THE ECOLOGICAL ROLE OF SEA HARES (OPISTHOBRANCHIA: ANASPIDEA) WITHIN TROPICAL INTERTIDAL HABITATS

Thesis submitted by:

Cathryn Lynne CLARKE

BSc (Zoology), BSc (Marine Biology), Dipl. Res. Methods

in March 2004

for the degree of Masters of Science by Research in the School of Marine Biology & Aquaculture James Cook University I, the undersigned, author of this thesis, understand that James Cook University will make it available for use within the University Library and, via t he Australian Digital Theses network, for use elsewhere. All users consulting this thesis will have to sign the following statement:

In consulting this thesis I agree not to copy of closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgement for any assistance which I have obtained from it.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

Beyond this, I do not wish to place any further restriction on access to this thesis.

Signature

Date

Abstract

Herbivory in temperate marine ecosystems has been the focus of widespread research attention. In comparison, little is known about tropical herbivores and the role they play in controlling the abundance of marine macrophytes. This study investigates the role of one group of invertebrate herbivores, the sea hares. Their role as herbivores in tropical intertidal habitats was examined at four study sites situated near Townsville, Queensland, Australia. The roles these animals play as herbivores were investigated by examining the variation in density and feeding specificity.

The variation in density was documented by surveying the distribution and abundance of sea hares and their food macrophytes for one year. In addition, the population dynamics of population irruptions of sea hares at a single site were examined in greater detail. There was extreme variation in the densities reached by each of the five local species recorded. *Aplysia dactylomela*, *Aplysia extraordinaria*, and *Petalifera petalifera* remained at low abundance throughout the sampling period. In contrast, *Bursatella leachii* and *Stylocheilus striatus* were found in extremely high density within the seagrass beds of Shelly Beach in association with a bloom of the cyanobacteria *Calothrix crustacea*. The population dynamics of *Bursatella leachii* showed that at this location, the population underwent continuous recruitment in contrast to the results of previous studies.

The relationships between each sea hare species and their preferred host macrophytes were species specific. The density of *Aplysia dactylomela* was positively correlated with a limited number of red algae species, but the clearest relationship was with the red macrophytic group (Rhodophyta), a finding consistent with previous feeding preferences experiments for this species. *Aplysia extraordinaria* density was correlated with several of the available red algae species however because of the low density, its feeding ecology remains unclear. *Petalifera petalifera* was found to maintain a very strict association with the brown calcified alga, *Padina tenuis*. The temporal distribution of all sea hares was restricted to the winter months, a relationship that may be related to environmental limitations, such as ultraviolet radiation. The abundances of *B. leachii* and *S. striatus* could not be quantitatively related to any of the algal species recorded because cyanobacteria bloom abundance was not estimated.

The feeding specificity of *Bursatella leachii* was more closely examined using two-way choice preference testing. This species preferred to consume the green alga, *Enteromorpha* sp. to the cyanobacterium *Calothrix crustacea*, the brown alga *Sargassum* sp., and the red alga *Pterocladia pinnata*. There was no difference in the feeding hierarchy obtained by examining edibility or attractiveness. Feeding specificity of the six local sea hares was also investigated by examining the morphology of the radula feeding organ. Scanning electron microscopy was used to examine the fine details of the radula teeth. Three radula teeth types were created based on the sea hare radulae examined: simple, bilobed and denticulate. These radula types correspond to the feeding preferences exhibited by each sea hare. Sea hares with complex radulae were more likely to be highly specialised feeders while those with simple radulae were more likely to be generalists. A theoretical framework was developed, based on these radula types, which can now be used to make testable predictions about feeding preferences of sea hare species with unknown feeding specificity.

The results of this study demonstrate that the density and feeding specificity of sea hares are species specific. Therefore care should be taken in extrapolating information from studies based on a small number of sea hare species to the entire sea hare group. Three of the sea hare species examined within (*A. dactylomela, A. extraordinaria* and *P. petalifera*) are not likely to play major roles as herbivores in their habitats as a result of their low densities. However, *Bursatella leachii* and *Stylocheilus striatus* would exert a strong influence on their seagrass habitats as a because of their high density and feeding specialisation on cyanobacteria. Sea hares may play an increased role in habitats, such as shallow intertidal ecosystems, unavailable to the primary herbivores.

Acknowledgments

This work could not have been undertaken and completed without the support and encouragement of many remarkable people. I would first like to thank my family and friends both in Canada and Australia for their encouragement in following my passion, especially Carol Clarke, Andrew Clarke, Peter Clarke and Ronald Brimacombe. I would like to extend my sincere, abounding gratitude to my supervisors, Dr. Gilianne Brodie, Associate Professor Dr. Rocky de Nys and Junior Professor Dr. Annette Klussmann-Kolb for their knowledge, confidence and advice.

Research facilities and technical support were kindly provided by John Morrison & the staff of MARFU and Kevin Blake of the Advanced Analytical Centre. I am grateful to John Collins, Jon Brodie and Laura Castell for their guidance. Specimens and technical advice were supplied by Barry Bendell, Josiah Pit, Erika Martinez-Fernandez, Jane Webb, Odette Ison, Katherine Thompson and Jackie Wolstenholme. Yvette Everingham and Tim Hancock offered statistical advice. Janice Lough allowed the use of weather information collected jointly by AIMS and GBRMPA. I would also like to thank the following people who served as my willing field and laboratory assistants: Andrew Graffen, Jessica Maddams, Raymond Bannister, Andrew Bauman, Ross Bauer, Luke Gardner, Heidi Ludwick, Andrew Clarke, Lachlan Barnes, and Even Moland. Two anonymous reviewers provided valuable comments on an earlier version of this composition

This work was supported by grants from the CRC Reef and the Malacological Society of Australasia. I would like to specifically thank Tim Harvey and Britta Schaffelke of the CRC Reef who were always available for both logistical and academic assistance. The School of Marine Biology and Aquaculture at James Cook University provided further practical and financial resources.

I would like to dedicate this work to my parents, who have given me the greatest gift, my love for the sea.

Table of Contents

Statement of Access		- 2 -
Abstract		- 3 -
Acknowledgements		- 5 -
List of Figures		- 7 -
List of Tables		- 11 -
Statement of Sources		- 12 -
Declaration on Ethics		- 13 -
Chapter 1:	Herbivory in tropical marine ecosystems	- 14 -
Chapter 2:	The spatial and temporal variation of sea hare populations in tropical intertidal habitats	- 24 -
Chapter 3:	The population dynamics of a <i>Bursatella leachii</i> population irruption at Shelly Beach	- 67 -
Chapter 4:	The feeding preferences of <i>Bursatella leachii</i> from Shelly Beach	- 83 -
Chapter 5:	The interaction of sea hare radula teeth morphology and feeding type	- 99 -
Chapter 6:	The role of sea hares as herbivores in tropical habitats	- 117 -
Literature Cited		- 124 -

List of Figures

Chapter 1

Figure 1.1: The sea hare species investigated during the current study: a) *Aplysia dactylomela*, scale bar = 50mm (Photo courtesy A. Klussmann-Kolb), b) two colour morphs of *Aplysia extraordinaria*, scale bar = 30mm (Photo courtesy A. Klussmann-Kolb). c) *Bursatella leachii* within *Sargassum* thalli, scale bar = 15mm (Photo courtesy A. Klussmann-Kolb), d) *Dolabella auricularia*, scale bar = 29.2mm (Photo courtesy A. Klussmann-Kolb), e) *Petalifera petalifera*, scale bar = 2.3mm and f) *Stylocheilus striatus*, scale bar = 4.4mm (Photo courtesy J. Collins).

- Figure 2.1: Map of study sites (*) around the Townsville (S 19° 10', E 146° 45') and Magnetic Island (S 19° 10', E 146° 50') area. Inset shows location of map within Queensland, Australia. Maps adapted from Geoscience Australia.
- Figure 2.2: Hierarchical nested sampling design for monitoring the spatial and temporal variation in algal species diversity and abundance.
- Figure 2.3: Hierarchical nested sampling design for monitoring the spatial and temporal variation in sea hare species diversity and density.
- Figure 2.4: The mean percent cover of each marine macrophyte present at Kissing Point during the October 2002-September 2003 sampling period. Only those species with a mean percent cover of greater than 0.1% are shown. For complete genus names see Table 2.1.
- Figure 2.5: The mean percent cover (+/- standard error) of the three seagrass species present at Shelly Beach during the October 2002-September 2003 sampling period.
- Figure 2.6: The mean percent cover (+/- standard error) of the marine macrophytes present at Picnic Bay during the October 2002-September 2003 sampling period. Only those species with a mean percent cover greater than 0.1% were included.
- Figure 2.7: The mean percent cover (+/- standard error) of the marine macrophytes present at Cockle Bay during the October 2002-September 2003 sampling period. Only those species with a mean percept cover greater than 0.1% were included.
- Figure 2.8: Temporal variation of marine macrophytes found at greater than 5% mean percent cover (+/- standard error) in any of the sampling months (October 2002 September 2003) at Kissing Point, a) *Halodule uninervis, Padina tenuis,* and Red sp.3, b) *Gelidium* sp., *Sacronema filiforme,* and Red sp. 1, c) *Acrocystis nana, Ceramium* sp., and *Hypnea pannosa.*
- Figure 2.9: The temporal variation in mean percent cover (+/- standard error) of the three seagrass species found at Shelly Beach between October 2002 and September 2003.

- Figure 2.10: The temporal variation in mean percent cover (+/- standard error) of those species with greater than 5% cover at any time during sampling at Picnic Bay between October 2002 and September 2003.
- Figure 2.11: Temporal variation in mean percent cover (+/- standard error) of those species with greater than 5% cover at any time during sampling at Cockle Bay between October 2002 and September 2003. Broken into two graphs for clarity where a) *Halophila ovalis, Halodule uninervis,* and *Acanthophora spicifera*, b) *Crouania* sp., *Halimeda opuntia,* and *Hydroclathratus clathratus.*
- Figure 2.12: Temporal variation of mean percentage cover (+/- standard error) of a) *Halodule uninervis* and b) *Halophila ovalis* at each of the four study sites between October 2002 and September 2003.
- Figure 2.13: The mean density of the four macrophytic groups (Rhodophyta, Chlorophyta, Heterokontophyta, and Seagrasses) at each of the four study sites where a) Kissing Point, b) Shelly Beach, c) Picnic Bay and d) Cockle Bay through time (October 2002 through September 2003).
- Figure 2.14: Scatter plots of the correlation between macrophytic groups where a) is Rhodophyta and Heterokontophyta, b) is Rhodophyta and Chlorophyta, c) is Heterokontophyta and Chlorophyta, d) is Seagrasses and Chlorophyta, e) is Seagrasses and Heterokontophyta and f) is Seagrasses and Rhodophyta.
- Figure 2.15: The temporal variation of the mean density (+/- standard error) of sea hare species between October 2002 and September 2003 at a) Kissing Point, b) Shelly Beach, c) Picnic Bay and d) Cockle Bay.
- Figure 2.16: The mean length (mm, +/- standard error) of *Aplysia extraordinaria* at each of the three sites this species was found within.
- Figure 2.16: The mean length (mm, +/- standard error) of *Aplysia extraordinaria* at each of the three sites this species was found within.
- Figure 2.17: The temporal variation of egg mass density (+/- standard error) at Picnic Bay, Kissing Point and Shelly Beach between October 2002 and September 2003.
- Figure 2.18: The fluctuation in the mean percentage cover of algae and sea hare density through time (October 2002 through September 2003) at a) Kissing Point, b) Shelly Beach, c) Picnic Bay and d) Cockle Bay.
- Figure 2.19: Principal components analysis of the months when sea hares were present.
 PC1 = 34.15% of variance, PC2 = 29.35%, PC3 = 19.78%. The arrows represent important component loadings: a) scatter plot of principal components 1 and 3, b) scatter plot of principal components 2 and 3. Legend: red circles are Picnic Bay, Blue diamonds are Kissing Point, green squares are Cockle Bay and purple triangles are Shelly Beach. Vectors: C = Chlorophyta, P = joint vector representing *P. petalifera* and Heterokontophyta, S = Seagrasses, A = joint vector representing *A. dactylomela*, *A. extraordinaria*, and the Rhodophyta.

- Figure 2.20: The temporal relationships between algae species and sea hare species at Kissing Point between October 2002 and September 2003 where a) *Aplysia dactylomela* and *A. extraordinaria* densities and *Pterocladia pinnata* percent cover, b) *A. dactylomela* and *Crouania* sp., c) *A. dactylomela* and *Solieria robusta*, d) *A. dactylomela* and *A. extraordinaria* densities and *Hypnea pannosa* percent cover, e) *A. extraordinaria* and *Gelidium* sp. and f) *A. extraordinaria* and *Sargassum* sp.
- Figure 2.21: The temporal relationships between algae species and sea hare species at Picnic Bay between October 2002 and September 2003 where a) *A. dactylomela* and *A. extraordinaria* mean densities and *Hypnea spinella* mean percent cover and b) *A. dactylomela* and *A. extraordinaria* mean densities and *Laurencia carolinensis*.
- Figure 2.22: The temporal relationships between algae species and *A. extraordinaria* at Cockle Bay between October 2002 and September 2003 where a) *Caulerpa racemosa*, b) *Sargassum* sp., c) *Pterocladia pinnata* and d) *Crouania* sp.
- Figure 2.23: Linear regression of the square root transformed Rhodophyta percentage cover and square root transformed *Aplysia dactylomela* density. The equation of the fitted line is: $\sqrt{(A. dactylomela)} = 0.0382*\sqrt{(Rhodophyta)} 0.170$, r value of 0.770.
- Figure 2.24: Linear regression of Heterokontophyta percentage cover and *Petalifera petalifera* density. The equation of the fitted line is *P. petalifera* = 0.001028*Heterokontophyta 0.0058, r value = 0.7283.
- Figure 2.25: The variation in air temperature, sea surface temperature (°C) and light intensity (microEinsteins m⁻² s⁻¹) over the sampling period (October 2002 to September 2003).
- Figure 2.26: The temporal relationship between light intensity (+/- standard error) and sea hare density (+/- standard error) between October 2002 and September 2003.

- Figure 3.1: The mean density (per 100m²) of *Bursatella leachii* and *Stylocheilus striatus* within the seagrass beds of Shelly Beach in 2002.
- Figure 3.2: The mean length (mm +/- standard error) of *Bursatella leachii* individuals during the four sampling trips to Shelly Beach in 2002. The letters denote significantly different subgroups as determined by Tukey's HSD post-hoc tests.
- Figure 3.3: Length-frequency histograms of *Bursatella leachii* present at Shelly Beach in 2002 during each of the four sampling trips where a) 25 June, b) 7 July, c) 21 July, and d) 27 July.
- Figure 3.4: The mean density of egg masses (per 100 m², +/- standard error) found during each sampling trip within the seagrass beds of Shelly Beach in 2002.

Chapter 4

- Figure 4.1: The mean percentage consumption (+/- standard error) of each pairwise choice trial by *Bursatella leachii*. Asterisks (*) indicate significant choices as evidenced by the respective statistical test. Each colour code represents a single two-way choice experiment denoted by letters A-F where A = *Enteromorpha* sp. vs. *Calothrix crustacea*, B = *Enteromorpha* sp. vs. *Pterocladia pinnata*, C = *Enteromorpha* sp. vs. *Sargassum* sp., D = *Calothrix crustacea* vs. *Sargassum* sp., E = *Calothrix crustacea* vs. *Pterocladia pinnata* and F = *Sargassum* sp. vs. *Pterocladia pinnata*.
- Figure 4-2: The mean number of times *Bursatella leachii* was recorded present on each alga within in each pairwise trial. Asterisks (*) indicate statistically significant differences. Each colour code represents a single two-way choice experiment denoted by letters A-F where A = *Enteromorpha* sp. vs. *Calothrix crustacea*, B = *Enteromorpha* sp. vs. *Pterocladia pinnata*, C = *Enteromorpha* sp. vs. *Sargassum* sp., D = *Calothrix crustacea* vs. *Sargassum* sp., E = *Calothrix crustacea* vs. *Pterocladia pinnata* and F = *Sargassum* sp. vs. *Pterocladia pinnata*.

- Figure 5.1: Scanning electron micrographs of the rachidian teeth of a) *Aplysia* dactylomela, scale bar = 50 μm, b) *Aplysia extraordinaria*, scale bar = 15μm, c) Bursatella leachii, scale bar = 10μm d) Dolabella auricularia, scale bar = 25 μm, e) Stylocheilus striatus, scale bar = 25 μm. Legend: Ba = tooth base, Lc = lateral cusp, Mc = median cusp.
- Figure 5.2: Scanning electron micrographs of the lateral teeth of a) *Aplysia dactylomela*, rachidian teeth are to left, scale bar = 50 μ m b) *Aplysia extraordinaria*, rachidian are to right, scale bar = 30 μ m; c) *Bursatella leachii*, rachidian are to left, scale bar = 25 μ m; d) *Dolabella auricularia*, rachidian teeth are to left, scale bar = 25 μ m; e) *Stylocheilus striatus*, rachidian is to right, scale bar = 25 μ m. Legend: L1 = primary lobe, L2 = secondary lobes.
- Figure 5.3: Scanning electron micrographs of the marginal teeth of a) *Aplysia dactylomela*, left edge of mounted radula, scale bar = 50μm; b) *Aplysia extraordinaria*, right edge of mounted radula, scale bar = 25 μm; c) *Bursatella leachii*, right edge of mounted radula, scale bar = 25 μm; d) *Stylocheilus striatus*, left edge of mounted radula, scale bar = 50μm. Legend: Ml = medial lobe, Ll = lateral lobe.
- Figure 5.4: Diagram demonstrating the generalist-specialist continuum and its associated predictions. Sea hares with simple radulae are more likely to be generalist feeders, large species and/or adults. At the other end of the spectrum, sea hares with complex radulae are more likely to be specialised feeders, small species and/or juveniles. In brackets are the species that exemplify the two extremes of this relationship.

List of Tables

Chapter 2

- Table 2.1: Species list of macrophytes present in each of the four study sites and their
designation as one of the four macrophytic groups: Rhodophyta, Seagrasses,
Heterokontophyta, and Chlorophyta.
- Table 2.2: The presence of sea hare species at each of the four study sites and within each of the months sampled. Legend: 0 = no recorded sea hares, P = Petalifera petalifera, E = Aplysia extraordinaria, S = Stylocheilus striatus, B = Bursatella leachii and D = Aplysia dactylomela.
- Table 2.3: The maximum length, minimum length, mean length (mm), and sample size of each sea species found at all sites, over all months sampled.

- Table 5.1: Collecting sites and locations in Queensland, Australia where sea hare specimens were obtained.
- Table 5.2: The classification of each local sea hare species according to radula type.
- Table 5.3: The classification of each local sea hare species according to radula type (determined in this study) and their reported feeding preferences (this study and other sources).
- Table 5.4: The phylogeny of the sea hare species investigated in the present study, as adapted from Klussmann-Kolb (in press).

Statement of Sources

I declare that this thesis is my own work and has not been submitted in any form for another degree of 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.

Signature

Date

Declaration on Ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics, Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology did not require clearance from the James Cook University Experimentation Ethics Review Committee for its work with Anaspideans. Animals were collected under the Great Barrier Reef Marine Park Authority permit #G02/1738.1.

Signature

Date