

This file is part of the following work:

**Bendell, Barry E. (2006) *Interactions amongst invertebrates, epiphytes, and seagrasses in tropical intertidal meadows*. PhD Thesis, James Cook University.**

Access to this file is available from:

<https://doi.org/10.25903/7rkw%2Dyt30>

Copyright © 2006 Barry E. Bendell

The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owners of any third party copyright material included in this document. If you believe that this is not the case, please email

[researchonline@jcu.edu.au](mailto:researchonline@jcu.edu.au)

**Interactions amongst invertebrates,  
epiphytes, and seagrasses in  
tropical intertidal  
meadows**

**Thesis submitted by**

**Barry E. BENDELL BSc (Carleton), MSc (McGill)  
in October 2006**

**for the degree of Doctor of Philosophy  
in the School of Marine Biology and Aquaculture  
James Cook University**

## **Electronic Copy**

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

31 March 2009

-----  
Barry E. Bendell

## Statement of Access

I, the undersigned, the author of this thesis, understand that James Cook University will make this thesis available for use within the University Library and, via the Australian Digital Theses network (unless granted an exemption), for use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do not wish to place any further restriction on access to this work.

31 March 2009

-----  
Barry E. Bendell

## **Statement on Sources**

### **Declaration**

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

31 March 2009

---

Barry E. Bendell

## **Acknowledgements**

Thanks, first and foremost, must go to all those along the way who gave me the opportunity to follow my interests. This project had its genesis in my experiences working in a marine national park in Thailand. I was living near an intertidal seagrass meadow, and I was allowed to sample the invertebrates there, with few questions asked. I am not sure what people thought I was doing, but they were willing to let me do it. I especially thank CUSO (the Canadian volunteer organization) and DTEC (Thailand's Department of Technical and Economic Cooperation) for placing and supporting me in my work at Had Chao Mai National Park. I also wish to thank the officials and staff of Thailand's marine national parks, and especially all the people at the Marine National Park Supporting Center, as it was then called. After acquiring some experience, and a limited understanding of tropical seagrasses, it was my good fortune to be accepted at James Cook University to pursue my interests in a more academic environment. For that, I must especially thank my supervisors, John Collins and Marcus Sheaves, who recognized something in my application that others had not, then took me on, and allowed me to do the project I had wanted to do. I also got helpful inputs from Michelle Waycott and Joe Holtum at James Cook University. It was Joe who showed me how to use his spectrophotometer. Dipali Ayling came out several times to assist with the sampling at the Magnetic Island sites.

## Contributions of others

Financial support was provided by an International Postgraduate Research Scholarship and a James Cook University Postgraduate Research Award for Overseas Students.

Matthew Knott and Basil Byrne sorted seagrass core samples from the plots at Picnic Bay and Cockle Bay.

Joe Holtum provided the spectrophotometer.

Identification of anemones was by Daphne Fautin of the University of Kansas. The identifications of *Alaba virgata* and *Electroma* were confirmed by Winston Ponder of the Australian Museum. The tiny jellyfish that walked along my seagrass leaves were identified as *Staurocladia* by Lisa Gershwin of James Cook University.

## Abstract

Interactions amongst invertebrates, epiphytes, and seagrasses were studied in intertidal meadows near Townsville, Australia. Data were collected to test the assumptions of the prevailing model of seagrass-epiphyte-grazer interactions. That model assumes that epiphytes have the potential to limit seagrass standing crop, but that invertebrate grazers limit epiphytes, and therefore indirectly benefit the seagrass. Furthermore, it is generally assumed that the community of epifaunal invertebrates is largely composed of epiphyte grazers, especially small gastropods and peracardian crustaceans, and that direct consumption of the seagrass is of minor importance.

One intertidal plot, 100 m<sup>2</sup> was established in each of three meadows; Shelly Beach, Cockle Bay and Picnic Bay. Samples of seagrass, invertebrates and epiphytic materials were taken at one to two month intervals over two years. Leaf samples were taken to estimate epifaunal invertebrate densities and epiphytic loads from the same leaves. The percent coverage of the leaves by epiphytic material was estimated, and the material was scraped from the leaves to estimate its ash-free dry weight (AFDW). The seagrass leaf area index (LAI), below ground dry weight (BGDW), and shoot or leaf density in each plot were estimated from core samples.

In June 2002, a sudden migration of the sea hare *Bursatella leachii* into the plot established at Shelly Beach was associated with a rapid reduction in epiphytic loads on *Halophila ovalis*, but not *Halodule uninervis*. Unexpectedly, the LAI of *H. ovalis* declined by two-thirds, while that of *H. uninervis* remained unchanged. It was hypothesised that the reduction in *H. ovalis* LAI was due to the sudden exposure of the leaves to high levels of irradiance, resulting in photodamage and photoinhibition. To test that hypothesis, a shading experiment was performed. Plots, 0.5 m<sup>2</sup>, were shaded with 70% shade cloth over 29 days, to adapt the seagrass to low light conditions, and were then exposed to ambient light. Chlorophyll concentrations increased significantly under shade, then fell to control levels in 4-10 days of exposure in both *H. ovalis* and *H. uninervis*. However, the former species did not show any significant increase in chlorophyll per area of substrate under shading, but appeared to trade-off increased chlorophyll production with a decrease in LAI and leaf density. There were no



significant differences directly related to the treatments other than the changes in chlorophyll concentrations and an increase in leaf length due to shading of *H. uninervis*.

During two years of sampling, occasions of high epiphytic loads, AFDW  $>2.0$  mg/cm<sup>2</sup>, on *Halodule uninervis* were followed by declines in shoot densities, BGDW, and LAI in the plots at Shelly Beach and Cockle Bay. However, within plots there were positive correlations between epiphytic cover and LAI. Those relationships suggested that *H. uninervis* benefited from epiphytic cover, up to a threshold, possibly because of protective effects against high irradiance. There may also have been reciprocal effects, such that increases in the seagrass canopy promoted development of epiphytic cover, which may have acted as a density-dependent limiter of *H. uninervis* production.

The littorid gastropod *Alaba virgata* was the commonest epifaunal epiphyte-grazer, especially at Shelly Beach. Its densities were negatively correlated with epiphytic cover. The positive relationship between epiphytic cover and *H. uninervis* LAI suggested that *A. virgata* was likely to have an indirect negative impact on the seagrass, contrary to the prevailing model of seagrass-epiphyte-grazer interactions. Likewise, the commonest amphipod, *Ericthonius*, had densities that were negatively correlated with epiphytic cover at Cockle Bay and Picnic Bay, but appeared most likely to have a negative impact on the seagrass.

The epifaunal communities were numerically dominated by suspension feeders; including the amphipods *Ericthonius* and *Podocerus*; the bivalve *Electroma*, and Anemones. Total epifaunal abundance was negatively correlated with seagrass LAI in each plot. Those relationships likely reflected a negative impact of the seagrass canopy on water flow, and thus food resources for suspension feeders. Therefore, epifaunal abundance and epiphytic cover had inverse relationships to seagrass LAI. A path analysis showed that a bottom-up model with separate effects from seagrass LAI to total epifaunal abundance and to epiphytic cover fit the data well. That analysis used data from all plots in a multigroup design, and suggested that the same processes were occurring in each of the plots despite other major differences among them.

There was conspicuous evidence of damage by invertebrate herbivores to the leaves of *Halophila ovalis*. The areas damaged were measured using image analysis techniques on leaf samples. Those animals found to be consuming seagrass included; a nerite gastropod, *Smaragdia souverbiana*; an undescribed species of sacoglossa; an amphithoid amphipod, *Cymadusa* sp.; and the sphaeromatid isopods *Cymodoce* spp. *H. ovalis* declined at Shelly Beach during the course of the study, and *Halodule uninervis* became more abundant, in a pattern suggesting successional change. However, changes in *H. ovalis* LAI and BGDW were correlated with measures of damage that were most likely caused by crustacean feeding.

The prevailing model of seagrass-epiphyte-grazer interactions does not provide a suitable description of those interactions in the intertidal meadows studied near Townsville. There was little evidence that epiphyte grazers would benefit the seagrass by removing epiphytic cover. On the contrary, there was evidence of negative impacts by exposing the leaves to high levels of irradiance, which are commonly experienced in tropical intertidal habitats. Suspension feeders, not epiphyte grazers, dominated the epifaunal community, which therefore had a very different relationship to the seagrass and its epiphytic cover from that anticipated in the current literature. Also unanticipated, consumption of seagrass appeared to play an important role in successional development. From this study, new models are proposed that will hopefully provide a better understanding from which to test and analyse interactions amongst invertebrates, epiphytes and seagrasses in tropical intertidal meadows.

# Contents

Statement of Access .....	ii
Statement on Sources .....	iii
Acknowledgements .....	iv
Contributions of others .....	v
Abstract .....	vi
Contents .....	ix
List of figures .....	xii
List of tables .....	xix
Chapter 1 General introduction .....	1
1.1 Background .....	1
1.1.1 The prevailing model of interactions .....	2
1.1.2 Invertebrates as consumers of seagrass .....	6
1.1.3 Limitations of support for prevailing model .....	10
1.2 Objectives .....	13
Chapter 2 General methods .....	15
2.1 Study area .....	15
2.2 Sampling plots .....	16
2.3 Sampling seagrass .....	18
2.4 Sampling invertebrates .....	19
2.5 Measuring epiphytic loads .....	22
2.6 Calculating leaf areas .....	24
2.7 Estimating herbivore damage .....	26
2.8 Abiotic parameters .....	30
2.8.1 Tidal exposure .....	30
2.8.2 Water and weather conditions .....	30
2.8.3 Sediment particle-size .....	30
2.9 Project design .....	31
2.10 Statistical analysis .....	32
2.10.1 Univariate .....	32
2.10.2 Multivariate .....	33
2.11 Definitions and abbreviations .....	33
Chapter 3 The impact of epiphyte grazing by sea hares on <i>Halophila ovalis</i> .....	35
3.1 Introduction .....	35
3.2 Results .....	36
3.2.1 Sea hare abundance .....	36
3.2.2 Epiphytic loads .....	38
3.2.3 Seagrass .....	39
3.3 Discussion .....	43
3.3.1 Changes in epiphytic material .....	43
3.3.2 Response of <i>Halophila ovalis</i> .....	44
3.4 Conclusions .....	48
Chapter 4 Seagrass epifauna and their relationships to epiphytic materials .....	49
4.1 Introduction .....	49
4.2 Methods .....	50
4.2.1 MDS and ANOSIM .....	50
4.3 Results .....	52
4.3.1 Epifaunal communities .....	52

4.3.1.1 Epifauna of <i>Halodule uninervis</i> .....	52
4.3.1.2 Comparison with <i>Halophila ovalis</i> .....	56
4.3.1.3 MDS and ANOSIM.....	57
4.3.2 Epiphytic material.....	58
4.3.2.1 Adjustments to AFDW measurements.....	58
4.3.2.2 Plot SB.....	58
4.3.2.3 Plot CB.....	61
4.3.2.4 Plot PB.....	63
4.3.3 Relationships between epiphytic loads and invertebrate densities.....	64
4.3.3.1 Plot SB.....	64
4.3.3.2 Plot CB.....	65
4.3.3.3 Plot PB.....	65
4.3.4 Comparisons among plots.....	66
4.4 Discussion.....	67
4.4.1 <i>Alaba virgata</i> .....	68
4.4.2 Amphipoda.....	70
4.4.3 <i>Electroma</i> .....	72
4.4.4 Anemones.....	72
4.4.5 Relationship to seagrass species and leaf morphology.....	73
4.4.6 Predatory interactions.....	74
4.5 Conclusions.....	76
Chapter 5 Standing crop of <i>Halodule uninervis</i> in relation to epiphytic materials.....	78
5.1 Introduction.....	78
5.2 Results.....	82
5.2.1 Plot SB.....	82
5.2.2 Plot CB.....	86
5.2.3 Plot PB.....	89
5.2.4 Comparisons among plots.....	92
5.3 Discussion.....	93
5.3.1 Light deprivation by high epiphytic loads.....	94
5.3.2 High irradiance as a limiter of seagrass standing crop.....	96
5.3.2.1 Photoinhibition and photodamage.....	96
5.3.2.2 Desiccation.....	97
5.3.3 Interactions.....	98
5.4 Conclusions.....	99
Chapter 6 Interactions between <i>Halodule uninervis</i> and its epifauna.....	101
6.1 Introduction.....	101
6.2 Statistical methods – path analysis.....	102
6.3 Results.....	105
6.3.1 <i>Alaba virgata</i> .....	105
6.3.2 <i>Erichthonius</i> .....	106
6.3.3 Total invertebrates.....	107
6.3.4 Overview.....	111
6.4 Discussion.....	112
6.5 Conclusions.....	117
Chapter 7 Herbivory and succession.....	118
7.1 Introduction.....	118
7.2 Results.....	119
7.2.1 Seagrass standing crop and epiphytic cover.....	119
7.2.2 Herbivory.....	121

7.2.2.1 Observations of feeding .....	121
7.2.2.2 Measurements of herbivore damage .....	123
7.3 Discussion .....	126
7.3.1 Herbivory .....	126
7.3.2 Succession .....	131
7.3.3 The role of invertebrate herbivores in seagrass ecosystems .....	133
7.4 Conclusions .....	135
Chapter 8 Abiotic factors .....	136
8.1 Introduction .....	136
8.2 Statistical methods – regression tree analysis .....	137
8.3 Results .....	138
8.3.1 Tidal exposure .....	138
8.3.2 Water and weather conditions .....	139
8.3.3 Regression trees .....	142
8.3.3.1 Plot SB .....	142
8.3.3.2 Plot CB .....	144
8.3.3.3 Plot PB .....	144
8.4 Discussion .....	147
8.5 Conclusions .....	150
Chapter 9 Shading experiment on the impact of high irradiance on intertidal seagrasses .....	151
9.1 Introduction .....	151
9.2 Methods .....	152
9.2.1 Design .....	152
9.2.2 Chlorophyll concentrations .....	153
9.2.3 Seagrass measurements .....	155
9.2.4 Epiphytic loads .....	156
9.2.5 Invertebrates .....	156
9.2.6 Statistical analysis .....	157
9.3 Results .....	157
9.3.1 Chlorophyll concentrations .....	157
9.3.2 Seagrass measurements .....	160
9.3.2.1 <i>Halophila ovalis</i> .....	160
9.3.2.2 <i>Halodule uninervis</i> .....	162
9.3.3 Epiphytic loads .....	164
9.3.4 Invertebrates .....	167
9.3.4.1 <i>Alaba virgata</i> .....	167
9.3.4.2 Herbivores .....	167
9.3.5 Interactions .....	169
9.4 Discussion .....	172
9.5 Conclusions .....	181
Chapter 10 General conclusions .....	183
10.1 New perspectives .....	183
10.2 Future directions .....	187
References .....	190
Appendix A: Epifaunal abundance tables .....	214
Appendix B: Comparison of invertebrate sampling techniques: coring versus leaf cutting .....	226
Appendix C: Changes in leaf morphology .....	228
Appendix D: Sediment characteristics .....	231

## List of figures

Fig. 1.1 A simple model of seagrass-epiphyte-grazer interactions. Light is reduced by water depth and turbidity. Epiphytic material on the leaf surface further reduces light. The impact of light is positive, as it is light used for photosynthesis. Epiphytes always have a negative impact, but epiphyte grazers limit that impact. .5	.5
Fig. 2.1 Map showing location of study sites near Townsville, Queensland, Australia. ....	17
Fig. 2.2 An example of the estimation of gastropod damage to a <i>Halophila ovalis</i> leaf. The 8-bit colour image (A) was converted to grayscale (B), then thresholded at a level that distinguished the pigmented areas with one or two layers of cells in black (C). That area was measured at 36.3 mm <sup>2</sup> . The missing area was painted (D) and the total area corresponding to the area of the undamaged leaf was measured at 42.2 mm <sup>2</sup> . The area of 2-layer damage is therefore the difference, 5.9 mm <sup>2</sup> . The density slice option was then used to identify the intermediate level of grey representing the area of 1-layer damage in red (E). Extra red pixels representing thin tissue at the edge of the leaf were erased. The desired area was selected (F), and the red pixels measured at 8.1 mm <sup>2</sup> . The percentage area of the leaf damaged by gastropods was therefore estimated at ((5.9 + (0.5*8.1))/42.2)*100=23.6%. ....	28
Fig. 2.3 Summary of the biological parameters measured from core samples and cut leaf samples. ....	29
Fig. 3.1 Mean estimates (± s.e.) of the density of sea hares (A) from quadrat samples, and the AFDW (B), DW (C), and ratio AFDW:DW (D) of epiphytic material on leaves of <i>Halophila ovalis</i> and <i>Halodule uninervis</i> from June to September 2002. ....	37
Fig. 3.2 Mean estimates (± s.e.) of leaf area index (LAI) and below ground dry weight (BGDW) of <i>Halophila ovalis</i> and <i>Halodule uninervis</i> in cores (n=10) from June to September 2002. ....	40
Fig. 3.3 Mean estimates (± s.e.) of the leaf area index (LAI) from cores (n=5), and AFDW of epiphytic material on <i>Halodule uninervis</i> leaves from June to September 2003. Also showing the approximate period when sea hares were active in the study plot. ....	41
Fig. 3.4 Mean densities and sizes (± s.e.) of <i>Halophila ovalis</i> leaves in core samples (n=10) from plot SB from June to September 2002. ....	42
Fig. 3.5 Relationship between AFDW of material on <i>Halophila ovalis</i> leaves and leaf area per gram of below ground dry weight. Regression line fitted for the period August 2002 to December 2003 (solid line, n=15, R <sup>2</sup> =0.27, p<0.05), excluding June and July 2002 (circled), and extrapolated (dashed line) to level of AFDW recorded in June 2002. ....	42
Fig. 3.6 A modification of the model of seagrass-epiphyte-grazer interactions (Fig. 1.1) to illustrate the impact of sea hare grazing on <i>Halophila ovalis</i> . In this case, light has a negative impact on the leaves. Epiphytic cover has a positive impact because it reduces damage by irradiance. Sea hares have a direct negative impact on epiphytes, but an indirect negative impact on the seagrass. ....	48

Fig. 4.1 Mean ( $\pm$ s.e.) densities of <i>Electroma</i> and <i>Alaba virgata</i> on <i>Halodule uninervis</i> leaves in plot SB.....	54
Fig. 4.2 Mean ( $\pm$ s.e.) densities of <i>Erichthonius</i> sp. and anemones on <i>Halodule uninervis</i> leaves in plot CB. ....	55
Fig. 4.3 Mean ( $\pm$ s.e.) densities of Amphipoda on <i>Halodule uninervis</i> leaves in plot PB. ....	56
Fig. 4.4 Comparison of the mean densities of major taxa as a proportion of the total density of animals on <i>Halodule uninervis</i> and <i>Halophila ovalis</i> leaves over 18 dates in plot SB and 6 dates in plot CB on which both seagrass species were sampled.....	57
Fig. 4.5 Non-metric multidimensional scaling of invertebrate communities on 79 sample dates among three plots (CB, PB, SB) and two seagrass species (Hu= <i>Halodule uninervis</i> , Ho= <i>Halophila ovalis</i> ). Vector lengths and angles correspond to the coefficients of correlations between the transformed abundance of the specified invertebrate taxa in each sample and the sample's value on the coordinate axes. ....	59
Fig. 4.6 Mean AFDW and percent cover ( $\pm$ s.e.) of epiphytic material on <i>H. uninervis</i> leaves from plot SB. Epiphytic material was sampled twice in June 2002, 11 days apart. Two samples in November 2002 are early and late in the month. ....	60
Fig. 4.7 Mean AFDW and percent cover ( $\pm$ s.e.) of epiphytic material on <i>H. ovalis</i> leaves from plot SB. Epiphytic material was sampled twice in June 2002, 11 days apart. Two samples in November 2002 are early and late in the month. ....	61
Fig. 4.8 Mean AFDW and percent cover ( $\pm$ s.e.) of epiphytic material on <i>H. uninervis</i> leaves from plot CB.....	62
Fig. 4.9 Mean AFDW and percent cover ( $\pm$ s.e.) of epiphytic material on <i>H. ovalis</i> leaves from the plot CB.....	63
Fig. 4.10 Mean AFDW and percent cover ( $\pm$ s.e.) of epiphytic material on <i>H. uninervis</i> leaves from plot PB. ....	64
Fig. 4.11 Mean ( $\pm$ s.e.) densities of <i>Alaba virgata</i> and mean percent cover of epiphytic material on <i>H. uninervis</i> leaves from plot SB. Samples were taken twice in June 2002, 11 days apart. Two samples in November 2002 are early and late in the month.....	65
Fig. 4.12 Mean ( $\pm$ s.e.) densities of <i>Erichthonius</i> and mean percent epiphytic cover on <i>H. uninervis</i> leaves in plot PB.....	66
Fig. 4.13 AFDW of epiphytic material on <i>Halodule uninervis</i> leaves calculated as 3-point moving averages in each plot.....	67
Fig. 5.1 Graphic representation of hypothesised changes in seagrass standing crop with changes in epiphytic cover (increasing to the left) and irradiance exposure (increasing to the right). The shape of the curve is taken from typical plots of photosynthetic rates versus irradiance (P-I curves) for plants with a photoinhibitory response, such as that shown by Drew (1979) for <i>Halophila stipulacea</i> . ....	80
Fig. 5.2 Mean LAI and BGDW ( $\pm$ s.e.) of <i>Halodule uninervis</i> in core samples, n=10 from June to September 2002, n=5 thereafter, from plot SB. ....	82

Fig. 5.3 Mean maximum lengths ( $\pm$ s.e.) of <i>Halodule uninervis</i> in cut leaf samples, n=10, and mean densities ( $\pm$ s.e.) of <i>H. uninervis</i> shoots in core samples, n=10 from June to September 2002, n=5 thereafter, from plot SB. ....	83
Fig. 5.4 <i>Halodule uninervis</i> LAI plotted against the mean AFDW ( $\pm$ s.e.) of epiphytic material on leaves in plot SB. ....	84
Fig. 5.5 <i>Halodule uninervis</i> shoot density and hours of tidal exposure of plot SB between 900 h and 1600 h over 14 days prior to sampling, following the methods in chapter 2 (section 2.8.1). ....	84
Fig. 5.6 <i>Halodule uninervis</i> LAI and hours of tidal exposure between 900 h and 1600 h over 14 days prior to sampling of plot SB. ....	85
Fig. 5.7 <i>Halodule uninervis</i> LAI and mean percent epiphytic cover ( $\pm$ s.e.) of leaves in plot SB. ....	86
Fig. 5.8 Relationship between <i>Halodule uninervis</i> LAI lagged forward one date and percent epiphytic cover over two years in plot SB. ....	86
Fig. 5.9 Mean LAI and BGDW ( $\pm$ s.e.) of <i>Halodule uninervis</i> in core samples, n=10, from plot CB. ....	87
Fig. 5.10 Mean densities ( $\pm$ s.e.) of <i>Halodule uninervis</i> shoots in core samples, n=10, from plot CB. ....	87
Fig. 5.11 <i>Halodule uninervis</i> LAI, epiphytic AFDW ( $\pm$ s.e.), and anemone densities per leaf area in plot CB. ....	88
Fig. 5.12 Relationship between <i>Halodule uninervis</i> LAI and percent epiphytic cover from June 2002 to July 2003 in plot CB, n=11. ....	89
Fig. 5.13 Mean LAI ( $\pm$ s.e.) of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> in cores from plot PB, n=5. ....	90
Fig. 5.14 Mean BGDW ( $\pm$ s.e.) of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> in cores from plot PB, n=5. ....	90
Fig. 5.15 Mean densities ( $\pm$ s.e.) of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> shoots in cores from plot PB, n=5. ....	91
Fig. 5.16 Relationship between <i>Halodule uninervis</i> BGDW and percent epiphytic cover over two years in plot PB. ....	92
Fig. 5.17 Relationship between combined LAI of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> , and percent epiphytic cover over two years in plot PB. ....	92
Fig. 5.18 Changes in LAI values calculated as 3-point moving averages in each plot. Total LAI in plot PB is the sum of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> . In plots SB and CB, only <i>H. uninervis</i> ( <i>H. u.</i> ) LAI is presented. Other similar seagrass species, <i>Zostera muelleri</i> and <i>Thalassia hemprichii</i> , in plots SB and CB respectively, made a minor contribution to the total LAI in those plots. ....	93
Fig. 5.19 A modified model of interactions among invertebrates, epiphytes, and the standing crop of <i>Halodule uninervis</i> . Light, as modified by the amount of epiphytic cover, may have both positive and negative impacts on the seagrass, but the leaf area of seagrass has a positive impact on epiphyte development. ....	100
Fig. 6.1 Path diagram representing the prevailing model of seagrass-epiphyte-grazer interactions. ....	103



Fig. 6.2 Path model of <i>Alaba</i> effects on <i>Halodule uninervis</i> in plot SB, n=21. *p<0.05. ....	106
Fig. 6.3 Path model of <i>Erichthonius</i> effects on LAI, calculated as the combined value of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> in plot PB (n=17), and <i>H. uninervis</i> in plot CB from June 2002 to June 2003 (n=10). †0.05<p<0.10, *p<0.05, ** p<0.01.....	107
Fig. 6.4 Relationships between epifaunal abundance and LAI (untransformed) in plots PB (r=-0.78, p<0.001), CB (r=-0.64, p<0.05), and SB (r=-0.64, p<0.005). LAI in plot PB is the combined value of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> , but in other plots is only <i>H. uninervis</i> . The relationship in plot CB was calculated for the period June 2002 to June 2003 (solid circles) only. Note the differences in the scale of LAI among the plots. ....	109
Fig. 6.5 Relationships between percent epiphytic cover and LAI (both untransformed) in plots PB (r=0.53, p<0.05), CB (R <sup>2</sup> =0.80, p<0.005), and SB (r=0.44, p<0.05). LAI in plot PB is the combined value of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> , but in other plots is only <i>H. uninervis</i> . The quadratic function in plot CB was calculated for the period June 2002 to July 2003 (solid circles) only. Note the differences in the scale of LAI among the plots. Plots PB and CB redrawn from Fig. 5.12 and Fig. 5.17.*** .....	110
Fig. 6.6 Path model of effects of LAI on the total density of epifauna and epiphytic cover (all variables transformed) in a multigroup analysis. LAI calculated as the combined value of <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> in plot PB (n=17), and <i>H. uninervis</i> in plot SB (n=21) and plot CB from June 2002 to June 2003 (n=10). †0.05<p<0.10, *p<0.05, ** p<0.01. *** p<0.001. ....	111
Fig. 6.7 Schematic diagram representing interactions among <i>Halodule uninervis</i> LAI, and cover by epiphytic material and epifauna. Coverage by epiphytic material or epifauna increases to a maximum that forces a fall in LAI, represented by the arrows. At maximum epiphytic cover, a fall in LAI results in a decrease in epiphytic cover, and an increase in epifauna. At maximum epifaunal cover, LAI falls to zero. ....	115
Fig. 7.1 Mean LAI (± s.e.) of <i>Halophila ovalis</i> in core samples from plot SB, n=10 from June to September 2002, n=5 thereafter. Letters A to D correspond to points of reduced <i>H. ovalis</i> LAI, as referred to in text. Percent epiphytic cover of leaves as in Fig. 4.7, with data for June and July 2002 estimated from regression relationship with AFDW. ....	120
Fig. 7.2 Patterns of change in LAIs of seagrass species in plot SB expressed as 3-point running averages. ....	121
Fig. 7.3 <i>Halophila ovalis</i> leaves showing various types of damage. Leaves collected from plot SB for epiphytes and epifauna on dates A to D as in Fig. 7.1.....	122
Fig. 7.4 <i>Halodule uninervis</i> leaf showing damage typical of isopod feeding. ....	123
Fig. 7.5 Percent surface (± s.e.) of <i>Halophila ovalis</i> leaves damaged by gastropods in samples collected to estimate epiphytic loads and epifaunal abundances in plots SB and CB. Letters A to D as in Fig. 7.1. ....	124
Fig. 7.6 Proportion of expected <i>Halophila ovalis</i> leaf area missing in core samples from plot SB. Letters A to D as in Fig. 7.1. ....	125

Fig. 7.7 <i>Halophila ovalis</i> LAI versus the proportion of leaves missing in core samples from plot SB. ....	126
Fig. 8.1 Hours of exposure of each plot by low tides, between 900 – 1600 h, over 14 days before sampling. ....	139
Fig. 8.2 Salinities of water samples taken at low tide in each plot. ....	140
Fig. 8.3 Cumulative rainfall over 14 days prior to sampling dates in each plot. ....	141
Fig. 8.4 Mean non-directional wind speed at 1500 h over 14 days prior to sampling dates in each plot. ....	141
Fig. 8.5 Water Temperatures in plots SB and CB during low afternoon tides. Complete data for plot PB are not available for the same time of day. Also shown are mean air temperatures at 1500 h over 14 days prior to sampling in plot SB. Similar air temperature curves were calculated for plots CB and PB, but are not shown. ....	142
Fig. 8.6 Regression trees of <i>Halodule uninervis</i> LAI and BGDW in plot SB. BGDW means were measured as g·m <sup>-2</sup> , temp. = temperature, invert. = invertebrates. ....	143
Fig. 8.7 Regression trees of <i>Halophila ovalis</i> LAI and BGDW in plot SB, where “missing” is the missing portion of <i>H. ovalis</i> leaves attributed to crustacean feeding, and <i>H. u.</i> = <i>Halodule uninervis</i> . ....	144
Fig. 8.8 Regression trees of <i>Halodule uninervis</i> LAI and BGDW in plot PB, where exp. = exposure, Epi. = epiphytic, and “All- <i>Elect</i> ” is the total density of all invertebrates excluding <i>Electroma</i> . ....	145
Fig. 8.9 Regression tree of total LAI, i.e. combine <i>Halodule uninervis</i> and <i>Cymodocea serrulata</i> LAI, in plot PB. Epi. = epiphytic, and “All- <i>Elect</i> ” is the total density of all invertebrates excluding <i>Electroma</i> . ....	146
Fig. 9.1 A shaded plot at Shelly Beach .....	153
Fig. 9.2 Mean ( $\pm$ s.e.) concentrations of chlorophyll <i>a</i> and <i>b</i> , and the ratio chlorophyll <i>a:b</i> , per cm <sup>2</sup> of <i>Halophila ovalis</i> leaf from treatment and control plots, n=9, except after 1 day of exposure when 5 plot pairs were sampled. Sampling occurred on the day shades were applied to the treatment plots (days of exposure = -29), the day shades were removed (days of exposure = 0), and subsequent days of exposure as indicated. ....	158
Fig. 9.3 Mean ( $\pm$ s.e.) concentrations of chlorophyll <i>a</i> and <i>b</i> , and the ratio chlorophyll <i>a:b</i> , per cm <sup>2</sup> of <i>Halodule uninervis</i> leaf from 9 treatment and control plots from the day shade treatment plots were exposed to ambient light (days of exposure = 0). ....	159
Fig. 9.4 Mean ( $\pm$ s.e.) values of LAI (A), leaf density (B), leaf size (C), and BGDW (D) of <i>Halophila ovalis</i> in core samples from 9 paired control and treatment plots before shading, after 29 days of shading treatment, and after 29 days of exposure to ambient light. ....	161
Fig. 9.5 Mean ( $\pm$ s.e.) values of LAI (A), shoots density (B), and BGDW (C) of <i>Halodule uninervis</i> in core samples from 9 paired control and treatment plots before shading, after 29 days of shading treatment, and after 29 days of exposure to ambient light. ....	163
Fig. 9.6 Mean ( $\pm$ s.e.) length of leaves measured in the field (A), and mean ( $\pm$ s.e.) of the lengths (B) and mean breadths (C) in scanned images of leaves cut to sample	

epiphytic material from 9 pairs of control and treatment plots after 29 days of shading, and after 29 days of exposure. ....	164
Fig. 9.7 Mean ( $\pm$ s.e.) AFDW of epiphytic material per cm <sup>2</sup> of <i>Halophila ovalis</i> leaf from 9 pairs of control and treatment plots after 29 days of shading, and after 29 days of exposure. ....	165
Fig. 9.8 Mean ( $\pm$ s.e.) AFDW of epiphytic material per cm <sup>2</sup> of <i>Halodule uninervis</i> leaf from 9 pairs of control and treatment plots after 29 days of shading and exposure, and from 4 pairs of plots after 4 days of exposure. ....	166
Fig. 9.9 Treatment plot four days after removal of shade. The dark material on seagrass surrounding the plot is epiphytic algae. ....	166
Fig. 9.10 Mean ( $\pm$ s.e.) number of <i>Alaba virgata</i> per cm <sup>2</sup> on <i>Halodule uninervis</i> leaves sampled to estimate the epiphytic loads in 9 pairs of control and treatment plots after 29 days of shading and exposure, and from 4 pairs of plots after 4 days of exposure. ....	167
Fig. 9.11 Estimated percent damage ( $\pm$ s.e.) of <i>Halophila ovalis</i> leaf surface areas by gastropods. Leaves sampled to estimate epiphytic loads in 9 paired control and treatment plots after 29 days of shading, and after 29 days of exposure. ....	168
Fig. 9.12 The estimated leaf surface area of <i>Halophila ovalis</i> missing as a proportion of the expected surface area ( $\pm$ s.e.) of leaves in core samples from 9 paired control and treatment plots before shading, after 29 days of shading treatment, and after 29 days of exposure to ambient light. ....	169
Fig. 9.13 Plot of the values of the contrast due to shading treatment effects on LAI versus the difference between treatment and control plots in chlorophyll <i>a</i> concentrations in <i>Halophila ovalis</i> leaves after 29 days of shading. Positive values on y-axis represent increases in LAI in shade plots relative to controls after shading. ....	170
Fig. 9.14 Plot of the values of the contrast due to exposure treatment effects on LAI versus the difference between treatment and control plots in chlorophyll <i>a</i> concentrations in <i>Halophila ovalis</i> leaves after 29 days of exposure. Positive values on y-axis represent increases in LAI of shade plots relative to controls after exposure. ....	171
Fig. 10.1 A modified model of interactions among invertebrates, epiphytes, and the standing crop of <i>Halophila ovalis</i> . Herbivores have a negative impact that can be modified by epiphytes. Light may have either a negative or positive impact, and the amount of light reaching the leaves is reduced by epiphytes. ....	186
Fig. 10.2 A modified model of interactions among invertebrates, epiphytes, and the standing crop of <i>Halodule uninervis</i> . The seagrass has a positive effect on epiphytes, but a negative impact on suspension feeders, both of which may reduce light reaching the leaves. ....	188
Fig. B.1 Mean number ( $\pm$ s.e.) of Amphipoda per cm <sup>2</sup> <i>Halodule uninervis</i> leaf in cut leaf and core samples in plots PB and plot CB (non-transformed data). ....	227
Fig. B.2 Mean number ( $\pm$ s.e.) of <i>Electroma</i> per cm <sup>2</sup> <i>Halodule uninervis</i> leaf in cut leaf and core samples in plots SB (non-transformed data). ....	227

Fig. C.1 Mean densities and mean size ( $\pm$ s.e.) of <i>Halophila ovalis</i> leaves in cores from plot SB. Data from the first four sampling dates are also presented in Fig. 3.4. Letters A to D as in Fig. 7.1. ....	228
Fig. C.2 Mean maximum lengths and mean breadths ( $\pm$ s.e.) of <i>Halodule uninervis</i> in cut leaf samples (n=10) from plot SB. Mean maximum lengths are also presented in Fig. 5.3. ....	229
Fig. C.3 Mean LAI values ( $\pm$ s.e.) of <i>Halodule uninervis</i> leaves <1.5 mm and >1.5 mm wide in core samples (n=5) from plot PB. ....	229
Fig. C.4 Mean maximum length ( $\pm$ s.e.) of <i>Halodule uninervis</i> leaves >1.5 mm wide in cut samples from plot PB. ....	230
Fig. D.1 Weight of material retained on sieves of different mesh sizes expressed as a proportion of the dry weight of sediment samples. ....	231

## List of tables

Table 1.1 Studies reporting experimental manipulations of epiphyte grazers on seagrass. In all studies, treatments with grazers had significantly reduced epiphyte biomass and/or chlorophyll <i>a</i> concentrations compared to treatments without grazers. Some also reported increases in measurements of seagrass abundance, growth, or productivity. Studies that also manipulated nutrients are indicated **. Invertebrates are identified as Gastropoda (G), Amphipoda (A), Isopoda (I), or Decapoda (D). .....	4
Table 4.1 Summary of the mean densities of major invertebrate taxa averaged over all sampling dates, presented as the number of animals per 100 cm <sup>2</sup> of leaf surface. Note that data from plot SB includes two sampling dates 11 days apart in June 2002. For more details see Appendix A. ....	53
Table 4.2 Mean measures of epiphytic load on <i>Halodule uninervis</i> leaves over the course of the study.....	67
Table A.1 Mean number of invertebrates per 100 cm <sup>2</sup> <i>Halodule uninervis</i> leaf surface in plot SB, n=10 on each sampling date. Part 1 of 2. ....	215
Table A.2 Mean number of invertebrates per 100 cm <sup>2</sup> <i>Halophila ovalis</i> leaf surface in plot SB on each sampling date. Part 1 of 2. ....	217
Table A.3 Mean number of invertebrates per 100 cm <sup>2</sup> <i>Halodule uninervis</i> leaf surface in plot CB, n=10 on each sampling date. Part 1 of 3.....	219
Table A.4 Mean number of invertebrates per 100 cm <sup>2</sup> <i>Halophila ovalis</i> leaf surface in plot CB, n=5 on each sampling date. ....	222
Table A.5 Mean number of invertebrates per 100 cm <sup>2</sup> <i>Halodule uninervis</i> leaf surface in plot PB, n=10 on each sampling date. Part 1 of 3. ....	223

# Chapter 1 General introduction

## 1.1 Background

Seagrass meadows are a common feature of tropical and temperate coastal regions. They harbour a diverse and abundant fauna, especially when compared to adjacent non-vegetated habitats (Lewis 1984, Orth et al. 1984, Connolly 1994a, Edgar et al. 1994, Edgar and Shaw 1995a, Heck et al. 1995, Connolly 1997). They provide food, shelter, and nursery areas for many commercially important species, including fish (e.g. Connolly 1994b, Gotceitas et al. 1997), decapods (Edgar 1990b, c, Haywood et al. 1995, Perkins-Visser et al. 1996), molluscs (Ambrose and Irlandi 1992, Ray and Stoner 1995), and holothurians (Mercier et al. 2000). And they provide food for some charismatic marine vertebrates: dugongs (*Dugong dugon*), manatees (*Trichechus manatus*), and green turtles (*Chelonia mydas*) (Lanyon et al. 1989). Compared with the Earth's other major biomes, seagrass meadows have an economic value per hectare that is greater than that of most other aquatic or terrestrial biomes, including coral reefs and mangroves (Costanza et al. 1997).

Seagrass meadows are among the world's most productive plant communities (Duarte and Chiscano 1999). Their high primary production comes from both seagrasses and the algae associated with them. The largest part of that algal production is usually epiphytic. Measurements of the primary production of the components of seagrass assemblages show that the production of epiphytic algae often exceeds that of the seagrasses on which they reside (Mazzella and Alberte 1986, Thom 1990, Moncreiff et al. 1992). Although epiphytic algae are typically a small part of the standing biomass in seagrass meadows, they have a high turnover. That high production is exploited by the most abundant animals in the seagrass meadow, usually gastropods (van Montfrans et al. 1982, Mazzella and Russo 1989) and amphipods (Zimmerman et al. 1979), which scrape epiphytic material from leaf surfaces. That material is preferred over refractory seagrass leaves and detritus (Kitting 1984). As a consequence, food webs in seagrass meadows appear to be more reliant on algal, as opposed to seagrass, production. Stable isotope analyses confirm the dominance of algal carbon sources in the diets of invertebrates inhabiting seagrass meadows, but differ as to the source of the algae.

Some have emphasised the importance of epiphytic algae (Kitting et al. 1984, Moncreiff and Sullivan 2001), while others (Smit et al. 2005, Behringer and Butler 2006) have found macroalgae to be more important. However, all isotope studies have been very consistent in finding minimal evidence of the direct consumption of seagrasses (Stephenson et al. 1986, Lepoint et al. 2004). Where seagrasses have been an important source of carbon (Kharlamenko et al. 2001), they were consumed as detritus.

### ***1.1.1 The prevailing model of interactions***

Epiphytes have faster growth rates than seagrasses, and have the potential to overgrow seagrass leaves and restrict their access to light for photosynthesis, and may also be a barrier to their uptake of inorganic carbon and nutrients (Sand-Jensen 1977, Twilley et al. 1985). The potential for epiphytes to limit seagrass standing crop appears to have been realized where nutrient enrichment has stimulated algal growth and resulted in the disappearance of seagrasses from polluted waters (Cambridge et al. 1986, Silberstein et al. 1986, Tomasko and Lapointe 1991). So, although epiphytes provide the greater base for food webs, their growth can threaten the survival of the seagrass, and the structural basis of the whole community. However, epiphyte growth does not normally dominate, which has been explained by the ability of epiphyte grazers to limit epiphyte biomass (Orth and van Montfrans 1984, van Montfrans et al. 1984). That capability has been well demonstrated in a number of experimental manipulations of epiphyte grazers on seagrass leaves (Table 1.1). In experimental treatments, seagrass with grazers have a lower biomass of epiphytes than treatments without grazers. Those experiments have involved a range of epiphyte grazers, including gastropods (Robertson and Mann 1982, Hootsmans and Vermaat 1985, Philippart 1995, Jernakoff and Nielsen 1997, Nelson 1997, Fong et al. 2000), gammaridean amphipods (Howard 1982, Jernakoff and Nielsen 1997, Duffy and Harvilicz 2001), a caprellid amphipod (Caine 1980), isopods (Hootsmans and Vermaat 1985, Williams and Ruckelshaus 1993, Duffy et al. 2001), decapods (Hays 2005), or a mix of invertebrate grazers (Howard and Short 1986, Neckles et al. 1993, Duffy et al. 2003). Among those studies, Williams and Ruckelshaus (1993), Neckles et al. (1993) and Hays (2005) also manipulated nutrient levels. Those studies found that grazers could limit the accumulation of epiphytic algae in nutrient enriched treatments. In other studies, water column nutrient

levels were not correlated to epiphyte biomass in the field (Nelson and Waaland 1997), or in mesocosms that contained a diverse fauna allowing for complex interactions (Lin et al. 1996). Therefore, it appears that seagrasses in polluted waters need not succumb to overgrowth by algae if there are healthy epiphyte grazer assemblages.

Many of the experimental manipulations of grazers that resulted in changes in seagrass epiphytes also reported changes in the seagrass (Table 1.1). In treatments with grazers, greater seagrass abundance, growth or productivity was reported compared to treatments without grazers. Therefore, it has been concluded that epiphyte grazers benefit seagrasses, which has been presumed to be a consequence of their consumption of epiphytes. Such conclusions have been drawn in a number of reviews specifically concerned with interactions amongst grazers, epiphytes and seagrasses (Orth and van Montfrans 1984, van Montfrans et al. 1984, Jernakoff et al. 1996), and broader reviews of seagrass ecosystems (Orth 1992, Williams and Heck 2001). Hughes et al. performed a meta-analysis of the effects on seagrasses of experimental manipulations of nutrients and grazers, and included many of those studies in Table 1.1. They concluded that those studies showed that grazers had positive effects on seagrasses, and that those effects were comparable in magnitude to the negative effects of water column nutrient enrichment.

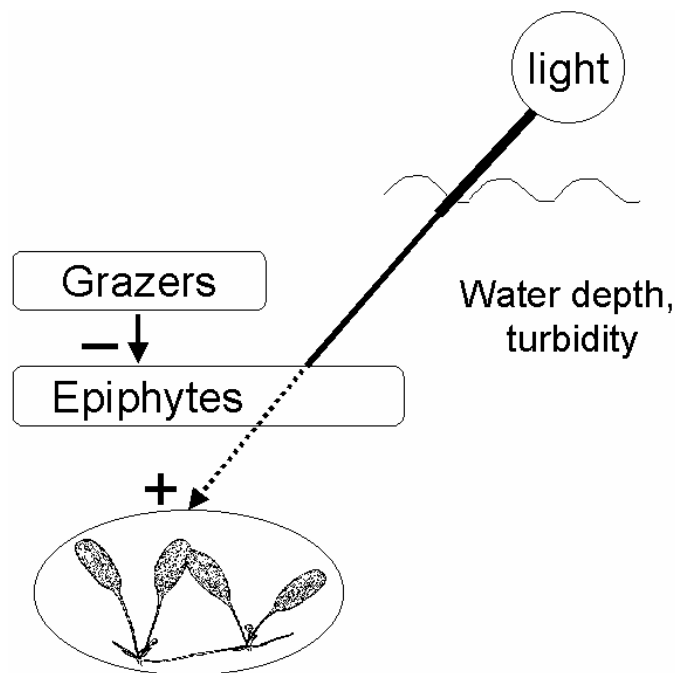
The results of experimental manipulations support a top-down model of grazer and epiphytes effects on seagrasses. Those interactions can be represented as a simple box and arrow diagram (Fig. 1.1). That model proposes that grazers affect the epiphytic cover of seagrasses to the benefit of seagrass production. An assumption of the model is that insolation is of immediate benefit to the seagrass because it provides light for photosynthesis. Any factor that limits light, such as epiphytic cover, is limiting to seagrass production. Therefore, the impact of epiphytes is always negative, and the ultimate impact of epiphyte grazers on seagrass production is positive because they limit epiphyte growth. This top-down view contrasts with the typical view of much of the seagrass literature, which has been most concerned with bottom-up factors that can limit seagrass production, especially light and nutrients (Hemminga and Duarte 2000).



**Table 1.1 Studies reporting experimental manipulations of epiphyte grazers on seagrass. In all studies, treatments with grazers had significantly reduced epiphyte biomass and/or chlorophyll *a* concentrations compared to treatments without grazers. Some also reported increases in measurements of seagrass abundance, growth, or productivity. Studies that also manipulated nutrients are indicated \*\*. Invertebrates are identified as Gastropoda (G), Amphipoda (A), Isopoda (I), or Decapoda (D).**

Study	Epiphyte Grazer(s)	Seagrass	Location	Increased seagrass measure(s) with grazers	Experimental units	Duration
Hays 2005**	Pagura maclaughlinae (D), Tozeuma carolinense (D)	Thalassia hemprichii	Florida	Growth	Greenhouse microcosms, subdivided 1 m <sup>2</sup>	5 weeks
Duffy <i>et al.</i> 2003	1 Gastropoda 3 Amphipoda 2 Isopoda	<i>Zostera marina</i>	Virginia	Biomass (depending on treatment)	Outdoor tanks 0.6 x 0.6 m	6 weeks
Duffy <i>et al.</i> 2001	<i>Gammarus macronatus</i> (A) <i>Idotea baltica</i> (I) <i>Erichsonella attenuata</i> (I)	<i>Zostera marina</i>	Virginia	Biomass ( <i>Erichsonella</i> treatment only, reduced with <i>Idotea</i> )	Outdoor mesocosms, 122 L	6 weeks
Duffy and Harvilicz 2001	amphithoids (A)	<i>Zostera marina</i>	Virginia	None	Outdoor microcosms, 22 L	4 weeks
Fong <i>et al.</i> 2000	<i>Clithon</i> spp. (G)	<i>Zostera japonica</i>	Hong Kong	Percent cover	Field exclosures, 40 x 40 cm	120 days
Nelson 1997	<i>Lacuna variegata</i> (G)	<i>Zostera marina</i>	Washington State	None	Blade segments in 500 mL flasks	10-12 days
Jernakoff and Nielsen 1997	<i>Thalotia conica</i> (G) Amphipoda	<i>Posidonia sinuosa</i>	West Australia	Leaf survival ( <i>T. conica</i> treatment)	Field exclosures, 6.5 L tubes	35 days
Philippart 1995	<i>Hydrobia ulvae</i> (G)	<i>Zostera noltii</i>	Netherlands	Density Biomass	Circular field exclosures, 1 m diameter	84 days
Williams and Ruckelshaus 1993 **	<i>Idotea resecata</i> (I)	<i>Zostera marina</i>	Washington State	Growth	Shoots in 2 treatment aquaria (not independent)	17 days
Neckles <i>et al.</i> 1993 **	Gastropoda Amphipoda Isopoda	<i>Zostera marina</i>	Virginia	Growth Production (depending on season)	Greenhouse microcosms, 110 L	1-2 months
Howard and Short 1986	Gastropoda Amphipoda Isopoda Caridea	<i>Halodule wrightii</i>	Florida	Biomass Shoot density Productivity	Pots in 2 treatment tanks (not independent)	3 months
Hootsman and Vermaat 1985	<i>Hydrobia ulvae</i> (G) <i>Littorina littorea</i> (G) <i>Idotea chelipes</i> (I)	<i>Zostera marina</i>	Netherlands	Growth	Indoor aquaria, 8 L	2 weeks
Robertson and Mann 1982	<i>Littorina neglecta</i> (G)	<i>Zostera marina</i>	Nova Scotia	Not reported	Field exclosures, 1.5 L tubes	26 days
Caine 1980	<i>Caprella laeviuscula</i> (A)	<i>Zostera marina</i>	Washington State	Not reported	Laboratory tanks, 22 L	3 weeks

Although epiphytic cover has usually been assumed to have negative impacts on seagrass, the literature does mention a few potential benefits. It has been proposed that epiphytic cover protects some seagrasses from high irradiance (Trocine et al. 1981), and from desiccation in intertidal areas (Penhale and Smith 1977). However, those possibilities have not usually been considered in further research.



**Fig. 1.1** A simple model of seagrass-epiphyte-grazer interactions. Light is reduced by water depth and turbidity. Epiphytic material on the leaf surface further reduces light. The impact of light is positive, as it is light used for photosynthesis. Epiphytes always have a negative impact, but epiphyte grazers limit that impact.

Models of interactions in seagrass meadows have been extended to include higher trophic levels. There is considerable evidence that small predatory fish and decapods have a significant impact on epiphyte grazers in seagrass meadows, especially amphipods (Nelson 1979b, Leber 1985, Duffy and Hay 2000). Those small predators are then prey for larger predators. Heck and Crowder (1991) proposed that top predators could impact the production of seagrass, and other macrophytes, via classic trophic cascades. In freshwater systems, there is strong evidence that predatory fish can limit the production of macrophytes by feeding on invertebrate epiphyte grazers (Martin et al. 1992) (Jones and Sayer 2003). However, an experimental manipulation of fish and nutrients in a seagrass system (Heck et al. 2000) did not demonstrate the operation of a simple trophic cascade. One likely reason is the diversity of epiphyte

grazers in seagrass systems, and the diffuse competition among them. There is considerable overlap in the diets of epiphyte grazers, and the removal of one grazer by a predator, may lead to the increased abundance of other equivalent grazers, and secondary production would remain unaffected. It has been argued that trophic cascades are not likely to operate in such complex, high-diversity ecosystems (Strong 1992), although they appear to operate in macrophyte-dominated freshwater lakes (Jones and Sayer 2003). The experiments of Edgar (1990a, 1993), and Edgar and Aoki (1993), suggest that marine epifaunal communities, both on seagrass and algal macrophytes, maintain constant levels of secondary production set by food resource limits, and not predation.

The major consumers of epiphytes on seagrasses are small invertebrates, especially gastropods, amphipods and isopods (Table 1.1). Some larger invertebrates, such as hermit crab (Tunberg et al. 1994) and shrimp (Morgan 1980), may also be important consumers of epiphytes; but their role has not been as well documented experimentally, with the exception of the recent work of Hays (2005). In contrast to their impact as grazers of epiphytes, small invertebrates are thought to have little impact as direct consumers of seagrass.

### ***1.1.2 Invertebrates as consumers of seagrass***

Herbivores, both invertebrate and vertebrate, are believed to consume only a small proportion of total seagrass production, and therefore have a minor impact on the flux of carbon and nutrients through seagrass ecosystems (Thayer et al. 1984). That belief is supported by isotope analyses (above). However, Valentine and Heck (1999) reviewed the literature and argued that the role of herbivores had been underestimated. They produce a large table showing many instances of high levels of consumption of seagrasses by vertebrates (waterfowl, fish, green turtles, dugongs, manatees) and urchins. Reports of the latter consuming large amounts of seagrass have become quite common (e.g. Klumpp et al. 1993, Valentine et al. 2000, Alcoverro and Mariani 2002). However, consumption by other invertebrates appears to be rare, as Valentine and Heck (1999) list only four cases. They included three instances of feeding on *Zostera marina*, by the isopod *Idotea chelipes* in Netherlands (Nienhuis and Groenendijk 1986), by the limpet *Tectura depicta* in California (Zimmerman et al. 1996, Zimmerman et al.

2001), and by the crab *Telmessus chieragonus* and three gastropods in Alaska, “at least under experimental conditions” (McConnaughey and McRoy 1979). One tropical study was listed, that of feeding on *Syringodium isoetifolium* by *Amphithoe* spp. in Fiji (Mukai and Iijima 1995). The same table has recently been repeated in Valentine and Duffy (2006), with some modifications, but adds only one more study of consumption of seagrass by invertebrates; the case of feeding on *Posidonia australis* by the crab *Nectocarcinus integrifons* in Victoria, Australia (Nichols et al. 1986) (but also see Klumpp and Nichols 1983).

The few cases of invertebrate consumption of seagrasses listed by Valentine and Heck (1999) might lead one to believe that they were anomalous, especially when compared to the greater apparent incidence of consumption by vertebrates. However, a more thorough re-examination of the literature finds many more cases. In fact, some invertebrates are highly adapted for living and feeding exclusively on seagrasses. The most specialized are species of limpets, Sacoglossa, and boring isopod crustaceans. Some limpets scrap the chlorophyll-containing epidermal layer from seagrass leaves. In one well-documented case, covered by Valentine and Heck (1999), unusually dense populations of the limpet *Tectura depicta* led to a dramatic decline of a local *Zostera marina* meadow (Zimmerman et al. 1996, Zimmerman et al. 2001). Other limpets with similar feeding habitats include *Notoacmea* (= *Tectura*) *paleacea* on *Phyllospadix* (Barbour and Radosevich 1979, Fishlyn and Phillips 1980), and *Lottia alveus*, on *Z. marina* (Carlton et al. 1991). Sacoglossa (Mollusca: Opisthobranchia) have specialized suctorial mouthparts, and two species feed only on seagrasses. They are *Elysia serca* which feeds on several Caribbean seagrasses, and *E. catulus* which feeds on *Z. marina* in the northwest Atlantic (Jensen 1982, 1983a, b). Ten species of limnoriid isopods have been identified, in the genera *Limnoria* and *Lynseia*, which feed by boring into living seagrass tissues, including leaves, sheathes, and rhizomes (reviewed in Gambi et al. 2003). The boring habits have been well described for several species from Australia (Brearley and Walker 1995), the Caribbean (van Tussenbroek and Brearley 1998), and the Mediterranean (Guidetti et al. 1997), and there are species occurring in a number of seagrass genera: *Amphibolis*, *Posidonia*, *Heterozostera*, *Zostera*, *Phyllospadix*. There are also a number of polychaetes, in the family Eunicidae, which bore into old leaf sheathes (Guidetti 2000, Gambi 2002). However, they do not

normally damage living tissue, and can be found on substrates other than seagrasses (Gambi et al. 2003).

Some generalist herbivores and omnivores include living seagrass tissue in their diets, and are not reported by Valentine and Heck (1999), or Valentine and Duffy (2006). The rock lobster, *Panulirus cygnus*, has been found to regularly feed on seagrasses in Western Australia (Joll and Phillips 1984), where at least four seagrass species were found in gut content samples, and were apparently consumed as living tissue. A sample of kelp crabs, *Pugettia producta*, from a *Zostera marina* meadow in Washington State USA, had stomach contents that were 23% seagrass (Caine 1980). The crab *Macrophthalmus hirtipes* is typically a deposit-feeder and burrower in mud flats; but in patches of *Z. novazelandica* (= *muelleri*) in New Zealand, it was found to get most of its diet directly from the seagrass, including leaves, sheathes, rhizomes, and roots (Woods and Schiel 1997). The blue manna crab, *Portuna pelagicus*, is a predator and scavenger; but 10% of its diet in a Western Australia study was seagrass (Edgar 1990c); although it is not clear how much of that was eaten as live material. Juveniles of the prawn *Penaeus esculentus* from *Z. capricorni* (= *muelleri*) meadows in Queensland, Australia, were found to ingest greater amounts of plant material as they grew, with the largest size class having seagrass in 85% of examined foreguts (O'Brien 1994). Snapping shrimp, Alpheidae, are common burrowers in tropical seagrass meadows, and are known to harvest living and detrital leaves that they drag into their burrows. Stapel and Erftemeijer (2000) estimated that alpheids removed 53% of the above-ground daily production from the surface of a *Thalassia hemprichii* meadow in Indonesia. The isopods *Idotea* spp. are common inhabitants of temperate meadows, and are very effective consumers of epiphytic algae. However, once they have deleted epiphytes, they will consume seagrass leaves, at least under experimental conditions (Robertson and Mann 1980, Hootsmans and Vermaat 1985, Nienhuis and Groenendijk 1986, Williams and Ruckelshaus 1993, Thom et al. 1995). In experimental treatments using *Idotea baltica* as an epiphyte grazer, Duffy (2001, 2003) (Table 1.1) found reduced seagrass biomass because of direct grazing of the seagrass leaves. Cebrián et al. (1996b) estimated that *I. basteri* accounted for <5% of herbivory occurring in *Posidonia oceanica* meadows on the Spanish Mediterranean coast. The gastropods *Lacuna* spp. are also important epiphyte grazers in temperate meadows (Nelson 1997). However, they have also been found to directly consume *Z. marina* (McConnaughey

and McRoy 1979, Stephenson et al. 1986). The amphipod *Cymadusa* is a common herbivore in seagrass meadows, and Kirkman (1978) found that it could severely reduce the biomass of *Z. capricorni* (=muelleri) in aquaria.

Invertebrate herbivores may also have an impact on seagrasses through seed predation. Nakaoka (2002) found that a tanaid crustacean, *Zeuxo* sp., at a site in Japan, bored into the seeds of *Zostera marina* and *Z. caulescens*, and consumed 14 and 23% of seeds of those species respectively. The prawn *Penaeus esculentus* consumes seeds of *Z. capricorni* (=muelleri) (Wassenberg and Hill 1987, O'Brien 1994), which were found to be as much as 13% of the diet of juveniles at some times of the year in Australia (Wassenberg 1990). Crabs and snails are potentially significant predators of *Z. marina* seeds (Wigand and Churchill 1988, Fishman and Orth 1996). Crabs were found to be the greatest potential consumers of *Phyllospadix torreyi* seeds, of which as much as 50% can be lost to predation (Holbrook et al. 2000). Alpheid shrimp have been found to harvest the seeds of *Enhalus acoroides* (Lacap et al. 2002). Seeds of *Posidonia australis* suffer high rates of predation in seagrass meadows in Western Australia. By using tethered seeds and video recording them, that predation was attributed to various invertebrate herbivores, including crabs, isopods, and amphipods (Orth et al. 2002, Orth et al. 2006, 2007).

In the current literature, invertebrates, other than urchins, have been assigned a minor role as consumers of seagrasses, as reflected in their low profile in reviews of herbivory (Valentine and Heck 1999, Valentine and Duffy 2006). However, the many recorded instances of direct consumption by invertebrate herbivores suggest that their role has been underestimated. More studies have been done on vertebrates because those animals are more conspicuous, more economically important (fish, waterfowl) or appealing for conservation efforts (dugongs, manatees, sea turtles). Urchins have received recent attention because their numbers have made them very conspicuous, and they can cause extreme defoliation. Few studies of smaller invertebrates have quantified their direct impact on seagrasses, and their role has been difficult to assess.

### ***1.1.3 Limitations of support for prevailing model***

The argument supporting the belief that seagrass-epiphyte-grazer interactions can control seagrass production relies almost exclusively on evidence from experimental manipulations of grazers (Table 1.1). Those experiments support a model of interactions whereby epiphyte grazers have an indirect positive impact on seagrasses as a consequence of their direct consumption of epiphytic material (Fig. 1.1). However, many of the experiments did not produce definitive results, as they suffered from design problems, and were done “under artificial conditions at small spatial and temporal scales” (Duffy and Hay 2000). Experimentation involving enclosures/exclosures are problematic in systems as open as the intertidal, and it is especially difficult to reproduce realistically natural conditions for small mobile invertebrates. The response to such problems has been a tendency to do experiments on a larger scale, with greater replication and more complexity that is presumed to reproduce more realistic conditions. However, such experiments can not overcome the logical problems inherent in applying an experimental approach to demonstrate indirect ecological effects. In the experiments on seagrass-epiphyte-grazer interactions, the positive effects on seagrass followed treatments with epiphyte grazers and were assumed to be a result of a measured reduction in epiphytes. In other words, it was assumed that the proximate cause of the change in seagrass was not the grazer treatment but the change in epiphytes, which had not been controlled. Such an indirect effect could never be conclusively demonstrated experimentally because there is no means of manipulating epiphytic growth on the leaves. There may be other causal paths from epiphyte grazers to seagrass that do not involve epiphytes and that can not be excluded as possible causes of the effects on seagrass. For example, suspension-feeding benthic mussels appear to benefit seagrasses by increasing the nutrient content of sediments with their feces and pseudofeces (Reusch et al. 1994, Peterson and Heck 2001). Epiphyte grazers could have similar effects, or other unknown effects, on seagrasses which can not be separated from the indirect effects through epiphytes because the grazer treatment was applied to both the epiphytes and the seagrass as a single experimental unit. The experiments clearly show an association between reduced epiphytes and benefits to seagrass, but do not demonstrate a causal relationship between them.

Randomized experiments provide the strongest evidence by which to infer cause and effect. However, in studies of ecological interactions, it is often very difficult, or impossible, to randomly assign values to the variables of interest. Furthermore, those variables can not be fixed in controlled experiments. Nonetheless, a strong preference has developed within marine biology for experimental studies, and against “observational” studies. In their analysis of trophic interactions, Heck and Valentine (2007) did not consider “papers that report observational or correlative evidence”, because they believed that “experimental evidence is the only conclusive way of evaluating the magnitude of indirect effects and of quantifying the relative importance of top-down and bottom-up factors on coastal ecosystems”. In fact, there is a strong case for the contrary argument. When it is not possible to control the variables directly causing an effect, then it is not possible to distinguish experimentally between direct and indirect effects. Such distinctions are critical to describing trophic interactions. Nonetheless, it is possible to use statistical controls on field data where variables have been allowed to vary freely. Patterns of correlation in the data reveal the underlying causal structure (Shipley 2004). Partial correlations between two variables control statistically for the effects of one or more additional variables. So, by examining patterns of correlation and partial correlation amongst variables one can distinguish direct from indirect effects. Path analysis provides a means of making inferences about the causal structure of interactions from correlation patterns.

Path analysis was initially proposed by the biologist Sewall Wright (1921), but has received little attention from biologists. It, and the related technique of structural equation modelling (SEM), have had their greatest development and use in the fields of economics and sociology; fields where the experimental manipulation of subjects is not a possibility. More recent attention from biologists (Shipley 1999) reflects a greater acceptance of the limitations of traditional experimental techniques, and theoretical developments (Pearl 1997). Those developments allow for the translation between causal models and statistical models used for hypothesis testing. The prevailing model of seagrass-epiphyte-grazer interactions can be represented as a causal model: grazers→epiphytes→seagrass. Such a model implies a set of correlations among the variables (Shipley 2004). Therefore, the correlations occurring in field data can be tested against the expected correlations assuming the operation of the causal model.



There have been surprisingly few studies which have used field data to relate the amount of algae, invertebrates, and seagrass within meadows, despite the many studies attempting to experimentally manipulate their interactions (Table 1.1). That is a reverse of the approach usually taken in other areas of ecology, where experiments have been developed to explain patterns first observed in the field (Underwood et al. 2000). A study by Nelson and Waaland (1997) in Washington State, attempted to relate field data on eelgrass, epiphytes, an epiphyte grazer, and various physical and chemical factors. They found that seasonal patterns in light availability best predicted changes in eelgrass biomass. They presented their analyses in the form of path diagrams, but those were actually representations of multiple regression analyses and do not test causal relationships. A recent study by Frankovich and Zieman (2005) examined various factors, including gastropod grazing, that affected epiphyte standing stock on *Thalassia testudinum* in Florida Bay. They found that gastropod abundance was negatively correlated with epiphyte standing stock; but the relationship was complicated, and they did not believe that grazing was a direct mechanism for controlling epiphytes in their study. Turbidity accounted for the largest part of the variation in the amount of epiphytic material on the leaves. Anecdotal evidence suggests that seagrass in parts of Chesapeake Bay USA declined following a decline of the epiphyte-grazing gastropod *Bittium varium* (van Montfrans et al. 1982). Similarly, in the Dutch Wadden Sea, observations of a decline in the mudsnail *Hydrobia ulvae* in the 1970s has been related to increased fouling and a decline in seagrass (Philippart 1995). The only well documented case of natural changes in a grazer population affecting epiphytic cover and seagrass abundance is provided by a recent study by Schanz et al. (2002) concerning *Hydrobia ulvae* in the German Wadden Sea. There, sheltered sites had a greater abundance of mudsnails, lower epiphyte biomass, and higher seagrass biomass, compared to exposed sites. Using experimental transplantations and enclosures, and manipulating water currents, they showed that the snails were washed from the seagrass at sites with greater water flow, leading to enhanced epiphyte growth and diminished seagrass development. Their study is the only one that experimentally manipulates an epiphyte grazer and relates its findings to patterns in the field.

Most of the experimental manipulations of seagrass fauna have involved the temperate fauna of *Zostera*, and it remains to be seen how well generalizations made from them will apply to tropical meadows. Those meadows typically support more species of

seagrass and macroalgal, and are more structurally complex than temperate meadows, which are often monospecific. In the tropical Indo-Pacific, it is not uncommon for meadows to support 5 or 6 species; including *Halophila* spp. that rise only a few centimetres above the substrate, and *Ehalus acoroides* with strap-like leaves more than 1 m long. As habitat for invertebrates, the smaller species form very different physical environments from that of the larger species. They provide less shelter from extreme weather conditions or predators, and produce less baffling of water flows and stabilisation of sediments. Larger species have been shown them to trap sediments and nutrients, but that effect was not found in monospecific meadows of small species in tropical Australia (Mellors et al. 2002). Nutrient levels are often low in tropical areas (Carruthers et al. 2002), but the turnover of seagrass biomass and nutrients is more rapid. Seasonal variation in seagrass biomass is typically less pronounced, but in some tropical intertidal areas there can be strong seasonal patterns related to changes in the tides (Stapel et al. 1997). Furthermore, those intertidal tropical meadows are more likely to suffer the effects of much higher levels of damaging irradiance (Ralph 1999a). Direct consumption of the seagrass by large herbivores, specifically dugongs, sea turtles, and urchins, is more common in the tropics (see review by Valentine and Heck 1999). Despite those differences, there was no reason to assume that the prevailing model of seagrass-epiphyte-grazer interactions would not generally apply in tropical areas.

The prevailing model of seagrass-epiphyte-grazer interactions is not well supported by field data. That lack of field evidence is a conspicuous gap in the literature, and is a challenge to the prevailing model, especially given the weakness of the experimental evidence. It was anticipated that the field data collected during this study would broaden the base of evidence for that model.

## **1.2 Objectives**

This study is concerned with seagrass-epiphyte-grazer interactions in intertidal meadows in tropical Australia. The study began with the hypothesis that top-down effects could determine the level of seagrass production in those meadows. It was assumed that variations in field measurements of seagrass, epiphyte, and epifaunal

abundances would reflect interactions among those elements of the community. It was considered beyond the scope of the study to measure the effects of nutrient inputs, or higher trophic inputs, e.g. fish predation. The assumed model of seagrass-epiphyte-grazer interactions was that of Fig. 1.1. Therefore, the principal objectives of the study were to test with field data the assumptions that:

- Invertebrate grazers limit the amount of epiphytic material on seagrass leaves.
- Epiphytic loads limit the standing crop of seagrass.
- Invertebrate grazers indirectly benefit the standing crop of seagrass as a result of their effects on epiphytic loads.
- Invertebrate grazers do not directly affect seagrass standing crop.

For the assumed model of seagrass-epiphyte-grazer interactions to be correct, those assumptions need to operate simultaneously as part of a system of interactions. The final objective was therefore to test the validity of that system as represent as a causal model in a path analysis.

## Chapter 2 General methods

### 2.1 Study area

The study was undertaken in intertidal seagrass meadows at Townsville (lat. 19° 15'S, long. 146° 50'E), on the northeast coast of Queensland, Australia. The climate of Townsville is dry tropical. Maximum temperatures occur in December and January, when the mean air temperature at 1500 h is 30.0°C. Minimum temperatures occur in July, when the mean air temperature at 1500 h is 23.8°C. Most of the rainfall occurs from December to March. The long-term average rainfall is 1121.5 mm; but in 2003, during the course of this study, less than half that amount was received. The area is occasionally subject to cyclones and severe tropical storms, but fortunately not during 2002-2004. Such storms have a history of causing considerable damage to local intertidal seagrass meadows (Birch and Birch 1984).

The Townsville area receives semidiurnal tides with a significant diurnal inequality. The diurnal pattern changes seasonally, with minimum low tides occurring during daylight hours from May to September, and during the night from November to March. The level of the highest astronomical tide is 4.0 m. The levels of the mean high water spring tides and mean high water neap tides are 3.1 and 2.2 m respectively.

Meadows in the Townsville area are often dominated by small-sized seagrasses, especially *Halodule uninervis* (Forsskål) Ascherson and *Halophila ovalis* R. Brown. Both species occupy a wide range of habitats and are widespread across the Indo-Pacific region (den Hartog 1970). *H. ovalis* occurs from the intertidal to depths > 30 m, and probably covers more surface area than any other single seagrass species. Both species are highly variable morphologically, with different forms appearing to occupy different habitat conditions (Waycott et al. 2004). They are species with rapid growth rates, and short leaf longevities; averaging only about 12 days in the case of *H. ovalis* (Duarte 1991a). Both species readily produce seeds, but also reproduce vegetatively, and are considered pioneer species. *H. uninervis* has slender strap-like leaves that are typically less than 15 cm long in the Townsville area. Leaf width varies greatly from <

0.5 mm to > 3.0 mm. *H. ovalis* has rounded leaves that vary greatly in dimensions, but most typically have a blade < 2 cm long on a shorter petiole.

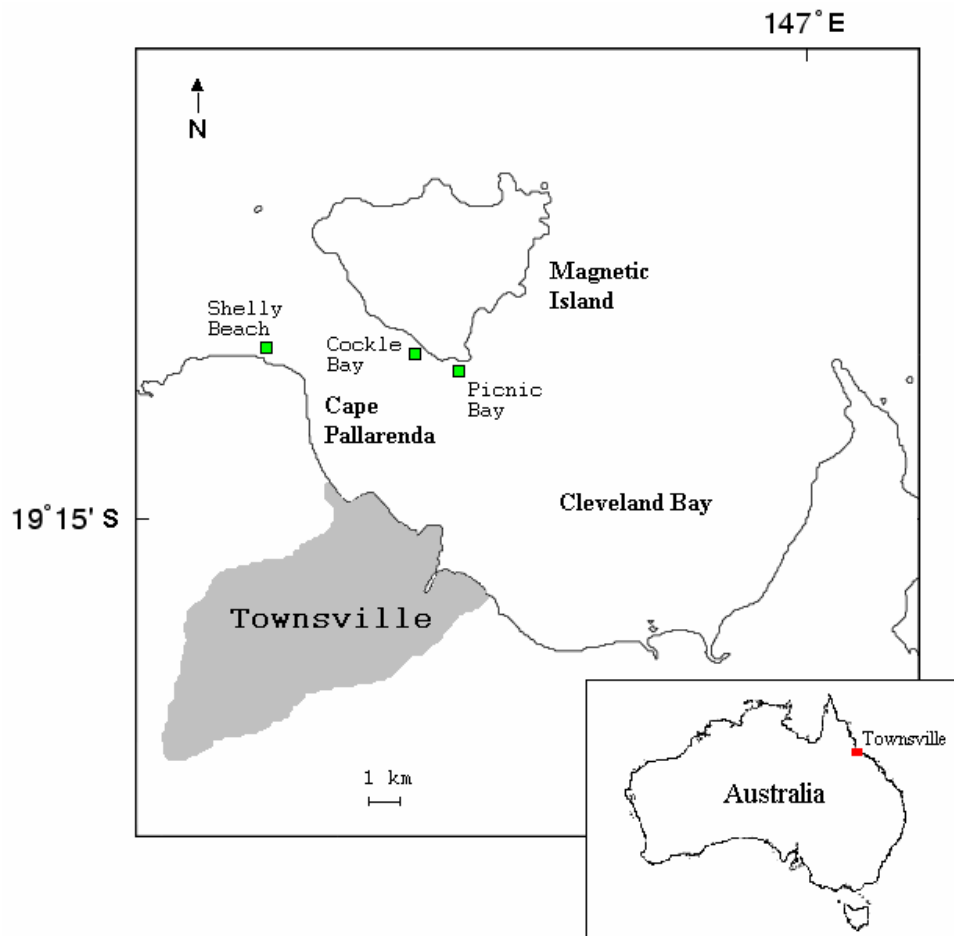
Small seagrasses create an environment for invertebrates that differs from that expected with large species. Smaller seagrasses are less able to trap sediments and nutrients (Mellors et al. 2002), and provide less shelter than larger species. The smaller physical scale of the habitat restricts the size of epifauna. Epiphytic macroalgae are uncommon, and the epiphytic material is more characteristic of periphyton. Thus, the epifauna are expected to be periphyton scrapers rather than feeders on larger algae. The absence of large fast-growing epiphytic algae likely reflects low nutrient availability. Waters around Townsville are oligotrophic (Alongi and McKinnon 2005), and the major source of nutrients is the wind-driven suspension of sediments (Walker 1981, Walker and O'Donnell 1981). Resettlement of suspended material likely contributes to the development of periphyton. Thus the environment of meadows around Townsville differs from that in other studies which have involved larger seagrasses, usually in temperate areas, with high levels of nutrient enrichment, and high production of macrophytic algae.

## 2.2 Sampling plots

Study plots were located in three separate meadows: Shelly Beach near Cape Pallarenda, and Cackle and Picnic Bays on the south side of Magnetic Island (Fig. 2.1). Those meadows were chosen because they were accessible from Townsville, and could be reached by a combination of inexpensive, environmentally friendly transportation techniques: cycling, walking, and riding the public ferry. However, they also represented a broad range of conditions under which the dominant intertidal seagrass, *Halodule uninervis*, grows.

The Shelly Beach meadow covers an extensive flat with a shallow gradient across soft inshore mud and offshore sand. The meadow was denuded of vegetation by Cyclone Tessie in April 2000 (J. Mellors, personal communications). At the time of this study, the dominant seagrasses in the intertidal area were *Halophila ovalis* and narrow-leaf *Halodule uninervis*. The morphology of the latter at Shelly Beach was consistent with

*Halodule pinifolia* (Miki) den Hartog, but that species is not universally recognized (Waycott et al. 2004). Broad-leaf *H. uninervis* was rare at Shelly Beach during this study. *Zostera muelleri* Irmisch ex Ascherson occurred in the muddy inshore areas, and *Halophila spinulosa* (R. Brown) Ascherson occurred sparsely in the lower intertidal. *Z. muelleri* has also been known as *Zostera capricorni* Ascherson in tropical areas. The seagrass taxonomy followed in this study is that of Waycott et al. (2004).



**Fig. 2.1** Map showing location of study sites near Townsville, Queensland, Australia.

Cockle Bay is a wide shallow bay protected from prevailing winds by Magnetic Island. It is fringed by mangroves and inshore mud, which gradually changes over 500 m to coarse coral debris behind a fringing reef. The mangrove and mud developed within the last 60 years in front of a former sandy shoreline (Wolanski 1994). The seagrass meadow has been denuded by cyclones on at least two occasions; Althea in 1971 (Birch and Birch 1984), and Tessie in 2000. The dominant seagrass at Cockle Bay during this study was broad-leaf *Halodule uninervis*, but there were significant patches of

*Halophila ovalis*, *Thalassia hemprichii* (Ehrenberg) Ascherson, *Cymodocea serrulata* (R. Brown) Ascherson and Magnus, and *Syringodium isoetifolium* (Ascherson) Dandy.

Picnic Bay is a smaller, deeper bay that is partially protected from the prevailing winds, but experiences greater exposure to waves. It has a jetty that was the terminus of the regular ferry service to Magnetic Island until 1 September 2003. The major intertidal seagrasses there were *Halodule uninervis*, *Halophila ovalis*, and *Cymodocea serrulata*.

One intertidal plot, 100 m<sup>2</sup>, was established in each meadow in an area with continuous cover by *Halodule uninervis*. Plots were located at 19° 10' 49.7"S, 146° 45' 30.6"E at Shelly Beach; 19° 10' 34.2"S, 146° 49' 38.2"E in Cockle Bay; 19° 10' 49.6"S, 146° 50' 35.2"E in Picnic Bay. The plots were marked out permanently with stakes at each corner. A single plot was established per site because this study was concerned with processes within plots, which necessitated intensive sampling of epiphytic loads and epifauna that could not be achieved on a larger scale.

Plots were visited every 4 to 8 weeks beginning in June 2002. At each visit, the plot was delineated on two sides with a rope marked in meter sections so that pre-selected random points could be located for sampling. Samples were taken to record the amount of seagrass, the epiphytic loads and the epifauna on the leaves. Sampling took place at low tides in the afternoon. However, the site at Picnic Bay was not exposed during daylight hours from about November to April, and sampling in that period took place at night. In April 2004 the plot was not exposed at night or day, and as a result there is a three-month interval between samples. Sampling continued until July 2004 at Cockle Bay and Picnic Bay, and until August 2004 at Shelly Beach. The precise dates of sampling events can be found in Appendix A.

### **2.3 Sampling seagrass**

Cores were taken at 10 randomly selected sites in each plot on each sampling occasion. The corer was made from a section of PVC pipe with a cross-sectional area of 45 cm<sup>2</sup>. Cores were taken to a depth of 10 cm, but at Picnic Bay and Cockle Bay coral rubble sometimes made it difficult to force the corer to that depth, and so shallower samples

were sometimes taken. Sediment was washed from the roots in a sieve, and the samples frozen until further analysis.

Leaves were removed from shoots by cutting them at the top of the sheath, or the top of the petiole in the case of *Halophila ovalis*. Counts were made of the number of *H. ovalis* leaves and shoots of other species. Leaves, and leaf fragments, of all species found in a sample were laid out on plastic transparencies for scanning and determination of leaf areas following the methods outlined below (section 2.6). The leaf area index (LAI) of each species in the plot on a sample date was calculated as the average leaf area per sample, calculated from one side of the leaf, divided by the area of substrate sampled, i.e. 45 cm<sup>2</sup>.

All remaining material, including roots, rhizomes, sheathes and leaf bases, were treated together as part of the below ground biomass. That material, separated by species, was oven dried at 80°C for 24 h, then weighed to determine the below ground dry weight (BGDW) of each species.

## **2.4 Sampling invertebrates**

*Halodule uninervis* leaves and their associated epiphytic loads and epifauna were sampled at 10 randomly selected sites in each plot on each sampling occasion. A sample of 10 leaves was taken at each site in the plots at Shelly Beach and Picnic Bay. Five leaves per site were taken at Cockle Bay, as leaves there were broader and thus had larger surface areas. Over the course of the sampling, *H. uninervis* leaves at Picnic Bay developed a greater average width, and so to reduce the variability of the leaf area sampled, only five leaves were taken per sample on the last three sampling dates at that site. During sampling, a small net was placed under each leaf, which was then cut at the base with a small pair of scissors. The leaf was quickly lifted out of the water and turned over into a 250 ml plastic container filled to the brim with filtered seawater. In that way, the associated invertebrates and epiphytic material was removed with a minimum of disturbance. The seawater used in the containers had been filtered through GF/C glass microfibre filters, also used to filter the epiphytic material (below).



*Halophila ovalis* leaves were also sampled from the Shelly Beach plot. Ten leaves were plucked at the petiole with a pair of forceps from each of the 10 randomly selected sites and placed in 70 ml containers filled with filtered seawater. *H. ovalis* distribution became patchy and leaf densities declined at Shelly Beach from August 2003, and it became impossible to find leaves at every site. So fewer than 10 samples were collected on some dates, and in April 2004, no *H. ovalis* samples were collected at all. The Shelly Beach plot was the only plot where *H. ovalis* was present over most of the two years of study. However, it became more abundant in the second year in the Cockle Bay plot, and was sampled there beginning in September 2003. The same procedure was followed as at Shelly Beach, but samples were taken at only five sites.

The cut leaves were brought back to the lab where they were examined under low-power magnification, and all macroinvertebrates removed within 30 hours of field sampling. Those animals were preserved in alcohol or formalin for later identification and counting. Animals typically considered part of the meiofauna, such as nematodes, copepods, and foramanifera were not removed. Hydroids, and the occasional ascidian, were treated as part of the epiphytic load. However, other attached animals, including the winged oyster *Electroma* (Pteriidae), spirorbid polychaetes, and anemones, were removed and counted. The area of leaves in each sample was calculated using the techniques described below, and animal abundances expressed as numbers per unit area of leaf.

The leaf-cutting technique provided a simple, efficient, and effective means of sampling epifaunal invertebrates from *Halodule uninervis*. During preliminary sampling, the technique was compared to the use of a corer (Appendix B), and was found to recover greater numbers of animals per area of seagrass sampled. Corers have often been used to sample invertebrates in seagrass meadows (Lewis 1984, Edgar et al. 1994, Lee et al. 2001), but they sample part of the substrate, and so do not specifically target epifaunal organisms. Recovery of small epifaunal organisms from core samples tends to be poor because the animals are mixed with a large quantity of sieve residue. As well, the sieve residue is typically preserved in the field for later sorting, and the recovery of dead animals is poorer than live ones. Leaf cutting is more time efficient than coring, as only epifaunal organisms are collected, and time is not expended sorting sieve residue and removing animals that may be exclusively benthic. The technique

Ch 2: General methods

was also advantageous in the context of this study because the same leaves used to sample invertebrates could be used to sample epiphytic material. Some other studies have used sampling techniques that specifically targeted the epifauna of larger seagrass species. The above ground portions of individual shoots have been sampled by enclosing them in a bag, and then cutting them at the substrate (Edgar and Robertson 1992, Attrill et al. 2000). Leaves have also been sampled using a specifically designed sampler with a hinged frame that closes shut on the leaves (Virnstein and Howard 1987a, Schneider and Mann 1991). However, neither of those approaches could be applied to the small seagrass species sampled here, but the leaf-cutting approach provided a simple alternative, expected to give similar results.

The leaf cutting technique was designed specifically to target small epifaunal organisms, especially gastropods and peracardian crustaceans, because those organisms are believed to limit the amount of epiphytic material on seagrass leaves, as suggested by a body of experimental evidence (Table 1.1). Other larger organisms may also feed on epiphytic material, e.g. decapod crustaceans; but there has been less experimental evidence to show that they limit epiphytic loads. They are also more likely to be feeders on macrophytic algae rather than on the periphyton-like material which dominated on the leaves in this study. So, they were not of concern for sampling. Samples were taken at low tide for ease of access to the plots, and there was no reason to believe that populations of small epifaunal invertebrates migrated out of seagrass meadow with the tides. Some of the more active epifaunal organisms may avoid capture with cut leaves, but there was no evidence of that for amphipods, the most important group of active epifaunal invertebrates (Appendix B). The most abundant amphipod in the meadows studied here, *Erichthonius*, builds domiciles on the leaves and does not readily abandon them when disturbed. Sphaeromatid isopods were the most active, and largest of the epifaunal animals, and the leaf-cutting technique may not have adequately sampled them. The technique was also applied to *Halophila ovalis* leaves, but the leaves were plucked at the petiole, and that may have caused more disturbance of the epifauna, and possibly reduced their likelihood of being captured.

Densities of larger animals in the plots were estimated, when necessary, using random tosses of a quadrat. Sea hares (Aplysiidae) were present in the plot at Shelly Beach on several dates, and their densities were estimated with 15 random tosses of a wire

quadrat 600 cm<sup>2</sup>. In July 2003, an aeolid nudibranch, *Limenandra fusiformis* became extremely abundant at Cockle Bay. Its density was estimated by 20 random tosses of a quadrat 266 cm<sup>2</sup>.

## 2.5 Measuring epiphytic loads

Epiphytic loads were measured on the same leaves used to calculate epifaunal densities. Two techniques were used: one estimated the weight of epiphytic material, and the other estimated the percentage area of leaves covered. Estimates were made of the weight of epiphytic material on all cut leaf samples collected at Shelly Beach and Cockle Bay. Weight estimates were not made on Picnic Bay samples collected after July 2003, because of a shortage of time. Estimation of percent leaf cover was made on all cut samples collected from Cockle Bay and Picnic Bay beginning in July 2002, and from Shelly Beach beginning in August 2002.

Estimates of the percent leaf cover were made immediately after the removal of epifauna. The leaves were examined under 10X magnification through an ocular 20X20 grid. One area of the grid, representing 0.25 mm<sup>2</sup>, was randomly selected from every 30 mm<sup>2</sup> of *Halodule uninervis* leaf surface area, considering both sides of the leaf. That area was then scored as covered (1), uncovered (0), or half-covered (0.5) with epiphytic material. At Picnic Bay, the leaf areas were large, and to save time, only every second 30 mm<sup>2</sup> leaf section was sampled, beginning in January 2003. The 30 mm<sup>2</sup> area represented a 1 cm length of the typical broad-leaf *H. uninervis* with a 3 mm width. *Halophila ovalis* leaves were scored once on either side of the leaf. The leaf was placed haphazardly beneath the grid, and the epiphytic cover scored for a pre-selected 0.25 mm<sup>2</sup> grid area. The sum of the scores was divided by the number of areas scored and converted to a percentage.

The technique for estimating the percent cover of leaf surfaces by epiphytic material was time-consuming, but more precise than qualitative scores based on assigning samples to categories of percent cover, such as the Braun-Blanquet technique (Kendrick and Lavery 2001, Sidik et al. 2001). In the technique used here, observer bias was reduced. There was equal weight given to the distal ends of *Halodule*

*uninervis* leaves and the newly emerged surfaces at the bases. The distal ends were more likely to have epiphytic cover. The technique also worked well for a variety of different types of epiphytic material.

Once the percent epiphytic cover had been estimated, the leaves were frozen in the containers with the water in which they had been collected. Later the samples were thawed, and the leaves moved to a petri dish where they were gently scraped with a razor blade to remove all epiphytic material. The scrapings were returned to the collection containers and the total contents filtered through a pre-weighed, pre-combusted, 47 mm diameter GF/C glass microfibre filter, which has a 98% particle retention rating of 1.2  $\mu\text{m}$ . The filtrate was washed through with 100 ml of distilled water to remove salts. Filters with filtrate were dried in an oven at 100°C for 24 h, and then weighed to determine the dry weight of epiphytic material per sample of cut leaves. The dried filters and filtrate were then ashed at 500°C for 3 hours in a muffle oven, and re-weighed. The difference between the dry weight (DW) and the weight after ashing gave the ash-free dry weight (AFDW), a measure of the organic matter in the epiphytic load. The area of the leaves scraped in each sample was determined using the techniques described below, and AFDW expressed as weight per unit area of leaf.

There were several potential sources of error in determining DW and AFDW of epiphytic material. Not unexpectedly, the epiphytic material on some leaves contained sand and pieces of calcareous shell and coral fragments, which all contribute to DW estimates. Unexpectedly however, salt crystals were precipitated as a result of freezing the samples, and were found in the filtrates of some samples. That problem was not recognized early, and there was no attempt to account for their presence. Salts made a contribution to DW and not AFDW, but only the latter has been used in analyses, except where the ratio AFDW:DW has been used in chapter 4. It was also realized that blank samples, containing only filtered seawater from the source used to fill the sample containers, had measurable AFDW. To determine that background weight of non-epiphytic material, two sets of 10 blank samples in 250 ml containers, and two sets of 5 blank samples in 70 ml containers were processed on different occasions in exactly the same way as samples containing epiphytic scrapings. AFDW can also be overestimated by the water of hydration driven off during combustion (American Public Health Association et al. 1995). The weight of the water of hydration was

measured after the ashing of two sets of 10 scraped samples from *Halodule uninervis*, and 2 sets of 5 from *Halophila ovalis*. The ashed samples were wetted with distilled water, re-dried at 100°C for 24 h, and weighed to determine the increase in weight as a proportion of AFDW. As well, AFDW can be underestimated by the incomplete combustion of organic material (Kendrick and Lavery 2001). To test for that error, small quantities of sucrose, approximately 0.1 g, were combusted at 500°C in each of the muffle ovens used.

## 2.6 Calculating leaf areas

Areas of leaves were needed for the calculation of the leaf area index from core samples, and to standardize estimates, with respect to leaf area of the amounts of epiphytic material and numbers of invertebrates in cutting samples. Leaf area estimates were made from images created with a desktop scanner. As all the leaves were wet, they were first arranged between two plastic transparencies before being placed on the glass of the scanner. All images were made in 24-bit colour at 300 dpi. The full colour image was not used for analysis, but the conversion to grey-scale or 8-bit colour was best handled by image analysis software. A resolution of 300 dpi is higher than needed for most applications, but narrow-leaved *Halodule* were typically only 0.6 mm wide, and there was high potential error in estimating its area at low resolutions. The image files were saved in TIFF format, as the image analysis software that was used did not accept compressed formats. Consequently, many large files were created that needed to be stored on CD for later analysis.

All scans that were used for analysis were done on a Hewlett-Packard Scanjet 4570c desktop scanner. Trials with other available scanners showed that performance varied greatly among them. Some produced shadows or coloured fringes around the images. Depending on the technology used, scanners may produce red, blue and green coloured fringes on images of 3D objects (Gann 1998). The problem was most apparent on scans of narrow-leaved *Halodule* that were placed horizontally on the scanner, i.e. with the long axis of the leaf parallel to the light source. Although a shadow-like fringe could be conspicuous, there was also evidence of extra green pixels on the edge of the image that increased the apparent width of the leaf, and therefore significantly

increased estimates of the area of very narrow leaves. The problem was reduced when the leaves were placed vertically on the scanner. For one machine, there was an almost 10% difference between the estimated areas of narrow strips of green paper placed between plastic transparencies in either a horizontally or vertically position. The use of the plastic transparencies increased the difference. For the Scanjet 4570c, the difference was less than 1%. That machine also produced images of copper pennies that accurately represented their areas. Nonetheless, scanning was always done with the leaves placed vertically, i.e. perpendicular to the light source.

Leaf areas in the digital images were estimated using Scion Image, an image analysis software for Windows developed from NIH Image for MacIntosh, and made available in the public domain at [www.scioncorp.com](http://www.scioncorp.com). The analysis of an image was always based on the indexed colour image, produced on a selected palette of 256 colours, i.e. 8 bits. A grayscale image was produced from that image using the convert to grayscale command in the process menu, rather than working with the grayscale image produced from the full colour image. The thresholding option was applied so that the dark gray pixels corresponding to the pigmented areas of the leaf were converted to black against a white background. The threshold level could be varied using the LUT tool, but the automatically set threshold was typically the correct one that defined the edge of the leaf. The part of the image with the leaves of interest was then identified with one of the selection tools. Using the measure command in the analyze menu, the area in black corresponding to the area of the leaves was calculated. The results were then pasted into an Excel spreadsheet for further manipulation.

There are a variety of methods in Scion Image for estimating the area of objects in images. However, the procedure outlined above gave consistent results, so that identical measurements would be obtained from repeat analyses of the same image. If the grayscale image from the full colour image was used, there was a gradient at the fringe of the image of the leaf that made the choice of an appropriate threshold less certain.

The petioles of *Halophila ovalis* leaves were erased from their images before calculating their areas. No other changes were made to the images of leaves from core samples. Areas of the leaves that were poorly pigmented, because the leaves were

immature, senescent, or damaged by herbivores, were not included in the leaf area index if they fell below the thresholding level that defined the margin of typical leaves. The areas of *Halophila ovalis* leaves used to calculate epiphytic loads were adjusted to include surfaces that were unpigmented as a result of gastropod feeding (see below). Those surfaces still supported epiphytic cover and often were not evident until that cover had been removed.

Images of entire, undamaged leaves in core samples were used to calculate mean sizes of *Halophila ovalis* leaves. However, to increase the sample size, small areas of damage that did not break up the integrity of leaves were painted in before measuring the area of the thresholded images. Leaves from core samples were used rather than leaves sampled to measure epiphytic loads and epifauna, as the latter may have been biased toward leaves that were larger and more visibly in the field.

To measure trends in the length of *Halodule uninervis* leaves in the Shelly Beach plot, the length of the longest leaf in each sample of cut leaves was measured in scanned images, and the mean of the longest leaves calculated for the 10 samples on each date. Leaf widths were measured by calculating the mean breadth of leaves, i.e. the area divided by the length, as typical *H. uninervis* leaves have a gradual taper. Mean breadth calculations at Shelly Beach were made for one randomly selected leaf per sample and averaged over the 10 samples on each date. At Picnic Bay, *H. uninervis* leaves were easily divided between two distinct width classes, those less than or greater than 1.5 mm. When the areas of leaves in core samples from the plot in Picnic Bay were estimated, the areas of the two width classes of *H. uninervis* leaves were also estimated.

## **2.7 Estimating herbivore damage**

*Halophila ovalis* leaves were subject to a variety of different types of herbivore damage. That damage was characterized as being from either gastropod or crustacean feeding. Gastropod damage removed the chlorophyll containing layers of the leaves, but usually left the form of the leaf intact. Some leaves appeared to have been chewed from the margins, and that damage was assumed to be due to crustaceans.

*H. ovalis* leaves have only two layers of pigmented cells aside from the midrib region (Tomlinson 1980). Where both layers were removed, a fragile transparent cuticle layer often remained. Where one layer was damaged, the leaf appeared paler green than the surrounding undamaged tissue. Therefore, in greyscale images different levels of damage were representing by different grey levels that could be separated using image analysis techniques.

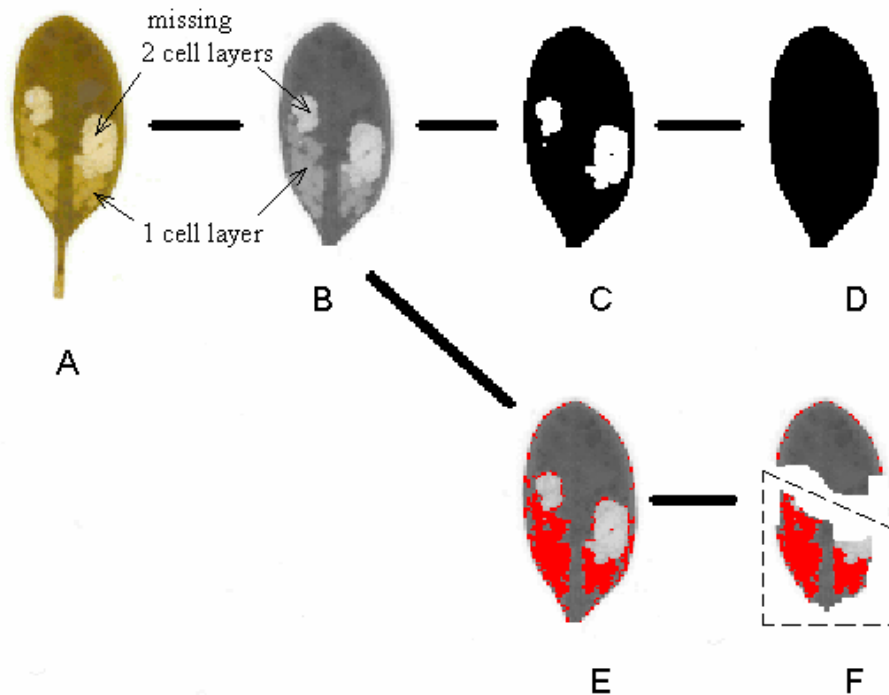
The percentage of *Halophila ovalis* leaf surfaces damaged by gastropods was estimated for the leaf samples collected for epiphyte analysis. The total area damaged in a sample was calculated as the sum of the area of 2-layer damage plus one-half the area of 1-layer damage, which was then expressed as a percentage of the undamaged sample leaf area, and averaged for all samples on a particular date. The areas of leaves where two layers had been removed were distinguished from pigmented areas using thresholding. Pale areas that represented the loss of a single layer of cells, or the loss of the pigmented content of the cells, were distinguished using the density slice option (Fig. 2.2). The limits of the density slice in the images were adjusted using the LUT tool to correspond to the limits of the damaged tissue as seen on individual leaves while they were being examined under 10X magnification. The 1-layer damage often had densities equal to undamaged tissue on the outer edge of the leaf, and it was necessary to erase or isolate those areas from the damaged areas in the image before calculations.

The type of procedure outlined above has been described as semi-automatic image analysis (Glasbey and Horgan 1995). Such an approach combines the human observer's qualitative abilities, in this case the ability to distinguish damaged and undamaged tissue, with the quantitative ability of the computer. The results can be far more accurate and efficient than those obtained by either one alone.

To quantify crustacean damage to *Halophila ovalis*, the leaf surface area observed from each core was compared to the expected areas of the leaves had each leaf been entire. The mean area of individual leaves in a sample was calculated from undamaged leaves and any leaves where the original leaf area was evident, as was often the case with gastropod damage. The mean leaf area per sample was then multiplied by the leaf count to estimate the expected leaf area without crustacean damage. The observed leaf surface with damage was calculated using thresholding, as described above, but areas



of 2-layer gastropod damage were painted in to prevent it from contributing to the crustacean damaged estimate. The amount of crustacean damage was then assumed to be the difference between the expected leaf area and the observed area with crustacean damage. That difference was then expressed as a percent of the expected leaf area, and averaged over all samples on a given date.



**Fig. 2.2** An example of the estimation of gastropod damage to a *Halophila ovalis* leaf. The 8-bit colour image (A) was converted to grayscale (B), and then thresholded at a level that distinguished the pigmented areas with one or two layers of cells in black (C). That area was measured at 36.3 mm<sup>2</sup>. The missing area was painted (D) and the total area corresponding to the area of the undamaged leaf was measured at 42.2 mm<sup>2</sup>. The area of 2-layer damage is therefore the difference, 5.9 mm<sup>2</sup>. The density slice option was then used to identify the intermediate level of grey representing the area of 1-layer damage in red (E). Extra red pixels representing thin tissue at the edge of the leaf were erased. The desired area was selected (F), and the red pixels measured at 8.1 mm<sup>2</sup>. The percentage area of the leaf damaged by gastropods was therefore estimated at  $((5.9 + (0.5*8.1))/42.2)*100=23.6\%$ .

The amount of damage to *Halodule uninervis* leaves was more difficult to estimate. The leaves are thicker, and the removal of tissue by gastropods is less evident in images from scanning. The percentage of *H. uninervis* leaf area damaged by gastropods in leaf samples from Shelly Beach was estimated on only two dates, 5 November 2002, and 18 March 2003, for comparison with gastropod damaged on *Halophila ovalis* leaves.

Those were the dates of maximum damage to *H. ovalis*. The length of leaf damage, as measured under 10X magnification, was expressed as a percentage of the total leaf length in each sample, and averaged over each sample on those dates.

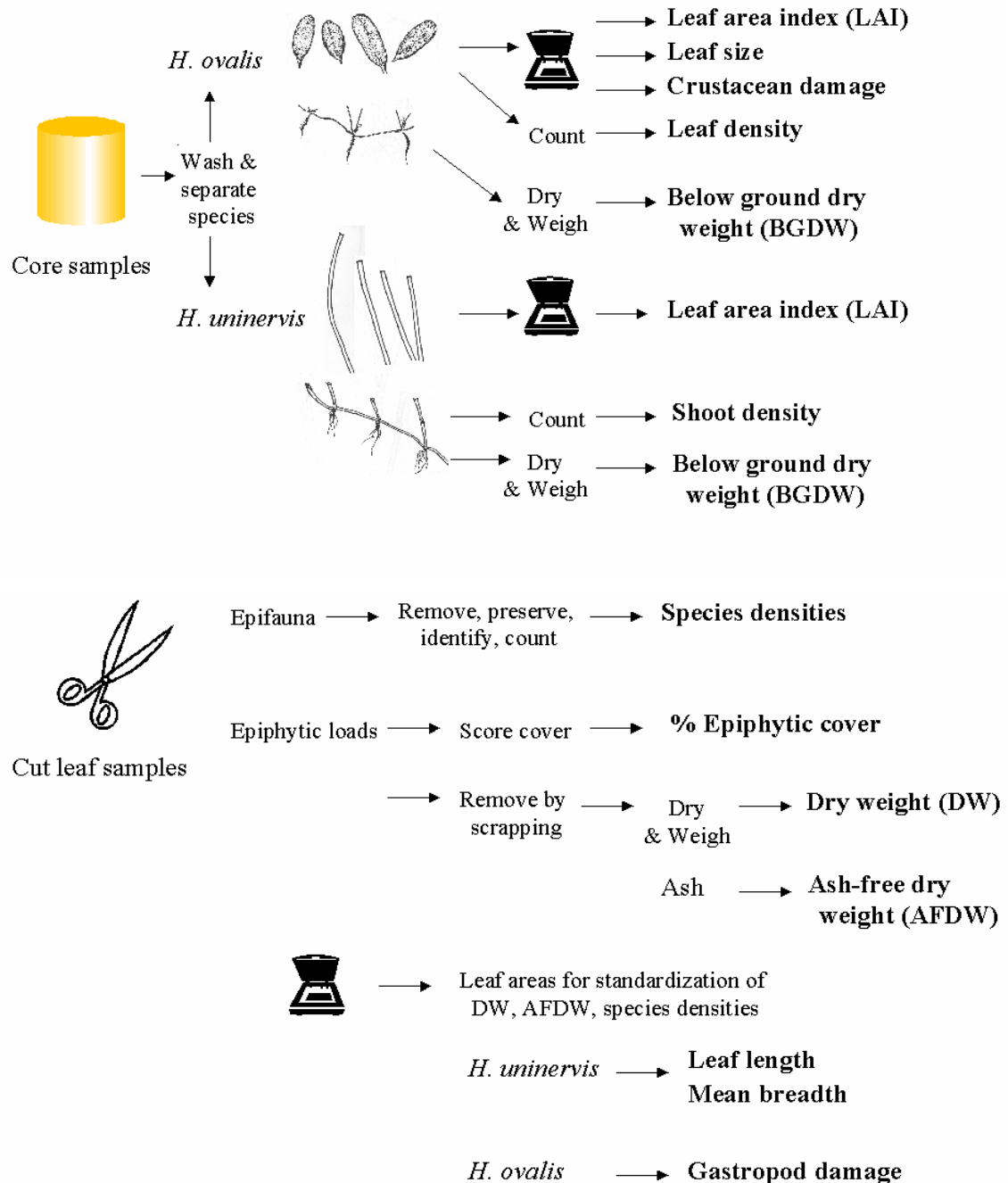


Fig. 2.3 Summary of the biological parameters measured from core samples and cut leaf samples.

## 2.8 Abiotic parameters

### 2.8.1 Tidal exposure

The height of each plot above chart datum was estimated by recording the times at which incoming tides entered the plots. Those times were compared with the times for predicted tidal heights in Seafarer Tides©, an enhanced electronic version of Australian National Tide Tables. Estimates of the tidal heights of the plots were based on averages of 17, 15 and 9 observations of the incoming tides at Shelly Beach, Cockle Bay and Picnic Bay, respectively.

The total hours of daylight exposure of the plots over 14 days were calculated for each sampling date. It was calculated as the sum of the time intervals when the tide level was equal to or below the estimated height of a plot, between 900 h and 1600 h, over 14 days prior to the date of sampling. Those time intervals were calculated from the tidal height predictions given in 20 minute intervals by the Seafarer Tides© charts.

### 2.8.2 Water and weather conditions

Salinity of the water in each plot was determined on each sampling occasion using a refractometer. Temperatures were taken of the water remaining in the plot at low tide, typically a few centimeters, on each sampling occasion, using an ordinary red spirit thermometer.

Daily weather readings from Townsville airport were obtained from the Bureau of Meteorology. Cumulative rainfall for 14 days prior to sampling was calculated for each sampling date in each plot. Mean air temperature and non-directional wind speed at 1500 h was also calculated over 14 days prior to each sampling date. The 14-day interval was chosen because it was the time of approximately one tidal cycle, and is within the time range calculated for a complete replacement of the standing crop of *Halophila ovalis* (Hillman et al. 1995).

### 2.8.3 Sediment particle-size

Three core samples (45 cm<sup>2</sup> X 10 cm deep) were taken at random from each plot in September and October 2004. Conspicuous seagrass elements were removed, and the

sediment oven dried to a constant weight, before being thoroughly mixed. A sub-sample, of approximately 100 gm, was accurately weighed, then washed through a stack of sieves with mesh sizes 2 mm, 1 mm, 500  $\mu\text{m}$ , 250  $\mu\text{m}$ , 125  $\mu\text{m}$ , and 63  $\mu\text{m}$ . The sediment retained on each sieve was transferred to pans and oven dried to a constant weight. The proportions of the sediment greater than each sieve size, and  $<63$   $\mu\text{m}$ , i.e. the proportion washed from the smallest sieve, was then calculated by comparison to the original weight of the sub-sample.

## 2.9 Project design

This project began with the understanding that there could be significant interactions between seagrasses and their associated invertebrate fauna, and more specifically that invertebrate grazers could benefit seagrasses by controlling epiphytic algae. Most importantly it was believed that those interactions could be demonstrated in the field, and not solely within the context of experimental manipulations. The current literature can be interpreted as supporting a single clear model of seagrass-epiphyte-grazer interactions. Such models are testable against field data. To evaluate the meaningfulness of a model, it needs to be tested against alternatives. Alternatives to the current prevailing model can be developed from existing knowledge. Furthermore, data used to test proposed models may suggest new models. It is through this process of developing models, testing them against alternatives, and proposing new and better ones that progress in scientific understanding can be made. That process is expressed in a more formal way by the developing and testing of path models. New models may also lead to better experiments that are grounded in the experience of natural phenomena. However, progress in understanding ecological interactions will most likely be made by taking them out of the lab, the mesocosm, and the field enclosure, and seeing how they are expressed under natural conditions.

In chapter 3, an alternative model is proposed based on observations of change in *Halophila ovalis* in one of the study plots. That model is then available to be tested against the prevailing model using field data for *Halodule uninervis* in chapter 6. The intervening chapters introduce and explore the complete set of data on seagrass, its epifauna, and epiphytic loads and cover. That data is then used in the tests of models

by path analysis in chapter 6. One assumption of the prevailing model is that there are no direct interactions between the seagrass and its epifauna, only indirect effects through grazing on epiphytic material. Direct effects, such as invertebrate herbivory, are assumed to be of minor consequence. However, during the course of this study there was conspicuous evidence of herbivory, especially on *H. ovalis*. So, the opportunity was taken to measure herbivore damage and to evaluate its potential impact, as reported in chapter 7. Abiotic factors have often been used to explain changes in local seagrass meadows, and their relative importance is considered with respect to the previously considered biotic factors using regression tree analysis in chapter 8. The alternative model proposed in chapter 3 suggested an experiment to demonstrate the proposed effects of epiphyte shading. That experiment is the subject of chapter 9

## **2.10 Statistical analysis**

### ***2.10.1 Univariate***

Simple correlations and univariate regressions were used to test those relationships that were *a priori* assumptions arising from the general model of seagrass-epiphyte-grazer interactions. Those tested assumptions were that changes in measurements of seagrass abundances were related to epiphytic loads and numbers of epiphyte grazers, and those epiphytic loads were related to the number of epiphyte grazers.

To demonstrate patterns of change in the data, differences among sampling dates in the mean values of the various measurements of seagrass, epiphyte, and invertebrate abundances were evaluated using single-factor analysis of variance (ANOVA) in each plot. The Tukey method was used to make *post hoc* pairwise comparisons between means. When necessary, the modified Tukey method for unequal sample sizes was used. Differences between means were accepted as significant where  $p < 0.05$ . For data from the Picnic Bay plot, a contrast of means was performed, comparing values after termination of ferry service ( $n=5$ ) with values from the same months, October to July, of the previous year ( $n=8$ ).

Invertebrate densities were often small values  $< 1.0 \text{ cm}^{-2}$ , and so were first expressed as numbers per  $100 \text{ cm}^{-2}$  leaf surface. Those numbers tended to increase in variance with the mean. Therefore, the  $\log_e (X+1)$  transformation has been applied to invertebrate data in order to stabilize the variance and improve normality. Analyses of data involving invertebrates were initially done with densities of separate taxa, and then repeated with the combined density of grazers, or all invertebrates.

Direct estimates of the percent leaf surface covered by epiphytic material were not made in June 2002 for any of the 3 plots, or in July 2002 for the plot at Shelly Beach. Indirect estimates of epiphytic cover on those dates were made from the strong linear relationship found between AFDW and percent epiphytic cover.

### **2.10.2 Multivariate**

Multivariate analyses of different types were used, but are unique to individual chapters, and so will be described only in those chapters. Non-metric multidimensional scaling (MDS) and analysis of similarities (ANOSIM) are described in chapter 4. Path analysis is described in chapter 6, and regression tree analysis in chapter 8.

## **2.11 Definitions and abbreviations**

In this study, **epifauna** refers to macroinvertebrates taken from samples of seagrass leaves, where macroinvertebrates are animals that would be retained on a  $0.5 \text{ mm}^2$  screen. It includes mobile forms, and sessile forms attached to the leaves, such as anemones, winged oysters (Pteriidae), and spirorbid polychaetes. It does not include hydroids, ascidians, or meiofauna. **Epiphytic material** is all of the remaining material on the leaves after the epifauna is removed, and may include algae, bacteria, fungi, detritus, soft flocculent matter, sand, and any remaining animal matter. **Epiphytic load** and **epiphytic cover** are measurements of the amount of epiphytic material by weight and percent cover respectively. **Epiphytes** are the organisms that make up the epiphytic material; but because there could be substantial amounts of non-living material on the leaves, it was preferable to use the broader terms when referring to the material on the leaves collectively. Many studies have referred to periphyton on the leaves, in reference to fine material composed of diatoms, bacteria, detritus, etc. Less often the term epiphyton has been used, and appears to refer to epiphytic periphyton.

Much of the material scraped from the leaves in this study could have been referred to as periphyton or epiphyton; but because larger attached algae have been included, those terms have not been used.

**Grazers** will refer to animals that feed on epiphytic material found on seagrass leaves. **Herbivores** will refer to animals that feed directly on the seagrass. **Grazing** and **herbivory** will likewise refer to feeding on epiphytic material and seagrass respectively. It is possible that an individual animal could behave at different times as both a grazer and an herbivore. In the current literature, it is common to refer to animals that feed on epiphytes as herbivores, but that will not be the usage here. Most of the animals discussed in this thesis are in a size range such that they would be referred to as mesograzers, and less often as mesoherbivores, in the current literature (Brawley 1992); but those terms have not been used here.

The study plots at Shelly Beach, Cockle Bay and Picnic Bay will be referred to as plots **SB**, **CB** and **PB** respectively, and the full names will refer to the locations in general and not specifically to the plots.

**AFDW** – Ash-free dry weight of epiphytic material (see section 2.5)

**DW** – Dry weight of epiphytic material (see section 2.5)

**BGDW** - Below ground dry weight of seagrass, including all plant parts other than the leaves (see section 2.3)

**LAI** – Leaf area index, calculated as the area of one side of the seagrass leaves over an area of substrate (see section 2.3).

## Chapter 3 The impact of epiphyte grazing by sea hares on *Halophila ovalis*

### 3.1 Introduction

In early June 2002, a plot was established at Shelly Beach in order to sample the seagrass, its epifauna, and epiphytic material using the methods outlined in the previous chapter. Shortly after initial samples were taken, there was a dramatic increase in the numbers of sea hares (Gastropoda: Aplysiidae), which were observed grazing epiphytic material from seagrass leaves. Most of the sea hares were the species *Bursatella leachii* de Blainville, but there were also a few *Stylocheilus striatus* (Quoy & Gaimard). The sea hares were up to 6 cm long, and large relative to the seagrass leaves or the typical epifauna at the Shelly Bay site. Both species are circumtropical in distribution, and have been reported occurring in dense migrating swarms in disparate parts of the globe (Marcus 1972, Lowe and Turner 1976) (see also the many observations and photos posted at [www.seaslugforum.net](http://www.seaslugforum.net)). The reasons for the swarming behaviour, or their sporadic occurrences, are not understood. Neither species has been associated with seagrass meadows, or been noted for feeding on epiphytic material from seagrass leaves. *B. leachii* feeds by ingesting surface mud or scraping epiphytic material from hard surfaces, and has been described as a benthic detritivore (Paige 1988). The species has typically been considered a feeder on blue-green algae, although it will feed on a variety of other algae, but not seagrasses (Clarke 2004). *S. striatus* is a very similar species, but is considered a more specialized feeder on blue-green algae, especially *Lyngbya*. It has been shown to sequester toxic secondary metabolites from *Lyngbya* that are deterrents to feeding by fish and crabs (Paul and Pennings 1991, Pennings et al. 1996).

The unusual circumstances of the sea hare incursion into the plot at Shelly Beach made it possible to examine the response of the seagrass to a sudden change in the amount of epiphyte grazing in a field situation. The prevailing understanding of seagrass-epiphyte-grazer interactions (chapter 1) suggests that sea hares should benefit seagrass production by removing the overburden of epiphytic material. That understanding assumes that epiphytic cover has negative impacts on seagrass, although there have



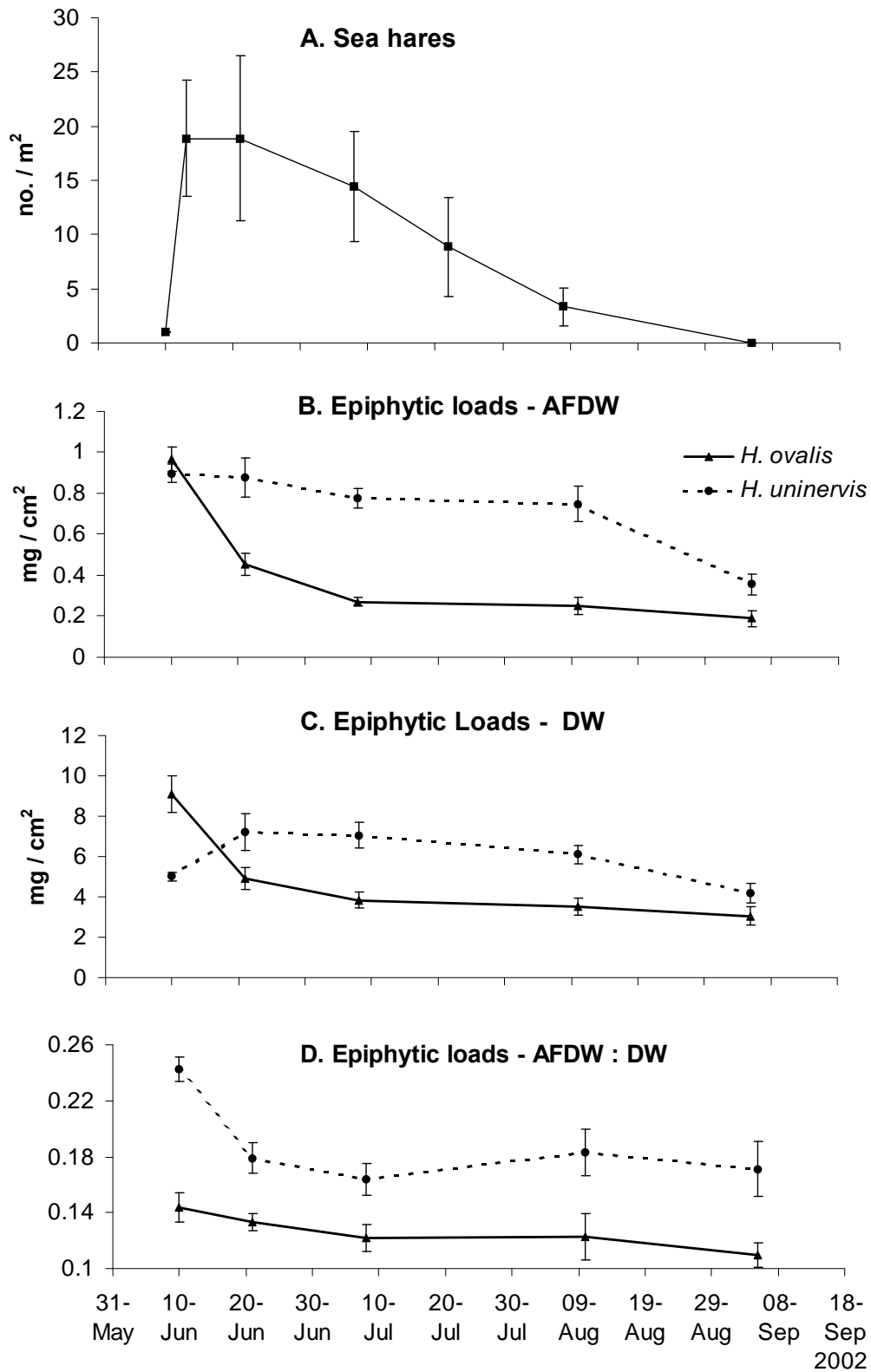
been some suggestions that epiphyte cover may have some positive effects. Trocine et al. (1981) proposed that epiphyte cover provides protection from UV-B radiation, and Penhale and Smith (1977) suggested that it provides protection against desiccation in intertidal habitats. However, those possibilities have never been considered with respect to seagrass-epiphyte-grazer interactions.

The hypothesis that an epiphyte grazer could provide measurable positive benefits for seagrass standing crop is examined in this chapter using data collected following the techniques outlined in the previous chapter. The data presented is largely limited to the first four months of the two years of sampling from one plot. The more limited set of data is considered here in order to establish a pattern that will be used to propose alternatives to the prevailing model of seagrass-epiphyte-grazer interactions. Alternative models proved useful in the analysis of the larger data set, which is presented elsewhere in the following chapters.

## 3.2 Results

### 3.2.1 *Sea hare abundance*

The first leaf samples were taken for epiphytes from the plot at Shelly Beach (plot SB) on 10 June 2002. Sea hares were present in the plot at that time, but in such low numbers that they were not detected in quadrat sampling. Their numbers in the plot were therefore  $<1 \text{ m}^{-2}$ , although they were observed to be more abundant elsewhere, especially on the outer edge of the meadow, where they appeared to be moving shoreward. By 13 June, their numbers had increased greatly in the plot (Fig. 3.1A), and there were estimated to be  $18.9 \pm 5.8$  (s.e.)  $\text{m}^{-2}$  substrate. By 21 June, when a second set of leaf samples for epiphytes was taken, the estimated density of sea hares was unchanged. During July and August, quadrat sampling showed a steady decline in sea hare numbers, and there were none in September. Overall changes in sea hare numbers were highly significant (Kruskal-Wallis ANOVA,  $H=23.6$ ,  $p<0.001$ ). The sea hares were observed scraping material from seagrass leaves, but there was never any evidence that they damaged those leaves. Sea hares were not observed in the other meadows studied.



**Fig. 3.1** Mean estimates ( $\pm$  s.e.) of the density of sea hares (A) from quadrat samples, and the AFDW (B), DW (C), and ratio AFDW:DW (D) of epiphytic material on leaves of *Halophila ovalis* and *Halodule uninervis* from June to September 2002.

The next year, in June 2003, large numbers of sea hares again appeared in the plot; but for a shorter period of time. On 15 June, their density was estimated at  $28.3 \pm 4.5$  (s.e.)  $\text{m}^{-2}$  substrate. By 29 June, their density had fallen to  $10.8 \pm 3.9$  (s.e.)  $\text{m}^{-2}$  substrate, and by 14 July there were none. *Stylocheilus striatus* was estimated to be 92% of the animals in 2003, and the remainder were *Bursatella leachii*, a reversal of the relative abundance of those species in the previous year. *S. striatus* were smaller, and were observed to do more climbing and feeding from *Halodule uninervis*.

### 3.2.2 Epiphytic loads

The ash-free dry weight (AFDW) of epiphytic material on *Halophila ovalis* leaves underwent a highly significant decline (ANOVA,  $F=49.2$ ,  $p<0.0001$ ) between June and September 2002 (Fig. 3.1B). Between 10 and 21 June, when sea hares first invaded the plot in large numbers, AFDW declined 53% (Tukey comparison,  $p<0.001$ ). Between 21 June and 8 July, it declined further ( $p<0.05$ ), so that AFDW was reduced 72% over four weeks. The decline in AFDW reflected a similar decline in the dry weight (DW) of material on *H. ovalis* leaves (ANOVA,  $F=18.5$ ,  $p<0.0001$ ), which was reduced 46% between 10 and 21 June (Tukey comparison,  $p<0.001$ ) (Fig. 3.1C). Therefore, there was no significant change in the ratio AFDW:DW (ANOVA,  $F=1.4$ ,  $p>0.05$ ) of epiphytic material on *Halophila ovalis* leaves (Fig. 3.1D).

In marked contrast, the quantity of epiphytic material on *Halodule uninervis* leaves did not show a decline in association with the increase in sea hares numbers, but appeared to undergo a change in composition. The DW of epiphytic material was actually greater when sea hares were present than when they were absent, either before or after the incursion (Fig. 3.1C). The overall changes in DW were significant (Kruskal-Wallis ANOVA used due to high variance heterogeneity,  $H=13.4$ ,  $p<0.01$ ), with a significant decline occurring between 8 July and September (multiple comparisons,  $p<0.05$ ). There was also an overall change in the AFDW of epiphytic material on *H. uninervis* (ANOVA,  $F=10.1$ ,  $p<0.0005$ ), but it remained almost constant until a decline between August and September, when sea hares were absent from the plot (Fig. 3.1B). Those changes reflected a change in the ratio AFDW:DW, which declined significantly immediately after sea hares entered the plot (ANOVA,  $F=5.0$ ,  $p<0.005$ ; Tukey comparison,  $p<0.05$ ) (Fig. 3.1D), indicating a decline in the organic content, and change in the composition of the epiphytic material.

*Halodule uninervis* leaves had a cover of crustose coralline algae overgrown with the blue-green algae *Calothrix crustacea* in 2002. In contrast, *H. ovalis* leaves were covered in a thick layer of flocculent material, mixed with blue-green algae and diatoms. Crustose coralline algae were uncommon on *H. ovalis*, and also became uncommon on *H. uninervis* after September 2002. In 2003, during the period 13 June to 9 September, which corresponded closely to the period examined the previous year, epiphytic AFDW on *H. ovalis* did not change significantly (ANOVA,  $F=2.8$ ,  $0.05 < p < 0.10$ ), but declined significantly on *H. uninervis* (Kruskal-Wallis ANOVA,  $H=17.8$ ,  $p < 0.001$ ).

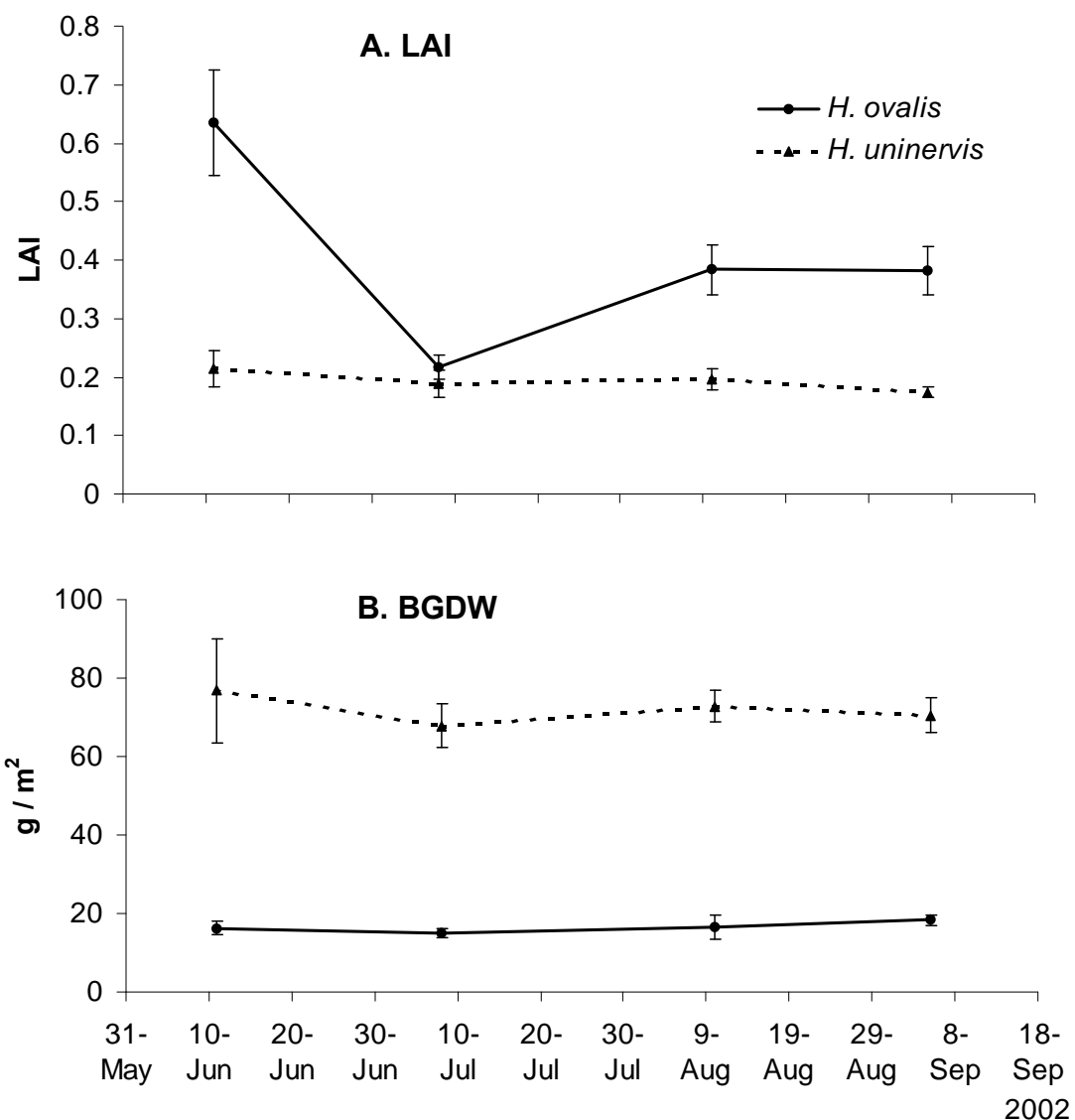
Measurement of percent cover had not begun at the beginning of the period being considered in this chapter, and there were no direct measures of the relative contributions of different kinds of epiphytic material. There are also no useful comparisons with other sites where sea hares were absent, as *H. ovalis* was also absent at that time from the plots at Picnic Bay and Cockle Bay, and measurements of the epiphytic material on *H. uninervis* did not begin there until after June 21.

### 3.2.3 Seagrass

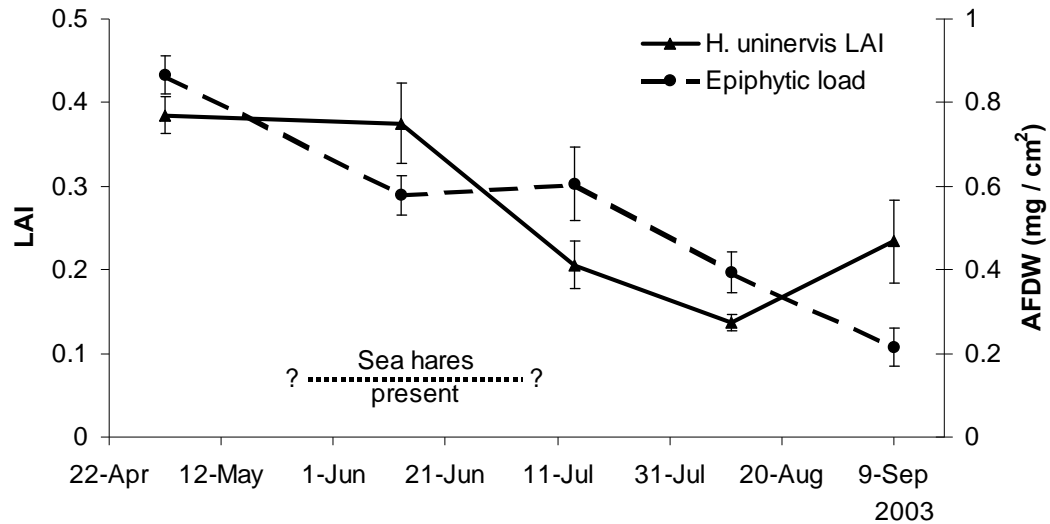
Unexpectedly, the leaf area index (LAI) of *Halophila ovalis* in plot SB fell significantly (ANOVA,  $F=9.8$ ,  $p < 0.0001$ ), by 65.9% between 10 June and 8 July 2002 (Tukey comparison,  $p < 0.001$ ) (Fig. 3.2A). Between July and August, there appeared to be some recovery of *H. ovalis* LAI, but it was non-significant. At the same time, *Halodule uninervis* LAI remained virtually constant from June to September 2002.

In marked contrast, during the same months in 2003, *H. uninervis* LAI declined significantly (ANOVA,  $F=7.0$ ,  $p < 0.005$ ) when its epiphytic AFDW was also declining (Fig. 3.3). *H. ovalis* LAI did not change significantly during that period (Kruskal-Wallis ANOVA,  $H=3.2$ ,  $p > 0.10$ ), although *H. ovalis* standing crop had fallen to very low levels. By August 2003, its LAI was only 2% of the value it had been in June 2002, and its leaves showed evidence of severe damage by invertebrate herbivores.

During the period June to September 2002, the estimate of the percentage of *Halophila ovalis* leaf areas missing, presumably due to crustacean feeding, ranged from 2.3 to 6.5%. The area of leaf surfaces damaged by gastropods ranged from <1% to 6.6%. On July 8, when the greatest decline in *H. ovalis* LAI was recorded, the damage attributed to crustaceans and gastropods was only 2.3% and 1.0% respectively. Those values were small and comparable to the general background level of damage recorded over two years of study (chapter 7). In contrast, the two types of damage later peaked at values of 36% and 43%.



**Fig. 3.2** Mean estimates ( $\pm$  s.e.) of leaf area index (LAI) and below ground dry weight (BGDW) of *Halophila ovalis* and *Halodule uninervis* in cores (n=10) from June to September 2002.



**Fig. 3.3** Mean estimates ( $\pm$  s.e.) of the leaf area index (LAI) from cores ( $n=5$ ), and AFDW of epiphytic material on *Halodule uninervis* leaves from June to September 2003. Also showing the approximate period when sea hares were active in the study plot.

The decline in *Halophila ovalis* LAI between June and July 2002 was related to a significant decline in both the mean density and the size of leaves (Fig. 3.4) (ANOVA,  $F=9.2$ ,  $p<0.001$ , and  $F=10.4$ ,  $p<0.0001$  respectively) between June and July (Tukey comparisons,  $p<0.001$ ). Leaf density then recovered significantly in August ( $p<0.025$ ), and was not significantly different than the initial value in June. However, leaf size did not recover, and remained constant until September, and so the lowered LAI in August and September can be largely attributed to the small size of the leaves in that period. Although leaf size declined in association with the decline in AFDW between June and July 2002, the mean sizes of *Halophila ovalis* leaves calculated over the period from June 2002 to December 2003 (data presented in Appendix C) were not correlated to AFDW ( $r=0.24$ ,  $p>0.10$ ,  $n=17$ ).

The below ground dry weight (BGDW) of both *Halophila ovalis* and *Halodule uninervis* did not change significantly over the period June to September 2002 (Fig. 3.2B). Therefore, the decline in *H. ovalis* LAI also represented a decline in the amount of leaf area supporting a given plant biomass below ground. Over all sampling dates from June 2002 to December 2003, the leaf area per gram of BGDW of *H. ovalis* was positively correlated with the AFDW of material on its leaves ( $r=0.71$ ,  $p<0.005$ ,  $n=17$ ). That relationship was still evident without the extreme data for June and July 2002 ( $r=0.52$ ,  $p<0.05$ ,  $n=15$ ), and so the change in LAI seen when sea hares entered the plot

appeared to be consistent with the relationship to AFDW found over the long-term (Fig. 3.5). *H. uninervis* did not show a similar relationship; and compared to *H. ovalis*, it always supported a much larger below ground biomass relative to its leaf area (compare Fig. 3.2A&B).

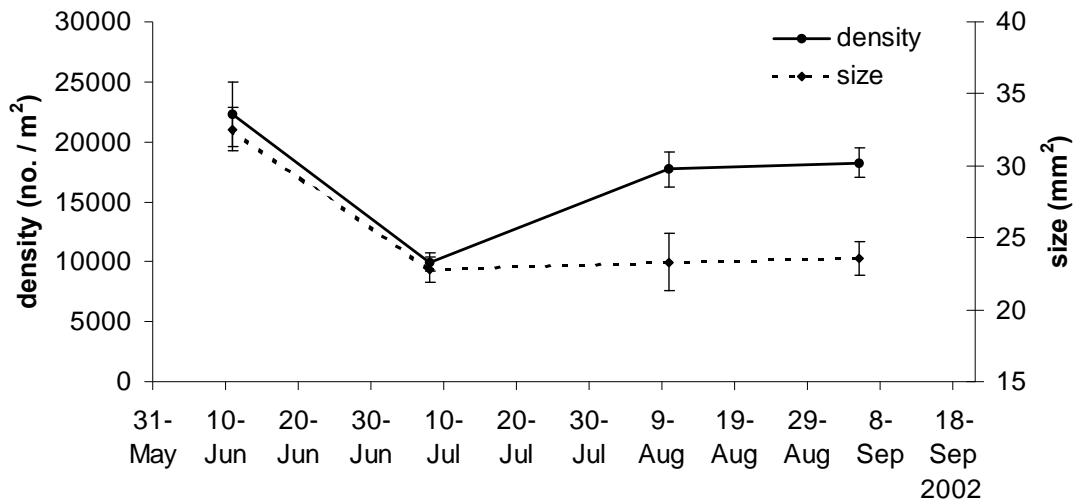


Fig. 3.4 Mean densities and sizes ( $\pm$  s.e.) of *Halophila ovalis* leaves in core samples (n=10) from plot SB from June to September 2002.

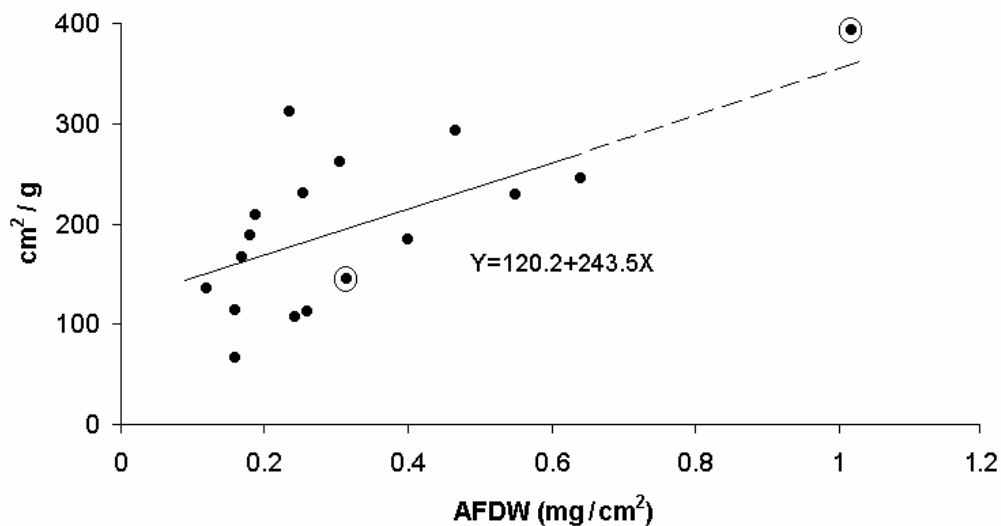


Fig. 3.5 Relationship between AFDW of material on *Halophila ovalis* leaves and leaf area per gram of below ground dry weight. Regression line fitted for the period August 2002 to December 2003 (solid line, n=15,  $R^2=0.27$ ,  $p<0.05$ ), excluding June and July 2002 (circled), and extrapolated (dashed line) to level of AFDW recorded in June 2002.

### 3.3 Discussion

#### 3.3.1 Changes in epiphytic material

Epiphytic loads on *Halophila ovalis* leaves underwent a highly significant decline in the study plot at Shelly Beach in a 10-day period when there was a dramatic increase in the numbers of a large epiphyte grazer, the sea hare *Bursatella leachii*. At the same time, epiphytic loads on *Halodule uninervis* did not decline, but experienced a significant change in composition, indicated by an increase in ash content. Before sea hares entered the plot in large numbers at Shelly Beach, there were much heavier loads, measured as DW of epiphytic material, on *Halophila ovalis* compared to *Halodule uninervis*. Epiphytic loads were greater than at any time in 1.5 years, and *Halophila ovalis* presented the largest ever leaf surface area. The rapid change in epiphytic loads on *H. ovalis* seems unlikely without the grazing of sea hares. Typically, changes in epiphytes are expected to reflect changes in temperature, light conditions, tides, and nutrients, which would be experienced on the surfaces of both seagrasses in common.

The divergent changes in the loads of the two seagrass species could be related to the behaviour of the sea hares, and differing characteristics of the seagrasses and their epiphyte covers. The sea hares species are benthic grazers, and not particularly associated with seagrass meadows. They would be expected to encounter *H. ovalis* leaves more often than *H. uninervis*, because the long leaves of the latter float away from the sediment surface, at least when submerged by the tide. The epiphytic material on *H. uninervis* included crustose coralline algae, which is not known to be a food source for *Bursatella leachii* or *Stylocheilus striatus* (Clarke 2004). In the presence of sea hares, there was an increase in DW and the proportion of ash in epiphytic material on *H. uninervis* leaves. Those changes likely reflect an increase in crustose coralline algae, and suggested that grazing by sea hares was favouring their increase relative to blue-green algae. The latter was an important element of the epiphyte assemblage on *H. uninervis*, and known to be a common food of the sea hare species (Paige 1988). Other gastropods that graze algae from seagrasses are also known to prefer non-coralline algae (Jacobs et al. 1983, Klumpp et al. 1992). In other habitats, crustose coralline algae have been found to be dependent on herbivores to prevent their overgrowth by fleshy algae (Dethier 1994), and such interactions may be important on seagrass leaves.



In 2003, epiphytic loads also declined on *Halodule uninervis* between June and September. Sea hares were present, but for a shorter period of time. Although they may have contributed to the decline, other factors were likely important. The epiphytic material also differed, as crustose coralline algae were absent from *H. uninervis* leaves in 2003.

### **3.3.2 Response of *Halophila ovalis***

The prevailing model of seagrass-epiphyte-grazer interactions assumes that epiphyte grazers benefit seagrasses by removing material that blocks light to the leaves and thus limits photosynthetic production (chapter 1). However, contrary to expectations, *Halophila ovalis* LAI declined in close association with the loss of a dense cover of epiphytic material, most likely due to grazing by sea hares. A variety of explanations might be proposed for that decline. Direct consumption of the leaves by herbivores seems an unlikely cause. At the time of the greatest loss of leaf area, the amount of herbivore damage to the leaves was small compared to the levels of damage seen at other times (chapter 8). Sea hares were never seen to damage the leaves, and there were no herbivores observed that could have removed entire leaves without a trace. It has been proposed that epiphyte cover protects intertidal leaves from desiccation (Penhale and Smith 1977), but there was no evidence of major desiccation of either *H. ovalis* or narrow-leaved *Halodule uninervis* in the plot during this study. Those seagrasses do not normally suffer desiccation, as they can lie prone on wet muddy substrates at low tide (Björk et al. 1999). It also seems unlikely that leaves were shed as a delayed response to the very high epiphytic loads experienced before sea hares entered the plot. *H. ovalis* often survives very low light conditions, and has been found growing while partially covered in sediment, and at depths >30 m (Lee Long et al. 1996, Erftemeijer and Stapel 1999).

The loss of epiphytic cover exposed *Halophila ovalis* leaves to increased levels of irradiance that may have had effects leading to a reduction in leaf area. Hillman et al. (1995) found that cultured *Halophila ovalis*, taken from a temperate estuary in Western Australia, were saturated with light at a level of approximately  $200 \mu\text{Em}^{-2}\text{s}^{-2}$ , meaning that increases in photosynthetic production did not occur at higher light levels.

Furthermore, *H. ovalis* is vulnerable to photoinhibition, which is the reduction in photosynthetic production at light levels greater than saturation. Ralph and Burchett (1995) observed photoinhibitory responses in *H. ovalis* at exposures to light of 500  $\mu\text{Em}^{-2}\text{s}^{-2}$  for greater than 10 minutes, and there was evidence of photodamage at exposures of 1000  $\mu\text{Em}^{-2}\text{s}^{-2}$  for 120 minutes. Tropical intertidal seagrasses can be exposed to light levels of  $>1500 \mu\text{Em}^{-2}\text{s}^{-2}$  (Ralph 1999b). Therefore, increasing exposure to light at those levels typical of intertidal habitats should not be expected to increase *H. ovalis* production, and may lead to its decline.

In one month in June and July 2002, *H. ovalis* experienced a highly significant 55% loss in the number of leaves, without a loss of below ground biomass, suggesting a direct impact on the leaves. Before sea hares arrived, *H. ovalis* leaves were densely covered in epiphytic material and probably adapted to shade conditions, which could have made them more vulnerable to irradiance damage. Importantly, the incursion of sea hares occurred at a time of year when daytime tidal depth over the study plot was lowest (see Fig. 8.1), and the possibility of exposure to damaging irradiance was greatest. Therefore, the immediate cause of the leaf loss could have been photodamage, premature senescence, and shedding of the leaves, which were then replaced by smaller leaves that were better adapted to high light conditions. The changes in the ratio of leaf area to below ground biomass seen when sea hares entered the plot were consistent with changes seen over the long-term with respect to epiphytic loads, but differed in magnitude.

Over 1.5 years, there was a positive relationship between the amount of epiphytic material on *Halophila ovalis* leaves and the amount of leaf area with respect to below ground biomass. That relationship occurred despite the continuous loss of *H. ovalis* biomass over that time, and occasionally high levels of herbivore damage to the leaves (chapter 7). The change in the proportion of leaf area may be seen as an adaptive response to changing light levels as a result of altered epiphytic shading. Under reduced irradiance, in experimental shadings, some seagrasses increase leaf surface area relative to below ground biomass to capture more light (Abal et al. 1994). Under high irradiance, the reverse can be expected, as the plants experience light levels that are potentially damaging to leaf tissues, but are in excess of needs for photosynthesis to maintain its below ground biomass. Hillman et al. (1995) found that *H. ovalis* grown

under low light conditions produced larger leaves and channelled a greater proportion of production into above ground biomass. Duarte et al. (2000) found that *H. ovalis* leaf area per shoot was significantly reduced when the canopy of larger species was experimentally removed in a Philippine mixed-species meadow. In Thailand, leaf density and size was significantly reduced during a period of reduced turbidity following the cessation of the monsoon (unpublished data). Thus, the relationship seen in this study between the relative proportion of the leaf area and epiphytic cover was consistent with changes with respect to light conditions seen in other studies. Changes in the ratio of leaf area to below ground biomass may be an effective indicator of changes in light conditions experienced by *H. ovalis*, whereas changes in leaf size or LAI alone may not be indicative.

*Halodule uninervis* epiphytic loads were not reduced when sea hares invaded the study plot in 2002, and LAI was not reduced. In contrast, epiphytic loads declined during the some period of greatest tidal exposure in 2003, and *H. uninervis* LAI declined. The reasons for the reduced loads in 2003 are not clear, but the observations suggest that *H. uninervis* may also have been protected from high irradiances by the greater epiphytic cover in 2002. Dawson and Dennison (1996) showed that both *Halophila ovalis* and *Halodule uninervis* were particularly sensitive to increases in UV radiation. Those species had the greatest decreases in photosynthetic efficiency and the smallest increases in UV-blocking pigment in response to UV radiation, when compared to other tropical Australian species. However, they were not especially sensitive to increases in photosynthetically active radiation (PAR), which may also cause photoinhibition. A number of studies have seen evidence of photoinhibition in other seagrass species (Libes 1986, Enríquez et al. 2002, Peralta et al. 2002). Durako et al. (2003) suggested that differences in the depth distributions of two *Halophila* spp. in Florida were related to differences in their tolerance of high irradiances.

Trocine et al. (1981) suggested there was a relationship between UV-B radiation damage and epiphytic cover. They studied photoinhibition in three Florida species of seagrass, and found that *Halophila engelmanni* Aschers was the most highly sensitive, and had no capacity for photorepair. However, photoinhibition in *H. engelmanni* was reduced by the presence of epiphytes, and they concluded that it was dependent on epiphytes as a shield from high irradiance in shallow water habitats. Despite those

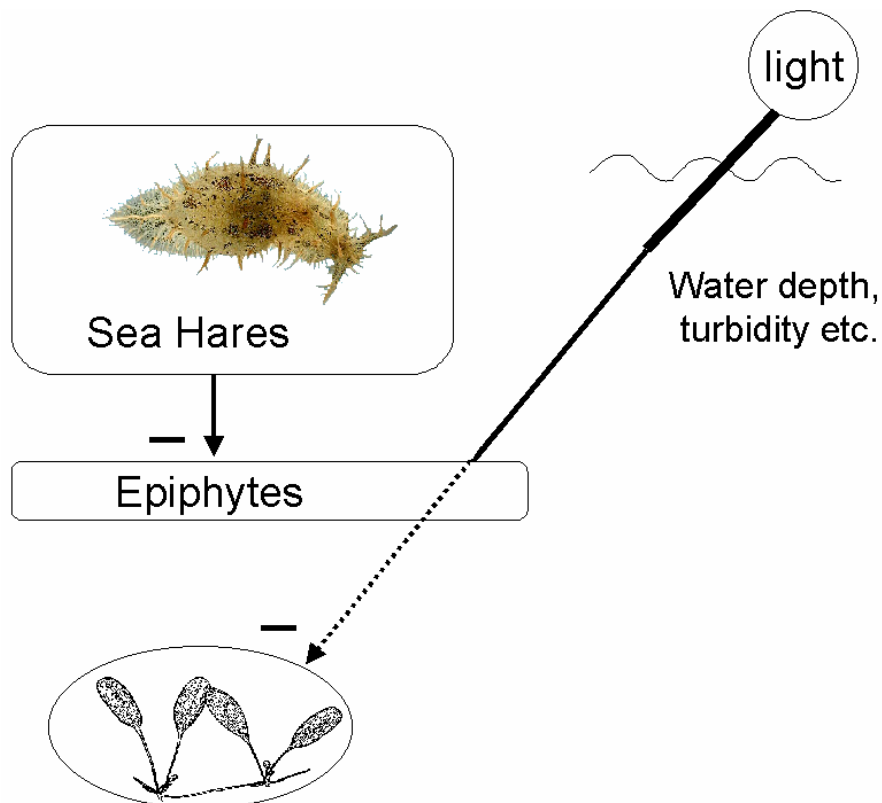
findings, there have been few attempts to relate epiphytic cover to the tolerance of high irradiance, or to relate such interactions to the distribution and abundance of seagrasses in tropical waters. Recently, Brandt and Koch (2003) have shown that periphyton cover on transparent artificial leaves provided an effective UV-B filter, while allowing proportionately more transmittance of PAR.

The circumstances described here represented a unique opportunity to examine the ability of a grazer to influence epiphytic loads and seagrass standing crops in a natural situation. There is considerable evidence from experimental manipulations of grazers, especially small gastropods and peracarid crustaceans (Table 1.1), that suggests they can control epiphytic cover, and benefit seagrass production. However, there is no field-based evidence, other than that of Schanz et al (2002), that supports that view. In this field study, a heavy burden of epiphytic material had developed on seagrass leaves before sea hares arrived, suggesting that resident grazers had not controlled epiphytic growth. The incursion of sea hares was expected to benefit the seagrass by relieving it of an overburden of epiphytic material. Although there was evidence that the sea hares could have a significant impact of epiphytic loads, there was no evidence of any benefit to the seagrass. On the contrary, there was evidence of a decline in conditions for *Halophila ovalis*.

Dense migrating populations of the sea hares *Bursatella leachii* and *Stylocheilus striatus* occur sporadically and unpredictably. This study was therefore opportunistic in nature, and represents a unique set of circumstances that developed in a singly seagrass plot. As such, the results have limited application. However, the circumstances illustrate that grazers may have impacts on seagrasses that are contrary to the expectation that appear in the current literature. Epiphytes are usually thought to have a negative impact on seagrasses because they block light for photosynthesis, which may be a critical factor in deep or turbid waters. However, in tropical intertidal habitats that experience saturating levels of light, epiphytes may have a function protecting seagrasses from high levels of irradiance. Therefore, grazers can potentially remove that protective layer and have negative impacts on seagrass production.

### 3.4 Conclusions

The removal of epiphytic cover from *Halophila ovalis* leaves by sea hares was associated with a significant loss of leaf area, contrary to the expectations found in the current literature on seagrass-epiphyte-grazer interactions. The most likely explanation was that the sea hares exposed the leaves to high levels of damaging irradiance by removing epiphytic cover, which appeared to have an important protective role, shielding seagrass leaves from the harmful effects of high irradiances. Therefore, sea hare grazing appeared to have a negative impact as an indirect consequence of their grazing. Those hypothesised interactions form the basis of an alternative model of seagrass-epiphyte-grazer interactions (Fig. 3.6) that will be considered when analysing other data (chapter 6). As a consequence of the preliminary analysis of the data presented in this chapter, an experiment was proposed and performed (chapter 9) to demonstrate the impact of shading and high irradiance exposure on the condition of seagrasses at Shelly Beach.



**Fig. 3.6** A modification of the model of seagrass-epiphyte-grazer interactions (Fig. 1.1) to illustrate the impact of sea hare grazing on *Halophila ovalis*. In this case, light has a negative impact on the leaves. Epiphytic cover has a positive impact because it reduces damage by irradiance. Sea hares have a direct negative impact on epiphytes, but an indirect negative impact on the seagrass.

## **Chapter 4 Seagrass epifauna and their relationships to epiphytic materials**

### **4.1 Introduction**

The prevailing model of seagrass-grazer-epiphyte interactions assumes that epiphyte-grazing animals have a significant negative impact on the amount of epiphytic material on seagrasses as a result of their direct consumption of that epiphytic material (chapter 1). In this chapter that assumption is examined. Firstly, the composition and abundances of the epifaunal communities are described, and major potential grazers of epiphytic material are identified. Secondly, changes in epiphytic coverage and loads are described. And thirdly, the anticipated correlative relationships between probable epiphytic grazers and the amount of epiphytic material are examined. The description of the elements of the system and their possible interactions are just the first steps toward the final goal of determining if the system as a whole, as manifested in field data, can support the prevailing model (chapter 6).

The prevailing model of seagrass-grazer-epiphyte interactions is supported by a body of experimental work (Table 1.1) showing that invertebrate epiphyte grazers are capable of controlling the abundance of epiphytes. That conclusion is supported by a number of reviews of the existing evidence (Orth and van Montfrans 1984, van Montfrans et al. 1984, Jernakoff et al. 1996, Valentine and Duffy 2006), and by the meta-analysis of Hughes et al. (2004). The animals used in the experiments were primarily small gastropods, amphipods, and isopods. They are often the most abundant animals in seagrass meadows, and their primary role in seagrass systems is believed to be that of epiphyte grazers (Valentine and Duffy 2006). As well as controlling epiphyte abundance, they may also affect the composition of the epiphytic algal assemblage by selectively grazing the most palatable species (Duffy and Hay 2000), or those that adhere most loosely to leaf surfaces (van Montfrans et al. 1982). Epiphyte abundance can be affected by a number of abiotic bottom-up factors, such as light, temperature, water flow, and nutrients (Borowitzka et al. 2006). In polluted waters, nutrients are thought to stimulate algal growth that limits seagrass production (Silberstein et al.

1986, Tomasko and Lapointe 1991). However, experimental manipulations involving both nutrients and epiphyte grazers have shown that the latter were still capable of limiting epiphyte growth and maintaining seagrass production under enriched conditions (Neckles et al. 1993, Williams and Ruckelshaus 1993). The expectation therefore was that under a wide range of field conditions one should see evidence of the negative impacts of epiphyte grazers on the amount of epiphytic material.

## **4.2 Methods**

The assemblages of invertebrates associated with intertidal seagrass near Townsville were sampled in three plots over two years following the methods and procedures outlined in detail in chapter 2. Those methods were specifically designed to target the small animals, especially small gastropods and peracardian crustaceans, associated with seagrass leaves, which are believed to be the major grazers of epiphytic material in the system. Invertebrate abundances were expressed as numbers per area of leaf. Statistical analyses of invertebrate abundances were done on log-transformed data. Where ANOVAs are performed, they test differences among means of transformed data over the specified dates. The same leaves sampled for invertebrates were used to quantify the amount of epiphytic material, measured as dry weight (DW) and ash-free dry weight (AFDW) of material per area of leaf, and as the per cent coverage of the leaf surface. Methods used for quantifying the amount of epiphytic material are also described in chapter 2.

### ***4.2.1 MDS and ANOSIM***

Observation suggested that the assemblages of epifaunal invertebrates differ substantially among the study plots. Non-metric multidimensional scaling (MDS) was used to demonstrate those differences, followed by analysis of similarities (ANOSIM) to test their significance (Clarke 1993). Those analyses were performed using the Primer 6 software package (Clarke and Gorley 2006), which is designed for analysis of ecological data using non-parametric techniques. MDS and ANOSIM are based on the matrix of species similarity (or dissimilarity) values between each pair of samples. In MDS, similarity values are used in an iterative procedure to construct a plot of the

samples in a few dimensions (usually 2), so that distances among the samples in the reduced space is as close as possible to the relative differences in the original similarity values. The fit of the plotted distances to the original similarities is measured by the stress statistic, where low values indicate a good fit. ANOSIM uses the ranks of the original similarity values to test if there are significant differences in similarities among pre-determined groups of samples. A test statistic (R) is calculated from the differences in average rank similarities within and between groups of samples. Its significance level is then determined with respect to values calculated from a large number of random permutations of the data.

The analyses were performed on the data in Appendix A. Those data give mean numbers of animals per 100 cm<sup>2</sup> of leaf area for each identified taxa on each sampling occasion. The analyses used combined data from 79 sets of samples from each date/plot/seagrass species combination. That included 21 sampling dates for *Halodule uninervis* at plot SB (excluding the second sampling from June 2002); 17 for *H. uninervis* at each of plots CB and PB (excluding the extra sampling in May 2002 at plot CB); 18 for *Halophila ovalis* at plot SB; 6 for *H. ovalis* at plot CB. Invertebrate taxa that did not occur in at least 5% of those samples were excluded, so that the analyses involved 39 unique taxa. The 4<sup>th</sup> root transformation was applied to the invertebrate data to reduce the weight of the most abundant taxa. Species similarities between the samples were calculated using the Bray-Curtis coefficient (Bray and Curtis 1957), the most commonly used measure of species similarity in ecological studies. The relationship between the resulting MDS ordination and the abundances of individual invertebrate taxa was investigated by multiple regressions. The transformed abundances of individual taxa were regressed against the samples scores on the two coordinate axes. Those taxa which occurred in greater than 40% of the samples and had  $R^2 > 0.60$  were shown on the MDS ordination as vectors with lengths equal to their multiple correlation coefficients (R), and directions given by the partial correlation coefficients. A two-way ANOSIM was performed with samples classified by seagrass species within plots.



## 4.3 Results

### 4.3.1 Epifaunal communities

The composition and abundance of invertebrates varied greatly over time. The epifauna on *Halodule uninervis* also differed greatly among the three plots, and are described separately. The epifauna on *Halophila ovalis* tended to differ from that on *H. uninervis*, and those differences are described for plots SB and CB where data on both species were collected.

#### 4.3.1.1 Epifauna of *Halodule uninervis*

##### 4.3.1.1.1 Plot SB

The epifauna on *H. uninervis* leaves in plot SB was predominantly molluscs, particularly the small gastropod, *Alaba virgata* (Philippi) (family Litiopidae), and the bivalve, *Electroma* sp. (family Pteriidae) (Table 4.1). The latter comprised 58% of the 1,495 animals collected from *H. uninervis* samples over two years, of which 92% were collected in the first 6 months. *Electroma* reached an extreme density of 10.6 cm<sup>-2</sup> leaf in October 2002, but was absent or rare on many sampling dates in 2003/4 (Fig. 4.1). *A. virgata* contributed 26% of animals collected on *H. uninervis*. Its population also varied considerably throughout the study. It was sometimes absent in samples, but it was always present in the meadow. *A. virgata* reached a maximum density of 2.7 cm<sup>-2</sup> leaf, also in October 2002, and had significant peaks (Tukey comparisons) in March 2003, September to October 2003, and August 2004 (ANOVA,  $F=15.2$ ,  $p<0.0001$ ). Densities of *A. virgata* and *Electroma* were positively correlated ( $r=0.55$ ,  $p<0.01$ ).

##### 4.3.1.1.2 Plot CB

Mean epifaunal densities were greater in plot CB than in other plots (Table 4.1). Over two years, 3,980 epifaunal animals were collected from *Halodule uninervis* leaf samples, at an overall mean density of 3.67 cm<sup>-2</sup> leaf. The most abundant animals were Amphipoda, comprising 51% of collected animals. *Ericthonius* sp. was the most abundant of those, with 36% of animals, and was present on all sampling dates. It reached peak densities of 8.1 cm<sup>-2</sup> leaf in April 2003, and 4.2 cm<sup>-2</sup> leaf in May 2004. The second most abundant amphipod was *Podocerus* sp., with 5% of animals. It had densities that were highly correlated with those of *Ericthonius* ( $r=0.78$ ,  $p<0.001$ ). In

contrast with plot SB, *Electroma* and *Alaba virgata* were uncommon in plot CB (Table 4.1).

**Table 4.1 Summary of the mean densities of major invertebrate taxa averaged over all sampling dates, presented as the number of animals per 100 cm<sup>2</sup> of leaf surface. Note that data from plot SB includes two sampling dates 11 days apart in June 2002. For more details see Appendix A.**

	Plot SB		Plot CB		Plot PB
	<i>H. uninervis</i>	<i>H. ovalis</i>	<i>H. uninervis</i>	<i>H. ovalis</i>	<i>H. uninervis</i>
No. sample dates	22	18	17	6	17
No. individuals	1495	380	3930	384	2017
Total Polychaeta	2.4	3.0	19.2	19.8	9.8
Total Mollusca	208.3	26.9	14.4	28.8	38.2
Gastropoda					
<i>Alaba</i> spp	59.3	7.8	5.8	0.0	11.0
Other Gastropoda	9.4	16.9	8.5	27.0	9.1
Bivalvia					
<i>Electroma</i>	139.5	1.8	0.1	1.0	18.1
Total Crustacea	17.1	18.4	218.6	83.9	80.9
Amphipoda					
<i>Erichthonius</i>	7.1	10.4	142.0	30.4	44.3
<i>Podocerus</i>	0.0	0.0	18.1	2.5	19.2
Other Amphipoda	9.3	4.9	32.9	18.4	9.6
Other Peracarida	0.7	3.1	24.1	32.6	7.7
Actiniaria	1.6	13.3	103.4	72.1	0.4
Other Epifauna	5.0	2.1	11.5	3.9	6.9
Total Epifauna	234.5	63.7	367.1	208.5	136.2

Plot SB - Epifauna

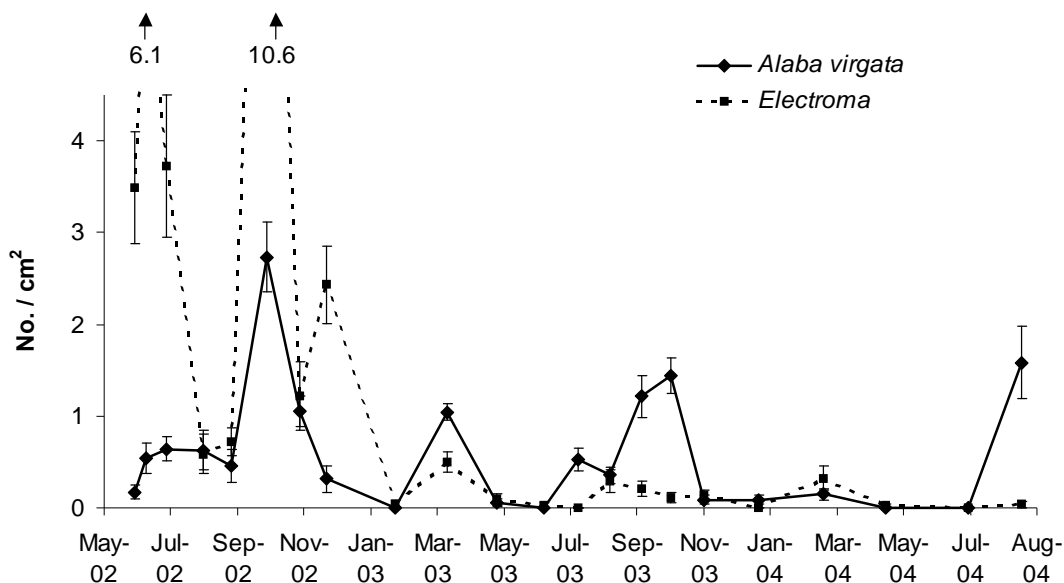
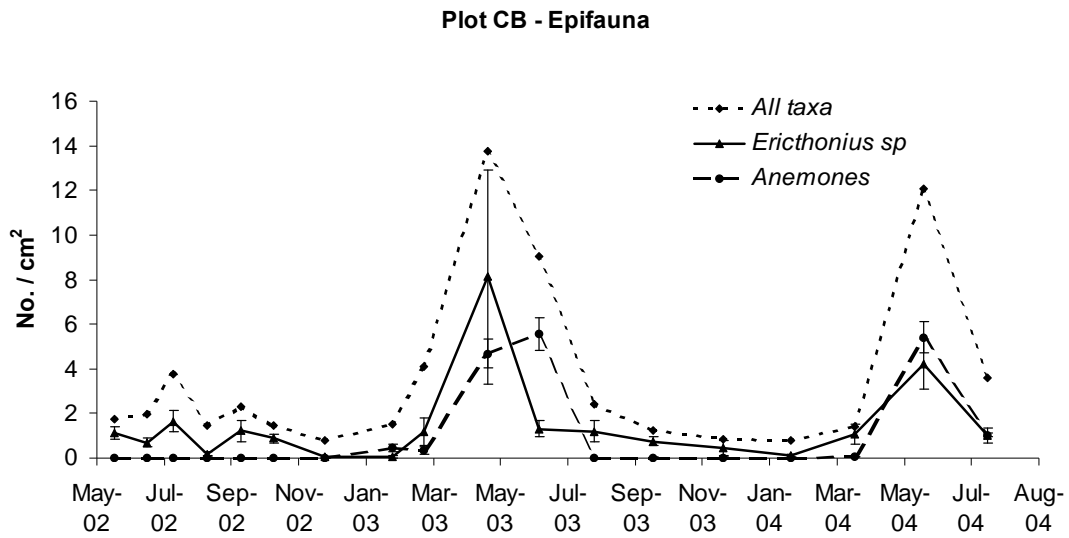


Fig. 4.1 Mean ( $\pm$  s.e.) densities of *Electroma* and *Alaba virgata* on *Halodule uninervis* leaves in plot SB.

Anemones were 29% of all animals collected from *Halodule uninervis* leaves over two years in plot CB. In 2003, at both Cockle Bay and Shelly Beach, the dominant anemone was *Bolocerooides mcmurrichi* (Kwietniewski), with small numbers of a second species, *Bunodeopsis australis* Haddon. In 2004, the relative abundances of the two species were reversed. Anemones reached very high densities in plot CB in April and June 2003, 4.8 and 5.5 cm<sup>-2</sup> leaf respectively; and again in May 2004, 5.4 cm<sup>-2</sup> leaf. In contrast, anemones were not found in sampling in May and June 2002 (Fig. 4.2). Peak densities of amphipods coincided with peak densities of anemones, and their densities were correlated ( $r=0.56$ ,  $p<0.025$ ).

The extremely high densities of anemones from April to June 2003 were followed by their total disappearance in July. Large numbers of an aeolid nudibranch, *Limenandra fusiformis* (Baba), which feeds on anemones, were found at that time. Its numbers were estimated from counts in quadrats at  $47.0 \pm 7.7$ (s.e.) m<sup>-2</sup> substrate. Only a few individuals of that species were seen in July 2004.

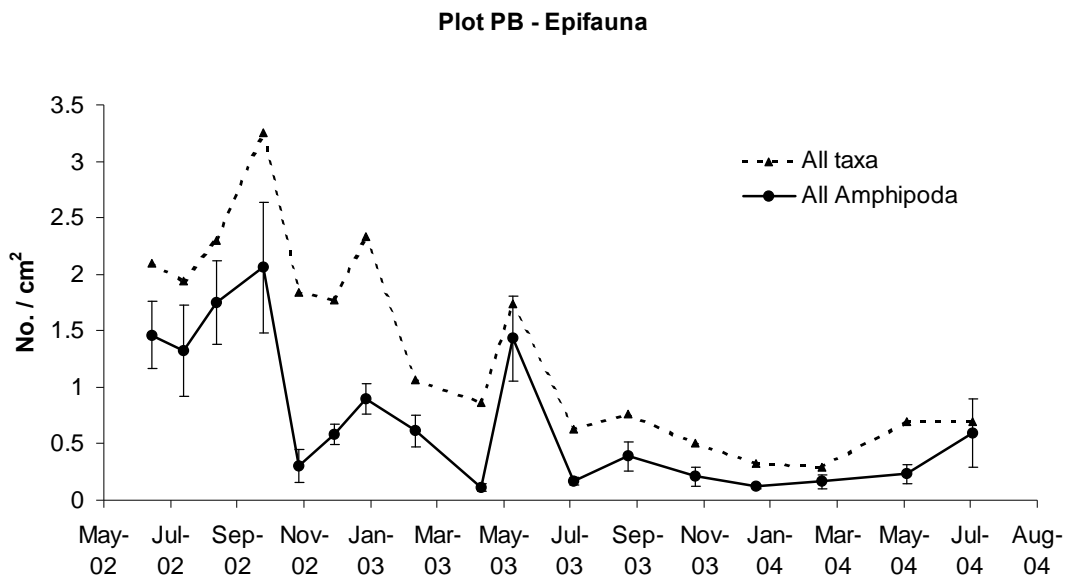


**Fig. 4.2** Mean ( $\pm$  s.e.) densities of *Ericthonius* sp. and anemones on *Halodule uninervis* leaves in plot CB.

#### 4.3.1.1.3 Plot PB

Overall mean epifaunal densities on *Halodule uninervis* were least in plot PB, at 1.36 cm<sup>-2</sup> leaf (Table 4.1). Densities declined during the two years of the study (Fig. 4.3). They were highest over the first four sampling dates in 2002, averaging 2.40 cm<sup>-2</sup> leaf. In contrast, they were only 0.56 cm<sup>-2</sup> leaf over the second year, from July 2003 to July 2004. Samples taken after the termination of the ferry service, from October 2003 to July 2004 (n=5), had significantly lower densities, 0.51 cm<sup>-2</sup> leaf, than samples from over the same period of the previous year (n=8), 1.69 cm<sup>-2</sup> leaf (contrast  $t=-9.33$ ,  $p<0.0001$ ).

The fauna in plot PB was dominated by Amphipoda, with 49.5% of 2,017 animals collected. The dominant amphipod was *Ericthonius* sp., with 27% of all animals; but *Podocerus* sp. was also common, with 14%. As in plot CB, densities of those two amphipods were highly correlated ( $r=0.76$ ,  $p<0.001$ ). *Alaba* spp. and *Electroma* sp. were 7.1% and 10.3% of animals collected; and as in plot SB, their densities were correlated ( $r=0.55$ ,  $p<0.025$ ).



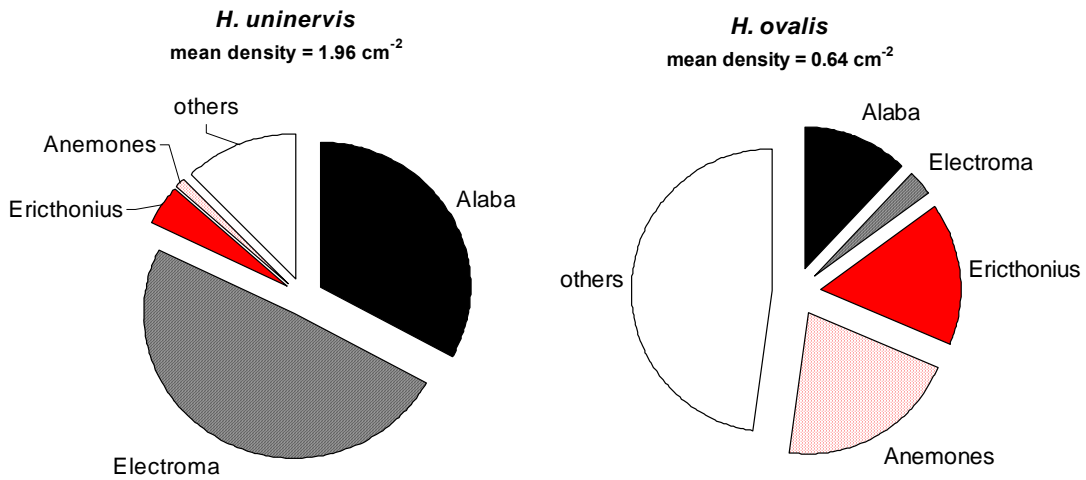
**Fig. 4.3** Mean ( $\pm$  s.e.) densities of Amphipoda on *Halodule uninervis* leaves in plot PB.

#### 4.3.1.2 Comparison with *Halophila ovalis*

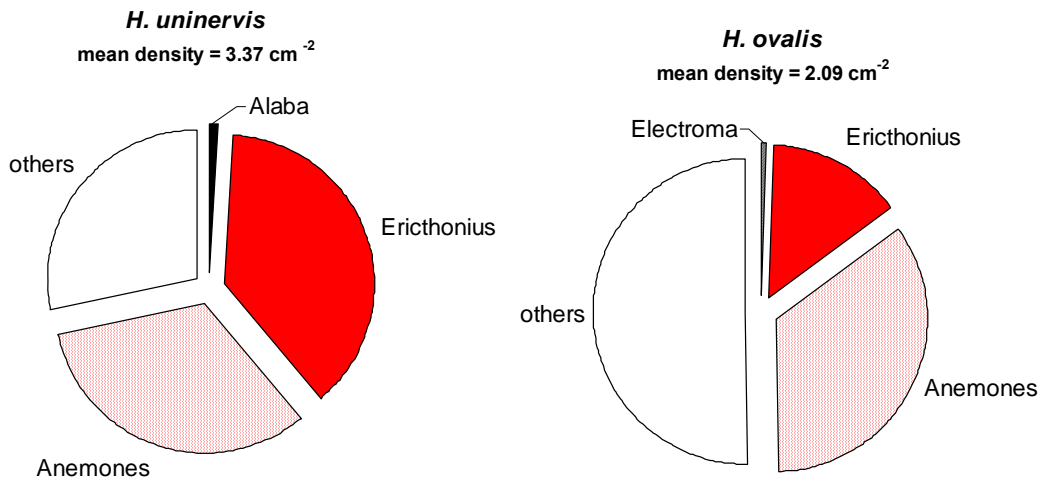
In both plots SB and CB, *Halophila ovalis* leaves supported fewer epifaunal invertebrates than *Halodule uninervis* leaves. In plot SB, only 380 animals were collected from *H. ovalis* samples on 18 sampling dates, at an overall mean density of 0.64 cm<sup>-2</sup> leaf. *H. uninervis* leaves on the same dates had a mean density of 1.96 cm<sup>-2</sup>. Differences in epifauna densities between the seagrass species in plot SB were significant (paired t-test,  $t=3.0$ ,  $p<0.01$ ). In plot CB, epifaunal densities on *H. ovalis* and *H. uninervis* were 2.1 cm<sup>-2</sup> and 3.4 cm<sup>-2</sup> respectively, averaged over the 6 dates on which leaf samples were taken of both species. Those differences were not statistically significant (paired t-test).

In plot SB, there were striking differences in the composition of the epifaunal communities associated with *Halodule uninervis* compared with *Halophila ovalis* (Fig. 4.4). *Electroma* and *Alaba virgata* were much more abundant on *H. uninervis* than on *H. ovalis* leaves. In contrast, *Erichthonius* and anemones (Actiniaria) were more abundant, and dominant, on *H. ovalis* in plot SB. In plot CB, *Erichthonius* and anemones were dominant on both species of seagrass, and *Electroma* and *A. virgata* uncommon (Fig. 4.4).

**Plot SB (n=18)**



**Plot CB (n=6)**



**Fig. 4.4** Comparison of the mean densities of major taxa as a proportion of the total density of animals on *Halodule uninervis* and *Halophila ovalis* leaves over 18 dates in plot SB and 6 dates in plot CB on which both seagrass species were sampled.

**4.3.1.3 MDS and ANOSIM**

The MDS ordination showed strong separation of the three plots and the seagrass species on the basis of their invertebrate community compositions (Fig. 4.5). The first axis separated plot SB samples and the samples of the other two plots. The second axis separated plot CB and PB samples, and separated *Halophila ovalis* and *Halodule uninervis*. The two-way ANOSIM found strong separation between the plots adjusted for seagrass species ( $R=0.63$ ,  $p<0.001$ ), and between the species adjusted for plot ( $R=0.35$ ,  $p<0.001$ ). The commonly occurring invertebrate taxa that were positively correlated with the first axis of the MDS ordination were all crustaceans, while those

negatively correlated with that axis were all molluscs. The crustaceans were associated with *H. uninervis* samples from plots CB and PB, and included the two suspension-feeding amphipods, *Erichthonius* and *Podocerus*; and two herbivores, the amphipod *Cymadusa*, and sphaeromatid isopods, which were mostly *Cymodoce*. The molluscs were separated on the second axis so the *Alaba virgata* and *Electroma* were associated with *H. uninervis* from plot SB, while Rissoidae sp 1 (a very tiny gastropod) was associated with *H. ovalis* from plot SB.

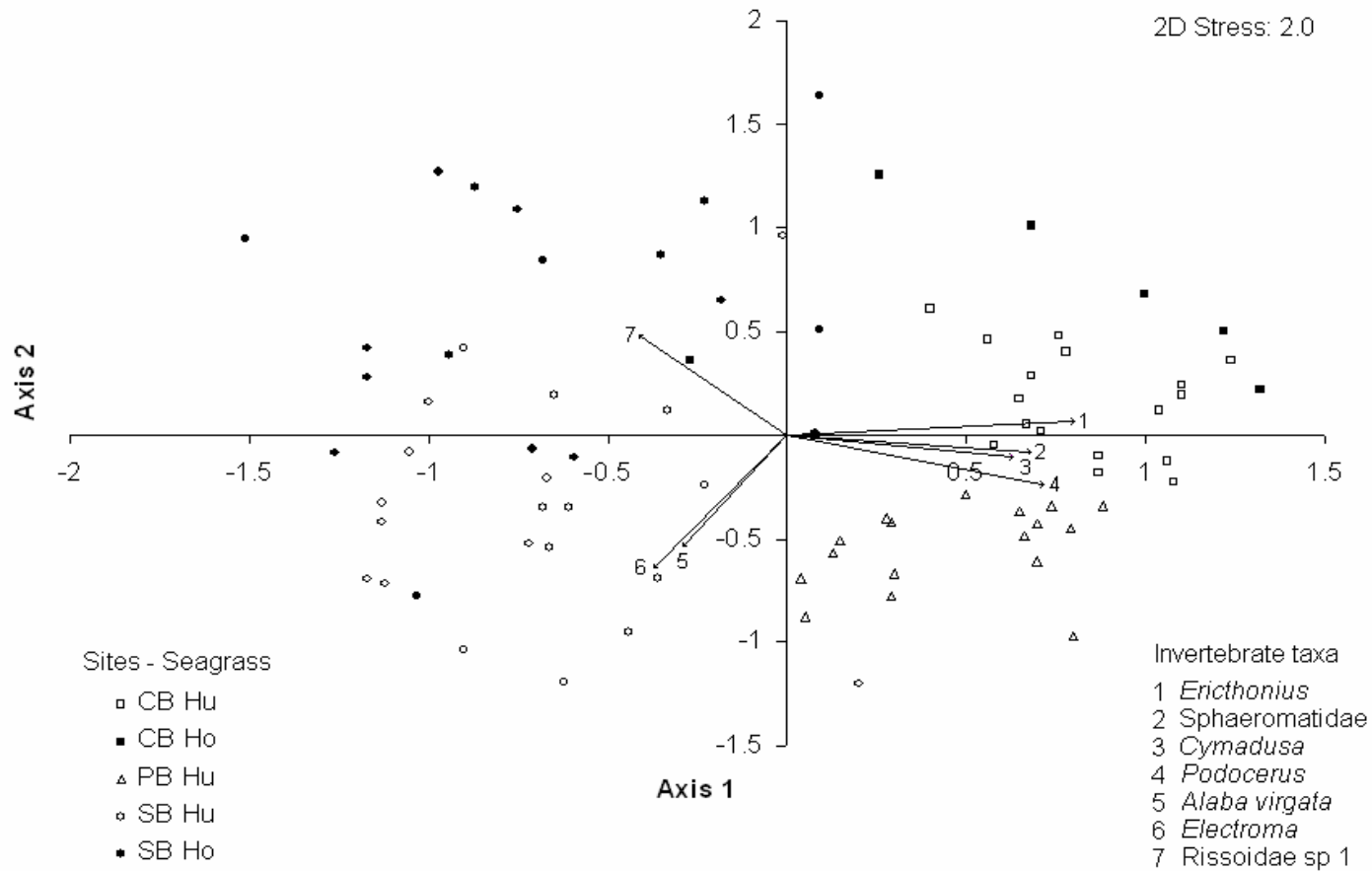
### **4.3.2 Epiphytic material**

#### **4.3.2.1 Adjustments to AFDW measurements**

Samples of filtered seawater in 250 ml and 70 ml containers had average background AFDWs of 0.6 and 0.4 mg respectively. Samples of combusted filtrate that were re-wetted and re-dried gained weight, due to the water of hydration, equal to approximately 10% of AFDW. Tests of the muffle ovens showed that sucrose was completely combusted at 500°C. Therefore, the final estimate of AFDW used in the analyses is 90% of the raw AFDW minus the background AFDW. In calculating the ratio AFDW:DW, the raw AFDW was used.

#### **4.3.2.2 Plot SB**

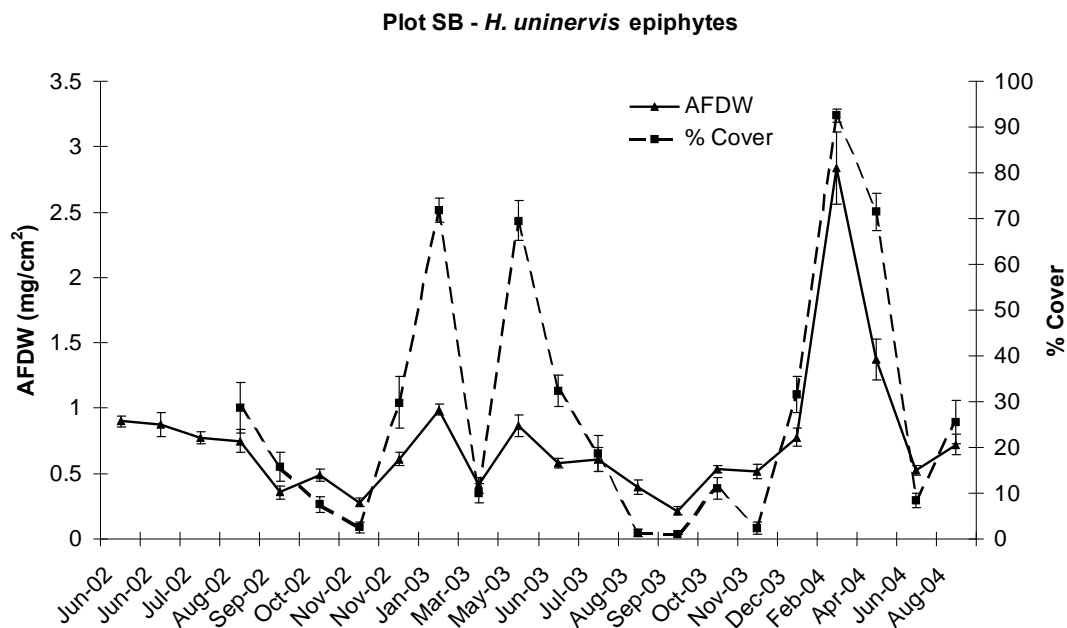
The AFDW of epiphytic material and the percentage epiphytic cover of the leaves showed very similar patterns of change. The two measures were highly correlated for both *H. uninervis* ( $r=0.84$ ,  $n=19$ ,  $p<0.001$ ) and *H. ovalis* ( $r=0.91$ ,  $n=18$ ,  $p<0.001$ ) in plot SB. Both measures of epiphytic loads were highly variable over time, on both seagrass species (Fig. 4.6 and Fig. 4.7) (all ANOVAs highly significant,  $p<0.0001$ ), and often changed significantly between successive sampling dates (Tukey comparisons,  $p<0.05$ ). However, the two measures sometimes differed in their performance. The percentage cover estimates detected significant differences between samples with 20-30% cover and those with almost no cover. AFDW measures did not differ significantly at those times. However, at times of very high cover, measures based on the weight of epiphytic material may better distinguish between thin and thickly layered epiphytic loads.



**Fig. 4.5 Non-metric multidimensional scaling of invertebrate communities on 79 sample dates among three plots (CB, PB, SB) and two seagrass species (Hu=Halodule uninervis, Ho=Halophila ovalis). Vector lengths and angles correspond to the coefficients of correlations between the transformed abundance of the specified invertebrate taxa in each sample and the sample's value on the coordinate axes.**



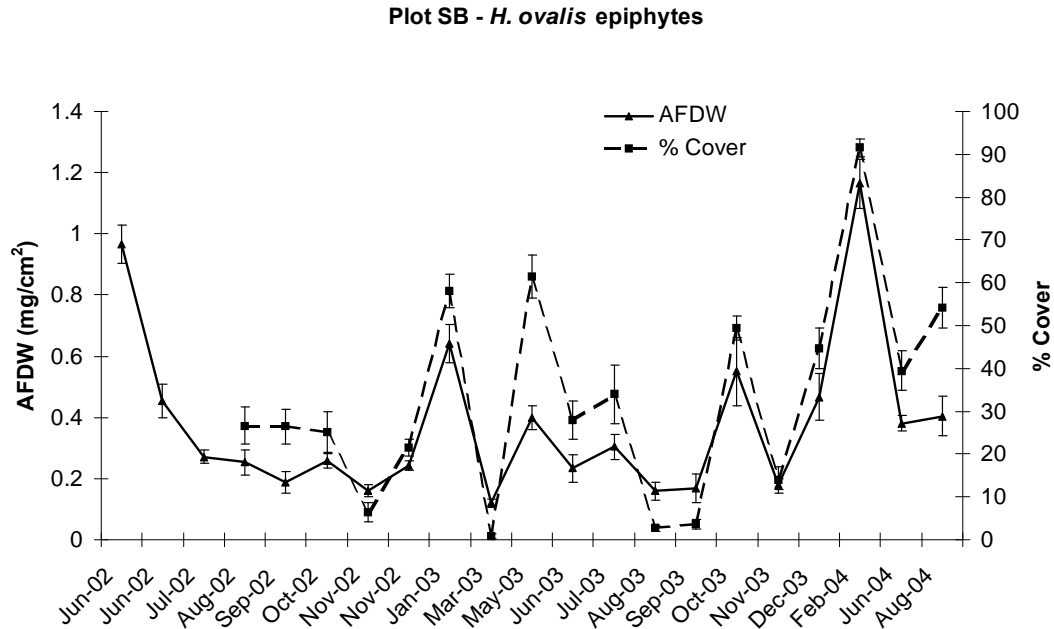
Epiphytic loads on *H. uninervis* leaves were much higher in February 2004 compared to any other sampling date in plot SB. High percent cover estimates were also obtained in January and May 2003 (Fig. 4.6), but AFDWs were about a third of the levels reached in February 2004. At Shelly Beach, the dominant epiphytic alga during those summer peaks in epiphytic cover was the centric diatom *Mastogloia* sp., which spreads as a broad, thin layer of cells, with each cell typically surrounded by a mucilaginous capsule. In February 2004, the layers were thicker, and included large amounts of soft flocculent material.



**Fig. 4.6** Mean AFDW and percent cover ( $\pm$  s.e.) of epiphytic material on *H. uninervis* leaves from plot SB. Epiphytic material was sampled twice in June 2002, 11 days apart. Two samples in November 2002 are early and late in the month.

Changes in percent cover and AFDW of epiphytic material on *Halophila ovalis* leaves (Fig. 4.7) usually reflected changes on *Halodule uninervis* leaves (Fig. 4.6). Strong peaks in those variables occurred on both species in February 2004, and January and May 2003. However, there was also a significant peak in epiphytic load due to a bloom of filamentous blue-green algae in October 2003 on *H. ovalis* that was not evident on *H. uninervis* (as also found at the same time in experimental shading plots, chapter 9). The most conspicuous difference between the two species occurred in June 2002, when loads on *H. ovalis*, but not *H. uninervis*, were reduced in association with an incursion

of sea hares (see chapter 3). The data for AFDW from June to September in Fig. 4.6 and Fig. 4.7 are the same as those already presented in Fig. 3.1.



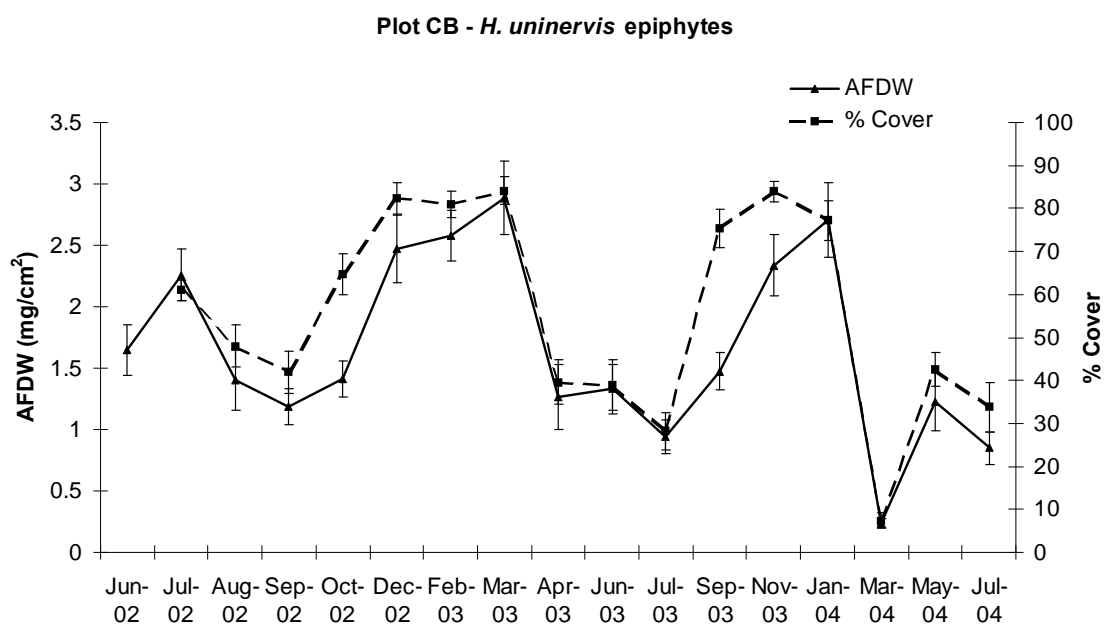
**Fig. 4.7** Mean AFDW and percent cover ( $\pm$  s.e.) of epiphytic material on *H. ovalis* leaves from plot SB. Epiphytic material was sampled twice in June 2002, 11 days apart. Two samples in November 2002 are early and late in the month.

The mean DW of epiphytic material on leaves of *Halodule uninervis* and *Halophila ovalis* averaged over two years were very similar, 5.27 mg cm<sup>-2</sup> and 5.12 mg cm<sup>-2</sup> respectively, and did not differ significantly on the same sampling dates (paired t-test,  $t=0.3$ ,  $n=21$ ,  $p>0.10$ ). However, the overall mean AFDW of epiphytic material was significantly higher on *H. uninervis* leaves than on *H. ovalis* leaves, 0.71 mg cm<sup>-2</sup> and 0.39 mg cm<sup>-2</sup> respectively (paired t-test,  $t=4.3$ ,  $p<0.001$ ). The ratio AFDW:DW, was greater on *H. uninervis* leaves than on *H. ovalis* leaves on every sampling date, with overall mean ratios 0.21 and 0.13 respectively (paired t-test,  $t=8.7$ ,  $p<0.001$ ). It should be noted that an unexpected precipitation of salts (see chapter 2) was more likely to contribute to DW of *H. uninervis* than *H. ovalis* because the former had been frozen in a larger volume of salt water, which should have reduced its ratio AFDW:DW.

#### 4.3.2.3 Plot CB

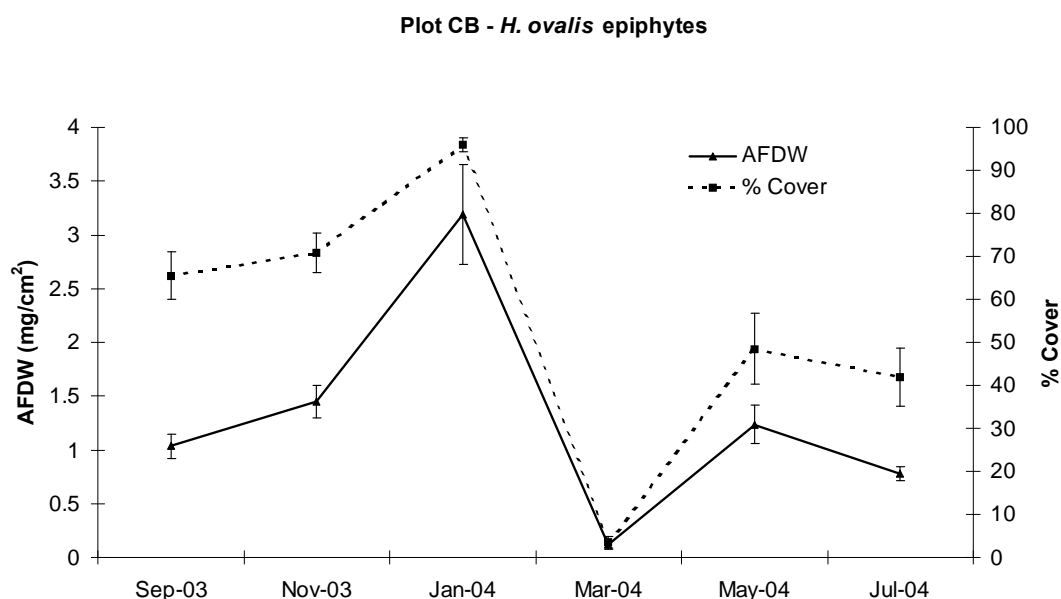
Very high epiphytic loads occurred in plot CB, with AFDW  $>2.0$  mg cm<sup>-2</sup> in July 2002, and from December 2002 to March 2003, and again from November 2003 to January

2004 (Fig. 4.8). In contrast, AFDW  $>2.0 \text{ mg cm}^{-2}$  occurred only once in plot SB. The extreme AFDWs in February and March 2003 were due to a bloom of the large centric diatom *Leptocylindricus* sp. In July 2002, there was an abundance of pinnate diatoms and red algae, especially *Ceramium* spp. However, at other times of high AFDW, December 2002, November 2003, and January 2004, most of the epiphytic load was soft flocculent material. AFDW of epiphytic material and percent epiphytic cover estimates were highly correlated in plot CB ( $r=0.91$ ,  $n=16$ ,  $p<0.001$ ).



**Fig. 4.8** Mean AFDW and percent cover ( $\pm$  s.e.) of epiphytic material on *H. uninervis* leaves from plot CB.

AFDW of epiphytic material and percent epiphytic cover on *Halophila ovalis* leaves followed the same pattern seen on *Halodule uninervis* leaves for the six sampling dates on which both were sampled. Extremely heavy AFDW,  $>3.0 \text{ mg cm}^{-2}$ , consisting of dense masses of flocculent material, occurred in January 2004, but was followed by a highly significant fall in epiphytic loads to March (Fig. 4.9). DW and AFDW of epiphytic material on the two seagrass species did not differ significantly on the six dates (paired t-tests,  $p>0.10$ ), but the ratio AFDW:DW was always greater on *H. uninervis* leaves (paired t-test,  $t=2.9$ ,  $p<0.05$ ).



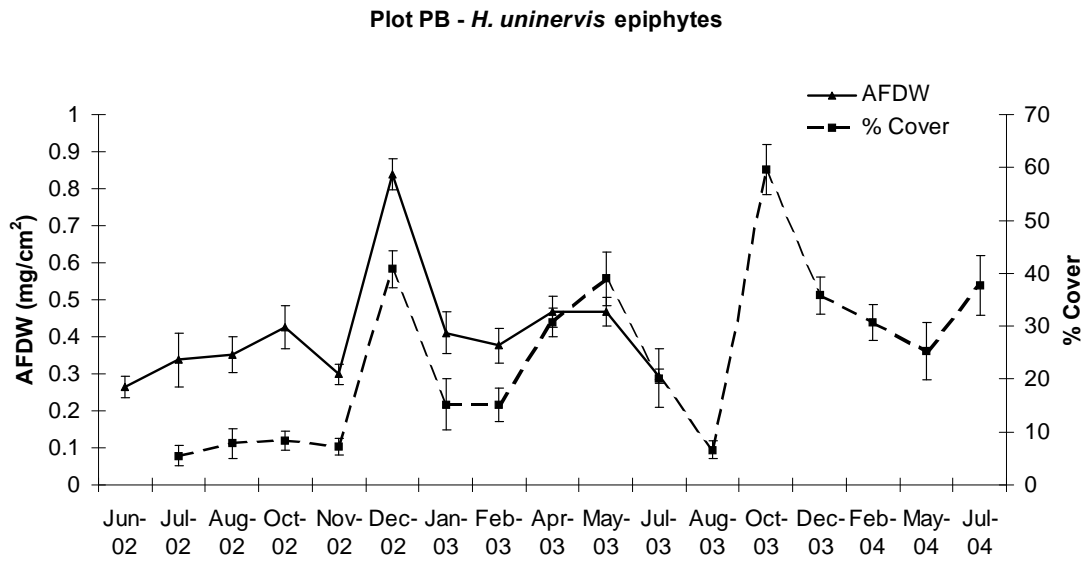
**Fig. 4.9** Mean AFDW and percent cover ( $\pm$  s.e.) of epiphytic material on *H. ovalis* leaves from the plot CB.

#### 4.3.2.4 Plot PB

Percent epiphytic cover began low, but rose over the two years of the study in plot PB (Fig. 4.10). In the earliest samples, from June to November 2002, percent epiphytic cover was consistently  $<10\%$ . In the final samples from December 2003 to July 2004, epiphytic cover was moderate, 25 to 38%. The greatest epiphytic cover was in October 2003, and was related to an increase in soft flocculent material on the leaves. In general however, there was less loosely adhering material on leaves in plot PB than in the other plots, and the commonest epiphytes were crustose coralline algae.

AFDW of epiphytic material and percent epiphytic cover were correlated ( $r= 0.74$ ,  $p<0.025$ ) for the dates on which both measures were obtained ( $n=10$ ). Epiphytic cover increased in the second year, when AFDW measurements were not made, but the correlation suggested that AFDW remained below  $1.0 \text{ mg cm}^{-2}$ , except in October 2003. Epiphytic cover never obtained the extremely high values seen in plots SB or CB.

Samples from October 2003 to July 2004 ( $n=5$ ), taken after the termination of ferry service to Picnic Bay, had significantly higher percent epiphytic cover, mean 37.8%, than samples over the same months in the previous year ( $n=8$ ), mean 22.1% (contrast  $t=7.15$ ,  $p<0.0001$ ).



**Fig. 4.10** Mean AFDW and percent cover ( $\pm$  s.e.) of epiphytic material on *H. uninervis* leaves from plot PB.

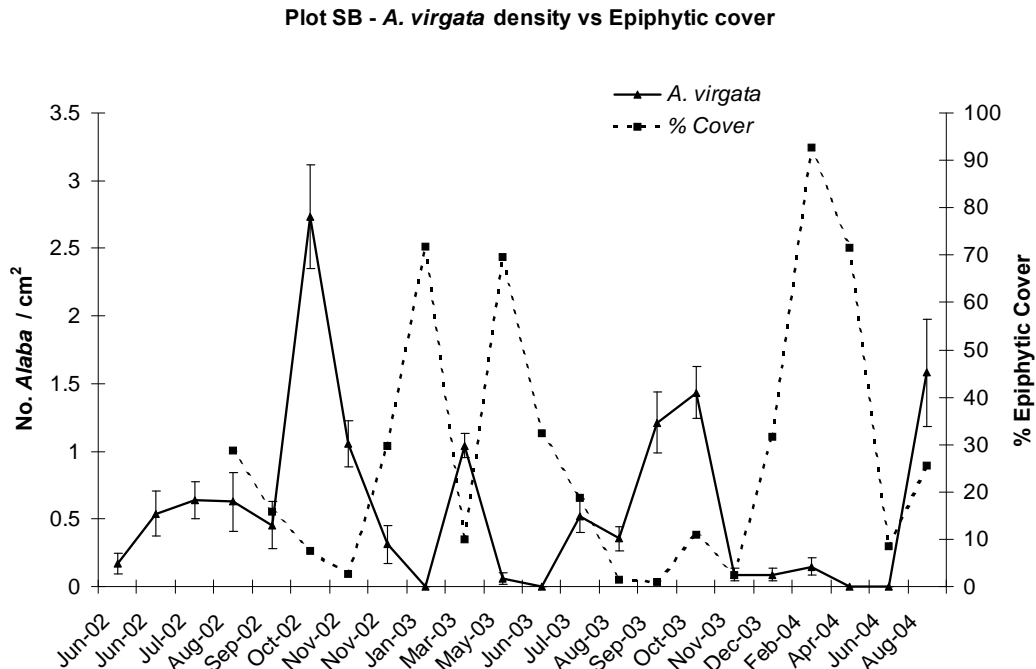
### 4.3.3 Relationships between epiphytic loads and invertebrate densities

#### 4.3.3.1 Plot SB

The small gastropod *Alaba virgata* was the most abundant potential epiphyte grazer in Plot SB. The percentage of *Halodule uninervis* leaf surfaces covered by epiphytic material in plot SB was inversely related to the density of *Alaba virgata* on the leaves ( $r=-0.51$ ,  $p<0.025$ ). Peaks in the abundance of *A. virgata* occurred when epiphytic cover was lowest (Fig. 4.11). However, the densities of *Alaba virgata* was not correlated to AFDW of epiphytic material on *H. uninervis* leaves ( $r=-0.30$ ,  $p>0.10$ ), which probably reflected the poor sensitivity of the AFDW measure to small changes in epiphytic cover at the lower value range, where *A. virgata* densities had their greatest changes. On *Halophila ovalis* leaves, densities of *A. virgata* were much lower than on *H. uninervis* leaves (Table 4.1), yet their densities were also negatively correlated to the percent cover by epiphytic material ( $r=-0.53$ ,  $p<0.025$ ).

A small number of fecal pellets were examined from *Alaba virgata* collected at Shelly Beach. They contained some of the most common epiphytes on *Halodule uninervis*.

Intact frustules of *Mastogloia* sp., fragments of other diatoms, and the blue-green alga *Calothrix crustacea* were found.



**Fig. 4.11** Mean ( $\pm$  s.e.) densities of *Alaba virgata* and mean percent cover of epiphytic material on *H. uninervis* leaves from plot SB. Samples were taken twice in June 2002, 11 days apart. Two samples in November 2002 are early and late in the month.

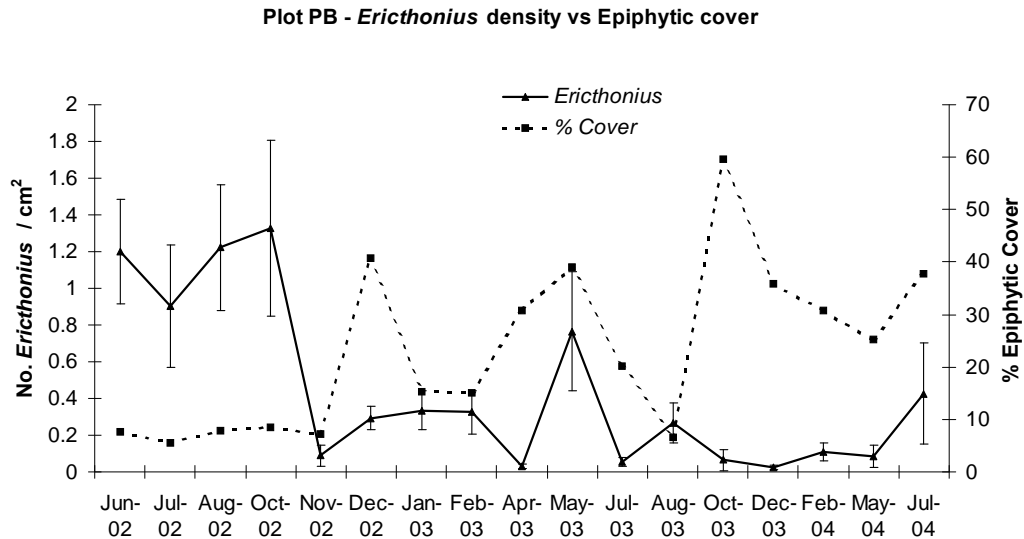
#### 4.3.3.2 Plot CB

Amphipoda were the most abundant animals on seagrass leaves in Plot CB. Numbers on *Halodule uninervis* increased in association with a decline of epiphytic cover from extremely high levels in March 2003, and were also associated with increasing numbers of anemones. Over two years, densities of *Erichthonius* were inversely correlated to the percent epiphytic cover ( $r=-0.55$ ,  $p<0.025$ ), and AFDW ( $r=-0.53$ ,  $p<0.05$ ). Densities of *Podocerus*, the second most abundant amphipod, were very strongly, and inversely correlated with epiphytic cover ( $r= -0.80$ ,  $p<0.001$ ), and AFDW ( $r= -0.73$ ,  $p<0.001$ ).

#### 4.3.3.3 Plot PB

Densities of invertebrates in plot PB were greatest during the period June to November 2002, when percent epiphytic cover was consistently low,  $<10\%$  (Fig. 4.12). The percent epiphytic cover on *Halodule uninervis* leaves was not significantly related to

densities of *Erichthonius* ( $r=-0.47$ ,  $0.05 < p < 0.10$ ), but was inversely correlated to densities of *Podocerus* ( $r=-0.54$ ,  $p < 0.05$ ), *Alaba* spp. ( $r=-0.71$ ,  $p < 0.001$ ), and *Electroma* ( $r=-0.61$ ,  $p=0.01$ ).



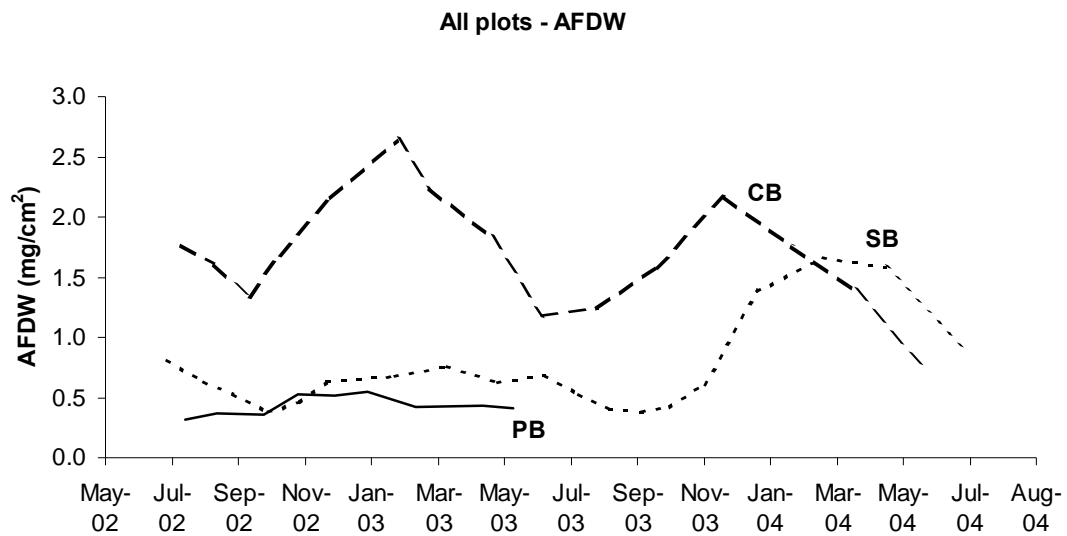
**Fig. 4.12** Mean ( $\pm$  s.e.) densities of *Erichthonius* and mean percent epiphytic cover on *H. uninervis* leaves in plot PB.

#### 4.3.4 Comparisons among plots

Highest loads of epiphytic material were found on seagrass leaves from plot CB. Over two years, the mean AFDW of epiphytic material on *Halodule uninervis* leaves in that plot was more than twice that on *H. uninervis* leaves in plot SB (Table 4.2). Only at the end of the study period did AFDW increase in plot SB to values comparable to those in plot CB (Fig. 4.13). Plot PB had the lowest epiphytic loads. Mean AFDW of epiphytic material sampled from plot PB, from June 2002 to July 2003 inclusive, was  $0.41 \pm 0.05$ (s.e.)  $\text{mg cm}^{-2}$ ; compared to means of  $0.65 \pm 0.06$ (s.e.)  $\text{mg cm}^{-2}$  and  $1.76 \pm 0.20$ (s.e.)  $\text{mg cm}^{-2}$  for plots SB and CB respectively over the same period.

**Table 4.2 Mean measures of epiphytic load on *Halodule uninervis* leaves over the course of the study.**

Plots	SB	CB	PB
% Epiphytic cover ( $\pm$ s.e.)	29.3 ( $\pm$ 5.9)	55.8 ( $\pm$ 5.0)	23.1 ( $\pm$ 3.5)
n	21	17	17
AFDW ( $\text{mg cm}^{-2}$ ) ( $\pm$ s.e.)	0.74 ( $\pm$ 0.12)	1.66 ( $\pm$ 0.16)	0.41 ( $\pm$ 0.05)
n	21	17	11



**Fig. 4.13 AFDW of epiphytic material on *Halodule uninervis* leaves calculated as 3-point moving averages in each plot.**

Plot CB also had the highest density of invertebrates per leaf area, and plot PB the lowest (Table 4.1). So comparisons among plots might suggest a positive association between epiphytic loads and epifaunal abundances. However, comparisons within plots were consistent in showing that the animals most suspected of being epiphyte grazers had abundances negatively correlated to epiphytic loads.

#### 4.4 Discussion

The dominant taxa on intertidal seagrass leaves were the small gastropod *Alaba virgata*, the bivalve *Electroma* sp., the amphipod *Ericthonius* sp. and anemones (Actiniaria). The molluscs dominated at Shelly Beach, while peracarid crustaceans dominated at Picnic Bay and Cockle Bay. Elsewhere, seagrass invertebrate fauna has



also been dominated by small gastropods and peracarid crustaceans (Valentine and Duffy 2006). The gastropods have been epiphyte grazers (van Montfrans et al. 1982, Mazzella and Russo 1989), and experimental manipulations of those animals show that they can limit the development of epiphytic cover on leaves (Robertson and Mann 1982, Philippart 1995, Nelson 1997, Fong et al. 2000). Likewise, many amphipods in seagrass meadows are epiphyte grazers (Zimmerman et al. 1979), and have been found to limit epiphyte development (Caine 1980, Howard 1982, Duffy and Harvilicz 2001). The prevailing model of seagrass-epiphyte-grazer interactions suggests that those animals control the production of epiphytic algae (chapter 1). Thus, the finding that *A. virgata* and amphipods generally dominated the epifaunal assemblage was consistent with expectations. As well, the finding of negative correlations between measures of the amount of epiphytic material on seagrass leaves and abundances of *A. virgata*, and the amphipods *Erichthonius* and *Podocerus*, were consistent with the hypothesis that they limit the development of epiphytes. However, the correlations are only supportive of that hypothesis and need to be evaluated in light of other evidence. As well, the amount of variation in measures of epiphytic material that were explained by those correlations tended to be low, even when significant. Unfortunately, little is known about the general biology of the animals found to dominate the epifauna in this study. Consideration of the limited information available on related animals, and observations of each of the major taxa, suggest that the relationships those animals have to epiphytic loads may be more complex than the simple epiphyte-grazer relationship that had been initially assumed.

#### **4.4.1 *Alaba virgata***

*Alaba virgata* was the most abundant gastropod encountered in this study. It has been found at high densities on seagrasses elsewhere, in the Gulf of Thailand (Swennen et al. 2001), and on the Andaman Sea coast of Thailand (personal observations). Despite its potential importance, nothing has been published on its biology. Examination of a small number of fecal pellets confirmed a suspicion that it is an epiphyte grazer.

In plot SB, percent epiphytic cover on *Halodule uninervis* was inversely related to densities of *Alaba virgata*. At very high densities,  $>1 \text{ cm}^{-2}$ , the feeding activity of *A.*

*virgata* was likely sufficient to explain the lack of epiphytic cover. However, when extremely high epiphytic loads occurred, there was no evidence of a positive numerical response from *A. virgata*, and the eventual decline of epiphytic cover cannot be attributed to increasing *A. virgata* numbers. Rather, sea hares were more likely to have been responsible for reducing epiphytic loads from its highest levels in 2002 and 2003. Once epiphytic loads were reduced to levels too low to support sea hares, *A. virgata* became more abundant, and likely reduced loads further.

*Alaba virgata* densities were also negatively correlated with percent epiphytic cover on *Halophila ovalis* leaves in plot SB and on *Halodule uninervis* leaves in plot PB; yet their densities were many times lower compared with densities on *H. uninervis* in plot SB (Table 4.1). Such correlations could arise for other reasons than the direct consumption of epiphytes. It is possible the *A. virgata* is more successful on leaf surfaces with low epiphytic cover because of differences in the type and quality of food resources. Such a situation would arise if *A. virgata* used clean leaf surfaces as a substrate for mucous feeding. Some gastropods use mucous trails to trap organic material or to stimulate the growth of microalgal food sources (Connor and Quinn 1984). Ingesting such enriched mucous can make a significant contribution to the nutrition of those gastropods. Holmes et al. (2001) estimated that the matter trapped in pedal mucous applied by *Calliostoma zizyphinum* (L.) to its own shell, supplied approximately one-fifth of its daily energy requirements.

Seagrass leaves would seem to be an ideal surface for the application of mucous for the purpose of trapping organic material, or promoting algal growth, for gastropod consumption. Davies and Beckwith (1999) showed that the intertidal periwinkle *Littorina littorea* (L.) fed on mucous trails enriched with microalgae. A related species, *Littorina neglecta* Bean, is an important epiphyte grazer on the leaves of *Zostera marina* in Nova Scotia (Robertson and Mann 1982). Fenchel et al. (1975) also suggested that *Hydrobia ulvae* (Pennant) ingests mucous that it uses to trap microorganisms. That species is a major epiphyte grazer in northern European *Zostera* meadows (Hootsmans and Vermaat 1985, Philippart 1995, Schanz et al. 2002). In Florida, the most abundant epifaunal animal found by Virnstein and Howard (1987a) on seagrasses was the gastropod *Crepidula convexa*, which likely uses mucous on its

gill lamella for suspension feeding (Chaparro et al. 2002). Despite its potential importance, the use of mucous for suspension feeding by gastropods in seagrass meadows has never been investigated. Such alternative feeding strategies could free gastropods from simply following the boom and bust of algal growth, and could potentially make them more effective controllers of algal growth. *Alaba virgata*, like many other small gastropods, produces sticky mucous trails. Those trails are easily observed inside collection jars, as they attract and hold loose flocculent material shaken off seagrass leaves.

#### **4.4.2 Amphipoda**

The dominant amphipod in this study was *Erichthonius*. It constructs domiciles made of silky material and detritus, which take the form of open-ended tubes, attached along their lengths to leaf surfaces. When densities of *Erichthonius* are high, domiciles can be found clustered together in colonies on broad-leaf *Halodule*. Often a large domicile can be found surrounded by small domiciles, which appear to be those of an adult female and her offspring.

Local *Erichthonius* were observed suspension feeding. They appeared to catch material with the long setae on their antennae, which they extended into the water column from the openings of their domiciles. Dixon and Moore (1997) described in detail that behaviour in *Erichthonius punctatus* (Hughes), a temperate species that they found on the holdfasts of kelp. That species captures particles from currents exterior to the domicile, and do not generate a flow of water through the domicile for feeding purposes, as described in some other corophioid amphipods. In calm water, *E. punctatus* can generate an exterior current using its antennae.

Although *Erichthonius* appeared to be primarily a suspension feeder, it may also feed by grazing epiphytic material. Dixon and Moore (1997) observed *Erichthonius punctatus* grazing epiphytic material from kelp, but only under poor conditions for suspension feeding. Other amphipods combine both types of feeding. Howard (1982) observed *Paradexamine curinga* J.L. Barnard on the seagrass *Heterozostera tasmanica* switching

between suspension feeding and grazing. The caprellid amphipod studied by Caine (Caine 1980), *Caprella laeviuscula* Mayer, did the same on *Zostera marina*.

Although negative correlations were found between *Erichthonius* abundance and epiphytic cover, the nature of the relationship is not clear. *Erichthonius* spp. are associated with biotic structures, such as seagrasses, macrophytic algae (Dixon and Moore 1997, Sotka et al. 1999), and hydroids (Bradshaw et al. 2003), that they use to elevate themselves above the substrate to exploit water currents for suspension feeding. Structures that are heavily covered in epiphytic material might not be suitable platforms for suspension feeding. So the negative correlation with epiphytic cover may reflect habitat preferences. Even so, epiphytes may be a useful alternative food source, and it may be advantageous to be able to suppress the development of epiphytes around the domicile. However, Duffy (1990) tested the ability of four species of amphipods to limit epiphyte growth on the seaweed *Sargassum*, and among the species tested, only *Erichthonius brasiliensis* did not significantly reduce the growth of epiphytes.

*Podocerus* was the second most abundant amphipod in this study, and had a strong negative association with epiphytic cover. It also uses its antennae for suspension feeding, and seeks the highest point from which to spread its long setose antennae into the water column (Barnard et al. 1988). It does not build domiciles, and could be found sitting exposed on the upper surfaces of *Halodule* leaves. Some other podocerids build structures to elevate themselves into the water column. Mattson and Cedhagen (1989) described how the podocerid *Dyopedos* constructs “masts” from which it feeds in passing currents. They described setae that secrete mucous in podocerids, and believed that another species, *Dulichia tuberculata* Boeck, used such mucous to cast a net for suspension feeding.

Among amphipods that inhabit seagrass meadows, suspension feeding appears to be common, but has received little attention. Corophioid amphipods, including *Corophium*, *Lembos*, and *Erichthonius* are often among the most abundant amphipods in seagrass meadows (Nelson 1979a, Lewis 1984, Stoner and Lewis 1985), and are principally suspension feeders (Dixon and Moore 1997). Several amphipods that have been found to limit epiphytic cover in experimental manipulations (Table 1.1) may also

suspension feed, including *Paradexamine*, *Caprella*, and *Gammarus* (Robertson and Mann 1980).

#### **4.4.3 *Electroma***

*Electroma* is a small suspension-feeding bivalve that attaches itself to seagrass leaves, and other upright structures, presumably to place itself in the water column for feeding. It occurred in very high numbers on *Halodule uninervis* leaves in plot SB in 2002, in a pattern consistent with the “irregular, intense peaks of recruitment” described by Keogh (1983) for *Electroma georgiana* Quoy and Gaimard in South Australia.

In plot PB, *Electroma* densities were negatively correlated to epiphytic loads on *Halodule* leaves, but there is no reason to believe that it could have any direct impact on epiphytic loads. Other causes must explain its association with low epiphytic cover. Epiphytic cover may interfere with recruitment, or reduce the feeding efficiency of *Electroma* by attenuating water flow near the leaf surface. As well, the soft flocculent material that settles out of the water column in the coastal boundary layer (Wolanski et al. 1997), and becomes incorporated in epiphytic material on leaf blades, may clog the filter-feeding apparatus of *Electroma*. Fabricius and Wolanski (2000) described how muddy marine snow smothers suspension-feeding organisms in coral reefs. The same considerations may also apply to the suspension-feeding amphipods and anemones on seagrasses, and contribute to the negative relationships seen with their densities and epiphytic cover.

#### **4.4.4 *Anemones***

At Cockle Bay, there were extraordinary densities of anemones, up to 5 cm<sup>-2</sup> leaf, of *Bolocerooides mcmurrici* in 2003, and *Bunodeopsis australis* in 2004. There was no apparent explanation for why blooms occurred at the same time in those years, but not in 2002. However, the population crash in 2003 was likely due to the nudibranch predator *Limenandra fusiformis*.

Large *B. mcmurrici* were 3 mm across the basal disc, and occupied the width of a broad-leaf *Halodule*. At high anemone densities, a large proportion of leaf surfaces

was covered. The animals would have reduced the amount of light reaching the leaves, and because they contain zooxanthellae, can be seen as competing with seagrass for light in much the same way as epiphytic algae. Percent epiphytic cover was estimated after anemones were removed, so the effective coverage of the leaves was underestimated during anemone blooms. High anemone densities could have negative impacts on seagrass production similar to those of high epiphytic cover. In California, a decline of *Zostera marina* was attributed to a bloom of *Bunodeopsis* sp. (thesis of A. Sewell 1996 cited in Williams and Heck 2001). In that case, the anemone's population bloomed after it was apparently introduced locally. It covered most of the surface of the seagrass, which subsequently died.

#### ***4.4.5 Relationship to seagrass species and leaf morphology***

*Halodule uninervis* always supported a higher density of epifauna than *Halophila ovalis*. There were two very conspicuous reasons for that difference. Firstly, epiphytic material on *H. uninervis* leaves always had a higher AFDW relative to DW, representing a greater concentration of organic material. Thus, an epiphyte grazer would find greater food resources per unit area, and feeding effort, on *H. uninervis* leaves. Secondly, *H. uninervis* rises higher above the substrate, and provides a better platform for suspension feeding invertebrates. Measures of the maximum leaf length in cut samples (Appendix B) showed that the *H. uninervis* canopy would typically be between 5 and 10 cm high, whereas *H. ovalis* would not be expected to stand more than 2 cm above the substrate.

The relative importance of the major invertebrate taxa varied greatly among the plots, and also differed between seagrass species within plot SB. In that plot, the epifauna on *Halodule uninervis* was dominated by *A. virgata* and *Electroma*; but on *Halophila ovalis*, anemones and *Ericthonius* dominated. In plot CB, anemones and *Ericthonius* dominated on both species (Fig. 4.4), while *Ericthonius* dominated on *H. uninervis* in plot PB. The differences were likely due to the difference in the width of the different leaves. At Shelly Beach, *H. uninervis* leaves average 0.6 mm wide (Appendix B), but at Cockle Bay and Picnic Bay they were approximately 3.0 mm wide. The effect of leaf width was most noticeable on the distribution of anemones. In plot SB, anemone

numbers increased on *H. ovalis* leaves in winter 2003 and 2004, but numbers on *H. uninervis* remained conspicuously low. Narrow-leaved *H. uninervis* at Shelly Beach did not appear to provide an adequate base of attachment for anemones. In contrast, large individual anemones could be found occupying one side of a single *H. ovalis* leaf at Shelly Beach. At Cockle Bay, both seagrass species were occupied, but greater densities occurred on broad-leaved *H. uninervis*, probably because it also provided higher points of attachment. In plot PB, anemones were uncommon, likely because of the greater exposure of the site to wave action.

A similar argument can be made to explain differences in *Ericthonius* numbers. Single small domiciles of *Ericthonius* were sometimes found on narrow-leaved *Halodule uninervis* in plot SB, but those leaves were too narrow to accommodate large domiciles, or clusters of domiciles. More *Ericthonius* were found on *Halophila ovalis* leaves in plot SB. However, *H. ovalis* provides a low point of attachment for suspension feeding. So in plot CB, much greater densities of *Ericthonius* were found on broad-leaved *H. uninervis* compared to *H. ovalis*.

#### **4.4.6 Predatory interactions**

*Alaba virgata* and *Electroma* on narrow-leaved *Halodule uninervis* at Shelly Beach, probably benefited from low numbers of epifaunal amphipods. In Thailand, there was a seasonal change in the epifaunal community in an intertidal *Halophila ovalis* meadow, from domination by gastropods, including *A. virgata*, to *Ericthonius* (unpublished data). That dichotomy between gastropod and amphipod dominated faunas, may arise because amphipods can predate or disrupt settling molluscan larvae. Dixon and Moore (1997) observed *Ericthonius punctatus* attacking smaller invertebrates, and gnawing on the carcasses of dead conspecifics. In the Baltic Sea, the distribution of an infaunal bivalve, *Macoma balthica* (L.), appears to be limited by the presence of deposit-feeding amphipods. Detailed work showed that the amphipods were capable of killing and consuming newly settled bivalve larvae, and that the rate of consumption could be great enough to control recruitment (Elmgren et al. 1986, Ejdung and Elmgren 1998, Ejdung et al. 2000). Thus, factors that influence the distribution and abundance of amphipods can potentially have cascading effects on other fauna.

Fish predation is considered a major factor controlling amphipod populations. In American seagrass meadows there is a pattern of decline in amphipod numbers during spring and summer, which has been attributed to the seasonal recruitment of juvenile fish (Nelson 1979b, a, 1980, Stoner 1980a). Similar seasonal declines in the numbers of caprellid amphipods on hydroids (Caine 1987) and sea whips (Caine 1983), and of a gammarid amphipod in salt marshes (Van Dolah 1978) have been related to fish predation. Edgar and Shaw (1995b) estimated that fish predation was capable of consuming the total production of crustaceans >1 mm size in a temperate Australian seagrass meadow. In addition, decapod predation may be an important limiter of amphipod numbers (Nelson 1981, Leber 1985).

Epifaunal amphipods are especially vulnerable to fish predation. Free-living or domicolous taxa on seagrass leaves show greater seasonal changes in abundance and greater susceptibility to fish predation than infaunal taxa (Nelson 1979b, Stoner 1979). *Ericthonius* and podocerids were common items in fish stomach contents from Sweden (Mattson and Cedhagen 1989). In the West Atlantic, *Ericthonius* was strongly selected over other amphipods by yellowtail flounder, *Limanda ferruginea*, probably because *Ericthonius* was more accessible because of its epifaunal habits (Collie 1985, 1987). In Florida, Sotka et al. (1999) used video cameras to record the feeding activity of fishes at segments of the macroalga *Halimeda tuna* with and without *Ericthonius brasiliensis* domiciles. Carnivorous fish showed a high selectivity for those plants with domiciles, even though the domiciles were made from calcareous *Halimeda* thalli that provided considerable protection for their inhabitants.

In light of the potential importance of fish predation, changes in abundance of *Ericthonius*, and its distribution among sites, ought to be considered with respect to information on fish populations. Unfortunately that sort of information was not available in this study. In the case of plot CB, it is tempting to believe that the increased abundance of epifaunal amphipods in association with anemone blooms was because the anemones provided some protection from predation.



## 4.5 Conclusions

Suspension feeders dominated epifaunal invertebrate assemblages associated with intertidal seagrass near Townsville. That finding was contrary to the expectation that epiphyte grazers would dominate the community. Of the dominant epifauna, only *Alaba virgata* is clearly a grazer of epiphytes. However, even it may be using a form of suspension feeding, if it is using sticky mucous to trap suspended material. The dominant amphipods, *Erichthonius* and *Podocerus*, are primarily suspension feeders, but may do some grazing of leaf surfaces. Other more sessile suspension feeders, *Electroma* and anemones, can be numerically dominant, but are extremely unpredictable, some years being extremely abundant, but not appearing in the next. If the dominant invertebrates are suspension feeders, then the relationship of the typical member of the community to the seagrass and its epiphytes is very different from the relationship expected with grazers. The limiting factors for the community are unlikely to be those that limit the amount of epiphytic material, but likely to be those that limit the supply of suspended material. Those factors would include the rate of flow of water over the leaves, the amount and size of particles in the water, and access to perches in the flow of water.

The negative relationships between epiphytic cover and the abundances of the major gastropods and amphipods found in this study are consistent with the hypothesis that those animals consume epiphytes and limit their production. However, there are other reasons why such correlations might arise. The water flow conditions that are advantageous for suspension feeders may not be so for epiphytes. Those animals may prefer substrates that are free of fouling. Flocculent materials that are part of the epiphytic load may interfere with suspension feeding. Overall, the limited information that is available on the biology of the invertebrates that dominated in the intertidal plots studied at Townsville suggests that their relationship to the epiphytes is more complex than the simple epiphyte-grazer relationship initially suspected.

The evidence from this study tends to contradict the current literature, which suggests that epifaunal communities in seagrass meadows are typically supported on epiphytic algae, which is principally consumed by small gastropods and peracarid crustaceans.

The importance of those animals has been emphasised by a number of experimental manipulations. However, many of the animals used in those experiments are not exclusively epiphyte grazers, but may also be suspension feeders. In the aquaria and mesocosms used for those experiments, there were likely few opportunities for suspension feeding, and the animals could have been more than normally reliant on epiphyte grazing. As a consequence, such experiments may have led to an over-estimation of the importance of epiphyte grazing.

## Chapter 5 Standing crop of *Halodule uninervis* in relation to epiphytic materials

### 5.1 Introduction

The prevailing model of seagrass-epiphyte-grazer interactions assumes that epiphytic cover has a negative impact on seagrass production because epiphyte growth on the leaves blocks light for photosynthesis (chapter 1). Increased epiphytic cover has often been attributed to nutrient enrichment of ambient waters (Borum 1985, Wear et al. 1999). The decline of seagrasses along developed coastlines in Western Australia (Cambridge et al. 1986, Silberstein et al. 1986), and the Caribbean (Tomasko and Lapointe 1991), has been attributed to increased epiphytic cover in association with nutrient enrichment. A relationship between seagrass decline and epiphytic cover, as a result of nutrient enrichment, has also been demonstrated in experimental manipulations (Twilley et al. 1985, Short et al. 1995). Nutrient enrichment may also lead to increased phytoplankton and macroalgal growth, which has similar effects on seagrass growth and production (Short and Burdick 1996, Hauxwell et al. 2001, McGlathery 2001, Hauxwell et al. 2003). A worldwide decline of seagrasses has been associated with eutrophication (Orth and Moore 1983, Shepherd et al. 1989).

Despite the strong association that has been found between high levels of nutrient enrichment and high epiphytic loads, there does not appear to be a good correlation between epiphytic loads and water column nutrient concentrations over a range of moderate nutrient values (Frankovich and Fourqurean 1997, Nelson and Waaland 1997). The absence of a strong relationship has been attributed to the very rapid uptake of nutrients from the water column, and the effects of epiphyte grazers (Lin et al. 1996). In experimental manipulations of both nutrients and grazers (Neckles et al. 1993, Williams and Ruckelshaus 1993), the latter may control epiphyte growth in nutrient enriched treatments.

Seagrasses in the Townsville area experience a broad range of epiphytic cover (chapter 4), including almost complete coverage of the leaves. However, in contrast with such situations described elsewhere, concentrations of soluble nutrients in the Townsville

area are generally low (Walker and O'Donnell 1981), and the coastal waters of the region have been characterized as oligotrophic (Alongi and McKinnon 2005). Nutrient levels at Townsville vary with the resuspension of shallow sediments by wind-driven waves, and with runoff during the rainy season (Walker 1981, Walker and O'Donnell 1981).

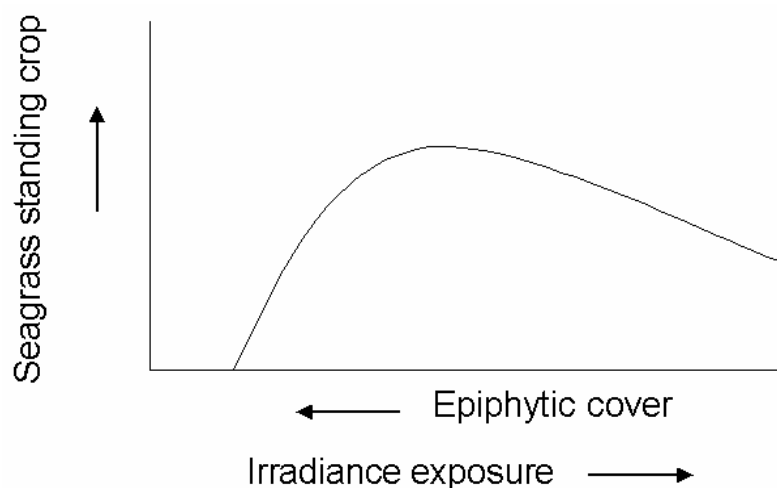
Since European settlement, muddy sediment discharges from rivers are estimated to have increased 5 to 10 fold as a result of clearing, grazing and cropping of land in local catchments (McCulloch et al. 2003). In addition, at the port of Townsville, sediment has been released by dredging of the local navigation channel (Wolanski and Gibbs 1992, Wolanski et al. 1992). Much of that nutrient-rich terrigenous material remains in the coastal zone and becomes trapped in mangroves and mud flats (Alongi and McKinnon 2005). At Cockle Bay, a broad mud bank and mangroves have developed since the 1930s, replacing the original sandy shore (Wolanski 1994).

Soft coastal sediments become resuspended during the ebbing of spring tides, producing a zone of high turbidity in the coastal boundary layer (Wolanski et al. 1997). The resuspended particulate matter remains trapped in the layer because it combines with biological material to form large sticky aggregates, termed muddy marine snow (Fabricius and Wolanski 2000). Those flocs settle rapidly, because of their large size, in a band up to a few hundred meters wide, and are not transported into offshore areas under calm conditions. When settled on the leaves, that material becomes a source of nutrients for epiphytic algae and bacteria, which produce mucous that causes further agglomeration. Thus, a thick layer of flocculent material and algae can accumulate on seagrass leaves in the near shore area.

Differences between the plots in the amount of epiphytic material were likely related to differences in local hydrological conditions, and location within bays with respect to sources of resuspended sediments. Both the Shelly Beach and Cockle Bay sites have inshore areas of very soft terrigenous mud that were the likely source of the soft flocculent material seen on the leaves. The width of the band over which flocculent material is deposited may depend on weather conditions at the time of spring tides. The amount of sediment available for resuspension may also vary over time. At Shelly

Beach, the area of very soft inshore sediment appeared to increase during the study, and may continue to do so until redistributed by a major tropical storm.

Epiphyte growth has usually been thought to have its major negative impact on seagrasses by shading the leaves, i.e. out-competing the seagrass for light; but may also compete with the seagrass for nutrients (Sand-Jensen 1977, Twilley et al. 1985). However, some positive impacts have also been proposed. Trocine et al. (1981) proposed that epiphyte cover provides protection from UV-B radiation, and Penhale and Smith (1977) suggested it provides protection from desiccation in intertidal areas. In chapter 3, a decline in the standing crop of *Halophila ovalis* in association with decreased epiphytic loads and grazing by sea hares (chapter 3) suggested that epiphytic loads benefited the seagrass, most likely by blocking harmful irradiance. Although maximum epiphytic cover is expected to have a negative impact on seagrass, it might be expected that greatest benefit, and therefore the maximum standing crop of seagrass, would occur at moderate levels of epiphytic cover/loads, particularly in intertidal habitats where the seagrass is exposed to high irradiance. The proposed relationship between seagrass standing crop and a range of epiphytic cover and irradiance exposure is represented graphically in Fig. 5.1.



**Fig. 5.1** Graphic representation of hypothesised changes in seagrass standing crop with changes in epiphytic cover (increasing to the left) and irradiance exposure (increasing to the right). The shape of the curve is taken from typical plots of photosynthetic rates versus irradiance (P-I curves) for plants with a photoinhibitory response, such as that shown by Drew (1979) for *Halophila stipulacea*.

Epiphytic cover is a more direct measure of exposure of the leaves to irradiance than measures of epiphytic load, although they are well correlated (chapter 4). However, AFDW measures organic content of the epiphytic material, and ought to be more sensitive to grazing pressure than epiphytic cover. It ought also to be more sensitive to nutrient enrichment. When most leaf surfaces are covered, the biomass of epiphytic material can still increase, and so weight measures may be the best indicators of that extreme condition. In chapter 3, AFDW was used as the critical measure of epiphytic material because of the lack of complete data on epiphytic cover when sea hares entered plot SB.

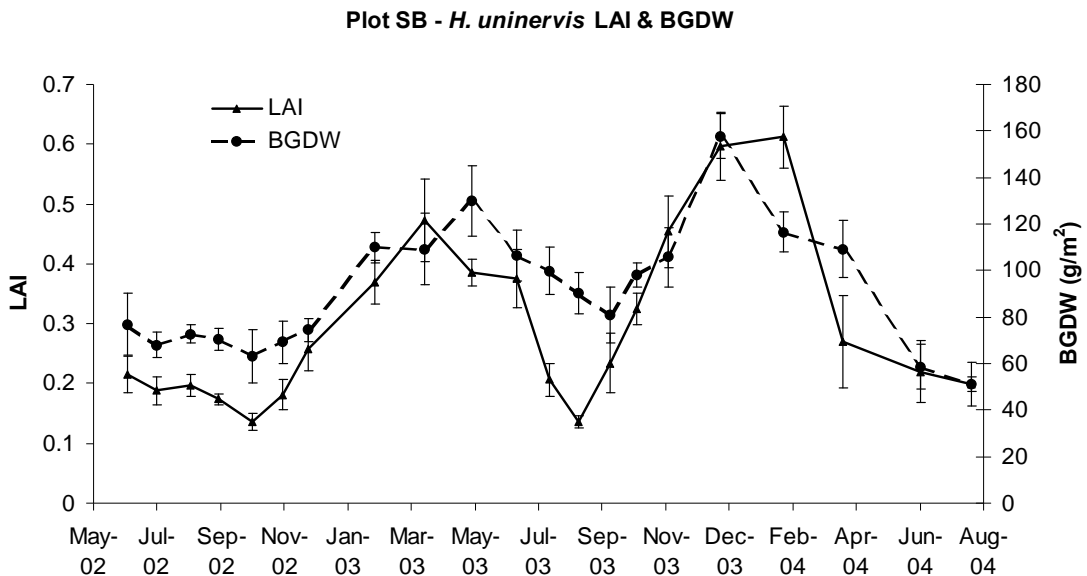
Epiphytes affect seagrasses; but it may also be true that seagrasses can affect the development of its epiphyte cover, especially through the size, shape and age of the leaves. The amount of epiphytic material on the leaves increases with the age of the leaves (Bulthuis and Woelkerling 1983, Borum 1987). On strap-like leaves, such as those of *Halodule uninervis*, which grow out from the sheath over time, there is usually less epiphytic cover on the basal tissue than on the older distal tissue. A slower leaf turnover rate would be expected to increase the total amount of material on the leaves. However, leaf turnover rate may not have a great influence on the amount of epiphytic material where it is much slower than epiphyte turnover rates, which may be determined by grazers. Tomasko and Lapointe (1991) found that a reduced leaf turnover rate was partly responsible for increased epiphytic loads on *Thalassia testudinum* at sites in the Caribbean experiencing nutrient enrichment. However, in the Florida study of Frankovich and Zieman (2005), *T. testudinum* leaf turnover rates did not explain any variation in epiphytic loads when other variables, including gastropod grazing, were considered. Seagrasses may also influence the development of epiphytic cover by releasing nutrients from the leaves, although the measured amounts of nutrients released from *Zostera marina* leaves and taken up by its epiphytes appears to be quite small (Penhale and Thayer 1980).

In this chapter, data are presented on the standing crop of *Halodule uninervis* sampled over two years in three plots near Townsville, using the methods presented in chapter 2. Changes in those measurements are considered with respect to the changes in epiphytic cover and loads presented in the previous chapter.

## 5.2 Results

### 5.2.1 Plot SB

Measurements of *Halodule uninervis* in plot SB varied considerably over the course of two years. The leaf area index (LAI) ranged from 0.14 to 0.61, and increased to peaks in March 2003 and December/February 2004, and thus appeared to have a seasonal pattern of change with maximum values in the austral summer (Fig. 5.2). BGDW (Below Ground Dry Weight) changes showed a similar pattern, although they appeared to be less seasonal, with peak values in May 2003 and December 2003. Shoot density closely followed BGDW, with peak values in the same months (Fig. 5.3). Maximum leaf length change was highly seasonal, with peaks in March 2003 and February/April 2004. In 2003, changes in below ground measures, i.e. BGDW and shoot density, appeared to lag changes in leaf measures, i.e. LAI and leaf length, but showed the reverse pattern in 2004. That difference between the years likely reflected differences in the causes of *H. uninervis* decline in the two years.



**Fig. 5.2** Mean LAI and BGDW ( $\pm$  s.e.) of *Halodule uninervis* in core samples,  $n=10$  from June to September 2002,  $n=5$  thereafter, from plot SB.

Plot SB - *H. uninervis* shoot density & leaf length

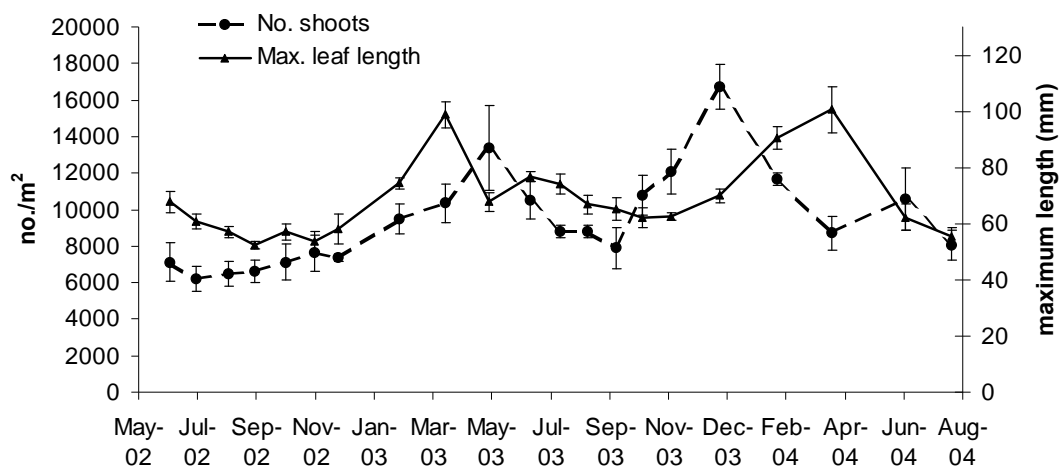


Fig. 5.3 Mean maximum lengths ( $\pm$  s.e.) of *Halodule uninervis* in cut leaf samples,  $n=10$ , and mean densities ( $\pm$  s.e.) of *H. uninervis* shoots in core samples,  $n=10$  from June to September 2002,  $n=5$  thereafter, from plot SB.

*Halodule uninervis* declined in early 2004 in association with extremely high epiphytic loads. The ash-free dry weight (AFDW) of epiphytic material on the leaves in February 2004 reached values almost three times those reached in the previous year, and remained above those values until at least April (Fig. 5.4). During that interval, *H. uninervis* LAI experienced its greatest and most significant decline between consecutive sampling dates (ANOVA,  $F=13.1$ ,  $p<0.00001$ ) (Tukey comparison,  $p<0.001$ ). Shoot density also declined significantly between December and April 2004 (ANOVA,  $F=6.7$ ,  $p<0.00001$ ) (Tukey comparison,  $p<0.0005$ ), and appeared to fall in advance of the decline in LAI. Those declines also occurred at the time of highest daytime tides during the year, i.e. least exposure (Fig. 5.5), so that light was reduced by a combination of both high epiphytic cover and high tides. Leaf length was shown to increase significantly at the site as a response to experimental shading (chapter 9). So the loss of shoots in advance of the loss of LAI, also at a time when leaf length was increasing (Fig. 5.3), suggested a loss due to light deprivation.



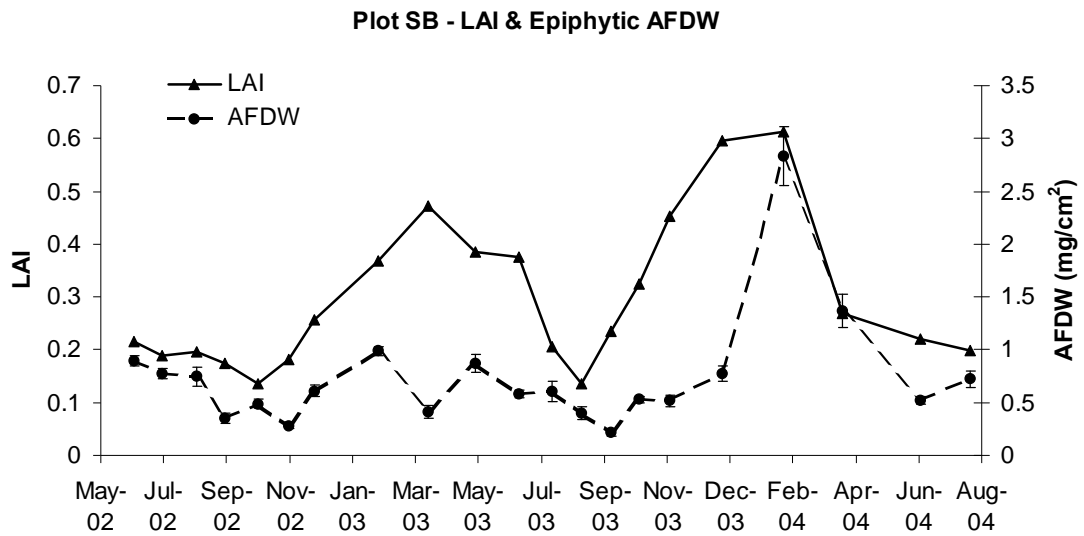


Fig. 5.4 *Halodule uninervis* LAI plotted against the mean AFDW ( $\pm$  s.e.) of epiphytic material on leaves in plot SB.

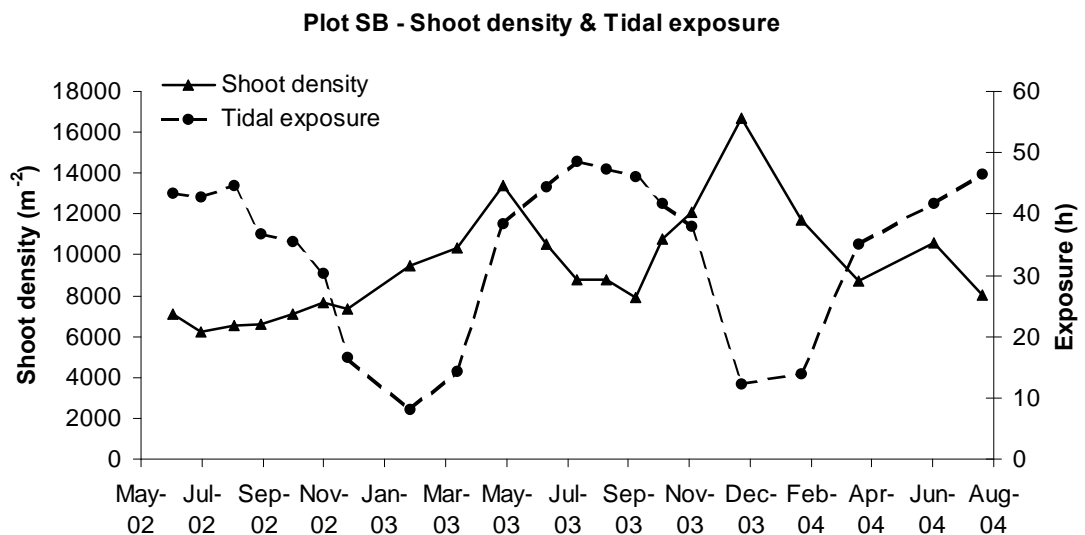
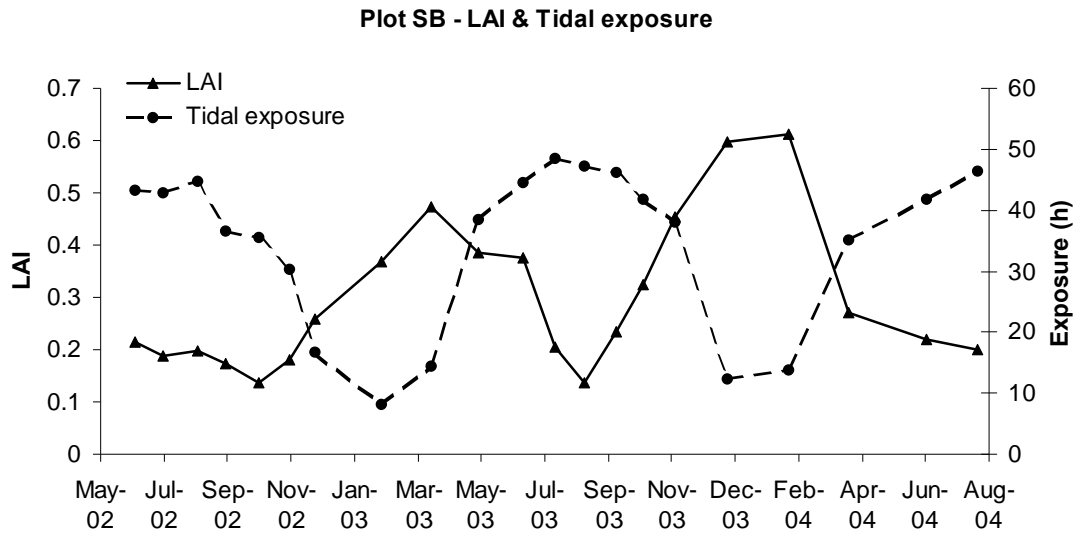


Fig. 5.5 *Halodule uninervis* shoot density and hours of tidal exposure of plot SB between 900 h and 1600 h over 14 days prior to sampling, following the methods in chapter 2 (section 2.8.1).

*Halodule uninervis* LAI also declined significantly in plot SB in mid 2003, between June and August (Tukey comparison,  $p < 0.025$ ). In marked contrast to early 2004, that decline was associated with the greatest daytime tidal exposure (Fig. 5.6), and declining epiphytic cover, which was virtually zero by August 2003 (Fig. 5.7). Shoot density and BGDW did not decline significantly during that period (Tukey comparison,  $p > 0.10$ ), and so appeared to be led by the loss in LAI. That pattern of decline was consistent with the loss of *Halophila ovalis* LAI, in the same plot, at the same time of year in 2002, without the loss of its BGDW, after the removal of epiphytic cover by sea hares

(chapter 3). It was suggested that those changes were related to high irradiance exposure. Therefore, there was evidence of a reduction in *H. uninervis* standing crop at both extremes of epiphytic cover in association with different tidal exposure regimes.



**Fig. 5.6** *Halodule uninervis* LAI and hours of tidal exposure between 900 h and 1600 h over 14 days prior to sampling of plot SB.

*Halodule uninervis* LAI was positively correlated with percent epiphytic cover ( $r=0.44$ ,  $p<0.05$ ) and AFDW ( $r=0.53$ ,  $p<0.025$ ) over two years, with high epiphytic cover during summer months associated with increased LAI (Fig. 5.7). In the second year, LAI appeared to lead changes in the amount of epiphytic material, so that a stronger correlation was obtained with epiphytic cover and AFDW if LAI was lagged forward one sampling date ( $r=0.70$ ,  $p<0.005$  for both variables) (Fig. 5.8). Shoot density was not correlated with percent epiphytic cover or AFDW ( $p>0.10$ ), but was also correlated with those variables when lagged forward ( $r=0.50$ ,  $p<0.025$  and  $r=0.68$ ,  $p<0.005$ , respectively).

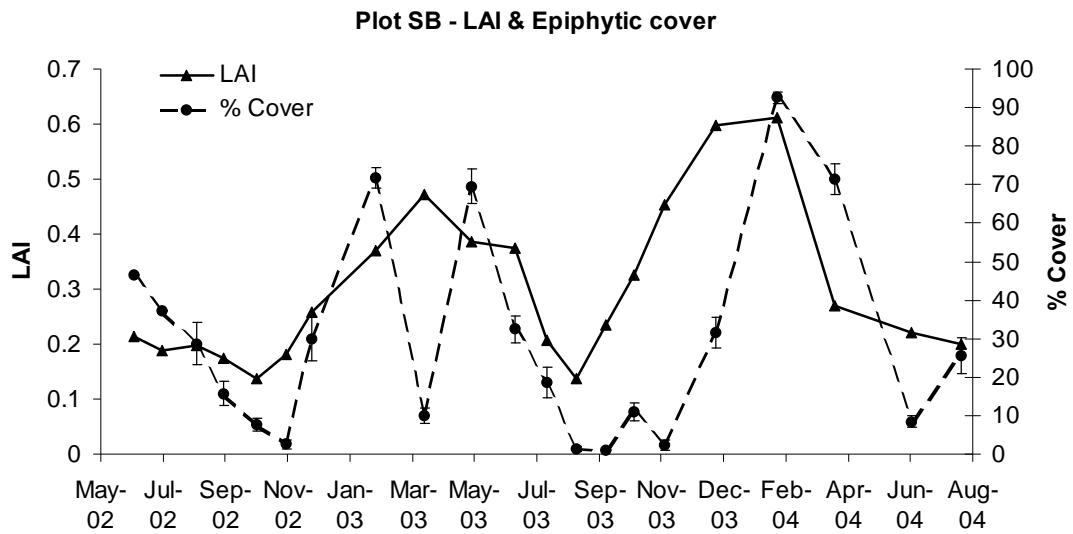


Fig. 5.7 *Halodule uninervis* LAI and mean percent epiphytic cover ( $\pm$  s.e.) of leaves in plot SB.

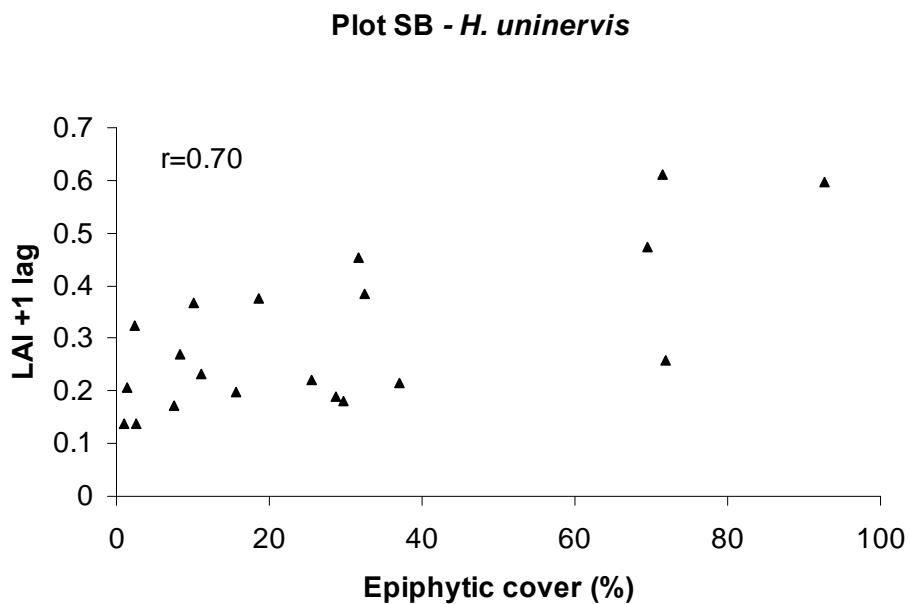
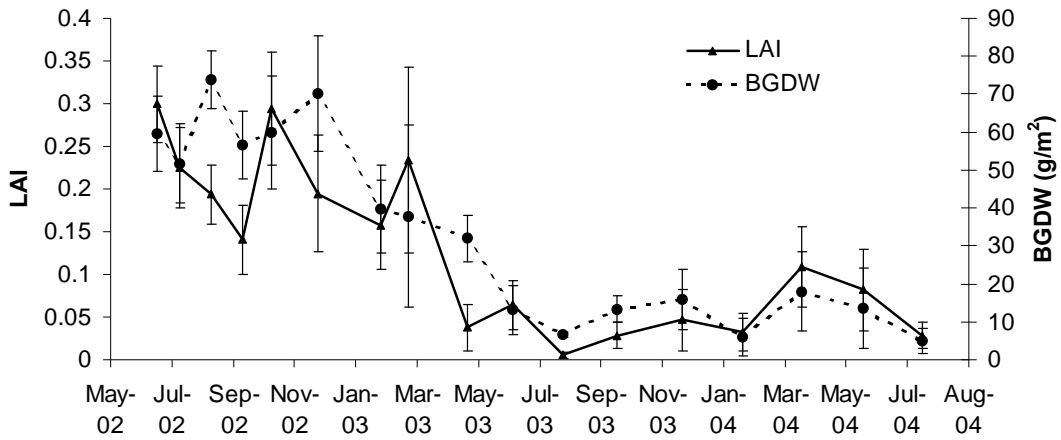


Fig. 5.8 Relationship between *Halodule uninervis* LAI lagged forward one date and percent epiphytic cover over two years in plot SB.

### 5.2.2 Plot CB

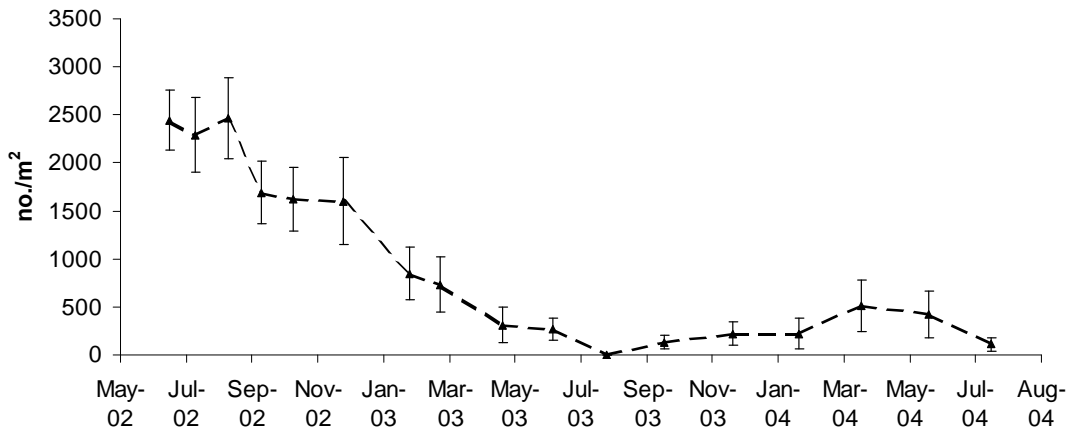
*Halodule uninervis* LAI in plot CB decreased over the first year of sampling, and remained at low values,  $<0.1$ , throughout the second (Fig. 5.9). In the first year, the seagrass became patchily distributed within the plot, and variance was large. LAI had its greatest decline between March and April 2003. By March, the density of *H. uninervis* shoots (Fig. 5.10) had already declined by 70%.

**Plot CB - *H. uninervis* LAI & BGDW**



**Fig. 5.9 Mean LAI and BGDW ( $\pm$  s.e.) of *Halodule uninervis* in core samples, n=10, from plot CB.**

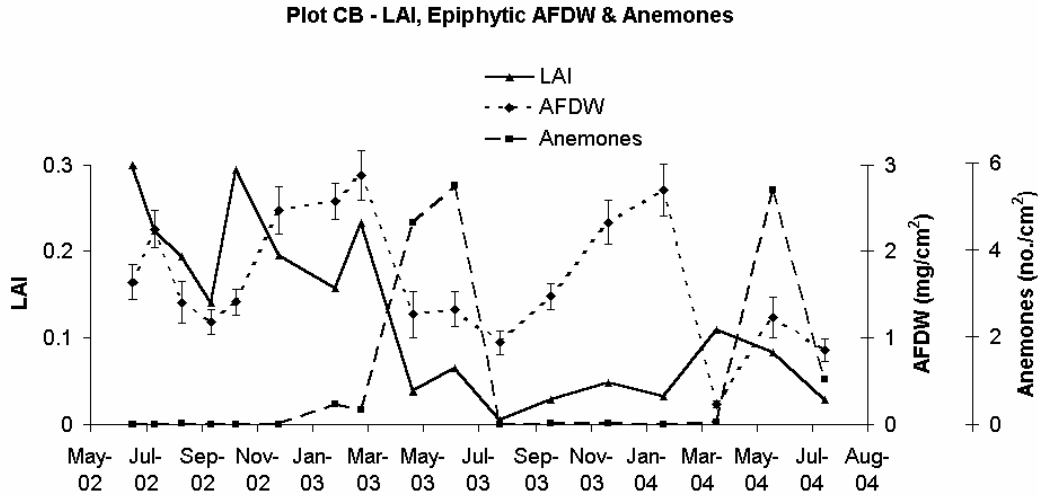
**Plot CB - *H. uninervis* shoots**



**Fig. 5.10 Mean densities ( $\pm$  s.e.) of *Halodule uninervis* shoots in core samples, n=10, from plot CB.**

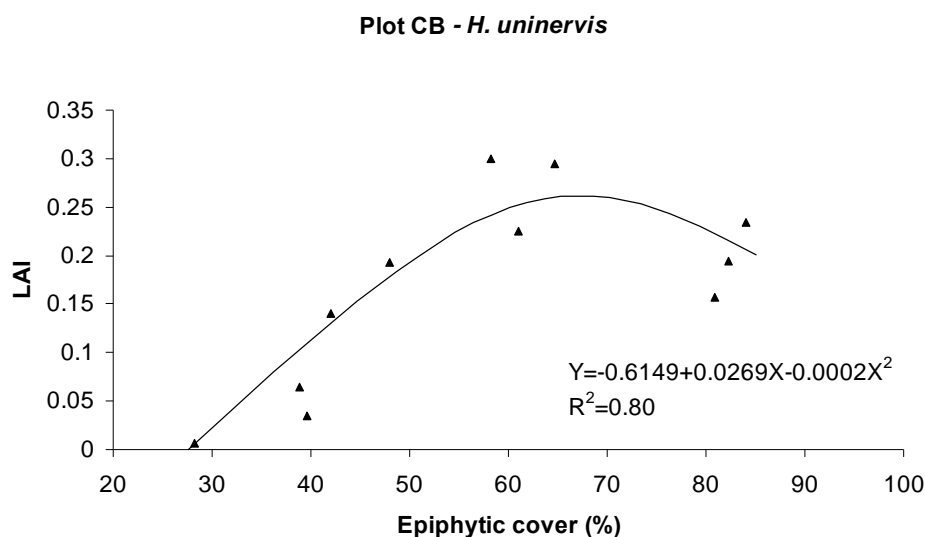
Epiphytic loads were much greater in plot CB than in other plots (for comparisons see Table 4.2 and Fig. 4.13), and were the likely reason for the reduced standing crop of *Halodule uninervis* in that plot. An extended period of extremely heavy loads, AFDW  $> 2 \text{ mg cm}^{-2}$ , from December 2002 to March 2003 was followed by a decline in LAI (Fig. 5.11). As in plot SB, the decline in LAI associated with high epiphytic loads was apparently led by a decline in shoot density. Epiphytic loads were more moderate from April to July 2003, but the reduction did not result in any recovery. Instead, anemones became extraordinarily abundant (Fig. 5.11), occurring at densities  $> 5 \text{ cm}^{-2}$ , occupying much of the open space on the leaves, and probably furthering the decline of *H.*

*uninervis*. By July, standing crop of *H. uninervis* was minimal, with zero shoots in the core samples (Fig. 5.10).



**Fig. 5.11** *Halodule uninervis* LAI, epiphytic AFDW ( $\pm$  s.e.), and anemone densities per leaf area in plot CB.

In the first year, the relationship between *Halodule uninervis* LAI and percent epiphytic cover was well described by a quadratic function ( $R^2=0.80$ ,  $p<0.005$ ) (Fig. 5.12) with maximum LAI at 67% epiphytic cover. That relationship reflected a moderate loss of LAI when epiphytic cover were highest, but a much reduced standing crop when anemone numbers were extremely high and epiphytic cover was moderate. A quadratic function also described the relationship between percent epiphytic cover and shoot density ( $R^2=0.67$ ,  $p<0.025$ , maximum value 61%). The relationships with AFDW were not as strong ( $p>0.05$ ). In the second year, epiphytic cover was more varied, but *H. uninervis* measurements were consistently low, probably because of the almost complete loss of shoots and BGDW, and the effects of high anemone densities. Therefore, the combined data for two years showed no significant correlations between those measures and epiphytic cover.



**Fig. 5.12 Relationship between *Halodule uninervis* LAI and percent epiphytic cover from June 2002 to July 2003 in plot CB, n=11.**

### 5.2.3 Plot PB

*Halodule uninervis* LAI values in plot PB were high, > 0.6, throughout most of the two year study (Fig. 5.13). Changes were small and non-significant (ANOVA,  $F=1.5$ ,  $p>0.10$ ), but suggested a seasonal pattern, with lowest values in July 2002 and 2004, and highest values in February 2003 and 2004. The lowest values occurred at times of the greatest daytime tidal exposure of the plot, and there was evidence of desiccation of the leaves. *Cymodocea serrulata* was a minor species in the plot at the beginning of the study, but its LAI increased to values comparable to those of *H. uninervis* by December 2003, thus doubling the total LAI of seagrass in the plot.

*Halodule uninervis* BGDW, in contrast to its LAI, changed significantly (ANOVA,  $F=6.1$ ,  $p<0.00001$ ) in plot PB, with a three-fold increase from initial values to a peak in October 2003 (Fig. 5.14). During that time, the density of shoots (Fig. 5.15) decreased gradually and non-significantly, but then rose significantly between January and May 2004 (ANOVA,  $F=2.0$ ,  $p<0.05$ ) (Tukey comparison,  $p<0.025$ ). *Cymodocea serrulata* BGDW and shoot density also increased during the second year, but remained low relative to those values for *H. uninervis*, even though LAI of the two species had risen to similar levels.

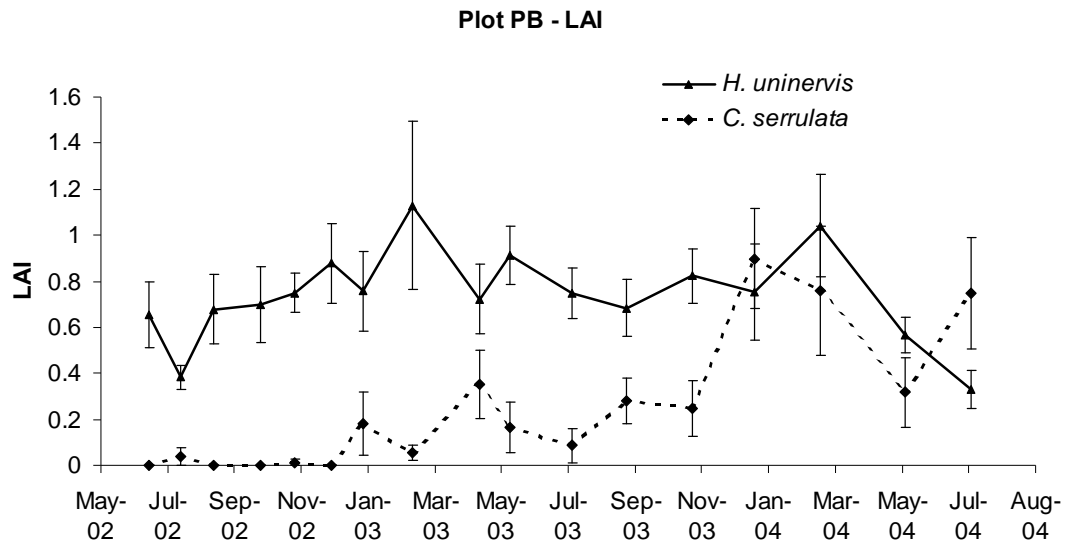


Fig. 5.13 Mean LAI ( $\pm$  s.e.) of *Halodule uninervis* and *Cymodocea serrulata* in cores from plot PB, n=5.

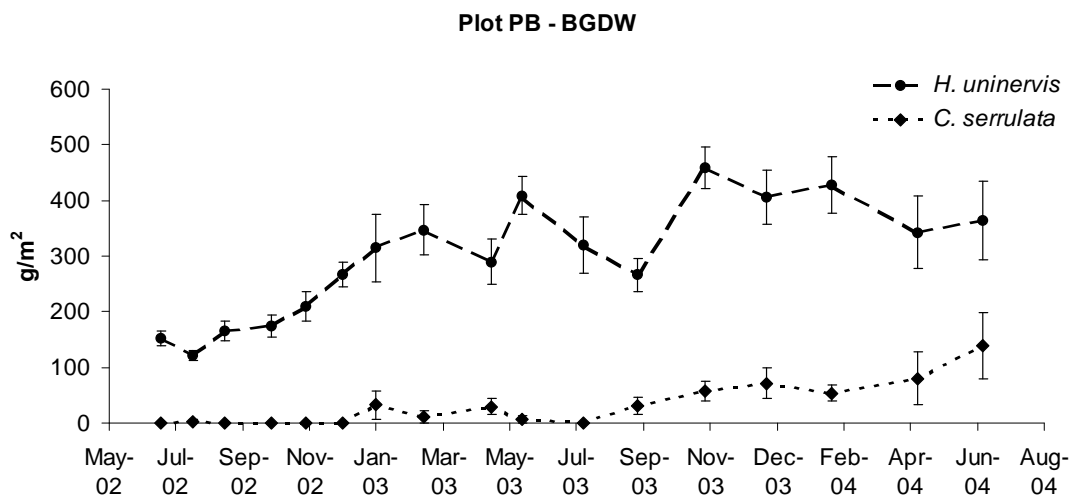
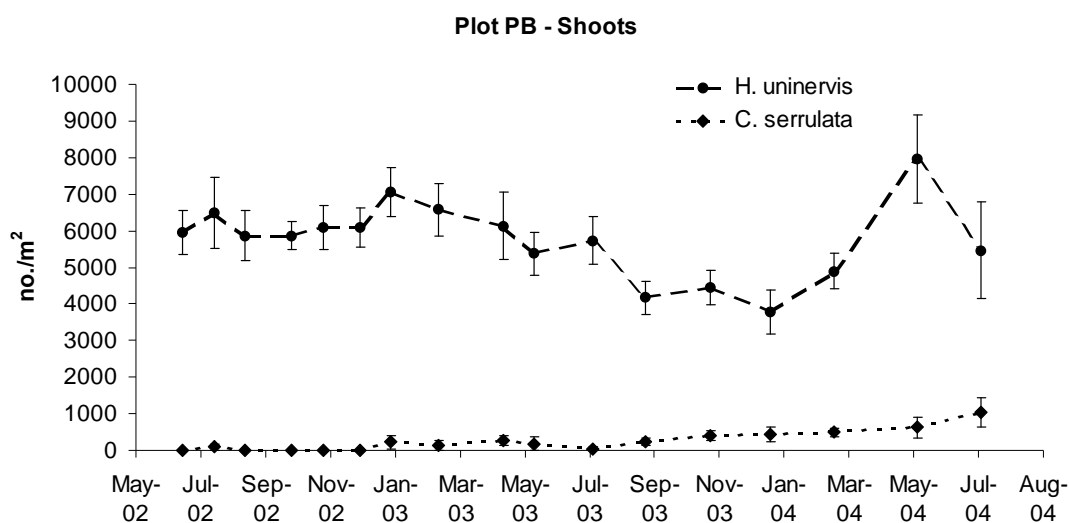


Fig. 5.14 Mean BGDW ( $\pm$  s.e.) of *Halodule uninervis* and *Cymodocea serrulata* in cores from plot PB, n=5.

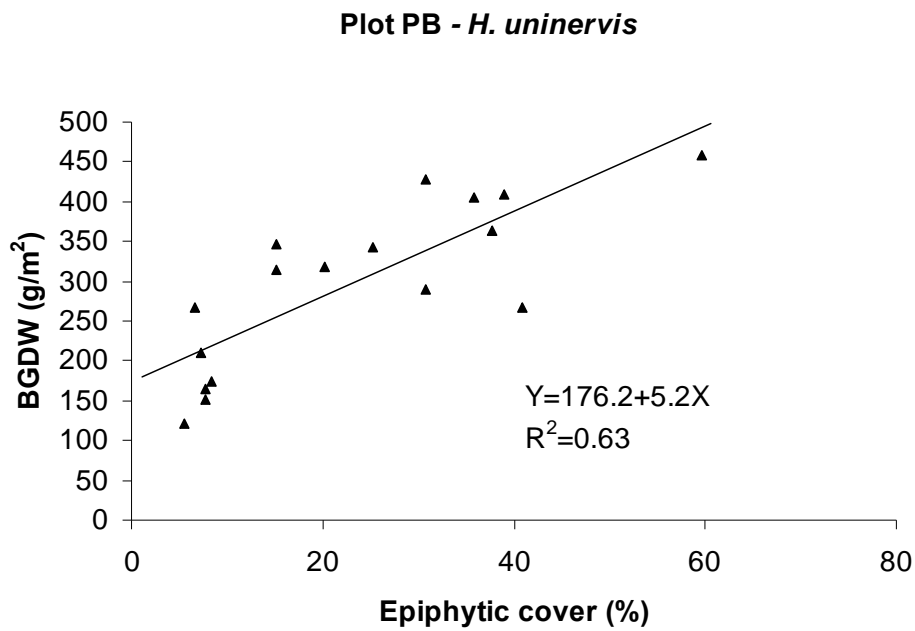
*Halodule uninervis* BGDW was significantly higher over the period October 2003 to July 2004, after termination of the ferry service, compared to the same period the year before ( $t=4.54$ ,  $p<0.0001$ ). In contrast, *H. uninervis* shoot density tended to be lower ( $t=-1.91$ ,  $0.05<p<0.10$ ), while LAI did not differ significantly ( $t=-1.30$ ,  $p>0.10$ ). At the same time, there were highly significant increases in all measures of *Cymodocea serrulata* abundance: LAI, BGDW, and shoot density ( $t=6.51$ ,  $t=5.56$ , and  $t=5.40$  respectively, all  $p<0.0001$ ).



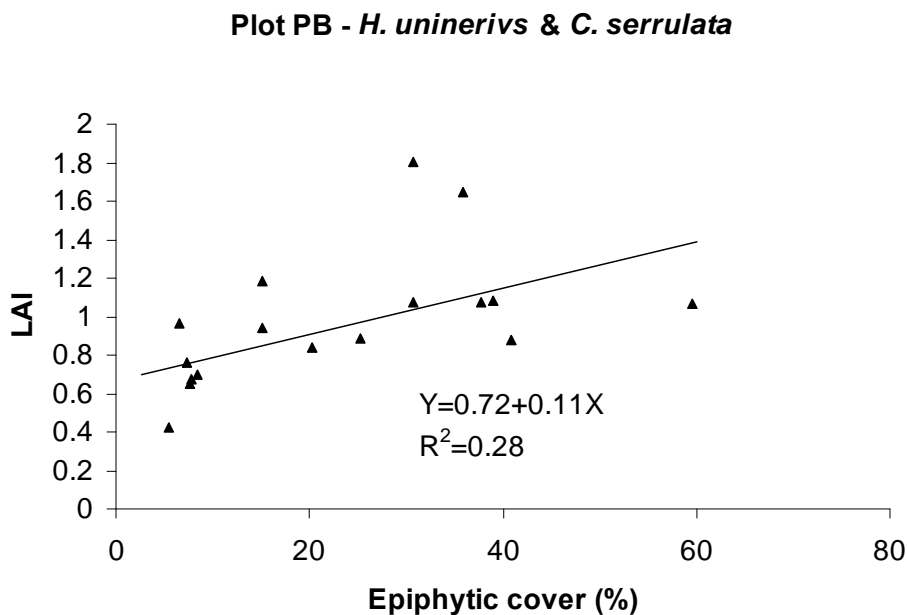
**Fig. 5.15** Mean densities ( $\pm$  s.e.) of *Halodule uninervis* and *Cymodocea serrulata* shoots in cores from plot PB, n=5.

Epiphytic loads in plot PB were low to moderate, and there was no indication of the declines in *Halodule uninervis* standing crop seen in other plots in association with high epiphytic loads. *H. uninervis* LAI in plot PB did not vary much over time, and was not correlated with changes in percent epiphytic cover ( $r=0.21$ ,  $p>0.10$ ). However, *H. uninervis* BGDW increased over time and was highly positively correlated with percent epiphytic cover ( $r=0.80$ ,  $p<0.001$ ) (Fig. 5.16). Likewise, *Cymodocea serrulata* LAI and BGDW increased with increasing epiphytic cover, as measured on *H. uninervis* leaves ( $r=0.48$ ,  $0.05<p<0.10$  and  $r=0.50$ ,  $p<0.05$  respectively). And as might be expected, the combined total LAI of all seagrass in the plot increased with increasing epiphytic cover ( $r=0.53$ ,  $p<0.05$ ) (Fig. 5.17).





**Fig. 5.16** Relationship between *Halodule uninervis* BGDW and percent epiphytic cover over two years in plot PB.

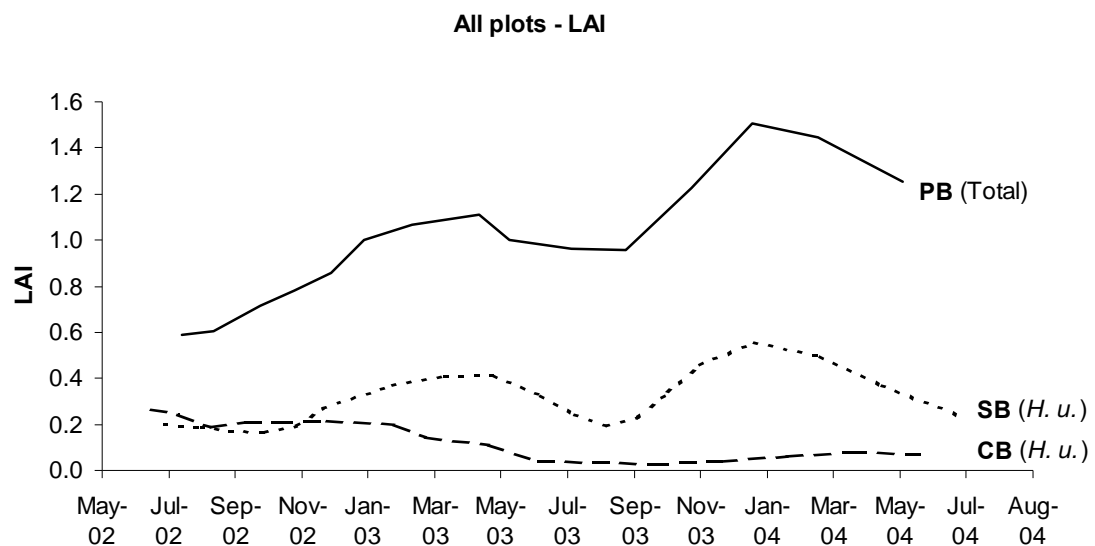


**Fig. 5.17** Relationship between combined LAI of *Halodule uninervis* and *Cymodocea serrulata*, and percent epiphytic cover over two years in plot PB.

#### 5.2.4 Comparisons among plots

Plot PB supported the greatest amount of seagrass throughout the course of the study (Fig. 5.18), and had the lowest average amount of epiphytic material on the leaves (see Table 4.2). Conversely, plot CB had the least amount of seagrass and consistently had

the greatest amount of epiphytic material. The total amount of seagrass in plot PB increased over the course of the study, while seagrass production in plot CB collapsed. Plot SB had intermediate average values of epiphytic measures, and seagrass standing crop. Epiphytic cover and loads varied greatly in plot SB, and when loads reached levels comparable to those in plot CB (Fig. 4.13) standing crop of *Halodule uninervis* declined. Therefore, comparison between plots supported the belief that epiphytic material had a negative impact on seagrass standing crop. However, within plots there were consistent positive correlations between seagrass standing crop and epiphytic cover (Fig. 5.8, Fig. 5.12, Fig. 5.16, Fig. 5.17). The mean values of LAI and the amount of epiphytic material differed between the plots, but they varied about those means in a similar way.



**Fig. 5.18** Changes in LAI values calculated as 3-point moving averages in each plot. Total LAI in plot PB is the sum of *Halodule uninervis* and *Cymodocea serrulata*. In plots SB and CB, only *H. uninervis* (*H. u.*) LAI is presented. Other similar seagrass species, *Zostera muelleri* and *Thalassia hemprichii*, in plots SB and CB respectively, made a minor contribution to the total LAI in those plots.

### 5.3 Discussion

Epiphytic cover may have very different and contradictory effects on seagrass depending on the circumstances. In tropical intertidal meadows, it may be blocking light that is needed for photosynthesis, but may also be blocking light that is damaging tissue and inhibiting photosynthesis. In this study of *Halodule uninervis*, field data was consistent with the occurrence of both sorts of effects. When the highest epiphytic

loads occurred there was evidence of negative effects, but over the broad range of loads there were consistent positive correlations between epiphytic cover and leaf area. Those findings suggest that there is a complex interaction between seagrass standing crop and its epiphytic loads.

### ***5.3.1 Light deprivation by high epiphytic loads***

Light has consistently been shown to be the major factor limiting seagrass growth and production. The attenuation of light through the water column limits the natural depth distributions of seagrasses (Duarte 1991b, Dennison et al. 1993). Widespread losses of seagrass have been blamed on reductions in underwater light levels as a result of the deteriorating quality of coastal waters. In polluted waters, nutrient inputs stimulate the production of algae, including macrophytes, phytoplankton and epiphytes, that obstruct light to seagrass, and leads to their decline (Twilley et al. 1985, Silberstein et al. 1986, Short et al. 1995, Short and Burdick 1996, McGlathery 2001, Hauxwell et al. 2003). Siltation and the resuspension of sediments increase turbidity and reduce underwater light levels, and likewise lead to the decline of seagrass (Onuf 1994, Preen et al. 1995, Terrados et al. 1998). The recognition of the importance of light has led to a profusion of experimental studies manipulating light levels to reveal their effects on the growth, productivity, and physiology of seagrasses (e.g. Dennison and Alberte 1982, Bulthuis 1983, Abal et al. 1994, Fitzpatrick and Kirkman 1995, Lee and Dunton 1997, Longstaff and Dennison 1999, Ruiz and Romero 2001, Major and Dunton 2002). Where other factors, including nutrients and herbivory have also been manipulated, light reduction was the most important limiter of growth and abundance (Ibarra-Obando et al. 2004).

Extremely heavy epiphytic loads,  $> 2 \text{ mg cm}^{-2}$ , in summer 2003 in plot CB, and summer 2004 in plot SB, were associated with declines in *Halodule uninervis*. Those declines were characterized by a loss of shoot density and BGDW in advance of a loss of LAI, while leaf length increased. That pattern of decline was consistent with loss due to light deprivation. Similar declines have been described in American *Halodule wrightii* Ascherson during light reduction by an algal bloom (Dunton 1994, Onuf 1996), and in light deprivation experiments in Australia with narrow-leaved *Halodule uninervis* (Longstaff and Dennison 1999), and other medium-sized species (Bulthuis 1983, Abal et al. 1994).

Longstaff and Dennison (1999) starved narrow-leaved *Halodule uninervis* of all light for 80 days using screens in the Gulf of Carpentaria. In the first 38 days, there was no loss of biomass, but canopy height increased. Subsequently, biomass and shoot density declined, but canopy height only fell below controls at 80 days. It was expected that *H. uninervis* would die-off completely after 100 days of darkness. In an experimental shading of *Heterozostera tasmanica* (Martens ex Ascherson), Bulthuis (1983) found that shading increased leaf length, but not leaf width, while shoot density declined. Leaf growth rates persisted under low light treatments, and appeared to be maintained by energy stores in the rhizomes. In an experimental shading of *Zostera muelleri*, Abal et al. (1994) found that shading reduced the number of shoots, but the average shaded shoot was larger than in controls, so that there was more photosynthetic tissue relative to below ground biomass in shaded plots.

Seagrasses may adapt to low light conditions by improving light harvesting capacities, through increasing the concentration of chlorophyll in the leaves (e.g. Dennison and Alberte 1982, Dennison and Alberte 1985, Lee and Dunton 1997) (see also chapter 9), or by increasing the relative size of the photosynthetic area (e.g. Abal et al. 1994). The costs of augmenting and maintaining a large leaf surface and high chlorophyll concentrations deplete carbohydrate reserves in roots and rhizomes. Consequently, the ratio of photosynthetic area to non-photosynthetic biomass increases as an initial response to decreased light levels. In this study, the loss of shoots and BGDW in advance of the loss of LAI, during periods of extremely heavy epiphytic loads, was consistent with those patterns. During periods of moderate epiphytic loads, BGDW was seen to accumulate, especially in plot PB.

In light deprivation experiments, the abilities of different seagrass species to survive low light conditions have been related to their ability to store carbohydrate reserves as below ground biomass (Czerny and Dunton 1995, Lee and Dunton 1997). *Halodule uninervis* has a large below ground biomass relative to its photosynthetic area, and can survive long periods of light deprivation, better than *Halophila ovalis* (Longstaff and Dennison 1999), but less well than some larger species, such as *Posidonia* spp. (Gordon et al. 1994, Ruiz and Romero 2001). Comparisons among species from different

studies, using diverse methods, should be made with caution. However, some larger seagrass species appear to have very different patterns of response to shading compared to the medium-sized species. In experimental shadings of *Thalassia testudinum* Banks ex König, leaf growth rates and biomass declined before below ground biomass, while leaves became narrower, but not longer (Lee and Dunton 1997, Ibarra-Obando et al. 2004). In shadings of *Posidonia* spp., leaf growth rates and length declined before shoot density and weight (Gordon et al. 1994, Fitzpatrick and Kirkman 1995, Ruiz and Romero 2001). Those species survive longer under severe shading than does *H. uninervis*, but are much slower to recover losses once shading is removed.

In the Townsville area, the period from late November to March, the austral summer, is the time of lowest light availability for the seagrass. It is the time of highest daytime tides, and when the most rainfall occurs, which would be expected to increase cloud cover, turbidity, and nutrient availability for algal growth. That is also the period of highest temperatures, and therefore likely to be the period of greatest metabolic requirements for the seagrass. So by the coincidence of those factors, the austral summer was when local seagrasses were most likely to experience low light stress. The greater length of *Halodule uninervis* leaves at that time clearly suggested that it was experiencing low light levels (Appendix C). Increased leaf length was the strongest response of *H. uninervis* to short-term experimental shading (chapter 9).

### **5.3.2 High irradiance as a limiter of seagrass standing crop**

#### *5.3.2.1 Photoinhibition and photodamage*

*Halodule uninervis*, like *Halophila ovalis*, may suffer from photoinhibition and photodamage as a result of the high irradiance levels experienced in tropical intertidal waters. Dawson and Dennison (1996) showed that both species were particularly sensitive to UV radiation, but not especially sensitive to elevated PAR. As suggested for *H. ovalis* (chapter 3), epiphytic cover may provide a barrier on seagrass leaves protecting them from the harmful effects of high irradiance (Trocine et al. 1981). The positive correlations found between epiphytic cover and measures of *H. uninervis* abundance within the study plots were consistent with that belief.

In plot SB, between June and August 2003, there was a significant decline in *Halodule uninervis* LAI in association with low epiphytic cover and low daytime tides, i.e. conditions of high irradiance exposure. BGDW and shoot density did not decline significantly during that time. That was a reverse of the pattern seen during the period of high epiphytic cover and high daytime tides. Thus, a decrease in leaf area relative to below ground biomass should provide a useful indicator of high irradiance stress, while an increase in leaf area relative to below ground biomass should indicate low irradiance stress.

#### 5.3.2.2 Desiccation

In plot PB, lowest *Halodule uninervis* LAI values occurred when tidal exposure was greatest in July 2002 and 2004, and there was evidence of desiccation of the leaves. Below ground biomass was not affected. The period of exposure was short, recovery was rapid, and desiccation did not appear to have a major long-term effect. De Iongh et al. (1995) documented a loss of above ground *H. uninervis* biomass during months of high daytime exposure in the Moluccas, Indonesia, also without the loss of below ground biomass. Erftemeijer and Herman (1994) found that seasonal variation in daytime tidal exposure was the main source of variation in biomass and productivity, as a result of desiccation, in a mixed meadow of *Enhalus acoroides* (L.f.) Royle and *Thalassia hemprichii* in Sulawesi, Indonesia. The latter species proved resilient to desiccation, as it could mobilize large stores of below ground carbohydrates to recoup the loss of the leaves (Stapel et al. 1997).

Broad-leaf *H. uninervis* in plot PB had robust shoots, which stood erect, making them vulnerable to desiccation. However, the plot had the least exposure of all the plots (see Fig. 8.1). In contrast, the narrow-leaved plants in plot SB, lay prone in the shallow skim of water that remained in the plot at low tide, and there was little evidence of desiccation, despite being exposed for much longer periods. Björk et al. (1999) found that intertidal *Halophila ovalis* and narrow-leaved *Halodule* in East Africa were actually less able to recover from desiccation than species found at greater depths, including *Cymodocea serrulata* and *Thalassia hemprichii*. However, the former

species were unlikely to suffer desiccation because they lay flat on the moist muddy substrate at low tide.

### **5.3.3 Interactions**

The positive correlations between epiphytic loads and measures of seagrass abundance are not easily explained solely by a protective effect of epiphytic material against high irradiance. The relationships were evident in all plots, irrespective of differences in mean epiphytic loads and tidal exposures. In plot SB, during the second year of sampling, LAI changes appeared to lead changes in epiphytic cover, suggesting a very different relationship. The best explanation was that the increase in the seagrass canopy attenuated water flow and allowed for greater trapping of suspended material in the meadow. Some of that material becomes trapped on leaf surfaces (Agawin and Duarte 2002). In a recent study, Frankovich and Zeiman (2005) found that turbidity accounted for the largest part of the variation in epiphytic loads on *Thalassia testudinum* in Florida Bay, probably because resuspended sediments were settling on the leaves. Settled material may then stimulate epiphyte growth, as suggested during the shading experiment (chapter 9). When loads become extremely heavy, shoot density and leaf area is lost and the seagrass canopy opens up, reducing conditions for the settlement of more material. Thus, the cycle of changing LAI and epiphytic loads in plot SB could be the result of reciprocal interactions, and changes in LAI could well appear to lead changes in epiphytic loads.

There are other ways in which a positive correlation between *Halodule uninervis* LAI and epiphytic loads might arise. An increase in epiphytic loads has been related to increased leaf age or reduced leaf turnover rates (Borum 1987, Tomasko and Lapointe 1991). However, an increase in leaf longevity may itself be a consequence of shading by epiphytes. In other plants, leaf life-span increases with decreasing light intensity, likely as a consequence of slower leaf aging (Vincent 2006). Likewise in intertidal seagrass, it can be expected that increased shading by epiphytic material will reduce damage by high irradiance, increase leaf longevity, reducing the turnover of leaf surfaces, and allowing greater time for the accumulation of more epiphytic material. LAI might also increase with shading because, although longevity increases, leaf

elongation rates are unaffected, at least in *Halodule wrightii*. In experimental shading of that species, leaf elongation rates were maintained by below ground reserves (Czerny and Dunton 1995). There was some suggestion of those sorts of effects during the shading experiment (chapter 9). Leaves of *H. uninervis* were significantly longer under shades, although LAI was not significantly different between shaded and non-shaded plots. That difference between the treatments suggested there were more leaves in the non-shaded plots, and that the shorter leaves in non-shaded plots were being turned over at a faster rate, assuming similar growth rates between the treatments. Another mechanism is suggested by Penhale and Smith (1977), who showed that leaves damaged by desiccation release increased amounts of organic material. If leaves damaged by high irradiance also increase their release of organic material, then epiphytic growth could be stimulated during periods of high irradiance, which would increase the protective cover of epiphytic material. However, neither of those effects would explain the way in which changes in LAI in plot SB appeared to lead changes in epiphytic cover. Whatever the cause, the relationship between epiphytic loads and LAI in plot SB appeared to contradict the causal relationship from epiphytes to seagrass postulated in models of seagrass-epiphyte-grazer interactions.

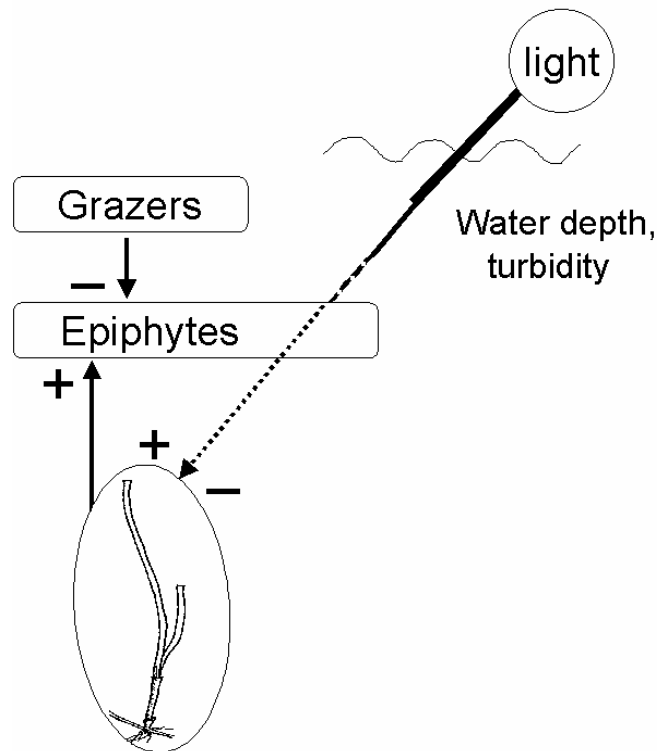
Epiphytic load appears to be a density-dependent factor limiting *Halodule uninervis* abundance. Moderate epiphytic cover may benefit the seagrass because it protects against irradiance damage; but as the canopy develops, more flocculent material is captured up to a maximum load that precipitates a decline in the seagrass. Maximum loads are reached at different leaf densities in different plots because the concentration of resuspended material in the water column differs between sites. The amount of resuspended material will be related the amount of soft terrigenous material in the bay, the distance from shore, currents and wind exposure. In plot PB, maximum loads were not approached, and other density-dependent limiting factors were more likely to become important, such as self-shading and the availability of sediment nutrients.

## 5.4 Conclusions

The standing crop of *Halodule uninervis* was reduced in association with extremely high epiphytic loads in two of the study plots. In one of those plots, *H. uninervis* LAI



was also reduced during a period of low epiphytic loads and high tidal exposure, which suggested possible negative effects from high irradiance exposure. Moderate epiphytic loads may have a positive impact on the standing crop because they provide some protection from irradiance. Negative impacts may occur at both extreme high or low loads. Within plots, there were positive relationships between epiphytic loads and the amount of seagrass that suggested a complex interaction between them. There may be reciprocal interactions, because the development of the seagrass canopy encourages greater epiphytic development. Epiphytic loads may behave as a density-dependent limiter of *H. uninervis* production. Models of seagrass-epiphyte-grazer interactions need to be modified to reflect those relationships (Fig. 5.19), including both potentially positive and negative impacts of epiphytic cover on seagrass standing crop.



**Fig. 5.19** A modified model of interactions among invertebrates, epiphytes, and the standing crop of *Halodule uninervis*. Light, as modified by the amount of epiphytic cover, may have both positive and negative impacts on the seagrass, but the leaf area of seagrass has a positive impact on epiphyte development.

## Chapter 6 Interactions between *Halodule uninervis* and its epifauna

### 6.1 Introduction

The prevailing model of seagrass-epiphyte-grazer interactions assumes that grazers indirectly benefit seagrasses by removing and controlling epiphytic cover (chapter 1). The model assumes 1) a negative effect of epiphyte grazers on epiphytic material, 2) a negative impact of the amount of epiphytic material on the standing crop of seagrass, and 3) no direct effects between epiphyte grazers and seagrass. That model is supported by a body of experimental work (Table 1.1). Although the results of those experiments are consistent with the model, they are not definitive. Their design can not distinguish between indirect grazer effects through the consumption of epiphytes and other unspecified direct or indirect effects. Those experiments do not demonstrate the causal process that is assumed to directly limit seagrass production, i.e. the effects of epiphytes. That is because it is not possible to directly manipulate epiphytic cover on the leaves. Although randomized experiments provide the best means of showing causal relationships, they are often not possible. However, causal processes can be tested using path analysis or structural equation modelling (SEM). Those techniques use the correlative relationships in field data to test their fit to pre-specified models, such as the prevailing model of seagrass-epiphyte-grazer interactions. It is also possible to use the patterns of correlation in the data to infer the underlying causal structural that gave rise to the data (Shiple 2004).

An alternative model of the effects of grazers was proposed as a result of the apparent effects of sea hare grazing on the epiphytic cover of *Halophila ovalis* (chapter 3). It was proposed that epiphytes benefited the seagrass by providing a protective shield against high levels of irradiance. Removing epiphytic cover appeared to have a negative impact on *H. ovalis*, possibly by exposing it to high levels of irradiance. As in the prevailing model, the impact of grazers on seagrass is indirect, and is a result of the effects of grazing on epiphytic cover. Like the prevailing model, this alternative model can be used as a pre-specified model against which to test the observed data using path analysis. New models can also be suggested by the data. As noted in chapter 4,

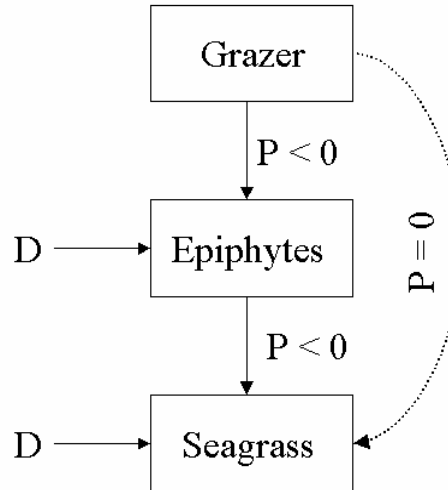
important elements of the epifaunal assemblage of *H. uninervis* are suspension feeders, and their relationship to the seagrass and its epiphytic cover can be examined.

This chapter concerns *Halodule uninervis* and its interaction with its epifauna through its epiphytic cover. It brings together the data on those elements previously presented in chapters 4 and 5 and considers them within the context of path models. The previous chapters considered the relationships that are parts of the overall model, i.e. interactions between epifauna and epiphytic material, and between epiphytic material and *H. uninervis*. However, to evaluate the fit of a particular model, it is not sufficient to look at interactions between individual variables. Rather, it is necessary to test how all the variables interact simultaneously as part of a pattern of correlations and partial correlations, i.e. as a single covariance structure.

## 6.2 Statistical methods – path analysis

Path analysis tests hypotheses about causal relationships among a set of observed variables (Pedhazur 1982, Kline 1998). The technique is most often used to test the fit of observed correlations and partial correlations to the values expected if pre-specified causal effects, represented in a model, were operating on the variables. The prevailing understanding of seagrass-epiphyte-grazer interactions provides a model that can be tested against observed data. That model can be represented as a path diagram (Fig. 6.1). The effect of the variable assumed to be a cause of change in a dependent (endogenous) variable is measured by a path coefficient (P). When there is only one predictor variable, the path coefficient is simply the regression coefficient (r) between the variables. When there are two or more predictors, the path coefficients are the standardized regression coefficients ( $\beta$ ) from the multiple regression of the endogenous variable on the predictors. The disturbance (D) is the proportion of the variance of an endogenous variable that remains unexplained. In the case of the prevailing model of seagrass-epiphyte-grazer interactions, grazers will have a negative effect on epiphytes ( $P < 0$ ), epiphytes will have a negative effect on seagrass ( $P < 0$ ), but grazers do not have any direct effect on seagrass ( $P = 0$ ), as their effect is completely indirect. The significance of the path coefficients can be tested with the same t-tests used for the corresponding regression coefficients. Path coefficients that are hypothesised to equal

zero are typically not represented in path diagrams, but the direct grazer-seagrass effect is represented in Fig 6.1 for illustrative purposes.



**Fig. 6.1 Path diagram representing the prevailing model of seagrass-epiphyte-grazer interactions**

The prevailing model of seagrass-epiphyte-grazer interactions is not the only model that can be proposed from existing knowledge of those systems. The results of chapter 3 suggest that epiphytic material may benefit seagrass. That possibility provides an alternative model that can be represented as a path diagram in the same way as the previous model except that epiphytes are assumed to have a positive effect on seagrass, i.e.  $P > 0$ .

The fit of the models to data was tested with a maximum likelihood chi-square ( $\chi^2$ ) statistic that tests the difference between the hypothesised model and the just-identified model. The just-identified model is the one that includes all parameters, and therefore perfectly fits the data. In the case of the path model in Fig 6.1, the just-identified model is one that includes direct effects between grazers and seagrass. It was tested against the reduced hypothesised (overidentified) model that does not include direct effects between grazers and seagrass. Therefore, the  $\chi^2$  test effectively tests the hypothesis that the path coefficient between grazers and seagrass is zero, i.e that the effects of grazers on seagrass are indirect. A non-significant ( $p > 0.1$ )  $\chi^2$  value suggests that the hypothesised model does not differ from the just-identified one, and that the reduced

hypothesised (overidentified) model fits the data. A significant value is cause to reject the model. A hypothesised model that fits the data is not accepted as the true model. It is just not rejected.

Many different models may fit the same data. One of those models may be preferred based on *a priori* knowledge or theory, but it is also necessary to consider other equivalent models (Kline 1998). Equivalent models are those that would produce the same correlations, but with different configurations of the paths. Some of those models may be implausible; but others may be credible, and it is necessary to show why the model that was first proposed should be preferred. In the case of the models proposed above, a top-down model was assumed, i.e. it was assumed that the effects were from the higher trophic level to the lower level, from grazers to epiphytes to seagrass. An equivalent bottom-up model with the same correlations can be produced by reversing the directions of the paths in Fig 6.1, from seagrass to epiphytes to grazers. In the following chapter, two top-down models of seagrass-epiphyte-grazer interactions are first considered. They will be referred to as the prevailing model, developed from the evidence of the current literature (chapter 1), and the alternative model, developed from the results of chapter 3. Their analyses will be followed by a consideration of the possibility of applying bottom-up equivalent models to the same data.

The three study plots differed greatly in their mean values of seagrass leaf area, epifaunal abundance and epiphytic cover (chapter 4 and 5). They also differed in epifaunal community composition, the type of epiphytic material, and the form of *Halodule uninervis* leaves (narrow-leaves versus broad-leaved). Nonetheless, one might expect the same causal processes to operate within the different plots. The fit of models can be tested simultaneously for the three locations using a multigroup analysis (Shipley 1999). It tests for differences in the covariance structural among the sites where the data is centered around different means. It is analogous to, but quite different from, an analysis of variance (ANOVA) which tests for differences among means.

In path analysis, the generally quoted rule concerning sample sizes is that there should be at least five times more observations than free parameters. The model in Fig. 6.1

contains five free parameters, including two covariances and three variances. So, there need to about 25 observations to test it. The number of observations in this study tended to be small, especially if testing within plots. However, small sample sizes result in conservative probability estimates, where the true probability will be larger than the value obtained when assuming a  $\chi^2$  distribution (Shipley 1999). There were 21 observations with complete data from plot SB, and 17 from plot PB. In plot CB, the standing crop fell to almost zero after the first year, and so the analysis is restricted to the first year, June 2002 to June 2003, with 10 observations.

Tests were performed using structural equation modelling in the Statistica software package (version 7.1) (StatSoft, Inc. 2005). All data used in path analyses were transformed to normalize their distributions. Mean invertebrate numbers per 100 cm<sup>2</sup> of leaf area were  $\log_e$  -transformed. The proportion (p) of leaf area covered by epiphytic material was transformed using the arcsine transformation ( $t(p) = \arcsin(\sqrt{p})$ ). The leaf area index (LAI) was normalized with the 4<sup>th</sup> root transformation. In graphs, some variables are not transformed for the ease of interpretation.

## 6.3 Results

### 6.3.1 *Alaba virgata*

*Alaba virgata* was the most common epiphyte-grazing invertebrate in plot SB. Its abundance on *Halodule uninervis* was negatively correlated with the percent epiphytic cover of the leaves (chapter 4), suggesting that *A. virgata* grazing had a direct negative impact on epiphytes. However, epiphytic cover was positively correlated with measures of seagrass abundance (chapter 5). Therefore *A. virgata* could not have an indirect positive impact on seagrass abundance as proposed by the prevailing model of seagrass-epiphyte-grazer. Rather, that positive correlation was in accordance with the alternative hypothesis that epiphytic cover has a positive effect on the seagrass, and epiphyte grazers have an indirect negative impact. Path analysis confirmed that the alternative model fit the data well (Fig. 6.2). There is no evidence in the data to suggest a direct relationship between *A. virgata* and *H. uninervis*, only an indirect one through epiphytic cover. However, an equivalent bottom-up model fits the data just as well, but reverses the presumed causation, so that increasing LAI (Leaf Area Index) is

represented as a cause of increasing epiphytic cover, and epiphytic cover reduces *A. virgata* numbers. Such a model would not have been proposed in advance of the study, but there is some evidence that gives it plausibility. For example, increases in LAI appeared to lead increases in epiphytic cover in plot SB (Fig. 5.8), and numbers of *A. virgata* were always negatively associated with epiphytic cover, even where their numbers seemed to be too few to control epiphyte abundance (chapter 4). The data fit both top-down and bottom-up explanations for the observed data, and there are no clear reasons to reject either of them. The reality may be that the variables are involved in reciprocal interactions.

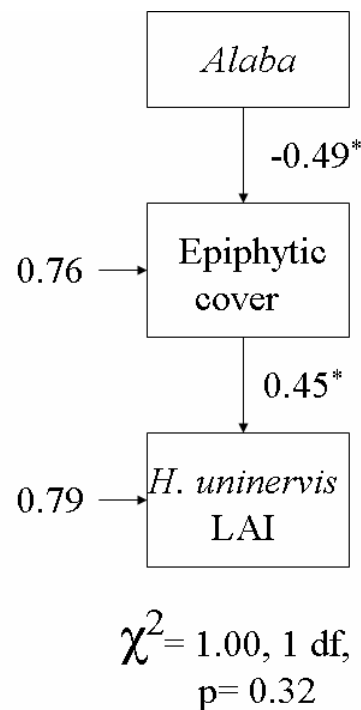
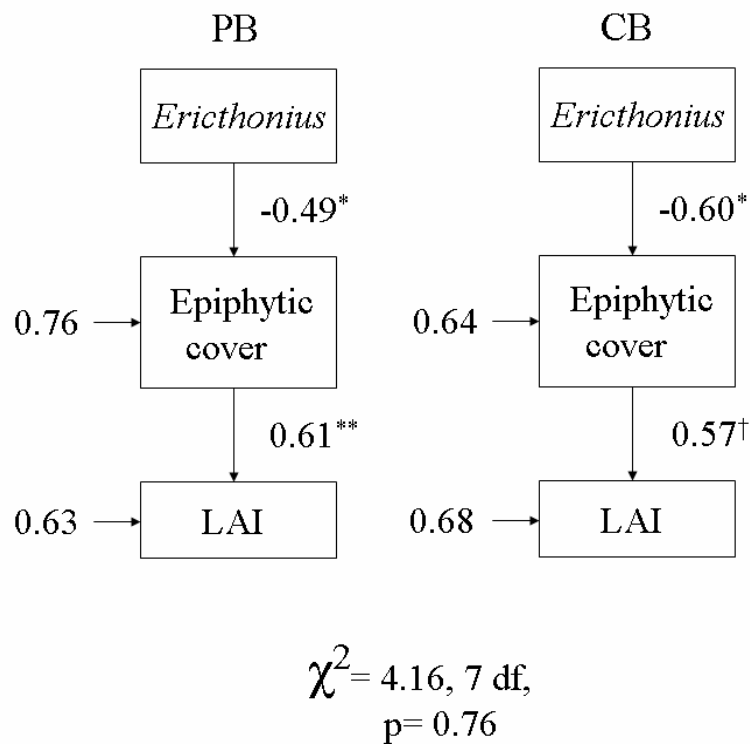


Fig. 6.2 Path model of *Alaba* effects on *Halodule uninervis* in plot SB, n=21. \*p<0.05.

### 6.3.2 *Ericthonius*

*Ericthonius* was the most abundant potential epiphyte grazer in both plots PB and CB. Their numbers were negatively correlated to epiphytic loads and cover (chapter 4). In plot PB, *Halodule uninervis* LAI did not vary, but *H. uninervis* BGDW was positively correlated to changes in epiphytic cover. Total LAI, calculated as a combination of *H. uninervis* and *Cymodocea serrulata* leaf areas, was also positively related to epiphytic cover (chapter 5). In plot CB, during the first year of the study, epiphytic cover tended

to be positively correlated to *Halodule uninervis*. Thus, if *Ericthonius* was having a negative impact on epiphytic cover, then it was most likely to be having a negative effect on the seagrass, consistent with the alternative model. Path analysis showed that the alternative model fit the data in a multigroup analysis using *Ericthonius* densities from plots PB and CB (Fig. 6.3). However, an equivalent model with reverse causation would fit the data equally well, and is not implausible. Epiphytic loads were extraordinary heavy in the plot CB, and it would seem unlikely that epiphytic cover was benefiting the seagrass. It also seems possible that the very heavy cover in that plot was potentially a deterrent to *Ericthonius* if it was primarily a suspension feeder.



**Fig. 6.3** Path model of *Ericthonius* effects on LAI, calculated as the combined value of *Halodule uninervis* and *Cymodocea serrulata* in plot PB (n=17), and *H. uninervis* in plot CB from June 2002 to June 2003 (n=10). †0.05<p<0.10, \*p<0.05, \*\* p<0.01.

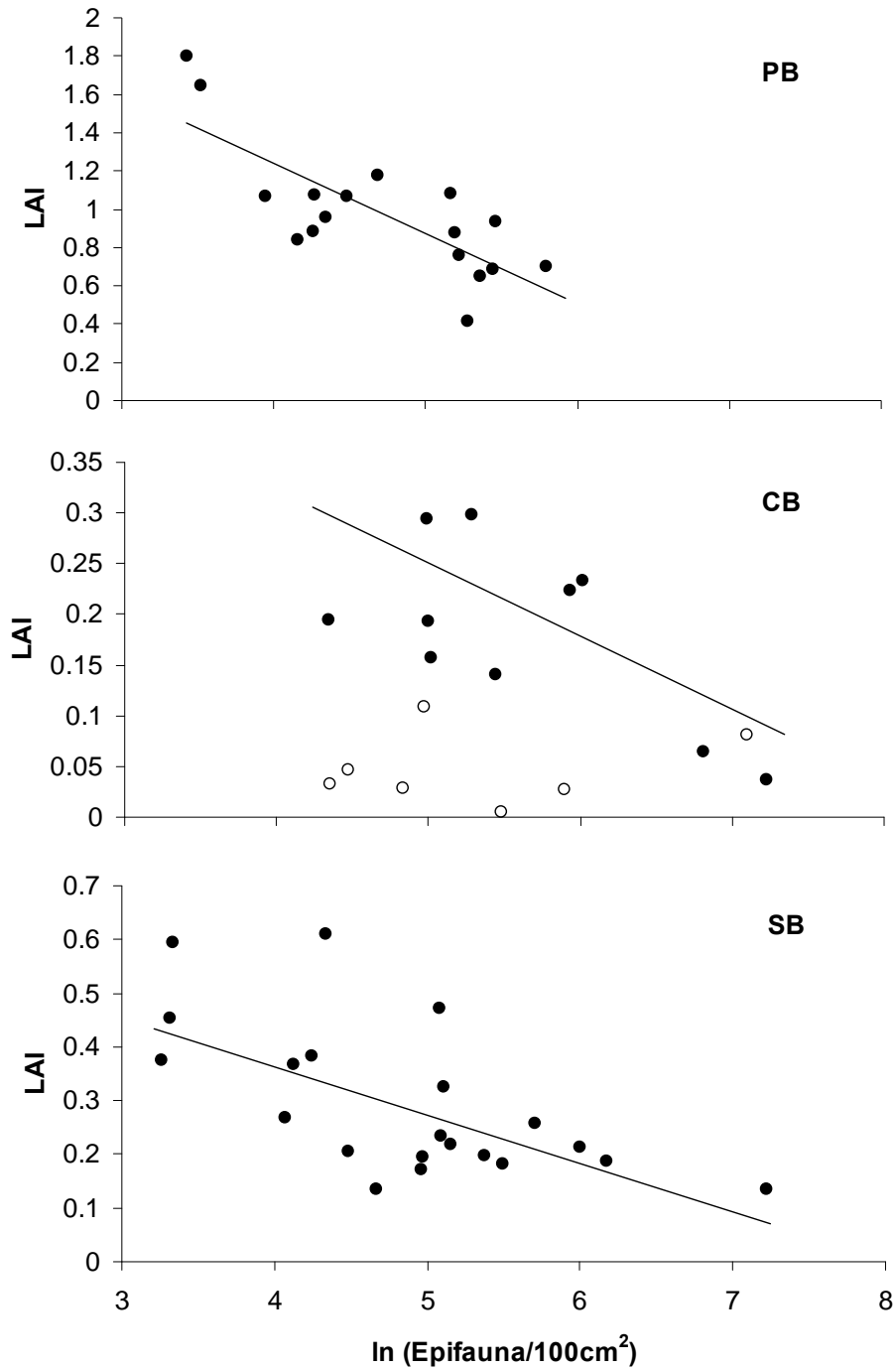
### 6.3.3 Total invertebrates

The density of invertebrates per leaf area on each sampling occasion was negatively related to the seagrass leaf area in each plot (Fig. 6.4). In plot PB, the density of epifauna was negatively correlated to *Halodule uninervis* BGDW ( $r=-0.74$ ,  $p<0.001$ ), but not its LAI ( $r=-0.16$ ,  $p>0.10$ ). However, changes in the canopy of plot PB were a

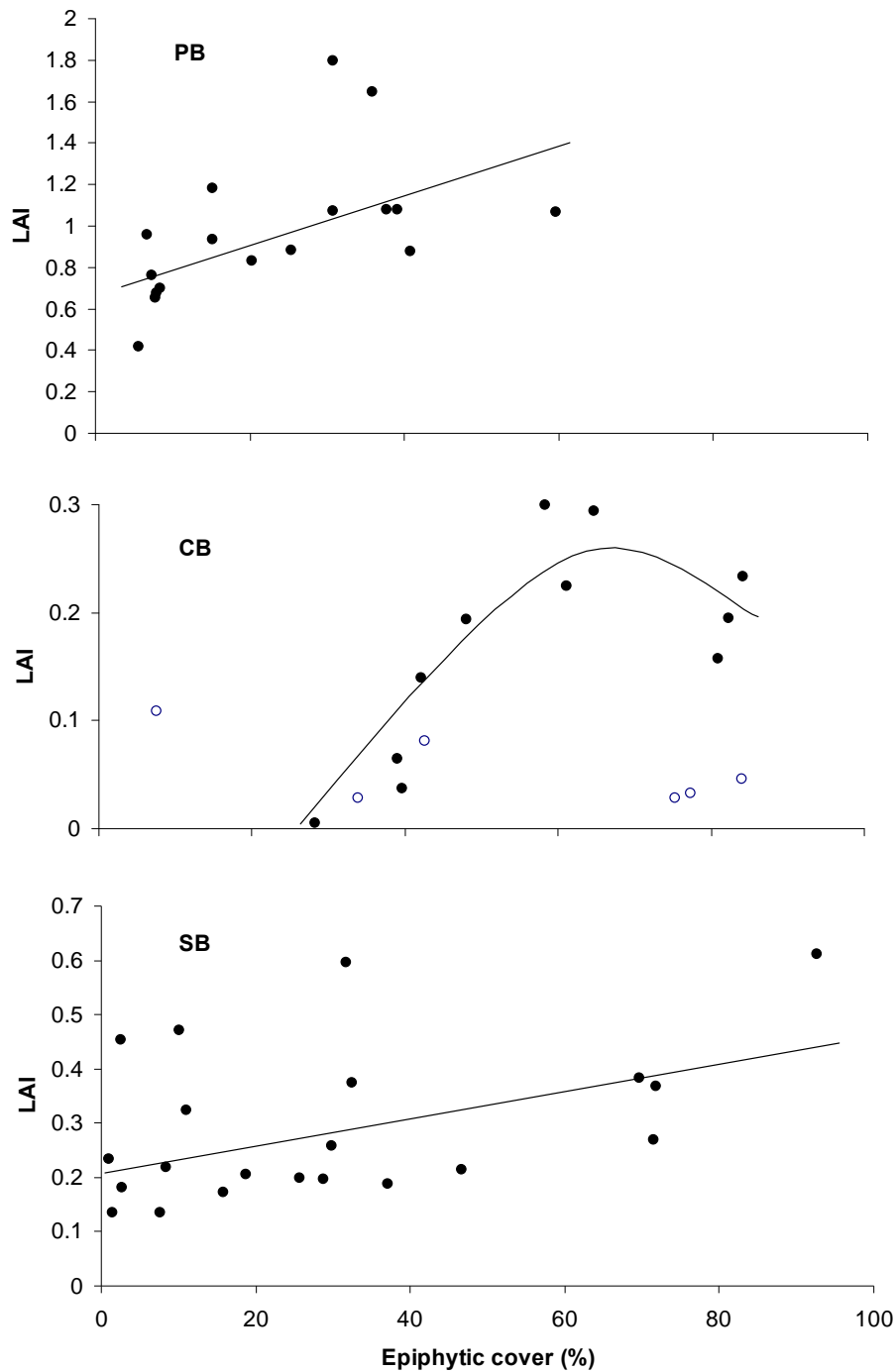


result of changes in *Cymodocea serrulata*, and invertebrate density was strongly related to the combined LAI of both species ( $r=-0.78$ ,  $p<0.001$ ). In plot CB, total epifaunal abundance was negatively correlated with *Halodule uninervis* LAI ( $r=-0.64$ ,  $p<0.05$ ) (Fig. 6.11) and BGDW ( $r=-0.81$ ,  $p<0.005$ ) from June 2002 to June 2003, but not from July 2003 to July 2004. The relationship during the first year reflected the dramatic rise in anemone densities as *H. uninervis* declined. There were very strong negative correlations between anemone densities and *H. uninervis* BGDW, and LAI, from June 2002 to June 2003 ( $r=-0.89$ ,  $p=0.001$ ; and  $r=-0.76$ ,  $p<0.025$ ). In plot SB, there were also negative correlations between the total invertebrates density and both *H. uninervis* LAI ( $r=-0.64$ ,  $p<0.005$ ) and BGDW (Below Ground Dry Weight) ( $r=-0.74$ ,  $p<0.001$ ) ( $r$  values for LAI and BGDW untransformed, invertebrate densities log-transformed).

Those strong correlations between total epifaunal density and the amount of seagrass suggest a more direct relationship between the seagrass and its epifaunal community than was hypothesised in the prevailing or alternative models. However, very few members of the epifauna are direct consumers of seagrass, and the general density of animals on the leaves was typically not great enough that they would be a physical burden, with the possible exception of anemones in Cockle Bay. However, the majority of the animals in each plot were suspension feeders, and it can be presumed that the development of the seagrass canopy limits their feeding opportunities. The data suggests a bottom-up relationship from the seagrass to its epifauna. As well, there were positive relationships between epifaunal densities and epiphytic cover (Fig. 6.5). The lag relationship between LAI and epiphytic cover in plot SB (Fig. 5.8) suggested that LAI predicted the cover as a bottom-up effect. A model assuming bottom-up relationships from seagrass LAI to invertebrate abundance and epiphytic cover, with no relationship between them, fit the data well for the three sites in a multigroup analysis. The data suggests that, although the sites differed greater in mean values amongst the variables, the same bottom-up causal processes were operating in each of the three sites. An equivalent model, with the causal relationships reversed between epiphytic cover and LAI, would fit the data equally well; but that model still implies a bottom-up explanation of epifaunal density, and no direct relationship between epifaunal density and epiphytic cover.



**Fig. 6.4 Relationships between epifaunal abundance and LAI (untransformed) in plots PB ( $r=-0.78$ ,  $p<0.001$ ), CB ( $r=-0.64$ ,  $p<0.05$ ), and SB ( $r=-0.64$ ,  $p<0.005$ ). LAI in plot PB is the combined value of *Halodule uninervis* and *Cymodocea serrulata*, but in other plots is only *H. uninervis*. The relationship in plot CB was calculated for the period June 2002 to June 2003 (solid circles) only. Note the differences in the scale of LAI among the plots.**



**Fig. 6.5** Relationships between percent epiphytic cover and LAI (both untransformed) in plots PB ( $r=0.53$ ,  $p<0.05$ ), CB ( $R^2=0.80$ ,  $p<0.005$ ), and SB ( $r=0.44$ ,  $p<0.05$ ). LAI in plot PB is the combined value of *Halodule uninervis* and *Cymodocea serrulata*, but in other plots is only *H. uninervis*. The quadratic function in plot CB was calculated for the period June 2002 to July 2003 (solid circles) only. Note the differences in the scale of LAI among the plots. Plots PB and CB redrawn from Fig. 5.12 and Fig. 5.17.\*\*\*

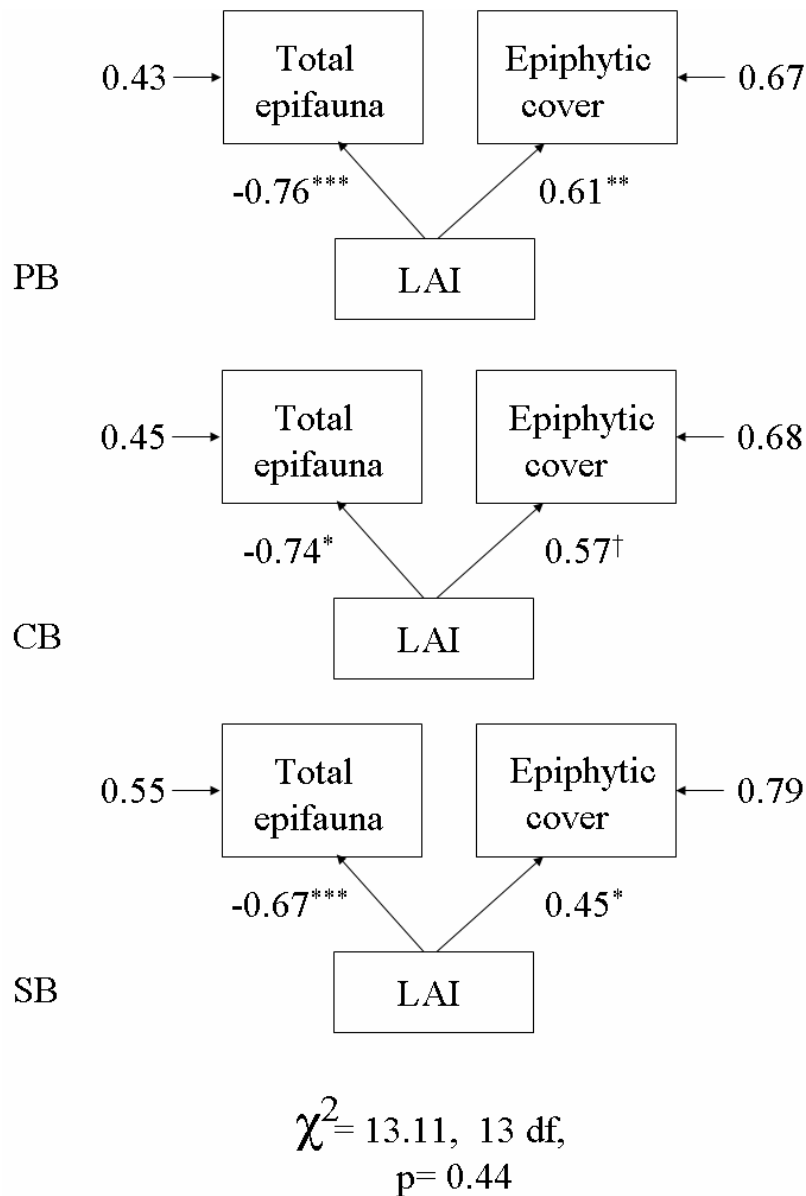


Fig. 6.6 Path model of effects of LAI on the total density of epifauna and epiphytic cover (all variables transformed) in a multigroup analysis. LAI calculated as the combined value of *Halodule uninervis* and *Cymodocea serrulata* in plot PB (n=17), and *H. uninervis* in plot SB (n=21) and plot CB from June 2002 to June 2003 (n=10). †0.05<p<0.10, \*p<0.05, \*\* p<0.01. \*\*\* p<0.001.

### 6.3.4 Overview

The prevailing model of seagrass-epiphyte-grazer interactions did not fit the data for individual species that were likely to be epiphyte grazers, nor did it fit the data on total epifaunal abundance. The lack of fit of that model reflected the positive relationships between epiphytic cover and seagrass LAI, and the negative relationships between total epifaunal abundance and LAI. However, the alternative top-down model provides an explanation for the positive relationship between epiphytic cover and seagrass LAI, and

fit data on likely epiphyte grazers, especially *Alaba virgata*. In the case of *Ericthonius* in plot CB, the data also fit the alternative top-down model, but did not seem as likely an explanation as a bottom-up model; nor could a bottom-up explanation be rejected in the case of *A. virgata*. Total epifaunal abundance in all plots appeared to be directly related to seagrass LAI, and could only be explained in a bottom-up fashion, reflecting the numerical dominance of the fauna by suspension feeders. Moreover, those bottom-up processes appeared to be operating in all plots irrespective of the great differences among the plots in mean values of the variables, as well as differences in the composition of the epifauna and epiphytic material.

## 6.4 Discussion

The prevailing model of seagrass-epiphyte-grazer interactions is not consistent with data from the tropical intertidal meadows examined in this study. That model suggests that epiphyte grazers benefit seagrass standing crop by limiting epiphytes that restrict light to the seagrass for photosynthesis. The data from plots SB and CB suggest that epiphytic loads did indeed reach levels that limited the standing crop of *Halodule uninervis*. However, contrary to expectations, epiphytic loads within each plot were positively correlated to standing crop. That relationship suggested that if epiphyte grazers were limiting epiphytic loads, then they were not having a beneficial impact on the seagrass. The positive correlation between epiphytic cover and seagrass standing crop leads to the rejection of the prevailing model.

The alternative top-down model presumes that epiphytes benefit seagrasses in shallow tropical waters by providing a protective cover from high levels of irradiance. Path analysis models show that the data for *Alaba virgata* and *Ericthonius* were consistent with that alternative hypothesis, and suggested that grazers had negative indirect effects on *H. uninervis*. As in the prevailing model, that alternative model presumes top-down effects. However, other equivalent models could also have fit the data, but presume bottom-up effects. In reality, it may be that there are reciprocal interactions amongst the seagrass, its epifauna, and epiphytes.

In all three plots, total epifaunal density was negatively correlated with the density of seagrass. There are a number of possible explanations for that relationship, including negative effects by epiphyte grazers and herbivores. However, suspension feeders dominated the fauna. They are dependent on a flow of water laden with edible particles, and the quantity and quality of that food is related to water flow rates (Muschenheim 1987, Fréchette et al. 1989). Most seagrass species have long strap-like leaves that cause a physical baffling that reduces flows relative to the density of the vegetation (Peterson et al. 2004). Flows are reduced within seagrass canopies (Gambi et al. 1990, Verduin and Backhaus 2000), which causes settling of suspended particulate matter transported from non-vegetated areas (Ward et al. 1984). The ability of a seagrass canopy to settle particulates has been correlated to LAI, specifically when  $LAI < 4$  (Gacia et al. 1999). Benthic mussels are suspension feeders that frequently occur in seagrass meadows, but have been found to have reduced growth and survival there compared to non-vegetated areas (Reusch 1998). That difference has been related to lower food supply in the canopy because of reduced water flow rates (Reusch and Williams 1999, Allen and Williams 2003). In this study, the reduce density of epifauna, mainly suspension feeders, in association with increasing LAI, is most easily explained by an association between increased LAI and reduced water flow and food resources for suspension feeders.

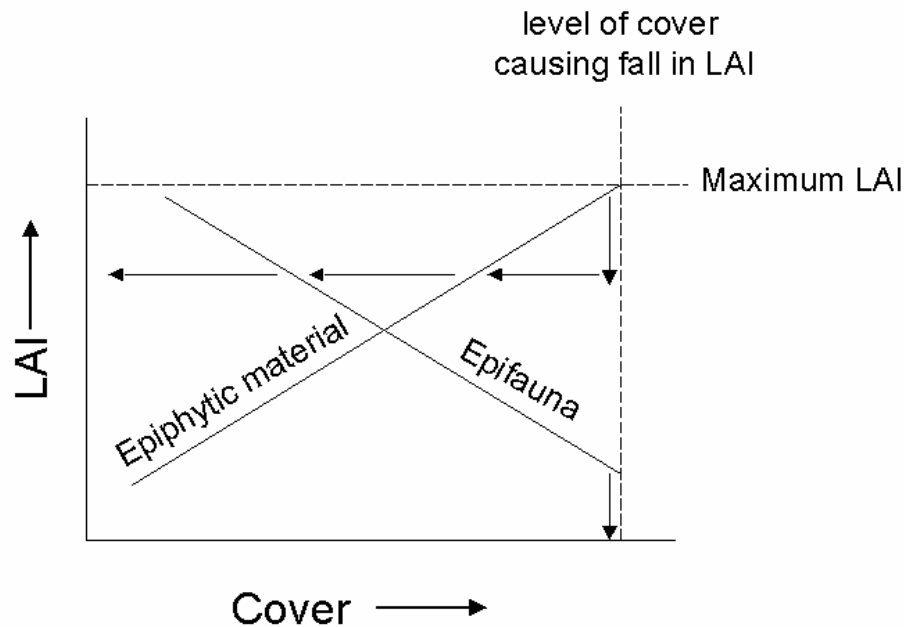
In all three plots, epiphytic loads were positively correlated with the density of seagrass, and that relationship could also be related to the effects of seagrass on water flow. More particulate material can be expected to settle out of the water column in seagrass meadows with higher LAI values because of the increased attenuation of flow. A portion of that material is trapped directly on the leaves (Agawin and Duarte 2002). In plots CB and SB, resettled material was an important part of observed epiphytic loads, and was likely an important stimulus for algal growth. During experimental shadings at Shelly Beach (chapter 9), an algal bloom appeared to be related to the settlement of material directly on leaves in non-shaded plots. Thus the observed changes in both epiphytic loads and invertebrate abundance on *Halodule uninervis* leaves can be related by the same mechanisms to changes in the plant's LAI.

Path models that presumed a direct relationship from *Halodule uninervis* LAI to total invertebrate densities fit the data well, but also suggested that there was no direct relationship to epiphytic loads. Negative correlations between total invertebrate densities and epiphytic loads appear to be the result of a common, but inverse, relationship to *H. uninervis* LAI. Bottom-up hypotheses appear to provide the best explanations of apparent seagrass-epiphyte-invertebrate relationships involving suspension feeders, which would include *Electroma*, anemones, and the dominant amphipods in this study, i.e. *Erichthonius* and *Podocerus*.

Grazers have a role in tropical intertidal *Halodule uninervis* meadows, but do not appear to be as important as they are presumed to be in other seagrass communities. *Alaba virgata* abundance was directly related to epiphytic cover, but it is uncertain to what extent their grazing limited it. Their numbers were greatest when daytime tidal exposure was greatest, so their likely impact on *Halodule uninervis* was negative. *Erichthonius* and *Podocerus* may have some impact on epiphytic cover, but it is unlikely to be significant, and less likely to affect the seagrass. Far greater information is needed on the feeding behaviour of those animals to assess their roles. Sea hares appeared to be the most capable of limiting epiphytic loads, but their numbers were unpredictable. In plot CB, and plot SB during the second year, epiphytic loads reached such high values that they must have escaped any control by grazers.

The positive relationships found between epiphytic loads and LAI suggests a positive feedback mechanism that allows epiphytic loads to increase to maximum levels. Increasing epiphytic loads might provide protection from high irradiance (as proposed in chapter 3) and allow for an increase in LAI which then traps more suspended material and further increases loads. During times of high daytime tides, the seagrass appears to suffer from low light stress, and in order to capture more light, LAI is increased, trapping still more epiphytic material. Finally, when the seagrass becomes severely light deprived, shoot density and LAI decrease, thinning out the canopy, allowing more water flow, reducing epiphytic cover, and increasing opportunities for suspension feeders. The LAI at which epiphytic loads reach a maximum would differ between sites because the amount of suspended material probably differs among sites because of hydrological and sediment differences. Near-shore sites receive greater

loadings of resuspended sediment, especially where there are deposits of fine terrigenous sediments, because of greater mixing in the coastal boundary layer (Wolanski et al. 1997).



**Fig. 6.7** Schematic diagram representing interactions among *Halodule uninervis* LAI, and cover by epiphytic material and epifauna. Coverage by epiphytic material or epifauna increases to a maximum that forces a fall in LAI, represented by the arrows. At maximum epiphytic cover, a fall in LAI results in a decrease in epiphytic cover, and an increase in epifauna. At maximum epifaunal cover, LAI falls to zero.

Decreases in the seagrass canopy increase the numbers of suspension feeders. When the coverage by epifauna is great enough, the canopy will further decline, eventually leading to its complete collapse. That appears to have happened at Cockle Bay during the first year of this study, when numbers of anemones, and *Ericthonius* with their domiciles, increased to extremely high densities after an initial reduction in the canopy following extremely heavy epiphytic loads. Cockle Bay supported both the greatest density of invertebrates and highest epiphytic loads, probably because of higher concentrations of resuspended sediments. The maximum LAI that *Halodule uninervis* could obtain at the site was not much greater than the minimum sustainable LAI that the epifaunal load would allow. Similar explanations can be applied to the data presented by Williams and Heck (2001) (from an unpublished thesis by Sewell 1996) of a bloom of the anemone *Bunodeopsis* on *Zostera marina* in California. There, the



seagrass initially declined during a period of high runoff from rains, but then declined further during the subsequent anemone bloom.

The negative relationships found between epifaunal densities and the densities of seagrass in this study were contrary to expectations. In many other studies, the abundances of invertebrates have been positively correlated with seagrass biomass or leaf surface area (Heck and Wetstone 1977, Stoner 1980b, Stoner and Lewis 1985, Ansari et al. 1991, Attrill et al. 2000). In North Queensland, similar positive correlations were found for fish and decapods in Cockle Bay (Kwak and Klumpp 2004), and for subtidal decapods at another site (Mellors and Marsh 1993). However, not all studies find positive relationships between seagrass and invertebrate abundance. The results of this study are consistent with those of Virnstein and Howard (1987b, a), who compared epifaunal abundances among three seagrass species in Florida. They found that the species with the lowest LAI, *Syringodium filiforme*, had the greatest density of epifaunal invertebrates per area of leaf, and the species with the greatest LAI, *Thalassia testudinum*, had the lowest density. Similarly, Lewis (1984) found greater numbers of amphipods per leaf surface area in low density *Halodule wrightii* than in high density *T. testudinum*. Comparing among studies over a range of latitude, Virnstein et al. (1984) concluded that seagrass biomass was a poor indicator of amphipod abundance. One of the reasons for the discrepancy among studies may be that those of Virnstein and Howard (1987b, a), and Lewis (1984), had larger numbers of suspension feeders in their samples, and used sampling techniques that targeted smaller epifaunal animals. Studies that focused on larger animals, such as decapods, are more likely to be sampling animals that enter seagrass meadows for shelter, and have a very different relationship to the seagrass canopy. There may also be important differences between intertidal versus subtidal, and temperate versus tropical faunas.

The positive relationships typically found between seagrass and invertebrate abundances have been explained by suggestions that the increase in the amount of seagrass provides more resources per area of substrate, and/or increases protection from predators (Heck and Wetstone 1977, Heck and Orth 1980, Orth et al. 1984). In the case of epiphyte grazers, an increase in above ground plant biomass would equate to an increase in the amount of surface area available for grazing. Thus, the number of

grazers should increase per area of substrate as seagrass biomass increases, but remain constant per area of leaf. A food resource ceiling occurs, as the surface area equates with the amount of food available to epiphyte grazers if other limiting factors (i.e. light and nutrients) remain unchanged, as suggested by the experiments of Edgar (1990a). However, in this study the relationship between invertebrate abundance and measures of seagrass abundance were quite different, and suggested that epifaunal abundance was not limited by epiphyte food resources, or predation.

## 6.5 Conclusions

The prevailing model of seagrass-epiphyte-grazer interactions, that presumes epiphyte grazers indirectly benefit seagrass production, does not provide a suitable description of interactions in intertidal *Halodule uninervis*. An alternative model, that presumes an indirect negative impact of epiphyte grazers on seagrass production, provides a better explanation, especially with respect to *Alaba virgata*. However, bottom-up explanations of the data cannot be rejected. Suspension feeders, rather than epiphyte grazers, are numerically dominant in the epifaunal community. The relationship of the seagrass to total invertebrate abundance and epiphytic loads is best explained by bottom-up processes that relate leaf area to the attenuation of water flow in the canopy. Epiphytic material appears to have a stabilising positive feedback response with respect to changing leaf area while epifauna has a destabilising response that could lead to the disappearance of the seagrass canopy. These models are not presented here as definitive explanations, but as original and testable models that may prove useful in developing a better understanding of seagrass-epifaunal interactions within the seagrass canopy. The models could be substantially refined by including, for example, measures of the relationship between leaf area and water flow, and between water flow and the activities of suspension feeders. Analyses at larger scales are also needed, as the analyses done here apply only to processes within plots. Differences amongst the plots suggest that other processes are operating at a larger scale.

## Chapter 7 Herbivory and succession

### 7.1 Introduction

Consumption of seagrasses by herbivores has traditionally been thought to have a minor impact on seagrass production and carbon budgets (Klumpp et al. 1989) (Thayer et al. 1984). The percentage of production removed by herbivores can be extremely varied (Cebrián et al. 1996a), and sometimes large. However, the total impact can still appear modest when averaged over many sites or studies (Cebrián and Duarte 1998).

Valentine and Heck (1999) reviewed the role of herbivores in seagrass ecosystems, and believed their role had been underestimated. In their review, and in a revision by Valentine and Duffy (2006), they found substantial evidence of vertebrate (fish, waterfowl, dugongs, manatees, green turtles) and urchin feeding on seagrasses, but little evidence of feeding by smaller invertebrates. The few invertebrates that were included suggest that they are rare exceptions to the general rule that such animals do not feed on seagrass. However, a more thorough re-examination of the literature (chapter 1) showed that there were many overlooked examples of small invertebrates feeding on seagrasses. They included highly specialized feeders, such as *Sacoglossa* (Jensen 1982, 1983a), limpets (Barbour and Radosevich 1979, Zimmerman et al. 2001), and boring isopods (Brearley and Walker 1995, van Tussenbroek and Brearley 1998, Gambi et al. 2003); as well as generalist herbivores, such as crabs (Klumpp and Nichols 1983, Woods and Schiel 1997), a prawn (O'Brien 1994), rock lobster (Joll and Phillips 1984), Alpheid shrimp (Stapel and Erftemeijer 2000), isopods (Robertson and Mann 1980, Nienhuis and Groenendijk 1986, Thom et al. 1995, Cebrián et al. 1996b), amphipods (Kirkman 1978), and gastropods (McConnaughey and McRoy 1979, Stephenson et al. 1986). It appears that there is more potential for invertebrates to affect seagrasses through direct feeding than is generally believed.

*Halophila ovalis* and *Halodule uninervis* are well known to be favoured food of dugongs (Preen 1995), but there are no reports of invertebrate feeding on those species. However, during the course of this study there was conspicuous evidence of damage to

the leaves of those species that was apparently caused by invertebrates. That damage was evident on leaves that were sampled to estimate invertebrate abundances and epiphytic loads following the sampling program and methods outlined in chapter 2. Methods were developed for quantifying that damage, and they have been described in detail in chapter 2. In this chapter, invertebrate consumers of *H. ovalis* and *H. uninervis* are identified, and the damage they cause is described and measured for the first time.

Tropical seagrass meadows have characteristic patterns of species succession. In the Indo-Pacific region, *H. ovalis* is usually the first species to become established after disturbance (Birch and Birch 1984). *H. uninervis* is also considered a pioneer species, but it will tend to dominate *H. ovalis* over time (den Hartog 1970). The Shelly Beach site was severely disturbed by Cyclone Tessie in April 2000, and changes in the species composition there were likely to reflect successional patterns. In this chapter, a possible relationship is examined between the patterns of herbivory found on the leaves and seagrass species succession over time at Shelly Beach.

## 7.2 Results

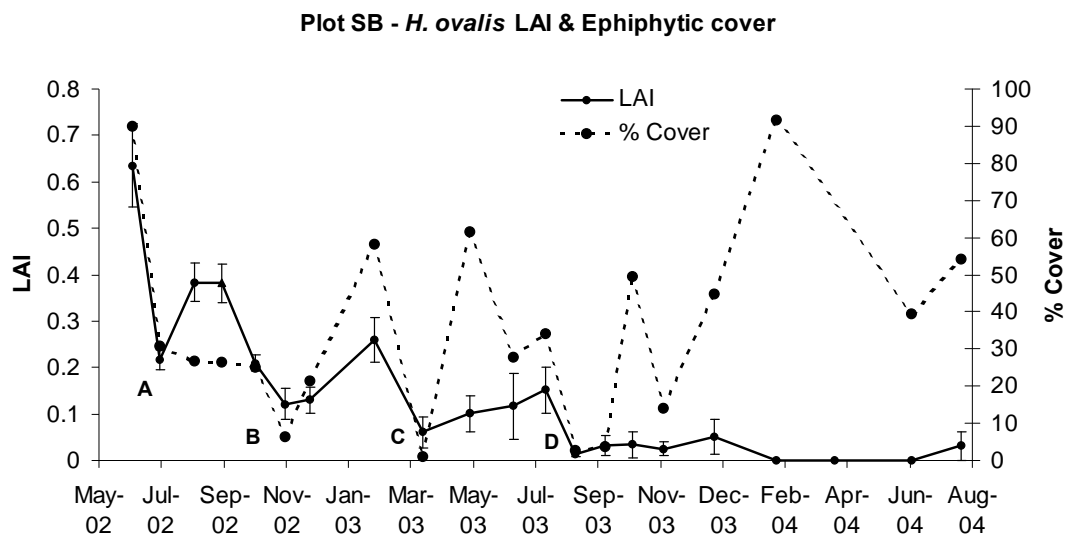
### 7.2.1 Seagrass standing crop and epiphytic cover

*Halophila ovalis* was the dominant species above ground in plot SB when sampling began in June 2002. Its LAI (Leaf Area Index) was 0.64, compared to 0.21 for *Halodule uninervis*. However, *H. ovalis* LAI fell in successive stages (Fig. 7.1, points A to D) over the first year of sampling, and by August 2003 (D) it was only 2% of its original value. Between August 2003 and August 2004, *H. ovalis* cover remained very sparse.

*Halodule uninervis* was the dominant species above ground by November 2002. *H. uninervis* LAI changed in a seasonal pattern (Fig. 7.2), with peaks of 0.47 and 0.62, in March 2003 and February 2004, respectively. It was always the dominant species below ground, with BGDW (Below Ground Dry Weight) always many times greater than that of *H. ovalis*. *H. uninervis* BGDW ranged from 51.2 to 157.8 gm m<sup>-2</sup> (Fig. 5.2), while *H. ovalis* peaked at 18.6 gm m<sup>-2</sup> in October 2002. *H. ovalis* had a large leaf

surface area relative to its below ground biomass. That ratio was lowest in August 2003 (D), at  $66.0 \text{ cm}^2 \text{ g}^{-1}$ , and highest in June 2002, at  $392.7 \text{ cm}^2 \text{ g}^{-1}$ . In contrast, the same ratios for *H. uninervis* ranged from a low of  $15.2 \text{ cm}^2 \text{ g}^{-1}$ , also in August 2003 (D), to  $52.5 \text{ cm}^2 \text{ g}^{-1}$  in February 2004. *H. ovalis* BGDW was negatively correlated with *H. uninervis* BGDW ( $r=-0.68$ ,  $p<0.005$ ) up to December 2003, after which *H. ovalis* was absent in core samples. The LAI values of the two species did not appear to be related over the same time period ( $r=-0.39$ ,  $p>0.10$ ).

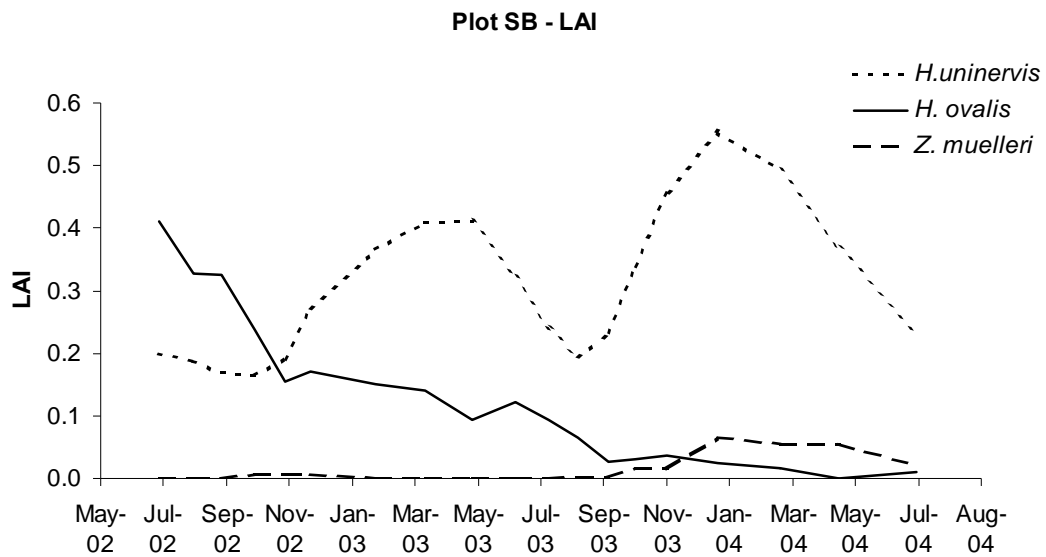
*Zostera muelleri* was present in plot SB throughout the two years, but often was not abundant enough to occur in samples. However, it became conspicuously more common by late 2003 (Fig. 7.2), and obtained an LAI of 0.15 in February 2004, the same date that *H. uninervis* LAI and its epiphytic loads peaked.



**Fig. 7.1** Mean LAI ( $\pm$  s.e.) of *Halophila ovalis* in core samples from plot SB,  $n=10$  from June to September 2002,  $n=5$  thereafter. Letters A to D correspond to points of reduced *H. ovalis* LAI, as referred to in text. Percent epiphytic cover of leaves as in Fig. 4.7, with data for June and July 2002 estimated from regression relationship with AFDW.

The largest decline in *H. ovalis* LAI occurred between June and July 2002 (A), in association with a sudden incursion of sea hares into the plot, and a loss of epiphytic cover (chapter 3). Subsequent declines in LAI also occurred at times of minimum epiphytic cover (Fig. 7.1). Over the first year, till August 2003, *H. ovalis* LAI was correlated to both percent epiphytic cover and epiphytic AFDW (Ash-Free Dry Weight)

( $r=0.68$ ,  $p<0.025$  and  $r=0.72$ ,  $p<0.01$  respectively,  $n=13$ ). However, that correlation was due almost exclusively to the very high LAI in association with high epiphytic cover on the first sampling date. Over the second year, epiphytic cover was also high, but LAI remained extremely low, or zero. Therefore, when the full two years data are considered, there was no correlation between *H. ovalis* LAI and epiphytic cover, or AFDW ( $p>0.10$ ,  $n=21$ ).

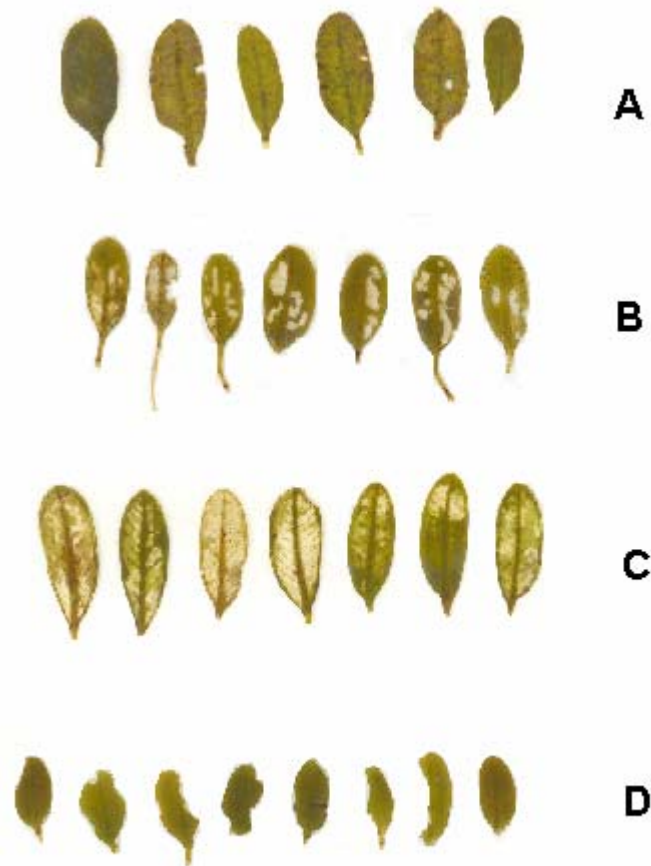


**Fig. 7.2** Patterns of change in LAIs of seagrass species in plot SB expressed as 3-point running averages.

## 7.2.2 Herbivory

### 7.2.2.1 Observations of feeding

Damage consistent with invertebrate feeding was often seen on seagrass leaves. *Smaragdia souverbiana* (Gastropoda: Neritidae) was regularly encountered on damaged leaves of both *Halophila ovalis* and *Halodule uninervis*. In the laboratory, specimens of *S. souverbiana* readily fed on leaves of *H. ovalis*. They usually removed both layers of cells from the leaves of that species, leaving only a thin, transparent cuticle. Such damage was often found in the field, and was extremely common at Shelly Beach in November 2002 (Fig. 7.3B). *H. uninervis* has a much thicker leaf, and damaged leaves had the epidermal layer removed, exposing the mesophyll, on one side.



**Fig. 7.3** *Halophila ovalis* leaves showing various types of damage. Leaves collected from plot SB for epiphytes and epifauna on dates A to D as in Fig. 7.1.

A tiny opisthobranch gastropod, <1 mm, from the order Sacoglossa was frequently found on the leaves of *Halophila ovalis*. Observations of animals brought back to the laboratory confirmed that it fed on *H. ovalis*. Feeding resulted in a mottled pattern on the leaves. Leaves were pale green where feeding had occurred on one layer of cells, and colourless where feeding had occurred on both layers. That type of damage was especially common on *H. ovalis* leaves from Shelly Beach in March 2003 (Fig. 7.3C), and Cockle Bay in January 2004. The Sacoglossa species observed feeding on *H. ovalis* leaves has not been described (K. Jensen, personal communications), and is probably in the family Limapontiidae. It was never seen on *Halodule uninervis* leaves, though other species of Sacoglossa were occasional seen on them.

*Halophila ovalis* leaves often appeared to have been chewed from the margins, a type of damage especially common in August 2004 (Fig. 7.3D) at Shelly Beach. Crustaceans were assumed to be the likely cause of that damage. The amphipod

*Cymadusa* sp. (Family Amphithoidae), which occurred regularly in sampling, was observed causing such damage in the laboratory. Similar damage was caused by the isopods *Cymodoce* spp. (Family Sphaeromatidae). The latter also feeds on *Halodule uninervis* and caused distinctive damage on broad leaves at both Picnic Bay and Cockle Bay. They typically break through the tough tissue of the vascular bundle on the outer margin of the leaf and remove the central portion, leaving the distal end of the leaf attached by the opposite margin (Fig. 7.4). The end later breaks off, usually leaving a trailing filament on the remainder. Species of *Cymodoce* are identified from mature males, and unfortunately almost all specimens encountered in routine sampling were juveniles or female. Mature males from Picnic Bay represented three species, as identified from the descriptions of Harrison and Holdich (Harrison and Holdich 1984); *C. longistylis*, *C. tribullis*, and *C. bipapilla*.



**Fig. 7.4** *Halodule uninervis* leaf showing damage typical of isopod feeding.

Despite the common observation of invertebrate damage on seagrass leaves, known seagrass consumers were a small proportion of the invertebrates taken in epifauna sampling (chapter 4 and appendix A). The combined number of *Smaragdia souverbiana*, Sacoglossa, amphithoid amphipods, and sphaeromatid isopods was only 6.7% of all animals collected over two years. *Halophila ovalis* leaf samples from plot SB had the greatest proportion of seagrass consumers, at 15% of 380 invertebrates.

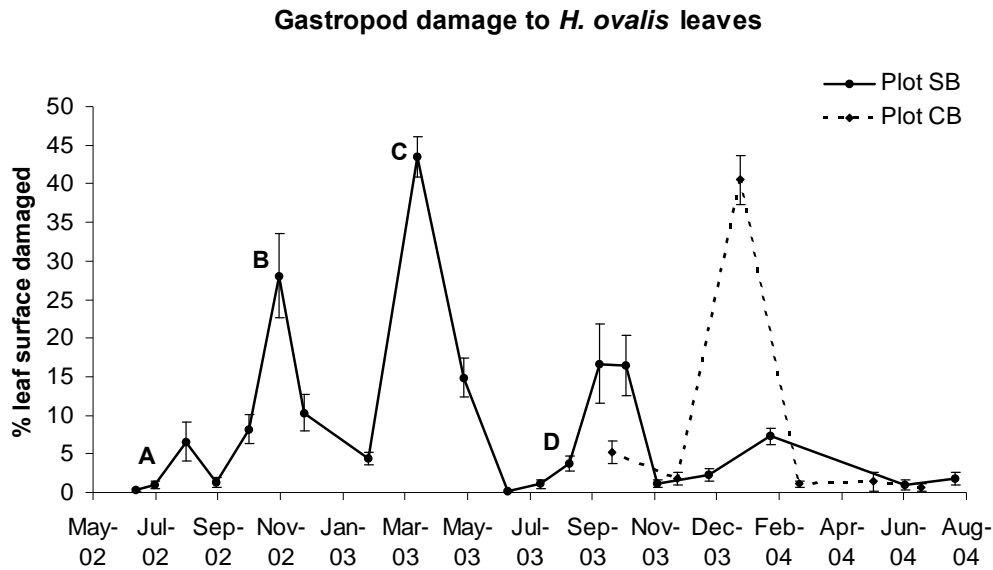
#### 7.2.2.2 Measurements of herbivore damage

In plot SB, estimates of the percent of *Halophila ovalis* leaf surfaces damaged by gastropods varied considerably over time, from near zero to a maximum of 43% in March 2003 (C) (Fig. 7.5). Estimates combined damage believed to be due to both *Smaragdia souverbiana* and Sacoglossa. The March 2003 (C) peak was believed due to the undescribed Sacoglossa. A peak in damage in November 2002 (B), 28%, was largely due to damage by *S. souverbiana*. In contrast, damage on *Halodule uninervis* leaves was much less. Damage, likely caused by *S. souverbiana*, was only 4.4% and



2.2% of the surface areas of *H. uninervis* in November 2002 and March 2003 respectively.

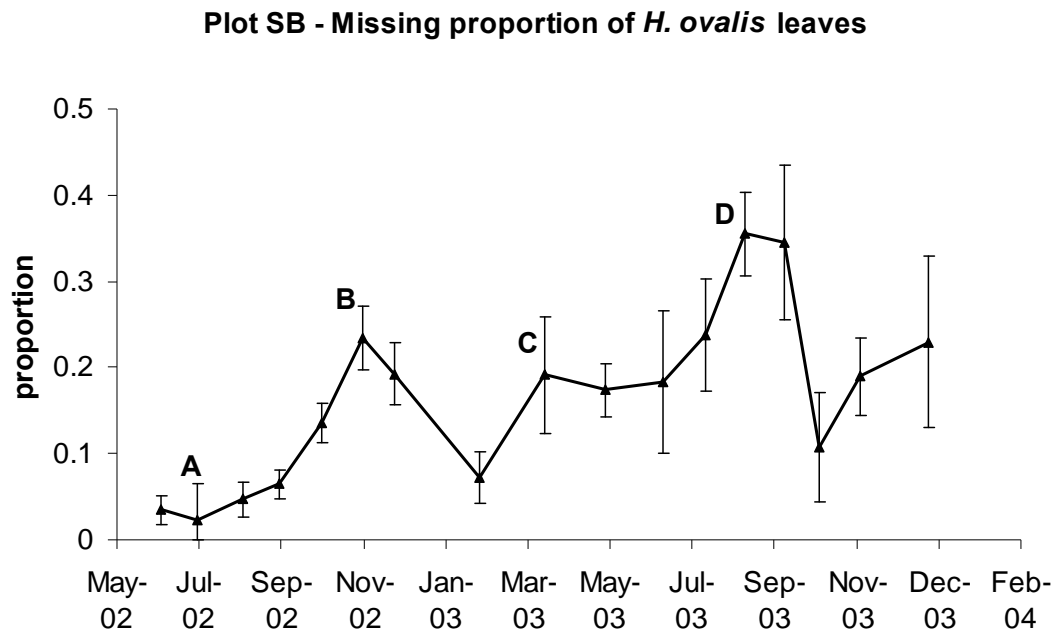
In plot CB, damage on *Halophila ovalis* leaf samples taken on 6 dates was greatest in January 2004, when 41% of surface areas were damaged, mostly by *Sacoglossa*. There was little damage on other sampling dates (Fig. 7.5).



**Fig. 7.5** Percent surface ( $\pm$  s.e.) of *Halophila ovalis* leaves damaged by gastropods in samples collected to estimate epiphytic loads and epifaunal abundances in plots SB and CB. Letters A to D as in Fig. 7.1.

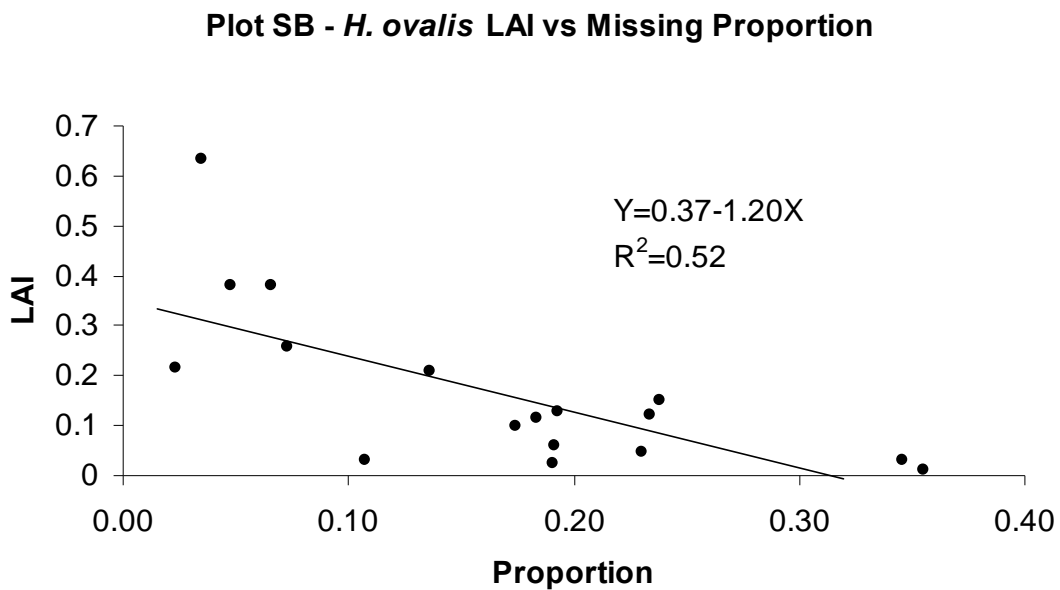
The proportion of *Halophila ovalis* leaves missing in plot SB, and presumed to be consumed by crustaceans, increased to a peak of 36% in August 2003 (Fig. 7.6, D). Those calculated missing proportions are minimum estimates of loss, as they do not account for leaves that may have been completely removed. There were no estimates after December 2003, when *H. ovalis* was absent in core samples. The error of the estimates increased over time as the number of cores with *H. ovalis*, and the number of leaves per core, decreased. From June 2002 to December 2003, *H. ovalis* LAI and BGDW were highly, negatively correlated to the estimate of the missing proportion ( $r=-0.72$ ,  $p=0.001$ ; and  $r=-0.68$ ,  $p=0.002$ , respectively) (Fig. 7.7). In contrast, there was no evidence of a relationship between *H. ovalis* LAI and the percentage of leaf damaged by gastropods over two years ( $r=-0.25$ ,  $p>0.10$ ), although during the first year

there tended to be decreases in *H. ovalis* LAI when the greatest gastropod damage occurred ( $r=-0.56$ ,  $0.05 < p < 0.10$ ).



**Fig. 7.6** Proportion of expected *Halophila ovalis* leaf area missing in core samples from plot SB. Letters A to D as in Fig. 7.1.

Estimates of invertebrate damage to *Halophila ovalis* leaves in plot SB were inversely related to epiphytic cover. Peaks in gastropod damage, in November 2002 (B) and March 2003 (C) (Fig. 7.5), corresponded to minimum estimates of percent epiphytic cover (Fig. 7.1). However, over the first year of the study, the correlation between the percent leaf surface area damaged by gastropods and the percent surface area covered by epiphytic material was not statistically significant ( $r=-0.55$ ,  $0.05 < p < 0.10$ ). In plot CB, peak gastropod damage to *H. ovalis*, probably caused by *Sacoglossa*, occurred when epiphytic cover on the leaves was at its greatest, in January 2004 (Fig. 4.9). In plot SB, peaks in estimates of the missing proportion of *H. ovalis* leaves, attributed to crustacean feeding, in November 2002 (B) and August 2003 (D) (Fig. 7.6), also corresponded to minimum estimates of percent epiphytic cover (Fig. 7.1). Estimates of the missing proportions were significantly and negatively correlated to percent epiphytic cover ( $r=-0.59$ ,  $p < 0.025$ ).



**Fig. 7.7** *Halophila ovalis* LAI versus the proportion of leaves missing in core samples from plot SB.

## 7.3 Discussion

### 7.3.1 Herbivory

*Halophila ovalis* declined by 98% in plot SB in just over one year. The decline was associated with damage to the leaves believed to be due to crustaceans. That damage increased over the course of the study, and was negatively correlated to *H. ovalis* LAI and BGDW over the period for which damage estimates could be obtained. Damage by gastropods was extremely high in November 2002 and March 2003, and may have accounted for the reduction of *H. ovalis* LAI on those dates. However, gastropod damage was much less on other dates, and it could not account for the overall decline of *H. ovalis*.

Declines in *Halophila ovalis* LAI during the first year of the study were associated with declines in epiphytic cover. Reduced epiphytic cover may have had a strong impact on certain dates, but the overall decline in *H. ovalis* LAI could not be explained by changes in epiphytic cover. Otherwise, one would have expected *H. ovalis* LAI to increase when epiphytic cover increased during the second year. Nonetheless, the single largest decline in *H. ovalis* LAI, between June and July 2002, may have been the result of the exposure of the leaves to high levels of irradiance following the removal of

epiphytic cover by sea hares, at a time when herbivore damage was minimal (chapter 3). Subsequent periods of low epiphytic cover were associated with high levels of herbivore damage to the leaves.

Epiphytic cover was inversely related to herbivore damage of *H. ovalis* leaves. That relationship likely occurs because many of the potential invertebrate herbivores of seagrass are generalists that also consume algae. Epiphytes provide an alternative, and possibly a preferred, food whose presence appears to reduce damage to the seagrass. It is also possible that some algae can act as a deterrent or a physical barrier to small seagrass herbivores. Epiphytic cover may have an indirect positive impact on the seagrass by reducing the impact of herbivores.

Some isopods and amphithoid amphipods are likely consumers of both seagrass and macroalgae. In north temperate *Zostera* meadows, the isopods *Idotea* spp. are capable of limiting the production of seagrass epiphytes (Williams and Ruckelshaus 1993), but may also consume the seagrass (Robertson and Mann 1980, Nienhuis and Groenendijk 1986, Thom et al. 1995). Epiphytes appear to be their preferred food resource (Boström and Mattila 1999), and the consumption of seagrass occurs when epiphytes have been reduced by grazing, at least in experimental settings (Hootsmans and Vermaat 1985, Duffy et al. 2001). In tropical meadows, the sphaeromatid isopods *Cymodoce* spp. appeared to cause extensive damage to seagrasses; but their feeding habits are otherwise unknown. The amphithoid amphipods *Cymadusa* spp. are well known as consumers of macroalgae (Zimmerman et al. 1979), but Kirkman (1978) also documented severe damage by an Australian *Cymadusa* sp. to a culture of *Zostera muelleri*. In Fiji, Mukai and Iijima (1995) found another amphithoid, *Amphithoe* sp., feeding mainly on blue-green algae attached to the leaves of *Syringodium isoetifolium*, but also consuming the seagrass. They estimated that it consumed as much as half the daily seagrass production.

The neritid gastropod *Smaragdia souverbiana* is common and widespread in the Indo-Pacific region (Loch 1994), but its feeding habits have never been examined. In this study, it appeared to cause extensive damage to *Halophila ovalis*, and to a lesser extent *Halodule uninervis*. The Caribbean species *Smaragdia viridis* is associated with

seagrass and has been observed feeding on the seagrass *Thalassia testudinum* (Thayer et al. 1984; B. van Tussenbroek, personal communications). It should be expected that *Smaragdia* spp. would also feed on epiphytic algae, as neritids are typically scrappers of algal films.

An undescribed Sacoglossa caused extensive damage to *Halophila ovalis*. Unlike *Smaragdia souverbiana* and the crustaceans, it is probably a highly specialized feeder. Sacoglossa have piercing suctorial feeding apparatus, and many are selective feeders on specific algae (Williams and Walker 1999). Only two other species, from the West Atlantic, are known to feed on seagrasses. The species found in this study did not appear to be deterred by epiphytic cover, as it caused extensive damage to *H. ovalis* at Cockle Bay when epiphytic cover was at its maximum.

*Halophila ovalis* appears to be more vulnerable to herbivores than *Halodule uninervis*. The percent surface area of *Halodule uninervis* leaves with damage, likely caused by the neritid *Smaragdia souverbiana*, was always <5%, and usually much less. Jensen (1983a) showed that the Atlantic seagrass-feeding sacoglossan, *Elysia serca*, had a preference for *Halophila engelmanni* over *Halodule wrightii* or *Thalassia testudinum*. She related that preference to the size of the seagrass cells, the thickness of the cell walls, and the size of the raptorial tooth that the sacoglossan uses to rasp them. *Halophila* was probably preferred because it has large thin-walled cells. The seagrass-feeding sacoglossan found in this study was never seen on *Halodule uninervis*. It is much smaller than *E. serca*, and may not have teeth large enough to feed on *H. uninervis*.

*Halodule uninervis* leaves contain more fibre than *Halophila ovalis* leaves (Aragones 1996), and should therefore be a less attractive food source. It has leaves with thickened margins containing fibrous vascular bundles. In contrast, *Halophila ovalis* leaves are thinnest at the margins. Crustaceans attack seagrass leaves from the lateral margins with chewing mandibles. Isopods appear to avoid the lateral margins of *H. uninervis* by biting through them at one point and consuming the median portions. However, even if the leaves of the two species were equally attractive as food, *H. ovalis* would be more vulnerable to leaf herbivores because a greater proportion of its tissue is

exposed above ground. *H. uninervis* can also use its large below ground nutrient reserves to replace leaves lost to episodes of herbivory, or desiccation.

The combined amount of *Halophila ovalis* leaf material removed by crustaceans and gastropods reached levels that were probably comparable to production. *H. ovalis* leaves have a short life-span, estimated at 12.4 days (Duarte 1991a). If it were assumed that the average age of the leaves in cores samples were half that value, then a loss of 50% of the leaf surface would represent a loss of approximately 8% day<sup>-1</sup>. Hillman et al. (1995) calculated the specific growth rate of *H. ovalis* shoots as 4-9% day<sup>-1</sup>. So, if the leaves in this study were assumed to have similar growth rates, then there were occasions when all new production could have been removed by invertebrate herbivores. However, such high rates of herbivory could not in themselves explain the collapse of *H. ovalis* biomass. It has rapid growth rates and is capable of recovering from episodes of severe herbivory, such as dugong feeding (Preen 1995). More important may be the size of below ground nutrient reserves, and how long the herbivory lasts. Heck and Valentine (1995) suggested that such reserves were critical for the recovery of *Thalassia testudinum* from episodes of severe herbivory by urchins. Sustained herbivory leads to the depletion of reserves, and the long-term loss of standing crop. In this study, herbivory by gastropods could be severe, but intermittent, and therefore less likely to reduce standing crop. Herbivory by crustaceans was more sustained, and more closely associated with the overall decline in *H. ovalis* standing crop.

Crustaceans appeared to be the most important consumers of *Halophila ovalis*. Unfortunately, it was not possible to get a measure of their damage on *Halodule uninervis* leaves during the course of this study. Crustacean damage, likely caused by the isopods *Cymodoce* spp., was often observed on broad-leaved *H. uninervis* at both Cockle Bay and Picnic Bay. *Cymodoce* spp. may be well adapted to live on broad-leaf *H. uninervis*. Adults are the same width as those leaves, and are well camouflaged against them. They could feed while sitting astride a leaf, which would be held in place by the vascular bundle opposite the one they had broken through. In contrast, narrow leaves would be broken off with a few bites, and might simply float away. More

observation needs to be done on the feeding preferences and behaviour of those animals.

Crustacean herbivores had a greater impact on *Halophila ovalis* than gastropods because they increased and maintained high levels of feeding over time, even as the abundance of *H. ovalis* decreased. The activity of those animals probably increased over time because the maturing meadow provided increased habitat. Greater canopy cover from *Halodule uninervis* would provide increased refuge from predators and a richer source of epiphyte foods for generalist herbivores. *H. uninervis* always supported more epifaunal animals per area of leaf than *H. ovalis* at Shelly Beach (chapter 4). Although the small epifaunal animals associated with *H. uninervis* leaves did not increase in abundance with increasing *H. uninervis* LAI (chapter 6), other studies in North Queensland (Mellors and Marsh 1993, Kwak and Klumpp 2004) have found that larger mobile fauna, i.e. fish and decapods, increased in abundance with increasing seagrass biomass. *Cymadusa* and *Cymodoce*, the crustaceans that were suspected of causing damage to *H. ovalis* leaves, are larger and more active than typical *H. uninervis* epifauna, and may benefit from increased seagrass cover. *H. ovalis* appears to provide less protection than the larger seagrass species, and so might not harbour as many of the larger generalist herbivores. A similar argument has been used to explain difference in seed predation compared within and outside of *Posidonia* meadows in Western Australia. Orth (1999) found that seedlings of *Posidonia* spp. were absent in *Posidonia* and *Amphibolis* meadows, but present in non-vegetated areas and *H. ovalis* meadows. Subsequent experiments with tethered *Posidonia* seeds (Orth et al. 2002) showed that they were more likely to be eaten in *Posidonia* meadows than in non-vegetated areas. Those results suggest that higher rates of herbivory can be expected in mature meadows than in early successional *H. ovalis* meadows.

The extent and variety of invertebrate feeding on *Halophila ovalis* in this study was unexpected, as there appears to be no mention in the literature of feeding on that species by any animal other than the dugong. Yet, measurements of leaf damage probably underestimated the extent of the feeding. No attempt was made to measure the loss of entire leaves, which could have been a sizeable proportion of the biomass removed, particularly by crustaceans. Nor was there an attempt to account for the

feeding by all the animals that are potential feeders on seagrass. At least two different groups of burrowing decapods, alpheid shrimp and the ocypodid crab *Macrophthalmus*, are common in intertidal tropical habitats, and are known to feed on seagrasses, but would leave little trace of their feeding. Stapel and Erftemeijer (2000) described and measured extensive harvesting of *Thalassia hemprichii* leaves by alpheid shrimp in an Indonesian meadow. Similar alpheid activity has been observed in meadows in the Townsville area (J. Collins, personal communications). Woods and Schiel (1997) found that local populations of *Macrophthalmus hirtipes* in *Zostera muelleri* patches in New Zealand took half their diet from living seagrass, both above and below ground.

### **7.3.2 Succession**

*Halophila ovalis* is typically the first species to colonize disturbed intertidal sites in tropical Indo-Pacific meadows, after cyclones (Birch and Birch 1984), dugong feeding (Preen 1995), or experimental removal (Rollon et al. 1998). *Halodule uninervis* also re-establishes itself quickly after disturbance, and with time tends to dominant *H. ovalis*. Depending on the site, *H. uninervis* may then be succeeded by other larger species (den Hartog 1970). Shelly Beach was denuded of seagrass by Cyclone Tessie in April 2000, and both species re-established themselves by early 2001 (M. Waycott and J. Mellor, personal communications). At the beginning of this study in 2002, *H. ovalis* was dominant, but rapidly declined, and was replaced by *H. uninervis*. *Zostera muelleri* became a more conspicuous, but minor, species in the plot after two years. The latter species formed dense patches on soft sediments inshore of plot SB. It is a slow colonizer, typical of sites with fine sediments and turbid waters, such as estuaries. The succession seen in plot SB was typical of meadows on soft-sediment intertidal sites along the Queensland coast (Carruthers et al. 2002). At sites with fine terrigenous sediments, *Halodule uninervis* tends to be succeeded by *Zostera muelleri*. At sites with coarse sediments, such as Picnic Bay and Cockle Bay, the succession is toward *Cymodocea serrulata*.

Invertebrate herbivory appeared to be a significant factor in the succession from *Halophila ovalis* to *Halodule uninervis* at Shelly Beach. However, succession of seagrasses has typically been explained by differences in the dispersal ability of the species and competition among them for light and/or nutrients. Both *H. ovalis* and *H.*



*uninervis* have good dispersal abilities, as they readily produce seeds and can take root from vegetative fragments. The former has faster growing rhizomes (Vermaat et al. 1995), and it rapidly reaches its maximum biomass, while the latter gradually increases in biomass over years (Birch and Birch 1984).

A decline of *Halophila ovalis* is not likely due to competition with other species for light. It is tolerant of low light conditions, and grows at depths > 40 m off the Queensland coast (Lee Long et al. 1996). Other evidence suggested that *H. ovalis* may have suffered from high irradiance stress in plot SB (chapter 3), and shading from larger seagrasses might actually benefit it. Duarte et al. (2000) found that the experimental removal of larger seagrasses resulted in a 30% decrease in the shoot size of *H. ovalis* in a mixed species Philippine meadow.

It seems more likely that *Halodule uninervis* could compete with *Halophila ovalis* for sediment nutrients. Meadows on the coast of Queensland have low sediment nutrient values, and the seagrasses are potentially nutrient limited (Mellors 2003). Competition for nutrients may be common in tropical meadows with mixed species. In the Caribbean, experimental manipulations (Williams 1990, Fourqurean et al. 1995) have shown that *Thalassia testudinum* is effective at exploiting sediment nutrients to the disadvantage of the earlier successional species, *Halodule wrightii* and *Syringodium filiforme*. In plot SB, the negative correlation between *H. uninervis* and *H. ovalis* BGDW, suggested that the former, with its much larger root biomass, could have exploited sediment nutrient to the disadvantage of the latter. Competition between those two species for sediment nutrients needs to be investigated to assess its role in succession. It is possible that the decline of *H. ovalis* was related to a combination of both herbivory and nutrient limitation.

Birch and Birch (1984) documented the succession of seagrasses in Cockle Bay over a decade following Cyclone Athea in 1971. *Halophila ovalis* rose and fell in abundance in the early years of their study, while *Halodule uninervis* rose over the whole decade. *Cymodocea serrulata* became abundant in the final years of their study, especially on deeper sediments. The latter never displaced *H. uninervis*, just as it did not displace it in plot PB during this study. *C. serrulata* grows at greater depths than *H. uninervis*

(Carruthers et al. 2002) and is probably more tolerant of low light conditions. The increased density of the canopy in plot PB produced conditions that were likely to favour *C. serrulata*, because of greater self-shading, and shading by an increase in epiphytic cover. Rasheed (2000) experimentally shaded plots cleared of vegetation in a Queensland meadow, and found that *C. serrulata* had a greater capacity to re-establish itself under low light conditions than did *H. uninervis*. At the end of their study, Birch and Birch (1984) found that *C. serrulata* was being displaced in Cockle Bay by *Halimeda opuntia*. That macroalga was often seen in plots CB and PB during the present study, but was never a significant part of the flora.

*Halophila ovalis* and *Halodule uninervis* are the preferred food of dugongs (De Iongh et al. 1995, Preen 1995). The effects of feeding by dugongs on seagrass successional development are the reverse of the effects of feeding by invertebrates. Dugongs often feed on the below ground biomass, and cause major disturbance. As *H. ovalis* is the major pioneer species in disturbed areas, dugongs can maintain the meadow at an early successional phase by regular feeding activity, a strategy described by Preen (1995) in Moreton Bay, Queensland. That “cultivation grazing” prevented meadows from succeeding to *Zostera muelleri*, the dugong’s least preferred seagrass food. *H. ovalis* is preferred by dugongs because it has the highest nitrogen content and lowest fibre (Preen 1995, Aragonés 1996), qualities that are also likely to make it a preferred food of invertebrate herbivores.

### ***7.3.3 The role of invertebrate herbivores in seagrass ecosystems***

Consumption of seagrass by invertebrates has received very little attention in the literature, except where it has involved urchins. The majority of herbivory studies reviewed by Valentine and Heck (1999) concerned vertebrates: waterfowl, fish, dugongs manatees, and green turtles. There have also been numerous studies of urchins that show them to be significant consumers of seagrass in the tropics (Valentine and Heck 1991, Klumpp et al. 1993, Valentine et al. 2000, Alcoverro and Mariani 2002, Peterson et al. 2002). However, they are uncommon in meadows on the Queensland coast, and were not encountered during this study. Studies of small invertebrate herbivores have been far fewer. The most significant impact of a small invertebrate on seagrasses has been reported for a limpet, *Tectura depicta*, on *Zostera*

*marina* in California (Zimmerman et al. 1996, Zimmerman et al. 2001). It removes the leaf epidermis. When it occurred at high densities locally, it was shown to be responsible for a dramatic decline in *Z. marina*. However, the circumstances in that case appeared to be anomalous, as the limpet is typically rare. In contrast, the decline of *Halophila ovalis* observed in this study was consistent with a successional decline. An assemblage of animals was responsible for the herbivory, and they represented taxa that are probably common and widespread in the tropical Indo-Pacific. They were also resident in the meadows. Most large seagrass herbivores are transient species that cause episodic declines in seagrass leaf area. Many seagrass can recover quickly from such losses (Valentine et al. 1997), as they have large below ground biomasses and fast growth rates. Sampling from other sites is needed to determine if the invertebrate herbivory described here is typical, and to determine if it is a common agent of successional change in tropical intertidal meadows.

Valentine and Duffy (2006) have recently argued that the role of herbivory in seagrass ecosystems has been underestimated because the main vertebrate herbivores have been reduced in abundance in recent times. They assume that small invertebrates are not significant consumers of seagrass, but are principally epiphyte grazers. However, on the evidence presented here, it would appear that the impact of smaller invertebrates as consumers of seagrass has been greatly underestimated. Dugongs have received considerable attention because they are large charismatic species, while the invertebrates that effectively compete with them for food have been ignored. Given the current lack of information, it is difficult to assess the relative importance of various herbivores. However, on the evidence presented here, it would appear that small invertebrates could have as great an impact as dugongs, at their present or historic abundances. Different types of herbivores have very different impacts on the structure and development of meadows. Herbivory by invertebrates promotes succession from *Halophila ovalis* to other species, but the disturbance caused by the feeding of dugongs retards that succession.

## 7.4 Conclusions

Invertebrate herbivory was a possible cause of a decline in *Halophila ovalis* at Shelly Beach. Crustaceans appeared to be the most important invertebrates contributing to that decline. Measures of damage attributed to crustaceans were correlated to changes in *H. ovalis* LAI and BGDW. Gastropod damage was sometimes extensive, and likely caused short-term reductions in *H. ovalis* LAI, but did not explain the overall decline. Epiphytic cover may provide some protection from herbivores, as measures of epiphytic cover were inversely related to measures of herbivore damage. The decline of *H. ovalis* and domination of the meadow by *Halodule uninervis* was consistent with recognized patterns of succession in tropical intertidal meadows. *H. ovalis* appears to be more vulnerable to herbivory than *H. uninervis*, and mature meadows may support more herbivores than early successional meadows. Thus, invertebrate herbivory may be an important agent of successional change in such meadows. Herbivory needs to be considered with respect to other factors that may play a role in seagrass succession, such as nutrient competition. Further research needs to identify the range of invertebrates feeding on seagrasses, what their feeding preferences are, and what their distributions and abundances are with respect to different successional stages. There have been few studies of herbivory by small invertebrates in tropical seagrass meadows, and their importance appears to have been greatly underestimated.

## Chapter 8 Abiotic factors

### 8.1 Introduction

On the Queensland coast, variation in the standing crop of seagrass has been studied previously with respect to abiotic environmental factors. Mellors et al.(1993) studied a mixed-species meadow at Green Island, and found that the standing crop was positively correlated to day length and temperature, and negatively correlated to the number of strong-wind days. McKenzie (1994) studied a *Zostera capricorni* (=muelleri) meadow near Cairns, and found that standing crop was influenced by light availability, temperature, salinity and tidal exposure. Lanyon and Marsh (1995) studied three meadows in the Townsville area, including Shelly Beach and Cockle Bay. They found positive correlations between seagrass standing crop and day length, temperature and rainfall. All three studies identified a seasonal pattern, with greatest standing crop during the months of the austral summer, and reduced standing crop during the winter. Factor analyses and correlations identified variables that were highly associated with each other, and with measures of seagrass abundance, and varied seasonally. Therefore, it was not possible to identify single factors responsible for the patterns. The austral summer was associated with longer days, higher temperatures, more rainfall, and less tidal exposure. Although significant relationships can be found between combinations of abiotic variables and measures of seagrass abundance, the amount of variation explained was generally poor, typically < 20%. There have been no attempts to explain local variation in seagrass standing crop with respect to biological parameters, such as epiphytic cover or grazer abundance.

In this chapter, some of the abiotic seasonal parameters usually considered to be important determinants of change in seagrass meadows are considered. They are compared with the biotic parameters previously presented to determine which factors best explain changes in seagrass abundance using the technique of regression tree analysis. Abiotic measurements were made following the methods presented in chapter 2.

## 8.2 Statistical methods – regression tree analysis

Regression trees were used to determine which variable, or combination of variables, including biotic and abiotic variables, best explained variation in the abundance of seagrasses in the sampled plots. Regression tree analysis is a nonparametric technique that is well suited for the analysis of complex ecological data (De'ath and Fabricius 2000). Trees are constructed by the repeated splitting of a single response variable into groups using combinations of explanatory variables. Each split is defined by a rule based on a single explanatory variable. For a numerical explanatory variable, the splitting rule is based on values less than or greater than some value. The splits are chosen so that the homogeneity of the response variable is maximized within each group. The analysis produces a series of nested trees of decreasing size, from which the “optimal” tree size can be selected using a cross-validation process (De'ath and Fabricius 2000). The selected tree provides a simple model based on the values of a few explanatory variables that best describe the variance of the response variable.

Trees were constructed for seagrass response variables, LAI (Leaf Area Index) and BGDW (Below Ground Dry Weight) of a particular species, for each plot using a suite of explanatory variables including: percent epiphytic cover and AFDW (Ash-Free Dry Weight), the densities of common invertebrates, tidal exposure and rainfall over 14 days, air temperature and wind speed at 1500 h averaged over 14 days, LAI and BGDW of other seagrass species. The data on biotic parameters are all presented in graphic form in previous chapters: *Halodule uninervis* LAI and BGDW in chapter 5, percent epiphytic cover and AFDW in chapter 4, densities of invertebrates in chapter 4, and in detailed tables in appendix A. *Halophila ovalis* LAI data from plot SB is presented in chapter 7. Measures of damage to the leaves by invertebrate feeding are also entered as explanatory variables for changes in *H. ovalis* in plot SB, and are presented graphically in chapter 7. Analyses of data from plot PB also included the presence/absence of ferry service to Picnic Bay as an explanatory variable. There was no need for transformation of any explanatory variables, as the splitting of the response variable is related to the rank order of the values of explanatory variables, so solutions are invariant to transformations of explanatory variables. V-fold cross-validation, with  $v=10$ , was repeated using different seeds to choose the best tree size, as single runs of

the cross-validation can sometime produce atypical results. Trees were also constructed for epiphytic cover and AFDW using the four abiotic variables and invertebrate densities as explanatory variables. Regression trees analyses were performed using the Statistica software package (version 7.1) (StatSoft, Inc. 2005).

Trees are labelled on each horizontal branch with the name of the explanatory variable and its values that determine the split of the response variable. The mean value of the response variable and the number of observations in each group produced by the splits is presented at each node. The relative lengths of the vertical axes of the tree are drawn proportional to the total sum of squares explained by each split.

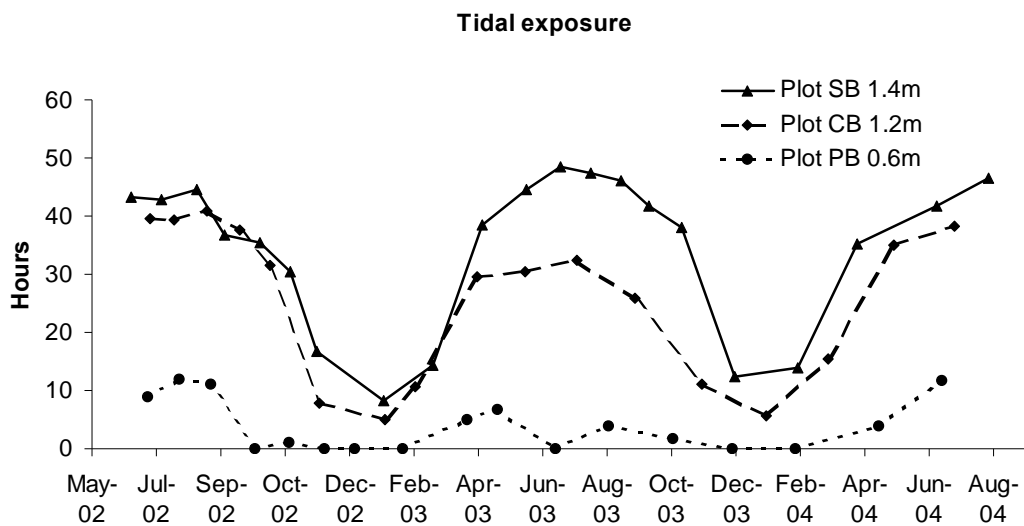
## 8.3 Results

### 8.3.1 Tidal exposure

The estimated heights above chart datum at Townsville of plots SB, CB and PB were 1.4, 1.2, and 0.6 m respectively. Estimates of the number of hours of exposure, between 900 h and 1600 h, over the 14 days before biological samples were taken, varied with height and season (Fig. 8.1). From December to March, the lowest spring tides occurred at night. From May to October, lowest spring tides occurred during the day. Plot PB was not exposed during daylight from December to March, and the daytime exposures in other months were similar to minimum values experienced in other plots. Plots SB and CB experienced some daytime exposure in every month. Plot SB had the greatest range of cumulative hours of exposure of over 14 days, from a minimum of 10.3 h in January 2003, to a maximum of 48.4 h in July 2003.

In plot SB, *Halodule uninervis* LAI was negatively correlated to the hours of daytime exposure over 14 days ( $r=-0.65$ ,  $p<0.001$ ). *H. uninervis* BGDW was also negatively correlated to daytime exposure, but less strongly ( $r=-0.48$ ,  $p<0.05$ ). In contrast, *H. ovalis* LAI and BGDW in plot SB was not correlated to daytime exposure ( $r=0.13$ ,  $r=0.02$ ,  $p>>0.10$ ). In plot CB, there were no significant relationships between daytime exposures and the LAI or BGDW of either species. In plot PB, *H. uninervis* LAI did not vary greatly over two years, but the small changes that did occur showed a strong seasonal pattern, and LAI was correlated with daytime tidal exposure ( $r=-0.72$ ,

$p < 0.001$ ). In July 2004, *H. uninervis* LAI in plot PB fell to its lowest level, and there was evidence of desiccation of the leaves after the longest ever 14-day exposure of the plot. *H. uninervis* BGDW was not correlated with daytime exposure in plot PB, nor was *Cymodocea serrulata* LAI or BGDW.



**Fig. 8.1** Hours of exposure of each plot by low tides, between 900 – 1600 h, over 14 days before sampling.

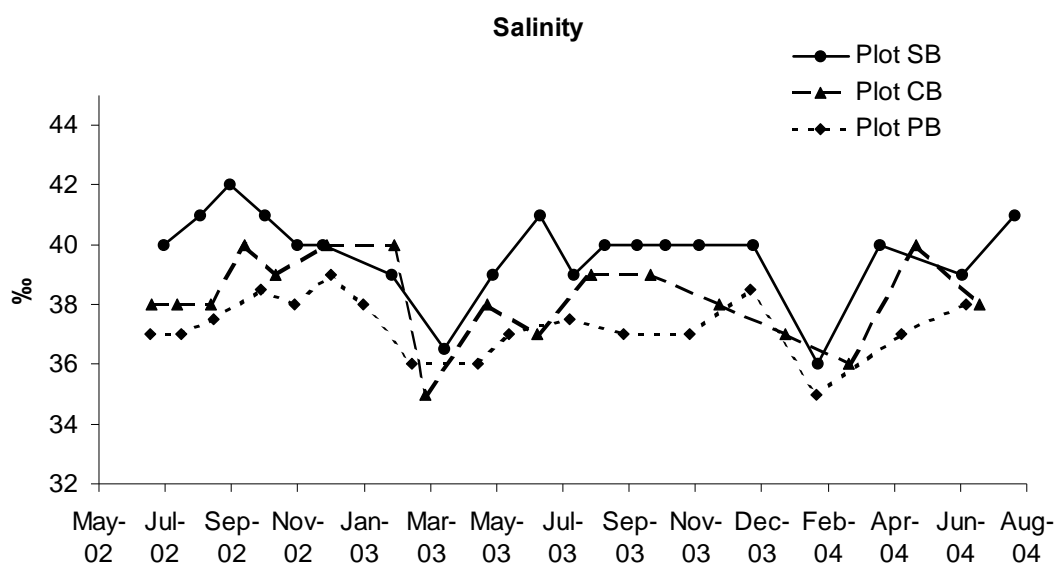
### 8.3.2 Water and weather conditions

Salinities ranged from 35 to 42‰. The lowest values were recorded in February and March (Fig. 8.2), at the time of greatest rainfall (Fig. 8.3). Salinity values were similar among the plots, but Shelly Beach consistently had higher values than Picnic Bay. The mean of all salinity values recorded at Shelly Beach, Cockle Bay and Picnic Bay were 39.7, 38.2 and 37.3‰, respectively. Changes in salinity were not biologically significant, and have not been included in further analyses.

Rainfall was greatest in February of both years (Fig. 8.3), and half the rain that fell during the study came in that month. Annual precipitation totals were well below long-term averages; but falls during February, typically the wettest month, were normal. Average non-directional wind speeds, measured at 1500 h, did not show a seasonal pattern (Fig. 8.4).



The recorded water temperatures in the plots during low afternoon tides ranged from 23°C in July and August 2002, to 38°C in February 2004 in plot SB (Fig. 8.5). Those water temperatures were usually elevated above maximum air temperatures recorded at Townsville airport. Air temperatures at 1500 h, averaged over 14 days, were used in the regression tree analyses. Those air temperatures were strongly and inversely correlated to tidal exposure (e.g.  $r=-0.82$  for plot SB), with the hottest months, December to March, having the lowest exposures of the plots. Consequently, air temperatures were correlated to seagrass measures in a way similar to tidal exposures; e.g. in plot SB, air temperatures were positively correlated to *Halodule uninervis* LAI ( $r=0.76$ ,  $p<0.001$ ).



**Fig. 8.2** Salinities of water samples taken at low tide in each plot.

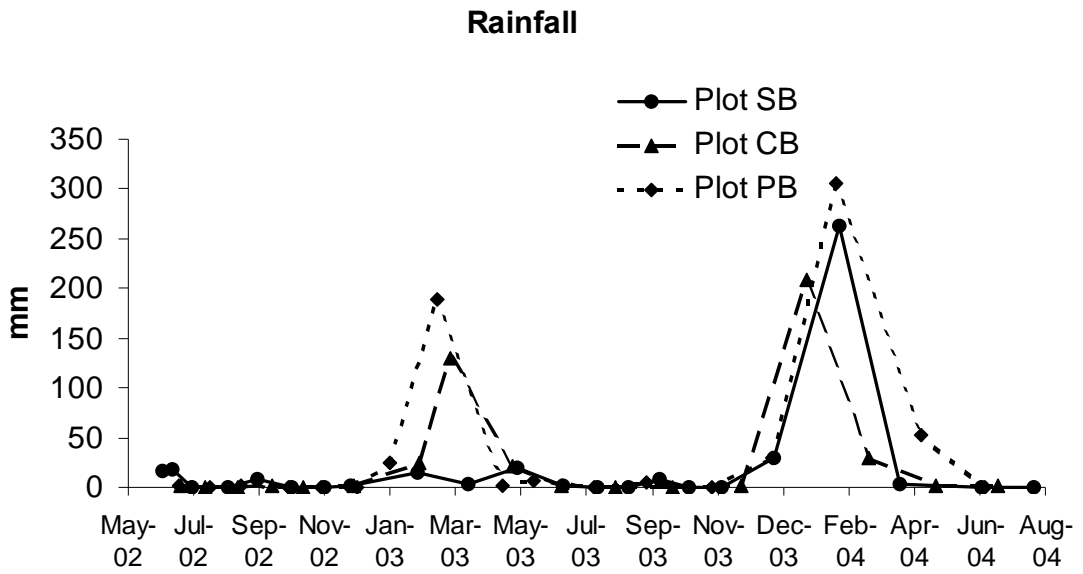


Fig. 8.3 Cumulative rainfall over 14 days prior to sampling dates in each plot.

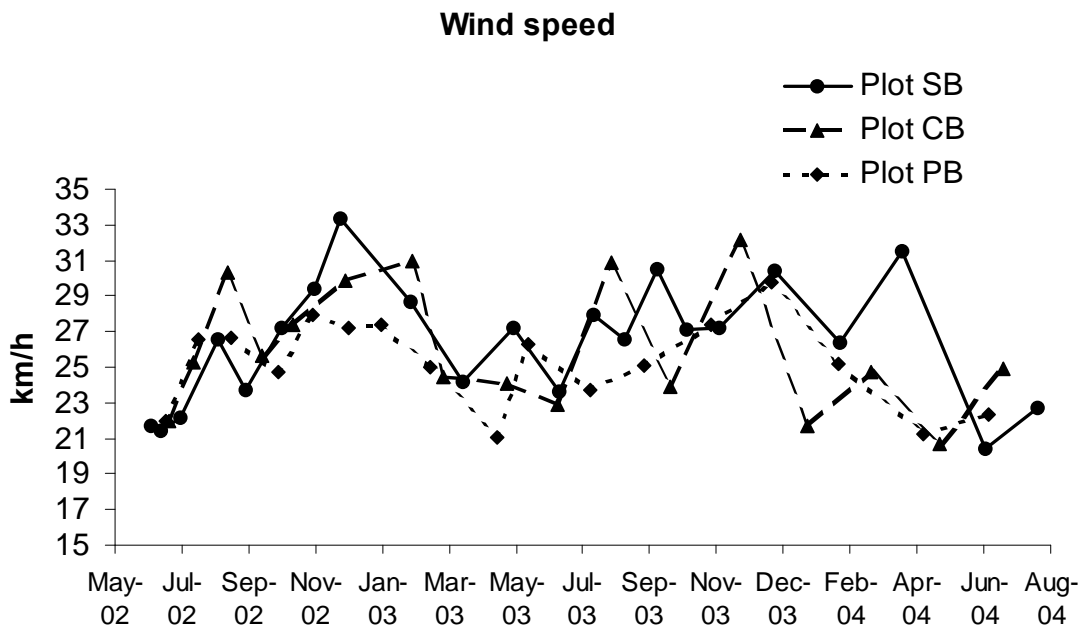
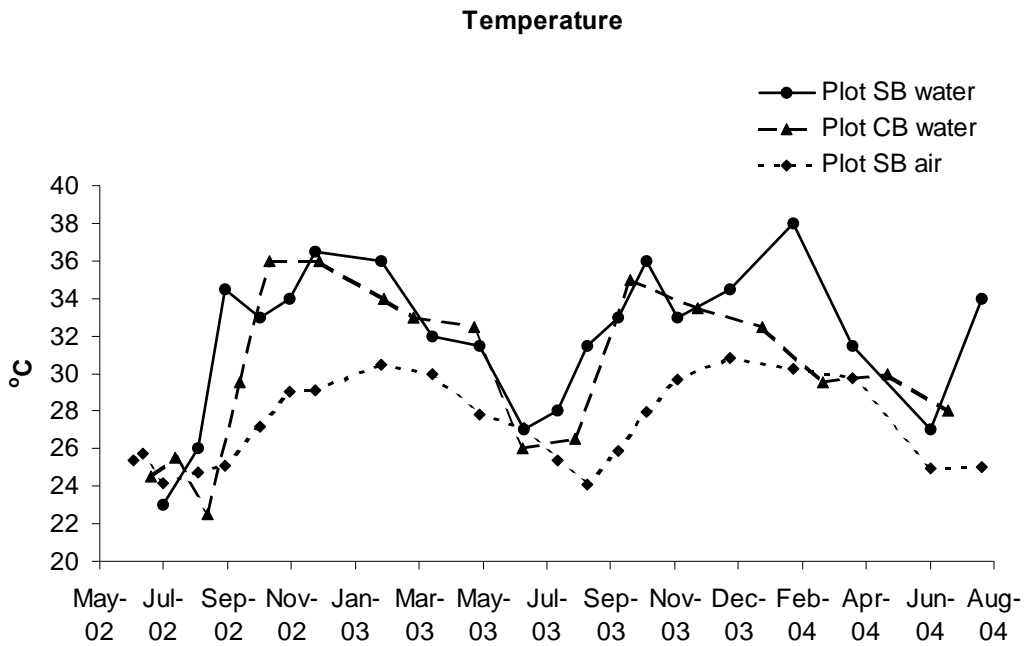


Fig. 8.4 Mean non-directional wind speed at 1500 h over 14 days prior to sampling dates in each plot.

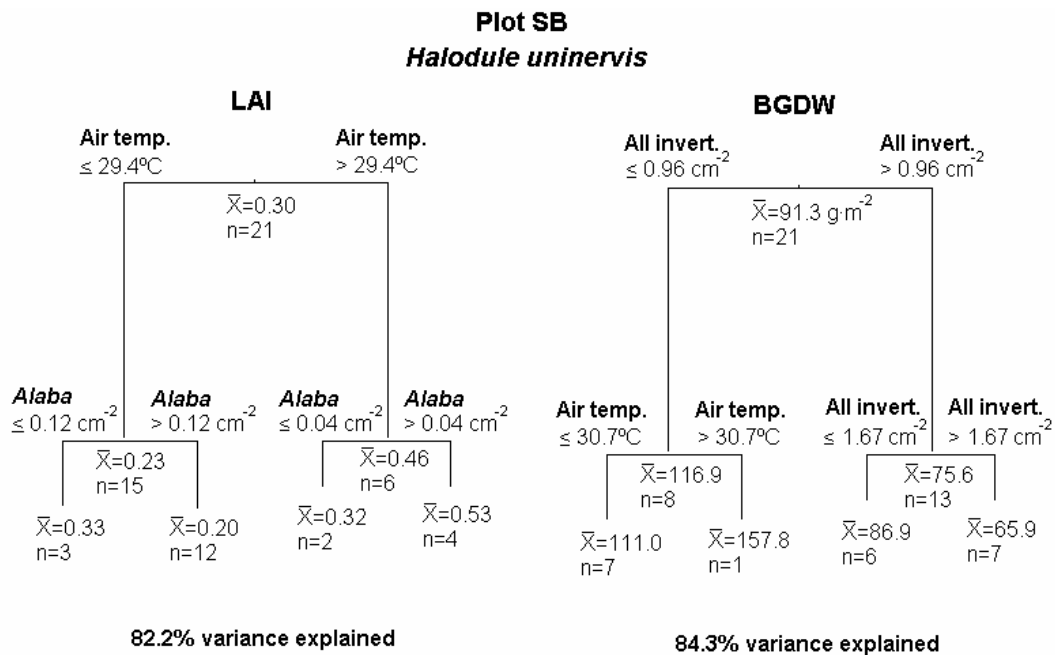


**Fig. 8.5** Water Temperatures in plots SB and CB during low afternoon tides. Complete data for plot PB are not available for the same time of day. Also shown are mean air temperatures at 1500 h over 14 days prior to sampling in plot SB. Similar air temperature curves were calculated for plots CB and PB, but are not shown.

### 8.3.3 Regression trees

#### 8.3.3.1 Plot SB

Changes in *Halodule uninervis* LAI in plot SB were most strongly related to seasonal factors. A division of LAI data based on mean air temperature alone accounted for 58.0% of the variation in the first spit of the regression tree analysis (Fig. 8.6). A similar division of LAI data could also be obtained on the basis of tidal exposure. Thus, the analysis suggests a division of the data between a hot season with low daytime tidal exposure, and a cooler season of high daytime exposure. The hot season was also the season of greatest rainfall, and greatest epiphytic cover. Division of *H. uninervis* LAI data on the basis of air temperature had the lowest cross-validation costs, but further divisions on the basis of *Alaba* densities accounted for an additional 24.2% of the variance (Fig. 8.6). The resulting tree shows trends in the data that suggest an interaction between seasonal factors and *Alaba* densities. During the cooler season, greatest *Alaba* densities were associated with decreased LAI, but in the hot season greater densities were associated with increased LAI.

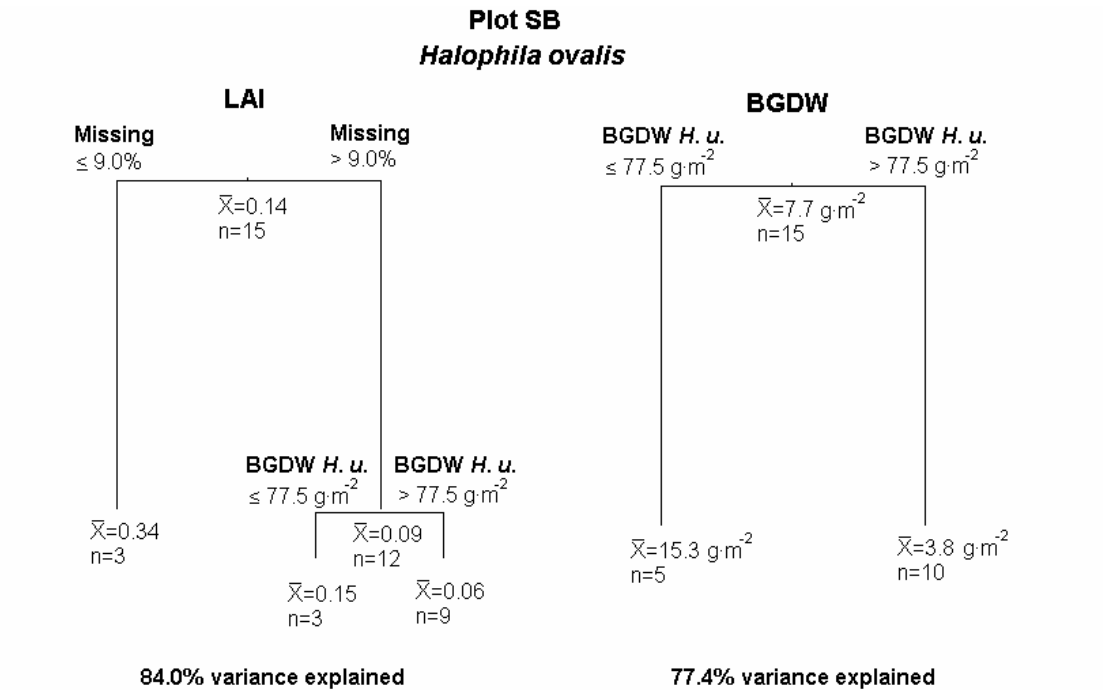


**Fig. 8.6** Regression trees of *Halodule uninervis* LAI and BGDW in plot SB. BGDW means were measured as  $\text{g}\cdot\text{m}^{-2}$ , temp. = temperature, invert. = invertebrates.

Changes in *Halodule uninervis* BGDW in plot SB were not closely related to seasonal factors, but were better explained by invertebrate densities. A satisfactory regression tree of the BGDW data was not obtained using individual invertebrate taxa. However, a good model was obtained using total invertebrate abundances, with 60.5% of the variance accounted for by a split based on the density of all invertebrates. Further variance was accounted for by another split of the same variable, and by splitting off one sample on the basis of temperature (Fig. 8.6).

Changes in *Halophila ovalis* LAI and BGDW in plot SB were not related to seasonal factors. LAI was closely related to the calculated missing leaf area attributed to crustacean feeding, which accounted for 75.4% of the variance in the regression tree (Fig. 8.7). A much smaller portion of *H. ovalis* LAI variance was accounted for by the BGDW of *Halodule uninervis*. In contrast, 77.4% of the variance in *H. ovalis* BGDW was explained by splitting based on *H. uninervis* BGDW (Fig. 8.7). Note that fewer samples were available for multivariate analysis of *H. ovalis* data than for *H. uninervis*,

because of the lack of *H. ovalis* in core samples on later dates, and the lack of invertebrate data on two earlier dates.



**Fig. 8.7** Regression trees of *Halophila ovalis* LAI and BGDW in plot SB, where “missing” is the missing portion of *H. ovalis* leaves attributed to crustacean feeding, and *H. u.* = *Halodule uninervis*.

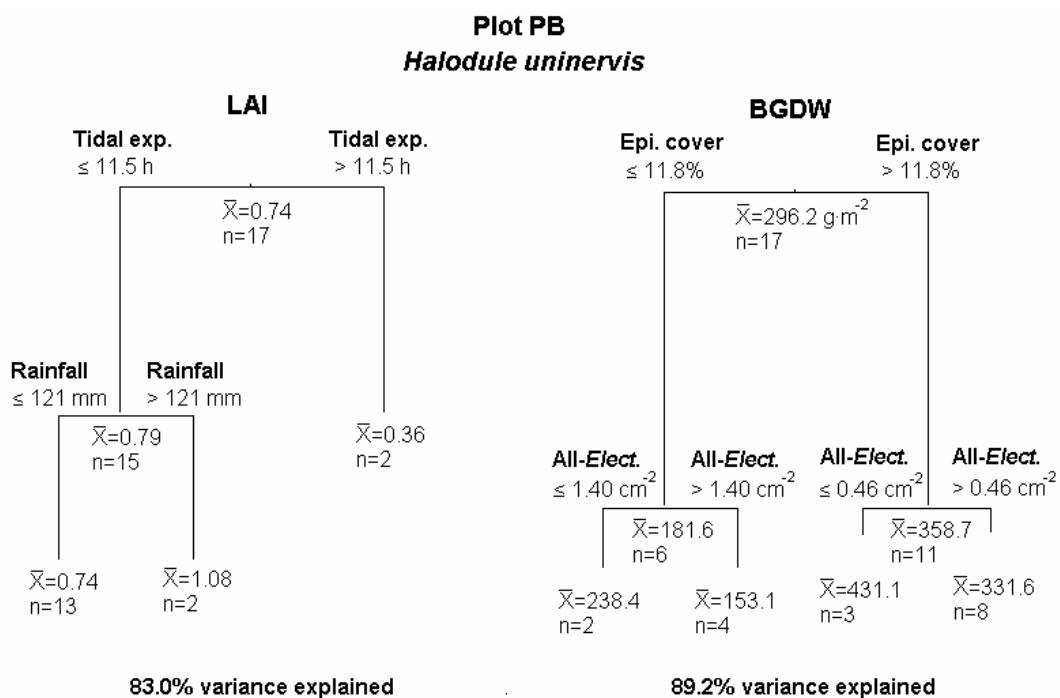
### 8.3.3.2 Plot CB

The regression tree analyses of data from plot CB did not find any variable that could be used as a satisfactory predictor of changes in *Halodule uninervis* LAI or BGDW in the plot. That likely reflects the collapse of *H. uninervis* abundance, and especially the almost complete loss of below ground biomass, making recovery difficult.

### 8.3.3.3 Plot PB

Changes in *Halodule uninervis* LAI in plot PB were modest and non-significant, but showed a seasonal pattern (Fig. 5.13). The regression tree analysis split off two samples on the basis of the highest tidal exposures, and another two samples on the highest rainfall. The highest exposure corresponded to the lowest LAI values in July 2002 and 2004, likely because of desiccation. The highest rainfall corresponded to the highest LAI values in February 2003 and 2004. Together those splits accounted for 83.0% of variance in LAI (Fig. 8.8).

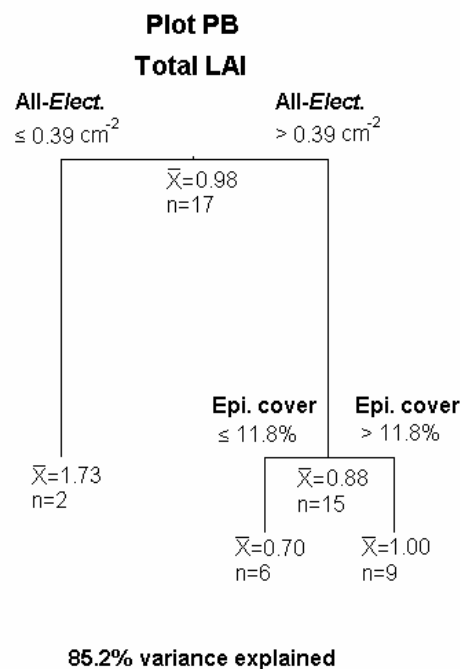
Changes in *Halodule uninervis* BGDW in plot PB were not related to climatic factors, but were closely related to epiphytic cover and invertebrate numbers. An initial run of the regression tree analysis, using separate invertebrate taxa as explanatory variables, produced a split in BGDW based on *Electroma* densities, explaining 71.5% of the variation. High *Electroma* densities were associated with low BGDW. As a relationship between *Electroma* and BGDW would be difficult to explain, the analysis was again run again without *Electroma*. That analysis split BGDW on the basis of percent epiphytic cover, explaining 71.0% of the variation (Fig. 8.8). Low epiphytic cover was associated with low BGDW. High *Electroma* densities appeared to be a good indicator of low epiphytic cover. Additional BGDW variance was explained by the densities of total invertebrates excluding *Electroma*. Invertebrate densities were greatest in association with the lowest BGDW.



**Fig. 8.8** Regression trees of *Halodule uninervis* LAI and BGDW in plot PB, where exp. = exposure, Epi. = epiphytic, and “All-Elect” is the total density of all invertebrates excluding *Electroma*.

Although *Halodule uninervis* LAI did not change significantly in plot PB, the seagrass canopy became denser during the second year because of an increase in *Cymodocea serrulata*. Changes in the total LAI, i.e. the sum of the LAI values of *H. uninervis* and *C. serrulata*, were not related to seasonal factors. A regression tree analysis showed

that differences in total LAI could be predicted from differences in the density of invertebrates and epiphytic cover. The analysis split two samples with lowest invertebrate density and greatest total LAI from the remainder, accounting for 67.3% of the variation in total LAI. The remainder were then divided on the basis of epiphytic cover, with greatest epiphytic cover associated with the greatest total LAI. An analysis based on individual invertebrate taxa did not produce satisfactory predictions, and it was necessary to use combined invertebrate densities. The analysis represented in Fig. 8.9 is based on total invertebrate densities excluding *Electroma*. When combined densities included *Electroma*, the same division of total LAI were achieved on the basis of invertebrate densities alone, i.e. without using epiphytic cover, which again suggested that *Electroma* densities were closely related to epiphytic cover. The analysis did not indicate that the presence/absence of ferry service to Picnic Bay was a good predictor of total LAI, or *H. uninervis* BGDW.



**Fig. 8.9** Regression tree of total LAI, i.e. combine *Halodule uninervis* and *Cymodocea serrulata* LAI, in plot PB. Epi. = epiphytic, and “All-Elect” is the total density of all invertebrates excluding *Electroma*.

## 8.4 Discussion

Seasonal factors explained much of the variation in *Halodule uninervis* LAI in plot SB, but were not good predictors of *H. uninervis* BGDW, or *Halophila ovalis* abundance in the same plot. Seasonal factors had a minor impact on seagrasses in plot PB. In those cases biological factors proved to be better predictors of seagrass abundance.

The greater seasonal variation of *Halodule uninervis* LAI in plot SB reflects the greater seasonal exposure of that plot to low daytime tides because of its greater elevation. Naturally, the leaves were more vulnerable to exposure than below ground biomass. However, the regression tree analysis selected air temperature rather than tidal exposure as the best predictor of *Halodule uninervis* LAI in plot SB. The two variables are closely correlated, but only the latter explains the difference with plot PB, which had much lower tidal exposure but could not have differed in air temperature. Water temperatures could have differed between the plots, but that would also have been the result of differences in tidal exposure.

The water in the plots during low daytime tides was only a few centimetres deep, and often became heated above normal sea and air temperatures. The highest water temperatures recorded in the field (38°C) approached levels great enough to produce thermal stress in *Halophila ovalis* (Ralph 1998), and those effects are likely to be greater when combined with high light stress (Ralph 1999b). However, the hottest temperatures occurred during the months of highest daytime tides, so the period of exposure to elevated temperatures was not great. Greatest seagrass standing crop of *H. uninervis* in plot SB was associated with highest temperatures, and so there was no evidence of thermal stress.

The increased abundance of seagrass during the hot season on the Queensland coast has been associated with various seasonal factors (Mellors et al. 1993, McKenzie 1994, Lanyon and Marsh 1995), including increased rainfall and increased day length. Rainfall is associated with an increase in nutrients from runoff. However, in the years of this study, significant rainfall did not occur until February. In plot SB, there was evidence of an increase in *Halodule uninervis* abundance and epiphytic cover before



February, and so rainfall seemed unlikely to have been a significant contributing factor. Changes in seagrass abundance are often interpreted with respect to changing light levels, and increased day length seems an obvious explanation for increasing seagrass abundance during the months of the austral summer. Ironically however, those months appeared to be times of decreasing light levels for the seagrass, because they are the months of highest daytime tides, and generally have higher levels of epiphytic cover (chapter 4). The longer average length of *H. uninervis* leaves in the hot season appeared to be a good indicator of lower environmental light levels. A shading experiment at Shelly Beach (chapter 9) showed that *H. uninervis* significantly increased its leaf length in response to shading. Longstaff and Dennison (1999) also showed an increase in leaf length as an early response to shading in *H. uninervis*. In this study, leaf length was positively correlated with epiphytic cover, and tended to be negatively related to tidal exposure, in both plots SB and PB (Appendix B). The reduced abundance of seagrass associated with the cool dry season on the Queensland coast, therefore appears to be related to increased light, due to increased tidal exposure during the day, combined with reduced epiphytic cover. It was hypothesised previously (chapter 3) that such conditions could lead to high irradiance stress, photoinhibition, and photodamage to the leaves. The observations in this study run contrary to the general explanation that reduced leaf cover at that time of year is the result of the shorter day lengths and lower temperatures of “winter” on the Queensland coast.

In general, more variation in seagrass abundance can be explained using biological variables than abiotic seasonal ones. However, interpreting the apparent relationships can be more difficult because of the complex interactions among the biological components and the seagrass. In the regression tree analyses, increased *H. uninervis* BGDW and total LAI in plot PB were associated with decreased numbers of epifaunal animals and increased epiphytic cover in a manner very consistent with the analyses presented elsewhere in this thesis. The positive association with epiphytic cover could be interpreted as a positive response of the seagrass from increased protection from high irradiance. However, there might also be a reciprocal relationship between the seagrass and epiphytic cover, as suggested in chapter 6, because increased canopy density improves conditions for acquiring epiphytic cover. Similarly, the negative

association with total epifaunal abundance, and *Electroma*, likely reflects the negative responses of the dominant suspension-feeding fauna to increases in canopy density.

The regression tree analysis for *H. uninervis* LAI in plot SB suggested that the effects of *Alaba virgata* were related to season. During the coolest time of the year, when tidal exposure was greatest, higher *A. virgata* densities were associated with lowest LAI, suggesting that epiphyte grazing was having a negative impact, increasing exposure to high levels of damaging irradiance. During the hottest time of the year, when tidal exposure was least, higher *A. virgata* densities were associated with highest LAI, suggesting that epiphyte grazing was having a positive impact, increasing exposure to light when levels were otherwise low. That split in the data was based largely on a single date, March 2002, when LAI peaked with low epiphytic cover and high *A. virgata* densities. That was the only instance when there was evidence supportive of the prevailing model of seagrass-epiphyte-grazer interactions, i.e. there were benefits to the seagrass from epiphyte grazing.

*Halophila ovalis* LAI was best predicted in regression tree analysis by damage to the leaves believed to be caused by crustacean feeding, confirming the conclusions of chapter 7. *H. ovalis* BGDW was best predicted by *Halodule uninervis* BGDW, reflecting the succession from the former species to the latter in the plot, but also suggesting the possibility that the species could be competing for below ground nutrients.

Regression tree analysis showed that termination of ferry service to Picnic Bay was not a good predictor of change in the seagrass in plot PB, despite significant differences in several biological parameters before and after the termination. The greatest change in plot PB over the two years was the increase in *Cymodocea serrulata* LAI, and it is not clear how that change could have been related to the ferry service. It is quite possible that the termination was coincident with the normal successional change from *Halodule uninervis* to *C. serrulata*.

## 8.5 Conclusions

Biotic factors were better predictors of seagrass abundance than abiotic factors, with the exception of *Halodule uninervis* LAI in plot SB. In that case, the seasonal change in the tidal exposure of the plot provided the best explanation for the strong seasonal pattern of variation. The pattern suggests greater seagrass abundance during the hottest time of the year in association with reduced light exposure, while lowest abundance occurs during the coolest time of the year in association with high light exposure. Regression tree analysis showed positive associations between measures of seagrass abundance and epiphytic cover, and negative associations with total epifaunal abundance in both plots SB and PB. Those results confirmed the patterns identified elsewhere in this study. Likewise, regression tree analysis confirmed the negative impact of herbivory on *Halophila ovalis* LAI, and a negative association with *H. uninervis* in plot SB, reflecting the species succession.

## Chapter 9 Shading experiment on the impact of high irradiance on intertidal seagrasses

### 9.1 Introduction

At Shelly Beach, between June and July 2002, there was a significant decline in the leaf area index (LAI) of *Halophila ovalis* following an invasion of the meadow by large numbers of the sea hare *Bursatella leachii*, concurrent with a significant decline in epiphytic loads on the leaves (chapter 3). It was hypothesized that epiphyte grazing by the sea hares led to the loss of epiphytic cover and exposed the leaves to damaging levels of irradiance, resulting in a loss of *H. ovalis* leaf area. Over the same period there was no evidence of a loss of epiphytic cover on *Halodule uninervis* leaves, or a decline in its leaf area.

Light intensity is one of the most important factors limiting the distribution and productivity of seagrasses. It is well appreciated that photosynthesis and growth are limited by low light levels related to a variety of factors, such as water depth (Duarte 1991b), transparency (Dunton 1994), and epiphytic shading (Silberstein et al. 1986). Many experimental studies have considered the responses of seagrass to reduced irradiance (e.g. Dennison and Alberte 1982, 1985, Lee and Dunton 1997, Longstaff and Dennison 1999, Moore and Wetzel 2000). That work has often been motivated by threats to seagrass from deteriorating water quality, which usually involves a reduction in light available for photosynthesis.

It is less appreciated that photosynthesis and growth of seagrasses may also be limited by high light intensities that result in photoinhibition or photodamage, an effect most likely in tropical intertidal habitats. Cultured *Halophila ovalis* has been shown to suffer photoinhibitory stress as a result of short-term increases in light intensity (Ralph and Burchett 1995, Ralph 1999a). Photoinhibition may occur as a result of excess photosynthetically active radiation (PAR) or ultra-violet (UV) radiation. Dawson and Dennison (1996) found that *Halophila ovalis* and *Halodule uninervis* were the most sensitive, amongst 5 Queensland species, to UV radiation; yet they were the least sensitive to differences in PAR. Trocine et al. (1981) found that *Halophila engelmanni*

Achers from Florida was highly sensitive to ultraviolet-B radiation. They also showed that epiphytic cover reduced photoinhibition of that species, and suggested that it was dependent on epiphytic material to shield it from UV-B radiation.

A shading experiment was therefore proposed to determine if reductions in shade cover and exposures to high levels of irradiance in an intertidal habitat could lead to the loss of *Halophila ovalis* leaf area in a way similar to the loss seen after the invasion of sea hares at Shelly Beach in 2002. It was proposed that moderate shading be provided during a period of high irradiance stress with the expectation of increased productivity of *H. ovalis*. After a period of shade-adaptation, those plants would be exposed to high irradiant ambient light, with the expectation of strong negative impacts on productivity. It was proposed that chlorophyll concentrations be used as an indicator of the plant's response to changing light conditions. Experimental shading of seagrasses has been shown to increase chlorophyll concentrations and reduce chlorophyll *a:b* ratios in several species (Wiginton and McMillan 1979, Dennison and Alberte 1982, 1985, Abal et al. 1994, Lee and Dunton 1997). Exposure to high irradiance and photoinhibition is associated with decreasing chlorophyll concentrations and increasing chlorophyll *a:b* ratios (Ralph 1999a).

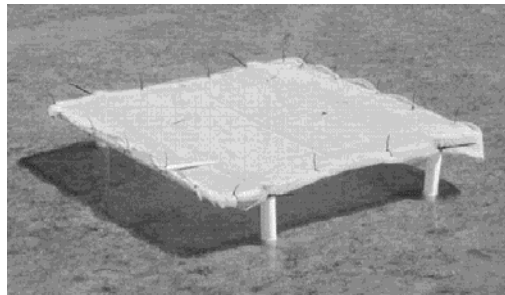
## 9.2 Methods

### 9.2.1 Design

Experimental plots were established at Shelly Beach on 25 and 26 August 2003. At that time of year, there was high daytime exposure of the meadow during spring tides. Plots were located where there was growth of both *Halophila ovalis* and *Halodule uninervis*. When the plots were established, there was nearly continuous coverage of *H. uninervis* over the meadow, but *H. ovalis* had become patchy in distribution. Nine pairs of plots were placed in a row approximately 200 m long, parallel to shore, and adjacent to the larger plot that was monitored over two years (chapter 2). All plots retained a thin layer of water at low tide.

A pair of plots, each 0.5 m<sup>2</sup>, was marked out at least two meters apart. Each was selected to have a similar area of *H. ovalis* cover. One member of the pair was

randomly selected for shading, and the other became a control. Shading was provided with 70% shade cloth attached with plastic cable ties to a 0.5 m<sup>2</sup> frame of 16mm PVC electrical conduit pipe. The sides of the frame, 0.7 m long, were connected with PVC elbows and fitted through holes drilled in posts of 30 mm PVC pipe driven into the sediment (Fig. 9.1). The frame stood 15 cm above the sediment, the maximum expected length of *Halodule uninervis*. The control plots were marked with PVC pipe at the corners.



**Fig. 9.1 A shaded plot at Shelly Beach**

Shade clothes were removed after 29 days, and the treatment plots exposed to ambient light for a further 29 days. That period was chosen to be similar to the period over which the changes in *Halophila ovalis* LAI had been observed the previous year following the incursion of sea hares. It was also longer than the time needed for a complete replacement of *H. ovalis* standing crop (Hillman et al. 1995).

Shade clothes were changed twice during the shading period to reduce fouling, which was found to be slight. Perimeters of all plots were cut with a knife to sever rhizomes and isolate plots from the surrounding meadow. They were cut on 5 occasions: when shade clothes were first installed, when they were changed, when they were finally removed, and then again after ten days.

### **9.2.2 Chlorophyll concentrations**

Leaves were sampled for chlorophyll analysis by spectrophotometry (Granger and Iizumi 2001). *Halophila ovalis* leaves were taken from the third pair, counting from the growing shoot tip. Those were generally the first fully emerged leaves on a shoot; and therefore the sample represented younger and more uniformly aged leaves than the general population of leaves. Three *H. ovalis* leaves were taken from different shoots

in each plot when the shades were first installed, again when they were removed, and after 1, 2, 4, 10, and 29 days of exposure. Due to time constraints, samples of *H. ovalis* leaves were obtained from only five of the plot pairs after one day of exposure.

Samples of *Halodule uninervis* leaves were not taken for chlorophyll analysis when the shades were first installed, as *H. ovalis* was the initial target species of the experiment. However, early in the experiment there was evidence of a response to shading by *H. uninervis*. Subsequently, three shoots of *H. uninervis* were taken for chlorophyll analysis on each occasion that *H. ovalis* leaves were sampled.

Each leaf of *H. ovalis* or shoot of *Halodule uninervis* was individually placed in a 1.5 ml plastic vial and immediately placed on dry ice in the field. They were stored at -70°C until analysed. Leaf material for chlorophyll analysis was taken from the eastern half of the plots, in order to minimize the impact of a gradient in sunlight exposure in the shaded plots. On days of the lowest tides, weak sunlight entered under the shades late in the day from the western side.

Before analysis for chlorophyll concentrations, leaves were removed from their vials, and any epiphytic material removed by gentle scraping or washing. Petioles of *Halophila ovalis* were removed. From shoots of *Halodule uninervis*, only the first fully extended leaf was used, so the leaf material analysed would be of uniform age. A 3 cm section was cut from that leaf, starting 0.5 cm above the base. Shorter sections were used when necessary to avoid areas of mechanical damage or invertebrate feeding on the leaf. Each leaf, or leaf section, was placed between transparencies, and images of them made with a desktop scanner at 300 dpi. Those images were later analysed using image analysis techniques to determine leaf tissue surface areas, as described in chapter 2.

Each leaf, or leaf section, was returned to its vial, which had been swabbed with tissue to remove droplets of seawater or detritus. The vial was partially filled with 80% acetone, and the leaf was finely ground with a pestle and a small quantity of clean sand. The vial was filled with 80% acetone to the 1.5 ml level, shaken, and refrigerated at -20°C for at least 10 minutes. The vial was spun in a centrifuge for 60 seconds and

then decanted into a 1 cm cuvette for analysis in a spectrophotometer (Agilent 8453 E UV-visible spectroscopy system).

The absorbency of the extract was measured at 647, 664 and 725 nm. Chlorophyll concentrations were calculated using the equations:

$$\text{Chl } a \text{ (}\mu\text{g ml}^{-1}\text{)} = 11.93E_{664} - 1.93E_{647}$$

$$\text{Chl } b \text{ (}\mu\text{g ml}^{-1}\text{)} = 20.36E_{647} - 4.68E_{664}$$

Where E = corrected absorbency, i.e. absorbency at the wavelength – absorbency at 725 nm. The chlorophyll concentration ( $\mu\text{g ml}^{-1}$ ) was then multiplied by the volume of the acetone (1.5 ml) and divided by the surface area of the tissue to express the chlorophyll concentration as  $\mu\text{g cm}^{-2}$  (Granger and Iizumi 2001).

### **9.2.3 Seagrass measurements**

The quantity of seagrass of both species was determined from core samples. On the day the shade clothes were installed, a core sample was taken at random from the west side of each plot using a section of PVC pipe, 45 cm<sup>2</sup> diameter, forced 10 cm deep into the sediment. Core samples were taken again 29 days later when the shades were removed, and after 29 days of exposure. Those cores were taken from the centres of the plots, to avoid any differences in shading across the plots. The first core was taken at random either north or south of the centre point of the plot, and the final core was taken opposite that. To prevent the collapse of sediment into the holes that were created by coring, a piece of PVC pipe of the same diameter and 10 cm length, was inserted into the hole and filled with sediment from outside the plot. The core samples were washed to remove sediment from the roots, and then frozen for later determination of the leaf area index (LAI) and below ground dry weight (BGDW), following the methods outlined in chapter 2. The numbers of leaves of *Halophila ovalis* and shoots of *Halodule uninervis* were counted at that time.

Leaf size differences between shaded and non-shaded plots were also examined. In the field, three leaves of *Halodule uninervis* were randomly selected from each plot and



their lengths measured by holding them against a small ruler. Those lengths were taken when the shades were removed and again after 29 days of exposure. *H. uninervis* leaf lengths and mean breadths, i.e. leaf surface area divided by length, were also calculated from images of the leaves sampled to measure epiphytic loads (see below). Leaf lengths measured in the field were always longer than lengths of leaves cut to measure epiphytic loads, as the former generally included part of the leaf covered by the sheath. The average areas of *Halophila ovalis* leaves were calculated from the images of the leaves in cores samples only, as there was expected to be a size bias among *H. ovalis* leaves collected to measure epiphytic loads.

#### **9.2.4 Epiphytic loads**

Observations suggested that the amount of epiphytic cover on the leaves differed between shaded and non-shaded plots. Therefore, epiphytic growth on the leaves was analysed from cuttings of five randomly selected leaves of each species taken from the west half of each plot on the day the shades were removed, and after 29 days of exposure. The percentage cover of epiphytic material was estimated using the techniques outlined in chapter 2. The leaves were frozen, and the epiphytic material was later removed by scraping, and its ash-free dry weight (AFDW) determined using the techniques outlined in chapter 2.

#### **9.2.5 Invertebrates**

Invertebrate animals were removed, and counted, from the leaves cut to measure the AFDW of epiphytic material. An increase in the number of *Alaba* was notice on *Halodule uninervis* leaves in the days immediately after the shades were removed. To document those changes, additional samples of *H. uninervis* leaves, 5 per plot, were taken from 4 pairs of plots on the fourth day of exposure. Those leaves were also scraped to measure epiphytic AFDW.

Estimates of areas of *Halophila ovalis* leaves damaged or missing because of invertebrate feeding were made using the methods described in chapter 2. The surface areas damaged by gastropods were calculated from images of the leaves taken to measure epiphytic material. The proportion of the leaves missing, mostly due to crustacean feeding, was calculated from the damage to leaves in core samples.

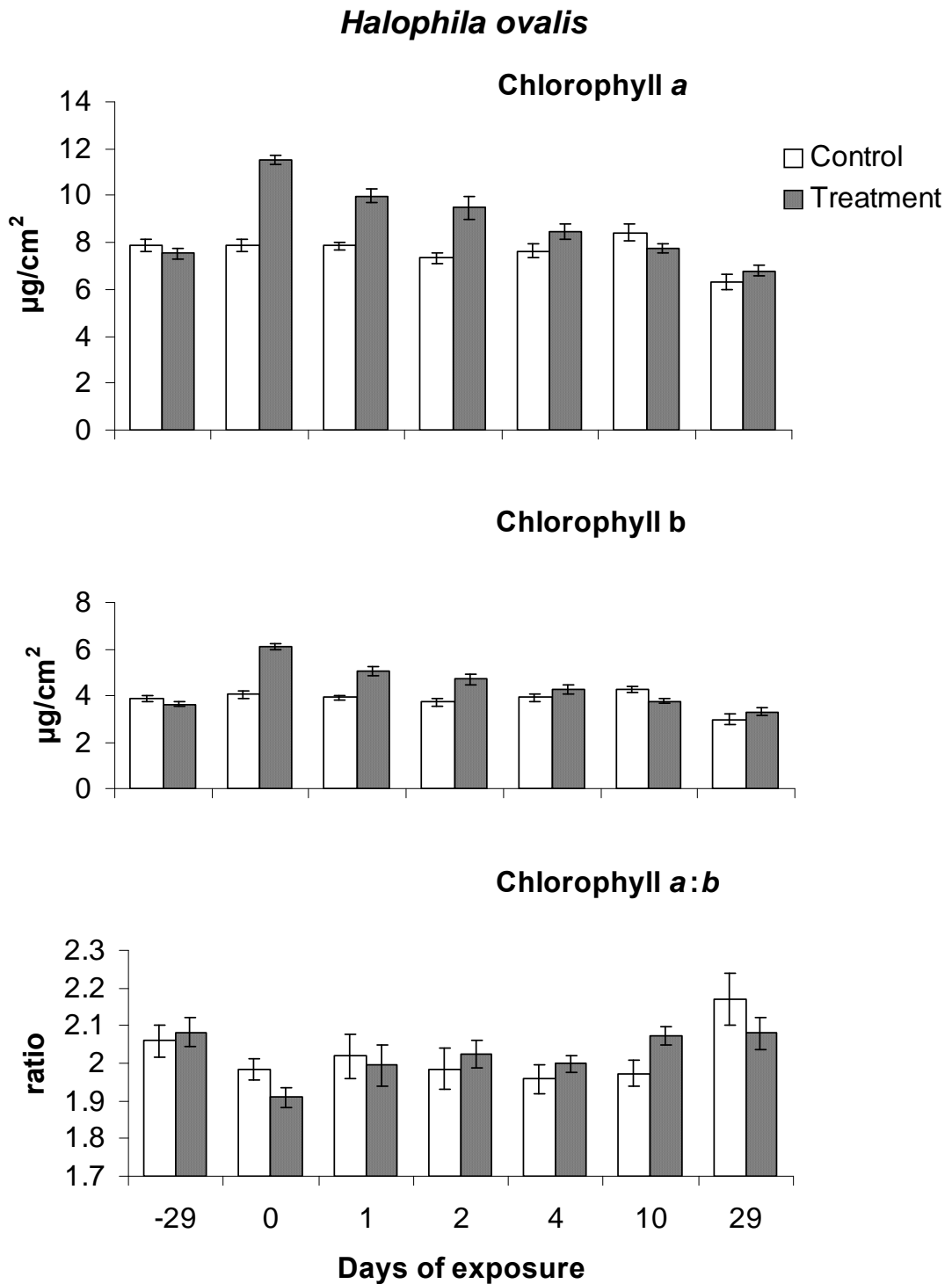
### **9.2.6 Statistical analysis**

Changes in seagrass measurements in response to the shading and exposure treatments were analysed using repeated measures analysis of variance (Johnson and Wichern 1982). The response variable was the difference between the treatment and control plot of each pair. The analysis simultaneously considered the response of two contrasts: the shading contrast representing the change in the difference between the control and shade plots over the time shades were in place, and the exposure contrast representing the change in the difference over the time of exposure. When no significant differences due to treatments were found, the measurements from each pair of treatment and control plots were averaged and differences in those values over the two time periods tested using repeated measures analysis. When an analysis indicated that there were significant differences, simultaneous 90% confidence intervals (CI) were calculated for each contrast to determine which contributed to those changes. The data has been presented as graphs of the means of values from control and treatment plots that do not accurately represent the paired experimental design or its analysis.

## **9.3 Results**

### **9.3.1 Chlorophyll concentrations**

*Halophila ovalis* leaves from plots shaded with 70% shade cloth for 29 days averaged 46% higher chlorophyll *a*, and 51% higher chlorophyll *b* concentrations, than non-shaded controls (Fig. 9.2). After exposure to ambient light levels, chlorophyll concentrations in treatment plots fell rapidly, and were only moderately higher than controls four days after the removal of the shades. The pattern of decline of chlorophyll *a* and *b* appeared similar, but the ratio chlorophyll *a:b* varied over time (Fig. 9.2). When the shades were removed, the ratio in shaded treatment plots was significantly lower than in control plots, as expected (paired t-test,  $t=2.54$ ,  $p<0.05$ ), although the difference was very small,  $< 0.10$ .



**Fig. 9.2** Mean ( $\pm$  s.e.) concentrations of chlorophyll *a* and *b*, and the ratio chlorophyll *a*:*b*, per  $\text{cm}^2$  of *Halophila ovalis* leaf from treatment and control plots,  $n=9$ , except after 1 day of exposure when 5 plot pairs were sampled. Sampling occurred on the day shades were applied to the treatment plots (days of exposure = -29), the day shades were removed (days of exposure = 0), and subsequent days of exposure as indicated.

### *Halodule uninervis*

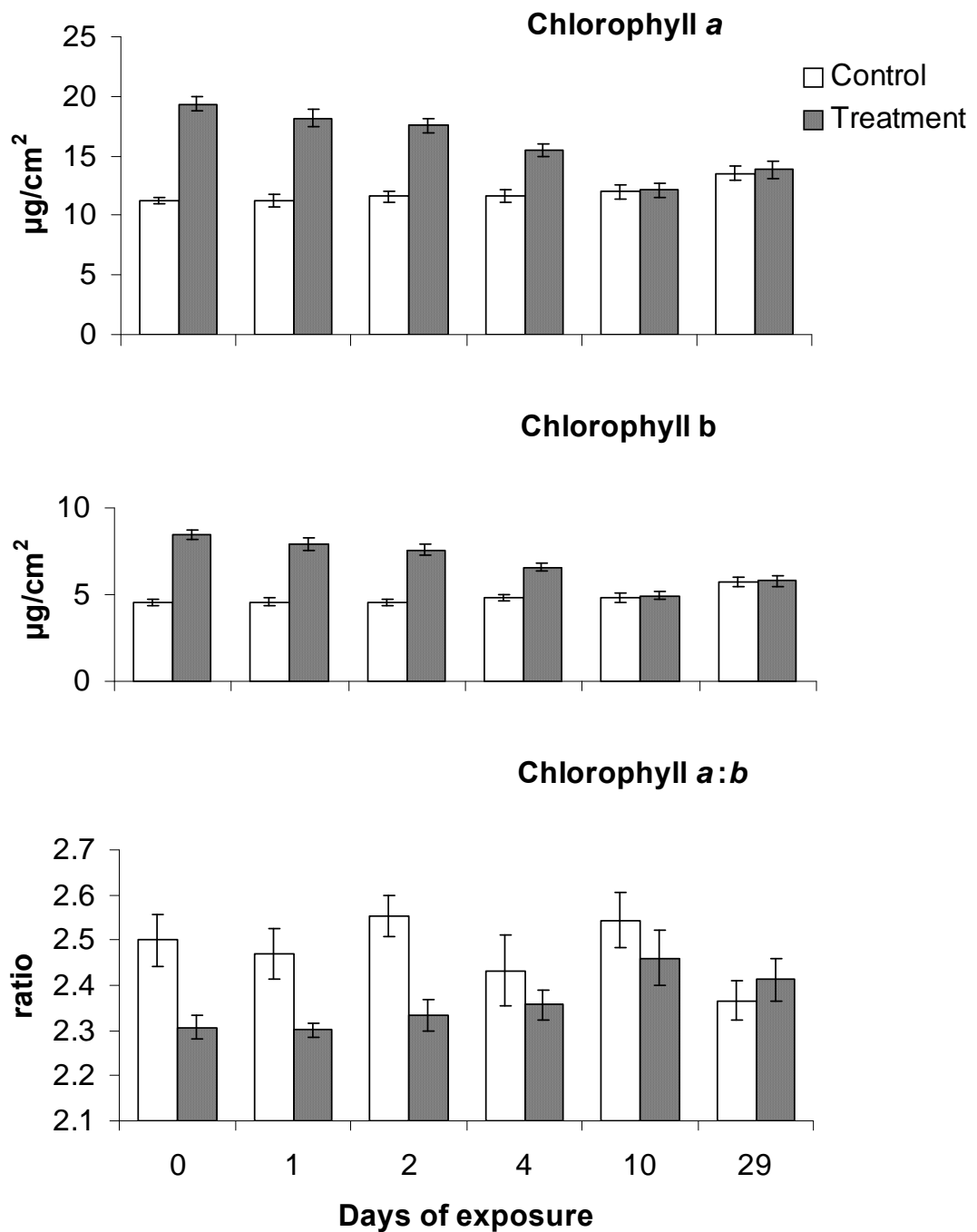


Fig. 9.3 Mean ( $\pm$  s.e.) concentrations of chlorophyll *a* and *b*, and the ratio chlorophyll *a*:*b*, per cm<sup>2</sup> of *Halodule uninervis* leaf from 9 treatment and control plots from the day shade treatment plots were exposed to ambient light (days of exposure = 0).

*Halodule uninervis* leaves averaged 62% higher chlorophyll *a* and 72% higher chlorophyll *b* concentrations than controls after 29 days of shading (Fig. 9.3). As with *H. ovalis*, chlorophyll *a* and *b* concentrations fell rapidly over succeeding days of

exposure, and were almost identical in control and treatment plots after 10 days. The ratio chlorophyll *a:b* (Fig. 9.3) was significantly lower in the treatment plots on the day the shades were removed (paired t-test,  $t=3.44$ ,  $p<0.01$ ), but rose to levels comparable to those in the controls after four days of exposure.

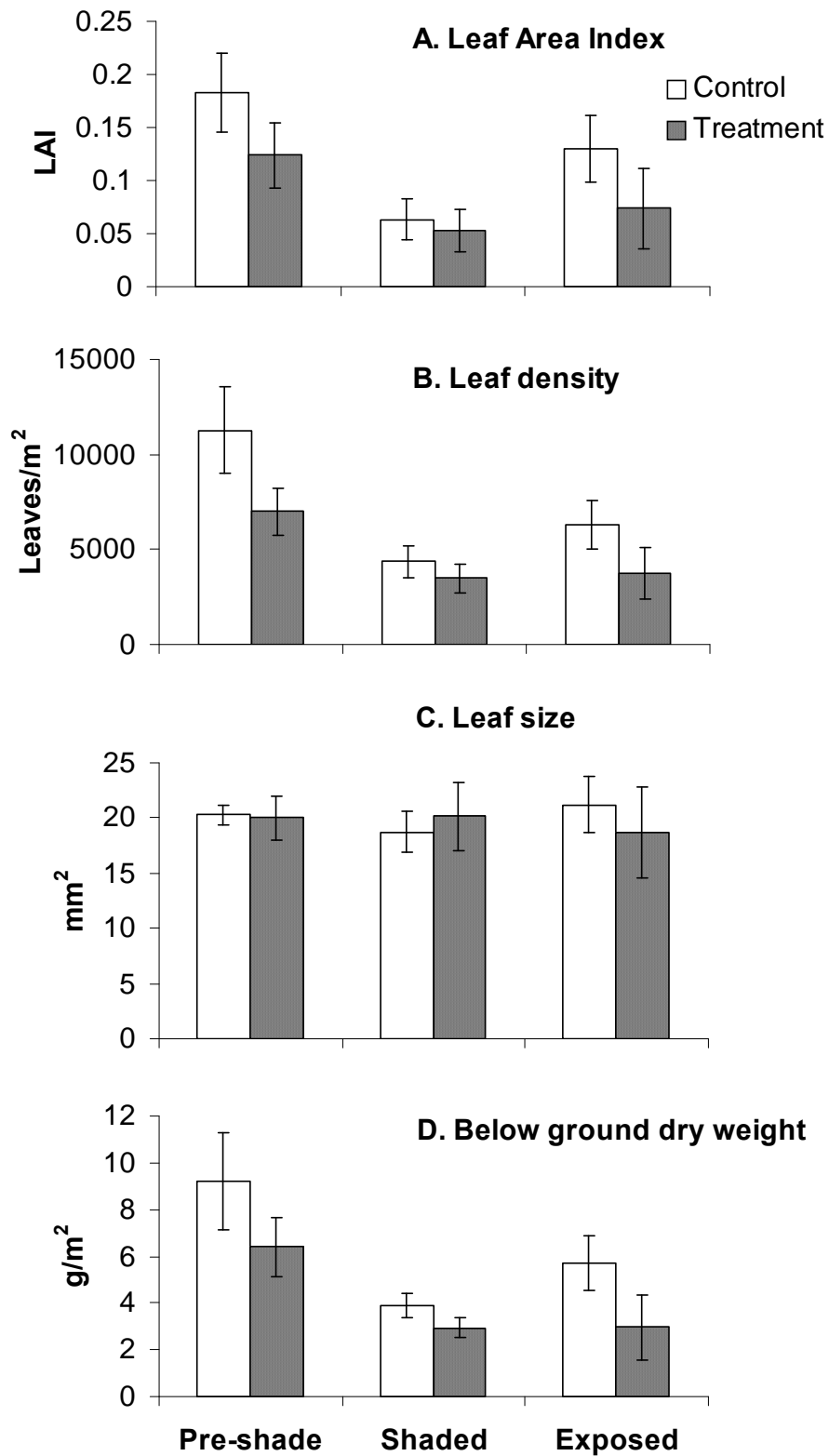
### 9.3.2 Seagrass measurements

#### 9.3.2.1 *Halophila ovalis*

The LAI of *Halophila ovalis* did not show any significant differences between control and treatment plots (repeated measures analysis, Hotelling's  $T^2=3.56$ ,  $p>0.10$ ). LAI decreased over the time of the experiment, irrespective of treatment (Fig. 9.4A), with an overall average LAI falling from 0.15 to 0.06 during the period of shading ( $T^2=19.90$ ,  $p<0.01$ ). Those changes were due to a significant fall in the value of the index during shading (90% CI  $-0.095\pm 0.058$ ), and no significant change during exposure (90% CI  $0.044\pm 0.058$ ). An identical pattern was seen in changes to *H. ovalis* leaf density (Fig. 9.4B), with no significant differences due to treatment ( $T^2=1.81$ ,  $p>0.10$ ), but significant changes in the average leaf density over time ( $T^2=19.25$ ,  $p<0.01$ ). Those differences were due to a significant change in leaf density over the shading period (90% CI  $-5208\pm 3160$  m<sup>-2</sup>) but not during exposure (90% CI  $1097\pm 2770$  m<sup>-2</sup>). Changes in average LAI were correlated to changes in average leaf density over both the shading and exposure periods ( $r=0.84$  and  $r=0.92$  respectively). The leaf size of *H. ovalis* (Fig. 9.4C) did not change with respect to treatments ( $T^2=2.21$ ,  $p>0.10$ ), and there was no evidence of changes in the average leaf size during the experiment ( $T^2=0.36$ ,  $p>0.10$ ).

Changes in BGDW of *H. ovalis* (Fig. 9.4D) were similar to those of LAI and leaf density. No significant differences were found due to treatments ( $T^2 = 1.72$ ,  $p>0.10$ ), but there was a tendency for average BGDW to change during the experiment ( $T^2=9.98$ ,  $0.05<p<0.10$ ). That tendency was due to a change in average BGDW during shading (90% CI  $-4.38\pm 3.78$  g m<sup>-2</sup>) and not during exposure (90% CI  $0.91\pm 2.41$  g m<sup>-2</sup>). There was no evidence of differences in the leaf area per gram BGDW due to the treatments ( $T^2 = 0.1$ ,  $p>0.10$ ).

*Halophila ovalis*



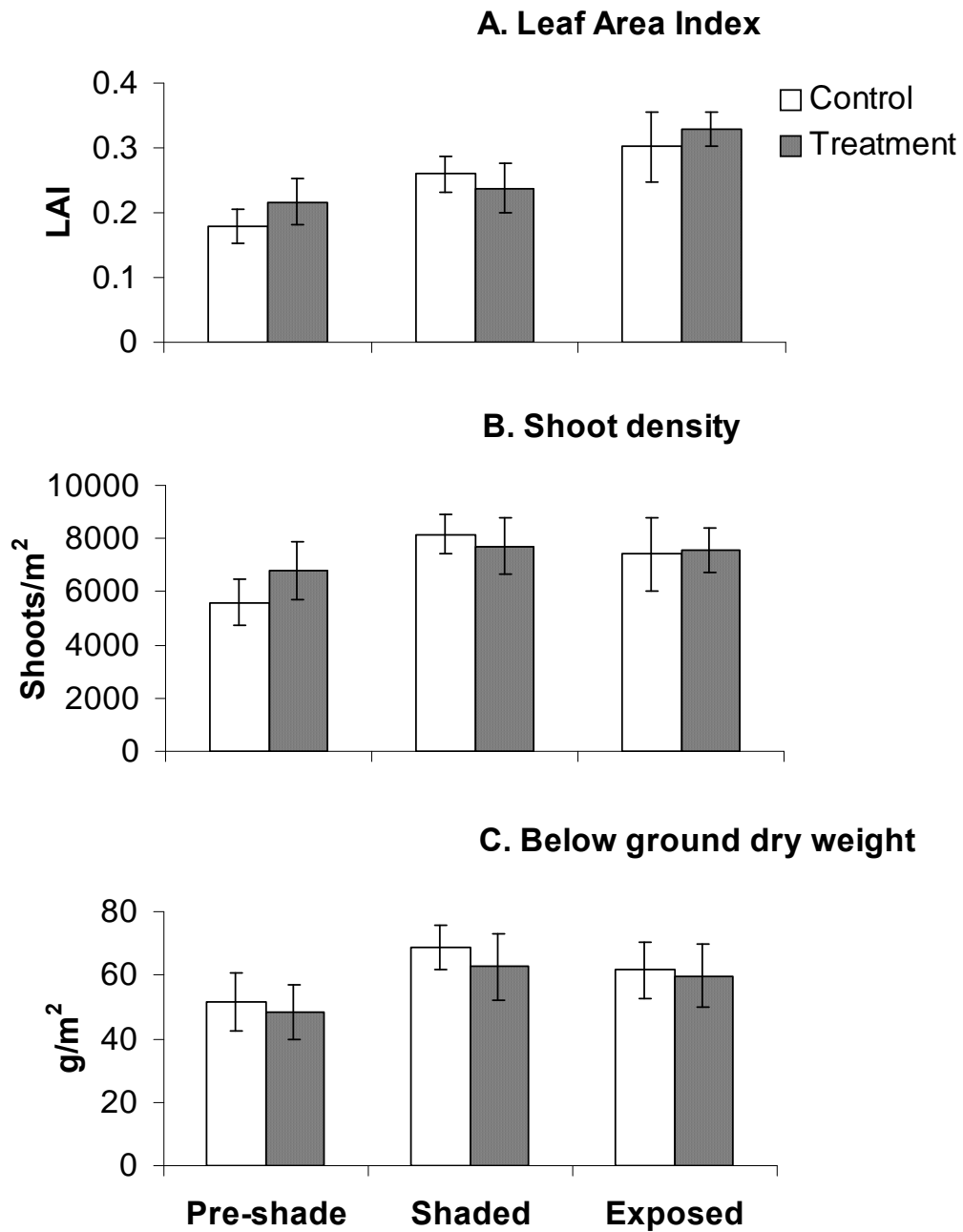
**Fig. 9.4** Mean ( $\pm$  s.e.) values of LAI (A), leaf density (B), leaf size (C), and BGDW (D) of *Halophila ovalis* in core samples from 9 paired control and treatment plots before shading, after 29 days of shading treatment, and after 29 days of exposure to ambient light.

### 9.3.2.2 *Halodule uninervis*

The LAI, number of shoots, and BGDW of *Halodule uninervis* (Fig. 9.5) did not show any significant differences between the control and treatment plots ( $T^2=1.84$ , 1.09, and 0.80 respectively,  $p>0.10$ ). Over the course of the experiment the average LAI rose from 0.20 to 0.32. That overall change was significant ( $T^2=13.26$ ,  $p<0.05$ ), but rises over the individual shading and exposure periods were very similar and non-significant (90% CI  $0.051\pm 0.079$  and  $0.066\pm 0.071$  respectively). The average number of shoots changed significantly during the experiment ( $T^2=12.62$ ,  $p<0.05$ ), with a significant rise during the shading period (90% CI  $1740\pm 1384$  m<sup>-2</sup>), but not the exposure period (90% CI  $-444\pm 1946$ ). There were highly significant changes in average BGDW during the experiment ( $T^2=43.32$ ,  $p<0.01$ ), due to a significant increase in BGDW during the shading period (90% CI  $15.6\pm 5.8$  g m<sup>-2</sup>), but no change during the exposure period (90% CI  $-4.9\pm 11.5$  g m<sup>-2</sup>).

Leaves of *Halodule uninervis* were conspicuously longer in shaded plots than in controls at the end of the shading period. Field measurements found that leaves in shaded plots averaged 1.8 cm longer than those in control plots (Fig. 9.6A) (paired t-test,  $t=-5.74$ ,  $p<0.001$ ). After the exposure treatment, differences in leaf length between plots were significantly reduced (paired t-test,  $t=-2.76$ ,  $p<0.05$ ), but those changes were due to an increase in leaf length in control plots. The same pattern was evident in length measurements of leaves cut for analysis of epiphytic material (Fig. 9.6B). Leaves from shaded treatment plots averaging 0.7 cm longer than those from controls (paired t-test,  $t=-4.14$ ,  $p<0.01$ ), and those differences were significantly reduced by the exposure treatment (paired t-test,  $t=-3.27$ ,  $p<0.05$ ), due to an increase in leaf lengths in control plots. Those same leaves had almost identical mean breadths in control and treatment plots at the end of the shading period (Fig. 9.6C), and there was no change in mean breadth due to the exposure treatment (paired t-test,  $t=-0.31$ ,  $p>0.10$ ).

## *Halodule uninervis*



**Fig. 9.5** Mean ( $\pm$  s.e.) values of LAI (A), shoots density (B), and BGDW (C) of *Halodule uninervis* in core samples from 9 paired control and treatment plots before shading, after 29 days of shading treatment, and after 29 days of exposure to ambient light.



## *Halodule uninervis*

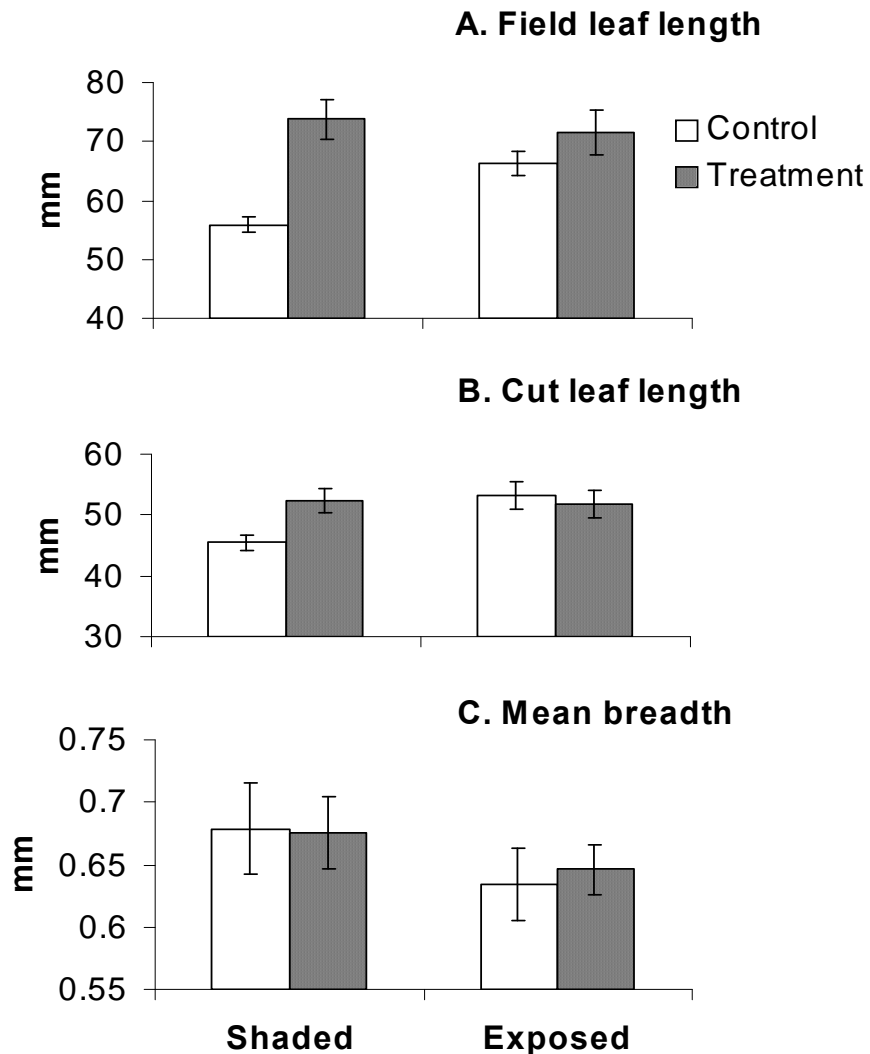
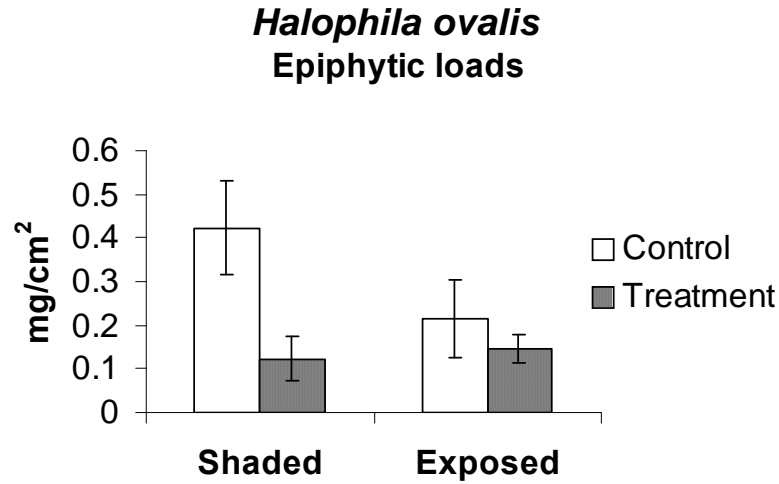


Fig. 9.6 Mean ( $\pm$  s.e.) length of leaves measured in the field (A), and mean ( $\pm$  s.e.) of the lengths (B) and mean breadths (C) in scanned images of leaves cut to sample epiphytic material from 9 pairs of control and treatment plots after 29 days of shading, and after 29 days of exposure.

### 9.3.3 Epiphytic loads

When shades were removed from treatment plots, there was a noticeable cover of soft flocculent material on seagrass leaves, especially on *Halophila ovalis* in control plots. They had 33% of their surfaces covered by epiphytic material, compared to 9% of *H. ovalis* leaf surfaces from shaded treatment plots. The AFDW of epiphytic material on *H. ovalis* leaves from control plots was 3.4 times greater than on leaves from shaded treatment plots (Fig. 9.7) (paired t-test,  $t=3.10$ ,  $p<0.01$ ). The AFDW of epiphytic

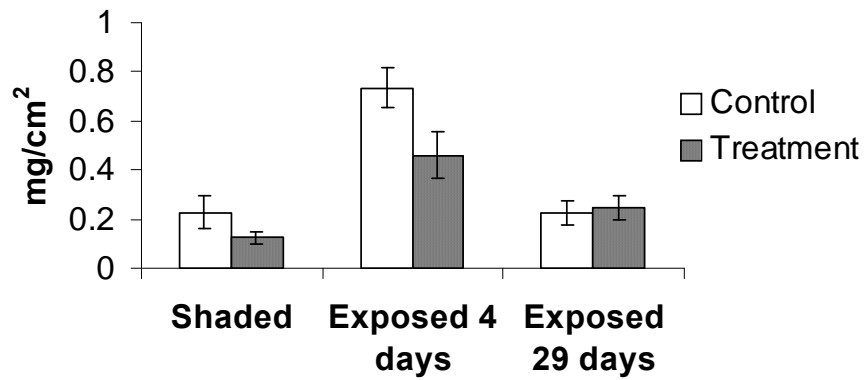
material on *Halodule uninervis* leaves was not clearly greater in control plots (Fig. 9.8) (paired t-test,  $t=1.59$ ,  $0.05 < p < 0.10$ ).



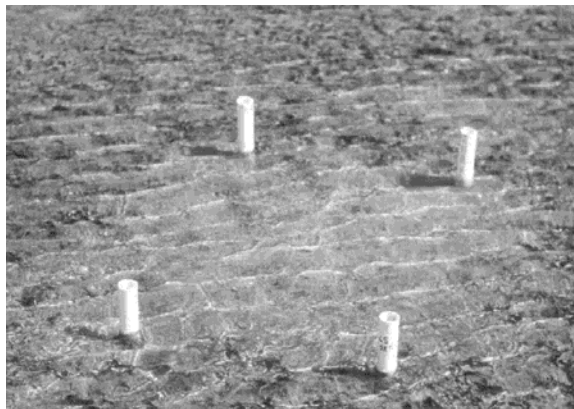
**Fig. 9.7** Mean ( $\pm$  s.e.) AFDW of epiphytic material per cm<sup>2</sup> of *Halophila ovalis* leaf from 9 pairs of control and treatment plots after 29 days of shading, and after 29 days of exposure.

Four days after the removal of the shades there was a conspicuous growth of filamentous blue-green algae over parts of the seagrass meadow, including control plots, but strikingly less developed in plots that had been shaded (Fig. 9.9). The growth was most developed on *Halophila ovalis* leaves, but also occurred on *Halodule uninervis* (Fig. 9.8). The AFDW of epiphytic material increased 2.4 times on *H. uninervis* leaves from four plot pairs re-sampled four days after the removal of the shades. After 29 days of exposure there was no evidence of differences between control and treatment plots in AFDW of epiphytic material.

***Halodule uninervis***  
**Epiphytic loads**



**Fig. 9.8** Mean ( $\pm$  s.e.) AFDW of epiphytic material per cm<sup>2</sup> of *Halodule uninervis* leaf from 9 pairs of control and treatment plots after 29 days of shading and exposure, and from 4 pairs of plots after 4 days of exposure.



**Fig. 9.9** Treatment plot four days after removal of shade. The dark material on seagrass surrounding the plot is epiphytic algae.

### 9.3.4 Invertebrates

#### 9.3.4.1 *Alaba virgata*

The experiment took place at a time of dense populations of *Alaba virgata* at Shelly Beach (see chapter 4, Fig. 4.1). However, only two individuals were found on leaves cut from treatment plots to measure epiphytic cover when shades were removed. The number of *A. virgata* per area of leaf cut from shaded plots was significantly lower compared to controls on the day shades were removed ( $p < 0.05$ , Wilcoxon test of the median=0 for paired differences). But after four days of exposure, numbers of *A. virgata* increased dramatically in both treatment and control plots on leaves cut from four plot pairs (Fig. 9.10).

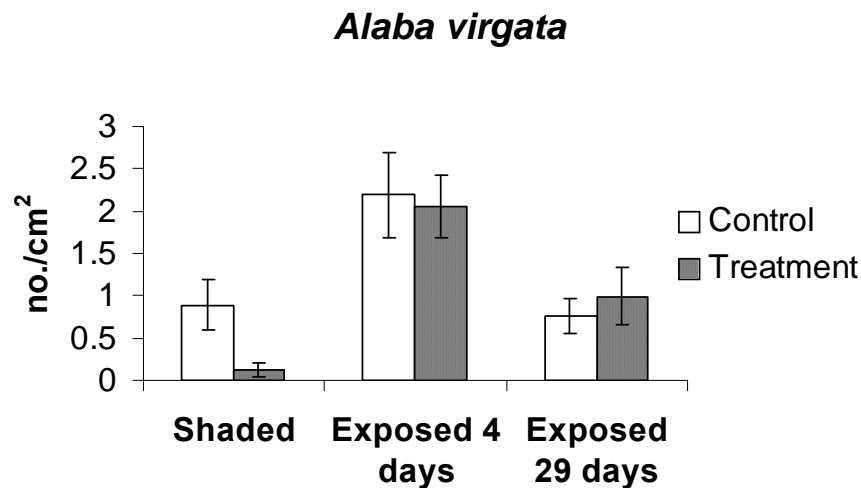
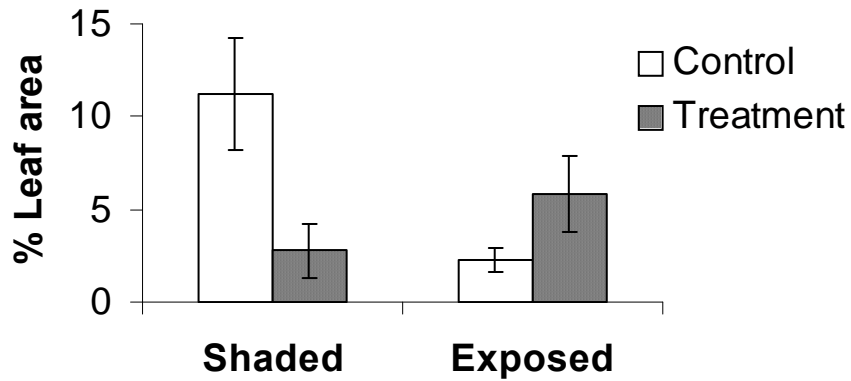


Fig. 9.10 Mean ( $\pm$  s.e.) number of *Alaba virgata* per  $\text{cm}^2$  on *Halodule uninervis* leaves sampled to estimate the epiphytic loads in 9 pairs of control and treatment plots after 29 days of shading and exposure, and from 4 pairs of plots after 4 days of exposure.

#### 9.3.4.2 Herbivores

Leaves of *Halophila ovalis* cut from control plots to measure epiphytic cover when shades were removed had significantly higher proportions of their surface areas damaged by gastropods than leaves from shaded plots (Fig. 9.11) (paired t-test,  $t=3.46$ ,  $p < 0.01$ ). Most of that damage was of the type attributable to the neritid *Smaragdia souverbiana*.

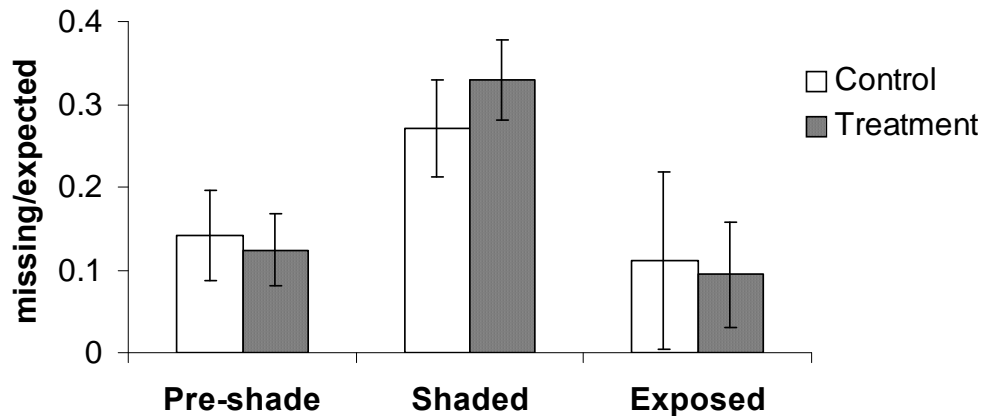
***Halophila ovalis***  
**Damage by gastropods**



**Fig. 9.11** Estimated percent damage ( $\pm$  s.e.) of *Halophila ovalis* leaf surface areas by gastropods. Leaves sampled to estimate epiphytic loads in 9 paired control and treatment plots after 29 days of shading, and after 29 days of exposure.

The proportion of *Halophila ovalis* leaves in core samples that appeared to be missing, probably due to crustacean feeding, did not differ due to treatment effects ( $T^2=0.95$ ,  $p>0.10$ ). However, the average proportion missing changed significantly during the experiment (Fig. 9.12) ( $T^2=31.29$ ,  $p<0.01$ ), with the proportion more than doubling during the shading treatment (90% CI  $20.1\pm 10.4\%$ ), and returning to former levels during exposure (90% CI  $-19.6\pm 12.6\%$ ). Damage due to gastropods on leaves from cores was small relative to damage attributed to crustaceans. The total area of the leaves missing or damaged in control plots when shades were removed was 28.8%, but was only reduced to 27.5% when damage due to gastropods was removed from the calculations.

***Halophila ovalis***  
**Missing proportion of leaf areas**



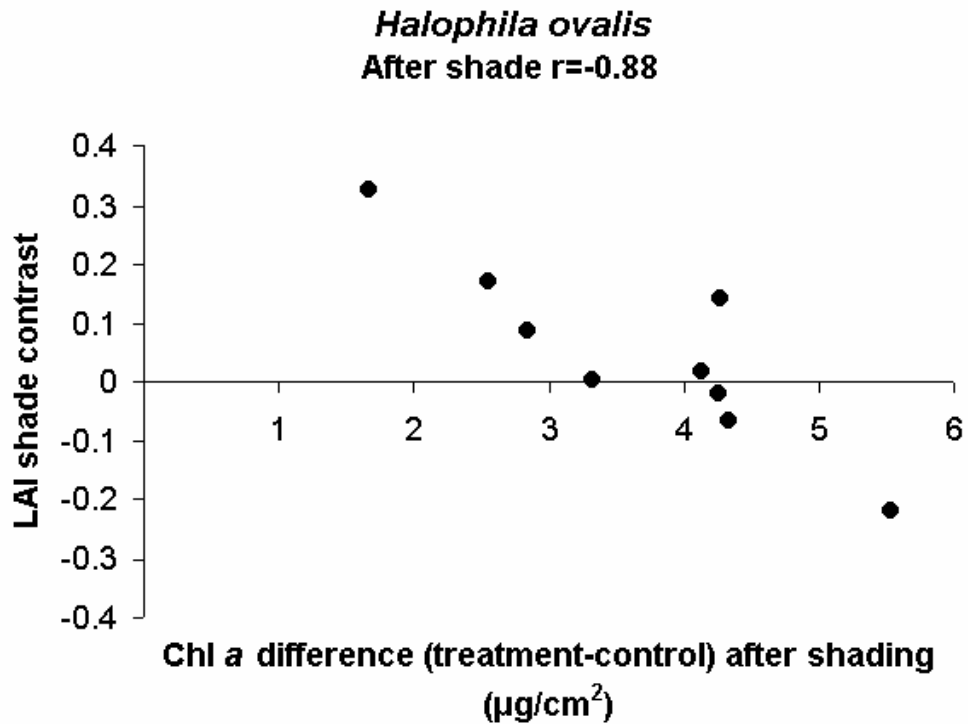
**Fig. 9.12** The estimated leaf surface area of *Halophila ovalis* missing as a proportion of the expected surface area ( $\pm$  s.e.) of leaves in core samples from 9 paired control and treatment plots before shading, after 29 days of shading treatment, and after 29 days of exposure to ambient light.

### 9.3.5 Interactions

There was evidence of a strong relationship between changes in *Halophila ovalis* LAI and differences in leaf chlorophyll content, after both shading and exposure treatments. The values of the contrasts representing changes in the differences between the control and treatment LAI have been plotted against the differences in chlorophyll *a* concentration after shading (Fig. 9.13) ( $r=-0.88$ ,  $p<0.005$ ), and after exposure (Fig. 9.14) ( $r=0.87$ ,  $p<0.005$ ). The contrasts have been calculated so that increasing values represent increasing LAI in treatment plots relative to control plots over the time period concerned.

After shading, all treatment plots had leaf chlorophyll concentrations greater than those in control plots, but the difference varied among the plot pairs. The plot pair with the greatest increase in chlorophyll *a* concentration in the shaded treatment plot had the most negative value in the LAI shading contrast, i.e. greatest decline in LAI in the shaded plot relative to the control over the shading period. At the opposite extreme, the plot pair with the least difference in chlorophyll *a* concentrations, had the greatest increase in LAI in the shaded plot compared to its control. Those changes in LAI reflected changes in the numbers of leaves in the plots and not changes in leaf size. A contrast based on the differences in the number of leaves, rather than LAI, was also

correlated to those differences in chlorophyll concentrations at the end of the shading period ( $r = 0.81$ ,  $p < 0.01$ ). There was no evidence of a relationship between BGDW and chlorophyll concentration during the shading period.



**Fig. 9.13** Plot of the values of the contrast due to shading treatment effects on LAI versus the difference between treatment and control plots in chlorophyll *a* concentrations in *Halophila ovalis* leaves after 29 days of shading. Positive values on y-axis represent increases in LAI in shade plots relative to controls after shading.

The amount of chlorophyll *a* produced by *Halophila ovalis* plants in the plots was calculated by multiplying the LAI by the leaf chlorophyll *a* concentration for each plot. Despite the much higher concentrations of chlorophyll *a* per unit area of leaf after shading (Fig. 9.2), the amount of chlorophyll *a* produced by *H. ovalis* per unit area of substrate did not differ significantly between treatment and control plots after shading (paired t-test,  $t = -0.36$ ,  $p > 0.10$ ), reflecting the relationship between LAI, leaf number and chlorophyll concentrations evident in Fig. 9.13.

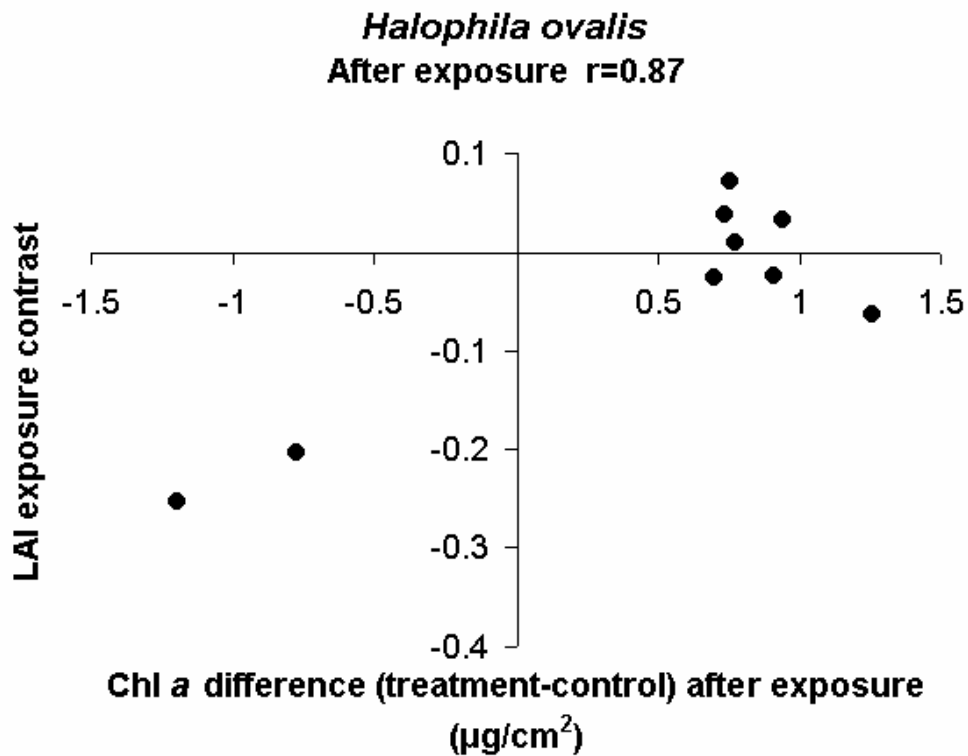


Fig. 9.14 Plot of the values of the contrast due to exposure treatment effects on LAI versus the difference between treatment and control plots in chlorophyll *a* concentrations in *Halophila ovalis* leaves after 29 days of exposure. Positive values on y-axis represent increases in LAI of shade plots relative to controls after exposure.

After exposure, differences in chlorophyll concentrations among plot pairs declined to near zero, as expected, but also varied among plot pairs. Two plot pairs were distinctive by having chlorophyll *a* concentrations lower in the shade plots compared to the controls, and having the lowest values of the LAI exposure contrast, i.e. LAI declined in those treatment plots relative to their controls over the time of exposure. Those plots also had the greatest declines in leaf number and BGDW. The BGDW exposure contrast was also correlated with differences in chlorophyll *a* concentration at the end of the exposure period ( $r = 0.79$ ,  $p < 0.025$ ).

The plot pair that showed the greatest decrease after exposure in chlorophyll *a* concentrations and LAI in its treatment plot (Fig. 9.14) also had the greatest difference in epiphytic load when the shades were removed, with highest epiphytic cover on leaves in the control plot. At the end of the exposure period, the differences between



control and treatment plots in chlorophyll *a* concentrations in *Halophila ovalis* leaves were correlated to differences in AFDW of epiphytic material on *H. ovalis* leaves when the shades were removed 29 days earlier ( $r=0.69$ ,  $p<0.05$ ).

There was no evidence of a relationship between changes in *Halodule uninervis* LAI or BGDW and differences in chlorophyll concentrations after either treatment. Calculations of the amount of chlorophyll *a* produced by *H. uninervis* after shading showed significantly more chlorophyll per unit area of substrate in treatment plots than in controls (paired t-test,  $t=-2.49$ ,  $p<0.05$ ).

## 9.4 Discussion

The experiment described here was undertaken as a test of the hypothesis that changes in the LAI of *Halophila ovalis* are related to changing light conditions, and specifically that a decrease in LAI could result from an exposure to high irradiance. That hypothesis arose out of data documenting a decline in *H. ovalis* LAI at Shelly Beach after the loss of its epiphytic cover, apparently due to the feeding activity of sea hares. It was initially anticipated that a decline in epiphytic cover would benefit the production of seagrass. Algal growth on leaves blocks light for photosynthesis, and effectively competes with seagrass for light (Sand-Jensen 1977, Bulthuis and Woelkerling 1983). That scenario is best illustrated where pollution increases nutrient levels, stimulates algal growth, and results in a decline of seagrasses (Cambridge et al. 1986, Silberstein et al. 1986, Short and Burdick 1996). The grazing of epiphytes by invertebrates has been shown to significantly reduce the negative impacts of algal growth on seagrasses in a number of experimental studies (Howard 1982, Hootsmans and Vermaat 1985, Neckles et al. 1993, Williams and Ruckelshaus 1993, Philippart 1995, Fong et al. 2000). Therefore, a decline in the standing crop of *H. ovalis* associated with the grazing activity of sea hares was unexpected.

The incursion of sea hares into the meadow at Shelly Beach in June 2002 was associated with a loss in epiphytic cover on *H. ovalis*, and a decline in its LAI with a loss of both leaf density and size, but no apparent decline in BGDW (chapter 3).

Similar changes were not found in *H. uninervis*. However, similar changes in *H. ovalis* had been seen in an intertidal meadow in Thailand, where a decline of LAI with a loss in leaf density and size occurred at a time of changing monsoons and increasing water transparency (unpublished data). In Philippines, Duarte et al. (2000) found that *H. ovalis* shoot size was reduced 30% when larger species were removed from experimental plots. The best explanation for those observations is that they result from a sudden exposure to high levels of irradiance. Increased irradiance exposure may occur due to reduced epiphytic cover, increased water transparency, or reduced canopy shading. The plants may suffer direct damage to leaf tissues, i.e. photodamage, or a reduction in rates of photosynthesis at high irradiance, i.e. photoinhibition, that may also be a consequence of photodamage. Those effects may be a consequence of either ultraviolet (UV) radiation or photosynthetically active radiation (PAR). Seagrasses growing in shallow tropical and subtropical waters are often exposed to intense irradiance and may be limited in productivity, growth and distribution by photoinhibition and photodamage. Peralta et al. (2002) found that *Zostera noltii* Hornem in Spain grew at slower rates when exposed to 100% of surface irradiance than did shaded plants exposed to 42%, suggesting photoinhibition.

*Halophila* spp. may be especially vulnerable to high irradiance. Trocine et al. (1981) studied the effects of UV-B radiation on three species of Florida seagrasses. They found that *Halophila engelmanni* Aschers was the most sensitive species, and *Halodule wrightii* Aschers the least. The thin epidermis of the former made it particularly vulnerable to damage, and there was no evidence that it had photorepair mechanisms. They showed that there was less photoinhibition in leaf tissue covered with epiphytic growth, and suggested that *H. engelmanni* was dependent on epiphytes and detritus to shield it from high UV-B radiation levels in intertidal habitats. Brandt and Koch (2003) used transparent artificial seagrass leaves to show that epiphytic cover was an effective UV-B filter, and blocked significantly more damaging UV-B radiation than useful PAR. *Halophila decipiens* has been shown to suffer photoinhibition at lower levels of irradiance, and have fewer UV-absorbing pigments than other *Halophila* spp. (Dawes et al. 1989, Durako et al. 2003), which might explain its more restricted distribution to deeper waters.

Ralph and Burchett (1995) found a significant inhibitory response to increased light intensity in leaf tissue of *Halophila ovalis* subjected to short-term exposure. Using plants cultured at  $100 \mu\text{mol m}^{-2}\text{s}^{-1}$ , there was evidence of photoinhibition at or above irradiances of  $500 \mu\text{mol m}^{-2}\text{s}^{-1}$ . At the highest irradiances used,  $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$  for 120 minutes, the tissue suffered from photodamage and was “virtually photosynthetically inactivated”. Under field conditions in the intertidal zone, plants can experience light intensities  $>1500 \mu\text{mol m}^{-2}\text{s}^{-1}$  for several hours during spring tides (Ralph 1999b).

*Halophila ovalis* and *Halodule uninervis* are likely to be more limited by UV radiation than by PAR. Dawson and Dennison (1996) investigated the responses to increased UV radiation and PAR among five species of seagrass from Moreton Bay, Australia. All species showed some sensitivity to increased UV, but *H. ovalis* and *H. uninervis* had the most significant responses. They showed the greatest decreases in chlorophyll concentrations and fluorescence, but the least increase in UV-blocking pigments. Those differences were significant whether comparing responses to increased UV levels over ambient levels, or comparing ambient levels to treatments screened to reduce UV. In contrast, there were no significant differences in either species to treatments of 100% and 50% of ambient PAR.

Changes in chlorophyll concentration are closely related to changes in irradiance. Leaf concentrations of chlorophyll *a* and *b* decrease with increases in light intensity, as an adaptive response that improves photosynthetic efficiency. Such changes are commonly seen in seagrasses sampled across a range of depths, and as a response to experimental shading (Wiginton and McMillan 1979, Dennison and Alberte 1982, 1985, Tomasko and Dawes 1990, Lee and Dunton 1997, Longstaff and Dennison 1999, Moore and Wetzel 2000, Major and Dunton 2002). Those studies usually find that the ratio chlorophyll *a:b* decreases with decreasing light intensity, but that has not always been the case (e.g. Major and Dunton 2002). Total chlorophyll concentrations decrease, and chlorophyll *a:b* typically increases, with increasing light intensity and photoinhibition (Ralph 1999a). Over the short-term, that may be a result of photodamage and the photo-oxidation of chlorophyll. Over the long-term, leaves are

produced with lower chlorophyll concentrations that are better adapted to high light conditions.

Changes in chlorophyll concentrations measured during the experiment described here were consistent with expectations. In both *Halophila ovalis* and *Halodule uninervis*, chlorophyll concentrations were raised significantly by the shading treatment, and fell to levels similar to controls within 4-10 days of the removal of the shades. Ratios of chlorophyll *a:b* were significantly lower for both species in shaded treatment plots.

Changes in leaf size or density were expected to occur in response to changes in irradiance. The decline in *Halophila ovalis* LAI after the incursion of sea hares was due to a decline in both the number and size of leaves. However, leaf density recovered, but leaf size remained constant over a period of several months with low epiphytic cover. That initial loss of leaves could be interpreted as the loss of photodamaged shade-adapted leaves that were exposed by the sea hares (chapter 3). Subsequently, new leaves were smaller and better adapted to high light conditions that remained constant due to the sustained feeding activity of the sea hares over two months. The expectation in the shading experiment was that exposure of the leaves by removal of the shade cloth would result in similar declines in *H. ovalis* LAI.

Contrary to expectations there was no evidence of a direct change in LAI, leaf or shoot density, BGDW, or the ratio of leaf area to BGDW, in response to either the shade or exposure treatments for either *Halophila ovalis* or *Halodule uninervis*. The only significant direct change due to treatments, other than the change in chlorophyll concentrations, was an increase in the length of *H. uninervis* leaves due to shading. As *H. uninervis* LAI, shoot density, and leaf width did not differ between the shade treatment and controls, there must have been more leaves per shoot, and possibly a higher leaf turnover rate, in control plots. Similarly, Longstaff and Dennison (1999) found that the canopy height of narrow-leaved *H. uninervis* in the Gulf of Carpentaria increased in the early stages of their light deprivation experiment. During two years of sampling in Shelly Beach and Picnic Bay, *H. uninervis* leaf length tended to correlate to both epiphytic cover and tidal exposure (Appendix 1). So, changes in leaf length in

that species appear to be a very consistent indicator of changes in the intensity of light experienced by the plant.

Irrespective of the experimental treatments, there were significant changes in the measurements of both seagrass species during the time of the experiment. LAI, leaf density, and BGDW of *Halophila ovalis* decreased over the first 29 days in both treatment and control plots. At the same time, shoot density and BGDW of *Halodule uninervis* increased, and its LAI increased over both 29-day periods in both control and treatment plots. Those changes were consistent with the changes seen in the larger plot monitored over two years (chapter 5). There, *H. uninervis* LAI reached a minimum in early August 2003, and then increased steadily until December; while *H. ovalis* LAI reached a minimum at the same time but did not significantly increase thereafter. That decline, and failure to recover, appeared to be related to high levels of invertebrate herbivory, especially by crustaceans (chapter 7). Damage attributed to both gastropods and crustaceans was at peak levels in September in the large monitored plot. High levels of damage were also found in the experimental plots at that time, and the decline in *H. ovalis* LAI in those plots also appears to be related to invertebrate herbivory, especially by crustaceans. It is possible that the patches of *H. ovalis* chosen for placement of the experimental plots were remnants that had escaped herbivores, but succumbed during the course of the experiment.

There was evidence that the shades affected activities of the gastropod herbivore *Smaragdia souverbiana*, but not crustacean herbivores. Gastropod damage to *Halophila ovalis* leaves was greater in control plots than treatment plots at the time the shades were removed. Other damage to *H. ovalis* leaves, most likely due to crustacean feeding, was much greater than gastropod damage, but did not differ between treatment and control plots.

Invertebrate herbivory was the most conspicuous source of change in *Halophila ovalis* LAI during the shade treatment, and likely to obscure treatment effects. The impact of herbivores could be affected by other factors, such as chlorophyll concentrations, which likely change C/N ratios, and affect the palatability of seagrasses (Valentine and Heck 2001). Experimental shading and changes in light intensity have been shown to change

C/N ratios (Ibarra-Obando et al. 2004) and palatability (Li et al. 2005). However, there was no evidence in the data that differences in the measurements of leaf damage, attributable to either gastropods or crustaceans, were correlated to differences in chlorophyll concentrations.

Changes in *Halophila ovalis* LAI over the course of the treatments were related to differences in chlorophyll concentrations, despite the absence of a direct relationship between LAI values and treatments. During shade treatment, chlorophyll concentrations increased in shaded plots relative to controls, but the differences between plot pairs varied greatly, likely due to unmeasured differences in epiphytic cover among controls in the weeks preceding the removal of the shades. The plot pair with the least difference in chlorophyll concentrations had the greatest increase in LAI in the shaded treatment plot compared to its control. At the other extreme, the plot pair with the greatest difference in chlorophyll concentrations had a decrease in LAI in the shaded treatment plot. It appears that a trade-off had occurred between chlorophyll production and leaf production. In order to increase light harvesting potential, *H. ovalis* may either increase the concentration of chlorophyll in its leaves, or increase the number of leaves, but one comes at the expense of the other. Although there appeared to be no relationship between measures of herbivore damage and chlorophyll concentrations, the possibility exists that the apparent relationship between the number of leaves and chlorophyll concentrations could have appeared because of the selective removal by herbivores of leaves with high chlorophyll concentrations. The measures of damage are based on sampled leaves, and do not take into account leaves that may have been completely removed by herbivores.

*Halophila ovalis* and *Halodule uninervis* differed in their response to shading. Both species increased leaf chlorophyll concentrations, but *H. ovalis* did not significantly increase total chlorophyll production, which was reflected in the relationship between the number of leaves and the concentration of chlorophyll. *H. ovalis* does not appear to have as many resources to respond to sudden changes in light conditions. The below ground biomass of seagrasses includes a store of nutrients which can buffer the plant against environmental stresses. However, *H. ovalis* has a small belowground biomass compared to its leaf surface area, which explains its inability to rapidly produce a net

increase in chlorophyll when subjected to shading. Prior to shading, *H. ovalis* in the 18 plots averaged five times more leaf area per weight of belowground material as *H. uninervis*,  $197.2 \text{ cm}^2 \text{ g}^{-1}$  compared to  $39.7 \text{ cm}^2 \text{ g}^{-1}$ . The differences between the species are consistent with those found during light deprivations experiments (Longstaff and Dennison 1999, Longstaff et al. 1999). *H. ovalis* was found to have low tolerance to light deprivation, and died after 38 days in the dark. In contrast, *H. uninervis* showed no biomass loss before 38 days, and could survive up 100 days in darkness. Over the short-term, *H. ovalis* appears to be more vulnerable than other species to the stresses of either low or high irradiance. That is not to say that it cannot adapt to those conditions over the long-term. *H. ovalis* lives over the greatest range of light conditions of any species, occurring at depths  $>40 \text{ m}$  (Lee Long et al. 1996), and intertidally.

The placement of shades had a significant impact on the development of epiphytic growth on seagrass leaves. When the shades were removed, soft flocculent material was observed on the leaves of *Halophila ovalis* in control plots, less in treatment plots, or on *Halodule uninervis*. That pattern was confirmed by the analysis of epiphytic material on the leaves. It appeared that the shade cloth had reduced the settlement of material from the water column. At that time, there were feeding trails of dugong, *Dugong dugon* (Muller), near the experimental plots. As dugongs feed on the whole seagrass plant, including the roots, they can resuspend large amounts of material, which could account for much of the material on the leaves. That material was more likely to settle on *H. ovalis* than *H. uninervis* because of differences in the shape and orientation of the leaves. *H. uninervis* floats up from the sediment, and was very thin at Shelly Beach (mean breadth approximately  $0.6 \text{ mm}$ ). *H. ovalis* is broad, and often parallel to the sediment. Several other studies that used experimental *in situ* shading have reported that epiphyte biomass is reduced in shaded plots relative to controls (Neverauskas 1988, Fitzpatrick and Kirkman 1995, Ruiz and Romero 2001). That reduction is usually believed to be a direct consequence of reduced light, but may be related to seagrass leaf defoliation, or increased epiphyte grazing (Ibarra-Obando et al. 2004). However, observations during this study suggested that an important contributing factor was the reduction in the settlement of material from the water column.

In the four days following the removal of the shades, there was a conspicuous increase in filamentous blue-green algae on the leaves. That growth was likely stimulated by nutrients available in the material that had settled on the leaves, and the intense light during that period. The removal of the shades and subsequent sampling had been timed to correspond to a period of very low tides. Four days after the removal of the shades, some of the formerly shaded treatment plots still had conspicuously less epiphytic material than the control plots and the surrounding meadow. A limited sample of the epiphytic material on *Halodule uninervis* leaves in four pairs of plots showed that there had been a substantial increase in the amount of material on that species over just four days in both control and treatment plots. The algal cover was unusual, as it was greater at the base of leaves because it was growing up the leaves from a mat. Typical algal growth on *H. uninervis* at Shelly Beach, including the diatom *Mastigloia* or the blue-green alga *Calothrix crustacea*, would be more developed at the distal ends of the leaves.

The placement of shades can also have a significant impact on epiphyte grazers. *Alaba virgata* was the most abundant epiphyte grazer on the leaves at the time of the experiment. Its numbers were reduced in shaded treatment plots compared to controls, possibly in response to differences in food resources. An increase in algal food resources may explain a very rapid rise in its numbers in both treatment and control plots over four days from the removal of the shades. Many of the animals recovered in the sampling at that time were small, suggesting a recent settlement.

After exposure, the relationship between LAI and chlorophyll concentrations in *Halophila ovalis* was consistent with changes hypothesised to occur as a response to exposure. However, the response seen here was more likely related to differences in shading by epiphytic material than to the differences in shading produced experimentally. The shaded treatment plot with the least epiphytic cover relative to its control had the lowest chlorophyll concentrations, smallest LAI, leaf density, and BGDW relative to its control after exposure. Surprisingly, differences in chlorophyll concentrations after exposure were correlated to differences in epiphytic cover 29 days earlier when shades were removed, and not to differences at the end of the exposure period. That relationship was not expected, as all the leaves would have been replaced



within the 29 days of exposure, but it does suggest that the differences in epiphytic cover measured when the shades were removed may have persisted for a period of weeks.

*Halophila ovalis* LAI failed to respond to the exposure treatment, and did not show a decline comparable to that seen after the sea hare incursion. That may reflect the inability to control shading by epiphytic material, the severity of the treatments, and the timing of the sampling. The deposition of material on leaves in control plots before the shades were removed reduced the difference in shading between control and treatment plots. Then the rapid growth of epiphytes after the removal of the shades reduced the potential impact of exposure on shade-adapted plants. When the sea hares had entered the meadow, they removed epiphytic material continuously over a period of two months, resulting in continuous high exposure.

The experimental treatment with 70% shade cloth probably changed light levels by an amount similar to natural changes. Shading increased leaf length in *Halodule uninervis*; but during the exposure period, leaf length in controls increased by an amount similar to that seen as a result of shading. During the exposure period, light levels in control would have been reduced compared to the period of shading because epiphytic cover increased and daytime tidal exposure decreased. When the first shades were put in place August 25, total previous 14-day exposure between 900-1600 h was 27 h. When the first shades were removed September 23, the comparable figure was 23.5 h. After 29 days of exposure, the figure was 17.2 h.

The timing of the sampling at 29 days after exposure may have missed the major impact on the leaves. The difference in the sampling dates that had covered the incursion of the sea hares had been 28 days. However, the sea hares entered the plot in large numbers three days after the initial sampling, and the average leaf may not have experienced exposure for several more days. The first sampling after the entry of the sea hares showed the greatest decline in LAI, and that sampling could have been from the generation of leaves that had directly experienced exposure and suffered photodamage and accelerated senescence. Subsequent sampling showed a recovery in leaf density, but the average leaf size remained small, reflecting adaptation to the

sustained change in light conditions. So by waiting 29 days to sample after the experimental exposure, a new generation of leaves were produced which had the opportunity to recover from the effects of the exposure. In the experimental shading, the plots that were most affected by exposure were those that may not have been able to recover because they had also lost below ground biomass.

The use of shade cloth in this experiment was intended to reduce light intensity to the seagrass in a manner similar to that experienced under a cover of epiphytic material. However, seagrass may not respond to those sources of shading in the same way, because of differences in the spectral composition of the light. Brandt and Koch (2003) showed that periphyton on seagrass leaves is an effective UV-B filter, as it allows the transmission of proportionately less UV-B radiation compared to PAR. Shade cloth would not change the composition of the light, and may not have been as effective at protecting the seagrass, especially because the amount of epiphytic material on the leaves was reduced in shaded plots relative to non-shaded plots. Tomasko (1992) found that the morphology of *Halodule wrightii* differed between treatments where light intensity was reduced by passing through a seagrass canopy, and treatments where light intensity was reduced by neutral density screens. The difference appeared to be due to changes in the spectral composition of light passing through seagrass leaves that allowed the plant to respond differently to shade caused by overgrowth by a competitor compared to shading caused by an inanimate object. Tomasko (1992) concluded that some morphological differences in *H. wrightii* were more strongly related to differences in the spectral composition of light than to differences in its intensity.

## 9.5 Conclusions

Chlorophyll concentrations changed in response to shading with 70% shade cloth and subsequent exposure to ambient light. Concentrations increased under shading, and rapidly decreased to control levels in 4-10 days after exposure in both *Halophila ovalis* and *Halodule uninervis*. Contrary to expectations, no significant direct effects of exposure were seen on leaf area or below ground biomass of either species. The only significant direct response was an increase in leaf length of *H. uninervis* due to shading.

However, significant interactions were found between changes in *H. ovalis* LAI and leaf density, and differences in chlorophyll concentrations. After shading, the difference in chlorophyll between shaded treatment and control plots was inversely related to changes in LAI and leaf density, suggesting a trade-off between leaf production and chlorophyll production in response to shading. After exposure, treatment plots with the greatest decrease in chlorophyll concentrations had the greatest decrease in LAI, leaf density, and below ground biomass. Differences in chlorophyll concentrations between control and treatment plots after exposure were correlated with differences in epiphytic cover measured the day shades were removed.

Experimental shading had significant impacts on the development of epiphytic loads, leading to unexpected differences between plots in the realized effective shading of the seagrass. Epiphytic loads were reduced in shaded plots because the shade cloth reduced the settlement of resuspended material. Shading also resulted in changes in algal grazer populations and differences in damage to seagrass by gastropod herbivores. Irrespective of treatments, LAI, leaf density, and belowground biomass of *Halophila ovalis* declined during the experiment, which took place during a period of high levels of damage by invertebrate herbivores, especially crustaceans, that were the most likely cause of the decline.

## Chapter 10 General conclusions

### 10.1 New perspectives

The prevailing model of seagrass-epiphyte-grazer interactions does not provide an adequate explanation for the observed changes in the abundances of invertebrates, epiphytes and seagrass in intertidal meadows at Townsville. Many of the underlying assumptions of the model are not supported by this study. At best, some elements of the model find qualified support, but other factors not anticipated by the model appear to dominate interactions among invertebrates, seagrass and epiphytes.

The basic assumptions of the prevailing model, as outlined in the introduction, were that:

- 1) Invertebrate grazers limit the amount of epiphytic material on seagrass leaves.
  - 2) Epiphytic loads limit the standing crop of seagrass.
  - 3) Invertebrate grazers indirectly benefit the standing crop of seagrass as a result of their effects on epiphytic loads.
  - 4) Invertebrate grazers do not directly affect seagrass standing crop.
- 
- 1) Invertebrate grazers did appear to limit the amount of epiphytic material on seagrass leaves in some circumstances. The sea hare *Bursatella leachii* appeared to have a major impact on the epiphytic loads of *Halophila ovalis* over a period of approximately two months in one of the plots. The small gastropod *Alaba virgata* probably also limited epiphytic loads on *Halodule uninervis* at several times during the study in the same plot. However, it is not possible to assert that epiphytic loads were generally being regulated by epiphyte grazers. In two of the plots, epiphytic cover appeared to escape any control by grazers; and in the third, grazers were not especially abundant. Contrary to expectations, the epifaunal community was dominated by suspension feeders, rather than epiphyte grazers.

- 2) Epiphytic loads did appear to limit the standing crop of *Halodule uninervis* in two of the three plots studied (chapter 5). Seagrass standing crop declined when the loads of epiphytic material reached extreme high values; but until those levels were reached, there was evidence of a positive relationship between epiphytic cover and standing crop. That relationship suggested that epiphytic cover was of benefit to the seagrass over a more moderate range of cover. One cannot assume that epiphytic cover always has a negative impact or is generally limiting for seagrass production, although there must always be some extreme level of cover at which basic light requirements of the plant cannot be met.
- 3) Invertebrate grazers did not benefit seagrass standing crop through their effects on epiphytic loads. On the contrary, when epiphyte grazing was greatest, in the cases of both *Bursatella leachii* and *Alaba virgata*, the effects on the seagrass were negative. The most likely explanation was that epiphytic cover has positive effects through shading of the seagrass from the harmful effects of the high irradiances.
- 4) Invertebrate herbivores directly consumed seagrass leaves and appeared to reduce the standing crop of *Halophila ovalis*.

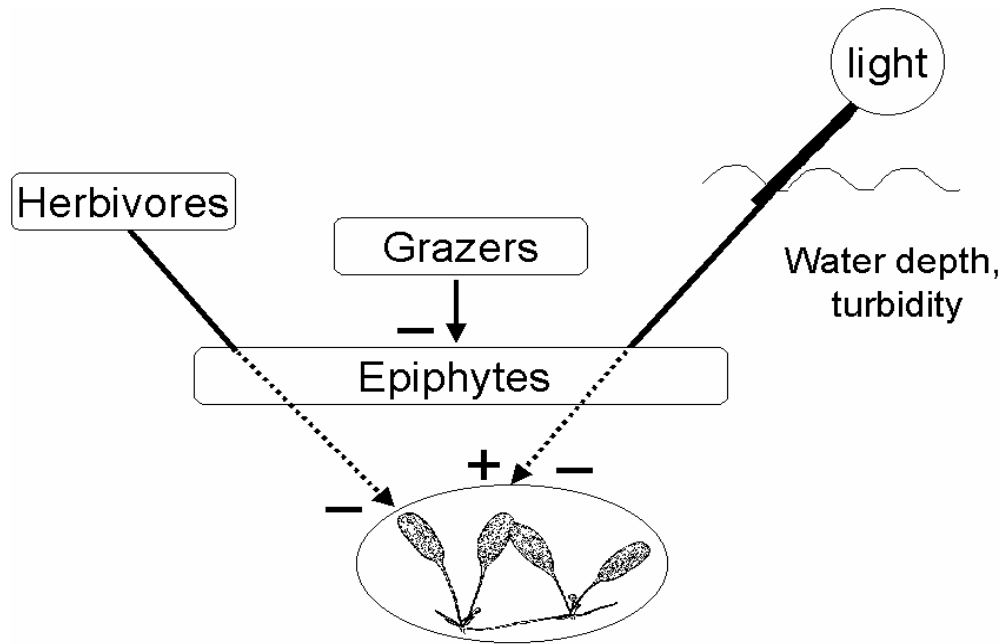
The epifaunal community of *Halodule uninervis* at Townsville differed fundamentally from the communities assumed to be generally characteristic of seagrass meadows. It has been assumed that the epifaunal communities are primarily composed of epiphyte grazers (van Montfrans et al. 1984, Jernakoff et al. 1996, Valentine and Duffy 2006); but unexpectedly, suspension feeders dominated in this study. Therefore, the relationships among the epifaunal animals, the seagrass, and its epiphytic cover, differed from expectations. Where epiphyte grazers dominate, one would expect epiphytes to be the major food resource for the community, and the surface area of the seagrass would be an indicator of the potential size of that resource. For suspension feeders, the food resource is carried by currents from outside the habitat, and the seagrass can be either an opportunity to exploit those currents, or an impediment to them. Feeding modes may tend to differ between tropical and temperate meadows. Resuspended sediments are possibly the major source of nutrients in tropical intertidal

habitats, and suspension feeding may be an especially profitable method for exploiting that resource. In contrast, epiphytic cover may be limited in tropical waters, in the absence of resuspended sediments, because of generally low concentrations of dissolved nutrients.

Consumption of seagrass by herbivores has been thought to account for a small proportion of production (Klumpp et al. 1989, Cebrián and Duarte 1998), or to be mainly due to vertebrates and urchins (Valentine and Heck 1999, Valentine and Duffy 2006). However, in this study, there was substantial evidence of severe invertebrate herbivory, particularly of *Halophila ovalis*. Although, herbivores may be only a small part of the total invertebrate community, they have the potential for a major impact on the structure and successional development of mixed-species tropical meadows.

A basic assumption of any seagrass study is that reduced light, for whatever reason, is limiting for seagrass production. However, tropical intertidal habitats are often saturated with light, and high irradiance is potentially a limiting factor because of photoinhibition and photodamage. As a consequence, seagrass production is likely to be greatest at intermediate light levels. That also implies that moderate levels of epiphytic cover can benefit the seagrass by protecting it from high levels of irradiance. Both *Halophila ovalis* and *Halodule uninervis* had reduced standing crop at times of high irradiance that combined low epiphytic cover and high tidal exposure.

More complete models of seagrass-epiphyte-grazer interactions can now be proposed for tropical intertidal meadows. In the case of *Halophila ovalis* (Fig. 10.1), herbivores are proposed to have a major negative impact. That impact may be mitigated by the presence of epiphytes, as suggested by negative correlations found between herbivore damage and epiphytic cover. Light may have either a negative or positive impact depending on the intensity reaching the leaves. Consequently, epiphytic cover can function as either a competitor with seagrass for light, or as a protector from high irradiance and herbivores.



**Fig. 10.1** A modified model of interactions among invertebrates, epiphytes, and the standing crop of *Halophila ovalis*. Herbivores have a negative impact that can be modified by epiphytes. Light may have either a negative or positive impact, and the amount of light reaching the leaves is reduced by epiphytes.

The relationships among *Halodule uninervis*, its epiphytic cover, and invertebrate epifauna differ from those of *Halophila ovalis* (Fig. 10.2). *H. uninervis* develops a canopy that modifies its environment, and that of its epibiota. Increases in *H. uninervis* leaf area had a positive impact on epiphytic loads, but a negative impact on its epifauna. Epiphytic cover modifies the light received by the leaves and may have either a positive or negative impact. Therefore, reciprocal interactions are possible between the seagrass and its epiphytic cover. Similarly, there may be reciprocal interactions with its epifauna. Most epifaunal animals were suspension feeders, and were more abundant when the canopy was reduced, but that greater abundance could further reduce light, and have a further negative impact on the seagrass, as seen in the case of anemones.

This study has a number of unique features. For the first time, invertebrates and epiphytes are studied from the small seagrass species that are ubiquitous across the tropical Indo-Pacific region. Nothing has been previously reported about the associated invertebrate fauna of *H. ovalis* or *Halodule uninervis*. They are the favoured foods of

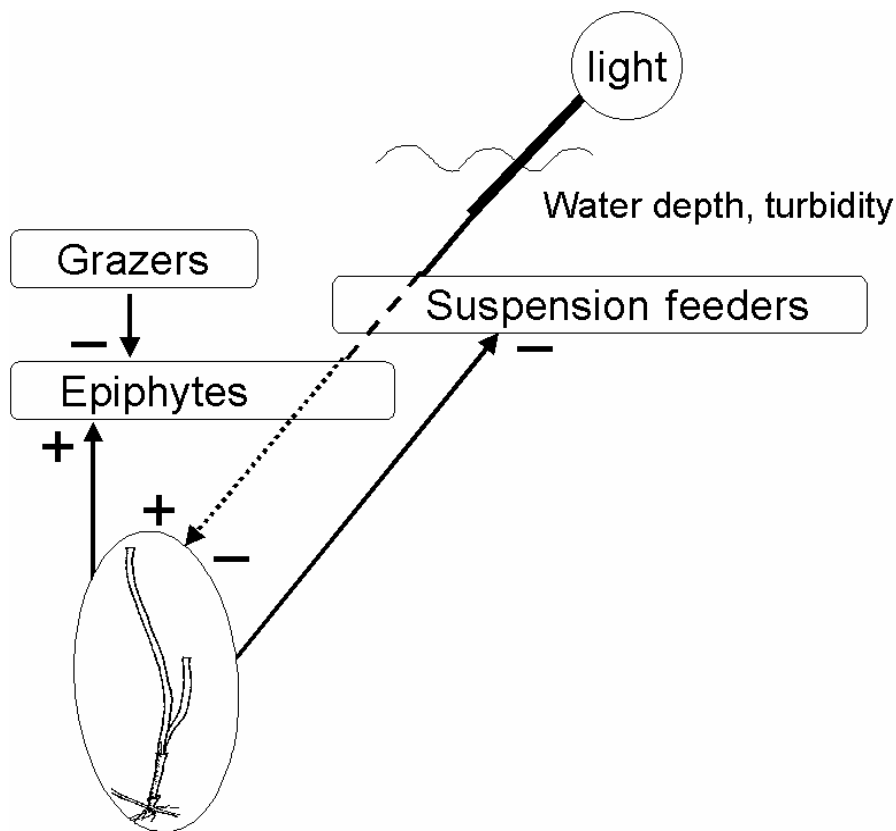
dugongs. Yet, there has been no evidence published of any animal feeding on them other than the dugong. This study identifies invertebrate herbivores, and measures their damage, and demonstrates their potential importance for the first time. Novel techniques have been developed to sample epifauna and measure epiphytic cover. Techniques are described for obtaining accurate estimates of the areas of leaves, and of herbivore damage, using easily available scanner technology. Most importantly, this study is almost unique in collecting and analyzing field data with respect to seagrass-epiphyte-invertebrate interactions. There has been a surprising lack of field data collected on invertebrates in seagrass meadows, with most studies on their interactions relying solely on experimental techniques. Experiments alone can not adequately demonstrate the operation of the hypothesized interactions involving epiphytic cover, although they have given rise to some important information. Path analysis provides a way of inferring relationships among variables where experimental techniques are not possible. Unlike any other study, this study has been able to test path models of interactions in seagrass meadows. The technique is especially useful in the study of trophic relationships because it allows one to make distinctions between direct and indirect effects, and between bottom-up and top-down effects. Moreover, the technique allows one to develop new models of interactions that can be tested with further research.

## **10.2 Future directions**

The alternative models of seagrass-epiphyte-grazer interactions proposed here need to be tested using a variety of techniques. The components of the models should be manipulated experimentally to demonstrate interactions that cannot be disentangled from field data alone. However, experiments with field enclosures and mesocosms have limitations in demonstrating relationships in open systems such as intertidal seagrass meadows. In particular, the hypothesis that the abundances of epiphytes and suspension-feeding invertebrates are related to water flow dynamics through the seagrass canopy needs to be tested with very different techniques. Manipulations of water flows using baffles or flumes in the manner of Schanz et al. (2002) appear to be a promising approach.



Mesocosm experiments strongly support the prevailing model of seagrass-epiphyte-grazer interactions, but it is not well supported by field data. The results of experiments should at least be consistent with patterns seen in the field, even though field data may not be adequate for interpreting causal mechanisms. However, there seem to have been few attempts to validate the prevailing model against field data. Of necessity, the analysis presented here is based on a small number of plots, and more broadly based data still needs to be collected to determine how widespread are the patterns identified from the intensive within plot approach taken in this study.



**Fig. 10.2 A modified model of interactions among invertebrates, epiphytes, and the standing crop of *Halodule uninervis*. The seagrass has a positive effect on epiphytes, but a negative impact on suspension feeders, both of which may reduce light reaching the leaves.**

The interpretation of field data is impeded by the lack of basic information on the life histories and feeding behaviours of the animals being sampled. This seems to be a greater problem in tropical areas, where even the taxonomy of some common animals can be in doubt. Some very basic studies need to be done to show which animals feed directly on the seagrass, and which are epiphyte grazers, or suspension feeders, or use a

combination of feeding strategies. The hypothesis that invertebrate herbivory is a factor in the succession of meadows, assumes that herbivores prefer to feed on early successional *Halophila ovalis* over late successional species, and that herbivores are more abundant in late successional meadows. Those assumptions need to be tested with feeding trials and abundance data, respectively.

More research is needed to understand the impacts of high irradiance on the distribution and abundance of seagrasses in shallow tropical waters. Trocine et al. (1981) demonstrated the damaging effects of UV radiation, and the likely mitigating effects of epiphytic cover, but those possibilities has received very little attention. In contrast, the effects of low irradiance have received considerable attention, and the techniques used to study them should be adapted to study high irradiance effects. The sources of the nutrients that control epiphytic growth are not well understood. During this study, it appeared that resuspended material was an important factor determining epiphytic loads, but work is needed to understand the source of the material, its abundance, and effects on the seagrass and its epifauna. It was clear in this study that epiphytic loads could be great enough to limit seagrass production, but it is not clear if that is a widespread phenomenon on the Queensland coast.

## References

- Abal, E. G. Z., N. Loneragan, P. Bowen, C. J. Perry, J. W. Udy, and W. C. Dennison. 1994. Physiological and morphological responses of the seagrass *Zostera capricorni* Aschers. to light intensity. *Journal of Experimental Marine Biology and Ecology* **178**:113-129.
- Agawin, N. S. R. and C. M. Duarte. 2002. Evidence of direct particle trapping by a tropical seagrass meadow. *Estuaries* **25**:1205-1209.
- Alcoverro, T. and S. Mariani. 2002. Effects of sea urchin grazing on seagrass (*Thalassodendron ciliatum*) beds of a Kenyan lagoon. *Marine Ecology Progress Series* **226**:255-263.
- Allen, B. J. and S. L. Williams. 2003. Native eelgrass *Zostera marina* controls growth and reproduction of an invasive mussel through food limitation. *Marine Ecology Progress Series* **254**:57-67.
- Alongi, D. M. and A. D. McKinnon. 2005. The cycling and fate of terrestrially-derived sediments and nutrients in the coastal zone of the Great Barrier Reef shelf. *Marine Pollution Bulletin* **51**:239-252.
- Ambrose, W. G. and E. A. Irlandi. 1992. Height of attachment on seagrass leads to trade-off between growth and survival in the bay scallop *Argopecten irradians*. *Marine Ecology Progress Series* **90**:45-51.
- American Public Health Association, American Water Works Association, and Water Environment Federation (U.S.). 1995. Standard methods for the examination of water and wastewater. 19th ed. edition. American Public Health Association, Washington, D.C.
- Ansari, Z. A., C. U. Rivonker, P. Ramani, and A. H. Parulekar. 1991. Seagrass habitat complexity and macroinvertebrate abundance in Lakshadweep coral reef lagoons, Arabian Sea. *Coral Reefs* **10**:127-131.
- Aragones, L. V. 1996. Dugongs and green turtles: grazers in the tropical seagrass ecosystem. PhD. James Cook University, Townsville.
- Attrill, M. J., J. A. Strong, and A. A. Rowden. 2000. Are macroinvertebrate communities influenced by seagrass structural complexity? *Ecography* **23**:114-121.
- Barbour, M. G. and S. R. Radosevich. 1979. <sup>14</sup>C uptake by the marine angiosperm *Phyllospadix scouleri*. *American Journal of Botany* **66**:301-306.
- Barnard, J. L., J. D. Thomas, and K. B. Sandved. 1988. Behavior of gammaridean Amphipoda: *Corophium*, *Grandidierella*, *Podocerus*, and *Gibberosus* (American *Megaluropus*) in Florida. *Crustaceana Supplement* **13**:234-244.

- Behringer, D. C. and M. J. Butler. 2006. Stable isotope analysis of production and trophic relationships in a tropical marine hard-bottom community. *Oecologia* **148**:334-341.
- Birch, W. R. and M. Birch. 1984. Succession and pattern of tropical intertidal seagrasses in Cockle Bay, Queensland, Australia: a decade of observations. *Aquatic Botany* **19**:343-367.
- Björk, M., J. Uku, A. Weil, and S. Beer. 1999. Photosynthetic tolerances to desiccation of tropical intertidal seagrasses. *Marine Ecology Progress Series* **191**:121-126.
- Borowitzka, M. A., P. S. Lavery, and M. van Keulen. 2006. Epiphytes on seagrasses. Pages 441-461 in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, The Netherlands.
- Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina*) along a nutrient gradient in a Danish estuary. *Marine Biology* **87**:211-218.
- Borum, J. 1987. Dynamics of epiphyton on eelgrass (*Zostera marina* L.) leaves: relative roles of algal growth, herbivory, and substratum turnover. *Limnology and Oceanography* **32**:986-992.
- Boström, C. and J. Mattila. 1999. The relative importance of food and shelter for seagrass-associated invertebrates: a latitudinal comparison of habitat choice by isopod grazers. *Oecologia* **120**:162-170.
- Bradshaw, C., P. Collins, and A. R. Brand. 2003. To what extent does upright sessile epifauna affect benthic biodiversity and community composition? *Marine Biology* **143**:783-791.
- Brandt, L. A. and E. W. Koch. 2003. Periphyton as a UV-B filter on seagrass leaves: a result of different transmittance in the UV-B and PAR ranges. *Aquatic Botany* **76**:317-327.
- Brawley, S. H. 1992. Mesoherbivores. Pages 235-263 in D. M. John, S. J. Hawkins, and J. H. Price, editors. *Plant-animal interactions in the marine benthos*. Clarendon Press, Oxford.
- Bray, J. R. and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* **27**:326-349.
- Brearley, A. and D. I. Walker. 1995. Isopod miners in the leaves of two Western Australian *Posidonia* species. *Aquatic Botany* **52**:163-181.
- Bulthuis, D. A. 1983. Effects of *in situ* light reduction on density and growth of the seagrass *Heterozostera tasmanica* in Western Port, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology* **67**:91-103.

- Bulthuis, D. A. and W. J. Woelkerling. 1983. Biomass accumulation and shading effects of epiphytes on leaves of the seagrass, *Heterozostera tasmanica*, in Victoria, Australia. *Aquatic Botany* **16**:137-148.
- Caine, E. A. 1980. Ecology of two littoral species of caprellid amphipods (Crustacea) from Washington, USA. *Marine Biology* **56**:327-335.
- Caine, E. A. 1983. Community interactions of *Caprella penantis* Leach (Crustacea: Amphipoda) on sea-whips. *Journal of Crustacean Biology* **3**:497-504.
- Caine, E. A. 1987. Potential effect of floating dock communities on a South Carolina estuary. *Journal of Experimental Marine Biology and Ecology* **108**:83-92.
- Cambridge, M. L., A. W. Chiffings, C. Brittan, L. Moore, and A. J. McComb. 1986. The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. *Aquatic Botany* **24**:269-285.
- Carlton, J. T., G. J. Vermeij, D. R. Lindberg, D. A. Carlton, and E. C. Dudley. 1991. The first historical extinction of a marine invertebrate in an ocean basin: the demise of eelgrass limpet *Lottia alveus*. *Biological Bulletin* **180**:72-80.
- Carruthers, T. J. B., W. C. Dennison, B. J. Longstaff, M. Waycott, E. G. Abal, L. J. McKenzie, and W. J. Lee Long. 2002. Seagrass habitats of northeast Australia: models of key processes and controls. *Bulletin of Marine Science* **71**:1153-1169.
- Cebrián, J. and C. M. Duarte. 1998. Patterns in leaf herbivory on seagrasses. *Aquatic Botany* **60**:67-82.
- Cebrián, J., C. M. Duarte, and N. Marbà. 1996a. Herbivory on the seagrass *Cymodocea nodosa* (Ucria) Ascherson in contrasting Spanish Mediterranean habitats. *Journal of Experimental Marine Biology and Ecology* **204**:103-111.
- Cebrián, J., C. M. Duarte, N. Marbà, S. Enríquez, M. Gallegos, and B. Olesen. 1996b. Herbivory on *Posidonia oceanica*: magnitude and variability in the Spanish Mediterranean. *Marine Ecology Progress Series* **130**:147-155.
- Chaparro, O. R., R. J. Thompson, and S. V. Pereda. 2002. Feeding mechanisms in the gastropod *Crepidula fecunda*. *Marine Ecology Progress Series* **234**:171-181.
- Clarke, C. L. 2004. The ecological role of sea hares (Opisthobranchia: Anaspidea) within tropical intertidal habitats. M.Sc. James Cook University, Townsville, Australia.
- Clarke, K. R. 1993. Non-parametric multivariate analysis of changes in community structure. *Australian Journal of Ecology* **18**:117-143.
- Clarke, K. R. and R. N. Gorley. 2006. Primer v6: user manual / tutorial. PRIMER-E, Plymouth.

- Collie, J. S. 1985. Life history and production of three amphipod species on Georges Bank. *Marine Ecology Progress Series* **22**:229-238.
- Collie, J. S. 1987. Food selection by yellowtail flounder (*Limanda ferruginea*) on Georges Bank. *Canadian Journal of Fisheries and Aquatic Sciences* **44**:357-367.
- Connolly, R. M. 1994a. A comparison of fish assemblages from seagrass and unvegetated areas of a southern Australian estuary. *Australian Journal of Marine and Freshwater Research* **45**:1033-1044.
- Connolly, R. M. 1994b. The role of seagrass as preferred habitat for juvenile *Sillaginodes punctata* (Cuv. & Val.) (Sillaginidae, Pisces): habitat selection or feeding? *Journal of Experimental Marine Biology and Ecology* **180**:39-47.
- Connolly, R. M. 1997. Differences in composition of small, motile invertebrate assemblages from seagrass and unvegetated habitats in a southern Australian estuary. *Hydrobiologia* **346**:137-148.
- Connor, V. M. and J. F. Quinn. 1984. Stimulation of food species growth by limpet mucus. *Science* **225**:843-844.
- Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton, and M. van den Belt. 1997. The value of the world's ecosystem services and natural capital. *Nature* **387**:253-260.
- Czerny, A. B. and K. H. Dunton. 1995. The effects of *in situ* light reduction on the growth of two subtropical seagrasses, *Thalassia testudinum* and *Halodule wrightii*. *Estuaries* **18**:418-427.
- Davies, M. S. and P. Beckwith. 1999. Role of mucus trails and trail-following in the behaviour and nutrition of the periwinkle *Littorina littorea*. *Marine Ecology Progress Series* **179**:247-257.
- Dawes, C. J., C. S. Lobban, and D. A. Tomasko. 1989. A comparison of the physiological ecology of the seagrasses *Halophila decipiens* Ostenfeld and *H. johnsonii* Eiseman from Florida. *Aquatic Botany* **33**:149-154.
- Dawson, S. P. and W. C. Dennison. 1996. Effects of ultraviolet and photosynthetically active radiation on five seagrass species. *Marine Biology* **125**:629-638.
- De'ath, G. and K. E. Fabricius. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* **81**:3178-3192.
- De Iongh, H. H., B. J. Wenno, and E. Meelis. 1995. Seagrass distribution and seasonal biomass changes in relation to dugong grazing in the Moluccas, East Indonesia. *Aquatic Botany* **50**:1-19.
- den Hartog, C. 1970. The sea-grasses of the world. North-Holland Publishing Company, Amsterdam.

- Dennison, W. C. and R. S. Alberte. 1982. Photosynthetic responses of *Zostera marina* L. (eelgrass) to *in situ* manipulations of light intensity. *Oecologia* **55**:137-144.
- Dennison, W. C. and R. S. Alberte. 1985. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). *Marine Ecology Progress Series* **25**:51-61.
- Dennison, W. C., R. J. Orth, K. A. Moore, J. C. Stevenson, V. Carter, S. Kollar, P. W. Bergstrom, and R. A. Batiuk. 1993. Assessing water quality with submersed aquatic vegetation. *BioScience* **43**:86-94.
- Dethier, M. N. 1994. The ecology of intertidal algal crusts: variation within a functional group. *Journal of Experimental Marine Biology and Ecology* **177**:37-71.
- Dixon, I. M. T. and P. G. Moore. 1997. A comparative study on the tubes and feeding behaviour of eight species of corophioid Amphipoda and their bearing on phylogenetic relationships within the Corophioidea. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **352**:93-112.
- Drew, E. A. 1979. Physiological aspects of primary production in seagrasses. *Aquatic Botany* **7**:139-150.
- Duarte, C. M. 1991a. Allometric scaling of seagrass form and productivity. *Marine Ecology Progress Series* **77**:289-300.
- Duarte, C. M. 1991b. Seagrass depth limits. *Aquatic Botany* **40**:363-377.
- Duarte, C. M. and C. L. Chiscano. 1999. Seagrass biomass and production: a reassessment. *Aquatic Botany* **65**:159-174.
- Duarte, C. M., J. Terrados, N. S. R. Agawin, and M. D. Fortes. 2000. An experimental test of the occurrence of competitive interactions among SE Asian seagrasses. *Marine Ecology Progress Series* **197**:231-240.
- Duffy, J. E. 1990. Amphipods on seaweeds: partners or pests? *Oecologia* **83**:267-276.
- Duffy, J. E. and A. M. Harvilicz. 2001. Species-specific impacts of grazing amphipods in an eelgrass-bed community. *Marine Ecology Progress Series* **223**:201-211.
- Duffy, J. E. and M. E. Hay. 2000. Strong impacts of grazing amphipods on the organization of a benthic community. *Ecological Monographs* **70**:237-263.
- Duffy, J. E., K. S. MacDonald, J. M. Rhode, and J. D. Parker. 2001. Grazer diversity, functional redundancy, and productivity in seagrass beds: an experimental test. *Ecology* **82**:2417-2434.
- Duffy, J. E., J. P. Richardson, and E. A. Canuel. 2003. Grazer diversity effects on ecosystem functioning in seagrass beds. *Ecology Letters* **6**:637-645.

- Dunton, K. H. 1994. Seasonal growth and biomass of the subtropical seagrass *Halodule wrightii* in relation to continuous measurements of underwater irradiance. *Marine Biology* **120**:479-489.
- Durako, M. J., J. I. Kunzelman, W. J. Kenworthy, and K. K. Hammerstrom. 2003. Depth-related variability in the photobiology of two populations of *Halophila johnsonii* and *Halophila decipiens*. *Marine Biology* **142**:1219-1228.
- Edgar, G. J. 1990a. Population regulation, population dynamics and competition amongst mobile epifauna associated with seagrass. *Journal of Experimental Marine Biology and Ecology* **144**:205-234.
- Edgar, G. J. 1990b. Predator-prey interactions in seagrass beds. I. The influence of macrofaunal abundance and size-structure on the diet and growth of the western rock lobster *Panulirus cygnus* George. *Journal of Experimental Marine Biology and Ecology* **139**:1-22.
- Edgar, G. J. 1990c. Predator-prey interactions in seagrass beds. II. Distribution and diet of the blue manna crab *Portunus pelagicus* Linnaeus at Cliff Head, Western Australia. *Journal of Experimental Marine Biology and Ecology* **139**:23-32.
- Edgar, G. J. 1993. Measurement of the carrying capacity of benthic habitats using a metabolic-rate based index. *Oecologia* **95**:115-121.
- Edgar, G. J. and M. Aoki. 1993. Resource limitation and fish predation: their importance to mobile epifauna associated with Japanese Sargassum. *Oecologia* **95**:122-133.
- Edgar, G. J. and A. I. Robertson. 1992. The influence of seagrass structure on the distribution and abundance of mobile epifauna: pattern and process in a western Australian *Amphibolis* bed. *Journal of Experimental Marine Biology and Ecology* **160**:13-31.
- Edgar, G. J. and C. Shaw. 1995a. The production and trophic ecology of shallow-water fish assemblages in southern Australia. I. Species richness, size-structure and production of fishes in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **194**:53-81.
- Edgar, G. J. and C. Shaw. 1995b. The production and trophic ecology of shallow-water fish assemblages in southern Australia. II. Diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **194**:83-106.
- Edgar, G. J., C. Shaw, G. F. Watson, and L. S. Hammond. 1994. Comparisons of species richness, size-structure and production of benthos in vegetated and unvegetated habitats in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **176**:201-226.
- Ejdung, G., L. Byrén, and R. Elmgren. 2000. Benthic predator-prey interactions: evidence that adult *Monoporeia affinis* (Amphipoda) eat postlarval *Macoma*



- balthica* (Bivalvia). Journal of Experimental Marine Biology and Ecology **253**:243-251.
- Ejdung, G. and R. Elmgren. 1998. Predation on newly settled bivalves by deposit-feeding amphipods: a Baltic Sea case study. Marine Ecology Progress Series **168**:87-94.
- Elmgren, R., S. Ankar, B. Marteleur, and G. Ejdung. 1986. Adult interference with postlarvae in soft sediments: the *Pontoporeia-Macoma* example. Ecology **67**:827-836.
- Enríquez, S., M. Merino, and R. Iglesias-Prieto. 2002. Variations in the photosynthetic performance along the leaves of the tropical seagrass *Thalassia testudinum*. Marine Biology **140**:891-900.
- Erfteimeijer, P. L. A. and P. M. J. Herman. 1994. Seasonal changes in environmental variables, biomass, production and nutrient contents in two contrasting tropical intertidal seagrass beds in South Sulawesi, Indonesia. Oecologia **99**:45-59.
- Erfteimeijer, P. L. A. and J. Stapel. 1999. Primary production of deep-water *Halophila ovalis* meadows. Aquatic Botany **65**:71-82.
- Fabricius, K. E. and E. Wolanski. 2000. Rapid smothering of coral reef organisms by muddy marine snow. Estuarine, Coastal and Shelf Science **50**:115-120.
- Fenchel, T., L. H. Kofoed, and A. Lappalainen. 1975. Particle size-selection of two deposit feeders: the amphipod *Corophium volutator* and the prosobranch *Hydrobia ulvae*. Marine Biology **30**:119-128.
- Fishlyn, D. A. and D. W. Phillips. 1980. Chemical camouflaging and behavioral defenses against a predatory seastar by three species of gastropods from the surfgrass *Phyllospadix* community. Biological Bulletin **158**:34-48.
- Fishman, J. R. and R. J. Orth. 1996. Effects of predation on *Zostera marina* L. seed abundance. Journal of Experimental Marine Biology and Ecology **198**:11-26.
- Fitzpatrick, J. and H. Kirkman. 1995. Effects of prolonged shading stress on growth and survival of seagrass *Posidonia australis* in Jervis Bay, New South Wales, Australia. Marine Ecology Progress Series **127**:279-289.
- Fong, C. W., S. Y. Lee, and R. S. S. Wu. 2000. The effects of epiphytic algae and their grazers on the intertidal seagrass *Zostera japonica*. Aquatic Botany **67**:251-261.
- Fourqurean, J. W., G. V. N. Powell, W. J. Kenworthy, and J. C. Zieman. 1995. The effects of long-term manipulation of nutrient supply on competition between the seagrasses *Thalassia testudinum* and *Halodule wrightii* in Florida Bay. Oikos **72**:349-358.
- Frankovich, T. A. and J. W. Fourqurean. 1997. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. Marine Ecology Progress Series **159**:37-50.

- Frankovich, T. A. and J. C. Zieman. 2005. A temporal investigation of grazer dynamics, nutrients, seagrass leaf productivity, and epiphyte standing stock. *Estuaries* **28**:41-52.
- Fréchette, M., C. A. Butman, and W. R. Geyer. 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. *Limnology and Oceanography* **34**:19-36.
- Gacia, E., T. C. Granata, and C. M. Duarte. 1999. An approach to measurement of particle flux and sediment retention within seagrass (*Posidonia oceanica*) meadows. *Aquatic Botany* **65**:255-268.
- Gambi, M. C. 2002. Spatio-temporal distribution and ecological role of polychaete borers of *Posidonia oceanica* (L.) Delile scales. *Bulletin of Marine Science* **71**:1323-1331.
- Gambi, M. C., A. R. M. Nowell, and P. A. Jumars. 1990. Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. *Marine Ecology Progress Series* **61**:159-169.
- Gambi, M. C., B. I. van Tussenbroek, and A. Brearley. 2003. Mesofaunal borers in seagrasses: world-wide occurrence and a new record of boring polychaetes in the Mexican Caribbean. *Aquatic Botany* **76**:65-77.
- Gann, R. G. 1998. Desktop scanners: image quality evaluation. Prentice Hall PTR, Upper Saddle River, N.J.
- Glasbey, C. A. and G. W. Horgan. 1995. Image analysis for the biological sciences. John Wiley & Sons Ltd., Chichester, England.
- Gordon, D. M., K. A. Grey, S. C. Chase, and C. J. Simpson. 1994. Changes to the structure and productivity of a *Posidonia sinuosa* meadow during and after imposed shading. *Aquatic Botany* **47**:265-275.
- Gotceitas, V., S. Fraser, and J. A. Brown. 1997. Use of eelgrass beds (*Zostera marina*) by juvenile Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **54**:1306-1319.
- Granger, S. and H. Iizumi. 2001. Water quality measurement methods for seagrass habitat. Pages 393-406 in F. T. Short and R. G. Coles, editors. *Global Seagrass Research Methods*. Elsevier Science B.V., Amsterdam.
- Guidetti, P. 2000. Invertebrate borers in the Mediterranean sea grass *Posidonia oceanica*: biological impact and ecological implications. *Journal of the Marine Biological Association of the United Kingdom* **80**:725-730.
- Guidetti, P., S. Bussotti, M. C. Gambi, and M. Lorenti. 1997. Invertebrate borers in *Posidonia oceanica* scales: relationship between their distribution and lepidochronological parameters. *Aquatic Botany* **58**:151-164.

- Harrison, K. and D. M. Holdich. 1984. Hemibranchiate sphaeromatids (Crustacea: Isopoda) from Queensland, Australia, with a world-wide review of the genera discussed. *Zoological Journal of the Linnean Society* **81**:275-387.
- Hauxwell, J., J. Cebrián, C. Furlong, and I. Valiela. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* **82**:1007-1022.
- Hauxwell, J., J. Cebrián, and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series* **247**:59-73.
- Hays, C. G. 2005. Effect of nutrient availability, grazer assemblage and seagrass source population on the interaction between *Thalassia testudinum* (turtle grass) and its algal epiphytes. *Journal of Experimental Marine Biology and Ecology* **314**:53-68.
- Haywood, M. D. E., D. J. Vance, and N. R. Loneragan. 1995. Seagrass and algal beds as nursery habitats for tiger prawns (*Penaeus semisulcatus* and *P. esculentus*) in a tropical Australian estuary. *Marine Biology* **122**:213-223.
- Heck, K. L., K. W. Able, C. T. Roman, and M. P. Fahay. 1995. Composition, abundance, biomass, and production of macrofauna in a New England estuary: comparisons among eelgrass meadows and other nursery habitats. *Estuaries* **18**:379-389.
- Heck, K. L. and L. B. Crowder. 1991. Habitat structure and predator-prey interactions in vegetated aquatic systems. Pages 281-299 in S. S. Bell, E. D. McCoy, and H. R. Mushinsky, editors. *Habitat structure: the physical arrangement of objects in space*. Chapman and Hall, New York.
- Heck, K. L. and R. J. Orth. 1980. Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. Pages 449-464 in V. S. Kennedy, editor. *Estuarine perspectives*. Academic Press, New York.
- Heck, K. L., J. R. Pennock, J. F. Valentine, L. D. Coen, and S. A. Sklenar. 2000. Effects of nutrient enrichment and small predator density on seagrass ecosystems: an experimental assessment. *Limnology and Oceanography* **45**:1041-1057.
- Heck, K. L. and J. F. Valentine. 1995. Sea urchin herbivory: evidence for long-lasting effects in subtropical seagrass meadows. *Journal of Experimental Marine Biology and Ecology* **189**:205-217.
- Heck, K. L. and J. F. Valentine. 2007. The primacy of top-down effects in shallow benthic ecosystems. *Estuaries and Coasts* **30**:371-381.

- Heck, K. L. and G. S. Wetstone. 1977. Habitat complexity and invertebrate species richness and abundance in tropical seagrass meadows. *Journal of Biogeography* **4**:135-142.
- Hemminga, M. A. and C. M. Duarte. 2000. *Seagrass ecology*. Cambridge University Press, Cambridge, UK.
- Hillman, K., A. J. McComb, and D. I. Walker. 1995. The distribution, biomass and primary production of the seagrass *Halophila ovalis* in the Swan/Canning Estuary, Western Australia. *Aquatic Botany* **51**:1-54.
- Holbrook, S. J., D. C. Reed, K. Hansen, and C. A. Blanchette. 2000. Spatial and temporal patterns of predation on seeds of the surfgrass *Phyllospadix torreyi*. *Marine Biology* **136**:739-747.
- Holmes, S. P., C. J. Sturgess, A. Cherrill, and M. S. Davies. 2001. Shell wiping in *Calliostoma zizyphinum*: the use of pedal mucus as a provendering agent and its contribution to daily energetic requirements. *Marine Ecology Progress Series* **212**:171-181.
- Hootsmans, M. J. M. and J. E. Vermaat. 1985. The effect of periphyton-grazing by three epifaunal species on the growth of *Zostera marina* L. under experimental conditions. *Aquatic Botany* **22**:83-88.
- Howard, R. K. 1982. Impact of feeding activities of epibenthic amphipods on surface-fouling of eelgrass leaves. *Aquatic Botany* **14**:91-97.
- Howard, R. K. and F. T. Short. 1986. Seagrass growth and survivorship under the influence of epiphyte grazers. *Aquatic Botany* **24**:287-302.
- Hughes, A. R., K. J. Bando, L. F. Rodriguez, and S. L. Williams. 2004. Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. *Marine Ecology Progress Series* **282**:87-99.
- Ibarra-Obando, S. E., K. L. Heck, and P. M. Spitzer. 2004. Effects of simultaneous changes in light, nutrients, and herbivory levels, on the structure and function of a subtropical turtlegrass meadow. *Journal of Experimental Marine Biology and Ecology* **301**:193-224.
- Jacobs, R. P. W. M., P. M. Hermelink, and G. Van Geel. 1983. Epiphytic algae on eelgrass *Zostera marina* at Roscoff, France. *Aquatic Botany* **15**:157-173.
- Jensen, K. R. 1982. Occurrence of *Elysia serca* Marcus in Florida, with notes on the synonymy and biology of the species. *Journal of Conchology* **31**:87-94.
- Jensen, K. R. 1983a. Factors affecting feeding selectivity in herbivorous Ascoglossa (Mollusca : Opisthobranchia). *Journal of Experimental Marine Biology and Ecology* **66**:135-148.

- Jensen, K. R. 1983b. Further notes on the ecology and systematic of *Elysia serca* Marcus (Opisthobranchia, Ascoglossa). *Journal of Molluscan Studies*, Supplement **12A**:69-72.
- Jernakoff, P., A. Brearley, and J. Nielsen. 1996. Factors affecting grazer-epiphyte interactions in temperate seagrass meadows. *Oceanography and Marine Biology: an Annual Review* **34**:109-162.
- Jernakoff, P. and J. Nielsen. 1997. The relative importance of amphipod and gastropod grazers in *Posidonia sinuosa* meadows. *Aquatic Botany* **56**:183-202.
- Johnson, R. A. and D. W. Wichern. 1982. *Applied multivariate statistical analysis*. Prentice-Hall, Inc., Englewood Cliffs, N.J.
- Joll, L. M. and B. F. Phillips. 1984. Natural diet and growth of juvenile western rock lobsters *Panulirus cygnus* George. *Journal of Experimental Marine Biology and Ecology* **75**:145-169.
- Jones, J. I. and C. D. Sayer. 2003. Does the fish-invertebrate-periphyton cascade precipitate plant loss in shallow lakes? *Ecology* **84**:2155-2167.
- Kendrick, G. A. and P. S. Lavery. 2001. Assessing biomass, assemblage structure and productivity of algal epiphytes on seagrasses. Pages 199-222 in F. T. Short and R. G. Coles, editors. *Global seagrass research methods*. Elsevier Science B.V., Amsterdam.
- Keough, M. J. 1983. Patterns of recruitment of sessile invertebrates in two subtidal habitats. *Journal of Experimental Marine Biology and Ecology* **66**:213-246.
- Kharlamenko, V. I., S. I. Kiyashko, A. B. Imbs, and D. I. Vyshkvartzev. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Marine Ecology Progress Series* **220**:103-117.
- Kirkman, H. 1978. Growing *Zostera capricorni* Aschers. in tanks. *Aquatic Botany* **4**:367-372.
- Kitting, C. L. 1984. Selectivity by dense populations of small invertebrates foraging among seagrass blade surfaces. *Estuaries* **7**:276-288.
- Kitting, C. L., B. Fry, and M. D. Morgan. 1984. Detection of inconspicuous epiphytic algae supporting food webs in seagrass meadows. *Oecologia* **62**:145-149.
- Kline, R. B. 1998. *Principles and practice of structural equation modeling*. The Guilford Press, New York.
- Klumpp, D. W., R. K. Howard, and D. A. Pollard. 1989. Trophodynamics and nutritional ecology of seagrass communities. Pages 394-457 in A. W. D. Larkum, A. J. McComb, and S. A. Shepherd, editors. *Biology of seagrasses. A treatise on the biology of seagrasses with special reference to the Australian region*. Elsevier, Amsterdam.

- Klumpp, D. W. and P. D. Nichols. 1983. Utilization of the seagrass *Posidonia australis* as food by the rock crab *Nectocarcinus integrifrons* (Crustacea : Decapoda : Portunidae). *Marine Biology Letters* **4**:331-340.
- Klumpp, D. W., J. S. Salita-Espinosa, and M. D. Fortes. 1992. The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community. *Aquatic Botany* **43**:327-349.
- Klumpp, D. W., J. T. Salita-Espinosa, and M. D. Fortes. 1993. Feeding ecology and trophic role of sea urchins in a tropical seagrass community. *Aquatic Botany* **45**:205-229.
- Kwak, S. N. and D. W. Klumpp. 2004. Temporal variation in species composition and abundance of fish and decapods of a tropical seagrass bed in Cackle Bay, North Queensland, Australia. *Aquatic Botany* **78**:119-134.
- Lacap, C. D. A., J. E. Vermaat, R. N. Rollon, and H. M. Nacorda. 2002. Propagule dispersal of the SE Asian seagrasses *Enhalus acoroides* and *Thalassia hemprichii*. *Marine Ecology Progress Series* **235**:75-80.
- Lanyon, J. M., C. J. Limpus, and H. Marsh. 1989. Dugongs and turtles: grazers in the seagrass system. Pages 610-634 in A. W. D. Larkum, A. J. McComb, and S. A. Shepherd, editors. *Biology of seagrasses. A treatise on the biology of seagrasses with special reference to the Australian region*. Elsevier, Amsterdam.
- Lanyon, J. M. and H. Marsh. 1995. Temporal changes in the abundance of some tropical intertidal seagrasses in North Queensland. *Aquatic Botany* **49**:217-237.
- Leber, K. M. 1985. The influence of predatory decapods, refuge, and microhabitat selection on seagrass communities. *Ecology* **66**:1951-1964.
- Lee, K.-S. and K. H. Dunton. 1997. Effects of *in situ* light reduction on the maintenance, growth and partitioning of carbon resources in *Thalassia testudinum* Banks ex König. *Journal of Experimental Marine Biology and Ecology* **210**:53-73.
- Lee Long, W. J., R. G. Coles, and L. J. McKenzie. 1996. Deepwater seagrasses in northeastern Australia - how deep, how meaningful? Pages 41-50 in *Seagrass biology: proceedings of an international workshop, Rottnest Island, Western Australia*.
- Lee, S. Y., C. W. Fong, and R. S. S. Wu. 2001. The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. *Journal of Experimental Marine Biology and Ecology* **259**:23-50.
- Lepoint, G., P. Dauby, and S. Gobert. 2004. Applications of C and N stable isotopes to ecological and environmental studies in seagrass ecosystems. *Marine Pollution Bulletin* **49**:887-891.

- Lewis, F. G. 1984. Distribution of macrobenthic crustaceans associated with *Thalassia*, *Halodule* and bare sand substrata. Marine Ecology Progress Series **19**:101-113.
- Li, Y., D. Yu, X. Xu, and Y. Xie. 2005. Light intensity increases the susceptibility of *Vallisneria natans* to snail herbivory. Aquatic Botany **81**:265-275.
- Libes, M. 1986. Productivity-irradiance relationship of *Posidonia oceanica* and its epiphytes. Aquatic Botany **26**:285-306.
- Lin, H.-J., S. W. Nixon, D. I. Taylor, S. L. Granger, and B. A. Buckley. 1996. Responses of epiphytes on eelgrass, *Zostera marina* L., to separate and combined nitrogen and phosphorus enrichment. Aquatic Botany **52**:243-258.
- Loch, I. 1994. *Smaragdia*. Australian Shell News **85/86**:1-2.
- Longstaff, B. J. and W. C. Dennison. 1999. Seagrass survival during pulsed turbidity events: the effects of light deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*. Aquatic Botany **65**:105-121.
- Longstaff, B. J., N. R. Loneragan, M. J. O'Donohue, and W. C. Dennison. 1999. Effects of light deprivation on the survival and recovery of the seagrass *Halophila ovalis* (R.Br.) Hook. Journal of Experimental Marine Biology and Ecology **234**:1-27.
- Lowe, E. F. and R. L. Turner. 1976. Aggregation and trail-following in juvenile *Bursatella leachii pleii*. Veliger **19**:153-155.
- Major, K. M. and K. H. Dunton. 2002. Variations in light-harvesting characteristics of the seagrass, *Thalassia testudinum*: evidence for photoacclimation. Journal of Experimental Marine Biology and Ecology **275**:173-189.
- Marcus, E. D. B.-R. 1972. On the Anaspidea (Gastropoda: Opisthobranchia) of the warm waters of the western Atlantic. Bulletin of Marine Science **22**:841-874.
- Martin, T. H., L. B. Crowder, C. F. Dumas, and J. M. Burkholder. 1992. Indirect effects of fish on macrophytes in Bays Mountain Lake: evidence for a littoral trophic cascade. Oecologia **89**:476-481.
- Mattson, S. and T. Cedhagen. 1989. Aspects of the behaviour and ecology of *Dyopedos monacanthus* (Metzger) and *D. porrectus* Bate, with comparative notes on *Dulichia tuberculata* Boeck (Crustacea : Amphipoda : Podoceridae). Journal of Experimental Marine Biology and Ecology **127**:253-272.
- Mazzella, L. and R. S. Alberte. 1986. Light adaptation and the role of autotrophic epiphytes in primary production of the temperate seagrass, *Zostera marina* L. Journal of Experimental Marine Biology and Ecology **100**:165-180.
- Mazzella, L. and G. F. Russo. 1989. Grazing effect of two *Gibbula* species (Mollusca, Archaeogastropoda) on the epiphytic community of *Posidonia oceanica* leaves. Aquatic Botany **35**:357-373.

- McConnaughey, T. and C. P. McRoy. 1979.  $^{13}\text{C}$  label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. *Marine Biology* **53**:263-269.
- McCulloch, M., S. Fallon, T. Wyndham, E. Hendy, J. Lough, and D. Barnes. 2003. Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. *Nature* **421**:727-730.
- McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. *Journal of Phycology* **37**:453-456.
- McKenzie, L. J. 1994. Seasonal changes in biomass and shoot characteristics of a *Zostera capricorni* Aschers. dominant meadow in Cairns harbour, northern Queensland. *Australian Journal of Marine and Freshwater Research* **45**:1337-1352.
- Mellors, J., H. Marsh, T. J. B. Carruthers, and M. Waycott. 2002. Testing the sediment-trapping paradigm of seagrass: do seagrasses influence nutrient status and sediment structure in tropical intertidal environments? *Bulletin of Marine Science* **71**:1215-1226.
- Mellors, J. E. 2003. Sediment and nutrient dynamics in coastal intertidal seagrass of north eastern tropical Australia. PhD. James Cook University, Townsville, Australia.
- Mellors, J. E. and H. Marsh. 1993. Relationships between seagrass standing crop and the spatial distribution and abundance of the natantian fauna at Green Island, northern Queensland. *Australian Journal of Marine and Freshwater Research* **44**:183-191.
- Mellors, J. E., H. Marsh, and R. G. Coles. 1993. Intra-annual changes in seagrass standing crop, Green Island, northern Queensland. *Australian Journal of Marine and Freshwater Research* **44**:31-44.
- Mercier, A., S. C. Battaglione, and J.-F. Hamel. 2000. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. *Journal of Experimental Marine Biology and Ecology* **249**:89-110.
- Moncreiff, C. A. and M. J. Sullivan. 2001. Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series* **215**:93-106.
- Moncreiff, C. A., M. J. Sullivan, and A. E. Daehnick. 1992. Primary production dynamics in seagrass beds of Mississippi Sound: the contributions of seagrass, epiphytic algae, sand microflora, and phytoplankton. *Marine Ecology Progress Series* **87**:161-171.
- Moore, K. A. and R. L. Wetzel. 2000. Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems. *Journal of Experimental Marine Biology and Ecology* **244**:1-28.



- Morgan, M. D. 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. *Limnology and Oceanography* **25**:896-902.
- Mukai, H. and A. Iijima. 1995. Grazing effects of a gammaridean amphipoda, *Ampithoe* sp., on the seagrass, *Syringodium isoetifolium*, and epiphytes in a tropical seagrass bed of Fiji. *Ecological Research* **10**:243-257.
- Muschenheim, D. K. 1987. The dynamics of near-bed seston flux and suspension-feeding benthos. *Journal of Marine Research* **45**:473-496.
- Nakaoka, M. 2002. Predation on seeds of seagrasses *Zostera marina* and *Zostera caulescens* by a tanaid crustacean *Zeuxo* sp. *Aquatic Botany* **72**:99-106.
- Neckles, H. A., R. L. Wetzel, and R. J. Orth. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* **93**:285-295.
- Nelson, T. A. 1997. Epiphyte-grazer interactions on *Zostera marina* (Anthophyta: Monocotyledones): effects of density on community function. *Journal of Phycology* **33**:743-752.
- Nelson, T. A. and J. R. Waaland. 1997. Seasonality of eelgrass, epiphyte, and grazer biomass and productivity in subtidal eelgrass meadows subjected to moderate tidal amplitude. *Aquatic Botany* **56**:51-74.
- Nelson, W. G. 1979a. An analysis of structural pattern in an eelgrass (*Zostera marina* L.) amphipod community. *Journal of Experimental Marine Biology and Ecology* **39**:231-264.
- Nelson, W. G. 1979b. Experimental studies of selective predation on amphipods: consequences for amphipod distribution and abundance. *Journal of Experimental Marine Biology and Ecology* **38**:225-245.
- Nelson, W. G. 1980. A comparative study of amphipods in seagrasses from Florida to Nova Scotia. *Bulletin of Marine Science* **30**:80-89.
- Nelson, W. G. 1981. Experimental studies of decapod and fish predation on seagrass macrobenthos. *Marine Ecology Progress Series* **5**:141-149.
- Neverauskas, V. P. 1988. Response of a *Posidonia* community to prolonged reduction in light. *Aquatic Botany* **31**:361-366.
- Nichols, P. D., D. W. Klumpp, and R. B. Johns. 1986. Lipid components and utilization in consumers of a seagrass community; an indication of carbon source. *Comparative Biochemistry and Physiology B* **83**:103-114.
- Nienhuis, P. H. and A. M. Groenendijk. 1986. Consumption of eelgrass (*Zostera marina*) by birds and invertebrates: an annual budget. *Marine Ecology Progress Series* **29**:29-35.

- O'Brien, C. J. 1994. Ontogenetic changes in the diet of juvenile brown tiger prawns *Penaeus esculentus*. Marine Ecology Progress Series **112**:195-200.
- Onuf, C. P. 1994. Seagrasses, dredging and light in Laguna Madre, Texas, U.S.A. Estuarine, Coastal and Shelf Science **39**:75-91.
- Onuf, C. P. 1996. Seagrass responses to long-term light reduction by brown tide in upper Laguna Madre, Texas: distribution and biomass patterns. Marine Ecology Progress Series **138**:219-231.
- Orth, R. J. 1999. Settling rates of *Posidonia coriacea* seeds and *Posidonia* spp. seedling abundance off Rottnest Island, Perth, Western Australia. Pages 51-61 in Proceedings of the ninth international marine biological workshop: the seagrass flora and fauna of Rottnest Island, Western Australia.
- Orth, R. J., K. L. Heck, and D. J. Tunbridge. 2002. Predation on seeds of the seagrass *Posidonia australis* in Western Australia. Marine Ecology Progress Series **244**:81-88.
- Orth, R. J., K. L. Heck, and J. van Montfrans. 1984. Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries **7**:339-350.
- Orth, R. J., G. A. Kendrick, and S. R. Marion. 2006. Predation on *Posidonia australis* seeds in seagrass habitats of Rottnest Island, Western Australia: patterns and predators. Marine Ecology Progress Series **313**:105-114.
- Orth, R. J., G. A. Kendrick, and S. R. Marion. 2007. *Posidonia australis* seed predation in seagrass habitats of Two Peoples Bay, Western Australia. Aquatic Botany **86**:83-85.
- Orth, R. J. and K. A. Moore. 1983. Chesapeake Bay: an unprecedented decline in submerged aquatic vegetation. Science **222**:51-53.
- Orth, R. J. and J. van Montfrans. 1984. Epiphyte-seagrass relationships with an emphasis on the role of micrograzing: a review. Aquatic Botany **18**:43-69.
- Paige, J. A. 1988. Biology, metamorphosis and postlarval development of *Bursatella leachii plei* Rang (Gastropoda: Opisthobranchia). Bulletin of Marine Science **42**:65-75.
- Paul, V. J. and S. C. Pennings. 1991. Diet-derived chemical defense in the sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). Journal of Experimental Marine Biology and Ecology **151**:227-243.
- Pearl, J. 1997. The new challenge: from a century of statistics to an age of causation. Computing Science and Statistics **29**:415-423.
- Pedhazur, E. J. 1982. Multiple regression in behavioral research. second edition. Holt, Rinehart, and Winston, New York.

- Penhale, P. A. and W. O. Smith. 1977. Excretion of dissolved organic carbon by eelgrass (*Zostera marina*) and its epiphytes. *Limnology and Oceanography* **22**:400-407.
- Penhale, P. A. and G. W. Thayer. 1980. Uptake and transfer of carbon and phosphorus by eelgrass *Zostera marina* and its epiphytes. *Journal of Experimental Marine Biology and Ecology* **42**:113-124.
- Pennings, S. C., A. M. Weiss, and V. J. Paul. 1996. Secondary metabolites of the cyanobacterium *Microcoleus lyngbyaceus* and the sea hare *Stylocheilus longicauda*: palatability and toxicity. *Marine Biology* **126**:735-743.
- Peralta, G., J. L. Pérez-Lloréns, I. Hernández, and J. J. Vergara. 2002. Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology* **269**:9-26.
- Perkins-Visser, E., T. G. Wolcott, and D. L. Wolcott. 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathbun). *Journal of Experimental Marine Biology and Ecology* **198**:155-173.
- Peterson, B. J. and K. L. Heck. 2001. Positive interactions between suspension-feeding bivalves and seagrass — a facultative mutualism. *Marine Ecology Progress Series* **213**:143-155.
- Peterson, B. J., C. D. Rose, L. M. Rutten, and J. W. Fourqurean. 2002. Disturbance and recovery following catastrophic grazing: studies of a successional chronosequence in a seagrass bed. *Oikos* **97**:361-370.
- Peterson, C. H., R. A. Luettich, F. Micheli, and G. A. Skilleter. 2004. Attenuation of water flow inside seagrass canopies of differing structure. *Marine Ecology Progress Series* **268**:81-92.
- Philippart, C. J. M. 1995. Effect of periphyton grazing by *Hydrobia ulvae* on the growth of *Zostera noltii* on a tidal flat in the Dutch Wadden Sea. *Marine Biology* **122**:431-437.
- Preen, A. 1995. Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. *Marine Ecology Progress Series* **124**:201-213.
- Preen, A. R., W. J. Lee Long, and R. G. Coles. 1995. Flood and cyclone related loss, and partial recovery, of more than 1000 Km<sup>2</sup> of seagrass in Hervey Bay, Queensland, Australia. *Aquatic Botany* **52**:3-17.
- Ralph, P. J. 1998. Photosynthetic response of laboratory-cultured *Halophila ovalis* to thermal stress. *Marine Ecology Progress Series* **171**:123-130.
- Ralph, P. J. 1999a. Light-induced photoinhibitory stress responses of laboratory-cultured *Halophila ovalis*. *Botanica Marina* **42**:11-22.

- Ralph, P. J. 1999b. Photosynthetic response of *Halophila ovalis* (R. Br.) Hook. f. to combined environmental stress. *Aquatic Botany* **65**:83-96.
- Ralph, P. J. and M. D. Burchett. 1995. Photosynthetic responses of the seagrass *Halophila ovalis* (R. Br.) Hook. f. to high irradiance stress, using chlorophyll *a* fluorescence. *Aquatic Botany* **51**:55-66.
- Rasheed, M. A. 2000. Recovery and succession in north Queensland tropical seagrass communities. PhD. James Cook University, Townsville, Australia.
- Ray, M. and A. W. Stoner. 1995. Growth, survivorship, and habitat choice in a newly settled seagrass gastropod, *Strombus gigas*. *Marine Ecology Progress Series* **123**:83-94.
- Reusch, T. B. H. 1998. Differing effects of eelgrass *Zostera marina* on recruitment and growth of associated blue mussels *Mytilus edulis*. *Marine Ecology Progress Series* **167**:149-153.
- Reusch, T. B. H., A. R. O. Chapman, and J. P. Groeger. 1994. Blue mussels *Mytilus edulis* do not interfere with eelgrass *Zostera marina* but fertilize shoot growth through biodeposition. *Marine Ecology Progress Series* **108**:265-282.
- Reusch, T. B. H. and S. L. Williams. 1999. Macrophyte canopy structure and the success of an invasive marine bivalve. *Oikos* **84**:398-416.
- Robertson, A. I. and K. H. Mann. 1980. The role of isopods and amphipods in the initial fragmentation of eelgrass detritus in Nova Scotia, Canada. *Marine Biology* **59**:63-69.
- Robertson, A. I. and K. H. Mann. 1982. Population dynamics and life history adaptations of *Littorina neglecta* Bean in an eelgrass meadow (*Zostera marina* L.) in Nova Scotia. *Journal of Experimental Marine Biology and Ecology* **63**:151-171.
- Rollon, R. N., E. D. De Ruyter Van Steveninck, W. Van Vierssen, and M. D. Fortes. 1998. Contrasting recolonization strategies in multi-species seagrass meadows. *Marine Pollution Bulletin* **37**:450-459.
- Ruiz, J. M. and J. Romero. 2001. Effects of *in situ* experimental shading on the Mediterranean seagrass *Posidonia oceanica*. *Marine Ecology Progress Series* **215**:107-120.
- Sand-Jensen, K. 1977. Effect of epiphytes on eelgrass photosynthesis. *Aquatic Botany* **3**:55-63.
- Schanz, A., P. Polte, and H. Asmus. 2002. Cascading effects of hydrodynamics on an epiphyte-grazer system in intertidal seagrass beds of the Wadden Sea. *Marine Biology* **141**:287-297.

- Schneider, F. I. and K. H. Mann. 1991. Species specific relationships of invertebrates to vegetation in a seagrass bed. I. Correlational studies. *Journal of Experimental Marine Biology and Ecology* **145**:101-117.
- Shepherd, S. A., A. J. McComb, D. A. Bulthuis, V. Neverauskas, D. A. Steffensen, and R. West. 1989. Decline of seagrasses. Pages 346-393 in A. W. D. Larkum, A. J. McComb, and S. A. Shepherd, editors. *Biology of seagrasses. A treatise on the biology of seagrasses with special reference to the Australian region*. Elsevier, Amsterdam.
- Shipley, B. 1999. Testing causal explanations in organismal biology: causation, correlation and structural equation modelling. *Oikos* **86**:374-382.
- Shipley, B. 2004. *Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference*. Cambridge University Press, Cambridge, UK.
- Short, F. T. and D. M. Burdick. 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. *Estuaries* **19**:730-739.
- Short, F. T., D. M. Burdick, and J. E. Kaldy. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. *Limnology and Oceanography* **40**:740-749.
- Sidik, B. J., S. O. Bandeira, and N. A. Milchakova. 2001. Methods to measure macroalgal biomass and abundance in seagrass meadows. Pages 223-232 in F. T. Short and R. G. Coles, editors. *Global seagrass research methods*. Elsevier, Amsterdam.
- Silberstein, K., A. W. Chiffings, and A. J. McComb. 1986. The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* Hook. F. *Aquatic Botany* **24**:355-371.
- Smit, A. J., A. Brearley, G. A. Hyndes, P. S. Lavery, and D. I. Walker. 2005. Carbon and nitrogen stable isotope analysis of an *Amphibolis griffithii* seagrass bed. *Estuarine, Coastal and Shelf Science* **65**:545-556.
- Sotka, E. E., M. E. Hay, and J. D. Thomas. 1999. Host-plant specialization by a non-herbivorous amphipod: advantages for the amphipod and costs for the seaweed. *Oecologia* **118**:471-482.
- Stapel, J. and P. L. A. Erftemeijer. 2000. Leaf harvesting by burrowing alpheid shrimps in a *Thalassia hemprichii* meadow in South Sulawesi, Indonesia. *Biologia Marina Mediterranea* **7**:282-285.
- Stapel, J., R. Manuntun, and M. A. Hemminga. 1997. Biomass loss and nutrient redistribution in an Indonesian *Thalassia hemprichii* seagrass bed following seasonal low tide exposure during daylight. *Marine Ecology Progress Series* **148**:251-262.

- Stephenson, R. L., F. C. Tan, and K. H. Mann. 1986. Use of stable carbon isotope ratios to compare plant material and potential consumers in a seagrass bed and a kelp bed in Nova Scotia, Canada. *Marine Ecology Progress Series* **30**:1-7.
- Stoner, A. W. 1979. Species-specific predation on amphipod crustacea by the pinfish *Lagodon rhomboides*: mediation by macrophyte standing crop. *Marine Biology* **55**:201-207.
- Stoner, A. W. 1980a. Abundance, reproductive seasonality and habitat preferences of amphipod crustaceans in seagrass meadows of Apalachee Bay, Florida. *Contributions in Marine Science* **23**:63-77.
- Stoner, A. W. 1980b. The role of seagrass biomass in the organization of benthic macrofaunal assemblages. *Bulletin of Marine Science* **30**:537-551.
- Stoner, A. W. and F. G. Lewis. 1985. The influence of quantitative and qualitative aspects of habitat complexity in tropical sea-grass meadows. *Journal of Experimental Marine Biology and Ecology* **94**:19-40.
- Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology* **73**:747-754.
- Swennen, C., R. G. Moolenbeek, N. Ruttanadukul, H. Hobbelink, H. Dekker, and S. Hajisamae. 2001. The molluscs of the southern Gulf of Thailand. *Thai studies in biodiversity* **4**:1-210.
- Terrados, J., C. M. Duarte, M. D. Fortes, J. Borum, N. S. R. Agawin, S. Bach, U. Thampanya, L. Kamp-Nielsen, W. J. Kenworthy, O. Geertz-Hansen, and J. Vermaat. 1998. Changes in community structure and biomass of seagrass communities along gradients of siltation in SE Asia. *Estuarine, Coastal and Shelf Science* **46**:757-768.
- Thayer, G. W., K. A. Bjorndal, J. C. Ogden, S. L. Williams, and J. C. Zieman. 1984. Role of large herbivores in seagrass communities. *Estuaries* **7**:351-376.
- Thom, R., B. Miller, and M. Kennedy. 1995. Temporal patterns of grazers and vegetation in a temperate seagrass system. *Aquatic Botany* **50**:201-205.
- Thom, R. M. 1990. Spatial and temporal patterns in plant standing stock and primary production in a temperate seagrass system. *Botanica Marina* **33**:497-510.
- Tomasko, D. A. 1992. Variation in growth form of shoal grass *Halodule wrightii* due to changes in the spectral composition of light below a canopy of turtle grass *Thalassia testudinum*. *Estuaries* **15**:214-217.
- Tomasko, D. A. and C. J. Dawes. 1990. Influences of season and water depth on the clonal biology of the seagrass *Thalassia testudinum*. *Marine Biology* **105**:345-351.
- Tomasko, D. A. and B. E. Lapointe. 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels:

- field observations and experimental studies. *Marine Ecology Progress Series* **75**:9-17.
- Tomlinson, P. B. 1980. Leaf morphology and anatomy in seagrasses. Pages 7-28 in R. C. Phillips and C. P. McRoy, editors. *Handbook of seagrass biology: an ecosystem perspective*. Garland STPM Press, New York.
- Trocine, R. P., J. D. Rice, and G. N. Wells. 1981. Inhibition of seagrass photosynthesis by ultraviolet-B radiation. *Plant Physiology* **68**:74-81.
- Tunberg, B. G., W. G. Nelson, and G. Smith. 1994. Population ecology of *Pagurus maclaughlinae* Garcia-Gomez (Decapoda: Anomura: Paguridae) in the Indian River Lagoon, Florida. *Journal of Crustacean Biology* **14**:686-699.
- Twilley, R. R., W. M. Kemp, K. W. Staver, J. C. Stevenson, and W. R. Boynton. 1985. Nutrient enrichment of estuarine submersed vascular plant communities. 1. Algal growth and effects on production of plants and associated communities. *Marine Ecology Progress Series* **23**:179-191.
- Underwood, A. J., M. G. Chapman, and S. D. Connell. 2000. Observations in ecology: you can't make progress on processes without understanding the patterns. *Journal of Experimental Marine Biology and Ecology* **250**:97-115.
- Valentine, J. F. and J. E. Duffy. 2006. The central role of grazing in seagrass ecology. Pages 463-501 in A. W. D. Larkum, R. J. Orth, and C. M. Duarte, editors. *Seagrasses: biology, ecology and conservation*. Springer, Dordrecht, The Netherlands.
- Valentine, J. F. and K. L. Heck. 1991. The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **154**:215-230.
- Valentine, J. F. and K. L. Heck. 1999. Seagrass herbivory: evidence for the continued grazing of marine grasses. *Marine Ecology Progress Series* **176**:291-302.
- Valentine, J. F. and K. L. Heck. 2001. The role of leaf nitrogen content in determining turtlegrass (*Thalassia testudinum*) grazing by a generalized herbivore in the northeastern Gulf of Mexico. *Journal of Experimental Marine Biology and Ecology* **258**:65-86.
- Valentine, J. F., K. L. Heck, J. Busby, and D. Webb. 1997. Experimental evidence that herbivory increases shoot density and productivity in a subtropical turtlegrass (*Thalassia testudinum*) meadow. *Oecologia* **112**:193-200.
- Valentine, J. F., K. L. Heck, K. D. Kirsch, and D. Webb. 2000. Role of sea urchin *Lytechinus variegatus* grazing in regulating subtropical turtlegrass *Thalassia testudinum* meadows in the Florida Keys (USA). *Marine Ecology Progress Series* **200**:213-228.

- Van Dolah, R. F. 1978. Factors regulating the distribution and population dynamics of the amphipod *Gammarus palustris* in an intertidal salt marsh community. *Ecological Monographs* **48**:191-217.
- van Montfrans, J., R. J. Orth, and S. A. Vay. 1982. Preliminary studies of grazing by *Bittium varium* on eelgrass periphyton. *Aquatic Botany* **14**:75-89.
- van Montfrans, J., R. L. Wetzel, and R. J. Orth. 1984. Epiphyte-grazer relationships in seagrass meadows: consequences for seagrass growth and production. *Estuaries* **7**:289-309.
- van Tussenbroek, B. I. and A. Brearley. 1998. Isopod burrowing in leaves of turtle grass, *Thalassia testudinum*, in a Mexican Caribbean reef lagoon. *Marine and Freshwater Research* **49**:525-531.
- Verduin, J. J. and J. O. Backhaus. 2000. Dynamics of plant-flow interactions for the seagrass *Amphibolis antarctica*: field observations and model simulations. *Estuarine, Coastal and Shelf Science* **50**:185-204.
- Vermaat, J. E., N. S. R. Agawin, C. M. Duarte, M. D. Fortes, N. Marb, and J. S. Uri. 1995. Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. *Marine Ecology Progress Series* **124**:215-225.
- Vincent, G. 2006. Leaf life span plasticity in tropical seedlings grown under contrasting light regimes. *Annals of Botany* **97**:245-255.
- Virnstain, R. W. and R. K. Howard. 1987a. Motile epifauna of marine macrophytes in the Indian River lagoon, Florida. I. Comparisons among three species of seagrasses from adjacent beds. *Bulletin of Marine Science* **41**:1-12.
- Virnstain, R. W. and R. K. Howard. 1987b. Motile epifauna of marine macrophytes in the Indian River lagoon, Florida. II. Comparisons between drift algae and three species of seagrasses. *Bulletin of Marine Science* **41**:13-26.
- Virnstain, R. W., W. G. Nelson, F. G. Lewis, and R. K. Howard. 1984. Latitudinal patterns in seagrass epifauna: do patterns exist, and can they be explained? *Estuaries* **7**:310-330.
- Walker, T. A. 1981. Dependence of phytoplankton chlorophyll on bottom resuspension in Cleveland Bay, Northern Queensland. *Australian Journal of Marine and Freshwater Research* **32**:981-986.
- Walker, T. A. and G. O'Donnell. 1981. Observations on nitrate, phosphate and silicate in Cleveland Bay, Northern Queensland. *Australian Journal of Marine and Freshwater Research* **32**:877-887.
- Ward, L. G., W. M. Kemp, and W. R. Boynton. 1984. The influence of waves and seagrass communities on suspended particles in an estuarine embayment. *Marine Geology* **59**:85-103.



- Wassenberg, T. J. 1990. Seasonal feeding on *Zostera capricorni* seeds by juvenile *Penaeus esculentus* (Crustacea : Decapoda) in Moreton Bay, Queensland. *Australian Journal of Marine and Freshwater Research* **41**:301-310.
- Wassenberg, T. J. and B. J. Hill. 1987. Natural diet of the tiger prawns *Penaeus esculentus* and *P. semisulcatus*. *Australian Journal of Marine and Freshwater Research* **38**:169-182.
- Waycott, M., K. McMahon, J. Mellors, A. Calladine, and D. Kleine. 2004. A guide to tropical seagrasses of the Indo-West Pacific. James Cook University, Townsville, Australia.
- Wear, D. J., M. J. Sullivan, A. D. Moore, and D. F. Millie. 1999. Effects of water-column enrichment on the production dynamics of three seagrass species and their epiphytic algae. *Marine Ecology Progress Series* **179**:201-213.
- Wigand, C. and A. C. Churchill. 1988. Laboratory studies on eelgrass seed and seedling predation. *Estuaries* **11**:180-183.
- Wiginton, J. R. and C. McMillan. 1979. Chlorophyll composition under controlled light conditions as related to the distribution of seagrasses in Texas and the U.S. Virgin Islands. *Aquatic Botany* **6**:171-184.
- Williams, S. I. and D. I. Walker. 1999. Mesoherbivore-macroalgal interactions: feeding ecology of sacoglossan sea slugs (Mollusca, Opisthobranchia) and their effects on their food algae. *Oceanography and Marine Biology: an Annual Review* **37**:81-128.
- Williams, S. L. 1990. Experimental studies of Caribbean seagrass bed development. *Ecological Monographs* **60**:449-469.
- Williams, S. L. and K. L. Heck. 2001. Seagrass community ecology. Pages 317-337 in M. D. Bertness, S. D. Gaines, and M. E. Hay, editors. *Marine community ecology*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Williams, S. L. and M. H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. *Ecology* **74**:904-918.
- Wolanski, E. 1994. *Physical oceanographic processes of the Great Barrier Reef*. CRC Press, Boca Raton.
- Wolanski, E. and R. Gibbs. 1992. Resuspension and clearing of dredge spoils after dredging, Cleveland Bay, Australia. *Water Environment Research* **64**:910-914.
- Wolanski, E., R. Gibbs, P. Ridd, and A. Mehta. 1992. Settling of ocean-dumped dredged material, Townsville, Australia. *Estuarine, Coastal and Shelf Science* **35**:473-489.
- Wolanski, E., S. Spagnol, and E. B. Lim. 1997. The importance of mangrove flocs in sheltering seagrass in turbid coastal waters. *Mangroves and Salt Marshes* **1**:187-191.

- Woods, C. M. C. and D. R. Schiel. 1997. Use of seagrass *Zostera novazelandica* (Setchell, 1933) as habitat and food by the crab *Macrophthalmus hirtipes* (Heller, 1862) (Brachyura: Ocypodidae) on rocky intertidal platforms in southern New Zealand. *Journal of Experimental Marine Biology and Ecology* **214**:49-65.
- Wright, S. 1921. Correlation and causation. *Journal of Agricultural Research* **10**:557-585.
- Zimmerman, R., R. Gibson, and J. Harrington. 1979. Herbivory and detritivory among gammaridean amphipods from a Florida seagrass community. *Marine Biology* **54**:41-47.
- Zimmerman, R. C., D. G. Kohrs, and R. S. Alberte. 1996. Top-down impact through a bottom-up mechanism: the effect of limpet grazing on growth, productivity and carbon allocation of *Zostera marina* L. (eelgrass). *Oecologia* **107**:560-567.
- Zimmerman, R. C., D. L. Steller, D. G. Kohrs, and R. S. Alberte. 2001. Top-down impact through a bottom-up mechanism. *In situ* effects of limpet grazing on growth, light requirements and survival of the eelgrass *Zostera marina*. *Marine Ecology Progress Series* **218**:127-140.

## **Appendix A: Epifaunal abundance tables**

The following tables show the densities of all taxa in leaf samples taken over the course of the study in three study plots. Densities are expressed as numbers of animals per 100 cm<sup>2</sup> of leaf surface.

Abbreviations used in the following tables:

*S. souverbiana* = *Smaragdia souverbiana*

*A. translucida* = *Alaba translucida*

*N. indicus* = *Notarcus indicus*

*L. fusiformis* = *Limenandra fusiformis*

**Table A.1 Mean number of invertebrates per 100 cm<sup>2</sup> *Halodule uninervis* leaf surface in plot SB, n=10 on each sampling date. Part 1 of 2.**

Date	10-Jun-02	20-Jun-02	8-Jul-02	10-Aug-02	5-Sep-02	7-Oct-02	5-Nov-02	29-Nov-02	30-Jan-03	18-Mar-03	2-May-03	13-Jun-03	
Mean sample leaf area (cm <sup>2</sup> )	2.9	2.6	2.6	2.4	2.4	2.8	2.7	3.0	4.0	5.0	3.1	4.3	
Nemertea													
Nereididae													
Trochidae													
Neritidae	<i>S. souverbiana</i>		4.2			3.1	4.1						
Rissoidae	sp. 1	3.2	3.2		8.5	13.7	7.1			1.9	9.3	17.1	
Litiopidae	<i>Alaba virgata</i>	17.0	54.1	63.9	62.8	45.4	273.1	105.3	31.2	103.9	6.0		
Haminoeidae		2.7											
Sacoglossa													
Aeolidiidae				6.5				4.3					
Pteriidae	<i>Electroma</i>	349.0	612.0	372.5	58.5	72.1	1060.7	121.5	243.3	4.7	49.3	9.2	2.7
Caprellidae													
Amphithoidae	<i>Cymadusa</i>	19.3	3.9	9.3	3.8		3.0			2.9	1.8	12.7	2.6
Eusiridae		13.3	10.1	17.3	3.1					1.7	15.8		
Ischyroceridae	<i>Erichthonius</i>				4.1	11.7	3.0					4.7	
	<i>Cerapus</i>			3.2	4.2								
	<i>Ischyrocerus</i>												
Paracalliopiidae													
other Amphipoda													
Munnidae		9.4											
Pycnogonida													
							4.0	14.3					
Chironomidae													
					3.5			5.9	53.0		7.6		
Actiniaria													
						3.9						2.5	
Total		401.3	692.6	473.6	143.0	141.2	1360.6	241.9	299.0	60.7	158.7	68.3	24.9

**Part 2 of Table A.1.**

Date	14-Jul-03	11-Aug-03	9-Sep-03	5-Oct-03	4-Nov-03	23-Dec-03	20-Feb-04	16-Apr-04	29-Jun-04	16-Aug-04	Mean
Mean sample leaf area (cm <sup>2</sup> )	3.8	3.1	3.2	3.0	3.4	4.4	5.1	5.1	3.0	2.4	
Nemertea								1.9			0.1
Nereididae								8.5	15.0	30.0	2.4
Trochidae		5.5									0.3
Neritidae	<i>S. souverbiana</i>		3.3	9.8	3.2		1.6				1.3
Rissoidae	sp. 1	8.2	21.5	15.8		14.7	10.9		6.8	9.6	6.9
Litiopidae	<i>Alaba virgata</i>	52.2	35.4	121.2	143.4	8.8	8.8	14.8		158.2	59.3
Haminoeidae											0.1
Sacoglossa		2.8									0.1
Aeolidiidae											0.5
Pteriidae	<i>Electroma</i>		28.8	21.2	10.7	14.4		32.5	2.3		3.7 139.5
Caprellidae								2.5		3.8	0.4
Amphithoidae	<i>Cymadusa</i>	5.5				3.4	7.5	18.0			4.3
Eusiridae		8.3						7.8	2.6		3.6
Ischyroceridae	<i>Erichthonius</i>								127.3	4.7	7.1
	<i>Cerapus</i>										0.3
	<i>Ischyrocerus</i>								7.7		0.3
Paracalliopiidae									4.0		0.2
other Amphipoda								2.3			0.1
Munnidae		2.9						3.3			0.7
Pycnogonida											0.8
Chironomidae		2.9					7.6	10.7			4.1
Actiniaria		4.9	13.3						7.6	4.0	1.7
Total		87.6	104.6	161.5	163.9	26.4	26.9	74.9	57.2	171.0	214.1 234.1

**Table A.2 Mean number of invertebrates per 100 cm<sup>2</sup> *Halophila ovalis* leaf surface in plot SB on each sampling date. Part 1 of 2.**

Date	10-Aug-02	5-Sep-02	7-Oct-02	5-Nov-02	29-Nov-02	30-Jan-03	18-Mar-03	2-May-03	13-Jun-03	14-Jul-03
n	10	10	10	10	10	10	10	10	10	10
Mean sample leaf area (cm <sup>2</sup> )	3.7	3.6	3.9	3	3.2	4.1	4.9	3.8	4.4	4
Nereididae			5.0				2.6			
Spirorbidae								2.2		
Trochidae									1.7	
Neritidae				5.3	3.5	6.0		2.8		
Rissoidae	2.7		1.8	3.3		7.5	1.8	13.1	11.5	17.8
Litiopidae			28.0	3.3	3.1		28.9			2.4
Columbellidae				2.8						
Sacoglossa			21.9	12.1		3.0	4.4			
Pteriidae			14.0	3.3	2.9		1.4			
Mytilidae										4.4
other bivalves			2.7							
Caprellidae		2.7						2.7		
Amphithoidae			2.3			4.8	3.8	2.8		
Eusiridae								13.3		
Ischyroceridae		10.3	3.2					34.3	13.2	54.8
Sphaeromatidae										2.2
Munnidae								22.3	6.2	
Pycnogonida										
Chironomidae					3.3	19.0	2.4	2.7		
Actiniaria						2.8		12.4	15.1	40.1
Total	2.7	13.0	84.1	28.1	15.2	37.0	45.3	108.6	47.7	121.7

**Part 2 of Table A.2.**

Date	11-Aug-03	9-Sep-03	5-Oct-03	4-Nov-03	23-Dec-03	20-Feb-04	29-Jun-04	16-Aug-04	Mean
n	10	7	7	8	9	7	9	10	
Mean sample leaf area (cm <sup>2</sup> )	2.7	2.9	2.3	3.5	4.3	3.6	3.7	3.8	
Nereididae	4.3			3.0	12.3	21.4		3.2	2.9
Spirorbidae									0.1
Trochidae	2.9								0.3
Neritidae	<i>S. souverbiana</i>	11.8	9.4	17.2	3.1	5.9	4.1		3.8
Rissoidae	sp. 1	20.0	27.6	11.8		13.1	23.3	3.9	8.8
Litiopidae	<i>Alaba virgata</i>		55.3	18.6					7.8
Columbellidae	<i>Zafra</i>								0.2
Sacoglossa	sp. n	7.6	4.4		4.2	10.9			3.8
Pteriidae	<i>Electroma</i>					5.8	4.3		1.8
Mytilidae								2.3	0.4
other bivalves									0.2
Caprellidae									0.3
Amphithoidae	<i>Cymadusa</i>					10.6	2.4	8.2	1.9
Eusiridae				3.0				28.9	2.5
Ischyroceridae	<i>Erichthonius</i>					2.5		69.2	10.4
	<i>Cerapus</i>								2.8
Sphaeromatidae		2.9							0.3
Munnidae						7.5	14.5		2.8
Pycnogonida			5.5						0.3
Chironomidae							2.9		1.7
Actiniaria		17.7	56.0	12.7			8.5	73.5	13.3
Total		67.2	152.6	65.7	13.4	62.8	52.9	140.5	85.7

**Table A.3 Mean number of invertebrates per 100 cm<sup>2</sup> *Halodule uninervis* leaf surface in plot CB, n=10 on each sampling date. Part 1 of 3.**

Date		29-May-02	27-Jun-02	20-Jul-02	19-Aug-02	19-Sep-02	17-Oct-02
Mean sample leaf area (cm <sup>2</sup> )		6.9	6.1	5.3	5.3	6.1	5.9
Nemertea							
Spionidae							
Cirratulidae						1.3	
Capitellidae							
Opheliidae							
Phyllodocidae							
Sigalionidae							
Syllidae		1.5		7.5	6.0	1.9	2.7
Nereididae	sp. 1		21.3	34.4			
	others		2.7	6.4	1.5	3.5	3.5
Sabellidae		1.1				1.5	
Spirorbidae							
Turbinidae	<i>Tricolia</i>						
Trochidae		2.9					
Neritidae	<i>S. souverbiana</i>			3.1			2.8
Rissoidae	sp. 1				1.5		
	sp. 2						
Litiopidae	<i>Alaba virgata</i>	9.2	17.1	1.8	15.2	1.5	5.6
	<i>A. translucida</i>			1.9		5.5	5.0
Scaliolidae	<i>Finella</i>						
Columbellidae	<i>Zafra</i>						
Rissoellidae	<i>Rissoella</i>						
Haminoeidae		1.4			2.6		
Sacoglossa				19.1			1.7
Aplysiidae	<i>N. indicus</i>						
Aeolidiidae	<i>L. fusiformis</i>						
Pteriidae	<i>Electroma</i>	3.8					
other bivalves							
Caprellidae							
Amphilochidae							
Amphithoidae	<i>Cymadusa</i>	8.1	27.9	19.5	11.8	9.5	8.8
Dexaminidae		1.4	7.9	58.5	35.3	2.2	
Eusiridae			12.0	1.9	4.3	3.1	
Ischyroceridae	<i>Erichthonius</i>	114.8	69.4	165.2	17.6	122.3	88.1
Podoceridae	<i>Podocerus</i>	23.4	11.3	18.2	3.2	13.0	3.5
Sphaeromatidae		3.5	2.7	1.9	3.0		2.7
Munnidae		1.4					
Leptocheiliidae			8.9	24.5	7.2	11.9	1.8
otherTanaidacea			1.6				
Cumacea			2.9		1.5	2.2	2.6
Brachyura megalops							1.3
Hippolytidae						1.9	1.8
Palaemonidae							
Chironomidae		1.6	10.3	11.1	17.3	37.9	9.8
Actiniaria					1.5		
Eleutheriidae	<i>Staurocladia</i>			1.4	17.1	11.1	4.2
Totals		174.1	196.1	376.4	146.6	230.2	146.0



**Part 2 of Table A.3.**

Date		2-Dec-02	1-Feb-03	1-Mar-03	27-Apr-03	11-Jun-03	30-Jul-03
Mean sample leaf area (cm <sup>2</sup> )		7.0	7.6	9.3	7.2	6.3	6.8
Nemertea							
Spionidae							
Cirratulidae							
Capitellidae							
Opheliidae				2.9		2.6	
Phyllodocidae				0.7			
Sigalionidae			1.6				
Syllidae		8.7	9.4	61.9	4.1	18.0	2.6
Nereididae	sp. 1	1.8					
	others	1.0	5.2	11.7		0.9	1.4
Sabellidae		1.4	1.3	7.0			
Spirorbidae							
Turbinidae	<i>Tricolia</i>				0.9		
Trochidae					0.9		
Neritidae	<i>S. souverbiana</i>						1.5
Rissoiidae	sp. 1	6.9	3.7		1.7		1.4
	sp. 2						
Litiopidae	<i>Alaba virgata</i>	1.4			2.9	4.7	18.3
	<i>A. translucida</i>						
Scaliolidae	<i>Finella</i>				2.2		
Columbellidae	<i>Zafra</i>				0.9		
Rissoellidae	<i>Rissoella</i>			3.5			
Haminoeidae				0.8			3.4
Sacoglossa				3.7			
Aplysiidae	<i>N. indicus</i>			2.5			
Aeolidiidae	<i>L. fusiformis</i>						11.9
Pteriidae	<i>Electroma</i>				1.9		
other bivalves				0.8			
Caprellidae						5.6	
Amphilochoidea							
Amphithoidae	<i>Cymadusa</i>	11.8	7.9	51.6	10.0	12.9	5.7
Dexaminidae			12.6			14.2	3.1
Eusiridae		1.3	16.1	59.8			
Ischyroceridae	<i>Erichthonius</i>	2.9	6.9	120.3	812.1	130.5	119.0
Podoceridae	<i>Podocerus</i>			1.7	20.8	83.0	49.5
Sphaeromatidae		3.3	4.2	1.5	21.0	51.5	16.0
Munnidae		2.8	1.2	4.5	5.8	12.0	1.5
Leptocheliidae		21.4	1.6	4.4			
other Tanaidacea							
Cumacea		4.2	27.4	34.1	20.1	15.1	3.8
Brachyura megalops		1.4					
Hippolytidae							
Palaemonidae			0.8	0.9			
Chironomidae		4.1	1.6				
Actiniaria			46.7	33.7	467.5	553.9	
Eleutheriidae	<i>Staurocladia</i>	1.4	0.8				
Totals		75.9	149.0	407.9	1372.7	904.9	239.0

**Part 3 of TableA.3.**

Date	21-Sep-03	23-Nov-03	22-Jan-04	19-Mar-04	19-May-04	15-Jul-04	Mean
Mean sample leaf area (cm <sup>2</sup> )	5.2	6.5	6.8	8.0	7.8	8.6	
Nemertea	2.3					1.1	0.2
Spionidae			1.9				0.1
Cirratulidae			1.0				0.1
Capitellidae			1.7				0.1
Opheliidae					2.5	2.4	0.6
Phyllodocidae			1.7				0.1
Sigalionidae							0.1
Syllidae	2.3	0.9	1.7		13.8	11.4	8.6
Nereididae						6.2	3.5
	2.1	1.9			1.4		2.4
Sabellidae	2.2		3.1		23.3	6.3	2.6
Spirorbidae				1.1	11.1	6.4	1.0
Turbinidae							<0.1
Trochidae					1.7		0.3
Neritidae							0.4
Rissoidae	12.9	5.5	14.3		1.1	2.7	2.9
			7.0				0.4
Litiopidae			3.3	1.2	2.6	8.5	5.2
						1.3	0.8
Scaliolidae							0.1
Columbellidae							<0.1
Rissoellidae					4.1		0.4
Haminoeidae			7.4			2.7	1.0
Sacoglossa			2.8		1.5	1.1	1.7
Aplysiidae							0.1
Aeolidiidae						3.2	0.8
Pteriidae							0.3
other bivalves							<0.1
Caprellidae	1.1				50.9		3.2
Amphilochidae	1.8					2.5	0.2
Amphithoidae	2.8	20.3	10.8	4.5	18.3	12.2	14.1
Dexaminidae	2.4				1.1	5.1	8.0
Eusiridae	1.1			1.1	3.1	4.9	6.0
Ischyroceridae	74.9	44.8	9.2	108.5	421.2	100.9	140.5
Podoceridae	5.1			17.3	62.4	18.7	18.4
Sphaeromatidae	2.1		2.7		9.6	3.1	7.2
Munnidae	1.6	1.2			4.3	5.3	2.3
Leptocheliidae	2.2		3.8				4.9
otherTanaidacea	1.1						0.2
Cumacea		4.6	2.3	1.7	27.3	20.7	9.5
Brachyura megalops							0.2
Hippolytidae		1.2					0.3
Palaemonidae							0.1
Chironomidae	3.9	3.8	1.7		3.0	30.8	7.6
Actiniaria	2.2	1.8		5.2	541.2	103.4	97.6
Eleutheriidae				1.8			2.1
Totals	124.2	86.0	76.5	142.5	1205.6	361.0	356.4

**Table A.4 Mean number of invertebrates per 100 cm<sup>2</sup> *Halophila ovalis* leaf surface in plot CB, n=5 on each sampling date.**

Date	21-Sep-03	23-Nov-03	22-Jan-04	19-Mar-04	19-May-04	15-Jul-04	Mean	
Mean sample leaf area (cm <sup>2</sup> )	4.6	8.2	4.8	5.4	8.9	4.9		
Phyllodocidae					3.6		0.6	
Syllidae	13.1		24.3		21.1	15.1	12.3	
Nereididae					3.6	3.9	1.2	
			4.9				0.8	
Sabellidae			13.6		10.8		4.1	
Spirorbidae	3.7				1.3		0.8	
Trochidae						5.2	0.9	
Rissoidae	3.9		31.8				6.0	
Rissoellidae					1.3		0.2	
Haminoeidae			3.6			50.7	9.1	
Sacoglossa		3.9	53.0				9.5	
Aplysiidae			4.9				0.8	
Gymnodoridae			3.6				0.6	
Pteriidae				6.0			1.0	
other bivalves			5.1				0.8	
Caprellidae					1.3		0.2	
Amphilochidae					5.5	5.2	1.8	
Amphithoidae		<i>Cymadusa</i>	14.1	13.8	3.4	9.7	11.6	8.8
Dexaminidae	8.8					30.5	6.6	
Eusiridae					1.3		0.2	
Ischyroceridae	3.9	6.2	15.0	17.2	49.5	90.7	30.4	
Podoceridae	4.9				5.1	5.2	2.5	
other Ampipoda						5.2	0.9	
Sphaeromatidae	3.9				6.1		1.7	
Munnidae		<i>Munna</i>			42.0	4.7	7.8	
Leptocheliidae	14.7	4.3	4.9				4.0	
other Tanaidacea					4.2		0.7	
Cumacea	9.7		13.0		47.4	40.4	18.4	
Chironomidae					6.1	4.2	1.7	
Ophiuroidea					3.6		0.6	
Actiniaria	9.7			9.6	380.9	32.6	72.1	
Eleutheriidae	8.6						1.4	
Totals	85.1	28.6	191.4	36.2	634.6	274.8	208.5	

**Table A.5 Mean number of invertebrates per 100 cm<sup>2</sup> Halodule uninervis leaf surface in plot PB, n=10 on each sampling date. Part 1 of 3.**

Date		25-Jun-02	24-Jul-02	22-Aug-02	4-Oct-02	4-Nov-02	6-Dec-02
Mean sample leaf area (cm <sup>2</sup> )		6.3	6.5	5.6	7.8	10.6	11.0
Nemertea					0.9		
Opheliidae							
Syllidae					9.7	3.2	10.1
Nereididae					2.9		1.4
Sabellidae							
Spirorbidae						15.3	14.3
Turbinidae	<i>Tricolia</i> <i>Phasianella</i>				2.4	4.0	1.6
Trochidae				0.8			
Neritidae	<i>S.</i> <i>souverbiana</i> sp. 2	1.7				4.9	1.9
Rissoidae		1.4			4.0		
Cerithiidae							
Litiopidae	<i>Alaba virgata</i> <i>A. translucida</i>	21.1	20.4	12.7	11.5	45.1	6.2 0.7
Scaliolidae	<i>Finella</i>						
Columbellidae							1.8
Rissoellidae	<i>Rissoella</i>						
Haminoeidae							
Sacoglossa							
Aplysiidae							2.5
other							
Gastropoda							
Pteriidae	<i>Electroma</i>	36.7	37.2	34.6	56.7	61.7	3.6
Mysidae	<i>Siriella</i>						
Caprellidae							
Amphilochoidea							
Amphithoidae	<i>Cymadusa</i>	2.9	3.9	7.5	2.8	0.9	14.2
Dexaminidae				1.6	2.7		1.8
Eusiridae		3.1	2.7	3.6	2.7		1.9
Ischyroceridae	<i>Erichthonius</i> <i>Ischyrocerus</i>	120.0	90.3	122.2	133.0	8.8	29.3
Podoceridae	<i>Podocerus</i>	18.7	35.6	37.2	64.7	20.9	11.0
other		1.5		2.6			
Amphipoda							
Sphaeromatidae		3.0	4.3	4.8		5.7	6.9
Munnidae					3.4		3.7
Leptocheliidae							7.2
otherTanaidacea							1.8
Cumacea					2.1		12.3
Hippolytidae							
Chironomidae					11.4	2.2	29.6
Actiniaria				1.8			
Eleutheriidae	<i>Staurocladia</i>				14.7	11.8	14.0
Totals		210.1	194.4	229.5	325.6	184.4	177.8

**Part 2 of Table A.5.**

Date		4-Jan-03	17-Feb-03	18-Apr-03	16-May-03	10-Jul-03	28-Aug-03
Mean sample leaf area (cm <sup>2</sup> )		12.8	11.9	16.0	13.0	11.2	10.2
Nemertea							
Opheliidae				0.6			
Syllidae		10.7	6.5	5.6	2.8		
Nereididae		0.5	2.9		1.5		0.8
Sabellidae				0.5			
Spirorbidae		47.3	3.6	2.5	0.7		
Turbinidae	<i>Tricolia</i>	25.8	5.6	20.5	3.0	16.2	0.8
	<i>Phasianella</i>					1.0	
Trochidae		0.7					
Neritidae	<i>S. souverbiana</i> sp. 2	2.6		0.9		1.0	
Rissoidae		0.7					
Cerithiidae			0.6	0.5			
Litiopidae	<i>Alaba virgata</i>	7.3	11.9	1.4	2.3	12.9	23.5
	<i>A. translucida</i>						
Scaliolidae	<i>Finella</i>						
Columbellidae							
Rissoellidae	<i>Rissoella</i>			8.0			
Haminoeidae		0.5	0.6				
Sacoglossa			0.6				
Aplysiidae							
other			0.6				
Gastropoda							
Pteriidae	<i>Electroma</i>	6.2	1.6	3.9	12.0	13.0	7.7
Mysidae	<i>Siriella</i>	1.2					
Caprellidae			1.0				
Amphilochidae		1.1					
Amphithoidae	<i>Cymadusa</i>	8.4	6.6	3.7	6.9	1.1	0.8
Dexaminidae							
Eusiridae		2.9	3.2	1.6	18.8	2.1	0.8
Ischyroceridae	<i>Ericthonius</i>	33.4	32.5	3.1	76.4	5.5	26.8
	<i>Ischyrocerus</i>		1.0			2.0	
Podoceridae	<i>Podocerus</i>	44.0	17.4	3.0	41.1	6.3	10.6
other Amphipoda							
Sphaeromatidae		16.6	7.1	2.2	5.7	1.7	1.9
Munnidae		9.3		2.8			1.5
Leptocheliidae					1.5		
OtherTanaidacea							
Cumacea		10.7	1.8	2.8			0.7
Hippolytidae							
Chironomidae		1.1	2.1	17.8			
Actiniaria				2.4	1.6		
Eleutheriidae	<i>Staurocladia</i>	2.8		2.9			
Totals		233.8	107.1	86.8	174.3	62.7	75.9

**Part 3 of Table A.5.**

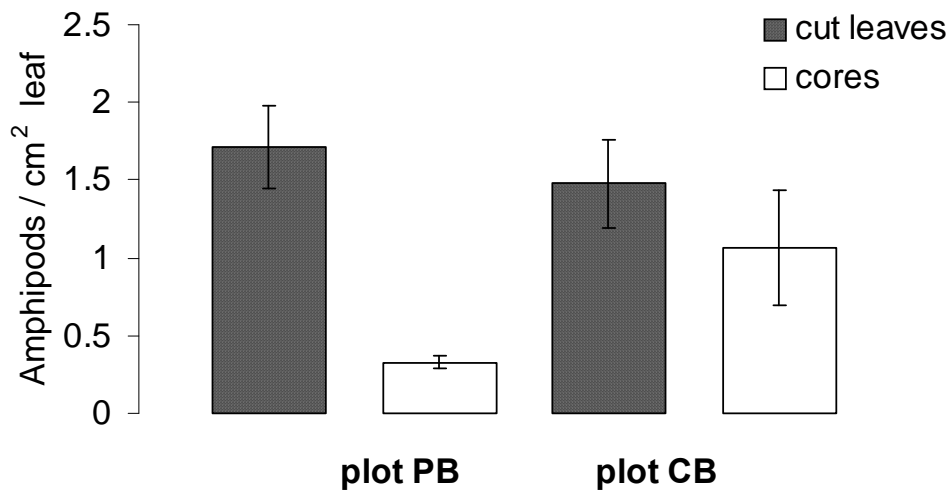
Date		27-Oct-03	21-Dec-03	18-Feb-04	5-May-04	3-Jul-04	Mean
Mean sample leaf area (cm <sup>2</sup> )		14.6	21.4	11.5	8.1	8.2	
Nemertea							0.1
Opheliidae							<0.1
Syllidae		2.5	3.1	1.5			3.3
Nereididae		1.1					0.7
Sabellidae							<0.1
Spirorbidae		3.9	3.6		7.0		5.8
Turbinidae	<i>Tricolia</i>		0.4	1.8	12.8	5.8	5.9
	<i>Phasianella</i>						0.1
Trochidae						1.1	0.2
Neritidae	<i>S. souverbiana</i>		0.7				0.8
	sp. 2		0.9	4.4			0.7
Rissoidae							0.1
Cerithiidae							0.1
Litiopidae	<i>Alaba virgata</i>	2.4	4.5		1.2	1.7	10.9
	<i>A. translucida</i>	0.5					0.1
	<i>Finella</i>		0.8				<0.1
Scaliolidae							0.1
Columbellidae							0.1
Rissoellidae	<i>Rissoella</i>		0.5				0.5
Haminoeidae		7.1	2.6				0.6
Sacoglossa							<0.1
Aplysiidae				0.9			0.2
other							<0.1
Gastropoda							
Pteriidae	<i>Electroma</i>	5.2		2.0	23.4	2.1	18.1
Mysidae	<i>Siriella</i>		0.4				0.1
Caprellidae							0.1
Amphilocheidae							0.1
Amphithoidae	<i>Cymadusa</i>	9.3	3.0			1.1	4.3
Dexaminidae		2.0					0.5
Eusiridae		1.2	2.8	4.8	14.4	4.1	4.2
Ischyroceridae	<i>Ericthonius</i>	6.4	2.5	11.0	8.7	42.7	44.3
	<i>Ischyrocerus</i>					2.3	0.3
Podoceridae	<i>Podocerus</i>	1.9	3.8	0.8		9.1	19.2
other Amphipoda							0.2
Sphaeromatidae		1.9	1.9	1.5	1.2		3.9
Munnidae			0.8				1.3
Leptocheliidae		0.8					0.6
OtherTanaidacea							0.1
Cumacea		0.5					1.8
Hippolytidae		0.8					<0.1
Chironomidae		1.4	0.4				3.9
Actiniaria					1.0		0.4
Eleutheriidae	<i>Staurocladia</i>	1.5		1.0			2.9
Totals		50.6	32.6	29.6	69.8	70.0	136.2

## **Appendix B: Comparison of invertebrate sampling techniques: coring versus leaf cutting**

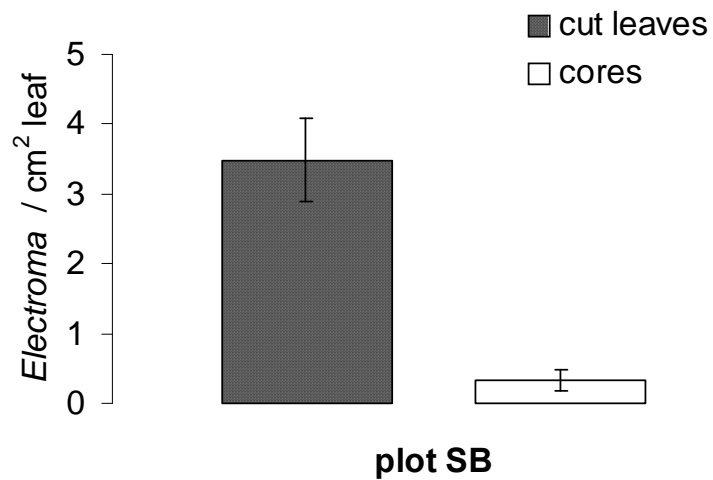
As part of preliminary sampling, invertebrates were sampled using a corer, and compared with samples taken using the leaf-cutting technique, which is described in chapter 2, section 2.3. Cores (45 cm<sup>2</sup>) were taken at 10 randomly selected sites in plot CB in May, and plot SB in early June. In plot PB, 6 cores were taken in May. The top centimetre of sediment in the core, and associated seagrass leaves with roots and rhizomes, was washed in a 0.5 mm sieve. The sieve residue was covered in clean seawater and brought back to the lab, where the animals were removed while still alive. The surface area of seagrass in those cores was estimated, using the technique described in chapter 2, section 2.5. The numbers of animals removed were expressed as numbers per cm<sup>2</sup> of *Halodule uninervis* leaf. Those numbers have been compared with the numbers of animals recovered from 10 samples of cut *H. uninervis* leaves taken in May in plots CB and PB, and early June in plot SB.

The results showed that greater numbers of animals were recovered per area of leaf in cut leaf samples than in core samples. Most notably, densities of amphipods were greatest on cut leaves in both plots CB and PB (Fig. B.1). Amphipods are highly mobile, and it was suspected that they might avoid being caught on cut leaves, but that did not appear to be the case. The greater numbers on cut leaves occurred despite the inclusion in cores of habitats other than the leaf surface, and some of the animals recovered were clearly benthic or epibenthic in origin.

Amphipods dominated the epifauna in plots CB and PB at the time of the sampling, but their numbers were low in plot SB. In that plot, *Electroma* dominated. Its density per area of *H. uninervis* leaf in cuttings from plot SB was 10 times greater than in cores (Fig. B.2). Many of those *Electroma* were delicate immature specimens, which were probably missed in the sieve residue of core samples once they became detached from the leaves. Likewise, small amphipods may become damaged when core samples are washed, and are not readily recovered from the residue. The small gastropod *Alaba virgata* was sometimes extremely abundant in plot SB; but at the time of the comparison sampling, it occurred in such low numbers that a meaningful comparison could not be made.



**Fig. B.1** Mean number ( $\pm$  s.e.) of *Amphipoda* per cm<sup>2</sup> *Halodule uninervis* leaf in cut leaf and core samples in plots PB and plot CB (non-transformed data).

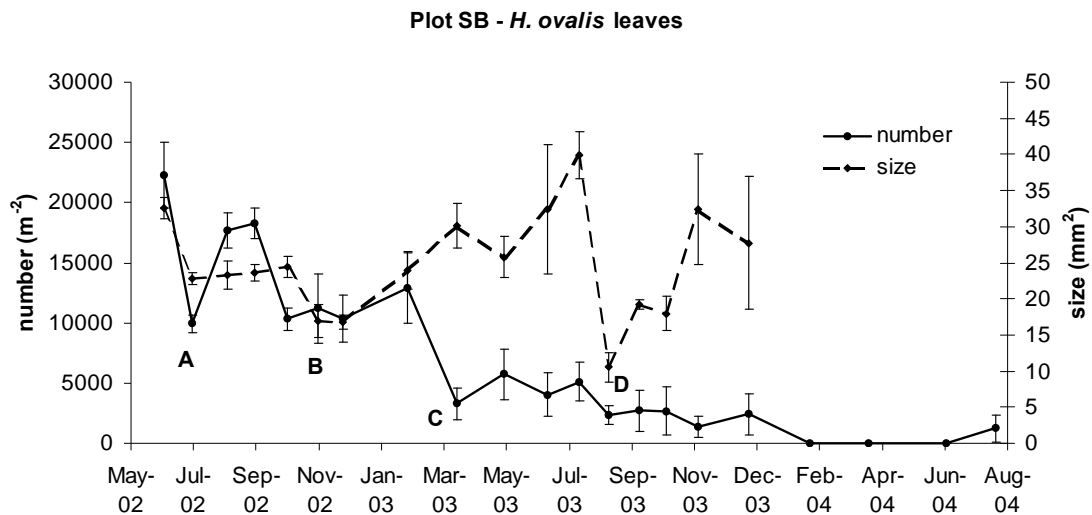


**Fig. B.2** Mean number ( $\pm$  s.e.) of *Electroma* per cm<sup>2</sup> *Halodule uninervis* leaf in cut leaf and core samples in plots SB (non-transformed data).



## Appendix C: Changes in leaf morphology

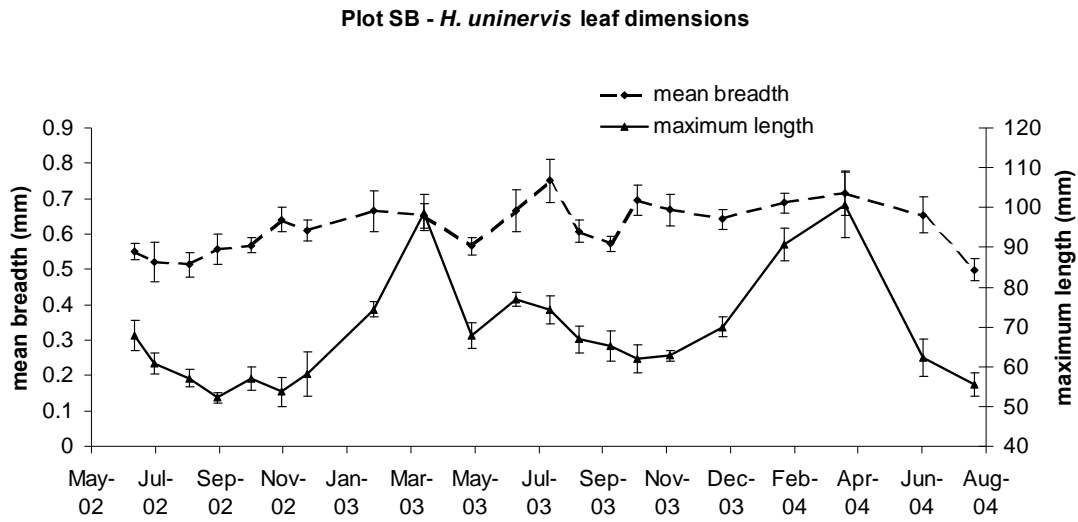
The density of *Halophila ovalis* leaves in plot SB declined overtime, closely paralleling the decline in LAI (Fig. 7.1). The average size of *H. ovalis* leaves, calculated as the area on one side, varied considerably (Fig. C.1). The variance of mean size estimates increased as the numbers of leaves available for measurement decreased.



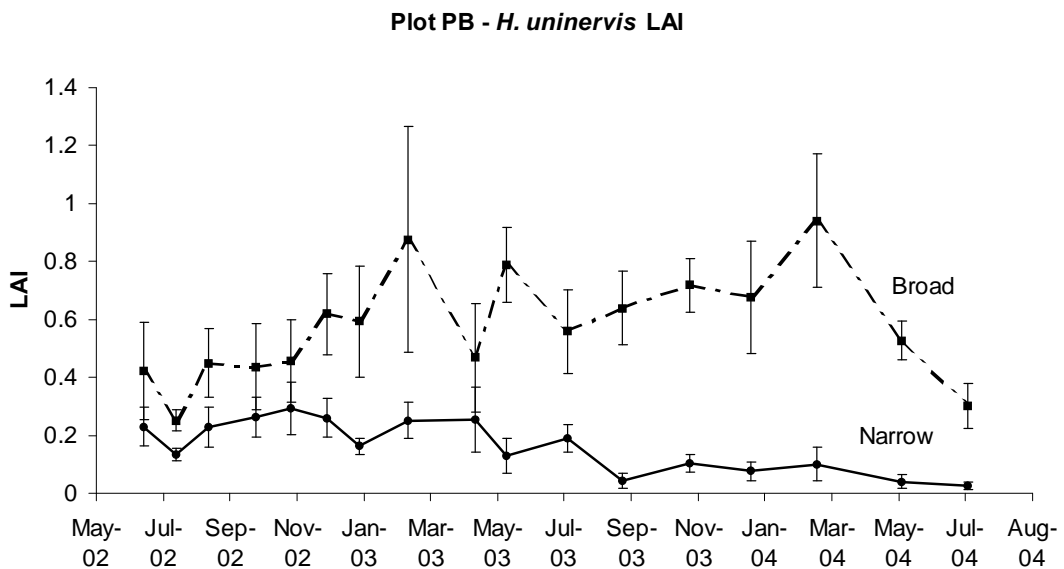
**Fig. C.1** Mean densities and mean size ( $\pm$  s.e.) of *Halophila ovalis* leaves in cores from plot SB. Data from the first four sampling dates are also presented in Fig. 3.4. Letters A to D as in Fig. 7.1.

In plot SB, *Halodule uninervis* leaves in samples of cut leaves were consistently very narrow, with mean breadths ranging from  $0.50 \pm 0.032$  to  $0.75 \pm 0.060$ (s.e.) mm. The changes in mean breadth did not show a seasonal pattern, in marked contrast to the highly seasonal changes in leaf length (Fig. C.2).

In plot PB, *Halodule uninervis* leaves were broader, but could be readily classified into two distinct forms. The broad leaf form had leaves approximately 3.0 mm wide, and dominated the site. The leaves were classified between those <1.5 mm wide, and those >1.5 mm for the calculation of LAIs for each form. The narrow form declined significantly over the course of the study (Fig. C.3). The narrow form tended to have longer leaves, so maximum leaf length in samples of cut leaves have been measured from among the broad leaves only (Fig. C.4).



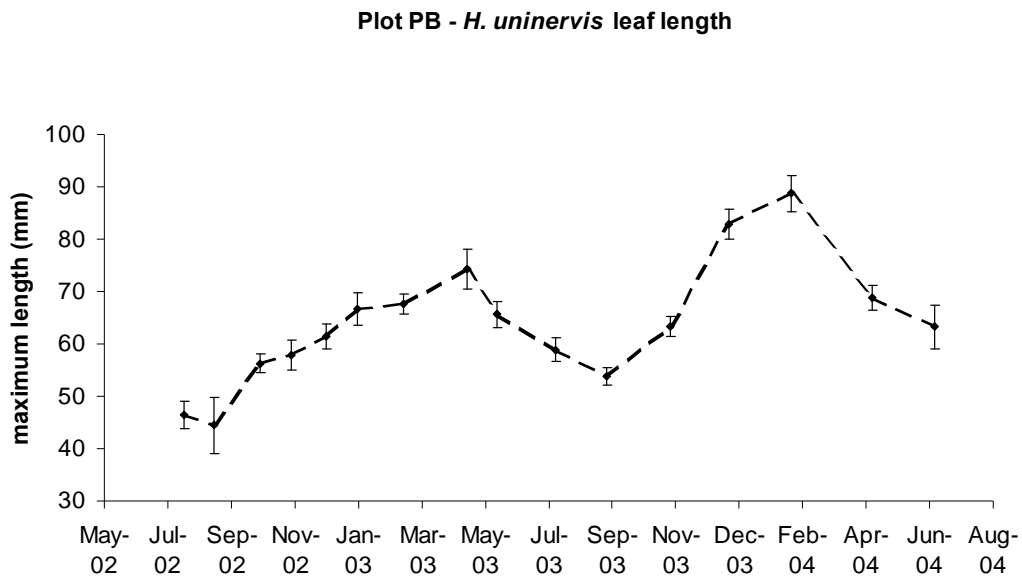
**Fig. C.2** Mean maximum lengths and mean breadths ( $\pm$  s.e.) of *Halodule uninervis* in cut leaf samples ( $n=10$ ) from plot SB. Mean maximum lengths are also presented in Fig. 5.3.



**Fig. C.3** Mean LAI values ( $\pm$  s.e.) of *Halodule uninervis* leaves  $<1.5$  mm and  $>1.5$  mm wide in core samples ( $n=5$ ) from plot PB.

The lengths of *Halodule uninervis* leaves were greatest during the hot season. Mean maximum lengths tended to be negatively correlated to the tidal exposures of both plots SB ( $r=-0.38$ ,  $0.05 < p < 0.10$ ) and PB ( $r=-0.49$ ,  $0.05 < p < 0.10$ ). However, those lengths were more clearly related to epiphytic cover of the leaves, with stronger positive

correlations in both plot SB ( $r=0.51$ ,  $p<0.025$ ) and PB ( $r=0.51$ ,  $p<0.05$ ). *H. uninervis* leaf length was also found to increase as a result of experimental shading at Shelly Beach (chapter 9), and in the experiments of Longstaff and Dennison (1999). Therefore, changes in *H. uninervis* leaf length appear to be a good indicator of changes in the light environment experienced by the seagrass over the short-term. In contrast, the size of *Halophila ovalis* leaves in plot SB was not correlated to either tidal exposure or epiphytic cover, and did not respond to shading.



**Fig. C.4** Mean maximum length ( $\pm$  s.e.) of *Halodule uninervis* leaves  $>1.5$  mm wide in cut samples from plot PB.

## Appendix D: Sediment characteristics

Sediment particle-size differed greatly among the sites. At Shelly Beach, sediments were deep, and consisted of very fine sand particles between 125 and 63  $\mu\text{m}$ , and mud <63  $\mu\text{m}$ . At Cockle Bay, the largest category of particles were coarse sands, >500  $\mu\text{m}$ ; but there was a heterogeneous mix of elements, including coral rubble, calcareous fragments of mollusc shell and *Halimeda*, and spicules of soft corals. Both the Shelly Beach and Cockle Bay sites had extensive areas of soft mud inshore of the plots, which had the potential to become re-suspended. At Picnic Bay, the sediments were shallow fine sands, >125  $\mu\text{m}$ , overlaying coral rubble.

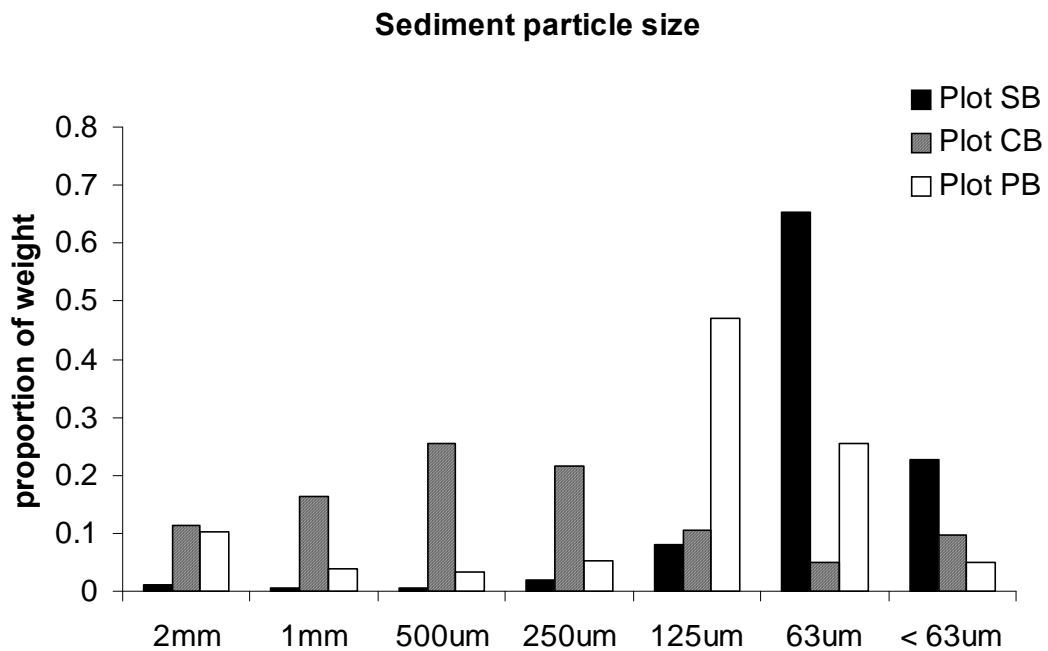


Fig. D.1 Weight of material retained on sieves of different mesh sizes expressed as a proportion of the dry weight of sediment samples.

