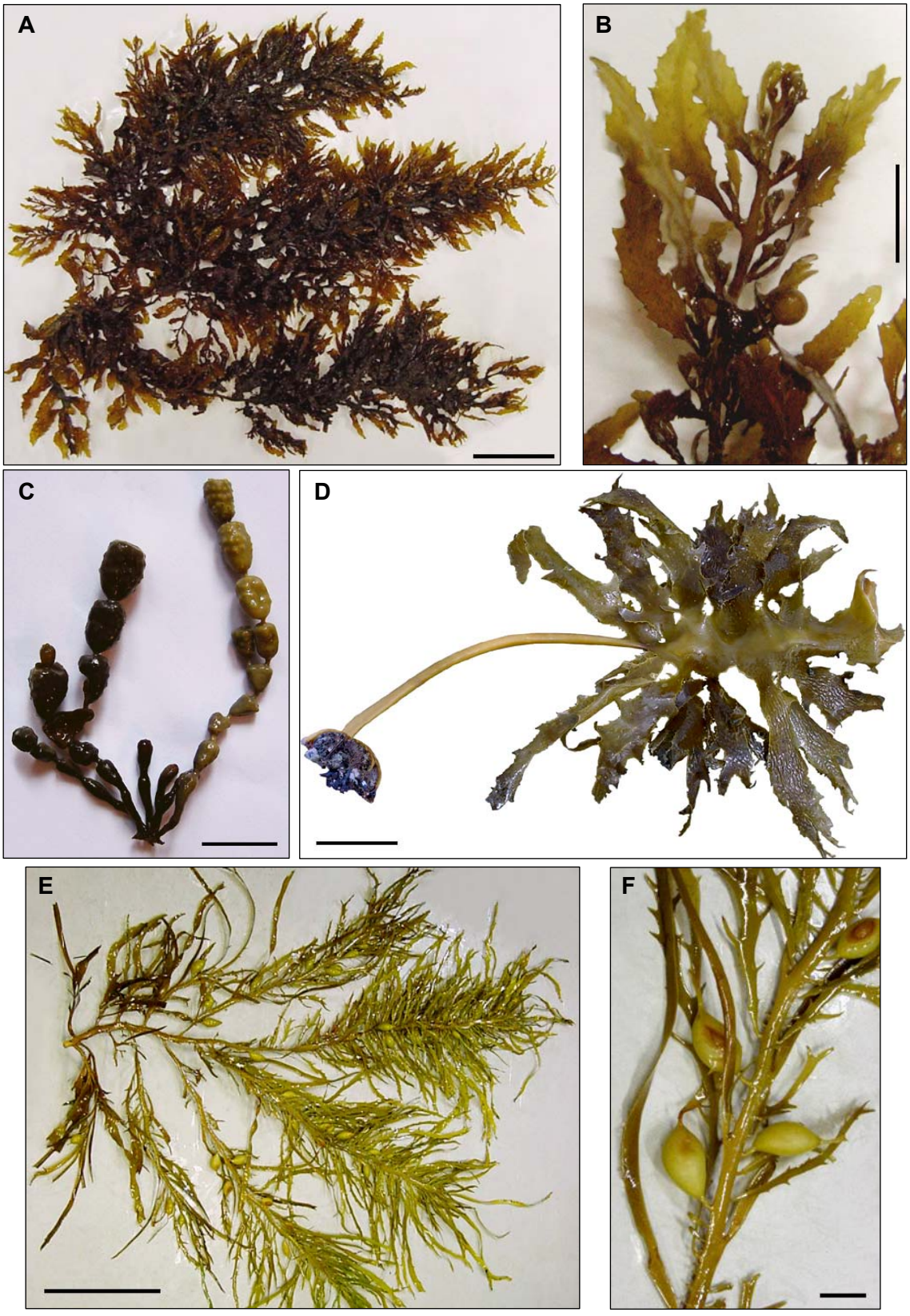


Plate 4-19: (A-B) *Sargassum* sp. (Yallah Bay, 8/7/01). **(A)** Habit. Scale = 5 cm. **(B)** Part of upper branch. Scale = 1 cm. **(C)** *Hormosira banksii* (drift, Lake Illawarra entrance channel, 20/7/01). Scale = 2 cm. **(D)** *Ecklonia radiata* (drift, Lake Illawarra entrance channel, 19/3/01). Scale = 10 cm. **(E-F)** *Phyllospora comosa* (drift, Lake Illawarra entrance channel, 17/1/01). **(E)** Habit. Scale = 20 cm. **(F)** Part of branch. Scale = 2 cm.

4.7 Results and Discussion (IV): Description of Red Macroalgae (Rhodophyta) in Lake Illawarra

The Rhodophytes are an abundant group of 650 - 700 genera, and over 4,000 species (Womersley, 1984). The eastern Australian coastline is particularly diverse in terms of Rhodophytes, with 174 genera and 381 species listed from New South Wales and Lord Howe Island (Millar and Kraft, 1993), while 284 genera and 800 species have been recorded from southern Australia (Womersley, 1994). Red algae are characterised by the dark red colour of the thallus, particularly from deep water collections, although material collected from shallow or intertidal regions is often bleached and yellow-brown in colour (Womersley, 1994). The plants vary from a few millimetres to a metre in length and they are predominately epilithic or epiphytic, but rarely free-floating or considered nuisance species. They typically feature some degree of branching, and are also found in a variety of forms, from filamentous to membranous, parenchymatous [45] or pseudoparenchymatous [46], cylindrical to compressed or inflated, or crustose (Womersley, 1994). Red algae, such as *Gelidium, Gracilaria* and *Pterocladia*, were harvested extensively (e.g., from Botany Bay) during the past century as a source of carrageenan and agar, which was used for a variety of scientific, commercial and industrial purposes (Wood, 1946; Womersley, 1984).

Red macroalgae were found throughout Lake Illawarra during the present study, attached to rocks, growing amongst seagrass beds, or as epiphytes on seagrass and other macroalgae. The following sections describe and illustrate 8 species from 8 different genera, including 6 newly recorded species, of benthic red macroalgae from Lake Illawarra. Note that all plates are listed at the end of this section.

4.7.1 Genus *Hypnea* Lamouroux 1813

Hypnea is a relatively common genera, with 47 species listed worldwide (Guiry, 2004), and 6 species from New South Wales (Millar and Kraft, 1993). *Hypnea* was found frequently in Lake Illawarra during the present study and all material examined appeared to belong to a single species, identified as *Hypnea boergesenii* (Dr. A.J.K. Millar, pers. comm., 2001). McConville (2000) also listed *Hypnea spinella* from Lake Illawarra, but this species was not found during the present study. *H. spinella* is distinguished from *H. boergesenii* by its much smaller size (thallus height up to 1.5 cm) and its habit, forming entangled, recumbent mats (Millar, 1990).

[45] **Parenchymatous:** the tissue of the thallus, composed of thin-walled cells of roughly equal dimensions, formed by cell division in different planes.

[46] **Pseudoparenchymatous:** resembling a parenchymatous structure, due to compactly interwoven filaments, but cell division has not occurred in all planes (Womersley, 1994).

Hypnea boergesenii Tanaka 1941

H. boergesenii was widely distributed around Lake Illawarra during the present study, found growing on rocks or epiphytically on larger macroalgae. It is probable that this species is the same as that listed in past studies (e.g., King *et al.*, 1997) as *Hypnea valentiae*, which is similar in appearance to *H. boergesenii*, but lacks the short lateral branchlets in the lower part of the axes (Millar, 1990). The thallus of *H. boergesenii* in Lake Illawarra is typically less than 15 cm high, erect, with a discoid holdfast, frequent branching, and with numerous short, lateral branchlets (Plate 4-20A, B). A cross-section through the axis shows prominent central axial cells encompassed by six larger periaxial cells (Plate 4-20E; refer to Millar, 1990, for full description).

4.7.2 Genus *Gracilaria* Greville 1830

Gracilaria is a widespread and common genus, with more than 160 species currently listed worldwide (Guiry, 2004), many of which are poorly defined (Withell *et al.*, 1994; Womersley, 1996). *Gracilaria* is a commonly used sea vegetable, the major source of food-grade agar, and has also been used as a source of research chemicals (e.g., agarose) and pharmaceutical chemicals (e.g., antiviral and anti-tumour compounds) (refer to Steentoft *et al.*, 1995, and references therein). In Australia, 21 species of *Gracilaria* are recognised (Withell *et al.*, 1994), including 8 species recorded from NSW (Millar and Kraft, 1993). The *Gracilaria* examined from Lake Illawarra during the present study appeared to belong to a singular species, identified as *Gracilaria edulis* (Dr. A.J.K. Millar, pers. comm., 2001).

Gracilaria edulis (Gmelin) Silva 1952

Gracilaria was found frequently throughout Lake Illawarra during the present study, occurring on a range of habitats, from rock platforms to sand flats, and amongst seagrass beds. However, *Gracilaria* is generally not considered a nuisance species in Lake Illawarra. As some confusion exists when attempting to distinguish between *G. edulis* and *G. verrucosa* (Withell *et al.*, 1994), it is possible that the *G. edulis* collected from Lake Illawarra represents the same plants previously referred to *Gracilaria verrucosa* by Yassini (1985) and King *et al.* (1997). Additionally, the form of *Gracilaria* in Lake Illawarra during the present study are similar to May's (1948) and Harris' (1977) descriptions of *G. confervoides* f. *ecortica*, which may be considered a synonym of *G. verrucosa* (Withell *et al.*, 1994).

G. edulis found in Lake Illawarra during the present study varies in colour from a medium red to brown when growing in deeper water, becoming bleached and yellow-red when found in shallow water. The thallus is typically erect, up to 50 cm in height and epilithic, with a small discoid holdfast (Plate 4-20D). Branching is irregular, with branches usually less than 1 mm in diameter, cylindrical and tapering towards the ends. A cross-section through the main axis showed a pseudoparenchymatous[46] structure, with cells up to 200 μm in diameter, gradually decreasing in

diameter towards the outer edge (Plate 4-20F). Cystocarps[47], when present, were prominent along the branches, less than 1 mm in diameter, sessile (i.e., without a stalk), subspherical and slightly basally constricted (Plate 4-20G).

4.7.3 Genus *Spyridia* Harvey 1833

Spyridia filamentosa (Wulfen) Harvey 1833

Spyridia filamentosa was identified using the descriptions of Womersley (1998), and was a common epiphyte of *Zostera* and *Ruppia* in Lake Illawarra during the present study. The thallus is a red to yellow-brown colour and less than 5 cm high (Plate 4-21A). Although *S. filamentosa* was only observed as a small seagrass epiphyte in Lake Illawarra, Womersley (1998) recorded this species from southern Australia as both epiphytic and epilithic, attaining a height of 7 - 18 cm. The axes of *S. filamentosa* specimens found in Lake Illawarra are 300 - 500 μm in diameter. Branching is irregular, occurring frequently on all sides of the axes, and tapering (Plate 4-21B). Segments on the branches are well-defined, with alternating bands of short nodal cells and longer internodal cells (Plate 4-21C). The ramelli (i.e., ultimate branches) are usually arranged spirally around the axis, with only one ramellus per segment (Plate 4-21B,C). The ramelli are up to 30 cells and 0.2 - 1.2 mm long, 25 - 45 μm wide, tapering to a mucronate (i.e., short and pointed) end cell, and with nodal bands 2 - 3 cells broad (Plate 4-21D).

4.7.4 Genus *Ceramium* Roth 1797

Ceramium sp.

Ceramium is a genus of approximately 170 species worldwide (Guiry, 2004), with 14 species recorded for New South Wales (Millar and Kraft, 1993). Past studies on Lake Illawarra (e.g., Harris, 1977; Yassini, 1985) had listed *Ceramium* sp. as a common seagrass epiphyte. As only two small specimens of *Ceramium* sp. were found during the present study, however, it could not be identified to species level. The thallus of *Ceramium* sp. in Lake Illawarra is less than 1 cm high, a reddish-brown colour, and epiphytic on *Zostera*. The axes are 80 - 100 μm in diameter, branching is irregularly subdichotomous[48], and the apices are tapering and involute[49] (Plate 4-22A). The genus *Ceramium* is distinguished by the rounded periaxial[50] and cortical[51] cells,

[47] **Cystocarps**: the reproductive vesicle-like structures in red macroalgae, which contain the carposporophyte and the pericarp (surrounding tissue) (Womersley, 2003).

[48] **Subdichotomous**: mostly dividing into two fairly equal sections near the apices.

[49] **Involute**: rolled inwards.

[50] **Periaxial cell**: surrounding an axial cell.

[51] **Cortical**: the outer layer of cells of a thallus, usually of smaller cells.

forming successive nodes of corticated bands, separated by internodal ecorticate[52] bands (Womersley, 1998; Plate 4-22A-C). The cortical bands of *Ceramium* sp. found in Lake Illawarra are 45 - 70 μm long, and 4 - 5 cells long (Plate 4-22C). Internodal spaces are present throughout the thallus and are approximately the same length as the nodal bands. Tetrasporangia[53] protrude from the cortex and are involucrate[54] (Plate 4-22C), with 1 - 3 sporangia per node, and are 35 - 50 μm in diameter, subspherical to ovoid, and have a persistent sheath. Further descriptions of the genus *Ceramium* in southern Australia are provided in Womersley (1998).

4.7.5 Genus *Centroceras* Kützing 1841

Centroceras clavulatum (Agardh) Montagne 1846

C. clavulatum was found frequently throughout Lake Illawarra in this study, as an epiphyte of *Zostera*, *Ruppia*, *Halophila* and larger macroalgae. The thallus of *C. clavulatum* in Lake Illawarra is a dark red to brown colour, 1 - 3 cm high, tufted and filamentous (Plate 4-23A). The axial filaments are 80 - 120 μm in diameter, branching is subdichotomous (i.e., mostly divided into fairly equal parts at the apex; Plate 4-23B), and the apices roll inwards, with short spines directed away from the axis (Womersley, 1998; Plate 4-23C). The periaxial and cortical cells are rectilinear, forming roughly parallel lines of rectangular cells (Plate 4-23C). Fertile material of *C. clavulatum* was not found in Lake Illawarra; refer to Womersley (1998) for further structural and reproductive information on *C. clavulatum*.

4.7.6 Genus *Polysiphonia* Greville 1823

Polysiphonia is a cosmopolitan and abundant genus, occurring on most of the coasts throughout the world (Womersley, 1979), and with more than 200 species currently listed worldwide (Guiry, 2004). New South Wales and Lord Howe Island have been credited with 17 species (Millar and Kraft, 1993), while 26 species have been recorded for southern Australia (Womersley, 1979, 2003). Several past studies (e.g., Harris, 1977, Yassini, 1985, King *et al.*, 1997) have documented the frequent occurrence of *Polysiphonia* sp. in Lake Illawarra, but the species of *Polysiphonia* was unknown prior to the present study. In this study, *Polysiphonia* was the most abundant of the Lake Illawarra epiphytes, found throughout the year attached to seagrasses, larger macroalgae, or rock. Samples of *Polysiphonia* with all reproductive features present were identified as *Polysiphonia sphaerocarpa* (Dr. A.J.K. Millar, pers. comm., 2001). Epilithic forms of *Polysiphonia*, up to 10 cm in height (Plate 4-23D), were also found frequently

[52] **Ecorticate**: without a cortex (the outer layer of small cells of a thallus).

[53] **Tetrasporangia**: a meiosporangium (i.e., structures for spores formed as a result of meiosis) with four spores, usually in a distinctive arrangement.

[54] **Involucrate**: basally surrounded by cortical filaments of a few cells (Womersley, 1998, 2003).

in Lake Illawarra. It is possible that this larger form of *Polysiphonia* is a different oligosiphonous[55] species of *Polysiphonia*, as *P. sphaerocarpa* is generally described as less than 2 cm high (Millar, 1990). However, the presence of more than one species of *Polysiphonia* could not be established as fertile material was not found for the larger form.

Polysiphonia sphaerocarpa Bøergesen 1918

The following description applies only to the small, tufted, epiphytic form of *P. sphaerocarpa* found in Lake Illawarra. These epiphytic forms were typically less than 2 cm in height, red to yellow-brown in colour, and filamentous with prolific branching (Plate 4-23E). The erect axes are 140 - 200 μm in diameter, arising from a discoid holdfast with digitate[56], unicellular rhizoids (Millar, 1990; Plate 4-23F). The branches are characterised by four pericentral cells (Plate 4-23F). Branching is alternate to irregular and is often dichotomous[57] near the apices (Plate 4-23H). Trichoblasts[58] are abundant and up to 500 μm in length (G, H). The cystocarps are spherical to ovoid, up to 420 μm in diameter, occurring frequently on the main axes and curving towards the axis (Plate 4-23G). Tetrasporangia are 40 - 90 μm in diameter, occurring in the swollen upper branches (Plate 4-23H). Spermatangial branches are a branch of a trichoblast near the apices, and terminated by a conspicuous sterile cap cell (Millar, 1990; Plate 4-23I).

4.7.7 Genus *Chondria* Agardh 1817

Chondria sp. has been recognised in Lake Illawarra by previous authors, such as Harris (1977) and McConville (2000), who also listed *Chondria lanceolata* from the Windang Peninsula. The most abundant form of *Chondria* in Lake Illawarra during the present study was identified as *Chondria angustissima* (Dr. A.J.K. Millar, pers. comm., 2001). Limited specimens resembling *C. lanceolata* were occasionally found along the Windang Peninsula during the present study, but these samples were insufficient for formal identification (Plate 4-24F, G). *C. lanceolata* is distinguished from *C. angustissima* by its slightly wider branches, with the lower branches compressed, 500 - 800 μm in diameter, and apices attenuated (i.e., gradually tapering; Womersley, 2003).

Chondria angustissima Gordon-Mills *et* Womersley 1987

C. angustissima is typically found in calm water habitats, such as estuaries and lagoons, attached to *Gracilaria*, *Zostera* or rock (Gordon-Mills and Womersley, 1987). In the present

[55] **Oligosiphonous**: with four pericentral cells (Millar, 1990).

[56] **Digitate**: branches resembling the fingers of a hand.

[57] **Dichotomous**: divided into two distinct parts.

[58] **Trichoblasts**: the hair-like filaments produced near the branch apices.

study, *C. angustissima* occurred attached to hard substrate in various locations around Lake Illawarra, or in loose-lying entangled masses. It was most commonly found as an epiphyte on *Zostera*, *Ruppia* and larger macroalgae, particularly along the Windang Peninsula *Ruppia* beds. The thallus of *C. angustissima* in Lake Illawarra is 1 - 6 cm high, a red-brown to yellowish colour, slender and much-branched, separating into one to several axes from the discoid holdfast (Plate 4-24A). The branches are spreading and often intertwined, terete, with axes 300 - 600 μm in diameter. Lesser branches are 250 - 350 μm in diameter, and ultimate branchlets are 150 - 300 μm in diameter (Plate 4-24B). Tetrasporangia are 60 - 100 μm in diameter and formed in the swollen lesser branches (Plate 4-24C). The spermatangial plates are irregularly discoid, up to 200 μm across (Plate 4-24D), and the plate margin contains both fertile and sterile cells (Gordon-Mills and Womersley, 1987). Cystocarps are ovoid, 300 - 500 μm in diameter, with a short stalk and a pointed basal spur (Womersley, 2003; Plate 4-24E).

4.7.8 Genus *Grateloupia* Agardh 1822

Grateloupia filicina (Lamouroux) Agardh 1822

Grateloupia was found periodically in Lake Illawarra during the present study. It was commonly found attached to rocks in shallow, sheltered bays, such as Yallah Bay, but was also found detached and free-floating along the eastern shoreline of the Lake. The species shown in Plate 4-25 was identified as *Grateloupia filicina* (Dr. A.J.K. Millar, pers. comm., 2001), and the following description is adapted from that of *G. filicina* in Womersley (1994). The thallus of *G. filicina* in Lake Illawarra was typically a dark red to reddish-brown colour, with a soft and mucilaginous[59] texture, and height varying from 10 - 50 cm (Plate 4-25A). It features a discoid holdfast, with the thallus separated into one or more fronds from the holdfast. The main axes are compressed, 1 - 3.2 mm in width, tapering upwards from the holdfast and narrowing towards the apices. Proliferous branchlets extend from the surface of the main axes, with numerous ramuli, 0.1 - 1 mm wide and 1 - 20 mm long (Plate 4-25A, B). A cross-section (Plate 4-25C, D) through the main axis shows a cortex (i.e., outer cell layer) of up to 8 cells wide, with elongate outer cells, 4 - 6.5 μm long and with a length by width ratio of 1.5 - 4. Inner cells of the cortex are largely ovoid, 4.5 - 6.5 μm long, and dispersing towards the medulla[60]. The medulla is composed of numerous intertwined and irregularly spaced filaments. Other Rhodophyte species, similar in appearance to *G. filicina*, have been recorded in Lake Illawarra in previous years. For example, King *et al.* (1990) recorded *Gelidium* sp. in Pithungnar Bay, King *et al.* (1997) listed *Pterocladia* sp. from Pithungnar Bay, and WBM (1998) recorded *Gelidiopsis variabilis* in Griffins Bay. These species may also exist in Lake Illawarra, but it is also possible that these records were actually *Grateloupia filicina*, due to the similarity in appearance.

[59] **Mucilaginous**: slimy, with surface mucilage.

[60] **Medulla**: central region of the axis, internal to the cortex (Womersley, 1994).

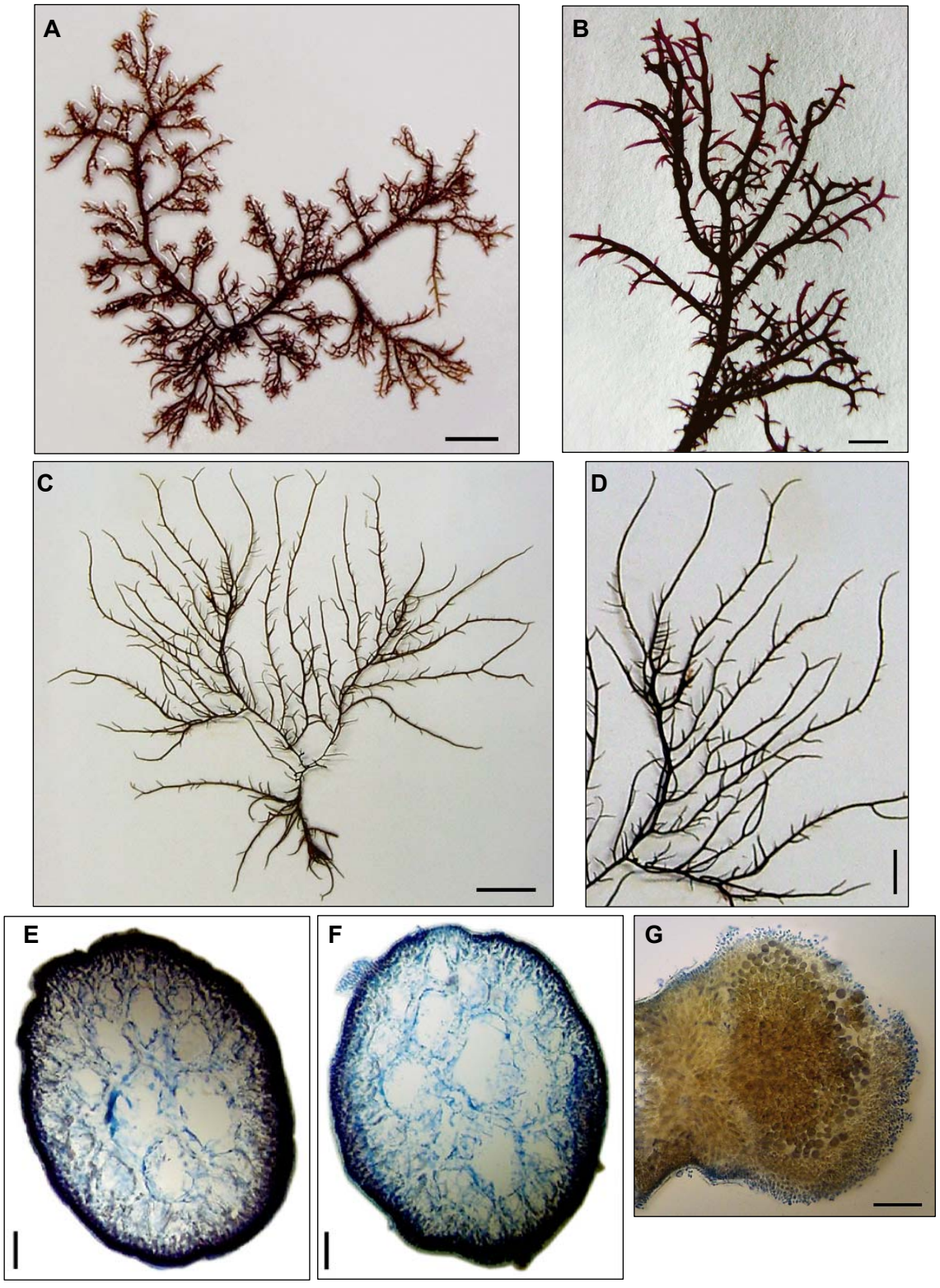


Plate 4-20: (A-B, E) *Hypnea boergesenii* (Yallah Bay, 23/1/01). **(A)** Habit. Scale = 1 cm. **(B)** Branch of dried plant. Scale = 2 mm. **(E)** Cross-section through main axis (aniline blue-stained). Scale = 100 μm. **(C-D, F-G)** *Gracilaria edulis* (Yallah Bay, 23/1/01). **(C)** Habit. Scale = 2 cm. **(D)** Upper branches. Scale = 1 cm. **(F)** Cross-section through main axis (aniline blue-stained). Scale = 100 μm. **(G)** Cross-section through cystocarp. Scale = 150 μm.

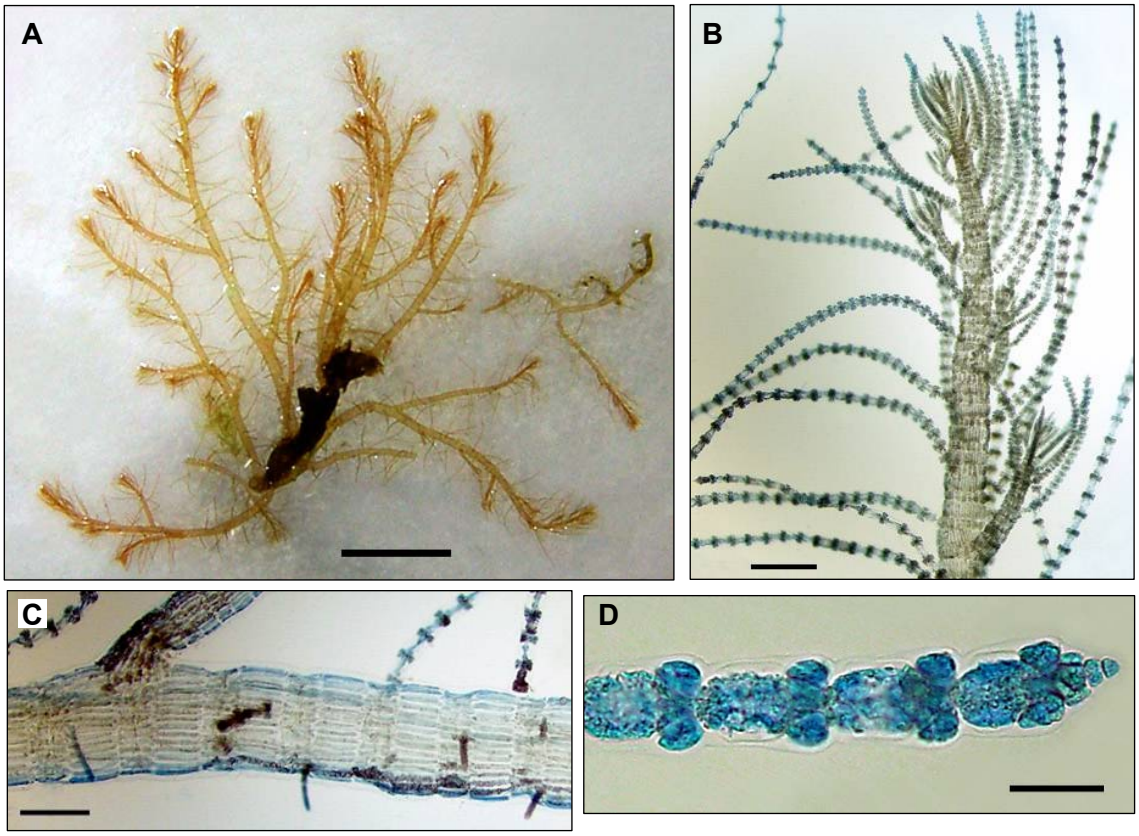


Plate 4-21: *Spyridia filamentosa* (PBP, 25/11/00). **(A)** Habit, epiphytic on *Zostera.* Scale = 3 mm. **(B)** Part of upper branch (aniline blue-stained). Scale = 200 μm. **(C)** Mid-section of branch, showing nodal bands (aniline blue-stained). Scale = 150 μm. **(D)** Apex of a ramellus (aniline blue-stained). Scale = 30 μm.

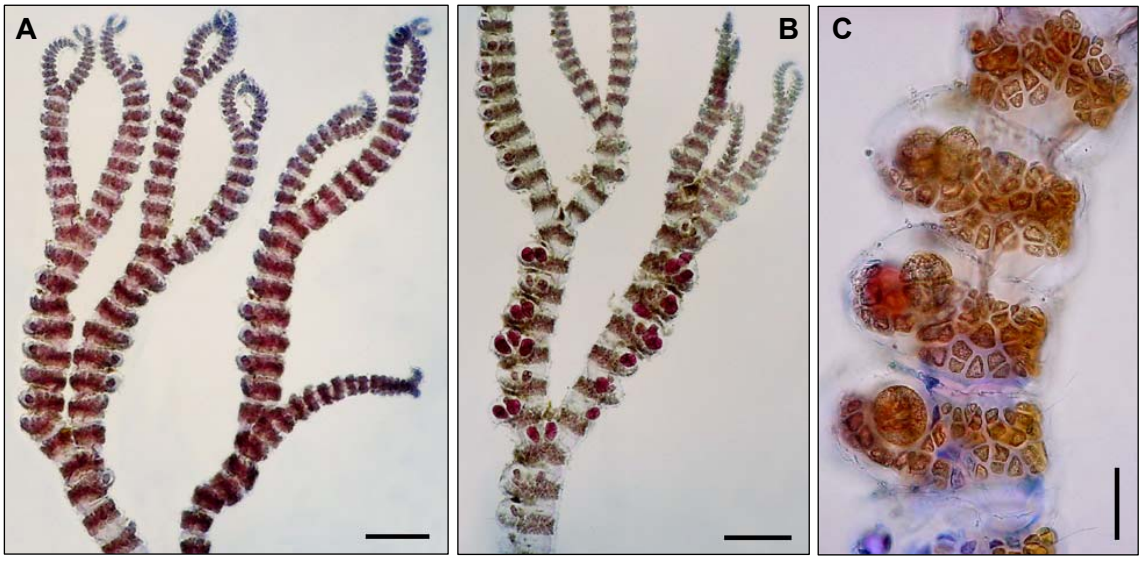


Plate 4-22: *Ceramium* sp. (Yallah Bay, 19/3/02). **(A)** Habit of upper branch (aniline blue-stained). Scale = 150 μm. **(B)** Branch with tetrasporangia. Scale = 200 μm. **(C)** Nodes with involucrate tetrasporangia. Scale = 50 μm.

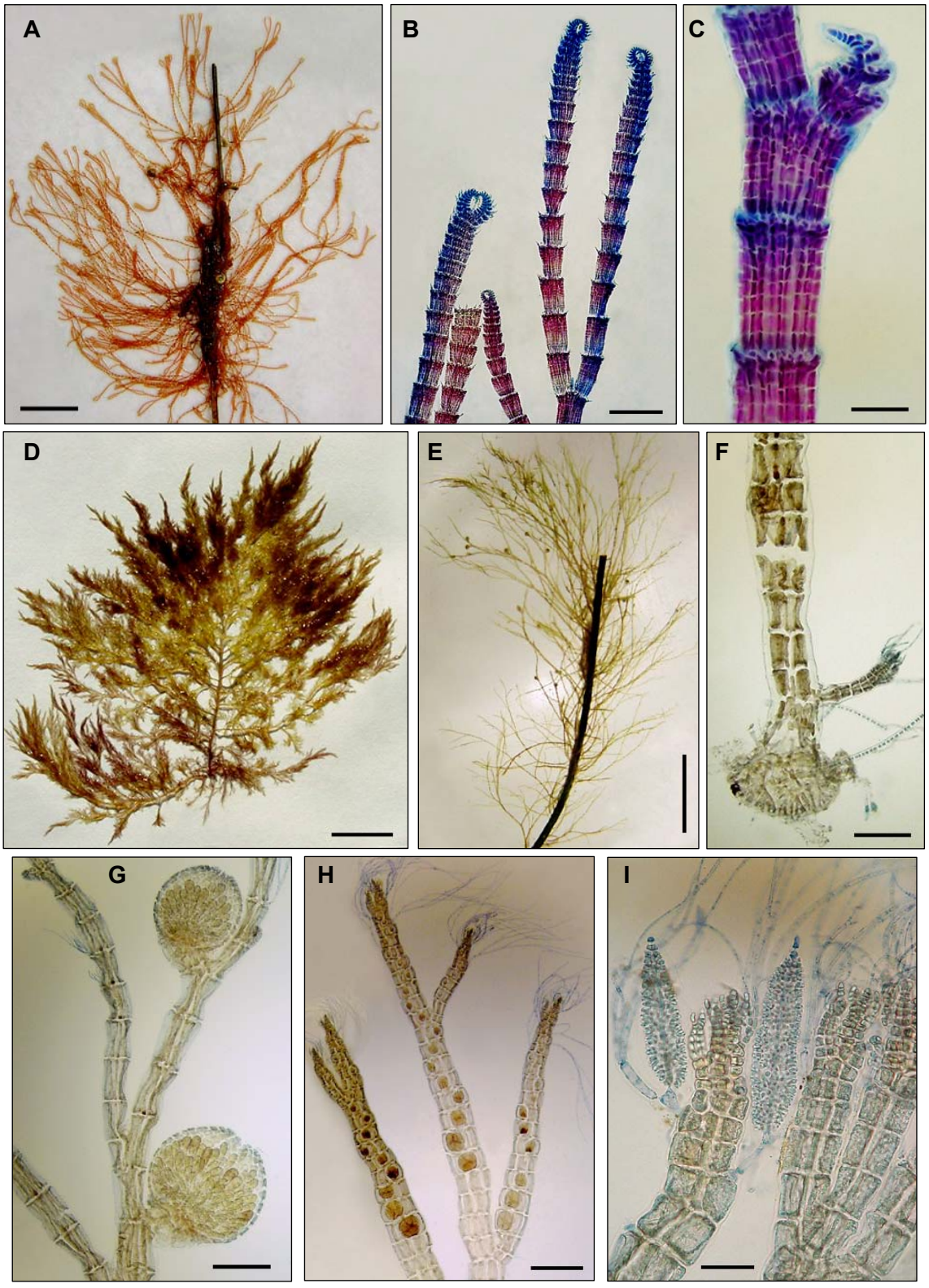


Plate 4-23: (A-C) *Centroceras clavulatum* (Purry Burry Point, 25/11/00). **(A)** Habit, epiphytic on *Ruppia*. Scale = 5 mm. **(B)** Habit of upper branches (aniline blue-stained). Scale = 150 μm. **(C)** Enlarged view of upper branch. Scale = 50 μm. **(D)** *Polysiphonia* sp., habit when epilithic (Yallah Bay, 8/7/01). Scale = 2 cm. **(E-I)** *Polysiphonia sphaerocarpa,* epiphytic on *Zostera* (Yallah Bay, 6/6/01). **(E)** Habit. Scale = 5 mm. **(F)** Discoid holdfast. Scale = 150 μm. **(G)** Mature cystocarps (aniline blue-stained). Scale = 200 μm. **(H)** Tetrasporangial branches (aniline blue-stained). Scale = 200 μm. **(I)** Spermatangial branches with sterile tips (aniline blue-stained). Scale = 50 μm.

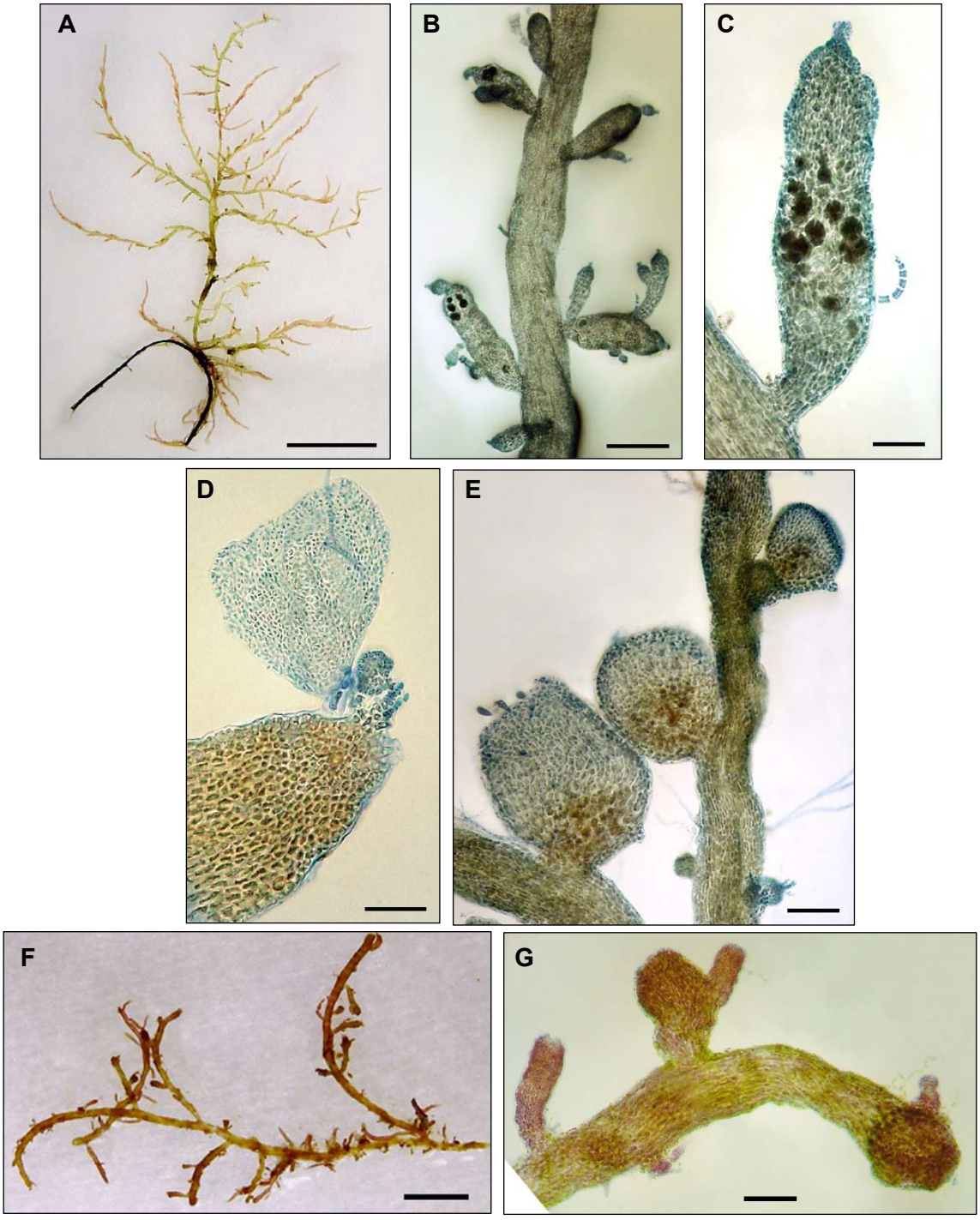


Plate 4-24: (A-E) *Chondria angustissima* (LIV, 25/11/00). **(A)** Habit, epiphytic on *Ruppia.* Scale = 1 cm. **(B)** Branches with tetrasporangia (aniline blue-stained). Scale = 200 μm. **(C)** Tetrasporangial branch with acute apex (aniline blue-stained). Scale = 150 μm. **(D)** Spermatangial plate (aniline blue-stained). Scale = 50 μm. **(E)** Cystocarps with short basal spur (aniline blue-stained). Scale = 150 μm. **(F-G)** Unidentified Rhodophyta, probably *Chondria* sp. (Lake Illawarra Village, 11/8/01). **(F)** Habit, epiphytic on *Ruppia.* Scale = 2 mm. **(G)** Part of branch. Scale = 200 μm.

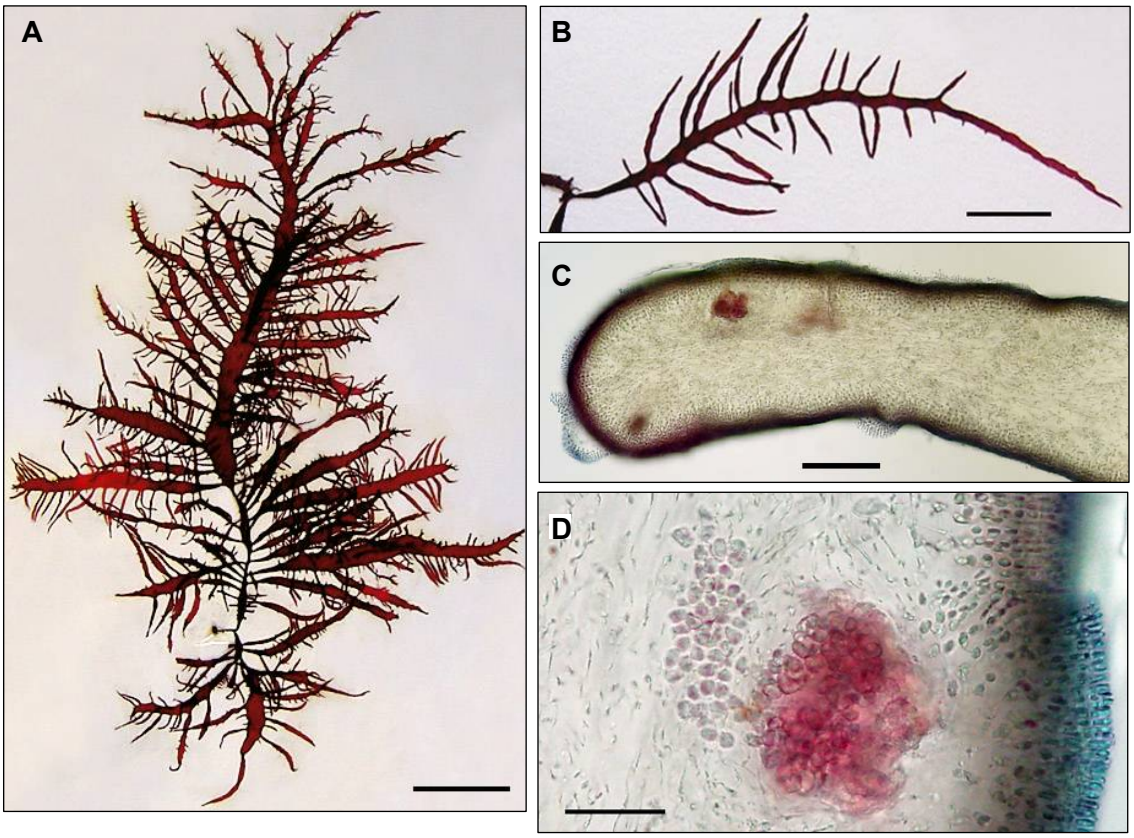


Plate 4-25: *Grateloupia filicina* (Yallah Bay, 3/9/01). **(A)** Habit. Scale = 2 cm. **(B)** Habit of branchlet. Scale = 5 mm. **(C-D)** Cross-section through main axis, with developing carposporophyte. Scale = 50 μm (aniline blue-stained). **(C)** Scale = 100 μm. **(D)** Scale = 50 μm.

4.8 Conclusions

During the present study of the macrophytes in Lake Illawarra, 4 seagrasses and approximately 35 species of macroalgae were recorded and described. Seagrasses found in Lake Illawarra are *Zostera capricorni*, *Ruppia megacarpa*, *Halophila ovalis* and *Halophila decipiens.* The macroalgae included: 14 species from 7 genera of green macroalgae; 9 species from 9 genera of brown macroalgae; and, 8 species from 8 genera of red macroalgae. Due to difficulties with taxonomic classification, morphological and inter-annual variability within and between species, and the impracticalities associated with sampling the entire Lake floor, it is possible that several more species of macroalgae exist in the Lake. The following chapter (5) discusses the localised distribution, biomass and nutrient relationships of the macrophytes described above.

CHAPTER 5 Studies on seagrass and macroalgal biomass and nutrients (carbon, nitrogen and phosphorus) in Lake Illawarra

5.1 Introduction

In eutrophic ecosystems, macroalgal blooms tend to occur as a result of increased nutrient availability. The monitoring of water column N and P concentrations is a traditional method of determining nutrient enrichment in marine and estuarine waters (see, e.g., Fong *et al.*, 1994). Previous studies (see, e.g., Fong *et al.*, 1993, 1994), however, have demonstrated that in warm temperate and subtropical coastal environments, there is little correlation between water column nutrient (N and P) concentrations and productivity or abundance of primary producers. Even in areas of high nutrient loading, macroalgae can sufficiently deplete the water column of N so that water quality still appears good (Valiela *et al.*, 1997). Concentrations of nutrients in macroalgal tissue are a function of nutrients assimilated from the water column from a certain length of time prior to collection, thus tissue nutrient contents and C:N:P molar ratios can be a useful indicator of *in situ* nutrient status (Wheeler and Björnsäter, 1992; Villares and Carballiera, 2003). Similarly, seagrasses typically take up nutrients *via* leaves and rhizomes over a longer time scale than macroalgae, so nutrient concentrations in seagrass tissue can reflect the long-term availability of nutrients (Fourqurean *et al.*, 1997).

Previous studies have used the stable isotopes, δ^{15}N and δ^{13}C, in seagrass and macroalgal tissue to indicate the source of C and N available for growth (e.g., Fourqurean *et al.*, 1997; McClelland *et al.*, 1997; Riera *et al.*, 2000). The δ^{13}C content of seagrasses reflects habitat influences, such as variations in photosynthetic metabolism, temperature, light intensity and oxygen (Smith *et al.*, 1976; McMillan *et al.*, 1980; McMillan and Smith, 1982), and therefore may be a useful indicator of seagrass productivity in estuaries (Grice *et al.,* 1996). Yamamuro *et al.* (2003) suggested that the δ^{15}N content of seagrass leaves reflects the assimilation of nitrogen over time and can therefore be used to monitor changes in dissolved inorganic nitrogen concentrations in the water column.

In Lake Illawarra, a number of previous studies have investigated the concentration of N and P in the water column (outlined in LIA, 1995; Sherman *et al.*, 2000) and sediment (Yassini, 1994), as well as the distribution and biomass of seagrasses (Harris, 1980; WBM 1993, 1996a, 1996b, 1998, 2000) and macroalgae (Yassini, 1985, 1986; Yassini and Clarke, 1986). Recent studies by University of Wollongong research students, conducted in Lake Illawarra concurrently with this study, included an estimate of macroalgal biomass and species content (McConville, 2000), the spatial distribution of seagrasses (Tadkaew, 2007), C, N, δ^{13}C and δ^{15}N contents of

seagrasses and macroalgae (Kuster, 2000), metal contents of *Zostera capricorni* (Howley, 2001) and nitrogen cycling processes (Qu, 2004a). However, little information is available about the spatial and temporal variations in the nutrient (C, N and P) content of both macroalgae and seagrasses in Lake Illawarra, and therefore the potential amount of carbon, nitrogen and phosphorus stored as macrophyte biomass in the estuary.

The work presented in this chapter examines the spatial and seasonal variations in the abundance and nutrient content of macrophytes at four key sites in Lake Illawarra. The main objectives of this study were to: a) conduct an in-depth analysis of the biomass, distribution and species-content of seagrass and macroalgae present at each study site; b) examine the elemental and isotopic contents of the seagrasses, *Zostera capricorni* and *Ruppia megacarpa*, and the most abundant macroalgae in Lake Illawarra; c) estimate the pools of C, N and P stored as seagrass and macroalgal biomass.

5.2 Results (I): Seagrass and Macroalgal Biomass

The results of biomass surveys of seagrass and macroalgae sampled at selected sites in Lake Illawarra, between 2000 and 2002, are presented in Figure 5-1 and Figure 5-2, with statistical analyses summarised in Table 5-1. The biomass and species composition of macroalgae measured at each site is listed separately for each sampling round (Table 5-2 - Table 5-6). Biomass data is presented in terms of dry weight (g DW m^{-2}). Macrophyte wet weight biomasses (g WW m^{-2}) are listed in Appendix 4.

5.2.1 Biomass of Seagrasses (*Zostera* and *Ruppia*)

Seagrass biomass at all sampling sites showed a significant correlation with the sampling season, with biomass typically declining during winter and increasing in summer (Figure 5-1 and Figure 5-2). To compare seasonal differences, seagrass biomass data from the five sampling rounds were grouped into winter and summer data sets as the two winter (2001 and 2002) sampling rounds were not significantly different ($p > 0.05$), nor were the spring (2000) and summer (2001 and 2002) sampling rounds (Table 5-1). ANOVA showed that at all sites, the total seagrass (leaf and root/rhizome) biomass was significantly higher in spring/summer than in winter ($p < 0.001$). The Oasis Caravan Park *Ruppia* beds consistently had the highest seagrass (leaf and rhizome) biomass over the entire sampling period, ranging from 231 ± 24.8 g DW m^{-2} in winter, to 440 ± 26.5 g DW m^{-2} in spring/summer. Total seagrass (*Zostera*) biomass at Purry Burry Point averaged 58.1 ± 6.38 g DW m^{-2} in winter and 226 ± 12.8 g DW m^{-2} in spring/summer. At Mullet Creek, total *Zostera* biomass averaged 145 ± 15.3 g DW m^{-2} in winter and 230 ± 22.9 g DW m^{-2} in spring/summer. The Nicolle Road *Ruppia* beds had the lowest seagrass biomass of all four study sites, with 54.8 ± 5.25 g DW m^{-2} in winter and 180 ± 12.1 g DW m^{-2} in spring/summer.

Comparisons across all sampling periods (spring 2000 - winter 2002), showed that *Ruppia* leaf biomass was significantly higher than root/rhizome biomass at both the Oasis Caravan Park and Nicolle Road sites (paired-t test; $p < 0.001$). *Ruppia* root/rhizome biomass at both OCP and NIC typically comprised 20 - 40 % and 30 - 45 % of the total seagrass biomass, respectively. At NIC, the proportion of *Ruppia* root/rhizome biomass increased slightly during winter, but this difference was not significant ($p > 0.05$). At Purry Burry Point, root/rhizome biomass typically comprised 40 - 44 % of the total seagrass biomass in all sampling rounds, except during winter 2001 where 67 % of the seagrass biomass was composed of roots-rhizomes (Table 5-4). At Mullet Creek, *Zostera* leaf biomass was equivalent to rhizome biomass throughout the study period; although rhizome biomass slightly exceeded leaf biomass during winter 2001, this difference was not significant ($p > 0.05$).

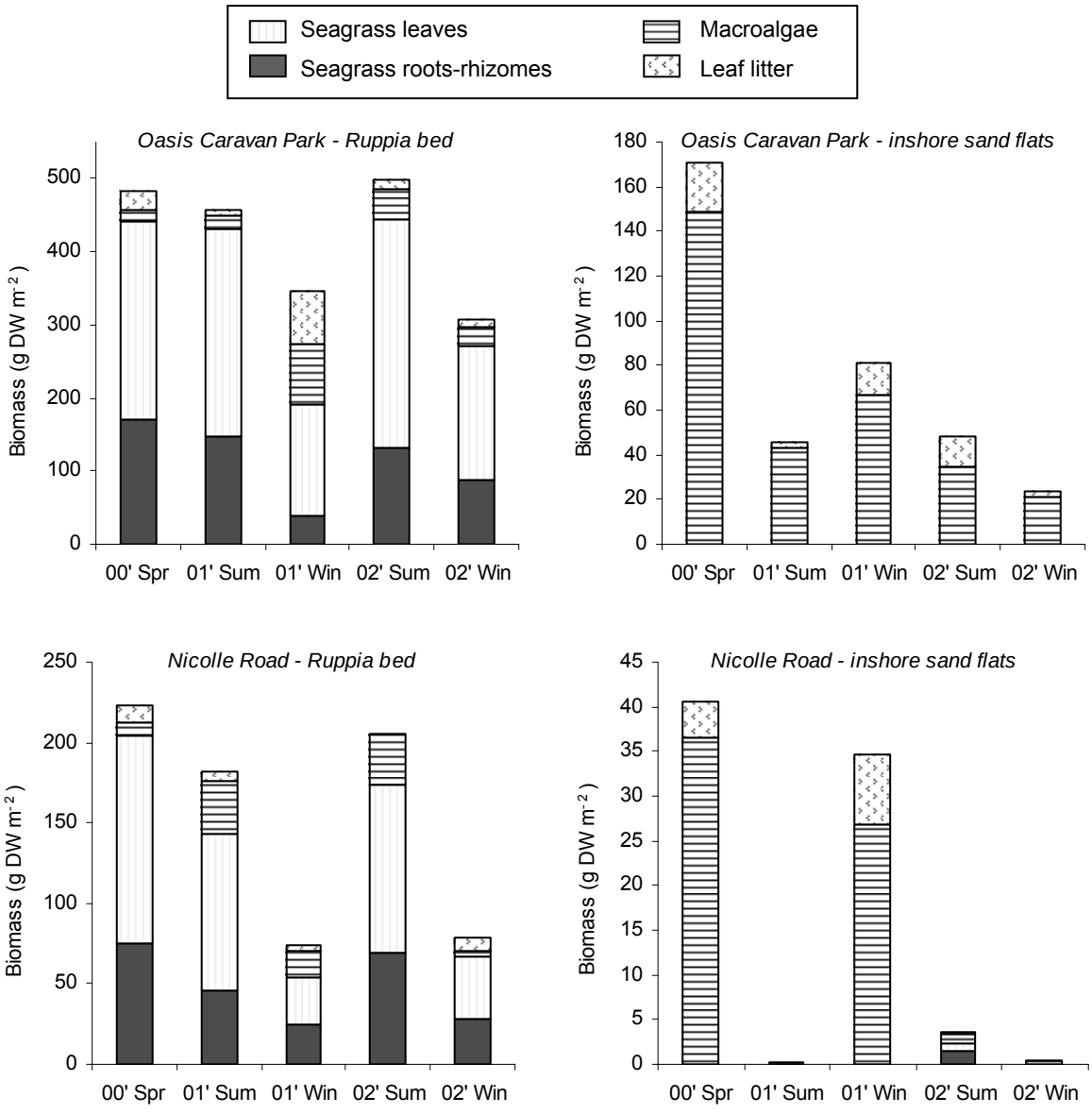


Figure 5-1: Seasonal variation in total macrophyte biomass (seagrass leaves, rhizomes, leaf litter and macroalgae) at the Oasis Caravan Park and Nicolle Road sites, Lake Illawarra.

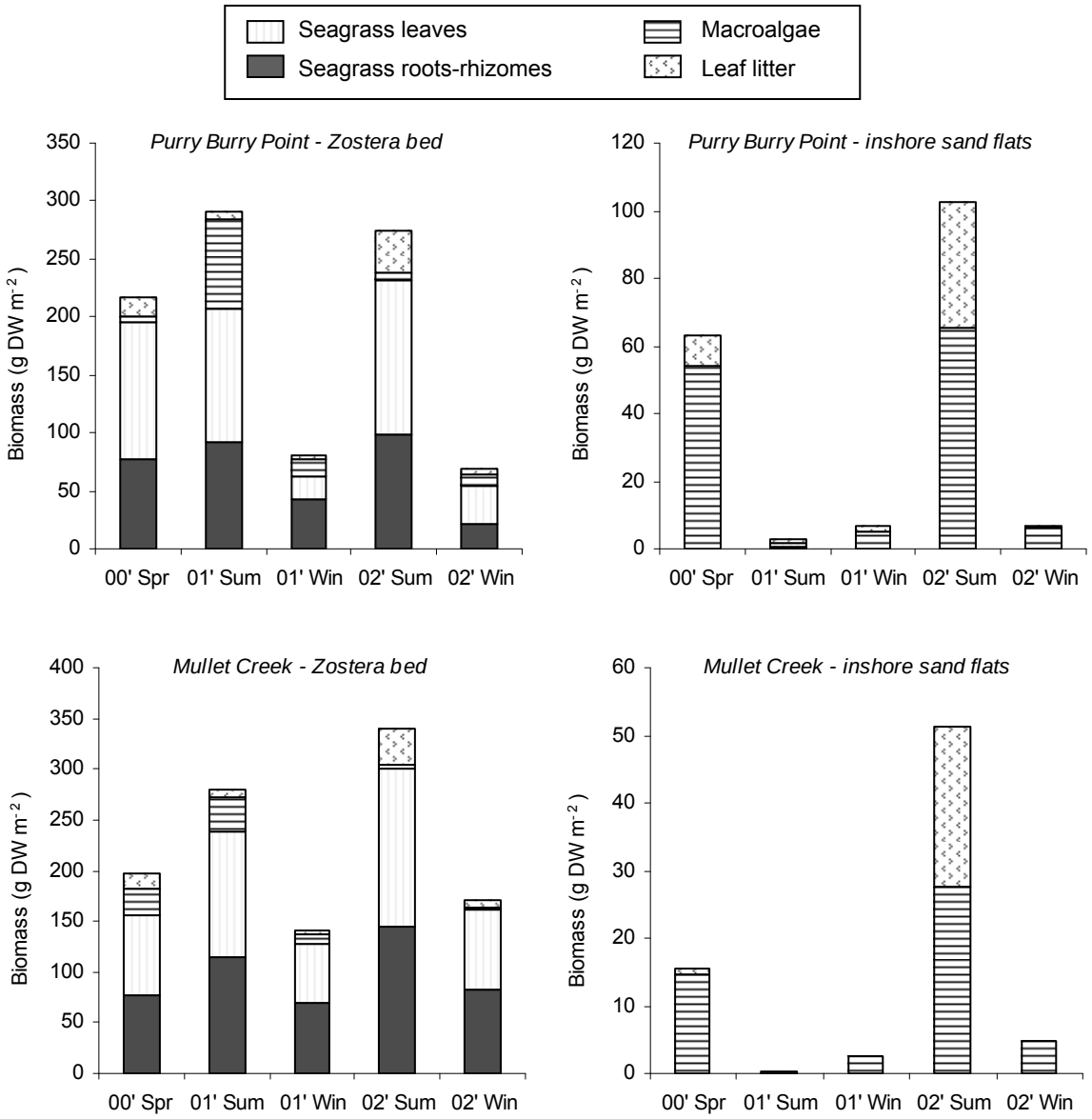


Figure 5-2: Seasonal variation in total macrophyte biomass (seagrass leaves, rhizomes, leaf litter and macroalgae) at the Purry Burry Point and Mullet Creek sites, Lake Illawarra.

It is interesting to note that during winter 2001, sparse populations of *Zostera capricorni* and *Halophila ovalis* were also recorded at the Oasis Caravan Park and Purry Burry Point sites, respectively. Very small populations of *Halophila decipiens* were also recorded at the Nicolle Road and Purry Burry Point sites in summer 2002.

A notable proportion of the total biomass (5 - 25 % per site) was categorized as “leaf litter”, referring largely to dead seagrass leaves and other organic material found entangled amongst the seagrass and algal beds. The biomass of this non-living material was usually highest at the sites with the densest algal blooms, Oasis Caravan Park and Primbee Bay. At the *Zostera* sites, Purry Burry Point and Mullet Creek, biomasses of this leaf litter material were often equivalent to that of macroalgae (Figure 5-2).

Table 5-1: Summary of multivariate ANOVA testing for differences in biomass of macrophytes collected from OCP, NIC, PBP, MC, spring 2000 - winter 2002.

	Parameter	Null Hypothesis	Factor	d.f.	F-Ratio	Probability	Power (α=0.05)	Differences (Tukey-Kramer multiple comparison $p < 0.05$)
Biomass over Seagrass Beds	Seagrass Leaf Biomass	H_0 = Biomass of seagrass leaves does not vary significantly between sites or seasons	Season	4	22.0	0.000000*	1.00	• 00-Spr, 01-Sum and 02-Sum > 01-Win and 02-Win
			Site	3	64.9	0.000000*	1.00	• OCP > NIC, PBP, MC
			Season x Site	12	1.3	0.242887	0.68	• No significant interactions
	Seagrass Rhizome Biomass	H_0 = Biomass of seagrass rhizomes does not vary significantly between sites or seasons	Season	4	17.5	0.000000*	1.00	• 00-Spr, 01-Sum and 02-Sum > 01-Win and 02-Win
			Site	3	20.6	0.000000*	1.00	• OCP and MC > NIC and PBP
			Season x Site	12	2.5	0.007338*	0.96	• Significant interactions
	Total Seagrass Biomass	H_0 = Total biomass of seagrass (leaves and rhizomes) does not vary significantly between sites or seasons	Season	4	32.6	0.000000*	1.00	• 00-Spr, 01-Sum and 02-Sum > 01-Win and 02-Win
			Site	3	65.2	0.000000*	1.00	• OCP > NIC, PBP and MC; MC > NIC
			Season x Site	12	2.4	0.011588*	0.94	• Significant interactions
	Total Macrophyte Biomass	H_0 = Total biomass over seagrass beds (incl. macroalgae and leaf litter) does not vary significantly between sites or seasons	Season	4	20.9	0.000000*	1.00	• 00-Spr, 01-Sum and 02-Sum > 01-Win and 02-Win
			Site	3	52.4	0.000000*	1.00	• OCP > NIC, PBP, MC; MC > NIC
			Season x Site	12	0.9	0.522170	0.50	• No significant interactions
Macroalgal Biomass	Macroalgae	H_0 = Total macroalgal biomass does not vary significantly between sites (OCP, NIC, PBP or MC), sampling seasons (spring 2000 - winter 2002), or site situation (seagrass beds versus inshore sand flats).	Site Situation	1	1.2	0.277320	0.19	• No significant differences between macroalgal biomass over seagrass beds vs inshore sand flats
			Season	4	5.6	0.000297*	0.98	• 00-Spr and 01-Sum > 02-Win
			Situation x Season	4	10.4	0.000000*	1.00	• Significant interactions: algal blooms occurred over sandflats in spring 2000, but biomass was higher over seagrass beds in later sampling rounds
			Site	3	18.0	0.000000*	1.00	• OCP > NIC, PBP and MC
			Situation x Site	3	3.4	0.019171*	0.76	• Significant interactions due to high variability in algal biomass between sampling sites and seasons
			Season x Site	12	2.5	0.004496*	0.97	
			Situation x Season x Site	12	4.5	0.000002*	1.00	• Significant interactions
Leaf Litter Biomass	Leaf Litter	H_0 = 'Leaf litter' biomass does not vary significantly between sites (OCP, NIC, PBP or MC), sampling seasons (spring 2000 - winter 2002), or site situation (seagrass beds versus inshore sand flats).	Site Situation	1	10.9	0.001152*	0.91	• Significantly more leaf litter over seagrass beds than inshore sand flats
			Season	4	7.3	0.000018*	1.00	• 02-Sum > 01-Sum and 02-Win
			Situation x Season	4	0.8	0.558843	0.24	• No significant interactions
			Site	3	7.6	0.000086*	0.99	• OCP > MC and NIC; PBP > NIC
			Situation x Site	3	1.7	0.177438	0.43	• No significant interactions
			Season x Site	12	5.1	0.000000*	1.00	• Significant interactions due to high variability in data
			Situation x Season x Site	12	1.7	0.080419	0.84	• No significant interactions

* Test significant at $p < 0.05$

Zostera and *Ruppia* beds at Lake Illawarra were adversely affected by the drought of late 2002-2003, which resulted in the Lake water level dropping by approximately 0.5 m, thus exposing substantial areas of seagrass beds along the eastern foreshore (Appendix 1B-C). Macrophyte surveys could not be conducted during summer 2003 due to the disappearance of much of the seagrass areas at the Oasis Caravan Park, Nicolle Road and Purry Burry Point sampling sites.

5.2.2 Biomass of Macroalgae

The maximum macroalgal biomass recorded during the study period were at Primbee Bay in spring 2000 (190 ± 15.4 g DW m^{-2}) and summer 2002 (369 ± 37.1 g DW m^{-2}), composed of mixed mats of *Chaetomorpha linum* and *Chaetomorpha billardierii*. Sampling was not undertaken at the Primbee Bay site during the remaining sampling rounds as only limited macroalgal and seagrass biomass was present. With the exception of Primbee Bay, the highest macroalgal biomasses recorded over the duration of the study occurred along the inshore area of the Oasis Caravan Park. At OCP, macroalgal biomass peaked at 148 ± 26.4 g DW m^{-2} with a *Chaetomorpha linum* bloom in spring 2000 (Table 5-2). Macroalgal biomass at the Windang Peninsular sites (OCP, NIC and PBP) dropped significantly (ANOVA: $p < 10^{-4}$) after the spring 2000 sampling round, due to foreshore harvesting being conducted in the inshore area during December, 2000.

ANOVA showed that the location of macroalgal biomass within sites (i.e., seagrass beds versus inshore sand flats) was generally not dependent on the sampling season ($p > 0.05$; Table 5-1). At the Nicolle Road and Purry Burry Point sites, there were generally no significant differences ($p > 0.05$) between the amount of macroalgal biomass over seagrass and inshore areas. The Oasis Caravan Park and Mullet Creek sites exhibited some significant variability with the abundance of algal biomass between locations (seagrass beds versus sand flats) over the sampling period, but these differences were not dependent on the sampling season (winter/summer).

Biomass of macroalgae associated with *Ruppia* beds at the Oasis Caravan Park site varied from 17.5 ± 9.92 g DW m^{-2} in summer 2001, to 82.9 ± 45.4 g DW m^{-2} in winter 2001 (small *Chaetomorpha* bloom); however, ANOVA showed that biomass did not change significantly between winter and summer sampling rounds ($p > 0.05$). At Nicolle Road, macroalgal biomass within the *Ruppia* beds ranged from 2.48 ± 1.08 to 33.7 ± 3.19 g DW m^{-2} over the sampling period, but the only significant differences detected were between the summer 2001 and winter 2002 sampling rounds ($F = 3.35$, $p < 0.05$, Tukey's $p < 0.05$). Macroalgae associated with Purry Burry Point *Zostera* beds also increased significantly to 77.7 ± 10.0 g DW m^{-2} during summer 2001 ($F = 36.3$, $p < 10^{-6}$), but averaged 6 - 15 g DW m^{-2} during all other sampling rounds. At Mullet Creek, macroalgae over seagrasses appeared to decrease significantly over the

sampling period (F = 7.51, $p < 0.001$), from 25.4 ± 9.48 g DW m^{-2} in summer 2001, to 0.84 ± 0.57 g DW m^{-2} in winter 2002.

Biomass of most macroalgal species present was clearly insignificant when compared to that of *Chaetomorpha* spp. (*C. linum* and *C. billardierii*), which represented 40 - 98 % of total algal biomass at each site. In summer 2001, the most abundant algal species were *Chaetomorpha* spp. and *Rhizoclonium riparium*, forming a bloom of approximately 40 g DW m^{-2} inshore of the Oasis Caravan Park (Table 5-3). The only significant macroalgal biomass documented during winter 2001 was at OCP, where *Chaetomorpha billardierii* formed a bloom of approximately 50 - 70 g DW m^{-2} over the *Ruppia* beds and inshore sand flats (Table 5-4). In summer 2002, a bloom of epiphytic and detached *Cladophora* sp. (35 g DW m^{-2}) covered much of the *Ruppia* beds at OCP (Table 5-5). Filamentous *Ulva* spp. (*U. intestinalis*, *U. compressa* and *U. ralfsii*) also accounted for a significant proportion of the total macroalgal biomass at each site, particularly at Purry Burry Point and Mullet Creek, where they were often found growing amongst, and just inshore, of the *Zostera* beds. At PBP, the benthic red alga, *Gracilaria*, constituted 85 % of the total algal biomass associated with seagrass beds in summer 2001, but *Gracilaria* is not considered a bloom-forming genus. No visible macroalgal blooms were documented at the Lake Illawarra sites (other than OCP) during winter 2001 and 2002. In winter, however, the majority of the macroalgal biomass at NIC, PBP and MC were species growing epiphytically on *Zostera* and *Ruppia* leaves (e.g., *Chondria, Cladophora, Polysiphonia, Centroceras* and *Ulva*). At Nicolle Road, for example, approximately 90 % of the total algal biomass in 2001-02 consisted of rhodophyte epiphytes growing on *Ruppia* leaves.

Table 5-2: Macrophyte biomass (dry weight) and species content recorded at four Lake Illawarra sites, spring 2000 (mean ± s.e.).

Site	Seagrass Beds			Sand Flats (Macroalgal Mats)		
	Genus/ Plant Part	**Biomass (g DW m^{-2})**	**Type ***	**Genus/ Plant Part**	**Biomass (g DW m^{-2})**	**Type ***
Oasis Caravan Park	*Ruppia* - leaves	271 ± 12	B	*Centroceras*	0.04 ± 0.04	E
	- rhizomes	170 ± 19		*Chaetomorpha*	113 ± 31	F
	Chaetomorpha	12.7 ± 6.1	F	*Chondria*	2.67 ± 1.0	E
	Cladophora	0.09	E	*Cladophora*	0.67 ± 0.6	F
	Ulva	4.06 ± 2.6	E	*Ectocarpus*	6.7 x 10^{-3}	F
	Leaf Litter	26.1 ± 12	-	*Ulva*	11.0 ± 5.0	F
				Hincksia	8.3 x 10^{-3}	B
				Lamprothamnium	0.14 ± 0.1	B
				Polysiphonia	0.08 ± 0.04	E
				Leaf Litter	22.4 ± 12	-
Nicolle Road	*Ruppia* - leaves	128 ± 10	B	*Chaetomorpha*	28.1 ± 4.3	F
	- rhizomes	75.4 ± 5.9		*Chondria*	0.31 ± 0.12	E
	Chaetomorpha	4.86 ± 3.2	F	*Codium*	3.3 x 10^{-3}	B
	Chondria	0.60 ± 0.5	E	*Gracilaria*	8.15 ± 1.4	B
	Gracilaria	3.00 ± 2.3	B	*Lamprothamnium*	1.7 x 10^{-3}	B
	Polysiphonia	0.03	E	*Polysiphonia*	6.7 x 10^{-3}	E
	Leaf Litter	10.0 ± 3.3	-	Leaf Litter	4.06 ± 0.84	-
Purry Burry Point	*Zostera* - leaves	117 ± 8.3	B	*Centroceras*	5.0 x 10^{-3}	E
	- rhizomes	77.4 ± 12		*Chaetomorpha*	31.3 ± 2.4	F
	Ruppia - leaves	21.7 ± 9.7	B	*Chondria*	0.05 ± 0.04	E
	- rhizomes	11.9 ± 5.5		*Cladophora*	1.7 x 10^{-3}	E
	Centroceras	0.14	E	*Codium*	1.7 x 10^{-3}	B
	Chaetomorpha	3.00 ± 1.5	F	*Ectocarpus*	5.0 x 10^{-3}	F
	Codium	1.33	B	*Ulva*	0.43 ± 0.3	F
	Ulva	1.69 ± 0.9	E	*Gracilaria*	22.0 ± 6.4	B
	Polysiphonia	0.07 ± 0.1	E	*Polysiphonia*	5.0 x 10^{-3}	E
	Spyridia	0.03	E	*Spyridia*	0.01 ± 0.01	E
	Leaf Litter	15.1 ± 3.7	-	Leaf Litter	9.5 ± 2.1	-
Mullet Creek	*Zostera* - leaves	80.3 ± 7.6	B	*Chaetomorpha*	7.50 ± 3.4	F
	- rhizomes	76.3 ± 7.1		*Chondria*	0.01	E
	Chaetomorpha	3.26 ± 1.1	F	*Cladophora*	6.7 x 10^{-3}	E
	Chondria	3.18 ± 1.8	E	*Codium*	0.46	B
	Cladophora	0.48 ± 0.5	E	*Ulva*	5.98 ± 2.3	F
	Codium	1.71	B	*Gracilaria*	0.71 ± 0.4	B
	Ulva	12.3 ± 5.0	B,E	*Polysiphonia*	0.02	E
	Gracilaria	1.91 ± 1.2	B	Leaf Litter	0.86 ± 0.39	-
	Hypnea	0.48 ± 0.4	B			
	Petalonia/Endarachne	0.65 ± 0.5	B			
	Polysiphonia	0.03	E			
	Sargassum	1.39 ± 1.0	B			
	Leaf Litter	14.5 ± 5.6	-			
	Primbee Bay			**Lake Illawarra Village**		
	Zostera - leaves	14.2 ± 5.0	B	*Ruppia* - leaves	0.23 ± 0.15	B
	- rhizomes	6.93 ± 2.9		- rhizomes	0.09 ± 0.06	
	Chaetomorpha	139 ± 13	F	*Centroceras*	1.7 x 10^{-3}	E
	Ulva	17.6 ± 11	F	*Chaetomorpha*	21.1 ± 1.7	A
	Gracilaria	33.5 ± 17	B	*Chondria*	3.3 x 10^{-3}	E
	Leaf Litter	32.9 ± 24	-	*Ulva*	0.52 ± 0.50	E
				Gracilaria	0.22	B
				Polysiphonia	1.7 x 10^{-3}	E
				Leaf Litter	5.07 ± 1.3	-

* B = Benthic, attached macrophyte; E = Epiphytic macroalgae; F = Free-floating macroalgae

Table 5-3: Macrophyte biomass (dry weight) and species content recorded at four Lake Illawarra sites, summer 2001 (mean ± s.e.).

Site	Seagrass Beds			Sand Flats (Macroalgal Mats)		
	Genus	Biomass (g DW m^{-2})	Type *	Genus	Biomass (g DW m^{-2})	Type *
Oasis	*Ruppia* - leaves	283 ± 12	B	*Centroceras*	0.08	E
Caravan	- rhizomes	148 ± 15		*Chaetomorpha*	34.3 ± 15	F
Park	*Centroceras*	7.68 ± 3.6	E	*Chondria*	3.3×10^{-3}	E
	Chaetomorpha	3.11 ± 1.6	F	*Cladophora*	0.56 ± 0.35	E
	Chondria	0.03	E	*Ulva*	0.19 ± 0.14	F
	Cladophora	0.91 ± 0.40	E	*Lamprothamnium*	0.22	B
	Ulva	0.06 ± 0.05	E	*Polysiphonia*	0.02	E
	Hincksia	0.05	E	*Rhizoclonium*	7.72 ± 4.0	F
	Lamprothamnium	5.60	B	Leaf Litter	2.77 ± 1.3	-
	Polysiphonia	0.04	E			
	Leaf Litter	10.0 ± 2.6	-			
Nicolle	*Ruppia* - leaves	97.1 ± 13	B	Negligible biomass	-	
Road	- rhizomes	45.7 ± 5.4		Leaf Litter	0.15 ± 0.08	-
	Centroceras	3.04 ± 2.7	E			
	Chaetomorpha	3.45 ± 0.55	F			
	Chondria	17.6 ± 4.7	E			
	Cladophora	7.67 ± 1.4	E,F			
	Ectocarpus	0.09 ± 0.01	E			
	Ulva	0.82 ± 0.42	E			
	Gracilaria	0.80 ± 0.72	B			
	Hypnea	0.07 ± 0.01	B			
	Polysiphonia	0.09 ± 0.01	E			
	Spyridia	0.06 ± 0.03	E			
	Leaf Litter	5.25 ± 2.5	-			
Purry	*Zostera* - leaves	115 ± 5.4	B	Negligible biomass	-	
Burry	- rhizomes	91.5 ± 7.9		Leaf Litter	1.03 ± 0.35	-
Point	*Chondria*	0.07 ± 0.02	E			
	Cladophora	9.76 ± 2.8	E,F			
	Ulva	1.84	F			
	Gracilaria	65.7 ± 12	B			
	Hypnea	0.11 ± 0.03	B			
	Polysiphonia	0.19 ± 0.04	E			
	Spyridia	0.02	E			
	Leaf Litter	6.61 ± 0.90	-			
Mullet	*Zostera* - leaves	124 ± 29	B	*Chaetomorpha*	0.10 ± 0.09	F
Creek	- rhizomes	114 ± 1.4		Leaf Litter	0.10 ± 0.07	-
	Chaetomorpha	0.70	F			
	Chondria	0.05 ± 0.01	E			
	Cladophora	2.78	E			
	Codium	1.18	B			
	Ulva	25.4 ± 5.0	E,F			
	Gracilaria	4.34 ± 2.9	B			
	Polysiphonia	0.08 ± 0.05	E			
	Spyridia	6.7×10^{-3}	E			
	Leaf Litter	6.11 ± 3.1	-			

* B = Benthic, attached macrophyte; E = Epiphytic macroalgae; F = Free-floating macroalgae

Table 5-4: Macrophyte biomass (dry weight) and species content recorded at four Lake Illawarra sites, winter 2001 (mean ± s.e.).

	Seagrass Beds			Sand Flats (Macroalgal Mats)		
Site	**Genus**	**Biomass (g DW m^{-2})**	**Type ***	**Genus**	**Biomass (g DW m^{-2})**	**Type ***
Oasis	*Ruppia* - leaves	141 ± 28	B	*Chaetomorpha*	66.5 ± 10	F
Caravan	- rhizomes	38.6 ± 8.2		*Chondria*	0.24	E
Park	*Zostera* - leaves	12.0	B	*Lamprothamnium*	0.41	B
	- rhizomes	1.30		Leaf Litter	14.2 ± 5.0	-
	Chaetomorpha	55.7 ± 43	F			
	Lamprothamnium	27.1 ± 15	B			
	Leaf Litter	74.1 ± 25	-			
Nicolle	*Ruppia* - leaves	30.0 ± 4.3	B	*Chaetomorpha*	0.32 ± 0.26	F
Road	- rhizomes	24.4 ± 2.7		*Chondria*	0.05 ± 0.04	E
	Chaetomorpha	0.04 ± 0.02	F	*Ectocarpus*	0.05	F
	Chondria	15.0 ± 4.6	E	*Ulva*	0.20 ± 0.11	F
	Ectocarpus	0.04 ± 0.02	F	*Gracilaria*	0.10 ± 0.05	B
	Hypnea	0.04 ± 0.02	B	*Hypnea*	0.03	B
	Lamprothamnium	0.06 ± 0.04	B	*Lamprothamnium*	0.01	B
	Polysiphonia	0.65 ± 0.46	E	*Polysiphonia*	0.29 ± 0.28	E
	Leaf Litter	16.0 ± 4.3	-	*Rhizoclonium*	25.8 ± 12.1	F
				Leaf Litter	6.46 ± 2.1	-
Purry	*Zostera* - leaves	20.5 ± 5.6	B	*Halophila* - leaves	1.05	B
Burry	- rhizomes	42.3 ± 7.2		- rhizomes	0.54	
Point	*Gracilaria*	14.2 ± 4.8	B	*Ruppia* - leaves	1.7×10^{-3}	B
	Hypnea	0.15 ± 0.08	B	- rhizomes	8.3×10^{-3}	
	Leaf Litter	2.66 ± 1.5	-	*Chaetomorpha*	0.18 0.11	F
				Ulva	2.71 ± 2.1	F, B
				Gracilaria	2.04 ± 1.1	B
				Hypnea	0.02	B
				Leaf Litter	1.64 ± 0.99	-
Mullet	*Zostera* - leaves	57.4 ± 13	B	*Chaetomorpha*	1.7×10^{-3}	F
Creek	- rhizomes	70.2 ± 9.3		*Chondria*	0.14 ± 0.09	E
	Chaetomorpha	0.06	F	*Cladophora*	0.25 ± 0.09	E
	Chondria	0.17	E	*Ectocarpus*	0.03	F
	Cladophora	0.40	E	*Ulva*	0.83 ± 0.44	E, B
	Ulva	3.33 ± 2.1	E	*Gracilaria*	0.83 ± 0.50	B
	Gracilaria	0.22 ± 0.20	B	*Hincksia*	0.04 ± 0.03	E
	Hypnea	0.02	B	*Hypnea*	3.3×10^{-3}	B
	Petalonia/Endarachne	0.05	B	*Petalonia/Endarachne*	1.7×10^{-3}	B
	Polysiphonia	4.19 ± 0.90	E	*Polysiphonia*	0.37 ± 0.24	E
	Sargassum	0.23	B			
	Leaf Litter	4.28 ± 2.3	-			

* B = Benthic, attached macrophyte; E = Epiphytic macroalgae; F = Free-floating macroalgae

Table 5-5: Macrophyte biomass (dry weight) and species content recorded at four Lake Illawarra sites, summer 2002 (mean ± s.e.).

	Seagrass Beds			Sand Flats (Macroalgal Mats)		
Site	**Genus**	**Biomass (g DW m^{-2})**	**Type ***	**Genus**	**Biomass (g DW m^{-2})**	**Type ***
Oasis	*Ruppia* - leaves	312 ± 46	B	*Chaetomorpha*	30.2 ± 15	F
Caravan	- rhizomes	131 ± 23		*Cladophora*	3.90 ± 1.8	F
Park	*Centroceras*	6.41 ± 3.6	E	*Gracilaria*	0.65	B
	Chaetomorpha	0.33	F	Leaf Litter	13.6 ± 3.7	-
	Chondria	0.05	E			
	Cladophora	34.8 ± 20	E,F			
	Leaf Litter	14.1 ± 5.2	-			
Nicolle	*Ruppia* - leaves	104 ± 25	B	*Zostera* - whole plant	0.12	B
Road	- rhizomes	69.1 ± 22		*Halophila* - leaves	0.92 ± 0.54	B
	Centroceras	0.05 ± 0.03	E	- rhizomes	1.45 ± 0.80	
	Chondria	31.2 ± 15	E	*Centroceras*	0.05	E
	Gracilaria	0.11 ± 0.07	B	*Gracilaria*	0.89 ± 0.54	B
	Hypnea	0.05	B	Leaf Litter	0.32	-
	Spyridia	0.29 ± 0.21	E			
	Leaf Litter	0.68 ± 0.45	-			
Purry	*Zostera* - leaves	117 ± 15	B	*Chaetomorpha*	2.03	F
Burry	- rhizomes	98.2 ± 15		*Ulva*	10.8 ± 4.9	F
Point	*Halophila* - whole plant	0.18	B	*Gracilaria*	52.4 ± 26	B
	Ruppia - whole plant	15.5	B	Leaf Litter	37.0 ± 15	-
	Centroceras	0.02	E			
	Cladophora	0.12	E			
	Ulva	1.24 ± 0.88	E			
	Gracilaria	5.56 ± 1.9	B			
	Leaf Litter	36.6 ± 15	-			
Mullet	*Zostera* - leaves	155 ± 22	B	*Chaetomorpha*	6.28 ± 3.4	F
Creek	- rhizomes	145 ± 25		*Chondria*	0.01 ± 0.01	E
	Ulva	3.58 ± 3.0	B,E	*Cladophora*	4.11 ± 3.9	E
	Gracilaria	0.21 ± 0.08	B	*Codium*	0.05	B
	Leaf Litter	36.0 ± 11	-	*Ulva*	7.64 ± 3.9	F
				Gracilaria	9.22 ± 4.1	B
				Lamprothamnium	0.20	B
				Spyridia	6.7×10^{-3}	E
				Leaf Litter	23.8 ± 2.6	-
Primbee	-			*Chaetomorpha*	369 ± 37	B
Bay						

* B = Benthic, attached macrophyte; E = Epiphytic macroalgae; F = Free-floating macroalgae

Table 5-6: Macrophyte biomass (dry weight) and species content recorded at four Lake Illawarra sites, winter 2002 (mean ± s.e.).

	Seagrass Beds			Sand Flats (Macroalgal Mats)		
Site	**Genus**	**Biomass (g DW m^{-2})**	**Type ***	**Genus**	**Biomass (g DW m^{-2})**	**Type ***
Oasis	*Ruppia* - leaves	183 ± 32	B	*Chaetomorpha*	18.2 ± 3.2	F
Caravan	- rhizomes	86.6 ± 15		*Chondria*	0.32 ± 0.22	E
Park	*Chaetomorpha*	20.1 ± 4.9	F	*Lamprothamnium*	2.45 ± 0.83	B
	Chondria	0.34 ± 0.25	E	*Polysiphonia*	2.84 ± 1.1	E
	Gracilaria	0.67	B	Leaf Litter	2.72 ± 1.1	-
	Lamprothamnium	4.49 ± 1.8	B			
	Polysiphonia	0.78 ± 0.77	E			
	Leaf Litter	10.3 ± 4.3	-			
Nicolle	*Ruppia* - leaves	31.8 ± 5.4	B	*Chaetomorpha*	0.41 ± 0.18	F
Road	- rhizomes	23.2 ± 3.4		*Chondria*	3.3×10^{-3}	E
	Chaetomorpha	0.28 ± 0.21	F	*Gracilaria*	6.7×10^{-3}	B
	Chondria	1.48 ± 0.79	E			
	Gracilaria	0.71	B			
	Polysiphonia	6.7×10^{-3}	E			
	Leaf Litter	8.1 ± 3.9	-			
Purry	*Zostera* - leaves	31.4 ± 5.7	B	*Chaetomorpha*	0.94 ± 0.70	F
Burry	- rhizomes	22.1 ± 3.3		*Ulva*	2.27 ± 1.9	E, B
Point	*Chaetomorpha*	1.89 ± 1.9	F	*Gracilaria*	2.81 ± 0.97	B
	Ectocarpus	3.3×10^{-3}	E	*Hypnea*	0.09	B
	Ulva	0.02	E, B	Leaf Litter	0.64 ± 0.21	-
	Gracilaria	8.04 ± 2.5	B			
	Hypnea	0.38 ± 0.35	B			
	Leaf Litter	4.52 ± 2.0	-			
Mullet	*Zostera* - leaves	79.4 ± 14	B	*Chaetomorpha*	1.17 ± 0.78	F
Creek	- rhizomes	82.2 ± 10		*Chondria*	0.66 ± 0.59	E
	Chaetomorpha	0.77 ± 0.58	F	*Cladophora*	0.49 ± 0.29	E
	Ulva	0.05 ± 0.04	E	*Codium*	1.24	B
	Gracilaria	0.02	B	*Ectocarpus*	8.3×10^{-3}	E
	Polysiphonia	6.7×10^{-3}	E	*Ulva*	0.49 ± 0.37	E
	Leaf Litter	9.20 ± 3.8	-	*Gracilaria*	0.62 ± 0.44	B
				Hincksia	0.02 ± 0.02	E
				Polysiphonia	6.7×10^{-3}	E

* B = Benthic, attached macrophyte; E = Epiphytic macroalgae; F = Free-floating macroalgae

5.2.3 Dry Matter Contents of Macrophytes

Average dry matter contents (% of wet weight biomass) were determined for each macrophyte species, over a range of sites and dates (Table 5-7). *Zostera* leaves and rhizomes typically contained about 76 - 92 % and 85 - 91 % water by weight, respectively. *Ruppia* leaves and rhizomes had similar water contents, with 80 - 90 % and 82 - 92 % water by weight, respectively. Wet to dry biomass ratios of all seagrass samples collected are listed in Appendix 5.

Macroalgae, particularly the most problematic genus, *Chaetomorpha*, exhibited a much wider variation in tissue water content than the seagrasses, ranging from 50 - 96 % water by weight. The proportion of wet to dry biomass in field-collected *Chaetomorpha* may give some indication of the growth conditions of this alga in Lake Illawarra; this is discussed further in Section 6.2.7.

Table 5-7: Dry matter contents of seagrasses and macroalgae, determined for all samples collected across all Lake Illawarra sites.

		No. of	Dry matter content (% of wet weight)		
	Genus	**samples**	**Mean ± s.e.**	**Min**	**Max**
Chlorophyta	*Bryopsis*	2	12.8 ± 0.3	12.5	13.0
	Chaetomorpha	138	10.5 ± 0.4	5.6	50.0
	Cladophora	60	17.3 ± 0.7	9.7	35.4
	Rhizoclonium	9	18.2 ± 1.4	13.0	26.7
	Ulva (sheet-like)	18	13.2 ± 0.5	10.9	18.5
	Ulva (filamentous)	91	12.2 ± 0.3	4.0	18.2
	Codium	16	14.7 ± 0.5	11.2	16.9
	Lamprothamnium	26	11.6 ± 0.4	9.2	19.3
Rhodophyta	*Centroceras*	28	13.1 ± 0.9	9.4	35.2
	Ceramium	1	11.1	-	--
	Polysiphonia	61	12.2 ± 0.2	8.3	18.0
	Chondria	82	9.5 ± 0.5	4.5	40.0
	Spyridia	15	12.6 ± 0.9	5.0	22.2
	Gracilaria	102	12.8 ± 0.4	1.2	33.6
	Hypnea	28	11.5 ± 0.4	7.3	15.6
	Grateloupia	1	13.6	-	-
Phaeophyta	*Ectocarpus*	16	12.1 ± 0.6	9.5	16.5
	Hincksia	7	15.6 ± 2.6	8.2	28.1
	Cystoseira	6	9.7 ± 0.3	8.5	10.5
	Sargassum	9	11.4 ± 0.7	9.3	16.1
	Scytosiphon	1	9.4	-	-
	Petalonia	5	14.0 ± 2.0	8.6	20.8
	Colpomenia	1	28.1	-	-
Seagrasses	*Halophila* - leaves	6	9.0 ± 0.7	5.6	10.9
	Halophila - rhizomes	5	9.7 ± 0.6	8.0	11.0
	Ruppia - leaves	65	13.0 ± 0.3	9.6	19.5
	Ruppia - rhizomes	60	11.9 ± 0.2	8.5	17.6
	Zostera - leaves	60	11.5 ± 0.3	8.5	24.4
	Zostera - rhizomes	59	11.7 ± 8.9	8.9	14.9

5.3 Results (II): Nutrient Analyses

5.3.1 Summary of Data and Statistical Analyses

Tissue nutrient analyses were conducted seasonally on *Ruppia megacarpa* at Oasis Caravan Park and Nicolle Road, and *Zostera capricorni* at Mullet Creek and Purry Burry Point. *Zostera capricorni* and *Ruppia megacarpa* were also sampled at Primbee Bay and Purry Burry Point, respectively, in spring 2000, but these species were not observed at the same sites in future surveys. Analyses were conducted periodically on macroalgae at the various sites, depending on availability of sufficient macroalgal biomass. The majority of macroalgae samples analysed for nutrient content were obtained from inshore sandy areas; the exception was *Lamprothamnium papulosum*, attached to the substrate amongst *Ruppia* beds at the Oasis Caravan Park in winter 2001.

One of the primary aims of this thesis was to fill a "gap" in knowledge regarding the nutrient content of macrophytes in Lake Illawarra, and the importance of these nutrient pools in terms of the Lake's nutrient budget. Detailed results of C, N, P, δ^{13}C and δ^{15}N analyses conducted on the most abundant macrophytes sampled in Lake Illawarra, between spring 2000 and winter 2002, are presented in Table 5-8 - Table 5-10. The laboratory results of all Lake Illawarra seagrass and macroalgal tissue samples analysed are provided in Appendix 6. Summaries of the statistical analyses conducted on the macrophyte nutrient data are present in Table 5-11 - Table 5-14. These results are discussed in more detail in the following sections.

Table 5-8: Tissue contents of seagrass leaves, roots-rhizomes and macroalgae at the Lake Illawarra Village (LIV), Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP), Primbee Bay (PRIM) and Mullet Creek (MC) sites, Lake Illawarra, spring 2000 (values are mean ± s.e., n = 3 - 6).

			Plant Tissue Contents					C:N:P Molar Ratios		
	Site	Genus and Plant Part	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C / P	C / N	N / P
Spring 2000	LIV	*Chaetomorpha linum*	39.66 ± 0.28	3.32 ± 0.28	0.14 ± 0.007	-15.65 ± 0.40	8.51 ± 0.39	716 ± 304	14.5 ± 1.56	50.9 ± 3.62
		Chaetomorpha aerea *	36.76	1.82	0.05	-18.22	9.30	1896	23.5	80.5
	OCP	*Ruppia megacarpa* - leaves	40.25 ± 0.37	2.74 ± 0.10	0.14 ± 0.003	-11.96 ± 0.04	2.08 ± 0.08	725 ± 20	17.2 ± 0.53	42.3 ± 1.85
		Ruppia megacarpa - rhizomes	38.61 ± 0.42	1.66 ± 0.12	0.12 ± 0.006	-10.76 ± 0.27	1.19 ± 0.16	833 ± 38	27.3 ± 1.83	30.6 ± 0.72
		Chaetomorpha billardierii	38.47 ± 0.28	3.28 ± 0.24	0.08 ± 0.010	-15.32 ± 0.22	10.55 ± 0.24	1411 ± 239	14.1 ± 1.02	98.4 ± 64.7
		Ulva intestinalis	31.73 ± 0.71	1.54 ± 0.11	0.04 ± 0.003	-10.35 ± 0.54	13.03 ± 0.20	1907 ± 131	24.2 ± 1.35	79.8 ± 9.76
	NIC	*Ruppia megacarpa* - leaves	38.39 ± 0.59	2.99 ± 0.01	0.19 ± 0.003	-12.77 ± 0.18	8.53 ± 0.33	531 ± 10	15.0 ± 0.27	35.5 ± 0.75
		Ruppia megacarpa - rhizomes	37.96 ± 1.06	1.95 ± 0.04	0.13 ± 0.007	-11.95 ± 0.07	7.17 ± 0.20	740 ± 56	22.7 ± 0.18	32.6 ± 2.19
		Chaetomorpha linum	37.49 ± 0.66	3.43 ± 0.16	0.12 ± 0.022	-15.48 ± 0.11	12.62 ± 0.59	919 ± 129	12.9 ± 0.39	70.7 ± 8.61
	PBP	*Zostera capricorni* - leaves	37.29 ± 0.82	2.97 ± 0.07	0.23 ± 0.003	-10.81 ± 0.25	2.18 ± 0.07	424 ± 13	14.6 ± 0.23	29.0 ± 0.67
		Zostera capricorni - rhizomes	33.94 ± 1.15	1.01 ± 0.06	0.12 ± 0.003	-10.27 ± 0.26	0.77 ± 0.05	751 ± 26	39.2 ± 1.57	19.2 ± 0.65
		Ruppia megacarpa - leaves	35.40 ± 0.50	2.00 ± 0.13	0.17 ± 0.003	-9.32 ± 0.21	3.65 ± 0.17	527 ± 10	20.9 ± 1.62	25.5 ± 1.43
		Ruppia megacarpa - rhizomes	31.87 ± 1.44	1.37 ± 0.55	0.21 ± 0.036	-9.69 ± 0.97	4.07 ± 2.32	392 ± 39	27.2 ± 0.31	14.4 ± 1.31
		Chaetomorpha linum	36.23 ± 0.71	3.00 ± 0.18	0.11 ± 0.017	-13.24 ± 0.38	9.92 ± 0.23	945 ± 127	14.3 ± 0.70	65.2 ± 6.44
	PRIM	*Zostera capricorni* - leaves	36.60 ± 1.29	2.96 ± 0.17	0.29 ± 0.007	-8.01 ± 0.25	1.67 ± 0.43	330 ± 15	14.5 ± 0.55	22.8 ± 1.15
		Zostera capricorni - rhizomes	31.03 ± 0.22	1.12 ± 0.10	0.20 ± 0.013	-9.91 ± 1.48	1.18 ± 0.54	410 ± 27	32.9 ± 2.96	12.5 ± 0.31
		Chaetomorpha linum	37.26 ± 0.30	2.09 ± 0.31	0.18 ± 0.026	-9.26 ± 0.30	7.56 ± 0.61	561 ± 82	22.0 ± 3.63	27.7 ± 7.04
		Ulva intestinalis	32.67 ± 1.02	0.90 ± 0.03	0.27 ± 0.013	-9.56 ± 0.14	4.85 ± 0.17	318 ± 25	42.2 ± 0.18	7.6 ± 0.62
		Ulva sp. (probably *U. lactuca*)	34.33 ± 1.27	2.62 ± 0.35	0.13 ± 0.015	-6.47 ± 1.03	9.61 ± 1.14	697 ± 71	15.8 ± 1.96	46.1 ± 8.16
	MC	*Zostera capricorni* - leaves	38.77 ± 0.26	3.13 ± 0.06	0.25 ± 0.003	-13.53 ± 0.46	4.35 ± 0.19	406 ± 4.2	14.5 ± 0.29	28.1 ± 0.63
		Zostera capricorni - rhizomes	34.25 ± 0.60	1.06 ± 0.07	0.17 ± 0.015	-11.58 ± 0.27	2.74 ± 0.18	536 ± 37	38.8 ± 2.93	14.0 ± 0.91
		Chaetomorpha linum	36.04 ± 0.91	3.27 ± 0.11	0.23 ± 0.053	-17.50 ± 1.17	9.75 ± 0.30	487 ± 163	12.8 ± 0.24	38.1 ± 13.0
		Ulva compressa	31.69 ± 0.60	2.05 ± 0.23	0.13 ± 0.012	-12.03 ± 0.40	11.09 ± 0.26	655 ± 54	18.4 ± 1.65	35.7 ± 0.86

***** Data for one sample of *Chaetomorpha aerea* only; all other samples at LIV were identified as *C. linum.* As *C. aerea* is morphologically different to *C. linum* (see Section 4.5.4) and only one specimen was available, the *C. aerea* data was considered anomalistic and removed from the statistical analyses.

Table 5-9: Tissue contents of seagrass leaves, roots-rhizomes and macroalgae at the Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP) and Mullet Creek (MC) sites, Lake Illawarra, summer - winter 2001 (values are mean ± s.e., n = 3 - 6).

			Plant Tissue Contents					C:N:P Molar Ratios		
	Site	Genus and Plant Part	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C / P	C / N	N / P
Summer 2001	OCP	*Ruppia megacarpa* - leaves	40.10 ± 0.51	2.83 ± 0.07	0.12 ± 0	-11.86 ± 0.21	2.04 ± 0.10	862 ± 11	16.5 ± 0.15	52.2 ± 1.21
		Ruppia megacarpa - rhizomes	38.34 ± 0.70	1.94 ± 0.03	0.10 ± 0.003	-10.71 ± 0.25	1.13 ± 0.12	959 ± 44	13.0 ± 0.62	41.6 ± 0.85
		Chaetomorpha billardierii	36.74 ± 1.46	2.23 ± 0.10	0.04 ± 0.003	-14.24 ± 0.43	5.50 ± 0.85	2611 ± 150	19.3 ± 0.20	136 ± 7.96
		Rhizoclonium riparium	33.69 ± 0.36	1.99 ± 0.03	0.07 ± 0	-9.02 ± 0.33	4.09 ± 0.08	1241 ± 13	19.8 ± 0.50	62.8 ± 1.01
	NIC	*Ruppia megacarpa* - leaves	37.93 ± 0.90	3.05 ± 0.03	0.16 ± 0.006	-12.16 ± 0.12	6.84 ± 0.09	612 ± 22	14.5 ± 0.20	42.2 ± 1.21
		Ruppia megacarpa - rhizomes	38.17 ± 1.33	1.98 ± 0.04	0.12 ± 0.003	-11.19 ± 0.16	6.96 ± 0.01	844 ± 5.8	22.5 ± 0.58	37.6 ± 0.75
	PBP	*Zostera capricorni* - leaves	36.21 ± 1.37	2.94 ± 0.11	0.24 ± 0.003	-10.54 ± 0.32	2.47 ± 0.67	395 ± 20	14.4 ± 0.27	27.5 ± 1.40
		Zostera capricorni - rhizomes	35.22 ± 1.66	1.06 ± 0.08	0.13 ± 0.003	-10.09 ± 0.17	3.29 ± 0.48	718 ± 36	39.6 ± 5.28	18.5 ± 1.72
	MC	*Zostera capricorni* - leaves	29.51 ± 1.31	2.23 ± 0.15	0.27 ± 0.032	-14.96 ± 0.31	5.64 ± 0.29	291 ± 23	15.4 ± 0.39	18.8 ± 1.25
		Zostera capricorni - rhizomes	32.81 ± 1.43	1.00 ± 0.05	0.15 ± 0.009	-12.16 ± 0.12	2.89 ± 0.24	579 ± 30	38.6 ± 2.03	15.0 ± 0.30
Winter 2001	OCP	*Ruppia megacarpa* - leaves	36.50 ± 0.87	1.97 ± 0.07	0.18 ± 0.006	-11.07 ± 0.11	2.82 ± 0.27	530 ± 27	21.7 ± 0.51	24.4 ± 0.94
		Ruppia megacarpa - rhizomes	36.82 ± 0.23	1.97 ± 0.06	0.18 ± 0.005	-11.48 ± 0.19	1.59 ± 0.26	543 ± 17	21.9 ± 0.54	24.9 ± 0.98
		Chaetomorpha billardierii	36.79 ± 0.43	2.41 ± 0.17	0.28 ± 0.010	-12.95 ± 0.22	7.61 ± 0.66	339 ± 10	18.0 ± 1.16	19.1 ± 1.42
		Lamprothamnium papulosum	36.60 ± 0.49	2.38 ± 0.07	0.15 ± 0.010	-14.20 ± 0.28	2.33 ± 0.27	622 ± 51	18.0 ± 0.49	34.7 ± 2.92
	NIC	*Ruppia megacarpa* - leaves	39.26 ± 0.40	2.67 ± 0.05	0.23 ± 0.008	-11.10 ± 0.11	2.32 ± 0.30	434 ± 14	17.2 ± 0.26	25.3 ± 0.81
		Ruppia megacarpa - rhizomes	38.22 ± 0.57	2.04 ± 0.05	0.24 ± 0.009	-10.98 ± 0.14	2.54 ± 0.30	421 ± 22	21.9 ± 0.54	19.3 ± 1.20
	PBP	*Zostera capricorni* - leaves	39.52 ± 0.57	3.69 ± 0.18	0.36 ± 0.011	-10.29 ± 0.10	4.05 ± 0.37	285 ± 6	12.6 ± 0.53	22.7 ± 0.72
		Zostera capricorni - rhizomes	31.05 ± 1.25	1.65 ± 0.05	0.31 ± 0.021	-10.56 ± 0.13	4.93 ± 0.32	263 ± 20	21.9 ± 0.49	11.9 ± 0.79
	MC	*Zostera capricorni* - leaves	40.55 ± 0.46	3.34 ± 0.20	0.30 ± 0.013	-12.31 ± 0.20	4.62 ± 0.37	353 ± 20	14.4 ± 0.88	24.6 ± 0.93
		Zostera capricorni - rhizomes	31.94 ± 1.60	1.28 ± 0.10	0.24 ± 0.014	-12.28 ± 0.35	4.34 ± 0.27	358 ± 33	29.7 ± 2.32	12.1 ± 0.70

Table 5-10: Tissue contents of seagrass leaves, roots-rhizomes and macroalgae at the Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP), Primbee Bay (PRIM) and Mullet Creek (MC) sites, Lake Illawarra, summer - winter 2002 (values are mean ± s.e., n = 3-6).

			Plant Tissue Contents					C:N:P Molar Ratios		
	Site	Genus and Plant Part	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C / P	C / N	N / P
Summer 2002	OCP	*Ruppia megacarpa* - leaves	32.63 ± 0.46	2.18 ± 0.06	0.32 ± 0.014	-10.20 ± 0.17	4.08 ± 0.42	262 ± 10	17.6 ± 0.58	15.0 ± 0.57
		Ruppia megacarpa - rhizomes	31.65 ± 0.73	1.38 ± 0.06	0.26 ± 0.020	-9.44 ± 0.11	2.29 ± 0.19	323 ± 21	26.8 ± 0.67	12.1 ± 0.98
		Chaetomorpha linum	28.99 ± 0.74	2.90 ± 0.09	0.17 ± 0.015	-11.71 ± 0.12	14.12 ± 0.38	461 ± 44	11.7 ± 0.18	39.4 ± 3.55
		Cladophora sp.	32.85 ± 0.20	1.76 ± 0.10	0.10 ± 0.007	-8.98 ± 0.20	7.72 ± 0.36	829 ± 52	21.9 ±1.21	38.0 ± 2.38
	NIC	*Ruppia megacarpa* - leaves	29.98 ± 0.74	2.27 ± 0.04	0.54 ± 0.017	-10.39 ± 0.05	3.89 ± 0.10	145 ± 6	15.4 ± 0.30	9.4 ± 0.29
		Ruppia megacarpa - rhizomes	30.75 ± 0.65	1.64 ± 0.04	0.49 ± 0.018	-9.81 ± 0.07	2.39 ± 0.19	163 ± 7	21.9 ± 0.49	7.4 ± 0.23
	PBP	*Zostera capricorni* - leaves	31.14 ± 0.13	2.40 ± 0.06	0.42 ± 0.028	-11.12 ± 0.24	4.53 ± 0.39	192 ± 11	15.2 ± 0.39	12.6 ± 0.57
		Zostera capricorni - rhizomes	28.19 ± 0.82	1.35 ± 0.08	0.25 ± 0.025	-9.60 ± 0.17	4.67 ± 0.41	306 ± 32	24.8 ± 1.39	12.2 ± 0.78
		Ulva compressa	33.35 ± 0.55	1.22 ± 0.02	0.08 ± 0.008	-6.70 ± 0.25	6.38 ± 0.40	1152 ± 146	31.9 ± 0.66	36.2 ± 4.68
		Gracilaria edulis	30.50 ± 0.49	3.49 ± 0.21	0.53 ± 0.035	-17.07 ± 0.38	9.34 ± 0.29	150 ± 11	10.3 ± 0.77	14.7 ± 1.03
	PRIM	*Chaetomorpha* spp.*	30.14 ± 0.62	1.70 ± 0.19	0.36 ± 0.022	-13.36 ± 0.29	6.85 ± 0.26	217 ± 11	21.9 ± 2.18	10.3 ± 0.93
	MC	*Zostera capricorni* - leaves	28.13 ± 1.11	2.15 ± 0.09	0.43 ± 0.022	-15.02 ± 0.20	5.58 ± 0.16	171 ± 3	15.3 ± 0.19	11.2 ± 0.21
		Zostera capricorni - rhizomes	32.66 ± 0.65	1.26 ± 0.04	0.26 ± 0.020	-12.74 ± 0.29	3.92 ± 0.71	339 ± 29	30.4 ± 0.46	11.2 ± 1.12
		Chaetomorpha linum	32.24 ± 1.33	2.58 ± 0.06	0.28 ± 0.015	-17.09 ± 1.57	7.18 ± 0.38	298 ± 14	14.6 ± 0.94	20.5 ± 1.55
		Ulva sp. (prob. *U. compressa*)	32.44 ± 0.94	2.25 ± 0.19	0.16 ± 0.014	-12.99 ± 0.53	9.63 ± 0.24	540 ± 52	17.0 ± 1.06	32.1 ± 3.79
Winter 2002	OCP	*Ruppia megacarpa* - leaves	31.08 ± 0.95	2.19 ± 0.08	0.28 ± 0.008	-10.60 ± 0.07	4.70 ± 0.34	288 ± 12	16.8 ± 0.87	17.4 ± 0.71
		Ruppia megacarpa - rhizomes	29.22 ± 0.80	1.53 ± 0.05	0.26 ± 0.008	-10.85 ± 0.17	2.86 ± 0.06	289 ± 12	22.4 ± 0.99	13.0 ± 0.65
		Chaetomorpha billardierii	36.11 ± 0.73	3.51 ± 0.09	0.25 ± 0.007	-11.86 ± 0.22	10.77 ± 0.46	379 ± 14	12.0 ± 0.50	31.6 ± 1.28
	NIC	*Ruppia megacarpa* - leaves	34.12 ± 0.69	2.14 ± 0.04	0.36 ± 0.014	-10.59 ± 0.15	3.56 ± 0.26	243 ± 7.9	18.6 ± 0.42	13.1 ± 0.53
		Ruppia megacarpa - rhizomes	31.46 ± 0.58	1.69 ± 0.04	0.29 ± 0.009	-10.35 ± 0.25	2.52 ± 0.07	279 ± 6.7	21.8 ± 0.41	12.8 ± 0.29
	PBP	*Zostera capricorni* - leaves	36.28 ± 0.65	2.78 ± 0.09	0.47 ± 0.018	-10.27 ± 0.16	5.64 ± 0.34	201 ± 5.4	15.3 ± 0.71	13.3 ± 0.70
		Zostera capricorni - rhizomes	25.67 ± 0.78	1.47 ± 0.05	0.29 ± 0.012	-10.56 ± 0.07	5.20 ± 0.24	226 ± 6.5	20.6 ± 1.22	11.2 ± 0.80
	MC	*Zostera capricorni* - leaves	36.55 ± 0.64	2.73 ± 0.12	0.33 ± 0.019	-12.82 ± 0.23	5.02 ± 0.33	288 ± 14	15.7 ± 0.48	18.4 ± 0.75
		Zostera capricorni - rhizomes	32.04 ± 1.11	1.19 ± 0.05	0.24 ± 0.016	-12.12 ± 0.15	4.88 ± 0.11	345 ± 12	31.6 ± 0.72	10.9 ± 0.34

* Samples collected from a mixed mat of *Chaetomorpha billardierii and Chaetomorpha linum* - separation of filamentous species for individual analyses was not possible.

Table 5-11: Summary of two-way ANOVA testing for differences in elemental and isotopic contents of seagrass leaves collected from the OCP, NIC, PBP and MC sites, spring 2000 - winter 2002 (n = 99).

	Parameter	Null Hypothesis	Factor	d.f.	F-Ratio	Probability	Power (α=0.05)	Differences (Tukey-Kramer multiple comparison $p < 0.05$)
Seagrass Leaves	Total C	H_0 = Total C contents of seagrass leaves do not vary significantly between sites or seasons	Season	4	82.4	0.000000*	1.00	• 00-Spr and 01-Win > 01-Sum, 02-Sum and 02-Win; • 02-Win > 01-Sum and 02-Sum
			Site	3	3.9	0.011761*	0.81	• No significant differences (Tukey's $p > 0.10$)
			Season x Site	12	14.0	0.000000*	1.00	• Significant interactions
	Total N	H_0 = Total N contents of seagrass leaves do not vary significantly between sites or seasons	Season	4	31.4	0.000000*	1.00	• 00-Spr, 01-Sum and 01-Win > 02-Sum and 02-Win
			Site	3	23.7	0.000000*	1.00	• PBP > MC > NIC > OCP
			Season x Site	12	12.2	0.000000*	1.00	• Significant interactions
	Total P	H_0 = Total P contents of seagrass leaves do not vary significantly between sites or seasons	Season	4	144.2	0.000000*	1.00	• 02-Sum > 02-Win > 01-Win > 01-Sum and 00-Spr
			Site	3	58.2	0.000000*	1.00	• PBP > MC, OCP and NIC; MC and NIC > OCP
			Season x Site	12	8.7	0.000000*	1.00	• Significant interactions
	$\delta_{13}C$	H_0 = $\delta_{13}C$ levels of seagrass leaves do not vary significantly between sites or seasons	Season	4	28.9	0.000000*	1.00	• 01-Win and 02-Win > 02-Sum > 01-Sum and 00-Spr
			Site	3	191.7	0.000000*	1.00	• PBP, NIC and OCP > MC; PBP > NIC
			Season x Site	12	12.5	0.000000*	1.00	• Significant interactions
	$\delta_{15}N$	H_0 = $\delta_{15}N$ levels of seagrass leaves do not vary significantly between sites or seasons	Season	4	8.0	0.000019*	1.00	• 00-Spr, 01-Sum, 02-Sum and 02-Win > 01-Win
			Site	3	37.9	0.000000*	1.00	• MC > PBP and NIC > OCP
			Season x Site	12	26.9	0.000000*	1.00	• Significant interactions
	C/P molar ratio	H_0 = C/P molar ratios of seagrass leaves do not vary significantly between sites or seasons	Season	4	453.3	0.000000*	1.00	• 01-Sum > 00-Spr > 01-Win > 02-Win > 02-Sum
			Site	3	283.7	0.000000*	1.00	• OCP > NIC and MC > PBP
			Season x Site	12	47.2	0.000000*	1.00	• Significant interactions
	C/N molar ratio	H_0 = C/N molar ratios of seagrass leaves do not vary significantly between sites or seasons	Season	4	4.9	0.001493*	0.95	• 01-Win and 02-Win > 01-Spr and 01-Sum
			Site	3	35.5	0.000000*	1.00	• OCP > NIC > PBP and MC
			Season x Site	12	7.4	0.000000*	1.00	• Significant interactions
	N/P molar ratio	H_0 = N/P molar ratios of seagrass leaves do not vary significantly between sites or seasons	Season	4	627.1	0.000000*	1.00	• 01-Sum > 00-Spr > 01-Win > 02-Win > 02-Sum
			Site	3	152.5	0.000000*	1.00	• OCP > NIC > MC and PBP
			Season x Site	12	62.9	0.000000*	1.00	• Significant interactions

* Test significant at $p < 0.05$

Table 5-12: Summary of two-way ANOVA testing for differences in elemental and isotopic contents of seagrass rhizomes collected from the OCP, NIC, PBP and MC sites, spring 2000 - winter 2002 (n = 99).

	Parameter	Null Hypothesis	Factor	d.f.	F-Ratio	Probability	Power (α=0.05)	Differences (Tukey-Kramer multiple comparison $p < 0.05$)
Seagrass Rhizomes	Total C	H_0 = Total C contents of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	37.0	0.000000*	1.00	• 00-Spr, 01-Sum and 01-Win > 02-Sum and 02-Win
			Site	3	21.4	0.000000*	1.00	• NIC, OCP and MC > PBP; NIC > MC
			Season x Site	12	3.8	0.000127*	1.00	• Significant interactions
	Total N	H_0 = Total N contents of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	16.2	0.000000*	1.00	• 01-Win > 00-Spr, 01-Sum, 02-Sum and 02-Win
			Site	3	122.3	0.000000*	1.00	• NIC > OCP > PBP > MC
			Season x Site	12	8.8	0.000000*	1.00	• Significant interactions
	Total P	H_0 = Total P contents of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	102.6	0.000000*	1.00	• 02-Sum > 02-Win > 01-Win > 00-Spr and 01-Sum
			Site	3	14.9	0.000000*	1.00	• NIC > PBP > MC and OCP
			Season x Site	12	12.8	0.000000*	1.00	• Significant interactions
	$\delta_{13}C$	H_0 = $\delta_{13}C$ levels of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	9.6	0.000002*	1.00	• 02-Sum > 00-Spr, 01-Sum, 01-Win and 02-Win
			Site	3	66.9	0.000000*	1.00	• PBP > NIC and OCP > MC
			Season x Site	12	7.2	0.000000*	1.00	• Significant interactions
	$\delta_{15}N$	H_0 = $\delta_{15}N$ levels of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	6.6	0.000133*	0.99	• 02-Win > 00-Spr, 01-Win and 02-Sum; 01-Sum > 00-Spr
			Site	3	89.9	0.000000*	1.00	• PBP > OCP and NIC; MC > OCP
			Season x Site	12	52.6	0.000000*	1.00	• Significant interactions
	C/P molar ratio	H_0 = C/P molar ratios of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	320.9	0.000000*	1.00	• 01-Sum > 00-Spr > 01-Win > 02-Sum and 02-Win
			Site	3	34.9	0.000000*	1.00	• OCP > NIC, MC and PBP
			Season x Site	12	14.6	0.000000*	1.00	• Significant interactions
	C/N molar ratio	H_0 = C/N molar ratios of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	24.4	0.000000*	1.00	• 00-Spr and 01-Sum > 01-Win, 02-Sum and 02-Win
			Site	3	56.0	0.000000*	1.00	• MC > PBP > NIC and OCP
			Season x Site	12	7.8	0.000000*	1.00	• Significant interactions
	N/P molar ratio	H_0 = N/P molar ratios of seagrass rhizomes do not vary significantly between sites or seasons	Season	4	256.3	0.000000*	1.00	• 01-Sum > 00-Spr > 01-Win > 02-Sum and 02-Win
			Site	3	179.4	0.000000*	1.00	• OCP > NIC > MC and PBP
			Season x Site	12	40.0	0.000000*	1.00	• Significant interactions

* Test significant at $p < 0.05$

Table 5-13: Summary of one-way ANOVA testing for differences in elemental and isotopic contents of seagrasses and macroalgae collected from OCP, NIC, PBP, MC, spring 2000 - winter 2002. *F*-ratios shown, with test significant at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Spatial Variance

H_0 = Tissue nutrient contents, isotope levels and C:N:P molar ratios of seagrasses (leaves and rhizomes) and macroalgae do not vary significantly between sites (one-way ANOVAs conducted for each sampling round)

Plant	Source of variance	d.f.	Plant Tissue Contents					C:N:P Molar Ratios		
			C	N	P	$\delta^{13}C$	$\delta^{15}N$	C / P	C / N	N / P
Seagrass Leaves	2000, spring	5	5.56*	5.53*	164.08***	8.51**	168.89***	181.93***	12.00***	44.49***
	2001, summer	3	17.92***	13.57**	31.76*** #	77.24***	39.77***	166.41***	13.36**	137.76***
	2001, winter	3	8.28***	40.20*** #	61.69***	19.44*** $	10.36***	20.24*** $	36.58*** #	1.64
	2002, summer	3	7.20**	2.77	17.01***	154.72***	6.26**	38.98***	8.51***	28.79***
	2002, winter	3	11.64***	15.82***	26.47***	51.85***	7.55**	16.39***	5.16*	16.40***
Seagrass Rhizomes	2000, spring	5	8.56**	30.50***	19.27* $	6.87**	211.04***	11.27**	9.21**	60.17***
	2001, summer	3	3.96	102.69***	12.00**	11.09**	79.27***	25.81***	18.28*** #	164.62***
	2001, winter	3	11.16***	26.38***	20.59*** #	16.05** $	28.80**	27.38***	51.10***	30.78*** #
	2002, summer	3	6.63**	7.80**	32.08***	75.48***	20.44***	11.04***	17.51***	10.10*** #
	2002, winter	3	11.80***	19.98***	4.61*	21.28***	94.30***	25.62***	32.24***	3.60*
Macroalgae (all species)	2000, spring	9	16.00***	12.56***	8.80***	49.65***	22.35***	9.77*** #	20.27*** #	9.89***
	2002, summer	6	4.31**	19.61***	81.25*** #	20.78** $	36.85*** #	83.48*** #	19.72** $	25.18***

Temporal Variance

H_0 = Tissue nutrient contents, isotope levels and C:N:P molar ratios of seagrasses (leaves and rhizomes) and macroalgae do not vary significantly between sampling rounds (one-way ANOVAs conducted on data collected at each site)

Site	Source of variance	d.f.	Plant Tissue Contents					C:N:P Molar Ratios		
			C	N	P	$\delta^{13}C$	$\delta^{15}N$	C / P	C / N	N / P
OCP	*Ruppia* leaves	4	24.41***	18.81***	118.72*** #	27.38***	10.99***	134.19*** #	11.27**	237.72***
	Ruppia rhizomes	4	34.80***	19.57***	53.46*** #	20.46***	14.06***	146.53***	7.94***	151.69***
	Chaetomorpha	4	21.08***	10.61*** #	111.41***	42.83***	33.68***	17.47** $	14.06***	80.21*** #
NIC	*Ruppia* leaves	4	36.11***	71.52***	170.48*** #	54.93***	83.28***	252.98***	24.53***	364.70***
	Ruppia rhizomes	4	28.61***	18.09***	206.70*** #	18.79***	109.19***	212.08*** #	0.48	21.76*** $
PBP	*Zostera* leaves	4	27.81***	18.18***	19.27*** $	3.75*	11.02***	98.68***	4.58**	92.45***
	Zostera rhizomes	4	11.54***	13.63***	17.27***	8.27***	20.41***	84.79***	23.23*** #	14.27***
MC	*Zostera* leaves	4	53.04***	17.72*** #	16.83***	16.34***	3.78*	70.01*** #	1.27 #	83.89***
	Zostera rhizomes	4	0.76	2.66	7.44***	2.51	20.36***	12.81***	4.89*	3.86*

Test performed on log-transformed data. $ Kruskal-Wallis ANOVA (*H*-ratio).

Table 5-14: Summary of paired-t testing for differences in C, N, P and isotopic contents of seagrass leaves and their respective roots-rhizomes, sampled at the Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP), Primbee Bay (PRIM) and Mullet Creek (MC) sites. *T*-values shown, with test significant at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

H_0 = Tissue nutrient contents, isotope levels and C:N:P molar ratios do not vary significantly between seagrass leaves and rhizomes										
			Plant Tissue Contents					C:N:P Molar Ratios		
	Site	Season	Total C	Total N	Total P	$\delta^{13}C$	$\delta^{15}N$	C/P	C/N	N/P
Ruppia	**OCP**	*All data*	2.48* (L)	7.22*** (L)	4.13*** (L)	-1.97	10.15*** (L)	-3.62** (R)	-6.96*** (R)	4.79*** (L)
		2000, spring	5.52* (L)	46.00*** (L)	3.50* (L)	-4.19	11.31** (L)	-2.48	-7.78* (R)	10.07** (L)
		2001, summer	6.11* (L)	10.29** (L)	5.00* (L)	-2.65	10.46** (L)	-2.95	-8.42* (R)	29.75** (L)
		2001, winter	-0.40	-0.002	0.83	2.23	9.87*** (L)	-1.17	-0.20	-0.60
		2002, summer	1.12	15.95*** (L)	6.19** (L)	-6.96*** (R)	5.81** (L)	-3.72* (R)	-12.56*** (R)	4.62** (L)
		2002, winter	1.66	11.24*** (L)	1.27	1.77	5.03** (L)	-0.02	-7.55*** (R)	8.10*** (L)
	NIC	*All data*	1.35	12.86*** (L)	5.24*** (L)	-5.16*** (R)	4.29*** (L)	-3.19** (R)	-12.07*** (R)	5.60*** (L)
		2000, spring	0.26	29.62** (L)	16.00** (L)	-3.33	3.59	-4.05	-18.88** (R)	1.96
		2001, summer	-0.11	15.53** (L)	6.50* (L)	-3.45	-1.27	-8.53* (R)	-9.97** (R)	5.81* (L)
		2001, winter	1.10	7.32*** (L)	-0.60	-0.86	-2.68* (R)	0.93	-7.66*** (R)	8.33*** (L)
		2002, summer	-0.68	24.22*** (L)	2.40	-5.46** (R)	9.59*** (L)	-1.86	-10.82*** (R)	5.12** (L)
		2002, winter	2.93* (L)	6.25** (L)	5.61** (L)	-2.03	4.79** (L)	-4.17** (R)	-4.41** (R)	0.50
	PBP	2000, spring	20.03** (L)	4.78* (L)	-2.31	1.57	-2.53	4.21	-4.33* (R)	4.10
Zostera	**PBP**	*All data*	7.02*** (L)	15.59*** (L)	9.10*** (L)	-2.05	-0.26	-3.77*** (R)	-7.03*** (R)	5.31*** (L)
		2000, spring	3.83	38.19*** (L)	46.86*** (L)	-3.27	22.21** (L)	-22.51** (R)	-18.14** (R)	13.58** (L)
		2001, summer	2.13	9.86* (L)	19.05** (L)	-1.39	-1.79	-15.30** (R)	-4.74* (R)	2.90
		2001, winter	8.65*** (L)	13.08*** (L)	1.73	1.40	-1.78	0.90	-17.16*** (R)	9.16*** (L)
		2002, summer	4.11** (L)	12.10*** (L)	9.84*** (L)	-6.90*** (R)	-0.24	-4.23** (R)	-7.39*** (R)	0.66
		2002, winter	8.70*** (L)	11.23*** (L)	11.32*** (L)	1.53	0.91	-5.14** R)	-3.07* (R)	2.17
	PRIM	2000, spring	4.86* (L)	25.52** (L)	7.79* (L)	1.28	3.16	-2.29	-7.18* (R)	12.31** (L)
	MC	*All data*	2.22* (L)	15.21*** (L)	8.44*** (L)	-6.27*** (R)	5.58*** (L)	-5.44*** (R)	-16.94*** (R)	6.88*** (L)
		2000, spring	8.98*** (L)	46.42*** (L)	7.10*** (L)	3.89** (R)	8.47*** (L)	-3.27* (R)	-8.46*** (R)	9.92*** (L)
		2001, summer	-1.21	6.92* (L)	3.33	-14.92** (R)	9.26* (L)	-10.34** (R)	-11.43** (R)	2.58
		2001, winter	5.67** (L)	14.26*** (L)	14.58*** (L)	-0.10	2.03	-0.27	-10.26*** (R)	16.45*** (L)
		2002, summer	-3.07* (R)	8.04*** (L)	5.53** (L)	-9.22*** (R)	7.12*** (L)	-5.68** (R)	-25.71*** (R)	-0.04
		2002, winter	3.34* (L)	12.02*** (L)	3.85* (L)	-2.76* (R)	0.49	-4.06** (R)	-19.93*** (R)	7.26*** (L)

Note: (L) = Leaves significantly higher than roots-rhizomes; (R) = Roots-rhizomes significantly higher than leaves.

5.3.2 Variations between Seagrass Leaves and Rhizomes

Paired-t tests were conducted to assess the differences between nutrient contents of seagrass leaves and rhizomes of the same plant. Where significant differences ($p < 0.05$) occurred in *Zostera* and *Ruppia* nutrient concentrations, seagrass leaves invariably had higher total C, N and P contents than roots-rhizomes (Table 5-14). Fewer significant differences occurred between *Ruppia* leaf and rhizome C contents, when compared to *Zostera*, but the single exception where rhizome C significantly exceeded leaf C was *Zostera* at Mullet Creek during the summer 2002 sampling round ($T = -3.1$, $p < 0.05$). Across all sites and all samples, seagrass leaf N contents significantly exceeded rhizome N ($p < 0.01$); *Zostera* samples showed a greater differentiation than *Ruppia*, with *Zostera* leaf N contents being approximately 1.5 - 3 times higher than rhizome N (Table 5-14). The only exception was OCP *Ruppia* samples collected during winter 2001, where no significant differences ($p > 0.05$) occurred between leaf N and rhizome N. Differences in total P contents were less pronounced than N; although leaf P typically exceeded rhizome P for the vast majority of samples, these differences were not always significant. Fewer significant differences occurred between seagrass leaf and root/rhizome $\delta^{13}C$ contents, particularly *Ruppia* samples (Table 5-14). Where significant differences did occur, leaf $\delta^{13}C$ contents were significantly more negative than rhizomes ($p < 0.01$). On the other hand, *Zostera* showed fewer significant differences between leaf and rhizome $\delta^{15}N$ contents than *Ruppia*. Where differences were significant, seagrass leaves exhibited higher $\delta^{15}N$ contents than rhizomes, with only one exception at Nicolle Road (winter 2001; $T = -2.6$, $p < 0.05$). In addition, where significant differences ($p < 0.05$) occurred between the molar ratios of seagrass leaves and their respective rhizomes, the C/P and C/N ratios of rhizomes exceeded leaves, but the N/P ratios of leaves invariably exceeded that of rhizomes ($p < 0.01$).

5.3.3 Intra-Spatial Variations in Nutrient and Isotopic Contents

The following section describes the variations in C, N, P and isotopic contents between seagrass leaves and macroalgae sampled from individual sites at the same time.

Oasis Caravan Park Site (OCP)

As various species of macroalgae were sampled at the Oasis Caravan Park throughout the study period, separate ANOVA tests were conducted to compare nutrient concentrations of *Ruppia* leaves and different macroalgal genera during each of the sampling rounds (Table 5-15). *Ruppia* leaves at OCP typically exhibited similar, or higher, total C contents than macroalgae, but N and P contents were highly variable between species. *Ruppia* leaf C concentrations ranged from 27.9 - 41.0 % C (mean: 35.1 ± 0.81 % C), while macroalgae ranged from 28.0 - 39.7 % C. Tukey-Kramer tests ($\alpha = 0.05$) showed that *Ruppia* leaf C

significantly exceed macroalgae C during spring 2000 and summer 2001, but macroalgae (*Chaetomorpha*) C contents significantly exceeded *Ruppia* leaf C on only occasion (winter 2002).

Ruppia leaves at OCP averaged 2.28 ± 0.07 % N across all OCP sampling rounds, whereas *Chaetomorpha* averaged 3.02 ± 0.13 % N. *Chaetomorpha* generally had significantly higher total N contents than *Ruppia* and other macroalgae (e.g., *Ulva, Cladophora*) collected during the same sampling round (spring 2000, winter 2001 - 2002). *Ruppia* leaf N exceeded *Chaetomorpha* N on only one occasion (summer 2001), when *Chaetomorpha* dropped to 2.23 ± 0.10 % N. On the other hand, total P contents of *Ruppia* leaves averaged 0.23 ± 0.02 % P (range: 0.12 - 0.37 % P) and significantly exceeded macroalgae collected from the same location during all sampling rounds except winter 2001. *Chaetomorpha* P averaged 0.16 ± 0.02 % P (range: 0.03 - 0.29 % P), and only exceeded *Ruppia* leaf P significantly during winter 2001, when it reached 0.28 ± 0.010 % P. *Chaetomorpha* generally had similar P contents to other macroalgal species present during the same sampling period; the only significant differences occurred between *Rhizoclonium* (summer 2001) and *Lamprothamnium* (winter 2001).

Chaetomorpha δ^{13}C contents ranged from -11.0 to -16.1 ‰ (mean: -13.3 ± 0.35 ‰) across the sampling period at the Oasis Caravan Park. During all sampling rounds, *Ruppia* leaves at OCP had significantly less negative δ^{13}C contents than *Chaetomorpha*, ranging from -12.2 to -9.64 ‰ (mean: -10.9 ± 0.14 ‰). The δ^{13}C contents of other species of macroalgae recorded at OCP varied somewhat; *Ulva* (spring 2000: -10.3 ± 0.54 ‰), *Rhizoclonium* (summer 2001: -9.02 ± 0.33 ‰) and *Cladophora* (summer 2002: -8.98 ± 0.20 ‰) had significantly more positive δ^{13}C contents than *Ruppia* leaves and *Chaetomorpha*. On the other hand, *Lamprothamnium* (winter 2001: -14.2 ± 0.28 ‰) had significantly more negative δ^{13}C contents than both *Chaetomorpha* and *Ruppia* leaves. Across all sampling periods, *Ruppia* leaves had significantly lower δ^{15}N contents (mean: 3.42 ± 0.26 ‰) than all macroalgae, except *Lamprothamnium* (winter 2001: 2.33 ± 0.27 ‰), collected at the same time. The δ^{15}N contents of *Chaetomorpha* at OCP varied considerably from 4.45 - 14.6 ‰ (mean: 9.98 ± 0.60 ‰), and were only significantly lower than *Ulva* (spring 2000: 13.0 ± 0.20 ‰).

Table 5-15: Summary of one-way ANOVA testing for differences in C, N, P and isotopic contents of seagrass leaves and macroalgae sampled at the Oasis Caravan Park (OCP), spring 2000 – winter 2002.

Site		Null Hypothesis	Parameter	d.f.	F-Ratio	Probability	Power (α=0.05)	Differences (Tukey-Kramer multiple comparison $p < 0.05$)
Oasis Caravan Park	Spring 2000	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves), *Chaetomorpha* or *Ulva*, collected at OCP in spring 2000	Total C	2	88.4	0.000001*	1.000	• *Chaetomorpha* and *Ruppia* > *Ulva*
			Total N #	2	25.8	0.000187*	1.000	• *Chaetomorpha* and *Ruppia* > *Ulva*
			Total P	2	23.4	0.000273*	1.000	• *Ruppia* > *Chaetomorpha* and *Ulva*
			$\delta^{13}C$	2	76.6	0.000002*	1.000	• *Ruppia* and *Chaetomorpha* are more negative than *Ulva; Chaetomorpha* is more negative than *Ruppia*
			$\delta^{15}N$	2	451.1	0.000000*	1.000	• *Ulva* > *Chaetomorpha* > *Ruppia*
	Summer 2001	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves), *Chaetomorpha* or *Rhizoclonium*, collected at OCP in summer 2001	Total C	2	12.6	0.007071*	0.934	• *Ruppia* > *Rhizoclonium*
			Total N	2	39.5	0.000351*	1.000	• *Ruppia* > *Chaetomorpha* and *Rhizoclonium*
			Total P $	2	7.2	0.027324*	1.000	• *Ruppia* > *Chaetomorpha*
			$\delta^{13}C$	2	61.4	0.000101*	1.000	• *Chaetomorpha* is more negative than *Ruppia*, and both species are more negative than *Rhizoclonium*
			$\delta^{15}N$	2	12.2	0.007623*	0.927	• *Chaetomorpha* > *Ruppia*
	Winter 2001	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves), *Chaetomorpha* or *Lamprothamnium*, collected at OCP in winter 2001	Total C	2	0.0	0.967706	0.054	• No significant differences
			Total N	2	7.1	0.014332*	0.812	• *Chaetomorpha* and *Lamprothamnium* > *Ruppia*
			Total P	2	53.2	0.000010*	1.000	• *Chaetomorpha* > *Ruppia* and *Lamprothamnium*
			$\delta^{13}C$	2	86.6	0.000001*	1.000	• *Lamprothamnium* is more negative than *Chaetomorpha*, and both species are more negative than *Ruppia*
			$\delta^{15}N$	2	47.3	0.000017*	1.000	• *Chaetomorpha* > *Lamprothamnium* and *Ruppia*
	Summer 2002	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves), *Chaetomorpha* or *Cladophora*, collected at OCP in summer 2002	Total C	2	14.5	0.001547*	0.985	• *Ruppia* and *Cladophora* > *Chaetomorpha*
			Total N	2	38.6	0.000038*	1.000	• *Chaetomorpha* > *Ruppia* > *Cladophora*
			Total P	2	68.0	0.000004*	1.000	• *Ruppia* > *Chaetomorpha* and *Cladophora*
			$\delta^{13}C$	2	75.9	0.000002*	1.000	• *Chaetomorpha* is more negative than *Ruppia*, and both species are more negative than *Cladophora*
			$\delta^{15}N$	2	128.4	0.000000*	1.000	• *Chaetomorpha* > *Cladophora* > *Ruppia*
	Winter 2002	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves) or *Chaetomorpha* collected at OCP in winter 2002	Total C	1	17.6	0.001846*	0.965	• *Chaetomorpha* > *Ruppia*
			Total N	1	119.0	0.000001*	1.000	• *Chaetomorpha* > *Ruppia*
			Total P	1	9.8	0.010812*	0.803	• *Ruppia* > *Chaetomorpha*
			$\delta^{13}C$	1	29.6	0.000283*	0.998	• *Chaetomorpha* is more negative than *Ruppia*
			$\delta^{15}N$	1	114.9	0.000001*	1.000	• *Chaetomorpha* > *Ruppia*

Test performed on log-transformed data. $ Kruskal-Wallis ANOVA (*H*-ratio). * Test significant at $p < 0.05$

Table 5-16: Summary of one-way ANOVA testing for differences in C, N, P and isotopic contents of seagrass leaves and macroalgae sampled at Nicolle Road (NIC), Purry Burry Point (PBP), Primbee Bay (PRIM) and Mullet Creek (MC).

Site		Null Hypothesis	Parameter	d.f.	F-Ratio	Probability	Power (α=0.05)	Differences (Tukey-Kramer multiple comparison $p < 0.05$)
Nicolle Road	Spring 2000	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves) or *Chaetomorpha*, collected at Nicolle Road in spring 2000	Total C	1	0.8	0.402098	0.121	• No significant differences
			Total N $	1	2.4	0.121335	0.348	• No significant differences
			Total P #	1	4.4	0.074643	0.419	• No significant differences
			$\delta^{13}C$	1	191.6	0.000002*	1.000	• *Chaetomorpha* is more negative than *Ruppia*
			$\delta^{15}N$	1	21.1	0.002521*	0.974	• *Chaetomorpha* > *Ruppia*
Purry Burry Point	Spring 2000	H_0 = Nutrient contents do not vary significantly between *Ruppia* (leaves), *Zostera* (leaves) or *Chaetomorpha*, collected at Purry Burry Point in spring 2000	Total C	2	1.2	0.351405	0.197	• No significant differences
			Total N	2	9.4	0.006284*	0.910	• *Zostera* and *Chaetomorpha* > *Ruppia*
			Total P	2	14.4	0.001572*	0.985	• *Ruppia* and *Zostera* > *Chaetomorpha*
			$\delta^{13}C$	2	30.5	0.000099*	1.000	• *Chaetomorpha* is more negative than *Zostera* and *Ruppia*
			$\delta^{15}N$	2	373.1	0.000000*	1.000	• *Chaetomorpha* > *Ruppia* > *Zostera*
	Summer 2002	H_0 = Nutrient contents do not vary significantly between *Zostera* (leaves), *Gracilaria* or *Ulva* collected at Purry Burry Point in summer 2002	Total C	2	17.4	0.000810*	0.995	• *Ulva* > *Gracilaria* and *Zostera*
			Total N	2	90.7	0.000001*	1.000	• *Gracilaria* > *Zostera* > *Ulva*
			Total P #	2	152.3	0.000000*	1.000	• *Gracilaria* and *Zostera* > *Ulva*
			$\delta^{13}C$	2	238.7	0.000000*	1.000	• *Gracilaria* is more negative than *Zostera*, and both species are more negative than *Ulva*
			$\delta^{15}N$	2	34.7	0.000059*	1.000	• *Gracilaria* > *Zostera* > *Ulva*
Primbee Bay	Spring 2000	H_0 = Nutrient contents do not vary significantly between *Zostera* (leaves), *Chaetomorpha*, sheet-like *Ulva* (S) or filamentous *Ulva* (F) collected at Primbee Bay in spring 2000	Total C	3	5.3	0.026544*	0.746	• *Chaetomorpha* > *Ulva* (F)
			Total N	3	11.4	0.002924*	0.976	• *Zostera* and *Ulva* (S) > *Ulva* (F)
			Total P	3	3.6	0.064479	0.570	• No significant differences
			$\delta^{13}C$	3	2.7	0.117684	0.442	• No significant differences
			$\delta^{15}N$	3	17.1	0.000775*	0.998	• *Ulva* (S), *Ulva* (F) and *Chaetomorpha* > *Zostera*; • *Ulva* (S) > *Ulva* (F)
Mullet Creek	Spring 2000	H_0 = Nutrient contents do not vary significantly between *Zostera* (leaves), *Chaetomorpha* or *Ulva* collected at Mullet Creek in spring 2000	Total C	2	50.7	0.000013*	1.000	• *Zostera* > *Chaetomorpha* > *Ulva*
			Total N	2	26.6	0.000167*	1.000	• *Chaetomorpha* and *Zostera* > *Ulva*
			Total P $	2	5.1	0.078988	0.826	• No significant differences
			$\delta^{13}C$	2	14.5	0.001550*	0.985	• *Chaetomorpha* is more negative than *Ulva* and *Zostera*
			$\delta^{15}N$	2	253.5	0.000000*	1.000	• *Ulva* > *Chaetomorpha* > *Zostera*
	Summer 2002	H_0 = Nutrient contents do not vary significantly between *Zostera* (leaves), *Chaetomorpha* or *Ulva* collected at Mullet Creek in summer 2002	Total C	2	4.5	0.044929*	0.609	• No significant differences (Tukey's $p > 0.05$)
			Total N	2	3.5	0.076918	0.497	• No significant differences
			Total P	2	40.9	0.000030*	1.000	• *Zostera* > *Chaetomorpha* > *Ulva*
			$\delta^{13}C$	2	6.4	0.018568*	0.772	• *Chaetomorpha* is more negative than *Ulva*
			$\delta^{15}N$	2	75.7	0.000002*	1.000	• *Ulva* > *Chaetomorpha* > *Zostera*

Test performed on log-transformed data. $ Kruskal-Wallis ANOVA (*H*-ratio). * Test significant at $p < 0.05$

Nicolle Road Site (NIC)

ANOVA testing between macrophyte genera analysed at the Nicolle Road site was only conducted for the spring 2000 sampling round (Table 5-16), as macroalgal biomass was negligible in subsequent sampling rounds. Tissue C and N concentrations of *Ruppia* and *Chaetomorpha* were fairly uniform, varying between 35.7 - 39.6 % C and 3.0 - 3.9 % N, and were therefore not significantly different ($p > 0.05$). Likewise, *Chaetomorpha* tissue P concentrations ranged from 0.07 - 0.22 % P (mean: 0.12 ± 0.02 % P), but were not significantly different ($p > 0.05$) to *Ruppia* leaf P (mean: 0.19 ± 0.003 %). *Chaetomorpha* had significantly more negative $\delta^{13}C$ contents ($F = 192$; $p < 10^{-5}$), and significantly higher $\delta^{15}N$ contents ($F = 21.1$; $p < 0.005$), than *Ruppia* leaves collected at the same time.

Purry Burry Point Site (PBP)

In spring 2000, mixed seagrass beds containing both *Zostera* and *Ruppia* were sampled at Purry Burry Point, but only sparse *Ruppia* was present in the same area in future surveys. ANOVA tests were conducted to compare nutrient and isotopic contents of macrophytes sampled at PBP in the spring 2000 and summer 2002 sampling rounds only (Table 5-16); macroalgal biomass was not sufficient for nutrient analyses during all other sampling rounds. Total C contents did not vary significantly between species in spring 2000 ($p > 0.05$), but in summer 2002, Tukey-Kramer tests ($\alpha = 0.05$) showed that tissue C contents of *Ulva* (33.4 ± 0.55 % C) were significantly higher than *Gracilaria* (30.5 ± 0.49 % C) and *Zostera* leaves (31.1 ± 0.13% C) ($F = 16.8$, $p < 0.001$). Tissue N and P contents varied markedly between species; *Ulva* had the lowest N (1.22 ± 0.02 % N), while *Gracilaria* had the highest (3.49 ± 0.21 % N). *Ruppia* leaves sampled during spring 2000 had significantly lower N contents than both *Zostera* leaves and *Chaetomorpha* collected at the same time. Additionally, the total P contents of the green macroalgae, *Ulva* and *Chaetomorpha* (0.08 - 0.11 % P), were significantly lower than those of the seagrasses (0.20 - 0.45 % P) and *Gracilaria* (0.53 ± 0.04 % P) analysed during the same sampling round (Tukey's $p < 0.05$). The $\delta^{13}C$ contents of *Zostera* and *Ruppia* leaves were similar at -10.8 ± 0.25 ‰ and -9.32 ± 0.21 ‰, respectively (spring 2000), and were significantly less negative than *Chaetomorpha* (-13.2 ± 0.38 ‰). The least and most negative $\delta^{13}C$ contents occurred in *Ulva* (-6.70 ± 0.25 ‰) and *Gracilaria* (-17.1 ± 0.38 ‰), respectively. The $\delta^{15}N$ contents of the seagrasses *Zostera* (range: 1.35 - 6.78 ‰) and *Ruppia* (range: 3.40 - 3.98 ‰) were also significantly lower than all macroalgae genera sampled; *Chaetomorpha* and *Gracilaria* had the highest $\delta^{15}N$ contents of 9.92 ± 0.23 ‰ and 9.34 ± 0.29 ‰, respectively.

Primbee Bay Site (PRIM)

Multiple comparisons between macrophyte genera were only conducted for the spring 2000 sampling round at Primbee Bay (Table 5-16); the summer 2002 sampling round included *Chaetomorpha* only. ANOVA comparisons showed that total C contents were similar across species, with the only significant differences being between *Chaetomorpha* (37.5 ± 0.30 % C) and *Ulva* (32.7 ± 1.02 % C) ($F = 4.37$; $p < 0.05$). The total N contents of *Ulva* (0.90 ± 0.03 % N) were also significantly lower than all other genera ($F = 13.12$; $p < 0.01$). However, the total P contents of filamentous *Ulva* (0.27 ± 0.01 % P) and *Zostera* leaves (0.29 ± 0.01 % P) were significantly higher than *Chaetomorpha* (0.18 ± 0.03 % P) and sheet-like *Ulva* (0.13 ± 0.02 % P) ($F = 8.75$; $p < 0.001$). The least negative $\delta^{13}C$ contents at Primbee Bay occurred in sheet-like *Ulva* (-6.47 ± 1.03 ‰), which were significantly less negative than both filamentous *Ulva* (-9.56 ± 0.14 ‰) and *Chaetomorpha* (-9.26 ± 0.30 ‰), but not *Zostera* leaves (-8.01 ± 0.25 ‰) (Tukey's $p < 0.05$). The most notable differences at Primbee Bay occurred between $\delta^{15}N$ levels of macroalgae (4.7 - 11.0 ‰), which were up to 5-fold higher than *Zostera* leaves (1.67 ± 0.43 ‰) collected at the same time.

Mullet Creek Site (MC)

ANOVA tests were conducted between *Zostera* leaves and the macroalgae (*Ulva* and *Chaetomorpha*) collected during spring 2000 and summer 2002 (Table 5-16). In spring 2000, *Zostera* leaves had significantly higher C contents than both *Chaetomorpha* and *Ulva* (Table 5-8), but the opposite trend occurred in summer 2002 (Table 5-10). *Ulva* had the lowest tissue N contents of 2.05 ± 0.23 % N in spring 2000, but no significant differences occurred between species in summer 2002. In both sampling rounds, *Ulva* had the lowest P contents of 0.13 - 0.16 % P, approximately half those of *Zostera* leaves, but Tukey-Kramer tests ($\alpha = 0.05$) showed that these differences were only significant in summer 2002 ($F = 40.9$, $p < 0.001$). In both spring 2000 and summer 2002, the $\delta^{13}C$ contents of *Ulva* (-12 to -13 ‰) were significantly less negative than *Chaetomorpha* (-17 to -18 ‰), but *Zostera* $\delta^{13}C$ levels were only significantly different to *Chaetomorpha* in spring 2000 (Tukey's $p < 0.05$). The $\delta^{15}N$ contents of the macroalgae were up to 2.5 times higher than those of *Zostera* leaves ($F = 75.7$, $p < 0.001$).

5.3.4 Inter-Spatial Variations in Nutrient and Isotopic Contents

Two-way ANOVAs showed significant interactions between the time of sampling and the site, for all seagrass nutrient parameters tested (Table 5-11 and Table 5-12). Significant interactions suggest that nutrient concentrations at each site may be dependent on the time of sampling, or vice-versa, thus making interpretation of the results difficult. Therefore, one-way ANOVA tests were conducted on each nutrient parameter to determine the source(s) of variance (Table 5-13). Temporal variations in nutrient and isotopic contents are discussed in Section 5.3.5, with graphical interpretations of the "site versus time" interaction effects

provided in Figure 5-3. Note that macroalgae were not included in the two-way ANOVA as nutrient analyses were only conducted on macroalgae when sufficient quantities were present. The following section describes the between-site variations in C, N, P and isotopic contents of seagrass leaves and macroalgae.

Seagrasses

Although *Zostera* and *Ruppia* belong to different families, it is useful to compare the trends in C, N and P analyses obtained for each species, despite the fact that these species occurred at two different sites. *Zostera* and *Ruppia* were rarely found to be co-occurring in Lake Illawarra, except for a transition zone in deeper water, so it was difficult to find a single location in which the two species could be feasibly sampled together. There were a number of interesting inter-spatial and inter-species differences in the nutrient contents of these seagrasses, which were worth noting. The Purry Burry Point, Nicolle Road and Oasis Caravan Park sites along the Windang Peninsula were very similar in terms of sediment composition, physical characteristics and overall aspect. If nutrient contents were not dependent on the species of seagrass, for example, one would expect to find few significant differences in the tissue C, N and P contents between the Windang Peninsula sites. In addition, C, N and P contents at the Windang sites should be significantly different to the Mullet Creek site on the opposite side of the Lake, as it received freshwater input and had finer-grained sediments than the Windang sites. However, this was rarely the case, as the C, N and P contents of *Zostera* at two physically different sites (PBP and MC) appeared to be more closely related than C, N and P of two different species at three physically similar sites (PBP, OCP and NIC). Also, *Zostera* rhizomes tend to grow at a greater depth than *Ruppia* rhizomes, which often tend to be mostly on or just below the sediment surface. Therefore, tissue nutrient contents of *Ruppia* rhizomes should be lower than *Zostera* rhizomes, which can presumably take up more nutrients from the sediment. However, the opposite trend often occurred, in which tissue nutrient contents of *Ruppia* rhizomes significantly exceeded those of *Zostera* (Figure 5-3), suggesting that *Ruppia* may also take up nutrients from the water column via the above-ground proportion of rhizomes.

Seagrass leaf C contents varied significantly between sites during all sampling rounds (Table 5-13), but these variations were not the same in each sampling round (Figure 5-3), nor could they be related consistently to the seagrass species (*Ruppia* or *Zostera*). One-way ANOVA testing of variations in nutrient concentrations between sites showed that *Ruppia* at the Oasis Caravan Park had significantly lower leaf C contents than seagrasses at NIC, PBP and MC in both winter 2001 and 2002 ($p < 0.001$; Tukey's $p < 0.05$). However, *Zostera* at Mullet Creek had significantly lower leaf C contents than all other sites in summer 2001 and 2002 ($p < 0.01$). Seagrass rhizome C contents exhibited a greater differentiation between *Ruppia* and *Zostera* sites than leaf C contents (Figure 5-3). The C concentrations of *Ruppia* rhizomes did not vary significantly between the Nicolle Road and Oasis Caravan Park sites during any sampling

round (Tukey's $p < 0.05$). However, the total C contents of *Ruppia* rhizomes exceeded (but not always significantly) those of *Zostera* rhizomes by approximately 10 % on all occasions, except Mullet Creek samples collected in 2002 (summer and winter).

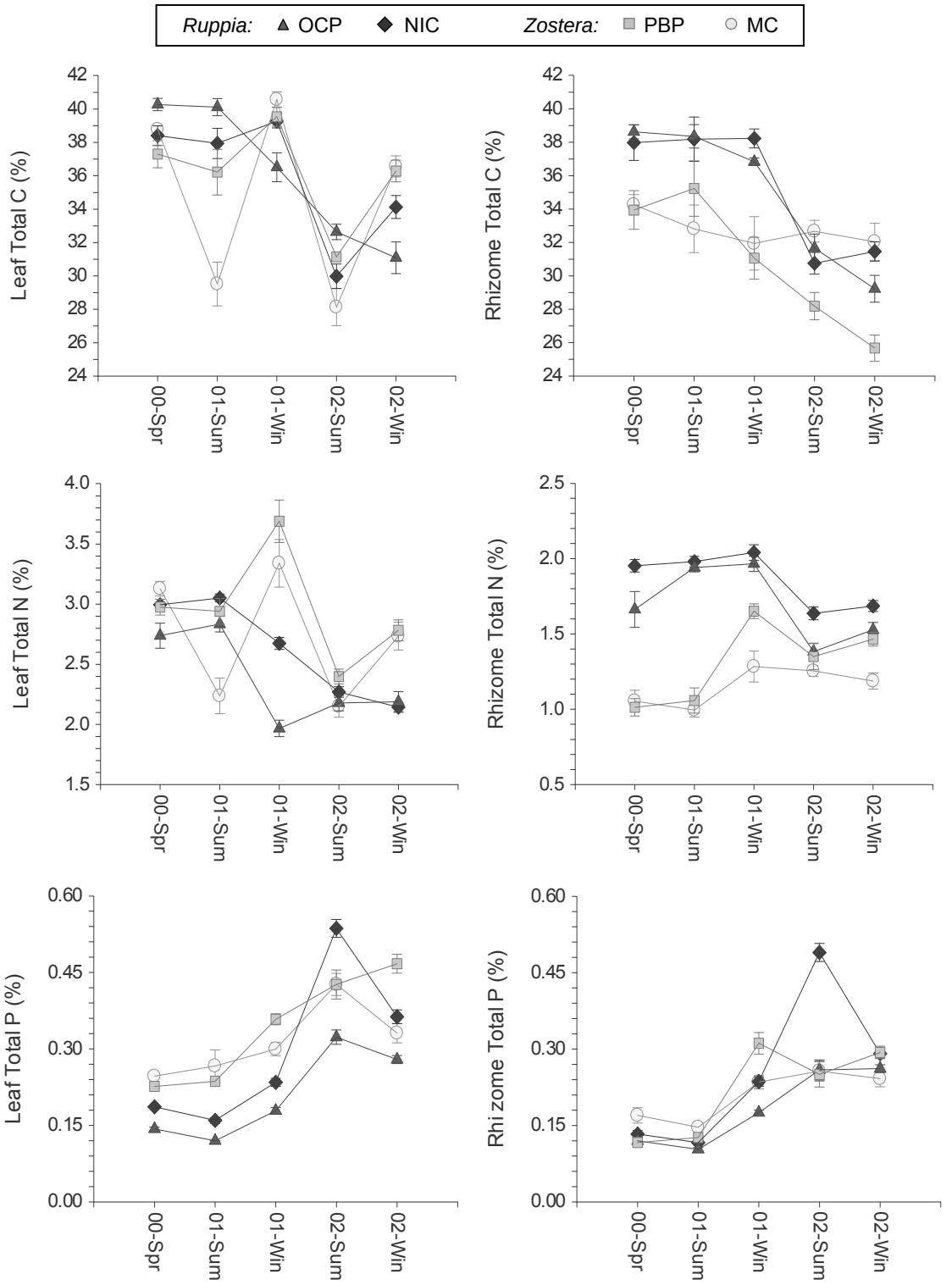


Figure 5-3: Spatial variations in C, N and P (% dry weight) concentrations of seagrasses (leaves and roots-rhizomes) at four Lake Illawarra sites (values are mean ± standard error).

Seagrass N contents varied markedly between sampling rounds, but only limited variations occurred between sites in each sampling round (Table 5-13; Figure 5-3). In spring 2000, the highest leaf N contents occurred at Mullet Creek (3.13 ± 0.06 % N), but the only significant differences occurred between sites MC and OCP (Tukey's $p < 0.05$). However, *Zostera* at Mullet Creek had significantly lower leaf N contents than all other sites in summer 2001 (2.23 ± 0.15 % N; $F = 13.6$, $p < 0.01$), whereas *Zostera* at both PBP and MC had significantly higher leaf N contents than both *Ruppia* sites in winter 2001 and 2002 ($p < 0.001$). Rhizome N contents at the *Ruppia* sites, NIC and OCP, significantly exceeded those of *Zostera* at PBP and MC between spring 2000 and winter 2001 ($p < 0.001$), but rhizome N was only significantly higher at NIC in summer 2002 and winter 2002 ($p < 0.01$).

Similarly, seagrass P contents varied significantly between sites, but not necessarily between species (Table 5-13; Figure 5-3). Leaf P concentrations at the *Ruppia* sites (NIC and OCP) were significantly (by approximately 20 - 40 %) lower than the *Zostera* sites (PBP and MC) during all sampling rounds, except summer 2002, where leaf P at NIC significantly exceeded all other sites ($p < 0.001$). In addition, *Ruppia* leaf P at NIC (range: 0.15 - 0.59 % P) significantly exceeded leaf P at OCP (range: 0.12 - 0.37 % P) in all sampling rounds, except summer 2001 (Tukey's $p < 0.05$). *Zostera* leaf P at Mullet Creek was significantly higher than leaf P at Purry Burry Point in spring 2000, significantly lower in winter 2001 and winter 2002, but not significantly different in summer 2001 and summer 2002 (Tukey's $p < 0.05$). Fewer significant differences occurred between root/rhizome P contents, and spatial variations were not static between sampling rounds. The significantly highest rhizome P contents occurred at Mullet Creek in summer 2001 (0.15 ± 0.01% P), PBP in winter 2001 (0.31 ± 0.02 % P), NIC in summer 2002 (0.49 ± 0.02 % P), and both PBP and NIC in winter 2002 (0.29 ± 0.03 % P).

The isotopic levels of seagrasses varied significantly between sites and sampling rounds, but the most dramatic between-site variations occurred with seagrass $\delta^{15}N$ contents (Figure 5-4). Tukey-Kramer comparisons showed that *Ruppia* at the Nicolle Road site had the highest leaf $\delta^{15}N$ contents in spring 2000 and summer 2001 (8.53 ± 0.33 and 6.84 ± 0.09 ‰, respectively), but was not significantly different to *Ruppia* at OCP during the remainder of the sampling period. Furthermore, seagrass leaf $\delta^{15}N$ contents at NIC and MC were significantly higher than both OCP and PBP in spring 2000 and summer 2001, but leaf $\delta^{15}N$ contents at NIC were significantly lower than MC and PBP between winter 2001 and winter 2002 ($p < 0.001$; Table 5-13). The $\delta^{15}N$ contents of seagrass root/rhizomes exhibited similar spatial trends to seagrass leaves (see Section 5.3.2). The Nicolle Road site had significantly higher rhizome $\delta^{15}N$ contents than all other sites in spring 2000 and summer 2001 (7.17 ± 0.20 and 6.96 ± 0.01 ‰, respectively), but was statistically similar to OCP thereafter. Rhizome $\delta^{15}N$ contents at both NIC and OCP were significantly lower than *Zostera* at PBP and MC in the winter 2001, summer 2002 and winter 2002 sampling rounds ($p < 0.001$).

Ruppia leaf $\delta^{13}C$ contents did not vary significantly between NIC and OCP throughout the sampling period ($p > 0.05$), and *Ruppia* rhizome $\delta^{13}C$ contents only varied significantly between NIC and OCP in winter 2001 ($p < 0.001$; Table 5-13). In spring 2000, summer and winter 2001, leaf $\delta^{13}C$ contents of *Zostera* at Purry Burry Point were significantly less negative than all other sites (NIC, OCP and MC), but in winter 2001, PBP *Zostera* had significantly more negative leaf $\delta^{13}C$ contents than *Ruppia* at NIC and OCP ($p < 0.01$). In all sampling rounds, the $\delta^{13}C$ contents of *Zostera* leaves at Mullet Creek (range: -15.9 to -10.7 ‰) were significantly more negative than all other sites (range: -13.0 to - 9.7 ‰) (Tukey's $p < 0.05$; Figure 5-4). Seagrass rhizome $\delta^{13}C$ contents followed similar patterns to leaves; *Zostera* at PBP had significantly less negative rhizome $\delta^{13}C$ contents than all other sites in spring 2000 and summer 2001, but seagrasses at both PBP and NIC had significantly less negative rhizome $\delta^{13}C$ contents than OCP and MC in winter 2001 ($p < 0.01$). *Zostera* at Mullet Creek had significantly more negative rhizome $\delta^{13}C$ contents than all other sites in summer (2001 and 2002), and winter 2002 ($p < 0.01$).

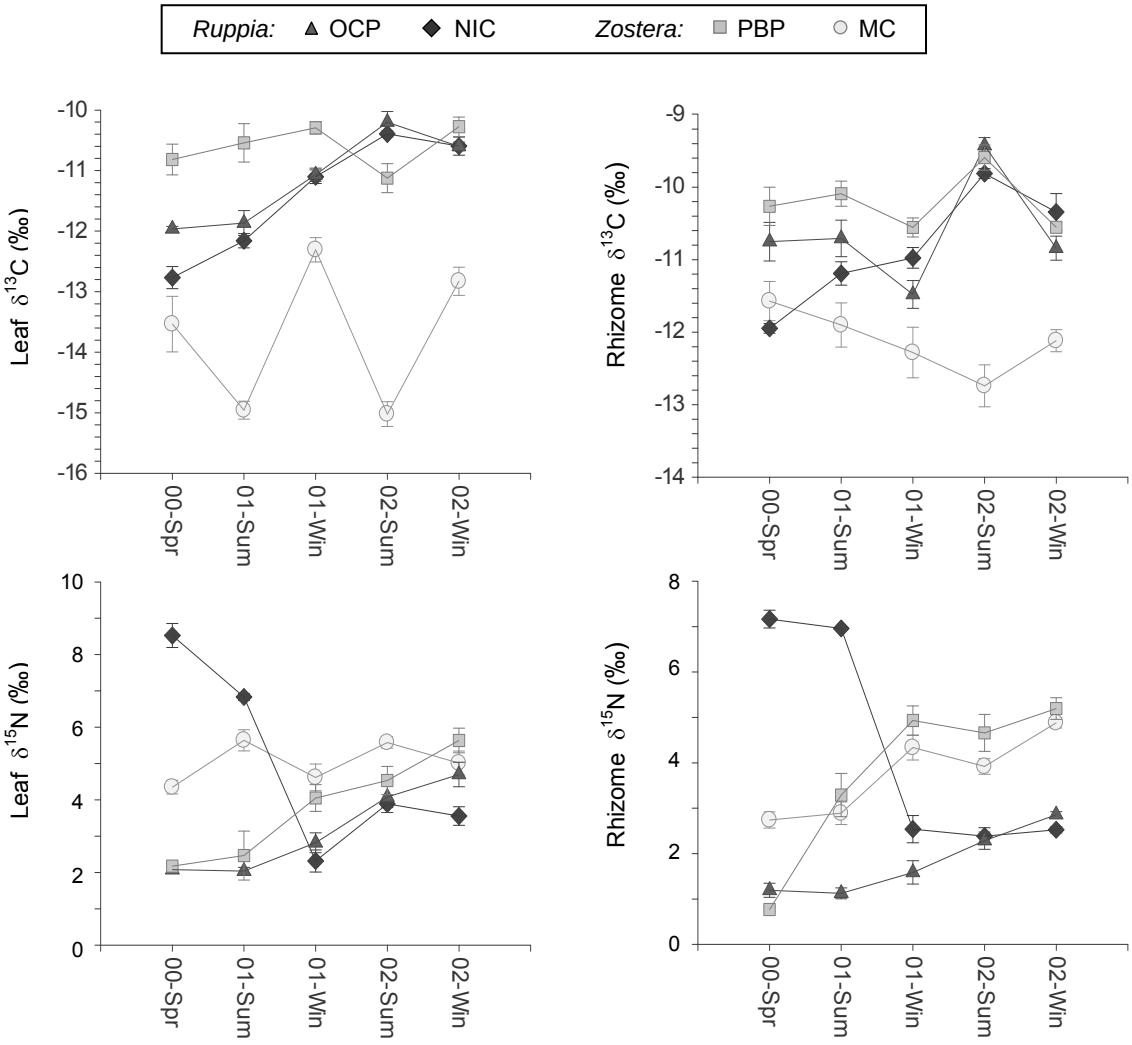


Figure 5-4: Spatial variations in $\delta^{13}C$ and $\delta^{15}N$ (‰) contents of seagrasses (leaves and roots-rhizomes) at four Lake Illawarra sites (values are mean ± standard error).

Macroalgae

Tukey-Kramer comparisons (α = 0.05) showed that macroalgae total C contents appeared to be more closely related to the genus than the site of collection. In spring 2000, C contents of *Ulva* ranged from 30.3 - 34.1 % C, but did not vary significantly between sites (OCP, PRIM and MC). However, all samples of *Ulva* had significantly lower C contents than all samples of *Chaetomorpha*, regardless of the sampling site (F = 15.1, $p < 0.05$). Similarly, total C contents varied little between macroalgae sampled at all Lake sites in summer 2002 (Table 5-13). The total N and P contents of macroalgae, however, varied significantly between sites, as well as within sites (Section 5.3.3). In spring 2000, *Chaetomorpha* N contents ranged from 2.1 % N at Primbee Bay, to 3.4 % N at Nicolle Road. *Ulva* N contents ranged from 0.9 % N at Primbee Bay, to 2.1 % N at Mullet Creek, and were significantly lower than the majority of *Chaetomorpha* samples (F = 12.6, $p < 0.05$). Macroalgae P contents (in spring 2000) ranged from 0.04 - 0.27 % P (Table 5-8), with genera at Nicolle Road and Oasis Caravan Park (*Chaetomorpha* and *Ulva*) typically having significantly lower P contents than those at Primbee Bay and Mullet Creek (F = 8.80, $p < 0.001$). P contents in spring 2000 generally did not vary significantly (Tukey's $p < 0.05$) between macroalgae genera collected at the same site. In summer 2002, however, *Gracilaria* at Purry Burry Point had significantly higher N and P contents (3.5 % N and 0.53 % P) than all other macroalgae sampled ($p < 0.001$), but the lowest N and P contents occurred in *Ulva* collected at the same site (1.2 % N and 0.08 % P). Interestingly, *Chaetomorpha* collected at Primbee Bay (the site with the highest macroalgal biomass in summer 2002) had significantly lower N contents and higher P contents than *Chaetomorpha* sampled at the Oasis Caravan Park and Mullet Creek sites (Tukey's $p < 0.05$).

Macroalgae exhibited a wider range of both δ^{15}N and δ^{13}C values than seagrasses across the Lake sampling sites, with values ranging from -4.89 to -19.8 ‰, and 1.80 to 14.6 ‰, respectively (Tables 4-8 - 4-10). The δ^{15}N and δ^{13}C contents of macroalgae appeared to show wider variations between different species collected at the same site, than the same species collected at different sites (Figure 5-5). In spring 2000, the isotopic signatures of macrophytes collected at Primbee Bay varied greatly from other sites, with the chlorophytes (*Chaetomorpha* and *Ulva*) having significantly less negative δ^{13}C contents (F = 49.7, $p < 0.001$), and significantly lower δ^{15}N contents (F = 22.4, $p < 0.001$), than macroalgae at all other sites. Some of this variation between macroalgae at Primbee Bay and other sites in spring 2000, however, may be due to laboratory differences (see Section 3.2.6). *Chaetomorpha* at Nicolle Road and *Ulva* at the Oasis Caravan Park had significantly higher δ^{15}N contents (~13 ‰) than the majority of macroalgae samples collected in spring 2000 (Tukey's $p < 0.05$). *Chaetomorpha* at Mullet Creek had the most negative δ^{13}C contents (~ -17 ‰) in both spring 2000 and summer 2002. Similarly, *Gracilaria* (PBP) also had quite negative δ^{13}C contents of about -17 ‰ in summer 2002, which were significantly more negative than those of *Ulva* (PBP, -6.7 ‰) and *Cladophora* (OCP, -9.0 ‰) (F = 39.8, $p < 0.001$). *Chaetomorpha* at the Oasis Caravan Park had significantly higher δ^{15}N contents than macroalgae at all other sites in

summer 2002 (14.1 ‰; F = 60.5, $p < 0.001$), while the lowest $\delta^{15}N$ contents occurred in *Ulva* at Purry Burry Point (6.38 ‰) and *Chaetomorpha* at Mullet Creek and Primbee Bay (Figure 5-5).

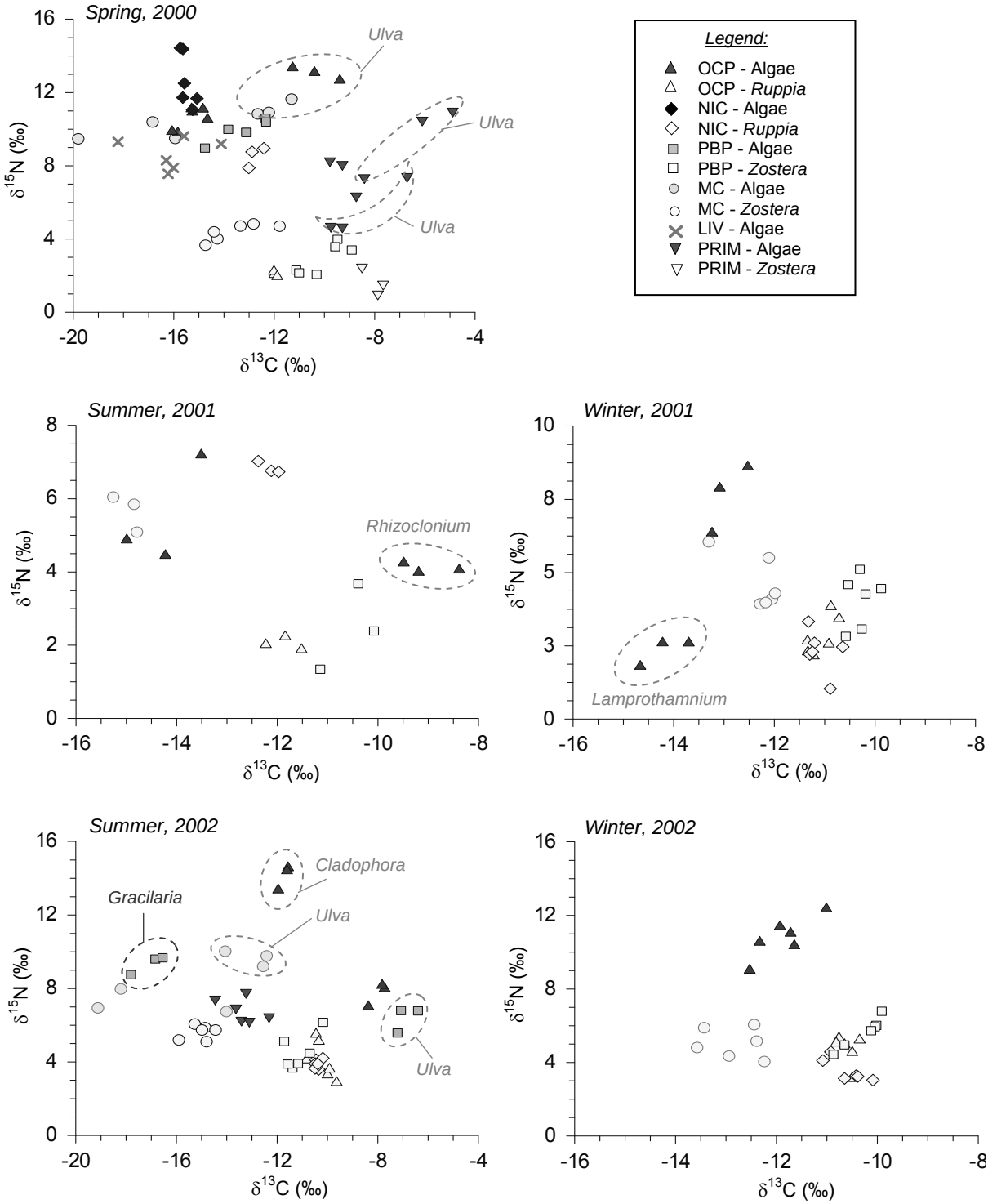


Figure 5-5: Scatter plots comparing $\delta^{15}N$ to $\delta^{13}C$ contents of seagrass (leaves only) and macroalgae at Lake Illawarra Village (LIV), Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP), Primbee Bay (PRIM) and Mullet Creek (MC). All macroalgae, except those circled, are *Chaetomorpha* spp.

5.3.5 Temporal Variations in Nutrient and Isotopic Contents

ANOVA testing showed significant variations in C, N and P contents of seagrasses and macroalgae between sampling rounds (Table 5-13), but few of these differences could be related directly to the sampling season (i.e., winter/summer).

Seagrasses

Two-way ANOVA comparisons showed significant interactions between sampling sites and the season, for seagrass leaf and rhizome total C, N and isotopic contents. The interaction between sampling sites and seasons was not significant ($p > 0.05$) for leaf or rhizome total P contents; in this instance, P contents were significantly higher in winter (leaves: $F = 4.36$, $p < 0.05$; rhizomes: $F = 17.2$, $p < 0.001$). Trends in the nutrient and isotopic concentrations of macrophytes at the Oasis Caravan Park, Nicolle Road, Purry Burry Point and Mullet Creek sites are shown in Figure 5-3 and Figure 5-4.

Ruppia leaves and rhizomes at both the Oasis Caravan Park and Nicolle Road sites exhibited very similar temporal trends with nutrient concentrations (see Section 5.3.2). Tukey-Kramer comparisons ($\alpha = 0.05$) showed that at both OCP and NIC, *Ruppia* leaf C and N contents declined significantly after summer 2001, whereas leaf P increased significantly ($p < 0.001$; Figure 5-3). At OCP, *Ruppia* leaf and rhizome C contents averaged 38 - 40 % C in spring 2000 and summer 2001, but declined significantly to 30 - 33 % C in summer/winter 2002 ($p < 0.001$). Similarly, the total C contents of *Ruppia* leaves and rhizomes at Nicolle Road averaged 38 % C between spring 2000 and winter 2001, but declined significantly to approximately 30 - 34 % C in 2002 ($p < 0.001$; Figure 5-3). At both OCP and NIC, *Ruppia* leaf and rhizome N contents in summer 2001 were about 1.5-fold higher than in summer/winter 2002 ($p < 0.001$; Figure 5-3). In contrast, *Ruppia* leaf and rhizome P contents at OCP were about 3-fold higher in summer 2002 (~0.3 % P), compared to spring 2000 and summer 2001 ($p < 0.001$). Likewise, *Ruppia* at Nicolle Road showed significant variations in leaf and rhizome P contents, increasing by 5-fold in summer 2002, compared to summer 2001 (~0.1 % P) ($F = 170$, $p < 0.001$).

Zostera, collected from the Purry Burry Point and Mullet Creek sites, displayed more seasonal trends in nutrient concentrations than *Ruppia*. *Zostera* leaf C contents showed a noticeable decline in both summer sampling periods, but these differences were more significant at Mullet Creek (Table 5-13; Figure 5-3). *Zostera* leaf N contents exhibited very similar temporal trends to leaf C contents, with leaf N contents increasing marginally in winter and decreasing in summer (Figure 5-3). This trend was most pronounced at Mullet Creek, with leaf N contents averaging 2.7 - 3.3 % N in spring 2000 and winter (2001, 2002), but decreased significantly to 2.2 % N in summer (2001, 2002) ($F = 17.7$, $p < 0.001$). *Zostera* rhizome C contents at Purry Burry Point showed a significant decline after winter 2001 ($F = 11.5$, $p < 0.001$), whereas

rhizome N contents increased significantly after summer 2001 (F = 13.6, $p < 0.001$). Rhizome C and N contents did not vary significantly between sampling periods at Mullet Creek ($p > 0.05$). At Purry Burry Point, *Zostera* leaf and rhizome P contents in winter 2002 were about double those of summer 2001 ($p < 0.001$; Figure 5-3). Similar trends occurred at Mullet Creek, with leaf P contents showing a significant peak at 0.43 % P in summer 2002 (F = 16.8, $p < 0.001$), and rhizome P contents increased significantly after summer 2001 (F = 7.4, $p < 0.001$).

The isotopic contents of *Ruppia* at the Oasis Caravan Park exhibited similar trends to total P contents at the same site; both $\delta^{13}C$ and $\delta^{15}N$ contents of *Ruppia* leaves at OCP increased significantly after summer 2001 ($p < 0.001$; Figure 5-4). Similarly, the $\delta^{15}N$ contents of OCP *Ruppia* rhizomes also showed a significant upwards trend after summer 2001, ranging from 1.2 ‰ in spring 2000, to 2.9 ‰ in winter 2002 (F = 14.1, $p < 0.001$). The $\delta^{13}C$ contents of *Ruppia* rhizomes at OCP averaged -11.5 to -10.7 ‰ throughout the study period, but showed a significant peak at -9.4 ‰ in summer 2002 (F = 20.5, $p < 0.001$). At Nicolle Road, *Ruppia* leaf and rhizome $\delta^{13}C$ contents were also significantly (by about 2 delta units) more negative in summer 2001, compared to summer 2002 ($p < 0.001$). In contrast, $\delta^{15}N$ contents of *Ruppia* leaves and rhizomes at NIC were about 1.7- and 2.9-fold higher, respectively, in summer 2001, compared to summer 2002 ($p < 0.001$).

Zostera leaf $\delta^{13}C$ contents at Mullet Creek followed similar trends to leaf total C and N contents, whereby $\delta^{13}C$ contents were significantly more negative in summer (2001, 2002; Figure 5-4). However, the $\delta^{13}C$ contents of *Zostera* rhizomes at Mullet Creek did not vary significantly over the sampling period, ($p > 0.05$). At Purry Burry Point, the $\delta^{13}C$ contents of *Zostera* leaves were significantly less negative in summer 2002, whereas $\delta^{13}C$ contents of rhizomes were significantly more negative at the same time ($p < 0.01$). PBP *Zostera* leaf and rhizome $\delta^{15}N$ contents increased significantly throughout the study period, from 2.2 and 0.8 ‰, respectively, in spring 2000, to 5.6 and 5.2 ‰, respectively, in winter 2002 ($p < 0.001$; Figure 5-4). Few significant seasonal differences occurred between *Zostera* leaf $\delta^{15}N$ contents at Mullet Creek, but rhizome $\delta^{15}N$ contents increased significantly throughout the study period, from 2.9 ‰ in summer 2001, to 4.9 ‰ in winter 2002 (F = 20.4, $p < 0.001$).

Macroalgae

Temporal variations in nutrient and isotopic contents were not assessed for the majority of macroalgal species collected, as different algal genera were sampled from each site during each consecutive sampling round. The nutrient contents of *Chaetomorpha* at the Oasis Caravan Park followed similar temporal trends to *Ruppia* collected at the same site (Section 5.3). Total C contents averaged 36 - 38 % C throughout the study period, but declined significantly to 29 % C in summer 2002 (F = 21.1, $p < 0.001$). *Chaetomorpha* total N contents

in winter 2002 (3.5 % N) were 1.8-fold higher than in summer 2001 ($F = 10.6$, $p < 0.001$). Similarly, *Chaetomorpha* total P contents increased by 4-fold in winter 2001 (0.28 % P), compared to summer 2001 ($F = 111$, $p < 0.001$). *Chaetomorpha* $\delta^{13}C$ contents became significantly less negative throughout the study period, increasing from -15.3 ‰ in spring 2000, to -11.9 ‰ in winter 2002 ($F = 42.8$, $p < 0.001$). The $\delta^{15}N$ contents of *Chaetomorpha* also varied significantly between sampling rounds, peaking at 14.1 ‰ in summer 2002 ($F = 33.7$, $p < 0.001$).

5.3.6 C:N:P Molar Ratios - Spatial and Temporal Variations

Seagrasses

Spatial and temporal variations in seagrass leaf and rhizome C/P, C/N and N/P molar ratios are presented in Figure 5-6. Notable interactions occurred between sites across the sampling period, but due to substantial heterogeneity in the molar ratio data sets, two-way ANOVA were not conducted on C:N:P data. Instead, one-way ANOVAs were used to examine the spatial variations between each site on each sampling occasion, and the temporal variations within each site across the sampling period (Table 5-13).

Both leaf and rhizome C/P and N/P molar ratios showed a notable decline over the course of the study, whereas fewer significant variations occurred in seagrass C/N ratios over the study period (Figure 5-6; Table 5-13). Trends in seagrass leaf and rhizome ratios did not appear to be directly related to the sampling season (winter or summer). The *Ruppia* sites (NIC and OCP), in particular, exhibited the most significant decline of up to 70 % in C/P and N/P molar ratios between summer 2001 and summer 2002 (Table 5-8 - Table 5-10). *Ruppia* at the Oasis Caravan Park exhibited significantly higher leaf C/P (range: 232 - 879) and N/P (range: 12.8 - 54.1) molar ratios than seagrasses at other sites during each sampling round ($p < 0.001$; Figure 5-6). Additionally, leaf C/P and N/P ratios at the Nicolle Road site also significantly exceeded those of the *Zostera* sites, but only during the 2000-01 sampling rounds. Rhizome molar ratios followed similar spatial trends to leaves, with the highest rhizome C/P and N/P molar ratios typically found in *Ruppia* at OCP, and the lowest in *Zostera* at Mullet Creek. Similarly, *Ruppia* at OCP typically had the highest leaf C/N molar ratios (range: 14 - 23) during each sampling round; this spatial difference was significant ($p < 0.05$) on the majority of occasions (Table 5-13). Spatial trends in C/N ratios of seagrass rhizomes were less distinctive than that of leaves; during each sampling round, *Zostera*, particularly at Mullet Creek, had significantly higher rhizome C/N ratios (range: 25 - 50) than *Ruppia* at Nicolle Road and Oasis Caravan Park (range: 19 - 31).

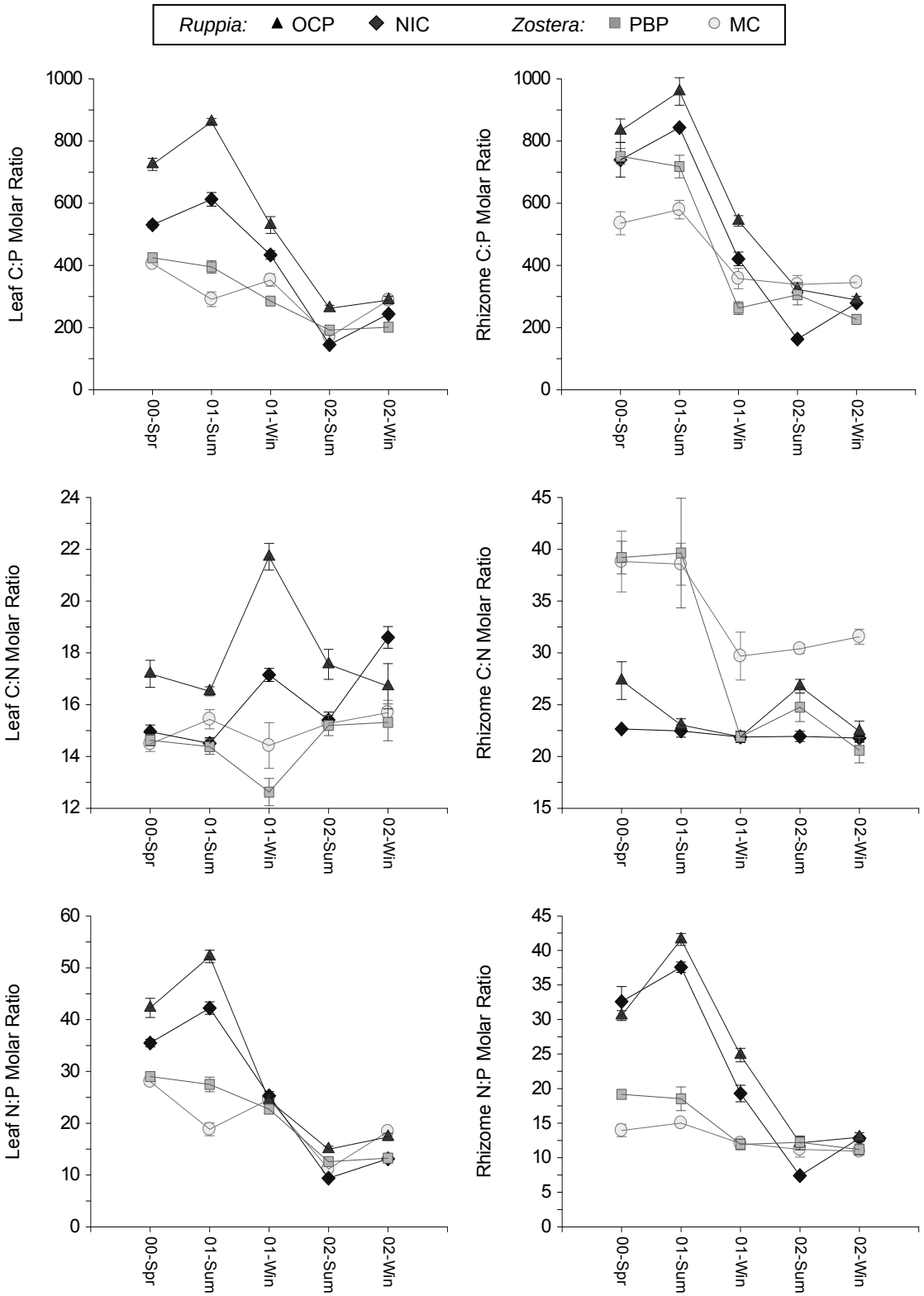


Figure 5-6: Spatial variations in molar C/P, C/N and N/P ratios of seagrasses (leaves and roots-rhizomes) at four Lake Illawarra sites (values are mean ± standard error).

Macroalgae

Macrophyte C:N:P molar ratios varied widely within and between both sites and species, but on all sampling occasions, the C/P and N/P molar ratios of macroalgal tissue greatly exceeded those of seagrass leaves, whereas macroalgae C/N ratios were usually similar to, or slightly less than, seagrass leaves collected at the same site (Tables 4-8 - 4-10). Over the entire study period, macroalgae C/P ratios ranged from a minimum of 130:1 in *Gracilaria* at PBP (summer 2002) to a maximum of 2910:1 in *Chaetomorpha* at OCP (summer 2001). Similarly, macroalgae C/N molar ratios ranged from a minimum of 9:1 in *Gracilaria* at PBP (summer 2002), to a maximum of 42:1 in *Ulva* at Primbee Bay (spring 2000). N/P ratios ranged from a minimum of 7:1 in *Ulva* (Primbee Bay, spring 2000), to a maximum of 151:1 in *Chaetomorpha* (OCP, summer 2001). Of the macroalgae analysed, the rhodophyte, *Gracilaria*, had the lowest average C/P (254 ± 64), C/N (10 ± 0.5), and N/P (24 ± 6) molar ratios, which were of a similar magnitude to the C:N:P ratios of the seagrasses.

The Oasis Caravan Park was only the only study site at which *Chaetomorpha* was analysed during each consecutive sampling round. Temporal comparisons showed a significant decline in the C/P and N/P ratios of *Chaetomorpha* at OCP after the spring 2000 - summer 2001 sampling rounds. C/P ratios peaked at 2611 ± 150 in summer 2001, but were 80 % lower in winter 2001 and subsequent sampling rounds ($H = 17.5$, $p < 0.001$; Kruskal-Wallis ANOVA). Similarly, *Chaetomorpha* N/P ratios were 3-fold higher in summer 2001 (137 ± 8.0) than in winter 2001-02 ($F = 18.2$, $p < 0.001$). In addition, the C/N ratios of *Chaetomorpha* in the 2001 (winter & summer: C/N ~18) rounds significantly exceeded the spring 2000 and 2002 (winter & summer, C/N ~12) sampling rounds ($F = 165$, $p < 0.001$). From the limited data available, it was tentatively concluded that the C/P and N/P ratios of the chlorophytes increased by approximately 2 - 3-fold during the summer months.

As expected from the literature (e.g., Duarte, 1990, 1992), clear patterns emerged between the total P contents of Lake Illawarra macrophytes, and their C/P and N/P molar ratios, as well as total N contents and C/N ratios (Figure 5-7). Both C/P and N/P ratios of macroalgae and seagrasses appeared to plateau at approximately 200 - 500 and 10 - 30, respectively, as P concentrations increased beyond ~0.2 % P. C/N concentrations of seagrasses (in particular, rhizomes) and macroalgae remained relatively constant at about 15 - 20, with N concentrations increasing above ~1.5 % N.

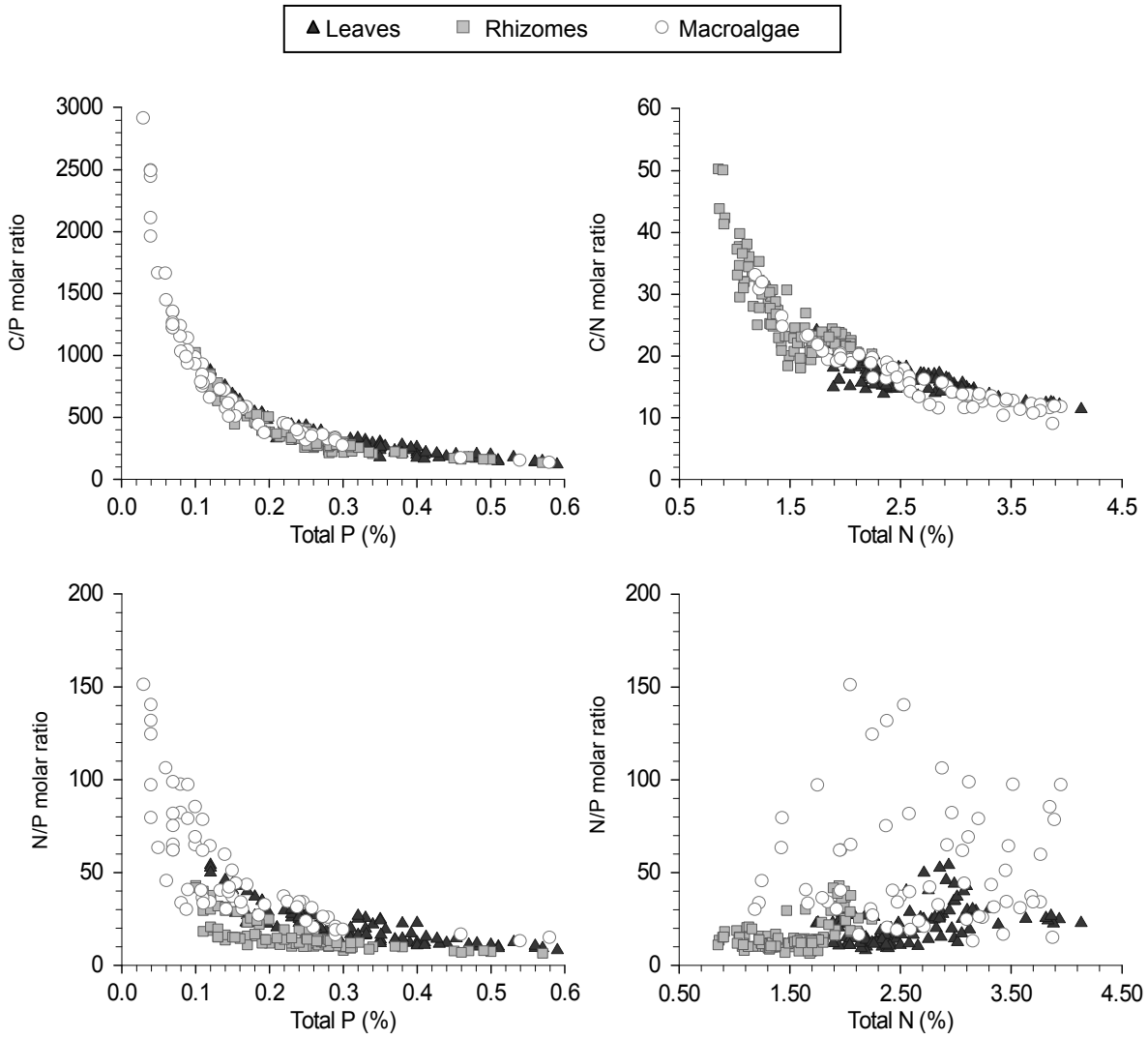


Figure 5-7: Scatter plots comparing the relationships between total P and N concentrations and C/P, C/N and N/P ratios of seagrasses (leaves and rhizomes) and macroalgae at Lake Illawarra, spring 2000 - winter 2002.

5.3.7 Macrophyte Nutrient Pools

Seagrasses

The seasonal variations in the "pools" of nitrogen (g N m^{-2}) and phosphorus (g P m^{-2}) associated with the *Ruppia* and *Zostera* beds at each of the Lake Illawarra study sites were estimated by combining seagrass biomass and tissue nutrient data. The above-ground proportion of the *Ruppia* bed at both OCP and NIC had significantly higher nutrient contents than the below-ground proportion, and contained approximately 70 - 80 % of the total nitrogen and phosphorus content of the seagrass bed. Similarly, for *Zostera* beds at the Purry Burry Point and Mullet Creek sites, the above-ground component of the seagrass accounted for 70 % of the total nitrogen, and 60 % of the total phosphorus in the bed. Total nitrogen contents (g N m^{-2}) of the *Ruppia* beds at both NIC and OCP declined significantly ($p < 0.001$) in winter,

increasing by approximately 2-fold in summer (Figure 5-8). Similarly, the total nitrogen contents of *Zostera* beds at Purry Burry Point were approximately 3-fold higher in the summer months ($p < 0.001$), but seasonal variations in total N contents of the *Zostera* bed at Mullet Creek were not significant ($p > 0.05$). At all sites, total phosphorus contents of the seagrass beds increased by up to 4-fold during summer 2002, but remained below 0.8 g P m^{-2} for the majority of the study period.

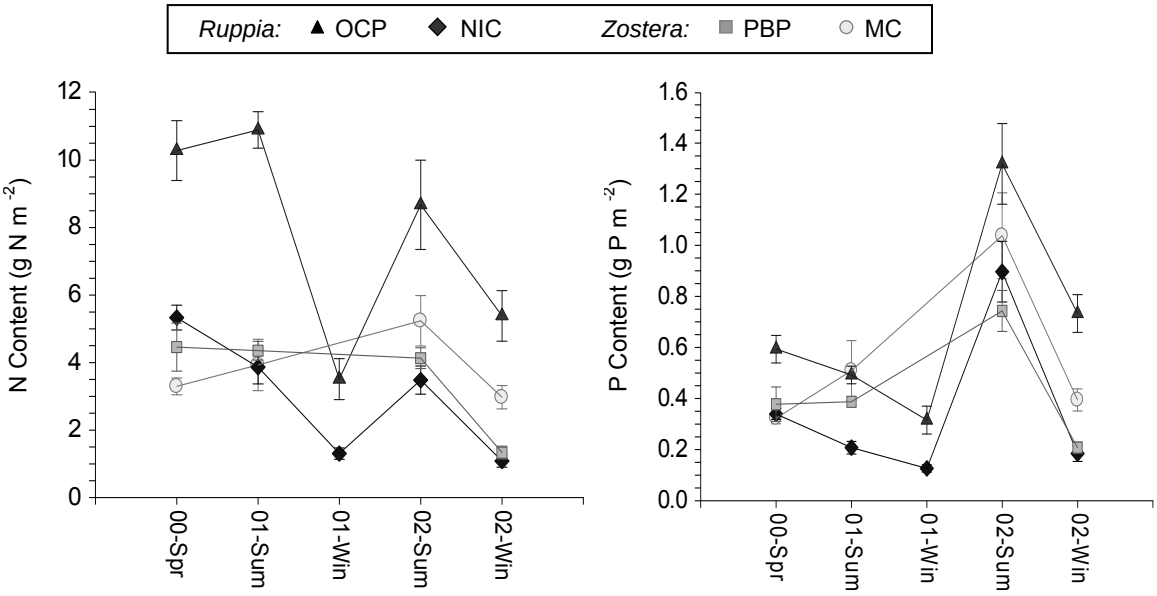


Figure 5-8: Seasonal and spatial variations in total N and P contents of seagrass beds (leaves and roots-rhizomes combined), at four Lake Illawarra sites (values are mean ± s.e.).

By combining biomass (above- and below-ground) and tissue nutrient data collected at each site, it was estimated that a one square metre section of *Ruppia* bed at the Oasis Caravan Park site contained between 3.5 - 10.9 g of nitrogen, 0.32 - 1.33 g of phosphorus and 65 - 170 g of carbon. Total *Ruppia* biomass at NIC was consistently lower than at OCP, and therefore the pools of nitrogen and phosphorus in *Ruppia* beds at NIC were about half those of OCP *Ruppia* beds (Figure 5-8). The average nitrogen and phosphorus contents of the *Ruppia* in Lake Illawarra, calculated by combining all N and P data collected from a dense *Ruppia* bed (OCP), and sparse *Ruppia* bed (NIC), were 4.82 ± 0.50 g N m^{-2} and 0.55 ± 0.06 g P m^{-2}. The total nitrogen and phosphorus contents of *Zostera* (above- and below-ground biomass) at Mullet Creek were similar to Purry Burry Point, and ranged from 1.2 - 5.2 g N m^{-2} and 0.2 - 1.0 g P m^{-2}, respectively. Combining data collected from PBP and MC over the entire study period (spring 2000 - winter 2002), the average total nitrogen and phosphorus contents of *Zostera* beds were 3.25 ± 0.23 g N m^{-2} and 0.47 ± 0.04 g P m^{-2}, respectively.

Macroalgae

The seasonal variations in biomass and calculated carbon, nitrogen and phosphorus contents of *Chaetomorpha* mats at five Lake Illawarra sites are listed in Table 5-17. The highest

combined N and P pools occurred at the Primbee Bay site, due to the higher *Chaetomorpha* biomasses and higher tissue N and P contents documented at that site. Biomass and nutrient tissue data collected over the entire study period were used to estimate the pools of N and P associated with macroalgae at each site. The average total biomass of macroalgae associated with seagrass beds and occurring in the inshore area of each site are listed in Table 5-18. Where nutrient data was unavailable (e.g., due to biomass being insufficient for nutrient analysis), an estimate of the N and P contents of each species was used, based on the average nutrient content for that genus, or similar genera, in the Lake. Of the four study sites (excluding Primbee Bay), the most significant pools of N and P appear to be at the Oasis Caravan Park, due to the higher seagrass and macroalgal biomasses found there.

Table 5-17: Seasonal variations in biomass (dry weight) and nutrient contents of *Chaetomorpha* spp. at the Oasis Caravan Park (OCP), Nicolle Road (NIC), Purry Burry Point (PBP), Primbee Bay (PRIM) and Mullet Creek (MC) sites, Lake Illawarra (values are mean ± s.e.).

Site	Season	Biomass (g DW m^{-2})	Total Carbon (g C m^{-2})	Total Nitrogen (g N m^{-2})	Total Phosphorus (g P m^{-2})
OCP	Spring 2000	113 ± 31	43 ± 12	3.8 ± 1.10	0.10 ± 0.030
	Summer 2001	68 ± 4	25 ± 2.3	1.5 ± 0.16	0.03 ± 0.003
	Winter 2001	67 ± 10	25 ± 3.9	1.6 ± 0.35	0.19 ± 0.028
	Summer 2002	60 ± 15	18 ± 4.4	1.8 ± 0.46	0.10 ± 0.030
	Winter 2002	13 ± 3	5 ± 1.2	0.5 ± 0.12	0.03 ± 0.008
NIC	Spring 2000	28 ± 4	11 ± 1.7	1.0 ± 0.18	0.03 ± 0.008
PBP	Spring 2000	31 ± 2	11 ± 1.0	0.9 ± 0.10	0.03 ± 0.006
PRIM	Spring 2000	139 ± 14	52 ± 5.1	2.9 ± 0.50	0.25 ± 0.050
	Summer 2002	369 ± 37	110 ± 12	6.4 ± 1.08	1.38 ± 0.23
MC	Spring 2000	15 ± 2	5 ± 0.8	0.5 ± 0.08	0.03 ± 0.005
	Summer 2002	12 ± 4	4 ± 1.5	0.3 ± 0.10	0.03 ± 0.012

Table 5-18: Averaged total biomass and nitrogen and phosphorus contents of macroalgae at the seagrass and inshore areas of four sites, Lake Illawarra (values are mean ± s.e.).

	Macroalgae over Seagrass Beds			Macroalgae over Inshore Sand Flats		
Site	Biomass (g DW m^{-2})	Total N (g N m^{-2})	Total P (g P m^{-2})	Biomass (g DW m^{-2})	Total N (g N m^{-2})	Total P (g P m^{-2})
OCP	37.1 ± 12	1.92 ± 0.6	0.094 ± 0.03	62.9 ± 12	0.77 ± 0.2	0.022 ± 0.01
NIC	18.6 ± 4.0	0.61 ± 0.2	0.063 ± 0.02	12.1 ± 3.8	0.20 ± 0.1	0.010 ± 0
PBP	23.1 ± 4.6	0.73 ± 0.2	0.061 ± 0.02	26.1 ± 7.4	0.46 ± 0.1	0.047 ± 0.02
MC	14.6 ± 8.4	0.31 ± 0.1	0.024 ± 0.01	9.9 ± 2.4	0.31 ± 0.1	0.008 ± 0

5.3.8 Sediment Grain Size and Nutrient Analyses

In each sampling round, sediment samples were collected along with seagrass samples from the top 0 - 10 cm of the rhizoidal zone beneath the seagrass beds. Grain-size analyses showed that the sediments at all the Lake sampling sites were composed primarily of fine to coarse-grained sand, in the 250 - 2000 μm fraction (Figure 5-9). The proportion of silt and clay

(< 63 μm) was typically less than 3 % at the eastern shore sites, Oasis Caravan Park, Nicolle Road, Purry Burry Point and Primbee Bay. Sediments at Mullet Creek, on the western shore, invariably contained a higher proportion of silt and clay than the eastern sites, averaging 25 - 35 % silt/clay in spring 2000 and summer 2001, and decreasing considerably to 5 - 12 % silt/clay from winter 2001-02. The higher silt/clay contents of sediment at the Mullet Creek site in spring 2000 were likely due to heavy rainfall in the preceding weeks, resulting in slightly increased river flow and deposition of fine sediments at the mouth of the Creek.

Nutrient analyses of sediments collected between spring 2000 and winter 2002 are presented in Figure 5-10. The analytical results of all sediment samples collected are listed in Appendix 7. Sediment samples collected at Mullet Creek had markedly higher total N, P and organic C contents than all other sampling sites in spring 2000 and summer 2001, but nutrient contents were similar to the eastern shore sites (OCP, NIC, PBP) in the winter 2001 - 2002 sampling rounds. Mean total N and P contents at Mullet Creek ranged from a maximum of 0.22 ± 0.1 % N and 0.03 ± 0.01 % P in summer 2001, to a minimum of 0.06 ± 0.01 % N and 0.01 % P in winter 2001. Similarly, mean organic C contents at MC varied by 3-fold between spring 2000 and winter 2001. This decrease in sediment nutrient content correlates with the lower proportion of fine-grained silt and clay at Mullet Creek in the latter sampling rounds.

Few significant differences were found between sediment nutrient contents at the sandy, eastern shore sites (OCP, NIC and PBP), and temporal variations in sediment nutrient contents were negligible (Table 5-19). The few exceptions were a significant increase in sediment organic C contents at both NIC and OCP in summer 2002 ($p < 0.01$, Kruskal-Wallis ANOVA). Likewise, total N contents at NIC also increased significantly in winter 2001 ($p < 0.01$, Kruskal-Wallis ANOVA).

Temporal differences in the isotopic contents of sediments at each site were also negligible. The only significant temporal differences noted were at OCP and PBP, where sediment $\delta^{13}C$ contents were significantly lower in the spring 2000 - summer 2001 sampling rounds (OCP: $F = 10.9$, $p < 0.001$; PBP: $F = 19.3$, $p < 0.001$). Throughout the sampling period, the least negative sediment $\delta^{13}C$ contents were recorded at the Oasis Caravan Park (about -17 ‰) and the most negative at Mullet Creek (about -23 ‰). Sediment $\delta^{13}C$ contents did not differ significantly between the three eastern Lake sites, but $\delta^{13}C$ contents at Mullet Creek were significantly lower ($p < 0.001$) than the eastern sites on all sampling occasions. Sediment $\delta^{15}N$ contents were similar across all sites, with most samples averaging about 3 - 4 ‰. The highest sediment $\delta^{15}N$ contents of 5.2 - 7.7 ‰ were recorded at the Nicolle Road *Ruppia* beds in spring 2000 and summer 2001, but it must be noted that isotopic analysis could not be conducted on the majority of samples from this group due to insufficient N contents. Sediment collected from the *Zostera* beds at Purry Burry Point had significantly higher ($p < 0.005$) $\delta^{15}N$ contents than the other sites in the final three sampling rounds (winter 2001-02).

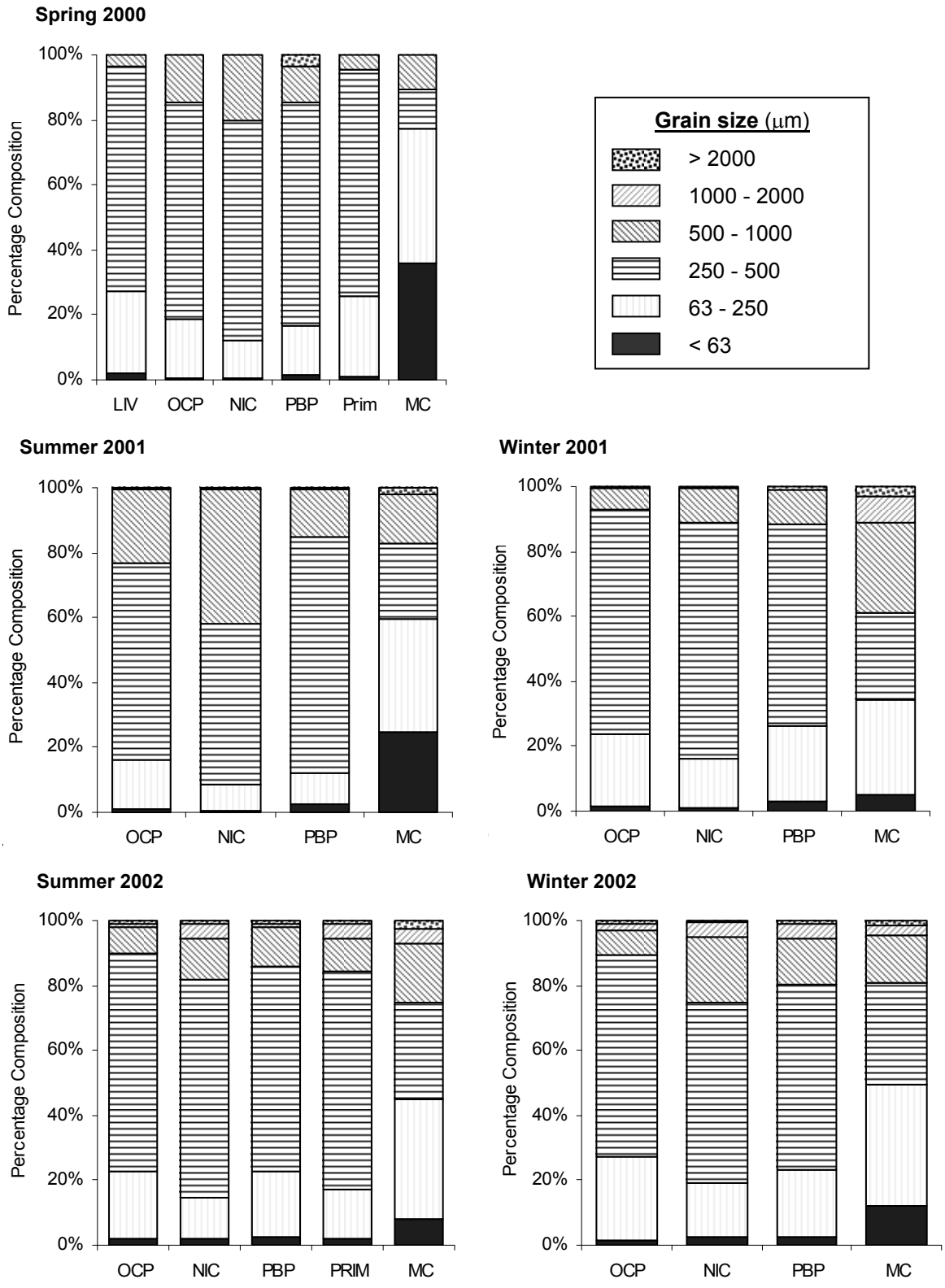


Figure 5-9: Percentage (average) grain size content of sediment (top 0 - 10 cm) collected from Lake Illawarra sampling sites, spring 2000 - winter 2002.

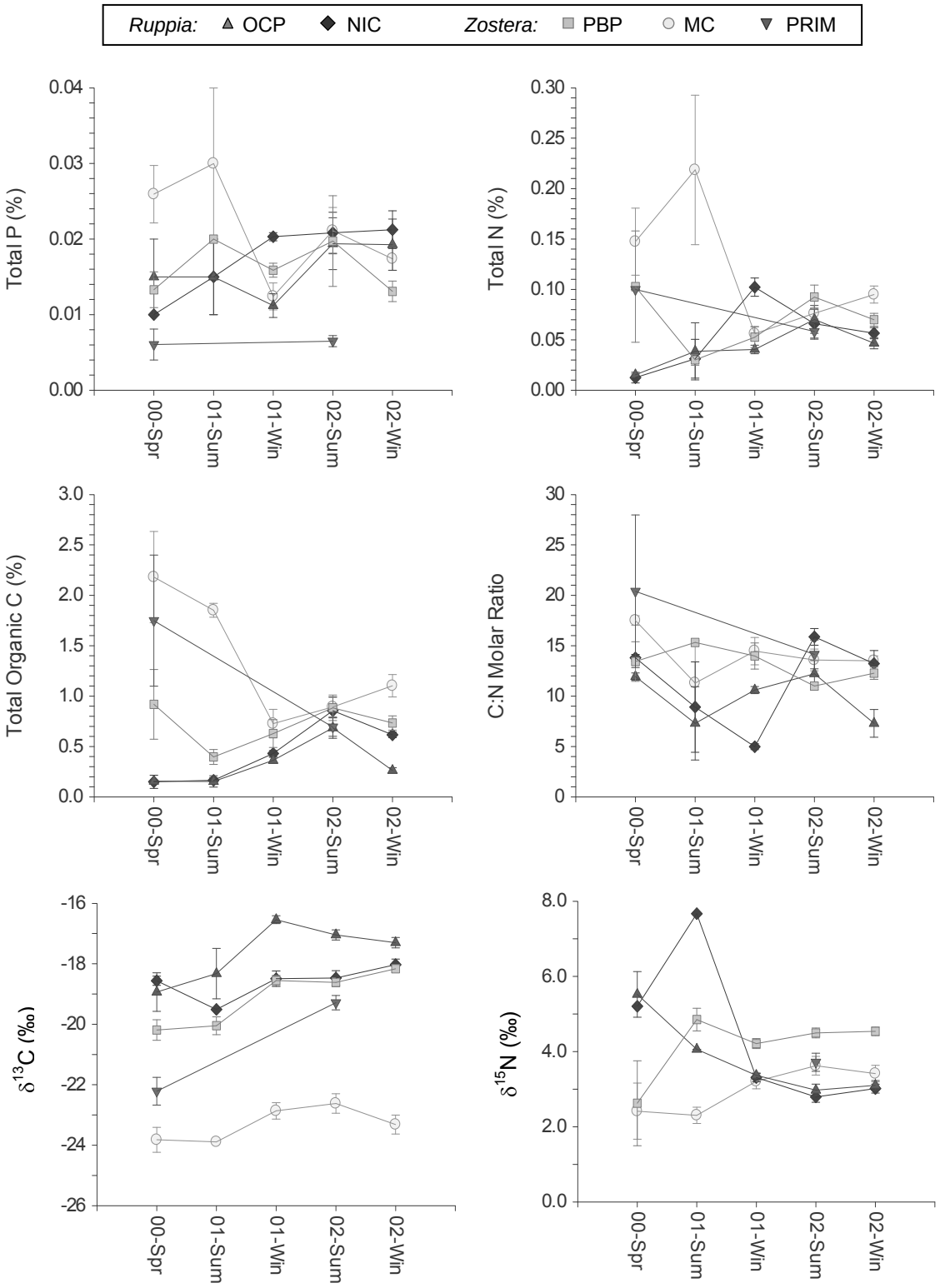


Figure 5-10: C, N and P contents, C/N molar ratios, $\delta^{13}C$ and $\delta^{15}N$ contents of sediment (top 0 - 10 cm) at five Lake Illawarra sites, 2000 (spring) - 2002 (winter) (values are mean ± s.e.). *Note:* several sediment samples collected from NIC and OCP (spring 2000 - summer 2001) did not contain sufficient N for isotopic analysis.

Table 5-19: Summary of ANOVA testing for differences in nutrient and isotopic contents of sediment collected from seagrass beds at OCP, NIC, PBP, MC, spring 2000 - winter 2002.

H_0 = Sediment nutrient and isotopic contents do not vary significantly between sampling rounds (spring 2000 - winter 2002) or sites (OCP, NIC, PBP and MC).						
Parameter	**Factor**	**d.f.**	**F-Ratio**	**Probability**	**Power (α=0.05)**	**Difference (Tukey-Kramer multiple comparison $p < 0.05$)**
Organic C #	A: Season	4	11.9	0.000000*	1.000	• 01-Summer < all other sampling rounds; 02-Summer > 01-Winter
	B: Site	3	72.2	0.000000*	1.000	• MC > PBP > NIC > OCP
	AB	12	10.6	0.000000*	1.000	• Significant interactions
Total N #	A: Season	4	6.5	0.000127*	0.989	• No significant differences (Tukey's $p > 0.05$)
	B: Site	3	25.1	0.000000*	1.000	• MC > PBP, NIC and OCP; • PBP > OCP
	AB	12	8.5	0.000000*	1.000	• Significant interactions
Total P #	A: Season	4	1.9	0.127385	0.539	• No significant differences
	B: Site	3	2.9	0.040051*	0.671	• No significant differences (Tukey's $p > 0.05$)
	AB	12	2.6	0.005306*	0.964	• Significant interactions
$\delta^{15}N$ #	A: Season	4	0.8	0.511324	0.253	• No significant differences
	B: Site	3	1.1	0.348587	0.289	• No significant differences
	AB	12	2.0	0.041037*	0.876	• Significant interactions
$\delta^{13}C$	A: Season	4	28.9	0.000000*	1.000	• 00-Spr and 01-Sum was more negative than 01-Win, 02-Sum and 02-Win
	B: Site	3	486.1	0.000000*	1.000	• (least negative) OCP > NIC > PBP > MC (most negative)
	AB	12	3.0	0.001529*	0.984	• Significant interactions

* test significant at $p < 0.05$

5.3.9 Macrophyte Biomass, Nutrient and Sediment Correlations

Correlation matrices were conducted to evaluate the relationships between all parameters analysed for both seagrass tissue and the underlying sediment (Table 5-20 and Table 5-21). To evaluate the relationships between the Lake Illawarra seagrass and sediment nutrient data, the Spearman-rank correlation was considered more appropriate than the Pearson's correlation matrix, as it is more tolerant of outliers, non-normality and unequal variances.

Seagrasses

The biomasses of seagrass leaves and rhizome were positively correlated ($p < 0.001$), as were a number of tissue nutrient parameters (Table 5-20). Seagrass rhizome biomass appeared to be negatively correlated with rhizome total N and P contents ($p < 0.001$), but there were no significant correlations between leaf biomass and leaf N and P contents ($p > 0.05$). At the *Zostera* sites, PBP and MC, total C contents of seagrass leaves increased in winter, with a corresponding decrease in plant biomass. Similarly, *Zostera* leaf N contents increased in winter, with a corresponding decrease in leaf biomass, indicating the storage (or no loss) of nutrients over winter. In contrast, *Zostera* leaf N contents decreased in summer with a significant increase in leaf biomass, suggesting stored nutrients were utilized to support increased growth over summer. *Zostera* rhizome C and N contents, however, did not appear

to vary according to rhizome biomass. These seasonal effects were less pronounced at the *Ruppia* sites, OCP and NIC. *Ruppia* leaf C and N contents declined over the entire sampling period, but the highest leaf N contents were documented in summer, with high leaf biomasses. At all sites, the P contents of seagrass leaves and rhizomes peaked in summer 2002, corresponding to the highest seagrass biomasses recorded during the study period.

Table 5-20: Spearman-rank correlation matrix between biomass (dry weight) and nutrient concentrations of seagrass leaves **(A)**, rhizomes **(B)** and leaves versus rhizomes **(C)**, collected from four sites (OCP, NIC, PBP and MC) (n = 102). Significant correlations are highlighted in bold.

(A)		Seagrass: above-ground component (leaves)					
	Test	**Biomass**	**Total C**	**Total N**	**Total P**	**$\delta^{13}C$**	**$\delta^{15}N$**
Leaves	**Biomass**	1	**-0.2745**	-0.1624	-0.1208	**-0.2035***	-0.0113
	Total C		1	**0.5912*****	**-0.3207****	-0.1550	0.0275
	Total N			1	**0.2403***	**-0.4279*****	**0.4049*****
	Total P				1	0.0701	**0.3110****
	$\delta^{13}C$					1	**-0.4179*****
	$\delta^{15}N$						1

(B)		Seagrass: below-ground component (roots-rhizomes)					
	Test	**Biomass**	**Total C**	**Total N**	**Total P**	**$\delta^{13}C$**	**$\delta^{15}N$**
Rhizomes	**Biomass**	1	0.0453	**-0.4046*****	**-0.3282*****	-0.0346	-0.0892
	Total C		1	**0.3853*****	**-0.5501*****	**-0.2836****	**-0.3221****
	Total N			1	0.0961	0.0864	-0.0896
	Total P				1	**0.2465***	0.1930
	$\delta^{13}C$					1	**-0.2331***
	$\delta^{15}N$						1

(C)		Seagrass: above-ground component (leaves)					
	Test	**Biomass**	**Total C**	**Total N**	**Total P**	**$\delta^{13}C$**	**$\delta^{15}N$**
Rhizomes	**Biomass**	**0.8912*****	**-0.2158***	-0.1343	-0.1199	-0.1938	0.0209
	Total C	0.0216	**0.4926*****	-0.0253	**-0.6825*****	**-0.2325***	**-0.2285***
	Total N	**-0.4437*****	**0.1963***	**-0.3186****	**-0.2694****	**0.3031****	**-0.2735****
	Total P	**-0.3554*****	**-0.3298*****	-0.0958	**0.7840*****	**0.3487*****	0.0591
	$\delta^{13}C$	0.0168	**-0.2552****	**-0.3486*****	0.1371	**0.8571*****	**-0.3943*****
	$\delta^{15}N$	-0.1300	0.1007	**0.3812*****	**0.3482*****	**-0.2064***	**0.8499*****

Note: test significant at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

A strong positive relationship occurred between leaf total P and rhizome total P contents ($p < 0.001$), and leaf total C and rhizome total C contents ($p < 0.001$), whereas rhizome N and leaf N were negatively correlated ($p < 0.01$). Duarte (1992) determined that concentrations of N and P in aquatic (marine and freshwater) macrophytes worldwide are positively related, and tend to form a linear relationship, but the relationship between N and P contents to C contents is limited. N and P contents of seagrasses and macroalgae varied widely throughout the present study, however, so relationships between these two key nutrients were difficult to define, and N and P contents did not appear to be linearly related. Correlations analyses of Lake Illawarra data showed a significant negative correlation between the N and P concentrations of macroalgae ($p < 0.001$), and the N and P contents of seagrass rhizomes ($p < 0.001$), but not seagrass leaves ($p > 0.05$) (Table 5-20).

Sediment and Seagrass Interactions

Correlation matrices showed significant relationships between each of the sediment nutrient parameters studied (Table 5-21). Sediment C and N contents were negatively correlated with rhizome C and N contents ($p < 0.05$), but few significant relationships occurred between sediment nutrient contents and seagrass leaf nutrient contents. Seagrass samples collected from the *Ruppia* sites (NIC and OCP) appeared to have slightly higher rhizome C contents than the *Zostera* sites (MC and PBP), but sediment C contents at NIC and OCP were marginally lower than PBP and MC on the majority of occasions. Similarly, samples collected from the seagrass beds at Mullet Creek appeared to have the highest sediment C contents, but not correspondingly high leaf or rhizome C contents. The majority of seagrass samples collected from Mullet Creek had lower rhizome N contents than the eastern Lake sites, but the N contents of sediments collected at the seagrass beds were noticeably higher than the eastern sites, especially in the spring 2000 - summer 2001 sample groups. Total P contents of seagrass leaves and rhizomes did not appear to be directly related to sediment P contents.

Table 5-21: Spearman-rank correlation matrix between biomass (dry weight) and nutrient concentrations of seagrass leaves, rhizomes and the underlying sediment, collected from four sites (OCP, NIC, PBP and MC), Lake Illawarra (n = 99). Significant correlations are highlighted in bold.

		Sediment at seagrass beds				
		Total C	**Total N**	**Total P**	**$\delta^{13}C$**	**$\delta^{15}N$**
Sediment	**Total C**	1	**0.8297*** **	**0.3557*** **	**-0.4496*** **	**-0.3459*** **
	Total N		1	**0.4517*** **	**-0.2829** **	**-0.3280** **
	Total P			1	-0.1601	**-0.2043***
	$\delta^{13}C$				1	0.0946
	$\delta^{15}N$					1
Leaves	**Biomass**	0.0512	0.0142	0.1446	**-0.2525***	0.0472
	Total C	**-0.2308***	-0.1846	**-0.2209***	**-0.2082** **	**0.2626** **
	Total N	**0.2440***	0.1116	-0.0662	**-0.5143*** **	**0.3766*** **
	Total P	**0.5062*** **	**0.3257*** **	0.1288	-0.1036	-0.0003
	$\delta^{13}C$	-0.1878	-0.1895	-0.0174	**0.5763*** **	-0.0203
	$\delta^{15}N$	**0.3650*** **	**0.2824** **	0.0030	**-0.5085*** **	0.0660
Rhizomes	**Biomass**	0.1012	0.0587	0.1883	-0.2684	-0.0372
	Total C	**-0.3978*** **	**-0.2341***	-0.0244	-0.0864	0.0391
	Total N	**-0.5389*** **	**-0.3082** **	**-0.2514***	**0.5707*** **	0.0823
	Total P	**0.2722** **	0.1675	0.1295	**0.2359***	-0.1710
	$\delta^{13}C$	-0.0310	-0.0529	0.0561	**0.4495*** **	0.0111
	$\delta^{15}N$	**0.2378***	0.1703	-0.1154	**-0.3235*** **	**0.2509***

Note: test significant at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Significant correlations also occurred with the isotopic contents of seagrass components and the underlying sediments. Both leaf and rhizome $\delta^{13}C$ contents were negatively correlated with leaf and rhizome $\delta^{15}N$ contents, respectively (Table 5-20), while sediment $\delta^{13}C$ contents were positively correlated with sediment $\delta^{15}N$ contents (Table 5-21). Similarly, a positive correlation occurred between sediment $\delta^{13}C$ contents and leaf and rhizome $\delta^{13}C$ contents, but only rhizome $\delta^{15}N$ contents were significantly correlated with sediment $\delta^{15}N$ contents. Figure 5-11 shows a strong relationship between the $\delta^{13}C$ contents of seagrass tissue and the underlying

sediment. Distinct clusters occurred at Mullet Creek, having more negative seagrass rhizome, leaf and sediment $\delta^{13}C$ contents than the eastern Lake sites, Oasis Caravan Park, Nicolle Road and Purry Burry Point.

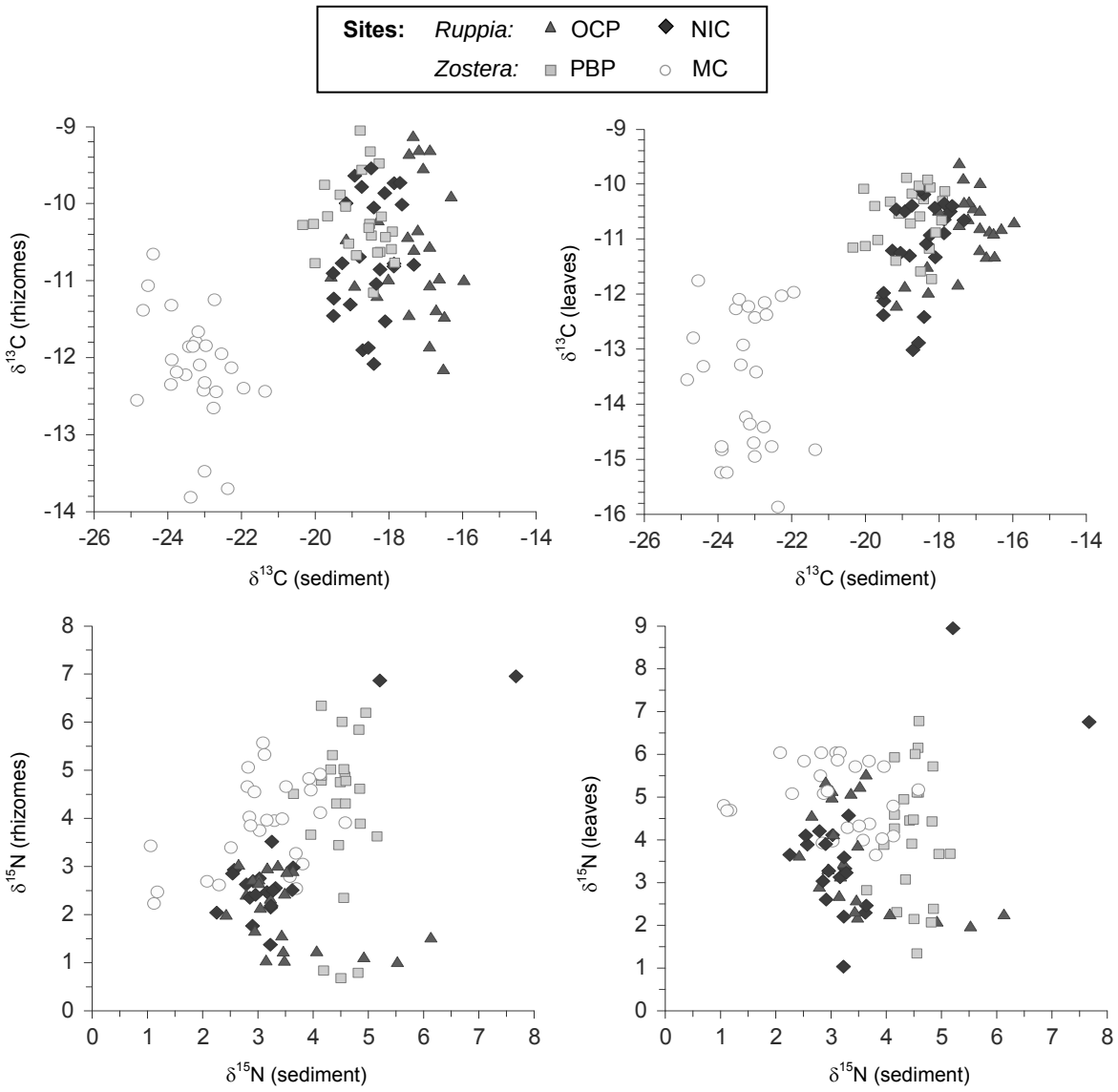


Figure 5-11: Scatter plots comparing the isotopic contents (‰) of seagrass leaves and rhizomes, and the underlying sediment at four sites, Lake Illawarra, spring 2000 - winter 2002.

Relationships between the $\delta^{15}N$ contents of seagrasses and sediment were less distinctive than $\delta^{13}C$ contents, but the samples generally appeared to be clustered by site. Little differentiation was found between the two *Ruppia* sites (NIC and OCP), which generally had lower seagrass and sediment $\delta^{15}N$ contents than the *Zostera* sites (PBP and MC). However, some outliers occurred in data collected from the Nicolle Road site in spring 2000 and summer 2001, having noticeably higher seagrass tissue and sediment $\delta^{15}N$ contents than at any other time during the study period. The remaining sediment samples collected from NIC during that

time period, however, were not analysed for $\delta^{15}N$ contents (due to insufficient N), and were thus removed from the analyses.

Macroalgae

Positive interactions occurred between the biomass of macroalgae and C and N contents of algal tissue, with the highest C and N contents often being associated with higher biomasses of macroalgae. Interactions between macroalgal biomass and total P contents, however, were less distinct. Correlation matrices conducted on macroalgae samples collected between spring 2000 and winter 2002 showed that macroalgal biomass (dry weight) was positively correlated with tissue total N contents ($p < 0.05$), but not total P ($p > 0.05$; Table 5-22). However, a strong positive correlation occurred between macroalgae total P and total N contents ($p < 0.001$). *Chaetomorpha* had significantly higher biomasses and higher P contents than other macroalgae, but P concentrations varied somewhat between sampling rounds. In general, the lowest tissue P contents of *Chaetomorpha* occurred during algal blooms (e.g., the Oasis Caravan Park site, spring 2000), whereas *Chaetomorpha* total P contents were often significantly higher in winter, when biomasses were comparatively lower than summer. Significant ($p > 0.05$) negative relationships also occurred between $\delta^{13}C$ levels and tissue C, N and P contents of macroalgae. More negative $\delta^{13}C$ levels of macroalgae, for example, were often associated with higher tissue N and P contents.

Table 5-22: Pearson's correlation matrix between biomass (dry weight) and nutrient concentrations of macroalgae, collected from four sites (OCP, NIC, PBP and MC), Lake Illawarra (n = 63). Significant correlations are highlighted in bold.

	Biomass	**Total C**	**Total N**	**Total P**	**$\delta^{13}C$**	**$\delta^{15}N$**
Biomass	1.0000	0.2126	**0.2973***	0.1364	**-0.3503****	-0.0025
Total C		1.0000	**0.4224*****	-0.2389	**-0.2978***	-0.0972
Total N			1.0000	**0.4229*****	**-0.6525*****	**0.3702****
Total P				1.0000	**-0.4049****	0.0012
$\delta^{13}C$					1.0000	-0.1388
$\delta^{15}N$						1.0000

Note: test significant at: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

5.3.10 Foreshore Cleanups

Foreshore cleanups were conducted (by the Lake Illawarra Authority) a number of times along the eastern Lake peninsula, throughout the course of this study. This process involved use of heavy machinery to remove floating organic material and the top-most surface layer of sediment along the inshore zone of the Windang Peninsula. The cleanup material was stockpiled at access points along the Lake foreshore, and later removed to landfill (Appendix 1D-E).

An estimated 200 tonnes (wet weight) of material was removed from the eastern Lake foreshore during the December 2000 foreshore cleanup (G. Clarke, pers. comm., 2001), shortly after the spring 2000 sampling survey had been conducted. Laboratory analyses showed that the material removed in December 2000 was composed of approximately 80 % (wet weight) sediment and fine organic material, often referred to as "black ooze", and 20 % (wet weight) macrophyte biomass, with equivalent amounts of macroalgae (primarily *Chaetomorpha*) and detached seagrass leaves. Samples collected from the July 2001 foreshore cleanup were composed of approximately 30 % (wet weight) "seagrass wrack" and some macroalgal biomass, and 70 % (wet weight) sediment. On average, approximately 5,000 tonnes of wet material was removed from Lake Illawarra each year, during foreshore cleanups conducted prior to the commencement of this study (G. Clarke, Lake Illawarra Authority, pers. comm., 2001). Using the average content of material determined during cleanup events in December 2000 and July 2001 (Table 5-23), it can be estimated that the amount of nitrogen and phosphorus removed by this practice annually would be approximately 5,900 kg dry wt. N and 600 kg dry wt. P, respectively.

Table 5-23: Average content of foreshore cleanup material removed from the Windang Peninsula, 2000 - 2001 (values are means ± standard error).

	Proportion of sample (% DW)	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
December 2000						
Organic material	9.0 ± 1.55	32.0 ± 1.21	2.20 ± 0.08	0.13 ± 0.009	-12.0 ± 0.26	8.48 ± 0.36
Sediment & fine material	91.0 ± 1.55	2.32 ± 0.21	0.29 ± 0.04	0.037 ± 0.003	-17.0 ± 0.30	9.02 ± 0.17
Mixed sample	100	5.39 ± 1.08	0.48 ± 0.07	0.046 ± 0.004	N/A	N/A
July 2001						
Organic material	22.0 ± 3.63	30.8 ± 1.09	1.48 ± 0.08	0.11 ± 0.005	-11.2 ± 0.21	3.88 ± 0.14
Sediment & fine material	78.0 ± 3.63	2.03 ± 0.42	0.20 ± 0.05	0.035 ± 0.006	-18.6 ± 0.40	7.20 ± 0.14
Mixed sample	100	7.33 ± 1.72	0.43 ± 0.10	0.047 ± 0.007	N/A	N/A

5.4 Discussion of Results

5.4.1 Biomass of Seagrass

The biomass of *Zostera* at both sampling sites (Mullet Creek and Purry Burry Point) was similar, despite differences in the physical characteristics of each site. The Mullet Creek site, on the western side of the Lake, was influenced by freshwater input and sediment with a much higher silt and clay content than the predominantly sandy Purry Burry Point site on the eastern side of the Lake. The biomass of *Zostera*, particularly above-ground material, declined significantly at both sites over winter. Previous investigations regarding seasonal effects on the growth and biomass of seagrass in Lake Illawarra also reported that *Zostera* biomass generally peaked in spring/summer and die-back of leaves occurred during winter (e.g., King *et al.*, 1997; White, 2003). In the present study, total *Zostera* biomass (above- and below-ground material) at Purry Burry Point averaged 226 ± 12.8 g DW m^{-2} in spring/summer and 58.1 ± 6.38 g DW m^{-2} in winter. Similarly, at Mullet Creek, total *Zostera* biomass averaged 230 ± 22.9 g DW m^{-2} in spring/summer and 145 ± 15.3 g DW m^{-2} in winter. Below-ground biomass of *Zostera* comprised 40 - 50 % of the total biomass at both sites, except for winter 2001, when biomass of roots and rhizomes comprised 55 % and 67 % of total biomass at MC and PBP, respectively.

The results obtained in the present study are comparable to data presented for similar areas of the Lake in previous studies (e.g., King *et al.,* 1997; WBM, 2000), but the above-ground biomasses of *Zostera* documented in the present study are somewhat higher than those reported by previous authors (Section 4.2.3). In January 2000, for example, WBM (2000) reported the average biomass of *Zostera* leaves to be 20.4 and 91.7 g DW m^{-2} at Mullet Creek and Purry Burry Point, respectively. In the present study, however, the average leaf biomasses found at similar sites during spring/summer (2000 - 2002) were 119 ± 13.5 and 117 ± 6.50 g DW m^{-2} at Mullet Creek and Purry Burry Point, respectively. It must be noted that one of the primary aims of the present study was to evaluate the nutrient contents of *Zostera* from healthy seagrass beds in the Lake (see, e.g., Section 2.6), rather than a broad range of seagrass beds of variable coverage. Sampling of seagrass biomass during the present study did, however, include a range of seagrass beds of varying density and spatial coverage, but differences in sampling procedures may also account for the higher leaf biomass documented in the present study (e.g., whether seagrass biomass was estimated by measuring and counting leaves, or by removing the entire biomass and weighing). This study highlights the fact that seagrass biomass can fluctuate significantly both temporally and spatially (within and between sites), thus caution must be exercised when comparing data collected by different authors, especially where different sampling seasons were used (see Section 4.2.3). In addition, the majority of past studies on seagrass biomass in the Lake included above-ground

(living leaf) material only, therefore temporal changes in the total seagrass biomass at those sites are difficult to assess.

Of the two *Ruppia* sampling sites in Lake Illawarra (NIC and OCP), total biomasses (leaves and rhizomes) were consistently higher at the Oasis Caravan Park, peaking at 440 ± 26.5 g DW m^{-2} in summer, and declining to 231 ± 24.8 g DW m^{-2} in winter. The decline in *Ruppia* biomass over winter was more noticeable at the Nicolle Road site, with biomass ranging from 54.8 ± 5.25 g DW m^{-2} in winter, to 180 ± 12.1 g DW m^{-2} in summer. This pattern in growth of *Ruppia* in Lake Illawarra is consistent with seasonal variation in the biomass of *Ruppia* in other parts of the World. For example, Menéndez (2002) reported that growth rates of *Ruppia cirrhosa* in the Ebro Delta, Spain, were noticeably lower in winter than summer; growth rates increased significantly in spring and summer, with stems reaching a maximum length during mid-summer. In Valle Smarlacca lagoon, Italy, Azzoni *et al.* (2001) documented the lowest biomass of *Ruppia cirrhosa* over winter (52 ± 10 g DW m^{-2}), with biomass peaking at 411 ± 94 g DW m^{-2} in mid-summer. These authors also noted that the total biomass, particularly below-ground material, of *R. cirrhosa* declined significantly in late summer.

It is interesting to note that *Halophila decipiens* was documented in the Lake Illawarra for the first time since the studies of Yassini (1985, 1986). It is possible that this species was present during previous surveys of Lake Illawarra seagrass beds (e.g., King 1990, 1997; WBM, 1996, 1998), but as microscopic examination is generally required to differentiate between *H. decipiens* and *H. ovalis*, it is possible that *H. decipiens* was labeled as *H. ovalis* during previous studies. Additionally, smaller seagrasses (e.g., *Halophila*) tend to have higher rates of photosynthesis and turnover of biomass, and respond faster to environmental conditions than larger seagrasses (e.g., *Zostera*), so that smaller seagrasses undergoing light deprivation or other stresses might only survive for a few weeks (Walker *et al.*, 1999). No attempts were made to quantify the distribution and biomass of *Halophila* in deeper areas of the Lake in the present study, and only patchy beds of *H. decipiens* and *H. ovalis* were observed in the shallow areas of Purry Burry Point and other sites. A previous study by WBM (2000) estimated that beds of *Halophila ovalis* typically occurred beyond the outer range of *Z. capricorni* and covered 54 ha, or approximately 7 % of the total seagrass area in Lake Illawarra.

5.4.2 Biomass of Macroalgae

Chaetomorpha was the dominant genus of macroalgae occurring in Lake Illawarra during the present study, with the highest biomasses accumulating in sheltered bays along the Windang Peninsula, particularly the Oasis Caravan Park site. The maximum biomasses of macroalgae recorded at the Windang sites, Oasis Caravan Park and Primbee Bay, were 150 and 370 g DW m^{-2}, respectively. Biomass of macroalgae at all other sites surveyed was typically less than 70 g DW m^{-2}, regardless of the sampling season. McConville (2000) recorded maximum

macroalgal biomasses of 140 - 14,000 g DW m^{-2} along the Windang Peninsula in March 2000, but macroalgal blooms of a similar magnitude were not observed during the present study. The largest blooms recorded along the Windang Peninsula during the present study were in spring 2000; after that survey, however, a considerable proportion of the biomass accumulating on the foreshore was mechanically removed by the Lake Illawarra Authority in December 2000 and July 2001. Macroalgal biomasses recorded between spring 2000 and winter 2002 were significantly lower than biomasses recorded by previous authors (see Section 4.2.6). For example, Yassini and Clarke (1986) documented average biomasses of 700 g DW m^{-2} at Griffins Bay, while the highest maximum average macroalgal biomasses recorded by King *et al.* (1990) were less than 2,000 g WW m^{-2} (approximately 200 g DW m^{-2}) in Koona Bay, Pithungnar Bay and along the Windang Peninsula. Yassini and Clarke (1986) attributed the excessive macroalgal blooms occurring in the 1980s to stormwater runoff, as well as leakages and wet weather overflows from the sewerage system. Since that period, however, the Lake Illawarra Authority, Wollongong City Council, Shellharbour City Council and Sydney Water have undertaken substantial works to improve water quality and the overall amenity of the Lake. With the Lake management practices currently in place it is unlikely that the levels of macroalgal biomass recorded during the 1980s and early 1990s will occur in the Lake again. The comparatively low macroalgal biomasses recorded during the present study are attributed largely to reduced rainfall during the study period (Figure 1-3), and therefore nutrients washed into the Lake from the catchment, as well as improvements in water quality since the early 1990s (see Section 1.5.5).

Previous studies on macroalgae in Lake Illawarra determined that the dominant genera were *Chaetomorpha*, *Ulva (U. lactuca* and *Enteromorpha intestinalis*), *Rhizoclonium*, *Gracilaria* and *Hypnea* (Yassini, 1985; King *et al.*, 1990; King *et al.*, 1997). During the present study, the dominant algal genus in Lake Illawarra was *Chaetomorpha* (identified as *C. linum* and *C. billardierii*), which was widely distributed in shallow, sheltered bays, with large masses often found entangled amongst seagrass beds. Other "nuisance" green algae, such as *Ulva* spp. and *Cladophora* spp., were found frequently in the Lake, particularly along rocky shorelines or as epiphytes on seagrass leaves, but biomasses were incomparable to that of *Chaetomorpha*. However, blooms of species other than *Chaetomorpha* occurred occasionally during the present study; for example in summer 2002, *Cladophora* attached to *Ruppia* leaves detached and formed floating masses (of about 35 g DW m^{-2}) over the seagrass beds at the Oasis Caravan Park site (Plate 4-13E).

Studies on various estuaries around the world have indicated that macroalgal blooms are likely to occur once a certain threshold for nutrient concentrations is exceeded by events such as freshwater runoff or sewage discharge (Lavery *et al.*, 1991). The Lake Illawarra Management Committee (1986, cited in Lavery *et al.*, 1991) previously defined this threshold limit as 15 - 30 $\mu g\ L^{-1}$ P (0.48 - 0.97 $\mu mol\ P\ L^{-1}$) and 36 - 68 $\mu g\ L^{-1}$ N (2.6 - 4.9 $\mu mol\ N\ L^{-1}$).

Recently, Davis and Koop (2006) suggested that stratification of the water column and the availability of light, rather than nutrients, are the key factors triggering algal blooms in enclosed freshwater bodies of southeastern Australia. Nutrient limitation, however, ultimately restricts the biomass of those blooms. In the Peel-Harvey Estuary, Western Australia, for example, Lavery *et al.* (1991) recorded a significant correlation between average annual macroalgal biomass (e.g., *Cladophora*, *Chaetomorpha* and *Ulva*) and average light attenuation during summer. These authors reported that growth rates of green macroalgae reached a maximum during summer and autumn, and they attributed the reduced growth rates in winter to lower temperatures and salinities, and a reduction in light availability.

The biomass of *Ulva* spp. in Lake Illawarra also tended to decline towards the end of summer, possibly due to higher water temperatures, but may also be due to increased evaporation resulting in higher salinities in shallow waters. On a number of occasions, the salinity at sampling sites during mid-summer was equivalent to, or slightly higher than, the average salinity of seawater (35 ppt) (see, e.g., Appendix 3). Martins *et al.* (1999) showed that the highest growth rates of *Enteromorpha intestinalis* (now *Ulva intestinalis*) in Mondego estuary, West Portugal, occurred between 10 - 22 ppt, but die-back occurred at salinities lower than 3 ppt and growth rates were significantly lower at salinities higher than 28 ppt. Rivers and Peckol (1995) determined that the optimal temperature for photosynthesis of *Ulva lactuca* was 15°C, with photosynthetic capacity and efficiency declining at 25°C and 5°C. Water temperature in Lake Illawarra is approximately 11 - 15°C during winter, which may explain the relative abundance of *Ulva* spp. in Lake Illawarra during winter, and absence of *Ulva* during the warmer summer months. However, some differences in the success of co-existing species in the field may not be due to differing morphology or environmental factors (e.g., salinity and temperature), but may instead be due to biological factors, such as preferences by grazers for one species over another (Lotze and Schramm, 2000). *U. intestinalis* has been cited as an important food source for lined shore crabs and topsmelt in southern Californian estuaries (Kamer and Fong, 2001) and *Ulva* spp. in Lake Illawarra may also be a preferred genus for grazers in the Lake.

5.4.3 C, N and P Contents of Seagrasses and Macroalgae

A summary of the nutrient contents (carbon, nitrogen and phosphorus) of the two Lake Illawarra seagrasses, *Zostera capricorni* and *Ruppia megacarpa*, and the most abundant macroalgae, *Chaetomorpha* spp., is provided in Table 5-24. The range of C, N and P values recorded in Lake Illawarra seagrasses and macroalgae were similar to those reported in the literature (Table 2-7 and Table 2-8). When comparing samples from all sites, collected over the entire study period, *Zostera* typically had higher leaf N and P contents than *Ruppia*. On the other hand, *Zostera* rhizome N and P contents were often similar to, or slightly lower than *Ruppia.* Tissue nutrient contents of macroalgae showed a wider variation than those of the

seagrasses; *Chaetomorpha* spp. had similar average nitrogen contents, but notably lower average P contents than the seagrasses. Consequently, the average C:N:P molar ratios calculated from all macrophyte samples showed that the C/P and N/P ratios of macroalgae were typically double those of the seagrasses, probably due to the low P contents of macroalgae compared to the seagrasses. This may be because the opportunistic macroalgae, such as *Chaetomorpha*, in Lake Illawarra have a lower requirement for P than the seagrasses, or that the algae quickly deplete internal P reserves at a faster rate than the seagrasses to sustain rapid growth. The batch culture experiments conducted on *C. linum* (Chapter 6) support the theory that this alga has a relatively low requirement for P. In addition, seagrasses are able to take up P from the sediment (via roots and rhizomes), and may have a competitive advantage over the algae when water column P concentrations are low.

Table 5-24: Average C, N and P concentrations and C:N:P molar ratios of dominant macrophytes, determined for all samples collected across six Lake Illawarra sites (mean ± s.e.).

Genus /	Plant Tissue Content			C:N:P Molar Ratios		
Plant Part	Total C (%)	Total N (%)	Total P (%)	C/P	C/N	N/P
Ruppia						
- leaves	35.2 ± 0.57	2.40 ± 0.05	0.28 ± 0.02	408 ± 30	17.3 ± 0.33	23.8 ± 1.84
- roots-rhizomes	34.3 ± 0.57	1.75 ± 0.04	0.24 ± 0.02	463 ± 36	23.1 ± 0.35	20.1 ± 1.54
Zostera						
- leaves	35.6 ± 0.62	2.86 ± 0.08	0.34 ± 0.01	288 ± 13	14.7 ± 0.20	19.8 ± 0.92
- roots-rhizomes	31.4 ± 0.51	1.27 ± 0.03	0.23 ± 0.01	399 ± 24	30.2 ± 1.10	12.9 ± 0.43
Macroalgae						
- all species	34.7 ± 0.36	2.56 ± 0.09	0.17 ± 0.01	808 ± 65	17.7 ± 0.72	47.9 ± 3.47
- *Chaetomorpha*	35.7 ± 0.46	2.82 ± 0.10	0.18 ± 0.01	796 ± 88	15.6 ± 0.60	52.5 ± 4.89

In a review of C, N and P concentrations of macrophytes worldwide, Duarte (1992) calculated average carbon concentrations (dry weight) of 24.8 ± 6.3 % C (range: 8.9 - 48.4 % C) for macroalgae, and 33.5 ± 4.4 % C (range: 23.6 - 43 % C) for seagrasses. Macroalgae typically have lower C contents than seagrasses, probably due to a higher proportion of structural carbon in seagrass leaves and rhizomes (Duarte, 1992). Additionally, faster growing macroalgae, such as the bloom-forming chlorophytes, often have lower C contents than slow-growing macroalgae, such as the phaeophytes, which tend to have a greater storage capacity for structural carbon (see, e.g., Lapointe *et al.*, 2005). This phenomenon was not always observed in the present study as *Chaetomorpha*, in particular, usually had similar or higher tissue carbon contents than both seagrasses and other macroalgae examined; macroalgae C contents in Lake Illawarra ranged from 28.0 - 39.7 % C, compared to 24.2 - 42.0 % C for the seagrasses. The higher C contents in *Chaetomorpha* may be because carbon concentrations of aquatic plants tend to increase with increasing nitrogen and phosphorus contents (Duarte, 1992). If P or N limits macrophyte growth, the products of photosynthesis may accumulate, leading to higher tissue C contents (Lapointe *et al.*, 2005). In the present study, C contents of Lake Illawarra seagrass leaves and macroalgae increased slightly with increasing N contents,

in particular, which may explain some of the high seagrass and macroalgae C contents documented in spring 2000 and summer 2001.

Although significant differences in the C contents of seagrass leaves and rhizomes occurred between sampling rounds, few of these differences could be related directly to the sampling season (winter or summer). It is important to note that all tissue C and N data used in the present study was obtained from the same laboratory; therefore, differences in C contents between the different sampling rounds should not be related to differences in analytical practices between laboratories. Menéndez (2002) noted that the carbon content of *Ruppia cirrhosa* from Tancada Lagoon, Spain, was relatively low in spring (about 34 % C) and highest in summer (about 40 % C), due to an increase in water temperatures and irradiance resulting in higher photosynthetic activity. In the present study, *Ruppia* leaf and rhizome C contents declined gradually between summer 2001 and winter 2002, but there were no obvious seasonal variations. *Zostera* samples, particularly those at Mullet Creek, exhibited a stronger seasonal variation, with leaf C contents declining in summer and increasing significantly in winter. The increase in *Zostera* leaf C contents during winter corresponded with a significant decline in leaf biomass during winter, particularly at the Mullet Creek site. Accordingly, *Zostera* leaf C contents decreased significantly in summer with increased plant biomass, but this seasonal trend was less pronounced with *Zostera* rhizome C contents. In contrast, Sfriso and Marcomini (1999) noted that leaf C contents of *Zostera marina* in the Venice Lagoon did not show a significant seasonal variation, but the C contents of roots-rhizomes increased in summer. In addition, Kaldy (2006) found that roots and rhizomes of *Zostera marina* in Yaquina Bay, Oregon, had higher C contents than leaves, probably due to higher structural demands and storage of non-structural carbohydrates. Where significant differences occurred between *Zostera* leaf and rhizome C contents in the present study, leaves had significantly higher C contents than rhizomes on all but one occasion (Mullet Creek, summer 2002). Similarly, few significant differences were found between *Ruppia* leaf and rhizome C contents, but where differences did occur, leaf C contents were significantly higher than rhizome C contents.

Several studies have shown that N and P contents in the above-ground proportions (leaves) of seagrasses are often significantly higher than the below-ground portions (roots-rhizomes) (Paling and McComb, 2000); this trend has been documented for *Zostera capricorni* (Queensland, Australia: Birch, 1976), *Zostera noltii* (Netherlands: Pérez-Lloréns *et al.*, 1991), *Zostera marina* (Venice lagoon: Sfriso and Marcomini, 1999; Yaquina Bay, Oregon: Kaldy, 2006) and *Zostera tasmanica* (Western Australia: Walker *et al.*, 2004). In the present study, paired t-tests showed that seagrass leaf N significantly exceeded rhizome N on all but one occasion (OCP, winter 2001). *Zostera* leaf N contents were approximately 1.5 - 3.5 times higher than rhizome N contents, whereas *Zostera* leaf P concentrations were 1.2 - 2.4 times higher than rhizome P contents. Similarly, *Ruppia* leaf N contents were approximately 1.1 - 1.7 times higher then rhizome N contents, but *Ruppia* leaf P contents were often similar to, or only

slightly higher than, rhizome P contents. Significant negative correlations between seagrass leaf biomass and N and P contents (Table 5-20) indicated that seagrass growth in Lake Illawarra was influenced by both nitrogen and phosphorus availability. *Zostera* leaf N contents increased significantly in winter, with a corresponding decrease in leaf biomass, indicating the storage, or no loss, of nutrients over winter. In contrast, *Zostera* leaf N contents decreased in summer with a significant increase in leaf biomass, suggesting stored nutrients were utilized to support increased growth over summer.

Seasonal trends in the N and P contents of seagrass samples were less pronounced in *Ruppia,* than *Zostera. Ruppia* leaf C and N contents at OCP and NIC declined over the entire sampling period, but the highest leaf N contents were documented in summer, with high leaf biomasses. At all sites, the P contents of seagrass leaves and rhizomes peaked in summer 2002, corresponding to the highest seagrass biomasses recorded during the study period. The seasonal variations in nutrient concentrations found in Lake Illawarra seagrasses is consistent with other studies suggesting some seagrasses store nutrients over winter, and may be limited by N or P during periods of higher biomass production (e.g., Walker *et al.*, 2004). Low tissue nutrient concentrations during summer are often due to nutrient utilisation exceeding uptake during periods of rapid growth; thus, seagrasses may become limited by nutrients when nutrient demands are greater than the nutrient supply (Walker *et al.*, 2004).

Tissue nutrient concentrations of macrophytes often vary considerably within and between sites, and according to the species, type of tissue, age of the plant, season and other environmental conditions (Touchette and Burkholder, 2000a). Differing N contents, for example, may be caused by variability in N demand and limitation as well as differing light intensities. Higher light intensities tend to enhance seagrass productivity, possibly resulting in a depletion of nitrogen in the sediment rhizoidal zone and, therefore, localised nitrogen limitation (Abal *et al.*, 1994, cited in Grice *et al.*, 1996). Carruthers *et al.* (1999) also noted that *Ruppia megacarpa* within Wilson Inlet, Western Australia, showed a significant between-site variation in growth; they suggested that variation in seagrass distribution and abundance may be related to interactions with fauna, such as fish and swans, competition between other macrophytes in the estuary, or storm events. All of these factors may reduce seagrass biomass to a different extent at different locations, which would explain some of the between-site variability exhibited by seagrasses. In the present study, differences between seagrass biomass and nutrient contents at each site may be related to varying availability of nutrients at each site. For example, the higher *Ruppia* biomasses at the Oasis Caravan Park, compared to the Nicolle Road site, may be related to influx of nutrients via the storm water drain located at the southern edge of the caravan park. However, a storm water drain was also located in the vicinity of the Nicolle Road site, and therefore runoff alone does not account for the significant difference in *Ruppia* biomass between the Nicolle Road and Oasis Caravan Park sites. In addition, differences in N and P concentrations of sediment collected at the OCP and NIC

Ruppia beds were rarely significant, but there were significant differences between nutrient concentrations of the *Ruppia* tissue at NIC and OCP. Groundwater seeping into the Lake from below the Windang golf courses and a former landfill site has also been suggested as a possible source of nutrients along the Windang Peninsula, particularly the southern end (I. Yassini, pers comm., 2003). A higher availability of nutrients at the OCP site is likely to influence the higher macrophyte biomasses found there.

The tissue nutrient concentrations, particularly N and P, of the macroalgae sampled showed a higher variability than those of the seagrasses in Lake Illawarra. Significant positive correlations were found between the biomass of macroalgae and C and N contents of algal tissue (Table 5-22), with the highest C and N contents often being associated with higher biomasses of macroalgae. However, macroalgal biomass and total P contents were not significantly correlated; whilst *Chaetomorpha* had significantly higher biomasses and higher P contents than other macroalgae, P concentrations varied somewhat. In general, the lowest tissue P contents of *Chaetomorpha* occurred during algal blooms (e.g., the Oasis Caravan Park site, spring 2000), indicating a possible exhaustion of nutrients following accelerated growth. In addition, *Chaetomorpha* total P contents were often significantly higher in winter, than summer, indicating storage of nutrients over winter. The exception was at the Primbee Bay site, summer 2002, where high *Chaetomorpha* biomasses (370 ± 37 g DW m^{-2}) corresponded with high tissue P contents (0.30 - 0.46 % P), but comparatively low N contents (1.1 - 2.5 % N). The higher tissue P contents at the Primbee Bay site in summer 2002 may be due to release of P from the sediment or decomposing algal biomass, higher water column P concentrations in summer (see, e.g., Figure 1-5), or lack of competition for available P with other macrophytes, such as *Ruppia*, compared to the Oasis Caravan Park site.

As some species of macroalgae, particularly green macroalgae, are capable of maintaining growth over winter, nutrients may be stored within plant tissue beyond the growing season (Kufel and Kufel, 2001). Opportunistic green macroalgae, such as *Ulva* and *Chaetomorpha*, have high nutrient uptake rates and large internal storage capacities; these algae, therefore, can take advantage of episodic nutrient pulses by rapidly taking up and storing nutrients and thus proliferating even when nutrient concentrations are low (Kamer and Fong, 2001). For example, Lavery *et al.* (1991) reported that *Ulva* in the Peel Inlet was often nutrient limited as it usually died-back by winter and required biomass to develop and nutrient stores to be replenished each spring to summer growing season. *Chaetomorpha*, however, was able to maintain biomass over winter, allowing it to accumulate and store nutrients during high nutrient loading events in winter; *Chaetomorpha*, therefore, was rarely nutrient limited, thus providing it with a competitive advantage over *Ulva* which had not stored nutrients over winter.

Lavery and McComb (1991a) suggested that *Chaetomorpha linum* in the Peel Inlet took up nutrients mostly from the water column, as indicated by seasonal fluctuations in tissue and

water column nutrient concentrations. As *Chaetomorpha* biomass was maintained throughout winter, this alga was able to perform luxury consumption and storage of N and P during winter when nutrient loads were higher in that estuary; this was indicated by the N and P levels being up to 2 and 5 times higher than the required nutrient levels, respectively. Stored nutrients would then be utilised in summer when nutrient loads are lower, thus the species rarely becomes nutrient limited. Lavery and McComb (1991b) determined that the critical nutrient levels for *Chaetomorpha linum* were 1.2 % N and 0.05 % P. In the present study, the N and P contents of *Chaetomorpha* ranged from 1.10 - 3.95 % N (mean ± s.e.: 2.83 ± 0.10 % N) and 0.03 - 0.46 % P (mean ± s.e.: 0.19 ± 0.02 % P), respectively. This data indicates that on the majority of sampling occasions, *Chaetomorpha* tissue nutrient concentrations were well above critical levels. *Chaetomorpha* tissue N concentrations below 1.5 % N only occurred at the Primbee Bay site, where biomasses of approximately 370 g DW m^{-2} were recorded. Similarly, *Chaetomorpha* P contents below 0.05 % P were only recorded on a few occasions, at the Oasis Caravan Park site (spring 2000 and summer 2001), but N contents of the same plants exceeded 2.0 % N, indicating a significant depletion of P, with respect to N.

Macroalgae attached to the substrate (e.g., *Gracilaria*) are likely to have higher N and P contents than free-floating varieties (e.g., *Chaetomorpha*) as they are able to take up nutrients regenerated from the sediment (Villares & Carballiera, 2003). In the present study, *Gracilaria* (Rhodophyta), attached to the substrate at Purry Burry Point, had the highest tissue nutrient concentrations of all macroalgae examined, with 3.49 ± 0.21 % N and 0.53 ± 0.04 % P in summer 2002. The N and P concentrations of *Gracilaria* were 2.8 and 6.6-fold higher, respectively, than those of drifting *Enteromorpha* (now *Ulva)*, found entangled amongst seagrass beds at the same site. Red macroalgae, such as *Palmaria palmata* along the Spanish coast, have been shown to have the highest concentrations of N and P during winter and autumn when water nutrient concentrations are higher, but biomass production is limited by light and temperature (Martínez and Rico, 2002). Compared to sheet-forming species, such as *Ulva*, *Gracilaria* has a low surface to volume ratio and takes up N relatively slowly, but has a comparatively large N storage capacity and can maintain growth even when water N concentrations are low (Anderson *et al.*, 1996; Fujita, 1985). Fujita (1985) also found that, although green macroalgae were more competitive and had higher growth rates than red macroalgae undergoing nutrient enrichment, *Gracilaria tikvahiae* could sustain growth at low N concentrations longer than *Ulva* spp.; this may explain why *Gracilaria* persists in Lake Illawarra throughout the year, yet other species dieback periodically.

5.4.4 Nutrient Limitation of Macrophytes

Phosphorus is often considered to be the nutrient that limits macrophyte primary production in fresh water, whereas nitrogen is often the limiting nutrient in marine and estuarine systems (Smith, 1984; Harding *et al.*, 2002). According to Fourqurean and Zieman (1992), P is often

the limiting nutrient in some lakes, which tend to have longer water residency times than frequently flushed estuaries and coastal ecosystems. In estuaries open to the sea, therefore, the products of N fixation and remineralization would be flushed out, resulting in N limitation. Phosphorus may also limit seagrass growth and biomass production in tropical estuaries dominated by carbonate sediments (Gras *et al.*, 2003). Previous studies have concluded that Lake Illawarra is a net sink for nitrogen and strongly nitrogen limited for phytoplankton primary production (Qu, 2004a). As nitrogen often limits macroalgal biomass production in coastal ecosystems, increases in nitrogen loading often result in increased macroalgal biomass, particularly blooms of opportunistic green macroalgae (Kamer and Fong, 2001). Phosphorus has also been reported to limit phytoplankton production in Australian estuaries and, in some systems, limitation by N or P may occur at different times (Davis and Koop, 2006, and references therein). In addition, different macroalgal species may be limited by different nutrients in the same estuary (Valiela *et al.*, 1997).

Tissue carbon, nitrogen and phosphorus contents and stoichiometric ratios of C:N:P have been widely used to indicate nutrient limitation of seagrasses (e.g., Duarte, 1990; Fourqurean, and Zieman, 1992; Johnson *et al.*, 2006) and macroalgae (e.g., Rhee, 1978; Hanisak, 1979; Fong, *et al.*, 1993; Pedersen and Borum, 1996). Previous studies have shown that while the tissue N and P contents of seagrasses can vary widely between species and sites, the carbon contents of seagrasses exhibit less variability; this suggests that limitation by carbon is rare due to a high availability of carbon in the water, and because the majority of carbon in seagrass leaves represents structural components (Atkinson and Smith, 1983; Duarte, 1990). However, seagrass carbon contents decline with very low nitrogen and phosphorus contents, of less than 1.2 % N and 0.13 % P, respectively (Duarte, 1990), but tissue nutrient contents of seagrass leaves sampled in Lake Illawarra during the present study rarely fell below 1.7 % N and 0.13 % P. According to Duarte (1990), plants limited by nitrogen or phosphorus should have low N and P contents, with respect to their C contents and, therefore, high C/N or C/P ratios. This author suggested that seagrasses with tissue nutrient contents below 1.8 % N and 0.20 % P are strongly nutrient limited.

Frequency distribution charts of all samples collected in this study showed that carbon contents of seagrass rhizomes were normally distributed, but seagrass leaf and macroalgae C contents were negatively skewed towards high carbon contents, of up to 40 % C (Figure 5-12). Nitrogen contents of all macrophyte samples collected appeared to follow a normal distribution, but P contents of both seagrasses and macroalgae were positively skewed towards P contents below ~0.3 % P.

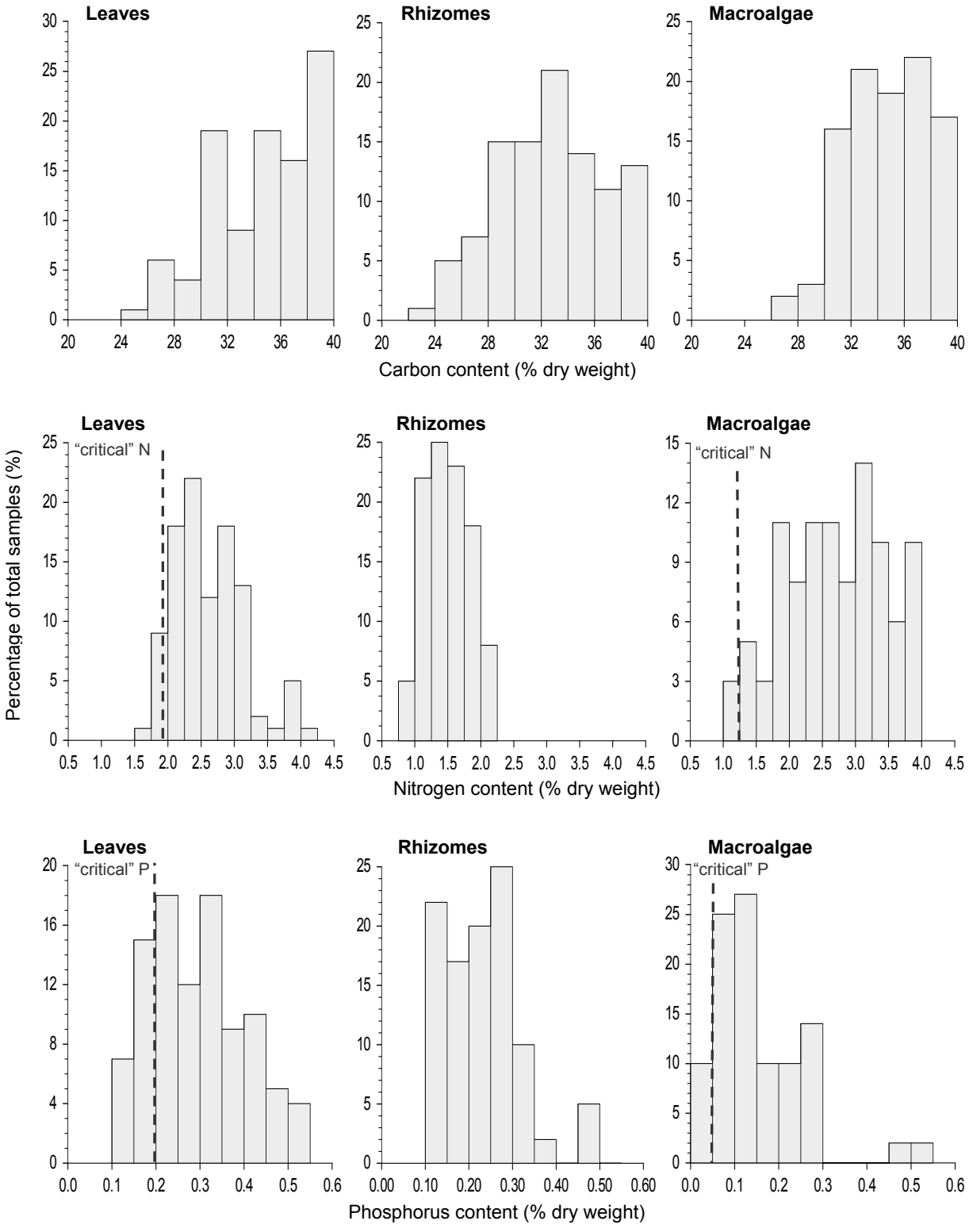


Figure 5-12: Frequency distribution of carbon, nitrogen and phosphorus contents of seagrasses (leaves and rhizomes; n = 99) and macroalgae (n = 63) in Lake Illawarra, 2000 - 2002. Red lines indicate critical concentrations of tissue N and P (see text for further details).

While only 6 % of *Ruppia* leaf samples contained phosphorus contents below the level considered critical for growth of angiosperms (0.13 % P: Gerloff and Krombolz, 1966), 43 % of

Ruppia leaf samples contained P contents below the level thought to indicate limitation of biomass production by P (0.20 % P: Duarte, 1990). In contrast, nitrogen contents of all *Ruppia* leaf samples were above the level considered critical for growth (1.3 % N: Gerloff and Krombolz, 1966), and only 4 % of *Ruppia* samples contained N contents below the level considered to indicate limitation by N (1.8 % N: Duarte, 1990). Furthermore, Thursby (1984) reported critical N and P contents of 2.5 - 3.0 % N and 0.25 - 0.35 % P for *Ruppia maritima*. Using these estimates would suggest that *Ruppia* in Lake Illawarra was deficient in P (< 0.25 % P), but N contents were sufficient, between spring 2000 and winter 2001. On the other hand, P contents were above 'critical' levels in 2002 (summer and winter) when *Ruppia* biomasses were slightly higher, but N contents were below 'critical' (< 2.5 % N) during that period.

All *Zostera* leaf samples contained nitrogen and phosphorus contents above 1.8 % N and 0.20 % P, respectively, suggesting that *Zostera* beds in Lake Illawarra were not N or P limited. As tissue analyses of Lake Illawarra macrophytes indicated that N concentrations were usually well above known critical concentrations, it is likely that internal phosphorus pools were exhausted in order to support growth. Therefore, any plant-available P in the water column or rhizosphere would be rapidly taken up by seagrasses or macroalgae, which may have resulted in localised P limitation. Certainly, the low concentrations of tissue P compared to N, particularly of *Ruppia* at the Oasis Caravan Park, indicated limitation by P at the time of sampling. This finding of P limitation in some Lake Illawarra macrophytes is particularly interesting as the majority of data indicates N limitation for microalgae production in Lake Illawarra (Qu, 2004a, b; Dongyan Liu, PhD Student, University of Wollongong, pers. comm., 2007).

The Redfield stoichiometric ratio of C:N:P (106:16:1) is often used to describe nutrient limitation of biomass production in marine phytoplankton; N/P ratios greater than 30:1, or less than 10:1, indicate deprivation of P or N, respectively. The Redfield ratio, however, is not always considered appropriate for use with macroalgae and seagrasses, which typically have C:N:P molar ratios around 550:30:1 (Atkinson and Smith, 1983; see, e.g., Section 2.6.2). Wheeler and Björnsäter (1992) estimated that N/P ratios in excess of 11 - 24 indicated P limitation in macroalgae, whereas ratios less than 8 - 16 indicated N limitation. In the present study, the N/P ratios of macroalgae ranged from 6.7 - 151 (overall mean ± s.e.: 47.3 ± 3.7). 15 % of all macroalgae samples had N/P molar ratios of 16 or below, indicating N limitation, but 77 % of macroalgae samples had tissue N/P ratios higher than 24, indicating limitation by P. In addition, 10 % of *Chaetomorpha* samples in Lake Illawarra had phosphorus contents below the level critical for growth of *C. linum*, but all samples were well above the critical N level (0.05 % P and 1.2 % N, respectively: Lavery and McComb, 1991b; Section 5.4.3). However, the use of tissue nutrient concentrations and C:N:P molar ratios to determine nutrient limitation can be problematic as algae of differing species and thallus morphologies can have different nutrient uptake and storage capacities and different seasonal patterns of growth (Rosenburg

and Ramus, 1982; Wallentinus, 1984; Fong *et al.*, 2003). For example, if there are high concentrations of both nitrogen and phosphorus in the water column, but an alga has a greater uptake and storage capacity for N than P, the subsequent elevated tissue N/P ratio would indicate limitation by P when it was not occurring (Fong *et al.*, 2003).

Previous studies have demonstrated that green macroalgae, such as *Chaetomorpha* or *Cladophora* have high storage capacities for N (e.g., Gordon *et al.*, 1981). For example, McGlathery *et al.* (1996) reported that internal N pools of *Chaetomorpha linum* varied from 1.2 - 4.6 % N (dry weight) when grown under N-limiting and N-saturating conditions. When grown under non-limiting conditions, internal N contents reflected uptake and storage of N that exceeded immediate growth requirements. The effect of nitrogen enrichment and deprivation on the growth of *Chaetomorpha linum* is discussed further in Chapter 6; culture experiments determined that N deprivation, rather than P deprivation, had a much greater effect on the growth rates of *C. linum* harvested from Lake Illawarra. *C. linum* maintained growth at rates of 10 % WW d^{-1} in unenriched and P-only treatments, but growth in N-only treatments averaged 15 - 20 % WW d^{-1}, and was often not significantly different to growth in N + P treatments. These experiments indicated that *C. linum* was far less reliant on P supply, than N supply.

It has been suggested that, for seagrasses, N/P ratios may be a more useful indicator of nutrient status than C/P or C/N ratios as they are not dependent on structural carbon, thereby reducing the variability between plants (Johnson *et al.*, 2006). In addition, tissue N/P ratios may be used to predict the supply of plant-available N and P in the water column (Wheeler and Björnsäter, 1992; Güsewell *et al.*, 2003). For seagrasses, N/P ratios greater than 30 indicate that P may limit biomass production, whereas N/P ratios less than 25 indicate N limitation (Fourqurean and Cai, 2001). The mean N/P contents of *Ruppia* leaves at the Oasis Caravan Park and Nicolle Road sites only significantly exceeded 30 in spring 2000 and summer 2001, indicating limitation by P at that time. However, mean *Ruppia* leaf N/P ratios averaged 25 in winter 2001, but decreased to less than 17 with increasing P contents in summer 2002 and winter 2002, indicating limitation by N in the latter sampling rounds. These N/P ratios support findings of occasional P limitation in *Ruppia,* based on low tissue P contents, as described above. *Zostera* leaves at Purry Burry Point and Mullet Creek typically had higher P contents than *Ruppia* leaves, and therefore N/P ratios did not exceed 30, but remained below 25 between winter 2001 - winter 2002, indicating N limitation.

As factors other than nutrient enrichment can limit seagrass growth and abundance (e.g., light, temperature or season), the degree of limitation due to nutrients is difficult to evaluate from tissue analyses alone (Touchette and Burkholder, 2000a). Differences in tissue nutrient concentrations between the two seagrass species (*Z. capricorni* and *R. megacarpa*) found in Lake Illawarra may be due to differences in external nutrient supply, as well as differences in nutrient acquisition or requirements, and growth strategies (Walker *et al.*, 2004). In addition,

internal retranslocation of nutrients from older to younger plant parts, especially during periods of peak biomass production, means that high nutrient concentrations are continuously supplied to the most metabolically active plant parts, thereby reducing the reliance on external nutrient supplies (Alcoverro *et al.*, 2000). Yamamuro *et al.* (2004) reported that N, P and $\delta^{15}N$ contents of *Enhalus acoroides* decreased with increasing distance along the leaf; the maximum ranges recorded in one leaf were 1.80 - 4.05 % N, 0.29 - 0.66 % P, and 1.28 - 2.93 ‰ for $\delta^{15}N$. Therefore, while tissue nutrient concentrations averaged over the whole plant part (i.e., leaves) may suggest nutrients may limit biomass production, seagrasses can still maintain high growth and productivity via internal remobilisation of nutrients to actively growing plant parts (Walker *et al.*, 2004).

Phosphorus limitation of *Ruppia* biomass production at the Oasis Caravan Park and Nicolle Road sites may be due to competition with other macroalgae (benthic and epiphytic) or phytoplankton. Average chlorophyll-*a* levels in Lake Illawarra were 3 - 7 $\mu g\ L^{-1}$ (Table 1-2), and average biomass of epiphytes on *Ruppia* leaves ranged from approximately 1 g DW m^{-2} in winter, to 30 DW m^{-2} in summer during the present study (Section 5.2). Algal epiphytes can successfully compete with seagrasses for nutrients and other available resources (see, e.g., Lin *et al.*, 1996; Gacia *et al.*, 1999). According to Plus *et al.* (2003), the light available to seagrasses (*Zostera noltii*) decreased by 80 % when the ratio of epiphyte to leaf biomass was at a maximum. In addition, epiphytes have been reported to remove P from the water at a faster rate than macrophytes (Pelton *et al.*, 1998). Dudley *et al.* (2001) reported that epiphytes attached to the surface of *Ruppia megacarpa* leaves in Wilson Inlet, Western Australia, removed more nitrate and ammonium from the water column than the seagrass leaves, despite having only 25 % of the biomass of *R. megacarpa.* These authors found that epiphytes took up nitrate and ammonium at approximately twice the rate of *R. megacarpa*, highlighting the importance of benthic macrophytes as a substrate for algal epiphytes which may act as a substantial sink for nutrients in estuaries.

P limitation of seagrass biomass production may also be due to seagrasses being unable to utilise P in the form that is present in the water column (Johnson *et al.*, 2006). It is estimated from Lake Illawarra water quality data that 10 - 90 % (mean: 50 %) of total P is in the form of orthophosphate-P (unpublished Lake Illawarra Authority data, pers. comm., 2007). Therefore, macrophytes in Lake Illawarra may be limited by P supply on occasions when the majority of total P is tied up as particulate phosphorus. In addition, arsenate (As(V), the dominant form of arsenic in estuarine waters) may interfere with uptake of phosphorus by seagrass and macroalgae (Johnson *et al.*, 2006). Plants take up arsenate via the phosphate uptake system; inorganic arsenate uncouples phosphorylation and inhibits phosphate uptake, and is therefore toxic to aquatic plants (Fourqurean and Cai, 2001). In Lake Illawarra, Howley (2001) noted that concentrations of arsenic in leaves of *Zostera capricorni* were generally at or below the detection limit (< 1 mg kg^{-1}). However, higher As concentrations were detected in rhizomes of

Z. capricorni at sites with elevated sediment metal concentrations, such as Yallah Bay and Primbee Bay (*Z. capricorni*: 4.75 ± 0.75 mg kg^{-1} and 2.50 ± 0.56 mg kg^{-1}, respectively). Concentrations of arsenic in leaves of *Ruppia megacarpa* in Lake Illawarra are not known.

In Lake Illawarra, molar ratios of dissolved inorganic N:P in the water column were generally below 5 throughout the year, indicating nitrogen should limit primary production (unpublished Pacific Power data, pers. comm., 2004). Concentrations of phosphate-P were typically 2 - 3-times higher in summer and autumn, compared to winter and spring (Figure 1-5) which correlates with the higher seagrass leaf P concentrations found in summer 2002 (see, e.g., Figure 5-3). LIA (1995) concluded that the release of N and P from the sediment was the most important source of nutrients to the water column, and is expected to have a significant impact on plant growth rates. Concentrations of P in the sediment rhizoidal zone of seagrass beds recorded during the present study were considerably lower than surface sediment P concentrations documented previously for other Lake Illawarra sites. Murrie (1994) and Payne (1994) recorded sediment total P concentrations of 317 - 1,020 μg g^{-1} in surface sediments across a range of Lake Illawarra sites. Yassini (1994) calculated mean total nitrogen and phosphorus concentrations of 777 μg P g^{-1} (~0.08 % P) and 0.29 % N in the top 0 - 10 cm of Lake Illawarra sediments. In the present study, sediment total P and N concentrations ranged from 40 - 420 μg P g^{-1} (mean: 180 ± 8 μg P g^{-1}) and 0.01 - 0.29 % N (mean: 0.07 ± 0.01 % N), with the highest nutrient concentrations recorded at Mullet Creek. These low nutrient concentrations are closely associated with the proportion of sand in the sediment, particularly along the eastern Lake sites (PBP, OCP and NIC), where the sediments were composed of approximately 97 % sand and coarse grains. As expected, sediment nutrient concentrations were higher at the source of freshwater input (Mullet Creek), where the proportion of silt/clay ranged from 5 - 60 %. The low sediment P concentrations may also be an indication that the majority of plant available P in the rhizosphere was exhausted by seagrasses. According to Sfriso and Marcomini (1999) *Zostera* will take up N and P from the sediment when water column nutrients are depleted, thus significantly reducing sediment P by about 14 % in their experiments.

Thus, it seems unlikely that the availability of P would limit the growth or biomass production of aquatic macrophytes in Lake Illawarra given that previous studies have concluded that the Lake is strongly nitrogen limited for phytoplankton production (e.g., Qu, 2004a) and that the Lake's sediments are a significant source of P (LIA, 1995). In addition, Lake Illawarra is reported to have high average total phosphorus concentrations of 0.12 mg L^{-1}, four times higher than the national guidelines (0.03 mg P L^{-1}); run-off into Lake Illawarra has naturally elevated phosphorus concentrations, due partly to the geology of the catchment (Scanes *et al.*, 2007, and references therein). However, localised P limitation may occur within macrophyte beds; for example, where N supply is high (e.g., due to stormwater inflows, groundwater seepage or sediment nutrient release), internal P stores may be subsequently depleted to

support growth. The high concentrations of N stored in seagrass and macroalgal tissue certainly implies storage of N that exceeds immediate growth requirements. In addition, nutrient enrichment experiments on *Chaetomorpha linum*, the dominant alga in Lake Illawarra determined that deprivation of P, at least in the short-term, had limited effects on the growth rates of that alga (Chapter 6).

5.4.5 Stable Isotopes of C and N

Many studies have recorded significant spatial and temporal variations in the isotopic ratios of aquatic macrophytes; Boon and Bunn (1994), for example, noted that significant variability (> 10 delta units) can occur in carbon and nitrogen isotopic values of the same species sampled at different sites at the same time, or at the same site at a different time of year. Localised variations in the isotopic signatures of macrophytes may be due to differences in productivity ($\delta^{13}C$), or the sources of nutrients ($\delta^{13}C$ and $\delta^{15}N$) (Guest *et al.*, 2004). The $\delta^{13}C$ values of seagrasses are usually around -10 ‰, but values ranging from -23 ‰ to -3 ‰ have been reported in the literature (Lepoint *et al.*, 2004; McMillan *et al.*, 1980; Table 2-10). In the present study, the $\delta^{13}C$ contents of seagrasses ranged from -7.7 to -15.9 ‰ (mean ± s.e.: -11.5 ± 0.16 ‰) for leaves, and -7.7 to -13.8 ‰ (mean ± s.e.: -10.9 ± 0.11 ‰) for roots-rhizomes. The $\delta^{15}N$ contents of seagrasses across all Lake Illawarra sites ranged from 1.0 - 9.0 ‰ (mean ± s.e.: 4.2 ± 0.16 ‰) for leaves, and 0.7 - 7.5 ‰ (mean ± s.e.: 3.4 ± 0.16 ‰) for roots-rhizomes. These $\delta^{15}N$ values are also typical of those cited in the literature for seagrasses, which typically lie between 0 ‰ and 8 ‰, but can vary from -2 ‰ to 12.3 ‰ (Lepoint *et al.*, 2004). The $\delta^{13}C$ and $\delta^{15}N$ values documented in the present study are also within the range of those previously reported by Kuster (2000) for Lake Illawarra seagrasses, at similar sites to those used in the present study. Macroalgae have similar $\delta^{13}C$ values to seagrasses, and are usually in the range of -12 ‰ to -23 ‰ (Smith and Epstein, 1971), but extreme values of -8.8 ‰ to -32.4 ‰ have been documented (Fry *et al.*, 1982). The $\delta^{13}C$ and $\delta^{15}N$ contents of macroalgae recorded across Lake Illawarra were more variable than those of the seagrasses, and ranged from -4.9 to -19.8 ‰ and 1.8 - 14.6 ‰, respectively.

The high variability in $\delta^{13}C$ and $\delta^{15}N$ contents of macrophytes recorded during the present study suggests that there are many environmental and other factors influencing the isotopic signatures of aquatic macrophytes; these include temperature, light availability, water movement, and the proximity to fresh-water and marine inflow and other sources of nutrients, such as groundwater, waste water or runoff (Smith *et al.*, 1976; McMillan and Smith, 1982; Hemminga and Mateo, 1996; France and Cattaneo, 1998; Boyce *et al.*, 2001). Variability occurs in $\delta^{13}C$ values due to fluctuations in the isotopic signature of carbon fixed during photosynthesis, the metabolic pathway used (e.g., C_3 or C_4) and differences in transport of carbon across the cell membrane (Smith and Epstein, 1971; Boon and Bunn, 1994; Coffin and Cifuentes, 1999). In the present study, variations in isotopic signatures between different

macrophyte species at the same site were often greater than variations between the same species at different sites (see, e.g., Figure 5-5). Variations in $\delta^{13}C$ contents between the same seagrass species at different Lake Illawarra sites were usually within 1 - 5 ‰, which is typical of the between-site variability reported for seagrasses (see, e.g., Papadimitriou *et al.*, 2006). Differences between species of macroalgae, however, showed greater variations; for example, $\delta^{13}C$ contents of *Gracilaria* (Rhodophyta) and *Ulva* (Chlorophyta) collected from the same site (Purry Burry Point, summer 2002) differed by 10 ‰. Similarly, the $\delta^{13}C$ and $\delta^{15}N$ contents of *Chaetomorpha* spp. at the Oasis Caravan Park often varied by 4 - 8 ‰ from other species of green macroalgae collected at the same time and location. Less negative $\delta^{13}C$ contents in macroalgae were usually associated with lower algal biomasses. Although the $\delta^{13}C$ contents of macroalgae were more variable than seagrasses, algal $\delta^{13}C$ contents generally did not vary more than 4 - 5 delta units from seagrasses at the same site. Macroalgae are usually depleted in ^{13}C compared to seagrasses, due partly to differences in fractionation of $^{13}C/^{12}C$ during photosynthesis (Raven *et al.*, 1995). In Western Australian coastal systems, for example, the $\delta^{13}C$ contents of macroalgae were about 8 - 10 ‰ more negative than seagrasses, *Amphibolis griffithii* (Smit *et al.*, 2005) and *Posidonia sinuosa* (Smit *et al.*, 2006).

In seagrasses, isotope values that are more negative suggest discrimination against the heavier isotope (^{13}C), and indicate that the supply of carbon exceeds the demand (Udy and Dennison, 1997a). When nutrients and light are non-limiting, seagrass photosynthesis and production may be limited by carbon availability. Under high productivity, the demand for carbon increases, and the ability to discriminate against ^{13}C is reduced (Udy and Dennison, 1997a); this results in enrichment of ^{13}C (Guest *et al.*, 2004). Vizzini *et al.* (2003) noted that intermediate leaves of Mediterranean *Posidonia oceanica* were slightly more enriched in ^{13}C (i.e., less negative $\delta^{13}C$ contents) than older leaves; the higher photosynthetic rates and, therefore, higher carbon demand, of younger leaves leads to reduced discrimination against ^{13}C. Likewise, the higher demand for carbon in summer, due to higher irradiance and higher photosynthetic demands, would also lower discrimination against ^{13}C, resulting in less negative $\delta^{13}C$ contents in summer.

In the present study, the $\delta^{13}C$ contents of *Ruppia* leaves were significantly less negative in summer 2002, in accordance with the higher leaf biomasses recorded during that period. *Zostera* leaves, however, appeared to show the opposite trend, with $\delta^{13}C$ contents being more negative in summer, than winter. The *Zostera* leaf and rhizome samples collected from the Mullet Creek site exhibited the greatest seasonal variability in $\delta^{13}C$ contents, with leaf $\delta^{13}C$ contents being more negative (by approximately 3 delta units) in summer, than in winter. Leaf $\delta^{15}N$ contents of *Zostera* at Mullet Creek also showed significant seasonal trends, with the highest $\delta^{15}N$ values recorded in summer, during periods of peak biomass. Similar trends were exhibited by temperate *Zostera noltii,* with the most negative $\delta^{13}C$ contents and highest $\delta^{15}N$

contents occurring during summer, when biomass was highest (Papadimitriou *et al.*, 2006). Additionally, *Zostera* rhizome and leaf samples at Mullet Creek were significantly more negative than the Windang Peninsula sites (*Zostera* and *Ruppia*); these differences are most likely due to the proximity to fresh water input, compared to the eastern Lake Illawarra sites, which are closer to the Lake's entrance to the sea. Similarly, Boyce *et al.* (2001) found significant within-site differences between the $\delta^{13}C$ contents of *Ruppia megacarpa* leaves, rhizomes and roots of Western Australian estuaries, but these differences tended to be site-dependent. They found that the pattern of spatial variability within *Ruppia* $\delta^{13}C$ values varied across lagoons and was possibly related to differences in water quality and proximity to freshwater and marine inputs. For example, Boyce *et al.* (2001) found that root and rhizome tissue samples of *Ruppia megacarpa* collected near freshwater sources in Nornalup Inlet, WA, had less negative $\delta^{13}C$ contents than those collected closer to the ocean. Therefore, these authors concluded that $\delta^{13}C$ values of *Ruppia* collected from one part of an estuary did not adequately represent the range of values exhibited across the entire estuary.

Differences between the isotopic signatures of plant parts (leaves and rhizomes) may also be related to storage of nutrients with distinct isotopic ratios (Boyce *et al.*, 2001). Differences between the $\delta^{13}C$ contents of seagrass leaves and rhizomes were rarely significant at the OCP, NIC or PBP sites (Table 5-14), but at Mullet Creek, *Zostera* leaves had significantly more negative $\delta^{13}C$ contents than rhizomes throughout the sampling period (except winter, 2001). Boyce *et al.* (2001) also found that *Ruppia megacarpa* leaves tended to be more negative than roots-rhizomes; they suggested that as seagrass leaves typically have faster turnover rates than below-ground material, the isotopic signatures of rhizomes may reflect carbon assimilation over a longer time frame than leaves. Differences between leaf and rhizome $\delta^{13}C$ contents at the Mullet Creek site would also be related to the freshwater input and the source of carbon assimilated (i.e., water column versus sediment). In addition, significant positive correlations were found between the values of $\delta^{13}C$ in leaves and rhizomes and sediment of the seagrass beds at each site (Table 5-21), indicating the incorporation of sediment-derived C. At each site, except for Mullet Creek, *Zostera* and *Ruppia* rhizome $\delta^{13}C$ contents were significantly more negative in winter, than in summer. This trend has also been documented for *Zostera marina* and *Posidonia oceanica*, and is partly due to a higher storage of soluble carbohydrates (enriched in ^{13}C) in seagrass rhizomes over winter (Vizzini *et al.*, 2003, and references therein).

Macrophyte $\delta^{15}N$ values fluctuate according to variations in the metabolic pathways used for nitrogen assimilation and the type of nitrogen assimilated, as well as the presence of nitrogen-fixing or denitrifying bacteria (Boon and Bunn, 1994). Udy and Dennison (1997a) suggested that the $\delta^{15}N$ ratios of seagrasses are primarily dependent on N supply, rather than other environmental factors, such as temperature and light. If seagrass growth is limited by nitrogen availability, there will be little discrimination between ^{14}N and ^{15}N, but if nitrogen availability is

high, discrimination will occur against ^{15}N, leading to negative $\delta^{15}N$ ratios. The predominantly positive $\delta^{15}N$ ratios of seagrass leaves and macroalgae recorded in the present study suggest that the supply of nitrogen to Lake Illawarra seagrasses was not excessive. This finding is supported by water column nitrogen concentrations (Figure 1-5); average dissolved inorganic nitrogen concentrations were 66 ± 9.7 $\mu g\ L^{-1}$ (unpublished Pacific Power data, pers. comm., 2004). The highest $\delta^{15}N$ values were recorded for *Ruppia* (6.7 - 8.9 ‰) and *Chaetomorpha* (11.1 - 14.4 ‰) at the Nicolle Road site in spring 2000 and summer 2001, but these high $\delta^{15}N$ contents were not replicated in later surveys. Kuster (2000) also reported high $\delta^{15}N$ contents at the same Nicolle Road site in autumn 2000; he documented $\delta^{15}N$ values up to 13.2 ‰ in *Ruppia* leaves and 14.9 ‰ in *Chaetomorpha* samples.

High $\delta^{15}N$ values in macrophyte tissue have been reported from areas subject to eutrophication or those receiving waste waters, and may be due to nitrification and denitrification processes resulting in isotopic fraction and thus enrichment of ^{15}N at the source of the wastewater (Hansson *et al.*, 1997; Grice *et al.,* 1996). The high $\delta^{15}N$ contents of seagrass tissue at the Nicolle Road site may be related to discharges from the storm water drain at that site; in the spring 2000 and summer 2001 sampling sites, the NIC seagrass sampling site was situated approximately 100 metres from the drain, but as the inshore seagrass beds were no longer present in the later sampling rounds, the NIC site was moved to a distance of at least 300 metres from the storm water drain for the winter 2001 - 2002 sampling rounds. Elevated $\delta^{15}N$ values in seagrasses may also be related to denitrification processes in marine and estuarine waters; denitrification leads to the loss of ^{14}N, and an enrichment of ^{15}N, from the inorganic N pool in the water column, and a subsequent enrichment of ^{15}N in the plants taking up that inorganic N from the water (Fourqurean *et al.*, 1997). Whereas $\delta^{15}N$ values close to 0 ‰ may be related to nitrogen fixation by organisms within the seagrass community (Yamamuro *et al.*, 2003; Meksumpun *et al.*, 2005). Qu (2004a) presents a more detailed review of denitrification and nitrification processes in Lake Illawarra.

5.4.6 Role of Macrophytes in Lake Illawarra Nutrient Budget

If the four Lake Illawarra sites surveyed (OCP, NIC, MC and PBP) are considered representative of the entire Lake, the total nutrient pools bound to macrophyte biomass can be tentatively estimated. WBM (2000) estimated the total area of seagrass beds in Lake Illawarra, using aerial photographs, whole-of-Lake surveys and biomass estimates at 15 sites of varying density and spatial coverage across the Lake (refer to Sections 4.2.1 - 4.2.4; Figure 4-3). These authors estimated the total area of *Zostera* and *Ruppia* beds in Lake Illawarra in summer 2000 to be 5.65 and 1.67 km^2, respectively. Using these areas of seagrass coverage and the average biomass of *Zostera* and *Ruppia* noted during the present study (all seasons), the total biomass of seagrasses was estimated at 970 T (dry weight) for *Zostera* (above-ground: 510 T), and 400 T (dry weight) for *Ruppia* (above-ground: 270 T). These biomass

estimates, however, are higher than calculations by previous authors; for example, WBM (2000) estimated the above-ground biomass of *Zostera* in the Lake to be 439 T, whereas King *et al.* (1997) reported 794 T (dry weight) of total *Zostera* biomass (above-ground: 324 T). In the present study, seagrass biomass in Lake Illawarra varied significantly between seasons; seasonal estimates of total seagrass (*Zostera* and *Ruppia*, above and below-ground) standing stock ranged from 800 T in winter, to 1,800 T in summer. Using the average N and P contents of Lake Illawarra seagrasses (Table 5-24), the average nutrient pools associated with seagrasses in 2000 - 2002, were estimated to be 8,800 kg dry wt. N and 1,100 kg dry wt. P for *Ruppia*, and 21,000 kg dry wt. N and 2,800 kg dry wt. P, for *Zostera*. It must be noted, however, that estimates of total nutrient pools are included as a general guide only; they are subject to variation according to tissue nutrient contents, spatial coverage and estimates of seagrass biomass, which is influenced by factors such as the sampling strategy used, the sampling season (e.g., winter versus summer) and the timing of sampling (e.g., before or after the seagrasses have shed their leaves).

Macroalgal distribution and abundance in Lake Illawarra was highly variable throughout the study period, and thus it was difficult to accurately quantify the pools of total nitrogen and phosphorus associated with algal biomass. *Chaetomorpha linum* and C. *billardierii* were the most abundant bloom-forming algae in the Lake, with biomasses peaking at 113 and 370 g DW m^{-2} at the Oasis Caravan Park (spring 2000) and Primbee Bay (summer 2002), respectively. At the Primbee Bay site, for example, macroalgal biomass covered approximately 90 % of a 10,000 m^2 area in summer 2002. Using the average N and P contents of *Chaetomorpha* spp. (6.42 ± 1.1 g N m^{-2} and 1.38 ± 0.23 g P m^{-2}, respectively) collected at Primbee Bay would give a total nutrient pool of approximately 58 kg N and 12 kg P stored as macroalgal biomass at that site in summer 2002. At the Oasis Caravan Park, the largest macroalgal bloom occurred in spring 2000, covering 60 % of an approximately 20,000 m^2 area, with an average *Chaetomorpha* spp. biomass of 113 ± 31 g DW m^{-2}. Using the average N and P contents of *Chaetomorpha* spp. (3.76 ± 1.1 g N m^{-2} and 0.10 ± 0.03 g P m^{-2}), collected at OCP during spring 2000, the nutrient pool stored as macroalgal biomass at that time was estimated at approximately 75 kg dry wt. N and 2 kg dry wt. P.

In order to provide an estimate of total macroalgal biomass in the Lake, it was tentatively assumed that the biomass and nutrient contents of macroalgae associated with seagrass beds at the four sites surveyed in the present study were indicative of macroalgae - seagrass interactions over the entire Lake. However, as the present study focussed primarily on shallow (less than 0.70 m depth) areas subject to macroalgal blooms, this assumption does not account for the decrease in macroalgal biomass in deeper areas, due to reduced availability of light. If we consider the total area of seagrass beds in the Lake, as stated above, the average pools of nitrogen and phosphorus contained within macroalgae (epiphytic and benthic) associated with seagrass beds in the Lake were estimated at 5,100 kg dry wt. N and 375 kg

dry wt. P, respectively. In addition, the area of Lake Illawarra which may be subject to macroalgal blooms in sheltered bays was estimated at 0.53 km^2 (Figure 4-5). Using the average macroalgal biomass determined for inshore areas of the Lake calculated during the present study and the average N and P contents of macroalgae (0.38 g N m^{-2} and 0.022 g P m^{-2}), the average pools of nitrogen and phosphorus associated with inshore macroalgal blooms were estimated at 200 kg dry wt. N, and 12 kg dry wt. P, respectively.

The pools of nitrogen and phosphorus contained within the macrophyte beds are compared to N and P in the water column and sediment in Table 5-25. The pools of N and P in the water column were calculated using the area of Lake Illawarra (35 km^2), the average water depth (1.8 m) (LIA, 1995), and the average total nitrogen and total phosphorus contents in the water column, between May 2005 and February 2007 (0.59 mg N L^{-1} and 0.10 mg P L^{-1}, respectively: Lake Illawarra Authority, pers. comm., 2007). These total N and P concentrations are similar to those recorded in Lake Illawarra between 1996 and 2001 (Table 1-2). The estimated pools of nitrogen and phosphorus in the macrophyte beds are within the same order of magnitude as the N and P in the water column. However, these pools of N and P are clearly insignificant when compared to the pools of N and P present in Lake Illawarra sediment. Sediment nutrient pools were estimated for the top 0 - 10 cm of sediment, using the average N and P contents (0.29 % N and 0.078 % P) and sediment bulk density (1,300 kg m^{-3}) reported for Lake Illawarra (Yassini, 1994); this gives nutrient loads of 3.8 kg N m^{-3} and 1.0 kg P m^{-3}. In addition, significant N and P contents are also recorded at greater depths; for example, Lake Illawarra sediment cores of 0.4 - 0.8 m depth had N and P contents of 0.12 % N and 0.06 % P (Yassini, 1994).

Table 5-25: Comparison between the estimated pools of nitrogen and phosphorus associated with macrophytes, water and sediment in Lake Illawarra (area = 35 km^2).

Component	Total Nitrogen Pool (kg DW)	Total Phosphorus Pool (kg DW)	Reference
Seagrass (leaves and rhizomes)	29,000	3,900	Present Study
Macroalgae	4,500	300	Present Study
Leaf litter ("seagrass wrack")	1,600	120	Present Study
Water column	37,000	6,300	LIA (unpublished data)
Sediment (top 0-10 cm)	13×10^6	3.5×10^6	After Yassini, 1994
Material removed annually during Foreshore Cleanups (2000-01)			
Sediment	2,900	400	Present Study
Seagrass wrack and/or macroalgal biomass	3,000	200	Present Study

The nutrient pools contained in leaf litter (referring to dead seagrass leaves) were calculated in the same way as macroalgae, using biomass data described in Section 5.2. The foreshore cleanups conducted by the Lake Illawarra Authority (2000 - 2001) also removed a significant proportion of the nitrogen and phosphorus pools contained within macroalgal biomass and

seagrass “wracks” (Table 5-25). Sediment typically comprised 70 - 80 % (dry weight) of the material removed during foreshore clean ups (see Section 5.3.10), but the sediment-bound nitrogen and phosphorus removed by this process is unlikely to impact the Lake’s sediment nutrient budget.

While the information presented in Table 5-25 gives an indication of the pools of nitrogen and phosphorus associated with macrophyte biomass during the present study, these nutrient pools in Lake Illawarra may vary considerably between years. In years with low macroalgal biomass and, therefore, low uptake of nutrients by macroalgae, there may be an excess of dissolved inorganic N and P available for phytoplankton growth. But in years with high macroalgal biomass, macroalgae may act as an important sink for nutrients in Lake Illawarra, as N and P bound to decomposing macroalgal biomass must first be remineralised before becoming available to phytoplankton (see, e.g., Martins *et al.*, 2007). In the present study, the seagrasses, *Zostera capricorni* and *Ruppia megacarpa*, were considered to be a more important sink for nutrients than macroalgae in Lake Illawarra.

5.4.7 Conclusion

The biomass of macroalgae in Lake Illawarra appeared much lower in 2000 - 03 than in previous decades. These lower algal biomasses may be due to drought, as well as improvements in Lake Illawarra management practices and, therefore, water quality. The maximum biomasses of macroalgae recorded in the present study were at the Windang sites, Oasis Caravan Park and Primbee Bay, with 150 and 370 g DW m^{-2}, respectively. Blooms of macroalgae were composed primarily of filamentous *Chaetomorpha* (identified as *C. linum* and *C. billardierii*). Chapter 6 examines the effect of nutrient enrichment and deprivation on the growth of the nuisance green alga, *Chaetomorpha linum*. Nutrient analyses showed high concentrations of N stored in the tissue of both seagrasses and macroalgae, which implied storage of N that exceeded immediate growth requirements. The concentrations of P in macroalgae and *Ruppia* were generally low, with respect to N, suggesting limitation by phosphorus. However, as previous studies have concluded that Lake Illawarra is strongly nitrogen limited, it appears unlikely that these low P concentrations would significantly limit the growth or biomass production of macrophytes in Lake Illawarra.

CHAPTER 6 Influence of nitrogen, phosphorus and temperature on growth of *Chaetomorpha linum*

6.1 Introduction

To achieve sustainable management of macroalgal issues in shallow coastal lagoons it is important to understand and define the key mechanisms controlling the growth and production of macroalgae. However, the nature of algal growth and distribution is inherently complex and may be influenced by a range of environmental factors, such as temperature and light (Morgan and Simpson, 1981a, 1981b; Duke *et al.*, 1989; O'Connor and West, 1991; Fong and Zedler, 1993; Matta and Chapman, 1995; O'Donohue and Dennison, 1997; Lee *et al.*, 1999), salinity regimes (Zablackis, 1987; Fong *et al.*, 1996; Imai *et al.*, 1997; Martins *et al.*, 1999; Wong and Chang, 2000; Kamer and Fong, 2000, 2001; Taylor *et al.*, 2001; Henley *et al.*, 2002), water movement and rate of exchange (Wheeler, 1982; Smit, 2002), plant density (Israel *et al.*, 1995), and competition with co-existing species and epiphytes (Friedlander *et al.*, 1991). It is also well established that the availability of nutrients, such as nitrogen and phosphorus, is one of the most significant factors regulating the growth and reproduction of macroalgae (e.g., Birch *et al.*, 1981; DeBoer, 1981; Hanisak, 1983; Owens and Stewart, 1983; Lundberg *et al.*, 1989; Björnsäter, and Wheeler, 1990; Valiela *et al.*, 1997; Larned, 1998; Bergamasco and Zago, 1999; Bowen, and Valiela, 2001).

Of particular importance is the relationship between macroalgal growth and the nutrient(s) limiting growth and productivity of the alga. A nutrient is considered to be limiting when the addition of that nutrient increases growth or photosynthesis (Fong *et al.*, 2003). As nitrogen often limits macroalgal growth and productivity in estuaries (Lotze and Schramm, 2000; Menéndez, *et al.*, 2002b; Fong *et al.*, 2003), increases in nitrogen loading will often result in increased macroalgal biomass, particularly blooms of opportunistic green algae such as *Ulva spp.* and *Chaetomorpha* spp.. These opportunistic green macroalgae may be a useful indicator for assessing and monitoring nutrient enrichment as they have fast nutrient uptake and growth rates and high internal nutrient storage capacities (Thomas and Harrison, 1987; Fong *et al.*, 1994; Viaroli *et al.*, 1996).

Fong *et al.* (2003) discussed three different methods typically employed to determine whether N or P limits the growth and productivity of macroalgae. First, N:P ratios in the water column have been used to determine nutrient availability and compared to the nutritional requirements of macroalgae (e.g., Wheeler and Björnsäter, 1992) and phytoplankton (reviewed by Hecky and Kilham, 1988) to estimate limitation. However, the nutritional requirements, including the optimal N:P ratio, of algae can vary within and between species and study sites (Rhee, 1978; Atkinson and Smith, 1983; Sterner and Grover, 1998; Fong *et al.*, 2003). For example, in the

same nutritional environment, growth and/or biomass formation of one species may be limited by N, while another species could be limited by P (Rhee, 1978; Fong *et al.*, 1994). It is difficult to draw conclusions about the amount of nutrients required for macroalgal growth as the form of N or P present, as well as the concentration in the water column, affects the growth of macroalgae (Waite and Mitchell, 1972) Also, water column nutrients vary over time, thus individual measurements may not provide an accurate determination of nutrient availability, especially in areas such as estuaries, which are subject to pulsed or fluctuating nutrient loads (Fong *et al.*, 1993, 2003).

Second, tissue nutrient concentrations and the N:P ratios in macroalgae have been used to determine nutrient limitation of biomass production (Wheeler and Björnsäter, 1992; Lyngby and Mortensen, 1994). For example, tissue N and P contents of *Ulva lactuca* provided a good indicator of the availability of nutrients over time, and were thus considered more suitable than water column data for long-term monitoring (Lyngby *et al.*, 1999). However, this approach can also be problematic as different species and functional forms of macroalgae may have different nutrient uptake and storage capacities and different seasonal patterns of growth (Rosenburg and Ramus, 1982; Wallentinus, 1984; Fong *et al.*, 2003). For example, if there are high concentrations of both nitrogen and phosphorus in the water column, but an alga has a greater uptake and storage capacity for N than P, the subsequent elevated tissue N:P ratio would indicate limitation by P when it was not occurring (Fong *et al.*, 2003). In addition, different measures of nutrient limitation can provide conflicting results; for example, in the same study of *Ulva rigida*, tissue N:P ratios (61:1) and low water column P concentrations (< 1 μmol P L^{-1}) suggested P limitation, whereas critical tissue N concentrations (< 2.0 % N) indicated N limitation (Sfriso, 1995).

Third, laboratory and *in situ* nutrient enrichment experiments have been used to determine limitation by examining the response of the alga through variables such as growth, reproduction, photosynthesis, or changes in tissue or water column nutrient concentrations (e.g., Steffensen, 1976; Gordon *et al.*, 1981; Brophy and Murray, 1989; Friedlander, *et al.*, 1991; Larned, 1998; Taylor *et al.*, 2001; Fong *et al.*, 2003). Controlled factorial experiments can be a more accurate method of predicting limitation than the aforementioned experimental approaches (Larned, 1998), and can provide direct, rather than indirect, evidence of nutrient limitation (Fong *et al.*, 2003). However, caution must be exercised when applying results obtained from single- or multi-factorial culture experiments to field conditions. It is difficult to draw conclusions of nutrient limited growth from culture experiments without taking into account the complex interaction with chemical and physical global environmental processes and those of the study site, such as nutrient fluxes, light, temperature, tidal exchange, regeneration processes at the sediment-water interface, competition with grazers, or plant density (Sfriso, 1995).

In the current investigation, the growth of a common species of macroalgae, *Chaetomorpha linum*, was examined in the laboratory under a range of temperatures and concentrations of nitrogen and phosphorus. Only one species of algae from Lake Illawarra was chosen for use in the culture experiments; although it would have been preferable to examine the growth of other algal species from Lake Illawarra, prevailing drought conditions meant that no other species of macroalgae could be found in sufficient quantities required for on-going experimentation. Attempts were also made to culture *Ulva* spp. collected from Lake Illawarra, but these algae did not survive the initial pre-conditioning period of laboratory culture. *C. linum*, however, is a hardy, resilient species, was relatively easy to locate in Lake Illawarra throughout the year, and was able to tolerate laboratory conditions for several months. *C. linum* has long-been cited as the most problematic, bloom-forming macroalgal species in Lake Illawarra, but little research has been conducted on the ecophysiology of this alga in NSW. This alga has fast nutrient uptake rates and a high internal storage capacity, and thus, it may be more competitive than other macroalgae as it can benefit from short-term or pulsed nutrient fluxes, such as rainfall or run-off events, by taking up excessive nutrients very quickly and storing them for later use (Lavery & McComb, 1991b). The growth of *C. linum* in these culture experiments was determined as a measurable change in biomass over time, which was used to calculate the relative growth rate (see Section 3.3 for the methodology relating to these experiments).

The primary objectives of this section of the study were:

- to determine the growth response of *Chaetomorpha linum* to ecologically relevant temperatures in Lake Illawarra;
- to examine the response of *C. linum* to varying concentrations of nutrients, and to determine whether nitrogen and/or phosphorus limits the growth of *C. linum*; and,
- to determine how these responses relate to ecological conditions in Lake Illawarra.

6.2 Experimental Results

Chaetomorpha linum cultured with a range of nutrient treatments and at temperatures of 10 - 30°C showed a significant increase in biomass after 14 days. Growth, which occurred as a measurable increase in filament length and biomass, was recorded in every treatment of every experiment conducted. There were no visible occurrences of epiphytic growth in any of the experiments. The results of each experiment are explained in further detail in the following sections.

6.2.1 Pilot Study (initial biomass comparison)

Two *C. linum* treatments of 1 g L^{-1} IM (initial mass) and 2 g L^{-1} IM were compared under non-limiting concentrations of N and P, supplied for the first 21 days, followed by a further 28 days

of growth in unenriched seawater. The length of the experiment was designed to assess the long term viability of the alga. Figure 6-1 shows that algal biomass in both treatments increased by up to 20 % WW d^{-1} immediately after enrichment, and the algae continued to grow at a decreasing rate for several weeks after enrichment was ceased. For both treatments, the relative growth rates for the first two weeks were 2 - 3 times higher than growth rates in the third week and 6 - 9 times higher than growth rates for the unenriched phase (Table 6-1); thus relative growth rates were significantly lower during the unenriched phase (RM ANOVA: $F = 1326$, $p < 10^{-6}$; Tukey's $p < 0.05$).

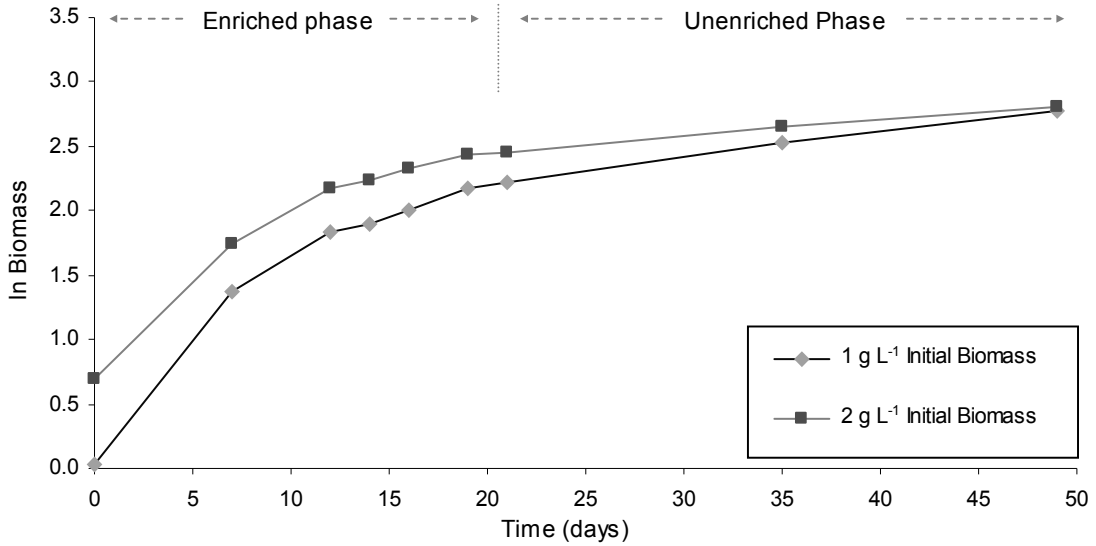


Figure 6-1: Pilot study comparing *Chaetomorpha linum* initial biomasses, conducted on a sunny windowsill for 7 weeks (mid-summer), with 30 μmol P L^{-1} and 70 μmol NH_4NO_3 L^{-1} added every 3 days for the first 3 weeks. Results are presented as the natural log of biomass (g WW L^{-1}) versus time.

While the biomass for the 2 g L^{-1} SM exceeded the 1 g L^{-1} SM throughout the experiment (Figure 6-1), the 1 g L^{-1} SM treatments had significantly higher relative growth rates (Table 6-1). Thus, the biomass of the 1 g L^{-1} SM approached that of the 2 g L^{-1} SM during the final weeks of the experiment, as growth rates were significantly (up to 40 %) slower in the treatments with higher initial biomass. These results show that the relative growth rates of *Chaetomorpha linum* were independent of the initial biomass used, especially during the exponential growth phase (i.e., the enriched phase). As the same nutrient concentrations were used for both treatments, nutrients would have been depleted faster in the 2 g L^{-1} SM treatments, thus lower growth rates in the treatments with higher biomass suggests that further formation of biomass was limited by available nutrients and/or restricted by space within the culture jars. It was concluded that an initial *C. linum* biomass of 1 g L^{-1} would be appropriate to use in future experiments.

Table 6-1: Results of ANOVA tests comparing relative growth rates of *Chaetomorpha linum* during nutrient enrichment and the succeeding unenriched phase, using initial biomasses (IM) of 1 g L^{-1} or 2 g L^{-1}.

H_0 = Initial biomass (1 or 2 g L^{-1}) has no effect on relative growth rates (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
Initial Biomass	29.82	0.000601*	• 1 g SM > 2 g SM
Time	602.99	0.000826*	• Week 1 > Week 2 > Week 3 > Unenriched Phase
Initial Mass x Time	7.81	0.000826*	• Significant interactions: differences in RGR between 'IM' treatments vary over time (see below)

H_0 = Initial biomass (IM) (1 or 2 g L^{-1}) has no effect on relative growth rates (one-way ANOVA conducted on each week of treatment)						
Treatment Phase	**Time (days)**	**RGR (% WW d^{-1}) (mean ± s.e.)**		**F-ratio**	**Probability**	**Tukey-Kramer ($p < 0.05$)**
		1 g L^{-1} IM	**2 g L^{-1} IM**			
Enriched	0 - 7	18.98 ± 0.99	15.08 ± 0.31	14.08	0.005602*	1 g IM > 2 g IM
	7 - 14	7.52 ± 0.16	6.92 ± 0.15	7.64	0.024491*	1 g IM > 2 g IM
	14 - 21	4.76 ± 0.30	3.37 ± 0.24	14.02	0.005671*	1 g IM > 2 g IM
Unenriched	21 - 49	2.01 ± 0.09	1.28 ± 0.09	31.82	0.000486*	1 g IM > 2 g IM

* term significant at alpha = 0.05

The primary objectives of the pilot studies were to determine whether *C. linum* could be cultured successfully under controlled laboratory conditions, and to establish the methodology to be used in later experiments. The high growth rates (up to 20 % WW d^{-1}) obtained during the first week of treatment indicated that *C. linum* originating from Lake Illawarra could be cultured successfully in the laboratory. *C. linum* continued to grow at an average rate of 2 % WW d^{-1} during the unenriched phase of treatment, indicating the algae could utilise stored nutrients for at least one month after saturation. Gordon *et al.* (1981) obtained similar results when culturing *Cladophora* sp. in a high nutrient medium for 7 days, after which the algae were returned to either P-free or N-free medium. Algae grown in P-free media (but with high N levels) showed minimal growth after 50 days, whereas algae grown in N-free media (but with high P) showed little growth after 40 days with no further N additions. *Cladophora* grown in high nutrient medium for 3 weeks then returned to P-free medium continued to show significant growth for 4 weeks with no additional P. The pilot study demonstrated that under non-limiting conditions (i.e., with sufficient nutrients, light and temperature), growth of *C. linum* may continue for many months.

6.2.2 Pilot Study (N-source)

In the second windowsill experiment comparing growth of *C. linum* with an N-source of either nitrate-N or ammonium-N, growth rates throughout the four-week study did not differ greatly between treatments (Figure 6-2A). Growth rates were typically high during the first week of culture (7 - 8 % WW d^{-1}), but slowed to a steady rate of about 2 - 3 % WW d^{-1} during the final weeks of the experiment (Table 6-2; Figure 6-2B). Analysis with repeated measures ANOVA showed that relative growth rates for the NO_3^--N treatment were 3 - 4 times higher in the first

week than in the following weeks (RM ANOVA: F = 94, $p < 10^{-6}$; Tukey's $p < 0.05$). Likewise, for the NH_4^+-N treatment, growth rates were 2 - 3.6 times higher in Week 1 than Weeks 2 - 4 (RM ANOVA: F = 67, $p < 10^{-6}$; Tukey's $p < 0.05$). In the first week of treatment, growth rates with NO_3^--N were slightly significantly higher than NH_4^+-N, whereas NH_4^+-N treatments had higher growth rates in the third week, but there were no significant differences between N-treatments in the second or fourth weeks (Table 6-2). Over 4 weeks of treatment combined, there were no significant differences in growth rates with nitrogen-N or ammonium-N ($p > 0.05$). It was subsequently concluded that *C. linum* could grow equally well with a nitrogen source of either NH_4^+-N or NO_3^--N. Further comparisons between the effect of nitrate-N and ammonium-N on *C. linum* growth are presented in Section 6.2.5.

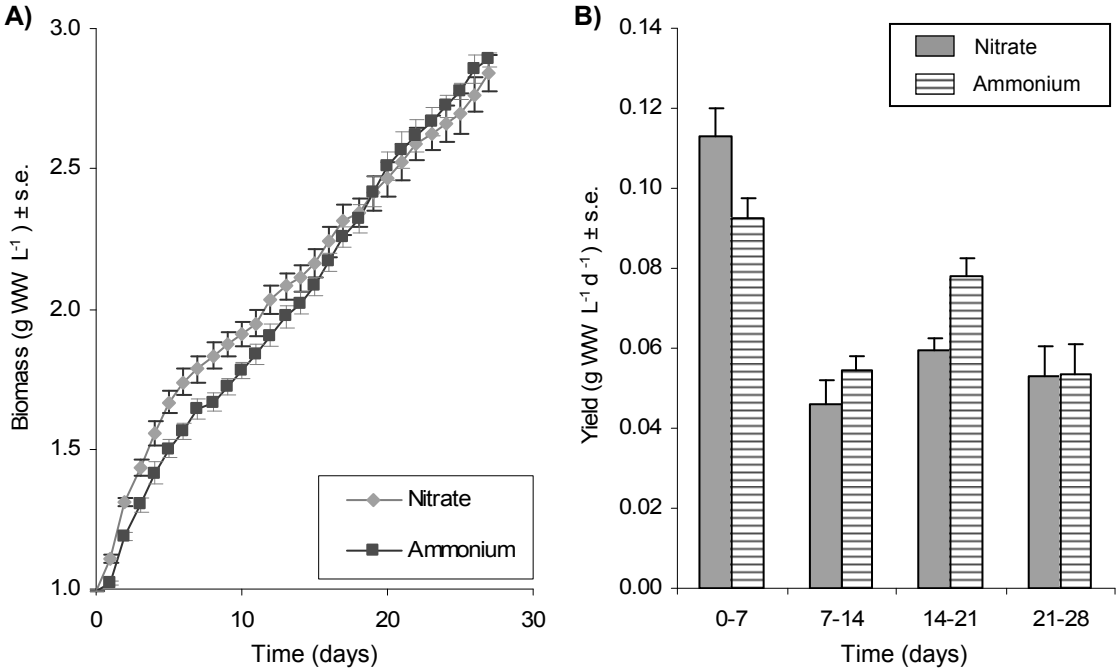


Figure 6-2: Preliminary nitrate-N versus ammonium-N windowsill experiment, using 30 μmol P L^{-1} d^{-1} and 100 μmol N L^{-1} d^{-1} as either NO_3^- or NH_4^+ for 4 weeks. Results are presented as biomass versus time (**A**) and yield versus time (**B**). Values are mean ± standard error.

C. linum growth rates in the second windowsill experiment were markedly slower than in the first pilot study. These lower growth rates of up to 9 % WW d^{-1} may have been due to the windowsill not receiving as much sunlight as earlier in summer, as well as cooler daily temperatures; the average water temperature for this experiment was 23°C, compared to 28°C for the first pilot study. The lowest growth rates were recorded in Week 2 of the experiment, which had cooler daily temperatures and significantly more cloud cover. Overnight declines in temperature would also have affected daily growth rates, as more time was required to heat the water during the day.

Table 6-2: Summary of ANOVA tests for the hypothesis of no significant differences in relative growth rates between nitrate-N or ammonium-N treatments.

H_0 = the form of nitrogen (nitrate or ammonium) does not have a significant effect on RGR (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
N-Treatment	0.49	0.505496	• No significant differences between RGR in nitrate-N or ammonium-N treatments
Time	159.36	0.000000*	• Week 1 > Weeks 2, 3 and 4 • Week 3 > Week 4
Treatment x Time	4.86	0.008823*	• Significant interactions: RGRs in different nitrogen treatments vary between weeks (see below)

H_0 = no significant differences in growth rates between nitrate-N and ammonium-N treatments (one-way ANOVA conducted on each week of treatment)					
Time	**RGR (% WW d^{-1})**		**F-ratio**	**Probability**	**Tukey-Kramer ($p < 0.05$)**
	NO_3^--N	**NH_3^--N**			
Week 1	8.36 ± 0.38	7.15 ± 0.29	6.39	0.035365*	Nitrate > Ammonium
Week 2	2.38 ± 0.31	2.98 ± 0.18	2.86	0.129321	No significant differences
Week 3	2.57 ± 0.10	3.41 ± 0.14	23.03	0.001358*	Ammonium > Nitrate
Week 4	1.98 ± 0.28	1.99 ± 0.30	0	0.988565	No significant differences

* term significant at alpha = 0.05

6.2.3 Effect of Mixing

Mixing by the aquarium bubblers had a significant effect on *C. linum* growth rates, even in the unenriched control treatments (Figure 6-3; Table 6-3; Table 6-4).

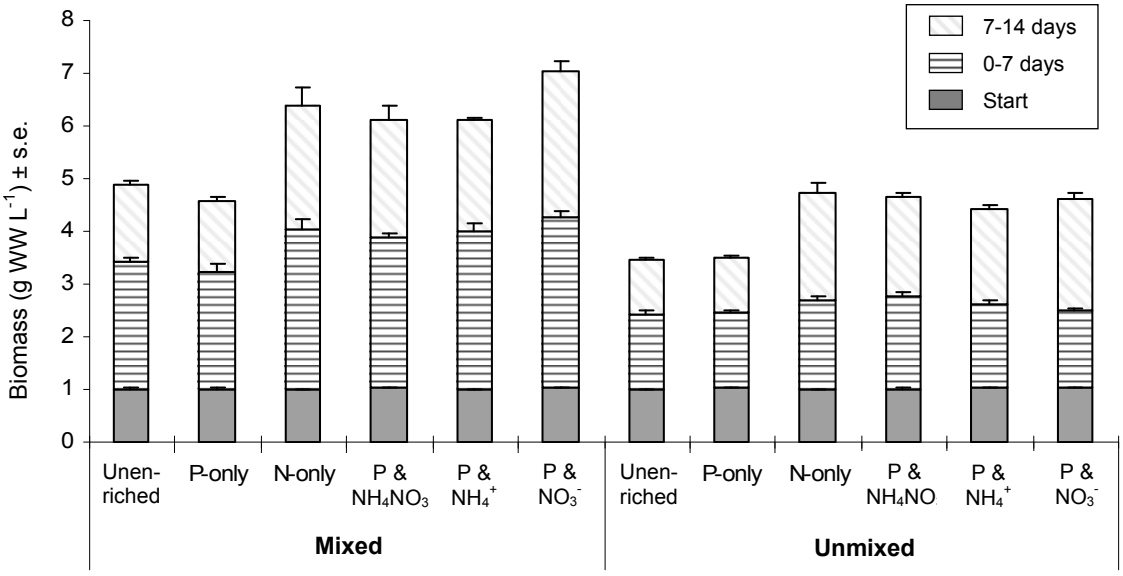


Figure 6-3: Biomass (wet weight) of *C. linum* (field collected) after 7 and 14 days of incubation at 15°C, with or without mixing by aquarium bubblers.

After 14 days of treatment, dry weight (DW) growth rates in mixed treatments were 20 - 35 % higher than unmixed treatments (RM ANOVA: F = 139, $p < 10^{-6}$). In the mixed treatments,

growth rates in the first week of treatment were up to 50 % higher than the second week (RM ANOVA: F = 44.2, p <10^{-4}). In the unmixed treatments, however, growth rates were significantly higher in the second week of treatment (RM ANOVA: F = 9.18, p < 0.05; Tukey's p < 0.05).

Table 6-3: Relative growth rates of *C. linum* after 7 and 14 days of incubation at 15°C, with or without mixing by aquarium bubblers (values are mean ± standard error).

		Relative Growth Rate (% DW d^{-1})		Dry Matter Content on Day 14 (%) [$]
	Treatment	**0-7 days**	**7-14 days**	
Mixed Beakers	Unenriched	15.2 ± 0.14	11.6 ± 0.45	11.4 ± 0.61
	P-only	15.8 ± 1.24	11.1 ± 0.45	10.4 ± 0.42
	NH_4NO_3-only	16.6 ± 0.81	15.8 ± 1.04	9.9 ± 0.42
	P & NH_4NO_3	16.9 ± 0.97	15.8 ± 0.43	10.6 ± 0.10
	P & NH_4^+	16.0 ± 0.68	15.0 ± 0.24	10.8 ± 0.14
	P & NO_3^-	17.4 ± 0.97	17.3 ± 0.42	10.2 ± 0.09
Unmixed Beakers	Unenriched	11.0 ± 0.30	9.9 ± 0.31	10.8 ± 0.83
	P-only	10.7 ± 1.01	9.8 ± 0.75	10.9 ± 0.61
	NH_4NO_3-only	10.7 ± 1.02	14.3 ± 0.72	9.8 ± 0.41
	P & NH_4NO_3	12.2 ± 0.50	14.0 ± 0.47	9.5 ± 0.30
	P & NH_4^+	10.6 ± 1.98	12.9 ± 0.79	9.8 ± 0.58
	P & NO_3^-	9.6 ± 1.01	13.6 ± 0.28	10.1 ± 0.37

[$] Initial dry matter content of *C. linum* on Day 0 = 13.6 ± 0.36 %.

Table 6-4: Summary of ANOVA testing for the hypothesis of no significant differences between relative growth rates (dry weight) after 14 days, using variable nutrient treatments in Mixed and Unmixed beakers.

H_0 = Mixing and nutrient enrichment do not have a significant effect on relative growth rates (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer (p < 0.05)**
Mixing	138.97	0.000000*	• Mixing significantly increases RGR
Treatment	10.73	0.000017*	• N-deprived treatments have lower RGRs than N-replete treatments
Mixing x Treatment	1.78	0.155953	• No significant interactions
Time	0.17	0.686324	• RGRs do not vary significantly between Week 1 and Week 2
Mixing x Time	26.29	0.000030*	• Significant interactions: RGRs of mixed treatments declined in Week 2, whereas unmixed treatments increased in Week 2
Treatment x Time	5.47	0.001699*	• Significant interactions: RGRs of N-deprived treatments decline more in Week 2 than N-replete treatments
Mixing x Treatment x Time	0.17	0.971430	• No significant interactions

H_0 = no significant differences in RGR (0-14 days) between nutrient treatments (one-way ANOVA)			
Experiment	**F-ratio**	**Probability**	**Tukey-Kramer (p < 0.05)**
Mixed	10.24	0.000527*	• P only, Unenriched < N-only, P & NH_4NO_3, P & NH_4^+, P & NO_3^-
Unmixed	3.39	0.038711*	• P only, Unenriched < P & NH_4NO_3 (only significant at Tukey's p < 0.10)

* term significant at alpha = 0.05

C. linum growth rates over 2 weeks were also significantly affected by the nutrient treatment used (RM ANOVA: F = 10.7, $p < 10^{-4}$). For the nitrogen-deficient treatments, growth rates in mixed beakers exceeded that of the unmixed beakers by 30 % (P-only) to 40 % (unenriched). Although growth rates in the unenriched treatments were slightly higher than the P-only treatments (Table 6-3), the difference was not significant for the mixed or unmixed group ($p > 0.05$; Table 6-4). For both mixed and unmixed beakers, growth rates in the nitrogen-replete treatments were up to 50 % higher than the nitrogen-deficient treatments, but there were no significant differences in growth rates between nitrogen-replete treatments (Table 6-4).

The effect of nutrient enrichment and deprivation on the dry matter contents of *C. linum* was also investigated to provide comparisons between field-collected data (Chapter 5) and provide a general assessment of the growth conditions in Lake Illawarra. During the 14 day experimental period, the dry matter content (DMC) decreased from an initial 13.6 ± 0.4 % to 9.2 - 11.7 % (RM ANOVA: F = 134, $p < 10^{-6}$; Table 6-5). DMC was typically higher in the nitrogen-deprived treatments than the nitrogen-replete treatments, and higher in the mixed than unmixed treatments, but these differences were not significant ($p > 0.05$).

Table 6-5: Summary of ANOVA testing for the hypothesis of no significant differences in dry matter contents after 14 days, using variable nutrient treatments in Mixed and Unmixed beakers.

H_0 = Mixing and nutrient enrichment do not have a significant effect on dry matter contents (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
Mixing	1.81	0.191517	• Mixing does not significantly affect DMC
Treatment	0.82	0.546491	• No significant differences in dry matter contents between nutrient treatments
Mixing x Treatment	1.21	0.332870	• No significant interactions
Time	133.58	0.000000*	• Week 1 > Week 2
Mixing x Time	0.02	0.876161	• No significant interactions
Treatment x Time	3.00	0.030425*	• Significant interactions: DMCs decline by varying degrees across nutrient treatments in Week 2
Mixing x Treatment x Time	0.72	0.617858	• No significant interactions

* term significant at alpha = 0.05

As expected, *C. linum* growth rates were significantly higher in treatments mixed by aquarium bubblers. This is because nutrient uptake is often greater when solutions are regularly mixed as water motion assists in moving ions to the plant surface (Lobban and Harrison, 1997). It is important to note that this experiment was conducted with *C. linum* that had been freshly collected from the field, so it is likely that the alga contained sufficient internal nutrient stores to sustain growth without the addition of external nutrients. Hence growth rates in the low nutrient treatments were far higher in this experiment than when the same experiment was repeated after the *C. linum* had undergone several weeks of pre-conditioning.

6.2.4 Effect of Phosphorus (Phosphate-P)

Experiments to determine the effect of phosphorus (as PO_4^{3-}) were conducted under conditions of optimal light, temperature and non-limiting concentrations of nitrogen (50 μmol L^{-1} d^{-1} of NH_4NO_3). The experiments were conducted at 15, 20 and 25°C. The first two attempts at the 20°C experiment (P1 and P2) were adversely affected by equipment malfunctions such as considerable temperature fluctuations and the light to day (L:D) photoperiod being approximately 16:8 hours (rather than the designated 12:12 hours), thus promoting excessive growth. Consequently the results presented in Figure 6-4 must be treated with some degree of caution and are included here to give some indication of the effect of light on *C. linum* growth. Statistical analyses showed that growth rates varied significantly between P experiments, and that that both P1 and P2 (L:D = 16:8 hours) were significantly higher than P3 (L:D = 12:12 hours; F = 311; $p < 10^{-6}$). Due to the incomplete and unreliable dataset for experiments P1 and P2, this data will not be discussed further.

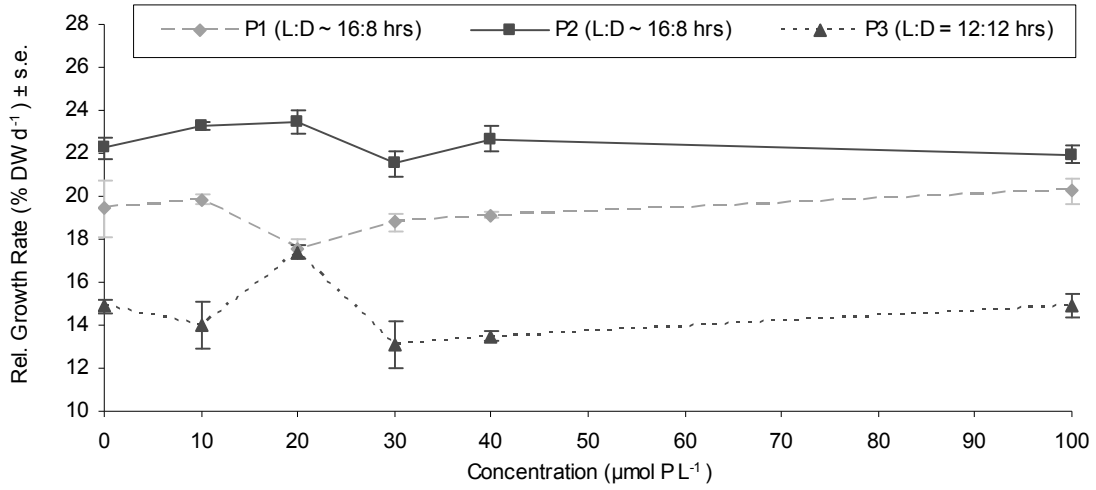


Figure 6-4: Relative growth rates (dry weight) of *C. linum* after 7 days of incubation at 20°C and a range of P concentrations with 100 μmol N L^{-1} d^{-1} (as NH_4NO_3).

Further experiments were conducted to evaluate the effect of PO_4^{3-}-P enrichment on *C. linum* growth over 14 days; both temperature and P enrichment had a significant effect on growth weights over the range investigated (Figure 6-5; Table 6-6; RM ANOVA: F = 35.0, $p < 10^{-6}$). Growth rates increased progressively from 15 to 25°C, with the highest growth rates of 25 % DW d^{-1} obtained at 25°C. In each experiment, biomass was the lowest in treatments without added P, such that all of the treatments with nutrients added exceeded the unenriched control treatment by at least 30 % (other than the 30 μM P treatment at 25°C). At 15 and 20°C, growth rates at 20 μM P were significantly higher than in treatments without added P (ANOVA: Tukey's $p < 0.05$; Table 6-7), and declined at concentrations above 20 μM P. Additionally, at 15°C, growth rates were significantly lower with 30 μM P than 10, 20 or 100 μM P (ANOVA: Tukey's $p < 0.05$). At 25°C, growth rates in the first week of treatment were up to 50 % higher

than the second week (RM ANOVA: F = 29.5, $p < 10^{-4}$). After 14 days at 25°C, growth rates peaked in the 10 μM P treatment (Figure 6-5), but were only significantly higher than the 30 μM P treatment, which resulted in significantly lower growth rates than all other P concentrations used (Table 6-7).

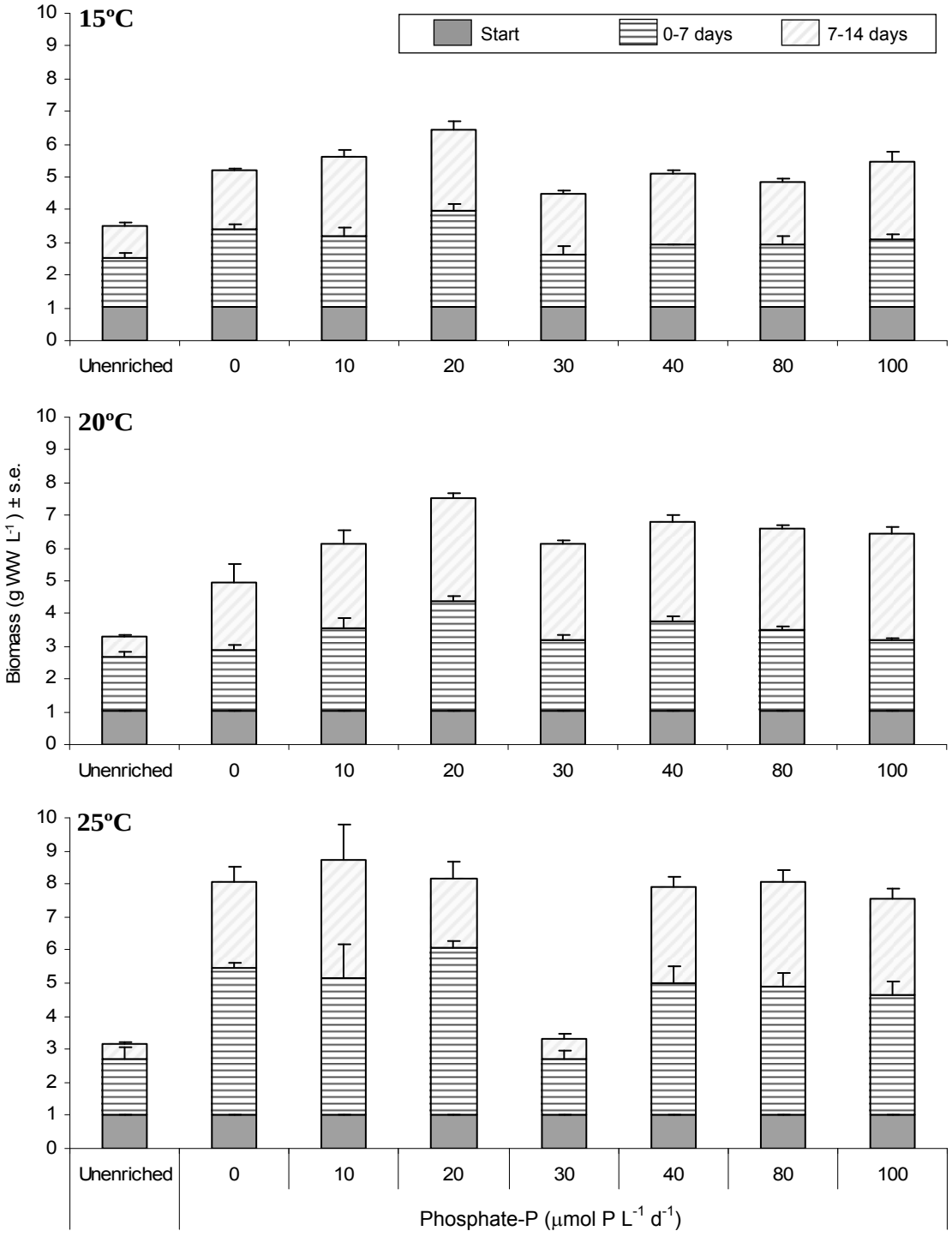


Figure 6-5: Biomass (wet weight) of *C. linum* after 7 and 14 days of incubation at 15, 20 and 25°C, with variable P and 100 μmol N L^{-1} d^{-1} (as NH_4NO_3, except for the unenriched treatments).

Table 6-6: Relative growth rates and dry matter content for *C. linum* after 14 days of incubation at 15, 20 and 25°C, and treatment with 100 μmol N L^{-1} d^{-1} (as NH_4NO_3) and 0 - 100 μmol L^{-1} d^{-1} of $P0_4^{3-}$-P (values are mean ± standard error).

Temp. (°C)	Treatment (μM P d^{-1})	Relative Growth Rate (% DW d^{-1})		Dry Matter Content on Day 14 (%) [$]
		0-7 days	7-14 days	
15°C	0	14.9 ± 0.54	15.3 ± 0.39	10.4 ± 0.27
	10	14.0 ± 1.10	17.6 ± 0.74	9.6 ± 0.19
	20	17.4 ± 0.73	19.6 ± 1.00	11.0 ± 0.74
	30	13.1 ± 1.19	14.0 ± 0.50	9.8 ± 0.20
	40	13.5 ± 0.10	16.1 ± 0.45	9.7 ± 0.27
	80	13.9 ± 1.30	14.7 ± 0.35	9.9 ± 0.14
	100	14.9 ± 0.76	16.2 ± 1.27	9.8 ± 0.20
	Unenriched	7.2 ± 0.72	8.1 ± 0.60	12.1 ± 0.11
20°C	0	13.8 ± 0.51	17.0 ± 1.88	11.7 ± 0.50
	10	16.9 ± 1.06	18.2 ± 2.00	10.7 ± 0.54
	20	19.9 ± 0.60	19.6 ± 0.38	9.4 ± 0.11
	30	16.1 ± 0.73	19.8 ± 0.42	10.8 ± 0.06
	40	17.1 ± 0.80	19.1 ± 0.66	9.8 ± 0.04
	80	15.8 ± 0.26	19.5 ± 0.13	9.5 ± 0.03
	100	14.6 ± 0.28	19.1 ± 0.52	9.5 ± 0.09
	Unenriched	17.4 ± 0.69	5.9 ± 0.20	13.8 ± 0.33
25°C	0	23.0 ± 0.72	19.6 ± 1.80	11.4 ± 0.19
	10	20.7 ± 2.97	20.9 ± 3.02	9.8 ± 0.18
	20	23.6 ± 0.66	16.1 ± 2.20	11.3 ± 0.05
	30	16.5 ± 0.65	8.1 ± 1.11	14.9 ± 0.26
	40	21.0 ± 1.69	19.0 ± 1.10	9.6 ± 0.16
	80	20.7 ± 1.23	20.3 ± 1.27	9.9 ± 0.06
	100	20.1 ± 1.29	19.1 ± 0.92	9.8 ± 0.09
	Unenriched	15.8 ± 1.73	6.2 ± 0.88	13.8 ± 0.51

[$] Initial dry matter content of *C. linum* on Day 0 = 11.1 ± 0.05 %

Repeated Measures ANOVA showed that enrichment with nitrogen and phosphorus combined had a significant effect on the dry matter contents of *Chaetomorpha linum* ($F = 49.3$, $p < 10^{-6}$; Table 6-7). At each temperature (15 - 25°C), dry matter contents (DMC) in the unenriched control treatment increased significantly after 14 days ($F = 32.3$, $p < 10^{-5}$, Tukey's $p < 0.05$), from an initial 11.1 % to 11.7 % on Day 14. In contrast, dry matter contents in the high P treatments (20 - 100 μM) declined significantly to about 9.5 - 9.8 % after 14 days (Tukey's $p < 0.05$). At 25°C, the lowest growth rates were observed in the 30 μM treatment, which may have been due to contamination of the solution used to prepare that treatment. Additionally, dry matter contents of *C. linum* in the 30 μM treatment exhibited the most significant increase, from an initial 11.1 % to 14.9 % on Day 14 ($F = 72.9$, $p < 10^{-6}$, Tukey's $p < 0.05$). It was generally concluded from these experiments that the dry matter contents of *C. linum* increased significantly when grown under stressful conditions. Dry matter contents of *C. linum* in the treatments with N added alone (i.e., 0 μM P), however, did not change significantly over the 14 day experimental period (Tukey's $p < 0.05$); this suggests *C. linum* undergoing P deprivation were not stressed (or stressed to a lesser degree) as long as sufficient N was present to support growth.

Table 6-7: Results of ANOVA tests for the hypothesis of no significant differences between relative growth rates or dry matter contents after 14 days, using variable concentrations of P (μmol P L^{-1} d^{-1}), at 15, 20 and 25°C.

H_0 = Temperature and phosphorus enrichment do not have a significant effect on relative growth rates (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
Temperature	42.44	0.000000*	• RGRs at 25°C > 20°C > 15°C
P-Treatment	35.02	0.000000*	• P-enrichment has a significant effect on RGR
Temp x Treatment	7.43	0.000000*	• Significant interactions: response of *C. linum* to P enrichment is not consistent across temperatures (see below)
Time	1.28	0.264157	• No significant differences between Weeks 1 and 2
Temp x Time	22.50	0.000000*	• Significant interactions: at 15-20°C, RGRs in Week 2 > Week 1, but at 25°C, RGRs in Week 1 > Week 2
Treatment x Time	6.15	0.000037*	• Significant interactions: RGRs in low-P treatments decline more than high P-treatments in Week 2
Temp x Treatment x Time	1.33	0.223031	• No significant interactions

H_0 = no significant differences in relative growth rates (DW, 0-14 days) between phosphorus concentrations (μmol P L^{-1} d^{-1}) (one-way ANOVA conducted at each temperature)			
Experiment	**F-ratio**	**Probability**	**Tukey-Kramer ($p < 0.05$)** [#]
P at 15°C	16.58	0.000003*	• 80, 40, 0, 100, 10, 20 > C • 20 > C, 30, 80, 40, 0, 100, 10
P at 20°C	11.08	0.000043*	• 0, 100, 10, 80, 30, 40, 20 > C; 20 > 0
P at 25°C	18.97	0.000001*	• 100, 20, 0, 40, 80, 10 > C, 30

H_0 = Temperature and phosphorus enrichment do not have a significant effect on dry matter contents (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
Temperature	23.40	0.000000*	• DMC at 25°C > 20°C > 15°C
P-Treatment	49.28	0.000000*	• Algae grown in low-P treatments have higher DMCs than those in high-P treatments
Temp x Treatment	13.34	0.000000*	• Significant interactions: variations in DMCs are not consistent across temperatures
Time	32.28	0.000001*	• Final DMC (day 14) < Initial DMC (day 0)
Temp x Time	19.18	0.000001*	• Significant interactions: dry matter contents in Week 2 increase with increasing temperature
Treatment x Time	43.35	0.000000*	• Significant interactions: DMCs in low-P treatments increase after 14 days, but DMCs in high-P treatments decrease after 14 days
Temp x Treatment x Time	9.00	0.000000*	• Significant interactions

H_0 = P-enrichment (μmol P L^{-1} d^{-1}) does not have a significant effect on dry matter contents (day 14) (one-way ANOVA conducted at each temperature)			
Experiment	**F-ratio**	**Probability**	**Tukey-Kramer ($p < 0.05$)** [#]
P at 15°C	7.03	0.000630*	• C > 0, 10, 30, 40, 80, 100
P at 20°C	26.66	0.000000*	• C > 0, 10, 30, 40, 80, 100 • 0 > 20, 40, 80, 100
P at 25°C	72.90	0.000000*	• 30 & C > 0, 10, 20, 40, 80, 100 • 0 & 20 > 10, 40, 80, 100

* term significant at alpha = 0.05; [#] C = unenriched control treatment

6.2.5 Effect of Nitrogen (Nitrate-N and Ammonium-N)

The growth of *C. linum* was significantly affected by the addition of nitrogen, as NO_3^--N or NH_4^+-N, to the culture media (RM ANOVA: F = 48.7, $p < 10^{-6}$), but not by the source of nitrogen used (RM ANOVA: F = 0.62, $p > 0.05$; Figure 6-6; Table 6-8; Table 6-9).

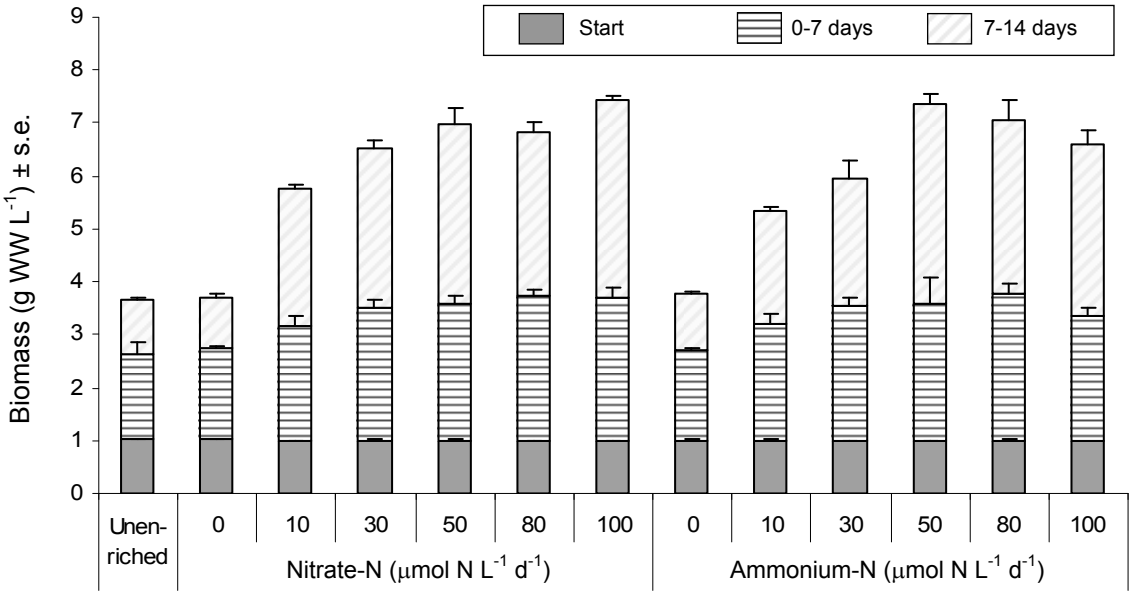


Figure 6-6: Biomass (wet weight) of *C. linum* after 7 and 14 days of incubation at 15°C, with 20 μmol P L^{-1} d^{-1} and nitrogen as NO_3^--N or NH_4^+-N (except for the unenriched treatment).

Table 6-8: Relative growth rates of *C. linum* during 2 weeks incubation at 15°C and treatment with either nitrate-N or ammonium-N. All treatments, other than the unenriched treatment, received 20 μmol P L^{-1} d^{-1} (values are mean ± standard error).

Treatment		Relative Growth Rate (% DW d^{-1})		Dry Matter Content on Day 14 (%) [$]
		0-7 days	7-14 days	
Nitrate-N (μM N d^{-1})	0	14.3 ± 0.21	9.5 ± 0.45	11.6 ± 0.07
	10	16.4 ± 0.80	18.1 ± 0.28	9.4 ± 0.07
	30	17.8 ± 0.63	19.7 ± 0.52	8.8 ± 0.20
	50	18.2 ± 0.54	20.9 ± 0.96	8.8 ± 0.19
	80	18.8 ± 0.46	20.0 ± 0.58	9.0 ± 0.11
	100	18.6 ± 0.70	22.0 ± 0.28	8.8 ± 0.08
Ammonium-N (μM N d^{-1})	0	14.0 ± 0.29	10.4 ± 0.18	11.4 ± 0.11
	10	16.5 ± 0.84	16.2 ± 0.42	10.1 ± 0.10
	30	18.0 ± 0.63	17.2 ± 1.46	9.4 ± 0.06
	50	17.9 ± 1.92	22.3 ± 0.53	9.1 ± 0.11
	80	18.8 ± 0.68	20.6 ± 1.28	9.0 ± 0.04
	100	17.1 ± 0.62	20.5 ± 0.90	8.9 ± 0.11
Unenriched	0	13.6 ± 1.08	10.0 ± 0.33	10.6 ± 0.13

[$] Initial dry matter content of *C. linum* on Day 0 = 10.2 ± 0.06 %

Growth rates were typically high across all treatments during the first week of incubation, ranging from about 14 % DW d^{-1} in the N-deficient treatments and 15 - 21 % DW d^{-1} in the treatments with nitrogen (Table 6-8; Table 6-9). During the second week of treatment, growth rates in the N-deficient treatments decreased by about 35 % (RM ANOVA: F = 13.3, $p < 0.005$; Tukey's $p < 0.05$), whereas growth rates in the 10 - 30 μM N treatments were similar to growth in the first week. Growth rates in the 50 - 100 μM N treatments were significantly higher during the second week of treatment (RM ANOVA: F = 30.3, $p < 10^{-3}$; Tukey's $p < 0.05$; Table 6-9).

Table 6-9: Results of ANOVA tests for the hypothesis of no significant differences between relative growth rates (dry weight) or dry matter contents after 14 days, using variable concentrations of nitrate-N or ammonium-N (μmol N L^{-1} d^{-1}).

H_0 = Nitrogen enrichment and the form of N used do not have a significant effect on relative growth rates (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
N-species (NO_3^- or NH_4^+)	0.62	0.437178	• No significant differences in RGRs between nitrate-N and ammonium-N treatments
N Concentration	48.68	0.000000*	• 100, 80, 50, 30, 10 > 0 μM N d^{-1} • 50, 80, 100 > 10 μM N d^{-1}
Species x Concentration	1.39	0.262248	• No significant interactions
Time	13.31	0.001273*	• RGRs in Week 1 > Week 2
N -species x Time	0.06	0.809868	• No significant interactions
Concentration x Time	6.56	0.000562*	• Significant interactions: RGRs in N-deprived treatments declined more in Week 2 than high-N treatments
N-species x Conc x Time	0.77	0.578002	• No significant interactions

H_0 = Nitrogen enrichment and the form of N used do not have a significant effect on dry matter contents (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Outcomes (Tukey-Kramer ($p < 0.05$)**
N-species (NO_3^- or NH_4^+)	3.77	0.064047	• No significant differences in DMCs between nitrate-N and ammonium-N treatments
N Concentration	76.32	0.000000*	• Low-N treatments have higher dry matter contents than high-N treatments: • 0 > 10, 30, 50, 80, 100 μM N d^{-1} • 10 > 30, 50, 100 μM N d^{-1} • 80 > 30, 50 μM N d^{-1}
Species x Concentration	1.46	0.239809	• No significant interactions
Time	80.55	0.000000*	• Final DMC (day 14) > Initial DMC (day 0)
N -species x Time	2.29	0.143155	• No significant interactions
Concentration x Time	21.27	0.000000*	• Significant interactions: DMC of low-N treatments increased significantly after 14 days, but DMC of high-N treatments declined significantly after 14 days
N-species x Conc x Time	0.89	0.504928	• No significant interactions

* term significant at alpha = 0.05;

Amongst the nitrogen-deficient treatments, there were no significant differences between growth rates in the unenriched treatment or the P-only treatments ($p > 0.05$). All of the algae that received even small additions of nitrogen (≥ 10 μmol L^{-1} d^{-1}) had growth rates up to 25 %

higher than the algae grown without added nitrogen (RM ANOVA: F = 48.7, $p < 10^{-6}$, Tukey's $p < 0.05$). For the ammonium-N treatments, biomass increased steadily with increasing nitrogen up to 50 μM NH_4^+-N, after which growth declined slightly (Figure 6-6). Similarly, biomass also increased with increasing NO_3^--N up to 100 μM NO_3^--N, but growth rates did not appear to be saturated at the highest NO_3^--N concentration used in this experiment (Figure 6-6). Statistical analysis, however, showed that *C. linum* growth rates were not significantly different when nitrogen was added at 30, 50, 80 or 100 μM NO_3^--N or 50, 80 or 100 μM NH_4^+-N (Tukey's $p > 0.05$;).

As the growth of *C. linum* did not appear to be saturated at the highest nitrate concentration of 100 μM used in the above experiment, the algae was cultured again at 25°C with variable nitrate concentrations, up to 1000 μmol L^{-1} d^{-1} (Figure 6-7; Table 6-10). At 25°C, *C. linum* was significantly affected by nitrate concentration (RM ANOVA: F = 13.8, $p < 10^{-4}$), with growth increasing up to 22 % DW d^{-1} with concentrations of 100 μM. Growth rates in the unenriched and low nitrate treatments (0 - 5 μM) were significantly lower than growth rates at the higher nitrate concentrations (50 - 1000 μM) (RM ANOVA: Tukey's $p < 0.05$), but there were no significant differences between growth rates at these higher concentrations (Tukey's $p > 0.05$; Table 6-11). Additionally, growth rates in each treatment (except for the 1000 μM treatment) declining significantly during the second week of treatment (RM ANOVA: F = 36.7, $p < 10^{-4}$).

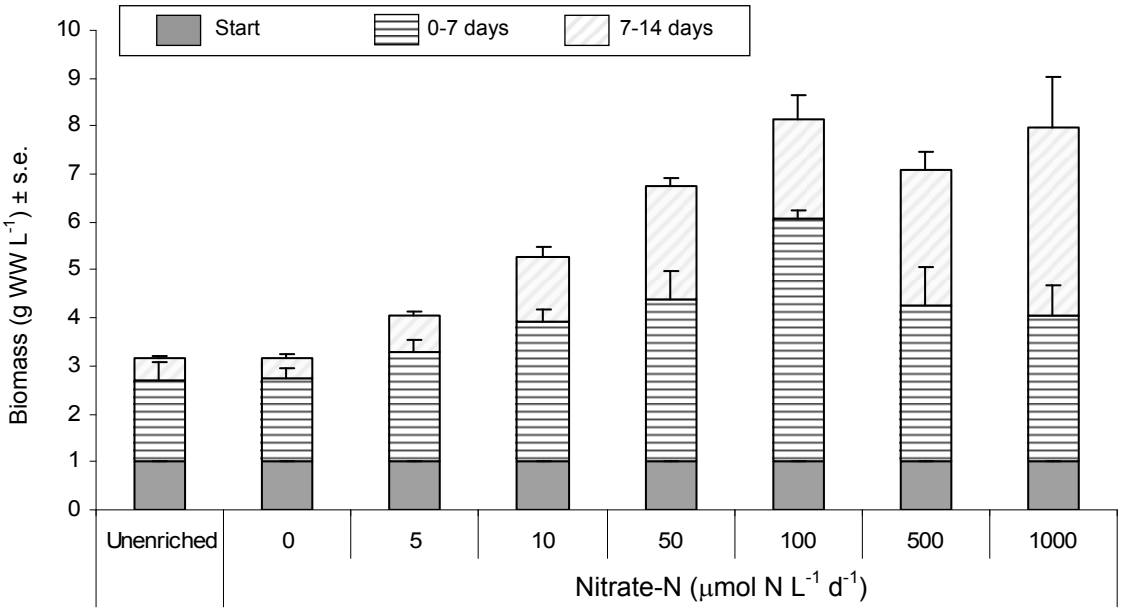


Figure 6-7: Biomass (wet weight) of *C. linum* after 7 and 14 days of incubation at 25°C, with variable nitrate-N and 20 μmol P L^{-1} d^{-1} (except for the unenriched treatment) (values are mean ± standard error).

Table 6-10: Relative growth rates of *C. linum* during 2 weeks incubation at 25°C and treatment with nitrate-N. All treatments, other than the unenriched treatment, received 20 μmol P L^{-1} d^{-1} (values are mean ± standard error).

Nitrate (μM N d^{-1})	Relative Growth Rate (% DW d^{-1})		Dry Matter Content on Day 14 (%)[$]
	0-7 days	7-14 days	
0	16.2 ± 1.09	6.9 ± 0.64	14.3 ± 0.18
5	17.6 ± 1.49	9.1 ± 0.85	13.0 ± 0.14
10	19.6 ± 1.16	12.8 ± 0.86	11.8 ± 0.57
50	20.0 ± 1.91	16.7 ± 0.59	10.1 ± 0.04
100	23.6 ± 0.66	16.1 ± 2.20	10.0 ± 0.05
500	18.7 ± 2.30	19.7 ± 1.78	10.5 ± 0.28
1000	18.5 ± 1.93	22.6 ± 2.61	10.4 ± 0.12
Unenriched	15.8 ± 1.73	6.2 ± 0.88	13.8 ± 0.51

[$] Initial dry matter content of *C. linum* on Day 0 = 11.1 ± 0.06 %

Table 6-11: Results of Repeated Measures ANOVA tests for the hypothesis of no significant differences between relative growth rates (dry weight) or dry matter contents of *C. linum* after 14 days, using variable concentrations of nitrate-N (0 - 1000 μmol N L^{-1} d^{-1})

RM ANOVA	Factor	F-Ratio	Probability	Tukey-Kramer ($p < 0.05$)[#]
H_0 = enrichment with nitrate-N does not have a significant effect on relative growth rates after 14 days	Treatment	13.81	0.000011*	• 10 > C; • 50 > C, 0; • 100, 500, 1000 > C, 0, 5;
	Time	36.71	0.000017*	• Week 1 > Week 2
	Treatment x Time	4.85	0.004279*	• Significant interactions: RGRs of low-N treatments decline more than high-N treatments in Week 2
H_0 = enrichment with nitrate-N does not have a significant effect on dry matter contents after 14 days	Treatment	23.95	0.000000*	• Dry matter contents are significantly affected by nitrate-N enrichment: ▪ 0 & C > 10, 50, 100, 500, 1000; ▪ 5 > 50, 100, 500, 1000; ▪ 10 > 50, 100
	Time	32.39	0.000033*	• Final dry matter contents > initial dry matter contents
	Treatment x Time	26.07	0.000000*	• Significant interactions: DMCs of low-N treatments increased, whereas DMCs of high-N treatments declined significantly after 14 days.

* term significant at alpha = 0.05; [#] C = unenriched control treatment.

6.2.6 Effect of Temperature

While varying temperature significantly affected the growth of *Chaetomorpha linum*, enrichment with nitrogen had the most significant impact. At each temperature of 10, 15, 20, 25 and 30°C, a significant increase in *C. linum* biomass occurred after 14 days, with this increase being highest in the treatments that received both nitrogen and phosphorus (Figure 6-8; Figure 6-9). Repeated Measures ANOVA was used to compare differences in growth rates between temperatures and nutrient treatments over the two week incubation period. As significant interactions were often found between these factors, individual one-way ANOVAs

were conducted on cumulative growth rates (0-14 days) to further assess the significant differences at each temperature and for each nutrient treatment (Table 6-12).

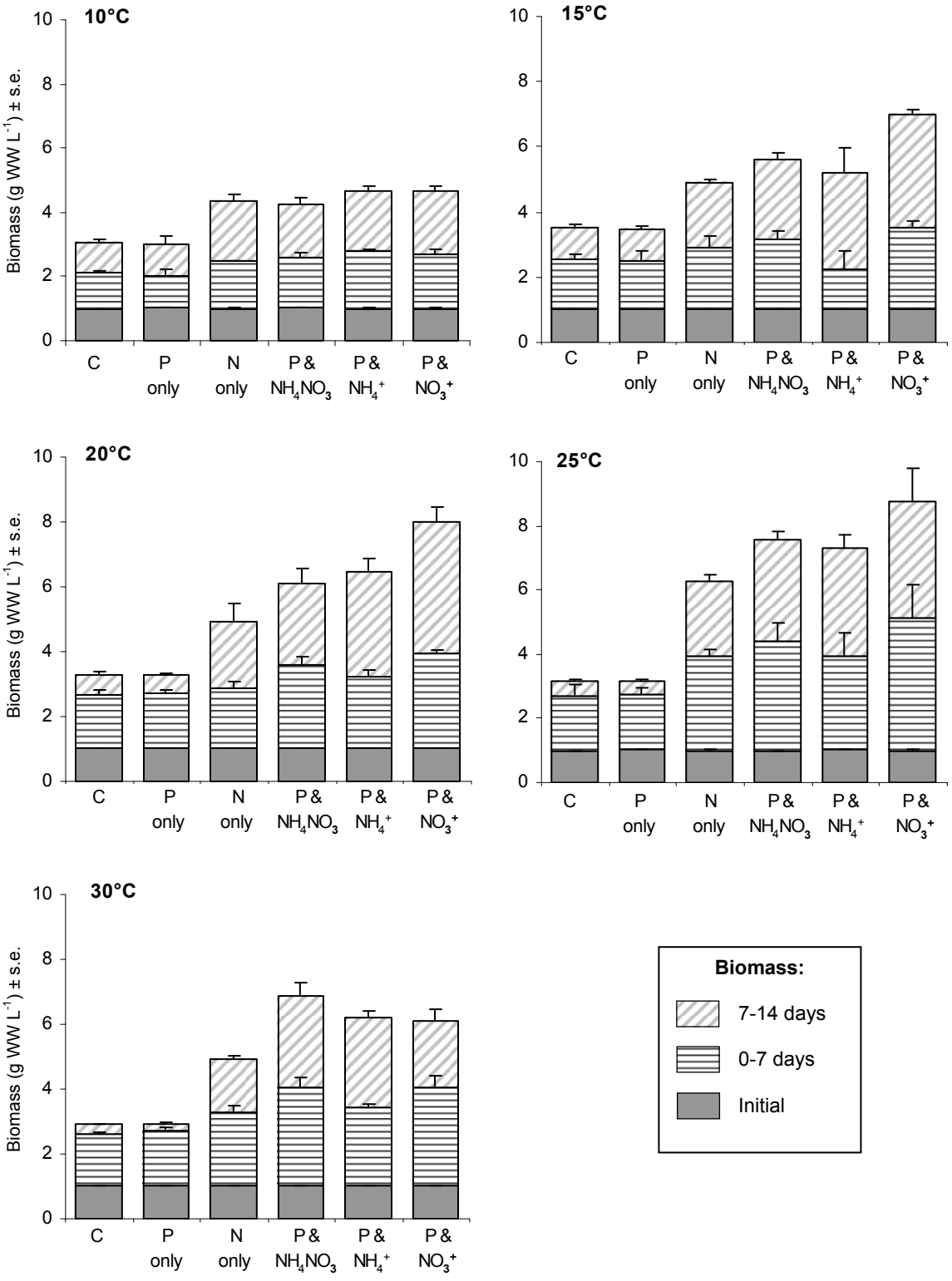


Figure 6-8: Biomass (wet weight) of *C. linum* after 14 days incubation at 10 - 30°C, under a range of nutrient treatments (C = unenriched control treatment).

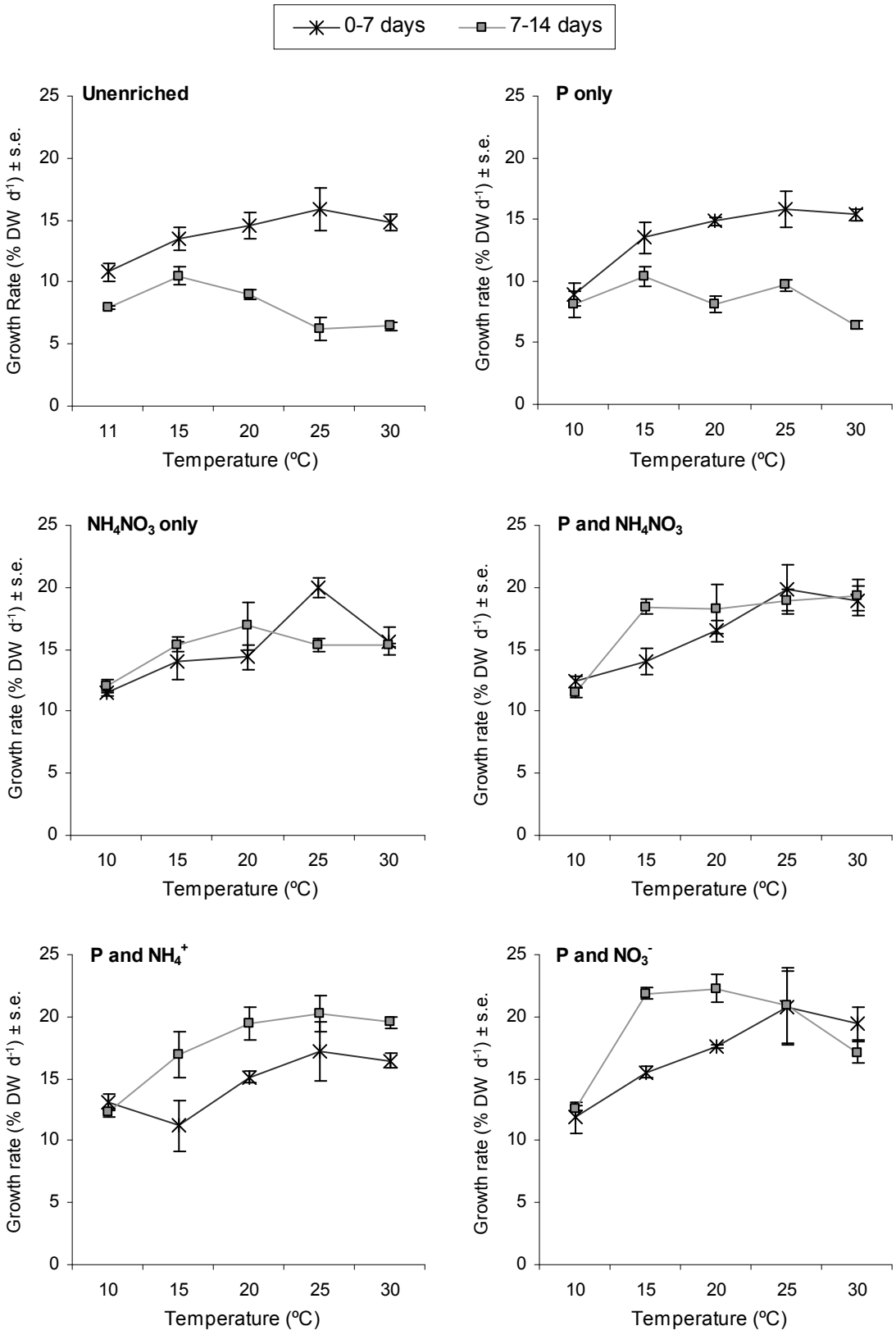


Figure 6-9: Relative growth rates (dry weight) of *C. linum* after 14 days of incubation at 10 - 30°C.

Table 6-12: Summary of ANOVA tests for the hypothesis of no significant differences between relative growth rates (dry weight) of *C. linum* after 14 days of laboratory culture at 10 - 30°C.

H_0 = Relative growth rates do not vary significantly according to temperature, nutrient treatment or time (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Multiple Comparisons (Tukey's $p < 0.05$)**
Temperature	58.43	0.000000*	• Growth rates at 25°C > 10, 15, 20, 30°C; • Growth rates at 10°C < 15, 20, 25, 30°C;
Treatment	93.21	0.000000*	• RGRs in N-replete treatments were significantly higher than N-deprived treatments: ▪ N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+ > P-only, C; ▪ P & NH_4NO_3, P & NO_3^- > N-only; ▪ P & NO_3^- > P & NH_4^+
Temp x Treatment	3.34	0.000157*	• Significant interactions between temperature and treatment (see one-way ANOVA tests below)
Time	6.51	0.013325*	• Growth rates in Week 1 > Week 2
Temperature x Time	7.31	0.000073*	• Significant interactions: at 25 & 30°C, RGRs decline in Week 2, but at 15 & 20°C, RGRs increase in Week 2. At 10°C, RGRs in Week 1 were not significantly different to Week 2.
Treatment x Time	20.15	0.000000*	• Significant interactions: all treatments have similar growth rates in Week 1, but N-deprived treatments decline in Week 2.
Temp x Treat x Time	1.41	0.153654	• No significant interactions

H_0 = Enrichment with N and/or P has no effect on relative growth rates after 14 days (one-way ANOVA conducted at each temperature)			
Temperature	**F-ratio**	**Probability**	**Tukey-Kramer Multiple Comparisons ($p < 0.05$)[#]**
10°C	10.02	0.000582*	• P & NH_4NO_3, P & NH_4^+, P & NO_3^- > P-only, C; • N -only > P-only
15°C	15.10	0.000081*	• P & NH_4NO_3, P & NO_3^- > P-only, C; • P & NO_3^- > P & NH_4^+, N-only
20°C	15.10	0.000081*	• P & NH_4NO_3, P & NO_3^- > P-only, C; • P & NO_3^- > P & NH_4^+, N-only
25°C	24.04	0.000007*	• N-only, P & NH_4NO_3, P & NH_4^+, P & NO_3^- > P-only, C
30°C	43.07	0.000000*	• N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+ > P-only, C; • P & NH_4NO_3, P & NH_4^+ > N-only

H_0 = Temperature has no effect on relative growth rates after 14 days (one-way ANOVA conducted for each nutrient treatment)			
Treatment	**F-ratio**	**Probability**	**Tukey-Kramer Multiple Comparisons ($p < 0.05$)**
Unenriched	7.37	0.004938*	• 15, 20, 25°C > 10°C
P-only	6.00	0.009980*	• 15, 20, 25°C > 10°C
N-only	8.99	0.002395*	• 15, 20, 25, 30°C > 10°C; 25°C > 15°C
P & NH_4NO_3	10.75	0.001209*	• 15, 20, 25, 30°C > 10°C
P & NH_4^+	15.04	0.000311*	• 20, 25, 30°C > 10, 15°C
P & NO_3^-	25.67	0.000031*	• 15, 20, 25, 30°C > 10°C

* term significant at alpha = 0.05; [#] C = unenriched control treatment

Across all temperatures, the treatments with nitrogen added had significantly higher growth rates than the unenriched control and the treatment with only phosphorus added to the culture media (RM ANOVA: F = 93.2, $p < 10^{-6}$, Tukey's $p < 0.05$). In the first week of treatment, growth rates were fairly similar across nutrient treatments, with few significant differences

found between nutrient treatments in Week 1, particularly at the lowest temperature of 10°C. A better differentiation between nutrient treatments occurred in the second week of treatment, however, when growth rates in the N-deprived treatments declined significantly (RM ANOVA: $F = 6.5$, $p < 0.05$; Tukey's $p < 0.05$), probably due to the depletion of internal nutrient stores. At the higher temperatures (15 - 30°C), internal nitrogen pools in the N-deprived treatments would have been depleted at a faster rate than at 10°C, resulting in lower growth rates in the second week of treatment at the higher temperatures. Internal nutrient pools at the start of these experiments, however, would have been minimal given the length of time in which the algae were starved of nutrients prior to conducting the experiments (see Section 3.3.2). By the second week of treatment, growth rates in the N-replete treatments (including the N-only treatment) were up to 5-fold higher than the N-deprived treatments (Figure 6-9). In general, growth rates in the P-only treatment were slightly lower than the unenriched control treatment, but these differences were not significant (Tukey's $p > 0.05$). Likewise, growth rates in the N-only treatment were often significantly lower than the P + N treatments, but there were generally few significant differences between growth rates in the P & NH_4NO_3, P & NH_4^+ or P & NO_3^- treatments (Tukey's $p > 0.05$; Table 6-12)

Growth rates typically increased with increasing temperature from 10 to 25°C, but growth in each treatment, particularly the N-replete treatments, declined at 30°C (Figure 6-9). Across all nutrient treatments, the highest growth rates of up to 27 % WW d^{-1} were recorded in the first week of treatment at 25°C, but these high growth rates were generally not sustained in the second week of treatment. Overall, growth rates were significantly higher at 25°C, and significantly lower at 10°C, than at all other temperatures (RM ANOVA: $F = 58.4$, $p < 10^{-6}$, Tukey's $p < 0.05$). In each of the nutrient treatments, growth rates were similar at 15, 20 and 25°C and declined slightly at 30°C, but these differences were rarely significant (Table 6-12). While *C. linum* growth rates at 10°C were significantly lower than at other temperatures (RM ANOVA, $F = 58.4$, $p < 0.05$), growth was still relatively consistent at 9 - 13 % DW d^{-1} and did not vary significantly between the first and second week of treatment (RM ANOVA: $F = 1.66$, $p > 0.05$). This suggests that, provided sufficient nutrients are present, *C. linum* can maintain steady growth and formation of new biomass at the lowest winter temperatures in Lake Illawarra.

It was concluded that 25°C was the optimum temperature for growth of *C. linum*, but only in the short term as growth rates at 25°C declined significantly in the second week of treatment (RM ANOVA: $F = 5.66$, $p < 0.05$, Tukey's $p < 0.05$) and are likely to have declined further if the experiment had been continued for a longer time period. At 30°C, growth rates were high in the first week of treatment (up to 19 % DW d^{-1}), but also declined significantly in the second week of treatment (RM ANOVA: $F = 47.6$, $p < 10^{-4}$; Tukey's $p < 0.05$). Additionally, when examined under a microscope, there was some evidence of cell damage to the *C. linum*

filaments, indicating that the algae would not survive for a long time at the highest temperature of 30°C.

6.2.7 Dry Matter Contents

The dry matter contents of *C. linum* biomass were significantly affected by nutrient deprivation and temperature (Figure 6-10; Table 6-13). The dry matter contents of all algae were significantly different after 14 days of laboratory culture (RM ANOVA: F = 10.2, $p < 10^{-4}$); in the N-deprived treatments, DMCs increased significantly to about 12 - 15 % of the wet weight biomass after 14 days, whereas DMCs in the N-replete treatments declined significantly to about 9 - 12 % after 14 days (Tukey's $p < 0.05$).

In addition, the N-deprived treatments (unenriched control and P-only) had significantly higher dry matter contents than the N-replete treatments at the end of the 14 day incubation period (RM ANOVA: F = 118, $p < 10^{-6}$; Tukey's $p < 0.05$). The highest dry matter contents of 17.5 ± 0.18 % were recorded at 25°C in the P-only treatment (ANOVA: F = 12.5, $p < 0.05$). In the N-enriched treatments, final dry matter contents (after 14 days) at 10°C were significantly higher than at 15 - 30°C, regardless of nutrient treatment (ANOVA: $p < 0.05$). The higher final dry matter contents recorded in the 10°C experiment, however, may have been partly due to the algae used having a significantly higher dry matter content at the start of that experiment (16.8 %, compared to approximately 11 % for the 15 - 30°C experiments).

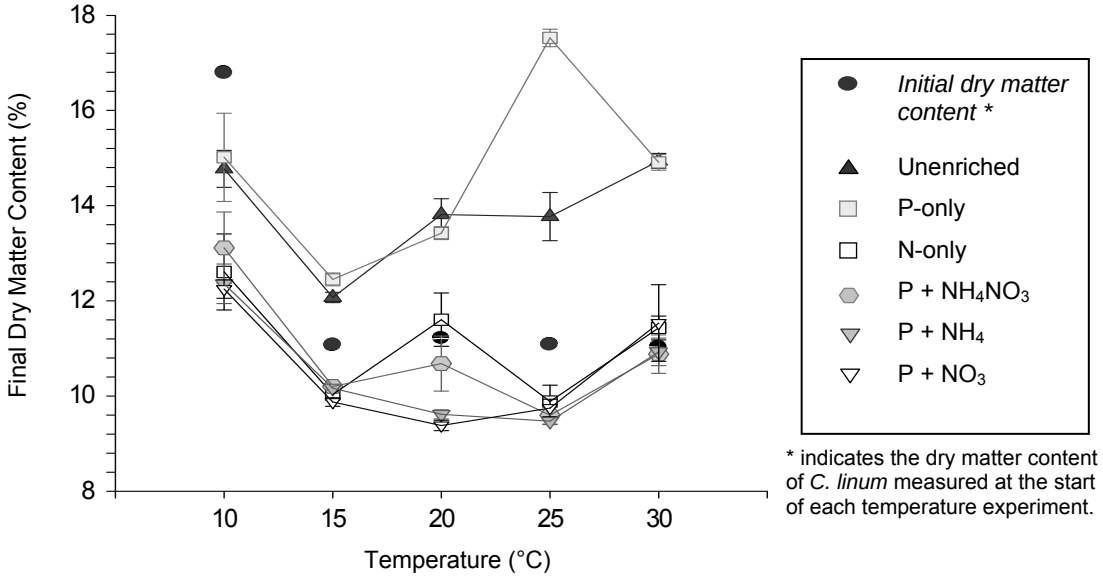


Figure 6-10: Final dry matter content of *C. linum* after 14 days of treatment at 10 - 30°C.

Table 6-13: Summary of ANOVA tests comparing the effect of temperature (10 - 30°C) and variable nutrient treatments on dry matter contents of *C. linum*.

H_0 = Dry matter contents do not vary significantly according to temperature, nutrient treatment or time (Repeated Measures ANOVA)			
Factor	**F-ratio**	**Probability**	**Multiple Comparisons (Tukey's $p < 0.05$)**
Temperature	591.81	0.000000*	• Dry matter contents were significantly affected by temperature: ▪ 10°C > 15, 20, 25, 30°C ▪ 25°C > 15°C ▪ 30°C > 15, 20°C
Treatment	117.66	0.000000*	• N-deprived treatments have significantly higher dry matter contents than N-replete treatments • P-only, C > N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+
Temp x Treatment	5.50	0.000000*	• Significant interactions between temp. and treatment
Time	10.24	0.000078*	• DMC at the start of the experiment is significantly different to DMC on Day 7 and Day 14.
Temperature x Time	38.27	0.000000*	• Significant interactions: DMCs decline after 14 days at 10°C, but increase at higher temperatures.
Treatment x Time	29.80	0.000000*	• Significant interactions: DMCs in N-deprived treatments increase in Week 2, whereas DMCs in N-replete treatments decline in Week 2
Temp x Treat x Time	2.77	0.000011*	• Significant interactions

H_0 = Enrichment with N and/or P has no effect on dry matter content after 14 days (one-way ANOVA conducted at each temperature)			
Temperature	**F-ratio**	**Probability**	**Tukey Kramer ($p < 0.05$)[#]**
10°C	3.77	0.027612*	• P-only > P & NO_3^-, P & NH_4^+
15°C	155.90	0.000000*	• P-only, C > N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+
20°C	26.64	0.000004*	• P-only, C > N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+; • N-only > P & NH_4NO_3, P & NH_4^+
25°C	135.98	0.000000*	• P-only, C > N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+
30°C	22.47	0.000010*	• P-only, C > N-only, P & NO_3^-, P & NH_4NO_3, P & NH_4^+

H_0 = Temperature has no effect on dry matter content after 14 days (one-way ANOVA conducted for each nutrient treatment)			
Treatment	**F-ratio**	**Probability**	**Tukey Kramer ($p < 0.05$)[#]**
Unenriched	11.78	0.000841*	• 10, 20, 25, 30°C > 15°C
P-only **	12.40	0.014612*	• 10, 25, 30°C > 15°C; 25°C > 20°C
N-only	5.76	0.011409*	• 10°C > 15, 25°C
P & NH_4NO_3	8.08	0.003551*	• 10°C > 15, 20, 25, 30°C
P & NH_4^+	25.88	0.000030*	• 10°C > 15, 20, 25, 30°C; 30°C > 20, 25°C
P & NO_3^- **	11.83	0.018635*	• 10°C > 15, 20, 25°C; 30°C > 20°C

* term significant at alpha = 0.05; ** Kruskal-Wallis ANOVA; [#] C = unenriched control treatment

This data shows a clear and consistent trend of *C. linum* dry matter contents increasing significantly at the lowest temperatures and when undergoing nitrogen deprivation. It has been generally concluded from these growth experiments that the dry matter content of *C. linum* increases (i.e., the plants lose water) when the algae are grown under stress (e.g., by temperature or nutrient limitation). The effect of nutrient enrichment and depletion on the dry matter content of *Chaetomorpha linum* has important implications for the field-collected data described in Chapter 5; high dry matter contents (e.g., > 13 - 14 %) may support the finding of nutrient limitation or other environmental stresses in field collected plants.

The dry matter contents of *Chaetomorpha* collected from Lake Illawarra between spring 2000 and winter 2002 ranged from 5.6 % to 25 % of the wet weight biomass (mean ± s.e.: 10.5 ± 0.4 %; Figure 6-11). It is important to note that tissue nutrient analyses were only undertaken when sufficient algal biomass was present and, therefore, tissue N and P data was only available for about one-third of the *Chaetomorpha* samples shown in Figure 6-11. The highest dry matter contents of 14 - 25 % shown here correspond with the *Chaetomorpha* samples with the lowest biomasses, for which nutrient analysis was not undertaken. In general, algae with low tissue P (≤ 0.05 % P dry wt.) and relatively low tissue N (≤ 2.7 % N dry wt.) had higher dry matter contents of 8 - 13 %. Whereas the lowest dry matter contents of 5.6 - 7 % often coincided with the highest tissue nitrogen contents of 3.5 - 3.8 % N dry wt., but this pattern was not consistent across the whole data set. Overall, dry matter contents of field-collected *Chaetomorpha* were lower than the average DMCs of 13 - 17 % recorded for *C. linum* undergoing nitrogen deprivation during laboratory culture (Figure 6-10). These predominantly low dry matter contents support the conclusion that *Chaetomorpha* collected from Lake Illawarra was generally not nitrogen-deprived during the study period.

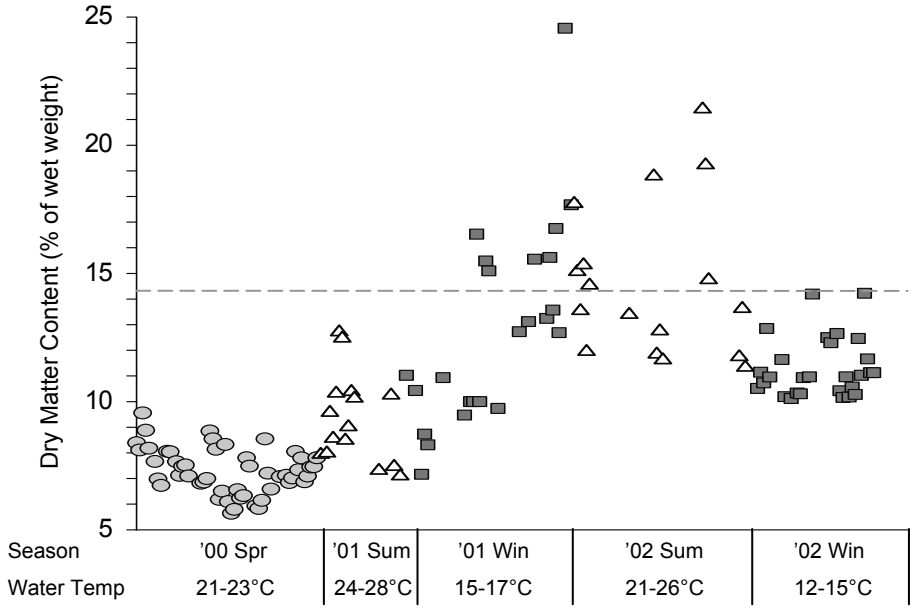


Figure 6-11: Dry matter contents of *Chaetomorpha linum* collected from Lake Illawarra, spring 2000 - winter 2002. Dashed line is the average dry matter content (after 14 days) of *C. linum* undergoing nitrogen deprivation in laboratory culture.

In addition, water temperatures during the study period ranged from about 12 - 15°C in winter, to 24 - 28°C in summer. These temperatures were within the range considered suitable to support growth of *C. linum*, although it is expected that the lower winter temperatures would limit the formation of biomass to some degree and result in slightly higher dry matter contents in winter.

6.3 Discussion of Results

Growth rate and nutrient uptake by macroalgae are regulated by factors such as light, temperature and nutrients, but not necessarily to the same degree by each of those factors (Duke *et al.*, 1989). Therefore, it is often more important to study the interactions between environmental factors, such as light, temperature, nutrients, rather than the individual factors (Matta and Chapman, 1995). Studies on green nuisance macroalgae, such as *Chaetomorpha linum*, however, can be difficult to assess as they often exhibit a broad tolerance to a wide range of irradiances, temperatures, salinities and nutrient concentrations (Taylor *et al.*, 2001). The experimental studies on the growth of *C. linum* could be considered highly successful in that a significant increase in biomass was recorded in all of the experiments and under every nutrient and temperature condition tested, including control treatments. However, this made interpretation of the results difficult as *C. linum* is a highly opportunistic alga which appears to be able to take advantage of a wide variety of conditions and, therefore, often only small differences were detected between nutrient or temperature treatments.

In nutrient enrichment experiments such as these, it is important to note that during the exponential phase of algal growth, nutrients would be considered saturating to growth rates according to Michaelis-Menten kinetics, at which point the algae are growing at the theoretical maximum rate obtainable (μ_{max}: see Section 2.5.3). When algal growth slows after the exponential phase, nutrients become limiting when the concentration falls below that required for maximum growth, so the measured specific growth rate (μ) is less than the theoretical maximum specific growth rate (μ_{max}). These experiments, therefore, are ultimately comparing the relative growth rates (μ or RGR) obtained at sub-optimum conditions, to the theoretical maximum growth rate obtained (μ_{max}) for *C. linum* when temperature, light and nutrients were at saturating levels. In the context of this study, a limiting factor is considered to be any factor whose absence, reduction or alteration results in a significant reduction in growth rates (μ) compared to the growth rate obtained when all other relevant factors are saturating. In phytoplankton studies, limitation is further defined in terms of Michaelis-Menten or Monod-type limitation, where low nutrient concentration limits algal growth rates, and Liebig-type limitation, where a single resource limits the formation of new algal biomass, usually at the end of the growth cycle (Kokum *et al.*, 2002; Williams, 2006). For example, if temperature, light and other nutrients, such as phosphorus, are at sufficient quantities, but nitrogen concentrations are low compared to the growth requirements of the alga, Liebig-type limitation (by nitrogen, in this case) may limit further formation of biomass.

The results of the present study regarding the effect of temperature and nutrient enrichment on the growth of *C. linum* are discussed further in the following sections.

6.3.1 Experimental Optima

The results obtained in the present study were within the range of experimental data documented in the literature. A temperature of 25°C was found to promote the highest growth rates of *Chaetomorpha linum* in the present study, which was similar to that found by King *et al.* (1990) and Taylor *et al.* (2001) for the same species (Table 6-14).

Table 6-14: Conditions found to promote the highest relative growth rates (% d^{-1}) for green macroalgae in laboratory culture.

Species	PO_4^{3-}-P (μmol L^{-1})	NO_3^--N (μmol L^{-1})	NH_4^+-N (μmol L^{-1})	Temp (°C)	References
Chaetomorpha linum	20	100-1000	50-100	25	Present Study
Chaetomorpha linum	-	-	-	20-25	King *et al.* (1990)
Chaetomorpha linum	30	400	80	20	Taylor *et al.* (2001)
Cladophora dalmatica	10	800	100	15	Taylor *et al.* (2001)
Cladophora aff. *albida*	8	29*	-	-	Gordon *et al.* (1981)
Enteromorpha clathrata	-	10.7	-	25	Fitzgerald (1978)
Enteromorpha compressa	20	1000	60	10	Taylor *et al.* (2001)
Ulva lactuca	14.5	43	-	-	Steffensen (1976)
Ulva rigida	30	400-800	80	15	Taylor *et al.* (2001)
Rhizoclonium tortuosum	100	1000	80	15-20	Taylor *et al.* (2001)

∗ μmol N L^{-1} as NH_4NO_3

When cultured with sufficient N (100 μmol N L^{-1} d^{-1}) the highest cumulative biomasses of *C. linum* (up to 8 g WW L^{-1} after 14 days) occurred at a phosphate-P concentration of 20 μmol P L^{-1} d^{-1}. After 14 days with sufficient P (20 μmol N L^{-1} d^{-1}), nitrogen enrichment in the form of 100 μmol NO_3^--N L^{-1} d^{-1} or 50 μmol NH_4^+-N L^{-1} d^{-1} promoted the highest *C. linum* biomasses of 7.5 g WW L^{-1}. Similarly, Taylor *et al.* (2001) determined that *Chaetomorpha linum* grown in culture responded best to a phosphate-P concentration of 30 μM and nitrogen in the form of either 400 μM nitrate-N or 80 μM ammonium-N.

6.3.2 Effect of Phosphorus Enrichment

In all experiments conducted, there were generally no significant differences between growth rates of *C. linum* in the P-only and unenriched control treatments. In addition, growth rates in the N-deprived treatments were always significantly lower than the N-only and N + P treatments. This indicates that deprivation of phosphorus has a limited effect on the growth of *C. linum*. However, as the algae continued to grow at a rate of approximately 10 % WW d^{-1} in the unenriched treatments it is possible that the alga utilised stored nutrients or that background levels of N in the unenriched substrates were sufficient to support slow growth. Lavery and McComb (1991b) also found that P starvation had a relatively mild effect on the growth of *Chaetomorpha linum* in culture and that growth was more severely inhibited by N deprivation than P deprivation; in the third week of their experiments the N-only treatment had

a relative growth rate of 7.3 % d^{-1}, compared to 3.2 % d^{-1} in the P-only treatment. In addition, Lotze and Schramm (2000) showed that enrichment with phosphate-P (30 μM) significantly affected growth rates of *Pilayella littoralis* (filamentous brown alga), but not *Enteromorpha intestinalis*. Enrichment with combined nitrate-N (500 μM) and phosphate-P (30 μM), however, increased growth rates of *E. intestinalis* and *P. littoralis* by 3.5-fold and 1.6-fold, respectively.

Phosphorus enrichment did have a significant effect on growth of *C. linum* in the present study, but only in combination with N enrichment. In experiments conducted with phosphate-P concentrations of 0 - 100 μmol P L^{-1} d^{-1}, algal growth rates increased steadily to approximately 18 - 20 % WW d^{-1} in the 20 μmol P L^{-1} d^{-1} treatment, but growth rates declined slightly at higher P concentrations. Similar results were reported by Taylor *et al.* (2001), who found that all green macroalgae they examined exhibited a broad tolerance to phosphate-P, growing in concentrations up to 200 μM. They reported that growth rates of *C. linum* were significantly affected by P enrichment, with growth rates increasing to about 22 % WW d^{-1} with increasing P, up to an optimal level of 30 μM.

In the present study, *C. linum* growth rates in the P-only treatments were often slightly lower than the unenriched treatments, but this difference was rarely significant. Menendez *et al.* (2002b) also found that *C. linum* growth rates after 10 days were higher in unenriched treatments (5.05 ± 0.4 % DW d^{-1}), than P-only treatments (3.39 ± 0.4 % DW d^{-1}). The slightly lower growth rates in the P-only treatments may be due to Michaelis-Menten type limitation of growth rates due to low N concentrations relative to the growth requirements of the plant. The high P concentrations (10 μM d^{-1} in the present study) may have also inhibited growth. For example, McClanahan *et al.* (2004) determined that P and N addition enhanced the growth of the filamentous *Enteromorpha prolifera*, but not frondose brown seaweeds (e.g., *Dictyota* sp. and *Sargassum* sp.); they concluded that high nutrient concentrations appeared to suppress the growth of large brown macroalgae. In addition, Lotze and Schramm (2000) showed that nutrient uptake rates of *Enteromorpha intestinalis* and *Pilayella littoralis* declined with elevated substrate concentrations (> 30 μM P and 500 μM N). Gordon *et al.* (1981) also found that growth rates of *Cladophora* were strongly influenced by P concentrations below 6 - 10 μmol P L^{-1}, but growth rates declined above this level.

6.3.3 Effect of Nitrogen Enrichment

Nitrogen enrichment had a significant effect on the growth of *Chaetomorpha linum*, with growth rates in all experiments increasing significantly in nitrogen-enrichment treatments, compared to P-only and unenriched control treatments. *C. linum* in the present study showed high growth rates in nitrate-N concentrations up to 1000 μmol L^{-1} d^{-1}, but growth appeared saturated at approximately 20 % WW d^{-1} in the 100 - 1000 μmol L^{-1} d^{-1} treatments. Enrichment with ammonium-N also resulted in significantly higher growth rates than control treatments,

with growth increasing to 22 % WW d^{-1} in the 50 μmol L^{-1} d^{-1} treatments, but declining very slightly at higher concentrations. Comparisons between the effect of enrichment with nitrogen-N and ammonium-N are discussed in Section 6.3.4.

Where P concentrations were high in the present study (20 μM), the highest growth rates of *C. linum* were achieved with a N:P molar ratio of 5:1 (as nitrate-N), but lower N:P ratios resulted in lower growth rates, as seen in the treatments receiving nitrate-N additions of 50 μmol N L^{-1} d^{-1} or less. The reduced growth rates in the unenriched and P-only treatments were not surprising given that both P and N are critical nutrients, but it was expected that growth would also be considerably reduced in the N-only treatment. For the majority of experiments conducted, however, growth rates in the N-only treatment, although slightly less, were often not significantly different to those in the N + P treatments, and were always significantly higher than N-deprived treatments. Similarly, Taylor *et al.* (2001) also documented *Chaetomorpha linum* growth rates of 12 % d^{-1} in treatments of 500 μM NO_3^--N, but without added P. These results indicate that *C. linum* has a very low P requirement or is able to rely on internal P stores to support growth, at least for the short duration of the experiment.

In the present study, *C. linum* growth rates in the first week of treatment were generally higher than in the second week, so that often little differentiation occurred between nutrient treatments in the first week. In nutrient enrichment experiments, the algae typically grow at the maximum rate obtainable (μ_{max}) during the exponential growth phrase, considered to be the first week of treatment in these experiments. Growth rates in nitrogen-deprived treatments slowed by the second week of treatment, presumably after internal stores were depleted and low nutrient concentrations limited growth rates according to Michaelis-Menten saturation kinetics. Menéndez (2002b), however, reported that *Chaetomorpha linum* growth rates were higher between 4 - 10 days than 0 - 4 days of treatment. This delay in growth following nutrient enrichment may be due to the algae replenishing internal nutrient stores prior to accelerated growth; for example, McGlathery *et al.* (1996) noted that following N-enrichment, the tissue N contents of *Chaetomorpha linum* increased by 4-fold in 4 days. In addition, nutrient uptake is often time-dependent; Lotze and Schramm (2000), for example, found that the highest uptake rates of nitrate, ammonium and phosphate for *Enteromorpha intestinalis* and *Pilayella littoralis* occurred during the first 15 - 30 minutes of incubation. Lavery and McComb (1991b) reported that *Chaetomorpha linum* had higher uptake rates of ammonium-N (~450 μmol g^{-1} DW hr^{-1}) than nitrate-N (~110 μmol g^{-1} DW hr^{-1}) or phosphate-P (~14 μmol g^{-1} DW hr^{-1}). McGlathery *et al.* (1996) also noted that *Chaetomorpha linum* took up ammonium 2 - 3 times faster than nitrate. In N-starved algae, the initial uptake rates of nitrate and ammonium were 30 - 50 % higher than subsequent uptake rates, lasting approximately 1 - 2 hours for ammonium and 0.5 - 1 hour for nitrate. These authors found that uptake of nitrate by *Chaetomorpha linum* occurred after an initial lag phase, possibly because nitrate uptake may be inhibited by the presence of internal nitrate or ammonium stores or by the presence of external ammonium.

Lopes *et al.* (1997) reported nitrate reductase activity in *Gracilaria tenuistipitata* exhibited a diurnal rhythm, with activity during the day being 30-fold higher than during the night. However, Menendez *et al.* (2002b) only found a diurnal cycle for *Chaetomorpha linum* when nitrate was added alone. These authors noted that when nitrate was added with P, the diurnal cycle only occurred nine hours after depletion of P, possibly because nitrate reductase is regulated by phosphorylation (Crawford, 1995), so that the algae had to first take up P, as ATP (adenosine triphosphate), formulated by phosphorylation, was required to initiate nitrate uptake.

Most species of macroalgae, particularly green algae, have the ability to take up nutrients rapidly, thus taking advantage of periodic nutrient pulses and sustaining growth during periods of low nutrient availability. For example, Fujita (1985) found that *Ulva lactuca* and *Enteromorpha prolifera,* when exposed to a 20 μM N pulse, could take up enough N in 10 hours to sustain growth for a further 10 days without external N. *Gracilaria tikvahiae* (Rhodophyta), however, required 27 hours to take up enough N to support growth for 14 days without external N, suggesting that *Gracilaria* was less-opportunistic than *Enteromorpha* and *Ulva.* McGlathery *et al.* (1996) reported that internal N pools of *Chaetomorpha linum* varied from 1.2 - 4.6 % N (dry weight) when grown under N-limiting and N-saturating conditions. When grown under non-limiting conditions, internal N contents reflected uptake and storage of N that exceeded immediate growth requirements. After the external N supply was removed, the algae continued to grow at similar rates even though internal N pools decreased exponentially, indicating the utilization of stored N. Preliminary experiments in the present study indicated that *Chaetomorpha linum* was able to take up and store enough nutrients to support growth for a lengthy period; after being cultured in enriched seawater media for 3 weeks, the algae maintained slow growth (~ 2 % d^{-1}) for a further 4 weeks in water without added N or P. This also indicates that any nutrient pulses in Lake Illawarra are likely to be rapidly taken up by *Chaetomorpha* spp. and support growth for several weeks afterwards.

Previous studies have shown that algae with nutrient enriched tissue take up nutrients slower than those with nutrient depleted tissue (e.g., McGlathery *et al.*, 1996). For example, Fong *et al.* (2003) showed that prior storage of tissue nutrients strongly influenced the growth response of tropical marine algae to nutrient enrichment; algae which were initially nutrient-depleted responded to N enrichment by increasing internal N stores. When tissue nutrients were initially depleted, growth of *Acanthophora spicifera* increased by 70 - 90 % after enrichment with N or P. In *A. spicifera* with initially nutrient-enriched tissue, growth doubled after enrichment with P-alone, but only increased by 30 % when N was added alone. These authors further suggested that mixed results in experiments designed to assess nutrient limitation in macroalgae may be due to internal nutrient stores not being taken into account. As the field-collected algae used in the present study was pre-conditioned in an unenriched culture media for at least 4 - 6 weeks prior to using in experiments, internal nutrient stores would have been considerably depleted, and variation in the initial tissue nutrient

concentrations between individual experiments is not likely to have influenced the results. Analyses conducted on *C. linum* samples randomly collected from the pre-conditioning tank showed that tissue nutrient concentrations were low (0.90 ± 0.04 % N and 0.03 ± 0.01 % P dry wt.) and well below the concentrations found for *Chaetomorpha* growing in Lake Illawarra (see Chapter 5).

The ranges of nitrogen and phosphorus concentrations used to evaluate *C. linum* growth in the present study are considerably higher than those commonly detected in Lake Illawarra. In the centre of the Lake, for example, phosphate-P concentrations ranged from 2 - 118 μg PO_4^{3-}-P L^{-1}, whereas dissolved inorganic nitrogen concentrations ranged from 10 - 270 μg L^{-1} for NH_4^+ and 1 - 296 μg L^{-1} for NO_3^- + NO_2^- (unpublished Pacific Power and Lake Illawarra Authority data, 1996 - 2001; Table 1-2). However, fluxes of orthophosphate from the catchment may exceed this range, particularly during wet weather, but the majority of plant-available P in the Lake is thought to be rapidly taken up by both macroalgae and phytoplankton, and therefore difficult to quantify (see, e.g., LIA, 1995). Release of nitrogen and phosphorus from sediment can be another significant source of nutrients for macroalgae in the Lake. For example, AWACS (1994, cited in LIA, 1995) estimated an average net orthophosphate release rate of 280 μmol P m^{-2} day^{-1} for an area along the Windang Peninsula without seagrass cover. This figure, however, was only considered representative of phosphorus release from the sediment at that particular area of the Lake at that time, and is included here as an example only. Benthic nitrogen fluxes in Lake Illawarra were analysed by Qu (2004a); he determined that DIN fluxes from the sediment showed seasonal and diel variations, with lower release rates during light due to photosynthetic activity of the aquatic plant and microphytobenthic communities. In general, unvegetated sediments exhibited a net efflux of DIN from the sediment, whereas seagrass areas showed net uptake of DIN over an annual cycle. In sediment cores from the Nicolle Road site, for example, NH_4^+ fluxes in the dark ranged from -13 μmol m^{-2} hr^{-1} in summer 2002 to 191 μmol m^{-2} hr^{-1} in summer 2003.

6.3.4 Variation in Response to Nitrate and Ammonium

Nitrate and ammonium are typically the most important sources of dissolved inorganic nitrogen for macroalgae. While some species of macroalgae can grow equally well with nitrate or ammonium as the N source, many species of macroalgae show a preference for nitrate over ammonium, and vice versa (Hanisak, 1983). Ammonium-N is usually the preferred form as it requires less biochemical energy for assimilation than other forms of dissolved nitrogen; assimilation of nitrate and nitrite require more energy than ammonium, as these must first be reduced to ammonium (Libes, 1992; Shaw *et al.*, 1998). Thus for many species of macroalgae, uptake of ammonium is more rapid than nitrate uptake at the same concentration (Hanisak, 1983). For example, McGlathery *et al.* (1996) found that uptake rates of ammonium by *Chaetomorpha linum* were always 2 - 3 times faster than nitrate uptake rates, and were

substantially faster when N-starved algae were resupplied with N, compared to algae with sufficient internal N stores.

In the present study *C. linum* responded equally well to a nitrogen source of nitrate-N or ammonium-N, but growth rates in the P + NO_3^- were fractionally higher than in P + NH_4^+ treatments, even though the nitrogen concentration was the same. When compared to unenriched control treatments, enrichment with 50 -100 μM nitrate-N increased *C. linum* growth rates by 1.3 - 1.4-fold, whereas enrichment with 50 - 100 μM ammonium-N increased growth rates by 1.3-fold. Similar to the present study, Menéndez *et al.* (2002b) also noted marginally higher growth rates of *C. linum* in P + NO_3^- treatments (Section 6.3.5), indicating that this species has a very high nitrate storage capacity. These authors also suggested that oxygen bubbling within the culture jars may aid the conversion of ammonium to nitrate via nitrification. Additionally, ammonium may be lost via volatilisation, due to the increase in pH within the algal mat; they noted that pH increased from 8.9 to 9.5 within 1.5 hours of exposure to light. Taylor *et al.* (2001), however, reported that *C. linum* had significantly higher growth rates (up to 22 % WW d^{-1}) when ammonium was the N-source, but this species was also highly tolerant of nitrate, with high growth rates (up to 19 % WW d^{-1}) recorded from 0 to 1000 μmol NO_3^--N L^{-1}. *C. linum* in the present study also exhibited a high tolerance to nitrate, with growth rates up to 15 - 22 % WW d^{-1} achieved in nitrate-N treatments of 50 - 1000 μmol N L^{-1} d^{-1}. Similarly, Lotze and Schramm (2000) determined that nitrate-N (500 μM) enrichment doubled the growth rates of *Enteromorpha intestinalis* and *Pilayella littoralis*, while ammonium-N (50 μM) enrichment increased growth rates by about 1.5-fold, compared to unenriched control treatments.

Although ammonium may be the preferred source of nitrogen, ammonium can be toxic to macroalgae at high concentrations (e.g., > 30 - 50 μM: Waite and Mitchell, 1972, cited in Lobban and Harrison, 1997; Topinka and Robbins, 1976). Ammonium toxicity in seaweeds has been related to an uncoupling of photosynthesis by inhibiting the electron transport chain, as well as inhibiting germling development in phaeophytes (Kevekordes, 2001, and references therein). The unionized ammonia, however, is the prevalent and more toxic form taken up by passive diffusion, and varies depending on temperature and pH (Kevekordes, 2001). Therefore, the growth media with concentrations of up to 100 μmol NH_4-N L^{-1} d^{-1} used in the present study may have contained sufficient unionized ammonia to be toxic to *C. linum*, but these solutions did not appear to inhibit the growth of the alga. In the majority or experiments conducted, growth rates of *C. linum* were approximately 10 - 15 % higher in P + NO_3^- treatments, than P + NH_4NO_3, but differences between N-enriched treatments were rarely significant. Previous studies have shown that uptake of nitrate may be inhibited in the presence of ammonium. For example, Smit (2002) reported that the uptake rate of NO_3^--N by *Gracilaria gracilis* was reduced by up to 38 % when NH_4^+-N was present in concentrations greater than 5 μM. Nitrate uptake rates of *Enteromorpha intestinalis* also decreased by 50 %

in the presence of 5 μM ammonium (Thomas and Harrison, 1987). In addition, O'Brien and Wheeler (1987) found that uptake of nitrate by *Enteromorpha prolifera* was inhibited at ammonium concentrations between 1 - 15 μmol L^{-1}; nitrate uptake was reduced by around 40 % in the presence of 1 μmol L^{-1} NH_4^+, and 50 % at ammonium concentrations of 7 and 15 μmol L^{-1}. They suggested that inhibition of nitrate uptake in the presence of ammonium may be important in coastal estuaries where ammonium fluxes from the sediment are often high.

6.3.5 Comparison to Previous Studies on Macroalgae

Growth rates recorded in the present study were within the range of those documented for green macroalgae in the literature (Table 6-15). Growth rates of macroalgae in culture are usually significantly higher than those achieved in field-based studies, averaging 5 - 20 % d^{-1}. *Chaetomorpha linum* typically attains a higher growth rate in culture than other green macroalgae, suggesting that this alga may also be more competitive than the majority of species when occurring under natural conditions. Comparing the results obtained in the present study to published values for other green macroalgae supports the hypothesis that *Chaetomorpha* spp. in Lake Illawarra is more competitive, in terms of nutrient uptake and rapid growth, than other nuisance macroalgal genera in the Lake. This explains why *Chaetomorpha* is presently the dominant bloom-forming genera in Lake Illawarra, and subsequently blooms of other nuisance genera, such as *Cladophora* and *Ulva,* are far less common (see Chapter 5).

Table 6-15: Relative growth rates (RGR) of macroalgae under various culture conditions.

Species	RGR (mean ± s.e.) (% d^{-1})*	Nutrients added (μM) P	N	Temp. (°C)	References
PO_4^{3-} and NO_3^-					
Chaetomorpha linum	17.6 ± 0.2	10	100	20	Present study
Chaetomorpha linum	18.3 ± 0.7	30	200	15	Taylor *et al.* (2001)
Chaetomorpha linum	8.4 ± 0.2	18	68	20-22	Menéndez *et al.* (2002b)
Enteromorpha intestinalis	12.6 ± 6.8	12	200	18	Björnsäter and Wheeler (1990)
Enteromorpha compressa	4.7 ± 0.5	30	200	15	Taylor *et al.* (2001)
Ulva fenestrata	14.1 ± 9.3	12	200	13	Björnsäter and Wheeler (1990)
PO_4^{3-} and NH_4^+					
Chaetomorpha linum	15.1 ± 0.5	10	100	20	Present study
Chaetomorpha linum	19.5 ± 0.7	30	100	15	Taylor *et al.* (2001)
Chaetomorpha linum	6.3 ± 1.5	18	120	20-22	Menéndez *et al.* (2002b)
Enteromorpha spp.	9.1 ± 0.2	20	200	20	Fujita (1985)
Enteromorpha compressa	1.0 ± 0.3	30	100	15	Taylor *et al.* (2001)
Ulva lactuca	9.3 ± 0.2	20	200	20	Fujita (1985)
Gracilaria tikvahiae	7.3 ± 0.6	20	200	20	Fujita (1985)
PO_4^{3-} and NH_4NO_3					
Chaetomorpha linum	17.7 ± 1.8	10	100	20	Present study
Cladophora sp.	13.2 ± 0.4	16	71	23	Gordon *et al.* (1981)

* all RGR values listed are wet weight, except those of Menéndez *et al.* (2002b), recorded as dry weight.

A comparison between growth rates achieved in the present study and a similar study on *Chaetomorpha linum* is presented in Table 6-16. Menéndez *et al.* (2002b) and Menéndez (2005) documented *C. linum* growth rates of 8.7 - 13.0 % d^{-1} under enrichment with nitrogen and / or phosphorus. Like the present study, Menéndez *et al.* (2002b) found that biomass of *Chaetomorpha linum* increased in all nutrient treatments (including unenriched control treatments) after 10 days, but growth rates of *C. linum* were significantly higher in enriched treatments, particularly the N + P treatments. *C. linum* growth rates in the present study, however, were approximately 2 - 3 times higher than those documented by Menéndez (2002b) using similar experimental parameters. These differences in growth rates may be due to physiological differences in the algae (e.g., Australian versus Mediterranean), a different light to day photoperiod (15:9 hours in Menéndez *et al.* (2002b) compared to 12:12 hours in the present study), or due to Menéndez *et al.* (2002b) using a higher initial biomass of approximately 5 g WW L^{-1}, compared to 1 g WW L^{-1} in the present study. Initial experiments showed that commencing the experiment with an algal biomass of 2 g WW L^{-1} resulted in significantly lower (up to 40 %) growth rates than a 1 g WW L^{-1} starting mass (see Section 6.2.1). Using a higher biomass may result in algal growth and biomass formation being limited by available nutrients and/or restricted by space within the culture jars. The biomass used in the current experimental work was designed to be non-limiting, but was considerably less than that which may occur in an algal bloom; maximum *Chaetomorpha* spp. biomasses of about 3 kg WW m^{-2} (in a water depth of 0.5 m) were recorded in Primbee Bay in summer 2002 (Chapter 5).

Table 6-16: Comparison between dry weight growth rates of *Chaetomorpha linum* recorded by Menéndez *et al.* (2002b) and the present study.

Present Study (0-14 days at 20°C)		**Menendez *et al.* (2002b) (0-10 days at 20-22°C)**	
Treatment	**$\dot{R}GR$ (% DW d^{-1})**	**Treatment**	**$\dot{R}GR$ (% DW d^{-1})**
Unenriched	11.79 ± 0.48	Unenriched	5.05 ± 0.4
10 μM PO_4^{3-}-only	11.49 ± 0.32	18 μM PO_4^{3-}-only	3.39 ± 1.6
50 μM NH_4NO_3-only	15.62 ± 0.83	120 μM NH_4^+-only	5.67 ± 0.9
50 μM NH_4NO_3 + 10 μM PO_4^{3-}	17.35 ± 1.40	68 μM NO_3^--only	6.73 ± 0.1
100 μM NH_4^+ + 10 μM PO_4^{3-}	17.29 ± 0.80	120 μM NH_4^+ + 18 μM PO_4^{3-}	6.33 ± 1.5
100 μM NO_3^- + 10 μM PO_4^{3-}	19.94 ± 0.61	68 μM NO_3^- + 18 μM PO_4^{3-}	8.41 ± 0.2

6.3.6 Effect of Temperature on Growth

Growth rates of *Chaetomorpha linum* increased significantly ($p < 0.05$) with increasing temperature between 10°C and 25°C, but declined markedly at 30°C. The highest relative growth rates of up to 27 % WW d^{-1} occurred in the first week of treatment at 25°C, but these high growth rates subsequently declined in the second week of treatment at 25°C. This optimal temperature range (20 - 25°C) for growth of *C. linum* correlates with water temperatures in Lake Illawarra measured during spring to late summer. *C. linum* growth rates

at 10°C averaged 13 - 15 % WW d^{-1} in the N + P treatments, suggesting that this species also has the ability to persist at the lowest temperatures (10 - 15°C), recorded in the Lake during winter. It must be noted that under the highest temperature and non-limiting nutrient conditions, individual plants with the highest growth rates in the first week of treatment were substantially less successful in the second week of treatment. Likewise, plants with slower growth rates in the first week of treatment usually had significantly higher growth rates in the second week. This indicates that the while *C. linum* can attain a fast increase in biomass in the short-term, the success of the plants may ultimately be short-lived.

Macroalgae, particularly bloom-forming species, are able to acclimatize to their environmental conditions (Lobban and Harrison, 1997). *Chaetomorpha* spp. in Lake Illawarra, for example, can tolerate and form blooms at a wide variety of water temperatures, from a minimum of 10°C in winter to about 30°C in summer, but the most extensive blooms are usually recorded in the warmer months. This ability to acclimatize to temperature was also observed in the experimental studies; *C. linum* was successfully grown at all temperatures when the temperature was kept constant at high or low temperatures (10 - 30°C) for two weeks, but on an occasion when the temperature fluctuated between 10 and 30°C within a 24-hour period (due to equipment malfunctions), the algae collapsed and did not recover. Taylor *et al.* (2001) suggested that tolerance to temperature may be related to their alga's natural habitat; shallow-water macroalgae, for example, would be exposed to higher temperatures during summer, and would have a higher tolerance to temperature than deep-water algae. As the *C. linum* biomass used in the present study was collected from a depth of 0.5 - 1 m, algae from shallower areas of the Lake may have an even higher tolerance to water temperatures above 30°C. In shallow (< 0.5 m depth) stagnant areas of Primbee Bay (Lake Illawarra), water temperatures up to 35 - 40°C were recorded within dense mats of *Chaetomorpha* spp. in summer, possibly related to photosynthesis-respiration processes within the algal mats. Attempts were made to ensure the algal samples collected were from independent populations and therefore representative of a wide range of genetic variants of *Chaetomorpha linum* from the Lake. However, as the algal biomass was collected over a 6 month period and from different locations in the Lake (depending on availability), some slight variation in responses between different algal populations used in the various experiments may be expected. King *et al.* (1990) concluded that at any particular growing temperature, *Chaetomorpha linum* appeared to have similar growth rates, regardless of the season in which they had been collected, indicating that response to temperature was not dependent on the recent history of the plant. Therefore, using algal biomass obtained during different seasons is not expected to have a significant effect on the experimental results.

Several studies on the growth of macroalgae in culture have concluded that temperature has a significant effect on macroalgal growth (e.g., Steffensen, 1976; Matta and Chapman, 1995). Some authors, however, have reported that temperature did not have a significant effect on

growth rates of certain species of macroalgae (e.g., *Dictyosphaeria cavernosa*: Stimson *et al.*, 1996). The results of the present study correlate with the findings of previous investigations into the growth response of *Chaetomorpha linum* and other green macroalgae to temperature. For example, King *et al.* (1990) investigated the effect of thermal enhancement from the Tallawarra Power Station (located on the Lake Illawarra foreshore) on seagrass and macroalgal growth dynamics. They studied the effect of temperature on the growth in culture of *Chaetomorpha linum* (collected from Lake Illawarra) over a 10 day period. These authors determined that temperatures of 20°C and 25°C were optimal for the growth of *C. linum*, with relative growth rates up to 30 % WW d^{-1} recorded. Growth rates declined to approximately 20 - 22 % WW d^{-1}, at 15°C and 30°C, with death or loss of biomass occurring at 35°C. Similarly, Taylor *et al.* (2001) found that *Chaetomorpha linum* growth rates increased from 10°C (8.5 % WW d^{-1}), with the highest growth rates recorded at 15°C and 20°C (11.5 and 12 % WW d^{-1}, respectively), and subsequently declined at 25°C and 30°C (9 and 5 % WW d^{-1}, respectively). Fong and Zedler (1985; 1993) also found that the highest biomass of *Enteromorpha* spp. occurred between a temperature of 18 and 22°C (3.9 g dry wt·aquarium^{-1}), whereas biomass was significantly lower at 12°C (0.8 g dry wt·aquarium^{-1}) and 25°C (0.6 g dry wt·aquarium^{-1}). These authors noted that the highest temperatures (25°C) enhanced the growth of cyanobacterial mats.

In a similar investigation to the present study, Taylor *et al.* (2001) found that macroalgal growth in culture was significantly affected by both irradiance and temperature. All macroalgae examined (*Chaetomorpha linum, Cladophora dalmatica, Enteromorpha compressa, E. linza, Rhizoclonium tortuosum, Ulva curvata* and *U. rigida*) grew successfully from the lowest to the highest irradiances (9 to 175 μmol m^{-2} s^{-1}), suggesting the ability to survive over darker winter months, or shading within algal blooms. However, the highest growth rates of *Chaetomorpha linum, Enteromorpha compressa* and *Cladophora dalmatica* were recorded at the highest irradiance (175 μmol m^{-2} s^{-1}), suggesting these species would tolerate even higher irradiances. The effect of irradiance on *Chaetomorpha linum* was not examined in the present study, but is likely to have a significant effect on the growth of this alga in Lake Illawarra, and is thus recommended for future research. Taylor *et al.* (2001) also reported that all of the above nuisance algae were significantly affected by temperature; all species grew at the lowest temperatures of 10°C, and the highest growth rates of *E. compressa* (8 % WW d^{-1}) were recorded at this temperature, suggesting that these algae would have the ability to survive over winter at even lower temperatures. For other green algal species examined, growth rates increased substantially at 15 - 20°C and were considerably lower at 25°C and 30°C. While all algae tolerated and grew at the higher temperatures of 25°C and 30°C, these authors reported that growth slowed and tissue damage became evident towards the end of the 15-day incubation period.

Tissue damage and a subsequent decline in plant health is likely to occur at extreme temperatures, as evidenced by the yellowing and breakage of *C. linum* filaments and reduction in water contents at the highest and lowest temperatures of 10°C and 30°C, particularly in the N-deprived treatments. The final dry matter contents of *C. linum* were approximately 9 - 10 % in the N-replete treatments at 15 - 25°C, but increased to 11.5 % and 13.2 % at 30°C and 11°C, respectively. Dry matter contents were always marginally higher after two weeks of treatment without added N (compared to N-replete treatments), regardless of temperature. Similarly, Gordon *et al.* (1981) reported that nutrient enrichment had a significant effect on *Cladophora* sp. dry matter contents; DMC increased by 17-fold in DIP treatments (up to 8.1 μmol P L^{-1}) after 3 weeks, and by 5-fold in DIN treatments (up to 357 μmol N L^{-1}) after 2 weeks, compared to unenriched control treatments.

Nitrogen deficiency often leads to a reduction in photosynthetic pigments, or chlorophyll (Menendez *et al.*, 2002); this was observed in the unenriched and P-only treatments, as the *C. linum* filaments became substantially lighter in colour after 1 - 2 weeks without added N. *C. linum* cultured without added nitrogen had substantially thinner and lighter, yellowish-green filaments after 14 days. Treatments with both N and P and the N-only treatment had noticeably thicker and brighter green filaments than the treatments without nitrogen (Appendix 1G). Menéndez *et al.* (2002b) found that P-enrichment had no effect on the chlorophyll content of *Chaetomorpha linum*, whilst N-enrichment significantly increased the chlorophyll content after 4 days but chlorophyll was then reduced to initial concentrations after 10 days. These authors suggested this was due to *C. linum* utilising chlorophyll to take up and store excess nitrogen, but N was subsequently lost from the chlorophyll pool once the external nitrogen supply had been depleted.

Reduced pigmentation or bleaching is a common characteristic of many species of macroalgae when placed under stress. Many macroalgae species, however, will regain a healthy appearance and normal colouring upon return to optimal growth conditions (e.g., *Gracilaria tenuistipitata*: Lee *et al.*, 1999). Morgan and Simpson (1981b) determined that irradiance and temperature conditions strongly affected the pigmentation of *Palmaria palmata* (red seaweed); plants cultured at lower temperatures (6 - 14°C) and low irradiance were a deep purple-red colour, whereas plants cultured at low temperatures and higher irradiance levels were paler (reddish-brown). At 18°C, plants were dark red at all irradiance levels. Additionally, *P. palmata* cultured at 6 - 14°C had better apical development and thinner tips than plants grown at 18°C. In addition, these authors reported that the dry matter content of *P. palmata* ranged from 12.6 - 25.6 % and increased with increasing temperature (6 - 18°C) and increasing irradiance.

Rooney and Kalff (2000) noted that the effect of temperature on macrophyte growth can be difficult to evaluate in laboratory and/or field studies as temperature and light effects often

interact and tend to vary over the growing season. In addition, seasonal fluctuations may only result in marginal differences in water temperatures. Lobban and Harrison (1997) further noted that the optimum temperature for algal growth will vary according to numerous biological and environmental parameters, such as habitat, species, strain, age or growth stage, interactions with other organisms (e.g., bacterial or epiphytic production), availability of light, salinity variations or fluctuating day and night temperatures. Subsequently, the optimum temperature determined under laboratory conditions, where the alga is isolated and temperature and light are often kept constant, may be narrower than that determined for their natural habitat and may not give a true indication of *in situ* optima. Therefore, it is difficult to extrapolate the experimental data collected during laboratory studies to conditions in Lake Illawarra because growth in culture removes the natural fluctuations in, and interactions between, environmental and biological factors, such as light, temperature, salinity and nutrient cycling processes.

6.3.7 Conclusions

Experiments have shown that Lake Illawarra *Chaetomorpha linum* is strongly N dependent and is capable of growing excessively with minimal concentrations of P in the water column or stored in tissue. In all experiments, at both optimal and sub-optimal conditions for growth, considerable biomass was achieved over the 14 day experimental period. The ability of *C. linum* to grow successfully in culture under a range of nutrient treatments, and without added phosphorus, in particular, correlates with the excessive growth of this alga in Lake Illawarra. These culture studies also correlate with previous studies suggesting Lake Illawarra is strongly nitrogen limited (Qu, 2004a; Chapter 5). While a temperature of 25°C was found to promote short term bursts of the fastest growth, *C. linum* grew equally well at temperatures of 15 and 20°C, but growth declined slightly at 10 and 30°C. These results indicate that *C. linum* is able to persist and acclimate to the range of temperatures and seasonal variations in the Lake. *C. linum* is also a long-lived species; the alga was kept in an aquarium filled with low nutrient seawater for up to 6 months and still remained viable; this finding supports Lavery and McComb's (1991b) conclusion that *C. linum* is able to persist over winter and store nutrients for later use in summer when conditions are optimal for growth.

McClanahan *et al.* (2004), however, concluded that results obtained from growth experiments on isolated algae cultured in containers are not likely to be a useful indication of the response of those alga to field situations. Growth rates estimated from container experiments are likely to be far higher than growth rates in the field as laboratory experiments generally do not account for loss of biomass due to natural circumstances such as predation, physical disturbance or competition. Therefore, the experimental data collected on the effect of temperature and nutrients on *C. linum* should be used to demonstrate the potential for growth in the Lake, and give an indication of the conditions (e.g., an increase in temperature above 20°C) which are likely to promote excessive growth. The most important information obtained

in the present study was the reliance of *C. linum* on nitrogen; whilst the form of nitrogen (nitrate-N or ammonium-N) did not significantly affect growth, the lack of nitrogen in the culture media had the greatest impact on growth of this alga. Hence future management practices regarding Lake Illawarra macroalgae should focus on reducing or removing nitrogen inputs and sources in the Lake.

CHAPTER 7 Conclusions and Recommendations

The present study has examined the distribution, biomass and nutrient relationships of seagrasses and macroalgae in Lake Illawarra, NSW, and some of the key factors relating to the excessive growth of macroalgae in the Lake. This information will assist with the long-term management of macroalgal problems in Lake Illawarra. This chapter summarises the major findings and conclusions drawn from the research. Recommendations for future work are also discussed.

7.1 Conclusions

7.1.1 Description of Macrophytes in Lake Illawarra

During the present study of shallow-water macrophytes in Lake Illawarra (Chapter 4), 4 seagrasses and approximately 35 species of macroalgae were recorded and described. Seagrasses found in Lake Illawarra are *Zostera capricorni*, *Ruppia megacarpa*, *Halophila ovalis* and *Halophila decipiens.* The macroalgae included: 14 species from 7 genera of green macroalgae; 9 species from 9 different genera of brown macroalgae; and, 8 species from 8 genera of red macroalgae. These macroalgae included 7 new records for Lake Illawarra: *Bryopsis* sp., *Gracilaria edulis, Hypnea boergesenii, Centroceras clavulatum, Polysiphonia sphaerocarpa, Chondria angustissima* and *Grateloupia filicina*. This study is the first of its kind for Lake Illawarra and the descriptions and photographs presented will significantly aid future research regarding macroalgae in Lake Illawarra.

7.1.2 Biomass of Seagrasses and Macroalgae

The major findings of the macrophyte biomass studies (Chapter 5) are outlined below.

Seagrasses

Biomass of seagrasses, *Zostera capricorni* and *Ruppia megacarpa*, at four Lake Illawarra sampling sites showed a significant correlation with the sampling season, with biomass typically declining during winter and increasing in summer. Over the entire sampling period, the highest total seagrass (leaf and rhizome) biomasses were recorded at the Oasis Caravan Park (OCP); total *R. megacarpa* biomass ranged from 231 ± 24.8 g DW m^{-2} in winter, to 440 ± 26.5 g DW m^{-2} in spring/summer. The Nicolle Road site (NIC) had the lowest *R. megacarpa* biomass, with 54.8 ± 5.25 g DW m^{-2} in winter and 180 ± 12.1 g DW m^{-2} in spring/summer. Total biomass of *Z. capricorni* at Purry Burry Point (PBP) averaged 58.1 ± 6.38 g DW m^{-2} in winter and 226 ± 12.8 g DW m^{-2} in spring/summer. At Mullet Creek, total *Z. capricorni* biomass averaged 145 ± 15.3 g DW m^{-2} in winter and 230 ± 22.9 g DW m^{-2} in spring/summer.

Comparisons across all sampling periods (spring 2000 - winter 2002), showed that *R. megacarpa* leaf biomass was significantly higher than root/rhizome biomass at both the Oasis Caravan Park (OCP) and Nicolle Road (NIC) sites; *R. megacarpa* root/rhizome biomass typically comprised 20 - 45 % of the total plant biomass. At Purry Burry Point, *Z. capricorni* root/rhizome biomass typically comprised 40 - 44 % of the total seagrass biomass in all sampling rounds, except during winter 2001 where 67 % of the seagrass biomass was composed of roots-rhizomes. At Mullet Creek, *Z. capricorni* leaf biomass was statistically equivalent to root/rhizome biomass throughout the study period.

The highest (summer) seagrass biomasses recorded during the present study appear higher than those recorded by previous authors for Lake Illawarra (e.g., WBM, 2000; King *et al.*, 1997). These higher biomasses may be related to the sampling season (summer versus autumn), as well as differences between the sites used. In addition, the aim of the present study was to sample "healthy" seagrass beds, rather than a broad spectrum of seagrass beds found in the lake, in order to obtain the nutrient contents of actively growing plants.

Macroalgae

The biomass of macroalgae in Lake Illawarra appeared much lower in 2000 - 03 than in previous decades (refer to Section 4.2.6). These lower algal biomasses may be due to drought, as well as improvements in Lake Illawarra management practices and, therefore, water quality. The maximum biomasses of macroalgae recorded in the present study were at the Windang Peninsula sites, Oasis Caravan Park (150 g DW m^{-2}) and Primbee Bay (370 g DW m^{-2}), in spring 2000 and summer 2002, respectively. Macroalgal biomass at the Windang sites (OCP, NIC and PBP) dropped significantly after spring 2000, due to foreshore harvesting being conducted in the inshore area during December 2000.

While the amount of macroalgal biomass recorded over inshore sand flats and seagrass beds at each site varied significantly between sampling rounds, algal biomass was generally not dependent on the sampling season (winter versus summer). Biomass of epiphytic, benthic and free-floating macroalgae associated with *R. megacarpa* beds at the Oasis Caravan Park site varied from 17.5 ± 9.92 g DW m^{-2} in summer 2001, to 82.9 ± 45.4 g DW m^{-2} in winter 2001. At Nicolle Road, macroalgal biomass associated with the *R. megacarpa* beds ranged from 2.48 ± 1.08 to 33.7 ± 3.19 g DW m^{-2} over the sampling period. Macroalgae associated with *Z. capricorni* at Purry Burry Point increased significantly to 77.7 ± 10.0 g DW m^{-2} during summer 2001, but averaged 6 - 15 g DW m^{-2} during all other sampling rounds. At Mullet Creek, macroalgae over *Z. capricorni* beds appeared to decrease significantly over the sampling period, from 25.4 ± 9.48 g DW m^{-2} in summer 2001 to 0.84 ± 0.57 g DW m^{-2} in winter 2002.

Blooms of macroalgae were composed primarily of filamentous *Chaetomorpha* spp. (identified as *C. linum* and *C. billardierii*). Other bloom-forming algae included *Cladophora* sp. and *Ulva* spp., but biomasses of these genera were very low compared to *Chaetomorpha* spp.

7.1.3 Nutrient Analyses of Macrophyte Tissue

The major findings of the macrophyte tissue nutrient analyses (Chapter 5) are outlined below:

Average C, N and P contents

Across all Lake Illawarra sampling sites, the average nitrogen content of *Zostera capricorni* leaves and roots-rhizomes was 2.90 ± 0.07 % N and 1.24 ± 0.03 % N, respectively. Average phosphorus contents of *Z. capricorni* leaves and root/rhizomes were 0.33 ± 0.01 % P and 0.23 ± 0.01 % P, respectively. *R. megacarpa* leaves had slightly lower average N and P contents than *Z. capricorni*, with 2.36 ± 0.05 % N and 0.27 ± 0.02 % P. Average N and P contents of *R. megacarpa* roots-rhizomes were 1.73 ± 0.04 % N and 0.24 ± 0.02 % P, respectively. Tissue nutrient analyses were only conducted on macroalgae when sufficient biomass was present at each of the sites; the majority of macroalgae samples analysed were *Chaetomorpha linum* and *Chaetomorpha billardierii. Chaetomorpha* spp. had a similar average nitrogen content, but notably lower average P content than the seagrasses, with 2.82 ± 0.10 % N and 0.18 ± 0.01 % P, respectively. The average C/P (808 ± 65) and N/P (47.9 ± 3.47) molar ratios of macroalgae were typically double those of the seagrasses.

Spatial Variations in C, N and P

Seagrass leaf and rhizome N contents varied markedly between sampling rounds, but only limited variations occurred between sites in each sampling round. Seagrass leaf N contents at PBP and MC were significantly higher than OCP and NIC in winter 2001 and 2002, but fewer spatial trends were found in other sampling rounds. On the other hand, *R. megacarpa* rhizome N contents at OCP and NIC were higher than *Z. capricorni* rhizome N contents (PBP and MC) throughout the study period, but these differences were not always significant. Leaf P concentrations of *R. megacarpa* were significantly lower (by approximately 20 - 40 %) than *Z. capricorni* for the majority of the sampling period. In addition, *R. megacarpa* leaf P at OCP (range: 0.12 - 0.37 % P) was significantly lower than at NIC (range: 0.15 - 0.59 % P) in all sampling rounds, except summer 2001. These lower leaf P concentrations of *R. megacarpa* at OCP are probably due to utilisation of P to support the higher leaf biomasses found at that site.

Seasonal Variations in C, N and P

Total leaf C contents of *Z. capricorni* at PBP and MC increased marginally in winter, with a corresponding decrease in plant biomass. Similarly, *Z. capricorni* leaf N contents increased in

winter and decreased in summer, corresponding to decreased leaf biomass in winter, and increased leaf biomass in summer, respectively. This suggests that *Z. capricorni* stored N (or did not lose N) over winter, and that stored nutrients were utilized in summer to support growth. *Z. capricorni* rhizome C and N contents, however, did not appear to vary according to rhizome biomass. Seasonal effects were less pronounced with *R. megacarpa* at OCP and NIC, but the highest leaf N contents corresponded to the highest biomasses in summer. Seagrass leaf P at all sites peaked in summer 2002, corresponding to the highest seagrass biomass recorded during the study period; high tissue P contents may be related to the higher water column P concentrations typically recorded in summer.

7.1.4 Nutrient Limitation of Macrophytes

Tissue C, N and P contents and molar ratios of C:N:P were used to assess the nutrient limitation of macrophyte growth and/or biomass production in Lake Illawarra. Duarte (1990) suggested that seagrasses with tissue nutrient contents below 1.8 % N and 0.20 % P are strongly nutrient limited. According to that recommendation, the majority of *R. megacarpa* leaf samples collected from Lake Illawarra would be P limited (< 0.20 % P). All *R. megacarpa* and *Z. capricorni* leaf samples contained N contents higher than 1.8 % N, and would, therefore, not be described as N limited according to Duarte's (1990) recommendation. Fourqurean and Cai (2001) further suggested that, for seagrasses, N/P atomic ratios greater than 30 indicated P limitation, whereas N/P ratios less than 25 indicated N limitation. Following their recommendation, *R. megacarpa* would only have been P limited in spring 2000 and summer 2001 (N/P > 30). *Z. capricorni* leaves at Purry Burry Point and Mullet Creek typically had higher P contents than *R. megacarpa* leaves, and therefore N/P ratios did not exceed 30, but remained below 25 between winter 2001 - winter 2002, indicating limitation by N according to Fourqurean and Cai's (2001) recommendation.

In the present study, macroalgae generally had high tissue N contents, but low P contents. For example, the bloom-forming *Chaetomorpha billardierii*, collected from the Oasis Caravan Park, typically had N contents of 2.3 - 3.9 % N, but P ranged from 0.03 - 0.29 % P. Thus, the N/P molar ratios of all macroalgae sampled ranged from 6.7 - 151 (overall mean: 47.3 ± 3.7). Wheeler and Björnsäter (1992) estimated that N/P ratios in excess of 11 - 24 indicated P limitation in macroalgae, whereas N/P ratios less than 8 - 16 indicated N limitation. By this recommendation, 15 % of all Lake Illawarra macroalgae samples would be considered N limited (N/P ratio < 16), whereas 77 % of macroalgae would be considered P limited (N/P ratio > 24). However, caution must be exercised when using N/P ratios of macroalgae to indicate limitation, as green macroalgae, in particular, often have higher uptake rates and storage capacities for N than P; therefore, elevated N/P ratios may indicate limitation for P when it is not occurring (Fong *et al.*, 2003).

In summary, nutrient analyses showed high concentrations of N stored in the tissue of both seagrasses and macroalgae, which implied storage of N that exceeded immediate growth requirements. The concentrations of P in macroalgae and *R. megacarpa* were generally low, with respect to N, suggesting localised limitation by phosphorus. However, as previous studies have concluded that Lake Illawarra is strongly nitrogen limited (Qu, 2004a), it appears unlikely that these low P concentrations would significantly limit the biomass formation of macrophytes in Lake Illawarra.

7.1.5 Macrophyte Nutrient Pools

By combining total seagrass biomass (above and below-ground) and tissue nutrient data collected at each site, it was estimated that the dense *R. megacarpa* beds at the Oasis Caravan Park site contained 3.5 - 10.9 g N m^{-2}, and 0.32 - 1.33 g P m^{-2}. Sparse to medium density *R. megacarpa* beds at the Nicolle Road site contained 1.1 - 5.3 g N m^{-2}, and 0.1 - 0.9 g P m^{-2}. For *Z. capricorni*, the total nitrogen and phosphorus contents of seagrass beds (above- and below-ground biomass) were 2.8 - 5.2 g N m^{-2} and 0.3 - 1.0 g P m^{-2} at Mullet Creek, and 1.2 - 4.5 g N m^{-2} and 0.2 - 0.8 g P m^{-2} at Purry Burry Point.

If the four seagrass sites surveyed (OCP, NIC, PBP and MC) are considered representative of the entire Lake, the total nutrient pools bound to macrophyte biomass can be tentatively estimated. In summer 2000, the total area of *Z. capricorni* and *R. megacarpa* beds in Lake Illawarra was estimated at 5.65 and 1.67 km^2, respectively (WBM, 2000). Using the average N and P contents of the seagrasses described above, the nutrient pools associated with seagrasses in 2000 - 2002, are estimated to be 8,800 kg dry wt. N and 1,100 kg dry wt. P for *R. megacarpa*, and 21,000 kg dry wt. N and 2,800 kg dry wt. P, for *Z. capricorni.* These figures are averaged over summer and winter data and assume that a significant proportion of the seagrass coverage described in WBM (2000) is sustained by below-ground biomass when winter die-back of above-ground material occurs. The estimates of total seagrass (*Zostera* and *Ruppia*) nutrient pools ranged from 16,600 kg N and 2,200 kg P in winter, to 36,000 kg N and 4,400 kg P in summer.

For *R. megacarpa*, roots/rhizomes comprised 27 % and 31 % of the total nitrogen and phosphorus pools, respectively. Similarly, for *Z. capricorni,* below-ground material comprised 29 % and 38 % of the total nitrogen and phosphorus pools, respectively. Also considered here is the proportion of non-living seagrass leaves, estimated using biomass data described in Section 5.2 (mean: 15 g DW m^{-2}), and the average nutrient contents of non-living seagrass leaves collected during the present study (0.11 % P and 1.48 % N). As *Zostera* sheds its leaves periodically throughout the year, considerable biomass (i.e., “seagrass wrack”) accumulates along the Lake’s foreshore, but the average N and P contents of dead and decaying seagrass leaves are approximately half those of living seagrass leaves. These

seagrass nutrient pools are shown in relation to the Lake Illawarra nutrient budget in Figure 7-1.

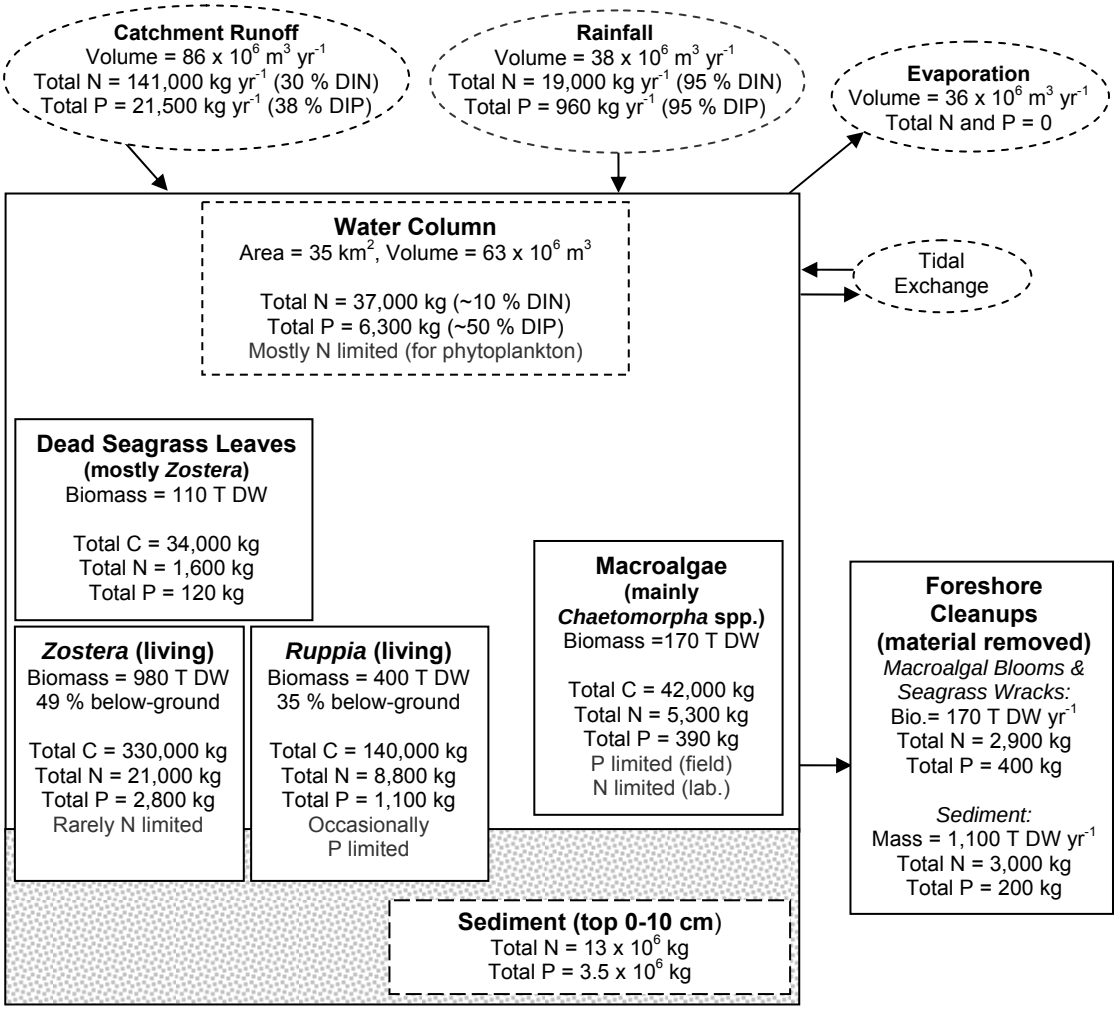


Figure 7-1: Averaged annual biomass (dry weight) and nutrient (total N and P) budgets for Lake Illawarra (see text for further details).

Macroalgal distribution and abundance in Lake Illawarra was highly variable throughout the study period, and thus it was difficult to quantify the pools of total nitrogen and phosphorus associated with algal biomass. At the Oasis Caravan Park, the largest macroalgal bloom occurred in spring 2000, covering 60 % of an approximately 20,000 m^2 area, with an average *Chaetomorpha* spp. biomass of 113 ± 31 g DW m^{-2}. Using the average N and P contents of *Chaetomorpha* spp. (3.76 ± 1.1 g N m^{-2} and 0.10 ± 0.03 g P m^{-2}, respectively), collected at OCP during spring 2000, the nutrient pool stored as macroalgal biomass at that time was estimated at approximately 75 kg dry wt. N and 2 kg dry wt. P.

If the proportion of macroalgal biomass (epiphytic and benthic) associated with seagrass beds during the present study is considered representative of the whole Lake, the average pools of nitrogen and phosphorus contained within macroalgae in seagrass areas were estimated at 5,100 kg dry wt. N and 375 kg dry wt. P, respectively. In addition, the area of Lake Illawarra

which may be subject to macroalgal blooms in sheltered bays was estimated at 0.53 km^2. Using the average macroalgal biomasses determined for inshore areas of the Lake calculated during the present study (~30 g DW m^{-2}), and the average N and P contents of macroalgae, the pools of nitrogen and phosphorus associated with inshore macroalgal blooms is estimated at 200 kg dry wt. N, and 12 kg dry wt. P, respectively. These algae-bound nutrient pools in the areas inshore of the seagrass beds are subject to wide variability throughout the year, depending on the magnitude of algal blooms, and the mechanical removal of algal biomass by the Lake Illawarra Authority.

It is estimated that the total pools of nitrogen and phosphorus contained within Lake Illawarra macrophytes (macroalgae, living and non-living seagrass biomass) during the present study were approximately 36,000 kg N and 4,400 kg P. The pools of N and P in the water column were calculated using the area of Lake Illawarra (35 km^2), the average water depth (1.8 m) (LIA, 1995), and the average total nitrogen and total phosphorus contents in the water column, between May 2005 and February 2007 (0.59 mg N L^{-1} and 0.10 mg P L^{-1}, respectively: Lake Illawarra Authority, pers. comm., 2007); this results in N and P pools of 37,000 kg N and 6,300 kg P. Evaporation for Lake Illawarra was estimated at 36 x 10^6 m^3 yr^{-1} (using 1,040 mm yr^{-1}: Miller, 1998), but loss of N or P from the system via evaporation is considered to be zero (Qu, 2004a). Rainfall was estimate at 38 x 10^6 m^3 yr^{-1}, using the average annual rainfall for Albion Park and Dapto (1,090 mm yr^{-1}: Section 1.5.3). The inflow of nutrients via rainfall was estimated using the method of Miller (1998, and references therein), using average total nitrogen and phosphorus concentrations of 0.5 mg N L^{-1} and 0.025 mg P L^{-1}, respectively. The loads of total nitrogen and phosphorus in catchment runoff were estimated at 141 x 10^3 kg N yr^{-1} and 21.5 x 10^3 kg P yr^{-1} (LIA, 1995; Miller, 1998). The effect of tidal exchange on the Lake's nutrient budget is uncertain due to recent construction works undertaken to maintain a more permanently open entrance to the sea (see Section 6.2).

It appears from these calculations that the pools of N and P in Lake Illawarra macrophytes are of the same magnitude of those in the water column. It must be noted, however, that these nutrient pools are insignificant when compared to those contained within Lake Illawarra sediment. Sediment nutrient pools were estimated for the top 10 cm of sediment, using the average N and P contents (0.29 % N and 0.078 % P) and sediment bulk density (1,300 kg m^{-3}) reported for Lake Illawarra (Yassini, 1994); this gives sediment nutrient pools of 13 x 10^6 kg N and 3.5 x 10^6 kg P. The foreshore cleanups conducted by the Lake Illawarra Authority during the present study removed a significant proportion of the macroalgal biomass and seagrass wracks accumulating along the inshore areas. It was estimated that approximately 600 kg of phosphorus and 6,000 kg of nitrogen were removed during foreshore cleanups, which may significantly affect the plant nutrient budget. The bulk of the material removed during this process, however, was sediment; the sediment-bound nutrients removed were minute when

compared to the Lake's sediment nutrient pools and are unlikely to affect the sediment nutrient budget.

In the present study, the seagrasses, *Zostera capricorni* and *Ruppia megacarpa*, were considered to be a more important sink for nutrients than macroalgae in Lake Illawarra. Qu (2004a) determined that Lake Illawarra is a net sink for nitrogen; using LOICZ budgets, he estimated there was a net sink for dissolved inorganic nitrogen in the Lake, removing 3003 x 10^3 mol N yr^{-1} (42,000 kg N yr^{-1}). This DIN sink is within the same order of magnitude as the pools of macrophyte-bound nitrogen (36,000 kg N) calculated during the present study; the macrophyte beds in Lake Illawarra, therefore, are likely to account for a significant proportion of the nitrogen sink calculated by Qu (2004a).

7.1.6 $\delta^{13}C$ and $\delta^{15}N$ Contents of Macrophytes

Across all sites, the $\delta^{13}C$ contents of seagrasses ranged from -7.7 to -15.9 ‰ (mean ± s.e.: -11.5 ± 0.16 ‰) for leaves, and -7.7 to -13.8 ‰ (mean ± s.e.: -10.9 ± 0.11 ‰) for roots-rhizomes. The $\delta^{15}N$ contents of seagrasses across all Lake Illawarra sites ranged from 1.0 - 9.0 ‰ (mean ± s.e.: 4.2 ± 0.16 ‰) for leaves, and 0.7 - 7.5 ‰ (mean ± s.e.: 3.4 ± 0.16 ‰) for roots-rhizomes. The $\delta^{13}C$ and $\delta^{15}N$ contents of macroalgae recorded across Lake Illawarra were more variable than those of the seagrasses, and ranged from -4.9 to -19.8 ‰ (mean ± s.e.: -12.6 ± 0.36 ‰) and 1.8 - 14.6 ‰ (mean ± s.e.: 8.9 ± 0.33 ‰), respectively.

Z. capricorni leaf and rhizome samples collected from the Mullet Creek site exhibited the greatest seasonal variability in $\delta^{13}C$ contents, with leaf $\delta^{13}C$ contents being more negative (by approximately 3 delta units) in summer, than in winter. In addition, $\delta^{13}C$ contents of *Z. capricorni* leaf and rhizome samples at Mullet Creek were significantly more negative than the Windang Peninsula sites (PBP, OCP and NIC); these differences are most likely due to the proximity to fresh water input, compared to the eastern Lake Illawarra sites, which are closer to the Lake's entrance to the sea.

The highest $\delta^{15}N$ values were recorded for *R. megacarpa* (6.7 - 8.9 ‰) and *Chaetomorpha linum* (11.1 - 14.4 ‰) at the Nicolle Road site in spring 2000 and summer 2001. These high $\delta^{15}N$ contents, however, were not replicated in later surveys, which may have been due to the NIC sampling site being located at a greater distance from the Nicolle Road storm water drain in later sampling rounds. High $\delta^{15}N$ values in macrophyte tissue have been reported from areas subject to eutrophication, or those receiving waste waters, and may also be related to denitrification processes in marine and estuarine waters; denitrification leads to the loss of ^{14}N, and an enrichment of ^{15}N, from the inorganic N pool in the water column, and a subsequent enrichment of ^{15}N in the plants taking up that inorganic N from the water (Fourqurean *et al.*, 1997).

7.1.7 Response of *C. linum* to Nutrient Enrichment and Temperature

Culture experiments on *Chaetomorpha linum*, the dominant alga in Lake Illawarra (Chapter 6), showed that this alga is strongly N dependent and is capable of growing excessively with minimal concentrations of P in the water column or stored in tissue. A significant increase in biomass occurred in all treatments, at both optimal and sub-optimal conditions for growth. The ability of *C. linum* to grow successfully in culture, under a range of nutrient treatments, and without added phosphorus, in particular, correlates with the excessive growth of this alga in Lake Illawarra.

Enrichment with nitrogen, rather than phosphorus, had the greatest effect on growth of *C. linum*; growth was significantly reduced in all treatments without added nitrogen. In all experiments conducted, there were generally no significant differences between growth rates of *C. linum* in the P-only and unenriched control treatments. *C. linum* maintained growth at rates of 10 % WW d^{-1} in unenriched and P-only treatments, but growth in N-only treatments averaged 15 - 20 % WW d^{-1}. For the majority of experiments conducted, growth rates in the N-only treatment, although slightly less, were often not significantly different to those in the N + P treatments, and were always significantly higher than N-deprived treatments. *C. linum* responded equally well to a nitrogen source of nitrate-N or ammonium-N, but growth rates in the P + NO_3^- were fractionally higher than in P + NH_4^+ treatments, even though the nitrogen concentration was the same. When compared to unenriched control treatments, enrichment with 50 -100 μM nitrate-N increased *C. linum* growth rates by 1.3 - 1.4-fold, whereas enrichment with 50 - 100 μM ammonium-N increased growth rates by 1.3-fold. Overall, these experiments indicated that *C. linum* was far less reliant on P supply, than N supply.

A temperature of 25°C was found to promote short term bursts of the fastest growth (up to 27 % WW d^{-1}), but *C. linum* grew equally well at temperatures of 15 and 20°C, and growth declined slightly at 10 and 30°C. This optimal temperature range (20 - 25°C) for growth of *C. linum* correlates with water temperatures in Lake Illawarra measured during spring to late summer. *C. linum* growth rates at 10°C averaged 13 - 15 % WW d^{-1} in the N + P treatments, suggesting that this species also has the ability to persist at the lowest temperatures (10 - 15°C), recorded in the Lake during winter. These results indicate that *C. linum* is able to persist and acclimate to the range of temperatures and seasonal variations in the Lake.

Growth rates estimated from enrichment experiments are likely to be far higher than growth rates in the field, as laboratory experiments generally do not account for loss of biomass due to natural circumstances, such as predation, physical disturbance or competition (McClanahan *et al.*, 2004). Therefore, the experimental data collected on the effect of temperature and nutrients on *C. linum* should be used to demonstrate the potential for growth in the Lake, and give an indication of the conditions (e.g., an increase in temperature above 20°C) which are likely to promote excessive growth. The most important information obtained in the present

study was the reliance of *C. linum* on nitrogen; whilst the form of nitrogen (nitrate-N or ammonium-N) did not significantly affect growth, the lack of nitrogen in the culture media had the greatest impact on growth of this alga. Hence, future management practices regarding Lake Illawarra macroalgae should focus on reducing or removing nitrogen inputs and sources in the Lake.

7.2 Recommendations for Future Work

In the present study, differences between seagrass biomass and nutrient contents at each of the study sites may have been related to varying availability of nutrients at each site. The highest seagrass (*R. megacarpa*) and macroalgal biomasses often occurred at the Oasis Caravan Park (OCP) site. These higher macrophyte biomasses at the OCP site may be related to influx of nutrients via the storm water drain located at the southern edge of the caravan park. Groundwater seeping into the Lake from below the Windang golf courses has also been suggested as a possible source of nutrients along the Windang Peninsula (I. Yassini, Wollongong City Council, pers comm., 2003). Additionally, nutrients in groundwater may be derived from the decomposition of organic matter at the former landfill site located in Primbee (I. Yassini, pers comm., 2005). A higher availability of nutrients, particularly nitrogen, at the OCP site is likely to influence the higher macrophyte biomasses found there; therefore, the source of nitrogen at the OCP site requires further investigation.

As macroalgal mats act as a sink for nutrients, mechanical harvesting of macroalgae is one method of removing nitrogen and phosphorus from Lake Illawarra and reducing the recycling of nutrients back into the system during decomposition of the algal mass. However, macrophyte harvesting may result in a further input of nutrients to the water column by disturbing sediment and resuspending organic matter which could, in turn, result in a reduction in dissolved oxygen (Calado and Duarte, 2000). The original aim of the present study was to investigate the impact of macroalgal harvesting on the ecology of Lake Illawarra. However, as macroalgal biomass in Lake Illawarra was generally low throughout the duration of this study, macroalgal harvesting did not take place. Therefore, the impact of macroalgal harvesting in Lake Illawarra should be investigated if this management practice takes place in future.

Dredging has also been suggested as a potential solution to the Lake Illawarra nutrient problem. Removing sediment-bound nitrogen and phosphorus will reduce the availability of these nutrients for algal growth, although it is highly likely that seagrass beds would be damaged by dredging. In the short-term, dredging may lead to a reduction in water transparency, an increase in sedimentation, and release of sediment-bound nutrients or contaminants, such as heavy metals (Erftemeijer and Robin Lewis, 2006). Macrophyte beds often recover from disturbance, but the recovery process can take several years. For example, following dredging in Tuggerah Lakes, NSW, macrophytes had recolonised the shallowest (1

m depth) areas within 4 months, but had not re-established in the deepest (> 1.4 m depth) areas 12 months after dredging (Collett *et al.*, 1981). Foreshore cleanups were conducted by the Lake Illawarra Authority a number of times along the eastern Lake Illawarra (Windang) peninsula, during 2000-01 (Section 5.3.10). This process involved the use of heavy machinery to remove floating organic material and the top-most surface layer of sediment along the inshore zone of the Windang Peninsula. Analyses showed that this material was composed of 20 - 30 % plant biomass ("seagrass wrack" and macroalgae) and 70 - 80 % sediment. This practice was particularly damaging to seagrass beds in the inshore area, and those seagrass areas removed due to foreshore cleanups at the Oasis Caravan Park and Nicolle Road sites in 2000-01 had not recovered by 2004. If practices such as the foreshore cleanups conducted during the present study are continued, it is recommended that the impact of these practices on seagrass beds and the Lake's ecology are further assessed.

Other management practices in Lake Illawarra have included substantial works programs to keep the Lake's entrance permanently open. However, while increasing the loss of nutrients to the ocean via the Lake's entrance is desirable, tidal exchange is unlikely to affect those areas where algal growth is most prolific (Yassini, 1986). In addition, Smith *et al.* (2003) consider that in many estuaries, particularly those with long residence times (> 15 - 20 days), about 95 % of nutrients (DIN and DIP) are retained or assimilated within the estuary. Although the opening of the Lake's entrance may reduce the residency time to 30 - 50 days, the majority of available nutrients will still be assimilated within the Lake (Morrison and West, 2004). A long-term solution to the Lake Illawarra nutrient problem is to employ appropriate catchment management procedures and ultimately reduce the influx of nutrients from the catchment. In addition, the area of seagrass beds in Lake Illawarra may fall if the Lake's entrance to the sea remains permanently open and is more affected by tidal fluctuations. Under conditions of limited freshwater inflow and shoaled entrance, average water levels in the Lake may be 0.20 - 0.25 m higher than sea level (LIA, 1995); this mean water level is likely to drop with a permanently open entrance. Seagrass areas, therefore, may be exposed when water levels drop due to tidal exchange or limited freshwater inflow, as documented in 2003 (see, e.g., Appendix 1C). An increase in salinity due to higher saltwater inflow may also have a deleterious effect on macrophytes in Lake Illawarra (see, e.g., Martins *et al.*, 1999).

The potential impact of climate change on macrophyte beds in Lake Illawarra also requires further examination; these impacts may include: rising sea level, higher surface and water temperatures, increased periods of drought followed by more intense storms, flooding and soil erosion, variability in oceanic currents, increased dissolved CO_2 concentrations and changes in oceanic pH (IPCC, 2007; CSIRO, 2007). Reduced rainfall (and a subsequent reduction in the amount of nutrients entering waterways via runoff) during drought periods should result in a reduction in the scale and biomass of nuisance macroalgal blooms occurring in the Lake, compared to previous decades. An increase in extreme flood intensity (CSIRO, 2007),

however, could result in higher pulses of nutrients and sediment being flushed into the Lake, enhancing eutrophication and nuisance algal growth, and resulting in the smothering of beneficial aquatic macrophytes, such as seagrasses. Decline in aquatic plants has important implications for loss of fish and crustacean habitat and juvenile nursery areas in Lake Illawarra. In addition, a decline in macroalgae and seagrass in the Lake would result in less biomass being available to act as a sink for C, N and P, leaving more nutrients available for uptake by phytoplankton and potentially toxic cyanobacteria (blue-green algae). Some aspects of climate change may be beneficial to aquatic plants; for example, increasing dissolved CO_2 concentrations (CSIRO, 2007) may enhance photosynthesis and growth in seagrasses and macroalgae (see, e.g., Section 2.3.2), but rising water temperatures, reduced oceanic pH and altered nutrient regimes may limit their biomass and distribution. A rise in sea surface temperature of about 0.5 - 2.5°C by 2070 (CSIRO, 2007; IPCC, 2007), for example, may affect the distribution of sensitive aquatic macrophytes, but is unlikely to greatly affect the abundance of opportunistic macroalgae. Bloom-forming macroalgae, such as *Chaetomorpha*, are highly adaptive and broadly tolerant of temperatures ranging from 10 - 25°C. Water temperatures above 30°C, however, are likely to exceed the tolerance threshold of many submerged macroalgae and seagrasses in the Lake, leading to photoinhibition, cell damage and biomass decline (see, e.g., Section 6.3.6).

The present study highlighted the importance of nitrogen and, to a less extent, water temperature, in limiting the biomass formation of Lake Illawarra *Chaetomorpha linum.* Preliminary investigations also indicated that light levels had a significant effect on the growth of *C. linum*; for example, an increase in the light to day photoperiod (from 12:12 to 16:8 hours) resulted in a substantial reduction in growth rates, and ultimately death of the alga. Recently, Davis and Koop (2006) suggested that stratification of the water column and the availability of light, rather than nutrients, are the key factors triggering algal blooms in enclosed freshwater bodies of southeastern Australia. Nutrient limitation, however, ultimately restricts the biomass of those blooms. With works being undertaken to keep the Lake's entrance to the sea permanently opened, water levels will fluctuate tidally and seasonally, which may subsequently result in variations in irradiance, particularly in the shallowest areas of the Lake where macroalgal blooms occur. High irradiances (e.g., during summer or at low tide) have a deleterious effect on productivity and growth of both seagrasses and macroalgae. Very high irradiance may result in photoinhibition, a mechanism that reduces photosynthetic rates, damages electron-transport proteins and impairs photophosphorylation (Touchette and Burkholder, 2000b). Therefore, the response of Lake Illawarra seagrasses and macroalgae to variations in light, tidal exchange, increasing salinity and fluctuating water levels should also be examined.

7.3 Summary

The information presented here has made a significant contribution to the understanding of seagrass and macroalgal growth, biomass and distribution in Lake Illawarra. Some of the key factors responsible for excessive growth of macroalgae in the Lake have been outlined, including the response to temperature, as well as nutrient enrichment and deprivation. This information will assist with the long-term management of macroalgal problems in Lake Illawarra.

CHAPTER 8 References

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APPENDICES

Appendix 1: Additional photographs of sampling sites and experimental apparatus.

Oasis Caravan Park (OCP) Sampling Site

Appendix 1A: Macroalgal bloom (*Chaetomorpha* sp.) near the Oasis Caravan Park, Lake Illawarra (4/12/00).

Oasis Caravan Park (OCP) Sampling Site

Appendix 1B: View from the Oasis Caravan Park: pre-drought, January 2002. The *Ruppia* bed sampling site can be seen in the middle-ground.

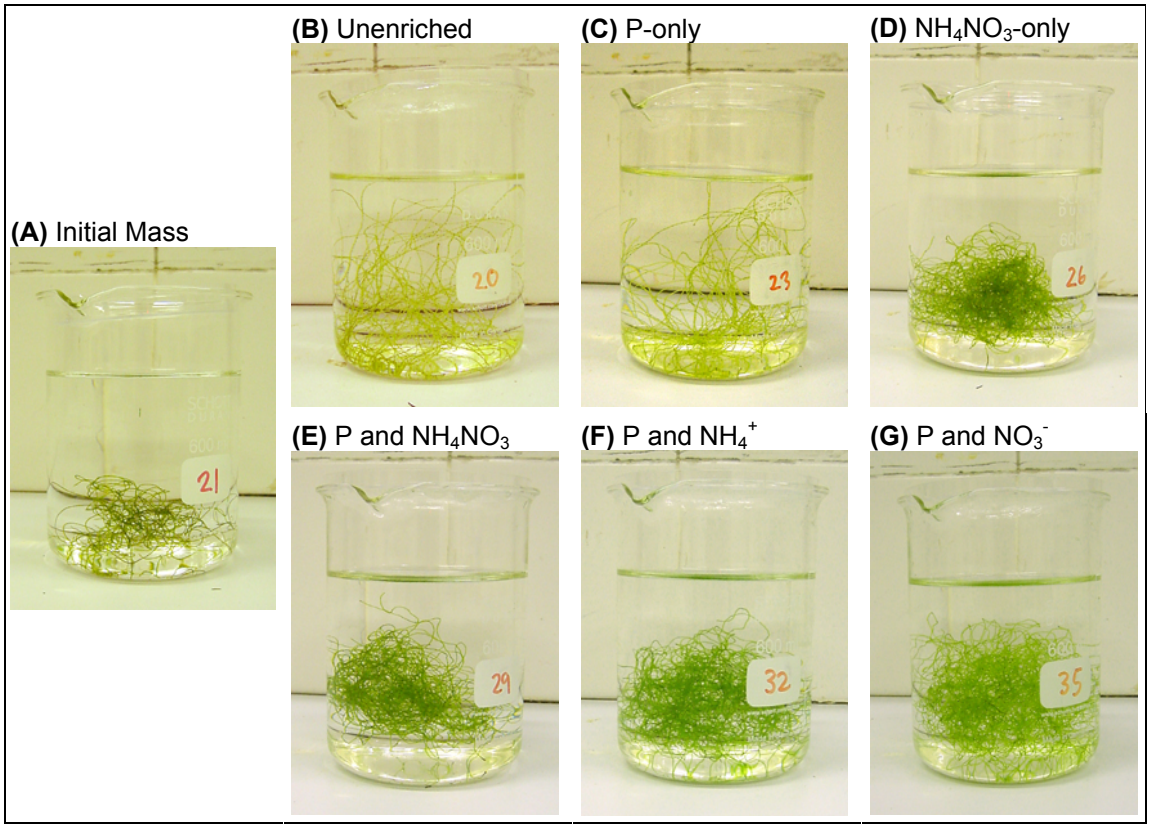
Appendix 1C: View from the Oasis Caravan Park: during drought conditions, January 2003. The reduced water-level completely exposed the *Ruppia* bed sampling site.

Appendix 1D: Machinery scraping sediment and organic material from the Lake shore, December 2000.

Appendix 1E: Stockpiles of cleanup material stored on the Windang foreshore, July 2001.

Growth experiments conducted on *Chaetomorpha linum*

Appendix 1F: *Chaetomorpha linum* growth experiments **(A)** and experimental apparatus **(B)** within the refrigerated incubator.



Appendix 1G: *Chaetomorpha linum* in various nutrient treatments, after incubation for 14 days at 20°C. **(A)** Typical starting mass after harvesting on Day 7. **(B-G)** *C. linum* masses grown between harvesting on Day 7 and Day 14.

Appendix 2: Glossary of taxonomic terms used in Chapter 4

(after Millar, 1990; Womersley, 1984, 1987, 1994, 1998, 2003)

Axillary: situated at an angle from the axis.

Bracts: leaf-like structures, unlike the normal foliage.

Cortical: the outer layer of cells of a thallus, usually of smaller cells.

Costate: with a midrib or central thickening of the branch.

Crustose: forming a thin crust over the substrate.

Cystocarps: the reproductive vesicle-like structures in red macroalgae, which contain the carposporophyte and the pericarp (surrounding tissue).

Dichotomous: divided into two distinct parts.

Digitate: branches resembling the fingers of a hand.

Dioecious: the male and female flowers are borne on separate plants.

Distromatic: a cross-section shows two cell layers.

Drift: refers to plants found free-floating or entangled amongst macrophyte beds, moved by wind or currents.

Ebracteate: without bracts.

Ecorticate: without a cortex (the outer layer of small cells of a thallus).

Elliptical: in the shape of an ellipse (e.g., egg-shaped).

Epilithic: attached to a hard substrate (e.g., rocks).

Entire: not serrated.

Gametophytic: the gamete-producing phase of a life history.

Gametangial branches: the sex organs which contain the gametes.

Glabrous: without hairs.

Globose: spherical or globular in shape.

Habit: refers to the characteristic or morphological form of a plant.

Holdfast: an attaching cell or organ, typically at the base of the plant.

Involucrate: basally surrounded by cortical filaments of a few cells.

Involute: rolled inwards.

Lanceolate: tapering from a rounded base to an apex.

Medulla: central region of the axis, internal to the cortex.

Monoecious: the male and female flowers may be borne on the same plant.

Monostromatic: single layered - a cross-section shows only one cell layer.

Mucilaginous: slimy, with surface mucilage.

Mucronate: (mucro) a short, sharp, pointed structure.

Mutic: without a terminal point.

Oblong: with a rounded apex and base.

Obovate: where the top of the leaf may be broader than the base (opposite of ovate).

Oligosiphonous: with four pericentral cells.

Ovate: egg-shaped, with a broader base.

Parenchymatous: the tissue of the thallus, composed of thin-walled cells of roughly equal dimensions, formed by cell division in different planes.

Pedicellate: growing on a cellular stalk.

Periaxial cell: surrounding an axial cell.

Petiole: the stalk of a leaf.

Petiolate: stalked leaves or vesicles.

Phaeoplast: the photosynthetic plastid of brown algae.

Plurilocular: with many cells, each cell containing a single spore.

Pseudoparenchymatous: resembling a parenchymatous structure, due to compactly interwoven filaments, but cell division has not occurred in all planes.

Pyrenoid: a distinguishable region of the chloroplast, associated with carbohydrate synthesis.

Ramelli: (singular = ramellus) lesser or ultimate branchlets.

Receptacle: the branch which holds the reproductive organs.

Rhizoid: a single-celled or few-celled filament, used for attachment or absorption.

Serrate: marginally toothed.

Sessile: without a stalk.

Sori: (singular = sorus) cluster of reproductive organs, occurring as a surface patch or raised group.

Spadix: (spadices) a spike-like structure, usually enclosed in a spathe (i.e., the large bracts surrounding an inflorescence).

Sporophytic: the spore-producing phase in the life history of a plant.

Stipe: the stalk, between the holdfast and the fronds, or bearing primary branches.

Subdichotomous: mostly dividing into two fairly equal sections near the apices.

Terete: cylindrical, but usually slightly tapering towards both ends.

Tetrasporangia: a meiosporangium (i.e., structures for spores formed as a result of meiosis) with four spores, usually in a distinctive arrangement.

Trichoblasts: the hair-like filaments produced near the branch apices.

Uniseriate: with the cells are arranged in a single row, and no more than one-cell wide.

Utricles: the swollen, terminal ends of cortical branches that form the outer layer of tissue in *Codium.*

Vesicular: with vesicles - sac-like structures, partly gas-filled, used for flotation, especially in kelps.

APPENDICES 3 - 7:

Data Collected at Lake Illawarra Sampling Sites, 2000 - 2002

Appendix 3: Water quality parameters at four Lake Illawarra seagrass sites, 2000 - 2002.

Season	Seagrass	Site*	Temp. (°C)	pH	Salinity (ppt)	DO (% Sat)	DO (mg/L)	Turbidity (NTU)
2000, Spring	*Ruppia* beds	OCP	23.3	8.4	29.8	88.7	5.3	3.2
		NIC	22.9	8.4	29.8	125.2	7.6	5.0
	Zostera beds	PBP	22.3	8.3	29.7	93.3	5.7	7.5
		MC	21.5	7.8	29.4	72.0	4.5	11.4
2001, Summer	*Ruppia* beds	OCP	27.9	7.8	28.3	118.2	7.9	1.7
		NIC	24.9	7.8	30.6	74.6	5.2	2.8
	Zostera beds	PBP	25.5	7.6	31.1	100.0	6.9	6.5
		MC	24.0	7.5	30.2	62.7	4.4	9.8
2001, Winter	*Ruppia* beds	OCP	16.1	8.1	28.1	99.3	8.3	3.4
		NIC	16.2	8.1	28.0	102.8	8.6	1.6
	Zostera beds	PBP	17.0	8.1	28.4	105.7	8.8	5.3
		MC	15.6	8.1	28.0	114.2	9.6	1.4
2002, Summer	*Ruppia* beds	OCP	21.8	8.8	37.8	78.3	5.5	1.8
		NIC	22.3	8.6	36.2	110.5	7.8	2.8
	Zostera beds	PBP	26.2	8.7	36.6	150.4	10.5	2.0
		MC	24.5	8.7	33.1	133.6	9.1	1.2
2002, Winter	*Ruppia* beds	OCP	13.4	8.1	29.3	140.3	9.6	3.4
		NIC	12.5	8.4	28.8	93.3	6.8	4.1
	Zostera beds	PBP	14.0	8.1	29.7	113.5	9.8	6.3
		MC	15.2	7.3	27.9	74.9	5.7	12.3

* Water depth = 0.5 - 0.6 m.

Appendix 4: Wet weight biomass of macrophytes at six sites, Lake Illawarra, 2000 - 2002.

	Biomass:	*Seagrass Beds* (g WW m^{-2})					*Inshore Sand Flats* (g WW m^{-2})		
Site	**Season**	**Seagrass Leaves**	**Seagrass Rhizomes**	**Macro-algae**	**Leaf Litter**	**Total Biomass**	**Macro-algae**	**Leaf Litter**	**Total Biomass**
Oasis Caravan Park (OCP) - *Ruppia*	2000, Spring	2598 ± 131	1489 ± 169	226 ± 77	190 ± 88	4313 ± 280	1995 ± 305	191 ± 32	2186 ± 326
	2001, Summer	2598 ± 38	1395 ± 112	165 ± 99	80 ± 16	4159 ± 214	99 ± 29	10 ± 4	110 ± 32
	2001, Winter	1050 ± 227	309 ± 65	497 ± 215	439 ± 148	1918 ± 433 *	562 ± 60	75 ± 24	637 ± 63
	2002, Summer	1970 ± 289	928 ± 174	195 ± 78	80 ± 35	3092 ± 471	276 ± 115	81 ± 22	357 ± 117 #
	2002, Winter	1391 ± 251	737 ± 121	217 ± 39	71 ± 31	2345 ± 204	200 ± 28	21 ± 8	221 ± 34
Nicolle Road (NIC) - ***Ruppia***	2000, Spring	1268 ± 99	686 ± 46	112 ± 47	68 ± 33	2134 ± 132	510 ± 77	34 ± 8	544 ± 81
	2001, Summer	924 ± 83	522 ± 61	308 ± 23	49 ± 23	1803 ± 146	0	1 ± 0.6	1 ± 1
	2001, Winter	231 ± 36	223 ± 29	135 ± 32	28 ± 16	618 ± 86	102 ± 43	50 ± 16	152 ± 54
	2002, Summer	718 ± 205	474 ± 126	201 ± 103	5 ± 3	1398 ± 203	6 ± 4	3 ± 3	102 ± 51
	2002, Winter	249 ± 41	205 ± 28	26 ± 12	34 ± 16	514 ± 93	3 ± 2	0	3 ± 2
Lake Illawarra Village (LIV)	2000, Spring	-	-	-	-	-	344 ± 35	42 ± 10	389 ± 36 $
Purry Burry Point (PBP) - *Zostera*	2000, Spring	1268 ± 109	676 ± 87	70 ± 21	124 ± 31	2443 ± 300 $	627 ± 53	158 ± 32	786 ± 75
	2001, Summer	1280 ± 118	769 ± 41	637 ± 110	63 ± 8	2749 ± 226	0	17 ± 6	17 ± 6
	2001, Winter	163 ± 52	356 ± 72	136 ± 49	18 ± 10	672 ± 115	58 ± 35	41 ± 25	134 ± 67 ^
	2002, Summer	1049 ± 140	870 ± 138	46 ± 14	326 ± 140	2379 ± 220 $	458 ± 137	564 ± 224	1022 ± 147
	2002, Winter	260 ± 41	203 ± 30	91 ± 30	31 ± 14	585 ± 41	49 ± 14	8 ± 3	57 ± 13
Mullet Creek (MC) - *Zostera*	2000, Spring	742 ± 49	615 ± 65	211 ± 77	104 ± 36	1671 ± 187	173 ± 33	6 ± 3	179 ± 32
	2001, Summer	1286 ± 251	1081 ± 24	237 ± 39	56 ± 28	2660 ± 287	1 ± 1	1 ± 1	2 ± 2
	2001, Winter	486 ± 92	599 ± 70	72 ± 37	28 ± 15	1185 ± 145	21 ± 5	0	21 ± 5
	2002, Summer	1442 ± 246	1151 ± 220	30 ± 24	322 ± 117	2945 ± 491	212 ± 39	194 ± 25	406 ± 43
	2002, Winter	722 ±127	702 ± 85	7 ± 5	58 ± 23	1490 ± 186	41 ± 6	0	41 ± 6
Primbee Bay (PRIM) *Zostera*	2000, Spring	119 ± 39	57 ± 24	-	-	-	2306 ± 104	263 ± 190	2745 ± 238
	2002, Summer	0	0	-	-	-	2560 ± 293	0	2560 ± 293

Note: Total macrophyte biomass includes: * *Zostera capricorni*; $ *Ruppia megacarpa*; # *Halophila decipiens*; ^ *Halophila ovalis.*

Appendix 5: Wet to dry biomass ratios of *Zostera capricorni* and *Ruppia megacarpa* at four Lake Illawarra sites, 2000 - 2002.

Season	Oasis Caravan Pk.		Nicolle Road		Purry Burry Pt.		Mullet Creek	
	Ruppia Leaves	*Ruppia* Rhiz.	*Ruppia* Leaves	*Ruppia* Rhiz.	*Zostera* Leaves	*Zostera* Rhiz.	*Zostera* Leaves	*Zostera* Rhiz.
2000, Spring	9.5	9.3	9.7	9.2	11.8	8.1	7.8	8.6
	9.7	9.3	9.6	9.1	11.6	8.8	9.0	9.4
	9.4	9.3	9.8	9.4	11.4	9.0	10.1	7.0
	9.9	9.1	10.2	9.0	10.4	8.5	10.3	6.7
	9.5	8.7	9.9	8.7	9.8	8.9	9.0	8.9
	9.3	7.3	10.0	9.4	9.7	9.8	10.0	7.6
2001, Summer	9.7	9.5	10.4	11.8	11.6	7.8	10.7	9.4
	9.6	9.8	8.5	11.3	11.6	8.7	11.3	9.6
	8.4	9.2	10.0	11.2	10.0	8.9	9.8	9.3
2001, Winter	6.9	8.1	7.9	9.7	7.9	9.2	9.2	9.5
	7.5	8.3	7.6	9.2	7.1	7.8	9.3	8.8
	7.2	7.8	8.2	9.0	7.3	8.1	8.1	7.4
	6.7	7.4	7.5	8.8	7.8	7.6	7.1	7.8
	7.9	8.3	7.5	9.6	8.9	9.1	9.4	9.8
	7.8	8.4	7.0	8.1	6.9	7.7	9.5	8.7
2002, Summer	6.2	7.2	6.2	8.2	8.4	8.7	7.5	7.8
	7.1	7.8	6.6	6.7	9.2	9.2	9.7	8.5
	6.1	6.8	6.9	8.1	9.1	9.6	10.0	7.9
	6.3	7.2	8.4	9.3	8.9	8.9	9.1	7.7
	5.8	6.0	6.0	6.2	8.9	8.0	9.9	7.5
	6.4	7.2	5.6	5.7	9.0	8.4	8.2	7.7
2002, Winter	7.0	8.1	7.8	9.2	7.6	9.0	9.2	9.0
	7.6	8.2	7.7	9.3	7.9	9.0	9.8	9.0
	7.4	8.9	8.0	8.7	9.0	9.0	8.9	8.3
	7.6	8.7	8.1	8.6	9.1	9.5	7.8	7.7
	7.9	8.7	7.7	9.0	8.5	9.4	9.5	8.8
	7.8	9.3	7.9	8.2	8.6	9.1	9.5	8.9
Mean ± s.e.	7.9 ± 0.25	8.3 ± 0.18	8.2 ± 0.26	8.9 ± 0.26	9.2 ± 0.28	8.7 ± 0.12	9.2 ± 0.19	8.4 ± 0.16

Appendix 6: Tissue nutrient analyses of macrophyte samples, Lake Illawarra, 2000-02

Appendix 6A: Nutrient analyses of *Ruppia megacarpa* samples, Lake Illawarra, 2000-02.

		***Ruppia megacarpa* - Leaves**					***Ruppia megacarpa* - Rhizomes**				
	Season	**Total C (%)**	**Total N (%)**	**Total P (%)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**Total C (%)**	**Total N (%)**	**Total P (%)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Oasis Caravan Park (OCP)	**2000, Spring**	39.8	2.55	0.14	-11.99	2.06	38.7	1.47	0.11	-10.23	1.09
		40.0	2.75	0.15	-12.01	2.23	37.9	1.64	0.12	-10.96	1.50
		41.0	2.91	0.14	-11.88	1.95	39.3	1.88	0.13	-11.08	0.99
	2001, Summer	40.9	2.93	0.12	-11.84	2.23	39.7	1.94	0.10	-10.45	1.22
		40.2	2.85	0.12	-12.23	2.02	38.0	1.89	0.10	-10.47	1.28
		39.2	2.71	0.12	-11.52	1.88	37.3	1.99	0.11	-11.21	0.89
	2001, Winter	35.4	1.89	0.18	-10.71	3.43	37.1	2.12	0.18	-11.01	2.31
		38.9	2.26	0.19	-10.88	3.84	36.6	1.79	0.17	-10.98	2.41
		36.5	2.00	0.20	-11.34	2.66	36.6	1.85	0.19	-11.40	1.03
		34.7	1.75	0.17	-11.22	2.15	36.0	2.00	0.17	-11.87	1.02
		34.3	1.93	0.18	-11.33	2.29	37.1	2.00	0.18	-11.48	1.54
		39.2	1.97	0.15	-10.92	2.56	37.6	2.04	0.16	-12.16	1.21
	2002, Summer	32.5	2.09	0.28	-10.46	5.50	30.0	1.39	0.21	-9.55	2.88
		31.0	2.03	0.29	-10.83	4.11	29.1	1.22	0.20	-9.92	2.12
		31.7	2.40	0.33	-10.34	5.12	34.3	1.63	0.25	-9.32	2.71
		33.2	2.18	0.32	-10.00	3.29	33.8	1.37	0.30	-9.32	1.64
		33.4	2.32	0.37	-9.92	3.60	31.3	1.32	0.31	-9.14	1.97
		34.0	2.03	0.35	-9.64	2.87	31.5	1.37	0.29	-9.37	2.39
	2002, Winter	29.3	1.89	0.26	-10.35	5.21	29.6	1.49	0.28	-10.61	2.86
		27.9	2.35	0.31	-10.50	3.12	30.0	1.53	0.23	-10.57	2.94
		32.7	2.01	0.29	-10.50	4.54	28.5	1.33	0.28	-11.00	3.01
		34.2	2.15	0.27	-10.76	5.32	32.5	1.54	0.27	-11.46	2.70
		32.0	2.32	0.28	-10.65	4.96	26.7	1.59	0.25	-10.36	2.63
		30.5	2.41	0.27	-10.82	5.05	28.0	1.69	0.27	-11.08	2.99
Nicolle Road (NIC)	**2000, Spring**	39.6	2.98	0.19	-12.41	8.95	35.9	1.87	0.14	-12.08	6.87
		37.6	2.99	0.19	-13.01	7.88	38.6	1.99	0.14	-11.90	7.09
		38.0	3.01	0.18	-12.88	8.76	39.4	2.00	0.12	-11.87	7.54
	2001, Summer	37.5	3.07	0.17	-12.38	7.02	39.7	1.97	0.12	-10.90	6.95
		36.6	2.98	0.15	-12.12	6.76	39.3	2.05	0.12	-11.23	6.96
		39.7	3.09	0.16	-11.97	6.73	35.5	1.93	0.11	-11.45	6.97
	2001, Winter	39.4	2.68	0.23	-11.20	2.60	39.1	1.91	0.22	-10.77	2.70
		40.9	2.80	0.25	-10.89	1.04	35.9	1.90	0.26	-10.81	1.38
		38.2	2.58	0.20	-11.32	3.33	39.0	2.24	0.20	-11.53	3.52
		38.7	2.78	0.25	-11.29	2.20	39.5	2.05	0.25	-10.69	2.15
		39.7	2.71	0.24	-11.24	2.29	37.2	2.10	0.25	-11.31	2.51
		38.6	2.48	0.24	-10.64	2.46	38.6	2.04	0.24	-10.78	2.98
	2002, Summer	31.0	2.19	0.48	-10.45	4.11	29.9	1.58	0.45	-9.99	2.76
		30.4	2.33	0.51	-10.49	3.90	31.4	1.69	0.50	-9.63	1.77
		29.8	2.37	0.56	-10.35	3.59	29.9	1.68	0.57	-9.73	2.18
		27.4	2.18	0.59	-10.49	3.65	33.5	1.66	0.47	-9.73	2.04
		32.6	2.39	0.57	-10.18	4.20	30.9	1.75	0.49	-10.05	2.63
		28.8	2.14	0.51	-10.38	3.89	28.9	1.46	0.46	-9.78	2.93
	2002, Winter	33.9	2.01	0.40	-11.08	4.10	31.9	1.80	0.32	-11.04	2.85
		35.8	2.13	0.39	-10.92	4.57	33.0	1.76	0.28	-10.85	2.55
		35.9	2.20	0.35	-10.65	3.13	29.6	1.57	0.26	-10.79	2.47
		32.3	2.05	0.31	-10.09	3.04	29.8	1.70	0.28	-9.54	2.35
		34.9	2.28	0.38	-10.42	3.28	31.7	1.60	0.29	-9.86	2.41
		32.0	2.19	0.35	-10.39	3.23	32.7	1.70	0.31	-10.01	2.50
PBP	**2000, Spring**	34.4	2.08	0.17	-9.48	3.98	30.6	1.34	0.24	-9.83	4.11
		36.0	1.74	0.17	-8.91	3.40	32.4	1.37	0.18	-9.65	4.10
		35.8	2.18	0.18	-9.57	3.58	32.6	1.39	0.22	-9.59	4.00

Appendix 6B: Nutrient analyses of *Zostera capricorni* samples, Lake Illawarra, 2000 - 2002.

		***Zostera capricorni* - Leaves**					***Zostera capricorni* - Rhizomes**				
	Season	**Total C (%)**	**Total N (%)**	**Total P (%)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**Total C (%)**	**Total N (%)**	**Total P (%)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Mullet Creek (MC)	**2000, Spring**	38.6	2.85	0.24	-12.80	4.79	32.3	0.86	0.14	-11.39	3.42
		38.5	3.12	0.25	-13.32	4.68	35.2	1.09	0.16	-10.66	2.46
		39.8	3.16	0.26	-11.76	4.67	33.6	1.04	0.14	-11.07	2.22
		39.1	3.30	0.24	-14.24	3.98	34.1	1.27	0.24	-11.80	2.78
		38.7	3.15	0.25	-14.71	3.63	33.7	1.22	0.17	-12.43	3.04
		37.9	3.18	0.24	-14.37	4.36	36.6	0.85	0.17	-12.10	2.53
	2001, Summer	26.9	1.94	0.21	-14.84	5.83	35.6	1.04	0.16	-12.03	3.38
		30.8	2.40	0.27	-15.25	6.03	31.9	0.90	0.13	-12.36	2.68
		30.9	2.37	0.32	-14.78	5.07	30.9	1.04	0.15	-11.32	2.61
	2001, Winter	42.0	2.85	0.25	-12.04	4.08	36.9	1.22	0.19	-12.14	4.11
		41.0	3.06	0.27	-11.97	4.27	29.7	1.08	0.20	-12.40	3.94
		39.1	2.82	0.31	-12.27	3.91	33.5	1.07	0.23	-12.23	4.02
		40.5	3.81	0.33	-13.29	6.03	25.8	1.20	0.26	-13.82	5.56
		39.4	3.63	0.32	-12.10	5.48	31.2	1.40	0.26	-11.86	4.65
		41.4	3.86	0.32	-12.16	3.96	34.6	1.74	0.27	-11.25	3.74
	2002, Summer	26.9	1.94	0.40	-14.84	5.83	32.0	1.30	0.18	-12.44	3.26
		30.8	2.40	0.50	-15.25	6.03	33.9	1.28	0.28	-12.19	3.95
		30.9	2.37	0.47	-14.78	5.07	32.6	1.24	0.23	-11.96	3.84
		29.7	2.26	0.43	-14.42	5.70	29.8	1.09	0.30	-12.66	4.58
		26.4	2.04	0.41	-14.96	5.70	33.6	1.32	0.24	-13.48	3.98
		24.2	1.89	0.35	-15.88	5.16	34.1	1.30	0.31	-13.71	3.90
	2002, Winter	36.5	2.65	0.29	-13.56	4.78	32.0	1.25	0.24	-12.56	4.91
		37.0	3.12	0.36	-12.23	4.01	29.0	1.02	0.19	-11.67	4.82
		34.2	2.46	0.26	-13.42	5.85	33.1	1.12	0.25	-11.85	5.32
		35.7	2.61	0.35	-12.43	6.02	35.3	1.34	0.30	-12.33	5.05
		38.9	3.05	0.39	-12.93	4.31	34.1	1.31	0.27	-11.86	4.65
		36.9	2.50	0.33	-12.38	5.12	28.6	1.08	0.21	-12.45	4.54
Purry Burry Point (PBP)	**2000, Spring**	35.7	2.88	0.23	-10.31	2.07	32.6	1.02	0.12	-9.88	0.79
		38.2	3.10	0.23	-11.12	2.31	36.2	1.11	0.12	-10.77	0.84
		38.0	2.94	0.22	-11.01	2.15	33.0	0.91	0.11	-10.16	0.68
	2001, Summer	38.4	3.15	0.23	-11.15	1.34	38.3	0.89	0.13	-10.27	2.35
		36.6	2.85	0.24	-10.39	3.68	34.9	1.13	0.12	-9.75	3.63
		33.7	2.81	0.24	-10.08	2.39	32.6	1.16	0.13	-10.26	3.89
	2001, Winter	38.6	3.38	0.34	-10.53	4.59	30.9	1.70	0.38	-10.52	6.35
		40.9	3.93	0.35	-9.88	4.45	31.9	1.71	0.37	-10.67	4.31
		41.1	3.88	0.38	-10.30	5.11	32.9	1.68	0.31	-10.36	4.31
		38.5	3.83	0.35	-10.27	3.07	25.6	1.49	0.28	-11.15	5.32
		40.3	4.13	0.40	-10.19	4.27	34.6	1.80	0.26	-10.41	4.79
		37.9	2.96	0.33	-10.58	2.83	30.3	1.52	0.27	-10.26	4.51
	2002, Summer	30.9	2.66	0.56	-11.39	3.67	27.3	1.54	0.34	-10.04	6.20
		31.6	2.41	0.44	-10.17	6.16	32.0	1.59	0.27	-9.56	4.87
		31.5	2.28	0.41	-10.71	4.48	28.6	1.34	0.23	-9.05	4.76
		31.0	2.46	0.38	-11.17	3.91	26.4	1.04	0.15	-9.47	3.45
		30.9	2.33	0.37	-11.72	5.11	26.9	1.42	0.26	-10.17	5.03
		30.9	2.24	0.40	-11.58	3.89	28.0	1.17	0.25	-9.32	3.66
	2002, Winter	34.9	2.86	0.41	-10.02	6.01	24.8	1.59	0.25	-10.31	6.01
		38.3	2.50	0.50	-10.87	4.43	23.3	1.48	0.28	-10.43	5.85
		35.4	2.99	0.45	-10.05	5.93	25.4	1.42	0.30	-10.62	4.89
		37.8	2.58	0.48	-10.65	4.95	24.6	1.59	0.29	-10.59	5.02
		34.6	2.75	0.43	-9.91	6.78	27.4	1.40	0.31	-10.63	4.78
		36.7	3.01	0.53	-10.12	5.72	28.5	1.32	0.33	-10.76	4.62
PRIM	**2000, Spring**	34.6	2.62	0.28	-7.87	1.00	30.6	0.92	0.17	-12.72	0.21
		39.0	3.12	0.28	-8.50	2.47	31.2	1.24	0.21	-9.30	2.09
		36.2	3.13	0.30	-7.66	1.54	31.3	1.19	0.21	-7.72	1.25

Appendix 6C: Tissue nutrient analyses of macroalgae samples, Lake Illawarra, 2000-02.

Season	Site	Description	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
2000, Spring	**PRIM**	*Ulva* sp. (formerly *Enteromorpha*)	34.1	0.95	0.24	-9.28	4.66
		Ulva sp. (formerly *Enteromorpha*)	30.7	0.85	0.28	-9.74	4.69
		Ulva sp. (formerly *Enteromorpha*)	28.0	1.20	0.08	-6.70	7.41
		Ulva sp. (sheet-like)	36.6	3.25	0.14	-6.10	10.49
		Ulva sp. (sheet-like)	32.2	2.57	0.10	-4.89	10.98
		Ulva sp. (sheet-like)	34.2	2.04	0.15	-8.41	7.35
		C. linum and *C. billardierii*	36.9	1.47	0.23	-8.73	6.35
		C. linum and *C. billardierii*	37.8	2.40	0.17	-9.78	8.27
		C. linum and *C. billardierii*	37.8	2.39	0.14	-9.28	8.07
	MC	*Chaetomorpha linum*	35.4	3.10	0.28	-15.92	9.46
		Chaetomorpha linum	34.9	3.24	0.28	-19.78	9.44
		Chaetomorpha linum	37.8	3.48	0.12	-16.82	10.35
		Ulva compressa	30.6	1.85	0.12	-11.28	11.61
		Ulva compressa	32.6	2.50	0.15	-12.63	10.79
		Ulva compressa	31.9	1.80	0.11	-12.19	10.87
	OCP	*Ulva intestinalis*	32.2	1.43	0.05	-9.39	12.66
		Ulva intestinalis	32.7	1.75	0.04	-11.27	13.34
		Ulva intestinalis	30.3	1.43	0.04	-10.40	13.09
		Chaetomorpha billardierii	37.6	2.92	0.10	-15.26	10.92
		Chaetomorpha billardierii	38.6	2.88	0.06	-14.84	11.09
		Chaetomorpha billardierii	38.6	2.53	0.04	-14.65	10.53
		Chaetomorpha billardierii	38.3	3.52	0.08	-15.29	11.10
		Chaetomorpha billardierii	38.1	3.85	0.10	-15.84	9.78
		Chaetomorpha billardierii	39.7	3.95	0.09	-16.07	9.87
	LIV	*Chaetomorpha linum*	39.3	3.22	0.14	-16.29	8.29
		Chaetomorpha linum	39.8	2.27	0.13	-14.11	9.19
		Chaetomorpha aerea	36.8	1.82	0.05	-18.22	9.30
		Chaetomorpha linum	39.7	3.87	0.16	-16.01	7.88
		Chaetomorpha linum	40.6	3.65	0.16	-15.60	9.61
		Chaetomorpha linum	38.9	3.59	0.13	-16.22	7.56
	NIC	*Chaetomorpha linum*	38.5	3.69	0.22	-15.64	11.72
		Chaetomorpha linum	38.8	3.77	0.14	-15.25	11.05
		Chaetomorpha linum	39.4	3.89	0.11	-15.08	11.67
		Chaetomorpha linum	35.7	2.97	0.08	-15.64	14.37
		Chaetomorpha linum	35.9	3.12	0.10	-15.58	12.49
		Chaetomorpha linum	36.5	3.12	0.07	-15.74	14.42
	PBP	*Chaetomorpha linum*	37.3	3.45	0.15	-13.83	9.98
		Chaetomorpha linum	35.9	3.06	0.11	-13.10	9.81
		Chaetomorpha linum	36.2	3.21	0.09	-13.12	9.82
		Chaetomorpha linum	38.3	3.32	0.17	-14.76	8.96
		Chaetomorpha linum	36.6	2.58	0.07	-12.34	10.59
		Chaetomorpha linum	33.1	2.37	0.07	-12.32	10.38
2001, Summer	**OCP**	*Chaetomorpha billardierii*	37.8	2.25	0.04	-14.99	4.87
		Chaetomorpha billardierii	38.5	2.38	0.04	-13.51	7.19
		Chaetomorpha billardierii	33.9	2.05	0.03	-14.22	4.45
		Rhizoclonium riparium	34.3	1.96	0.07	-9.49	4.24
		Rhizoclonium riparium	33.0	2.05	0.07	-8.38	4.05
		Rhizoclonium riparium	33.8	1.96	0.07	-9.19	3.99

Appendix 6C (cont): Tissue nutrient analyses of macroalgae samples, Lake Illawarra, 2000-02.

Season	Site	Description	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
2001, Winter	**OCP**	*Chaetomorpha billardierii*	37.6	2.72	0.29	-13.09	7.88
		Chaetomorpha billardierii	36.7	2.13	0.29	-13.24	6.34
		Chaetomorpha billardierii	36.1	2.38	0.26	-12.53	8.60
		Lamprothamnium papulosum	36.0	2.23	0.16	-13.70	2.59
		Lamprothamnium papulosum	36.3	2.48	0.16	-14.22	2.60
		Lamprothamnium papulosum	37.6	2.43	0.13	-14.67	1.80
2002, Summer	**MC**	*Ulva compressa*	31.6	2.25	0.19	-12.53	9.17
		Ulva compressa	31.4	1.93	0.14	-12.39	9.74
		Ulva compressa	34.3	2.58	0.15	-14.04	9.99
		Chaetomorpha linum	34.8	2.47	0.29	-18.18	7.93
		Chaetomorpha linum	31.5	2.59	0.30	-13.99	6.70
		Chaetomorpha linum	30.4	2.67	0.25	-19.10	6.90
	OCP	*Cladophora* sp.	32.5	1.65	0.09	-8.38	7.00
		Cladophora sp.	33.2	1.67	0.11	-7.73	7.99
		Cladophora sp.	32.8	1.96	0.11	-7.82	8.16
		Chaetomorpha linum	30.4	3.08	0.16	-11.57	14.57
		Chaetomorpha linum	28.0	2.84	0.19	-11.96	13.35
		Chaetomorpha linum	28.5	2.77	0.15	-11.61	14.40
	PBP	*Ulva intestinalis*	32.3	1.23	0.08	-7.21	5.58
		Ulva intestinalis	33.6	1.19	0.09	-7.08	6.79
		Ulva intestinalis	34.1	1.25	0.06	-6.40	6.78
		Gracilaria sp.	29.8	3.88	0.58	-16.84	9.60
		Gracilaria sp.	30.2	3.43	0.46	-17.81	8.75
		Gracilaria sp.	31.4	3.16	0.54	-16.55	9.66
	PRIM	*C. linum* and *C. billardierii*	27.7	1.10	0.30	-12.32	6.46
		C. linum and *C. billardierii*	30.4	1.51	0.34	-13.64	6.94
		C. linum and *C. billardierii*	32.3	1.67	0.35	-13.23	7.78
		C. linum and *C. billardierii*	30.7	1.50	0.36	-13.41	6.27
		C. linum and *C. billardierii*	29.4	2.45	0.37	-13.10	6.22
		C. linum and *C. billardierii*	30.5	1.98	0.46	-14.46	7.42
2002, Winter	**OCP**	*Chaetomorpha billardierii*	35.7	3.77	0.25	-12.53	9.01
		Chaetomorpha billardierii	37.9	3.21	0.27	-11.93	11.38
		Chaetomorpha billardierii	38.3	3.46	0.22	-11.65	10.35
		Chaetomorpha billardierii	34.6	3.58	0.26	-11.01	12.35
		Chaetomorpha billardierii	33.8	3.70	0.24	-12.33	10.53
		Chaetomorpha billardierii	36.3	3.35	0.24	-11.72	11.02

Appendix 7: Nutrient analyses of sediment samples collected at seagrass sites, Lake Illawarra, 2000 - 2002.

Appendix 7A: Nutrient analyses of sediment samples collected at *Ruppia megacarpa* sites, Lake Illawarra, 2000-02.

Season	Oasis Caravan Park (OCP)					Nicolle Road (NIC)				
	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
2000, Spring	0.153	0.015	0.020	-18.29	4.92	0.216	0.018	0.010	-18.41	5.21
	0.160	0.016	0.010	-19.57	6.13	0.085	0.007	0.010	-18.71	n/a*
	0.156	0.015	0.010	-18.93	5.52	0.150	0.013	0.010	-18.56	n/a*
2001, Summer	0.210	0.067	0.020	-17.49	4.06	0.138	0.012	0.020	-19.51	n/a*
	0.097	0.010	0.010	-19.16	n/a*	0.192	0.050	0.010	-19.50	7.67
	0.154	0.039	0.015	-18.32	4.06	0.165	0.031	0.015	-19.51	7.67
2001, Winter	0.493	0.057	0.012	-15.96	3.23	0.330	0.075	0.021	-19.27	2.91
	0.320	0.031	0.010	-16.63	3.48	0.457	0.123	0.019	-17.86	3.22
	0.302	0.032	0.007	-16.72	3.15	0.545	0.133	0.020	-18.10	3.25
	0.345	0.037	0.010	-16.89	3.48	0.379	0.096	0.022	-18.80	3.23
	0.317	0.037	0.010	-16.49	3.43	0.416	0.102	0.019	-19.05	3.62
	0.402	0.049	0.018	-16.52	3.46	0.456	0.084	0.020	-17.86	3.64
2002, Summer	1.181	0.130	0.031	-17.07	3.63	0.718	0.050	0.020	-19.16	3.03
	0.676	0.082	0.025	-16.31	3.04	0.514	0.033	0.010	-18.93	2.90
	0.596	0.053	0.010	-17.19	3.02	1.493	0.134	0.020	-17.86	3.24
	0.515	0.056	0.020	-16.88	2.94	0.780	0.065	0.030	-17.70	2.25
	0.498	0.032	0.010	-17.34	2.42	0.795	0.058	0.020	-18.41	2.79
	0.647	0.069	0.020	-17.45	2.79	0.832	0.057	0.025	-18.74	2.57
2002, Winter	0.298	0.030	0.018	-17.32	3.52	0.512	0.050	0.019	-18.34	2.54
	0.201	0.070	0.034	-16.89	3.17	0.708	0.060	0.021	-18.24	3.32
	0.188	0.050	0.022	-18.01	2.65	0.663	0.050	0.019	-17.32	3.16
	0.253	0.050	0.013	-17.45	2.90	0.612	0.040	0.013	-18.48	2.85
	0.267	0.040	0.015	-17.21	3.02	0.593	0.060	0.024	-18.11	2.96
	0.370	0.040	0.012	-16.89	3.36	0.607	0.080	0.031	-17.64	3.27

* N contents of samples were not sufficient for $\delta^{15}N$ analyses.

Appendix 7B: Nutrient analyses of sediment samples collected at *Zostera capricorni* sites, Lake Illawarra, 2000-02.

Season	Purry Burry Point (PBP)					Mullet Creek (MC)				
	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Total C (%)	Total N (%)	Total P (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
2000, Spring	0.386	0.030	0.020	-19.32	4.81	2.860	0.200	0.031	-24.66	1.07
	0.290	0.021	0.010	-20.00	4.19	3.065	0.210	0.034	-24.38	1.19
	0.338	0.026	0.015	-19.66	4.50	2.963	0.205	0.032	-24.52	1.13
	n/a	n/a	n/a	n/a	n/a	1.454	0.090	0.019	-23.23	3.59
	n/a	n/a	n/a	n/a	n/a	1.350	0.090	0.020	-23.01	3.82
	n/a	n/a	n/a	n/a	n/a	1.402	0.090	0.019	-23.12	3.71
2001, Summer	0.323	0.025	0.020	-20.34	4.55	1.922	0.145	0.040	-23.88	2.52
	0.471	0.035	0.020	-19.75	5.16	1.783	0.293	0.020	-23.90	2.09
	0.397	0.030	0.020	-20.04	4.85	1.853	0.219	0.030	-23.89	2.31
2001, Winter	0.534	0.032	0.013	-19.09	4.15	0.537	0.050	0.011	-22.26	4.14
	0.272	0.027	0.018	-18.88	4.42	0.296	0.034	0.011	-21.93	3.31
	0.410	0.039	0.014	-17.90	4.58	1.159	0.083	0.020	-23.50	2.86
	0.599	0.063	0.017	-18.42	4.35	1.135	0.068	0.016	-23.37	3.10
	1.249	0.096	0.016	-18.48	4.15	0.666	0.049	0.010	-23.41	2.82
	0.709	0.060	0.019	-18.52	3.64	0.572	0.052	0.008	-22.72	3.04
2002, Summer	1.275	0.132	0.042	-19.17	4.95	0.904	0.086	0.019	-21.34	3.70
	0.897	0.087	0.021	-18.74	4.57	1.264	0.079	0.026	-23.75	3.17
	0.707	0.083	0.031	-18.78	4.49	0.954	0.076	0.012	-22.53	2.88
	1.030	0.104	0.013	-18.27	4.46	0.781	0.074	0.027	-22.74	3.97
	1.020	0.104	0.007	-18.20	4.55	0.529	0.052	0.013	-22.99	3.45
	0.377	0.044	0.004	-18.50	3.96	0.925	0.091	0.030	-22.36	4.59
2002, Winter	0.752	0.080	0.010	-18.54	4.52	0.713	0.070	0.013	-24.82	4.14
	0.832	0.090	0.010	-18.09	4.83	1.510	0.120	0.013	-23.16	3.94
	0.633	0.060	0.011	-18.24	4.15	1.020	0.080	0.022	-22.95	3.13
	1.020	0.080	0.018	-17.93	4.32	1.182	0.110	0.021	-22.98	2.83
	0.553	0.050	0.017	-18.31	4.59	0.962	0.080	0.017	-23.30	3.52
	0.611	0.060	0.013	-17.85	4.84	1.234	0.110	0.019	-22.67	2.95