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Nutrient fluxes in the meadow forming seagrasses Posidonia *and* Amphibolis *from the Adelaide metropolitan coast*

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Nutrient fluxes in the meadow forming seagrasses Posidonia *and* Amphibolis *from the Adelaide metropolitan coast.*

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Executive overview

Seagrasses occupy a narrow band of sandy seabed close to the coast and are therefore vulnerable to anthropogenic influences, particularly for meadows near large population centres. Over 5000 Ha of seagrasses have been lost from Adelaide coastal waters over the last 70 years and much of this loss has been attributed to nutrient inputs from wastewater, industrial and stormwater discharges. So as to manage these inputs into the future, we need better understanding of the fate of nutrients, particularly their biological uptake in this system. This study represents an attempt to quantify some of the important uptake rates of the biotic components of the system and, through a modeling approach, place these rates in the broader context of the assimilative capacity of the whole region in relation to its nutrient inputs.

This study used an *in-situ* isotope-labelling and nutrient spike approach to obtain ecologically relevant estimates of seasonal variability in ammonium, nitrate, carbon and phosphorus uptake and the allocation of resources in two species of temperate seagrass common to this coast (*Amphibolis antarctica* and *Posidonia angustifolia*).

The biomass standardized uptake rate of ammonium by plankton was higher than that of other biotic components (seagrass leaf, seagrass root, attached epiphytes). It peaked in winter (0.98 mg N. g^{-1} DW. h^{-1}) in the plankton community associated with the *Posidonia* beds. Leaves, roots and epiphytes registered significantly higher uptake rates of ammonium in the *Amphibolis* complex than *Posidonia*. Uptake of ammonium by *Amphibolis* leaves ranged from 0.08 mg N. g^{-1} DW. h^{-1} (winter and spring) to 0.14 mg N. g^{-1} DW. h^{-1} (summer). Ammonium uptake rates by *Posidonia* leaves ranged from 0.03 mg N. g^{-1} DW. h^{-1} (summer) to 0.08 mg N. g^{-1} DW. h^{-1} (spring). Overall, root uptake rates were lower than other biotic components. Epiphytes on *Amphibolis* had higher uptake rates than those on *Posidonia*. The effect of season was not significant for leaves, roots or epiphytes of *Amphibolis* and *Posidonia*. However, plankton uptake rates did vary seasonally with much higher uptake rates in winter that were not found at other times of the year (nearly 3 folds higher than in spring).

In contrast to the general trend in ammonium uptake, nitrate uptake rates for biotic components were significantly affected by seasons. Among the various biotic components, plankton accounted for the highest nitrate uptake rates ranging from 0.003 mg N. g^{-1} DW. h^{-1} in summer (*Amphibolis* bed) to 0.69 mg N. g^{-1} DW. h^{-1} in winter (*Posidonia* bed). Nitrate uptake rates of leaves were relatively low and were greatest in spring of 0.009 and 0.011 mg N. g^{-1} DW. h^{-1} for *Posidonia* and *Amphibolis* respectively. Uptake of nitrate by the root component was negligible and did not differ between species or across seasons. The biotic uptake rates for nitrate were an order of magnitude slower than ammonium. It is evident that there was a clear affinity for ammonium over nitrate as a preferred inorganic nitrogen source by the seagrass complex (seagrass leaves, seagrass roots and epiphytes).

Uptake of carbon by the seagrass complex was affected by both season and species. Carbon uptake rates of plankton were generally higher than other components of the system. Uptake rates ranged from 0.01 mg C. g^{-1} DW. h^{-1} (summer) to 0.61 mg C. g^{-1} DW. h^{-1} (spring) in *Posidonia* and 0.02 mg C. g^{-1} DW. h^{-1} (summer) to 0.93 mg C. g^{-1} DW. h^{-1} (winter) in *Amphibolis*. Carbon uptake by the *Amphibiolis* complex was higher than in the *Posidonia* complex. The *Amphibolis* complex had higher uptake rates in summer whereas the *Posidonia* complex was higher in spring.

Total uptake of phosphorus by biological components was negligible, never exceeding 0.5% of the total resource. Phosphorus uptake rate varied seasonally with higher rates in winter

(0.05 mg PO_4. g^{-1} DW. h^{-1}) and lower rates in spring (0.02 mg PO_4. g^{-1} DW. h^{-1}) for *Amphibolis* and highest in winter (0.07 mg PO_4. g^{-1} DW. h^{-1}) and least in spring (0.004 mg PO_4. g^{-1} DW. h^{-1}) for *Posidonia*.

Using a modeling approach, uptake rates were scaled to the level of the Adelaide coast by taking into consideration the biomass specific uptake rates and multiplying them by the estimated biomass of each of the components. This allowed a comparison of the annual input with the annual uptake rates for the different components. Uptake was far greater prior to 1978 due to a larger biomass of seagrass, and the greater ambient concentrations (which cause more rapid uptake) than is the case today. In 2005, we estimate that uptake of ammonium by the seagrass complex in the Adelaide region (seagrass and associated epiphytes) represents 465 tonnes of ammonium per year and 3.04 tonnes of nitrate. This accounts for 31% of the ammonium and less than 1% of the nitrate which is currently discharged into Adelaide's waters. Of the ammonium and nitrate taken up by the biotic components, 99% and 88% respectively was accounted for by the seagrass and its associated epiphytes. Thus, whilst the model has demonstrated that the seagrass complex is responsible for a significant portion of the uptake, there are clearly other important sinks and processes which remain unaccounted for. The role of loss processes from the seagrass also requires quantification.

1. General introduction

Estimates of seagrass loss along the Adelaide metropolitan coast have been reported to be nearly 5000 ha since the 1940's (Neverauskas, 1987a-c; Hart, 1997; Shepherd et al., 1989). Previous studies have established a relationship between seagrass degradation along the Adelaide metropolitan coast with elevated nutrients, increased turbidity due to suspended particulates, toxicants, decreased salinity due to storm water inputs and substrate instability (Johnson, 1981; Clarke, 1987; Shepherd et al., 1989; Steffensen et al., 1989; Edyvane, 1996; Seddon, 2002). The work of Shepherd (1970) was the first to link elevated nutrient levels from sewage effluent to degradation of seagrasses along the metropolitan coast. Most of the losses started in shallow waters close to the shore and have then progressed outwards from the coast (see review by Westphalen et al. 2005). More recently Tanner (2005) reported the disappearance of deep-water seagrass *Heterozostera* from lower Gulf St Vincent between the 1970's and 2001 and suggested that the loss could be attributed to coastal inputs from the metropolitan area leading to a long-term increase in turbidity in the coastal waters.

Seagrasses are highly productive marine angiosperms that grow in shallow coastal waters (Harlin 1993) providing critical habitat and a nutritional base for finfish, shellfish, and herbivorous animals (Klumpp et al. 1989). Coastal urbanization and nearshore developments have resulted in declines in water quality affecting seagrasses (Shepherd et al., 1989; Seddon, 2000). Such activities, in recent decades, have resulted in increased nutrient loading and turbidity in nearshore systems dominated by seagrasses (Shepherd et al., 1989; Short and Wyllie-Echeverria, 1996; Dixon 1999) affecting the distribution and composition of seagrass meadows (Hansen *et al.* 2000; Welsh *et al.* 2000; Hemminga and Duarte 2000; Erftemeijer and Middelburg 1995). Excessive nitrogen loading, in particular, has been reported to have detrimental effects on seagrass-dominated estuaries by inhibiting seagrass growth and survival through the stimulation of phytoplankton and epiphytic and benthic microalgal growth (Hillman et al. 1989; McComb 1995; Touchette and Burkholder 2000). Eutrophication is also considered to be a major cause for the loss of seagrass in Australia (Gabric and Bell, 1993; Campbell and Miller, 2002; Bryars et al., 2003). Eutrophication not only has an indirect effect by stimulating algal overgrowth and consequently reducing available light, but for some species a direct physiological effect (Touchette and Burkholder 2000; Welsh *et al.* 2000; van Katwijk *et al.* 1997). For example, ammonium toxicity, relatively common in vascular plants, has been reported in the seagrasses *Ruppia drepanensis* and *Zostera marina* (Touchette and Burkholder 2000). *Z. marina* also suffers from excess nitrate levels as it appears to lack a "shut-off" mechanism for water-column uptake (Touchette and Burkholder 2000; Short and McRoy 1984; Burkholder et al., 1994).

Various studies have identified ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) as the largest sources of nitrogen for seagrass (Touchette and Burkholder 2000; Pedersen, Paling and Walker 1997; Paling 1991; Marba *et al.* 2002; Thursby and Harlin 1984; Erftemeijer and Middelburg 1995). There is limited knowledge on uptake rates of organic nitrogen sources in seagrass beds but most evidence indicates that they are relatively insignificant. Since nitrate and ammonium are considered the most significant sources of nitrogen, most studies assume that they are the only sources (Pedersen et al., 1997; Touchette and Burkholder 2000; Lee and Dunton 1999; Pedersen and Borum 1992; Iizumi and Hattori 1982). Evidence suggests this to be a valid assumption, with nitrate and ammonium supplying over 90% of external nitrogen to seagrass. Consequently, the present study only looked at the uptake and resource allocation of ammonium and nitrate in *Amphibolis* and *Posidonia*.

The nitrogen pool in sediments has a large capacity to supply the majority of nitrogen to rooted marine plants; therefore, nutrient cycling in sediments is a critical process. Unlike most species of algae that are dependent on nutrient concentrations in the water column,

seagrasses are rooted plants that meet a majority of their nutrient requirement from the sediment or substrate (Nybakken 1997). Sediments have the capacity to act as a source or sink for nitrogen from the water column and this is often quantified by measuring nitrate and ammonium fluxes across the sediment-water interface (Lavery et al., 2001). Seagrasses are therefore capable of recycling nutrients into the ecosystem that would otherwise be trapped in the sediment and become unavailable. Although sediment pore water is generally considered to be the primary source of nitrogen for seagrass, there is evidence that suggests that uptake of both nitrogen and phosphorus by below ground biomass is insufficient to meet the total nutrient requirement of the plant (Stapel et al., 1996; Lee and Dunton, 1999). Some species such as *Amphibolis antarctica* and *Phyllospadix torreyi* that are commonly found on rocky substrates, with little or no sediment around the roots, meet a majority of their nutrient demands from the water column by uptake through leaves (Pedersen et al., 1997; Terrados and Williams 1997).

Uptake mechanisms of nutrients by seagrass across cell membranes may be either active or passive (Hemminga and Duarte, 2000; Touchette and Burkholder, 2000). Nitrate uptake is an active process and is relatively less complex than ammonium uptake, which involves dual processes of low and high affinity systems (Figure 1.1). In the low affinity system, ammonium is passively transported through membrane channels, while the high affinity system works on a transmembrane transport protein across the plasma membrane (Ourry et al., 1997). The uptake of ammonium is regulated by feedback mechanisms dictated by tissue ammonia levels (Lee and Ayling, 1993). Ammonium that is taken up does not accumulate in the tissues because of its toxicity; instead ammonium is rapidly processed into organic compounds (Touchette and Burkholder, 2000). Young, actively growing roots have been reported to account for most of the nitrogen taken up by the below-ground biomass, with a minimal uptake by the rhizomes (Short and McRoy, 1984; Stapel et al., 1996).

Conversely, nitrate is highly soluble and relatively abundant in the water column (Burkholder et al., 1994). Levels of carbohydrate reserves in seagrass and the ambient nitrate concentrations regulate the activity of the enzyme nitrate reductase (NR). This in turn dictates nitrate uptake. Seagrass leaves have been reported to expend up to 25% of their total respiratory energy on nitrate uptake and assimilation (Touchette and Burkholder, 2000). Without a feedback mechanism to control the uptake, excessive uptake can lead to depletion of carbohydrates, thereby compromising growth (Lee and Dunton, 1999). Nitrate is translocated for storage in vacuoles in the leaves for subsequent assimilation (Pedersen et al., 1997; Hemminga and Duarte, 2000; Marba et al., 2002). This offers an explanation as to why assimilated nitrate levels in the leaf tissues are higher than in the root/rhizome complex. Nitrate reductase is a key regulatory enzyme responsible for nitrate assimilation and metabolism (Hemminga et al., 1991; Touchette and Burkholder, 2000). The activity of this enzyme is regulated by water temperature, ambient ammonium and nitrate concentrations, dissolved oxygen and carbon dioxide concentrations (Touchette and Burkholder, 2000; Welsh et al., 2000). The byproduct of enzyme activity, nitrite, is further reduced to ammonium by nitrite reductase. The ammonium thus formed, and that taken up by the seagrass, enters the GS/GOGAT cycle [(Glutamine synthetase (GS) / glutamate synthase (glutamine-oxoglutarate amidotransferase or GOGAT)], resulting in the synthesis of glutamine, which is then transaminated to form glutamate molecules after the addition of two carbon skeletons. Glutamate molecules are eventually used for the production of amino acids and other organic molecules (Touchette and Burkholder, 2000).

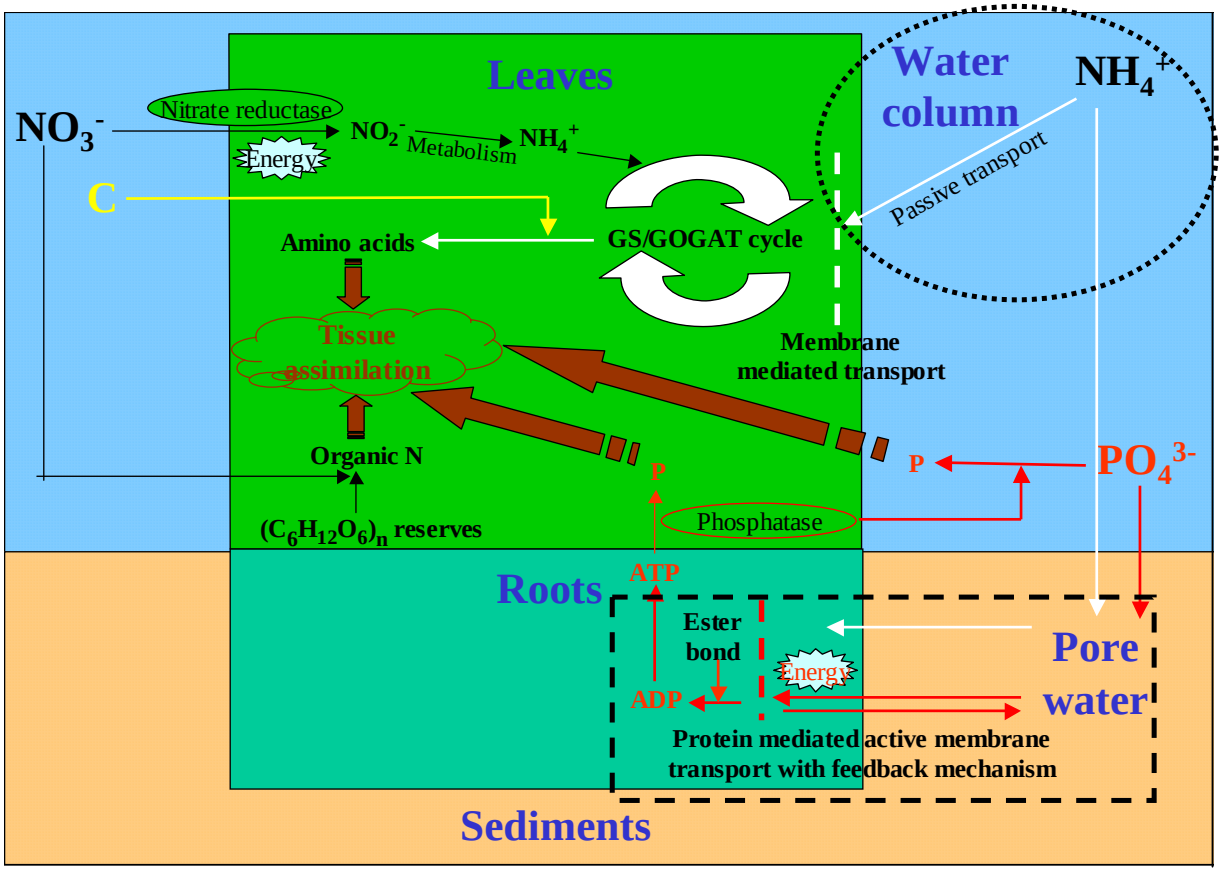

Figure 1.1 : A schematic representation of the nutrient uptake mechanisms in seagrasses. Low affinity system (marked by a dotted line circle) refers to passive transport across a membrane while the high affinity system (marked by a dashed line square) is a transmembrane protein mediated active transport.

Phosphate (PO_4^{3-}) is the common form of phosphorus in seagrass beds. As with nitrate, seagrasses take up phosphorus by active transport through the roots. Inorganic phosphorus has a low solubility and is readily adsorbed by particulates (McRoy et al., 1972; Touchette and Burkholder, 2000). The uptake of phosphorus by seagrass depends on factors such as diffusion between pore and column waters, seagrass species, and environmental conditions (Lee and Dunton, 1999; Brix and Lyngby, 1985). After uptake, phosphorus is translocated to other tissues. Inorganic phosphorus uptake is also achieved by protein mediated active membrane transport with a feedback mechanism (Muchhal-Umesh and Raghothama, 1999). The phosphorus taken up in this manner is esterified to form adenosine diphosphate (ADP) and further to adenosine triphosphate (ATP) (Taiz and Zeiger, 1991). The intracellular phosphatase activity increases under lower concentrations of intracellular phosphorus or high phosphorus demand, to maximize the use of internal phosphorus by releasing it from phosphorus containing compounds (Vincent and Crowder, 1995). Phosphorus uptake and assimilation has a direct bearing on carbon and nitrogen metabolism through the energy transfer pathways or ATP / NADP (H) cycle (Touchette and Burkholder, 2000).

Seagrasses do not utilize HCO_3^- as efficiently as macroalgae and cyanobacteria (Beer et al., 1980). Most of the carbon fixation occurs in the leaf mesophyll cells through an enzyme (carbonic anhydrase) mediated process (Goodwin and Mercer, 1983 Bjork et al., 1997). In some species, the uptake is an active energy intensive process (Beer and Rehnberg, 1997), while in others the carbonic anhydrase works as an extracellular or membrane enzyme to convert HCO_3^- to CO_2 prior to its uptake (Beer et al., 1980). The fixed carbon is then stored in the leaf bundle sheath cells where it is transformed into amino acids (Abel and Drew, 1989) and assimilated into the tissues.

While nutrient dynamics, uptake and resource allocation are well documented in tropical seagrass systems, there is a greater need to improve understanding about temperate oligotrophic systems. This need becomes more critical as there is little information on the assimilative capabilities of seagrasses found in these regions where a comparatively small increase in nutrient loads, particularly nitrogen, has a greater influence on the health of seagrasses than those found in mesotrophic systems. Based on the detailed literature review of Westphalen et al (2005), a conceptual model describing the fate of nutrients in the Adelaide coastal waters was constructed (Figure 1.2). This model illustrates all the major and minor nutrient pathways. From the viewpoint of the present study, however, this model was further simplified to show only the significant pathways (Figure 1.3). The compartments in the modified model were quantified from the results of the uptake and resource allocation components of the present study, which a focus on the seasonal fluxes and resource allocation of carbon and nitrogen in *Posidonia* and *Amphibolis* commonly found off the Adelaide metropolitan coastline. The experiments involved isolating the seagrass in chambers and incubating them with a known concentration of nitrogen, phosphorus and / or carbon in the water column over time. Changes in the water column / pore water concentration of these nutrients over time were measured to determine fluxes. Uptake rates of the various compartments were measured to quantify resource allocation in *Posidonia* and *Amphibolis*.

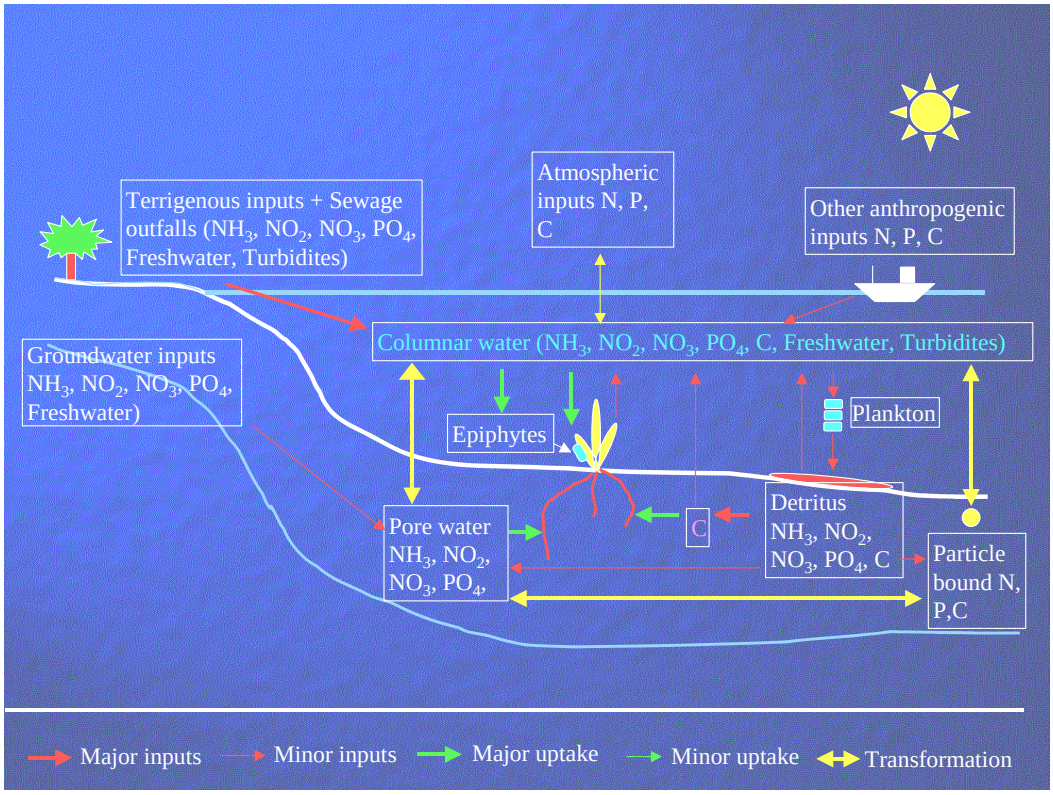

Figure 1.2 : A detailed conceptual model showing the fate of nutrients in Adelaide coastal waters.

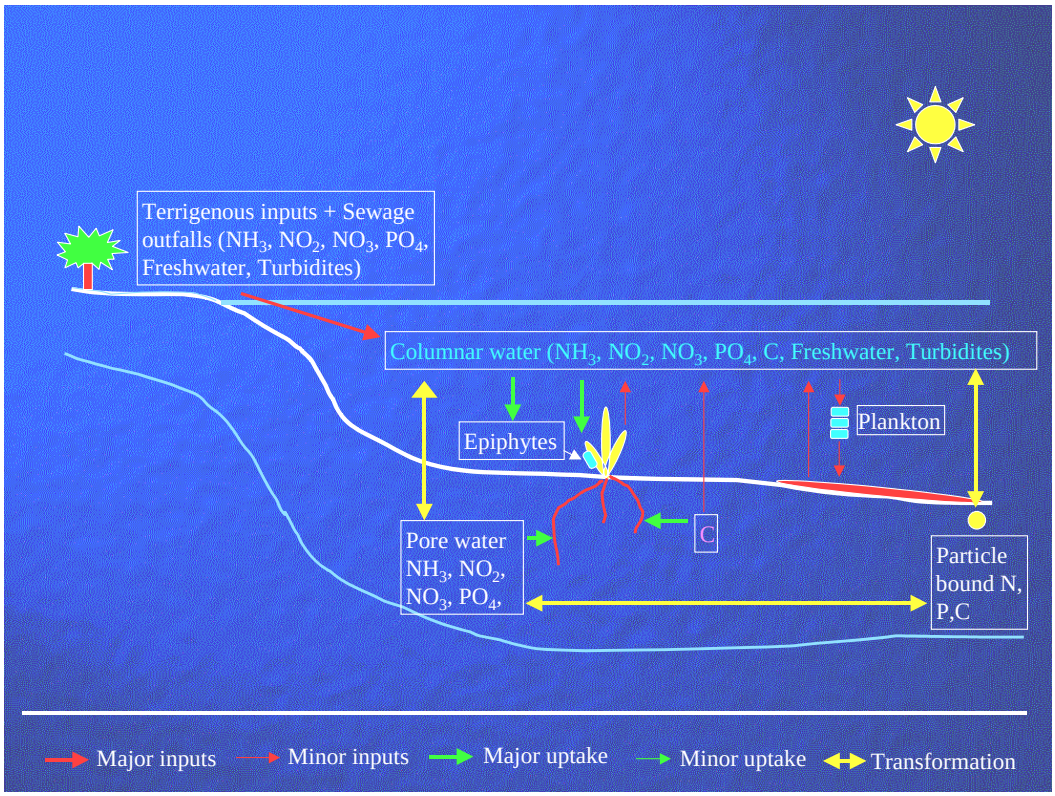

Figure 1.3 : A simplified conceptual model used in this study showing the fate of nutrients in Adelaide coastal waters.

2. Uptake and resource allocation of anthropogenic inputs of ammonium and nitrate in temperate seagrass beds of *Posidonia* and *Amphibolis*.

2.1. Introduction

Seagrass communities are composed of a diverse assemblage of primary producers that take up nutrients from the water column. These primary producers include the seagrasses, epiphytes attached to the seagrass leaves, macroalgal communities and phytoplankton. Although there is some published literature on whole community uptake (eg. Thomas et al., 2000), there is a paucity of information available on nutrient uptake for individual components of the community (Cornelisen and Thomas, 2002). These components vary in their morphology and physiology, thus warranting studies to fill the void on information pertaining to nutrient uptake, resource allocation and the factors influencing nutrient metabolism in seagrasses.

Seagrasses take up inorganic nitrogen through both leaf and root tissues (Iizumi and Hattori, 1982; Thursby and Harlin, 1982; Thursby and Harlin, 1984; Short and McRoy, 1984; Stapel et al., 1996; Pedersen et al., 1997; Terrados and Williams, 1997; Lee and Dunton, 1999). It is recognized that the major inorganic nitrogen sources for seagrasses are ammonium and nitrate for uptake by leaves from the water column and ammonium from porewater by roots (Lee and Dunton, 1999). However, in some seagrass environments with rocky substratum (eg. some *Amphibolis antarctica* beds), almost all the inorganic nutrient requirements are met through leaf uptake (Terrados and Williams, 1997).

Epiphytes play an integral role in the ecology of seagrass communities, including food web dynamics (Fry and Parker 1979) and nutrient cycling (Harlin, 1973; McRoy and Goering, 1974). In addition, epiphytes are a major contributor to the overall productivity of seagrass meadows (Moncreiff et al., 1992) and are considered an important factor influencing the distribution and abundance of seagrasses (Kuo and McComb, 1989). Although the significance of epiphytes in seagrass ecosystems is well documented, few published accounts are available, especially in temperate waters, describing the uptake of inorganic nitrogen by epiphytes in seagrass ecosystems (Hemminga et al., 1991; Cornelisen and Thomas, 2002) and their interaction with seagrasses for nutrient acquisition.

Since seagrasses are able to utilize inorganic nitrogen from sediments and the water column, N-cycles in seagrass beds are complex. Interaction with other components in a seagrass ecosystem, such as epiphytes and plankton, makes the nutrient dynamics process in the system more complex. In order to have a better understanding of the processes there is a need to develop whole-plant nitrogen budgets, based on the uptake dynamics of leaves, roots, epiphytes and plankton (Lee and Dunton, 1999). The present study adopted the *in-situ* isotope-labeling approach to obtain ecologically relevant estimates of seasonal nitrogen uptake rates and allocation of resources in various components of two species of temperate seagrass, *Amphibolis antarctica* and *Posidonia angustifolia*.

2.2. Materials and methods

2.2.1. Description of the sampling equipment

The benthic chambers used in this study comprised 6 identical cylindrical units made of clear perspex (Appendix 1), each with an overall volume of 0.0106 m^3. Each chamber has an inflow and an outflow connection onto which a pump line is connected. The outflow (chamber's outlet) is a PVC screw type connector glued on to the chamber. The inflow (chamber's inlet) is a spout on to which the pump line outlet could be pushed in. Inside the

chamber the inflow spout opens into a flow indicator with a few coloured beads that float when there is a flow of water into the chamber. The chambers have sampling straws glued on, serving as sampling ports for pore water and chamber water collections using a syringe. These sampling ports are terminated with a two-way valve that isolates the chamber from the surrounding water. A pore water sampler made with an air stone diffuser was hooked to the pore water sampling straw with a tygon tube internally in the chamber.

The stainless steel cutters to which the chambers were bolted had a sharp cutting edge with a square platform. Rubber washers were glued on to the platform to provide a tight seal between the chamber and the cutter after the chamber is bolted down (Appendix 1). Each cutter has a volume of 0.0045 m^3 and covered an area of 0.0453 m^2 when pushed into the sediment.

The pump line consists of a PVC connector on one end that mates with the outlet connector glued on the chamber. A fiber reinforced PVC hose links this connector to the intake of a submersible inline pump (LMV Amazon) through a flow control valve. The outlet of the pump is connected through a hose to a pressure compensator. The pressure compensator is a collapsible bag that compensates for the reduction in the volume of water contained in the chamber as a result of samples being drawn by syringes. It provides a pressure relief and prevents pore water from being upwelled into the chamber due to syringe sampling. The outlet of the compensator feeds through a hose into the outflow spout of the chamber.

The 6 pumps connected to the chambers are powered by a 6V DC, 144 Ah underwater battery pack. The switch on the battery pack for power is encased in a flexible polythene tubing (Appendix 2).

2.2.2. Field sampling

2.2.2.1. Sampling location

The site chosen for the experiment was located off Tennyson (34°52.532' S 138°27.797' E, Appendix 3). All deployments were carried out within 100 m radius of the site. The field site comprised beds of *Posidonia angustifolia* and *Amphibolis antarctica* alongside each other at an average water depth of about 8 m during high tide.

2.2.2.2. Chamber deployment and sample collection

Stainless steel cutters were driven into seagrass beds by SCUBA divers at least 48 hours prior to the experiment to allow for stabilization of the sediments and recovery of seagrass. Three of these cutters were driven into *Amphibolis* and the remaining 3 into *Posidonia*. Care was taken to ensure minimal damage to seagrass, at the same time ensuring that the cutters were driven at least 10 cm into the sediment. During the deployment of the cutters, samples of *Posidonia* and *Amphibolis* were obtained using a 24 cm diameter corer for the measurement of background levels of ^{15}N in leaves, roots and epiphytes. Seagrass core samples were transported in mesh bags under dark conditions. Approximately 1.5 L of water sample was collected in a polyethylene bottle about 0.5 m above the seagrass bed for determination of background levels of ^{15}N in phytoplankton and bacteria, qualitative and quantitative analysis of phytoplankton, and measurement of ambient water quality. Water quality parameters viz., water temperature, dissolved oxygen (DO), salinity, and pH were measured using a Hach SensIon 156 multi-parameter probe immediately after collection on board the vessel. Upon taking the ambient water quality measurements, approximately 100 ml of the water samples were fixed with Lugol's iodine for qualitative and quantitative phytoplankton analysis. The remaining water sample was then transported to the laboratory on ice under dark conditions for the measurement of background levels of ^{15}N in phytoplankton and bacteria.

All field deployments were carried out at around 10 am in the morning on the day of the trial. The dates for the uptake and resource allocation trials for $^{15}NH_3$ and $^{15}NO_3$ are provided in Appendix 4. On the day of the field trial, a clean glass bottle was used to collect about 20 g of sediments contained in each of the 6 cutters for background levels of ^{15}N in the sediments and transported to the laboratory on ice under dark conditions. After collection, the rubber seals on the cutters were cleaned of all debris and sand. Divers then positioned and aligned the chambers over the stainless steel cutters and bolted them down to the cutter to ensure a water-tight seal between the chamber and the cutter. Pump lines with the pressure compensators were then connected to each of the six chambers. Pumps were then connected to the underwater battery pack and powered on to maintain water flow in the chambers.

Nutrient stock solutions (1000 ppm) for spiking were prepared from labelled salts of $^{15}NH_4Cl$ (^{15}N, 98%, Novachem Pty Ltd) and $K^{15}NO_3$ (^{15}N, 99.22%, Novachem Pty Ltd) for ammonium and nitrate uptake and resource allocation trials, respectively. Nutrient spike solution was loaded into 20 mL syringes sealed with an end cap. Each chamber was then spiked with the nutrient solution contained in the syringes to yield a final concentration of 13.5 ppm of the nutrient in each of the six chambers.

Chambers were then incubated for 2 hours. At the end of the incubation, about 120 ml of water sample was drawn from each chamber using an end capped syringe for water quality measurements and to measure uptake of nutrients by phytoplankton and bacteria. The protocol adopted for measuring water quality was identical to that for chambers, described in Section 2.2.2.2. Seagrass samples from each chamber were cored out in the manner described previously and transported to the laboratory in a mesh bag under darkness for biomass and nutrient uptake measurements. Data on photosynthetically available radiation (PAR) levels during each trial were obtained from an Odyssey light logger.

2.2.3. Laboratory analysis

2.2.3.1. Treatment of equipment and glassware

All glassware used in the study was rinsed in AR grade Methyl alcohol and then ‘baked’ in a furnace at 150° C prior to use. The work-bench and all equipment coming in contact with the samples were cleaned with AR grade methyl alcohol prior to use.

2.2.3.2. Biomass estimation

Seagrass samples for biomass estimation were rinsed in clean, filtered seawater, and cleaned of epiphytes, dead leaves and sediments. Wet weight measurements of the total biomass, above-ground biomass and below-ground biomass from the 6 chamber and 6 background samples were made. Moisture content in sub-samples of the above- and below-ground biomass was measured gravimetrically after freeze-drying the samples in a Thermo Savant Micro Modulyo freeze-drier. Both the above ground biomass and below ground biomass were expressed on a dry weight basis. Epiphyte loading was deducted from the above ground biomass to obtain the corrected above ground biomass values on a dry weight basis, which were then used for subsequent calculations.

Qualitative and quantitative analysis of phtytoplankton was done by pipetting a 1 ml aliquot of the Lugol fixed sample onto a Sedgewick-Rafter cell. A Leica DME binocular light microscope was used for identification up to genus level. The abundance of plankton was expressed as the number of cells per unit volume of the sample.

2.2.3.3. Nutrient uptake rate measurements

Particulate nitrogen (PON) was measured in triplicate for every deployment by filtering 200 ml through a 47 mm diameter Whatman GF/F filter paper. Upon filtration, the filter papers with suspended particulates were stored in clean glass bottles at –40° C until freeze-drying. Frozen samples were directly freeze-dried. Total suspended particulate concentration was measured gravimetrically adopting standard procedures (Strickland and Parsons, 1972). The filter papers were then used for the analysis of PON by alkaline persulphate digestion colourimetric procedure (Grasshoff et al., 1983). A Lachat Quickchem 8000 autoanalyser was used for colourimetric analysis.

Both background and enriched seagrass samples were processed in the laboratory immediately after collection. Great caution was exercised to ensure no cross contamination of the samples. Epiphytes were carefully scraped off the seagrass leaves (15 leaves for *Amphibolis* and 10 for *Posidonia*) using a clean scalpel. Scraped epiphytes were collected and transferred into a clean glass scintillation vial. The scraped seagrass leaves were weighed and their length and width recorded. The leaves were then transferred into a clean glass bottle. Likewise, the below ground biomass was weighed and stored in a clean glass bottle. A known volume of background and enriched water samples, for the quantification of phytoplankton and bacterial uptake rates, was filtered through a Whatman GF/F filter paper (25 mm diameter, nominal pore size 0.4 μm) under vacuum. The filter papers with suspended particulates were transferred into clean bottles for storage. Since it was practically impossible to segregate bacterial uptake from plankton uptake, what is described in this study as plankton uptake is in fact a combined uptake by phytoplankton and bacteria. Because of high spatial and temporal variabilities associated with plankton distribution, plankton measurements in this study have been made for mass balance budgets. All samples were stored under dark at –40° C. Upon thawing, the samples were immediately freeze-dried in a Thermo Savant Micro Modulyo freeze-drier. Dry-weight of epiphytes was recorded to calculate epiphyte loading, expressed as dry-weight biomass per unit dry weight and unit area of seagrass leaves.

To measure background levels and uptake of labelled nutrients from the water column by various biotic compartments viz., leaf, root, epiphytes and phytoplankton, dried samples were pulverized using a Pulveriser Fritsch Pulverisette 7. A sub-sample of the pulverized sample was analysed in a Europa Scientific continuous flow mass spectrophotometer Geo 20-20 for the determination of nitrogen content (mg) and atom % ^{15}N in the tissues. Uptake rates of various compartments were then calculated with assumptions outlined by Cornelisen and Thomas (2002) using formulae modified from Mateo et al. (2001).

Calculation of uptake rates ($^{15}NH_3$ or $^{15}NO_3$) for seagrass tissues and epiphytes used the equation :

$$U = \frac{N \times (\text{At. \% } {}^{15}\text{N } E_T - \text{At. \% } {}^{15}\text{N } B_T)}{W \times t \times (\text{At. \% } {}^{15}\text{N } E_W - \text{At. \% } {}^{15}\text{N } B_T)}$$

Where,

U = Uptake rates in (mg N. g^{-1} DW. h^{-1})

At. % ^{15}N E_T = atom % ^{15}N in the enriched tissue

At. % ^{15}N B_T = atom % ^{15}N in the background tissue

At. % $^{15}N\ E_W$ = atom % ^{15}N in the enriched water (based on the amount of atom % ^{15}N and background atom % ^{15}N concentration)
N = Total nitrogen content in tissues in (mg)
W = dry weight of tissue in (g DW)
t = duration of incubation in (h)

Calculation of uptake rates ($^{15}NH_3$ or $^{15}NO_3$) for plankton used the equation :

$$U = \frac{PON \times V \times (At.\ \%\ ^{15}N\ E_T - At.\ \%\ ^{15}N\ B_T)}{W \times t \times (At.\ \%\ ^{15}N\ E_W - At.\ \%\ ^{15}N\ B_T)}$$

Where,
U = Uptake rates in (mg N. g^{-1} DW. h^{-1})
At. % $^{15}N\ E_T$ = atom % ^{15}N in the enriched tissue
At. % $^{15}N\ B_T$ = atom % ^{15}N in the background tissue
At. % $^{15}N\ E_W$ = atom % ^{15}N in the enriched water (based on the amount of atom % ^{15}N and background atom % ^{15}N concentration
PON = Total nitrogen content in tissues in (mg)
V = Total volume of the chamber (13.5 L)
W = dry weight of tissue in (g DW)
t = duration of incubation in (h)

2.2.3.4. Resource allocation

Uptake rates of nutrients in the previous section were biomass standardized. Biomass standardized nutrient uptake have been expressed in terms of uptake per gram of the component in question. This section investigates the uptake rate of the component without regard to its biomass. This measure, for the purposes of the study is known as “total component uptake” and reflects the greater contribution of the overall uptake of a component whose biomass is greater. The component here refers to leaf, root or epiphyte. These values for total component uptake and the total input were used to calculate percentage of resource allocated to each component.

Percent uptake of total available resource of NH_3 and NO_3 by biotic components at the end of the experiment is given in Appendix 5. These data show that there was no limitation of nutrients in the chamber at the termination of the experiment. Since the ambient levels of NH_3 and NO_3 were close to undetectable levels, the concentrations of $^{15}NH_3$ and $^{15}NO_3$ spiked were taken as total inputs / resource of NH_3 and NO_3 in the chamber.

2.2.4. Data analysis

Ammonium and nitrate uptake rate data were analysed by a non-parametric permutation based MANOVA (PERMANOVA Ver. 1.6; Anderson 2005a). A two way MANOVA (2 species x 3 seasons) was conducted with 4 dependent variables (uptake by leaves, roots, epiphytes and plankton) as illustrated in Figure 2.1. Separate analyses were carried out for ammonium and nitrate, as the experiments were conducted on different days and using different patches of seagrass. As PERMANOVA is a randomization / permutation based method, it is non-parametric, and therefore makes no assumptions of normality. However, non-parametric methods still make the assumption of homogeneity of variances, and where this is not satisfied, there is an increased chance of occurrence of a Type I error (Manly 1997). A balanced design such as that used in this study improves the accuracy of the result to some degree in the event of heterogeneity of variance (McArdle and Anderson 2004). In order to assess the level of homogeneity of variance, the nonparametric test "PERMDISP" (Anderson

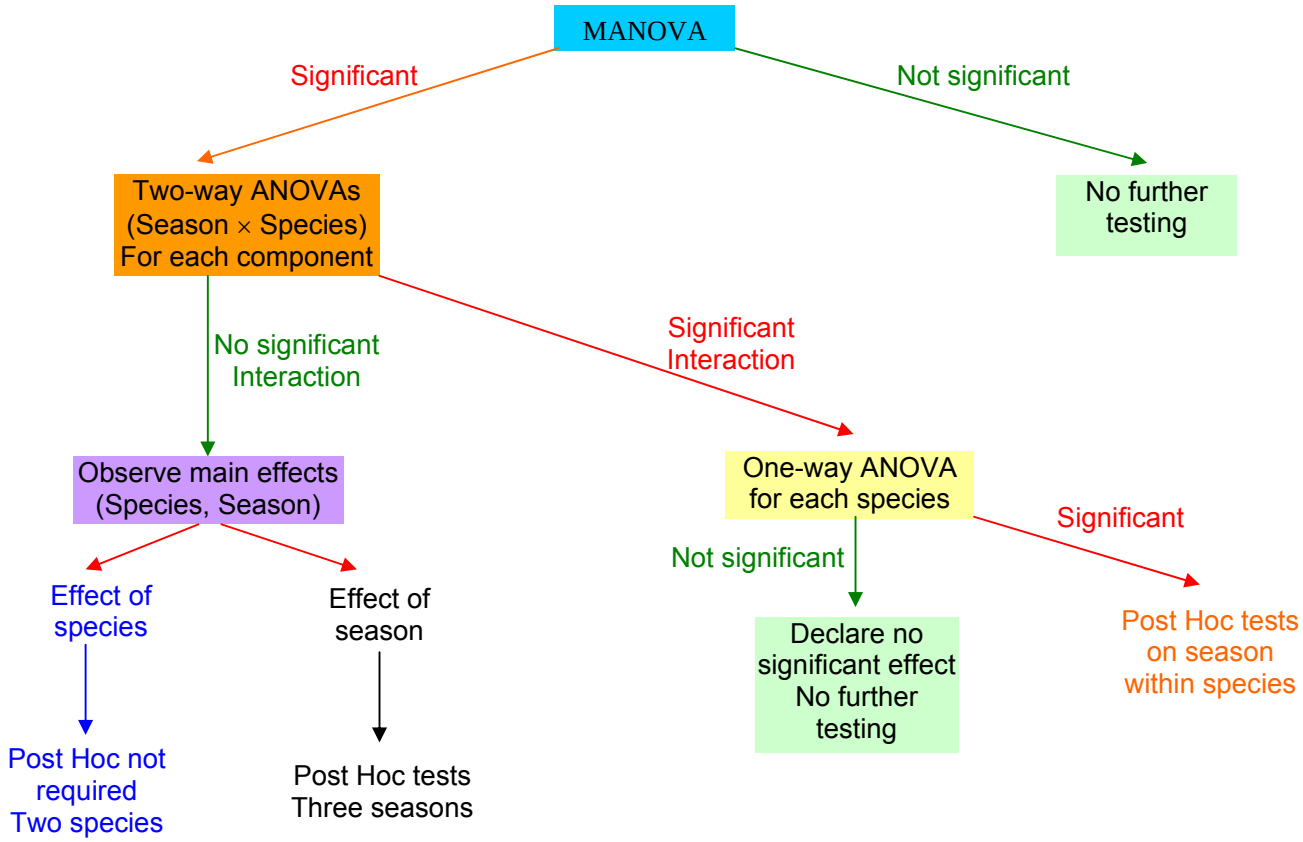

Figure 2.1 : Flow chart representation of the statistical tests used for ammonium and nitrate uptake rate studies.

2004b) was used to make an assessment. This test is a permutation based analogue of a Levene's test applied to multivariate data. Whilst homogeneity of variance would not always be achieved, the test was carried out (as there are no real alternatives) but caution must be applied to interpreting the results where the assumption was not met.

When the MANOVA was significant, separate univariate analyses were applied to test the effects of season and species on each of the leaf, root, epiphyte and plankton uptake components. Main effects were tested only where there was no interaction. Where an interaction occurred, the effect of season was examined within each species separately. In each case, wherever an ANOVA was conducted, the homogeneity of variances was assessed using PERMDISP. Post-hoc tests in PERMANOVA are not corrected for multiple tests, so the significant P value was adjusted in the manner of Bonferroni to account for this. In all cases, this meant a critical P value of 0.0166. Data were transformed to Ln (x+1) to meet the assumption of homogeneity of variance.

2.3. Results

2.3.1. Background physicochemical and biological data

Mean values of ambient and chamber water quality for various physicochemical parameters measured during chamber deployments for the three seasons are summarized in Appendix 6. Seasonal differences were very pronounced for most parameters, with the exception of salinity and pH. The differences between ambient levels and levels in the chamber were insignificant, with the exception of dissolved oxygen where mean concentrations ranged from 6.6 - 10.3 $mg.L^{-1}$ in ambient and 5.9 - 6.9 $mg.L^{-1}$ in chamber water. Details of the seasonal variations in biological parameters in *Posidonia* and *Amphibolis* meadows monitored during the study are summarized in Appendix 7. The above-ground and below-ground biomass for both species registered a peak in spring, and a reduction in summer. Epiphytes registered highest loading in summer (*Posidonia* : 0.57 ± 0.04 $g.g^{-1}$; *Amphibolis* : 5.03 ± 0.88 $g.g^{-1}$). Plankton abundance was highest in winter (29.3 ± 0.7 $cells.ml^{-1}$) and least in spring (5.0 ± 2.5 $cells.ml^{-1}$).

2.3.2. Uptake rates

2.3.2.1. Ammonium

Uptake of ammonium by the different components of the seagrass bed demonstrated a complex response to the effects of season and species, as indicated by a significant interaction effect in the MANOVA testing these effects (Table 2.1). When each of the components (seagrass leaves, roots, epiphytes, and plankton) were examined individually, the general trend was for a difference between species, but no effect of season. Leaves, roots and epiphytes all demonstrated significantly higher uptake in the *Amphibolis* complex than in *Posidonia* (Figure 2.2; Table 2.2*)*. Unsurprisingly, plankton had similar uptake regardless of the species of seagrass. Plankton did, however demonstrate different uptake rates according to season (Figure 2.1; Table 2.2). The seasonal difference in uptake by plankton was due to high uptake rates in winter that was not evident at other times of the year.

Uptake rates of ammonium by *Amphibolis* leaves ranged from 0.08 (winter and spring) to 0.14 (summer) mg $N.g^{-1}DW.\ h^{-1}$ (Figure 2.2). *Posidonia* leaves had uptake rates of 0.03 (summer) to 0.08 (spring) mg $N.g^{-1}DW.\ h^{-1}$. Roots of *Amphibolis* demonstrated mean ammonium uptake rates ranging from 0.01 mg $N.g^{-1}DW.\ h^{-1}$ in winter and spring to 0.03 mg $N.g^{-}DW.\ h^{-1}$ in summer. *Posidonia* root uptake rates were negligible, not exceeding 0.01 mg $N.g^{-1}DW.\ h^{-1}$.

Table 2.1 : Summarised MANOVA table for species and seasonal variabilities in ammonium and nitrate uptake rates of leaves, roots, epiphytes and plankton. The two forms of nitrogen were analysed separately. **Bolded** figures are significant at P=0.05.

Nutrient	Source	Degrees of freedom	Mean Sum of Squares	F	P
Ammonium	Species	1	6005.9	9.471	**0.003**
	Season	2	24412.4	38.497	**<0.001**
	Species × Season	2	2268.4	3.5771	**0.029**
Nitrate	Species	1	2124.2	4.391	**0.041**
	Season	2	29096.4	60.148	**<0.001**
	Species × Season	2	427.6	0.884	0.452

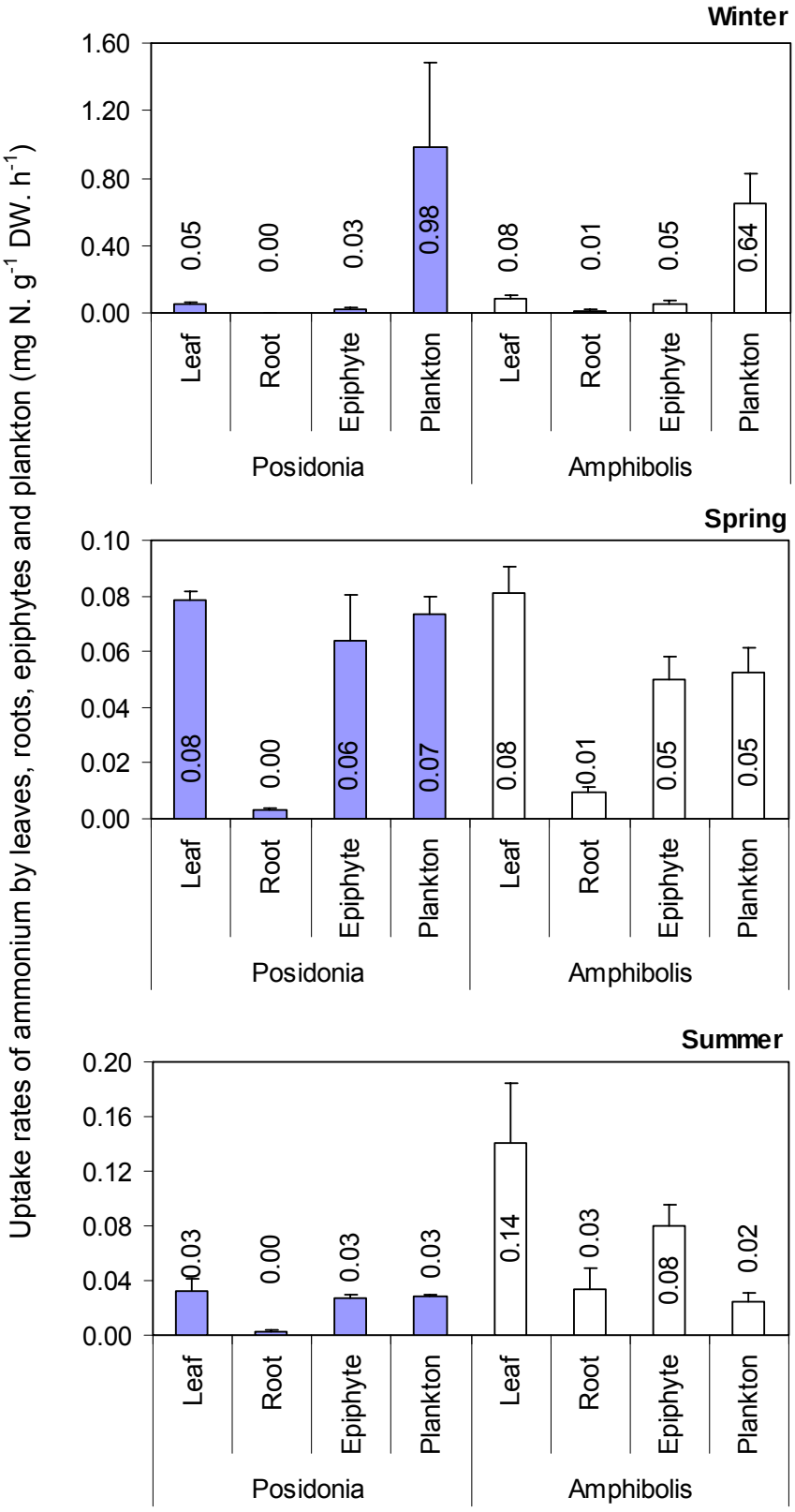

Figure 2.2 : Mean seasonal uptake rates of ammonium by leaves, roots, epiphytes and plankton in *Posidonia* and *Amphibolis*. Error bars depict standard error of means (n=3). The Y-axis scales on the three graphs differ.

Table 2.2 : Summarised results of two-way ANOVA for uptake rates of ammonium and nitrate by leaves, roots, epiphytes and plankton for species, season and their interactions. Data were transformed to ln (n+1) prior to analyses. **Bolded** figures are significant at P=0.05.

Nutrient	Dependent variable	Fixed factor	Degrees of freedom	Mean Sum of Squares	F	P
Ammonium	Leaves	Species	1	12587.3	4.215	**0.021**
		Season	2	5440.2	1.822	0.116
		Species × Season	2	3834.6	1.284	0.271
	Roots	Species	1	28926.8	12.998	**<0.001**
		Season	2	4145.9	1.863	0.125
		Species × Season	2	1526.4	0.686	0.632
	Epiphytes	Species	1	14960.1	6.088	**0.017**
		Season	2	4518.3	1.839	0.180
		Species × Season	2	6745.4	2.745	0.078
	Plankton	Species	1	44.5	0.060	0.997
		Season	2	28375.1	38. 454	**<0.001**
		Species × Season	2	708.4	0.960	0.410
Nitrate	Leaves	Species	1	2550.8	1.756	0.179
		Season	2	21322.9	14.679	**<0.001**
		Species × Season	2	2174.3	1.497	0.239
	Roots *	Species	1	26758.4	17.748	**<0.001**
		Season	2	4278.9	2.838	**0.048**
		Species × Season	2	6784.3	4.499	**0.009**
	Epiphytes	Species	1	7276.5	2.802	0.052
		Season	2	10103.6	3.891	**0.006**
		Species × Season	2	4167.8	1.605	0.175
	Plankton	Species	1	603.8	1.354	0.251
		Season	2	30093.8	67.487	**<0.001**
		Species × Season	2	417.4	0.936	0.422

* Failed homogeneity of variance test

Overall, root uptake rates were lower than those of the other biotic components when compared on a per gram basis. Epiphytic uptake rates ranged from 0.03 to 0.06 mg $N.g^{-1}DW.\ h^{-1}$ for *Posidonia* and 0.05 to 0.08 mg $N.\ g^{-1}DW.\ h^{-1}$ for *Amphibolis*. Ammonium uptake by plankton peaked in winter (0.98 and 0.64 mg $N.g^{-1}DW.\ h^{-1}$ for *Posidonia* and *Amphibolis* respectively) decreasing in spring and dropping further in summer (0.03 and 0.02 mg $N.\ g^{-1}DW.\ h^{-1}$ for *Posidonia* and *Amphibolis* respectively). Ammonium uptake by plankton revealed significant differences between seasons ($P<0.001$; ANOVA), with post-hoc test revealing all seasons to be significantly different from each other ($p<0.01$).

2.3.2.2. Nitrate

In general, nitrate was taken up an order of magnitude slower than ammonium (Figure 2.3 c.f. 2.1). In contrast to the general trend in ammonium uptake, nitrate uptake in the biotic components was significantly affected by season, but not species (Figure 2.3, Table 2.2; two way ANOVAS conducted after significant MANOVA; Table 2.1). Only the root component differed from this trend. Plankton uptake varied considerably, with an order of magnitude difference between each of the three seasons (0.690 and 0.458 mg $N.g^{-1}DW.\ h^{-1}$ in winter down to 0.05 and 0.06 mg $N.g^{-1}DW.\ h^{-1}$ in spring to 0.005 and 0.003 mg $N.g^{-1}DW.\ h^{-1}$ in summer; in all cases *Posidonia* presented before *Amphibolis*). Post-hoc tests identified all seasons as being different to one another ($P<0.001$ all cases). Leaf uptake, whilst low, was greatest in spring, demonstrating uptake rates of 0.009 and 0.011 mg $N.g^{-1}DW.\ h^{-1}$ for *Posidonia* and *Amphibolis* respectively (c.f. 0.003 - 0.005 mg $N.g^{-1}DW.\ h^{-1}$at other times of the year). Epiphyte uptake also differed between seasons, with highest uptake in spring (0.012 and 0.058 mg $N.g^{-1}DW.\ h^{-1}$ for *Posidonia* and *Amphibolis* respectively). Summer registered lower mean values, although not significantly different to spring, while winter was significantly lower (Post hoc test, $P=0.0052$; 0.003 and 0.006 mg $N.g^{-1}DW.\ h^{-1}$ for *Amphibolis* and *Posidonia* respectively). Uptake of nitrate by the root component did not differ with species or season (see earlier) and was almost negligibly low. A significant interaction effect of season and species on root uptake (Table 2.2) made it necessary to examine the effect of season individually for each species. For neither species was uptake significantly different between species ($P>0.05$ one way ANOVA).

2.3.3. Resource allocation

It is evident that there was a clear preference for the uptake of ammonium over nitrate by the biotic components. *Amphibolis* utilised ammonium more efficiently than *Posidonia*, taking up 85% of the total resource in spring to about 22% in summer (Appendix 5). *Posidonia* on the other hand, utilised between 4% in summer and 8.6% in spring. Nitrate utilisation was also higher in *Amphibolis* than *Posidonia*. *Amphibolis* took up about 17% of the total resource in spring to 1.4% in summer. Uptake by *Posidonia* ranged between 8.7% in spring down to 0.3% in summer.

2.3.3.1. Ammonium

Total component uptake of ammonium in *Posidonia* was highest in spring followed by summer and least in winter (Figure 2.4). Uptake by leaves dominated all three seasons, with resource allocation ranging from 61% in winter to 73% in summer. Uptake by roots remained consistent at 21% in all three seasons. Winter saw an epiphytic uptake of 15% of the total uptake. The contribution of epiphytes reduced from 11% in spring to 3% in summer.

Biotic uptake of ammonium by *Amphibolis* was highest in spring, followed by winter and summer (Figure 2.5). Resource allocation into leaves was at its peak in summer (79%), followed by winter (39%) and spring (34%). Root uptake also peaked in summer (19%), remaining consistent in winter and spring (4%). Epiphytes accounted for the bulk of the ammonium resource in winter (57%) and spring (61%).

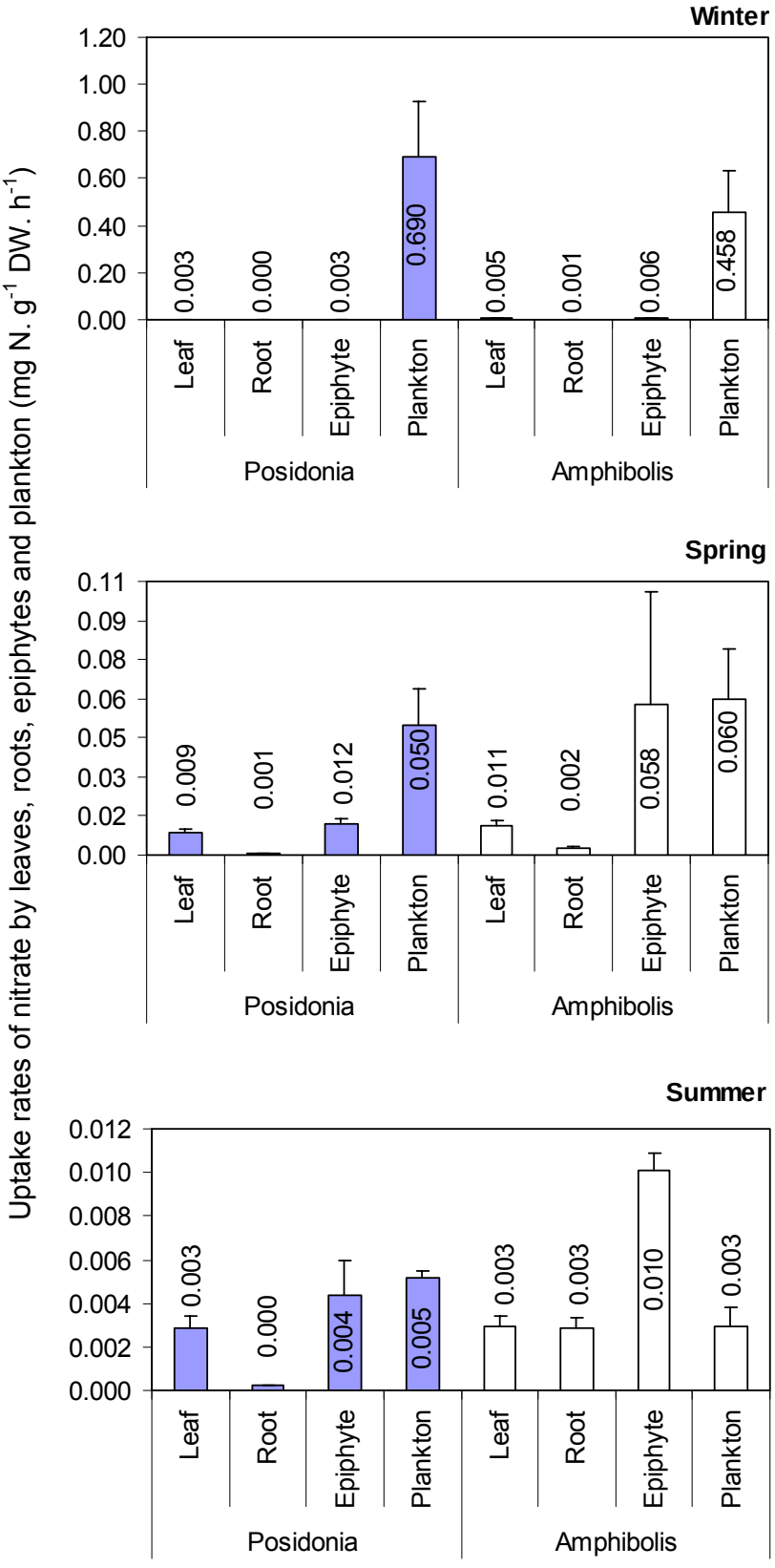

Figure 2.3 : Mean seasonal uptake rates of nitrate by leaves, roots, epiphytes and plankton in *Posidonia* and *Amphibolis*. Error bars depict standard error of means (n=3). The Y-axis scales on the three graphs differ.

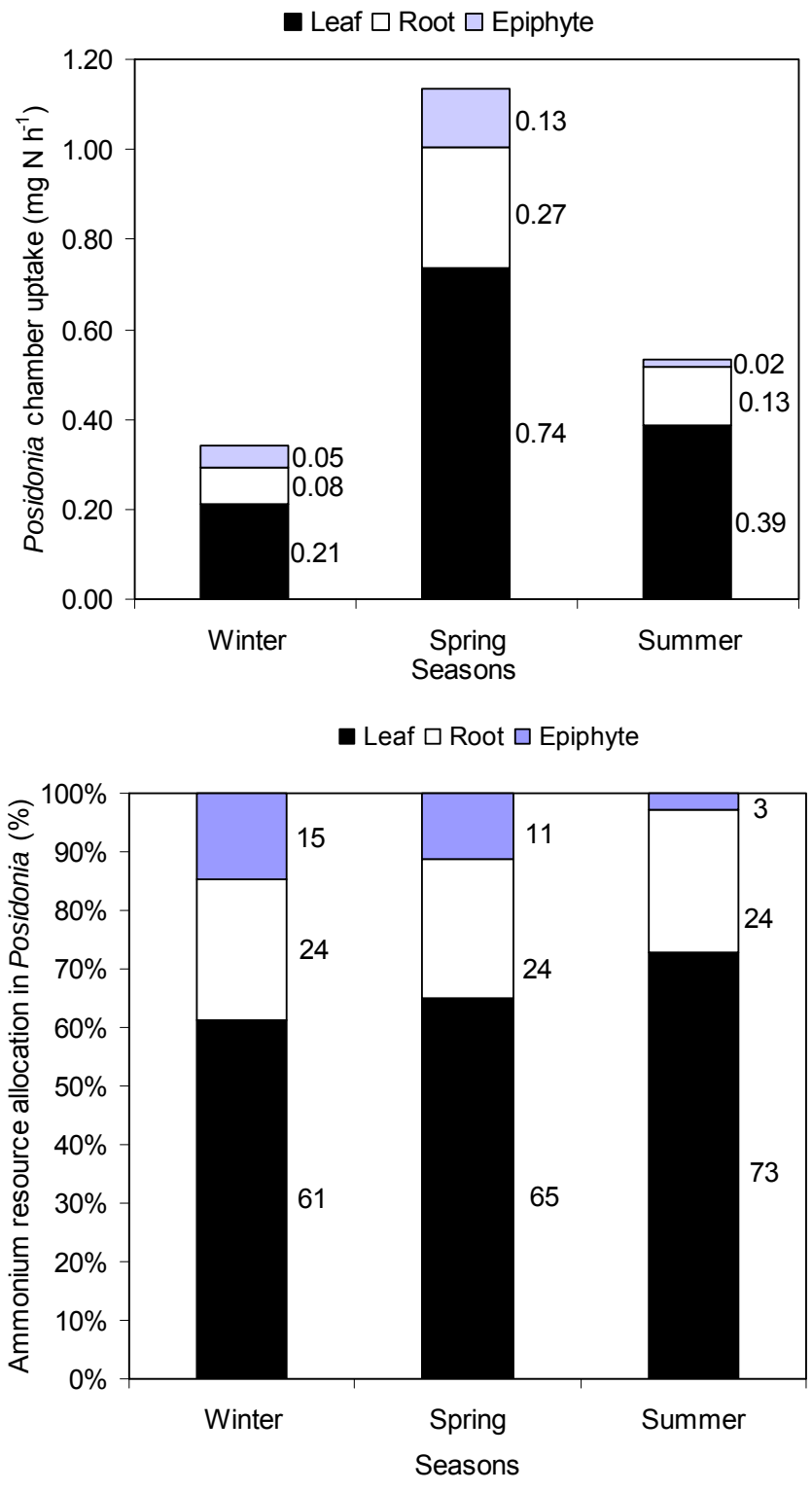

Figure 2.4 : Seasonal variation in allocation of ammonium resources in leaves, roots, and epiphytes in *Posidonia*. The total component uptake rates take into account the effect of the different biomass of each component.

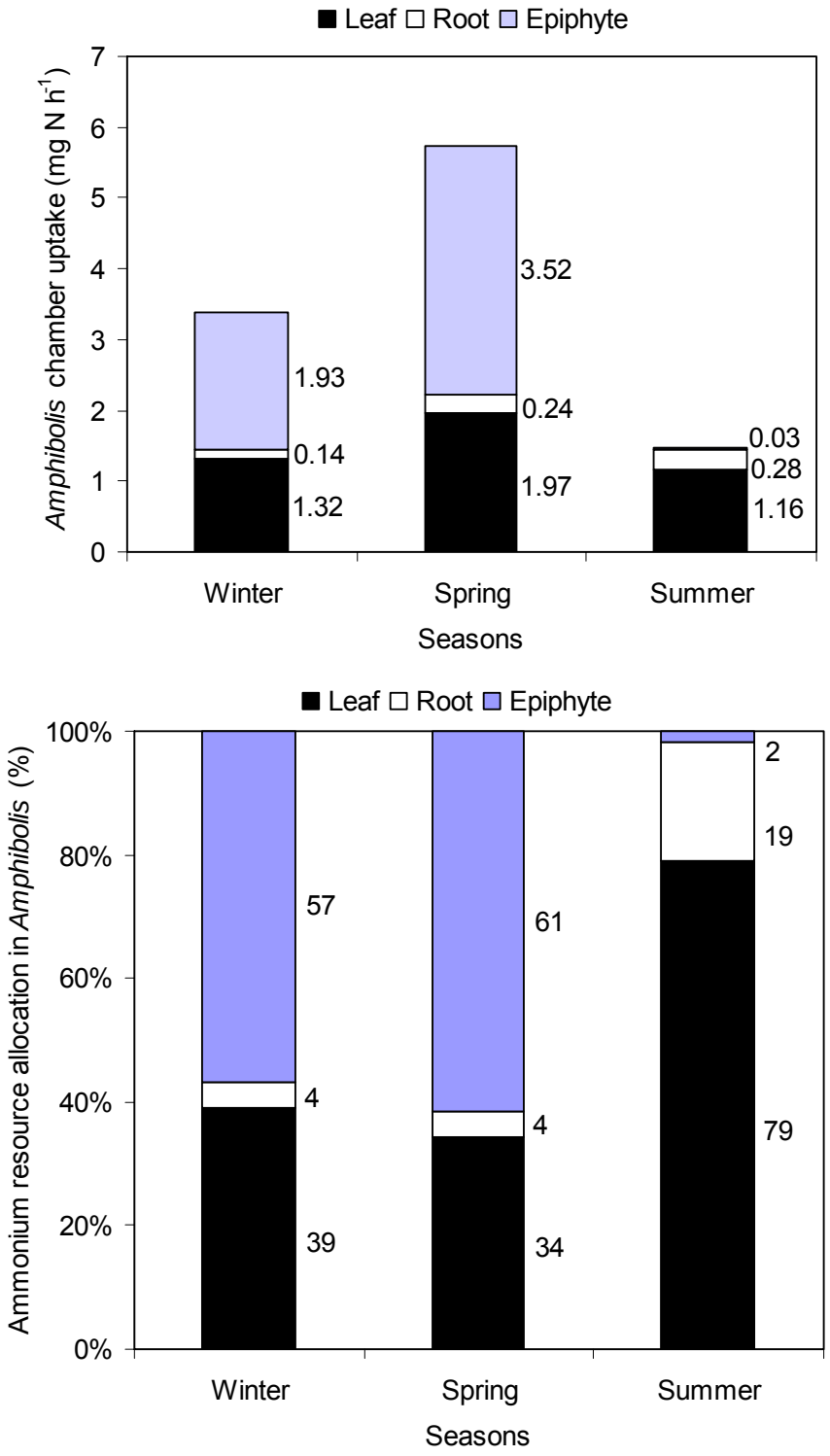

Figure 2.5 : Seasonal variation in allocation of ammonium resources in leaves, roots, and epiphytes in *Amphibolis*. The total component uptake rates take into account the effect of the different biomass of each component.

2.3.3.2. Nitrate

As with ammonium, total component uptake of nitrate in *Posidonia* peaked in spring (Figure 2.6). A very distinct seasonal pattern was observed with the allocation of nitrate resources in a *Posidonia* bed. Leaf accounted for nearly 49% of the total resource in winter, followed by a near equal contribution of roots and epiphytes (26% each). However, in spring epiphytes accounted for over 75% of the total resources of nitrate, followed by leaves (16%) and roots (9%). Leaves accounted for the bulk of the resources in summer (64%), followed by roots (30%) and epiphytes (6%). Highest total component uptake rates of nitrate in *Amphibolis* were observed in spring, followed by winter and summer (Figure 2.7). As in *Posidonia*, a distinct seasonal pattern in resource allocation was observed. Winter was characterized by high allocation of resources into epiphytes (59%), followed by leaves (34%) and roots (7%). Allocation of resources into epiphytes growing on *Amphibolis* dominated the total biotic uptake in spring amounting to 61%, with leaves 36% and roots 3%. In summer, seagrass uptake accounted for the bulk of the total resources with leaves accounting for 52% and roots 37%, while epiphytic uptake was about 11% of the resources.

2.4. Discussion

The results of this study indicate that *Amphibolis* and *Posidonia*, the two dominant seagrass taxa found off the Adelaide metropolitan coast (Westphalen et al., 2005) showed an increased affinity for ammonium over nitrate. *Amphibolis* was observed to utilise both ammonium and nitrate more efficiently than *Posidonia*. Highest utilisation of inorganic nitrogen was in spring and least in summer.

Tissue nitrogen uptake rates are partially dependent on photosynthesis, which supplied the required energy (Turpin et al., 1990; Huppe and Turpin, 1994). Seagrass photosynthetic efficiency is closely correlated to water temperature and underwater irradiance, both of which exhibit seasonal trends (Herzka and Dunton, 1997), thereby affecting seasonal inorganic nitrogen acquisition rates. The condition of the seagrass, especially *Amphibolis*, appeared to be relatively poor in summer based on visual observations. Fine sediments were observed to have settled on the seagrass, possibly originating from Outer Harbour dredging operations. Under these conditions, seagrass photosynthesis may be lowered and as a result, density, biomass, nutrient uptake processes and the aerial extent of seagrasses might be affected (Walker and McComb, 1992; Fitzpatrick and Kirkman, 1995; Bondsorff et al., 1997; Short and Neckles, 1999), offering a possible explanation for reduced biological uptake of inorganic nitrogen in summer. However, photosynthetically available radiation measured at an adjacent site (Collings et al. 2006a) did not register a decline in summer when compared to winter or spring.

It was interesting to see higher uptake of inorganic nitrogen by leaves of both *Posidonia* and *Amphibolis* than by roots. Although seagrass roots are exposed to dissolved inorganic nitrogen (DIN) concentrations that are an order of magnitude greater than water column concentrations, their leaves account for a significant portion of total nitrogen acquisition (Izumi and Hattori, 1982; Short and McRoy, 1984; Lee and Dunton, 1999). In a whole plant nutrient budget developed for *Thalassia testudinium*, Lee and Dunton (1999) reported that leaves and roots contributed equally to the total nitrogen budget. However, the results of this study revealed that the roots contributed only a small percentage to the inorganic nitrogen taken up when compared to the leaves of both species. Higher inorganic nitrogen uptake affinities of the leaves have been reported to be an adaptation to maximize nutrient assimilation in oligotrophic environments (Burkholder et al., 1994; Lee and Dunton, 1999). Published leaf uptake rates for ammonium (5-270 μmol g^{-1} DW h^{-1}) are comparable to the uptake rates reported in this study, while, nitrate uptake rates of this study are nearly 10 orders of magnitude lower than the rates reported by Touchette and Burkholder (2000) of 3-75 μmol g^{-1} DW h^{-1}.

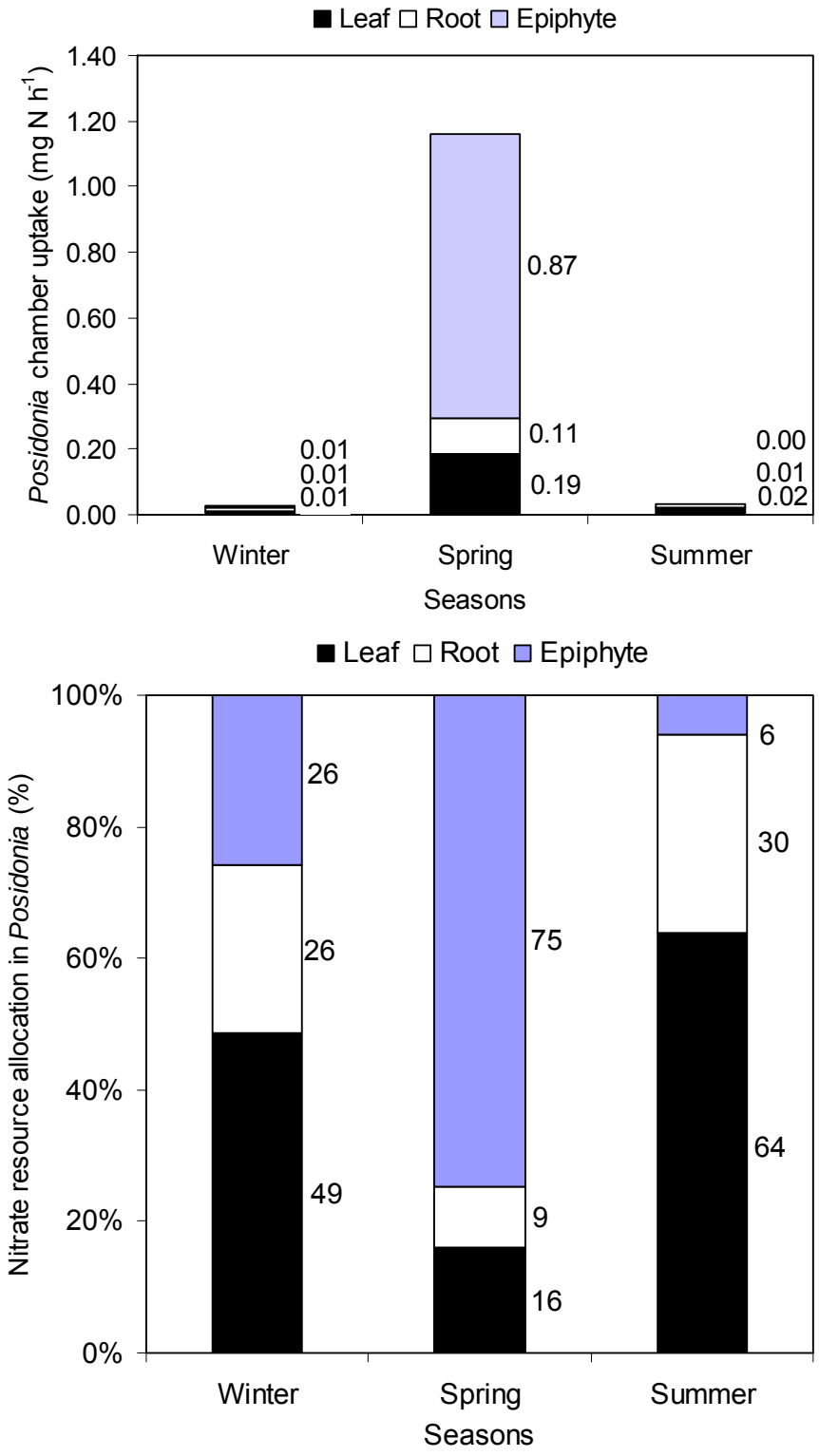

Figure 2.6 : Seasonal variation in allocation of nitrate resources in leaves, roots and epiphytes in *Posidonia*. The total component uptake rates take into account the effect of the different biomass of each component.

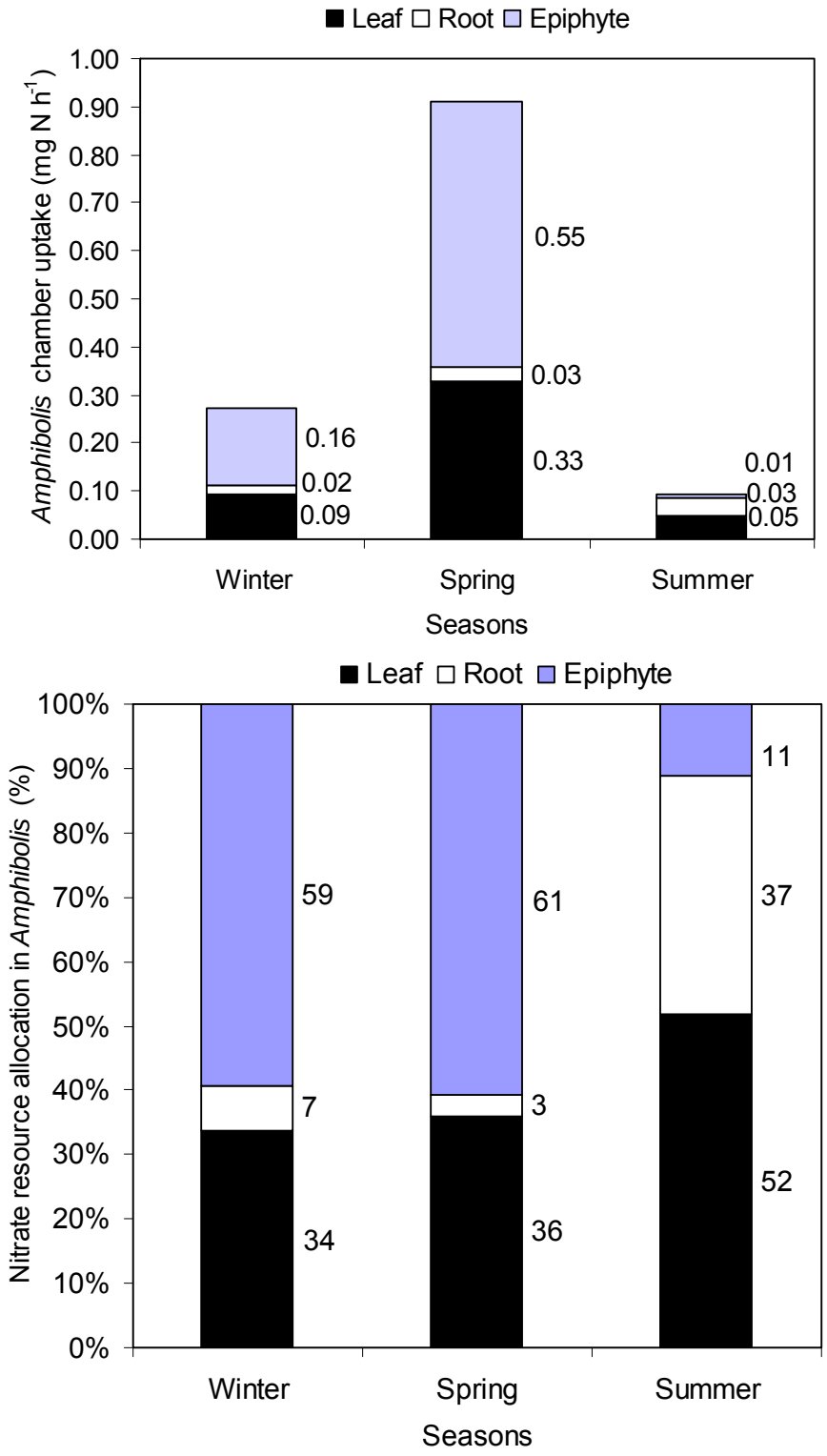

Figure 2.7 : Seasonal variations in allocation of nitrate resources in leaves, roots and epiphytes in *Amphibolis*. The total component uptake rates take into account the effect of the different biomass of each component.

This variation could be attributed to the fact that some seagrass species show a lower affinity for uptake of nitrate over ammonium (Touchette and Burkholder, 2000). Paling and McComb (1994) also reported significantly lower uptake rates for nitrate than ammonium by *Amphibolis* seedlings, suggesting a higher affinity for ammonium in the water column. Studies on other species have demonstrated a higher uptake affinity for ammonium than nitrate for seagrass leaves (eg. Short and McRoy, 1984; Terrados and Williams, 1997; Lee and Dunton, 1999), which has been attributed to physiological demands associated with uptake of nitrate (Roth and Pregnall, 1988; Turpin et al., 1991; Touchette and Burkholder, 2000). Thus the findings from this study were consistent with earlier studies that demonstrated a preference for the reduced form of nitrogen (i.e., ammonium) over nitrate.

In a comparative study on uptake rates of ammonium and nitrate by *Amphibolis antarctica* and macroalgae in Western Australia, *Amphibolis* seedlings and adults were reported to assimilate nutrients at a comparable rate as algae at higher background levels of nutrients (Paling and McComb, 1994). However, the authors reported that at lower ambient concentrations of nutrients, seagrass leaves were less efficient at taking up water column nutrients than some of the structurally complex algae. Microalgae and macroalgae are competitively at an advantage over seagrass as they are known to be far more efficient in assimilating nutrients. When background concentrations of nutrients are high, the growth of epiphytes and phytoplankton are favoured at the expense of seagrass production (Kemp et al., 1983; Borum, 1985). Previous studies have provided evidence that epiphytes can either physically inhibit uptake of nutrients by seagrass leaves (Johnstone, 1979; Sand-Jensen et al., 1985; Cornelisen and Thomas, 2004) or out-compete seagrasses for water column nutrients because of their superior uptake kinetics (Sand-Jensen, 1977; Wallentinus, 1984; Sand-Jensen et al., 1985).

Higher epiphytic loading and therefore higher epiphyte biomass is also a likely explanation for higher resource allocation of ammonium and nitrate to epiphytes on *Amphibolis* over *Posidonia*. That loading difference could be attributed to the differences in morphology and growth characteristics of the species (Shepherd et al., 1989). The terete, woody stems of *Amphibolis* offer more surface area for settlement of large epiphytes. In contrast, the blades of *Posidonia* support far lower standing crops of much smaller algae, explaining lower resource allocation of ammonium in epiphytes on *Posidonia* (Shepherd, 1973; Borowitzka et al., 1990; Lavery and Vanderklift, 2002). Smothering of epiphytes by resuspended sediments from the dredging operations is the most likely cause for reduced epiphytic uptake of inorganic nitrogen during summer.

3. Modelling the fate of anthropogenic inputs of nitrogen in seagrass meadows off the Adelaide metropolitan coast.

3.1. Introduction

Seagrasses are critical components of near-shore systems as they contribute to productivity, stability and biodiversity. Seagrass beds also provide habitats to a large group of organisms including fish and invertebrates, and act as effective nutrient sinks (Harris, 1999; Dudley et al., 2001; Harris, 2001; MacFarlane and Booth 2001). It has been reported that increased nutrient loading in a seagrass habitat beyond a critical threshold is often accompanied by large declines in seagrass cover (Shepherd et al., 1989). Very often these declines are reported to be irreversible (Harris, 1999). Since the 1940's, approximately 5000 ha of seagrass meadows have been lost from the Adelaide metropolitan coast. Most of the seagrass loss along the Adelaide metropolitan coast occurred close to shore advancing seaward (Westphalen et al., 2005). Being an effective sink for nutrients, the loss of seagrass and its associated components are likely to have long-term deleterious effects on the ecosystem. Simple numeric models or ecosystem models are effective management tools that often assist in predicting impacts and assisting in more effective management.

One of the primary purposes of modeling is to use knowledge gleaned at one scale to extrapolate and make predictions at another scale. In this study, a simple model has been created to examine the uptake of nutrients within the context of the Adelaide coast. The model essentially takes the results elicited from small-scale experiments and extrapolates them to describe the situation for the much larger area represented by the entire metropolitan coast.

Numerical models are predictive tools developed to understand relevant variables, interactions and ecosystem processes. Conceptual models allow a framework to be provided, outlining the important processes, sinks and sources. Numerical models represent an attempt to quantify these frameworks to some degree. How far such quantification proceeds is a reflection of both pragmatic and theoretical considerations. Appropriate simplification is an important feature of a model. Increased complexity often fails to result in increased accuracy or understanding. Hence, sensible decisions have to be made as to the level of complexity that is to be incorporated. In some instances, these decisions need to be made for pragmatic reasons, and this needs consideration when assessing the conclusions. In this study, important assumptions have been made regarding the relationship between ambient nutrient concentrations and uptake rates, and biomass estimations of each of the components. There are a number of studies where numerical models have been developed to measure responses of seagrass communities to increased nutrient loading (McEwan et al., 1998). In the Australian context, they have been successfully applied to other coastal water studies (Harris et al., 1996; McEwan et al., 1998; Murray and Parslow, 1999).

The model proposed here is based on measured seasonal uptake rates of nitrogen in *Amphibolis* and *Posidonia* seagrass complexes comprising leaves, roots, epiphytes and plankton, and identifies compartments that are significant in the cycling of nitrogen in both species. The two scenarios presented in the model cover a period during peak impacts (1978), and the current conditions (2005) in the Adelaide Coastal Waters Study region (Figure 3.1). The model provides a coarse estimate of total ammonium and nitrate assimilation, as it is based on numerous assumptions highlighted in section 3.2. Nevertheless, it serves to put the small scale results identified in the uptake experiments into the broader context of the Adelaide metropolitan coast and its associated inputs.

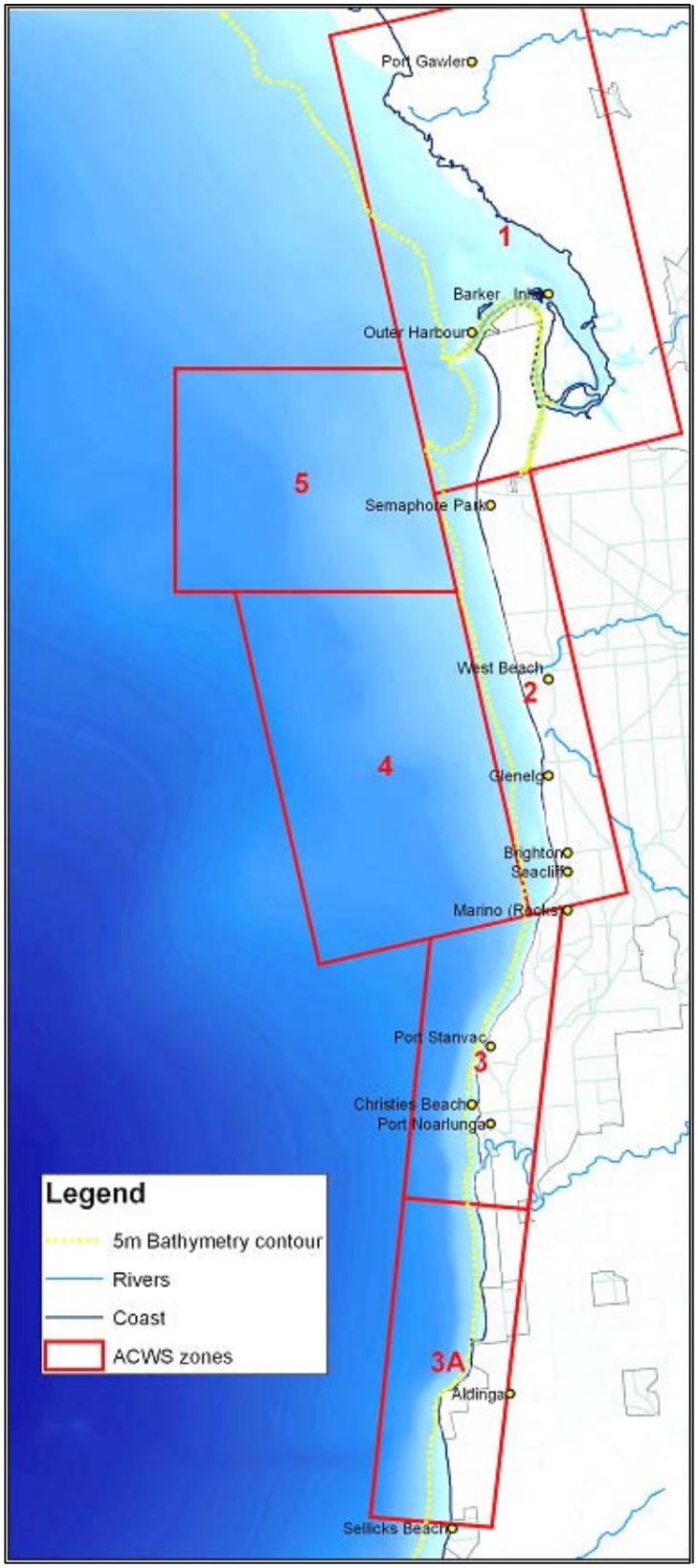

Figure 3.1 : The ACWS study zone referred to in the nutrient model (from Wilkinson et al., 2005).

3.2. Model description, parameters used and assumptions

Nutrient uptake rates are dependent not only on the physiology of the plant, but also on the ambient level of the nutrients in the water column. Increased concentration in the water column results in faster uptake rates (Romero et al. 2006). However, the shape of the relationship between ambient concentration and uptake is variable. As the concentrations of nutrients used in the chamber experiments (1 mg L^{-1}) were well beyond what is naturally experienced in the field, if we are to construct a real-world model, it is necessary to consider what the uptake rate should be under natural, rather than elevated conditions. A Michaelis-Menten type curve is typically evident across the entire range of ambient nutrient concentrations. However, several authors have demonstrated a linear relationship between uptake rate and ambient nutrient concentration at nutrient levels in the range we were working with (Iizumi and Hattori, 1982; Thursby and Harlin, 1984; Paling and McComb, 1994; Pedersen et al., 1997; Lee and Dunton, 1999; Rossier, 2004; Cornelisen and Thomas, 2006). This observation implies that saturation rates are much higher than those used in these experiments where concentrations were presumably at or below the half-saturation values (Km). Thus, based on a linear relationship, an equation was constructed to calculate the uptake rate as it would be expected under ambient (natural) nutrient concentrations :

$$Uptake\ Rate_{[Natural]} = Uptake\ Rate_{[Experimental]} \times \frac{[Natural\ Concentration]}{[Experimental\ Concentration]}$$

Note that this relationship assumes an intercept of zero, which equates to an assumption that at an ambient concentration of zero that there is no uptake. The linear relationship of uptake rates to ambient concentration used in this study provide more conservative estimates than Michaelis-Menten uptake.

Having estimated the uptake rates that are likely to be operating under natural conditions for each of the major biotic components, it was necessary to quantify the biomass of each of those components. Experimental uptake rates are expressed on a biomass standardized (i.e. per gram) basis. Therefore, an estimation of the biomass involved allows us to extrapolate these results to the scale of the Adelaide metropolitan coast. Parameterisation of these components was based on the values presented in Table 3.1.

Daily uptake across the entire region for a day may then be calculated as :

$$Daily\ Uptake = Uptake\text{-}Rate_{[Natural]} \times Biomass \times 24hrs$$

Daily uptakes for each day are summed to provide an annual picture. Note that daily rates will differ between seasons (as experimentally determined) and this is incorporated within the model. As uptake rates were available for only 3 seasons, the autumn rate was calculated as an average of those three seasons.

The major assumptions inherent in this model are:

a) That the experimental uptake rates accurately represent the seasonal uptake.
b) That the linear relationship between ambient concentration and uptake rate is constant.
c) That biomass has been accurately estimated across the Adelaide metropolitan region.
d) That the boundaries used to describe the Adelaide coastal region (Figure 3.1) accurately represent the extent of the movement of nutrients.

e) That ambient concentrations are even across the zone such that up-take kinetics remain within the linear bounds described by the model across the whole region. This is unlikely to be true because concentrations around major point sources are known to be elevated and seagrass systems in these regions will almost certainly experience loadings above the Km value.

Modeling nitrogen uptake by seagrass is a complex process as physical characteristics such as wind exposure, hydrodynamics, exposure to run-off, nutrient levels, turbidity, tidal changes and surrounding vegetation affect uptake processes on a seagrass bed (Morgan and Kitting, 1984), often leading to large variations in nutrient uptake characteristics in space and time. There are a variety of unquantified processes which we have had to assume do not contribute significantly to the model. Notably, denitrification is discounted based on the assumption that, whilst variable, it is typically important only when ammonium levels exceed the primary producers demand (Romero et al. 2006). In these oligotrophic waters, ammonium levels are generally low (as measured in this study), and therefore denitrification is considered of minor importance to this model, as it is only likely to occur in the region of plume discharge (not measured in this study). Translocation between roots and leaves is discounted as both components are considered in this model and an increase in one because of translocation will be balanced by a decrease in the other. Leaching of organic nitrogen by the seagrasses is of relatively minor (<10%; Borum et al. 1989) importance. As uptake rates were measured for two hours during the day, it is assumed that this rate is an accurate representation of uptake across the entire 24 hour cycle. The assumption made is not unreasonable as Lee and Dunton (1999) demonstrated no difference in ammonium and nitrate uptake in *Thalassia testudinum* between night and day. Uptake by algal components is also ignored as macroalgae cover a very small proportion of the substrate in comparison to seagrass as very little of the substrate is rocky.

For the scenario where a historical comparison is made, the model assumes that the biological uptake rates and the average biomass were constant. The model covers the current scenario and compares the current annual biotic assimilative capacity with the scenario in 1978 when all the sludge pipes were operational in the study area (Wilkinson et al., 2005). At this stage there was more seagrass and higher nutrient levels. *Posidonia* cover decreased 14% between 1978 and 2005, and *Amphibolis* decreased 99%. However, biomass specific uptake rates also differed because of the higher ambient concentration in 1978. It is worthy to note that the estimation of Blackburn and Decker (2005) is used to estimate current *Posidonia* and *Amphibolis* cover. There is some concern with this due to the very low cover of *Amphibolis* (Table 3.1), particularly in light of the fact that they record no *Amphibolis* from the Grange area; this study was carried out in that locality on *Amphibolis* beds, which are still surviving. However, the effect on the model is unlikely to be severe as their account indicates *Posidonia* being present which will at least have some effect on uptake. Furthermore, *Amphibolis* has always represented a far lesser component of the system than *Posidonia* in terms of coverage (Table 3.1). The integration of leaf, root and epiphytic compartments is referred to as the seagrass complex (*Posidonia* or *Amphibolis*). When historical loss of seagrass is modeled, it involves the loss of this whole complex, resulting in bare sand. However, it specifically excludes the phytoplankton component, making the implicit assumption that the phytoplankton biomass is independent of the seagrass bed.

This model is designed to put our experimental uptake rates into the context of the larger area and estimate the effect, on gross nitrogen uptake, of various historically relevant losses of seagrass. As it is a simple deterministic model, no attempts were made to statistically analyse the outcomes.

Table 3.1 : Parameters used for the nutrient model and their literature source.

Parameters used in the model	Value	Source
Adelaide metropolitan coast study area	224 km^2	Blackburn and Dekker, 2005
Posidonia cover in the study area in 2005	131 km^2	Blackburn and Dekker, 2005
Amphibolis cover in the study area in 2005	0.17 km^2	Blackburn and Dekker, 2005
Posidonia cover in the study area in 1978	152.32 km^2	Shepherd et al., 1989; Edyvane, 1999
Amphibolis cover in the study area in 1978	38.08 km^2	Shepherd et al., 1989; Edyvane, 1999
Average Depth (assumes depth grades uniformly from 0 to edge of seagrass at 18 m)	9 m	Westphalen et al., 2005
Posidonia leaf biomass in winter spring and summer	130, 415 & 270 $g.m^{-2}$	Lill, 2005
Posidonia root biomass in winter spring and summer	1700, 2230 & 2000 $g.m^{-2}$	Lill, 2005
Epiphyte loading on *Posidonia* in winter spring and summer	65, 43 & 55 $g.m^{-2}$	Lill, 2005
Amphibolis leaf biomass in winter spring and summer	100, 213 & 150 $g.m^{-2}$	Lill, 2005
Amphibolis root biomass in winter spring and summer	150, 566 & 200 $g.m^{-2}$	Lill, 2005
Epiphyte loading on *Amphibolis* in winter spring and summer	130, 144 & 105 $g.m^{-2}$	Lill, 2005
Plankton biomass in the study area during winter spring and summer	0.085, 0.042 & 0.023 $mg.L^{-1}$	Bryars et al., 2006
Mean ambient ammonium concentrations in 2005	0.017 $mg.L^{-1}$	Bryars et al., 2006
Mean ambient nitrate concentrations in 2005	0.001 $mg.L^{-1}$	Bryars et al., 2006
Mean ambient ammonium concentrations in 1976	0.05 $mg.L^{-1}$	Steffensen, 1985
Mean ambient nitrate concentrations in 1976	0.03 $mg.L^{-1}$	Steffensen, 1985

3.3. Results and discussion

3.3.1. Assimilation rates at the present time

Modeled data showed high ammonium assimilation by the *Posidonia* seagrass complex when compared to the *Amphibolis* complex for all seasons in the study area (Figure 3.2a). Although measured biomass-standardized ammonium uptake rates of the *Amphibolis* complex are a lot higher than the *Posidonia* complex (see chapter 2), in terms of overall assimilation, contribution by the *Amphibolis* complex was insignificant. Since *Amphibolis* has been reported to be more sensitive to the effects of sludge than *Posidonia*, the loss of *Amphibolis* stands from the Adelaide metropolitan coast is likely to have been more rapid than that of *Posidonia* (Neverauskas, 1985). Currently, *Amphibolis* cover is less than 0.01% of the total seagrass cover in the ACWS study area (Blackburn and Dekker, 2005), making the contribution by the *Amphibolis* complex to the total assimilation of ammonium relatively insignificant. Assimilation by plankton was significant only in winter, when relatively higher plankton biomass is found to occur in these waters. Biotic components assimilated approximately 0.8, 2.3 and 0.8 tonnes of ammonium per day during winter, spring and summer, respectively. The seagrass complex accounted for nearly 90% in winter and 100% percent in spring and summer of the total ammonium assimilated per day by biotic components.

Seasonal differences in biotic nitrate assimilation were evident, with peak values in spring (0.018 tonnes. day^{-1}), followed by winter (0.006 tonnes. day^{-1}) and summer (0.005 tonnes. day^{-1}). Seagrass complexes accounted for 49% in winter and 99% in spring and summer, of the total biological uptake of nitrate. Plankton accounted for nearly 50% of the total biotic assimilation of nitrate in winter, but was not a significant contributor in spring and summer (Figure 3.2b). As with ammonium assimilation, only the *Posidonia* seagrass complex took up significant amounts of nitrate. Leaves of *Posidonia* assimilated about 65% and 55% of the total nitrate assimilated, followed by roots (25 and 29%) and epiphytes (9% and 17%) in spring and summer respectively.

In summary, only a third of the current input of ammonium (1509.3 t yr^{-1}) to the Adelaide coastal waters (Wilkinson et al., 2005) was taken up by modeled biotic uptake. The seagrass complexes accounted for 98% of the total biological assimilation from the metropolitan coastline. On the other hand, the modeled biotic nitrate assimilation of 3.44 t yr^{-1} accounted for less than 1% of the total nitrate input of 473.6 t yr^{-1} to the coastal waters of Adelaide. Of the total nitrate assimilated, the seagrass complex accounted for nearly 88%.

3.3.2. Historical comparison

Between 1978 and 2005, the ACWS study area lost about 31% of its seagrass. According to the model this decline in seagrass is associated with a reduction in the quantity of ammonium and nitrate assimilated (Figure 3.3). Nearly 1600 tonnes less of ammonium and 164 tonnes less of nitrate are assimilated under the 2005 scenario than the 1978 scenario. Whilst it may be tempting to attribute decrease in assimilative capacity to the loss of seagrass, it is important to remember that the total uptake is dependent not only on the quantity of seagrass present, but also on the ambient nutrient levels (uptake rates in the model are linearly proportional to the ambient concentration in the water column).
To quantify the proportion of lost assimilative capacity as the result of seagrass loss, the model was run keeping ambient nutrient concentrations constant at 2005 levels, and comparing assimilative capacity with 1978 seagrass bed size to that evident with 2005 seagrass bed size. This effectively identifies what proportion of the assimilative capacity loss is due to seagrass loss (as opposed to decreased uptake rates caused by decreased ambient nutrient concentrations).

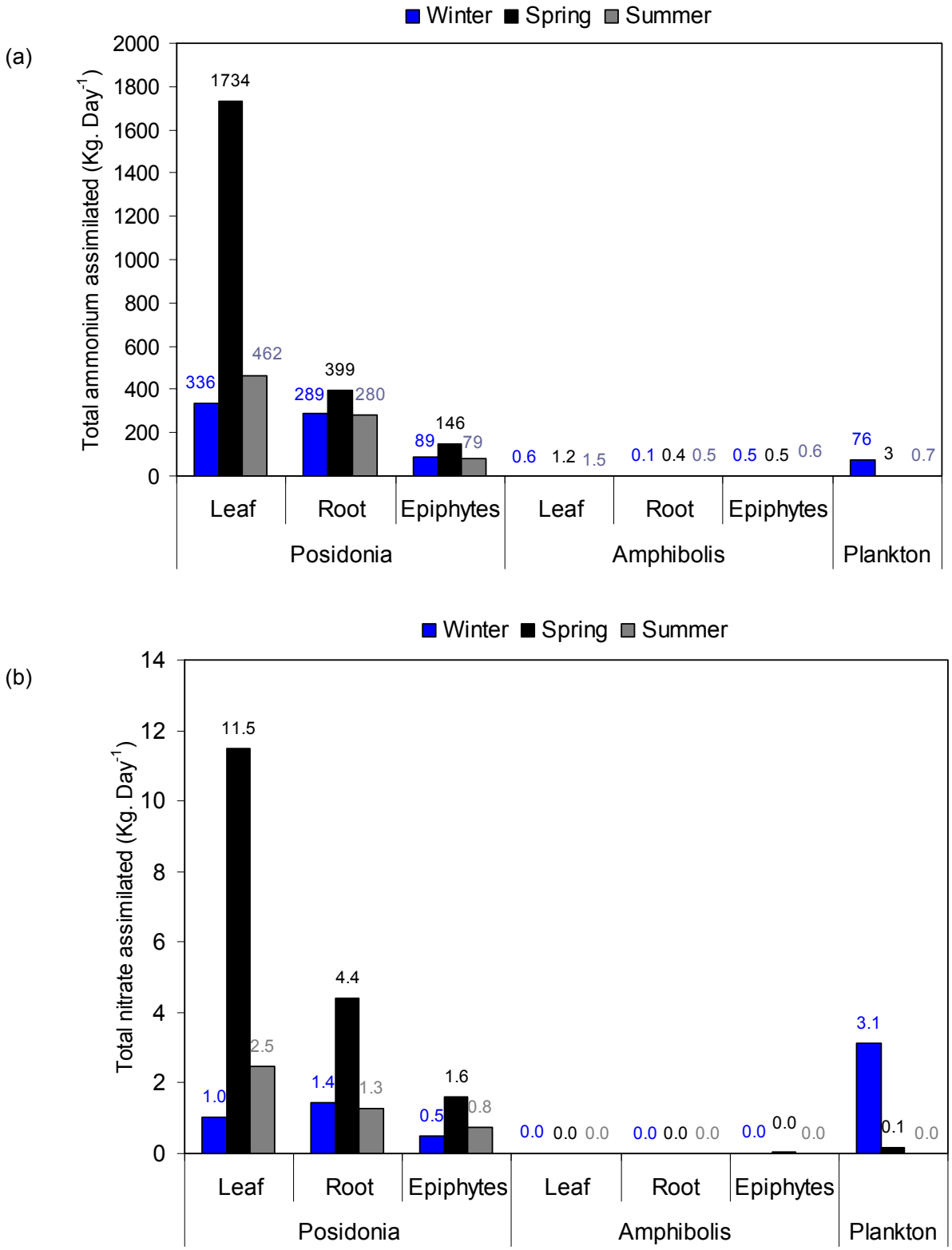

Figure 3.2 : Modeled data showing (a) total daily ammonium assimilation and (b) total daily nitrate assimilation during various seasons in 2005 by biological components in the study area.

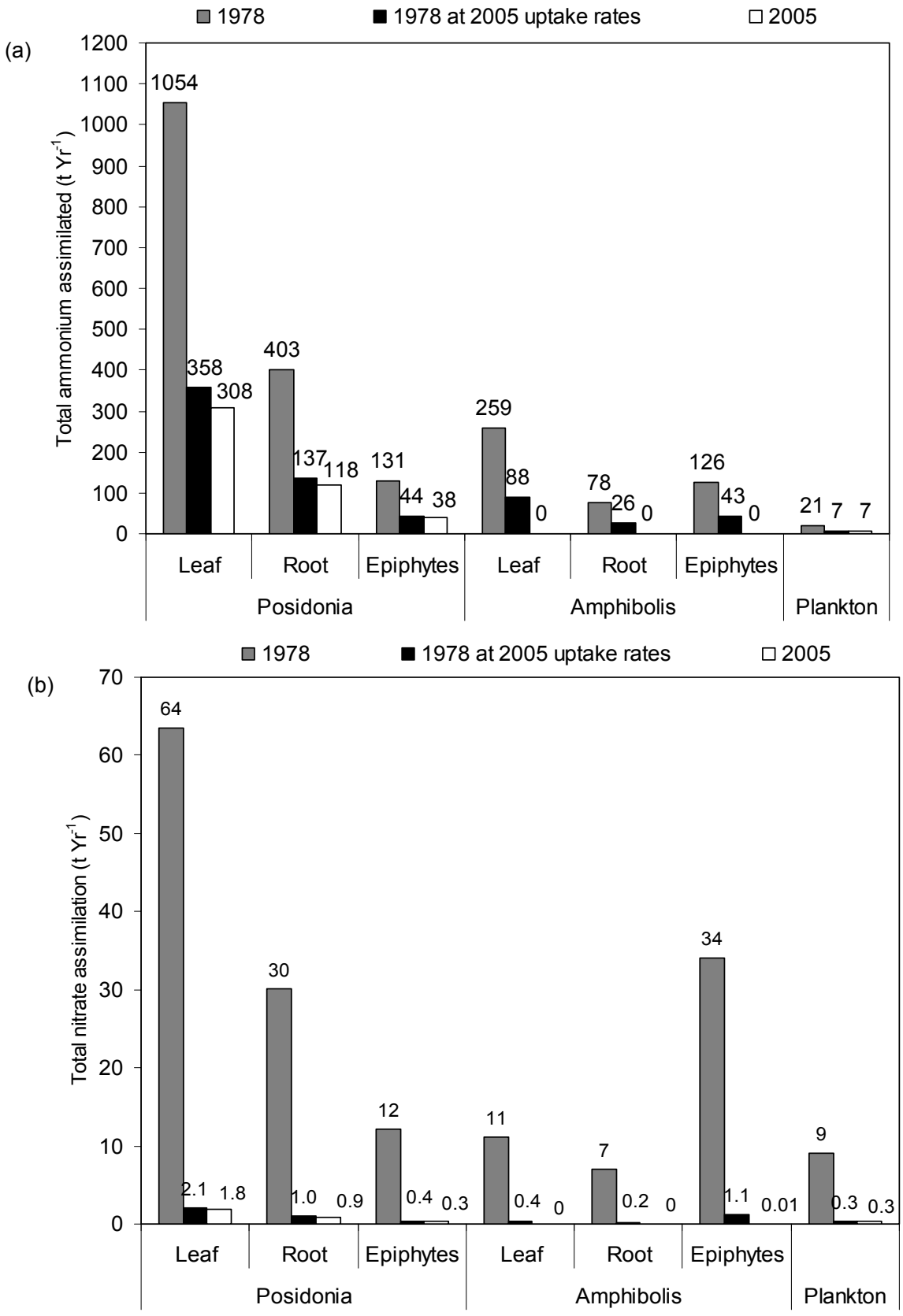

Figure 3.3 : A comparison of the modeled total yearly biotic (a) ammonium assimilative capacity and (b) nitrate assimilative capacity in the study area for 1978 and 2005. In 1978, annual input levels were estimated as 2165 t ammonium and 1221 t nitrate. In 2005 levels were 1509 t ammonium and 474 t nitrate (Wilkinson et al 2005).

The comparison revealed that the major proportion of the decreased assimilative capacity was due to decreased uptake rates caused by lower ambient concentrations. Of the decrease in ammonium assimilative capacity of 1600 t yr^{-1}, only 232 t yr^{-1} (14%) was attributable to seagrass loss, and the remainder to decreased uptake rates caused by the lower ambient concentrations. In the case of nitrate, even less of the loss in assimilative capacity is caused by seagrass loss. Of the 164 t yr^{-1} loss in assimilative capacity, only 2.2 t yr^{-1} (1.3%) is attributable to seagrass loss and the remainder to lower ambient nitrate levels.

Comparing the annual uptake to the inputs to the zone at each point in time, it is evident that seagrass uptake had the potential to take up a far greater proportion of the load in 1978 than it currently does. In 1978, potential ammonium uptake represented 96% of the anthropogenic input, whilst in 2005, it represents only 31% (Figure 3.3a). Similarly uptake of nitrate represented 13% of input in 1978 and less than 1% in 2005. These figures might be interpreted as an indication that the higher levels of nutrients are not problematic because the seagrasses have the capacity to increase their uptake rate to mop up the excess nitrogen. However, we must remain cognizant of the fact that it is considered very likely that these high nutrient levels were a major cause of the original decline, so an indicated high uptake rate may not offset the fact that the seagrass bed is diminishing because of the effects of ammonium. Whilst uptake, and an ability to mop up excess nutrients, appears to be decreased by our current, relatively low, ambient nutrient state, this does not, in any way indicate that higher nutrient conditions represent a healthy situation for the seagrasses. Indeed, Collings et al. 2006b experimentally demonstrate that very low increases in ambient nutrients are highly detrimental to seagrass beds.

Furthermore, high uptake rates do not indicate a permanent sequestering of the nutrients merely that it can be taken up. What has not been studied here is the fate of nutrients after uptake. If the biomass of the seagrass beds does not increase, nor the percentage of plant biomass represented by nitrogen, then increased uptake will be balanced by increased rates of loss, as the nitrogen is lost to detrital pools and re-released.

The seagrass complex (i.e. seagrass and associated epiphytes) accounted for 98% of the total biological assimilation. Thus it is clearly a very important component of the uptake, and consequently any degradation causing continuing loss of seagrass beds should be viewed with concern. Thus, despite the decrease in nitrogen inputs since 1978, there is a need to be cautious. Even though there have been significant improvements in recent years in wastewater treatment and catchment management (Butler et al., 1997), seagrass loss is still continuing on the Adelaide coast largely through the expansion of ‘blowouts’ and increased fragmentation of the seagrass meadows (Clarke, 1987; Hart, 1997; Seddon, 2002; Westphalen et al., 2005).

This modeling exercise does not represent a definitive formula for the fate of nitrogen in the Adelaide coastal system. It is rare that a model is able to manage this. Rather, models like this one represent a step in an iterative process to refine our understanding of the system. In this case, we have attempted to put some context on the experimental uptake values obtained through the chamber experiments. In extrapolating these values to the scale of the Adelaide coast, we have allowed the rates to be viewed in terms of the inputs to the system. However, conclusions beyond this are to be made with caution. To pretend that all the important factors have been included in the model would be naïve. In particular, this relatively simple model includes only uptake rates, and ignores the effects of nutrient cycling, the contributions of eroding or dying seagrass, and the spatial dynamics of nutrient inputs, biogeochemical processes occurring in the sediment and losses across the arbitrarily defined system boundary (i.e. interactions with the wider gulf environment and beyond). These are the areas that will require refinement in the future if we are to construct a more comprehensive model for nutrient dynamics of the Adelaide metropolitan coast.

4. Uptake and resource allocation of inorganic carbon by the temperate seagrasses *Posidonia* and *Amphibolis*.

4.1. Introduction

An understanding of the production ecology of seagrasses is important to the Adelaide Coastal Waters Study as earlier studies speculated that poor light regimes and nutrient loadings were possible causes for the decline of seagrasses along the Adelaide metropolitan coast (eg. Shepherd et al., 1987; Seddon, 2000). Light drives photosynthesis in autotrophs and the energy derived from photosynthesis is utilized for various metabolic processes and growth. Changes in metabolic processes over seasons, from measured estimates of carbon uptake or gas exchange rates, yield a good measure of productivity dynamics in seagrasses. Productivity measurements from carbon uptake have been suggested to be good indicators of the physiological health of seagrasses (Touchtte and Burkholder, 2000; Larkum et al., 2006), the basis on which this study was undertaken.

Seagrasses, like terrestrial plants, require carbon, which they assimilate in the form of inorganic carbon through the Calvin Cycle (Beer and Koch, 1996). Inorganic carbon in the marine environment occurs in four forms, carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). Carbon dioxide dissolves in seawater to form carbonic acid, which dissociates further form bicarbonates and carbonates (Hemminga and Duarte, 2000). Bicarbonate is 150 times more abundant than carbon dioxide and 6 times more abundant than carbonates in seawater at 15° C (Beer and Rehnberg, 1997). Although most marine macrophytes can utilize bicarbonates from seawater for their photosynthetic requirements, seagrasses have been reported to be less efficient than many macrophytes (Beer, 1994). While the ability of seagrasses to utilise bicarbonates for their photophysiological need has been debated for over 2 decades, recent research has shown that seagrasses do take up bicarbonates from seawater (Beer, 1989; Durako, 1993; Larkum and James, 1996). Although the affinity for carbon dioxide as an inorganic carbon source is much higher, the fact that bicarbonates are a lot more abundant in seawater means it is safe to assume that utilization of this anion is of paramount importance to seagrass for obtaining high photosynthetic rates *in-situ* (Beer and Rehnberg, 1997). Some seagrasses have been reported to directly utilise bicarbonates as an inorganic carbon source for photosysnthesis (Sand-Jensen and Gordon, 1984; Durako, 1993; Beer and Rehnberg, 1997; Bjork et al., 1997).

There is a paucity of literature on carbon use by seagrass, especially bicarbonate uptake mechanisms and utilization by seagrass (Beer and Rehnberg, 1997). seagrasses acquire inorganic carbon from seawater by converting bicarbonate to carbon dioxide extracellularly with the help of membrane bound carbonic anhydrase (Millhouse and Strother, 1986; James and Larkum, 1996; Beer et al., 2002). Millhouse and Strother (1986) have also suggested direct uptake of bicarbonates by seagrass, but this requires further experimental validation. In order to understand the basic functioning of an ecosystem, it is often essential to understand the complex processes involved in flow and allocation of nutrients such as carbon to various compartments involved in the uptake and assimilation of these nutrients (Leopold, 1949; Mateo et al., 2006).

Isotope labeling techniques based on radioactive tracers ($H^{14}CO_3$) or stable isotopes ($H^{13}CO_3$) have been commonly used to measure carbon uptake rates, fixation and allocation to various tissue compartments in seagrasses. Because of the hazards associated with the use of radioactivity in the field, attempts have been made to switch to the use of stable carbon isotopes for *in-situ* studies. Detailed studies by Mateo et al. (2001) and Miller and Dunton (2006) have demonstrated good agreements between ^{14}C and ^{13}C techniques in

experiments with seagrass and large macroalgae respectively, thus providing a good substitute for ^{14}C, and overcoming the limitations in using radioactive tracers, especially in *in-situ* experiments.

In the present work, we report seasonal variations in uptake rates and resource allocation of inorganic carbon in the temperate seagrasses *Amphibolis* and *Posidonia* using stable isotope carbon spike experiments *in-situ*. The study also investigated the rate of translocation of inorganic carbon from incubated leaves to roots. Although some literature pertaining to carbon uptake and utilization in tropical species and a few temperate species exists, there is a paucity of literature on *Amphibolis* and *Posidonia*, a gap in knowledge this study is likely to fill.

4.2. Materials and methods

The methodologies adopted in this experiment are identical to that described in chapter 2 (please see section 2.2), with the exception that:

1) Samples of seagrasses and water were collected for background levels of ^{13}C in leaves, roots, epiphytes and plankton and transported to the laboratory as detailed in section 2.2.2.2.

2) The dates for the uptake and resource allocation trials for $H^{13}CO_3$ for the three seasons studied are given in Appendix 4.

3) Bicarbonate stock solution (1000 ppm) for spiking was prepared from a labelled salt of $NaH^{13}CO_3$ (^{13}C, 99%, Cambridge Isotope laboratories Inc) for carbon uptake and resource allocation trials, respectively. Each chamber was then spiked with the stock solution to yield a final concentration of 13.5 ppm of bicarbonate.

4) Particulate organic carbon (POC) was measured in triplicate for every deployment by filtering 200 ml through a 47 mm diameter Whatman GF/F filter paper. Upon filtration, the filter papers with suspended particulates were stored in clean glass bottles at – 40° C. Frozen samples were freeze-dried. Total suspended particulate concentration was measured gravimetrically adopting standard procedures (Strickland and Parsons, 1972). The filter papers were then used for the analysis of POC. POC was analysed by high temperature combustion non-dispersive infrared gas analysis method using a Shimadzu TOC 5000A organic carbon analyser and Shimadzu solid sample module SSM 5000.

5) All freeze-dried and pulverized samples were analysed in a Europa Scientific continuous flow mass spectrophotometer Geo 20-20 for the determination of carbon content (mg) and atom % ^{13}C in the tissues. Uptake rates of various compartments were calculated with assumptions outlined by Cornelisen and Thomas (2002) using formulae modified from Mateo et al. (2001).

Calculation of carbon uptake rates for seagrass tissues and epiphytes used the equation:

$$U = \frac{C \times (\text{At. \% } ^{13}C\ E_T - \text{At. \% } ^{13}C\ B_T)}{W \times t \times (\text{At. \% } ^{13}C\ E_W - \text{At. \% } ^{13}C\ B_T)}$$

where,
U = Uptake rates in (mg C. g^{-1} DW. h^{-1})
At. % $^{13}C\ E_T$ = atom % ^{13}C in the enriched tissue
At. % $^{13}C\ B_T$ = atom % ^{13}C in the background tissue
At. % $^{13}C\ E_W$ = atom % ^{13}C in the enriched water (based on the amount of atom % ^{13}C

and background atom % ^{13}C concentration)
N = Total carbon content in tissues in (mg)
W = dry weight of tissue in (g DW)
t = duration of incubation in (h)

Whole plant uptake rate was calculated as the sum of leaf uptake rates and root translocation rates.

Calculation of carbon uptake rates for Plankton used the equation:

$$U = \frac{POC \times V \times (\text{At. \% } ^{13}C\ E_T - \text{At. \% } ^{13}C\ B_T)}{W \times t \times (\text{At. \% } ^{13}C\ E_W - \text{At. \% } ^{13}C\ B_T)}$$

Where,
U = Uptake rates in (mg C. g^{-1} DW. h^{-1})
At. % $^{13}C\ E_T$ = atom % ^{13}C in the enriched tissue
At. % $^{13}C\ B_T$ = atom % ^{13}C in the background tissue
At. % $^{13}C\ E_W$ = atom % ^{13}C in the enriched water (based on the amount of atom % ^{13}C and background atom % ^{13}C concentration
POC = Total nitrogen content in tissues in 'mg'
V = Total volume of the chamber (13.5 L)
W = dry weight of tissue in (g DW)
t = duration of incubation in (h)

Percent uptake of total available resource of carbon by biotic components at the end of the experiment is given in Appendix 5. These data show that there was no depletion of dissolved inorganic carbon in the chamber at the termination of the experiment. Total carbon inputs were calculated as the sum of the background levels of dissolved inorganic carbon and the $H^{13}CO_3$ added. Ambient and chamber water quality parameters monitored during the study are summarised in Appendix 6.

Data analysis

Parametric analysis was applied to the uptake of carbon because missing values created an unbalanced design that could not be analysed by NPMANOVA. Therefore a standard two-way parametric Multivariate Analysis of Variance (MANOVA; 2 species × 3 seasons) with four dependent variables (uptake by whole plant, epiphytes and plankton) was utilised. Statistical software SPSS Ver. 14 (SPSS Inc., Illinois, USA) was used to test for significant differences, between seasons and species for the dependent variables. The assumption of homogeneity of variances was tested with a Levene's test.

If the MANOVA was significant, separate univariate analyses were applied to test the effects of season and species on whole plant, epiphyte and plankton uptake. This was done by a two-way Analysis of Variance (ANOVA). Main effects were tested only where there was no interaction. Where an interaction occurred, the effect of season was examined within each species separately. The homogeneity of variances was tested, whenever an ANOVA was conducted. Whenever significant differences were observed from ANOVA, a suitable *post-hoc* test was run to identify dependent variables that were significantly different. Either a Tukey HSD or Games-Howell *post-hoc* test was applied to identify the significantly different component. Games-Howell nonparametric test was used when Levene's test failed. Whenever the assumptions of the test were not met, the data were transformed using a natural log (ln) transformation. All the statistical tests were assessed at α= 0.05 (i.e. 95% confidence level).

4.3. Results

4.3.1. Background physicochemical and biological data

Results of the various physico-chemical and biological parameters monitored during the study are described in section 2.3.1.

4.3.2. Carbon uptake rates

The general tendency was for highest uptake rates for each component to occur in summer or spring (Figure 4.1), but this was not entirely consistent. Carbon uptake by the components of the seagrass ecosystem was influenced by a significant interaction of species x season (MANOVA, Pillai's Trace: P=0.02). Analysis was subsequently carried out on each component (whole plant, epiphytes and plankton) using separate 2 way univariate ANOVAs, testing for the effects of season and species. In using these analyses, the assumption of homogeneity of variances was violated only in the case of phytoplankton (P <0.001). In the absence of an appropriate transformation, the test continued, but a Games Howell *post-hoc* test was used rather than the parametric Tukey test (see later).

4.3.2.1. Whole plant uptake

Whole plant uptake of carbon was influenced by a significant interaction term for the effects of species and season (Table 4.1; P=0.007), indicating that uptake by *Posidonia* and *Amphibolis* reacted to the effect of season in different ways. The effect of interaction necessitated individual analyses for each species. While, *Posidonia* did not register any seasonal differences, whole plant uptake in *Amphibolis* was influenced by seasons (ANOVA, P=0.003). Tukey's HSD registered significant differences in whole plant uptake rates for *Amphibolis* between winter and summer (P = 0.003) and spring and summer (P = 0.04). Mean carbon uptake rates by *Posidonia* and *Amphibolis* during winter, spring and summer were 0.45, 0.47 and 0.45 mg C. g^{-1} DW. h^{-1} and 0.25, 0.55 and 0.94 mg C. g^{-1} DW. h^{-1}, respectively (Figure 4.1).

4.3.2.2. Epiphytic uptake

Uptake rates for epiphytes were consistent for *Posidonia* and *Amphibolis*, with mean values ranging from 0.08 (winter) to 0.12 mg C. g^{-1} DW. h^{-1} (summer) for *Posidonia* and from 0.08 (winter) to 0.32 mg C. g^{-1} DW. h^{-1} (spring). Carbon uptake rates of epiphytes were not influenced by either species or seasons (Table 4.1; Figure 4.1).

4.3.2.3. Plankton uptake

Carbon uptake rates of plankton (when biomass standardised) were generally higher than other components of the system in winter and spring but not in summer. Uptake rates ranged from 0.01 (summer) to 0.61 mg C. g^{-1} DW. h^{-1} (spring) in *Posidonia* and from 0.02 (summer) to 0.93 mg C. g^{-1} DW. h^{-1} (winter). While plankton uptake of carbon was not significantly affected by species, it was affected by season (P = 0.040), with the lowest uptake rates observed in summer and significantly different to spring (Games-Howell P = 0.009). Winter was not significantly different to either season. Note that *post-hoc* tests in this instance were non-parametric Games Howell tests as the Levene's test of homogeneity failed for the plankton component.

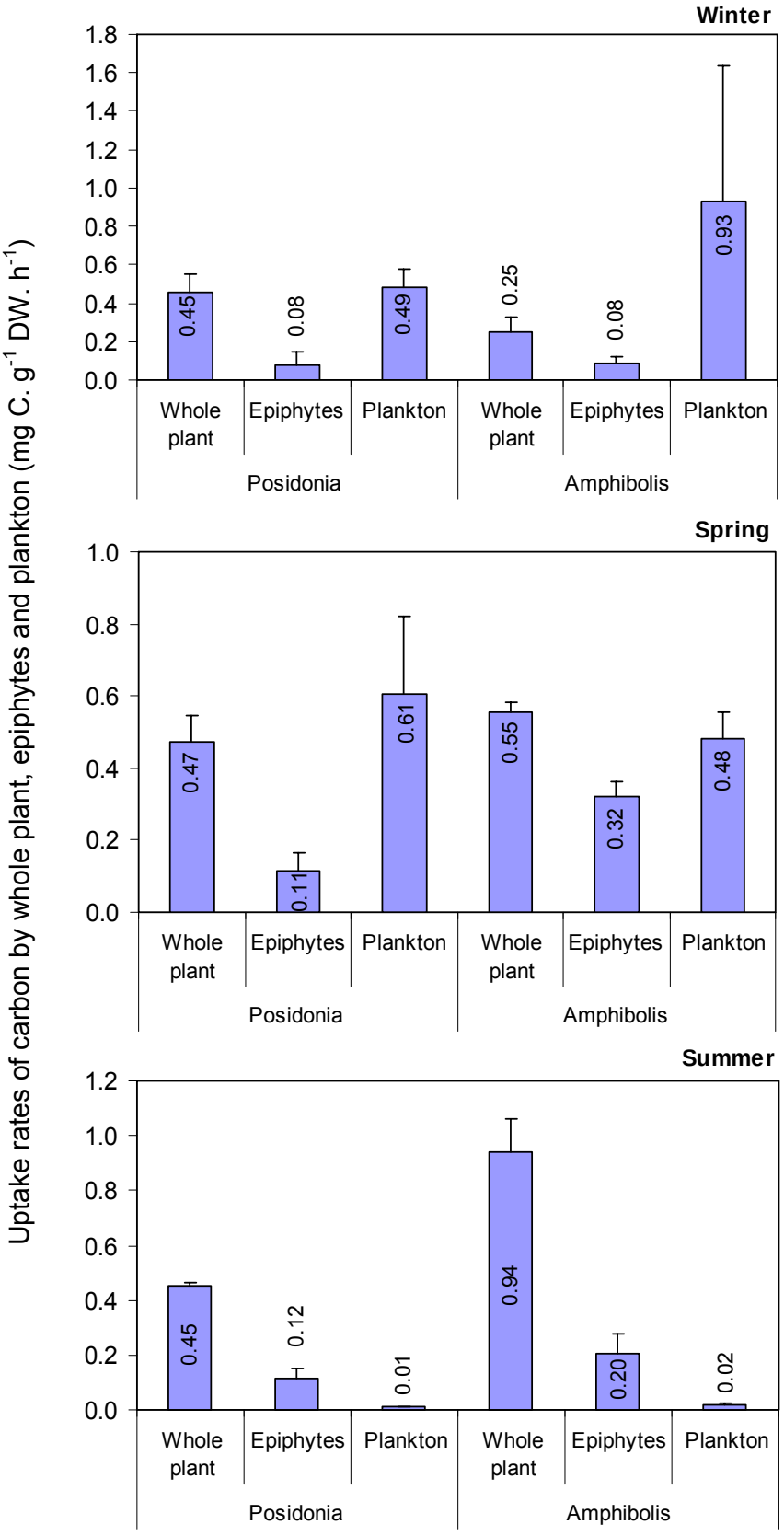

Figure 4.1 : Mean seasonal uptake rates of carbon by whole plant, epiphytes and plankton in *Posidonia* and *Amphibolis*. Error bars depict standard error or means (n=3). The Y-axis scales on the three graphs differ.

Table 4.1 : Summarised two-way ANOVA table for species and seasonal variabilities in carbon uptake rates of whole plant, epiphytes and plankton. **Bolded** figures are significant at P=0.05.

Source	Dependent variable	Degrees of freedom	Mean Sum of Squares	F	P
Species	Whole plant	1	0.081	4.445	0.059
	Epiphytes	1	0.039	4.506	0.057
	Plankton	1	0.049	0.292	0.600
Season	Whole plant	2	0.141	7.738	**0.008**
	Epiphytes	2	0.026	2.979	0.092
	Plankton	2	0.737	4.358	**0.040**
Interaction term Species × Season	Whole plant	2	0.146	8.049	**0.007**
	Epiphytes	2	0.015	1.754	0.218
	Plankton	2	0.116	0.685	0.525

4.3.2.4. Root translocation rates

The complex effects of species and season on root uptake are evidenced by the significant interaction term in the analysis (Table 4.2; P<0.001), indicating that the different species reacted to the effect of season in different ways, and necessitated individual analyses for each species. Carbon translocation rates in *Amphibolis* roots registered significant seasonal differences (P = 0.007; Figure 4.2). Spring (mean: 0.10 mg C. g^{-1} DW. h^{-1}) was the time of highest translocation of carbon into the root for this species, differing significantly from winter (mean: 0.01 mg C. g^{-1} DW. h^{-1}; p= 0.006, Tukeys HSD). Summer (mean: 0.05 mg C. g^{-1} DW. h^{-1}) was intermediate and not considered significantly different to either spring or winter. Translocation rates in *Posidonia* roots registered significant seasonal differences between all seasons, with highest rates of translocation in winter (mean: 0.28 mg C. g^{-1} DW. h^{-1}) followed by spring (mean: 0.21 mg C. g^{-1} DW. h^{-1}) and least in summer (mean: 0.03 mg C. g^{-1} DW. h^{-1}). Tukey's HSD registered significant differences in root translocation rates for *Posidonia* between winter and spring (P = 0.03), winter and summer (P<0.001) and spring and summer (P < 0.001).

4.3.3. Resource allocation

Percent carbon uptake in this study was a fraction of the total carbon resource pool available for utilisation. Percent uptake of the total resources ranged from 3.5% in winter to about 21% in spring in *Amphibolis* beds (Appendix 5).

In *Posidonia* meadow, whole plant accounted for the bulk of carbon taken up during all seasons, accounting for nearly 99%, 98% and 91% respectively, of the total carbon resource during winter, spring and summer (Figure 4.3). Allocation of carbon resources to epiphytes remained consistently low during winter (1%) and spring (2%), increasing to 9% during summer.
Whole plant accounted for 70% of the bulk of the total carbon resource allocation in *Amphibolis* in winter, followed by epiphytes (30%) (Figure 4.3). However, in spring and summer epiphytes took up 45% of the total resource when compared to whole plant uptake of 55% of the total carbon resource in the chamber.

4.4. Discussion

In temperate and subtropical waters, seagrass biomass, especially leaf biomass, shows seasonal trends, increasing in spring and summer and decreasing in autumn and winter (Vermaat et al., 1987; Dunton, 1994). In spring, when water temperature and daylight hours

increase, production of above-ground biomass also increases due to growth of new leaves, reported to originate from stored carbon reserves in the rhizomes (Dawes and Lawrence, 1980; Dawes and Guiry, 1992; Tussenbroek, 1995). The results for carbon assimilation, above-ground biomass and below-ground biomass in spring from this study are comparable to what has been reported in literature. However, contrary to what has previously been reported, inorganic carbon uptake rates in summer were lower than in spring.

Water temperature and underwater irradiance have been reported to play a critical role in regulating seagrass productivity (especially leaf biomass) and metabolism during late spring and summer (Bulthuis, 1987; Lee and Dunton, 1996). The condition of the seagrass, in this study especially *Amphibolis*, appeared to be relatively poor in summer based on visual observations. Fine sediments, which might have originated from Outer Harbour dredging operations, were observed on seagrass leaves. Under these conditions, seagrass photosynthesis may be lowered and as a result, density, biomass, nutrient uptake processes and the aerial extent of seagrasses might be affected (Walker and McComb, 1992; Fitzpatrick and Kirkman, 1995; Bondsorff et al., 1997; Short and Neckles, 1999), offering a possible explanation for reduced biological uptake of inorganic carbon in summer.

Table 4.2: Summarised two-way ANOVA table for species and seasonal variabilities in translocation rates of carbon in roots. **Bolded** figures are significant at P=0.05.

Source	Degrees of freedom	Mean Sum of Squares	F	P
Species	1	0.055	83.533	**<0.001**
Season	2	0.024	35.958	**<0.001**
Interaction term Species × Season	2	0.030	45.382	**<0.001**

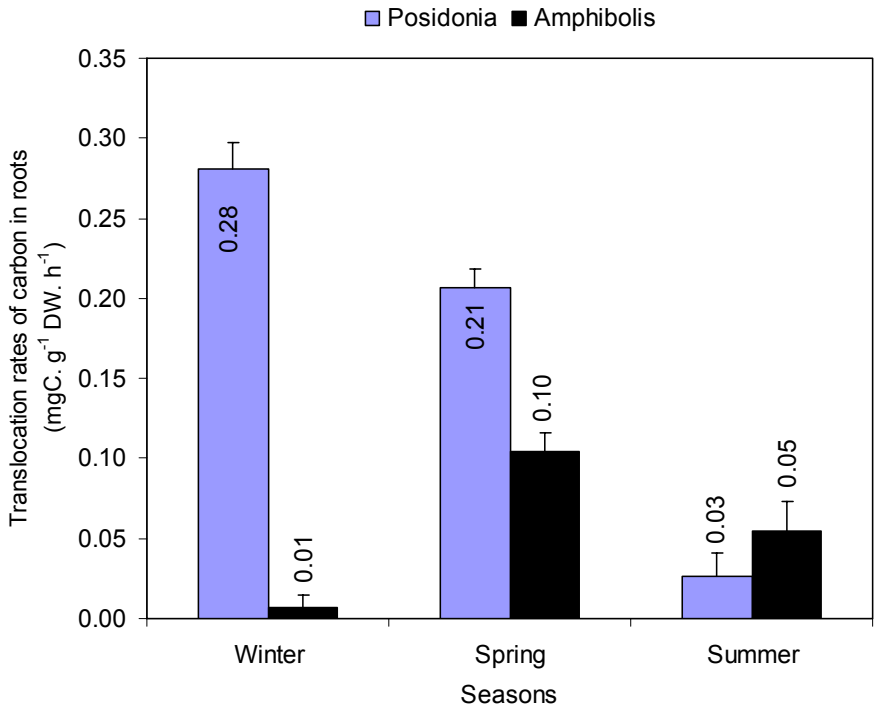

Figure 4.2: Mean seasonal rates of carbon translocation in the roots of *Posidonia* and *Amphibolis*. Error bars depict standard error or means (n=3).

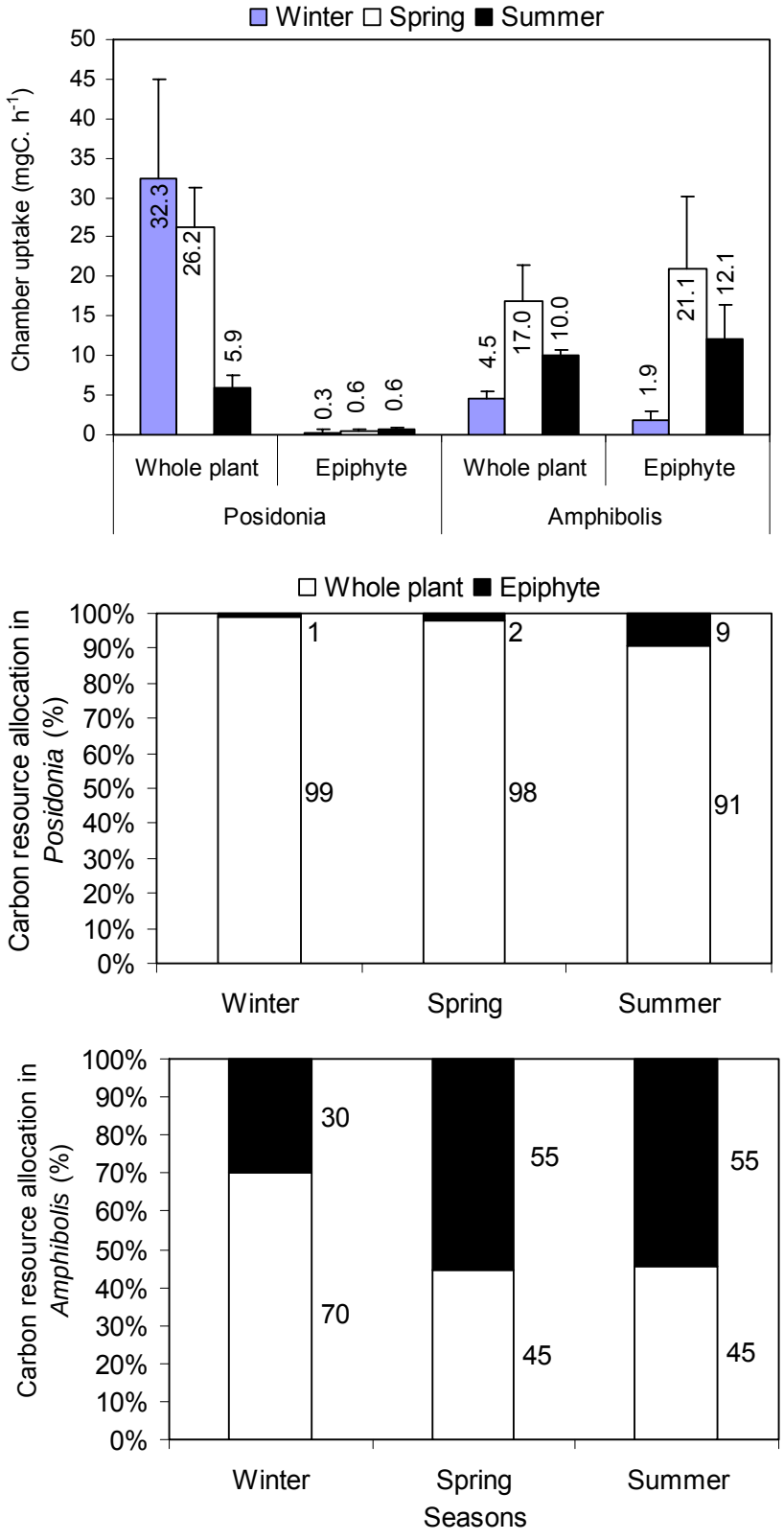

Figure 4.3: Seasonal variation in allocation of carbon resources in whole plant and epiphytes in *Posidonia* and *Amphibolis*. The total component uptake rates take into account the effect of the different biomass of each component.

Compared to macroalgae, seagrasses are far more sensitive to human introduced perturbations (Mercado et al., 2003). This favours macroalgae competing with seagrass for resources such as nutrients and carbon (Hernandez et al., 1997; Clavero et al., 1999).

There is demonstrated evidence that seawater dissolved inorganic carbon (DIC) concentrations are never limiting, and are sufficient to saturate *in-situ* photosynthesis in seagrass under optimal light conditions (Schwarz et al., 2000). However, shaded conditions, as observed in this study, could limit photosynthesis by affecting DIC acquisition mechanisms (Schwarz et al., 2000). Mateo et al (2006) gives a detailed account of the effect of light attenuation on nutrient uptake by seagrass and overall seagrass productivity. In their study in northwestern Gulf of Mexico, a reduction in surface irradiance to less than 18% led to the production of less oxygen for below-ground tissue respiration, resulting in the build up of sulphides and ammonium. Reduced oxygen supply to the root tissue resulting in reduced nutrient uptake, coupled with the toxic effect of sulphide and ammonium build up, results in significant loss of productivity and biomass (Onuf, 1994; Hauxwell et al., 2003). Also, high epiphytic load together with sediment deposition on leaves as observed in this study during summer inhibits carbon and nutrient uptake by seagrass leaves, thus limiting seagrass growth (Shepherd et al., 1989). It is likely that a combination of some of these factors might be responsible for the low inorganic carbon uptake and significant reduction in leaf (*Posidonia* and *Amphibolis*) and root biomass (*Amphibolis*) at the study site in summer.

Inorganic carbon resource allocation in this study revealed a significant pool of carbon being allocated to whole plants of *Amphibolis* and *Posidonia*. Whole plants of *Posidonia* took up carbon at a significantly higher rate than *Amphibolis* during all seasons. It was, however, interesting to observe that the inorganic carbon allocation to *Amphibolis* decreased with an increase in epiphytic uptake during spring and summer. Epiphytes on seagrass leaves have been reported to compete with seagrass for available carbon (Mateo et al., 2006) and in some instances observed to hamper the uptake of inorganic carbon by seagrass leaves (Kiswara et al., 2005).

While seagrasses have been reported to meet their nutrient requirements through roots from sediment porewater and through leaves from the water column (Maier and Pregnall, 1990; Lee and Dunton, 1999), inorganic carbon is acquired by seagrasses from the water column only, through the leaves (Bjork et al., 1997; Marba et al., 2002) and to below-ground modules. Harrison (1978) and Libes and Boudouresque (1987) demonstrated that carbon was indeed mobilised in *Posidonia oceanica* and transferred from leaves to roots. Libes and Boudouresque (1987) further reported allocation of carbon from incubated shoots to neighbouring shoots on the same rhizome. Translocation of carbon even under short incubations, as in this study, has been reported before (Harrison, 1978; Abel and Drew, 1989). Translocation mechanisms in other seagrass species have also been reported (for eg. Bittaker and Iverson, 1976; Penhale and Thayer, 1980; Zimmerman and Alberte, 1996; Marba et al., 2002). These authors concluded that translocation might be an important mechanism for young seagrass ramets to acquire resources and for seagrass clones to expand and persist. Marba et al. (2002) reported that 27.1 to 80.6% of the carbon acquired by the leaves of different seagrass species could be exported to adjacent shoots to contribute to the growth of new and colonising shoots. The role of below-ground tissues as critical carbohydrate storage organs was further emphasised by Touchette and Burkholder (2000) in their review paper. These tissues have been reported to serve as a photosynthetic reservoir supporting growth and maintaining other tissues during periods of low photosynthetic production (Pirc, 1989; Burke et al., 1996; Alcoverro et al., 2001).

Generally, algae (micro- and macroalgae) are more efficient assimilators of carbon and nutrients than seagrasses, as observed in this study. When background concentrations of nutrients are high, the growth of epiphytes and phytoplankton are favoured at the expense of seagrass production (Kemp et al., 1983; Borum, 1985). Mateo et al. (2006) emphasised the

importance of planktonic organisms to carbon fixation in seagrass ecosystems. Even though high carbon fixation rates by plankton were observed in this study, the total biomass of plankton in relation to other biological compartments was too small to make a significant contribution to the overall uptake.

While inorganic carbon uptake rates for epiphytes on *Posidonia* and *Amphibolis* were consistent, uptake by epiphytes on *Amphibolis* was particularly high accounting for more than 55% of the total carbon resource in spring and summer. Higher epiphytic loading on *Amphibolis* when compared with *Posidonia* could be attributed to the differences in morphology and growth characteristics of the two species (Shepherd et al., 1989). The terete, woody stems of *Amphibolis* offers more surface area for settlement of large epiphytes, whereas the blades of *Posidonia* can only support far lower standing crops of much smaller algae (Shepherd, 1973; Borowitzka et al., 1990; Lavery and vanderklift, 2002). Algal epiphytic contribution has been reported to range from 20% to 60% of the biomass (Borrowitzka and Leithbridge, 1989; Borowitzka et al., 2006). Other researchers have similarly reported large contributions of algae in seagrass beds (Danuby. 1989; Moncreiff et al., 1992; Yamamuro, 1999; Moncreiff and Sullivan, 2001).

5. Temporal variations in biological uptake rates of inorganic phosphorus in a temperate *Posidonia* and *Amphibolis* meadow.

5.1. Introduction

Nutrient sources in nearshore coastal systems include oceanic waters, terrigenous inputs, nutrient recycling within the system and atmospheric fixation (Mann, 1982). In coastal environments, such as the Adelaide coastal waters, human activities contribute significantly to the nutrient loading (Nixon, 1993), stimulating phytoplankton and macroalgae (Short, 1987), often leading to eutrophication. Nutrients such as phosphorus are dispersed through the system by a number of nutrient cycling processes occurring in the water column and sediments (McMahon and Walker, 1998). These nutrients eventually become available to primary producers through columnar water, pore water and sediments (Lavery et al., 1993; McMahon and Walker, 1998).

Rooted macrophytes such as seagrass have been reported to meet their phosphorus requirements by uptake from sediments (Barko et al., 1986) and the water column (Brix and Lyngby, 1985). However, dissolved inorganic phosphorus easily binds to carbonate rich sediments (Jensen et al, 1998), limiting its availability for biological uptake (Fourqurean et al., 1992; Touchette and Burkholder, 2000). The redox potential of the sediments and the sediment water interface also plays an important part in dictating nutrient bioavailability. Oxic sediments may act both as a sink (Bostrom and Petterson, 1982) and a source (Bortelson, 1970) of phosphorus. However, under anoxic conditions these sediments act as a source of phosphorus. When phosphorus, in the form of orthophosphate ions (HPO_4^{2-}, $H_2PO_4^-$) and organic compounds is available, phytoplankton have evolved mechanisms to sequester it from waters. This subsequently results in water column concentrations often below the limits of analytical detection (<0.3 $\mu g.L^{-1}$), making them unavailable to macrophytes such as seagrass. These processes are particularly significant from the viewpoint of seagrass meadows off the Adelaide metropolitan coastline, where oxic carbonate sediments make phosphorus cycling in the water column, pore water and sediments a complex process - a challenge for seagrasses in acquiring these nutrients from the system.

Different seagrass species complexes have different nutrient requirements. Unfortunately, few published works exist that report relative nutrient uptake rates from temperate waters. This is of significance for *Posidonia* and *Amphibolis*, two dominant seagrass taxa found in South Australian waters (Westphalen et al., 2005).

As a part of the broader study on cycling and quantification of seasonal budgets for various nutrients in these waters, it was necessary to obtain reliable estimates of nutrient uptake rates in various seasons. In the following investigation, total phosphorus uptake rates in a *Posidonia* and *Amphibolis* dominated seagrass complex were measured in three seasons. Since no stable isotope for phosphorus exists, it was not possible to measure individual uptake rates for the individual biological components of the seagrass complex, as was done for carbon and nitrogen. Instead, total biological uptake rates in the seagrass complex were measured and reported in this section.

5.2. Materials and methods

The description of equipment, sampling location and chamber deployment was identical to that in chapter 2 (section 2.2).

Stock nutrient solution of 1000 ppm for spiking was prepared from AR grade salt of KH_2PO_4 and loaded into 20 mL syringes. These syringes were sealed with an end cap. Each chamber was then spiked with the stock solution carried in syringes to yield a final concentration of 13.5 ppm of the nutrient in each chamber. Samples for initial concentrations of phosphorus in pore water (C_{ip}) and columnar water (C_{ic}) were collected in 60 mL syringes and sealed immediately with an end cap. The samples were filtered, immediately after collection, through a 0.45 μm pore size membrane cartridge filter (Millipore) into a 60 mL polyethylene bottle pre-rinsed with de-ionised water and the sample. The bottles were then stored in ice under dark conditions.

Chambers were incubated for 2 hours. Pore water (C_{fp}) and chamber water samples (C_{fc}), constituting final concentrations, were collected and processed the same way as 'initial samples'. About 120 ml of water sample from each chamber was collected along with two ambient samples for water quality measurements. Water quality parameters viz., water temperature, dissolved oxygen (DO), salinity, pH were measured using a Hach SensIon 156 multi-parameter probe almost immediately after collection on board the vessel. At the termination of the experiment, seagrass samples from each chamber were cored out and transported to the laboratory for biomass analysis. Data for photosynthetically available radiation (PAR) was obtained from an Odyssey light logger deployed on site.

Water samples collected and filtered aboard the vessel were stored in sample bottles and frozen at –20° C until they could be analysed. The frozen samples were shipped to the Water Studies Centre, Monash University for the analysis of soluble reactive phosphorus.

Concentrations of phosphate (PO_4) in the chamber water and pore water were analysed by Flow Injection Analysis using a Lachat QuickChem 8000 automated flow injection ion analyser. In the test for phosphate, the orthophosphate present in the sample was made to react with molybdate in an acid medium to form phosphomolybdate. This intermediate product then reacted with ascorbic acid, with an antymonium catalyst, to yield molybdenum blue that is measured at 880 nm. The absorbance of this solution was proportional to the concentration of soluble reactive phosphate.

The seagrass samples collected in the field were processed in the laboratory immediately upon arrival. The samples were rinsed in clean seawater and cleaned of dead leaves, debris and sand. Wet weights of the total biomass of above- and below-ground biomass of seagrass collected from each of the 6 chambers deployed were measured. The moisture content of a sub-sample of each biomass component was measured gravimetrically by freeze-drying the samples. The moisture content of the samples was then used to work out the dry weight of the total biomass (W) contained in each chamber.

Total biotic phosphate uptake rate, a consolidated value for the uptake rates of seagrass above ground biomass, below ground biomass and epiphytes, was calculated using the formula:

Phosphorus uptake rate (mg PO_4. g^{-1} DW. h^{-1}) = $\frac{(TIC - TFC)}{W \times t}$

Where,
TIC (Total initial concentration in $mg.L^{-1}$) = $[(C_{ic} \times 13.5) + (C_{ip} \times 1.211)]$
TFC (Total final concentration in $mg.L^{-1}$) = $[(C_{fc} \times 13.5) + (C_{fp} \times 1.211)]$
13.5 = Volume of water column contained in the chamber in (L)
1.211 = Volume of pore water contained in the steel cutter of the chamber in (L)
W = Total biomass in (g DW)
t = duration of incubation in (h)

Data for phosphorus uptake were analysed using a two-way analysis of variance (ANOVA) with Tukey's pair-wise comparison *post-hoc* test to determine significant differences between seasons. The assumption of homogeneity of variance was tested using Levene's test, and was met. The statistical analysis was carried out using Minitab Ver 13.2 with a significance level of α= 0.05.

5.3. Results and discussion

Results of the various physico-chemical and biological parameters monitored during the study are described in section 2.3.1.

Total uptake of spiked inorganic phosphorus by biological components was negligible during the study, never exceeding 0.5% of the total resource (Appendix 5). In carbonate rich sediments, dissolved inorganic phosphorus is retained by the sediments (Jensen et al, 1998), limiting its availability for biological uptake (Fourqurean et al., 1992; Touchette and Burkholder, 2000). Sediments of the Adelaide metropolitan coast are predominantly carbonate (Shepherd and Sprigg, 1976), limiting the bioavailability of phosphorus in this study. Also, inorganic phosphorus uptake affinities and rates for seagrass are often much lower than ammonium and depend on the nutritional status of the plant and the prevailing environmental conditions (Touchette and Burkholder, 2000) and is consistent with the low biotic uptake rates recorded in this study. Results revealed highest uptake rates in winter (0.05 mg $PO_4.g^{-1}$ DW. h^{-1}) and least in spring (0.02 mg $PO_4.g^{-1}$ DW. h^{-1}) for *Amphibolis* and highest in winter (0.07 mg $PO_4.g^{-1}$ DW. h^{-1}) and least in summer (0.004 mg $PO_4.g^{-1}$ DW. h^{-1}) for *Posidonia* (Figure 5.1). These uptake rates were similar to the rates reported by Paling and McComb (1994) in Western Australia. While there was a significant difference in the seasonal uptake rates, there was no difference in the uptake rates in meadows of the two species (Table 5.1). Tukey's test revealed significant differences in the uptake rates between winter and spring. Factors such as light and temperature have been reported to play an important role in regulating phosphorus uptake by seagrass (McRoy and Barsdate, 1970; Patriquin, 1972; Penhale and Thayer, 1980; Touchette and Burkholder, 1999). While high nutrient uptake rates in summer might be expected, fine suspended sediments settling on seagrass leaves might have been a reason for reduced uptake during that season. The fine suspended sediments might have originated from Outer Harbour dredging operations. Seagrasses were observed to be in relatively poor conditions based on visual observations. Under these conditions, seagrass photosynthesis may be lowered, explaining the reduced uptake rates (Patriquin, 1972; Perez et al., 1994). Overall phosphorus uptake rates by *Posidonia* and *Amphibolis* complex were quite low, which is in agreement with the findings of Paling and McComb (1994).

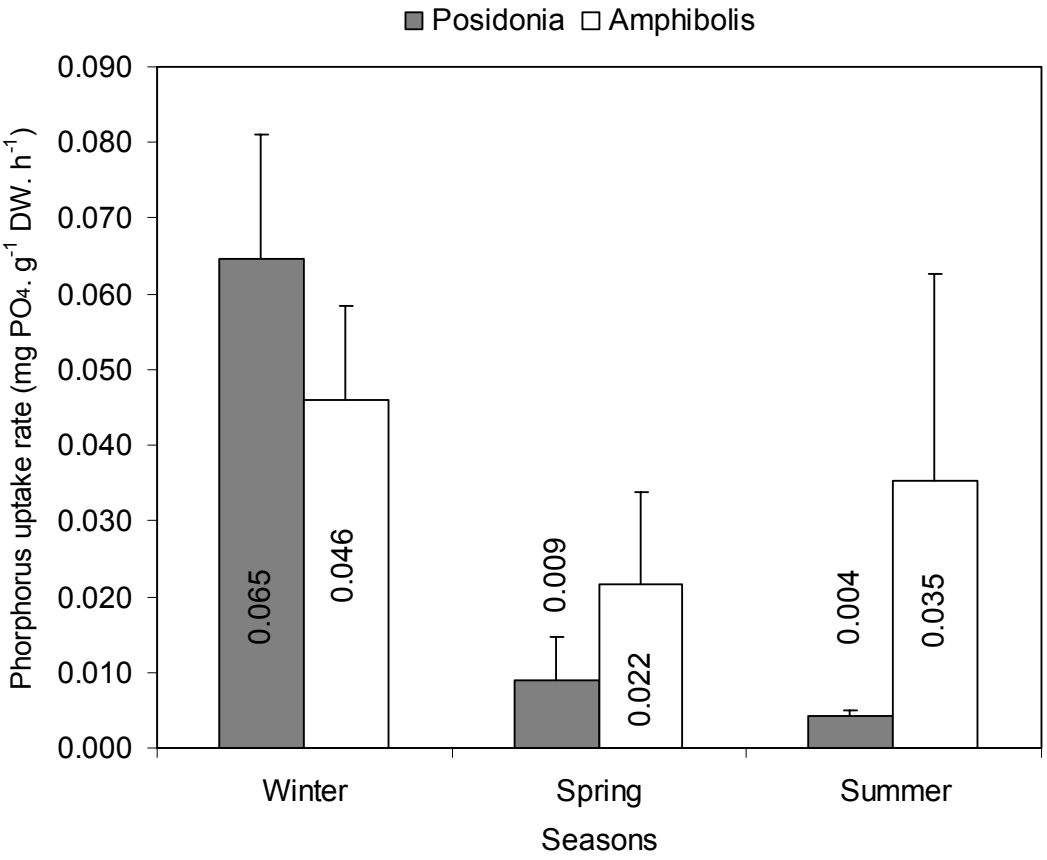

Figure 5.1: Mean seasonal biotic phosphorus uptake rates in a *Posidonia* and *Amphibolis* meadow. The error bars denote standard error of means (n=3).

Table 5.1: Two way ANOVA table for biological phosphorus uptake rates in *Posidonia* and *Amphibolis* beds. Results of Tukey's pair-wise comparison are arranged in the ascending order of their means and lines are drawn over treatment groups that are not significantly different from each other (P>0.05). The abbreviations W, Sp and Su represents winter, spring and summer respectively. **Bolded** figures are significant at p=0.05.

Source	Degrees of freedom	F	p	Tukey's pair wise comparison
Seasons	2	4.24	**0.04**	W > Su > Sp
Species	1	0.47	0.51	
Season * Species	2	1.39	0.29	

6. Conclusions

6.1. Nitrogen uptake and resource allocation

Seagrass loss often results in substrate instability resulting in larger areas of seagrass loss called 'blowouts' (Clarke and Kirkman, 1989). Seagrass loss along the Adelaide metropolitan coastline has been historically related to elevated nutrients from the release of treated wastewater (Shepherd, 1970; Shepherd et al., 1989) and increased turbidity leading to light attenuation (Shepherd et al., 1989; Edyvane, 1996). Higher concentrations of ambient nutrients promote the proliferation of fast growing species, including phytoplankton, epiphytes and opportunistic macroalgae that compete with seagrass for resources (Sand-Jensen and Borum, 1991; Duarte, 1995). This study provides data on seasonal dynamics in uptake rates and allocation of inorganic nitrogen in various biological compartments of a *Posidonia* and *Amphibolis* seagrass complex. This information could be used as part of an effective management plan for nutrient inputs into the Adelaide coastal waters so as to prevent further loss of seagrass and to indicate potential effects of that loss. These results are of special significant since *Posidonia*, the dominant seagrass taxa found to occur along the Adelaide metropolitan coastline, are slow-growing and may take centuries to re-colonise (Kirkman, 1997).

6.2. Nitrogen model

6.2.1. Adelaide coastal waters annual ammonium biotic assimilation capacity

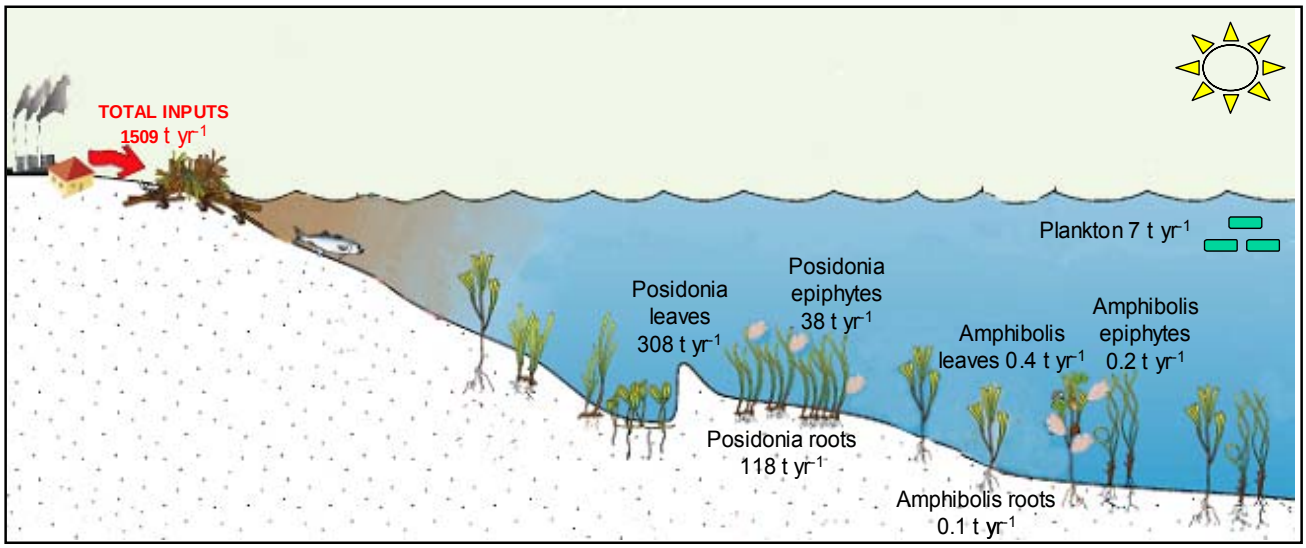

Figure 6.1: A simplified summary of the current annual ammonium biotic assimilation capacity in relation to the total anthropogenic inputs for the Adelaide coastal waters. Figures in tonnes ammonium / year

Of the total ammonium assimilated by biotic components, the seagrass complex accounts for nearly 90% of the total in winter and 100% in spring and summer. Assimilation by plankton accounted for the remaining 10% in winter, when higher plankton biomass is found in these waters. Currently, the *Posidonia* seagrass complex accounts for most of the ammonium assimilated for all seasons in the study area. *Amphibolis* cover is less than 0.01% of the total seagrass cover in the ACWS study area (Blackburn and Dekker, 2005), making the contribution by the *Amphibolis* complex to the assimilation of ammonium insignificant.

The highest assimilation of ammonium was modeled in spring (2.3 t day^{-1}) followed by spring and summer (0.8 t day^{-1})

In 2005 the total inputs of ammonium to the Adelaide coastal waters were reported to be 1509.3 t yr^{-1} (Wilkinson et al., 2005). The modeled biotic uptake was just a third of the total inputs. The seagrass complexes accounted for 98% of the total biological assimilation from the metropolitan coastline. A summary of the fate of the anthropogenic inputs of ammonium and annual biotic assimilation rates is highlighted in Figure 6.1.

6.2.2. Adelaide coastal waters annual nitrate biotic assimilation capacity

Seasonal differences in biotic nitrate assimilation were evident, with highest assimilation rates in spring, followed by winter and least in summer. Plankton accounted for nearly 50% of the total biotic assimilation of nitrate in winter, with the seagrass complex assimilating most of the nitrate in spring and summer. As with ammonium assimilation, only the *Posidonia* seagrass complex took up a significant amount of nitrate, with leaves accounting for the bulk of the assimilation followed by roots. Epiphytic assimilation was significant in spring and summer.

It is worth noting here that the current annual biotic nitrate assimilation of 3.44 t yr^{-1} accounts for less than 1% of the total nitrate input of 473.6 t yr^{-1} to the coastal waters of Adelaide. Of the total nitrate assimilated, the seagrass complex accounted for nearly 88%. A summarised version of the biotic assimilation capacity for nitrate along the Adelaide metropolitan coast is highlighted in figure 6.2.

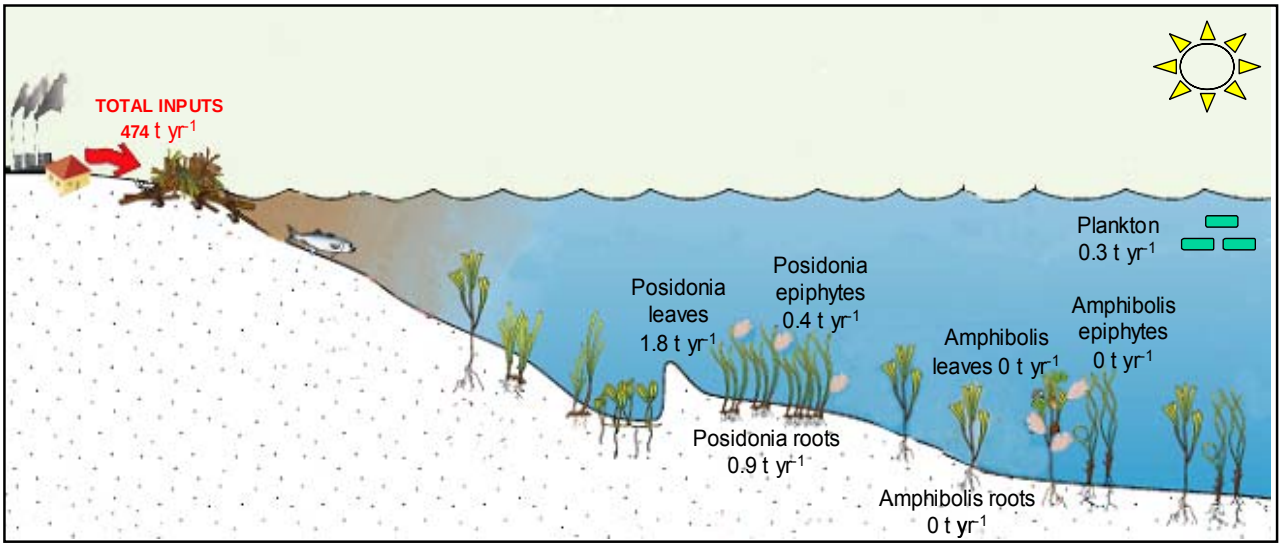

Figure 6.2: A simplified summary of the current annual nitrate biotic assimilation capacity in relation to the total anthropogenic inputs for the Adelaide coastal waters. Figures in tonnes nitrate / year.

Our model clearly indicates that the seagrasses on the coast of Adelaide represent an important component in the nitrogen cycle of the region. Given its importance, not only to nitrogen cycling, but also to the stability of the ecosystem (Shepherd et al., 1989), further loss of these seagrass beds is likely to have important ramifications. To some degree, the model suggests that a decrease in seagrass may result in elevated nutrient levels. Whilst this may, in turn increase uptake rates, the indications are that this would be outweighed by a continuing loss of seagrass caused by the effects of eutrophication.

A critical appraisal of the model indicates that input levels are still in excess of the apparent ability of the biotic component to take up ammonium and nitrate, despite the fact that ambient levels are considerably reduced compared with historical levels. Clearly, there are sinks for nitrogen that have not yet been accounted for viz., pore water, columnar water, sand, benthic microalgae, herbivores, etc. Quantifying these is an important future direction for this work. Another important advance for our understanding of nutrient cycling in this system is to go beyond uptake rates and quantify turnover rates of nutrients within each of the components.

6.3. Carbon uptake and resource allocation

Carbon is an essential structural component of photosynthetic organisms such as macrophytes and microphytes. Carbon uptake studies are often used as a good measure of the physiological state of these organisms. Water temperature and underwater irradiance are known to play a critical role in regulating seagrass productivity (especially leaf biomass), metabolism and carbon uptake. Fine sediments probably from the Outer Harbour dredging operations, are likely to have resulted in lower carbon uptake and a reduction in the above-ground and below-ground biomass in summer. Shaded conditions from suspended particulates in the water column coupled with high epiphytic load and sediment deposition on leaves in summer may be responsible for reduced carbon uptake by seagrass leaves thereby limiting seagrass growth. A combination of some of these factors might be responsible for the significant reduction in leaf (*Posidonia* and *Amphibolis*) and root biomass (*Amphibolis*) at the study site in summer. Whilst epiphytes may compete with seagrass for “resources”, especially in *Amphibolis* where epiphytic loading is usually high, it is apparent from this study that inorganic carbon is not a limiting nutrient, thereby excluding the possibility of competition for this resource.

6.4. Phosphorus uptake

Total uptake of spiked inorganic phosphorus by biological components was negligible in the study, never exceeding 0.5% of the total resource. Low biological uptake rates of inorganic phosphorus could be attributed to carbonate sediments and particulates in the water column binding inorganic phosphorus, limiting its availability for biological uptake. Highest uptake rates were in winter and lowest in spring. As with carbon, smothering of the seagrass complex by suspended sediments probably resulted in reduced uptake during summer, as the chamber deployments during that season coincided with dredging operations. Overall phosphorus uptake rates reported here for *Posidonia* and *Amphibolis* complexes were comparable to the findings of Paling and McComb (1994) in Western Australia.

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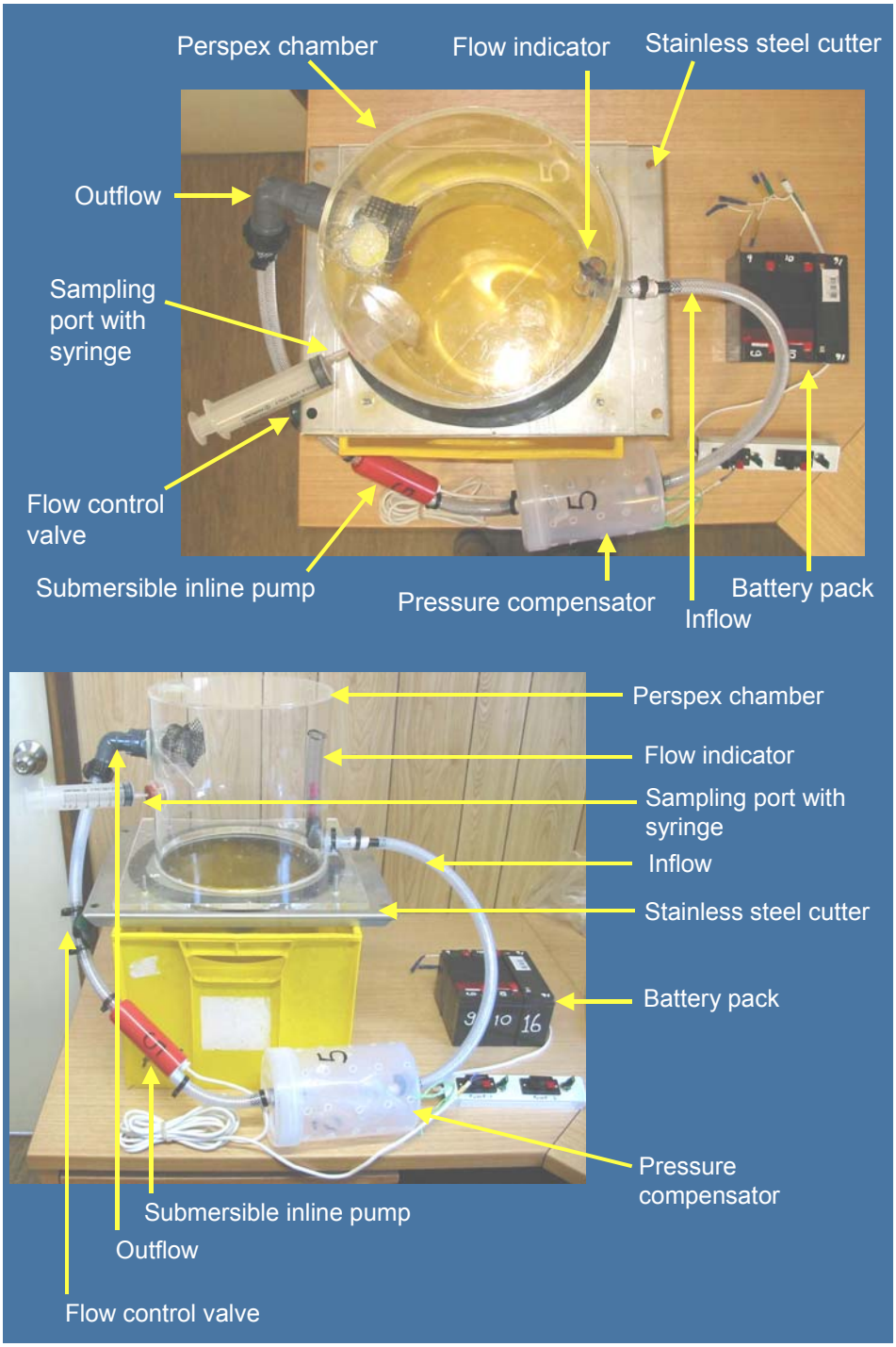
8. Appendices

Appendix 1: Benthic chamber and its components

Top and side view of a benthic chamber showing all the components

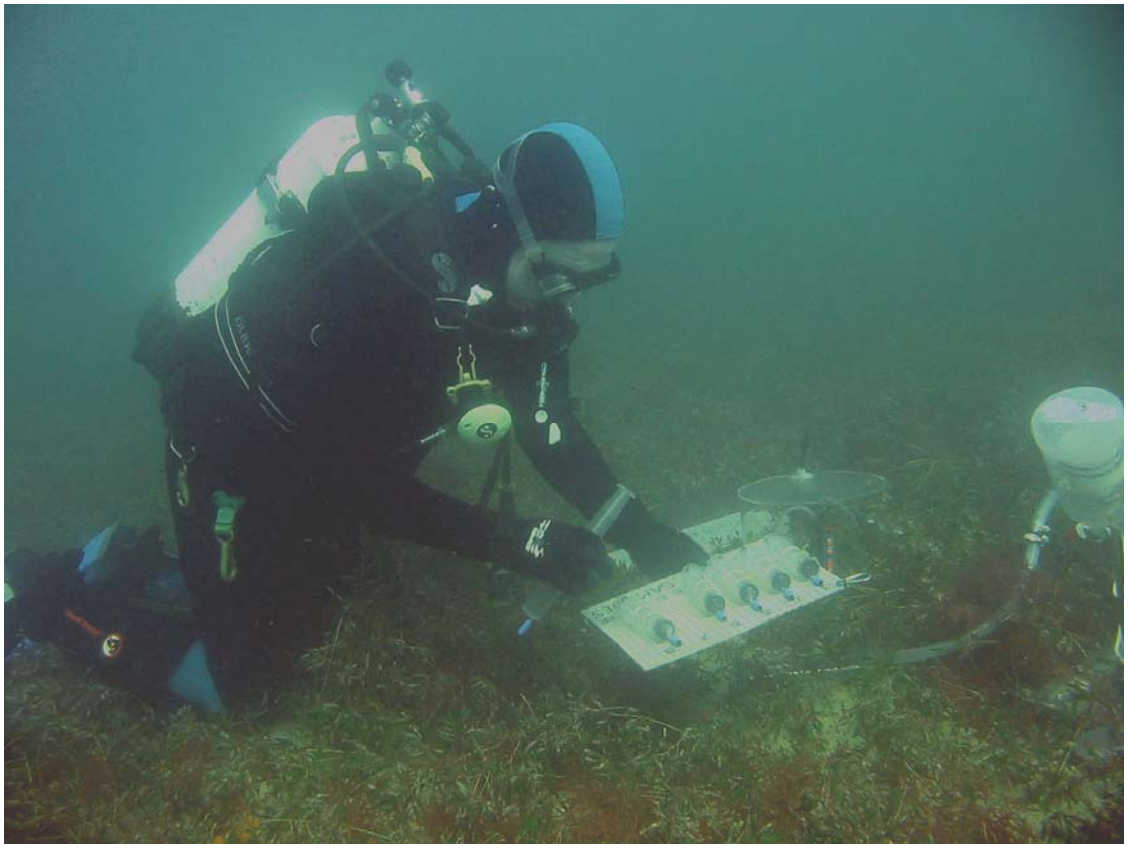


Appendix 2 : Deployment of benthic chambers

Chambers deployed over *Posidonia* beds

Close-up of chambers deployed over *Amphibolis* beds

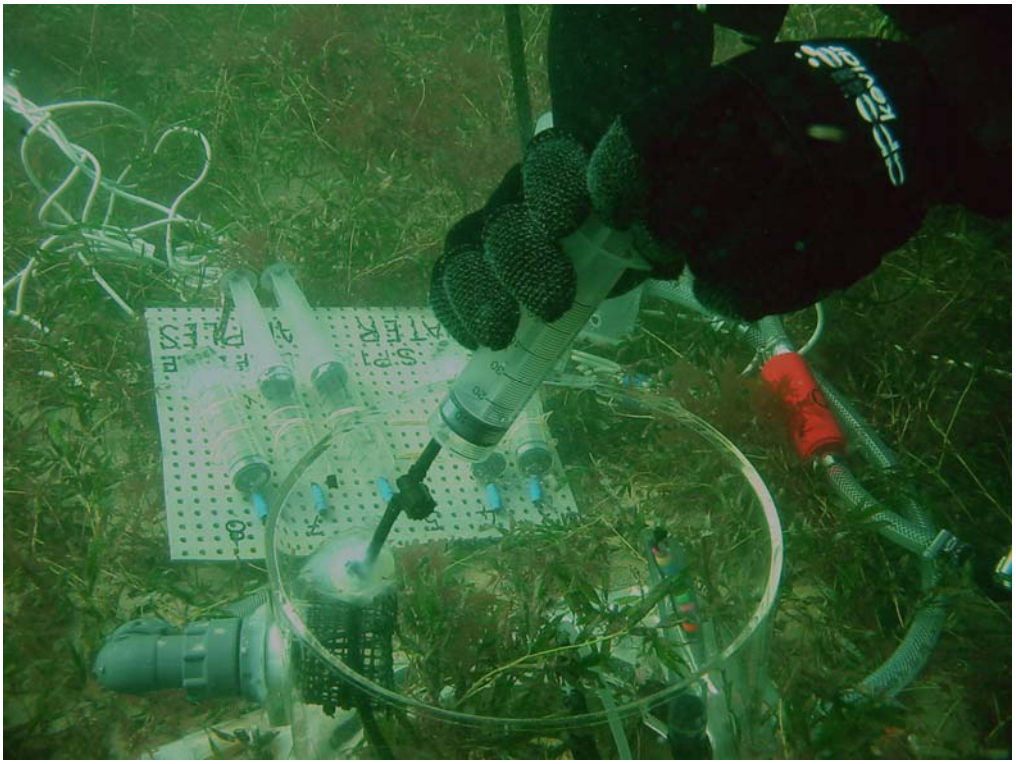
Close-up of the high amperage underwater battery pack



Syringe holder and syringes used for collecting water samples

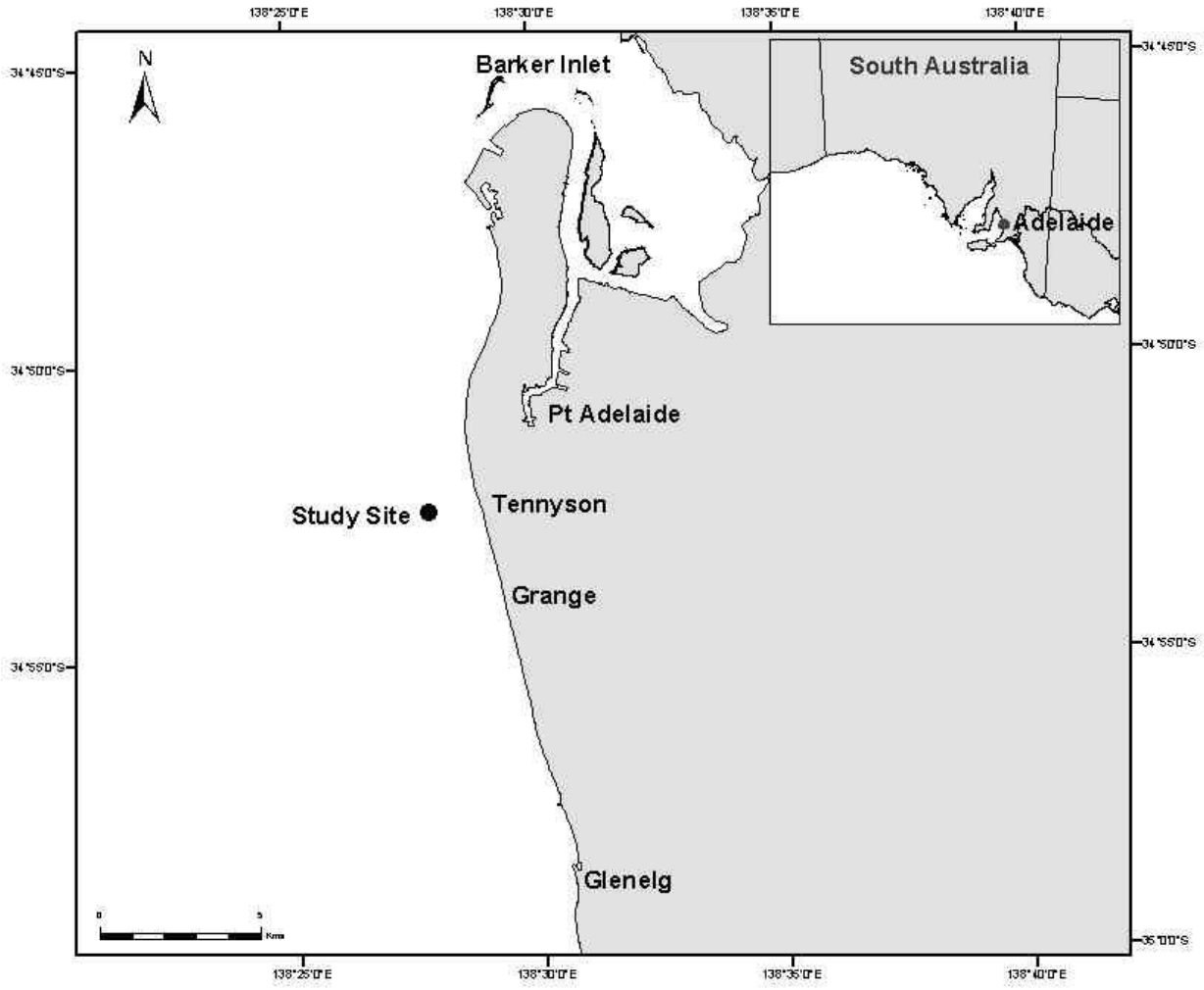
Pore water sampler used in the study

Diver collecting chamber water sample



Collection of pore water samples

Appendix 3: Map showing location of chamber deployment. All chamber deployments were carried out within a 100 m radius of the point marked off Tennyson.



Appendix 4: Chamber deployment dates

Details of the study	Winter deployment	Spring deployment	Summer deployment
$^{15}NH_3$ fate and resource allocation study	29.06.2005	14.10.2005	22.02.2006
$^{15}NO_3$ fate and resource allocation study	01.08.2005	16.10.2005	23.02.2006
$H^{13}CO_3$ fate and resource allocation study	03.08.2005	20.10.2005	27.02.2006
PO_4-P uptake study	05.08.2005	29.11.2005	21.02.2006

Appendix 5: Percent uptake of total resource by the biotic components at the end of the incubation. The data shows adequate availability of nutrients and that there was no limitation of nutrients in the chamber at the termination of the experiment. Values represent mean ± standard error of means (n=3).

Resource	Species	Winter	Spring	Summer
Ammonium	*Posidonia* sp.	5.8 ± 0.1	8.6 ± 0.2	4.0 ± 0.1
	Amphibolis sp.	51.1 ± 3.7	85.2 ± 0.6	21.8 ± 0.3
Nitrate	*Posidonia* sp.	1.8 ± 3.8	8.7 ± 0.3	0.3 ± 0.0
	Amphibolis sp.	4.9 ± 1.4	17.2 ± 0.5	1.4 ± 0.0
Carbon	*Posidonia* sp.	18.0 ± 12.9	14.8 ± 5.4	3.6 ± 1.4
	Amphibolis sp.	3.5 ± 1.8	21.0 ± 13.6	12.1 ± 4.7
Phosphate	*Posidonia* sp.	0.48 ± 0.12	0.07 ± 0.04	0.03 ± 0.01
	Amphibolis sp.	0.34 ± 0.09	0.16 ± 0.09	0.26 ± 0.20

Appendix 6: Ambient and chamber water quality during the deployments in winter, spring and summer. All values are means ± standard error of means.

Parameter		Winter	Spring	Summer
Dissolved Oxygen ($mg.L^{-1}$)	Ambient (n=3)	8.1 ± 2.3	10.3 ± 0.4	6.6 ± 0.4
	Chamber (n=12)	6.9 ± 0.3	6.4 ± 1.0	5.9 ± 0.9
Salinity	Ambient (n=3)	37.4 ± 0.0	37.4 ± 0.3	37.0 ± 0.4
	Chamber (n=12)	37.4 ± 0.0	36.8 ± 0.4	37.4 ± 0.4
Temperature (°C)	Ambient (n=3)	12.5 ± 0.7	16.6 ± 0.7	21.2 ± 0.3
	Chamber (n=12)	12.5 ± 0.5	16.6 ± 0.6	21.5 ± 0.4
pH	Ambient (n=3)	8.6 ± 0.9	7.8 ± 1.1	8.6 ± 0.0
	Chamber (n=12)	8.3 ± 0.0	9.0 ± 0.7	8.6 ± 0.1
Photosynthetically Available Radiation ($\mu mol.m^{-2}. s^{-1}$)	Ambient (n=20)	59.4 ± 1.6	165.5 ± 6.1	191.8 ± 11.2

Appendix 7: Table summarising background biological data during the chamber deployments. All values are means ± standard error of means.

Parameters	Winter			Spring			Summer		
	n	*Posidonia*	*Amphibolis*	n	*Posidonia*	*Amphibolis*	n	*Posidonia*	*Amphibolis*
Seagrass									
Above-ground biomass (g DW. m^{-2})	9	119.4 ± 15.9	407.6 ± 37.8	12	342.3 ± 35.6	545.9 ± 70.7	12	202.0 ± 36.3	281.2 ± 32.3
Below-ground biomass (g DW. m^{-2})	9	1571.9 ± 379.5	378.6 ± 50.9	12	2378.0 ± 361.4	1232.1 ± 432.7	12	2516.9 ± 509.2	382.7 ± 82.0
Moisture in leaves (%)	12	81.8 ± 0.5	70.3 ± 0.8	15	79.7 ± 0.7	71.7 ± 0.5	11	78.8 ± 1.0	72.1 ± 0.4
Moisture in roots (%)	12	55.7 ± 4.2	45.8 ± 6.0	15	64.5 ± 0.9	68.2 ± 1.2	11	74.3 ± 1.5	76.1 ± 1.7
Epiphytes									
Loading per leaf weight (g DW.g DW^{-1})	9	0.47 ± 0.04	1.59 ± 0.24	15	0.37 ± 0.04	2.78 ± 0.53	11	0.57 ± 0.04	5.03 ± 0.88
Loading per leaf area (g DW.cm^{-2})	9	0.38 ± 0.07	1.45 ± 0.67	15	0.21 ± 0.03	0.8 ± 0.2	11	0.29 ± 0.2	1.32 ± 0.26
Moisture	12	56.0 ± 3.5	62.2 ± 5.8	15	64.6 ± 2.1	79.2 ± 1.6	11	72.0 ± 1.3	75.7 ± 1.7
Phytoplankton									
Total cell counts (no.cells.ml^{-1})	3	29.3 ± 0.7		3	5.0 ± 2.5		3	12.3 ± 2.7	
Dominant species (%)	*Thalassiosira* sp. 48.2, *Nitzschia* sp. 9.5, *Navicula* sp. 9.2, *Coscinodiscus* sp. 8.6, *Prorocentrum* sp. 4.5, *Tintinid* sp. 4.2, *Protoperidinium* sp. 2.4, *Bacillaria paradoxa* 2.1, Misc. dinoflagellates 6.8 and Misc. diatoms 4.5			*Thalassiosira* sp. 41.1, *Coscinodiscus* sp. 23.3, *Nitzschia* sp. 11.1, *Leptocylindricus* sp. 11.1, *Bacillaria paradoxa* 6.7, *Prorocentrum* sp. 3.3 and *Tintinid* sp. 3.3			*Leptocylindricus* sp. 54.5, *Nitzschia* sp. 23.7, *Protoperidinium* sp. 5.8, *Navicula* sp. 4.5, *Ceratium tripos* 4.5, *Tintinid* sp. 1.2, and Misc. dinoflagellates 5.7		