DUGONGS AND GREEN TURTLES: GRAZERS IN THE TROPICAL SEAGRASS ECOSYSTEM

Thesis submitted by

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Dugong feeding on Halaphila in the Philippines (photo provided by Henriette Schlüpman and Duane Yates).

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Dedication

to

Santos G. Aragones
Alice V. Aragones
Consuelo Habito
Edgardo Gomez
John McManus
Angel Alcala

ABSTRACT

This study examined aspects of the interactions between dugongs, green turtles and their tropical seagrass food. In order to examine the effects of herbivory on the community structure, productivity, and nutritional composition of seagrass, experiments simulating intensive and light dugong grazing (uprooting whole plants) and intensive turtle cropping (removal of aboveground biomass) were carried out in intertidal seagrass beds at Cardwell (18 ° 14 ' S, 146 ° E) and Ellie Point (16 ° 53 ' S, 145 ° 46 ' E) on the northeast Queensland coast. Grazing experiments at Cardwell and Ellie Point were monitored monthly for a year before the seagrass samples were harvested. An additional short-term experiment was also carried out at Cardwell only, wherein samples were harvested one month and two months after cropping, while those from the grazing plots were harvested after four months. Seagrasses were harvested opportunistically from eight sites and from four depths at one site to investigate specific and spatial variation in nutrient composition. The effect of artificial nitrogen and phosphorus fertiliser treatments on seagrass nutrients was investigated experimentally at Shelley Beach (19 ° 19 ' S, 146 ° 50 ' E). Determinants of the nutritional composition of tropical seagrasses and the nutritional basis of the observed feeding preference of these herbivores were also considered.

Two techniques were used in seagrass ecology for the first time:

(1) Video recording was used for monitoring temporal changes in the species composition and abundance in tropical seagrass communities;

(2) Near infra-red reflectance spectroscopy (NIRS) was used to measure the concentrations of the following: nitrogen, organic matter, neutral detergent fibre, acid detergent fibre, lignin, water soluble carbohydrate, and starch and *in vitro* digestibility of dry matter.

The development of the NIRS technique involved the collection of 10 species of seagrasses: *Halophila ovalis, H. minor, H. spinulosa, H. decipiens, H. trichostata, Halodule uninervis, Cymodocea serrulata, C. rotundata, Syringodium isoetifolium,* and *Zostera capricorni* (with *H. uninervis* and *Z. capricorni* exhibiting two varieties). From this collection, a seagrass database consisting of 1,165 samples of leaves (n = 556), roots/rhizomes (n = 552), whole plant (n = 11), seeds (n = 3), and detrital matter (n = 43), including the samples from the grazing experiments, was developed. Then, using NIRS, the spectra of all samples were collected. From this spectral population, some 200 spectra representative of the whole population were selected, using a computer algorithm package (NIRS 3) as the calibration set and prediction equations (multivariate models) developed for the above seagrass nutritional components.

The nature and extent of the effects of grazing and cropping were related to:

(1) the intensity of the grazing impact; and

(2) the nature of the seagrass community, including its species composition and location.

In a mixed-species bed at Ellie Point, intensive grazing altered the species composition by promoting the growth of a more opportunistic (short-lived) species, *Halophila ovalis* in the spaces created by the grazing disturbance at the expense of a long-lived species, *Zostera capricorni*. Grazing also reduced the amounts of detrital matter. The species composition of a monospecific bed of *Halodule uninervis* was not affected by grazing. Both light and intensive grazing, and cropping increased the net above-ground biomass productivity of *H. ovalis* and *Halodule uninervis*. Recovery times varied from months for *H. ovalis* and *Zostera/Cymodocea* at Ellie Point to more than one year for *H. uninervis* at Cardwell. In both cases, grazing improved the seagrass bed as grazing habitat for dugongs and green turtles.

Simulated dugong grazing improved the nutritional composition (nitrogen and water soluble carbohydrate) of *H. ovalis* and *H. uninervis*. This improvement was detectable 10 to 12 months later. In short-term experiments, both grazing and cropping increased the leaf nitrogen concentration of *H. uninervis*. The digestibility (*in vitro*) of dry matter of *H. uninervis* moderately increased after grazing and cropping. Grazing and cropping had variable effects on the fibre and lignin contents of *H. uninervis* depending on the plant part, nature and intensity of herbivory and duration of the recovery. Enhanced nutrients in the sediments increased the concentrations of nitrogen, starch, and fibre of *H. minor* and *H. uninervis*.

The nutritional composition of seagrasses also varied between plant parts, among species, between varieties, among depths, and among locations (sites). *Halophila* species, together with *Syringodium isoetifolium*, were more digestible than *Z. capricorni*, *C. serrulata*, and *C. rotundata*, while *Halodule uninervis* had the highest nitrogen and starch concentrations of any of the species.

Dugongs and green turtles appear to optimise their diet by selecting food species that maximise digestible nutrients. This is achieved by selecting seagrass species that are more digestible and have higher nutrients (e.g. nitrogen and carbohydrates/starch) and/or species which can compensate for grazing.

Changes in feeding habitats due to herbivory by dugongs and green turtles affect the functional dynamics of tropical seagrass ecosystems through the alteration of resource availability and sediment redox conditions resulting from grazing disturbance. Consequently, mosaics of patches of varying species and nutritional compositions are produced at a local scale.

A major and long-term reduction in the number of dugongs and green turtles in some areas may lead to an irreversible degradation of their habitats as preferred food species are replaced by less-preferred species. In other areas, other forms of natural disturbance and environmental constraints probably maintain the community at a low seral stage.

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CHAPTER 1.

GENERAL INTRODUCTION

Seagrasses form one of the most productive and important coastal ecosystems (Hillman *et al.* 1989; Larkum *et al.* 1989; Poiner and Peterken 1995). They provide food and shelter for commercially and recreationally important fisheries species, and in the tropics and subtropics, food for endangered large herbivores. These herbivores include the green turtle (*Chelonia mydas*), and three of the four extant sirenians (sea cows): the dugong (*Dugong dugon*) and two of the three species of manatee (*Trichechus manatus* and *Trichechus senegalensis*) (Thayer *et al.* 1984; Lanyon *et al.* 1989).

Only the dugong is a seagrass specialist (Marsh *et al.* 1982; Lanyon *et al.* 1989; Preen 1993, 1995). Green turtles feed on both seagrasses and algae (Garnett *et al.* 1985; Bjorndal *et al.* 1991; Read 1991; Brand 1995). Florida and West African manatees eat a wide range of aquatic vegetation (Reynolds and Odell 1991).

Despite the sizes of adult dugongs (up to about 350 - 400 kg), manatees (about 350 - 1600 kg) and green turtles (up to 200 kg), Thayer *et al.* (1984) assumed that their densities are too low for them to make any significant impacts on their feeding habitats. However, this assumption is not justified, at least in Australia, where the densities of dugongs and green turtles may be high (e.g. Moreton Bay Preen 1993, 1995a; Australian region Marsh *et al.* 1995a). For example, Preen (1993) estimated that about 600 dugongs consumed 28% of the total seagrass production in favoured areas in Moreton Bay, Queensland, the southern limit of the dugong's range on the east coast of Australia.

Dugongs graze destructively by uprooting whole plants (Wake 1975; Heinsohn *et al.* 1977; Preen 1993) while green turtles crop leaves (Bjorndal 1980; Lanyon *et al.* 1989). Green turtles in the Caribbean maintain 'grazing plots', wherein individuals regularly recrop patches of seagrasses for the younger leaf regrowth (Bjorndal

1980). These modes of foraging can have important effects on the structure of seagrass communities. Preen (1993, 1995) and Kuiper-Linley (1994) found that grazing by dugongs and green turtles alters the structure of a sub-tropical seagrass community in Moreton Bay. Preen suggested that the grazing disturbance created by dugongs may enhance the quality of their habitats because the growth of a more preferred species (e.g. *Halophila ovalis*) is promoted while the expansion of a lesser preferred (e.g. *Zostera capricorni*) is controlled.

Diet selection by dugongs and green turtles may be affected by spatial and temporal variation in the nutrient content of their food supply. In Moreton Bay, Preen (1993, 1995b) suggested that seasonal omnivory by dugongs on invertebrates helps them cope with the supposedly poor nutritional quality of food available in the area during winter. Foraging theory (Westoby 1978; Stephens and Krebs 1986) suggests that these large marine herbivores might be expected to maximise nutrient intake, particularly in times of greatest energy requirements such as when females are lactating (*sensu* Price and White 1985; Minson 1990). There are also indications that reproduction is limited by food in both dugongs (Marsh 1995) and green turtles (evident from El Niño years; Limpus and Nicholls 1988). However, studies which examine and compare the nutritional variation of seagrasses according to location (including water depth), season, species, plant part, leaf morphology and age are wanting (Wake 1975; Lanyon 1991).

The value of seagrasses to a herbivore is mainly determined by their nutrient content, and the herbivores' digestive capabilities and requirements (Lanyon and Marsh 1995b). There is very little information on the digestive physiology of green turtles (but see Bjorndal 1980, 1985; Bjorndal *et al.* 1991) or dugongs (except for Murray *et al.* 1977; Marsh *et al.* 1978; Lanyon 1991; Lanyon and Marsh 1995b). The digestive physiology of these animals is very difficult to study because they are not easily held under controlled conditions for feeding experiments. An alternative approach is to carry out a comprehensive evaluation of the nutritional composition of several seagrasses (ranging from the most to the least preferred

species) and to use these data to make predictions of their importance to the dugong and green turtle by comparing them to the reported preferences.

The nutritional composition of food plants may critically influence the survivorship, fecundity and distribution of their herbivores (Crawley 1983; Van Soest 1994). The low growth rates and low lifetime fecundity of green turtles (Bjorndal 1980, 1982a, 1985) have been attributed to the low nutritive value of seagrasses. Captive-reared green turtles grow faster when fed a high protein, animal diet (Caldwell 1962). There are, however, very few studies which examine the nutrient composition of seagrasses from the perspective of herbivores. Bjorndal (1980, 1985) examined the nutritional ecology of green turtles feeding on *Thalassia testudinum*; similarly, Lanyon (1991) examined the nutritional ecology of dugongs feeding on some tropical species such as *Halophila ovalis, Halodule uninervis, Cymodocea serrulata*, and *Zostera capricorni*. Neither of these studies is comprehensive enough for robust generalisations. Additionally, there is very little information on the nutrient basis of food selection by either dugongs or green turtles (but see Bjorndal 1980, 1985 for adult green turtles; Brand 1995 for juvenile green turtles; Preen 1993 for dugongs). Based on limited data, Lanyon (1991) and Preen (1993) suggested that dugongs select their food primarily on the basis of high nitrogen and low fibre content.

The main objective of this study is to determine by simulation, the effects of various levels of grazing and cropping on tropical intertidal seagrass communities in northern Australia. As this region harbours relatively large populations of dugongs and green turtles (Marsh *et al.* 1995a and references cited therein), it is an ideal site to test whether grazing and cropping by large herbivores changes the community dynamics, structure, and nutritional composition of seagrasses. I also aimed:

(1) To determine whether dugongs and green turtles gain nutritional advantages from eating:
   a) a particular species of seagrass,
   b) deepwater rather than intertidal seagrasses,

c) regrowth from uprooted and cropped seagrasses, and

d) narrow rather than broad leaf varieties of seagrasses;

(2) To determine the extent of spatio-temporal variability in seagrass nutrients and whether nutritional differences exist between similar species of seagrasses from tropical and subtropical regions;

(3) To determine the recovery time of seagrasses from various levels of grazing impact; and

(4) To develop a conceptual model which synthesises the interactions between large marine herbivores and tropical seagrasses.

In order to accomplish these aims, I applied cost-effective techniques for:

(1) monitoring the biomass of tropical seagrasses through time; and
(2) predicting the nutritional composition of seagrasses.

This General Introduction (Chapter 1), is followed by a Literature Review (Chapter 2), which provides a more detailed review of previous works and places the thesis in a theoretical and historical context. Chapter 3 summarises the rationale for the study approach and methods used in this research. Chapter 4 details the application and evaluation of a visual estimation technique based on video recorded images which I used for monitoring seagrass biomass during the simulation experiments. Chapter 5 examines the effects of simulated grazing on the community structure of tropical seagrasses. Chapter 6 describes the development, use and evaluation of near infra-red reflectance spectroscopy (NIRS) for predicting the nutrient composition of seagrasses. Chapter 7 details the effects of species, plant parts, location and season on seagrass nutrients. The effects of grazing on the nutritional composition of tropical seagrasses are described in Chapter 8. Chapter 9 synthesises the whole study and outlines a conceptual model of the interaction between green turtles and dugongs and their seagrass food in order to provide some insights into the critical conservation question:

Will a major and long-term reduction in the numbers of dugongs and green turtles in an area lead to an irreversible degradation of their habitat?

To assist the reader, I have separated the chapters in this thesis by green pages. The tables and figures are placed at the end of the corresponding chapter. The front page of each set of tables is marked by a yellow page; the front page of the set of figures is blue.

CHAPTER 2.

LITERATURE REVIEW

This chapter surveys the literature related to: (1) the dynamics and structure of tropical seagrass communities, including how their growth form and life history strategies influence their response to disturbances such as grazing; (2) grazing ecology, including the impacts of grazing on community structure, productivity and nutritional composition of plants, in general, and seagrasses, in particular; (3) the nutritional content and quality of plants in relation to the effects herbivory may have on tissue nutrient content and quality of seagrasses; and (4) the two largest strictly marine herbivores– the dugong (*Dugong dugon*) and the green turtle (*Chelonia mydas*), and what is known about their feeding and nutritional ecology.

2.1. SEAGRASS COMMUNITY AND DYNAMICS

2.1.1. Seagrass distribution and taxonomy

Seagrasses are vascular marine angiosperms that cover extensive areas in coastal waters from tropical to temperate regions. Worldwide, there are about 12 genera consisting of approximately 57 species (Kuo and McComb 1989). Over 30 species can be found within Australian waters (Poiner and Roberts 1986; Walker and Prince 1987). Australia is therefore an ideal site to conduct research on seagrass ecology, including seagrass-herbivore interactions.

The highest seagrass species diversity in the tropics is found within the coastal waters of the Malesian region enclosed by Indonesia, Borneo, Papua New Guinea, and the Torres Strait in northern Australia (Mukai 1993). Mukai (1993) recognised that the most diverse seagrass communities are found in the waters of north-eastern Queensland, Australia (8 genera with 14 species; Lee Long, *et al.*, 1993). Despite this diversity, very few researchers have examined the nutritional qualities of seagrasses in the tropics (Birch 1975; Lanyon 1991) and their importance to

their direct consumers which include dugongs (Lanyon 1991; Preen 1993) and green turtles (Read 1991; Kuiper-Linley 1994; Brand 1995).

2.1.2. Role and importance of seagrasses to herbivores

Seagrass communities are one of the most productive and dynamic ecosystems (McRoy and McMillan 1977; Thayer *et al.* 1984; McRoy 1996). They provide critical habitats for many species, including small (e.g. prawns, sea urchins) and large marine herbivores (i.e. dugongs and green turtles, Larkum *et al.* 1989). Organic matter derived from seagrass production may enter the trophodynamic system either directly, or indirectly. The direct route occurs when live plant matter is consumed by herbivores. The indirect route involves dead plant particulate matter, together with its decomposers (i.e. bacteria, fungi and associated animals), being consumed by detrivores (Thayer *et al.* 1984). Seagrass primary production is known to be high and rivals that of cultivated crops (Thayer *et al.* 1975). Most of the studies on the trophodynamics of seagrasses have focused on the detrital cycle. Very few studies have concentrated on the 'herbivory cycle' in tropical seagrass communities (but see Bjorndal 1980; Zieman *et al.* 1984; Klumpp *et al.* 1993; Preen 1993, 1995a; Kuiper-Linley 1994).

The value of tropical seagrass communities as habitats for commercially and recreationally important species of fish and crustaceans and large marine herbivores has been appreciated for at least two decades (e.g. Thayer *et al.* 1984; Coles *et al.* 1987; Poiner *et al.* 1987; Bell and Pollard 1989; Lanyon *et al.* 1989; Poiner and Peterken 1995). However, few studies have examined plant-herbivore interactions directly, particularly for the large herbivores (but see Bjorndal 1980, 1985; Preen 1993, 1995a). Plant-herbivore interactions are important as they can affect primary and secondary production, energy flow and nutrient cycling (Mooney and Gordon 1983; Sprugel 1984).

2.1.3. Megaherbivores in seagrass communities

Herbivores obtain energy by feeding on primary producers and are the main link in the trophic flux between autotrophs and secondary consumers (Begon *et al.* 1990). Herbivores are taxonomically and ecologically diverse (Huntly 1991). Crawley (1989b) divided herbivores into invertebrates and vertebrates, but suggested that the fundamental difference between these groups is in the size of their bite (which reflects their body mass), and their population density relative to the food resource. Owen-Smith (1986) called plant-feeding mammals (that typically attain adult body mass in excess of one metric tonne), including the sirenians, 'megaherbivores'. I have extended this definition to include the green turtles. The adults of this species may weight up to 200 kg. From here on, I will refer to dugongs and green turtles collectively as 'marine megaherbivores'. Although these large marine herbivores weigh below one metric tonne, I adopted the term 'megaherbivore' to emphasise that, among the seagrass herbivores, they have the greatest capacity to have considerable impacts as a consequence of their large size. I must emphasise though, that the application of the term 'megaherbivore' in this context does not imply that the dugong and/or the green turtle are generalist feeders that can feed on any plant food irrespective of quality.

2.1.4. Life history strategies and community structures of seagrasses

2.1.4.1. *Seagrasses are modular plants*

In order to understand the interactions between seagrasses and their herbivores, an understanding of the unique properties of seagrasses is required. Seagrasses are rhizomatous plants that undergo continuous production and loss of modules (Tomlinson 1974). These modules are made up of: (1) leaves, (2) roots, and (3) rhizomes (Fig 2.1). The leaves form the aboveground biomass, while the roots and rhizomes form the below-ground biomass. The rhizomes, which ensure the vegetative spread of these modular organisms, may be either horizontal (long shoot) or vertical (short shoot), spreading the plant upwards or sidewards (Tomlinson 1974; Bell and Tomlinson 1980; Brouns 1987; Duarte *et al.* 1994).

The growth of these modular plants can be complex. The degree of differentiation between horizontal and vertical rhizomes increases (see Tomlinson 1974; Bell and Tomlinson 1980) from species with monomorphic rhizomes (e.g. genera *Zostera* and *Enhalus*) to those with distinct major (horizontal rhizome) and minor (vertical rhizome) axes (e.g. genera *Cymodocea* and *Thalassia*) (Duarte *et al.* 1994). Major or primary axes have faster growth rates than minor axes (Brouns 1987). This may be a consequence of the strong apical dominance typical of rhizomatous plants (Bell and Tomlinson 1980), an observation supported by Tomasko *et al.* (1991), who reported that the survival of transplanted *Thalassia testudinum* increased with the number of short shoots, and that more rapid reproduction of new short shoots occurs in parts of the rhizomes with intact apical meristem. The importance of modularity to seagrass ecology has only been appreciated recently (Duarte *et al.* 1995). The population biology of modular plants is different from unitary plants. Duarte *et al.* (1995) suggested that the reconstruction of seagrass dynamics by age determination is probably one of the most important tools to further seagrass ecology. Similarly, meristematic or modular growth of seagrasses may be an important factor in the observed variability in recovery of seagrass from disturbances like grazing by marine megaherbivores. Preen (1993, 1995a) suggested that the disturbance on a subtropical seagrass bed following dugong grazing can drive determinate shoots to indeterminate growth, leading to proliferative growth and rapid regrowth.

2.1.4.2. *Life history strategies*

The response of seagrasses to disturbances such as grazing may reflect their variable life history strategies. Growth patterns and growth rates, together with persistence, reproductive effort/output, propagule dispersability and length of life cycles, are some of the factors that determine the life history strategies of seagrass species (Clarke and Kirkman 1989). In the tropics, the rhizomes of the members of the Cymodoceoideae (e.g. *Cymodocea*), and the Halophiloideae (e.g. *Halophila*) have relatively fast linear growth rates and are frequent colonizers (Birch and Birch 1984; Brouns 1987; Williams 1988). *Halophila ovalis* apparently flowers and fruits

all year round in the tropics (den Hartog 1970). This species has also been found to develop 'hair-like' structures during its seedling development which may act as an anchor for the seedling (Kuo and Kirkman 1992). Additionally, the Halophilae have one of the longest rhizome extension rates (70- 335 cm/year) (Clarke and Kirkman 1989). Seagrasses with such short life-cycle features are usually referred to as opportunistic or fast-growing ('pioneering') species. Other species, like *Enhalus acoroides* and members of the genus *Thalassia*, usually have longer life cycles, tall canopy height and presumably slower growth rates (Clarke and Kirkman 1989) and are often referred to as robust ('climax') species.

Successional theory applied to aquatic macrophytes is now regarded as simplistic, lacking rigorous documentation, and useful only in a conceptual sense (e.g. Kautsky 1988; Stevenson 1988; Barrett *et al.* 1993). I will, however, occasionally use the terms, 'pioneering' and 'climax', when discussing life strategies in order to differentiate short-lived, fast growing species from long-lived, slow growing ones as no new, better, and acceptable terminology exists. Further, studies in seagrass ecology deal mostly with species rather than populations. I caution that most studies in plant population ecology deal with unitary plants and may not be appropriate models for communities of modular plants. This becomes important when testing theories in seagrass ecology developed with unitary plant models from terrestrial systems (Duarte *et al.* 1995). Such a perspective is important in understanding the interactions between seagrasses and their herbivores because the results may consequently be misinterpreted.

2.1.4.3. *Seagrass communities*

Unlike temperate seagrass species, tropical seagrasses tend to occur in mixed-species stands (Poiner and Peterken 1995). They also tend to be very patchy in distribution. Some species seem to be ephemeral (e.g. *Halophila trichostata*) (Kuo *et al.* 1993). Studies on the effects of herbivores on plants should be carried out at the levels of plant population or plant community, so that the patterns inferred are appropriate to such higher levels of organisation (Huntly 1991).

Seagrasses are mostly found in coastal nearshore waters. However, recent surveys have shown that seagrasses in the tropics can also be found in deep water (between 15 and 58 m) (Lee Long *et al.* 1996). The importance of these deepwater seagrasses for herbivores has not yet been examined carefully. Anderson (1994) proposed that carbohydrate-rich rhizomes of *Halophila spinulosa* might compensate for deepwater foraging of dugongs observed in eastern Shark Bay, Western Australia. However, his suggestion was not supported by any measurements of carbohydrate concentrations. Anderson extrapolated from suggested similar species from another study (Masini 1982) which examined the non-structural carbohydrates of some seagrasses in Shark Bay. However, Masini did not examine *H. spinulosa*.

Subtropical seagrasses are distributed between temperate ($30^\circ < 60^\circ$, latitudes) and tropical ($<30^\circ$ latitudes) regions, and tend to have density (abundance), and species diversity and composition similar to the tropical species. However, in subtropical regions, seagrasses probably experience more significant temperature changes than in the tropics, and exhibit more pronounced seasonality in growth (Poiner 1985; Preen 1993). The significance of these discrete environments to herbivores, which are distributed in both systems, has not yet been considered.

2.1.5. Seagrass community dynamics and natural disturbance

Seagrass communities are dynamic at both spatial and temporal scales (Clarke and Kirkman 1989). Most studies of seagrass community dynamics (changes in productivity, species composition, distribution, age structure, and so on) have been based on qualitative observation. Only recently has a more quantitative approach emerged. Data on the changes in productivity, composition, and distribution over time are lacking (Duarte *et al.* 1994; Poiner and Peterken 1995). Several factors influence the primary production, growth, tissue composition and distribution of seagrasses: light (e.g. Dennison 1987; Zieman *et al.* 1989; Abal *et al.* 1994); temperature (e.g. Kirkman *et al.* 1982; Bulthius 1987); salinity (e.g. Walker 1985); water movement (e.g. Fonseca and Kenworthy 1987); nutrient availability (e.g.

Short 1987; Erftemeijer and Middelburg 1993) and herbivory (Preen 1993, 1995a). These factors vary temporally and spatially (e.g. Hillman *et al.* 1989; Preen 1993) and consequently influence the dynamics of seagrass communities.

The effects of natural disturbances on the structural dynamics of communities are important in understanding the ecology of natural disturbance and communities (Pickett and White 1985). Tropical communities are mainly structured by seasonal biotic and abiotic disturbances; in contrast, temperate communities are mainly structured by seasonal abiotic disturbances and physiological extremes. Some of the common sources of biotic disturbances in tropical seagrass communities are grazing, species competition, and senescence of the organisms (Clarke and Kirkman 1989; Kenworthy *et al.* 1989). Abiotic causes include increased water movement, erosion, and smothering by sediment during the seasonal monsoons (Clarke and Kirkman 1989; Coles *et al.* 1989). Regular and periodic natural disturbances influence the changes in the structure of seagrass communities (Clarke and Kirkman 1989; Preen 1993). However, information on tropical seagrass dynamics is recent and very limited (Phillips and Meñez 1988; Williams 1988, 1990; Mellors *et al.* 1993; Preen 1993; Tomasko *et al.* 1993; Lanyon and Marsh 1995; McKenzie 1994). Likewise, interest on the significance of herbivory by dugongs and green turtles on seagrass communities has only been appreciated recently (Preen 1993, 1995; and see Section 2.4). Consequently, a better knowledge of the ecology of tropical seagrasses and their responses to disturbances such as grazing may contribute to understanding coastal processes and proper management of these resources. The only study on the response of seagrasses to grazing disturbance was carried out in a subtropical system (Moreton Bay, Queensland, Preen 1993). Another study (de Iongh *et al.* 1995) which primarily monitored the seagrass distribution and seasonal biomass changes of *Halodule uninervis* in relation to dugong grazing in the East Indonesia area. It was, however, limited only to tracking the frequencies of feeding trails and was not able to measure the biomass removed nor the net primary productivity in relation to dugong grazing. Thus, it lacked the data necessary to support the conclusions.

2.2. GRAZING ECOLOGY

2.2.1. Herbivory and grazing

Herbivory is defined as the consumption of living plant materials (Begon *et al.* 1990). However, it has also been defined to include any form of ingestion of plant material, not necessarily its assimilation, and thus includes the effect of all major herbivores (Cyr and Pace 1993). Grazing is one form of herbivory. It is the act of ingesting grasses and other monocotyledonous plants. Browsing is the act of ingesting leaves and branches of trees, shrubs and forbs. This distinction is important as these two forms of herbivory have different effects on plants. Grazing is considered a regular source of disturbance and part of the normal community process in plants (e.g. Grime 1979; McNaughton 1986; Huntly 1991; Hixon and Brostoff 1996). Browsing has similar effects (e.g. Bryant *et al.* 1983, 1989; Tuomi *et al.* 1984; McNaughton and Georgiadis 1986); however, as a consequence of the relative difference in the plant sizes (bulkier, trees *vs* fragile, grasses), the effects of grazing are more obvious.

Studies on the impacts of herbivory on natural systems are few, and recent, in comparison to studies on the impacts of herbivory on cultivated plants. This has stimulated an increase in the number of studies, reviews, conferences and textbooks solely dedicated to herbivory or herbivore-plant interactions. In this account, I refer to herbivory in the context of natural systems unless it is necessary to refer specifically to herbivory in cultivated systems.

2.2.2. Grazing in aquatic and terrestrial ecosystems

Food sources for herbivores vary. Herbivores in marine ecosystems feed on seagrasses, algae and phytoplankton (Cyr and Pace 1993). Herbivores in terrestrial ecosystems feed on all sorts of plants (from grasses, forbs, shrubs to trees including woody species) (e.g. McNaughton *et al.* 1989, 1991; Bryant *et al.* 1989; Coughenour 1991a, 1991b; McNaughton and Banyikwa 1995).

Even though there are fewer studies on herbivory in aquatic systems than in terrestrial ones, some comparisons can be made. Levels of herbivory vary between ecosystems. Generally, the level of herbivory is assumed to be greater in aquatic ecosystems than terrestrial ecosystems (Petrusewicz and Grodzinski 1975; Whittaker 1975; Ricklefs 1990), because aquatic herbivores maintain a higher biomass and/or graze more per unit of net primary production biomass than terrestrial systems (Cyr and Pace 1993). Variations in mass-specific herbivory rates in aquatic systems, particularly those which are algal-based, have been attributed to features of the plants grazed, such as nutrient content and/or chemical or physical defences (e.g. Lubchenco and Gaines 1981; Hay and Fenical 1988; Padilla 1993; Meyer *et al.* 1994). However, the effects of these and other factors on herbivory in a seagrass-based system have only been recognised recently and remain to be detailed and confirmed (Sand-Jensen *et al.* 1994).

2.2.3. Grazing methods and plant demography

Foraging methods vary depending on the herbivores involved. One of the groups most studied are the ungulate grazers of pastures or grasslands (e.g. for livestock, Hughes 1990, 1993; Burlison *et al.* 1991; Illius *et al.* 1992; for wild ungulates, Sinclair and Norton-Griffiths 1979; McNaughton 1979a, 1984, 1989; Frank and McNaughton 1993; Hartvigsen and McNaughton 1995). Like most browsers (except some species like the elephant which removes whole trees, e.g. Edroma 1989), the feeding of ungulate grazers is basically parasitic rather than predatory; i.e. they reduce the rate of plant growth but tend not to kill their food plants (Crawley 1983). Other groups of herbivores include root/rhizome-feeders, sap-suckers, bark-feeders, seed-feeders and the like (see Crawley 1983, 1989a; Bernays and Chapman 1994). Very few studies deal with animals which tend to consume whole plants. This is an essentially predatory activity (e.g. Edroma 1989; Dublin 1995) and can have dramatic effects on the fecundity, survivorship and recovery of plant communities. Although dugong grazing removes both above- and below-ground biomass, it does not necessarily kill the whole plant (Preen 1993) as seagrasses are modular plants (see Section 2.1.4.1). However, the uprooting nature

of dugong grazing is destructive (Preen 1993) and could have significant effects on the fecundity, survivorship and recovery of seagrass communities.

2.2.4. Focus of studies on grazing impacts

Studies on the impacts of grazing have focused mainly on: (1) their effects on the structure and function of communities (or the dynamics of populations) or ecosystems; and/or (2) their influence on plant strategies including tolerance (i.e. compensatory growth), defence and the partitioning of nutritional resources (i.e. plant nutrients) in response to herbivory. However, this approach has been rarely applied to seagrass communities (but see Bjorndal 1980; Preen 1993; Kuiper-Linley 1994).

2.2.4.1. *Impacts on the structure of communities*

Several aspects of the structure of plant communities could be influenced by grazing. Grazing has been shown to influence the age structure, species diversity or composition and abundance of plant communities (e.g. Huntly 1991; Frank and McNaughton 1993; Gauthier *et al.* 1995; Heck and Valentine 1995 and references cited therein). Grazing is considered a major environmental influence on grasslands, interacting in complex ways with other environmental factors (e.g. resource availability, physiological factors and biochemical factors) (e.g. McNaughton 1976, 1979, 1983a, 1983b, 1984, 1989, 1990; Dyer *et al.* 1982; Noy-Meir *et al.* 1989; Hartvigsen and McNaughton 1995). Studies in terrestrial ecosystems have shown that gregariousness or group formation in grazing animals can increase foraging efficiency by modifying vegetation structure in order to maintain herbage of high biomass and quality (e.g. McNaughton 1984, 1989; McNaughton and Georgiadis 1986; Post and Klein 1996). Similarly, gregariousness in grazing dugongs at Moreton Bay increases foraging efficiency by modifying the seagrass community structure to maintain more of the preferred species (Preen 1993, 1995a). In algal-based marine systems, herbivory has been shown to influence plant morphology, productivity, distribution, community structure, and nutrient relations and tissue nutrient content (e.g. Hay *et al.* 1983, 1987a;

Lewis *et al.* 1987; Valentine and Heck 1991; Hixon and Brostoff 1996). Grazing has also been shown to influence the vegetation structure of a sub-Arctic salt marsh (Cargill and Jefferies 1984; Jefferies 1988). Additionally, it has been suggested that by selectively feeding on specific genotypes or populations of plants, herbivores have the potential to affect the genetic composition of communities (McNaughton 1983a; Herms and Mattson 1992; Houle and Simard 1996). Incidentally, plant genetic identity influences two aspects of herbivory: plant susceptibility to and tolerance of herbivory (Crawley 1983; Marquis 1984; Rosenthal and Kotanen 1994; Houle and Simard 1996). Plant susceptibility occurs when plant quality induces further herbivory, while plant tolerance reduces plant quality presumably discouraging further herbivory. In seagrass-based systems, similar studies, particularly in the tropics, are limited (Bjorndal 1980; Preen 1993; Kuiper-Linley 1994; de Iongh *et al.* 1995).

2.2.4.2. *Impacts on the nutritional quality of plants*

The nutritional quality of food plants may improve or deteriorate under herbivore exploitation (Crawley 1983). Grazing has been shown to improve the potential nutritional qualities (for the animals' benefit) of terrestrial plant communities (e.g. Coppock *et al.* 1983; McNaughton 1984, 1985a; Jaramillo and Detling 1988, McNaughton and Banyikwa 1995), salt-marsh communities (Cargill and Jefferies 1984), algae (e.g. Wilkinson and Sammarco 1983; Hay *et al.* 1987b; Hixon and Brostoff 1996), and seagrasses (e.g.Dawes and Lawrence 1979; Bjorndal 1980; Zieman *et al.* 1984; Kuiper-Linley 1994). In the case of livestock, moderate grazing in grasslands tends to improve plant quality because leaf senescence is prevented (Crawley 1983). However, as grazing intensity increases (i.e. overgrazing), plant quality is reduced as regrowth leaves become smaller and more fibrous (Benz 1974), and generally less nutritious (Ralphs *et al.* 1990), because the more palatable species are outcompeted by the less palatable ones (i.e. weeds) (e.g. Wilson and Macleod 1991; Green and Kauffman 1995). Cropping or clipping the leaves of the seagrass *Thalassia testudinum* increased nitrogen concentration and reduced lignin and ash in new leaves (Dawes and Lawrence 1979; Bjorndal 1980; Zieman *et al.* 1984).

Herbivory is also considered a constraint on the evolution of leaf morphologies and secondary chemicals as plant defences against herbivory (e.g. Bryant *et al.* 1983; Rhoades and Cates 1983; Edwards 1989; Karban and Myers 1989; Brown and Lawton 1991; Haukioja 1991). Information on the effects of grazing on the nutritional attributes of seagrasses has been limited to turtle cropping and to only a few species and nutritional components (see Bjorndal 1980; Kuiper-Linley 1994). de Iongh *et al.* (1995) monitored only the total organic carbon (or what they referred to as the ratio of ash-free dry weight to dry weight) in relation to dugong grazing in the Moluccas, East Indonesia. They did not realise that what they actually measured was organic matter. Organic matter is an insufficient measure of nutritive value for it is simply a parameter delineating the organic from the inorganic fraction of the dry matter.

2.2.4.3. *Other determinants of plant nutrients*

There are many other, non-grazing determinants of plant nutrients. The nutrient content of plants is typically phenotypic, determined by the interactions between the plants inherent requirements (genotype) and their environment. It may vary between species, age, part, location and season (e.g. Etherington 1978; Crawley 1983; Salisbury and Ross 1992). These factors have rarely been considered in nutritional studies of any seagrass-herbivore system (Lanyon 1991). Lanyon (1991) examined the nutrient variation of some seagrasses (*Halophila ovalis*, *Halodule uninervis, Cymodocea serrulata*, and *Zostera capricorni*) and found that their nutrient content varies by species, plant parts, location and season.

Resource availability potentially affects the variability in the responses of plants to herbivory (e.g. Bryant *et al.* 1983, 1989; Tuomi *et al.* 1984; Coley *et al.* 1985; Myers 1987; McNaughton *et al.* 1989). All plants are dependent on the availability of light, water and nutrients for growth, and these factors vary spatially and temporally. Coley *et al.* (1985) suggested that the relative limitation of resources constrains the types of plant defences. The importance of resource availability to the possible variability of seagrass response to herbivory has never been addressed directly. Most studies focused on temporal variability of nutrients in the sediments

in relation to plant growth dynamics and tissue composition (e.g. Boon 1986; Pedersen and Borum 1993; Hemminga *et al.* 1994). Spatio-temporal variability of plant nutrients may affect the timing and the intensity of herbivory. Similarly, the consequences of herbivory as a disturbance could also affect plant nutrients by contributing to their spatio-temporal heterogeneity.

2.2.4.4. *Implications of grazing impacts*

Grazing has ecological and evolutionary implications. There is increased acceptance of the notion that 'herbivory is beneficial to plants' through compensatory growth (Owen and Wiegert 1976, 1981; McNaughton 1979b; Owen 1980; McNaughton 1983a, 1984; Crawley 1987; Paige and Whitham 1987; Karban and Myers 1989; Westoby 1989; Post and Klein 1996). This idea has contributed to the interest in the coevolution of plants and herbivores initiated by Ehlrich and Raven in 1965. Since then, many studies have developed interest in the coevolution of plant-herbivore systems (e.g. Southwood 1973; Gilbert and Raven 1975; Gilbert 1979; Janzen 1980; Domning 1981; Stebbins 1981; Thompson 1982; Cottrell 1986; Ivany *et al.* 1990).

McNaughton (1983a, p. 331) proposed that "mechanisms have evolved that lead to compensatory growth of plants following herbivore damage and that these mechanisms are a major components of plants' evolutionary responses to their long coexistence with animals". The internal mechanisms of compensation involve modifications of plant metabolism resulting in changes in tissue (nutrient) composition. The external mechanisms of compensation involve modifications of plant environment that are favourable to plant growth and productivity (McNaughton 1983a).

2.3. NUTRITIONAL CONTENT AND QUALITY OF PLANTS FOR HERBIVORES

2.3.1. General

Plant dry matter can be divided into soluble and insoluble fractions (Van Soest 1994). The soluble fraction consists mainly of cell contents which are essentially digested by enzymes secreted by all animals. The insoluble fraction consists mainly of cell wall polysaccharides and lignin, collectively referred to as fibre, which are essentially indigestible to some animals (Van Soest 1994) which cannot produce cellulase. However, cellulase which is produced by microflora found in the digestive tracts of some herbivores which enables them to digest fibre.

Several nutrients in the soluble fraction (cell contents) are required by herbivores. Nitrogen is one of the most important plant nutrients because it is essential for protein synthesis (Mattson 1980; Crawley 1983). It is often used as an index of the nutritional value of plants as it can ultimately influence the nutritional status of herbivores (Mattson 1980; Crawley 1983) but even abundant nitrogen is only poorly used by animals if available energy is lacking (Van Soest 1994). Plant materials are generally low in nitrogen for they are primarily made up of carbon as opposed to animals which are primarily made up of protein. Consequently, nitrogen is often considered limiting in the diet of herbivores (Mattson 1980; Crawley 1983; Van Soest 1994). Some nitrogen is also bound in the cell wall (Van Soest 1994).

Other factors affect the nutritive value of a particular food species. These include the digestive (structure and function) capability of animals to process a particular food species and the accessibility of the nutrients within such food species. For example, some animals such as hindgut fermenters (e.g. dugongs and green turtles) maximise their intake of digestible nutrients on poor quality diets (Bjorndal 1979, 1980a; Lanyon and Marsh 1995b), and some chemicals like tannins can combine with other nutrients reducing their digestibility or availability (Mould and Robbins

1982). Limited studies have examined the nutrient contents of seagrasses, particularly from a megaherbivore's perspective (Bjorndal 1980; Lanyon 1991).

2.3.2. Nutritional attributes of seagrasses as food for herbivores

From the herbivores' perspective, the nutritional quality of plants (including seagrasses) depends on their nutrient content and the ability of an animal to extract those nutrients. The nutrient content of a plant is based on the concentrations of nutrients *per se* (e.g. nitrogen, starch, water soluble carbohydrates) or anti-nutrients (fibre, phenolics). The level of food intake and the ability of an animal to extract those nutrients determines the nutritional quality of the plant.

The level of food intake is difficult to measure for most wildlife species. In most herbivores, digestibility is measured by *in vitro* or *in vivo* techniques, where it is usually expressed as the percentage of a component that disappeared from the initial materials (see Van Soest 1994; Choo *et al.* 1982). Studies on seagrass-herbivore interactions have focused on nutrient content (Bjorndal 1980; Lanyon 1991) but no study has examined nutritional quality. The determination of nutritional quality is so important to animal nutrition that digestion trials are now standard in livestock production studies (see Van Soest 1994). However, attempts to measure the nutritional quality of seagrasses or the relevance and applicability of nutritional quality for marine megaherbivores are still lacking.

The calorific and nutritional contents of seagrasses are usually considered to be lower than those of most terrestrial grasses (Birch 1975; Bjorndal 1980; Lanyon 1991). However, low calorific content does not necessarily mean low nutritional quality. Terrestrial grasses have high calorific content (gross energy) but this is bound in fibre and lignin, and, therefore, has low digestible energy (Van Soest 1994). Murray *et al.* (1977) suggest that seagrasses have high digestibility (~84%). This is because aquatic plants in general, predictably have less lignin because they require fewer (cell wall) structural components as a consequence of living in an aquatic environment. Wake (1975), Murray *et al.* (1977), Dawes (1979, 1980),

Best (1981), Zieman *et al.* (1984) and Lanyon (1991) noted that several tropical seagrasses have nutrient contents which are comparable to terrestrial vascular plants. Several studies have examined the nutrient contents of seagrasses. However, most of these studies have been directed towards resource availability and growth constraints (e.g. Short 1987; Zimmerman *et al.* 1987; Duarte 1990; Perez *et al.* 1994), phylogeny and biochemistry (e.g. McMillan 1983, 1984, 1986; Masini 1982; Gillan *et al.* 1984; Waldron *et al.* 1989). Comparison of data from different authors is often difficult because of variations in the analytical methods employed, inconsistencies in the plant part used (whole or separate roots, rhizomes, and leaves), and low replication preventing calculation of variability (*sensu* Dawes and Lawrence 1983).

Lanyon's (1991) work on the nutritional attributes of some tropical seagrasses is one of the most comprehensive accounts. Lanyon reported temporal differences in the nutrient contents of some tropical seagrass species. She found that *Halophila ovalis* and *Halodule uninervis* (narrow-leaf variety) were lowest in fibre concentration and highest in nitrogen concentration, and that *Zostera capricorni* has significantly higher concentrations of fibre than any of the four species she worked with (*Cymodocea serrulata* was the fourth species). In addition, Lanyon also found that the leaf and root/rhizome fractions of the plants were usually chemically distinct, the leaf fraction containing higher levels of fibre and nitrogen while the root/rhizome fraction had higher levels of soluble carbohydrates and phenolics (when they occur).

Seagrass nutrients can also be influenced by other factors such as: variety (leaf morphology), location (i.e. region, locality and depth), sediment types (nutrient availability) and age. The narrow-leaf variety of *H. uninervis* generally had a higher concentration of nitrogen and lower concentration of fibre than the wide-leaf variety (Lanyon 1991). Unfortunately, Lanyon was not able to examine whether there was any chemical difference between the two varieties of *Z. capricorni*. Preen (1993) claimed that dugongs in Moreton Bay mainly avoided the wide-leaf variety and speculated that the narrow-variety must be nutritionally superior to the

wide-variety. Preen, who worked on a subtropical system, used the seagrass nutritional data of Lanyon (1991), which dealt with samples collected only from the tropics. There may be a considerable difference between these regions, as seagrasses in the subtropics experience more defined seasons. Additionally, the variation in nutrient content of seagrasses from different regions or locations is probably confounded by intrinsic spatio-temporal differences in nutrient availability, which are usually a function of sediment type, water quality and season (see Moriarty and Boon 1989; Erftemeijer and Middelburg 1993; Erftemeijer *et al.* 1994). The age of food plants, particularly that of the leaves, has been suggested to be important to herbivores because young leaves have more nutrients (Coley 1980; Mooney and Gulmon 1982; Crawley 1989a; Harper 1989). In marine systems, it has been reported that green turtles prefer the younger seagrass leaves (regrowth) which presumably have higher nitrogen contents and lower fibre (Bjorndal 1980; Ogden *et al.* 1983; Zieman *et al.* 1984). Unfortunately, Lanyon (1991) lacked enough replication in her samples for comparisons within, and between sites and species. There is a need to address these deficiencies in order to better understand the interactions between megaherbivores and the seagrass ecosystem.

2.3.3. Strategies on how herbivores extract nutrients in plants

Nutritional variation in plant quality has important implications for the distribution of herbivores because the availability of both quality and quantity of food affects animal survivorship (Crawley 1983; Jarman 1993). This is probably more critical for sessile herbivores than for mobile species. Most megaherbivores are mobile and capable of travelling considerable distances (e.g. African elephants, dugongs and green turtles). There are far fewer sessile herbivores than mobile ones. Most importantly, the spatial scale of the distribution of herbivores is smaller than the spatial scale of the variability in the distribution of plants (Crawley 1983; Jarman 1993). However, it is not clear whether mobile marine megaherbivores merely track changes in seagrass abundance and quality within their home ranges, emigrating to other sites as food supplies decline, or whether they have preferred

sites which could have been maintained through changes brought about by their grazing activities.

Jarman (1993) summarised the ways in which terrestrial mammalian herbivores can mitigate spatio-temporal variability in their food supply (see Table 7.4, p 117): (1) mobility; (2) manipulation of plant community by making it more homogeneous; (3) timing of life history events (e.g. parturition, lactation) to match availability; (4) storage of metabolites (laying down reserves of fats); and (5) manipulation of food plants to prolong productive growth. Studies of the interactions between megaherbivores and seagrass in such herbivore-plant systems should also consider those factors, particularly the possibility that these herbivores manipulate their plant communities.

As mentioned earlier, nitrogen is limiting for most herbivores as plant materials are generally low in nitrogen. Mattson (1980) suggests seven possible mechanisms by which herbivores may cope with an inadequate nitrogen supply. The first mechanism is by regulation of plant chemistry. Nitrogen concentration in plant tissues is usually increased by all levels of simulated or real herbivory (McNaughton 1979a, 1979b, 1984). As the seagrass megaherbivores are believed to feed on low-quality food (Birch 1975; Bjorndal 1980; Lanyon 1991), improving its nutritional attributes by grazing (and re-grazing) on preferred feeding sites may address that problem. The other mechanisms which Mattson suggests are: (1) increased consumption rates; (2) prolonged periods of feeding, digestion, and development; (3) specialized digestive systems that rely on endosymbionts and/or ectosymbionts; (4) occasional carnivory; (5) switching among plant parts and plant species; and (6) evolution of larger body size, which provides mechanical, thermoregulatory, hydroregulatory, and energetic advantages.

2.4. THE MARINE MEGAHERBIVORES

2.4.2. The main groups

Two groups of large herbivores dominate tropical marine ecosystems: three species of sirenian or sea cow and the one species of sea turtle. Of the four modern species of sirenians (three manatees and one species of dugong; Domning 1976; Savage 1977; Marsh *et al.* 1986; Marsh and Lefebvre 1994), only the dugong (*Dugong dugon*) is strictly marine (Domning 1976; Reynolds and Odell 1991; Marsh and Lefebvre 1994). The West Indian manatee, *Trichechus manatus*, and the West African manatee, *Trichechus senegalensis,* are both riverine and marine, and eat a wide variety of terrestrial, freshwater, and marine vegetation (Lefebvre *et al.* 1989; Reynolds and Odell 1991; Marsh and Lefebvre 1994). A third manatee species, the Amazonian manatee, *Trichechus inunguis*, is strictly riverine (freshwater), being confined to the Amazon River Basin (Reynolds and Odell 1991). However, seagrass is an important component of the food of *T. manatus* (Campbell and Irvine 1977; Etheridge *et al.* 1985) and perhaps, *T. senegalensis* (Reynolds and Odell 1991). The green turtle, *Chelonia mydas* is the only strictly herbivorous marine turtle (Thompson 1980; Mortimer 1982a; Bjorndal 1985). It can be found at large population densities in seagrass habitats and is known to eat considerable amounts of seagrasses (Ogden 1980; Bjorndal 1982b; Garnett *et al.* 1985; Lanyon *et al.* 1989; Marsh *et al.* 1995a). Another sea turtle, the hawksbill, *Eretmochelys imbricata,* is known to ingest seagrass occasionally but is usually associated with coral reefs (Lanyon *et al.* 1989) and is essentially carnivorous (Mortimer 1982a).

2.4.2. The dugong

2.4.2.1. *Distribution and status*

The dugong's range extends throughout the tropical and subtropical coastal and island waters of the Indo-Pacific, between 27 ° N and 27 ° S (Nishiwaki and Marsh 1985; Marsh and Lefebvre 1994). However, quantitative assessments of the size of dugong populations are only available for the Arabian Gulf (Preen 1989) and Australia (e.g.

Marsh and Saalfeld 1989b, 1990; Bayliss and Freeland 1989; Marsh *et al.* 1990, 1991b, 1994; Marsh and Lawler 1992; Marsh *et al.* 1995a, 1995b). In Australia, it is estimated that there are about 80,000 dugongs (Marsh *et al.* 1995a, and see Fig 2.2). Only anecdotal information exists for most of the dugong's range, which is now believed to be represented only by relict populations (Marsh and Lefebvre 1994). For this reason, the dugong is listed as vulnerable to extinction in the *IUCN Red List of Threatened Animals* (IUCN 1990).

2.4.2.2. *Food, preferences and feeding physiology*

The dugong is the only large herbivore which is a seagrass specialist (Wake 1975; Marsh *et al.* 1978, 1982; Marsh 1991a; Reynolds and Odell 1991), taking significant amounts of algae only if seagrasses are scarce (Spain and Heinsohn 1973; Heinsohn and Spain 1974; Heinsohn 1981; Marsh *et al.* 1982). In some areas (e.g. Shark Bay, Moreton Bay), they may also intentionally take some invertebrates seasonally to augment their diet (Anderson 1989; Preen 1995b). Information on the food preferences of dugongs is limited. Based on nutritional composition of seagrasses recorded in the literature (mainly Lanyon 1991) and observations on dugong grazing in Moreton Bay, Preen (1993) ranked the dugong's species preference as follows: *H. ovalis* ≥ *H. uninervis* (narrow-leaf) > *H. spinulosa* ≥ *S. isoetifolium* > *Z. capricorni* (wide-leaf). Based on nutrient content, Lanyon (1991) suggested equivalent rankings although she was not able to examine *H. spinulosa* and *S. isoetifolium*. Marsh *et al.* (1982) reported that 95% of the stomach contents of 95 dugongs examined from the north Queensland area contained *Halodule*, 89% contained *Halophila*, 61% contained *Cymodocea*, and 39% contained *Thalassia*. They also reported large quantities of rhizomes of *Halodule* and *Halophila* in the stomach contents. Aragones (1994) reported that dugongs in the Philippines fed on *Halophila ovalis*, *Halodule uninervis, C. rotundata*, *C. serrulata*, and *Thalassia hemprichii* but apparently avoided *Enhalus acoroides*. Both Lanyon (1991) and Preen (1993) suggested, that if dugongs are to optimise feeding, they must select seagrass species that are high in nitrogen and low in fibre. In contrast, Anderson (1994) suggested that dugongs in Shark Bay, Western Australia, selectively feed

on supposed carbohydrate-rich rhizomes of *H. spinulosa*, to compensate for the supposedly higher costs of deepwater seagrass foraging. Similarly, de Iongh *et al.* (1995) suggested that dugongs in East Indonesia feed on *H. uninervis* because of the presence of high amounts of "total organic carbon" in its below-ground biomass. Additional nutritional data are needed to better understand the observed preference (Preen 1993) and deviations from this patterns seen in other areas (e.g. dugongs in the Gulf of Carpentaria feed extensively on leaves of *S. isoetifolium*, A.R. Preen 1995, pers. comm.).

Very little is known about the physiology of dugongs, particularly their nutrition and digestion (Murray *et al.* 1977; Murray 1981; Lanyon and Marsh 1995b). Only Murray *et al.* (1977) investigated the dugong's capability to digest different nutrients. They found high concentrations of short chain fatty acids (SCFA) in the caecum and large intestine (183 and 236 mM, respectively), suggestive of the importance of fermentative digestion of the cell wall constituents in the nutrition of the dugong. However, only one dugong was examined. Most research has been restricted to the anatomy and histology of the digestive tract (Osman-Hill 1945; Meinertz 1956; Kenchington 1972; Spain and Heinsohn 1975; Marsh *et al.* 1977; Lanyon 1991). In general, the importance of nutrients and their required concentration, the amount of food required; and the physiology of assimilation are still unknown. Nonetheless, the extensive information available from animals with similar or related digestive morphology and functions (e.g. manatees, horse) may help explain the nutritional ecology or, even be used for modelling the nutritional ecology of dugongs.

2.4.2.2. *Dugong grazing*

Dugong grazing disturbs the substrate by uprooting whole plants (Preen 1993, Perry manuscript). A variety of feeding trails produced by grazing dugongs has been described. A serpentine feeding trail is most common and characteristic of those described in Australia (e.g. Heinsohn and Birch 1972; Wake 1975; Heinsohn *et al.* 1977; Anderson and Birtles 1978; Anderson 1981; Preen 1993). Circular

patches have been observed in the Philippines (Aragones 1994) and northern Australia (Preen 1995, pers. comm.), and a form intermediate between circular and typical meandering trails has been reported from Kenya (Jarman 1966). The wide muzzles of dugongs, together with their habit of grazing along meandering feeding trails, may prevent them from selectively feeding on individual plants (Preen 1993, 1995a). This implies that dugongs must select feeding areas based on community (structure) characteristics, such as species composition and biomass or abundance rather than individual plant characteristics.

Preen (1993) reported that dugongs in subtropical Moreton Bay have significant impacts on the community structure of seagrasses. He postulated that dugongs in such a system undertake 'cultivation grazing', grazing in large herds at the same location for some time. The effect of such grazing is to prevent the expansion of *Z. capricorni* while increasing the abundance of *H. ovalis*. Preen also reported that despite the destructive nature of dugong gazing, recovery of the seagrasses was usually rapid (months). In the tropics, the importance of dugong-seagrass interactions is less obvious and has been little studied. This may partially be because the impacts are less obvious, because of the smaller group and/or they are more dispersed. Group sizes reported from aerial surveys of the tropics are variable (maximum herd size ranges from 4 (Townsville) to 182 (east Cape York); see Preen 1993 for a summary of all references on dugong aerial surveys until 1990, Table 7.5 p 290). In contrast, consistently large concentrations of up to several hundred dugongs have been seen by Preen (1993) and others in subtropical Moreton Bay at all times of the year. This difference is one of grouping rather than abundance as there are many more dugongs in tropical areas, such as Torres Strait, than in subtropical areas such as Moreton Bay (see Fig 2.2, and Preen 1993 for summary of references, Table 7.5). However, the variable group sizes seen in the tropics could partially be an artefact of the aerial survey technique (survey height) used, time of year of surveys and concentration of aggregation. Because the observers are limited by the field of vision (strip transect) from low altitude aircraft, the distinction between small groups and diffuse herds of dugongs may not be obvious. Big herds (~150 - 200) of dugongs have recently been observed in the Gulf of Carpentaria and Shoalwater Bay

(Preen 1996, pers. comm.) and were reported by Marsh *et al.* (1980) within the Gulf of Carpentaria region (Wellesley Islands). Recent data suggest that dugong herds in the Gulf of Carpentaria disperse before and during the wet season (Preen, 1996 pers. comm.). Most surveys in the tropics have been conducted during the late dry season (see Preen 1993 for summary, Table 7.5), after herd structure has supposedly broken down.

There are differences and similarities in the ways manatees and dugongs feed on seagrasses. West Indian manatees can produce feeding scars which resemble a dugong's feeding trail (Provancha and Hall 1991) but tend to produce elliptical feeding patches of varying sizes (up to an average area of 27 m^2; Powell and Lefebvre 1990). Hartman (1979), and Provancha and Hall (1991) have reported that manatees crop only on the leaves of seagrasses and only seldom feed on rhizomes. This has been attributed to the angle of deflection of the manatees' rostrum being less than that in the dugong (Domning 1982), which is strongly deflected (Domning 1976) allowing the dugong to feed more easily on the sea bottom (Lanyon 1991). However, other authors (Packard 1981; Lefebvre and Powell 1990) have reported that most manatees also graze by uprooting plants. Similarly, dugongs have been reported to vary their foraging methods depending on the food species available (Anderson 1981; Preen 1993). Some undertake surface grazing (cropping only). This is probably indicative of plant abundance (biomass), height, plant morphology, and substrate (Anderson 1981; A.R. Preen 1995, pers. comm.; pers. observ.) or even nutrient status. Dugongs also tend not to take the roots/rhizomes of *Enhalus*, *Cymodocea*, and *Thalassia* (Marsh *et al.* 1982; Erftemeijer *et al.* 1993). This may, however, be more of an artefact of the morphology of the species and substrate. *Enhalus, Cymodocea* and *Thalassia* have well developed root systems usually forming mattress-like root/rhizome systems (Brouns 1985a, 1987b; Brouns and Heijs 1986) which, together with the type of substrate, may impede the capacity of the dugong to take their roots/rhizomes.

Manatee grazing, like dugong grazing, seems to have considerable impacts on the community structures of seagrasses. Lefebvre *et al.* (1995) postulated that manatee

grazing may help to maintain mixed-species seagrass beds. This may also be the case for dugong grazing.

2.4.3. The green turtle

2.4.3.1. *Distribution and status*

The green turtle's range extends throughout the tropical and sub-tropical seas of the Pacific, Indian and Atlantic Oceans (Bjorndal 1982a; Groomebridge and Luxmore 1989). Green turtle hatchlings spend their developmental stage in the open ocean, while the juvenile and adult turtles are mainly associated with coastal shallow waters (Limpus *et al.* 1984).

Migratory distances between nesting beaches and feeding areas vary and may exceed 3000 km (Bjorndal 1982b) or be as little as tens of kilometres (Limpus *et al.* 1992). In eastern Australia, green turtles have shown high fidelity in their choice of both feeding and nesting areas (Limpus *et al.* 1992). The absolute size estimate of some sea turtle breeding units in Australia is in the tens of thousands to hundreds of thousands (Marsh *et al.* 1995). Up to 11, 500 green turtles were recorded nesting at Raine Island, northern Australia on a single night in 1984 (C. Limpus, unpublished data cited in Marsh *et al.* 1995). IUCN (1990) lists the green turtle as an endangered species because of its excessive exploitation (for eggs, meat, and carapace) throughout its range, particularly in the East Pacific region, resulting in dramatic declines in numbers over the 1900s.

2.4.3.2. *Food, preferences and feeding physiology*

Green turtles are carnivorous during their oceanic (post-hatchling) stage (Hirth 1971, 1993; Limpus *et al.* 1984), and do not begin to enter benthic feeding areas and eat seagrasses until they are about a decade old (Mortimer 1981a, Bjorndal 1982b; Limpus and Reed 1985; Lanyon *et al.* 1989). The green turtle is a generalist feeder and eats both algae and seagrass during the post-oceanic stage of its life cycle (Bjorndal 1985, Garnett *et al.* 1985; Read 1991; Brand 1995). Green turtles eat different vegetation

types over their geographical range (Mortimer, 1981b; Garnett *et al.* 1985; Lanyon *et al.* 1989). This variation in feeding may be associated with the latitudinal distribution of algae and seagrasses along continental coasts and coral reefs (Garnett *et al.* 1985; Lanyon *et al.* 1989). For example, in the Galapagos Islands, green turtles feed mainly on green algae of the genera *Ulva* and *Caulerpa* (Prichard 1971). In the Ogasawara Islands, Japan, they also feed predominantly on algae (Bjorndal *et al.* 1991). In Mosquito Lagoon, Florida, they seem to graze exclusively on several species of seagrasses (Mendonca 1983). Likewise, in Nicaragua, they have been found frequently to ingest only seagrass (Mortimer 1981b).

In Australian waters, green turtles have also been observed to vary their diet (Garnett *et al.* 1985; Read 1991; Brand 1995). Green turtles feeding in seagrass beds in inshore bays and estuaries in Australia are thought to feed non-selectively on the seagrass genera available, including *Halophila, Halodule, Cymodocea, Thalassia* and *Thalassodendron* (Limpus *et al.* 1984; Lanyon *et al.* 1989). Green turtles feed mainly on algae on coral reefs in the southern Great Barrier Reef region (Limpus and Reed 1985a). In contrast, they feed almost exclusively on seagrass in some coastal areas (e.g. Shoalwater Bay) (Limpus and Reed 1985b). In Moreton Bay, juvenile green turtles ingest both algae and seagrasses (Read 1991; Brand 1995).

In other areas such as the Caribbean, green turtles feed very selectively on only one seagrass species, *Thalassia testudinum*, even though algae are also abundant in their feeding grounds (in Nicaragua, Mortimer 1981b; in the Bahamas, Bjorndal 1980; and St Croix, Virgin Islands, Ogden *et al.* 1983). In Moreton Bay, juvenile turtles seem to prefer to feed on the seagrasses *H. ovalis* and *H. uninervis*, and on the algae *Gracilaria spp.* (Read 1991; Brand 1995). In Mosquito Lagoon, Florida, green turtles have been reported to feed exclusively on the seagrasses *Syringodium filiforme, Halodule wrightii*, and *Halophila sp.* (Mendonca 1983). Green turtles in Torres Strait appear to prefer a wider range of food: five genera of algae (*Hypnea, Laurencia, Caulerpa, Vidalia*, and *Sargassum*) and one seagrass species (*Thalassia hemprichii*) (Garnett *et al.* 1985).

Similarly to dugongs, information about the physiology of green turtles is limited, particularly regarding nutrition and digestion (Bjorndal 1985; Bjorndal *et al.* 1991; Bjorndal, in press). One way of gaining a better understanding of the nutritional ecology of green turtles in some areas would be to examine the nutritional attributes of a range of seagrass species, and then relate those data to the nutrient composition of seagrass leaves and observed turtle grazing preferences.

2.4.3.3. *Impacts of green turtle cropping*

Unlike dugongs, green turtles, crop only leaves when feeding on seagrasses (Lanyon *et al.* 1989). Such foraging can significantly influence the physiology and community structure of seagrasses at a local scale (Bjorndal 1980; Kuiper-Linley 1994). In the Caribbean, green turtles are known to exhibit an unique feeding behaviour. They maintain 'grazing plots' (Bjorndal 1980), where individuals cut seagrass blades at the basal portion (which then floats away), subsequently returning to the same spot and maintaining these plots through regular recropping (Bjorndal 1980). By feeding on young *Thalassia testudinum* regrowth, the turtles increase the quality of their food, i.e. the young regrowth has higher nitrogen and lower fibre contents (Bjorndal 1980; Zieman *et al.* 1984; Bjorndal 1985). In Moreton Bay, simulated turtle cropping has shown that leaf biomass, leaf regrowth rates and water-soluble carbohydrate concentrations of the leaves of *H. ovalis*, *Z. capricorni*, and *C. serrulata* were increased by simulated turtle grazing (Kuiper-Linley 1994). Further, the leaf width of *Z. capricorni* and *C. serrulata* decreased following simulated turtle cropping (Kuiper-Linley 1994). However, Kuiper-Linley did not measure the concentrations of nitrogen or fibre, which are essential to gain a better understanding of the nutritional ecology of green turtles (*sensu* Bjorndal 1985). Turtle grazing, like dugong grazing, may also increase the rate of recycling of nutrients to the seagrass community by short-circuiting the detrital cycle (Thayer *et al.* 1982, 1984; Lanyon *et al.* 1989).

2.4.4. *Some comparisons between dugongs and green turtles*

Green turtles have a relatively low ingestion rate when compared to dugongs. When eating *Thalassia testudinum*, the average daily consumption of an adult green turtle (~ 66 kg) is equivalent to only 0.24-0.33% of its body weight, i.e. approximately 2.2 kg wet weight of seagrass/day (Bjorndal 1980). Green turtles are poikilotherms with low metabolic rates (Fenchel *et al.* 1979; Bjorndal 1980). An adult dugong's average daily consumption (assuming an average body weight of 350 kg) is equivalent to 8.1% of its body weight, i.e. approximately 28.5 kg wet weight of seagrasses/day (Preen, 1993). Preen's measurements of daily consumption rates were, however, indirect as they were based on the premise that he was able to account for all the dugongs grazing in the particular seagrass bed he was monitoring. Using the daily intake of about 20 kg wet weight of seagrasses/day (combined average from two immature dugongs held under captivity) (Lanyon and Marsh 1995b), the daily consumption rate of a dugong could also be as high as 15% of its body weight (based on a combined average body weight of 130.5 kg of the two immature dugongs). Aragones (1994) estimated the daily consumption of a solitary dugong to be about 30.5 kg wet weight of seagrasses/day by mapping feeding scars and estimating the biomass removed (by harvesting the adjacent ungrazed patch).

Both species, however, use the soluble components of their diet which they digest enzymatically (Marsh *et al.* 1978; Bjorndal 1979, 1982, 1985; Lanyon *et al.* 1989; Lanyon 1991). The teeth of dugongs may assist in mechanical breakdown (Lanyon *et al.* 1989; Lanyon 1991) but there is no mechanical breakdown of food by turtles except through the action of their muscular stomachs (Bjorndal 1985; Lanyon *et al.* 1989).

The relatively high population estimates of dugongs and green turtles in northern Australia and their estimated daily consumption rates, suggest that they have a huge, potential grazing impact. Although dugongs are more specialised seagrass feeders than green turtles, and an individual dugong also consumes 10 times more seagrass than an individual green turtle, there are many more green turtles than dugongs in Australia (H. Marsh 1995, pers. comm.). Limpus (1996, pers. comm.) estimates that there are more

than a million green turtles in northern Australia alone. I must emphasise though that this is only an estimate and that not all green turtle eat seagrasses. However, assuming that only 50% of the estimated number of green turtles (> 500,000) eat seagrasses, they could collectively consume 1,100 tonnes (wet weight) of seagrasses per day, which is about 401,500 tonnes per year. Similarly, 80,000 dugongs could collectively consume about 2,400 tonnes (wet weight) of seagrass per day, about 876,000 tonnes per year. Therefore, even though it is difficult to quantify the absolute impacts of dugong and green turtle grazing on seagrass communities, particularly in tropical Australia, it is apparent that it is potentially huge.

The grazing impact of these species is certainly a very significant plant-herbivore relationship in tropical seagrass ecosystems. A summary comparison between dugongs and green turtles as grazers in seagrass systems is shown in Table 2.1. Both species have been observed to maintain 'cultivated sites' (Bjorndal 1980; Preen 1993). However, no study has yet examined the effects of grazing by dugongs and green turtles in the same seagrass community. This is necessary in order to understand resource partitioning between these species.

Studies involving large herbivores such as dugongs and green turtles have emphasised the basic biology of these animals and more recently their feeding and nutritional ecology (i.e. how nutrition affects their biology and determines their interactions with the environment) (e.g. Heinsohn and Birch 1972; Wake 1975; Heinsohn *et al.* 1977; Mortimer 1982a, 1982b; Bjorndal 1980, 1985; Lanyon 1991; Preen 1993). However, the response of the seagrass community to herbivory has received little attention. The only investigations of megaherbivore-seagrass interactions are those of Preen (1993, 1995a) and Kuiper-Linley (1994), who both worked in a subtropical seagrass system (in Moreton Bay, 27° S latitude). Provancha and Hall (1991), and Lefebvre and her colleagues (1995), are examining the effects of grazing by manatees in the seagrass beds in east central Florida (28° N latitude). The only study in the tropics (de Iongh *et al.* 1995) examined the relationship between the pattern of grazing by dugongs and the distribution and seasonal biomass changes in an intertidal seagrass bed dominated by *Halodule uninervis*. de Iongh *et al.* (1995) found that the frequency

of dugong grazing was positively correlated with the total organic levels in the below-ground biomass, from which they were able to conclude that dugongs' preference for *H. uninervis* is based on a strategy involving a high net rate of energy intake. More information, including the effects of grazing on species composition on a mixed-species bed, amounts of detrital matter and primary productivity, is necessary to better understand the habitat requirements of these marine megaherbivores, their feeding ecology and ecological roles.

2.5. CONCLUSIONS

Tropical seagrass communities are different from temperate ones in many ways, particularly their support of megaherbivores. The dugong and green turtle, particularly in Australia, have a large impact on seagrasses as a consequence of their herbivory. Seagrass-megaherbivore interactions have received very little attention and have only recently been recognized as an important process in the seagrass ecosystem. In addition, information on the nutritional composition of seagrass relevant to herbivores, such as variation by species, variety, and location (e.g. depth) is limited. Many similar studies on plant-herbivore interactions in other systems have shown that such interactions contribute to the structuring of plant communities and their dynamics. Study of particular plant-herbivore system in the marine environment is, however, very different from other systems, as the investigator is constrained by accessibility of sites and the imposition of tidal cycles. Consequently, several innovations were necessary to examine these plant-herbivore interactions with statistical power. This will be discussed further in the following chapter.

Table 2.1. Comparison of the dugong and green turtle as grazers in the seagrass system (modified after Lanyon *et al.* 1989).

	Dugong	Green turtle
Age classes eating seagrasses	all	post oceanic phases only
Composition of diet	predominantly seagrass leaves and roots/rhizomes	algae and seagrass leaves only
Average ingestion rate (kg wet weight of seagrass/day)	30	2.2
Method of plant breakdown	mechanical (teeth) hindgut fermenter enzymatic digestion (small and large intestines) and microbial digestion (caecum and colon)	muscular stomach hindgut fermenter enzymatic digestion (small and large intestines) and microbial digestion (caecum and colon)
Animal-seagrass interaction	'cultivation grazing' in at least some areas; large quantities of whole plants removed; nutrients returned as faeces, excretion and decomposition	maintain 'grazing plots' in some areas; young leaves cropped; nutrients returned as faeces, excretion and decomposition, or exported from systems when adults migrate to breed

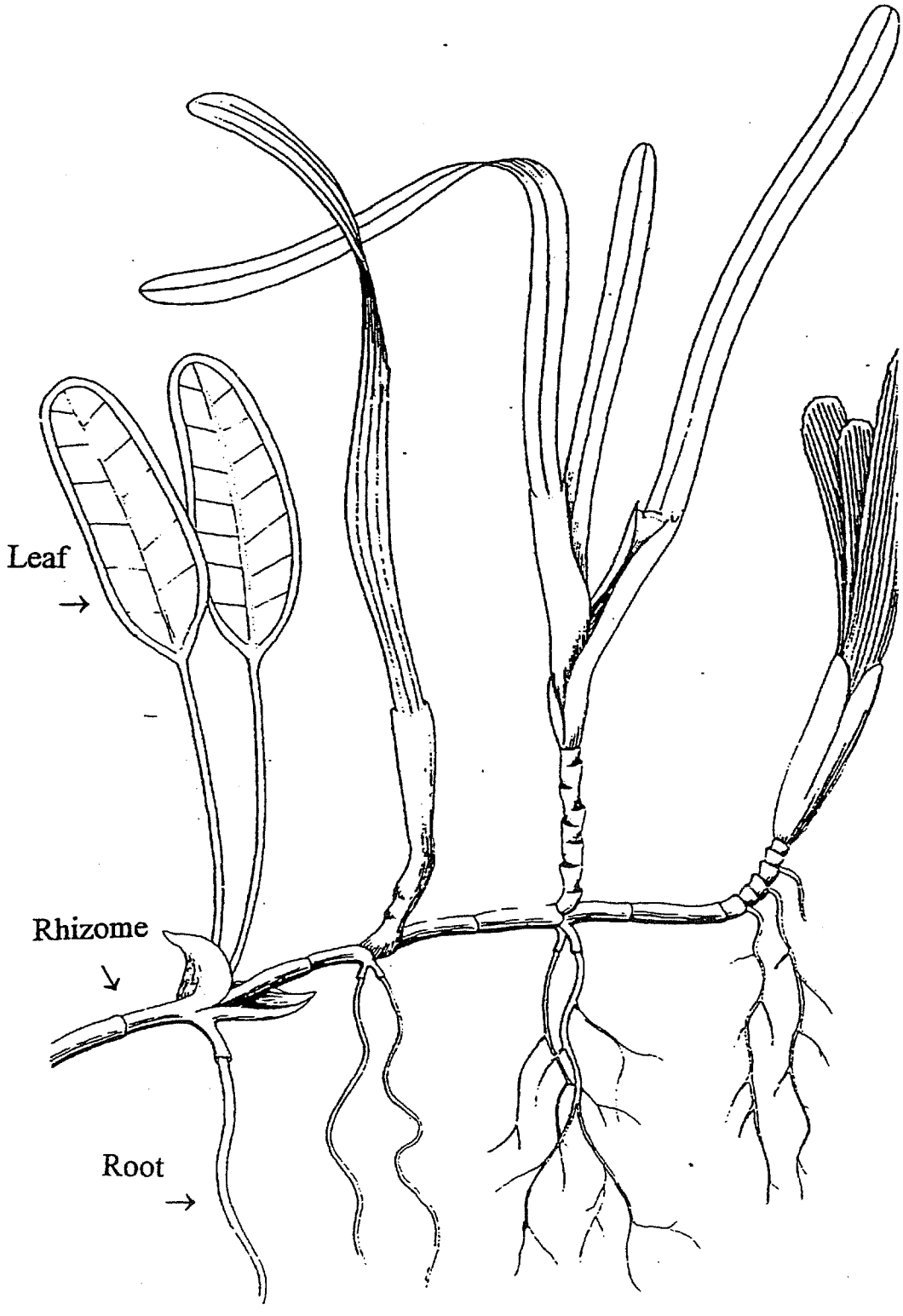

Fig. 2.1. A diagrammatic representation of a 'composite' seagrass showing its three modular features: leaf, root and rhizome (source: Lanyon 1986).

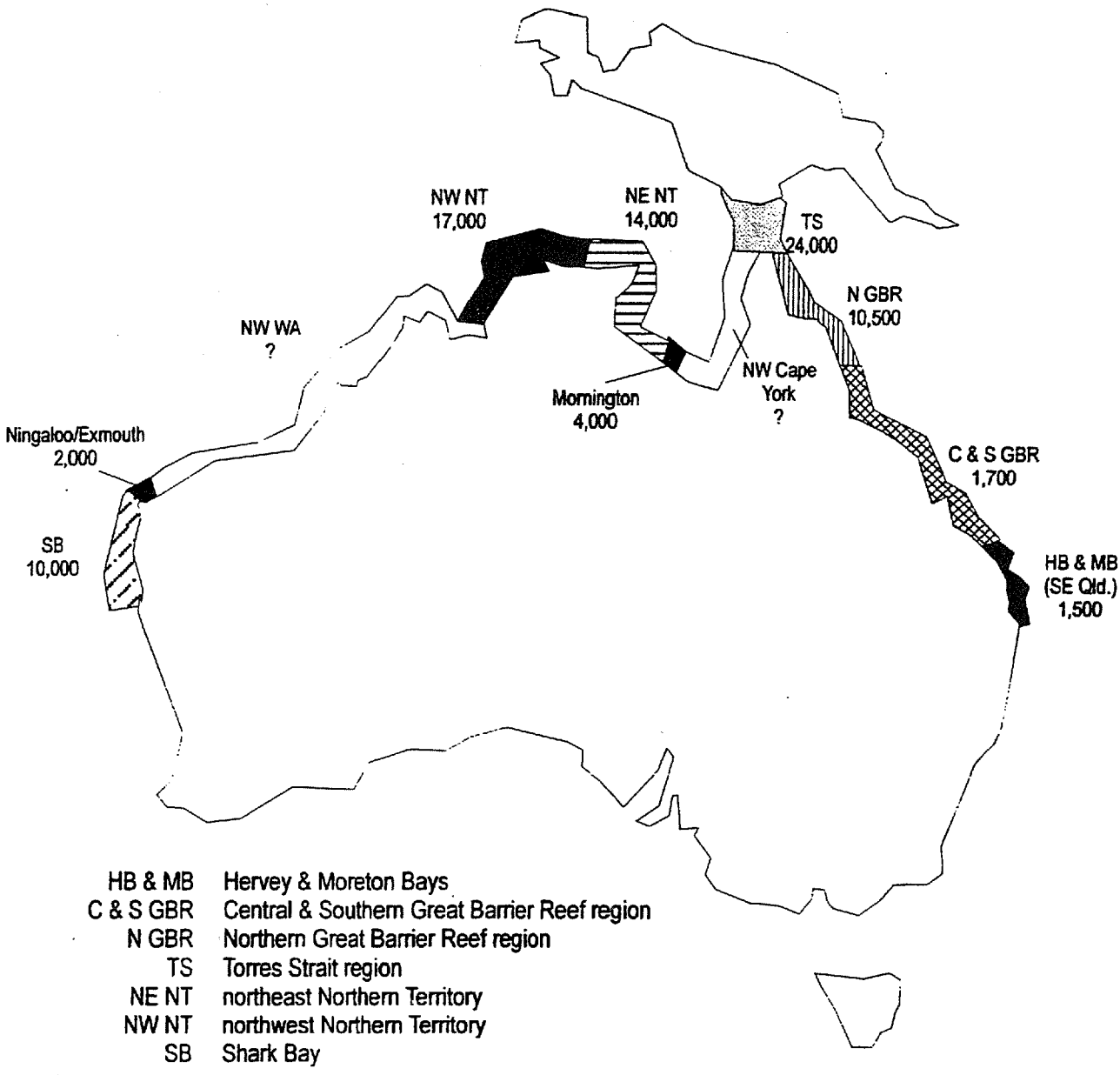

Fig. 2.2. Estimates of dugong populations along the Australian coastline (total ~ 80,000). The northwest Cape York Peninsula (MW Cape York) and northwest Western Australia (NW WA) regions have not yet been surveyed. (References: Marsh *et al.* 1995a, 1995b and references cited therein).

CHAPTER 3.

THE RATIONALE FOR MY STUDY APPROACH

3.1. INTRODUCTION

This chapter presents the rationale behind the approach and methods used in this research. More specific reasons for choosing a particular method are presented in each of the methods chapters (Chapters 4 and 6). I used video recording to monitor changes in seagrass biomass, and near infra-red reflectance spectroscopy to predict the nutrient contents of seagrasses. This enabled me to increase replications and consequently statistical power despite the limitations of my budget.

3.2. STATISTICAL POWER AND REPLICATION

Statistical power is widely recognised as important in ecological studies (Quinn and Dunham 1983; Andrew and Mapstone 1987; Eberhardt and Thomas 1991; Taylor and Gerrodette 1993). The power (1 - ß) of a test of a statistical hypothesis is the probability that the null hypothesis (H_o) will be rejected when it is false. To improve the power of a test, either the probability of Type I error, α (probability that the null hypothesis is true but is rejected), or the sample size must be increased (Sokal and Rohlf 1995; Zar 1996). As it is undesirable to increased the probability of Type I error, it is preferable to increase the sample size (Zar 1996). In order to determine if treatment effects are significant, they need to be replicated. These replicates should be statistically independent (to avoid pseudoreplication, see Hulbert 1984). However, these ideals are often difficult to achieve in field-based studies, due to limitations of personnel, time, and money.

To test the hypotheses outlined in Chapter 1, I had to overcome several problems. Firstly, although tropical Queensland, Australia has high densities of dugongs and green turtles (Section 2.5.2.4), poor visibility in the coastal waters prevents

underwater observations of grazing by these herbivores, and similarly makes working underwater to simulate grazing impacts difficult. Secondly, this region is notorious for harbouring dangerous animals such as crocodiles and sharks. These factors and the shyness of dugongs make direct observations of new feeding trails almost impossible especially in subtidal habitats.

3.3. APPROACH ADOPTED

I avoided these problems by performing simulation experiments instead of monitoring actual feeding trails, and by limiting my experiments to intertidal seagrass beds. The experimental plots simulating several grazing regimes, were left 'open' (non-enclosed). I conducted a comprehensive monitoring program for any natural grazing disturbances by dugongs (as cropping by turtles are very difficult to distinguish) on these plots (1 m^2). The following sections detail the advantages and disadvantages of the different potential approaches, and the main reasons why I adopted the particular methodologies.

3.3.1 Advantages of simulated grazing

Several methods are available for studying the effects of grazing by dugongs and green turtles on the community structure and nutritional attributes of seagrasses. These include monitoring the recovery of seagrasses from natural or simulated feeding events. The use of natural as opposed to simulated feeding events has advantages and disadvantages (see Appendix 1). Natural feeding trails are authentic but their age is difficult to estimate. In addition, the feeding trails of green turtles are usually less conspicuous (except in some areas in the Caribbean, see Bjorndal 1985) than those of dugongs, which typically leave serpentine scars on seagrass beds (e.g. Heinsohn *et al.* 1977; Anderson and Birtles 1978; Preen 1993). Simulations can be controlled and monitored more closely and the changes or response of the seagrasses can be followed through time from the initial simulation until complete recovery. Simulation also allows the treatment to be kept uniform and repeatable. For these reasons, I monitored the recovery of seagrasses from simulated feeding events. There is some precedence for

this approach. Preen (1993) monitored both natural and simulated feeding events for his study of dugongs and seagrass interactions at Moreton Bay. Kuiper-Linley (1994) used simulation to investigate the effects of the different frequencies of cropping by green turtles on seagrasses in Moreton Bay. I employed a simulation approach as it has been proven to work and to allow comparisons with these previous studies. A comparison of potential methods to investigate grazing by dugongs and cropping by green turtles on seagrass is summarised in Appendix 1.

3.3.2. Advantages of intertidal seagrasses

Although dugongs have been reported to graze in subtidal areas (Marsh 1989; Lee Long *et al.* 1989, 1995; Preen 1993; Anderson *et al.* 1994), they regularly graze in intertidal seagrasses as evidenced by the abundance of feeding trails seen in this area (Heinsohn 1972; Heinsohn and Birch 1972; Wake 1975; Heinsohn *et al.* 1977; Anderson and Birtles 1978; Heinsohn 1981; Preen 1993; Aragones 1994). In comparison with the intertidal area, environmental variables in subtidal areas are more uniform, however, this region is less accessible and has poor visibility. In contrast, even though the intertidal area is exposed to more variable environmental factors, it can be accessed on foot at low tides. I decided to work in the intertidal area primarily for reasons of access or to avoid the problems outlined above.

3.3.3. The utility of exclusion experiments

In terrestrial systems, hypotheses regarding effects of grazing and/or herbivore-plant interactions are traditionally tested using exclusion experiments (e.g. McNaughton 1979b; Rawes 1981; Watt 1981; Illius *et al.* 1992). In marine systems, exclusion experiments are usually carried out on a small scale due to the costs of the enclosures and impacts of the marine environment on the exclusion structures, such as fouling and corrosion. Most exclusion experiments in marine ecosystems have been used to test effects of grazing by microherbivores such as invertebrates (e.g. Underwood 1981; Underwood and Jernakoff 1981) and fish (Sammarco 1983; Kamura and Choonhabandit 1986; Hinds and Ballantine 1987). Exclusion experiments to test the

effects megaherbivores such as dugongs and green turtles need to be carried out at a larger scale (at least ~ 10 m^2). Preen (1993) used overlapping ropes over plots to discourage grazing by dugongs in his exclusion experiment. Lefebrve and her colleagues (1995) used large cages (~ 10 m^2) to exclude manatees from grazing on submerged aquatic vegetation at Florida (Lefebvre *et al.* 1989). I did not adopt this approach as it requires extensive replication to be statistically powerful. Apart from the different regimes of grazing by dugongs, the effects of the enclosure materials on seagrasses should be tested as well. Treatment is a fixed factor (with and without enclosures), which has to be randomly replicated at several sites (site within treatment), with the number of sites representing the error degrees of freedom for the test. Thus, in order to be powerful enough to be meaningful, exclusion experiments on dugongs and green turtles must be replicated at several sites.

The construction costs for each enclosure are high (> $ 100's) and considerable time and effort are required to build and maintain them. In addition, areas with a 3.0 m tidal range, such as in Central Section of the Great Barrier Reef (GBR) will require very high enclosures. In addition, the shading effects of the enclosures on the seagrasses and fouling can confound the results of such exclusion experiments. The use of enclosures is possible in Florida as the tidal range is small. However, Lefebvre and her colleagues were unable to build sufficient enclosures to test the effect of enclosures independently of the effect of treatment. In addition, their experiment was limited to one site only.

Monitoring 'open' experiments is cheaper because it requires less infrastructure, and is consequently easier to replicate. I chose this approach for my experiments, because the disadvantages of exclusion outweighed the advantages. Kuiper-Linley (1994) also adopted the 'open' experimental approach in her study. However, Kuiper-Linley's design was pseudoreplicated because she harvested on several occasions from the same plots, and hence, her replicates were not statistically independent (see Hulbert 1984).

CHAPTER 4.

USING VIDEO FOR MONITORING SEAGRASS BIOMASS: DEVELOPMENT, USE AND EVALUATION

4.1. INTRODUCTION

One of the major objectives of this study was to investigate the effects of grazing by dugongs and green turtles on the structure of tropical seagrass communities. In Chapter 3, I explained why simulation was the most appropriate technique for my purpose. This approach required monthly monitoring of the changes in several parameters such as species abundance (leaf biomass) and composition, as seagrasses recovered from the simulated grazing events. A monthly monitoring program was required to observe the effects of the different treatment levels and other disturbances such as natural grazing.

A range of visual methods for monitoring the community composition and abundance of tropical seagrass meadows is available. Mellors (1991) developed a visual estimation technique for seagrasses by adapting the BOTANAL procedure (Tothill *et al.* 1978) which estimates pasture yield and composition in the field. Mellors' technique allows the investigator to estimate the above-ground biomass of seagrasses in a given area visually, using calibration and validation quadrats in which the corresponding dry weights of seagrass are measured directly by harvesting. Photo-quadrat analysis (Lanyon 1991; Lanyon and Marsh 1995) is an adaptation of point-quadrat estimation (Foster *et al.* 1991) to seagrasses. This method, which combines the use of photography and modified point quadrat techniques, is also calibrated in terms of the dry weight of harvested quadrats adjacent to those being monitored on each occasion. Counting shoots (Dennison 1990) in a given area is a traditional technique for monitoring seagrass above-ground biomass abundance in the field (e.g. Poiner 1984; Preen 1993). Most if not all of these methods (Poiner 1984; Dennison 1990; Preen 1993) require considerable time and effort in the field. Hence, I used a combination of the first two techniques.

I modified Mellors (1991) technique, by using video images to track changes in the above-ground biomass (as described in Section 4.2.3). I chose the video technique rather than photography to record seagrass plots as it is more convenient, cheaper (no film and print processing required), reliable, fast and practical, and allowed me to check the images in the field. I did not use direct visual estimation because of the limited window of time available at low tide. It would be almost impossible for one person to estimate the seagrass biomass in 64 x 1 m^2 or 144 x 0.4356 m^2 plots in less than three hours. I rejected the option of employing several observers as this would have been expensive and introduce inter-observer variability. In addition, such an approach would necessitate harvesting additional samples as each observer requires a calibration set to account for individual scaling and biases. Video recording took all the time available between tides but allowed me to cope with the replication required at the plot level.

Methods for estimating seagrass biomass are becoming less intrusive. For example, Long *et al.* (1994) recently estimated seagrass biomass in Moreton Bay (see Fig 5.1) at a broadscale. They applied a combination of Geographic Information System (GIS) and Computer Aided Drafting (CAD) for sample design, a Global Positioning System (GPS) for locating sample sites in the field and a grab for taking samples. This technique is more appropriate for regional rather than local-scale monitoring because, it has limited capacity to detect small changes. Broad-scale monitoring can only pick large changes and hence will not be able to detect chronic declines in seagrass.

This section describes and evaluates the methodology employed to monitor the seagrasses in the grazing experiments (see Chapter 5).

4.2. MATERIALS AND METHODS

4.2.1. Study sites

The experiments referred to in the following sections were carried out in the intertidal seagrass beds at Ellie Point (along the Cairns Harbour area, 16 ° 53 ' S, 145 ° 46 ' E), and Cardwell (18 ° 14 ' S, 146 ° E) in tropical north Queensland, Australia . These study sites are described in detail in Section 5.2.1.

4.2.2. Field of vision for video monitoring

I used 1 m^2 and 0.4356 m^2 (0.66 x 0.66 m) quadrats (plots) for various experiments. Although these quadrat size were smaller than the areal extent of the grazing impacts of a single dugong or green turtle, their size allowed appropriate replication. Each quadrat was systematically divided into 0.33 x 0.33 m (= 0.11 m^2) subsquares using colour-coded strings (see Fig. 4.1) to allow resolution of the details of the seagrass patch in view, without losing valuable information (species composition and above-ground biomass). This size was chosen after analyses of the resolution of different sizes of subsquares. Sequential numbers were assigned to the subsquares of the quadrats to ensure systematic video recording (see Fig 4.1).

I used a handheld Sony video 8 (Handycam, CCD- TR305E) camera and Sony video tapes (8 mm, MP 120). A splash-proof Sony video casing (SPK-TRX2) was used to protect the equipment from seawater. A polarizing lens filter (Marum) was installed to minimise the effects of bright light. During December and January, when the low tides occur at night, spotlights were used to highlight the visual field during video recording.

4.2.3. Estimation of seagrass above-ground biomass via video recorded images

4.2.3.1. Pilot study: Development of calibration set and the scoring scale

Calibration set

Quadrats of *H. ovalis*, *Z. capricorni*, and *H. uninervis* at Ellie Point, and *H. uninervis* at Cardwell were filmed using a video. A wide range of above-ground biomasses spanning the upper and lower limits encountered for each species at each site were filmed. Some subquadrats were also photographed (Nikonos IV-A) using Kodakchrome films. The corresponding above-ground (leaf) biomass dry weight (= grams dry weight, g dw) of each image was determined directly by harvesting the seagrass biomass of each subquadrat immediately after filming.

Development of an appropriate scoring scale and regression analysis of the calibration set

A score for each dry-weight category (in half integers, to reduce the range of numbers in the scoring scale) was assigned to each video image. A regression between dry-weights (x-axis) and the equivalent scores (y-axis) was carried out for each species, for each site, following Mellors (1991). The scoring scale was designed so that the relationship between score and above-ground biomass (in dry weight) was linear.

After plotting the above-ground dry-weight values and their equivalent scores, I determined my discrimination threshold, i.e. the smallest dry-weight range that I could distinguish on the basis of video images. It was 0.20 to 0.40 g dw/0.11 m^2 independent of species. I then standardised the scoring scale for each species using a discrimination threshold of 0.40 g dw/0.11 m^2 as the basis of the dry-weight range interval for the scoring scale as follows:

score	dry-weight (g)
0.00	< 0.01
0.50	0.01 < 0.40
1.00	0.40 < 0.80
1.50	0.80 < 1.20
2.00	1.20 < 1.60
2.50	1.60 < 2.00
etc.	

The use of a uniform scale for all species facilitated parametric statistical comparisons among species.

Any missing values on the scoring scales were defined by purposive sampling, i.e. selecting images estimated to represent these missing scores and recording them in video, and then harvesting them. Similarly, the range of the scoring scale for each species was extended as necessary when an estimated biomass was outside the existing range. The regressions between dry-weights (x-axis) and scores (y-axis) were recalculated to incorporate the additional samples. The final equation thus defined the conversion of scores to biomass. The highest score assigned in the estimation for the whole study was 15 (for *Zostera capricorni* and *H. ovalis*).

4.2.3.2. Application and expansion of scoring scale

Viewing and estimating the seagrass biomass via the video recorded images
The video tapes containing the images of the seagrass plots from the monthly monitoring (see Section 5.2.3.3) were viewed and enhanced using a computer connected to a framegrabber (VideoVue Image Capture, Video Associates Labs Inc 1993). Each image was previewed (through a video preview windows), frozen and then captured using the VideoVue Windows-driven functions. Some images were enhanced with the Paint Shop Pro Software (e.g. apply filter or sharpen image) (JASC Inc 1995). The leaf biomass of each species in each image was visually estimated by assigning a score (based on the scale described in Section 4.2.2.1). The set of images for each

species, for each site compiled for the scoring scales were used as 'reference/calibration photos' to ensure consistency of scoring. The estimated above-ground biomass of each plot was calculated as the sum of the corresponding subsquares (nine for 1.0 m^2 and four for the 0.4356 m^2 plots).

4.2.4. Monitoring leaf biomass

The scoring scales were used to estimate monthly changes in the above-ground biomass of the different seagrass species in control and experimental plots at Ellie Point and Cardwell, through video images as described in Section 5.2.4.2. The estimation for *Cymodocea rotundata* at Ellie Point, was based on the scoring scale developed for *Z. capricorni* as the two species are morphologically similar.

4.2.5. Collection of samples

Seagrass samples from the experiments were harvested at the end of the monitoring periods at each site (see Table 5.2, for dates) to determine total biomass (Chapter 5) and nutritional attributes (Chapter 6), and to assess the accuracy[1] (= reliability) of my visual estimates of the above-ground biomass from video recorded images.

Two to three subsquares were randomly subsampled from each quadrat and harvested. It would have been logistically impossible, and inappropriate from a conservation perspective to harvest entire quadrats. The latter would have involved harvesting and sorting the seagrass from approximately 300 quadrats (130 x 1 m^2 and 170 x 0.4356 m^2).

The seagrass within each 0.11 m^2 sub-quadrat was removed with a hand trowel to a depth of approximately 8 to 10 cm. Each sample was placed in a mesh collection bag. After removing the sediments by gentle washing with seawater, each sample was placed in separate clickseal plastic containers and labelled. Additional samples were harvested whenever the biomass of the seagrass collected for a particular quadrat was very low.

[1] Accuracy is the closeness of a measured or computed value to its true value.

4.2.6. Processing of samples

The samples collected were washed using filtered seawater and sorted by hand according to species (following Den Hartog 1970; Lanyon 1986; and Kuo and McComb 1989), into leaf (above-ground) and root/rhizome (below-ground) fractions. Detritus was separated from the samples collected from Ellie Point. Most of the detrital matter resulted from robust and taller species shedding their leaf sheaths. Due to limited time, not all of the subsamples harvested for each quadrat were sorted unless the total biomass was low. Separated seagrass samples were placed in labelled paper bags, and air-dried to constant weight in a ventilated oven at 60 ° C (to minimise loss of volatiles) for at least 48 to 72 hours. Biomass was recorded as dry-weight (dw) of herbage in milligrams (g) per 0.11 m^2.

4.2.7. Assessment of score accuracy

All the samples collected from the final harvest (see Section 4.2.4.) were included in the assessment of the accuracy of the video estimates (scores) for each species (validation set). For comparison with predicted scores, the biomass recorded for the various samples collected for each species was categorised into their corresponding weight ranges and converted into actual scores. A linear regression through the origin between my visually-estimated predicted scores (x-axis) versus actual scores (y-axis) was performed for each species present at each study site and all species combined (for Ellie Point only).

4.2.8. Conversion of scores into biomass

After verifying that my scores were reliable and suitable for predicting above-ground biomass, the final above-ground biomass scores for each plot (sum of the nine subsquares for 1 m^2 and four for 0.4356 m^2) from the experiments were converted into above-ground dry weights using the linear relationship of the calibration set:

$$y = bx \qquad \textbf{(Equation I)}$$

Where **y** is the equivalent above-ground biomass (g dw); **x** is the score; and **b** is the slope. The relationship was forced through the origin because the scale included zero. The median values of the dry-weight range (0.20 intervals) for each score was used in the regression. The slope of the equation between the known weights and their corresponding scores was 0.4. For the 0.4356 m^2 plots, each biomass was multiplied by 2.2956841 to convert it to a metre-square equivalent.

The below-ground (root/rhizome) biomass was calculated after defining its relationship (ratio) with the above-ground (leaf) biomass. However, because below-ground biomass of tropical seagrasses varies temporally (Lanyon and Marsh 1995), it was only possible to calculate the root/rhizome biomass from the leaf scores for the final harvest (at the end of the experiments). The mean above- and below-ground ratio of two (sometimes three) subsamples was used to convert the above-ground biomass scores of each plot into the corresponding below-ground biomass.

4.3. RESULTS AND DISCUSSION

VISUAL ESTIMATION OF SEAGRASS BIOMASS VIA VIDEO IMAGES

4.3.1. Accuracy and precision[2]

The relationships between the actual scores and the visually estimated leaf biomass scores for the various species for Ellie Point and Cardwell were generally excellent, with r^2 always greater than 0.90 (see Figs 4.3a - d to 4.5a - b). The slopes of the regression lines were always close to 1 and had small standard errors (see Table 4.1). The 95% CI of the slope excluded one for only one result which was driven by the leverage of a single point (see Fig 4.5b). The 95% CI only narrowly excluded 1, and so the scores from this experiment were used without corrections. The precision (s.e. slope/slope) of the predicted scores was also good (see Table 4.1).

[2] Precision is the closeness of repeated measurements to the same quantity.

My scoring scheme accounted for the inherent variation in morphology, which prevails in multispecies seagrass meadows. The biomass of each of the morphologically distinct species was estimated separately. Taller seagrasses, like *Zostera* or *Cymodocea* have a higher biomass per unit area than smaller species like *H. ovalis*. The consistency of the scoring process was ensured by using a set of pictures/images with known weights as 'reference photos' for each separate species and for each site. In Mellors' original work (1991), an observer ranks or scores the standing crop in each quadrat (correct to one decimal place) according to a pre-determined scale. Morphological differences between seagrasses (mixed-species bed) are difficult to account for using her technique. This may have been one of the main reasons why the r^2 values (0.65 to 0.96; see Figs 4.3 and 4.4 for comparison with my results) using her technique tend to be lower than mine. Furthermore, her technique was also compromised by using different observers, a disadvantage which I overcome by using video.

Nonetheless, Mellors' technique (1991) has several advantages over mine. Her results were soon available after each field trip, while I had to process video images in the laboratory. In addition, Mellors' was able to validate the reliability of the visual estimates for each sampling occasion by harvesting and sorting at least 10 calibration quadrats. In contrast, I my scores were assessed against the same 'reference photos' throughout the study.

Whereas my method used a pre-determined scale (the intervals of which were determined by the discrimination threshold) Mellors used a continuous scale (based on current biomass range). The development of a uniform scoring scale for all species and the determination of a discrimination threshold are actually the most crucial aspects of this method as everything depended on the robustness of the scores. The selection of the smaller sampling unit (0.11 m^2) was also critical in the efficiency of this method.

The precision obtained using my technique (see Table 4.1) was comparable to that of Mellors' (1991) monthly sampling census (0.05 to 0.13). Both were within the

recommended target range of 0.10 to 0.20 level for field programs (Thresher and Gunn 1986). The precision of my technique for estimating leaf biomass is also very high (see Section 4.3.1), rivalling that of the harvesting technique (Poiner 1984; Downing and Anderson 1985).

There is species bias in estimating the dry weights of a mixed-species bed since not every species is equally available to the observer. The trends in my scoring suggests that I may have underestimated the scores of smaller species when biomass was high and overestimated them when biomass was low. This is mainly due to the 'canopy' effect which occurs when smaller and less conspicuous species like *H. ovalis* and *H. uninervis* are interspersed with *Zostera/Cymodocea*, which are much taller and more conspicuous and tend to overshadow the smaller species. In addition, not all of the leaf fraction is above the ground. This is one of the reasons why most scores tended to be underestimated at the higher end of the scale, and overestimated at the lower end of the scale producing a systematic bias.

Mellors (1991) suggested that it is better to have more samples estimated with acceptably lower accuracy than a few samples measured exactly, provided that there is no systematic error. This is basically what my method achieved and was why I used the sum of the nine subsquares as representative dry weights rather than the means of the randomly sampled subsquares. This resulted in more accurate biomass estimates because tropical seagrasses vary from patchy to dense stands, a feature often neglected when monitoring patchy beds (Brouns 1987a).

4.3.2. Advantages of biomass in dry-weight

My method, like that of Mellors (1991), estimates biomass as dry weight rather than percent cover. Estimates of biomass in dry-weight are more ecologically and biologically relevant to productivity than those of the percentage cover (e.g. Foster *et al.* 1991; Tomasko *et al.* 1993; Lanyon and Marsh 1995) or relative ranking (Coles *et al.* 1985, 1987). In addition, with biomass expressed in dry weight,

comparisons with other studies are possible because drying to constant weight is a standard procedure (Dennison 1990).

4.3.3. Non-intrusive nature of the technique

There is a need to develop techniques which limit the destruction of seagrasses. Destructive harvesting may contribute to further declines in seagrass biomass especially in areas recovering from catastrophic disturbances such as cyclones. The estimation of leaf biomass via video recorded images is less destructive than most other methods used for monitoring biomass (e.g. Downing and Anderson 1985; Dennison 1987, 1990; Mellors 1991; Lanyon and Marsh 1995).

4.3.4. Practicality and cost-benefits of the video technique

For monitoring tropical seagrasses, the video recording technique rivals visual estimation and photo-quadrat analysis (see Table 4.2). Video recording technique incorporates the harvesting method efficiently. Field time and labour requirements are relatively low. The video and photography methods destroy less seagrass than direct harvesting or Mellors' (1991) visual estimation technique. The cost of the video is an initial disadvantage but this is quickly overcome in a long-term monitoring program (Table 4.3). The cost of the video camera (including all its accessories), framegrabber + software and the construction of the platform was about $ A 3,000. A computer is standard equipment in a laboratory. In comparison to field counting and harvesting, over 12 months of monitoring (with a high sampling intensity, e.g. see Section 5.2.3.), I estimate that an investigator will save at least $ A 33,600 based on a monthly saving of about $ 2,800 (see Table 4.3). Visual estimation using video images was also at least 35 times faster than the other field techniques. Allowing $15,000 for salaries for sorting the calibration/validation samples, the savings would still be about $18,600.

The video technique is also more cost-effective than photography. For example, in my grazing experiments, two sites, each with 64 x $1m^2$ plots divided into 9

subsquares (= 576 shots/site), require at least 32 rolls of 36-shot slide films. Given that, one will have to allot at least $ 832 monthly for a single shot per subsquare (since $ 26 is required to buy and process each roll of film in Australia). Using two shots per subsquare (in case something goes wrong with the first shot), the expenditure becomes $ 1,664 per month. This translates into an expenditure of close to $20,000 for 12-monthly monitoring, if the photographic technique is employed.

Estimation via video images is cheaper than the alternative techniques used to monitor seagrasses because: (1) it is not necessary to harvest a calibration set on each sampling occasion (c.f. Mellors 1991); (2) it does not use photographic prints; and (3) it avoids most of the hundreds of hours of laboratory work needed to sort harvested samples. Permanent records can also be kept and a close comparison of previous images with more recent ones is possible by using a set of 'reference' photos/images. It took only two to three hours to video 64 x 1 m^2 quadrats, a rate impossible to attain if one was to harvest. The scoring process was slow, particularly at the start of the project because, I kept on looking at the 'reference' photos. However, I eventually scored faster as I gained more experience (see Table 4.3).

Both video and photographic methods provide permanent records. These can be used for other purposes, e.g. future comparisons of the status of the seagrass beds of particular sites or even to look at the temporal changes among associated organisms like algae. This is the main advantage of my method over that of Mellors (1991) visual estimation technique because her visual estimates of seagrass biomass in the field are not accompanied by any visual proof.

The time required and labour intensity in the field are also relatively low in comparison to the rest of the methods. It requires less time in the field than the visual estimation and is less destructive as it does not require either destructive sampling (as in harvesting) or the sampling calibration set (10 samples for each sampling) harvested as required for Mellors' (1991) technique.

4.3.5. Problems with this technique

This method cannot estimate below-ground biomass of seagrasses. Extrapolating the below-ground biomass from the above-ground biomass is only possible if one harvests regularly as the ratio of above- and below-ground biomass varies seasonally (Lanyon and Marsh 1995; McKenzie 1995).

This technique will have limited application in subtidal seagrass beds which have murky or turbid waters because visibility (e.g. light requirements) for video recording may be impaired. Similarly, visual estimation from video recorded images may not work in temperate seagrass beds, where a canopy of tall species dominates.

Identification of morphologically similar species is also a problem. For example, young plants of *Z. capricorni* and *C. rotundata* can be very similar and difficult to differentiate. This was why *C. rotundata* was only identifiable in the latter stages of my study at Ellie Point. *C. rotundata* was very difficult to distinguish from *Z. capricorni* even in harvested samples. Lee Long (pers. comm.) believes that all the species from Ellie Point were *Z. capricorni*.

4.3.6. Recommendations for the further improvement of this technique

This technique could be improved further. In situations where species identification is difficult, a continuous program of harvesting seagrass samples (of various species and stages) outside the plots to confirm taxonomic identification is advisable. Similarly, knowledge of the inherent changes in the leaf morphologies or characteristics of certain species during their growth is ideal. This technique still has to be tested using several scorers (observers) at the same plots or images before it is adopted by research institutions which expect to have several people working on a project.

I recommend that the scorers of the video images in the computer laboratory are the same individuals that do the field work. Detailed knowledge of the field conditions at each study site is advisable because variations of substrate and species composition of the bed may affect the level of exposure of the above-ground biomass. Ignorance of these differences may result in systematic errors in the estimates.

This technique should be re-calibrated or validated over time, particularly if it is to be employed for long-term monitoring. In addition, the images should be estimated in batches, so as to reduce the possible effect of observer drift. Thus, whenever possible, the spatial and temporal scales of the sampling design, should be used to group the images, e.g. all the data for a site for a sampling occasion, should be estimated in a batch, before starting on another site or time.

This method is recommended for long term monitoring programs as it requires expensive equipment. It is recommended that every individual scorer (observer) develops his/her own calibration set, discrimination threshold (weight interval) and scoring scale if research personnel is limited. In such case, randomly selected images should be re-scored every now by the same observer to check repeatability of scale. Accuracy of the scale should also be simultaneously monitored by regularly harvesting 'scored' samples for calibration purposes. For monitoring programs which employ several people, it is advisable to optimise the scoring scale by developing appropriate discrimination thresholds which should be acceptable to all observers or developed by all potential observers. I had a very small discrimination threshold (0.4 g dw/0.11 m^2) as I was primarily interested in small changes. Few monitoring programs will require such fine discrimination.

Table 4.1. Validation statistics for the estimation using the video images technique for samples harvested for each species, for each site and precision of estimates (s.e. of slope/slope).

site	species	N	slope[1]	s.e. of slope[2]	95% CI of slope[3]	precision (s.e. slope/slope)
Ellie Point	*H. ovalis*	103	1.0145	0.0229	0.9689 - 1.0601	0.02
	Z. capricorni	80	1.0289	0.0281	0.9726 - 1.0852	0.03
	H. uninervis (n[4])	40	0.9585	0.0512	0.8546 - 1.0625	0.05
	C. rotundata	32	1.0366	0.0305	0.9744 - 1.0989	0.03
Cardwell	*H. uninervis* (n[4])					
	1st Experiment	120	0.9799	0.0137	0.8949 - 1.0528	0.01
	2nd Experiment	100	1.1349	0.0176	1.0995 - 1.1704[5]	0.02

[1] The slope of the regressions between the predicted and actual scores.
[2] Standard errors (s.e.) of the slopes of the regressions.
[3] The 95% confidence interval (CI) of the slopes of the regressions.
[4] Narrow-leaf variety.
[5] Excluded 1, however only slightly.

Table 4.2. A comparison of the different methods used to monitor tropical seagrass biomass over time. See text for discussion.

Data	Video estimation (this study)	Visual estimation (Mellors 1991)	Photo-quadrat (Lanyon 1991; Lanyon & Marsh 1995b)	Harvesting (Preen 1993)
Permanency of records	high	low	high	low
Type of information	leaf only	leaf only	leaf only	leaf and root + rhizome fractions
Resolution of information	high	high[2]	high	high
Labour intensity in the field	low	low	low	high
Time required in the field	low	medium	low	high
Labour in the laboratory	medium	low	medium	high
Time required in the laboratory	medium	low	medium	high
Destructiveness	low	medium	low	high
Expenditures	high[1]	low	high[3]	medium

[1]Video requires high initial investment.
[2] Precision and accuracy of the visual estimation may vary with observer.
[3] Expenditure is continuously incurred for processing the photos.

Table 4.3. A comparison between the costs of video and field counting/harvesting techniques for monitoring of tropical seagrass biomass once per month for 12 months at two sites with 16 by 1 m^2 plots (each plot with 9 subsamples).

Component	Video	Field Count/ Harvest	Savings (Field count/harvest) - (Video)
1) FIELD WORK Field time (days)	5	10	5
Maintenance costs (A $) (e.g. accommodation & food)	500	1,000	500
Labour costs (A $) (at $ 100/day)	500	1,000	500
2) LABORATORY WORK Time (Hrs)	24	144	120
Labour Costs (A $) (at $ 15/Hr)	360	2,160	1,800
Total savings per month = $ 2800 and was 35x faster			

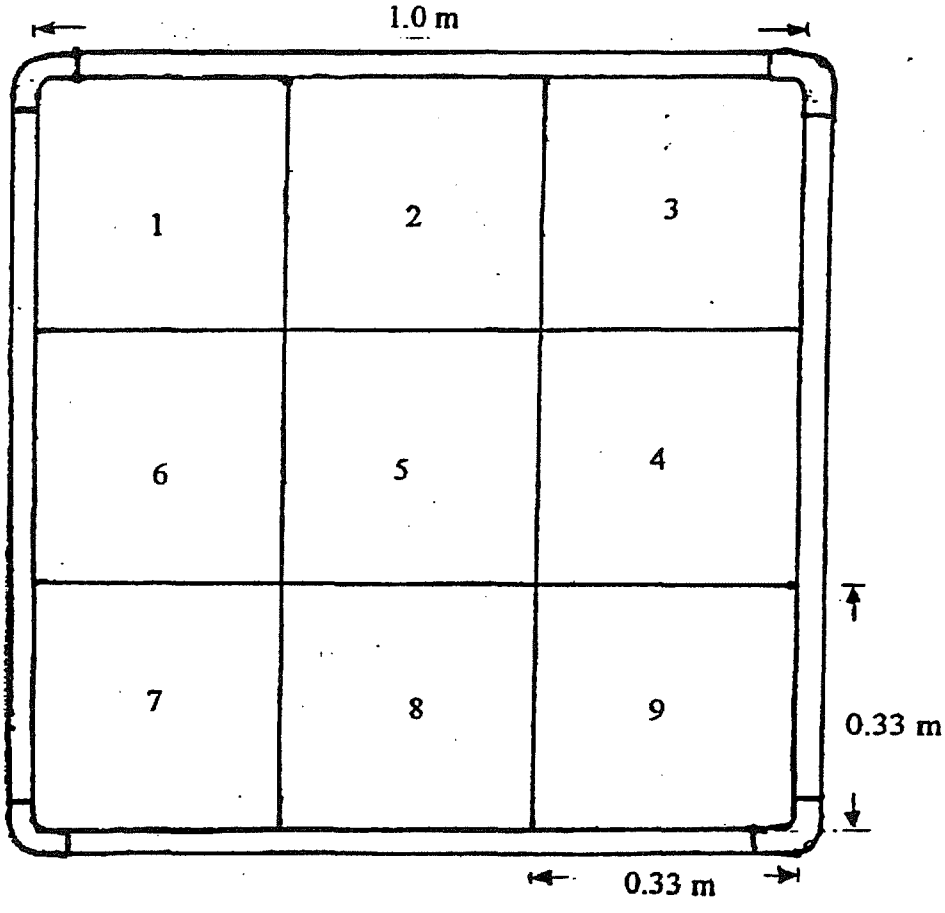

Fig. 4.1. Schematic diagram of the 1.0 m^2 quadrat showing the positions of the nine subsquares.

Fig. 4.2. The position of the platform and quadrat, and the handheld video camera during the shooting of the individual subsquares of a plot.

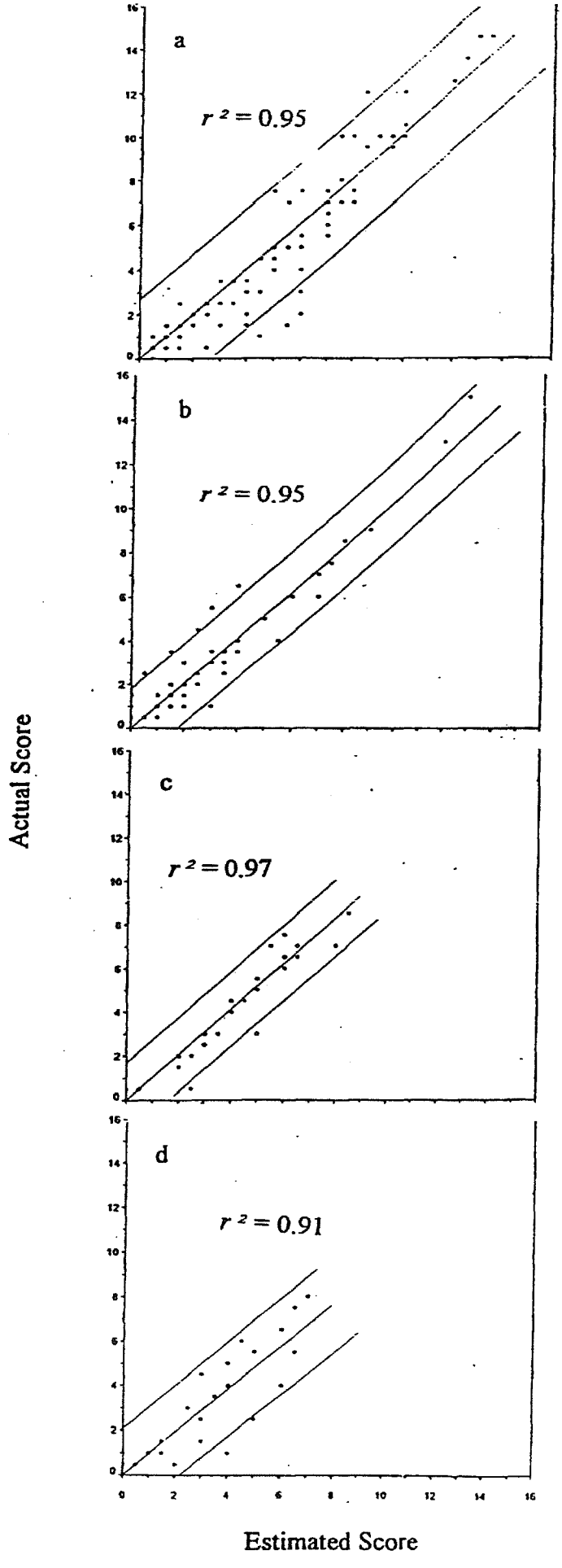

Fig. 4.3. Plots of the estimated scores versus the actual scores obtained from harvested samples for (a) *H. ovalis*, (b) *Z. capricorni*, (c) *C. rotundata*, (d) *H. uninervis* at Ellie Point; showing the regression line passing through the origin, r^2 values and 95% CI limits.

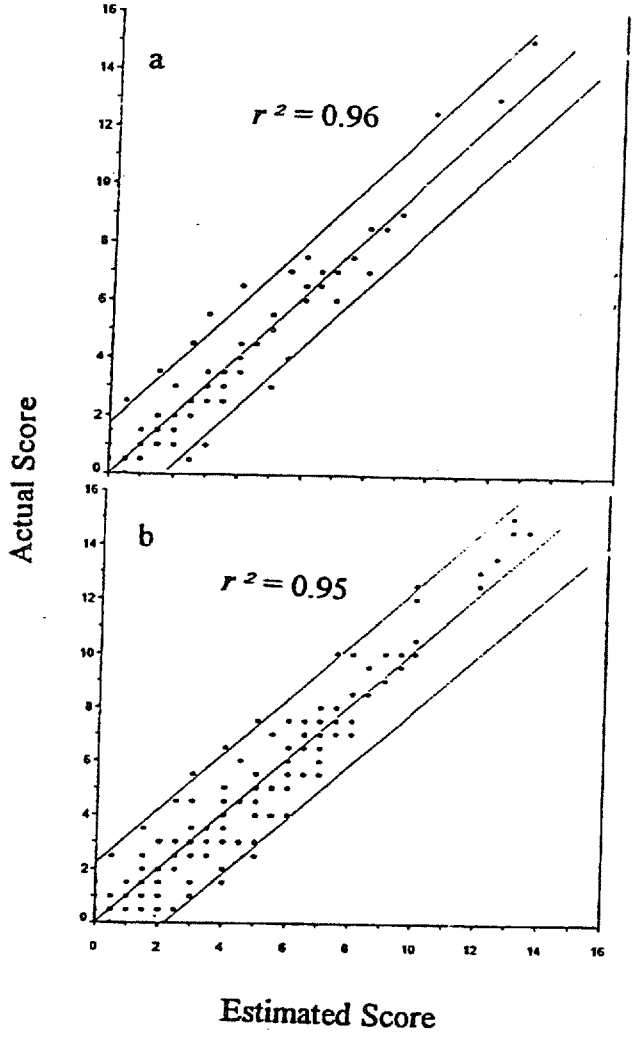

Fig. 4.4. Plots of the estimated scores versus the actual scores obtained from harvested samples for (a) combination of *Zostera* and *Cymodocea*, (b) all species combined at Ellie Point; showing the regression line passing through the origin, r^2 values and 95% CI limits.

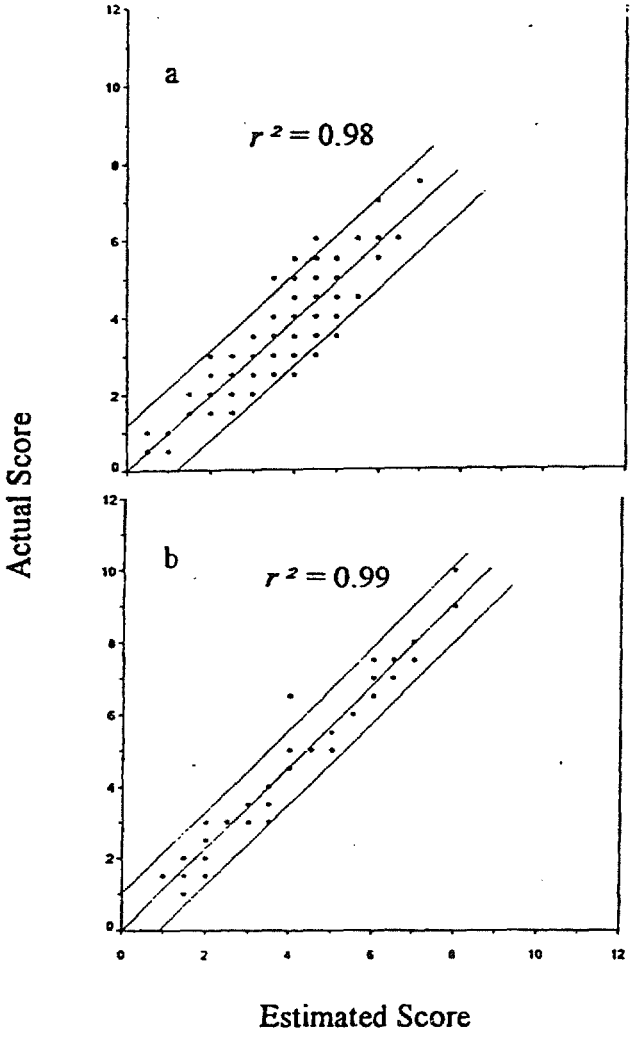

Fig. 4.5. Plots of the estimated scores versus the actual scores obtained from harvested samples for *Halodule uninervis* at Cardwell: (a) first experiment, (b) second experiment; showing the regression line passing through the origin, r^2 values and 95% CI limits.

CHAPTER 5.

EFFECTS OF GRAZING ON THE STRUCTURE AND DYNAMICS OF TROPICAL SEAGRASS COMMUNITIES

5.1. INTRODUCTION

McNaughton (1986) argued that a "lack of grazing and not its occurrence is abnormal for many grasslands". Herbivory influences plant morphology, productivity, distribution, and community structure in terrestrial systems (e.g. McNaughton 1983, 1985; Crawley 1983; McNaughton and Banyikwa 1995) and in algal-based marine systems (e.g. Lewis *et al.* 1987; Valentine and Heck 1991; Wahl and Hay 1995). Limited information exists on the direct response of seagrass ecosystems to herbivory. Most studies deal with invertebrate or fish herbivory (e.g. Underwood 1980; Bell and Westoby 1986; Valentine and Heck 1991; Klumpp *et al.* 1993) or examine herbivory indirectly (i.e. through trophic flow, detrivory and the like, e.g. Klumpp *et al.* 1989; Poiner *et al.* 1992). Herbivory by marine megaherbivores has rarely been studied (but see Preen 1993, 1995; de Iongh *et al.* 1995, for dugongs; Provancha and Hall 1991, Lefebvre *et al.* 1995, for manatees; Bjorndal 1980, Ogden *et al.* 1983, Read 1991, and Kuiper-Linley 1994, for green turtles). These studies reveal that herbivory by marine megaherbivores can have considerable impacts on the dynamics of seagrass communities.

During the early stages of research on dugongs in Australia, Heinsohn *et al.* (1977) suggested that grazing by dugongs can influence the community structure of tropical seagrasses. However, little is known of the seagrass plants' response to herbivory in spite of considerable study of dugongs and green turtles in sub-tropical and tropical seagrass systems in the Australian region (Wake 1975; Heinsohn *et al.* 1977; Anderson and Birtles 1978; Heinsohn 1981; Johnstone and Hudson 1981; Anderson 1982a, 1982, 1986, 1994; Marsh *et al.* 1982, 1984; Lanyon 1991; Preen 1993; Lanyon and Marsh 1995, for dugongs; Limpus and

Walter 1980; Garnett *et al.* 1985; Read 1991; Limpus 1992; Limpus *et al.* 1993; Brand 1995, for sea turtles). Preen's (1993) work is the most comprehensive, and the first to examine the grazing impacts of dugongs. Kuiper-Linley (1994) carried out a study on the effects of simulated turtle cropping on seagrasses. However, both these studies were carried out in Moreton Bay, a subtropical system. The studies conducted to investigate the direct effects of turtle cropping on seagrass communities have mostly been carried out in the Caribbean region (e.g. Bjorndal 1980, 1985; Ogden *et al.* 1983; Zieman *et al.* 1984). Similar studies are lacking for other tropical systems, particularly in the Australasian region (except de Iongh *et al.* 1995), where dugongs and green turtles are found in large numbers (Ogden 1980; Thayer *et al.* 1984; Marsh *et al.* 1995). The only study in the tropics (de Iongh *et al.* 1995) examined the relationship between grazing by dugongs, and seagrass distribution and seasonal biomass changes in an intertidal seagrass bed dominated by *Halodule uninervis*. More information including the effects of grazing on species composition of a mixed-species bed, amounts of detrital matter, and primary productivity, is needed for a better understanding of the habitat requirements of these marine megaherbivores, their feeding ecology and ecological roles.

In contrast to this dearth of studies into the ecological roles of dugongs and green turtles in the seagrass ecosystem, several relatively sophisticated models explaining the roles of herbivores in terrestrial systems have been developed. These models are characterised by complex dynamics (e.g. grazing, depending on the intensity, can modify the physiology and structure of grazed plants variably, profoundly affecting the interactions between the plant and its abiotic and biotic environments, see Hilbert *et al.* 1981; Dyer *et al.* 1982; McNaughton 1983). Most of these models use variables which are very difficult to quantify in the field. Consequently, based on what is known of terrestrial animal-plant interactions and dugong grazing in a subtropical seagrass system (Preen 1993), I infer that grazing in the tropical seagrass ecosystem is a complex phenomenon involving complicated dynamics. However, few empirical data explain or support this suggestion.

The studies reported in this chapter were designed to obtain information on tropical seagrass dynamics as a consequence of grazing. The major objective of this study was to investigate experimentally whether grazing by the two largest marine herbivores affects the community structure of tropical seagrasses over two time frames: short-term (monitored one to four months) and long-term (monitored 10 to 12 months), and at several levels of grazing intensity: intensive dugong grazing, light dugong grazing, and turtle cropping.

These grazing experiments showed that the intensity of herbivory by dugongs and green turtles influenced the structure and dynamics of tropical seagrass communities by altering the species composition, biomass, amounts of detrital matter, and net above-ground biomass productivity. Most importantly, they showed that even light grazing can have significant effects on the community structure by altering the species and biomass, and that in general recovery from grazing disturbances in the tropics, is rapid. I conclude that quality (Chapter 7), quantity and botanical composition of vegetation, the three most important properties used in assessing feeding niches (Bell 1971; Jarman 1974) are positively correlated with the intensity of vertebrate herbivory in tropical seagrass ecosystems.

5.2. MATERIALS AND METHODS

5.2.1. Study Sites

Two grazing experiments were carried out in intertidal seagrass beds in tropical Queensland, Australia (see Fig 5.1). A tropical area was chosen, as detailed in Chapter 2, because most dugongs and green turtles occur in the tropics. I chose an intertidal area since many dugongs feed on near-shore seagrasses (Lipkin 1975; Wake 1975; Johnstone and Hudson 1981; Marsh *et al.* 1982; Preen 1993).

5.2.1.1. Ellie Point (Cairns Harbour)

Ellie Point (16 ° 53'S, 145 ° 46'E) is located in Trinity Bay, approximately 3.5 km north of Cairns, northern Queensland (Fig 5.2), immediately east of the Cairns International Airport. The seagrass bed is primarily intertidal and was dominated by *Zostera capricorni* (McKenzie 1995). Patches of other species, such as *Halophila ovalis, Halodule uninervis, Cymodocea rotundata* and *C. serrulata*, also occur in this bed. Ellie Point was chosen because it was representative of a mixed-species bed dominated by a robust ("climax") species (*Zostera capricorni*, wide variety).

The positions or locations of the Latin squares for the grazing experiments (referred to as sites) were chosen haphazardly in mixed-species regions of the bed. The substrate was mainly terrigenous mud, approximately 5 -10 cm deep, with the deeper layers probably anoxic (as reflected by their blackish colour), covered by sand. The chosen sites were partially protected from the easterly waves by a sandbank, 150 m away from the edge of the bed (McKenzie 1995). Tides in the region are semi-diurnal; mean sea level is 1.643 m and mean low spring tide reaches 0.74 m (Queensland's Official Tide Tables and Boating Guide 1994). The bed was relatively exposed to winds from the north, east, and south and protected by a mountain range to the west. The sites selected for my experiments were exposed at a tidal height of approximately 0.85 m (elevation threshold).

5.2.1.2. Cardwell/Hinchinbrook Channel

Cardwell (18 ° 15'S, 146 ° 01'E) is located at the northern end of the Hinchinbrook Channel (Fig 5.3), approximately 195 km north of Townsville, Queensland, Australia. The intertidal seagrass bed south of Meunga Creek, where I established my experiments, primarily consisted of *Halodule uninervis. Halophila ovalis* was interspersed with *H. uninervis* in the subtidal regions of the bed. The seagrass beds in this area occur as narrow strips, parallel to the shore, and up to 150 m wide. These beds are predominantly patchy, typical of tropical seagrass meadows. The substrate

ranges from fine silt and mud, to sand. The study site is relatively exposed to winds from the north, but relatively protected from easterly winds by Hinchinbrook Island, and a mountain range to the west and south. Tides are semi-diurnal, with a mean sea level of 1.86 m and a mean low spring tide at 0.75 m (Queensland's Official Tide Tables and Boating Guide 1994). The sites of the grazing experiments were chosen haphazardly. Those selected for the first experiment were exposed at a tidal height of approximately 0.85 m; those for the second experiment were exposed at 1.0 m (elevation threshold). A higher elevation was necessary for the second experiment to allow me a longer window of time to complete monitoring an increased number of experimental treatments.

I will refer to Ellie Point and Cardwell as 'beds', the positions of the Latin squares for the grazing experiments as 'sites' and the locations of the experimental treatments as 'plots'.

5.2.2. Details of Experiment

5.2.2.1 Simulation of grazing and cropping

Grazing simulations were conducted from May to June 1993 at Ellie Point and Cardwell/Hinchinbrook Channel. At each site, grazing and cropping by dugongs and green turtles were as outlined below and summarised in Table 5.1. I refer to 'dugong grazing' as grazing and 'green turtle grazing' as cropping to avoid confusion between these two distinct forms of herbivory. Natural grazing and cropping may vary in areal extent and frequency (e.g. grazing, Heinsohn *et al.* 1977; Anderson and Birtles 1978; Heinsohn 1981; Preen 1993; cropping, Bjorndal 1980; Ogden *et al.* 1983). However, for logistical reasons I confined the areal extent of the herbivory treatments to 1.0 m^2 plots.

All simulations were carried out carefully so that they replicated each level of herbivory as closely as possible. The treatments were completed in the shortest possible time to avoid confounding temporal effects. To keep the intensive grazing treatment (see

Table 5.1) uniform and repeatable, I simulated intensive dugong activity by removing all seagrass (above-ground biomass) from each plot leaving only remnant amounts of roots and rhizomes (see Preen 1995). The measurements of Heinsohn (1972), Heinsohn and Birch (1972), Heinsohn *et al.* (1977), Anderson and Birtles (1978), Heinsohn (1981), and Preen (1993) indicate that natural feeding trails of dugongs are from 2 to 15 m long. I could not simulate such long trails in a replicated design. Instead, I simulated three short (1.0 m long) feeding trails approximately 150 mm wide and 275 mm apart within 1.0 m^2 plots for the light dugong grazing treatment. Intensive turtle cropping was simulated by removing all leaves from the seagrasses within the plots (Bjorndal 1980; Ogden *et al.* 1983). All simulations were performed during low tides because it was impossible to work in the murky waters at these sites even with SCUBA. The simulations were staggered (Table 5.2), as the low tides allowed only three to five accessible days per fortnight.

5.2.2.2. Plot preparation

In each Latin square, each plot was established in an area with an approximately uniform seagrass cover. Each corner of each plot was marked by a peg (untreated wooden garden stake), with no more than 3 cm protruding to allow relocation while avoiding a 'halo' effect around the peg. A movable quadrat of PVC pipes and elbows fitted tightly onto these markers ensuring that I went back to the exact location each time.

5.2.3. Experimental Design

5.2.3.1. Long-term experiments (Ellie Point and Cardwell)

Long-term experiments were performed at Ellie Point (monitored over 11 months) and at Cardwell (monitored for 14 months, Table 5.2). A third experiment at Bolger Bay, Magnetic Island, was set up in July 1993, but was abandoned after the intertidal seagrass disappeared.

These experiments used a 4 x 4 Latin square design with a 1.0 m^2 quadrat (= plot) (see Fig 5.4) and four treatments: (I) intensive grazing by dugongs, (II) light grazing by dugongs, (III) cropping by turtles, and (IV) undisturbed (control) (Table 5.1a), and four replicates per treatment. The treatments of the first row and column of each Latin square were independently assigned by lottery. The remaining treatments were assigned so that each occurred only once per row and column. An aluminium strip tied to one of the corner pegs was used to identify the assigned treatment for each plot. Four Latin squares per site were established about 25 to 35 m apart (total plots = 64), approximately 200 m from and parallel to the shoreline. Buffer zones of at least 1.0 m wide were maintained along all sides of each plot to prevent edge effects (see Fig 5.4).

5.2.3.2. Short-term experiment (Cardwell)

A short-term experiment was monitored over four months at Cardwell. This experiment used four 6 x 6 Latin squares with the following treatments: (I) light dugong grazing, (II) undisturbed or control for Treatment I, (III) turtle cropping 1, (IV) control for Treatment III, (V) turtle cropping 2, and (VI) control for Treatment V (Table 5.1b). Each plot was 0.4356 m^2 (0.66 m x 0.66 m). The sampling unit was reduced to allow an expanded number of plots (N = 144). High intensity dugong grazing was not included because the time available to monitor the recovery was insufficient. The Latin squares were established following the procedures of the long-term experiments (Section 5.2.3.1.). Each treatment and its control were sequentially harvested (see Table 5.2 for dates) to test the short-term effects of grazing on the nutritional attributes of seagrasses.

5.2.3.3. Parameters monitored

Changes in species composition and abundance (leaf biomass only) were monitored monthly for the long-term experiments. The short-term (second) experiment was monitored bi-monthly for the first two months, then once a month until the last pair of treatments was harvested. Monthly and bi-monthly (for second experiment)

monitoring was carried out to ensure that any natural disturbances (e.g. dugong grazing) on seagrasses were identified, recorded and quantified (leaf biomass removed estimated). Grazing from fish and invertebrates was considered negligible.

5.2.4. Measurement of parameters

5.2.4.1. Initial status of quadrats

The status of each quadrat before and after the application of the treatments was recorded on a video tape to allow the assessment of species present and estimated above-ground biomass. Each subsquare of each quadrat was recorded by video photography following the procedures described in Section 5.2.2.1. Pre- and post-treatment video recording was done on the day of application of the treatments to avoid any confounding effects of time.

5.2.4.2. Monitoring seagrass biomass via video recording

I systematically video recorded each plot of each Latin square, commencing with those plots furthest from the shoreline and ending with those nearest the shore (or from highest to lowest number, see Fig 5.4 for an example). Before video recording each plot, I placed the quadrat over the permanent pegs at each corner. A platform (0.25 m x 1.2 m x 0.20 m) of timber plates was placed across the middle portion of each plot (see Fig 4.2) to enable me to shoot the video without disturbing the treatments and make the shots as vertical as possible. I recorded by video each subsquare within a quadrat in a chronological order as described in Section 4.2.2. (and see Fig 4.1). The focal distance of the video recording was my standing height (1.6 m plus height of platform, ~ 0.10 - 0.15 m). The presence/absence of feeding trails and other relevant information (e.g. relative abundance of algae) were recorded for each quadrat and its surrounds at each site onto video or in a field notebook. All sampling was done during low tides.

5.2.4.3. Temporal variation

All the subsquares of each plot were recorded by video every month using the procedures described in Section 5.2.4.2. The estimated scores for each site were recorded on separate spreadsheet files. To avoid any bias in the scoring, each subsquare was scored without reference to the previous scores. I found it difficult to distinguish *Z. capricorni* from *C. rotundata* in the video images taken at Ellie Point, consequently their scores were pooled (*Zostera/Cymodocea*) in the final analysis. The above-ground biomass of each plot was estimated as the sum of the scores from the nine corresponding subsquares.

Neither study site was monitored in December 1993. The day tides did not allow access and I was not yet prepared for night monitoring. Data for December 1993 were estimated in the graphical presentation as the mean (median value) leaf biomass scores of the plots by extrapolating between the November and January data. These estimates were excluded from the statistical analyses.

5.2.4.4. Estimation of seagrass removed from natural dugong feeding trails

Natural disturbance by dugongs occurred only at the monospecific bed of *H. uninervis* at Cardwell. The leaf biomass of seagrass removed by natural dugong grazing within each plot was estimated visually via the video images following the same principle employed in estimating the standing leaf biomass. The feeding scars were very distinct, though some were very difficult to follow, particularly when another trail overlapped in the next sampling. Previous feeding trails were distinguished from new ones by comparing the images. This was done by viewing the two images simultaneously (side by side) using Paint Shop Pro Software (JASC Inc, 1995). In these cases, the scores refer to the amount of above-ground biomass removed by the dugongs. These scores were recorded simultaneously with the estimated standing crop (remaining above-ground biomass) from the video recorded images of the subsquares. These scores were then added to the leaf biomass estimates so that the 'undisturbed' standing crop could

be estimated and converted into dry weights following the procedures detailed in Section 4.2.8.

5.2.5. Seagrass harvesting

Seagrass samples from the experiments were harvested (see Table 4.2 for actual dates) following the procedures explained in Section 4.2.4.

5.2.6. Biomass determinations

The biomass of each species in each plot was converted and recorded following the procedures described in Section 4.2.5.

Whole plant (total biomass) refers to both leaf and root/rhizome fractions, whereas standing crop refers to above-sediment materials (= leaf fraction) (after Zieman and Wetzel 1980). I use the terminology, leaf fraction, as opposed to above-ground biomass, as not all leaves are above ground.

5.2.7. Above-ground biomass production

A 'minimal' estimate of the net above-ground biomass production (NABP) was determined by summing the biomass accumulation per month over the duration of experiment using Equation 5.1.

$$\textbf{NABP (g dw/m}^2\textbf{)} = \sum_{i=1}^{n} \left(x_{ai} - x_{ai-1} \right) \qquad \textbf{(Equation 5.1)}$$

Where x_{ai} is either (1) above-ground biomass for species a at time i or (2) above-ground biomass for all species combined at time i (starting from the first month of recovery) and n is the number of months of the experiment.

An exact measurement of net primary productivity could not be obtained because below-ground biomass production, biomass losses to excretion, death and decomposition rates, or harvest by other smaller herbivores like fish, were not measured.

5.2.8. Statistical Analyses

5.2.8.1. Response of the seagrasses to the different treatments over time

The temporal response of the leaf biomass (g dw/m^2) of seagrass at Ellie Point and Cardwell to the various grazing and cropping treatments was examined using repeated measures univariate ANOVA. Site was treated as a random factor; treatment and time (number of months from day 1) were fixed factors. The values of the replicates (plots) for each treatment, for each Latin square were aggregated to account for the random factor, site (G. Dea'th 1996, pers. comm.). Univariate ANOVAs were used because the number of degrees of freedom meant that the multivariate test had low power (G. De'ath 1995, pers. comm.). At Ellie Point, species was considered as another repeated factor, and separate analyses were carried out for all species combined and each species separately. Epsilon corrected average F values (Greenhouse-Geisser Epsilon) were used to compensate for possible deviations from univariate assumptions. All F ratios were compared against the within residuals mean square using SPSS Release 6.1.3 (1994).

5.2.8.2. Effects of grazing on above- and below-ground parts of seagrasses at harvest

The leaf and root/rhizome fractions of each species, for each plot, of each treatment were calculated for each study site (Ellie Point and Cardwell) after harvesting at the end of the experiment following the procedures detailed in Section 4.2.8. The ratio of the root/rhizome to leaf fraction was calculated for each plot by using the values from the harvested subsamples (0.11 m^2 of 1 m^2 plots). General factorial-design ANOVAs were

performed to test for the effects of treatment (trt), site, species (sp) and their interactions on the proportions of the different plant parts, the whole plant, ratio of roots/rhizomes to leaves and amounts of detritus (species pooled), upon harvesting. Ratio was transformed (log of ratio + 1) before analysis. Site was treated as a random factor; the rest as fixed factors. At Ellie Point, *Halodule univervis* occurred at low biomass in some plots only and was excluded from the regression (unique sums of squares) analyses of these experiments. The resulting design (=model) was:

Design I

trt vs 1, site vs wr , sp vs 2, trt*sp vs 3, site*trt=1 vs wr, sp*site=2 vs wr, site*sp*trt=3 vs wr.

Where trt stands for treatment which was tested against the site by treatment interaction (1); site was tested against within residual mean square (wr); species (sp) was tested against the species by site interaction (2), which was tested against the within residual mean square; the treatment by species (trt*sp) was tested against the site by species by treatment (site*sp*trt) interaction, which was in turn tested against the within residual mean square.

At Cardwell, a similar factorial-design ANOVA was performed except, that it lacked the species factor. Therefore the design for Cardwell was:

Design II

trt vs 1, site vs wr, site*trt=1 vs wr.

Where the treatment tested against the site by treatment interaction (1), which was then tested against the within residual mean square; and the site was also tested against the within residual mean square.

In the second experiments, general factorial-design ANOVAs were also performed with the paired treatments-controls (pair 1= Trts I and II, 2 = Trts III and IV, and 3 = Trts V and VI) to test the effects of treatment, site, and their interactions on the proportions of the different plant parts, whole plant and the ratio of roots/rhizomes to

leaf fractions. Site was treated as a random factor; the remainder as fixed factors. The model for the analysis for each paired-treatments was similar to Design II of the first experiment (detailed above).

5.3. RESULTS

5.3.1. Grazing effects: long-term experiments

5.3.1.1. Ellie Point (mixed-species seagrass bed)

Initial differences among sites

There were differences in the species composition and leaf biomass between sites prior to the experiment (Table 5.3 and Appendix 2 Figs 1 to 8). The leaf biomass of *H. ovalis* and *Zostera/Cymodocea* were almost equal at site A, where smaller amounts of *H. uninervis* also occurred. Site B was dominated by *H. ovalis* interspersed with some *Zostera/Cymodocea* with traces of *H. uninervis*. Sites C and D were dominated by *Zostera/Cymodocea* with very sparse *H. ovalis* and no *H. uninervis*.

Temporal changes in the seagrasses at control plots

The combined leaf biomass of all species declined during the winter months to reach a low in June and increased after the wet summer months (see Figs 5.5, 5.6d and Appendix 2 Fig 9). The leaf biomass of *H. ovalis* declined during the winter-spring months to a low in November and increased during the summer months from December until the March harvest. The leaf biomass of *Zostera/Cymodocea* declined during the winter months, increased during the spring months, was lowest in February, and increased again the following month (Fig 5.6d). The leaf biomass of *Halodule uninervis* was very low and almost constant over time.

Repeated measures analysis of variance

Overall

The results of the repeated measures ANOVA, which considered all species simultaneously, were complex and hard to interpret (Table 5.4). Almost all factors and interactions were significant. The treatment effect was highly significant. The treatment by species interaction was fairly similar across the species. I ignored effects of site as it was a random factor. The factor of most interest, the species by time by treatment interaction, was difficult to interpret (Appendix 2 Fig 1). To interpret these results separate repeated measures ANOVAs were carried out for each species (see below).

By species

The treatment by time interaction was highly significant for both *H. ovalis* and *Zostera/Cymodocea* but not for *Halodule uninervis* (see Fig 5.6, Table 5.5 and Appendix 2 Fig 1). The effect of intensive grazing treatment on each species through time was exactly opposite: the leaf biomass of *H. ovalis* increased as *Zostera/Cymodocea* deceased in response to intensive grazing (Appendix 2 Fig 1). My inability to detect change in *H. uninervis* may have been confounded by the low biomass and high spatial variability of this species at this site (see Fig. 5.6 and Table 5.6).

Overall recovery

The overall recovery of the seagrasses (all species combined) from the different regimes of simulated herbivory at Ellie Point was relatively rapid (Fig 5.5). Even the intensive grazing plots recovered after five months. Recovery from light grazing took about two months despite a slight decline in the leaf biomass after the first month of recovery. Many of the injured plants (particularly the roots/rhizomes) at the edges of the light grazing trails died, resulting in a reduction

in the leaf biomass and slight widening of the feeding tracks. The recovery of the leaf biomass of seagrasses in the intensive cropping treatment varied according to species as explained below (Fig 5.6).

Specific responses to intensity of herbivory

Halophila ovalis

The recovery of *H. ovalis* from the different treatments was very rapid (Figs 5.6). The plots exposed to intensive cropping had the most rapid recovery (approximately one month, see Fig 5.6c). Those exposed to light dugong grazing were similar to the controls after one month, even though their biomass declined subsequently due to the drastic decline in leaf biomass in June which was also evident in the controls (see Table 5.6). The plots exposed to high intensity dugong grazing had the slowest recovery (three months).

The responses to treatments were relatively similar from the third month of recovery (August) onwards. The recovery from the high intensity dugong grazing, however, was greater than for any of the other treatments. Even after eleven months, the leaf biomass of *H. ovalis* subjected to simulated intensive grazing was significantly higher than that of the control (see Table 5.6 and 5.7). The other treatments were similar to the controls.

Zostera/Cymodocea

Zostera/Cymodocea did not recover from the treatments as rapidly as *H. ovalis* (Fig 5.6). Recovery from cropping took two months; from light dugong grazing, three months. As for *H. ovalis*, the leaf biomass of *Zostera/Cymodocea* continued to decline after the first month of recovery from light grazing. *Zostera/ Cymodocea* took five months to recover from simulated intensive grazing.

Intensity of herbivory affects species composition and abundance

Intensive grazing

The species composition of the seagrass plots exposed to intensive grazing switched in dominance from *Zostera/Cymodocea* to *H. ovalis* (see Figs 5.6a and 5.6b). The leaf biomass of *H. ovalis* was half that of *Zostera/Cymodocea* in May (1993). By the following March (1994) leaf biomass of *H. ovalis* was twice that of *Zostera/Cymodocea* (see also Table 5.6). This switch was also reflected in the significant treatment by time interactions for *H. ovalis* and *Zostera/Cymodocea* (see Table 5.5).

Light grazing

The seagrass plots exposed to light grazing also switched from being dominated by *Zostera/Cymodocea* to *H. ovalis* (Fig 5.6b). This was reflected in the significant treatment by time interactions for *H. ovalis* and *Zostera/Cymodocea* (see Table 5.5).

Cropping

There was no significant change in the species composition of the seagrass plots exposed to intensive turtle cropping (Fig 5.6c). *Halophila ovalis* and *Zostera/Cymodocea* both recovered rapidly after the first month, but from the third month (August) onwards *Zostera/Cymodocea* dominated except when it declined slightly in February (1994).

Biomass upon harvesting

After 11 months, both the leaf fraction and the ratio of leaf to roots/rhizomes were significantly affected by the treatments, however, the roots/rhizomes and total biomass were similar across treatments (Table 5.7 and see Fig 5.7). The treatment

by species effect was significant only for the leaf fraction, i.e. the leaf biomass of *H. ovalis* from intensive grazing was significantly higher than that of the control (Table 5.7 and Fig. 5.7a). The ratio of the roots/rhizomes to the leaves at the intensive grazing plots was significantly lower than that of the controls. The ratio also varied according to species. The *Zostera/Cymodocea* group had a higher ratio of roots/rhizomes to leaves than the other two species (Fig. 5.7d).

Amount of detritus upon harvesting

Ten months after recovery the treatment plots had significantly different amounts of detritus than the controls (Table 5.8 and Fig 5.8). The intensive grazing plots had significantly less detrital matter; the light grazing and cropping plots had more.

Grazing and net above-ground biomass production

The intensity or level of herbivory affected the net above-ground biomass production (NABP) variably, depending on species (Fig 5.9). The NABP of *H. ovalis* was significantly higher than the controls and increased with the intensity of herbivory (Table 5.9). In contrast, the NABP of *Z. capricorni* decreased as the level of herbivory increased.

5.3.1.2. Cardwell (monospecific seagrass bed)

Repeated measures analysis of variance

The results for Cardwell were very similar to those at Ellie Point (Table 5.10). The most interesting effect was the significant treatment by time interaction (see also Fig 5.10a).

Overall profile ignoring natural grazing

The leaf biomass of *Halodule uninervis* in the control plots changed unimodally over time (Fig. 5.10a). It was lowest during the winter months, gradually increased in spring, peaked in summer (February 1994) and declined again from March to July. The trends for the leaf biomass of *H. uninervis* were generally similar across all treatments (Fig 5.10a). The period from one low peak (June 1993) to another suggests that the turnover time in *H. uninervis* was between eight to twelve months (see Fig 5.10a). (The use of turnover time here is not comparable to studies which specifically determined turnover times by employing tagging techniques.)

Response of H. uninervis to grazing

The rate of recovery of *H. uninervis* from the different grazing regimes varied from rapid to slow. The leaf biomass of the plots exposed to light grazing and intensive cropping recovered quickly and was similar to that of the control plots after the third month (Fig 5.10a). The leaf biomass of the plots exposed to intensive grazing was significantly lower than that of the control almost throughout the duration of the study (one year). The biomass recovered to a level similar to the controls after eight months, coincident with the estimated turnover time of *H. uninervis* in such site and the peak of the above-ground biomass (February), then declined again, and was significantly lower than the remaining treatments after a year (see Fig 5.10a). However, this recovery could have been prolonged by the occurrence of repeated natural dugong grazing within the study sites (see Fig 5.10b).

Amount of leaf biomass removed by dugongs

The leaf biomass removed by dugongs over time at Cardwell during the study is shown in Fig 5.10c. Dugongs grazed within the study area from August (1993) to

May (1994), inclusive (see also Fig 5.10b). The major grazing events, based on the levels of estimated amount of leaf biomass removed, occurred during the months of November (1993), February, March and May (1994).

Natural grazing by dugong may have been the major contributor to the decline in November 1993, and February and May 1994 (Fig 5.10b). However, other environmental factors such as daytime tidal exposure[1] and the seasonality of *H. uninervis* may also have contributed to these declines (particularly in May, start of winter, when biomass is at its lowest).

Biomass upon harvesting

After 12 months, all the factors examined: the leaf, root/rhizome fractions, whole plants, and the ratio of leaf to roots/rhizomes of *H. uninervis* were still significantly affected by the treatments (Table 5.11). The leaf, root/rhizome and whole plant biomass, including the ratio of the leaves to roots/rhizomes from intensive grazing were significantly lesser than that of the control (Fig. 5.11).

Grazing and net above-ground biomass production

The intensity or level of herbivory affected significantly the net above-ground biomass production (NABP) of *H. uninervis* (Table 5.12 and see Fig 5.12). The NABP was significantly higher in the treatments than the controls, with the cropping treatment exhibiting the highest levels followed by intensive grazing and light grazing (Fig 5.12 and see Table 5.12).

5.3.2. Grazing effects: short-term experiments

The results of the repeated measures ANOVA for the three experiments showed highly significant treatment, time, and treatment by time interaction effects (Table

[1] Daytime tidal exposure in northern Queensland is greatest from June to August.

5.13). The effects of the grazing regimes were similar across the sites, and the site by time interactions. The undisturbed plots in the short-term grazing experiments always had more leaf biomass than any of the other treatments as the experiments were concluded before complete recovery (see Tables 5.2 and 5.11, and Fig 5.13). After four months of recovery, the effect of the light dugong grazing treatment was still significant as evidenced by a lower biomass than its control for both plant parts and whole plants, and a smaller ratio of roots/rhizomes to leaves (Figs 5.13 to 5.14 and Table 5.14). There was significantly less leaf biomass and a smaller ratio of roots/rhizomes to leaves in the cropping treatment harvested after one month than in the controls. However, the biomass in the root/rhizome fraction and in the whole plants was similar to the controls (Fig 5.14 and Table 5.14). The effect of the cropping treatment after two months was significant only for the leaves; the leaf biomass was considerably less than the corresponding undisturbed plots. The biomass in the roots/rhizomes fractions, whole plants and the ratio of roots/rhizomes to leaves was similar to the controls.

5.4. DISCUSSION

GRAZING DISTURBANCE AND DYNAMICS OF TROPICAL SEAGRASSES

My simulated grazing experiments demonstrated that the levels of grazing by large marine herbivores influenced the community structure of tropical seagrasses by affecting the biomass, species composition and amount of detritus, and the community dynamics by altering the net above-ground biomass productivity. These empirical data support the hypothesis of Heinsohn *et al.* (1977) that dugong grazing affects the structure of tropical seagrass communities. The nature and extent of the effects were related to: (1) the intensity (and timing) of the grazing impact; and (2) the nature of the seagrass community, including its species composition and location. Recovery times varied from months for *H. ovalis* and *Zostera/Cymodocea* at Ellie Point (see Fig. 5.2 for location) to more than one year for *H. uninervis* at Cardwell (see Fig 5.3 for location).

Scale of disturbance

Despite the intense and subsurface impact of grazing by dugongs, the scale of disturbance from grazing is different from non-grazing disturbances on seagrass beds. The scale of disturbance from dugong's grazing is limited by their feeding behaviour. Dugongs generally feed in linear, serpentine feeding trails that are about as wide as their muzzle (Heinsohn *et al.* 1977; Anderson and Birtles 1978; Preen 1993). However, despite the great density of these serpentine and overlapping feeding trails, small tufts of seagrass survive (Preen 1993, 1995a). Patches of seagrasses which survive even the most intensive regime of grazing, are an "ungrazable reserve" and the key to the resilience of seagrass recovery (Preen 1993). Preen (1995a) observed that a seagrass patch size of even less than 1 m^2, interspersed across quite large areas (> 50 ha) severely disturbed by dugongs, is enough to facilitate rapid recovery (months). In contrast, the scale of disturbance of seagrass from sedimentation, water scouring, or "die-off", including the impacts of flooding or cyclones is often more severe because large and uniform loss of seagrass areas occurs (Short 1983; Poiner *et al.* 1989; Preen 1993; Preen *et al.* 1995c; Poiner and Peterken 1995). For example, recently, Preen *et al.* (1995c) reported that 1,000 km^2 of seagrass lost at Hervey Bay in 1992 died presumably as a result of light deprivation caused by a persistent plume of turbid water and resuspended sediments following two major floods and a cyclone which all occurred within three weeks. Most recovery, after two years, was limited to water deeper than 10 m. The sediment disturbance associated with the cyclone in shallow areas presumably buried or killed the seeds as a result of abrasion by the churning sediment (Preen *et al.* 1995c).

The amount of seagrass removed by dugong grazing varies with species. In the light grazing treatment plots, I removed an average of 69%, 79% and 84% of the above-ground biomass of *H. ovalis*, *Zostera/Cymodocea* and *H. uninervis*, respectively. In tropical Queensland, dugong grazing has been reported to remove from feeding trails an average of 63 - 86% of total seagrass biomass for various

species including, *H. ovalis*, *Z. capricorni*, and *H. uninervis* (Wake 1975). In subtropical Queensland (Moreton Bay), intensive dugong grazing removed an average of 90%, 92% and 79% above-ground biomass in a seagrass bed dominated by *H. ovalis*, *Z. capricorni* and *H. uninervis*, respectively (Preen 1993). In my intensive grazing plots, I removed uniformly all above-ground biomass in the 1 m^2 plots. This is close to the level of removal recorded in favoured feeding sites in Moreton Bay, wherein dugongs reportedly removed 96% of the above-ground biomass and 95% of the shoot density of seagrass (Preen 1993). Dugongs feed on both above and below-ground biomass. However, the below-ground component removed during the simulations in this study was not measured. But, I found it noticeably harder to remove the below-ground biomass of *Zostera/Cymodocea* than *H. uninervis*, with *H. ovalis* the easiest. In Moreton Bay, dugongs remove 78% of the below-ground biomass of *H. ovalis* from feeding trails, 46% of *H. uninervis*, and 36% of *Z. capricorni* (Preen 1993). Apart from the species, the type of substrate also affects how much below-ground biomass could be removed by dugongs. Seagrass growing in a hard substrate, e.g. compact sand or reef pavement, will be more difficult to remove than that growing in a soft substrate e.g. loose sand or mud. A comprehensive analysis of the stomach contents of dugongs from Queensland showed that most of the rhizomatous materials present was predominantly *Halophila* and *Halodule* only, while the other species identified (*Thalassia, Cymodocea, Enhalus* and *Zostera*) were present mainly as leaves (Marsh *et al.* 1982).

In some areas in the tropics, dugong disturbance presumably occurs at an intensity similar to that in Moreton Bay. Herds of dugongs up to 150 have been sighted in Shoalwater Bay, and up to 200 in the Gulf of Carpentaria (Preen 1996, pers. comm.). Recent aerial survey data from the Gulf of Carpentaria show some indications that these herds break up before and during the wet season (Preen 1996, pers. comm.). The aerial surveys in the tropics have been held largely during the late dry season (see Preen 1993 for a summary, Table 7.2) when weather conditions are better. Therefore, timing of the surveys could be one of the reasons

why large herds have rarely been reported in this region (e.g. see Heinsohn 1972; Marsh *et al.* 1980). Additionally, the small group sizes seen in the tropics could be an artefact of the aerial survey technique (survey height) used, and concentration of aggregation. Because the observers are limited by the field of vision (strip transect) which is due to low-level altitude of the aircraft. Under these circumstances, it is difficult to decide whether small groups of dugongs are part of a large diffuse herd. Spatial analysis of the distribution in Torres Strait based on aerial surveys conducted at 137 m and 274 m suggest that the dugongs are aggregated in diffuse herds over a scale of several square kilometres in November and March (H. Marsh 1996, pers. comm.).

Changes in community structure

Species composition

The results of this study accord with Preen's (1993) results from the subtropics. Grazing influences the relative abundance of tropical seagrasses in a mixed-species seagrass bed. At Ellie Point, which was dominated by *Zostera/Cymodocea*, grazing disturbance enabled the more opportunistic species, *H. ovalis*, to increase in biomass and occupy more space than the longer-lived species, *Zostera/Cymodocea*. The net effect was to shift the species composition of the community in favour of *H. ovalis* at the expense of *Zostera/Cymodocea* (see Fig 5.6a, see also Chapter 9).

Halophila ovalis

The above-ground biomass of *H. ovalis* increased under both simulated intensive and light grazing in comparison to the control plots (Fig 5.7). This contrasts with Preen's (1993) findings from experiments which were also conducted in winter (but in subtropical Moreton Bay) and which showed no significant changes in the relative abundance of *H. ovalis* as a result of light grazing. Preen suggests that,

within the seagrass community he tested, light grazing disturbance by dugongs does not alter the relative abundance of *Z. capricorni* and *H. spinulosa*, but may reduce the relative abundance of *H. ovalis* in Moreton Bay. The discrepancy between our results could be due to regional differences in growth. *Z. capricorni* in Moreton Bay, has a winter-spring growth period, while at Ellie Point, it has a spring growth period (McKenzie 1994; and this study). This enabled the *H. ovalis* at Ellie Point to colonise the 'feeding trails' during the first month after grazing even during winter (see Fig 5.6b and Appendix 2 Fig 1a). Thus, timing of grazing disturbance may influence the course of recovery as certain species may only be seasonally available to colonise. The relationship of the timing of grazing disturbance to species recovery is discussed below.

In general, after 11 months, the above-ground seagrass biomass was greater than below-ground in grazed plots (see Fig 5.7b). This could be advantageous to dugongs and green turtles. *H. ovalis* is one of the food species most preferred by dugongs and juvenile green turtles in Moreton Bay (Preen 1993 for dugongs; Read 1991, Brand 1995 for green turtles). Leaves of *H. ovalis* are the most digestible among the species examined in this study (Chapter 7) and leaves in general have more nitrogen than the root/rhizome fraction (Lanyon 1991; this study, see Chapter 7). Thus, by grazing on seagrass beds with *H. ovalis*, growth of *H. ovalis* leaves increases, inducing further herbivory (or regular regrazing). Most feeding trails in the tropics have been reported from stands dominated by *H. ovalis* (e.g. Wake 1975; Anderson and Birtles 1978, tropical Queensland; Aragones 1994, Philippines; Supanwanid 1996, Thailand), implying that this species must be an important food item. *Halophila* was found in 89% of the dugong stomachs examined from Queensland (Marsh *et al.* 1982).

Halodule uninervis

In a monospecific bed of *H. uninervis* at Cardwell, no changes in species composition were detected even after intensive grazing. This is consistent with the

results of Preen (1993) who reported no changes in species composition in monospecific stands of *H. ovalis* and *H. uninervis* in Moreton Bay (Area 3). Preen argued that these species are more adapted to disturbance. This is because these species have life history strategies characteristic of opportunistic or pioneering species. Both species have faster growth rates and shorter turnover times than *Zostera capricorni* and *Cymodocea rotundata* which seem to have much slower growth rates and longer turnover times. In Australia, the recorded specific growth rate (% per day; Hillman *et al.* 1989) and turnover time (days) of *H. ovalis* ranged from 4.0-9.0% and 11-24 days (Hillman and McComb 1988), respectively; in *Zostera capricorni* the specific growth rate and turnover time have been estimated to vary from 0.8-3.5% and 32-125 days (Kirkman *et al.* 1982; Larkum *et al.* 1984; King and Holland 1986), respectively. The only available estimates of specific growth rate and turnover time for *Cymodocea rotundata* are from Papua New Guinea (2.5-4.0% and 25-40 days, respectively; Brouns 1987a). These results clearly show why *H. ovalis* is more able to respond to disturbance than *Z. capricorni* or *C. rotundata.* I was not able to find any corresponding data for *H. uninervis*, but it has always been considered as an opportunistic species (e.g. see Den Hartog 1970; Lanyon 1991; Lee Long *et al.* 1993; Preen 1993). I predict that its specific growth rate and turnover time will be closer to those of *H. ovalis* than those of *Z. capricorni* and *C. rotundata. H. uninervis* together with *Cymodocea serrulata* have been observed to colonise a seagrass bed at Cockle Bay destroyed by a cyclone after initial colonisation by *H. ovalis* (Birch and Birch 1984).

Detritus

This study showed that grazing can alter the relative abundance of detrital matter in seagrass beds (see Fig 5.8), confirming that dugong grazing reduces the amounts of detritus available to the detrital chain (Heinsohn *et al.* 1977). The intensive grazing plots had considerably less detrital matter, presumably because most of the plant material was from new growth. Thus, grazing changes the age structure of seagrass communities by increasing populations of young plants. This suggests that

dugongs short-circuit the detrital cycle of seagrass beds. During their annual cycle, seagrass plants produce organic matter, undergo senescence, the leaves die and are exfoliated to decompose *in situ* or be exported from the system (Thayer *et al.* 1975). The process of decomposition usually takes a long time. In the Caribbean, Thayer *et al.* (1975) reported that *Thalassia testudinum* leaves may require more than 8 weeks for 60% of their initial weight to be lost during decomposition. An even longer period is required for this detrital material to be of a size and nutritional state beneficial to fish (detrivores). The nutritional composition of living seagrass blades is altered significantly during the processes of senescence and death (Klumpp *et al.* 1989). The nitrogen concentration in senescent blades of *T. testudinum* was half that of the living material (Zieman *et al.* 1984). Thus, by short-circuiting the detrital cycle, dugongs improve the nutritional state of the seagrass, particularly the leaves. It is also likely that dugongs defecate in the same areas where they graze, thus returning the nutrients in those areas. Therefore, the generalisation that most of the energy flow in the seagrass ecosystem is through the detrital chain (e.g. Robertson and Mann 1980; McRoy and Helfferich 1980; Klumpp *et al.* 1989), may not be true in areas where megaherbivores are present in high densities (e.g. Moreton Bay). In the Caribbean, repeated cropping by green turtles short-circuits the time course of normal decomposition, and defecation enriches the seagrass beds of *T. testudinum* as faeces are supposedly nutritionally better than the food source (higher nitrogen concentration; Thayer *et al.* 1975). Interestingly, both light grazing and cropping plots contained more detritus than the controls after 11 months of recovery (Fig 5.8). This was due to the stimulated growth (compensatory growth) followed by the deaths of seagrass (leaves) in these plots resulting from the simulated grazing (see below and also Chapter 9).

Changes in net above-ground biomass productivity

According to the grazing optimization hypothesis developed for terrestrial grazers, above-ground net primary productivity is maximised at some optimal grazing level (Fig 5.15, and see McNaughton 1979b; Hilbert *et al.* 1981; Dyer *et al.* 1982). The

results at Ellie Point clearly showed that, as grazing intensity increased, the net above-ground biomass productivity (NABP) of *H. ovalis* increased, while that of *Zostera/Cymodocea*, decreased (see Fig. 5.9). The proponents of the grazing optimization hypothesis suggested that plants growing near to their maximum potential relative growth rate have less opportunity to respond positively to grazing and potentially can sustain less grazing than plants with realised growth rates far below maximum. Therefore, the differential NABP response observed in the two main species of seagrass studied at Ellie Point, may mean that under natural conditions (wherein resources are limited), growth rates of *H. ovalis* will be far below their maximum, while in *Zostera/Cymodocea*, it will be near maximum. Grazing, reduces competition for limited resources by opening patches and enabling the more opportunistic of the different seagrass species to thrive. Birch and Birch (1984) observed that *H. ovalis* was one of the first species to colonise a seagrass bed at Cockle Bay after a cyclone, but eventually decreased exponentially as it was excluded by *H. uninervis* and *Cymodocea serrulata*.

These results suggest that *H. ovalis* has a greater capacity to compensate for dugong grazing than any of the other species studied. It is a herbivory-tolerant species. In terrestrial systems, many plants have been shown to be capable of compensatory growth in response to mammalian herbivory (e.g. McNaughton 1983; Paige and Whitman 1987; Houle and Simard 1996). Likewise, plant growth rate is suggested to be undercompensated when overgrazed (McNaughton 1983; Wilson and Macleod 1991), i.e plant growth declines as the intensity of herbivory increases (see Fig 5.15). This is because plants can sustain optimum growth through compensatory mechanisms up until only a certain intensity of grazing (see Fig 5.15, and see McNaughton 1979b; Hilbert *et al.* 1981; McNaughton 1983; Wilson and Macleod 1991). Repeated defoliation could deplete the plants' carbohydrate and protein reserves (Paige and Whitham 1987). Details on the resulting changes in the nutrient content of seagrasses after grazing, and the mechanisms by which dugong grazing increases seagrass productivity will be discussed in Chapter 8. An integration of the effects of grazing on the community

structure and dynamics, including productivity and nutritional composition of tropical seagrasses is presented in Chapter 9.

Recovery

Timing and intensity of grazing

Recovery of seagrasses from grazing disturbance depends on the timing and intensity of the grazing disturbance, and the species composition and location of the beds. Table 5.15 compares the results of this study to previous studies of recovery of seagrasses from grazing. At Ellie Point, both *H. ovalis* and *Zostera/Cymodocea* recovered rapidly from intensive and light grazing, despite the treatments being applied in autumn-winter, when their growth is lowest (see Fig 5.6b and Appendix 2 Fig 1b). In Thailand, recovery of *H. ovalis* was also rapid even though its recovery occurred during the dry season (Supanwanid 1996), when its growth is supposedly at its lowest. The effect of timing of grazing disturbance on the recovery period of seagrasses in subtropical system of Moreton Bay, was more pronounced than suggested by studies in the tropics. Recovery of *H. ovalis* from intensive grazing was relatively faster when it occurred in autumn than in winter (see Table 5.15). From the data available (Table 5.15), it appears that the timing of the grazing disturbance is a more important determinant of the recovery period of seagrasses in the subtropics than in the tropics presumably because seasons are characteristically more pronounced (see Preen 1993 for subtropical seagrasses; Lanyon 1991; McKenzie 1994; Lanyon and Marsh 1995a; this study, for tropical seagrasses).

Recovery can vary

Among the three species in Table 5.15, *H. ovalis* recovered most quickly, followed by *H. uninervis*, then lastly *Z. capricorni*. As mentioned earlier, *H. ovalis* has faster specific growth rate and turnover time than *H. uninervis* and *Z. capricorni*.

The recovery of *H. uninervis* at Cardwell took longer (eight months) than in simulations in Indonesia and Moreton Bay. However, I conducted my experiments in winter (dry season), whereas de Iongh *et al.* (1995) conducted their research over the wet season, while Preen (1993) did his experiments during the summer and autumn growing seasons (see Table 5.15 and Fig 5.10). Another confounding factor was the repeated grazing that occurred within the study sites at Cardwell during the experiments. In Moreton Bay, Preen suggested that the recovery of *H. uninervis* beds from dugong grazing can be suppressed, if recovery is followed by even low levels of sustained grazing pressure. In the Moluccas, East Indonesia, dugong feeding trails on a bed dominated by *H. uninervis* did not show any significant recovery during the dry season (de Iongh *et al.* 1995).

In contrast, dugong feeding trails on *H. ovalis* beds in Thailand recovered rapidly (two months) even though the grazing disturbance occurred during the dry season (Supanwanid 1996). Likewise, dugong feeding trails on *H. ovalis*, at Ellie Point, recovered rapidly (one months), despite grazing occurring in winter. This further supports the notion that that *H. ovalis* has a more opportunistic strategy than *H. uninervis*. Therefore, recovery of seagrass after grazing disturbance depends not only of timing of disturbance but also on the characteristics of the species present.

Effects of spatial variability

Effects of location or regional difference on recovery from grazing was clearly shown by *Z. capricorni* (see Table 5.15). Despite similar timing of grazing experiments, *Z. capricorni*, in the tropics (Ellie Point), recovered more rapidly (3-5 mo) than the same species in the subtropics (Moreton Bay, 6.5-10 mo) despite *Z. capricorni* having a winter-spring growth season in Moreton Bay.

Overall, the recovery of seagrasses from grazing varies according to species, location and timing of the disturbance. As my experiments were conducted during the autumn-winter season, which is the period of low growth, I predict that

tropical seagrasses will recover rapidly from grazing disturbance which occurs at other times of the year.

Conclusions

1) The scale of disturbance from grazing is different from non-grazing disturbances.

2) Grazing changes the structure of tropical seagrass communities by altering their species abundance and composition, and short-circuiting the detrital cycle.

3) Grazing also changes the net above-ground biomass productivity of tropical seagrasses. The magnitude of these effects (# 2 and # 3) depends on nature of the seagrass community, including its species composition, and the nature, intensity and timing of the disturbance caused by herbivory.

4) Among the species examined, *Halophila ovalis* showed the greatest capacity to compensate for herbivory.

5) Recovery of seagrasses from grazing disturbance depends on its timing and intensity, and the species composition and location of the seagrass bed.

6) Herbivory by dugongs and green turtles probably contributes to the spatial heterogeneity of tropical seagrass communities.

Table 5.1. Description of the different treatment levels for the (A) long-term and (B) short-term grazing experiments.

Treatment Level	Description
A. Long-term	
(I) Intensive dugong grazing	all plants uprooted, resulting in bare plots (all above-ground materials removed; some below-ground left, as in intensive dugong grazing)
(II) Light dugong grazing	3 evenly separated feeding strips (about 15 cm wide), all perpendicular to the shoreline
(III) Turtle cropping	leaves cut approximately 1-2 cm above ground (except in *H. ovalis* for which entire above ground biomass was removed)
(IV) Control	undisturbed
B. Short-term	
(I) Light dugong grazing	2 evenly separated feeding strips (each about 15 cm wide), harvested after 4 months
(II) Control 1	undisturbed, harvested with Treatment I
(III) Turtle cropping 1	leaves cut approximately 1-2 cm above ground, harvested after 1 month
(IV) Control 2	undisturbed, harvested with Treatment III
(V) Turtle cropping 2	similar to Treatment III, but harvested after 2 months
(VI) Control 3	undisturbed, harvested with Treatment V

Table 5.2. Dates of the (A) long-term and (B) short-term experiments.

Experimental Site	Date	
	Simulation	Harvest
(A) Long-term		
1) Ellie Point	May 3 - 6, and 19 - 22, 1993[1]	March 27 - 28, and April 25 - 26, 1994[1]
2) Cardwell	June 1 - 5, and 17, 1993[1]	July 7 - 9, and 21 - 23, 1994[1]
(B) Short-term		
Cardwell	August 5 - 7, 1994	Trt[2] I & II, December 1 - 2, 1994
		Trt III & IV, September 17 - 18, 1994
		Trt V & VI, October 16 - 17, 1994

[1] The time difference between the first and second simulation/harvesting events was ignored on the basis of t-tests comparing the biomass of the two sets of harvested samples. Only the first simulation dates and second harvesting dates were used in the final analysis.
[2] Treatment

Table 5.3. Mean and standard errors of leaf dry-weights (g dw/m^2) of each species and mean total leaf biomass (all species combined) at each Latin square at Ellie Point on May 3 - 6, and May 19 - 22, 1993 before the treatments were applied.

Site	*H. ovalis*	*Zostera capricorni* + *Cymodocea rotundata*[*]	*H.uninervis*	Total Leaf Biomass
A	13.91 (0.86)	12.31 (0.54)	3.19 (1.21)	9.80 (1.50)
B	15.11 (1.75)	6.33 (1.63)	0.13 (0.05)	7.19 (1.99)
C	6.15 (0.20)	19.34 (0.61)	0.00	8.50 (2.44)
D	0.50 (0.38)	20.54 (1.33)	0.00	7.01 (2.91)

[*] were combined due to the difficulties in their identification from video images

Table 5.4. Results of the univariate or multivariate repeated measures ANOVA of the grazing experiments at Ellie Point which examined the mean response of the leaf biomass of three different seagrass species (species treated as repeated measures). There were four treatments: three regimes of simulated grazing (intensive and light intensity dugong grazing, and turtle cropping) and control. Included are the results from the Epsilon corrected averaged F using Greenhouse-Geisser (GG)[1]. All F ratios were compared to the within residuals. Random factors are italicised, other factors are fixed. Significant p values are in bold.

Factor	SS	df (GG adjusted)	MS	F	p (GG corrected)
between-subjects[2]					
Site	92.84	3	30.95	9.66	**0.004**
Treatment	229.89	3	76.63	23.92	**0.000**
within-subject[3]					
Time	1573.67	10 (4.00)	157.37	146.77	**0.000 (0.000)**
Species	4640.49	2 (1.17)	2320.24	75.22	**0.000 (0.000)**
Species*Time	1453.92	20 (2.54)	72.70	24.47	**0.000 (0.000)**
Treatment*Species	389.38	6 (3.52)	64.90	2.01	0.103 (0.155)
Treatment*Time	691.78	30 (11.99)	23.06	21.51	**0.000 (0.000)**
*Site**Time	450.91	30 (11.99)	15.03	14.02	**0.000 (0.000)**
*Site**Species	4651.75	6 (3.52)	775.29	25.13	**0.000 (0.000)**
*Site**Species*Time	1192.34	60 (7.62)	19.87	6.69	**0.000 (0.000)**
Treatment*Species*Time	486.51	60 (7.62)	8.11	2.73	**0.000 (0.030)**

[1] Greenhouse-Geisser is a very conservative test which corrects for the correlation through time which contradicts the assumptions for univariate repeated measures ANOVA. Therefore, results from this test are considerably more reliable than unadjusted (Norusis 1993).
[2] Between-subjects effects are averaged over time.
[3] Within-subjects effects are dependent on time.

Table 5.5. Results of the univariate or multivariate repeated measures ANOVAs of the grazing experiments at Ellie Point which examined the mean response of the leaf biomass of the individual seagrass species from the four treatments. Included are the results from the Epsilon corrected averaged *F* using Greenhouse-Geisser (GG)[1]. All *F* ratios were compared to the within residuals. Random factors are italicised, other factors are fixed. Significant *p* values are in bold.

Factor	SS	df (GG adjusted)	MS	*F*	*p* (GG corrected)
(1) *Halophila ovalis*					
between-subjects[2]					
Site	1971.68	3	657.23	31.78	**0.000**
Treatment	7.37	3	2.46	0.12	0.947
within-subjects[3]					
Time	1540.21	10 (2.99)	154.02	54.91	**0.000 (0.000)**
*Site**Time	586.22	30 (8.98)	19.54	6.97	**0.000 (0.000)**
Treatment*Time	595.19	30 (8.98)	19.84	7.07	**0.000 (0.000)**
(2) *Zostera/Cymodocea*					
between-subjects					
Site	2232.75	3	744.25	19.67	**0.000**
Treatment	594.17	3	198.06	5.23	**0.023**
within-subjects					
Time	1443.40	10 (1.94)	144.34	40.69	**0.000 (0.000)**
*Site**Time	924.63	30 (5.82)	30.82	8.69	**0.000 (0.000)**
Treatment*Time	562.90	30 (5.82)	18.76	5.29	**0.000 (0.003)**
(3) *Halodule uninervis*					
between-subjects					
Site	540.16	3	180.05	28.24	**0.000**
Treatment	17.73	3	5.91	0.93	0.466
within-subjects					
Time	43.99	10 (2.57)	4.40	6.66	**0.000 (0.003)**
*Site**Time	132.40	30 (7.72)	4.41	6.68	**0.000 (0.000)**
Treatment*Time	20.20	30 (7.72)	0.67	1.02	0.455 (0.448)

[1] Greenhouse-Geisser is a very conservative test which corrects for the correlation through time which contradicts the assumptions for univariate repeated measures ANOVA. Therefore, results from this test are considerably more reliable than unadjusted (Norusis 1993).

[2] Between-subjects effects are averaged over time.

[3] Within-subject effects are dependent on time.

Table 5.6. A comparison of the mean leaf biomass (g dw/m^2) of each species at selected times at Ellie Point in response to the different treatments: (1) intensive grazing, (2) light grazing, (3) cropping, and (4) undisturbed. In parentheses are the standard errors of the mean.

Treatment	Before simulation (May 1993)	After 1 Month (June)	After 4 Months (September)	After 10 Months (March 1994)
Halophila ovalis				
Treatment 1	8.03 (3.17)	0.21 (0.15)	6.84 (2.15)	17.42 (2.61)
Treatment 2	8.38 (3.27)	2.80 (1.11)	6.16 (2.41)	14.99 (3.60)
Treatment 3	9.85 (3.53)	3.76 (1.55)	7.34 (2.76)	11.14 (3.46)
Treatment 4	9.43 (4.17)	4.36 (1.97)	5.46 (1.64)	9.49 (3.33)
Z. capricorni + *C. rotundata*				
Treatment 1	16.29 (3.13)	0.09 (0.05)	6.84 (2.15)	8.51 (2.84)
Treatment 2	14.98 (3.53)	4.35 (1.24)	6.16 (2.41)	14.99 (3.60)
Treatment 3	14.01 (2.88)	4.64 (1.14)	11.89 (2.51)	8.39 (3.62)
Treatment 4	13.24 (4.08)	9.23 (3.29)	12.11 (3.56)	11.28 (4.12)
Halodule uninervis				
Treatment 1	0.80 (0.75)	0.00	0.87 (0.87)	1.55 (1.44)
Treatment 2	0.63 (0.54)	0.30 (0.30)	1.11 (1.11)	2.50 (2.45)
Treatment 3	0.25 (0.22)	0.38 (0.23)	0.84 (0.84)	0.96 (0.85)
Treatment 4	1.64 (1.64)	0.62 (0.62)	1.40 (1.40)	2.20 (2.18)

Table 5.7. Results of the multi-factor analysis of variance which examined the mean response of the biomass of separated (leaf and roots/rhizomes fractions) and combined (whole plant) plant parts, and ratio of the biomass of roots/rhizomes to leaves to the different treatment levels (intensive and light grazing, cropping and undisturbed) of two seagrass species (*H. ovalis* and *Zostera/Cymodocea*) harvested from Ellie Point. Random factors are italicised, other factors are fixed. Significant *p* values are in bold.

Factor	Error Term	SS	df	MS	*F*	*p*
1) Leaves						
Site	Residual	242.63	3	80.88	1.43	0.237
Treatment	*Site**Treatment	246.44	3	82.15	7.67	**0.008**
Species	Species**Site*	432.18	1	432.18	0.36	0.590
*Site**Treatment	Residual	96.35	9	10.71	0.19	0.995
Species**Site*	Residual	3589.17	3	1196.39	21.22	**0.000**
Species*Treatment	*Site**Species*Treatment	599.11	3	199.70	4.04	**0.045**
*Site**Species*Treatment	Residual	444.99	9	49.44	0.88	0.549
Residual[1]		5412.12	96	56.38		
2) Roots/Rhizomes						
Site	Residual	11967.60	3	3989.20	11.19	**0.000**
Treatment	*Site**Treatment	1790.14	3	596.71	1.66	0.246
Species	Species**Site*	19793.66	1	19793.66	1.51	0.306
*Site**Treatment	Residual	3254.61	9	361.62	1.01	0.434
Species**Site*	Residual	39256.12	3	13085.37	36.72	**0.000**
Species*Treatment	*Site**Species*Treatment	4088.25	3	1362.75	2.08	0.174
*Site**Species*Treatment	Residual	5907.08	9	656.34	1.84	0.070
Residual[1]		34213.70	96	356.39		

[1] = Site*Sp*Trt*Replication

Factor	Error Term	SS	df	MS	F	p
3) Whole Plant						
Site	Residual	14503.51	3	4834.50	8.49	**0.000**
Treatment	*Site**Treatment	840.91	3	280.30	0.68	0.589
Species	Species**Site*	14376.25	1	14376.25	0.65	0.479
*Site**Treatment	Residual	3734.91	9	414.99	0.73	0.681
Species**Site*	Residual	66425.40	3	22141.80	38.89	**0.000**
Species*Treatment	*Site**Species*Treatment	7813.87	3	2604.62	2.57	0.119
*Site**Species*Treatment	Residual	9126.67	9	1014.07	1.78	0.082
Residual		54656.58	96	569.34		
4) Ratio of roots/rhizomes to leaves[2]						
Site	Residual	0.06	3	0.02	0.54	0.655
Treatment	*Site**Treatment	0.39	3	0.13	6.41	**0.013**
Species	Species**Site*	3.97	1	3.97	37.28	**0.009**
*Site**Treatment	Residual	0.18	9	0.02	0.51	0.863
Species**Site*	Residual	0.32	3	0.11	2.68	0.051
Species*Treatment	*Site**Species*Treatment	0.02	3	0.01	0.21	0.889
*Site**Species*Treatment	Residual	0.31	9	0.03	0.88	0.548
Residual		3.82	96	0.04		

[2] transformed into log(ratio +1) before analysis

Table 5.8. Results of the multi-factor analysis of variance of the mean response of the detrital matter (g dw/m^2) from the different treatment levels (simulated intensive and light grazing and cropping, and undisturbed) after 11 months of recovery of the seagrasses (species pooled together) at Ellie Point. Significant *p* values are in bold.

Factor	Error	SS	df	MS	*F*	*p*
Site	Residual	31.00	3	10.33	4.89	**0.006**
Treatment	Treatment*Site	62.28	3	20.76	8.41	**0.006**
Treatment*Site	Residual	22.21	9	2.47	1.17	0.345
Residual[1]		73.95	35	2.11		

[1] = Treatment*Site*Replication

Table 5.9. Results of the univariate or multivariate repeated measures ANOVA of the grazing experiments at Cardwell which examined the mean response of the leaf biomass of *Halodule uninervis* over time to four treatments: three regimes of simulated grazing (simulated intensive and light dugong grazing, and turtle cropping) and control. Enclosed in parentheses are the results from the Epsilon corrected averaged F using Greenhouse-Geisser (GG)[1]. All F ratios were compared to the within residuals. Random factors are italicised, other factors are fixed. Significant p values are in bold.

Factor	SS	df (GG adjusted)	MS	F	p (GG corrected)
Between-subjects[2]					
Site	546.24	3	182.08	21.37	**0.000**
Treatment	2326.80	3	75.60	91.05	**0.000**
Within-subject[3]					
Time	5875.73	13 (3.67)	451.98	189.52	**0.000 (0.000)**
Treatment*Time	1419.35	39 (11.02)	36.39	15.26	**0.000 (0.000)**
*Site**Time	457.77	39 (11.02)	11.74	4.92	**0.000 (0.000)**

[1] Greenhouse-Geisser is a very conservative test which corrects for the correlation through time which contradicts the assumptions for univariate repeated measures ANOVA. Therefore, results from this test are considerably more reliable than unadjusted (Norusis 1993).
[2] Between-subjects effects are averaged over time.
[3] Within-subject effects are dependent on time.

Table 5.10. Results of the multi-factor analysis of variance of the mean response of separated (leaves and roots/rhizomes) and combined (whole plant) (g dw/m^2) plant parts, and ratio of roots/rhizomes to leaves of *H. uninervis* after 12 months of recovery from the different treatment levels (simulated intensive and light grazing, cropping, and undisturbed) at Cardwell. Random factors are italicised, other factors are fixed. Significant *p* values are in bold.

Factor	Error	SS	df	MS	*F*	*p*
(1) Leaves						
Site	Residual	137.83	3	45.94	7.60	**0.000**
Treatment	Treatment**Site*	171.46	3	57.15	16.14	**0.001**
Treatment**Site*	Residual	31.88	9	3.54	0.59	0.802
Residual[1]		290.04	48	6.04		
(2) Roots/rhizomes						
Site	Residual	818.35	3	272.78	3.94	**0.014**
Treatment	Treatment**Site*	3595.18	3	1198.39	56.61	**0.000**
Treatment**Site*	Residual	190.52	9	21.17	0.31	0.969
Residual		3326.96	48	69.31		

[1] = Treatment**Site**Replication

Factor	Error	SS	df	MS	F	p
(3) Whole Plant						
Site	Residual	1530.49	3	510.16	4.89	**0.005**
Treatment	Treatment**Site*	5288.85	3	1762.95	48.57	**0.000**
Treatment**Site*	Residual	326.69	9	36.30	0.35	0.953
Residual		5004.58	48	104.26		
(4) Ratio of roots/rhizomes to leaves[2]						
Site	Residual	0.02	3	0.01	2.55	0.067
Treatment	Treatment**Site*	0.16	3	0.05	48.18	**0.000**
Treatment**Site*	Residual	0.01	9	0.00	0.34	0.959
Residual		0.15	48	0.00		

[2] transformed into log (ratio +1) before analysis

Table 5.11. Results of the univariate or multivariate repeated measures ANOVA for the short-term grazing experiments at Cardwell which examined the mean response of the leaf biomass of *Halodule uninervis* over time involving three-paired treatments (see footnotes). Enclosed in parentheses are the results from the Epsilon corrected averaged F using Greenhouse-Geisser (GG)[1]. All F ratios were compared to the within residuals. Random factors are italicised, other factors are fixed. Significant p values are in bold.

Factor	SS	df (GG adjusted)	MS	F	p (GG corrected)
A) Treatments I & II[2]					
between-subjects[3]					
Site	92.45	3	30.82	3.15	0.186
Treatment	542.72	1	542.72	55.43	**0.005**
within-subject[4]					
Time	1619.72	7 (2.53)	231.39	313.06	**0.000 (0.000)**
Treatment*Time	73.36	7 (2.53)	10.48	14.18	**0.000 (0.002)**
*Site**Time	24.28	21 (7.58)	1.16	1.56	0.157 (0.276)
Residuals[5]	15.52	21 (7.58)	0.74		
B) Treatments III & IV[6]					
between-subjects					
Site	108.82	3	36.27	1.65	0.345
Treatment	496.69	1	496.69	22.61	**0.018**

[1] Greenhouse-Geisser (GG) is a very conservative test which corrects for the correlation through time which contradicts the assumptions for univariate repeated measures ANOVA. Therefore, results from this test are considerably more reliable than unadjusted (Norusis 1993).
[2] Simulated low intensity dugong grazing and its controls
[3] Between-subjects effects are averaged over time
[4] Within-subject effects are dependent on time
[5] = *site**treatment*replication
[6] Simulated turtle cropping harvested after one month and its controls

Factor	SS	df (GG adjusted)	MS	F	p (GG corrected)
B) Treatments III & IV					
within-subject					
Time	173.78	3 (1.17)	57.93	24.04	**0.000 (0.010)**
Treatment*Time	168.90	3 (1.17)	56.30	23.36	**0.000 (0.011)**
*Site**Time	15.07	9 (3.52)	1.67	0.69	0.702 (0.625)
Residuals	21.69	9 (3.52)	2.41		
C) Treatments V & VI[7]					
between-subjects					
Site	66.15	3	22.05	2.97	0.198
Treatment	491.35	1	491.35	66.19	**0.004**
within-subject					
Time	295.91	5 (1.73)	59.18	114.64	**0.000 (0.000)**
Treatment*Time	130.63	5 (1.73)	26.13	50.61	**0.000 (0.000)**
*Site**Time	9.00	15 (5.19)	0.60	1.16	0.387 (0.435)
Residuals	7.74	15 (5.19)	0.52		

[7] Simulated turtle cropping harvested after two months and its controls

Table 5.12. Results of the analysis of variance which examined the responses of the different plant parts (leaf and root/rhizome biomass), whole plant and ratio of roots/rhizomes to leaves of *Halodule uninervis* harvested from the short-term grazing experiments (see footnotes) conducted at Cardwell. Random factors are italicised, other factors are fixed. Significant *p* values are in bold.

Factor	Error Term	SS	df	MS	*F*	*p*
1) Treatments I & II[1]						
a) Leaves						
treatment	site*treatment	21.03	1	21.03	53.05	**0.005**
site	residual	34.27	3	11.42	12.07	**0.000**
*site**treatment	residual	1.19	3	0.40	0.42	0.741
residual[2]		37.87	40	0.95		
b) Roots/Rhizomes						
treatment	site*treatment	136.45	1	136.45	82.87	**0.003**
site	residual	273.33	3	91.20	13.15	**0.000**
*site**treatment	residual	4.94	3	1.65	0.24	0.870
residual		277.33	40	6.93		
c) Whole Plant						
treatment	site*treatment	12215.16	1	12215.16	36.97	**0.009**
site	residual	4797.76	3	1599.25	3.50	**0.024**
*site**treatment	residual	991.21	3	330.40	0.72	0.544
residual		18254.72	40	456.37		
d) Ratio of roots/rhizomes to leaves[3]						
treatment	site*treatment	0.02	1	0.02	25.06	**0.015**
site	residual	0.05	3	0.02	4.35	**0.010**
*site**treatment	residual	0.00	3	0.00	0.19	0.903
residual		0.16	40	0.00		

[1] simulated light grazing and its controls
[2] = *site**treatment*replication
[3] transformed to log (ratio + 1) before analysis

Factor	Error Term	SS	df	MS	*F*	*p*
2) Treatments III & IV[4]						
a) Leaves						
treatment	*site**treatment	41.47	1	41.47	35.59	**0.009**
site	residual	1.28	3	0.43	3.22	**0.033**
*site**treatment	residual	3.50	3	1.17	8.76	**0.000**
residual		5.32	40	0.13		
b) Roots/Rhizomes						
treatment	*site**treatment	17.02	1	17.02	4.92	0.113
site	residual	12.36	3	4.12	4.10	**0.013**
*site**treatment	residual	10.38	3	3.46	3.44	**0.026**
residual		40.24	40	1.01		
c) Whole Plant						
treatment	site*treatment	5924.29	1	5924.29	8.08	0.066
site	residual	5370.08	3	1790.03	28.69	**0.000**
*site**treatment	residual	2200.56	3	733.52	11.76	**0.000**
residual		2495.58	40	62.39		
d) Ratio of roots/rhizomes to leaves[3]						
treatment	*site**treatment	0.58	1	0.58	68.69	**0.004**
site	residual	0.09	3	0.03	6.62	**0.001**
*site**treatment	residual	0.03	3	0.01	1.84	**0.156**
residual		0.18	40	0.00		

[3] transformed to log (ratio + 1) before analysis
[4] simulated cropping harvested after one month and its controls

Factor	Error Term	SS	df	MS	*F*	*p*
3) Treatments V & VI[5]						
a) Leaves						
treatment	*site**treatment	20.63	1	20.63	66.75	**0.004**
site	residual	1.50	3	0.50	0.93	0.435
*site**treatment	residual	0.95	3	0.31	0.58	0.635
residual		21.50	40	0.54		
b) Roots/Rhizomes						
treatment	*site**treatment	2.12	1	2.12	0.53	0.521
site	residual	27.98	3	9.33	3.47	**0.025**
*site**treatment	residual	12.07	3	4.02	1.50	0.230
residual		107.63	40	2.69		
c) Whole Plant						
treatment	site*treatment	921.99	1	921.99	7.23	0.074
site	residual	3206.17	3	1068.72	6.44	**0.001**
*site**treatment	residual	382.40	3	127.47	0.77	0.519
residual		6640.47	40	166.01		
d) Ratio of roots/rhizomes to leaves[3]						
treatment	*site**treatment	0.19	1	0.19	7.84	0.068
site	residual	0.08	3	0.03	6.64	**0.001**
*site**treatment	residual	0.07	3	0.02	5.90	**0.002**
residual		0.17	40	0.00		

[3] transformed to log (ratio + 1) before analysis
[5] simulated cropping harvested after two months and its controls

Table 5.13. Results of the multi-factor analysis of variance which examined the mean response of the net above-ground biomass production (NABP) of three seagrass species (*H. ovalis*, *Z. capricorni* + *C. rotundata*, and *H. uninervis*) to the different treatment levels (intensive and light grazing, cropping and undisturbed) at Ellie Point. Random factors are italicised, other factors are fixed. Significant *p* values are in bold.

Factor	Error Term	SS	df	MS	*F*	*p*
Site	Residual	104.59	3	34.86	2.16	**0.098**
Treatment	*Site**Treatment	43.69	3	14.56	4.64	**0.032**
Species	Species**Site*	736.96	1	736.96	3.01	0.181
*Site**Treatment	Residual	28.25	9	3.14	0.19	0.994
Species**Site*	Residual	733.66	3	244.55	15.15	**0.000**
Species*Treatment	*Site**Species*Trt	257.93	3	85.98	10.54	**0.003**
*Site**Species*Trt[1]	Residual	73.43	9	8.16	0.51	0.867
Residual[2]		1549.85	96	16.14		

[1] Trt = Treatment
[2] = Site*Species*Treatment*Replication

Table 5.14. Results of the multi-factor analysis of variance which examined the mean response of the net above-ground (NABP) of *H.uninervis* from long-term experiment at Cardwell to the different treatment levels (high and low grazing, cropping and undisturbed). Random factors are italicised, other factors are fixed. Significant *p* values are highlighted in bold.

Factor	Error Term	SS	df	MS	*F*	*p*
Site	Residual	2.25	3	0.75	24.99	**0.000**
Treatment	*Site**Treatment	10.41	3	3.47	32.09	**0.000**
*Site**Treatment	Residual	0.97	9	0.11	2.11	**0.047**
Residual[1]		2.46	48	0.05		

[1] = Site*Treatment*Replication

Table 5.15. Results of the multi-factor analysis of variance which examined the mean response of the net above-ground biomass productivity (NABP) of *H.uninervis* from short-term experiments (see footnotes) conducted at Cardwell. Random factors are italicised, other factors are fixed. Significant *p* values are highlighted in bold.

Factor	Error Term	SS	df	MS	*F*	*p*
Treatments I & II[1]						
Site	Residual	2.77	3	0.92	1.04	0.385
Treatment	*Site**Treatment	0.47	1	0.47	3.23	0.170
*Site**Treatment	Residual	0.44	3	0.15	0.16	0.919
Residual[2]		35.54	40	0.89		
Treatments III & IV[3]						
Site	Residual	36.24	3	12.08	11.99	**0.000**
Treatment	*Site**Treatment	0.23	1	0.23	0.03	0.878
*Site**Treatment	Residual	24.88	3	8.29	8.23	**0.000**
Residual		40.31	40	1.01		
Treatments V & VI[4]						
Site	Residual	3.03	3	1.01	1.71	0.180
Treatment	*Site**Treatment	14.91	1	14.91	132.99	**0.001**
*Site**Treatment	Residual	0.34	3	0.11	0.19	0.903
Residual		23.66	40	0.59		

[1] simulated low grazing and its controls
[2] Residual = Site*Treatment*Replication
[3] simulated cropping harvested after one month and its controls
[4] simulated cropping harvested after two months and its controls

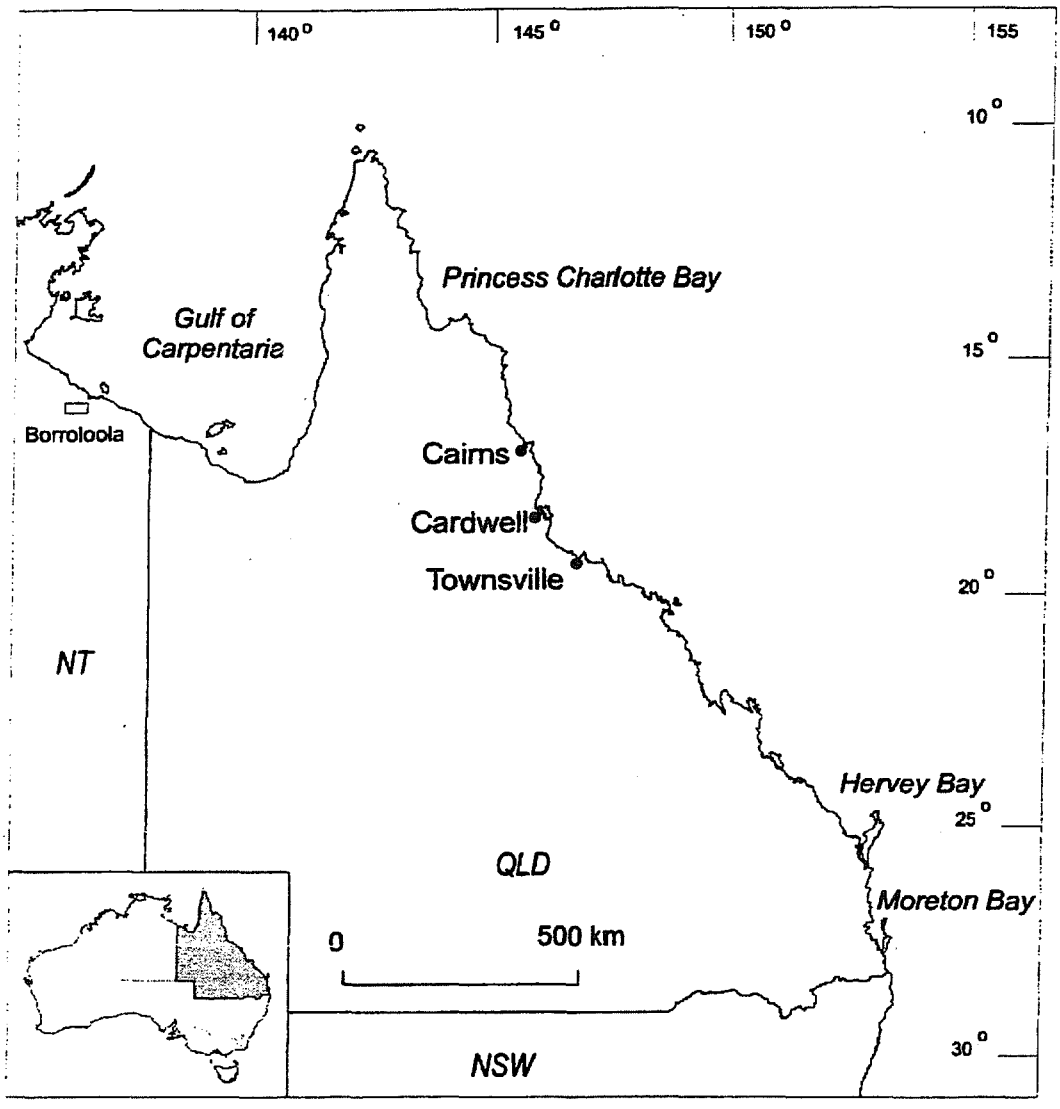

Fig. 5.1. Map showing locations of Cairns and Cardwell, where the grazing experiments were conducted. Locations of other sites relevant to this study are also shown. They include Moreton Bay, Hervey Bay, Townsville, and Princess Charlotte Bay in Queensland, Boroloola in the Northern Territory.

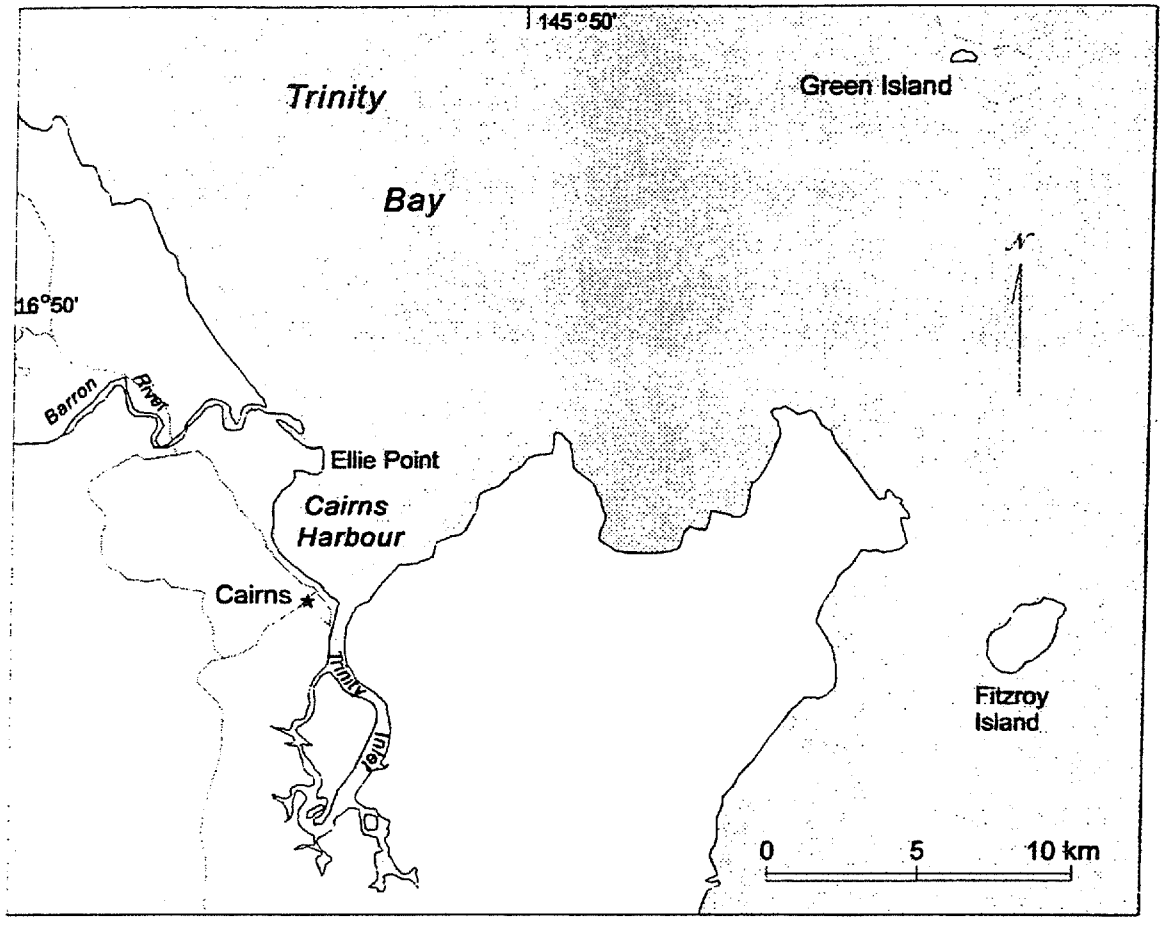

Fig. 5.2. Map showing the location of Ellie Point in relation to Cairns, as well as the location of Green Island (Chapter 7).

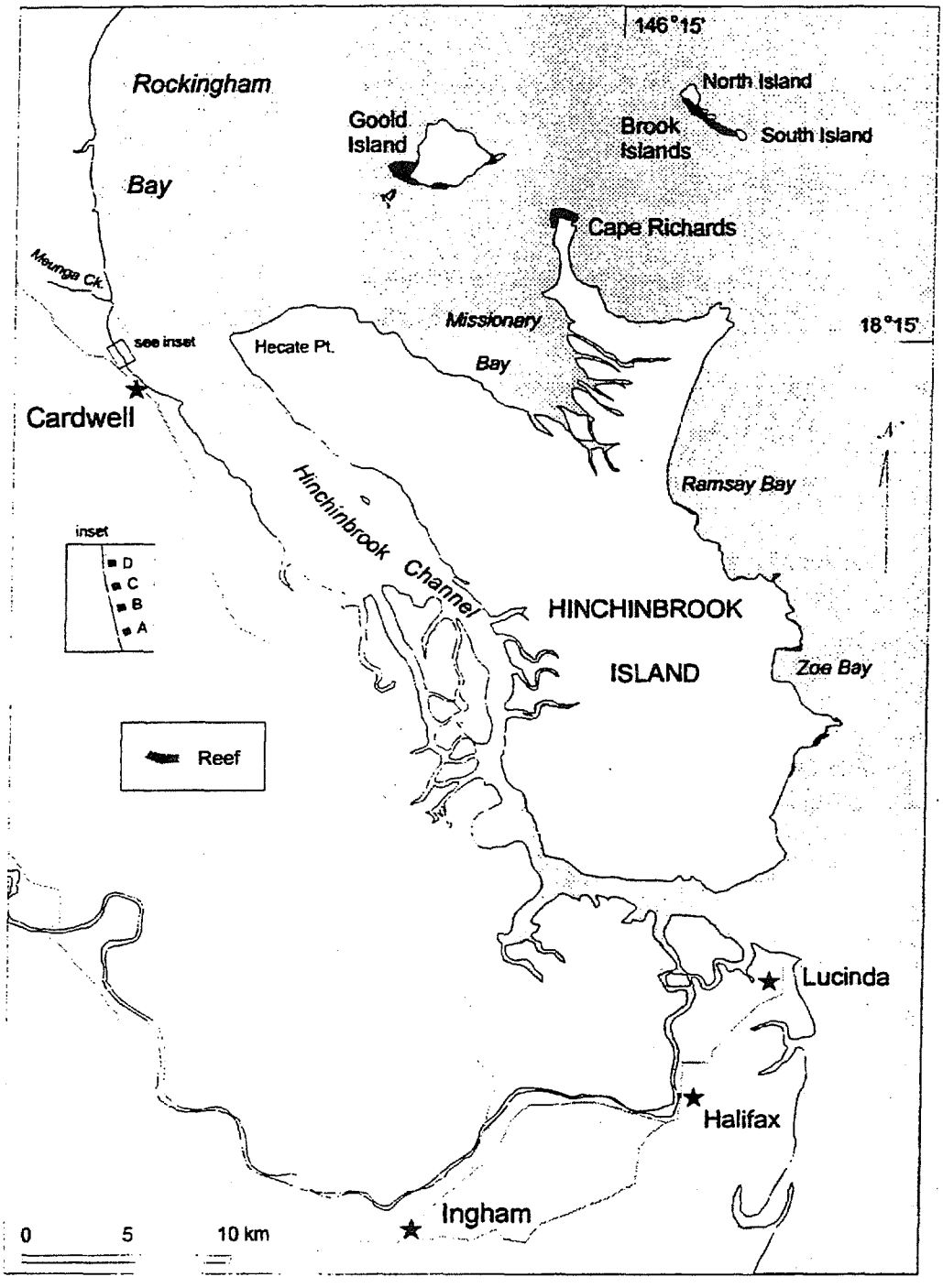

Fig. 5.3 Map showing location of Cardwell and other sites relevant to this study such as North Brook Island (Chapter 7). Inset shows the distribution of the four study sites (Latin squares A to D).

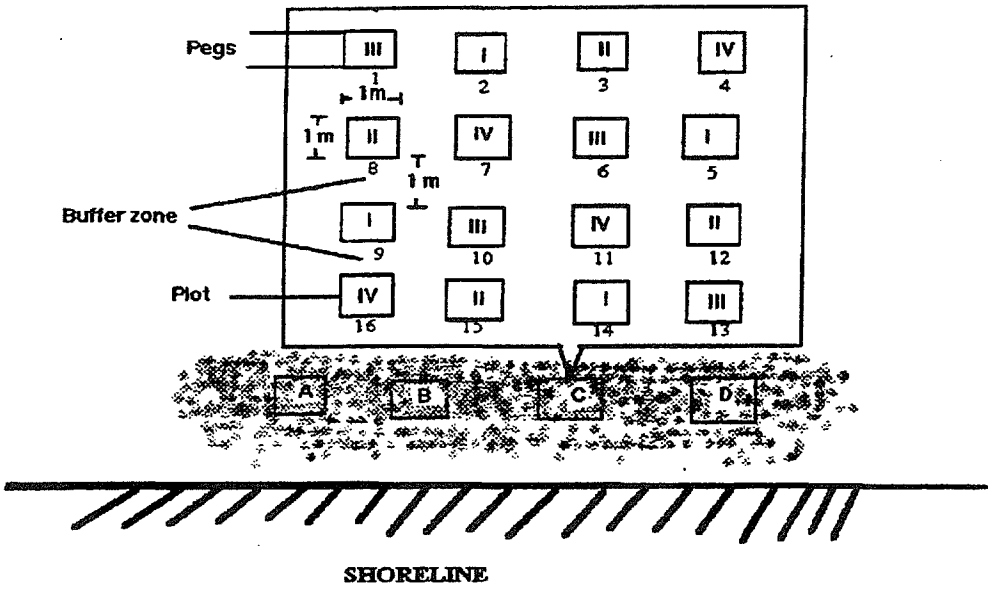

Fig. 5.4. Diagram of the arrangement of each of the four Latin squares (= sites) on the seagrass beds at Ellie Point and Cardwell for the long-term experiments. Inset shows a detailed arrangement of a 4 x 4 Latin square design, dimension of plots and buffer zones, and locations of pegs for markers. The different treatments were: (I) intensive grazing, (II) light grazing, (III) cropping, and (IV) undisturbed.

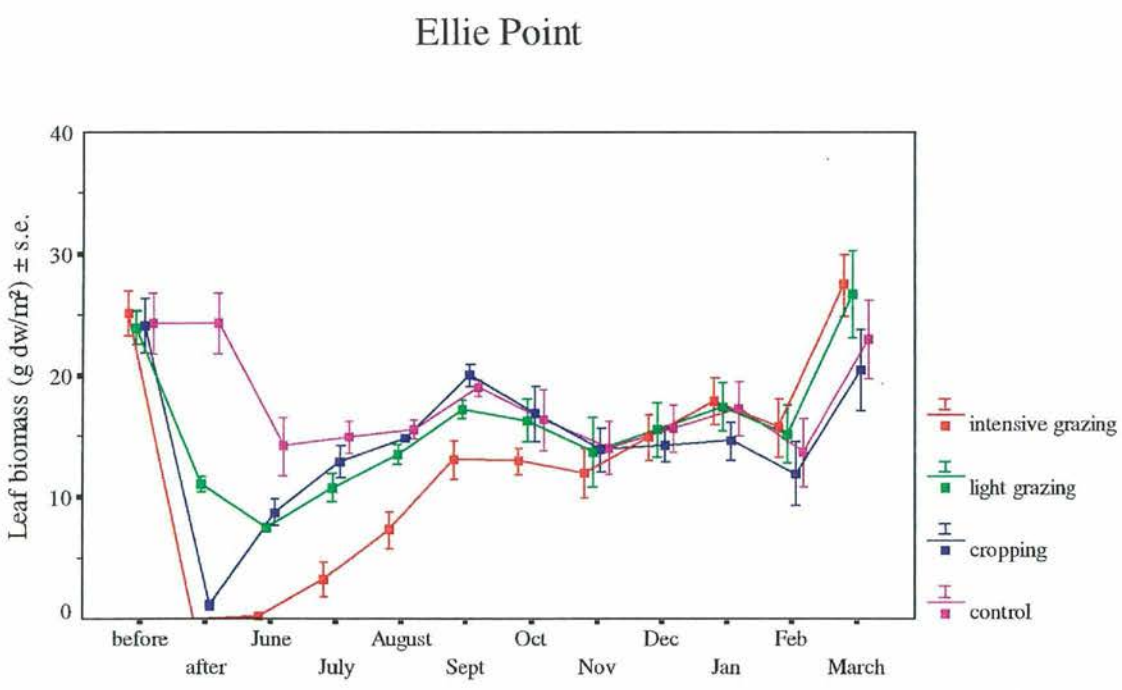

Fig. 5.5. The response of the leaf biomass (g dw/m^2) of all seagrass species combined to the different treatment levels (simulated high and low intensity dugong grazing, turtle cropping and control) over time at Ellie Point; showing the means (n=4) with standard errors. Both before and after simulation measurements were carried out on the same days in May 1993.

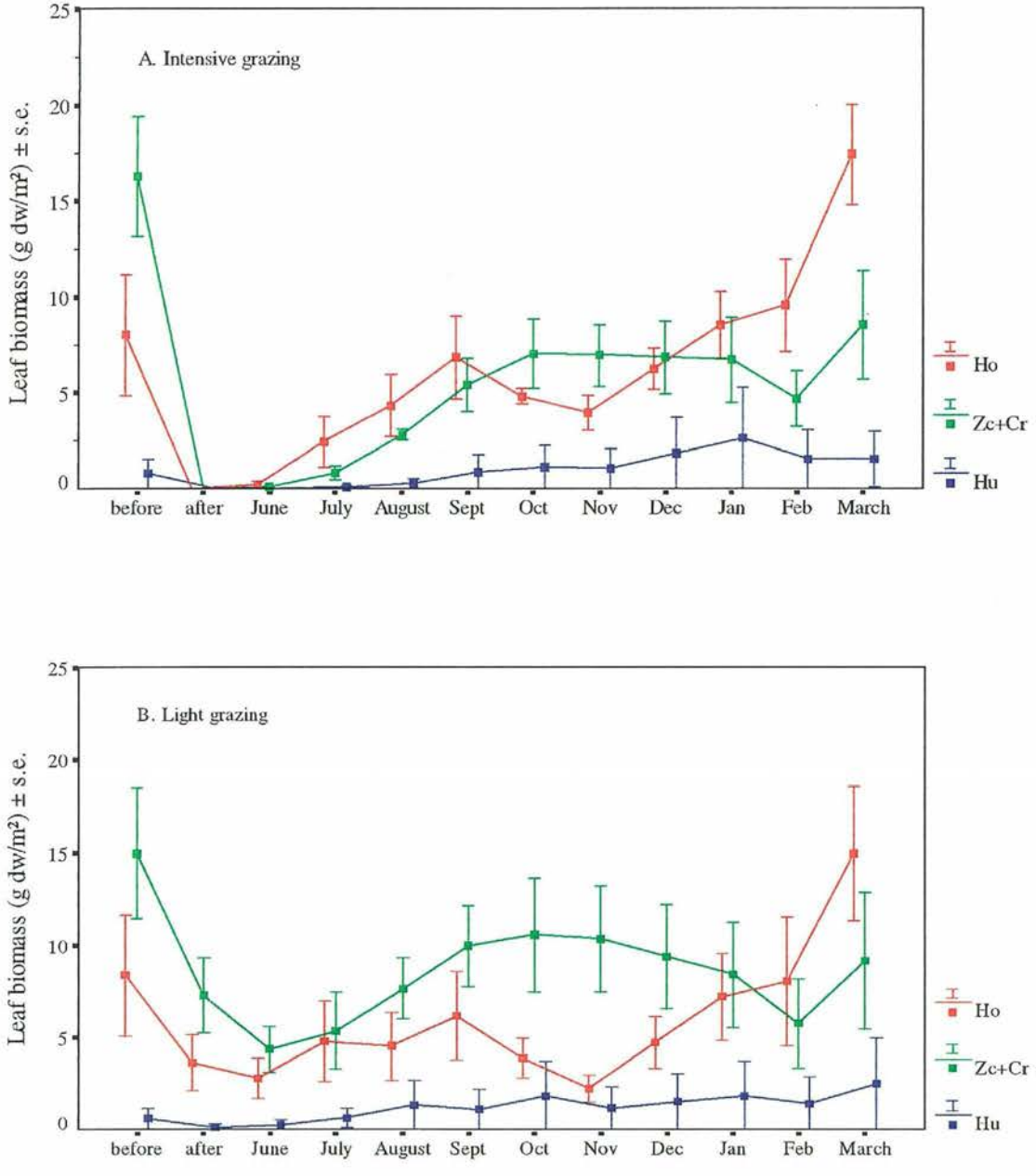

Fig. 5.6a & b. The response of the leaf biomass (g dw/m^2) of the three seagrass species (Ho = *H. ovalis*, red colour; Zc+Cr = *Zostera/Cymodocea,* green colour; and Hu = *H. uninervis*, blue colour) to the different treatment levels: (**a**) intensive grazing and (**b**) light grazing, over time at Ellie Point; showing the means (n=4) with standard errors. Both before and after simulation measurements were carried out on the same days in May 1993.

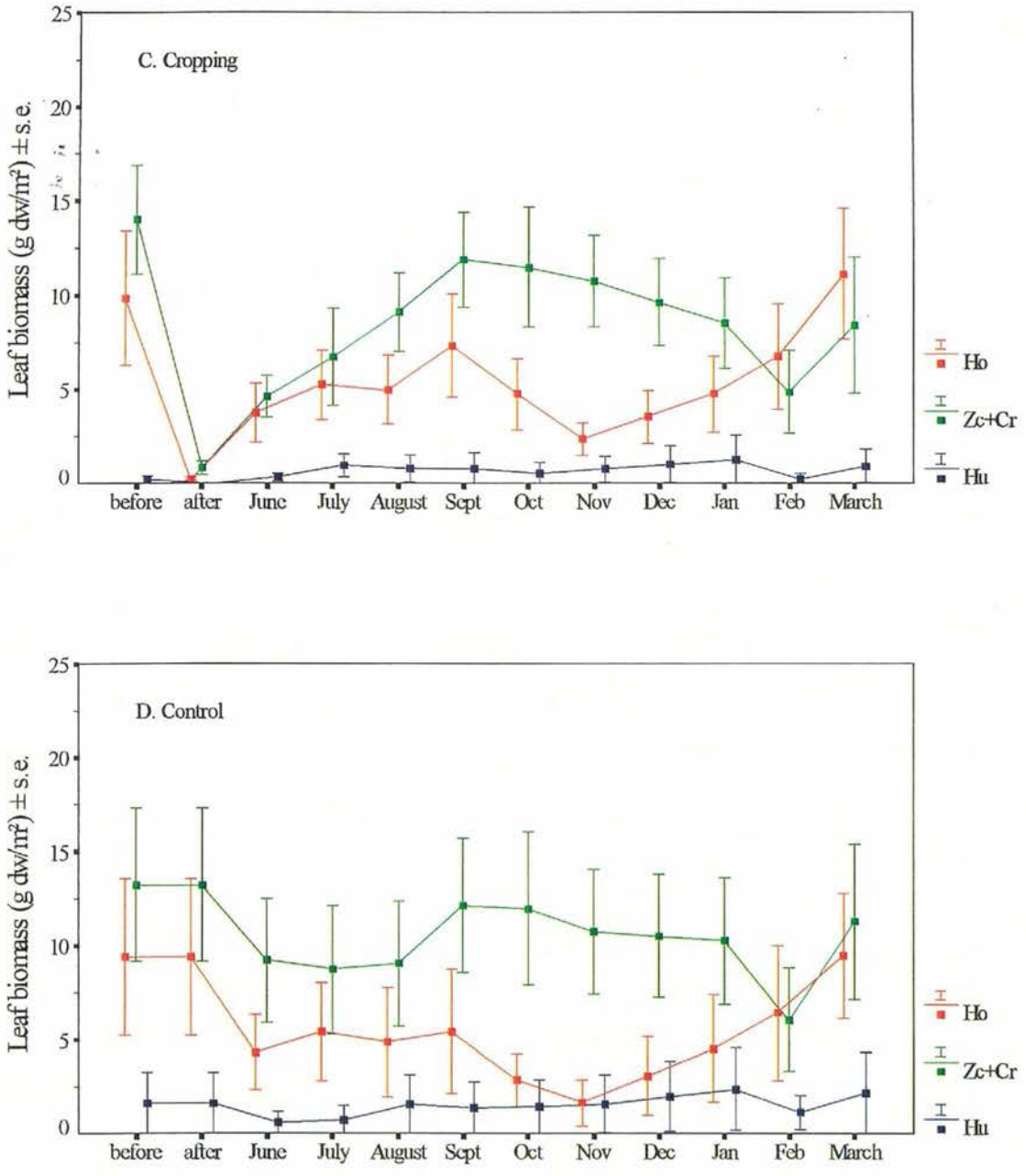

Fig. 5.6c & d. The response of the leaf biomass (g dw/m^2) of the three seagrass species (Ho = *H. ovalis*, red colour; Zc+Cr = *Zostera/Cymodocea*, green colour; and Hu = *H. uninervis*, blue colour) to the different treatment levels: (**c**) cropping, and (**d**) control, over time at Ellie Point; showing the means (n=4) with standard errors. Both before and after simulation measurements were carried out on the same days in May 1993.

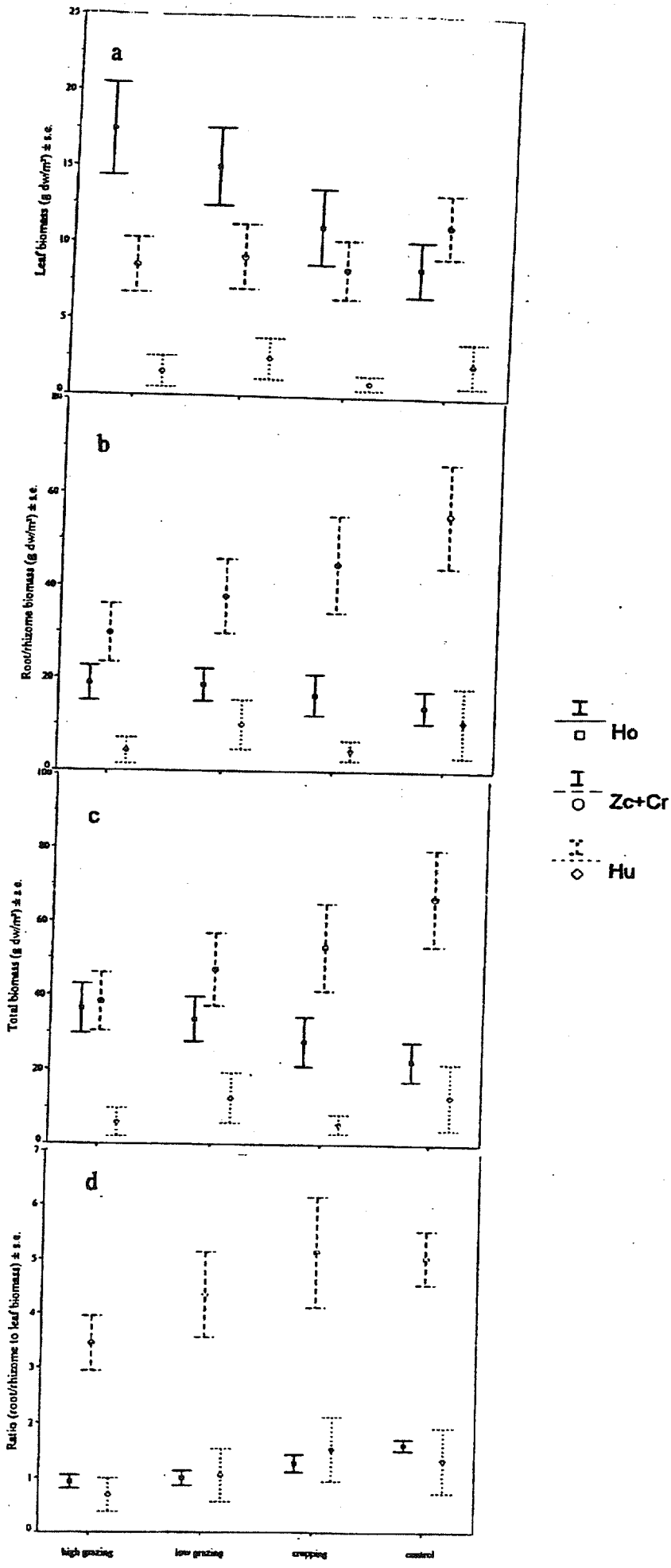

Fig 5.7. Plots of the mean response (g dw/m ^{2}, with standard errors) of (a) leaves, (b) roots/rhizomes (c) total biomass and (d) ratio of the roots and rhizomes biomass to leaves of (Ho) *Halophila ovalis*, (Zc + Cr) *Zostera capricorni* plus *Cymodocea rotundata*, and (Hu) *Halodule uninervis* to the different treatment levels after 10 months at Ellie Point.

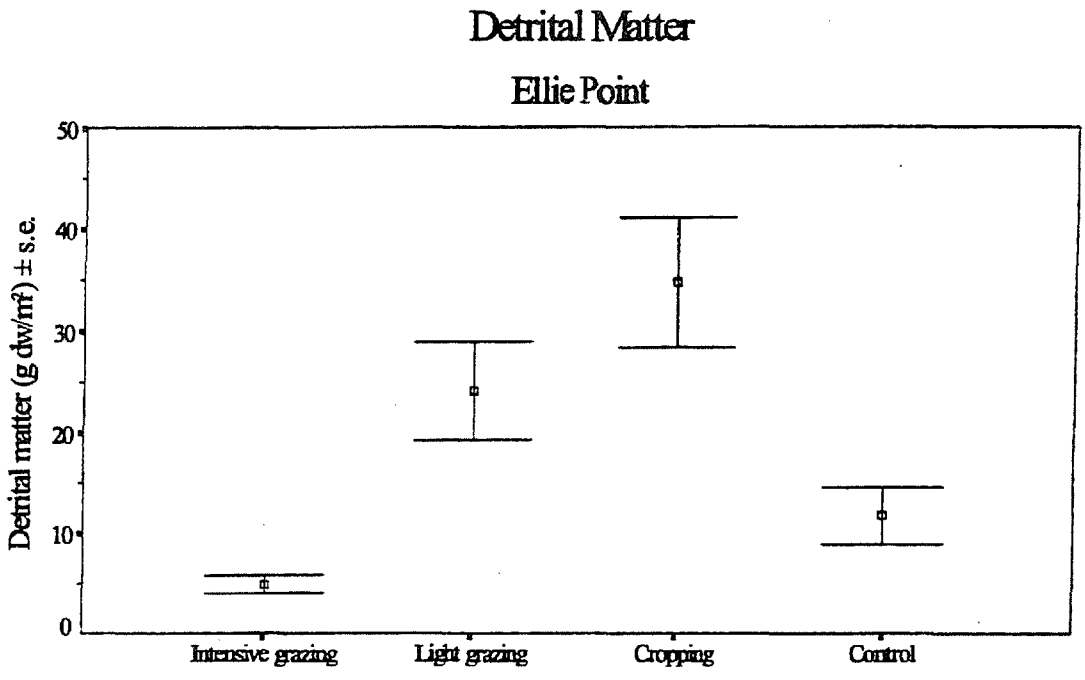

Fig. 5.8. The mean levels of detrital matter with standard errors (g dw/m^2) after 10 months of recovery from the different treatments (simulated intensive and light grazing and turtle cropping, and undisturbed) at Ellie Point.

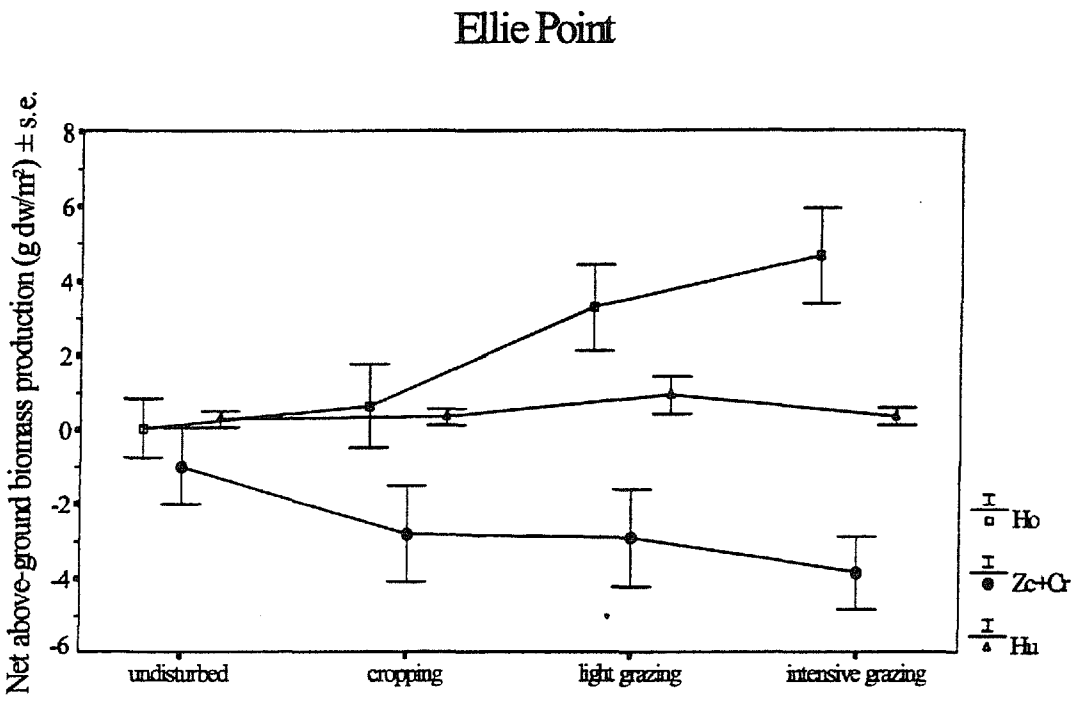

Fig. 5.9. The response of the net above-ground biomass production (g dw/m^2, with standard errors) of (Ho) *Halophila ovalis*, (Zc + Cr) *Zostera capricorni* plus *Cymodocea rotundata*, and (Hu) *Halodule uninervis* to the different treatments (simulated intensive and light dugong grazing, turtle cropping and control) at Ellie Point.

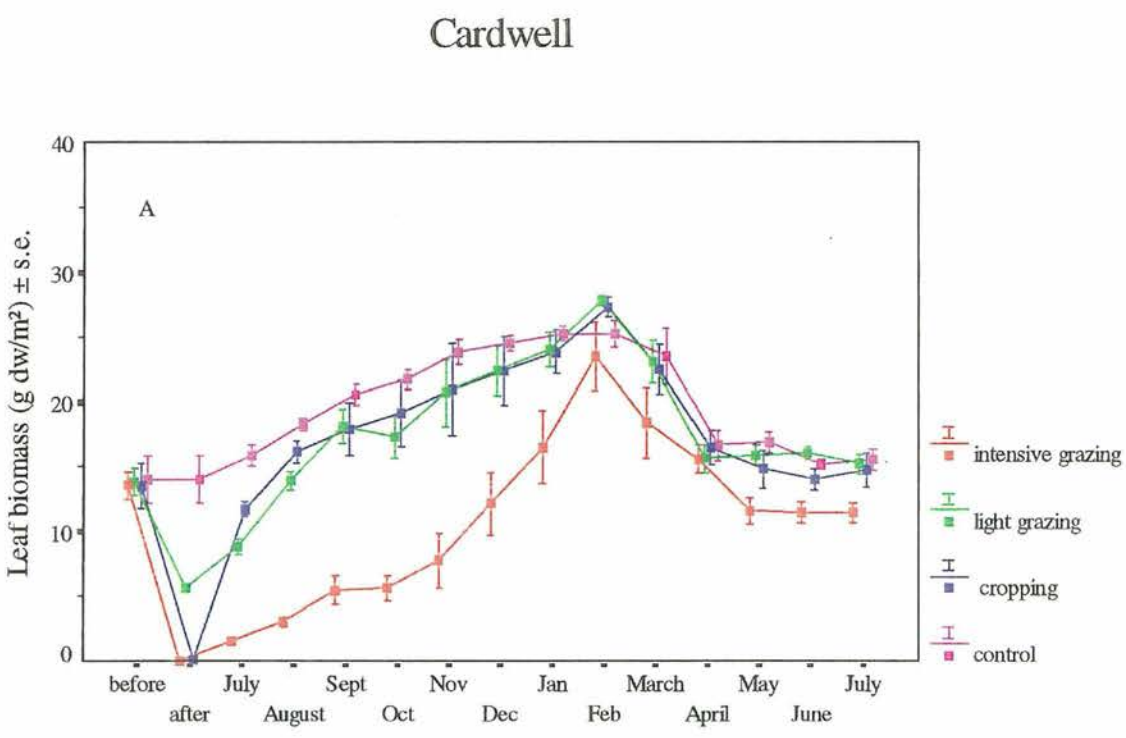

Fig. 5.10a. The response of the leaf biomass (g dw/m^2) of *H. uninervis* to the different treatment levels (simulated intensive and light dugong grazing, turtle cropping and control) corrected for natural grazing over time at Cardwell; showing the means (n=4) with standard errors. Both before and after simulation measurements were carried out on the same day in June 1993.

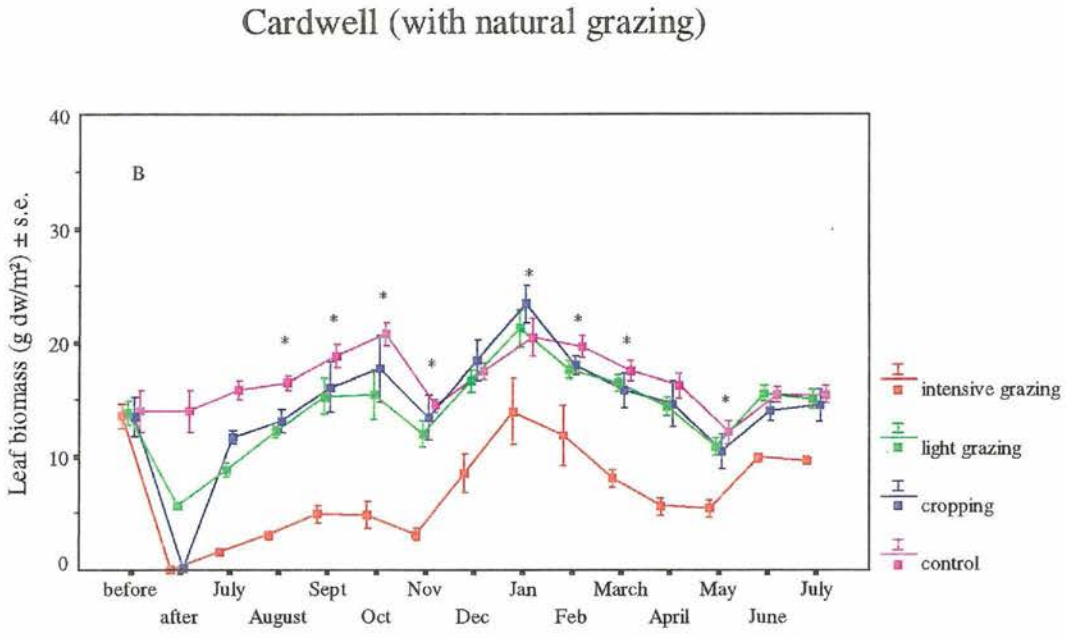

Fig. 5.10b. The response of the *H. uninervis* leaf biomass (g dw/m^2) to the different treatment levels (simulated intensive and light dugong grazing, turtle cropping and control) not corrected for natural grazing over time at Cardwell; showing the means (n=4) with standard errors. Both before and after simulation measurements were carried out on the same day in June 1993. (*) occurrence of natural dugong grazing.

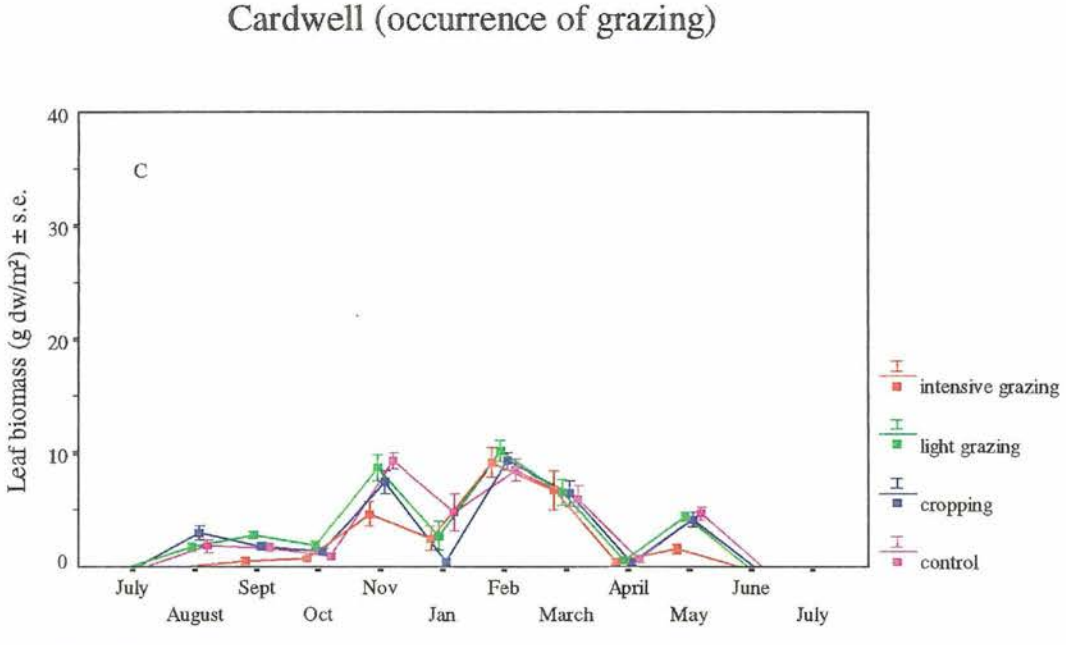

Fig. 5.10c. The amounts (mean ± s.e.) of *H. uninervis* leaf biomass (g dw/m^2) removed as a result of natural grazing by dugongs over time at Cardwell. The values were grouped according to treatments to show how much was added to the standing crop (shown in Fig 4.4b) and that dugongs grazed on all types of plots.

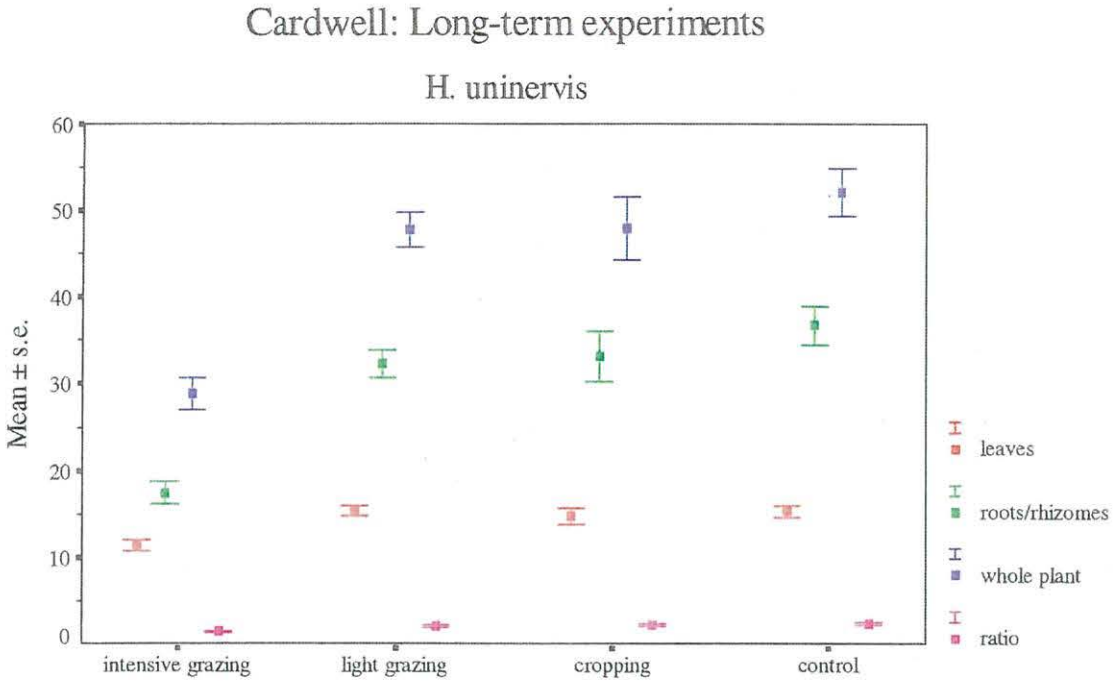

Fig 5.11. Plots of the mean response (g dw/m^2, with standard errors) of the leaves (red), roots/rhizomes (green), whole plant (blue) and ratio of the roots and rhizomes biomass to leaves (pink) of *Halodule uninervis* to the different treatment levels (intensive grazing, light grazing, cropping, and control) from the long-term experiments conducted at Cardwell.

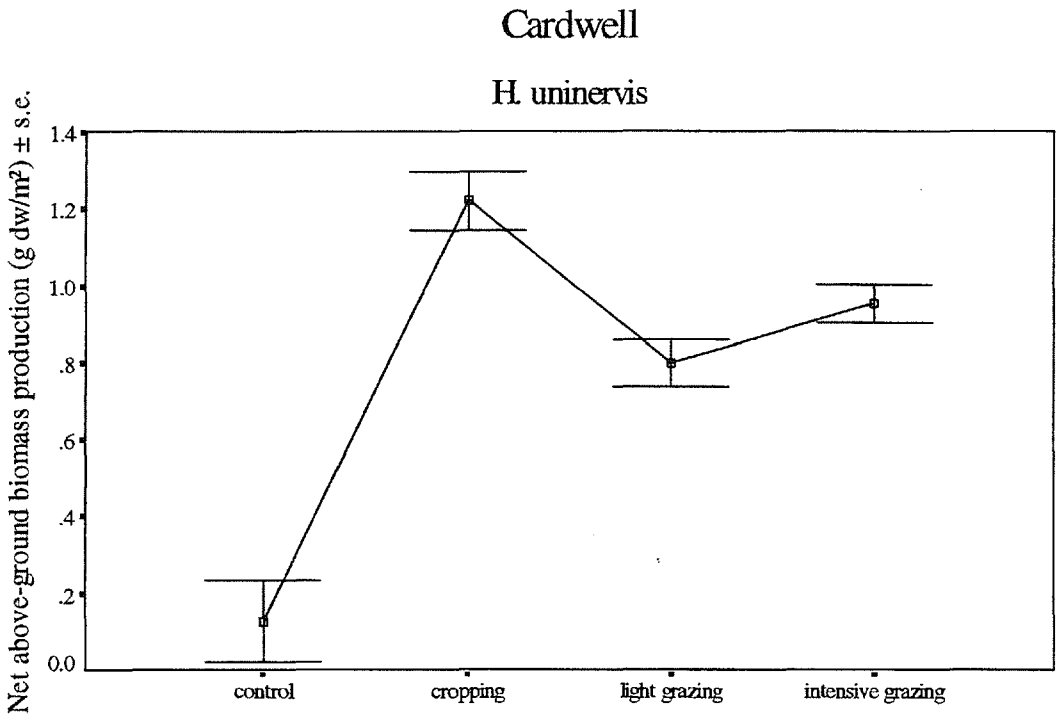

Fig. 5.12. The response of the net above-ground biomass production (g dw/m^2, with standard errors) of *Halodule uninervis* to the different treatments (simulated intensive and light dugong grazing, turtle cropping and control) at Cardwell from the long-term experiments.

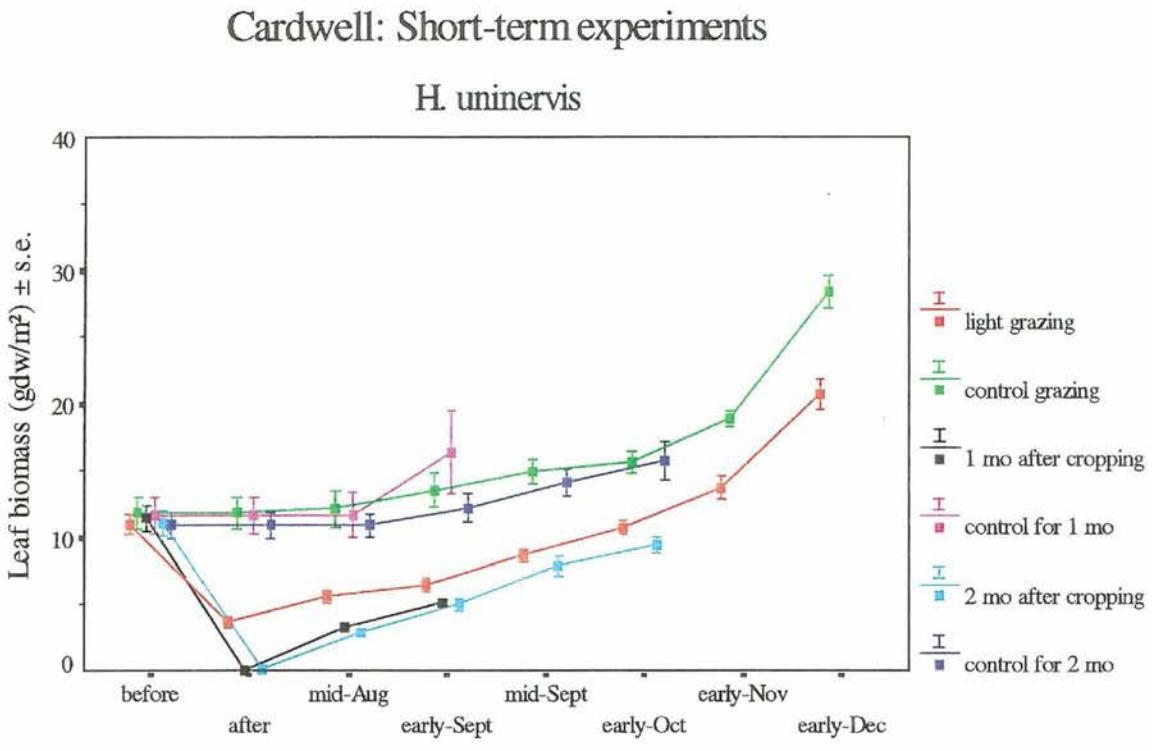

Fig. 5.13. The response of the leaf biomass (g dw/m^2) of *H. uninervis* from the different treatment levels (simulated light grazing and its control, turtle cropping harvested after 1 month and its control, and turtle cropping harvested after 2 months and its control) over time at Cardwell; showing the means with standard errors. Both before and after simulation measurements were carried out on the same day in August 1994.

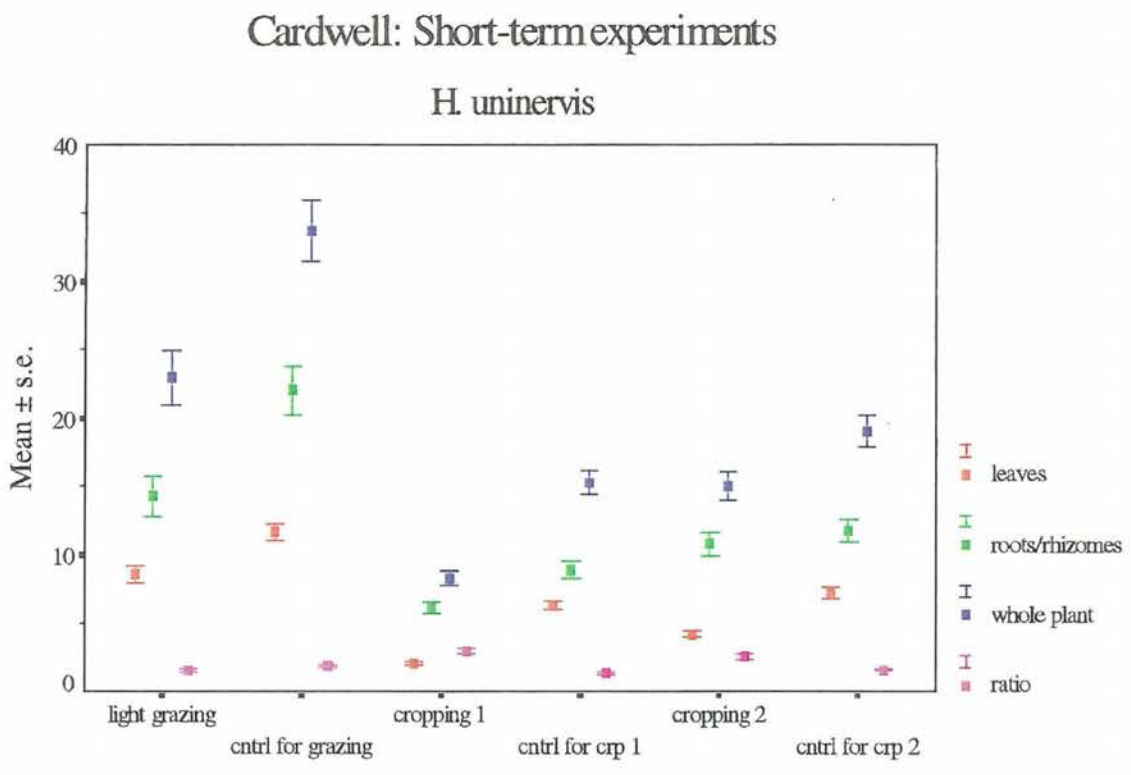

Fig 5.14. Plots of the mean response (g dw/m^2, with standard errors) of leaves (red), roots/rhizomes (green), whole plant (blue), and ratio of the roots and rhizomes biomass to leaves (pink) of *Halodule uninervis* to the different treatment levels (simulated light grazing and its control, turtle cropping harvested after 1 month and its control, and turtle cropping harvested after 2 months and its control) from the short-term experiments conducted at Cardwell.

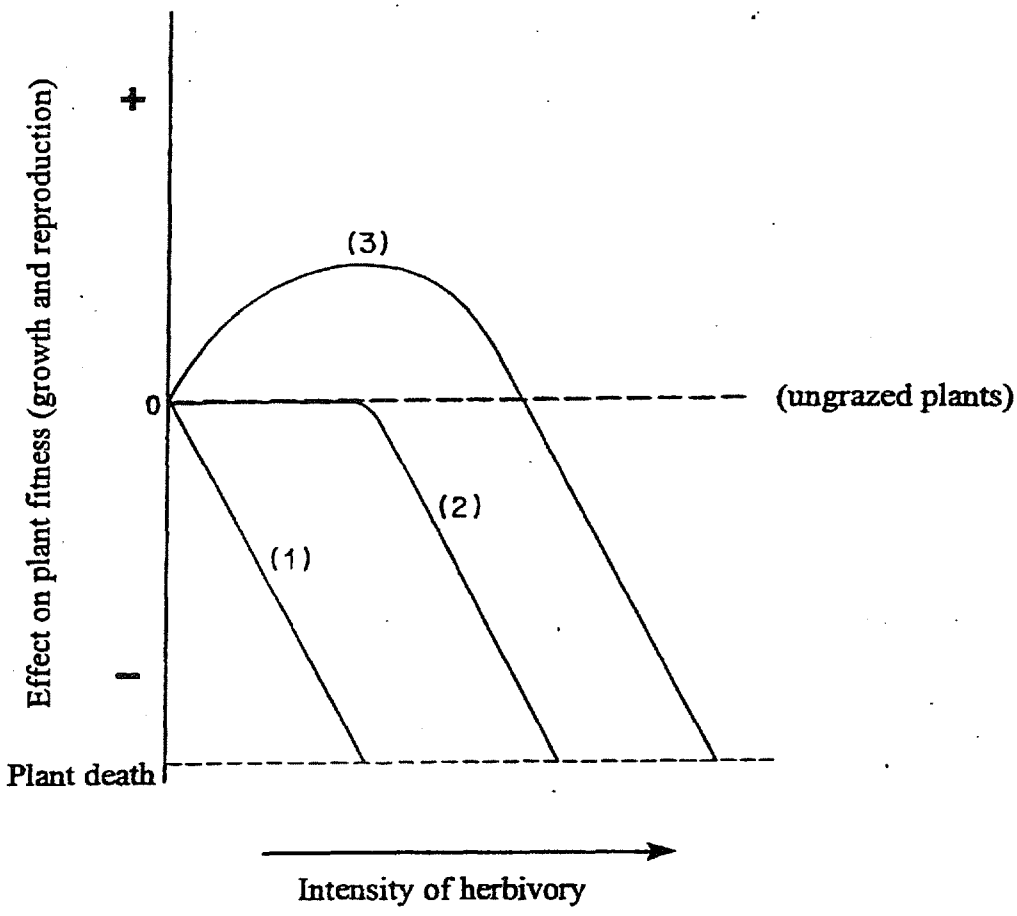

Fig. 5.15. Three predictions of the grazing optimization hypothesis. (1) Plant fitness declines as the intensity of herbivory increases; (2) plants are able to compensate up to a certain level of herbivory, then fitness declines as the intensity of herbivory increases; (3) as the intensity of grazing increases, fitness of plants increased at moderate levels of herbivory above that of ungrazed plants. However, at higher intensities fitness declines. (Modified after Dyer 1975; McNaughton 1979a; Dyer *et al.* 1982; and Jefferies 1988).

CHAPTER 6.

NEAR INFRA-RED REFLECTANCE SPECTROSCOPY (NIRS) AS A PREDICTOR OF SEAGRASS NUTRIENTS

6.1. INTRODUCTION

Conventional methods for analysing the chemical composition of plant material, particularly methods for nutrient contents are laborious and expensive, consequently limiting replications. This constraint is particularly serious for studies of the nutritional ecology of wildlife which may eat a variety of food plants growing under a variety of conditions. Near infra-red reflectance spectroscopy (NIRS) offers the potential to solve this problem. Near infra-red reflectance spectroscopy has become a widely used method for analysing agricultural and food products because it is very rapid and requires little or no sample preparation (Osborne *et al.* 1993; Shenk and Westerhaus 1993).

I used the NIRS analytical technique as this allowed me to analyse 1,165 samples for eight nutritional components. The required 9,320 determinations would have been expensive and laborious using conventional methods. The importance of statistical power and how it was achieved despite the limitations of my budget was discussed in Chapter 3.

An extensive body of literature on near infra-red spectroscopy exists. In this introduction I will, however, mainly refer to Marten *et al.* (1989), Osborne *et al.* (1993), and Shenk and Westerhaus (1993), unless cited otherwise, in order to present the history, definition and principles of NIRS, important terms and methods used in NIRS. Further information on NIRS can be obtained from the references listed in the main references cited above.

History of NIRS

Karl Norris and his collaborators in the 1950s initiated the application of spectroscopic methods, including visible, and near infra-red (NI) transmittance and reflectance for the rapid assessment of the quality of agricultural products. They experimented with a very small segment of the electromagnetic spectrum – the near infra-red – (between 700 and 1200 nm) and found that reflectance spectra could be obtained for opaque biological samples such as leaves and grains (Butler and Norris 1960; Norris and Butler 1961; Massie and Norris 1965 in Osborne *et al.* 1993). Since then, the NIRS technique has been refined and applied to forages (Norris *et al.* 1976), and food products including baked food (Osborne 1991), dairy food (Frankhuizen 1992), and vegetables (Tsou 1995). This development was made possible by concomitant technological advances in optics, electronics, computer hardware and software, and especially chemometrics.

Definition and principle of the NIRS analysis

Karl Norris (1989) defines NIRS analysis as "an instrumental method for rapidly and reproducibly measuring the chemical composition of sample with little or no sample preparation". The principle of this technique is that near infra-red spectrum contains information on the composition of the materials exposed to such light, based on the fact that each of the major chemical components (methyl, CH; amino, NH; hydroxyl, OH and carbonyl, CHO) of a sample has unique near infra-red absorption properties which can be used to differentiate one component from the others. The sum of these absorption properties, combined with the radiation-scattering properties of the sample, determines the diffuse reflectance of a sample. The compositional information can be resolved by proper treatment of the reflectance data. For example, for a particular component to be analysed, the simplest procedure is to measure the reflectance at two wavelengths, with one of the wavelengths chosen to be at a maximum absorption and the other at a minimum point. The ratio of these two reflectance values measured on different samples can be correlated to the concentration of that specific component in those samples. By performing this correlation, a calibration equation can be developed

to predict the concentration of the component in samples from their reflectance measurements.

More commonly, calibration equations are, however, developed from more than two wavelengths of a spectrum, usually 5 - 250 wavelengths and so calibration equations are, in these cases, multivariate models. The selection of the calibration set is also important. Ideally, a calibration set is a selection of samples, representative of the whole spectral population (Windham *et al.* 1989). These samples are then analysed for the particular components of interest and the relationship between these values and the spectral characteristics is modelled. The result is an equation which can be used to predict the composition of new, unknown samples. Calibration equations specify the mathematical relationships between the information in the NIR spectrum and the laboratory reference data. Presently, NIR reflectance spectroscopy is useful only when quantified in terms of current conventional chemical methods. Thus, NIRS remains a secondary method of measurement which must be calibrated against primary laboratory reference methods.

In this section, I describe the development of calibration equations for multiple nutrients in a wide range of seagrasses found in tropical and subtropical Australia. In addition, I evaluate the implications of my results for further applications of this technique to seagrass-herbivore interactions and seagrass ecology, and other similar (wildlife) nutritional ecological studies. The formulae for the calibration equations, including the constants, are not given here because they are calculated using a computer program. In this study I used the NIRS 3 software package (Infrasoft International, Port Matilda, Penn. 1992) to monitor instrument performance, collect spectral data, develop calibration equations, and evaluate the performance of calibration equations.

Statistical terminology

The statistical terms used in NIRS vary among authors. The terms used here are adapted from Smith and Flynn (1991), Kellaway and Stimpson (1993), and Shenk

and Westerhaus (1993). These terms defined below relate to NIRS determinations (terms 1 to 3), and the laboratory reference methods (chemical analyses) (term 4).

1) **Standard error of calibration (SEC),** also called standard error of performance of the calibration, SEP(C), or standard error of estimates or residual standard deviation, is the error due to differences between routine laboratory analysis (RLA) values and NIRS- predicted values within the calibration set:

$$\mathbf{SEC} = \sqrt{(\text{error mean square})}.$$

2) **Standard error of performance (SEP),** also called the standard error of prediction or standard error of validation, is the error due to the differences between RLA and NIRS- predicted values outside the calibration:

$$\mathbf{SEP} = \sqrt{(\text{error mean square})}.$$

3) **Standard error of cross validation (SECV),** is the best estimate of prediction accuracy available from the calibration samples. It is the error due to differences between RLA values and NIRS-predicted values within the cross validation sets:

$$\mathbf{SECV} = \sqrt{(\text{error mean square})}.$$

This is obtained by combining the validation errors i.e., the errors obtained from the prediction of samples removed from the calibration set which are predicted by the remaining samples during cross validation (Stone 1974). This is mainly used instead of SEP (see below) in cases where a separate validation set is not available or possible, e.g. if the project's resources are restricted by limited sample size or volume, or funds for laboratory analyses.

4) **Standard error of the laboratory (SEL)** is the error due to sampling and analytical errors in the primary analyses or NIRS determinations:

$$\mathrm{SEL} = \sqrt{(\sum(y_1 - y_2)^2/N)}$$

where y_1 and y_2 are duplicate analyses for a sample and N is number of samples.

Precision is indicated by the size of SEC and SEP or SECV, and SEL. Accuracy is indicated by the coefficient of determination (r^2), slope and intercept in regression equations relating NIRS determinations to primary analyses.

6.2. MATERIALS AND METHODS

6.2.1. Seagrass database collection

Samples of seagrasses were collected in tropical north Queensland including the Gulf of Carpentaria, and subtropical south Queensland (Moreton and Hervey Bays) (see Fig 5.1 and Table 6.1 for locations). The collection comprised 10 species (with some species having two varieties), representing the diversity of regional seagrasses: *Halophila ovalis, H. minor, H. spinulosa, H. decipiens, H. trichostata, Halodule uninervis* narrow- and wide-leaf varieties, *Cymodocea rotundata, C. serrulata, Syringodium isoetifolium,* and *Zostera capricorni* narrow (< 2 mm) - and wide (≥ 2 mm)-leaf varieties. Most samples were collected in areas where grazing by dugongs and green turtles was likely.

The samples collected were classified into two groups: those collected from experiments and those collected for other reasons (Table 6.1). Samples collected from experiments were those exposed to different regimes of herbivory and fertilisation. This included the samples collected from the initial simulations and final harvesting of seagrasses from the grazing experiments at Ellie Point and Cardwell (Chapter 5). Seagrass samples collected at Bolger Bay during the initial simulation (abandoned after the intertidal seagrasses disappeared) were included also. A handful of seagrasses (approximately 100 g wet-weight) was collected from the initial simulations. At least three replicates were collected for each species. Collection of the samples from the final harvesting of seagrasses from the grazing experimental plots is described in Chapter 4 (Section 4.2.6). Seagrass samples were also collected from experimental plots exposed to different regimes

of fertilisation at Shelley Beach, Townsville (see Fig 6.1a). This experiment was done in collaboration with Ms C. Roder and Ms J. Mellors in early 1995 and is described further in Chapter 8. The remaining seagrass samples were collected at the Turtle Group of Islands off Starcke area and Pipon Island off Cape Melville (Fig 6.1b), a coastal site (Borroloola) at the Gulf of Carpentaria, Green Island (see Fig 5.2), North Brook Island (see Fig 5.3), Moreton Bay, and Hervey Bay (see Table 6.1 for locations of these sites). These samples were collected opportunistically on trips to those areas. At some sites among these areas, samples were collected at different times of the year, depths, and from several locations within such sites. Except for the samples from the experiments, the rest of the samples were harvested from subtidal beds. Some samples of *H. spinulosa* mixed with some *H. ovalis* were harvested from several depths (either by spot diving with SCUBA or grab) at Pipon Island (see Appendix 6). At least 100 to 200 g wet-weight of mixed samples were collected from each site, depending on its depth.

6.2.2. Sample preparation

The samples were placed in a refrigerated container (approximately 5-10 ° C) immediately after collection. The samples were then washed and cleaned of remaining substrate in clean seawater, sorted by hand into species, and into leaf and root/rhizome fractions (except for *H. trichostata* and *H. decipiens*, where leaves and roots/rhizomes were combined because the leaves of these species are small), placed in labelled paper bags, and dried to constant weight for at least 48 - 72 hours in an oven at 60 ° C. Each sample was then ground in a cyclone mill (Udy Corporation, Fort Collins, CO) through a 1 mm aperture mesh. Each ground sample was placed in a labelled plastic jar. Before each sample was scanned in the NIRS spectrophotometer, the jars containing the samples were placed in a constant humidity and temperature chamber (15% RH, 4 - 8 ° C) with their lids off at the BSES (Bureau of Sugar Experiment Station), Meringa for at least 72 hours. This was carried out to ensure that the moisture content was constant and to minimise noise in the Oxygen-Hydrogen (O-H) band in the NIR spectra (Shenk and Westerhaus 1993).

6.2.3. Routine NIRS scanning and analyses

6.2.3.1. *Collection of spectral data*

Reflectance (*R*) measurements of monochromatic light were made from the visible (400 - 1100 nm) range and the near infra-red (1100 - 2500 nm) range at 2 nm intervals. Reflectance spectra of the samples were collected using an NIRSystems Inc (Silver Springs, MD) Model 6500 fitted with a spinning cup module. The cup type used was the small ring cup. The instrument was housed in a room maintained at 22 - 24 ° C and 55- 60 % RH. A set of 30 to 40 samples was scanned each time. Each sample was stirred using a spatula to ensure a homogeneous mixture. Then, using a spatula, a subsample was taken from several parts of the sample and packed evenly into the back of the NIRS sample cell. A prefabricated backing wad (made of thick paper card) slightly larger than the interior of the sample cell was then placed on top of the sample to keep it in place.

The instrument was set to average two complete scans per sample and to ensure that these two complete spectra were similar, a statistic referred to as root mean square (RMS) error was generated by NIRS 3. The RMS is the standard error of the difference between two scans collected from the same instrument; expressed as log $(1/R)/10^6$. RMS error in this context, is an index of spectral similarity, which measures the deviations in the optical (log $1/R$) data at each wavelength (= photometric noise) (ISI 1992) and ensures that duplicate spectra from subsamples are statistically similar before they are averaged. An RMS error value of 50 was used. Westerhaus (1989) suggests that an RMS value of 50 will not affect the repeatability of the analysis. Whenever subsample spectra exceeded this preset RMS error, they were scanned again. Large RMS (> 80, N. Berding 1995 pers. comm.), often implies significant photometric noise or poor NIRS repeatability, and may reflect instability in instrument room temperature or RH (Shenk and Westerhaus 1993a).

Modified sample cells

The validity of the NIR spectroscopy as a predictor of nutrient contents of seagrasses was assessed in a range of preliminary measurements using a large-volume cell (see Fig 6.2). However, most of my samples were too small (about 0.2 - 0.5 g) to fill the whole cell (which required about 2.0 - 3.0 g). This required some modifications to the sample cell. New sample cells for routine NIRS scanning were fabricated from a black anodised aluminium alloy of T6 hardness. Spectroscopic-grade quartz windows (General Electric, Dayton, OH) were spectrally matched and glued in front of each cell (see Fig 6.2). These new sample cells considerably reduced the amount of sample required (~ 0.2 - 0.35 g), and enabled me to scan even very small samples (≤ 0.2 g). A small experiment was performed to test whether the sample size affected the results. Several large samples were scanned using both the regular cell and the new small-volume cell. The predicted values from both procedures were compared. There was no significant difference between the results (see Section 6.3), so the small cell was used for scanning the rest of the samples.

NIR spectroscopy is non-destructive and so all samples were retained after scanning. All 1,150 samples (see Table 6.1) were scanned. All sample information (including the sample number and spectra collected) were recorded via a computer connected to the NI spectrophotometer through ISI software package.

6.2.3.2. *Diagnostic tests*

A diagnostic test of the NIR spectrophotometer's accuracy was performed before commencing scanning each day and at two hour intervals using the "Analyse Check Cell" options in the NIRS 3 Program (ISI 1992). A calibration check cell of carefully packed ground sugarcane leaf of known nitrogen composition was provided by BSES, Meringa. This check cell reading verified that the instrument was performing satisfactorily within defined limits (preset by NIRS 3). If the instrument had not been used for at least one month, additional diagnostic tests were carried out before scanning. These included photometric (NI) response, wavelength accuracy using an

internal polystyrene reference, and instrument repeatability (noise) using an internal ceramic reference through the NIRS 3 Diagnostics options (ISI 1992).

6.2.3.3. *NIRS analysis*

The reflectance (R) readings were converted to absorbance (A) values using the following equation: $A = \log(1/R)$ (ISI 1992). The samples were scanned using the SCAN program of the NIRS 3 software. Figure 6.3 shows the various steps involved in the prediction of seagrass constituents and *in vitro* dry matter digestibility using NIRS.

6.2.4. Selection of the calibration set for chemical analyses

6.2.4.1. *Selection*

A broad (or "universal") calibration set of approximately 17 % (n = 200) of the total number of seagrass spectra (N = 1,165) was selected by using the CENTER and SELECT algorithms in the NIRS 3 software. The CENTER algorithm ranked the spectra according to their distance from the center of the population (global 'H'), while SELECT was used to identify the representatives for the calibration set based on the distance between a sample and its neighbours, called neighbourhood 'H' (Shenk and Westerhaus 1993a). The SELECT Program chose the most 'typical' samples according to the spectral variability of the whole population and these were regarded as a calibration set. Global and neighbourhood 'H' values were computed with 20 to 30 factors using the Mahalanobis distance instead of the Euclidean distance because a spectrum has high dimensional data and does not necessarily conform to Euclidean (plane) geometry (Shenk and Westerhaus 1993).

The samples for the calibration set were selected over several months because the sample collection was designed to be cumulative through time due to the limitations of the study. There was a slow return (and turnover) of samples because the samples had to be sorted by hand individually into plant parts leading to an accumulation of unsorted samples. Most experimental samples were only available at the conclusion of the experiments in the latter stage of the study. Fortunately,

the first 200 samples represented a diversity of seagrasses of various species, of morphs, from various sites, and depths. The initial calibration set of 70 (out of 200) was expanded by using the CENTER and SELECT algorithms a second time on the whole population, after all the 1,165 samples were scanned. The SELECT algorithm was used to choose 190 samples which represented the spectral variability of the population. From these, only 120 new samples were considered because 65 of the 70 samples in the initial calibration set were either chosen a second time or represented by a close neighbour (based on the 'H' distance calculated and generated by NIRS 3). Unfortunately, not all samples selected in the second SELECT run were large enough for the full set of eight chemical analyses. In such cases, the closest neighbour that contained sufficient material was used instead. Some 10 outliers from the initial predictions (for nitrogen and organic matter) were included in the final calibration set, bringing the final calibration set to 200 samples. This expansion of the original calibration was necessary to predict accurately the chemical composition of the outliers and other samples related to them. Shenk and Westerhaus (1993) recommend that outliers identified in this way be examined carefully and incorporated into a revised calibration set.

6.2.4.2. *A comparison between leaf or root/rhizome- and whole plant-based calibrations*

The samples selected for the calibration set were divided into two groups: leaves and roots/rhizomes. Thus, two options were possible for calibration: a separate calibration each for leaf and roots/rhizomes or a calibration combining both plant parts (whole plant). A short experiment was performed to test predictions from single-plant part or narrow-based calibration equations against those of multi-plant parts or broad-based calibration equations. Single-plant part calibrations were developed separately for the leaves (n = 88) or roots/rhizomes (n = 105) selected, then a broad-based calibration was developed combining both plant parts, including a few detrital matter samples (n = 88 + 105 + 7 detritus samples = 200). I assumed that the leaves and roots/rhizomes selected in the broad-based calibration would have also been chosen in separate selections for each plant part because they were

part of the same population, only separated. Thus, they would have still been representative of each group. Grouping by plant part rather than species was the simplest possible division as my samples included a diversity of species (see Table 6.1). The development of the calibration equation is described in Section 6.2.6. Predictions from single-product calibrations were compared to those of the broad-based calibration equations by comparing their SECV (see Section 6.1 for definition), r^2 (coefficient of determination, which represents the total variability in the NIRS predicted values explained by the regression), slope (of the regression), and bias (calculated using NIRS 3). Bias is an estimate of the mean difference between the laboratory and NIRS measurements for the calibration or monitoring sets (Smith and Flynn 1991). The bias confidence limits were set to distinguish between no bias and a bias no greater than 1.0 x SEC (standard error of the calibration, see Section 6.1 for definition) with 90% confidence when using a two-tailed Type 1 error probability of 0.10 (ISI 1992). Shenk and Westerhaus (1993) suggests that if the bias is greater than the confidence limit, the calibration may be insufficient and should be expanded (i.e. recalibrated).

6.2.5. Routine chemical analysis (Laboratory reference methods)

Samples in the calibration set were analysed for organic matter (OM), total nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid lignin (AL), water-soluble carbohydrates (WSC), total starch (TS), and *in vitro* dry matter digestibility (IVDMD). All routine chemical analyses were carried out in duplicate using dried, ground materials at the Animal Physiology Laboratory of the Zoology Department of James Cook University. Duplicates in any analysis which had poor agreement: > 2% for N and > 5 % for others, were repeated. Additionally, a randomly chosen subset of the laboratory samples was repeated in duplicate to estimate the precision of the analyses (= validation set). All analyses were expressed as a percentage (%) of dry weight of sample. Correlation analysis between the two sets of analyses (calibration and validation sets) was performed for each reference method.

These procedures were carried out to ensure high precision and accuracy for each analysis, because the accuracy of NIRS analysis ultimately depends on the accuracy of the reference chemical methods.

6.2.5.1. Total Nitrogen (Nitrogen)

Total nitrogen was analysed on 250 to 300 mg of dried, ground materials by a semi-micro Kjeldahl technique with selenium as a catalyst. Distillation and titration were performed using an automated a Vapodest Gerhardt analyser. Recovery of $(NH_4)_2SO_4$ standards was 99-101 %.

6.2.5.2. Organic Matter (OM)

Residual moisture was determined by drying 250 mg sample overnight at 80 ° C in a forced draught oven. The organic matter content of the samples was measured by burning it in a muffle furnace at 550 ° C for four hours. When the muffle furnace cooled to below 100 ° C ashed samples were transferred to the desiccator for weighing. The ash weight was expressed as percent ash using the formula: percent ash = (ash weight ÷ sample dry weight) x 100. Organic matter was then expressed as percentage of dry weight of sample (burnt off during the ashing) using the formula: organic matter = 100 - percent ash. The dry matter of any biological material can be divided into two components: organic matter representing the total organic components, and ash representing the inorganic matter.

6.2.5.3. Neutral Detergent Fibre (NDF)

Sequential NDF and ADF analyses using the Filter Bag Technique (Komarek *et al.* 1994) and following Van Soest *et al.* (1991) were performed on 250 mg dried ground samples. Each sample was placed in individual filter bags (size #F57, ANKOM Co., Fairport, NY, USA) and sealed by an electric heat sealer. The analysis was carried out using the ANKOM^{200} Fiber Analyzer (ANKOM Co., Fairport, NY, USA).

Neutral detergent (ND) residue essentially consists of cell wall components including cellulose, hemicellulose, lignin, and silica. Some structural protein associated with the cell wall (Van Soest 1982), and some starch (Schweizer and Würsch 1979; Morrison 1980) may possibly be included in this fraction.

The samples were agitated for 80 minutes in ND solution maintained at 100 ° C in the digestion vessel. Then, the ND solution was drained and the samples rinsed with hot water (~ 85 ° C) five times. Heat stable α-amylase was used in each of the first three rinses. Residual water was gently squeezed from each bag before it was soaked in acetone for five minutes, air-dried, and then oven-dried overnight at 60 ° C.

6.2.5.4. Acid Detergent Fibre (ADF)

The acid detergent fibre (ADF) analysis was performed on the NDF residue, using the ANKOM^{200} Fiber Analyzer following the procedures of Van Soest *et al.* (1991) and Komarek *et al.* (1994). The amount of ADF was determined after soaking the samples in the acid detergent (AD) solution following the procedures outlined in Section 6.2.5.3.

The AD residue consists of lignin, cellulose, cutin, and acid-insoluble ash (largely silica) (Robertson 1978; Van Soest 1994).

6.2.5.5. Acid Lignin (Lignin)

The acid lignin (lignin) was measured on the ADF residue (Section 6.2.5.4) following the method of Van Soest *et al.* (1991). Samples were soaked in a flat-bottom container filled with 72% sulphuric acid in an ice bath for 3 h to dissolve the cellulose. After three hours, the samples were washed three times in hot water, once in cold water, air-dried, and then dried overnight in the oven at 60 ° C.

6.2.5.6. Water Soluble Carbohydrates (WSC)

Water soluble carbohydrates were extracted from 100 mg dried ground samples by boiling each with 8 ml of 80% aqueous ethanol (Association of Official Analytical Chemists 1980) in a capped 10 ml centrifuge tube for one hour at 80 ° C. The sample was centrifuged at 3000 rpm for 10 minutes and the supernatant decanted into a container, and then sealed. Two additional extractions (each with 8 ml of distilled water, heated at 60 ° C for one hour; after Radojevic *et al.* 1994) were performed on the residue, centrifuging, decanting and combining supernatants after each extraction. Each individual residual pellet was stored in the freezer for starch assay (see below). The anthrone assays were boiled for 15 minutes as glucose and fructose display a similar response after this time. The optical density of each sample was determined spectrophotometrically at 630 nm using fructose as a standard (Jermyn 1975). The WSC composition was expressed as percentage of dry weight[1] (see Appendix 4 for equation formula).

6.2.5.7. Total Starch (Starch)

The concentration of starch was measured enzymatically using a commercial total starch assay kit (Megazyme Total Starch Kit [2], Megazyme Australia). The assay was performed on residual pellets remaining after the WSC analysis. The "Standard Assay Procedure (AA/AMG)" of the Megazyme Kit including the "Modification to Standard Procedure for Samples Containing Resistant Starch (DMSO/AA/AMG)" was used. This procedure relies on DMSO (dimethyl sulphoxide) to solubilise the starch, and the enzymes α-amylase and amyloglucosidase to convert the starch to glucose. Glucose was measured using a glucose oxidase procedure as described by McCleary *et al.* (1994). This assay procedure is an adaptation of the methods of AACC Method 76-11[3], Karkalis (1985) and McCleary *et al.* (1994). The starch concentration was expressed as percentage of sample dry weight (see Appendix 4

[1] WSC are often expressed in mg/g of fructose equivalents. The values reported here could be converted to fructose equivalents by multiplying the values by 0.1.
[2] "Megazyme, Total Starch Assay Procedure (AA/AMG 10/94)".
[3] American Association of Cereal Chemists: "Approved methods of AACC". Method 76-11, approved October 1976.

for equation formula). Some modifications were performed and are outlined as follows:

Step 1. Tube and pellet, and residual water remaining after WSC extraction were weighed and recorded. The volume of water in pellet (VWP) was calculated using this equation: VWP = (wet weight of tube + pellet) - (initial dry weight of tube + pellet).

Step 5. Exactly 4.1 ml of amyloglucosidase in acetate buffer (200 mM, pH 4.5) were added after 3 ml of α-amylase were added in each sample and incubated at 50 ° C for 30 minutes.

6.2.5.8. *In Vitro* Dry Matter Digestibility (IVDMD)

The *in vitro* dry matter digestibility (IVDMD) was measured on 250 to 300 mg dried, ground samples sealed inside ANKOM filter bags (see Section 6.2.5.3) by a modification of the method of Choo *et al.* (1982). These samples were incubated for 24 hours in an oven at 40 ° C with acid pepsin solution (6 g pepsin in 3 litres of 0.1 N HCl) in conical flasks (3000 ml). The flasks were stirred continuously. After 24 hours, the pepsin solution was removed, the samples thoroughly rinsed with tap water (10 to 15 times), and replaced with cellulase solution (20.4 g sodium acetate and 9.1 ml acetic acid, 18.75 g cellulase enzyme[4], and 375 mg chloramphenicol, all in 3 litres of deionised water). The samples were then incubated for 48 hours at 40 ° C with same level of agitation as the pepsin solution. Following incubation, the cellulase solution was removed and the samples thoroughly rinsed with tap water, air-dried, oven-dried overnight at 60 ° C, and weighed.

6.2.6. Calibration and statistical methods

Modified partial least squares (MPLS) regressions were developed for the full spectrum calibrations of OM, N, NDF, ADF, lignin, hemicellulose, WSC, TS, and IVDMD (see Equation 6.1 below). The raw spectral data (see Fig 6.4a for an example) were transformed into the first derivative (see Fig 6.4b for an example)

[4] From *Trichoderma viride* (Sigma Ltd.)

function (Marten *et al.* 1989). Derivatives are used partly because they substantially reduce, albeit not entirely, possible particle size differences among samples. Additionally, they reduce the high correlations between spectral data at different wavelengths (Osborne *et al.* 1993). To fully remove particle size effects and emphasise the more relevant chemical data, detrending and standard normal variate (SNV) transformations were also applied to the derivatized spectra before calibration (*sensu* Shenk and Westerhaus 1993). Fig 6.4c shows an example of the effects of detrend and SNV transformations on a derivatized spectrum. In theory, all of these procedures make the calibration more robust (Westerhaus 1989). Then, the values for each nutritional attribute from the calibration set were correlated with 259 terms (every 8 nm from a total 2072 nm) for each MPLS equation (see Appendix 5 for an example prediction equation). The ISI software (NIRS 3) calculates a regression equation in the form of:

$$Y = \beta_0 \pm \beta_1 X_1 \pm \beta_2 X_2 \pm \beta_3 X_3 \pm \beta_4 X_4 \ldots\ldots \textbf{(Equation 6.1)}$$

Where Y is a specific nutritional attribute predicted by NIRS: X_1, X_2, X_3, and X_4 are absorption measurements or derivatives at wavelengths λ_1, λ_2, λ_3, and λ_4, respectively; β_0 is the regression constant; and β_1, β_2, β_3, and β_4 are partial regression coefficients. An example prediction equation for a particular nutritional attribute (nitrogen) is presented in Appendix 5.

The algorithm of the MPLS regression provides a more stable and accurate NIRS calibration for the full spectrum than the standard partial least squares (PLS) regression (Shenk and Westerhaus 1991a, 1993) or classical multiple linear regression (Meuret *et al.* 1993). The modification in MPLS is that the NIR residuals at each wavelength, obtained after each factor is calculated, are standardized (i.e. divided by the standard deviations of the residuals at each wavelength) before calculating the next factor (Shenk and Westerhaus 1993). Furthermore, MPLS or PLS reduces the large set of raw spectral data into a small number of orthogonal factors, a procedure which is particularly efficient when data

are significantly intercorrelated (Meuret *et al.* 1993). The different NIRS calibration methods are summarised in Marten *et al.* (1989), and Shenk and Westerhaus (1993). In addition, utility and limitations of curve fitting and derivative techniques were reviewed by Maddams (1980).

Modified partial least squares or PLS require cross validation to prevent overfitting (i.e. using too many terms in the equation) and to select the optimum number of terms for each calibration equation (Osborne *et al.* 1993). Cross validation drops one sample from the calibration set and performs a calibration from the remaining samples, and makes a prediction of the value for the sample left out. This exercise is repeated until all samples have been cross validated and an average squared prediction error calculated by combining these validation errors into what is known as a standard error of cross-validation (SECV, see also Section 6.1). Standard error of the cross validation (SECV) gives a more realistic estimate of accuracy than the standard error of calibration (SEC) (Meuret *et al.* 1993). The number of PLS factors associated with the minimum SECV is all that is retained from the cross validation procedure. Then, calibration is performed on all samples using the number of factors determined by cross validation. Cross validation is an efficient procedure because all samples are used for both calibration and validation, and avoids the need to set aside samples for a validation set (Osborne *et al.* 1993; Shenk and Westerhaus 1993). Another advantage of cross validation is that outliers from the prediction residuals are identified readily (Shenk and Westerhaus 1991a, 1993). In practice, samples with cross validation residuals greater than 2.5 (= critical 'T' outlier values) were identified and omitted, and the cross-validation performed again. I set the critical 'H' outlier value at 4.0. so that samples with a spectral distance greater than 4.0 were considered too far from the population mean and were eliminated. Two passes were made to remove outliers before each calibration equation was finalised. Shenk and Westerhaus (1993) suggested that cross validation works best for full spectrum calibration methods because these methods do not change drastically when a few samples are omitted as outliers.

The number of samples in the calibration set (17 % of the total number of the sample population) was limited by analytical resources (sample volume and money). As a result, I estimated the standard error of prediction (SEP) by cross validation following Meuret *et al.* (1993).

6.2.7. Predictions from calibration equations

The calibration equations obtained from MPLS regressions with cross-validation for each nutritional attribute were used to predict the constituent values of the entire sample using the PREDICT Algorithm of the NIRS3 software. These calibrations were monitored for statistical comparisons of laboratory values against predicted values. Linear regressions between each set of predicted values and laboratory reference values were plotted for each constituent (see Appendix 3). Other statistics (r^2, SEC, SECV, bias and bias confidence limits calculated as percentage of means, and slope) were computed through NIRS 3 (ISI 1992).

6.3. RESULTS AND DISCUSSION

Small- versus large-volume sample cells

The small- and large-volume sample cells produced similar and highly correlated predictions of the seven seagrass constituents and IVDMD (Table 6.2). This made sample preparation easier as a smaller volume (~ 0.35 g) sufficed and enabled me to scan even very small samples (~ 0.2 g). Blakeney and Batten (1995) also demonstrated that reliable results can be obtained from samples as small as 0.2 g using 'microcells' (special compartment cells under constant pressure using soft sponge). This capability of NIRS to use a small sample volume is very valuable, particularly in seagrass ecology, where sampling can be limited by low biomass and/or by the logistics of sampling in deep water sites.

Whole plant-based versus single-plant part calibration equations

Whole plant-based (broad-based) calibration equations compared well with the single-plant part calibration equations in predicting the different seagrass constituents and IVDMD. There was very little difference between their precision and accuracy (compare Tables 6.3 and 6.4). Only two constituents (OM and NDF) had consistently lower cross-validation errors using both single-product calibrations than those determined from whole plant-based calibrations. The rest of the constituents and IVDMD were only slightly lower in only one of these single-plant part calibrations. Therefore, a whole plant-based calibration equation is as accurate as narrow-based or single-plant part calibrations requiring lesser chemical analyses. Furthermore, narrow-based calibrations have limited use only, while broad-based equations, although more difficult to develop, have more uses (Shenk 1983). Broad-based equations have more applications because they were developed from a wide diversity of samples and usually a large number samples. In contrast, narrow-based equations are usually developed for a particular group (e.g. species or samples of a particular species harvested from a particular season) and thus have limited applications to such populations only.

The calibration of starch concentration in leaves was very poor – reflected by r^2 = 0.26, and the bias exceeded its control limits (see Table 6.3). This means that the single-plant part (leaf) equation for starch is a 'poor' predictor of starch in the leaves because its predictions were beyond the 90% confidence interval (using a two-tailed Type 1 error probability of 0.10). This was primarily due to the small range of values for starch in the leaves of seagrasses (0.2 - 0.8 % starch as dry matter). This case also reflects the dichotomy in plant parts of seagrasses, as some constituents seem to be more abundant or present only in a particular part of the plant (e.g. starch and WSC abundant mainly in roots/rhizomes only, see Chapter 7). This reemphasises the need for seagrass samples for nutritional studies to be sorted by plant parts, as some herbivores may target certain plant parts only. This is also important for understanding dugong nutrition as we can determine where the particular nutrients of interest may be coming from.

Recent studies (e.g. Meuret *et al.* 1993; Shenk and Westerhaus 1993) have also shown that whole plant-based calibrations differ only slightly from single-product calibrations. Smith and Flynn (1991) suggested that although development of broad-based calibrations are tedious, once developed, broad-based equations are more cost-effective than conventional techniques. This suggests that an initial broad-based calibration is more useful to expand than to gather another set of samples for a new calibration each time the data set has to be extended. Shenk and Westerhaus (1993) noted that broad-based calibrations were made possible only recently by technological improvement in desktop computers and software. This advantage is very important for wildlife nutritional studies.

Precision and accuracy of reference laboratory analysis

Generally, the precision of the laboratory determinations (SEL) was exceptional (see Table 6.4). The outstanding precision of the nitrogen (SEL = 0.06) reflects the nature of micro-Kjeldahl analysis, i.e. minimal operator involvement (weighing samples only) and because it is a well-defined component. Similarly, the use of ANKOM Filter bag technology (ANKOM Co., Fairport, NY, USA) for measurements of fibre content (Komarek *et al.* 1994), lignin and digestibility of plant materials further simplified the long, tedious filtration step that used to limit the speed and precision of these kinds of gravimetric analyses (see Section 7.2). The highest SEL of 3.18 (lignin) was relatively low, considering that this analysis was one of the most difficult as reflected by a low accuracy (r^2 = 0.73). Additionally, lignin has the highest SEL because it is a poorly defined material; lignin is one of the most difficult plant components to define chemically (Van Soest 1994).

The accuracy of all laboratory analyses was good with r^2 close to 1 (0.73 - 0.99) for the validation set for each method (see Table 6.5 and Appendix 3). This level of accuracy was achieved only after modifying some of the conventional techniques (as discussed above), making the methods less tedious and operator reliant, and the results more repeatable.

Precision and accuracy of the NIRS prediction equations

The overall performance of the prediction equations for the different constituents and *in vitro* digestibility of seagrasses was excellent (Table 6.4). This is best reflected by the closeness of the SECV and SEL values (SECV/SEL). The SEL values were usually lower than the SECV values confirming that the reference methods were accurate. In one case, WSC, the SECV (0.06) was even better than the SEL (0.09). This confirms that the NIRS predictions were robust. Furthermore, most of the r^2 values exceeded 0.90. However, the value for lignin was 0.73 (see Table 6.4) which implies that NIRS could only predict 73% of the variation in lignin measurements (c.f. Shenk and Westerhaus 1993). The SECV values of the seagrass constituents and IVDMD were all comparable or even better than those found in the constituents for terrestrial foliage (c.f. Meuret *et al.* 1993), and their biases were all within the limits. Predictions of nitrogen were the most precise as reflected by the smallest SECV, slope of 1.0 and r^2 value (closest to 1). In contrast, lignin has the largest SECV and lowest r^2 value (see Table 6.4). This is, as mentioned earlier, due to the nature of the poor chemical definition of lignin. Lignin is the only major plant polymer whose subcomponents are not clearly known, as amino acids are for proteins and sugars for polysaccharides (Van Soest 1994). The physical properties of lignin are considerably altered by strong acids, promoting further polymerization and condensation and may cause initially soluble matter to become insoluble products (Van Soest 1994). Additionally, these differences may also be due to the techniques involved in the analyses, i.e. automated micro-Kjeldahl analysis for nitrogen compared with gravimetric analysis for lignin. However, the predictions for lignin are still within acceptable limits (Shenk and Westerhaus 1993) and are comparable to the values reported for lignin for terrestrial forage (r^2 = 0.79, slope = 0.81, bias = 0.18%, Kellaway and Stimson 1993).

NIRS has fewer sources of errors than conventional methods

Williams (1974) concluded that NIRS has fewer sources of errors than conventional methods even Kjeldahl nitrogen. However, it is important to be aware of the possible sources of errors in NIRS. Biologists/ecologists are aware that possible sources of errors in the laboratory such as weighing samples and preparation of reagents are difficult to identify and monitor. In contrast, sample preparation (e.g. particle size and moisture status) and presentation (i.e. sampling) to the spectrophotometer which are the potential sources of errors in NIRS, are easier to identify and monitor (Osborne *et al.* (1993).

Studies have showed that samples milled to finer particle sizes are better than those milled to coarse and medium sizes in that they have higher NIR spectral repeatability (e.g. Casler and Shenk 1985; Kellaway and Stimson 1993). Future NIRS users should be aware of the of the possible repercussions of changing the grinding technique (e.g. mesh size). The homogeneous particle size of my samples was achieved by using a 1 mill mesh grinding; while moisture was kept constant by dehumidification before scanning (see Section 6.2.2). However, the fine particle size which is good for NIRS does not necessarily correlate with animal performance (e.g. digestibility) nor gives the precision required for repeatable gravimetric analyses (e.g. Casler and Shenk 1985). The fine particles of the samples tend to be lost in the conventional gooch crucible filtration technique resulting to poor repeatability of the methods. These problems were apparent in the initial stages of this study but were eventually addressed and solved by using the ANKOM Filter bag technology for fibre, lignin, and IVDMD analyses.

Questions relating to potential sources of errors in sampling are how representative were the spectra gathered or how representative were the samples submitted to analysis (Williams 1975; Osborne *et al.* 1993). To ensure that a sample is representative, at least two complete scans (subsamples) should be obtained with a low RMS error and particularly ensuring that the samples are well mixed.

The technique of packing the samples into the sample cell is also critical. Samples should be mixed thoroughly and an aliquot scooped with a spatula. Subsamples should never be poured or tamped down (Osborne *et al.* 1993). Additionally, samples should always be packed with consistent compactness, and the sample cells, including the quartz window, thoroughly cleaned after every sample.

The maintenance of constant temperature and humidity in the instrument room is also very critical to the performance of the instrument. It is well accepted that NIRS instruments, if properly maintained, contribute few sources of error to the analysis unless they malfunction (Osborne *et al.* 1993). Nowadays, programs in NIRS 3 such as CENTER and SELECT ensure that the samples selected for the calibration are representative of the spectral variability of the population (Shenk and Westerhaus 1991a, 1993).

Future use of NIRS in nutritional ecology

The use of NIRS technology in ecology or biology is currently impeded by the expensive price of the instrument (e.g. NIRSystems Inc Model 6500, ~ $ US 100,000). However, arrangements with institutions which have this instrument can be explored such as leasing instrument time. Furthermore, research institutions involved in large projects involving nutritional analyses can justify buying an NIRS instrument as it is more cost-effective in the long run than conventional techniques.

Conclusion

Near infra-red reflectance spectroscopy has been used successfully to analyse multiple seagrass constituents and IVDMD. This study showed that broad-based calibrations can be made on extremely diverse sets of data, including leaves and roots/rhizomes and detrital matter from about 10 species of seagrasses using recognised calibration procedures. Even the calibrations for complex constituents and IVDMD were very satisfactory, confirming the appropriateness of the modifications applied to some of the conventional chemical methods used. Thus

NIRS prediction equations have been established for N, OM, NDF, ADF, lignin, starch, WSC, and IVDMD across 10 species of seagrass.

As a result of this study, studies on seagrass-herbivore interactions can employ the calibration equations developed in this work, expanding them by incorporating ≥ 10% of each new set of samples to the calibration set and then re-calibrating (using CENTER, SELECT and CALIBRATE, see also Fig 6.3). It will be necessary to ensure that all new samples are processed in a manner similar to the previous (calibration) samples.

The promising results of this study show that NIRS can be a powerful tool for nutritional ecologists. Near infra-red reflectance spectroscopy has several advantages over chemical analyses in terms of speed, simplicity and the capacity to increase replication. Wildlife nutritional ecologists, unimpeded by laborious and expensive chemical analyses, can now focus on answering questions such as why particular herbivores select for particular plant species. However, an important point to emphasise is that NIRS relies on the precision and accuracy of the reference, or routine, chemical analyses, so these must be monitored regularly.

Table 6.1 A break down of the seagrass samples collected in this study. Including locations of collection sites (latitude-longitude), date of collection, a list of species collected, and number of samples (*n*, subtotal enclosed in parentheses) which is further divided into leaves, roots, whole plants (leaves and roots/rhizomes combined), seeds, and detrital matter (seagrass matter only).

Site	Month/Year of Collection	Species	*n*	Leaves	Roots and Rhizomes	Whole Plants	Seeds	Detritus
Ellie Point (16°53'S, 145°46'E)	May 1993	*Cymodocea serrulata*	4	2	2			
		Halophila ovalis	7	4	3			
		Zostera capricorni(w[6])	13	5	5			
		Halodule uninervis (n[7])	4	2	2			
	March 1994[1]	*H. ovalis*	86	43	43			
	(see also Table 5.2)	*Z. capricorni*(w[6])/*C. rotundata*	138	54	55			33
		H. uninervis (n[7])	19	9	10			
			(271)	(116)	(122)			
Cardwell (16°53'S, 145°46'E)	June 1993	*H. uninervis/pinifolia*[8] (n[7])	12	8	4			
	July 1994[1]		128	64	64			
	Aug 1994[1]		9	5	4			
	Oct 1994[1]		96	48	48			
	Sept 1994[1]		96	48	48			
	Dec 1994[1]		96	48	48			
	Jan 1995		12	6	6			
	(see also Table 5.2)		(449)	(227)	(222)			
Shelley Beach (19°19'S, 146°50'E)	March 1995[1]	*H. minor*	46	23	23			
		H. uninervis/pinifolia[8] (n[7])	70	35	35			
			(116)	(58)	(58)			
Bolger Bay (19°19'S, 146°50'E)	June 1993	*H. minor*	8	4	4			

Table 6.2. Results of paired t-test (2-tailed, n=20) comparing predictions using regular and small sample cells in NIRS.

Component	Correlation (*r*)	t-value	*p*
Nitrogen	0.998	-1.45	0.164
Organic Matter	0.999	0.65	0.525
Neutral Detergent Fibre	0.995	-0.06	0.950
Acid Detergent Fibre	0.996	1.04	0.313
Hemicellulose[a]	0.961	-0.77	0.449
Lignin	0.997	-1.67	0.111
Starch	1.000	-0.18	0.856
Water Soluble Carbohydrate	1.000	-1.91	0.072
In Vitro Dry Matter Digestibility	0.999	0.23	0.821

[a] derived by calculating the difference between NDF and ADF values

Table 6.3. Standard errors of the cross validation (% of mean), coefficient of determination (r^2), slope of the regression, bias[a] (% of mean) of the prediction and limits of the bias[a] (% of mean) associated with NIRS analyses using single-plant part prediction equations for the different nutritional components of seagrasses. Compare with Table 6.4.

	Leaf Fraction						Roots and Rhizomes Fraction					
Component	*n*	SECV	r^2	Slope	Bias	Bias limit	*n*	SECV	r^2	Slope	Bias	Bias limit
Nitrogen	85	0.099	0.99	0.98	0.01	0.04	102	0.065	0.92	0.93	0.00	0.03
Organic Matter	70	1.923	0.96	0.98	0.09	0.88	94	1.920	0.98	1.00	0.14	0.90
Neutral Detergent Fibre	78	2.270	0.96	1.02	0.38	1.01	102	2.518	0.89	0.99	0.19	1.25
Acid Detergent Fibre	78	2.820	0.90	1.00	0.00	1.11	102	1.610	0.95	0.97	-0.20	0.75
Water Soluble Carbohydrate	70	0.05	0.92	1.07	0.04	0.23	95	0.08	0.88	1.00	0.05	0.40
Starch	64	0.128	0.26	0.05	0.23[b]	0.07	89	1.434	0.98	0.91	-0.45	0.69
Lignin	74	3.968	0.72	0.97	0.44	1.67	100	2.755	0.79	0.94	-0.23	1.48
In Vitro Dry Matter Digestibility	74	2.025	0.78	0.88	-0.26	0.96	104	2.776	0.91	1.01	-0.07	1.34

[a] see Section 6.2.4.2 for definition
[b] bias > than its limit

Table 6.4. Nutrient composition of seagrass samples (% dry matter) within the calibration set, number of samples used in the calibration (*n*), standard errors (% of mean) of laboratory analysis (SEL), calibration set (SEC), cross validation (SECV), ratio of SECV to SEL, and slope, bias[a] (% of mean) and limits of the bias[a] (% of mean) associated with NIRS equations developed using a combined population of plant parts (leaf + root/rhizome). Compare with Table 6.3.

Component	*n*	Mean	Range	SEL	SEC	SECV	SECV/SEL	r^2	Slope	Bias[a]	Bias limit[a]
Nitrogen	198	1.15	0.38-3.60	0.06	0.07	0.08	1.33	0.99	1.00	-0.01	0.04
Organic Matter	192	61.41	31.97-77.40	2.00	1.93	2.17	1.08	0.96	1.00	0.01	1.16
Neutral Detergent Fibre	198	34.17	19.17-59.79	2.54	2.14	2.79	1.10	0.94	1.00	0.00	1.28
Acid Detergent Fibre	198	24.98	12.86-41.16	2.02	1.89	2.15	1.06	0.91	1.00	0.02	1.13
Water Soluble Carbohydrate	184	1.67	0.01-7.95	0.55	0.58	0.60	1.09	0.90	1.00	0.02	0.35
Starch	175	5.60	0.20-30.60	0.90	1.08	1.25	1.40	0.98	0.95	-0.36	0.65
Lignin	195	12.95	2.09-30.18	3.18	3.11	3.35	1.05	0.73	1.02	0.31	1.86
In Vitro Dry Matter Digestibility	198	86.66	62.68-98.05	2.48	2.52	2.76	1.11	0.86	1.04	-0.19	1.45

[a] See Section 6.2.4.2 for definition

Table 6.5. Summary statistics between the validation (blind run) and calibration (first run) samples for the different laboratory analyses.

Analysis	*n*	r^2	slope
Nitrogen	12	0.99	1.00
Organic Matter	11	0.93	1.08
Neutral Detergent Fibre	10	0.98	0.90
Acid Detergent Fibre	10	0.97	1.08
Water Soluble Carbohydrate	14	0.90	1.04
In Vitro Dry Matter Digestibility	10	0.98	0.97

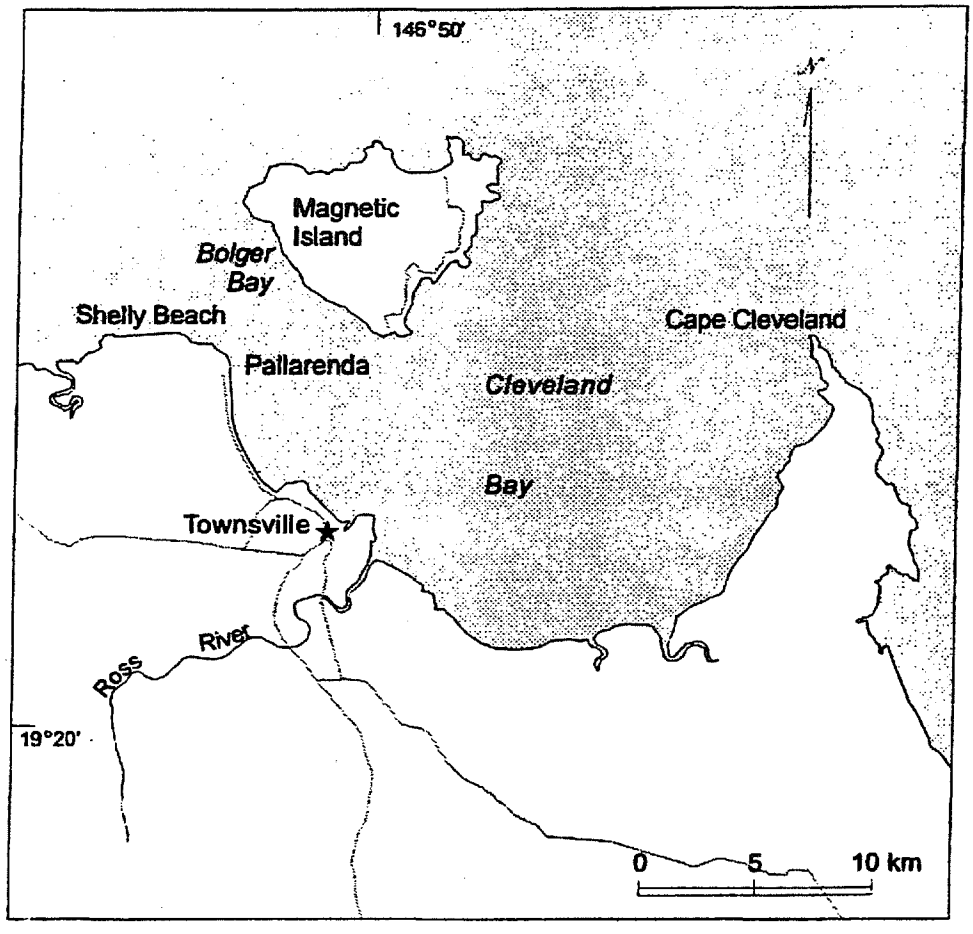

Fig. 6.1a Location of Shelly Beach, Bolger Bay, Magnetic Island in relation to Townsville.

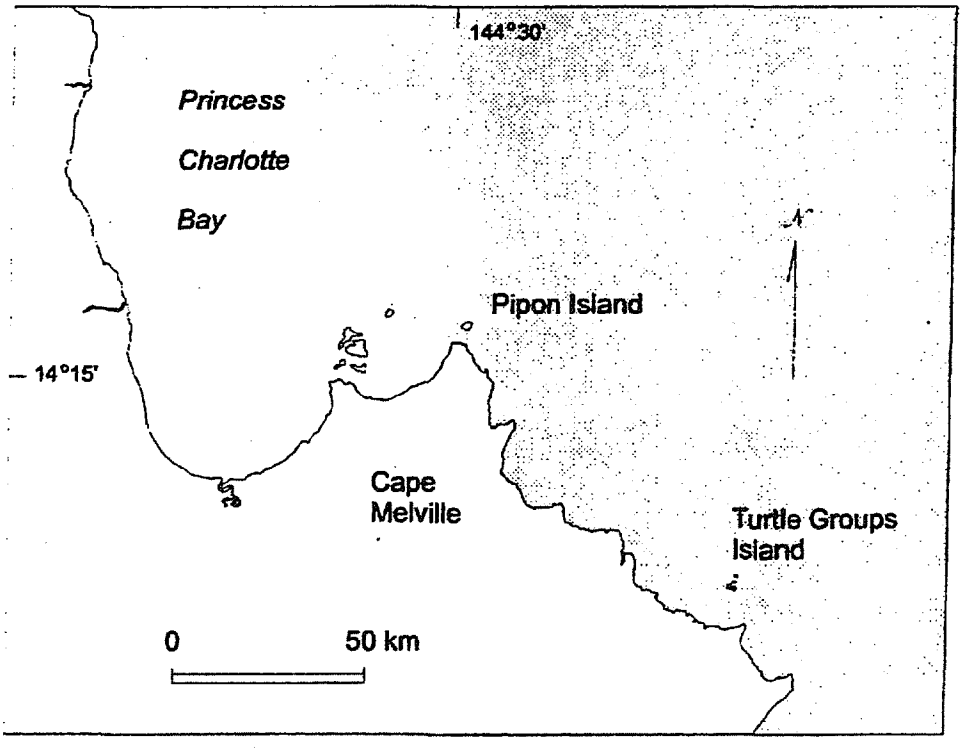

Fig. 6.1b. Location of Pipon Island and the Turtle Group Island off Cape Melville.

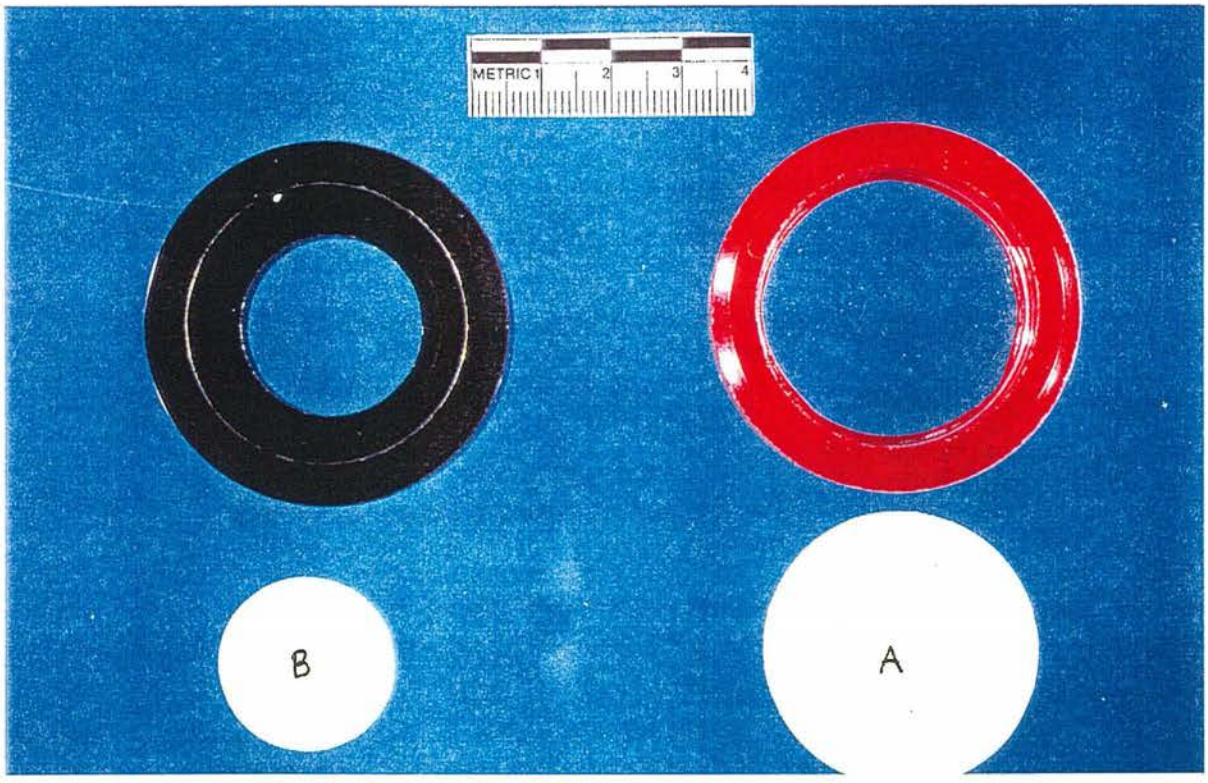

Fig. 6.2. A comparison of the size difference between a regular sample cell (A) and a modified one (B) used in NIRS.

① Sample Presentation

Dried
↓
Ground
↓
Dehumidified
↓
Pack in sample cell
↓

② SCAN
diagnostics tests, check instrument set-up, and check cell (every two hours)
sample analysis
return sample to container
repeat ① & ② until all samples scanned

↓

③ CENTER
the whole population of seagrass samples in the file structured according to their Mahalanobis or r^2 distance from the average spectrum
↓

④ SELECT
used to choose a representative group of samples for calibration

↓

⑤ Reference Laboratory Methods
samples "selected" analysed in duplicate for constituents of interest and IVDMD

↓

⑥ CALIBRATION
recommended "regression method" is modified partial least squares
Cross-validation is then recommended to estimate the optimal number of terms for calibration
MPLS equation should be chosen on the basis of the lowest SECV rather than SEC
calculate SEL

↓

⑦ PREDICT
seagrass constituents and IVDMD predicted using the calibration equations "developed" in ⑥
↓

⑧ Monitor Calibrations
examine performance of equations by examining the slope, bias, and r^2

Fig 6.3. Flowchart of the prediction of the nutritional composition of seagrasses using NIRS (NIRS 3, ISI 1992).

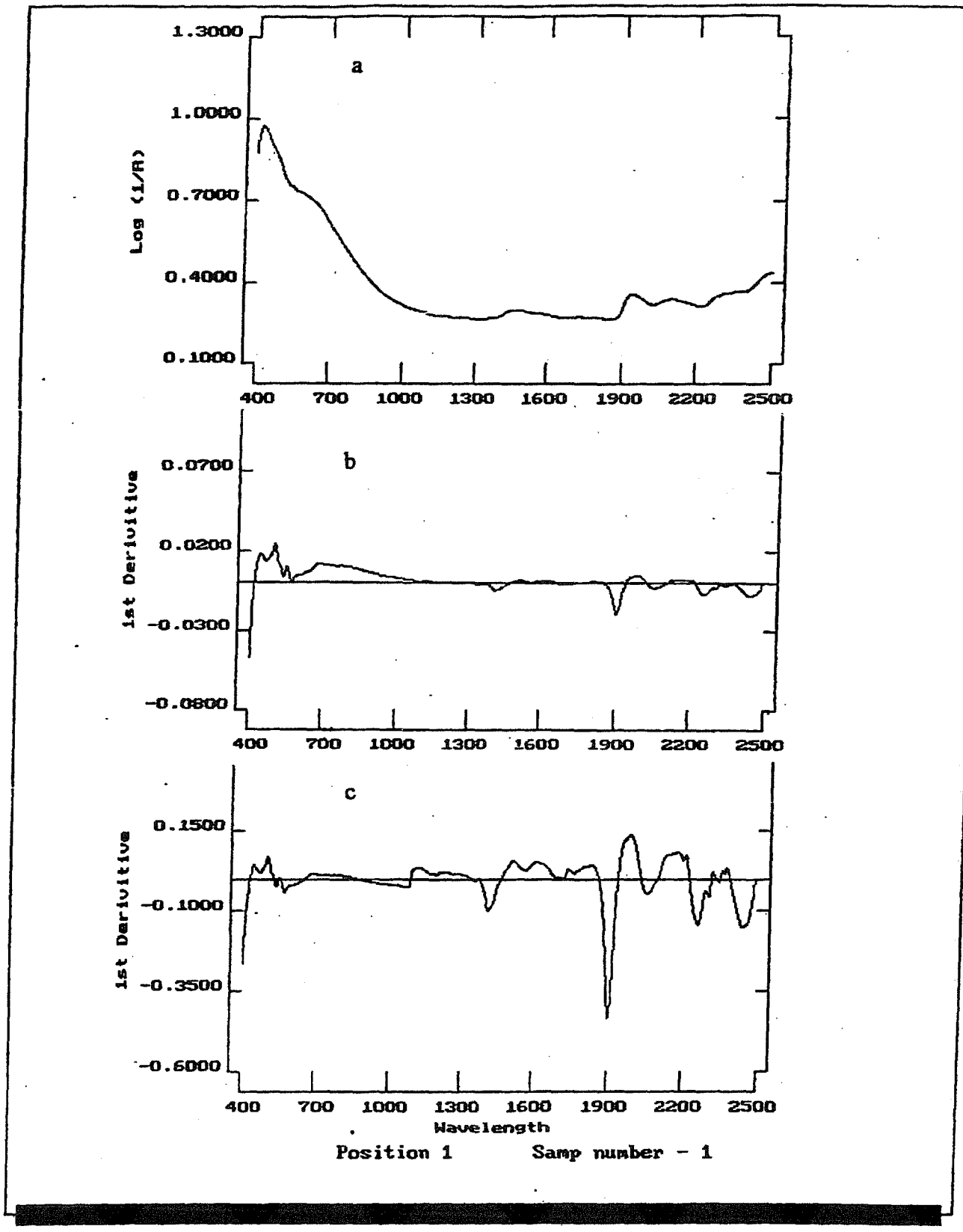

Fig. 6.4. An example raw spectrum (A), derivatized (B), and detrended and standard normal variate (C) transformed seagrass sample. Sample # 1 is a root/rhizome fraction of *Zostera capricorni*.

CHAPTER 7.

DETERMINANTS OF THE NUTRITIONAL ATTRIBUTES OF TROPICAL SEAGRASSES

7.1 INTRODUCTION

As discussed in Chapter 2, the nutritional quality of a plant to a consumer depends on its nutrient content and the ability of the animal to extract those nutrients. Nutrient content describes the concentrations of nutrients (e.g. nitrogen, water soluble carbohydrates) and anti-nutrients (e.g. fibre, phenolics). The level of food intake and the capacity of the animal to extract those nutrients determines the nutritional quality of the plant.

The nutrient content of seagrass as forage can be conveniently divided into those components making up the structure of the plant (cell wall) and those contained inside the cell wall (cell contents). Most of the nutrients in the soluble fraction (cell contents) are required by herbivores. The components of the cell contents examined in this study were total nitrogen, water soluble carbohydrate and starch. Nitrogen is considered one of the most important plant nutrients because it is essential for protein synthesis (Mattson 1980; Crawley 1983). Water soluble carbohydrate in forage represents the most rapidly digestible part of the non-structural carbohydrate of the plant (Van Soest 1994). Starch, from the plant perspective, is regarded as a reserve carbohydrate often classified with soluble carbohydrates because of its partial solubility to hot water (Van Soest 1994).

The components of the cell wall studied here were neutral detergent fibre, acid detergent fibre, hemicellulose, and lignin. Neutral detergent residues comprise the total cell wall constituents, while acid detergent divides the residue into fractions soluble and insoluble to 1N acid (Van Soest 1994). The major components of the neutral detergent residue include lignin, cellulose and hemicellulose; the minor components include some protein and bound nitrogen, minerals and cuticle. The

acid-soluble fraction includes hemicellulose and cell wall proteins, while the residue recovers the least digestible fraction, including lignin and cellulose (Van Soest 1994). Hemicellulose in plants is found mostly in lignified walls and is generally insoluble, but it does become water-soluble when delignified (Van Soest 1994). Lignin is the most significant factor limiting the availability of plant cell wall material to animal herbivores because of its consistent association with indigestibility.

The digestibility of food materials, including those of plants, is measured to assess their quality for the consumers. The significance of digestibility is that it represents that part of the food material available for digestion by the animal or microbial enzymes (Van Soest 1994). This could either be evaluated by *in vivo* or *in vitro* digestibility techniques. *In vitro* dry matter digestibility (IVDMD) measures the proportion of the total dry matter that disappears from the initial sample after incubation with pepsin, HCl and fungal cellulase (Choo *et al.* 1982; Van Soest 1994). Measuring IVDMD is an appealing alternative technique to feeding experiments in order to measure an animal's performance on a particular food plant because *in vivo* techniques are very difficult to perform especially since most wild herbivores are very difficult to hold under captive conditions. Even though IVDMD may not always give the same answer as *in vivo* experiments, similarities can be expected (Van Soest 1994).

Secondary compounds of seagrasses (e.g. tannins and phenols; Lanyon 1991) were not measured in this study since the methods available to measurr them are poor (see Waterman and Mole 1994), and since no nutritional study has yet convincingly argued their validity in impeding herbivory. One of the species most preferred by dugongs, *H. uninervis* has higher concentrations of total phenols than *Z. capricorni*, a less preferred species (Lanyon 1991). A herbivore may preferentially feed on a tannin-rich food source because the benefit of high energy may offset the cost of consuming the tannins (e.g. Smallwood and Peters 1986).

The nutritional components of seagrasses may vary by species, variety, part, location, and season (Lanyon 1991). Lanyon provides the most comprehensive data on nutrient contents (total nitrogen, organic matter, soluble carbohydrates, total phenols, condensed tannins, NDF, ADF, and lignin) of some tropical seagrass species [*H. ovalis, H. uninervis* (narrow- and wide-leaf varieties), *Zostera capricorni, Cymodocea serrulata*] according to plant parts. Lanyon found that *H. ovalis* and *H. uninervis* narrow-leaf variety had more nitrogen and less fibre than *Z. capricorni*, *C. serrulata* and *H. uninervis* wide-leaf variety. Similarly, the nutritional status of plants, including seagrasses, can be affected by injury such as that inflicted by grazing (McNaughton 1983b; Myers and Karban 1989). In this chapter, I will describe the variations in the nutrient contents and IVDMD of some tropical seagrasses, emphasising the effects of species, sites, plant parts, and varieties, and comment on their relevance to green turtles and dugongs. The effects of species and sites as determined by the grazing experiments will also be considered. The significant effects of the grazing treatments will be considered in Chapter 8. The effects of experimentally enhancing the levels of nutrients (nitrogen and phosphorus) in sediments on the nutritional composition of tropical seagrasses will also be considered (Section 7.3.5).

Optimal foraging theory predicts that herbivores optimise grazing by maximising the nutrients or by mixing their diet (Westoby 1974, 1978; Belovsky 1986), and that an optimal forager may show preferences when there are nutrient constraints (Pulliam 1975). I will explore the relevance of these suggestions in relation to the nutrient composition of tropical seagrasses and proposed dietary preferences of dugongs and green turtles.

This study shows that the nutritional components of tropical seagrasses vary by species, variety, and plant part, and that additional sediment nutrients variably alter their nutritional composition. I conclude that the nutritional properties of seagrass species may drive diet selectivity in the dugong and green turtle, and that levels of nitrogen, IVDMD, fibre, lignin and starch are relevant to their food selection and preference.

7.2. MATERIALS AND METHODS

7.2.1. Collection and preparation of samples

All seagrass samples collected, including those from the experiments, were pooled according to species, plant parts, location (and by study sites in the experiments only), time (month and year) of collection, whenever applicable, and into narrow- and wide-leaf varieties for *Halodule uninervis* and *Zostera capricorni* (see Table 6.1).

The collection of samples from the grazing experiments at Ellie Point and Cardwell was described in Chapter 5. The collection of samples from the nutrient enhancement experiments conducted at Shelley Beach (see Fig. 6.1a) is detailed below. The remainder of the samples was opportunistically collected from Moreton Bay, Hervey Bay, Bolger Bay (Magnetic Island, Townsville), North Brook Island, Green Island, Turtle Group Islands (Princess Charlotte Bay), Pipon Island, and Borroloola (Gulf of Carpentaria) (for locations see Table 6.1 and Fig 6.1). Samples of *Halophila spinulosa* were collected at various depths within the Pipon Island area (14°7'S, 144°32'E) to investigate whether the nutritional composition of a tropical seagrass varies by depth. A summary table of information on the samples collected at different depths off Pipon Island is presented in Appendix 6. The dates of collection of the samples are summarised in Table 6.1. In general, each sample was sorted by hand into individual species, separated into leaf and roots/rhizomes fractions, dried, and ground as detailed in Sections 4.2.6 and 6.2.2.

7.2.2. Nutrient enhancement experiments

This experiment was carried out as an Honours project of Ms Chantal Roder at the Department of Tropical Environment Studies and Geography, James Cook University. Roder (1995) used a randomised block design with 30 quadrats (3 replicate blocks each of ten treatments) to investigate the effects of enhanced sediment nutrients on a tropical seagrass bed at Shelley Beach, Townsville. This

seagrass bed is dominated by *H. uninervis*[1] with sparse *H. minor*[2] (Lanyon 1991). Using Osmocote® slow release fertilisers, nitrogen (N only - containing 23% nitrogen) and phosphorus (P only - containing 18% phosphorus) were added to the sediments of marked 0.7 x 0.7 m quadrats both separately and in combination, and in low and high concentrations (Table 7.1). The loadings used in these experiments were based on the quantities used in a similar experiment conducted at Moreton Bay (69 g/m^2 of nitrogen and 9 g/m^2 of phosphorus). The loadings used in the Moreton Bay experiment were estimated assuming that *Syringodium isoetifolium* grew at a rate of 10 g m^{-2} d^{-1} and that its plant tissue had a concentration of nitrogen of 1.5% and a concentration of phosphorus of 0.45% (J. Udy, 1994, pers. comm. to C. Roder). To examine whether the physical disturbance involved in applying fertilisers has any effects on seagrasses, an experimental control was included in addition to a field control for each block. The experimental control involved digging up plugs of seagrass, and replacing them within the same quadrat. A buffer zone of at least 1 m separated each quadrat from the next. The experiment lasted for 105 days, after which the seagrass samples were harvested using a 0.11 m^2 quadrat to gather subsamples haphazardly from each of the 0.49 m^2 plots. The seagrasses collected were rinsed, cleaned, sorted, dried and ground following the procedures described in Section 6.2.2.

7.2.3. Routine NIRS scanning and selection of calibration set

All samples were scanned in the near infra-red region using an NIRSystems monochromator (Model 6500) following the procedures detailed in Section 6.2.3. A calibration set was selected from the whole spectral population following the procedures described in Section 6.2.4.

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[1] Exhibited leaf tips characteristic of both *H. uninervis* and *H. pinifolia*.

[2] *Halophila minor* was previously regarded as *Halophila ovalis* and *Halophila ovata*. Sachet and Fosberg (1973) promoted *Halophila minor* to species level from a subspecies in the *H. ovalis* complex of den Hartog (1970), replacing *H. ovata* Gaud in Freyn. (Kuo and McComb 1989).

7.2.4. Nutritional attributes analysed

Samples selected for the calibration set were analysed for total nitrogen, organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid lignin, water soluble carbohydrate (WSC), total starch, and *in vitro* dry matter digestibility (IVDMD). Details of these methods are described in Section 6.2.5. The values for each nutrient component analysed in the calibration set were used to develop a predictive equation for each determination following the procedures described in Section 6.2.6. Hemicellulose was assumed to be the difference between ADF and NDF values (Van Soest *et al.* 1991). Thus, caution should be taken in interpreting hemicellulose values as they could have twice the error of the other components.

7.2.5. Routine predictions from NIR spectra

The nutritional composition (see Sections 7.1 and 7.2.4) of each sample was predicted using NIRS. The prediction equations were used to estimate the values of the different nutritional composition of each sample using the algorithms and routines detailed in the NIRS 3 (ISI 1992) software package (see Section 6.2.7).

7.2.6. Statistical analyses

7.2.6.1. Comparison between species in the whole data set

Multivariate analysis of variance (MANOVA)

Multivariate analysis of variance (MANOVA) (using SPSS 6.1.3, 1994) was used to test whether the eight nutritional variables (nitrogen, OM, NDF, ADF, lignin, WSC, starch and IVDMD) varied by species and varieties within species. Hemicellulose was not included as a nutritional variable as it was not independently determined and would, therefore, correlate with NDF and ADF values since it was derived from their difference. The nutritional composition of the different species was confounded by sites and time of collection because the samples outside the

experiments were opportunistically collected (see Sections 6.2.1 and 7.2.1). As a result, the examination of the variations in the nutritional composition among the species was possible only when the values for each nutrient for each species were aggregated over site and time of collection. Before running each MANOVA, the homoscedasticity (equality of variance) was checked by plotting the standardised residuals of the data against predicted values of the model, while homogeneity of covariance matrices and multivariate (and univariate) normality assumptions were assessed using Box's *M* test and q-q plots. Whenever assumptions were violated, logarithmic transformations were performed. The different nutritional components measured (% dm) were the dependent variables. Species was the independent variable. In the MANOVAs which tested the effects of variety, species and variety were used as crossed factors.

Linear discriminant analyses (LDA)

A linear discriminant analysis (LDA) was performed (using SPSS 6.1.3, 1994) on the nutrient data for each plant part to determine how close or far apart from each other the eight species of tropical seagrasses (*H. ovalis, Z. capricorni, H. uninervis, H. spinulosa, H. minor, C. rotundata, C. serrulata, S. isoetifolium*) and varieties (narrow- and wide-leaf) are on the basis of the different nutritional components (nitrogen, OM, NDF, ADF, lignin, WSC, starch and IVDMD). *Halophila trichostata* and *Halophila decipiens* were not included in the species list as they were not sorted into plant parts. LDA automatically standardises the nutrient values to *Z* scores (mean = 0 and s.d. = 1) to equalise the effect of variables on different scales, in this case, those components (WSC and starch) log transformed. The cell means were weighted by number of samples since the replication level for each species varied (2 - 64). In addition to a linear discrimination involving the two varieties (wide- and narrow-leaf) of *H. uninervis* and *Z. capricorni*, another analysis was performed using a third category of seagrass: neither wide- nor narrow-leaf (for those species which did not manifest any distinct variation in leaf morphology, e.g. *Halophila* species). The other species which have leaf width wider than 2 mm were classified as wide, e.g. *C.*

serrulata and *C. rotundata*. This was carried out mainly to examine whether the groups would remain distinct in a two-dimensional system. This is biologically and ecologically justified as this may be a possible criterion for initial discrimination or assessment of seagrasses by herbivores. The important nutritional components separating species were identified by: (1) their correlation coefficients with the discriminant functions, (2) rank of variables in the stepwise selection, and by (3) plotting their vectors in a 2-dimensional discriminant space.

7.2.6.2. Grazing experiments

General factorial-design ANOVAs using Designs I [trt vs 1, site vs wr , sp vs 2, trt*sp vs 3, site*trt=1 vs wr, sp*site=2 vs wr, site*sp*trt=3 vs wr] and II [trt vs 1, site vs wr, site*trt=1 vs wr] (see Section 5.2.10.2) were performed to test for the effects of grazing treatment, site, species (the last factor at Ellie Point only) and their interactions on the concentrations of each nutritional component (nitrogen, OM, NDF, ADF, hemicellulose, lignin, water soluble carbohydrates, starch, and IVDMD) for both separated and combined plant parts (whole plant), for each of the long-term grazing experiment. Site was treated as a random factor; other factors were fixed. The ANOVAs were performed for the nutritional attributes measured for *Halophila ovalis* and *Zostera/Cymodocea* from Ellie Point, and for *Halodule univervis* from Cardwell. Whenever effects were significant, simple contrasts were performed (using SPSS 6.1.3, 1994) to determine which treatments differed from the controls. Similarly, deviation contrasts were performed whenever species effects were significant to determine which species were affected.

For the short-term experiments, general factorial-design ANOVAs were performed with paired treatments-controls (pair 1= Trts I and II, 2 = Trts III and IV, and 3 = Trts V and VI, see Table 5.1) to test the effects of treatment and site, and their interactions on the proportions of the separated and combined plant parts as described in Section 5.2.10.2. The model used in analysis of each paired-treatment was similar to Design II of the first experiment and is detailed in Section 5.2.10.2.

This chapter will consider only the effects of species, sites, and variation by plant parts. The effects of the treatments will be discussed in Chapter 8.

7.2.6.3. Nutrient enhancement experiments

General factorial-design ANOVAs (see Appendix 7) were performed to test the effects of the levels sediment nutrients, species, and block, and their interactions, on each nutritional component (nitrogen, OM, NDF, ADF, hemicellulose, lignin, water soluble carbohydrates, starch, and IVDMD) in both separated and combined plant parts (whole plant) of *Halophila minor* and *Halodule uninervis* collected from the nutrient enhancement experiments conducted at Shelley Beach. Nitrogen [3 levels: N_0, N_1 and N_2] and phosphorus [3 levels: P_0, P_1 and P_2] were treated as fixed factors, while block (3 replicates) was treated as a random factor. Paired t-tests were performed between the experimental control and field control to check whether they differed. Results showed that the experimental control and the field control were never significantly different. Therefore the experimental control was used as the N_0P_0 treatment in the subsequent analyses. A sequential sums of squares regression was employed because of the uneven replication between species. Preliminary analyses tested for interactions between treatments and blocks. The results were not significant and so those terms were pooled in the final ANOVA model (see Appendix 7). This was to ensure that the testing of a random factor (block) over a composite error term in the final analyses was robust (as there was no replication within blocks). Whenever significant effects were detected, contrasts were carried out (using SPSS 6.1.3, 1994) to identify the levels at which the factor differed (Norusis 1993). Simple contrasts were performed to compare the effects of N and P to their respective controls. Deviation contrasts were used to compare the mean response measurements from each block with the overall mean.

7.2.6.4. Effects of depth on the nutritional composition of *H. spinulosa*

To test whether the nutritional composition of *H. spinulosa* collected at Pipon Island varied by depth, four depths (m, based on Port Datum) were defined as follows: 10, 16.5 - 18, 20 - 20.5, and 27 m (see Appendix 6). The samples from the 16.5 to 18 m,

and 20 to 20.5 m depths were pooled to define a larger gradient between depths and to balance the replication. A MANOVA was performed for each plant part (leaf and root/rhizome fraction) and whole plant to test the effect of depth using the eight nutritional components (nitrogen, OM, NDF, ADF, lignin, WSC, starch, and IVDMD) as dependent variables.

7.3. RESULTS

7.3.1. Variations in seagrass nutrients by species and plant parts

7.3.1.1. All species combined

In general, the leaves of the seagrasses studied had higher concentrations of nitrogen, OM, NDF, ADF, hemicellulose, and lignin, and had higher digestibility (*in vitro*) of dry matter (IVDMD) than the roots/rhizomes (Table 7.2). The roots/rhizomes, however, contained more water soluble carbohydrate and starch than the leaves. A table summarising the means and ranges of values of the different nutritional components of the different species examined and separated according to plant parts is presented in Appendix 8 Table 1.

The results of the MANOVA (using the eight nutritional components) for the effect of species on the nutritional composition of: (1) leaf and (2) root/rhizome fractions, and (3) the whole plant were highly significant (Table 7.3). The accompanying (optional) univariate tests also showed that the effect of species for each plant part was highly significant for each nutritional component (Appendix 9). This was clearly demonstrated by the linear discrimination analyses (LDA) which showed distinct species groups for both the leaf and root/rhizome fractions using the first two functions (Fig 7.1 and Table 7.4). The large eigenvalues associated with the functions indicate that there was greater variability between species than within species, and that these first two functions explained most of the variance (Table 7.4). This is further shown by the excellent classification (using all functions) into species for both the leaf and root/rhizome fractions (based only on the data used to define these LD functions). Using the eight nutritional variables,

the samples of a particular species in the data set were classified in the right group 98% of the time for the leaf fraction and 97% of the time for the root/rhizome fraction.

The first discriminant function in the leaf fraction was correlated largely with the NDF, ADF and lignin components (Table 7.5). The second discriminant function in the leaf fraction was mainly correlated with nitrogen content and IVDMD level. In the root/rhizome fraction, the first function discriminated between species largely on the basis of their starch and IVDMD concentration, while the second function discriminated the species mainly according to the levels of ADF and NDF (see Table 7.5). The importance of these variables in discriminating the species is further evident in the order of how these variables were entered in the stepwise selection (see Table 7.6). The variable contributing the largest variability with respect to the total variability (as indicated by Wilk's lambda) is selected first. In the leaf fraction, the NDF, ADF and nitrogen were the first three variables selected followed by OM and IVDMD (Table 7.6a). In the root/rhizome, starch was first chosen, followed by NDF, WSC, IVDMD and nitrogen (Table 7.6b).

Correlations between the discriminant functions and the important nutritional components became more evident when the vectors of each nutritional component were plotted (Fig 7.1). For example, in the leaf fraction, five groups were distinguishable: (1) *H. ovalis* and *H. minor*; (2) *C. rotundata*, *C. serrulata* and *H. uninervis* and; the last three groups, (3) *S. isoetifolium*, (4) *H. spinulosa* and (5) *Z. capricorni* were singularly distinguishable. The first group of species had higher levels of IVDMD and lignin, and lower concentrations of ADF than the second group. This is illustrated by the relative positions of these species on the vectors for IVDMD and lignin (positively correlated), and ADF (negatively correlated, as the ADF vector was at the opposite position of the IVDMD and lignin). The second group has lower IVDMD but higher concentrations of nitrogen, WSC, OM, and NDF (see Fig 7.1a). The leaves of *S. isoetifolium* have high IVDMD and starch concentrations, and low concentrations in nitrogen and NDF. The leaves of *H. spinulosa* were positively correlated with the starch vector (see also Appendix 8

Fig 8) and negatively with the NDF vector. The almost central location of *Z. capricorni* suggests intermediate concentrations on most of the nutritional components discriminating these groups. The largest variability in the nutrient content of the leaf fraction was attributed to NDF, ADF, lignin, IVDMD and nitrogen, as evidenced by vectors being longer than rest. However, nitrogen correlated positively with NDF, suggesting that species with high concentration of nitrogen may also have high concentration of NDF. Likewise, those species with high levels of IVDMD may have low OM content. In contrast, OM, WSC, and starch had the smallest variability as indicated by their short vectors.

In the root/rhizome fraction, three groups could be distinguished (see Fig 7.1b): (1) *H. ovalis*, *H. minor* and *H. spinulosa* and *Z. capricorni* formed a big group which had high IVDMD and lignin (*Z. capricorni* had the least IVDMD and intermediate levels of most of the nutritional components, because of its almost basal positioning to the IVDMD vector and almost central location, see also Appendix 8 Fig 9); (2) *C. rotundata*, *C. serrulata,* and *S. isoetifolium* had higher levels of both WSC and ADF (see Fig 7.1b); (3) *H. uninervis* correlated strongly with the starch vector; its starch concentration was the highest among the species examined here (see Appendix 8 Fig 8). It is interesting to note that the species preferred by dugongs as whole plants, such as *Halodule* and *Halophila* (Marsh *et al.* 1982), were high in IVDMD (*Halophila*) and nitrogen and starch (*Halodule*) in both plant parts. The linear discrimination analysis (LDA) plot for the roots/rhizomes (Fig 7.1a) showed also that IVDMD, ADF, lignin, NDF and WSC had large variance as indicated by the long vectors. In contrast, the short vectors starch, nitrogen and OM, suggest that there was not much variability among the species in these components.

7.3.1.2. Differences in nutrient composition between varieties

The nutritional composition of the wide- and narrow-leaf varieties of *H. uninervis* and *Z. capricorni* were significantly different (Table 7.7). Multivariate analysis showed that the effects of species and variety and their interaction were highly

significant. The main factor of interest is the species by variety interaction. The distinct groupings by varieties for each species were clearly demonstrated in the LDA and by the large eigenvalues (Table 7.8). This is further shown by the classification (using all functions) into varieties for both the leaf (97%) and root/rhizome (96.5%) fractions (based only on the data used to define these LD functions). The LDA plots clearly showed that the narrow-leaf variety of *H. uninervis* was nutritionally distinct from the wide-leaf variety with a higher concentration of nitrogen, WSC and IVDMD, and lower lignin and ADF concentrations (see Fig. 7.2a). In contrast, the leaf fractions of the narrow- and wide-leaf varieties of *Z. capricorni* only approached significance ($p = 0.073$) as evidenced by their overlapping distribution in the discriminant space.

The same trends were seen in the LDA for the root/rhizome fraction (Fig 7.2b). The narrow-leaf and wide-leaf varieties of *H. uninervis* were distinctly different. The narrow-leaf variety of *H. uninervis* had higher concentrations of starch and nitrogen, while the wide-leaf variety had higher levels of OM and ADF. The wide-leaf variety of *Z. capricorni* had a slightly higher concentrations of NDF and lignin in the roots/rhizomes than the narrow variety.

As in the first two linear discriminant functions, the third function indicated that the leaves and roots/rhizomes of the narrow- and wide-leaf varieties of *Z. capricorni* were only slightly separated. However, the variance explained by this function was only 3.7% for the leaves and 1.3% for the roots/rhizomes (see Fig 7.2c).

It is interesting to note that the LDA using "neither" as an additional variety for the rest of the species that do not manifest wide- nor narrow-leaf varieties, clearly showed distinct groupings (see Fig 7.3 and Table 7.9). The leaves of the wide-leaf morphology were separated by having higher concentration of NDF, while the narrow-leaf had higher concentrations of nitrogen, OM, lignin, starch, and IVDMD. The leaves of the 'neither' group had higher concentration of ADF. The largest variability in the leaves was attributed to ADF and NDF; intermediate variance was attributed to nitrogen, lignin, and OM; the least variance was

attributed to starch, IVDMD, and WSC (shortest). Even the roots/rhizomes were nutritionally distinct (Fig 7.3b). The narrow-leaf group separated from the wide-leaf by having higher concentrations of nitrogen, NDF, lignin, and IVDMD, and lower ADF. The 'neither' group had higher concentrations of WSC (and organic matter), and lower concentrations of starch. Overall, this means that seagrasses, based on these three general leaf morphologies, are nutritionally distinct. A comparison of the nutritional components between narrow-leaf and wide-leaf varieties of *H. uninervis* and *Z. capricorni* is presented in Appendix 8 Table 2.

7.3.2. Species differences in nutrient composition detected by the grazing and nutrient enhancement experiments

7.3.2.1. *H. ovalis* vs *Zostera/Cymodocea* (Grazing experiments)

Despite no significant difference being detected between the concentrations of nitrogen, water soluble carbohydrate, and starch in *H. ovalis* and *Zostera/Cymodocea*, overall the results showed that *H. ovalis* was nutritionally superior to *Zostera/Cymodocea* (Table 7.10 and see Appendix 8 Table 1). This is because *H. ovalis* contained less NDF, ADF, hemicellulose and lignin in both the separated and combined plant parts (lesser by 25.2% to 61.8%), but had higher IVDMD in the whole plants (+10.6%). IVDMD levels were the most significant, indicated by a high significance level ($p = 0.001$) and the largest F ratio (219.40) among the different nutritional components significantly affected (see Table 7.10). This suggests that IVDMD is the most important nutritional component distinguishing between the leaves of *H. ovalis* and *Zostera/Cymodocea*. Additionally, the leaves of *H. ovalis* were 5.0% more digestible (*in vitro*) than those of *Zostera/Cymodocea*. The OM content of *H. ovalis* was, however, moderately lower than that of *Zostera/Cymodocea* both in separated and combined plant parts. This also suggests that OM is not an important parameter for detecting food selectivity nor should it be interpreted as a nutritional parameter.

Overall, the levels of the nutrients such as nitrogen, OM, NDF, and ADF, and IVDMD in the leaf fraction were higher than in the roots/rhizomes for both *H. ovalis* and *Zostera/Cymodocea*. The root/rhizome fraction, on the other hand, has more starch and water soluble carbohydrate than the leaves.

7.3.2.2. *H. minor* vs *H. uninervis/pinifolia* (Nutrient enhancement experiments)

The differences in the concentrations of the nutritional components of *Halophila minor* and *Halodule uninervis* were highly significant (see Table 7.11). *H. uninervis* had higher concentration of starch in its roots/rhizomes (+168.8%) than *H. minor*. This is probably the most important nutritional difference between these species as indicated by a large *F* ratio (2104.44) and the largest among the nutritional components which also showed significant effects. Consequently, the starch concentration in the whole plants of *H. uninervis* was also significantly higher (+158.0%) than in *H. minor*. *H. uninervis* also had a higher concentration of WSC in the leaves, nitrogen in the leaves and roots/rhizomes, and OM in both separate and combined plant parts (see Table 7.11). However, *H. minor* had higher IVDMD than *H. uninervis*, particularly in its roots/rhizomes (+14.2%) and in the whole plants (+6.4%). The *F* ratio (632.73) also suggests that the digestibility of dry matter of the roots/rhizomes is an important difference between these species. In contrast, there was little difference between the levels of IVDMD in the leaves (0.9%) of these two species.

7.3.3. Site differences in nutrient composition detected by the grazing and nutrient enhancement experiments

7.3.3.1. Between Latin squares (Grazing experiments)

Except for the slight effects on water soluble carbohydrate ($p = 0.043$), all of the nutritional components in the leaves from the long-term grazing experiments conducted at Ellie Point showed high significant site (Latin square) effects (see

Table 7.12). In the root/rhizome fraction, only the hemicellulose and WSC concentrations were similar across sites. In the whole plants, only the concentrations of hemicellulose, WSC, and starch were similar across sites. At Cardwell, significant effect of site was identified for three of nine components (nitrogen, OM, and NDF) in the leaf fraction, but only one (nitrogen) in the root/rhizome fraction. The nutrient contents of the whole plants of *H. uninervis* at Cardwell were similar across sites. Fig 7.4 illustrates the variable effect of sites on the nitrogen concentration.

In the short-term grazing experiments, all of the nutritional components and plant parts showed significant variations across sites (Table 7.13). For example, the leaf nitrogen and OM content varied across sites in the root/rhizome fraction.

7.3.3.2. Between blocks (Nutrient enhancement experiments)

The concentrations of NDF, ADF, hemicellulose, and lignin in the leaves of *H. uninervis* and *H. minor* varied across blocks (Table 7.14). In the roots/rhizomes, the concentrations of OM, NDF, hemicellulose, WSC, starch and IVDMD varied across blocks; in the whole plants only OM and WSC varied across blocks. Only the nitrogen concentration showed no block effects.

7.3.4. Variations in nutrient contents of *Halophila spinulosa* across depth

The results of the MANOVA showed that depth had significant effects on the nutritional composition of *H. spinulosa* (Table 7.15 and Fig 7.5). The leaf nitrogen concentration in the 16-18 m depth was the lowest along the depth gradient; the leaf nitrogen concentrations at 10 and 20-20.5 m depths were similar and at intermediate levels; while at 27 m depth it was the highest. The starch concentration in the leaves was highest in the 20-20.5 m depth followed by that at 16-18 m, then 27 m, while the leaves at 10 m had the least (nil concentration). The water soluble carbohydrate concentration in the root/rhizome was highest at 10 m

depth followed by that at 20-20.5 m and least at 16-18 and 27 m. The concentration of lignin was highest at 10 m depth.

7.3.5. Effects of enhanced sediment nutrients on the nutritional composition of tropical seagrasses

The proportional (%) changes in the concentrations of the nutritional components of *H. minor* and *H. uninervis* resulting from significant effects of nutrient enhancement treatments (N_1, nitrogen concentration is one fold more than the ambient; N_2, nitrogen concentration is twice of the ambient; and P_1, phosphorus concentration is one fold more than the ambient; P_2, nitrogen concentration is twice of the ambient) are summarised in Table 7.16. Three and a half months after applying the fertilisers, the nitrogen concentrations in the leaf and root/rhizome (N_2 only) fractions of *H. minor* and *H. uninervis* were significantly higher in N_1 and N_2 treatments than the controls (see Table 7.16, and Appendix 11 Fig 1). The concentrations of NDF, hemicellulose and lignin significantly increased in the leaf fraction of both *H. minor* and *H. uninervis* as a result of the N_1 and N_2 treatments (see Table 7.16). Likewise, lignin concentration in the root/rhizome fraction of *H. minor* significantly increased (see Appendix 11 Fig 6 and Table 7.16). Starch concentration in the leaf fraction of *H. minor* also increased (see Table 7.16). The IVDMD of the roots/rhizomes of *H. uninervis* moderately increased. The only significant effect of phosphorus was under P_2 treatment where the hemicellulose concentration decreased in the leaves of *H. minor* (see Table 7.16 and Appendix 11 Fig. 5).

7.4. DISCUSSION

General comments on the nutritional composition of tropical seagrasses

In comparison to tropical terrestrial grasses, tropical seagrasses contain greater concentrations of nutrients and less fibre (cf Tables 7.17 and 7.18). Van Soest (1994) reports that the digestibility (*in vitro*) of tropical grasses in general, ranges

from 30-65% of dry matter. The IVDMD of four species of tropical grasses harvested at Sri Lanka, ranged from 50.8-62.5% (Senanayake 1995, see Table 7.18). These values are considerably lower than those recorded here for tropical seagrasses (78-99%). This may be due to the higher levels of lignin, fibre, and silica associated with terrestrial grasses (Van Soest 1994). The levels of NDF, ADF, and hemicellulose of four tropical grasses were considerably higher than those of tropical seagrasses (see Tables 7.17 and 7.18). This is because aquatic macrophytes, including seagrasses, in general, have lesser structural requirements than terrestrial grasses, as a consequence of living in an aquatic environment.

This study, like Lanyon's (1991), showed that the range of the mean values for the leaf nitrogen concentration of seagrasses (1 to 3% dm) falls within the range of values reported for terrestrial grasses (~ 1 to 4% dm, Stobbs 1973; Mattson 1980). The values of leaf nitrogen concentrations of several tropical grasses collected at Rockhampton, Queensland, ranged from 0.3 to 2.2% dm (A. Woolnough 1996, pers. comm.). With the higher digestibility of dry matter, lower fibre and lignin in seagrasses, dugongs are feeding on nutritionally superior food plants than terrestrial herbivores. This contradicts the earlier suggestion by Birch (1975) that tropical seagrasses are generally nutritionally poor but, as mentioned earlier, Birch's suggestion was limited by the small number of samples (only two to five samples per species). Furthermore, Birch also equated calorific content with nutritional content, and concluded that since seagrasses have the lowest calorific contents of any vascular vegetation, they are nutritionally poor. However, low calorific content does not necessarily mean low nutritional quality. Terrestrial grasses have high calorific content (gross energy) but it is actually bound in lignin and fibre, and therefore has low digestible energy (*sensu* Van Soest 1994). The lignin and fibre content of seagrasses are lower (see Table 7.17 and Appendix 8 Table 1) than terrestrial grasses; probably a consequence of living in an aquatic environment, requiring less structural components.

In general, the results reported in this study are comparable to those of Lanyon (1991). A comparison of our results for similar species and nutritional components

is summarised in Table 7.17. However, there are some differences between our results. The relatively low OM values (44-73.5% dm) reported in this study contrast with the high OM values (70-98% of dm) values reported by Lanyon. Birch (1975) and Wake (1975) reported low OM values (40-80% dm) similar to my results. This discrepancy is due to the different methods employed. Lanyon determined OM by ashing the NDF residue, while I (like Birch and Wake) ashed the actual dry matter. The neutral detergent solution would have dissolved some of the soluble inorganic matter such as salts (e.g. NaCl) associated with ash (see Appendix 12) which would have resulted in less ash and thus higher levels of organic matter. Inorganic materials are part of the ash and not OM. Lanyon did not measure OM according to any officially recognised method and she is therefore incorrect in calling these values OM.

The NDF and ADF values reported in this study were slightly lower than those reported by Lanyon (see Table 7.17). This is, again, due to methodological differences. I used a heat stable α-amylase (Van Soest *et al.* 1991) for my NDF determination to minimise the effect of unwanted side activities, e.g. inclusion of unwanted starch leads to difficulties in filtering the fibre and increases the analytical error which results to higher NDF values. Lanyon measured NDF and ADF following traditional procedures (e.g. Van Soest 1967) but without α-amylase. Lanyon also did not analyse NDF and ADF sequentially. As a result, her ADF residue may have been contaminated by hemicellulose, pectin and cell solutes (Van Soest 1982) resulting in slightly higher ADF values.

I employed an acid lignin method while Lanyon utilised a permanganate lignin method. As a result, the lignin values reported in this study were moderately higher than Lanyon's. Acid (72% sulfuric acid) would have dissolved more lignin than permanganate. The permanganate method is a shorter (and alternative) procedure for lignin determination (Van Soest 1982) but the acid lignin method is currently more accepted (Van Soest 1994). These differences could also be an artefact of the analytical errors due to the intractable nature of lignin to biochemical elucidation (*sensu* Van Soest 1994).

The leaf nitrogen concentrations reported in this study are comparable with those reported in the literature (Birch 1975; Wake 1975; Lanyon 1991). Birch (1975) reported mean values for some tropical seagrasses harvested from Townsville (Magnetic Island) as 1.58%, 1.60%, 1.48% (dm) for *Z. capricorni, S. isoetifolium*, and *C. serrulata*, respectively (cf Table 7.17 and Appendix 8 Table 1). Lanyon (1991) reported very similar nitrogen concentrations for *Z. capricorni* and *C. serrulata* harvested also from Townsville (see Table 7.17). The mean value of 0.92% (dm) for the leaf nitrogen concentration of *H. uninervis* (variety not specified) reported by Birch is unusually low. That value is, however, based on four samples only. The values I recorded for *H. uninervis* (narrow-leaf variety) from Townsville are comparable to those reported by Lanyon (see Table 7.17). The values I recorded for the *H. uninervis* from all of the different sites ranged from 1.6 to 3.8 % (mean = 2.89% dm). Therefore, the leaf nitrogen concentrations of tropical seagrasses are not really low as earlier thought.

Effects of species and plant parts

This study, like Lanyon's (1991), showed that the nutritional composition of tropical seagrasses varies with different plant part and species (see Tables 7.3 and 7.19, and Appendix 8 Table 1). Like Wake (1975) and Lanyon (1991), I found that *H. uninervis* (narrow-leaf variety) stood out among the other species as one with the highest level of leaf nitrogen (see Fig 7.1a). Based on feeding observations and gut content analyses, *H. uninervis* is a species preferred by dugongs (Marsh *et al.* 1982; Preen 1993). However, the results of the linear discriminant analysis (LDA) (Fig 7.1a) showed that the leaves of *C. rotundata* and *C. serrulata* were similar to *H. uninervis*. But *C. rotundata* and *C. serrulata* have seldom been seen in dugong gut content samples. Why? The answer may lie in the different nutritional composition and structure of their root/rhizome fraction. *H. uninervis* is also starch-rich (see Fig 7.1b). The apparent preference of dugongs for *H. uninervis* suggests: (1) that dugongs can detect or differentiate the species, probably by leaf morphology (Preen 1993; and see below), or (2) they optimise their diet not only in respect to higher concentrations of nitrogen but also carbohydrates (water soluble

carbohydrates and starch). Optimal foraging theory predicts that herbivores maximise nutrients by mixing their diet (Westoby 1974, 1978). Starch is the main carbohydrate reserve of seagrasses (Dawes and Lawrence 1979; Masini 1982). Starch concentration was highest in the roots/rhizomes of *H. uninervis*; the other species having moderate concentrations only (see Fig 7.1b). Waldron *et al.* (1989) report that *H. uninervis* contained twice as much structural carbohydrate than *Halophila stipulacea* and *H. ovalis*. Incidentally, Preen (1996, pers. comm.) reported that dugongs in the Gulf of Carpentaria graze extensively on *Syringodium isoetifolium*, which I found to have high levels of WSC (see Appendix 8 Table 1 and Fig 7). In contrast to *C. serrulata* and *C. rotundata*, *H. uninervis* does not rely on monopodial and continuous horizontal rhizome extension to maintain meadows but repetitively branches its vertical shoots to form clusters of erect shoots (Brouns 1987b). The horizontal extension of rhizomes usually forms strong root/rhizome mattresses which consequently may impede the capabilities of dugongs to harvest *C. rotundata* and *C. serrulata*. In contrast, *H. uninervis* does not form strong root/rhizome mattresses and is probably easier to harvest. As discussed in Chapter 5, the type of substrate is another determinant of the accessibility of below-ground biomass to dugongs.

Lanyon (1991) and Preen (1993) suggest that dugong preference (for *H. uninervis* and *H. ovalis*) is driven mainly by the amount of nitrogen and fibre in a particular seagrass species. Brand (1995) suggests that preference of juvenile green turtles at Moreton Bay for *H. ovalis* is driven by the amount of fibre. However, my results indicate that the preferred nutritional menu includes more than just nitrogen and fibre. From the LDA, we could say that the variables which varied most were more important in terms of selectivity since they were the components the animals could presumably detect easily as opposed to those components with only small variations. I, therefore, suggest that IVDMD, nitrogen, NDF, and lignin (for the leaves and roots), together with ADF and WSC/starch (for the roots/rhizomes) are the more important variables for group separation as indicated by their large variations. This in turn could be considered as a better set of nutritional variables in predicting preferences of dugongs and green turtles. For example, in the LDA, *H.*

ovalis and *H. minor* separated consistently from the rest of the species (both in the leaves and roots/rhizomes) as more digestible and with low ADF concentrations (see Fig 7.1). Meanwhile, *H. uninervis* leaves, correlated with WSC, NDF, and nitrogen, while its roots strongly correlated with starch (see Fig 7.1).

H. ovalis and *H. uninervis* are not the only nutritionally outstanding seagrasses tested. The other members of the Halophilae also characteristically contained more nutrients and less fibre than the rest of the species examined in this study. *S. isoetifolium* also showed that it was a good food species, as indicated by its close association with the Halophilae in its leaf content and with its root/rhizomes recording the highest levels of WSC. Intensive grazing by dugongs on *S. isoetifolium* beds in the Gulf of Carpentaria (Borroloola) has been observed by Preen (1996, pers. comm.). Dugongs in captivity, at Jaya Ancol Oceanarium in Jakarta, Indonesia, were also fed leaves of *S. isoetifolium* (Lanyon and Marsh 1995b).

Effects of variety

The nutritional composition of the varieties of *Z. capricorni* and *H. uninervis* was very distinct. These varieties maybe distinguishable by herbivores. For example, in dugongs, this could possibly be achieved by the initial contact of their muzzles on the seagrass. Preen (1993) reported apparent avoidance by dugongs of the wide leaf morph of both species in Moreton Bay. Most of the feeding trails reported from tropical Queensland have been on beds of narrow-leaf *H. uninervis* (e.g. Heinsohn and Birch 1972; Wake 1975; Anderson and Birtles 1978; Preen 1993). Narrow-leaf *H. uninervis* has the highest leaf nitrogen concentration of the seagrass species investigated (see also Appendix 8 Fig 1), a result in accordance with Lanyon (1991). There was, however, little difference between the leaves of the two varieties of *Z. capricorni* (Fig 7.2a). This could, however, be confounded by spatio-temporal variations, as the samples of wide variety *Z. capricorni* were mainly from Ellie Point, while the samples for the narrow variety were largely from Moreton Bay. In Shoalwater Bay, narrow-variety *Z. capricorni* plants are

important food for dugongs (Anderson and Birtles 1978) and green turtles (Limpus, unpublished data, cited in Lanyon *et al.* 1989; Brand 1995). However, wide-variety *Z. capricorni* plants rank low in the preferences of dugongs in Moreton Bay (Preen 1993). Both *Z. capricorni* and *H. ovalis* have lower concentrations of phenolics than *H. uninervis* and *C. serrulata* (Lanyon 1991).

H. ovalis* vs *Z. capricorni

H. ovalis had relatively 25 to 62% less 'fibre' (including hemicellulose and lignin) than *Z. capricorni*. Even though *Z. capricorni* has more OM, *H. ovalis* had considerably higher IVDMD than *Z. capricorni*. These results explain Preen's (1993) observation that dugongs prefer *H. ovalis* to *Z. capricorni*.

H. minor/ovalis* vs *H. uninervis

Although *H. minor/ovalis* and *H. uninervis* are usually the two most preferred species (Preen 1993), they are still very distinct nutritionally (see Table 7.11), a result supported by Waldron *et al.* (1989). *H. uninervis* and *Halophila* species differed in their concentrations of nitrogen and carbohydrate. The most marked difference was starch content (as indicated by the largest relative difference) of +158-169% in *H. uninervis*. The difference between their nitrogen content was also considerable with *H. uninervis* having higher concentrations (+22-30%) than *H. ovalis*. However, this advantage could be negated by the higher levels of fibre (+23.5-65%) and the lesser IVDMD (-0.9 to 14%) in *H. uninervis*. Condensed tannins have been reported to be present in *H. uninervis* and absent in *H. ovalis* (Lanyon 1991).

Effects of enhanced nutrients

Enhanced nitrogen variably affected the nutritional composition of tropical seagrasses (see Table 7.16). Enrichment caused an increase in nitrogen content of seagrasses as in previous works (e.g. Duarte 1992; McGlathery 1995). The

nitrogen content of seagrass tissues has been used as an indicator of the nutritional status of seagrasses, and Short (1987) and Duarte (1990) have suggested that seagrass growth is nitrogen limited when the average nitrogen concentration in the leaves is below 1.8% of dry matter. Thus, plants with nitrogen content similar or larger than 1.8% (dm) will be expected to show smaller responses. This explains why there was a smaller increase in nitrogen content in *H. uninervis* compared with *H. minor*. This was also evident in the smaller increase in nitrogen concentration of *H. uninervis* compared to *H. ovalis* following grazing (see Chapter 8). Also the nutritional composition of plants is a phenotypic trait, determined by the interactions between the plants' inherent requirements (genotype) and their environment (Mattson 1980; Antonovics *et al.* 1988; Van Soest 1994). *H. ovalis* is short-lived and its nutrient uptake and growth are presumably faster than in *H. uninervis*.

The results are consistent with the notion that seagrasses growing in terrigenous sediments are limited in nitrogen but not in phosphorus (Short 1987). The elevated nitrogen had greater impact than phosphorus which was significant only in the moderate reduction in hemicellulose content of *H. minor* (see Table 7.16). The elevated nitrogen levels in the sediment increased starch content in *H. ovalis*, suggesting that when the limiting nutrients are abundantly available, *H. ovalis* can grow more and store more carbohydrate as starch. However, this increase in growth also meant that the seagrass plants have to invest more in their structural framework resulting in higher fibre and lignin, and may consequently have lower digestibility. Thus, elevated nutrients can have both positive and negative effects with respect to seagrass herbivores. The resulting increase in biomass from enhanced growth (Roder 1995) and the higher concentrations of nitrogen and starch suggest the potential benefits. Apart from the increase in fibre content in seagrass tissues, another potential danger from enhanced nutrients is the increase in the amounts of sediment trapped by the enhanced growth (Roder 1995). This could impede the efficiency of the grazers and even be detrimental to seagrasses.

Effects of sites and depth

This study, like those of Dawes and Lawrence (1980, 1983), and Lanyon (1991), showed significant variations in nutrient contents of seagrasses across sites. This has important implications to seagrass herbivores which do not only have to contend with the nutritional variability of plant parts and species, but also with the spatial variability at local and regional scales. The significant local variations in the nutrient content of seagrass tissues were evident from the significant block effects in the nutrient enhancement experiments which were only 5 to 10 m apart (see Table 7.14). The effects of variations in the nutrient content of seagrass tissues in a larger patch were evident in grazing experiments from the significant effect of sites which were about 25 to 50 m apart (see Table 7.12). This may mean that large-scale spatial variation in the nutritional composition of seagrasses is not uncommon. This was apparent in the observed discrepancies between the means of the leaf nitrogen concentration of *H. ovalis* from Moreton Bay (1.4 ± 0.04 % dm), Ellie Point (2.1 ± 0.08 % dm), and Turtle Group Islands (1.1 ± 0.06 % dm). I could not test these differences statistically since these values were confounded by depth and the time of collection. Further studies into the variation of large spatial scales of nutritional composition of tropical seagrasses would be fruitful because such huge spatial variation may influence the feeding ecology, nutritional ecology and distribution of seagrass herbivores. This variability may be reduced by maintaining favourite feeding sites (see Chapter 8). Dugongs at Cardwell (pers. observ.) and Moreton Bay (Preen 1993) have been observed to return to sites to regraze (Chapter 8).

This study has shown that the nutritional composition of a tropical seagrass, *H. spinulosa*, varied significantly with depth. The nitrogen concentration in the leaves and roots/rhizomes was greatest in the samples collected from 27 m, while WSC concentration was highest at 10 m and lowest at 27 m. This contradicts the suggestion that the supposedly higher costs of deep water foraging by dugongs in Shark Bay, Western Australia are compensated for by the carbohydrate-rich rhizomes of *H. spinulosa* (Anderson 1994). Anderson assumed that the

roots/rhizomes of *H. spinulosa*, like *H. ovalis* and *H. uninervis*, have high starch concentrations. Anderson's assumptions were based on Masini's (1982) data for *H. ovalis* (19.5 mg starch/gm of dry weight) and *H. uninervis* (124.5 mg starch/gm of dry weight). When converted to percentage of dry weight by multiplying them by 0.1, resultant starch values are 1.95 and 12.45% dm, respectively. These values are comparable to my results. However, my results showed that the concentrations of starch in the roots/rhizomes of *H. spinulosa* (mean = 0.57% dm) and *H. ovalis* (0.98% dm) were much lower than the corresponding values for *H. uninervis* (13.7% dm) (see Appendix 8 Table 1). Dugongs presumably feed on deep water seagrass because they are selecting for species which live in deep water.

Conclusions

1) Tropical seagrasses, are more digestible than tropical terrestrial grasses and contain greater concentrations of nutrients and less fibre.

2) The nutritional composition of seagrasses varies by plant parts, species, sites, and depths.

3) The different species of seagrass can be classified on the basis of nutritional components such as nitrogen, OM, NDF, ADF, lignin, WSC, starch and IVDMD.

4) The leaves of *H. ovalis, H. minor, H. spinulosa* and *S. isoetifolium* species are more digestible than those of *Z. capricorni, H. uninervis, C. rotundata* and *C. serrulata*, and the largest variability in the nutritional composition of the leaves is attributed to the levels of NDF, lignin, nitrogen and IVDMD.

5) The roots/rhizomes of *H. uninervis* contained more starch than the rest of the species. The largest variability in the nutrient contents of roots/rhizomes was attributed to IVDMD, WSC, and ADF.

6) Enhanced nutrients in the sediments affected the nutritional composition of two tropical seagrasses by increasing their concentrations of nitrogen, starch, and fibre.

7) I suggest that IVDMD, nitrogen, ADF, NDF, WSC, and lignin are a better set of nutritional variables for predicting feeding preferences of dugongs and green turtles than nitrogen and fibre *per se*.

Table 7.1. The different treatments used in the nutrient enhancement experiments conducted at Shelley Beach, Townsville by Ms C. Roder and the quantities of nutrients (Osmocote® fertilizer) used for each treatment.

Treatment	Concentration of nitrogen added* (g/m^2)	Concentration of phosphorus added* (g/m^2)
Field control (N_0P_0)	none	none
Experimental control (N_0P_0)	none	none
Low nitrogen (N_1P_0)	32.76	none
Low phosphorus (N_0P_1)	none	4.50
High nitrogen (N_2P_0)	65.51	none
High phosphorus (N_0P_2)	none	9.00
Low nitrogen and low phosphorus (N_1P_1)	32.76	4.50
Low nitrogen and high phosphorus (N_1P_2)	32.76	9.00
High nitrogen and low phosphorus (N_2P_1)	65.51	4.50
High nitrogen and high phosphorus (N_2P_2)	65.51	9.00

* The calculations of the levels of nutrients used were based on the nutrient-enhancement experiments of J. Udy (1994, pers. comm. to Roder) in terrigenous sediments of a seagrass bed at Moreton Bay.

Table 7.2. The pooled means and range (minimum and maximum) of values for nine nutritional components (% dm) of the different plant parts (including detrital matter*) of eight tropical seagrass species (*Halophila ovalis, H. minor, H. spinulosa, Halodule uninervis, Zostera capricorni, Cymodocea serrulata, C. rotundata*, and *Syringodium isoetifolium*).

	Leaves n = 556		Roots/Rhizomes n = 552		Detrital Matter* n = 43	
	mean	range	mean	range	mean	range
Nitrogen	2.28	0.72 - 3.76	0.75	0.12 - 1.66	0.77	0.35 - 1.48
Organic Matter	64.80	41.07 - 79.28	61.44	32.04 - 89.34	45.17	32.06 - 61.54
Neutral Detergent Fibre	42.56	18.16 - 62.15	30.19	16.90 - 46.91	38.94	27.52 - 53.22
Acid Detergent Fibre	29.04	7.72 - 45.52	22.46	9.43 - 38.02	30.09	20.04 - 41.37
Hemicellulose	13.53	3.87 - 21.55	7.76	0.46 - 15.28	8.84	3.13 - 14.35
Lignin	15.74	1.17 - 29.43	9.75	1.58 - 24.64	19.71	10.31 - 28.36
Water Soluble Carbohydrate	0.13	0.00 - 4.49	0.32	0.00 - 10.93	0.07	0.00 - 0.21
Starch	1.13	0.00 - 7.0	8.21	0.00 - 30.70	2.68	0.20 - 6.30
In Vitro Dry Matter Digestibility	90.45	78.34-99.90	86.46	63.18 - 99.90	79.34	68.49 - 87.13

* The detrital matter was collected mainly from *Z. capricorni* only.

Table 7.3. Results of the MANOVA test of the differences between concentrations of nutritional components among tropical seagrass species using Pillai's trace*. Significant p values are highlighted in bold.

Plant part	Value	Approximate F	Numerator df	Error df	p
Leaf	3.40784	64.74779	56.0	3822.0	**0.000**
Root/rhizome	3.92686	86.73949	56.0	3808.0	**0.000**
Whole plant	2.73001	87.83032	56.0	7693.0	**0.000**

*Pillai's trace is the most powerful and robust of the multivariate test statistics for evaluating multivariate differences because the significance level based on it is reasonably correct even in the presence of mild violations of homogeneity of covariance matrices or multivariate normality (Norusis 1993).

Table 7.4. Summary table of linear discrimination analysis involving eight tropical seagrass species (*H. ovalis, Z. capricorni, H. uninervis, H. spinulosa, H. minor, C. rotundata, C. serrulata, S. isoetifolium*) using aggregated data and weighted cell mean values of eight nutritional components (nitrogen, OM, NDF, ADF, lignin, WSC, starch, and IVDMD); showing the first two (of seven) discriminant functions.

Plant Part	Function	Eigenvalue	Percentage of Variance	Cumulative Percentage	Canonical Correlation
Leaf	First	18.3470	66.82	66.82	0.9738
	Second	5.2026	18.95	85.77	0.9158
Root/rhizome	First	23.7698	60.15	60.15	0.9796
	Second	8.8125	22.30	82.45	0.9477

Table 7.5. Pooled within-groups correlations between the discriminating variables and the first two discriminant functions. Variables (nutritional components) are ordered by size of correlation within function.

Nutritional Component	First Function	Second Function
1) Leaf		
Neutral detergent fibre	0.69798*	0.18462
Acid detergent fibre	0.59235*	-0.13231
Lignin	0.40800*	0.18530
In Vitro Digestibility (dm)	-0.11426	0.17698*
Organic Matter	0.49026	-0.30532
Nitrogen	0.41957	0.46361*
Starch	0.01309	-0.09335
Water soluble carbohydrate	0.00868	-0.00233
2) Roots/Rhizomes		
Starch	-0.51238*	-0.29314
In Vitro Digestibility (dm)	0.39188*	0.05506
Lignin	0.01622	0.13159
Neutral detergent fibre	0.07888	0.50720*
Acid detergent fibre	0.15277	0.40877*
Water soluble carbohydrate	-0.04234	0.26031
Organic Matter	-0.18707	0.34479
Nitrogen	-0.25671	0.01921

* denotes largest absolute correlation between each variable and any discriminant function; shown are the first two functions only.

Table 7.6. The order of entry of the different variables (nutritional components) in the discriminant analysis of the species groups for each plant part using a stepwise selection method; also shown are Wilk's lambda and significance values. All variables for each plant were acceptable to the selection rule (i.e. for each step: minimise Wilk's lambda, minimum tolerance level = 0.001, minimum F value to enter = 3.84, maximum F value to remove = 2.71).

Step	Variable Entered	Wilk's Lambda	Significance
A) Leaf Fraction			
1	Neutral detergent fibre	0.098110	**0.000**
2	Acid detergent fibre	0.03176	**0.000**
3	Nitrogen	0.01452	**0.000**
4	Organic Matter	0.00761	**0.000**
5	*In Vitro* Digestibility (dm)	0.00331	**0.000**
6	Lignin	0.00218	**0.000**
7	Water soluble carbohydrate	0.000109	**0.000**
8	Starch	0.00078	**0.000**
B) Root/Rhizome Fraction			
1	Starch	0.11524	**0.000**
2	Neutral detergent fibre	0.02720	**0.000**
3	Water soluble carbohydrate	0.00714	**0.000**
4	*In Vitro* Digestibility	0.00187	**0.000**
5	Nitrogen	0.00077	**0.000**
6	Organic Matter	0.00029	**0.000**
7	Lignin	0.00020	**0.000**
8	Acid detergent fibre	0.00012	**0.000**

Table 7.7. Results of the two-factor MANOVA testing the effects of variety on *H. uninervis* and *Z. capricorni* using Pillai's trace*. Significant *p* values are highlighted in bold.

Factor	Value	Exact *F*	Numerator df	Error df	*p*
1) Leaves					
Species	0.75140	125.81365	8.0	333.0	**0.000**
Variety	0.40127	27.89693	8.0	333.0	**0.000**
Species*Variety	0.60353	63.36434	8.0	333.0	**0.000**
2) roots/rhizomes					
Species	0.93108	565.72243	8.0	335.0	**0.000**
Variety	0.46963	37.07878	8.0	335.0	**0.000**
Species*Variety	0.67367	86.44738	8.0	355.0	**0.000**
3) whole plant					
Species	0.67962	180.04571	8.0	679	**0.000**
Variety	0.23366	25.87848	8.0	679	**0.000**
Species*Variety	0.49906	85.55500	8.0	679	**0.000**

*Pillai's trace is the most powerful and robust of the multivariate test statistics for evaluating multivariate differences because the significance level based on it is reasonably correct even in the presence of mild violations of homogeneity of covariance matrices or multivariate normality (Norusis 1993).

Table 7.8. Summary table of linear discrimination analysis involving two varieties (narrow- and wide-leaf) for both *H. uninervis* and *Z. capricorni* using aggregated data and weighted cell mean values of eight nutritional components (nitrogen, OM, NDF, ADF, lignin, WSC, starch, and IVDMD); since there were only two groups only one function is generated.

Plant Part	Function	Eigenvalue	Percentage of Variance	Cumulative Percentage	Canonical Correlation
Leaf	First	4.1272	100	100	0.8972
Root/rhizome	First	3.4005	100	100	0.8791

Table 7.9. Summary table of linear discrimination analysis involving three categories for varieties: narrow-leaf and wide-leaf (for both *H. uninervis* and *Z. capricorni*), and neither (for the rest of the species which neither manifest such variety) using aggregated data and weighted cell mean values of eight nutritional components (nitrogen, OM, NDF, ADF, lignin, WSC, starch, and IVDMD).

Plant Part	Function	Eigenvalue	Percentage of Variance	Cumulative Percentage	Canonical Correlation
Leaf	First	8.6655	89.35	89.35	0.9469
	Second	10.0326	10.65	100	0.7127
Root/rhizome	First	11.2569	82.17	82.17	0.9583
	Second	2.4421	17.83	100	0.8423

Table 7.10. Summary of significant ($p < 0.05$) effects of species on the nutritional components of *H. ovalis* and *Zostera/Cymodocea* harvested from grazing experiments conducted at Ellie Point (see Appendix 10 Tables 1 - 9 for complete ANOVA tables), showing relative (%) magnitude of differences in nutrient concentration in plant parts affected, and corresponding p values and F ratios (enclosed in parentheses). Nitrogen, water soluble carbohydrates and starch were similar across species.

Nutritional Component	Plant part	% difference of nutrient concentration in *H. ovalis* relative to *Zostera/Cymodocea*	p (F ratio)
Organic Matter	whole plant	-16.9	**0.017 (23.00)**
	leaf	-15.5	**0.009 (36.24)**
	root/rhizome	-18.6	**0.037 (13.00)**
Neutral Detergent Fibre	whole plant	-34.0	**0.011 (32.63)**
	leaf	-33.0	**0.006 (50.20)**
	root/rhizome	-36.0	**0.021 (19.84)**
Acid Detergent Fibre	whole plant	-27.9	**0.011 (31.21)**
	leaf	-30.5	**0.009 (37.92)**
	root/rhizome	-25.2	**0.016 (23.92)**
Hemicellulose	whole plant	-50.3	**0.004 (65.10)**
	leaf	-44.8	**0.003 (76.78)**
	root/rhizome	-61.8	**0.015 (25.99)**
Lignin	whole plant	-32.7	**0.005 (59.66)**
	leaf	-50.2	**0.001 (160.06)**
	root/rhizome	-41.9	**0.008 (40.10)**
In Vitro Dry Matter Digestibility	whole plant	+10.6	**0.001 (219.40)**
	leaf	+ 5.0	**0.050 (10.06)**
	root/rhizome	+16.3	**0.008 (41.65)**

Table 7.11. Summary of significant ($p < 0.05$) effects of species on the nutritional components of *H. minor* and *Halodule uninervis* (narrow-leaf variety) harvested from the fertilization experiments conducted at Shelley Beach (see Appendix 7 for complete ANOVA tables), showing relative (%) magnitude of differences in nutrient concentration in plant parts affected, and corresponding p values and F ratios (enclosed in parentheses).

Nutritional Component	Plant part	% difference of nutrient concentration in *H. minor* relative to *H. uninervis*	p (F ratio)
Nitrogen	leaf	-21.9	**0.000 (70.59)**
	root/rhizome	-30.0	**0.000 (19.34)**
Organic Matter	whole plant	-36.4	**0.000 (325.78)**
	leaf	-23.5	**0.000 (171.44)**
	root/rhizome	-49.0	**0.000 (744.23)**
Neutral Detergent Fibre	whole plant	-33.9	**0.000 (46.19)**
	leaf	-39.7	**0.000 (458.42)**
	root/rhizome	-25.6	**0.000 (156.06)**
Acid Detergent Fibre	whole plant	-26.0	**0.000 (31.37)**
	leaf	-39.1	**0.000 (277.75)**
	root/rhizome	-8.5	**0.025 (5.61)**
Hemicellulose	whole plant	-50.0	**0.000 (58.68)**
	leaf	-40.9	**0.000 (441.73)**
	root/rhizome	-65.1	**0.000 (260.21)**
Lignin	whole plant	-38.5	**0.000 (43.37)**
	leaf	-40.6	**0.000 (104.83)**
	root/rhizome	-35.3	**0.000 (58.82)**
Water Soluble Carbohydrate	leaf	-40.6	**0.014 (6.82)**
Starch	whole plant	-158.0	**0.000 (27.07)**
	root/rhizome	-168.8	**0.000 (2104.44)**
In Vitro Dry Matter Digestibility	whole plant	+6.4	**0.000 (25.69)**
	leaf	-0.9	**0.016 (5.88)**
	root/rhizome	+14.2	**0.000 (632.73)**

Table 7.12. Summary of significance levels (p) of the effect of sites on the different nutritional components of seagrasses harvested from long-term grazing experiments (intensive grazing, light grazing, cropping and control) conducted at Ellie Point and Cardwell. Sites (Latin squares) were at about 40 to 50 m apart. Please refer to Appendix 10 Tables 1-18 complete ANOVA tables. Significant p values are highlighted in bold.

Nutritional Component	Ellie Point			Cardwell		
	leaf	root	whole	leaf	root	whole
Nitrogen	**0.000**	**0.000**	**0.049**	**0.001**	**0.000**	0.956
Organic Matter	**0.000**	**0.017**	**0.004**	**0.005**	0.386	0.768
Neutral Detergent Fibre	**0.000**	**0.001**	**0.000**	**0.040**	0.565	0.923
Acid Detergent Fibre	**0.001**	**0.000**	**0.000**	0.205	0.365	0.874
Hemicellulose	**0.000**	0.120	0.225	0.074	0.002	0.723
Lignin	**0.001**	**0.000**	**0.000**	0.540	0.086	0.800
Water Soluble Carbohydrate	**0.043**	0.147	0.102	0.180	0.963	0.903
Starch	**0.026**	**0.018**	0.214	0.164	0.086	0.727
In vitro dry matter digestibility	**0.000**	**0.000**	**0.002**	0.402	0.421	0.941

Table 7.13. Summary of significance levels (p) of the effect of blocks on the different nutritional components of seagrasses harvested from short-term grazing experiments conducted at Cardwell. Sites were about 40 to 50 m apart. Please refer to Appendix 10 Tables 19-27 for complete ANOVA tables. Significant p values are highlighted in bold.

Nutritional Component	Light intensity dugong grazing and its control			Turtle cropping harvested after 1 month			Turtle cropping harvested after 2 months		
	leaf	root	whole	leaf	root	whole	leaf	root	whole
Nitrogen	**0.038**	0.059	0.997	**0.014**	**0.000**	0.905	**0.000**	**0.000**	0.712
Organic Matter	0.139	**0.000**	**0.005**	**0.010**	**0.002**	0.225	**0.008**	**0.000**	**0.000**
Neutral Detergent Fibre	**0.004**	**0.001**	0.739	0.180	0.103	0.939	**0.033**	0.308	0.987
Acid Detergent Fibre	**0.009**	**0.000**	0.628	0.508	**0.000**	0.291	**0.000**	0.066	0.821
Hemicellulose	**0.011**	**0.014**	0.560	0.568	**0.003**	0.764	**0.000**	0.541	0.823
Lignin	**0.004**	0.072	0.861	0.397	**0.009**	0.818	0.920	0.053	0.979
Water Soluble Carbohydrate	**0.041**	**0.012**	**0.001**	0.568	**0.003**	0.764	**0.000**	0.541	0.823
Starch	0.092	0.699	0.699	0.135	**0.000**	**0.000**	**0.003**	**0.000**	**0.002**
In vitro dry matter digestibility	**0.000**	**0.011**	0.057	0.208	0.871	0.975	**0.001**	**0.004**	**0.003**

Table 7.14. Summary of significance levels (p) of the effect of sites on the different nutritional components of seagrasses harvested from nutrient enhancement experiments conducted at Shelley Beach. Blocks were about 5 to 10 m apart. Please refer to Appendix 7 for complete ANOVA table. Significant p values are highlighted in bold.

Nutritional Component	Leaf	Root/rhizome	Whole plant
Nitrogen	0.100	0.058	0.848
Organic Matter	0.085	**0.000**	**0.001**
Neutral Detergent Fibre	**0.000**	**0.001**	0.201
Acid Detergent Fibre	**0.001**	0.193	0.258
Hemicellulose	**0.001**	**0.001**	0.220
Lignin	**0.020**	0.086	0.156
Water Soluble Carbohydrate	0.127	**0.044**	**0.010**
Starch	0.457	**0.000**	0.516
In vitro dry matter digestibility	0.454	**0.001**	0.529

Table 7.15. Results of the MANOVA testing effects of depth on the nutritional attributes of *Halophila spinulosa* using Pillai's trace*. Significant *p* values are highlighted in bold.

Plant part	Value	Approximate *F*	Numerator df	Error df	*p*
Leaf	2.90625	23.25079	24.0	18.0	**0.000**
Root/rhizome	2.77997	9.47606	24.0	18.0	**0.000**
Whole plant	2.07148	5.85623	24.0	63.0	**0.000**

*Pillai's trace is the most powerful and robust of the multivariate test statistics for evaluating multivariate differences because the significance level based on it is reasonably correct even in the presence of mild violations of homogeneity of covariance matrices or multivariate normality (Norusis 1993).

Table 7.16. Summary of significant ($p < 0.05$) effect of treatments on the nutritional components of *Halophila minor* and *Halodule uninervis* harvested from the enhanced sediment nutrient experiments (three levels of nitrogen and phosphorus: N_0, N_1, N_2 and P_0, P_1, P_2) conducted at Shelley Beach: the table shows proportional (%) changes in concentration relative to N_0; treatment (enclosed in parentheses); and plant parts affected. The whole plant was not affected; and the acid detergent fibre and water soluble carbohydrates were similar across treatments. See Appendix 7 for complete ANOVA tables.

Species	Plant Part	Nitrogen	Organic Matter	Neutral Detergent Fibre	Hemicellulose	Lignin	Starch	IVDMD
Halophila minor	leaf	+ 22.7 (N_1)		+ 8.4 (N_1)	+ 15.1 (N_1)	+ 24.9 (N_1)	+ 64.9 (N_1)	
		+ 26.1 (N_2)		+ 8.5 (N_2)	+ 13.5 (N_2)	+ 25.0 (N_2)	+ 46.2 (N_2)	
					- 7.7 (P_2)			
	root/rhizome	+ 32.0 (N_2)	- 7.5 (N_1)			+ 26.6 (N_1)		
						+ 22.0 (N_2)		
Halodule uninervis	leaf	+ 8.4 (N_1)		+ 6.3 (N_1)	+ 12.8 (N_1)	+ 5.2 (N_1)		
		+ 14.5 (N_2)		+ 6.2 (N_2)	+ 16.6 (N_2)	+ 4.7 (N_2)		
	root/rhizome	+ 10.1 (N_2)						+ 2.2 (N_1)
								+ 3.8 (N_2)

Table 7.17. A comparison of the mean values (number of samples, n, is enclosed in parentheses) of some nutritional components of some similar species of tropical seagrasses examined by Lanyon (1991) and this study.

	Organic Matter		(Total) Nitrogen		NDF		ADF		Lignin		Location	Reference
Species	leaves	roots	leaves	roots	leaves	roots	leaves	roots	leaves	roots		
Halophila ovalis	89.96 (3)	77.30 (3)	1.57 (7)	0.88 (14)	42.60 (5)	46.40 (5)	24.74 (3)	31.18 (4)	6.41 (3)	5.99 (4)	Townsville	Lanyon 1991
Halophila ovalis (n= 81 leaf; 77 root)	56.73	49.00	1.73	0.62	32.26	26.48	21.89	21.41	10.78	8.97	Qld[1]	this study
H. minor[2] (n= 27 leaf; 27 root)	50.86	44.20	1.95	0.56	31.86	24.32	20.72	19.75	11.52	8.75	Townsville	this study
Halodule uninervis (n)	91.95 (21)	94.05 (21)	1.91 (47)	0.67 (47)	50.96 (28)	44.62 (28)	30.89 (6)	21.32 (6)	11.39(13)	6.39(13)	Townsville	Lanyon 1991
Halodule uninervis (n) (n= 30 leaf; 30 root)	64.62	73.32	2.33	0.69	47.89	31.21	30.91	20.13	16.87	11.03	Townsville	this study
Halodule uninervis (w)	93.37 (35)	93.06 (36)	1.45 (74)	0.59 (70)	53.75 (43)	49.85 (44)	32.59 (8)	23.44 (8)	11.98 (8)	6.38 (8)	Townsville	Lanyon 1991
Halodule uninervis (w) (n= 10 leaf; 10 root)	70.27	73.20	1.68	0.63	44.99	32.05	30.62	22.65	14.86	11.59	Qld[1]	this study
Zostera capricorni (w)	90.07 (6)	82.68 (6)	1.60 (6)	0.56 (6)	62.57 (6)	63.52 (6)	36.55 (6)	36.58 (6)	10.60 (6)	5.66 (6)	Townsville	Lanyon 1991
Zostera capricorni (w) (n= 53 leaf; 61 root)	63.30	55.66	1.92	0.66	43.64	34.88	28.08	26.77	15.24	15.59	Qld[3]	this study
Cymodocea serrulata	92.81 (36)	97.55 (35)	1.41 (71)	0.62 (70)	51.67 (45)	34.30 (44)	29.94 (9)	24.69 (9)	7.55 (9)	5.86 (9)	Townsville	Lanyon 1991
Cymodocea serrulata (n= 4 leaf; 4 root)	71.23	73.40	1.67	0.75	46.23	39.47	29.15	30.22	15.28	15.79	Ellie Point	this study

[1] pooled samples collected at Moreton Bay, North Brook Island, Ellie Point, and Turtle Group Island (Queensland)
[2] *H. minor* at Shelley Beach, Townsville has been identified by earlier studies as *H. ovalis* by Lanyon (1991), and as *H. ovata* by Newling (1994).
[3] pooled samples collected at Moreton Bay and Ellie Point (Queensland).

Table 7.18. Nutritional composition (% dm) of four tropical grass species harvested in Sri Lanka, showing mean values only (from Senanayake 1995).

	NDF	ADF	Hemicellulose	IVDMD
Axonopus compressus	72.1	42.3	29.4	62.5
Imperata cylindrica	71.4	45.3	26.1	50.8
Cynodon dactylon	76.3	44.2	32.6	51.6
Pennisetum polystachyon	75.8	46.3	29.3	52.7
overall mean	73.9	44.5	29.4	54.4

Leaf fraction - Species

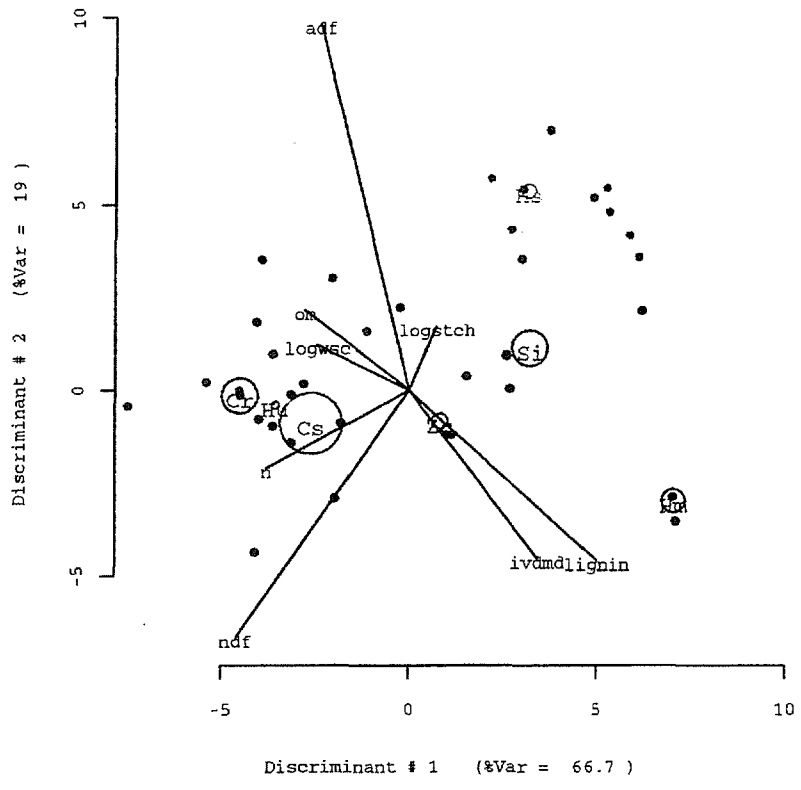

Fig 7.1a. Linear discriminant analysis plots of species groupings for the leaf fraction, showing only the first two functions. The set of dots (colour-coded) show the spread of each species across the discriminant space; the location of the species code represents the centroid for each species, while the area of the circle represents the standard error. (Legend: *Halophila ovalis*, Ho, yellow; *Zostera capricorni*, Zc, dark green; *Halodule uninervis*, Hu, pink; *Halophila spinulosa*, Hs, light blue; *Halophila minor*, Hm, dark blue; *Cymodocea serrulata*, Cs, light green; *Cymodocea rotundata*, Cr, orange; and *Syringodium isoetifolium*, Si, red).

Root/rhizome fraction - Species

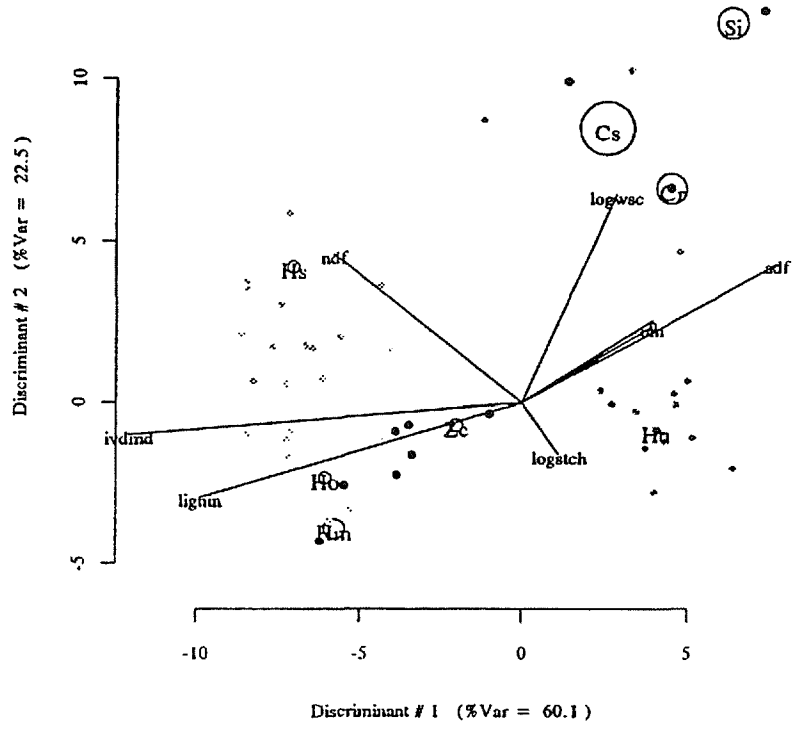

Fig 7.1b. Linear discriminant analysis plots of species groupings for the root/rhizome fraction, showing only the first two functions. The set of dots (colour-coded) show the spread of each species across the discriminant space; the location of the species code represents the centroid for each species, while the area of the circle represents the standard error. (Legend: *Halophila ovalis*, Ho, yellow; *Zostera capricorni*, Zc, dark green; *Halodule uninervis*, Hu, pink; *Halophila spinulosa*, Hs, light blue; *Halophila minor*, Hm, dark blue; *Cymodocea serrulata*, Cs, light green; *Cymodocea rotundata*, Cr, orange; and *Syringodium isoetifolium*, Si, red).

Leaf fraction - Variety

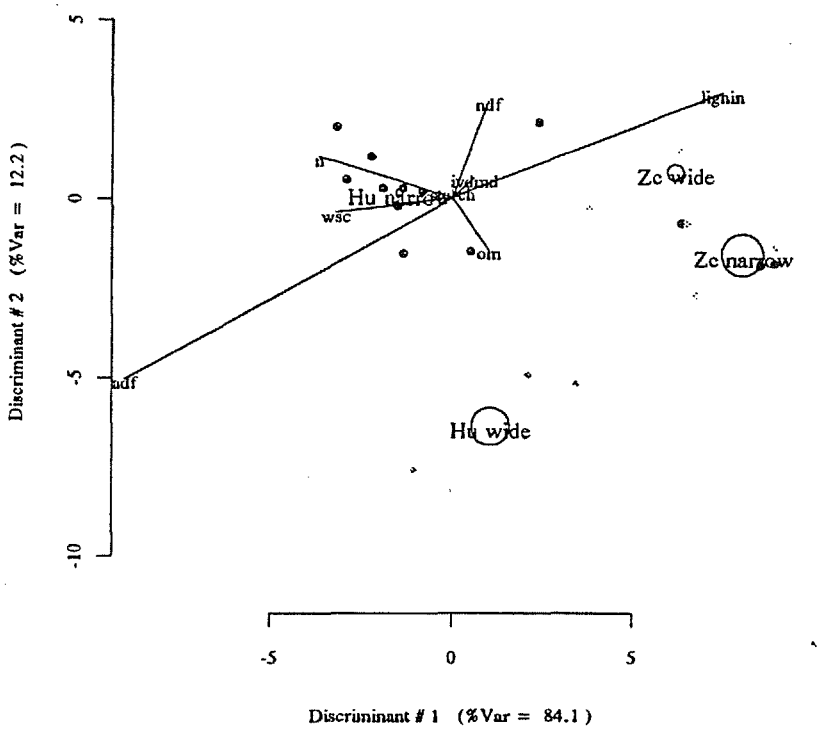

Fig 7.2a. Linear discriminant analysis plots of variety groupings (wide or narrow) for the leaf fraction of *Halodule uninervis* (Hu) and *Zostera capricorni* (Zc), showing only the first two functions. The set of dots (colour-coded) show the spread of each variety of each species across the discriminant space; the location of the species/variety code represents the centroid for each variety of each species, while the area of the circle represents the standard error. (Colour-code: *Z. capricorni* wide, yellow; *Z. capricorni* narrow, blue; *H. uninervis* wide, green; *H. uninervis* narrow, red).

Root/rhizome fraction - Variety

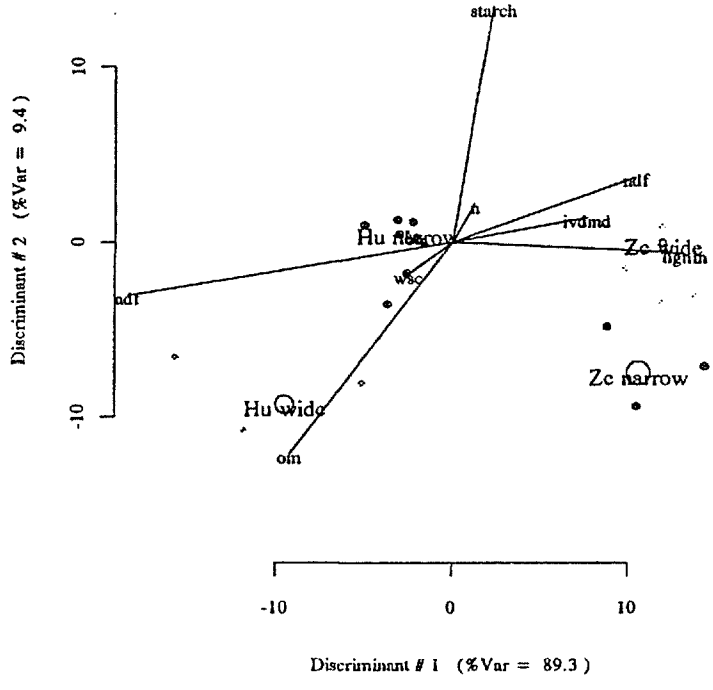

Fig 7.2b. Linear discriminant analysis plots of variety groupings (wide or narrow) for the root/rhizome fraction of *Halodule uninervis* (Hu) and *Zostera capricorni* (Zc), showing only the first two functions. The set of dots (colour-coded) show the spread of each variety of each species across the discriminant space; the location of the species/variety code represents the centroid for each variety of each species, while the area of the circle represents the standard error. (Colour-code: *Z. capricorni* wide, yellow; *Z. capricorni* narrow, blue; *H. uninervis* wide, green; *H. uninervis* narrow, red).

Leaf fraction - Variety

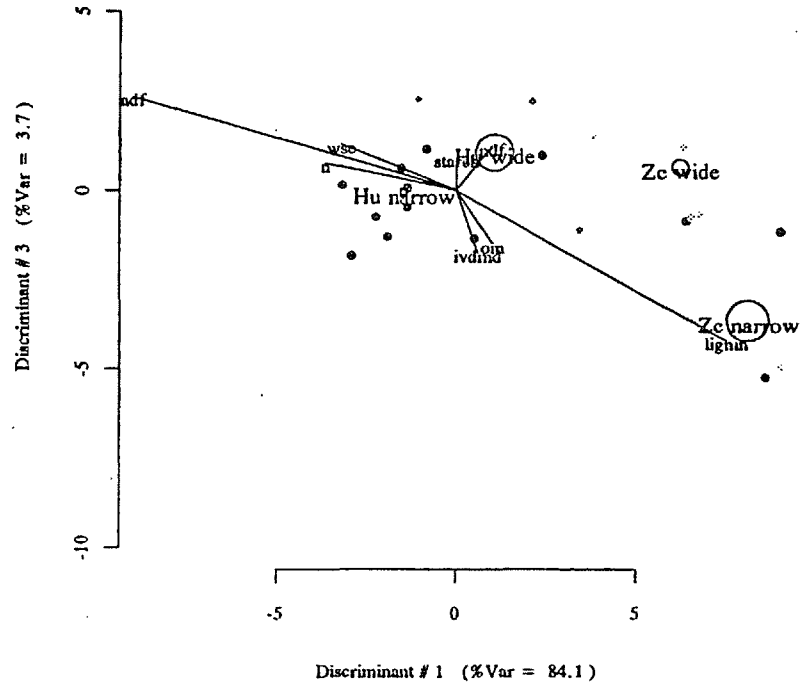

Fig 7.2c. Linear discriminant analysis plots of variety groupings (wide or narrow) for the leaf fraction of *Halodule uninervis* (Hu) and *Zostera capricorni* (Zc), showing only the first and third functions. The set of dots (colour-coded) show the spread of each variety of each species across the discriminant space; the location of the species/variety code represents the centroid for each variety of each species, while the area of the circle represents the standard error. (Colour-code: *Z. capricorni* wide, yellow; *Z. capricorni* narrow, blue; *H. uninervis* wide, green; *H. uninervis* narrow, red).

Leaf fraction - Variety

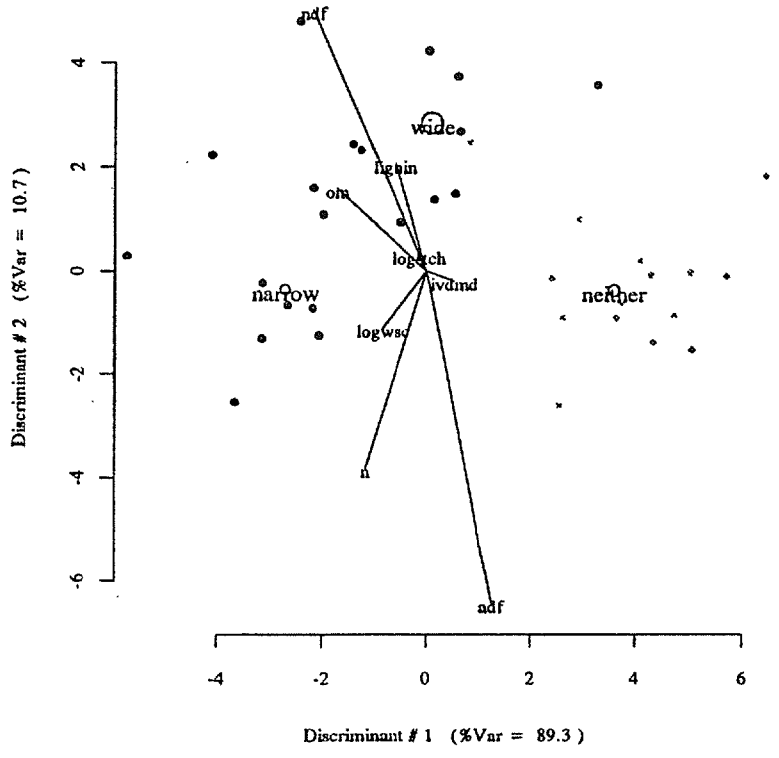

Fig 7.3a. Linear discriminant analysis plots for the leaf fraction of the wide-leaf species, narrow-leaf species and neither narrow- nor wide-leaf species, showing only the first two functions. The set of dots (colour-coded) show the spread of each morphology across the discriminant space; the location of the morphology code represents the centroid of each group, while the area of the circle represents the standard error. (Colour-code: wide-leaf varieties, blue; narrow-leaf varieties, red; neither, green).

Root/rhizome fraction - Variety

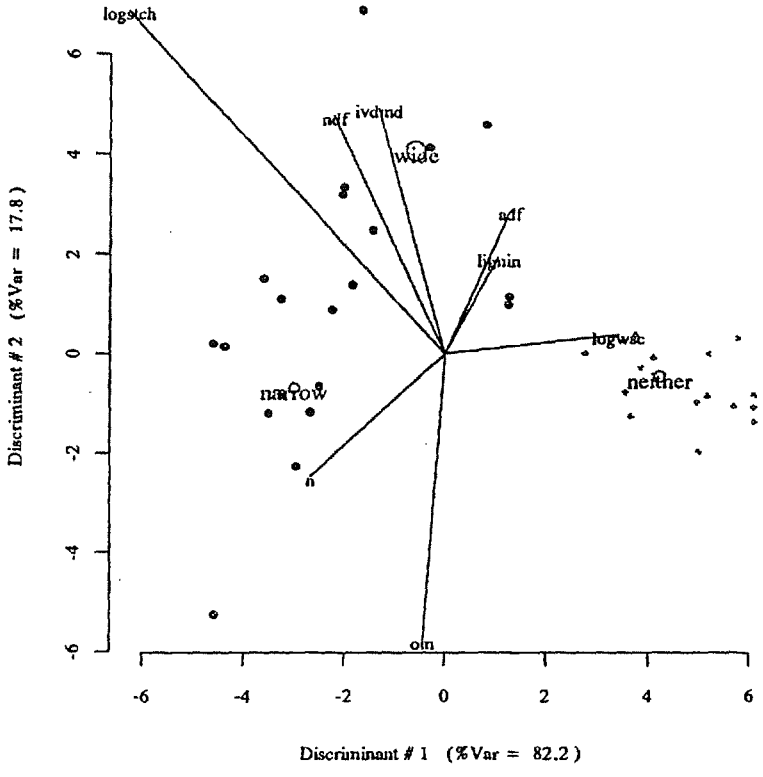

Fig 7.3b. Linear discriminant analysis plots for the root/rhizome fraction of the wide-leaf species, narrow-leaf species and neither narrow- nor wide-leaf species, showing only the first two functions. The set of dots (colour-coded) show the spread of each morphology across the discriminant space; the location of the morphology code represents the centroid of each group, while the area of the circle represents the standard error. (Colour-code: wide-leaf varieties, blue; narrow-leaf varieties, red; neither, green).

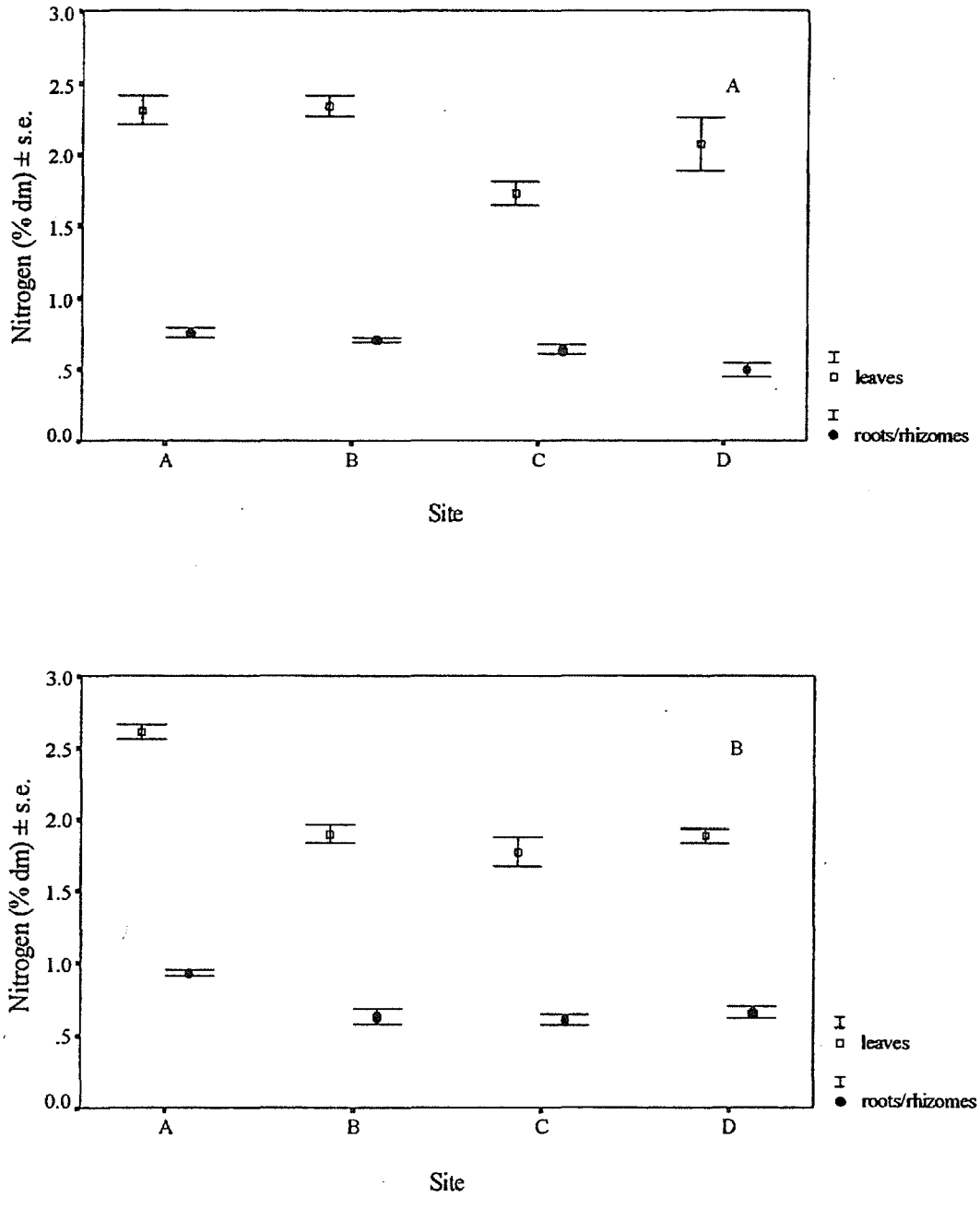

Fig. 7.4. Plots of the mean values of the concentration of nitrogen in *Halophila ovalis* (A) and *Zostera capricorni* + *Cymodocea rotundata* (B) from the control plots of the grazing experiments conducted at Ellie Point; showing the variation from four sites (A to D).

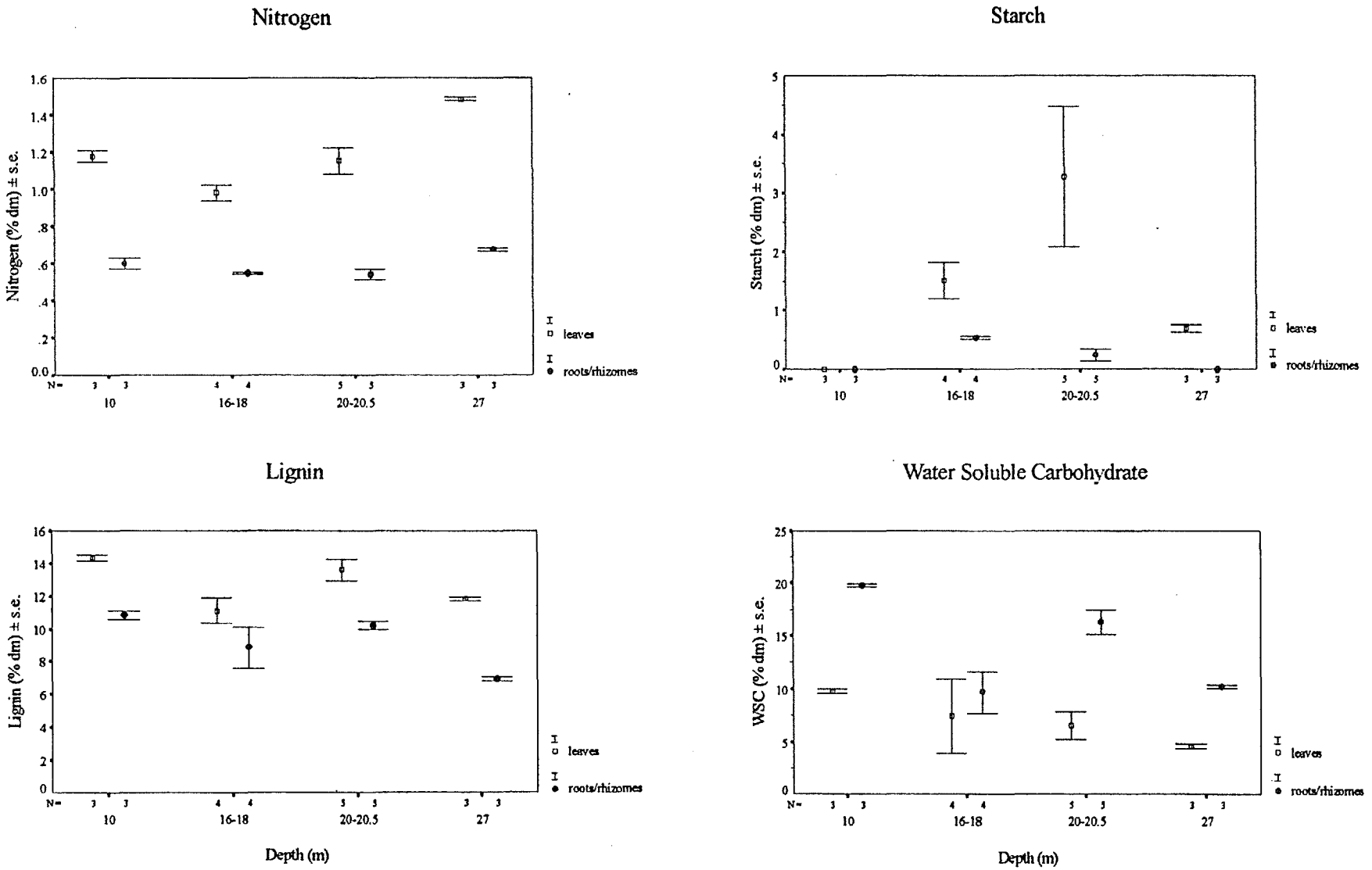

Fig 7.5. Plots of the mean response of some nutritional components of the separate plant parts of *Halophila spinulosa* collected at Pipon Island along 4 depths (10, 16-18, 20-20.5, and 27 m). Please note the different scales in the y-axes.

CHAPTER 8.

EFFECTS OF GRAZING ON THE NUTRITIONAL COMPOSITION OF TROPICAL SEAGRASSES

8.1. INTRODUCTION

Herbivory has been shown to influence plant-nutrient relationships and tissue nutrient content in terrestrial systems (e.g. McNaughton 1983, 1985; Jaramillo and Detling 1988; McNaughton and Banyikwa 1995) and in algal-based marine systems (e.g. Wilkinson and Sammarco 1983b; Hay *et al.* 1987; Hay 1991; Hixon and Brostoff 1995). However, effects of herbivory by large marine herbivores on the nutritional attributes of seagrasses have rarely been studied (but see Bjorndal 1980, 1985, and Kuiper-Linley 1994, for green turtles). Such information is important to elucidate the interactions between dugongs/green turtles and seagrasses, and better understand their nutritional ecology. Bjorndal's studies (1980a, 1985) which describe the impacts of cropping on seagrass nutrient content were limited to *Thalassia testudinium* and to a few nutrient components only: total nitrogen, NDF, ADF and lignin. Kuiper-Linley (1994) investigated other nutrient components such as water soluble carbohydrates and starch in a subtropical seagrass system. Kuiper-Linley found that the concentration of water soluble carbohydrates in the leaves of *Z. capricorni, H. ovalis*, and *C. serrulata* increased after repeated simulated cropping. Similar studies on dugongs are, however, limited to de Iongh *et al.* (1995), which was restricted to a study on the seasonal changes in the total organic carbon in *H. uninervis* in East Indonesia in relation to dugong grazing. de Iongh *et al.* found that the frequency of dugong grazing was positively correlated with total organic carbon.

Most nutrient-related studies on seagrass deal with the C:N:P ratio or other similar nutrient parameters in relation to nutrient availability and plant nutrient requirements (e.g. Atkinson and Smith 1983; Short *et al.* 1985; Duarte 1990; Erftemeijer 1993). Nutrients measured in these sorts of studies have very limited

applications (nitrogen only) in the nutritional ecology of these megaherbivores. Only one study (Bjorndal 1980) has indirectly examined the effects of herbivory on the nutritional quality of seagrasses. This distinction is important (see Chapter 7). The nutrient content of a plant refers to the concentrations of nutrients (e.g. nitrogen, carbohydrates) or anti-nutrients (phenolics) present in a particular plant. The nutritional quality of a plant describes how well a herbivore can use the total plant or a specific plant part. Thus, the nutritional quality of a plant is specific to a particular herbivore and is determined by the level of food intake and how well that animal can extract the metabolizable energy (e.g. *in vivo* or *in vitro* digestibility). Bjorndal calculated the apparent digestibility coefficient (ADC) by the lignin ratio technique (Van Dyne 1968 cited in Bjorndal 1980) to measure whether cropped seagrass blades are of better nutritional quality than uncropped blades. This was an indirect measure of nutritional quality. Previous studies dealt mainly with seagrass nutrient content, even though some authors claim that they were dealing with nutritional quality (e.g. de Iongh *et al.* 1995). More measurements of nutritional quality are needed to better understand the interactions between dugongs/green turtles and seagrasses and the nutritional ecology of these megaherbivores.

In Chapter 5, I experimentally demonstrated the effects of herbivory by dugongs and green turtles on the structure and dynamics of tropical seagrass communities. In this chapter, I will examine the effects of different grazing treatments (simulated grazing by dugongs and cropping by green turtles) on the nutrient content and *in vitro* dry matter digestibility (IVDMD) – one measure of nutritional quality of some tropical seagrasses. The effects of different species, plant parts, and site (local spatial variability) as determinants of nutrients in seagrasses, including those from the grazing experiments have already been discussed in Chapter 7.

Plants that have been grazed by dugongs and green turtles may compensate for the biomass removed. As mentioned in Chapter 2, McNaughton (1983) proposed two mechanism for compensatory growth. The internal mechanisms of compensation involve modifications of plant metabolism resulting in changes in tissue (nutrient)

composition. The external mechanisms of compensation involve modifications of plant environment that are favourable to plant growth and productivity (McNaughton 1983). Using this as a line of argument, I believe that compensatory growth in seagrasses may also be due to the extrinsic and intrinsic consequences of grazing.

This study showed that grazing and cropping can have varying effects on the nutrient content and nutritional quality of some tropical seagrass species. My results offer a biochemical basis for the preferences dugongs and green turtles exhibit for particular seagrass species and feeding sites, and the reasons why they may vary their feeding strategies. These empirical data also supports the theory that herbivory benefits the grazer and grazed plants (Owen and Wiegert 1981; McNaughton 1983).

8.2. MATERIALS AND METHODS

Grazing experiments were conducted at Ellie Point and Cardwell (see Section 5.2). The collection and preparation, and analysis and estimation of the nutritional composition of seagrasses, including nitrogen, OM, NDF, ADF, hemicellulose, lignin, WSC, starch, and IVDMD were described in Sections 7.2.1, and 6.2.5 and 6.2.7, respectively. The statistical analyses of these data were described in Section 7.2.4.

8.3. RESULTS

8.3.1. Effects of grazing on the nutritional composition of some tropical seagrasses

8.3.1.1. Nitrogen

The intensive and light grazing treatments significantly increased nitrogen concentrations in the leaves of *H. ovalis*, and in the whole plants and leaves and

roots/rhizomes of *H. uninervis* (summarised in Table 8.1 and detailed in Appendix 10 Tables 1, 10 & 19 and Appendix 10 Figures 1 & 10). Cropping increased nitrogen concentration in the roots/rhizomes of *H.uninervis*. None of the treatments had a significant effect on *Zostera/Cymodocea*.

The magnitude of the increase varied with species and plant part and was greatest (27.4%) in the leaves of *H. ovalis*. In *H. uninervis*, the increase was greater in the roots/rhizomes than in the leaves. In all cases, the effect was positively correlated with the intensity of the grazing treatment (i.e. the intensive grazing being the greatest and cropping the least).

In the short-term experiments, nitrogen concentration increased in the leaves of *H. uninervis* after a four-month recovery from the light grazing treatment, and in the leaves and whole plant after a two-month recovery from the cropping treatment.

8.3.1.2. Organic Matter (OM)

The intensive grazing treatment significantly increased organic matter concentrations in the roots/rhizomes of *H. ovalis* and *Zostera/Cymodocea* in the long-term experiments (Table 8.2, and Appendix 10 Tables 2, 11 & 20 and Appendix 10 Figures 2 & 11). None of the grazing treatments in the long-term experiments had a significant effect on the concentration of organic matter in *H. uninervis*.

In the short-term experiments, the OM concentration increased in the leaves and roots/rhizomes of *H. uninervis* after a two-month recovery from the cropping treatment.

8.3.1.3. Neutral Detergent Fibre (NDF)

The intensive grazing treatment significantly increased neutral detergent fibre concentrations in the whole plant and root/rhizome fractions of *H. uninervis*

(Table 8.3, and Appendix 10 Tables 3, 12 & 21 and Appendix 10 Figures 3 & 12). The grazing treatments had no significant effect on NDF concentrations in *H. ovalis* and *Zostera/Cymodocea* in the long-term experiments at Ellie Point, and in the short-term experiments on *H. uninervis* at Cardwell.

8.3.1.4. Acid Detergent Fibre (ADF)

The intensive grazing treatment significantly increased acid detergent fibre concentrations in the whole plant, leaves and roots/rhizomes of *H. uninervis* (Table 8.4, and Appendix 10 Tables 4, 13 & 22 and Appendix 10 Figures 4 & 13). In the short-term experiments, the concentrations of acid detergent fibre in *H. uninervis* significantly decreased in the leaves by 7.3% after a one-month recovery from the cropping treatment but increased in the root/rhizome after the second month recovery from the cropping treatment by 12.2%.

8.3.1.5. Hemicellulose

The intensive grazing treatment in the long-term experiments and light grazing treatment in the short-term experiments significantly reduced hemicellulose concentrations in the roots/rhizomes of *H. uninervis* (Table 8.5, and Appendix 10 Tables 5, 14 & 23 and Appendix 10 Figures 5 & 14).

8.3.1.6. Lignin

The intensive and light grazing, and cropping treatments significantly increased lignin concentrations in the roots/rhizomes and whole plant (intensive grazing only) of *H. uninervis* (Table 8.6, and Appendix 10 Tables 6, 15 & 24 and Appendix 10 Figures 6 & 15). In the long term-experiments, the magnitude of the increase varied with plant part with the whole plant being the greatest (45.2%). In all cases, the effect was positively correlated with the intensity of the grazing treatment with the intensive grazing being the greatest and cropping the least. In

contrast, in the short-term experiments, lignin concentration decreased in the roots/rhizomes of *H. uninervis* under the light grazing treatment.

8.3.1.7. Water Soluble Carbohydrates (WSC)

The intensive grazing treatment significantly increased WSC concentrations in the whole plant and roots/rhizomes of *H. ovalis*, and significantly reduced the WSC concentration of *Zostera/Cymodocea* (Table 8.7, and Appendix 10 Tables 7, 16 & 25 and Appendix 10 Figures 7 & 16). None of the treatments from both the long-term and short-term experiments had a significant effect on *H.uninervis.*

8.3.1.8. Starch

The cropping treatment significantly reduced starch concentrations in the whole plant, leaves, and roots/rhizomes of *H.uninervis* in the short-term experiments only (Table 8.8, and Appendix 10 Tables 8, 17 & 26 and Appendix 10 Figures 8 & 17). The magnitude of reduction was negatively correlated with the period of recovery, i.e. reduction in starch concentration declines as recovery is prolonged. None of the dugong grazing treatments from both the long-term and short-term experiments had a significant effect on starch concentrations of *H. ovalis* and *Zostera/Cymodocea* and *H. uninervis.*

8.3.1.9. *In Vitro* Dry Matter Digestibility

The intensive grazing treatment in the long-term experiments and cropping treatment in the short-term experiments significantly increased the digestibility of dry matter (*in vitro*) of the leaves of *H. uninervis* (Table 8.9, and Appendix 10 Tables 9, 18 & 27 and Appendix 10 Figures 9 & 18). The grazing treatments had no significant effect on the digestibility of dry matter (*in vitro*) of *H. ovalis* and *Zostera/Cymodocea.*

8.4. DISCUSSION

HERBIVORY BY DUGONGS AND GREEN TURTLES IMPROVED SEAGRASS NUTRIENTS AND *IN VITRO* DRY MATTER DIGESTIBILITY

These experiments showed that simulated grazing by dugongs and cropping by green turtles influenced the nutritional composition of the following tropical seagrasses: *Halophila ovalis, Zostera capricorni/Cymodocea rotundata,* and *Halodule uninervis* (Table 8.10). The results support the following hypotheses: (1) that seagrass nutrients and nutritional quality improve as a result of dugong grazing (cultivation grazing, Preen 1993), and (2) that green turtle cropping improves nutrient content and the nutritional quality of seagrass blades after cropping (Bjorndal 1980).

Overall, the results of my short- and long-term grazing experiments were similar. The concentrations of nitrogen, organic matter and water soluble carbohydrates, and the digestibility of dry matter (*in vitro*), all increased (see Tables 8.1-8.9). The concentrations of ADF, hemicellulose, and lignin in *H. uninervis* responded variably (see Table 8.10). These empirical data suggest that different seagrass species may have different responses to herbivory depending on their life history characteristics.

Effects of grazing on nutrient content and nutritional quality

Herbivory can have positive effects on the nutritional composition of seagrasses by increasing plant nutrient content (see Table 8.10). In the case of nitrogen, the magnitude of the effects was positively correlated with the intensity of simulated herbivory. As discussed in Chapter 7, nitrogen is believed to be a limiting nutrient for most herbivores (Mattson 1980). To date, all studies on simulated or real cropping, and the limited studies on simulated and real grazing on seagrass have reported increases in nitrogen concentration following herbivory (see Table 8.11).

This study and Perry's (manuscript), showed that the leaf nitrogen content of *H. ovalis* and *H. uninervis* increased significantly following grazing (see Table 8.11). In Moreton Bay, Perry (manuscript) showed that dugong grazing increased nitrogen concentration in seagrass tissues. Perry found that the leaf nitrogen concentration increased from 1.8% to 2.9% in grazed patches of *H. ovalis, H. uninervis*, and *Z. capricorni*, when compared with ungrazed patches of *H. uninervis* and *Z. capricorni*. The nitrogen concentration in the roots/rhizomes also increased from 0.53% to 0.85%. These increases (45 and 46%) are higher than those reported in this study (see Table 8.11). This discrepancy may be due to one or more of the following factors: (1) spatial and temporal variation in seagrass nutrients (Chapter 8); (2) the absence of *H. ovalis* from Perry's ungrazed site; and (3) sampling differences. Perry chose only young shoots and only the first five internodes from her seagrass samples. I did not discriminate between young and old shoots. Neither did I specifically collect samples from regrowth in the feeding trails only. I consider this inappropriate as dugongs cannot be selective at that spatial scale in the wild (Heinsohn *et al.* 1977; Preen 1993) as they are limited by the width of their muzzle (22 cm, Spain and Heinsohn 1975). The dugongs most probably assess feeding areas at the level of community characteristics (Wake 1975; Preen 1993). Furthermore, Perry performed her measurements on a pooled sample of seagrass (*H. ovalis, H. uninervis*, and *Z. capricorni*) and did not examine individual species.

This study and others show that cropping can have considerable effects on the leaf nitrogen and organic matter concentrations of seagrasses (see Table 8.11). Further comparisons between this study and others are impeded by: (1) the different species involved, and (2) the potential influence of spatial and temporal variability in the response of seagrass to herbivory. My results support Bjorndal (1980a), who showed that turtle cropping does not affect the NDF concentration in the leaves of seagrass (*T. testudinum*). However, I found a significant reduction in ADF concentration in the leaves of *H. uninervis* one month after cropping (see Table 8.10). After two months, the ADF concentration in the leaves was similar to those of the ungrazed. In contrast, Bjorndal (1980a) did not find any significant changes

in the ADF concentration in the tissues of *T. testudinum* after cropping, but showed a 50% reduction in lignin concentration. Acid detergent fibre is associated with maturing plant tissues (Van Soest 1994), and my result may be indicative of young leaves during the first month of regrowth.

Although the nitrogen and organic matter content of some species increased in response to herbivory, the effects, particularly of dugong grazing were not always positive. The negative effects included the significant increase in fibre and lignin in both the roots/rhizomes and the whole plants of *H. uninervis* and the significant reduction in the water soluble carbohydrates in the rhizomes and whole plants of *Zostera/Cymodocea* (see Table 8.10).

Among these negative effects, the increase in lignin was largest suggesting that the digestibility of seagrasses is not necessarily related to lignin, contradicting Bjorndal's (1980a) apparent digestibility measurements which used lignin as a reference marker of digestibility. This may also suggest that lignin in forage is difficult to interpret as its chemical properties are poorly defined. The increased NDF, ADF and lignin concentrations in *H. uninervis* may, however, be insignificant to the animal because most of the negative effects were restricted to the roots/rhizomes, with the leaves slightly increasing in ADF concentration only (+3.1%, see Table 8.10). This may explain why dugongs appear to surface graze (crop) rather than uproot (graze) the *H. uninervis* beds at Cardwell on some occasions (pers. observ.). Furthermore, the dugong is a large hindgut fermenter and hindgut fermenters generally have the capacity to maximise fibre digestion (*sensu* Van Soest 1994). Therefore, this moderate increases in fibre and lignin probably make little difference to dugongs. The apparent digestibilities (using lignin as a reference marker of digestibility) of NDF and ADF of a dugong's digesta samples, consisting mainly of *H. ovalis* (98.8%) and some *H uninervis* (1.2%) were 84% and 82%, respectively (Murray *et al.* 1977). These digestibility values are relatively high for fibre (e.g. 34% in greater glider, Foley 1987). Furthermore, the relatively small increase in fibre concentration in the leaves of *H. uninervis* following grazing is insignificant in comparison to the 30.5% to 36% relative

difference in leaf fibre concentration between *H. ovalis* and *Z. capricorni* (see Chapter 7). Overall, the properties of *H. uninervis* like high digestibility and high concentrations of nitrogen and starch presumably override these negative effects of herbivory (Chapter 7). With increased nitrogen, WSC, OM and IVDMD, *H. uninervis* would still be nutritionally better to most seagrasses (except *H. ovalis*, see Chapter 7). It is not surprising that it is a preferred species of dugongs (see also Chapter 7).

Mechanism of changes in nutrients

Tillage: extrinsic consequence of grazing

Perry (manuscript) suggests that the increase in nitrogen concentration in seagrass tissues in Moreton Bay, may be due to an increase in benthic nitrogen fixation, as a result of intensive dugong grazing. She reports that the rates of microbial nutrient cycling in seagrass sediments are higher in grazed than ungrazed areas and proposes that such rises are due to increased bacterial growth and availability of energy substrates from: (1) carbohydrate leakage from broken plant parts; (2) more rapid decay of plant matter from both aerobic and anaerobic decay resulting from the aeration of sediments due to grazing; (3) increased light and higher rates of photosynthesis in benthic cyanobacteria or recolonising seagrasses; and (4) indirectly increasing photosynthate release via changes in seagrass species composition and age structure. In this respect, I postulate that dugong grazing resembles the agricultural practice of tillage ("ploughing"), which is routinely performed by farmers to increase crop productivity (Cornish and Pratley 1987).

In terrestrial systems, however, repeated "tillage" in a particular site results in saturation of sediment nutrients causing a reduction in yield. How is this avoided by dugongs? Firstly, they use relatively large home ranges (Marsh and Rathbun 1990; Preen 1993, and 1996 pers. comm.). Secondly, in marine systems, other regular processes such as tides and waves may contribute to the cyclic distribution of 'new' (imported) nutrients into seagrass communities, particularly intertidal beds

(Klumpp *et al.* 1989). Thirdly, internal recycling of nutrients in seagrass beds, primarily from decomposing roots and rhizomes, is common (Pedersen and Borum 1993). In addition, seagrasses, unlike terrestrial plants, can assimilate nutrients both from the water column and sediments (Short and McRoy 1984; Hemminga *et al.* 1991, 1994). However, it has been reported that turtles abandon 'grazing plots' in the Caribbean (Bjorndal 1908a) when the sediment ammonium concentration is reduced, leading to reduced growth rates of *T. testudinum* (Zieman *et al.* 1984). This may be due to the nature of turtle cropping which, unlike grazing by dugongs, has little effect on sediments. By influencing the sediment redox conditions, dugong grazing is likely to make an important contribution to the function of seagrass ecosystems in the tropics.

Translocation

Carbohydrates are the main repository of photosynthetic energy in plants (Van Soest 1989). In plants, starch is the most important of the storage carbohydrates (Van Soest 1994). In seagrasses, carbohydrates were found in the form of starch or as soluble carbohydrate in the rhizomes of the major taxa of seagrasses in Shark Bay (Masini 1982). Dawes and Lawrence (1979) propose that the mobilisation of carbohydrates stored in rhizomes supports blade regeneration after defoliation in the field and laboratory, and during seasonal growth. The significant decline in the concentration of starch in the roots and rhizomes after cropping (see Appendix 10 Fig 17) indicates that starch in the roots/rhizomes is the main storage compound enabling leaf regrowth in *H. uninervis*. After the second month of recovery from cropping, the leaf nitrogen of *H. uninervis* increased while starch decreased. Dawes *et al.* (1979) also showed that nitrogen concentration increased while carbohydrates decreased in regrowth blades of *T. testudinum* cropped every 2 weeks. In contrast, Kuiper-Linley (1994) suggests that water soluble carbohydrate is an important storage compound in *C. serrulata*. However, not all reductions in starch and water soluble carbohydrates are necessarily translocated for leaf growth (e.g. respiration).

Implications for dugongs and green turtles of the changes in nutritional composition of seagrasses

Dugong

Most of the positive effects of dugong grazing resulting from the long-term experiments were evident in *H. ovalis* and *H. uninervis* only. The only positive effects in *Zostera/Cymodocea* was a slight increase in organic matter (+4.6%) in the roots/rhizomes. However, an increase in OM could mean so many things, e.g. it could mean that the cell wall materials or cell contents moderately increased. However, in short-term cropping experiments in Moreton Bay, the leaves of *Z. capricorni* and *C. serrulata* showed significant increases in water soluble carbohydrate concentrations (Kuiper-Linley 1994), suggesting that the important changes in the nutrient contents of *Zostera/Cymodocea* could have occurred in the early stages of the experiments and not been apparent after about 10 months. The results of both the short- and long-term simulation experiments clearly showed that grazing on *H. ovalis* and *H. uninervis* could be beneficial (see Table 8.11). Field observations and stomach content analyses indicate that *H. ovalis* and *H. uninervis* are preferred food of dugongs, and that *Z. capricorni* is the least preferred species at least in subtropical Moreton Bay (Preen 1993). However, narrow-variety *Z. capricorni* plants are important food for dugongs (Anderson and Birch 1978) and green turtles (Limpus, unpublished data, cited in Lanyon *et al.* 1989; Brand 1995) at Shoalwater Bay (see Chapter 7).

If nitrogen is indeed one of the more important nutrients for dugongs (Lanyon 1991; Preen 1993; this study), then one would expect they would maximise foraging efficiencies by selectively feeding on species containing more total nitrogen (Chapter 7) and return to feeding sites both to exploit the preferred species and to benefit from any increases in the concentration of nitrogen following grazing. Dugongs in certain feeding sites in Moreton Bay (Preen 1993, 1995a) and Cardwell (pers. observ.) return to regraze after 4 to 6 months, which would enable them to take advantage of these opportunities. In the Moluccas, east Indonesia, the

frequency of dugong feeding trails correlated positively with the ratio of ash-free dry weight to dry weight of the root/rhizome fraction of *H. uninervis* (de Iongh *et al.* 1995). de Iongh *et al.* suggest that this is indicative of the dugongs' preference for higher levels of total organic carbon in the roots/rhizomes of *H. uninervis*, and further conclude that this supports the optimal foraging strategy for energy maximisation. However, their conclusion is flawed as it failed to use relevant nutritional parameters. They actually measured organic matter only (ratio of ash free dry weight to dry weight), and organic matter nor total organic carbon, do not have any nutritional meaning. As discussed earlier, extrapolating nutritional meaning from OM is inappropriate and should not be interpreted more than what it means *per se*. Therefore, appropriate supportive data should be gathered first to make any conclusions regarding the possible foraging strategy dugongs select (see Chapter 9).

The digestibility of dry matter (*in vitro*), and the concentrations of organic matter and water soluble carbohydrates also increased after grazing. Therefore, by returning to previously grazed areas, dugongs potentially get more digestible energy per bite while maintaining the 'quality' of their feeding areas. However, it is interesting to note that the IVDMD was only slightly affected by grazing (see Table 8.10 and Fig 18). I suspect that this is because the values for digestibility of the seagrasses examined (see Chapter 7) were already very high (90-98%), leaving little scope for improvement (see Appendix 10 Fig 18).

Green turtles

My experiments showed that cropping seagrasses in the tropics has long-term and short-term effects (see Table 8.10). The first two months after cropping were the optimum times for the animals to recrop if nutritional benefits are to be maximised. The cropped plots were visually distinct from the surrounding uncropped seagrass beds (pers. observ.) until the second month of recovery. The leaves were significantly more digestible in the first month of recovery after cropping (see Appendix 10 Fig 18b), while the concentrations of nitrogen and organic matter in

the leaves in the second month of recovery were significantly higher (see Table 7.3 and Appendix 10 Figs 10b & 11b). A significant reduction in the ADF concentration in the leaves of *H. uninervis* was recorded from the first month's regrowth (see Table 8.10). This ties in well with the suggestion that ADF is associated with maturing plant tissues (Van Soest 1994). These results clearly provide a biochemical basis for the maintenance of "grazing plots" by green turtles in the Caribbean, and show how seagrasses cope with such herbivory.

An integration of the effects of dugong grazing and turtle cropping on the structure (e.g. species composition), dynamics and nutritional composition of tropical seagrass communities is presented in Chapter 9.

CONCLUSIONS

1) Simulated dugong grazing and turtle cropping altered the nutritional composition of tropical seagrasses.

2) The concentrations of nitrogen, OM, WSC, and IVDMD, all increased after herbivory, while the response of anti-nutrients including ADF, hemicellulose, and lignin were variable in *H. ovalis, H. uninervis*, and *Zostera/Cymodocea*.

3) The improvement in nutrients and quality was positively correlated with the intensity of herbivory and mostly seen in *H. ovalis* and *H. uninervis*, the species favoured by both dugongs and green turtles (*sensu* Preen 1993 for dugongs; Read 1991, Brand 1995).

4) Light intensity grazing can also result to the improvement of the nutritional composition of tropical seagrasses.

5) By influencing sediment redox conditions, dugong grazing is likely to make an important contribution to the resultant improvement of the nutritional composition

of seagrass tissues, and consequently the function of seagrass ecosystems in the tropics.

6) Dugongs and green turtles may be "cultivating" their feeding sites. The results of this study support the theory that herbivory benefits both the grazer and the grazed plants.

Table 8.1. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on nitrogen concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on nitrogen concentrations in *Zostera/Cymodocea* (tested at Ellie Point only).

Treatment	Species	Plant Part	% change relative to control	*p*	Reference to Appendix 10 Tables 1, 10 & 19 and Appendix 10 Figures 1 & 10
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. ovalis*	leaf	+ 27.4	0.004[1]	Appendix 10 Table 1, species*treatment interaction; Appendix 10 Fig 1a
	H. uninervis	whole plant	+ 5.8	0.000[1]	Appendix 10 Table 10, whole plant, treatment effect; Appendix 10 Fig 10a
		leaf	+ 4.6	0.006[1]	Appendix 10 Table 10, leaf, treatment effect; Appendix 10 Fig 10a
		root/rhizome	+ 12.7	0.000[1]	Appendix 10 Table 10, root/rhizome, treatment effect; Appendix 10 Fig 10a
Light grazing	*H. ovalis*	leaf	+ 14.5	0.042[1]	Appendix 10 Table 1, species*treatment interaction; Appendix 10 Fig 1a
	H. uninervis	whole plant	+ 3.4	0.013[1]	Appendix 10 Table 10, whole plant, treatment effect; Appendix 10 Fig 10a
		leaf	+ 3.1	0.037[1]	Appendix 10 Table 10, leaf, treatment effect; Appendix 10 Fig 10a
		root/rhizome	+ 6.0	0.005[1]	Appendix 10 Table 10, root/rhizome, treatment effect; Appendix 10 Fig 10a
Cropping	*H. uninervis*	root/rhizome	+ 6.0	0.010[1]	Appendix 10 Table 10, root/rhizome, treatment effect; Appendix 10 Fig 10a
Short-term experiments[2] (one or two months, cropping; four months, light grazing)					
Light grazing	*H. uninervis*	leaf	+ 10.1	0.049	Appendix 10 Table 19, leaf, treatment effect (Trt I & II); Appendix 8 Fig 10b
Cropping (1 month)	*H. uninervis*	whole plant	+ 8.4	0.028	Appendix 10 Table 19, whole plant, treatment effect (Trt V & VI); Appendix 10 Fig.10b
		leaf	+ 8.9	0.045	Appendix 10 Table 19, leaf, treatment effect (Trt V & VI); Appendix 10 Fig 10b

[1] Determined using contrasts, performed only when main or interaction effects were statistically significant ($p < 0.05$).
[2] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.2. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on organic matter concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on organic matter concentrations in the long-term experiments on *Halodule uninervis*.

Treatment	Species	Plant Part	% change relative to the control	*p*	Reference to Appendix 10 Tables 2, 11 & 20 and Appendix 10 Figures 2 & 11
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. ovalis*	root/rhizome	+ 3.6	0.036	Appendix 10 Table 2, root/rhizome, treatment effect; Appendix 10 Fig 2b
	Zostera/Cymodocea	root/rhizome	+ 4.6	0.036	Appendix 10 Table 2, root/rhizome, treatment effect; Appendix 10 Fig 2b
Short-term experiments[1] (one or two months, cropping; four months, light grazing)					
Cropping (2 months)	*H. uninervis*	leaf	+ 2.6	0.041	Appendix 10 Table 20, leaf, treatment effect (Trt V & VI); Appendix 10 Fig. 11b
		root/rhizome	+ 6.9	0.007	Appendix 10 Table 20, root/rhizome, treatment effect (Trt V & VI); Appendix 10 Fig 11b

[1] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.3. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on neutral detergent fibre concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on neutral detergent fibre concentrations in *H. ovalis* in the long-term experiments (tested at Ellie Point only) and in *H. uninervis* in the short term-experiment[1].

Treatment	Species	Plant Part	% change relative to the control	*p*	Reference to Appendix 10 Tables 3, 12 & 21 and Appendix 10 Figures 3 & 12
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. uninervis*	whole plant	+ 5.3	0.004[2]	Appendix 10 Table 12, whole plant, treatment effect; Appendix 10 Fig 12a
		root/rhizome	+ 9.6	0.001[2]	Appendix 10 Table 9, root/rhizome, treatment effect; Appendix 10 Fig 12a

[1] Light grazing intensity and cropping treatments only and at Cardwell only.
[2] Determined using contrasts, performed only when main effects were statistically significant ($p < 0.05$).

Table 8.4. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on acid detergent fibre concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on acid detergent fibre concentrations in *H. ovalis* and *Zostera/Cymodocea* (tested at Ellie Point only).

Treatment	Species	Plant Part	% change relative to the control	p	Reference to Appendix 10 Tables 4, 13 & 22 and Appendix 10 Figures 4 & 13
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. uninervis*	whole plant	+ 8.9	0.000[1]	Appendix 10 Table 13, whole plant, treatment effect; Appendix 10 Fig 13a
		leaf	+ 3.1	0.030[1]	Appendix 10 Table 13, leaf, treatment effect; Appendix 10 Fig 13a
		root/rhizome	+18.5	0.000[1]	Appendix 10 Table 13, root/rhizome, treatment effect; Appendix 10 Fig 13a
Short-term experiments[2] (one or two months, cropping; four months, light grazing)					
Cropping (1 month)	*H. uninervis*	leaf	- 7.3	0.048	Appendix 10 Table 22, leaf, treatment effect (Trt III & IV); Appendix Fig. 13b
Cropping (2 months)		root/rhizome	+12.2	0.009	Appendix 10 Table 22, root/rhizome, treatment effect (Trt V & VI); Appendix 10 Fig 13b

[1] Determined using contrasts, performed only when main effects were statistically significant ($p < 0.05$).
[2] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.5. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on hemicellulose concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on hemicellulose concentrations in *H. ovalis* and *Zostera/Cymodocea* (tested at Ellie Point only).

Treatment	Species	Plant Part	% change relative to the control	p	Reference to Appendix 10 Tables 5, 14 & 23 and Appendix 10 Figures 5 & 14
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. uninervis*	root/rhizome	-12.6	0.005[1]	Appendix 10 Table 14, root/rhizome, treatment effect; Appendix 10 Fig 14a
Short-term experiments[2] (one or two months, cropping; four months, light grazing)					
Light grazing	*H. uninervis*	root/rhizome	- 5.7	0.046	Appendix 10 Table 23, leaf, treatment effect (Trt III & IV); Appendix 10 Fig. 14b

[1] Determined using contrasts, performed only when main effects were statistically significant ($p < 0.05$).
[2] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.6. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on lignin concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on lignin concentrations in *H. ovalis* and *Zostera/Cymodocea* (tested at Ellie Point only).

Treatment	Species	Plant Part	% change relative to the control	*p*	Reference to Appendix 10 Tables 6, 15 & 24 and Appendix 10 Figures 6 & 15
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. uninervis*	whole plant	+45.2	0.000[1]	Appendix 10 Table 15, whole plant, treatment effect; Appendix 10 Fig 15a
		root/rhizome	+16.0	0.000[1]	Appendix 10 Table 15, root/rhizome, treatment effect; Appendix 10 Fig 15a
Light grazing	*H. uninervis*	root/rhizome	+16.3	0.026[1]	Appendix 10 Table 15, root/rhizome, treatment effect; Appendix 10 Fig 15a
Cropping	*H. uninervis*	root/rhizome	+14.2	0.048[1]	Appendix 10 Table 15, root/rhizome, treatment effect; Appendix 10 Fig 15a
Short-term experiments[2] (one or two months, cropping; four months, light grazing)					
Light grazing	*H. uninervis*	root/rhizome	- 7.9	0.045	Appendix 10 Table 24, leaf, treatment effect (Trt III & IV); Appendix 10 Fig.15b

[1] Determined using contrasts, performed only when main effects were statistically significant ($p < 0.05$).
[2] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.7. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on water soluble carbohydrate concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on water soluble carbohydrate concentrations in *H. uninervis* in both the short-term[1] and long-term experiments at Cardwell.

Treatment	Species	Plant Part	% change relative to the control	p	Reference to Appendix 10 Tables 7, 16 & 25 and Appendix Figures 7 & 16
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. ovalis*	whole plant	+55.5	0.000[2]	Appendix 10 Table 7, whole plant, species*treatment effect; Appendix Fig 7c
		root/rhizome	+70.4	0.009[2]	Appendix 10 Table 7, root/rhizome, species*treatment effect; Appendix 10 Fig 7b
	Zostera/Cymodocea	whole plant	-30.6	0.018[2]	Appendix 10 Table 7, whole plant, species*treatment effect; Appendix Fig 7c
		root/rhizome	-29.2	0.028[2]	Appendix 10 Table 7, whole plant, species*treatment effect; Appendix Fig 7b

[1] Light grazing intensity and cropping treatments only and at Cardwell only.
[2] Determined using contrasts, performed only when main (or interactions) effects were statistically significant ($p < 0.05$).

Table 8.8. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on starch concentrations in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on starch concentrations in the long-term experiments in *Halophila ovalis* and *Zostera/Cymodocea* at Ellie Point (10 months) and in *Halodule uninervis* at Cardwell (12 months).

Treatment	Species	Plant Part	% change relative to the control	*p*	Reference to Appendix 10 Tables 8, 17 & 26 and Appendix 10 Figures 8 & 17
Short-term experiments[1] (one or two months, cropping; four months, light grazing)					
Cropping (1 month)	*H. uninervis*	whole plant	-87.7	0.018	Appendix 10 Table 26, whole plant, treatment effect; Appendix 10 Fig 17b
		leaf	-84.8	0.041	Appendix 10 Table 26, leaf, treatment effect; Appendix 10 Fig 17b
		root/rhizome	-88.2	0.015	Appendix 10 Table 26, root/rhizome, treatment effect; Appendix 10 Fig 17b
Cropping (2 months)		whole plant	-65.3	0.001	Appendix 10 Table 26, whole plant, treatment effect; Appendix 10 Fig 17b
		root/rhizome	-75.0	0.002	Appendix 10 Table 26, root/rhizome, treatment effect; Appendix 10 Fig 17b

[1] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.9. Summary of significant ($p < 0.05$) effects of the various treatments (intensive grazing, light grazing, turtle cropping) on digestibility of dry matter (*in vitro*) in seagrass leaves, roots and rhizomes, and whole plants. The experiments on *Halophila ovalis* and *Zostera/Cymodocea* were conducted at Ellie Point only, those on *Halodule uninervis* at Cardwell only. The grazing treatments had no significant effect on digestibility of dry matter (*in vitro*) in *H. ovalis* and *Zostera/Cymodocea* (tested at Ellie Point only).

Treatment	Species	Plant Part	% change relative to the control	*p*	Reference to Appendix 10 Tables 10, 18 & 27 and Appendix 10 Figures 9 & 18
Long-term experiments (*H. ovalis* & *Zostera/Cymodocea*, 10 months; *H. uninervis*, 12 months)					
Intensive grazing	*H. uninervis*	leaf	+ 0.6	0.007[1]	Appendix 10 Table 18, leaf, treatment effect; Appendix 10 Fig 18a
Short-term experiments[2] (one or two months, cropping; four months, light grazing)					
Cropping (1 month)	*H. uninervis*	leaf	+ 1.4	0.028	Appendix 10 Table 27, leaf, treatment effect (Trt III & IV); Appendix 10 Fig. 18b

[1] Determined using contrasts, performed only when main effects were statistically significant ($p < 0.05$).
[2] Light grazing intensity and cropping treatments only and at Cardwell only.

Table 8.10. Summary of the proportional (%) changes (+ increased, - decreased, relative to the control) in the nutritional composition of some tropical seagrasses significantly ($p < 0.05$) affected by simulated herbivory (in parentheses). The nutritional composition of the leaves of *Z. capricorni/C. rotundata* was not affected.

		Nitrogen	Organic Matter	Neutral Detergent Fibre	Acid Detergent Fibre	Hemicellulose	Lignin	Water Soluble Carbohydrate	Starch	*In Vitro* Dry Matter Digestibility
1) Long-term experiments										
H. ovalis	Leaves	+27.4 (HG)[1] +14.5 (LG)[2]								
	Roots/ Rhizomes		+3.6 (HG)[1]					+70.4 (HG)[1]		
	Whole plant							+55.5 (HG)[1]		
Z.capricorni/ C. rotundata	Roots/ Rhizomes		+4.6 (HG)[1]					-30.6 (HG)[1]		
	Whole plant							-29.2 (HG)[1]		
H. uninervis	Leaves	+ 4.6 (HG)[1] + 3.1 (LG)[2]			+ 3.1(HG)[1]					+0.6 (LG)[2]
	Roots/ Rhizomes	+12.7 (HG)[1] + 6.0 (LG)[2] + 6.0 (C)		+9.6 (HG)[1]	+18.5 (HG)[1]	-12.6 (HG)[1]	+45.2 (HG)[1]	+52.3 (HG)[1]		
	Whole plant	+ 5.8 (HG)[1] + 3.4 (LG)[2]		+5.3 (HG)[1]	+ 8.9 (HG)[1]		+16.0 (HG)[1]			
2) Short-term experiments										
H. uninervis	Leaves	+10.1 (LG)[2] +8.4 (C)[4]	+2.6 (C)[4]		-7.3 (C)[3]				-84.8 (C)[3]	+1.4 (C)[3]
	Roots/ Rhizomes		-6.9 (C)[4]		+12.2 (C)[4]	- 5.7 (LG)[2]	-7.9 (C)[3]		-88.2 (C)[3] -75.0 (C)[4]	
	Whole plant	+8.9 (C)[4]							-87.7 (C)[3] -65.3 (C)[4]	

[1] HG = Intensive dugong grazing
[2] LG = Light dugong grazing
[3] C = Turtle cropping harvested after 1 month
[4] C = Turtle cropping harvested after 2 months

Table 8.11. Effects of grazing or cropping on some nutritional components of seagrass from this study and others.

Grazing/ Cropping	Species	Plant Part	Location (latitude, longitude)	Nutritional component	% change relative to control	Reference
Intensive Grazing	Mixed (*H. ovalis*, *H. uninervis*, *Z. capricorni*)	leaves	Moreton Bay (27.5°S, 153.3°E)	Nitrogen	+45.0	Perry (manuscript)
Intensive Grazing	Mixed (*H. ovalis*, *H. uninervis*, *Z. capricorni*)	roots/ rhizomes	Moreton Bay	Nitrogen	+46.0	Perry (manuscript)
Intensive Grazing	*H. ovalis*	leaves	Ellie Point (16°53'S, 145°46'E)	Nitrogen	+27.4	this study
Intensive Grazing	*H. ovalis*	roots/ rhizomes	Ellie Point	Nitrogen	+6.0	this study
Intensive Grazing	*H. uninervis*	leaves	Cardwell (18°14'S, 146.°E)	Nitrogen	+4.6[1]	this study
Intensive Grazing	*H. uninervis*	roots/ rhizomes	Cardwell	Nitrogen	+12.7[1]	this study
Light Grazing	*H. uninervis*	leaves	Cardwell	Nitrogen	+3.1[1]	this study
Light Grazing	*H. uninervis*	roots/ rhizomes	Cardwell	Nitrogen	+6.0	this study
Light Grazing	*H. uninervis*	leaves	Cardwell	Nitrogen	+10.1[2]	this study
Cropping	*T. testudinum*	leaves	Caribbean (21.1°N, 72.5°E)	Nitrogen	+6.0 to +11.0*	Bjorndal 1980a
Cropping	*T. testudinum*	leaves	Florida (28.1°N, 82.4°E)	Nitrogen	+7.0 to +13.5*	Dawes and Lawrence 1979
Cropping	*T. testudinum*	leaves	Caribbean (17.3°N, 64.5°E)	Nitrogen	+13.0 to +47.0*	Zieman *et al.* 1984

Cropping	*H. uninervis*	leaves	Cardwell (18°14'S, 146.°E)	Nitrogen	+8.4	this study
Cropping	*T. testudinum*	leaves	Florida (28.1°N, 82.4°E)	Organic Matter	+19.0	Dawes and Lawrence 1979
Cropping	*T. testudinum*	leaves	Caribbean (21.1°N, 72.5°E)	Organic Matter	+9.3 to +16.8	Bjorndal 1980a
Cropping	*T. testudinum*	leaves	Florida (28.1°N, 82.4°E)	Organic Matter	+3.9[3] +1.8[4] +12.7[5]	Dawes *et al.*1979
Cropping	*H. uninervis*	leaves	Cardwell	Organic Matter	+2.6	this study
Cropping	*C. serrulata*	rhizomes	Moreton Bay (27.5°S, 153.3°E)	Water soluble carbohydrate	-71.0	Kuiper-Linley 1994
Cropping	*H. uninervis*	roots/ rhizomes	Cardwell	Starch	-75.0 to -88.2[2]	this study

° data taken at several different seasons
[1] long-term experiments
[2] short-term experiments
[3] winter sampling
[4] spring sampling
[5] fall sampling

CHAPTER 9.

GENERAL DISCUSSION

DUGONG, GREEN TURTLE AND SEAGRASS INTERACTIONS

A general model

This study has shown through experiments that grazing by dugongs and cropping by green turtles affects the species composition and nutritional quality of tropical seagrass communities (Chapters 5 and 8). These effects are dependent on the nature, location, timing and intensity of herbivory, and the life history characteristics of the target species, and other disturbances affecting the seagrass community.

Dugongs usually uproot the whole plant, while turtles take only the leaves (Lanyon *et al.* 1989). Thus, dugongs have greater impacts on the demography of seagrass communities than turtles (Chapter 5). Dugong grazing promotes the growth of more opportunistic species of seagrass at the expense of longer-lived species (Chapter 5). The opportunistic species (e.g. *Halophila* spp) have higher concentrations of nitrogen and higher digestibility than the longer-lived ones (e.g. *Z. capricorni*) (Chapters 7 and 8). Thus, dugong grazing in an area presumably promote further herbivory (cultivation grazing *sensu* Preen 1995) leading to periodic disturbances of the seagrass community and the development of localised areas which are favoured by, and nutritionally favourable to dugongs.

In influencing sediment redox conditions, dugong grazing is likely to be an important contribution to the functioning of seagrass ecosystems in the tropics. Perry (manuscript) found that sediments in dugong feeding trails contain higher nutrients than the sediments of ungrazed areas of seagrass beds of *H. uninervis* and *Z. capricorni* enabling dugongs to increase the turnover rates of nitrogen in seagrass communities, and sustain the net primary productivity of preferred

species. The creation of 'open' spaces as a result of grazing disturbances reduces competition by altering availability of resources, enabling more opportunistic species of seagrass to thrive.

Altering the availability of resources, together with the effects of removing tissues on plant growth, may result in short- and long-term demographic and genetic changes to populations and the coevolution of forage species and herbivores (Jefferies 1988). I believe this occurs in the dugong-seagrass ecosystem as well as terrestrial systems and have developed a conceptual model of the interaction (Fig 9.1).

In contrast to dugong grazing, turtle cropping cannot alter the sediment nutrient cycle. In fact, extensive repeated cropping in the same area could lead to ammonium reduction resulting in lower net primary productivity (see Zieman *et al.* 1984). Zieman's research was carried out on *Thalassia testudinum* in the Caribbean. I predict that the situation may be different in seagrass beds in much of tropical Australia for the following reasons:

- the seagrass beds in north-eastern Australia are much more diverse than those in the Caribbean, so it is probably a better strategy for turtles to sample a variety of food plants rather than to maintain plots which are repeatedly cropped;
- most of the seagrass biomass comprises pioneer species such as *Halophila* and *Halodule* rather than long-lived species such as *Thalassia hemprichii* and *Enhalus acoroides* (Coles *et al.* 1989; Lee Long *et al.* 1993);
- disturbance generated by dugong grazing (Preen 1995a) enhances the microbial activities in the sediments, increasing sediment nutrients (Perry, manuscript) which then enables the remaining plants to recover rapidly and increase productivity. However, the density of manatees in the Caribbean is too low (Marsh and Lefebrve 1995) to generate similar effects.

Tropical seagrasses as a food resource

Nutritional composition

The nutritional data obtained from this study showed that tropical seagrasses contain greater concentrations of nutrients and less fibre and that they are generally more digestible than terrestrial grasses (Chapter 8). The data also showed, like Wake (1975) and Lanyon (1991), that the nutritional composition of tropical seagrass varies between plant parts, species, and location. Generally, seagrass leaves have higher concentrations of nitrogen than the roots/rhizomes, but roots/rhizomes have higher concentrations of starch and WSC than leaves (Chapter 8). Opportunistic species of the genus *Halophila* were the most digestible of the different species examined, while *Halodule uninervis*, another opportunistic species, had the highest concentrations of nitrogen in its leaves and starch concentrations in its roots/rhizomes (Chapter 8). *Syringodium isoetifolium* also showed promising nutrient properties like high IVDMD levels in its leaves and high concentrations of WSC in its roots/rhizomes. Although more robust, *Cymodocea serrulata* and *C. rotundata* also had higher concentrations of nitrogen in their leaves and WSC in their roots/rhizomes than a similarly robust species, *Z. capricorni*, which had intermediate levels of nutrients in its leaves and moderate levels of starch and IVDMD in its roots/rhizomes. However, *Cymodocea* and *Zostera* have notably higher levels of fibre than the other species examined. Based on the preferences of dugongs (Preen 1993) and green turtles (at least juveniles, Read 1991 and Brand 1995), it appears that these animals optimise their diet by selecting food species that maximise digestible nutrients. Likewise, dugongs may preferentially feed on *H. uninervis*, a tannin-rich food source (Lanyon 1991), because the benefit of high energy may offset the cost of consuming the tannins (*sensu* Smallwood and Peters 1986).

Lanyon (1991) reported that seagrass nutrients vary seasonally, and are low during the dry season and high during the wet season. Lanyon found that the mean leaf nitrogen concentration of *H. uninervis* in February was 3%, while in August it was

1.1%. Relatively, this is about a 93% reduction. Lanyon, also reported huge interannual variations (~ 56%) in leaf nitrogen concentrations of *H. uninervis* along the Townsville area (from 2.3% in June 1984 to 1.3% in June 1985).

The nutritional composition of tropical seagrasses also varies spatially (Chapter 7). Apart from the inherent local scale variation of nutrients (e.g. variations in nutrient availability due to sediment types), large-scale (e.g. between bays or regions) variations are most likely not uncommon. This is also suggested by the results of the nutrient enhancement experiments (Chapter 7), which could be considered as a simulation of the variation in sediment nutrients at a regional scale.

Specific variation in seagrass nutrients also increases spatial variability, as the species composition of tropical seagrass communities, unlike those of temperate areas, is patchy (Poiner and Peterken 1995). The complexity of plant community structure and composition means that herbivores such as dugongs or green turtles cannot maximise nutrient intake by restricting their diet to seagrasses with the highest nutrient content. They have to sample at both plant and patch level (*sensu* Illius *et al.* 1992; O'Reagain and Schwartz 1995), to detect changes in forage quality and availability. Feedback mechanisms in foraging behaviour suggest that post-ingestive feedback and long-term memories increase the chances of detecting those species with high nutrients (Provenza and Cincotta 1993). Most ungulates appear to have a well developed, long-term spatial memories allowing them to remember both the location and amount of food present in the environment (Bailey *et al.* 1989; Laca 1993). This may also be true for dugongs and green turtles, which coupled with the presumably intimate knowledge of their home range (in dugongs, evidenced by satellite tracking data, Marsh and Rathbun 1990, Preen 1993, Preen 1996, pers. comm.; in green turtles, evidenced by the high site fidelity, Limpus *et al.* 1989, Read 1991, Limpus *et al.* 1992) would also enable them to locate spatially dispersed patches in complex environments like tropical seagrass communities. Thus, these animals may be monitoring the quality and availability of the different patches.

Distribution, accessibility and availability

Halophila and *Halodule* species are the most common genera off the coast of Queensland (Coles *et al.* 1987; Lee Long *et al.* 1993). Species richness is highest at depths of less than 6 m deep and all of the 14 species from this region have been found in this zone (Lee Long *et al.* 1993). Accessibility to these seagrass beds is affected by tidal cycles (e.g. Queensland coastal waters have a 1-6 m tidal range). Additionally, increasing boat traffic (both recreational and commercial), particularly in nearshore areas, may hinder the access of dugongs and turtles to shallow seagrass beds (Preen 1993). At depths between 6 m and 11 m, species of *Halophila* and *Halodule* are the most frequently sampled seagrasses (Lee Long *et al.* 1993). The availability of *Halophila* and *Halodule* at these depths presumably provides food for dugongs and green turtles which is not dependent on tidal access. This is presumably, particularly important for dugongs as their metabolic maintenance is higher than turtles. Only *Halophila* species are common in areas deeper than 11 m (Lee Long *et al.* 1993). Monospecific meadows are rare but occur mainly in areas of high exposure or high energy (e.g. open coastlines) or in deep water (at depths > 11 m) (Lee Long *et al.* 1993). These patterns are based on broad-scale surveys using research vessels and it is possible that the extensive beds of *Halophila* and *Halodule* at 6 m to 11 m extend further inshore to the intertidal region in some areas (Preen 1996, pers. comm., and pers. observ.). It is possible that the extensive beds of *Halophila* and *Halodule* extend even into the nearshore (intertidal) areas.

Anderson (1994) and others (Lanyon 1991) have assumed that deep water foraging is energetically expensive. To date, there is no information available on the comparative energetics of diving by dugongs or turtles under different conditions of depth, water movements, and temperature. However, we do know that dugongs avoid low temperatures (<18 ° C) (Anderson 1986; Marsh *et al.* 1991; Preen 1993). Dugongs in Moreton Bay in winter, offset the effects of cold water, by regularly migrating out of the Bay to warm oceanic waters (Preen 1993). As a result, dugongs presumably minimises energy expenditure by fasting and/or feeding on

plant species (or even selected invertebrates) that are more accessible or available than their preferred food items (Preen 1993). The least energetically expensive scenario for a dugong would presumably be to feed in calm warm waters where the drag effects of waves are reduced. This scenario could be achieved by feeding in: intertidal areas at slack water at high tide; and/or (2) subtidal (deep water) seagrass areas, especially in calm conditions.

Seagrass availability to herbivores is partially dependent on morphology. All species are available to green turtles, since they take only the leaves, however, some species are presumably cropped more efficiently than others and one would expect that species with small leaves and a low above-ground biomass would be the most energetically expensive to crop. The accessibility of seagrass plants to dugongs is presumably a function of rhizome morphology. Among the six species (*H. ovalis, H. uninervis, C. serrulata, C. rotundata, S. isoetifolium,* and *T. hemprichii*) studied by Brouns (1987b), *H. uninervis* and *H. ovalis* had the shallowest and least complex root system (see also den Hartog 1970 and Tomlinson 1974) and which Tomlinson (1974) described as creeping. All tropical Cymodoceoidea (*C. serrulata, C. rotundata, S. isoetifolium,* and *H. uninervis*), except *H. uninervis* are robust and exhibit reiterative branching of the rhizomes (Brouns 1987b) forming a mattress-like root/rhizome system. Several authors (den Hartog 1970; Tomlinson 1974; Brouns 1987b) have reported similarly that *Thalassia* species have the most prolific root/rhizome system. This means that not all species of seagrasses are available as whole plants to dugongs. *Amphibolis antartica* is an important component of the food supply of dugongs in Shark Bay in winter and they are able to take only its leaves (Anderson 1986) as it is a robust temperate seagrass species of Cymodoceoidea with a prolific root/rhizome system (Kuo and McComb 1989).

Sediment type also presumably plays an important role in determining the accessibility of the below-ground biomass of seagrasses to dugongs. Plants are much harder to dig up when they are growing in hard sediments. Dugongs appear to produce shorter feeding trails (mean length = 2.3 m) when feeding on

seagrasses on sandy substrate (Moreton Bay, Preen 1993) than when feeding on seagrasses on sandy mud (mean feeding trail lengths of 2.9 and 8 m from two beds at Shoalwater Bay, Anderson and Birtles 1978). Similarly, the feeding trails in Moreton Bay were narrower (12-23 cm, Preen 1993) than those at Shoalwater Bay (19-25 cm, Anderson and Birtles 1978). In Moreton Bay, Preen (1993) found that most of the favoured feeding sites of dugongs were located in areas where sediments had a grain size of medium to fine sand.

Combinations of these factors could ultimately determine the accessibility of below-ground biomass to dugongs, a hypothesis supported with evidence from analyses of the gut contents of dugongs. Examination of dugong stomach contents indicates that rhizomatous materials is largely from *Halophila* and *Halodule* species. The other species often occur only as leaf materials (Marsh *et al.* 1982; Erftemeijer *et al.* 1993; Preen 1993). This is further corroborated by the abundant reports of dugong feeding trails on beds of *Halodule* (narrow-variety) and/or *Halophila* species (e.g. Wake 1975; Heinsohn *et al.* 1977; Anderson and Birtles 1978; Preen 1993; Aragones 1994; de Iongh *et al.* 1995; Supanwanid 1996). This enhances the notion that some species (e.g. *Halophila* and *Halodule*) would be easier to uproot as whole plants than others.

Dugongs have also been reported feeding on whole plants of *Z. capricorni* (narrow-variety) in some areas (e.g. Cleveland Bay, Townsville, Wake 1975; Shoalwater Bay, Wake 1975; Anderson and Birtles 1978) but in sparser and soft substrate (mud to sandy mud). In Moreton Bay, less rhizomatous materials are taken when dugongs feed on *Z. capricorni* (Preen 1993), presumably because of the more compact sandy substrate. In the Gulf of Carpentaria dugongs appear to take only the leaves when feeding on *Syringodium isoetifolium* (Preen 1996, pers. comm.). Leaves rather than rhizomes of *Thalassia hemprichii* have also been reported in the gut contents of dugongs from Torres Strait (D. Domning, pers. comm. to H. Marsh).

Basis of diet selection

I believe that the feeding choices made by dugongs, and green turtles are based on: (1) higher digestible nutrient intake; (2) ease of harvesting (accessibility and availability of below-ground components for dugongs); and (3) the capacity of the species harvested to compensate for herbivory. Selection for the species which are highly digestible (*Halophila*), and have high nutrients (*Halodule*), suggests that these herbivores make choices resulting in maximised nutrient intake rather than merely maximised bulk intake. Juvenile green turtles feed on these small-leaf species even though it may be energetically costly, because the presumably higher concentrations of digestible nutrients is a more cost-effective tradeoff than opting for bulkier species with lower digestibility.

Both energy maximisation (bulk, generalist) and nutrient maximisation (selection for limiting nutrients, e.g. nitrogen) are foraging strategies likely to be achieved by tactics which maximise the intake rate of digestible plant tissues (Illius and Gordon 1993). The ease of dugongs harvesting low biomass seagrass species rather than bulky and robust species is apparent from the difference in the complexity of their root/rhizome. Similarly, the small low biomass species are presumably easier to distinguish from the taller and morphologically more robust species (e.g. *Thalassia hemprichii*, *Enhalus acoroides*, *Z. capricorni*). The *Halophila* species (e.g. *H. decipiens, H. minor, H. ovalis*) are some of the smallest and most fragile of the tropical seagrasses. These structural properties, together with their distinctly shorter height presumably enables the meadows they dominate to be easily detected by dugongs and green turtles. In terrestrial grasslands, it has been suggested that vegetational properties such as height (sward), density, and the vertical distribution of biomass, are the major variables that determine intake rate (e.g. Hodgson 1985; Illius and Gordon 1991). Thus, these cues may be important in facilitating easy recognition by dugongs and green turtles for patch quality, as their habitat is highly variable over space and time.

de Iongh *et al.* (1995) attempts to explain the feeding strategy of dugongs on sparse *H. uninervis* meadows with low above-ground biomass and high below-round biomass in the Moluccas, East Indonesia, on the basis of the energy maximisation theory, which states that large herbivores may aim at high net rate of energy intake (Pyke *et al.* 1977).

They observed more dugong feeding trails when the above-ground biomass was low and below-ground biomass higher than when the above-ground biomass was high and the below-ground biomass lower, and suggested that dugongs must prefer sparse *H. uninervis* because of the significant positive correlation between frequency and total organic carbon in the below-ground biomass. However, their conclusion is flawed (or they may have misinterpreted their results) for two reasons.

(1) They did not use nutritional parameters relevant to herbivores, measuring organic matter only (ratio of ash free dry weight and dry weight), not carbohydrate. Organic matter in plants will always correlate positively with total organic carbon since plants are predominantly made up of carbon in contrast to animals which are based on protein.

(2) They assumed that the density of feeding trails was a reliable index of feeding preference whereas it is an index of feeding activity. Grazers such as dugongs, have to work more when the biomass is low (*sensu* van de Koppel *et al.* 1996) resulting in higher frequencies of feeding trails. Thus, frequency of feeding trails is not an index of preference. At Cardwell, numerous dugong feeding trails were seen intermittently in areas of low biomass (pers. observ.). In Townsville, several reports (Wake 1975; Anderson and Birtles 1978; Heinsohn *et al.* 1978) have cited abundance of feeding trails on sparse beds of *Z. capricorni* and/or *H. uninervis*. Mattson (1980) argues that one of the many ways herbivores mitigate limited nutrients (e.g. nitrogen) is by having longer feeding bouts. I suspect this was the case here and that the higher organic matter (total organic carbon) in the below-ground biomass is only a consequence of its biomass being

considerably higher than the above-ground biomass. I would argue instead that the dugong, an optimal forager, was maximising not only energy but nutrients (nitrogen and carbohydrate), particularly, digestible nutrients.

Results of my grazing experiments on meadows of *H. uninervis* at Cardwell showed that the control plots contained twice as much root/rhizome biomass as the leaf fraction as the intensively grazed plots (see Fig 5.11). However, the seagrasses from the intensively grazed plots were nutritionally better than those in the controls (see Appendix 10 Figs 1-18). I suspect that the dugongs in those areas regraze because they acquire better nutritional benefits (like higher nitrogen concentration and better digestibility of dry matter) from regrowth than old standing crop.

Implications for future research

This study has identified directions for future research into the ecology and biology of dugongs, green turtles and tropical seagrasses. First and foremost is the need to have more information about the actual behaviour of free-ranging individuals especially, rates of intake for various species of seagrass. This will not only help us elucidate what the animals are actually doing, but also give a more direct estimate of how much disturbance could be attributed to herbivory. The nutrient value of forage could then be estimated using the NIRS technology based on samples of seagrass. This could be done by following feeding trails left by dugongs and harvesting samples close to these trails to estimate biomass and nutritional composition. This may be more difficult for green turtles as their feeding marks are less conspicuous. It would also be useful, although difficult if not impossible at present, to have estimates of the energetics and costs of feeding under various environmental conditions.

In vivo digestibility tests are also needed to rate the accuracy of the *in vitro* measurements. To gain more insight into the importance of nutritional quality, diet selection and grazing intake for dugongs and green turtles, measurements using natural and dosed *n*-alkenes or *n*-alkanes as markers could be applied. This could

be done by using the ratio of natural (odd-chain) *n*-alkanes or *n*-alkenes in plant cuticular waxes and synthetic (even-chain) *n*-alkanes or *n*-alkenes (by species), with which animals are dosed to measure faecal output (Mayes *et al.* 1986). NIR calibrations could also be derived for organic matter intake (g/day OMI) using freeze-dried and ground faeces samples.

The energetic and nutritional consequences of different diets dictated by differences in distributions of tropical seagrasses should also be examined. This could be done by comparing the nutritional composition of the different species available for various sites of interest and by relating this to some index or measure of animal fitness. It would be useful to relate a measure of reproductive fitness in terms of reproductive outputs (e.g. mother calf ratio) which could then be related back to nutrient availability (or even palatability) based on the nutritional composition and species composition of particular seagrass beds or regions. This could indicate whether some areas are better sites than others by virtue of different nutrient availability due to different species compositions of seagrass meadows. A palatability map of seagrasses could also be developed using NIRS. This could be done by incorporating the nutritional data obtained from NIRS and relating it to the species composition and abundance of the (community structure) of the seagrass community of interest, which in turn would be related back to the known feeding preferences of dugongs and green turtles.

CONCLUSIONS

My results confirm that grazing by dugongs can disturb tropical seagrass communities leading to conditions beneficial to pioneering and/or opportunistic species, and retarding the taller and more robust species. As a result, species composition of seagrass communities is altered at least at a local scale. In addition, by influencing the sediment redox conditions, dugong grazing is likely to make an important contribution to the function of seagrass ecosystems in the tropics. Consequently, I predict that areas which support sizeable numbers of dugongs provide better "quality" food than areas which have no megaherbivores such as

dugongs and green turtles and rely only on natural turnover rates for recycling and redistribution of nutrients. Conversely, regions which support only small and fragmented dugong populations as a result of over-exploitation are probably less favourable to herbivores than regions with comparable levels of natural disturbance with high densities of herbivores. This contrasts with terrestrial grasslands where intensive grazing typically lowers the carrying capacity by converting pastures to a lower seral stage dominated by less palatable, grazing-resistant species (Willms *et al.* 1985; Edroma 1989; Ralphs *et al.* 1990).

I predict that the impact of a given level of dugong grazing in seagrass meadows depends on other environmental factors and the frequency, intensity and timing of other forms of natural disturbances. Thus, the answer to the question posed in my introduction: *will a major and long-term reduction in the numbers of dugongs and green turtles in an area lead to an irreversible degradation of their habitat*, is likely to be locality dependent.

I predict that cropping by green turtles and grazing by dugongs will alter the species composition of at least some intertidal and shallow tropical areas at local scales, as Preen (1995a) showed for subtropical, Moreton Bay. The impact is likely to be less in deeper water in which the Halophilae are the only species able to tolerate the low light intensities (Lee Long *et al.* 1993, 1996). At a regional scale, I predict that the major changes in the distribution and abundance of seagrasses are more likely to depend on the frequency and severity of extreme weather events such as cyclones and floods (Coles *et al.* 1989; Poiner *et al.* 1989; Poiner and Peterken 1995) which in turn have major impacts on the distribution and abundance of dugongs and green turtles. Although cyclones and floods are natural events, their impact can be exacerbated by soil erosion as a result of poor farming practices in catchments draining into the sea as shown for Hervey Bay (Preen and Marsh 1995; Preen *et al.* 1995). The greatest challenge of dugong conservation in Australia may be the conservation and/or preservation of inshore seagrass beds in the region. It will be hard to convince the farmer that erosion from his property

may threaten the survival of sea cows grazing on submarine pastures many kilometers downstream!

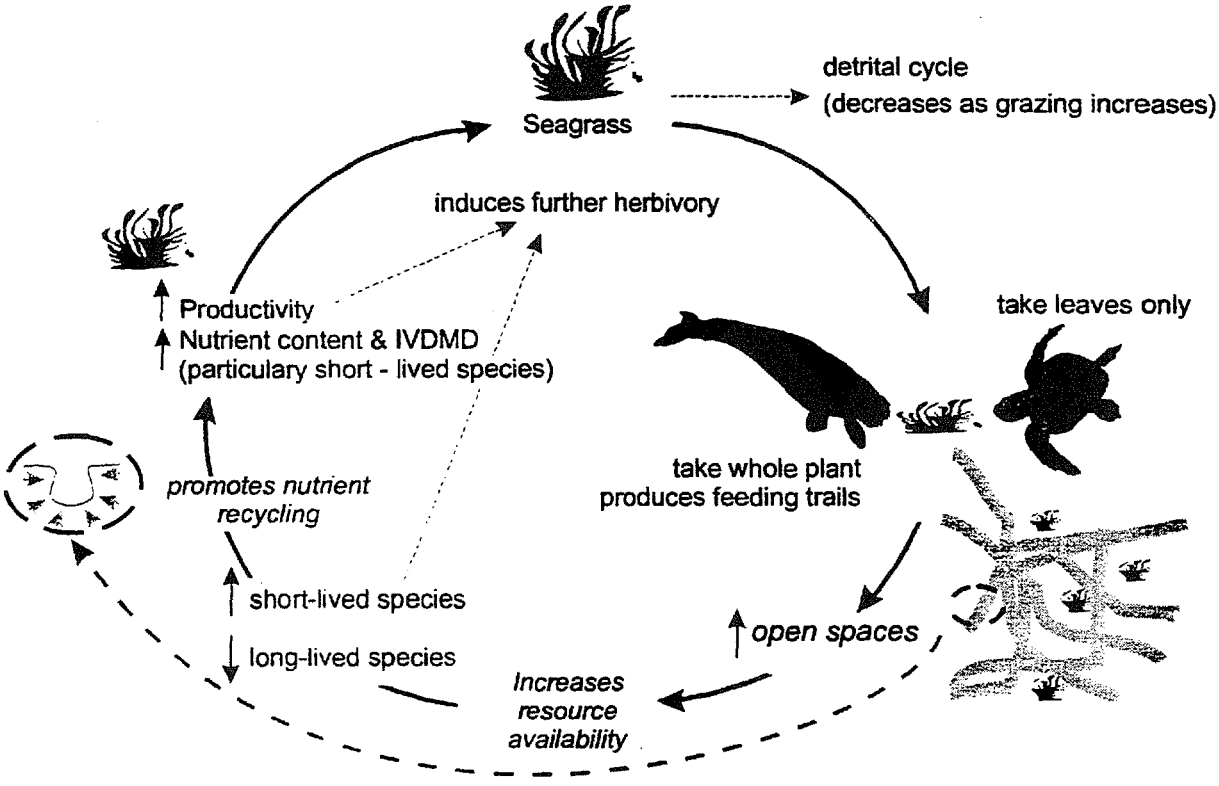

Fig. 9.1. A model of the interactions between dugongs, green turtles and tropical seagrass communities.

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