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

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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.

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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 *Bursatella leachii* population irruption at Shelly Beach - 67 -

Chapter 4: The feeding preferences of *Bursatella leachii* 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).

Chapter 2

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: $\sqrt{}(\textit{A. dactylomela}) = 0.0382*\sqrt{}(\text{Rhodophyta}) - 0.170$, 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 (^{o}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.

Chapter 3

Figure 3.1: The mean density (per 100m^{2}) 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 m^{2}, +/- 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*.

Chapter 5

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 µm b) *Aplysia extraordinaria*, rachidian are to right, scale bar = 30 µm; c) *Bursatella leachii*, rachidian are to left, scale bar = 25 µm; d) *Dolabella auricularia*, rachidian teeth are to left, scale bar = 25 µm; e) *Stylocheilus striatus*, rachidian is to right, scale bar = 25 µm. 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) S*tylocheilus 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.

Chapter 5

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.

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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

Chapter 1: Herbivory in tropical marine ecosystems

Herbivory

In general, herbivores are a special group of animals that feed on macrophyte material (Crawley 1983). They are distinct from detritivores in that they consume living macrophytes and possess the ability to influence the rate at which their food macrophytes are produced. Herbivores are the primary link between an ecosystem's primary productivity and the remainder of the food web (Choat 1991). When only the macrophytes and animals are considered, an average ecosystem consists of one-third herbivorous species which consume 10% of the total primary productivity (Crawley 1983). However, large variation in the amount of primary productivity removed by herbivores has been documented. For example, forest insects consume only 2% of the net productivity in most years but this increases to almost 100% in years when conditions are favourable (Rafes 1970). Herbivores have a profound effect on the macrophyte communities through direct removal of biomass, altering the habitat structure, and aiding nutrient cycling (Crawley 1983). In addition, by removing certain macrophytes, herbivores can influence the competitive abilities of macrophytes, and thereby affect species richness.

There is a large body of literature surrounding terrestrial herbivory. However, marine herbivores are linked to a vegetation type that is fundamentally different to that found in terrestrial habitats (Choat 1991). Thus terrestrial herbivory theories may not be particularly relevant to marine herbivores and marine ecosystems. On coral reefs, the rate of herbivory is greater than that for any other marine or terrestrial habitat (Carpenter 1986). In spite of this, the importance of the study of herbivory in coral reefs has only been recently recognized (Hughes 1994; Hixon & Brostoff 1996; Hay 1997). This may result from the apparent lack of algae on a reef, because algal species are not the most obvious components of reef ecosystems. This occurs because of the high rate of algal removal by herbivores and results in high algal turnover and productivity (Carpenter 1986). This high productivity is the basis for the diversity of coral reefs and their associated habitats, including tropical seagrass beds, reef flats and coastal rocky reefs.

On shallow reefs, nearly all algal biomass is removed by herbivores, with the exception of crustose-coralline algae, the bases of filamentous algae and chemically and physically defended macroalgae (Hay 1991). In tropical communities where there is reduced spatial heterogeneity eg. seagrass beds, reef flats and mangroves, a more diverse and abundant array of marine algae may be present (Hay 1981, 1984b, 1991). These shallow-water habitats are thought to serve as refuges for palatable algae because the predation risk is too high for large herbivorous fish (Hay 1997). Marine herbivores (mostly invertebrates) that inhabit these areas thus have an abundance of algae available and can generally escape competition from a large guild of herbivores, the herbivorous fish.

The major herbivores on coral reefs are the fish and in some locations, the sea urchins. Either of these groups alone can remove almost 100% of the available algal primary productivity (Hatcher & Larkum 1983; Carpenter 1986; Klumpp & Polunin 1989). When herbivorous fish are removed from these habitats, sea urchin populations often expand as a result of an absence of predators and competitors (Hay 1984b; Carpenter 1986). Removal of these two major herbivore groups has been shown to have dramatic consequences for coral reefs. Hughes (1994) documented how the effect of overfishing and a sea urchin mass mortality event dramatically increased algal cover on Jamaican reefs. Subsequently, these algae prevented the re-establishment of the coral community and ultimately caused an ecosystem coral-algal phase shift. Recent researchers have speculated that the resilience of an ecosystem to disturbance (its ability to recover) depends on the presence of redundant species (Peterson *et al.* 1998). Redundant species have the ability to increase their functional role following the removal of the primary species, or group of species, and effectively replace the primary species. For example, sea urchins may act as redundant species in Jamaican coral reef ecosystems. They increased in number when herbivorous fish were removed and became largely responsible for the removal of algal biomass. When disease removed this redundant species, the ecosystem failed and shifted to an algal-dominated community (Hughes 1994).

With the increasing frequency of anthropogenic disturbances within marine ecosystems, for example: overfishing, pollution, nutrient enrichment and sedimentation, the importance of a resilient ecosystem is crucial. Shallow-water coastal habitats are at increased risk from disturbance because of their proximity to human populations. If the dominant herbivorous fishes are removed from tropical ecosystems we must ask: what redundant species are available to prevent the occurrence of coral-algal phase shifts and what is their capacity to remove algal biomass from the community?

One such herbivorous group that should be examined for their role as redundant species are the sea hares (Gastropoda: Opisthobranchia: Anaspidea: Aplysiidae). These opisthobranch molluscs are large herbivores that occupy a diverse array of shallow-water habitats, from coral reefs, intertidal reef flats, and rocky reef to seagrass beds, mud flats and sandy beaches. Sea hares have massive individual appetites, large reproductive output and feed upon a range of macroalgal species and cyanobacteria (Carefoot 1987).

Sea Hares

The Aplysiidae, or sea hares, are relatively well-studied organisms. Rhinophores and cephalic tentacles on the head, laterally extended parapodia and two unique defensive glands, the ink and opaline glands, characterize this family (Beesley 1998). Sea hares have been used in numerous studies as models for testing neurophysiological questions because of their large nerve cords and nerve cells. There are many studies on all aspects of their anatomy, physiology, taxonomy, ecology and behaviour. However, there is not an equal representation of all species within these studies. While there are a small number of taxa that have been used repeatedly in ecological studies, the majority of species have not been investigated and there is a lack of basic information about them. There are approximately 100 species of sea hare worldwide, and 20 of these are found in Australian waters (Carefoot 1987; Beesley 1998; Klussmann-Kolb in press). The majority of sea hare species are found in tropical or subtropical waters, however most studies have been performed in temperate habitats (Eales 1960; Switzer-Dunlap & Hadfield 1979). There is very little ecological data recorded for Australian sea hare species (Beesley 1998) and barring anecdotal reports, there are no published accounts of tropical populations.

The life history characteristics of sea hares suggest that they would have a large impact on the associated algal communities. The few studies specifically performed to examine the role they play as herbivores in ecosystems have focused on species of the genus *Aplysia.* These accounts largely concluded that sea hares are not likely to affect the algal community to any great degree. In New Zealand, Willan (1979) used cages to exclude sea hares from intertidal habitats and observed an increased standing crop of *Laurencia* within the cages. However, the lack of cage controls meant that this effect was difficult to evaluate and could not be separated from the simultaneous exclusion of herbivorous fish. Similarly, problems with cage effects in a study by Carefoot (1985) confounded possible effects of sea hares on algal biomass however the results indicated that fish were the dominant herbivores on the Barbados reefs examined. Carefoot (1987) concluded that the low densities generally reported for *Aplysia* spp. were never high enough to affect the overall algal communities. Similar studies have not been undertaken for other sea hare genera with higher densities, so their possible impact on marine habitats is currently unknown.

In addition to the density of herbivores, the degree of feeding specialization will influence the effect that herbivores have on their environment. Crawley (1983) divided herbivores into three groups based on the number of macrophyte species they consume. Monophagous herbivores are restricted to a single food macrophyte, oligophages consume a limited number of macrophyte species and polyphagous herbivores can consume a wide variety of macrophyte species. Monophages can be further divided into 1) absolute monophages, which eat a single macrophyte species over their entire geographical range and 2) functional monophagy, where the herbivore feeds on a single macrophyte species in each habitat, but the macrophyte species may vary in different parts of the species' range. Polyphagy and monophagy are likely the result of contrasting selection conditions (Crawley 1983). Polyphagy is thought to evolve under circumstances where food is spatially and temporally variable and/or when food search time is highly correlated with predation risk. The evolution of monophagy is attributed to conditions where specialization allows the herbivore to exploit a different food to other competing species and thus escape competition. Monophagy is also thought to be prevalent when the food species is highly abundant with single species stands so that search time is reduced.

While there are a large number of terrestrial specialist herbivores, marine specialists are relatively rare (Hay & Fenical 1988; Rogers *et al.* 1995). In contrast, many sea hares have specialised diets where they consume a single or a few species of algae from a single algal group (Carefoot 1987; Rogers *et al.* 1995). In his review of the ecology of sea hares, Carefoot (1987), suggested that broad feeding preferences are widespread in *Aplysia*. However, most *Aplysia* spp. are reported to consume only a few red algae species from the local habitat and polyphagy may only be concluded for the species as a whole. Individual populations are selective in their feeding but animals of the same species from different habitats may consume a different range of algae. Body size may be an important factor in the degree of specialization in herbivores where smaller, less mobile species are more specialized (Steneck 1982). Small sea hare species were hypothesized to follow this trend by Hay and Fenical (1988). This trend has also been demonstrated within a species, as the feeding preferences expand through ontogenetic development in a species of *Aplysia* (Pennings 1990b). It is unknown whether this ontogenetic variation in feeding preferences occurs in other sea hare species.

Study Organisms

Six local sea hare species were investigated in the present study. In alphabetical order they are: *Aplysia dactylomela* (Rang, 1828), *A. extraordinaria* (Allan, 1932), *Bursatella leachii* (de Blainville, 1817), *Dolabella auricularia* (Lightfoot, 1796), *Petalifera petalifera* (Rang, 1828), and *Stylocheilus striatus* (= *S. longicauda*) (Quoy & Gaimard, 1832).

Aplysia dactylomela is a member of the sub-family Aplysiinae (Klussmann-Kolb in press) and has the greatest body of literature devoted to it. This species is a relatively large sea hare visually distinguished by thick black rings on the parapodia (Figure 1.1a). The maximum recorded size of *A. dactylomela* is 1400g in weight and 450mm in length and this species is known to live for 10-11 months (Willan 1979; Carefoot 1987; Marshall & Willan 1999). *Aplysia dactylomela* has been recorded from a variety of shallow water habitats including seagrass beds, rocky shores, sandy reef lagoons, reef flats, reef crests and amongst coral rubble from warm temperate and tropical waters worldwide (Marcus & Hughes 1974; Willan 1979; Carefoot 1987; Marshall & Willan 1999; Rudman 1999a). Often the habitats contain red algae (Rhodophyta), such as species of *Laurencia* and *Acanthophora*, and occasionally, green and brown algae (Carefoot 1987). *Aplysia dactylomela* is considered a broadly oligophagous herbivore. Its feeding preferences include a range of predominantly red algae and more rarely, green algae (Carefoot 1987). The algae *A. dactylomela* is reported to feed upon are

Laurencia, *Corallina*, *Centroceras*, *Cladophora, Gracilaria*, *Acanthophora spicifera*, *Spyridia filamentosa*, *Delisea pulchra*, *Stypopodium zonale* and *Ulva* (Carefoot 1970; 1985; 1987; 1991; Switzer-Dunlap & Hadfield 1979; Gerwick & Whatley 1989; Rogers *et al.* 2000a) . *Aplysia dactylomela* has been shown to sequester secondary metabolites from *D. pulchra, L. obtusa,* and *S. zonale* (Gerwick & Whatley 1989; Rogers *et al.* 2000a).

Aplysia extraordinaria is much less well studied and understood. It is recognized by small spots and broken lines on the thin parapodia (Figure 1.1b). It is also a member of the sub-family Aplysiinae. The lifespan and maximum size are unknown for this species. Its known distribution is limited to the east coast of Australia, northern New Zealand, and Japan but it may have a wider distribution under other names (see Spence & Willan 1996; Rudman 1999b; Hamatani & Baba 2003). This species is known for its active swimming locomotion and is reported to grow to 400mm in length (Rudman 1999b). The only published reports on *A. extraordinaria* list its habitat as *Zostera* seagrass beds (Allan 1932; Rudman 1999b) although in the present study it was regularly found on fringing reef flats with abundant macroalgae and sparse seagrass, and never in seagrass beds. There is no published literature known to the author on the feeding preferences of this species.

Bursatella leachii is commonly called the ragged sea hare because of its numerous projections of the parapodia (Figure 1.1c). This species is found in warm temperate and tropical waters throughout the world (Paige 1988). *Bursatella leachii* is a member of the sub-family, Notarchinae, and seven subspecies are currently recognized (Paige 1988; Rudman 1998). Published accounts of the maximum size are not available for *Bursatella leachii* although this species was found in excess of 75mm in the present study. Reported habitats of *B. leachii* include muddy bays, seagrass beds, and shallow, macroalgae-dominated harbours (Lowe & Turner 1976; Wu 1980; Paige 1988). In Japan, *B. leachii* was shown to prefer *Enteromorpha*[1] to other macroalgal species tested (Wu 1980). However, in Florida, gut content analysis indicated that this species is a benthic detritivore based on the large amount of sediment in the gut (Paige 1988).

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[1] The genus *Enteromorpha* has recently been merged with the genus *Ulva* based on the following study: Hayden, H. S., J. Blomster, C. A. Maggs, P. C. Silva, M. J. Stanhope and J. R. Waaland (2003). Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. European Journal of Phycology **38**: 277-294. For convenience throughout the present work '*Enteromorpha'* has been used to designate the filamentous growth form of the genus *Ulva.*

These conflicting results may represent the feeding preferences of different subspecies or reflect the differences in methodology used in these studies. Further work by Paige (1988) using food choice experiments showed the preference of *B. leachii* for cyanobacteria from the family Oscillatoriaceae, including *Lyngbya majuscula*. *Bursatella leachii* also consumed the mat-forming xanthophyte, *Vaucheria* and was shown to feed on *Enteromorpha* under starvation conditions in the laboratory (Paige 1988). These reports suggest that *B. leachii* is a restricted oligophage. The chemical properties of *B. leachii* have been overlooked so it is unknown whether this species sequesters cyanobacteria secondary metabolites in the same manner as *S. striatus*. Large aggregations of *B. leachii* have been documented in Florida although these are ephemeral in their appearance (Paige 1988). A maximum density of 24 300 m^{-2} was observed on the Florida shoreline by Lowe & Turner (1976). Aggregative behaviour has been attributed to reproductive activities for this species (Lowe & Turner 1976).

Dolabella auricularia is a large sea hare found circumglobally in shallow sandy habitats (Pennings *et al.* 1993). Its life span is approximately 16 months and attains mass in excess of 1000g (Switzer-Dunlap & Hadfield 1977, 1979). The posterior end of *D. auricularia* is flattened (Figure 1.1d) with a heavily calcified shell lying under the tissue. Because of this character, *D. auricularia* is considered the most primitive sea hare species and is a member of the sub-family Dolabellinae (Klussmann-Kolb in press). In Guam, Pennings and Paul (1992) found *D. auricularia* in habitats containing *Acanthophora spicifera, Dictyota cervicornis*, *Galaxaura oblongata, Halimeda macroloba* and *Padina tenuis*. *Dolabella auricularia* is the only true generalist feeder of those sea hare species investigated thus far. This species grew fastest when fed a mixture of *Dictyota cervicornis*, *Enteromorpha clathrata*, *Padina tenuis,* and *Sargassum cristaefolium* (Pennings *et al.* 1993). Feeding history and relative algae abundance were found to influence feeding preferences in *D. auricularia.* Pennings *et al.* (1993) found that the preferred alga was always the species that the sea hares were not maintained upon when fed monospecific diets or the one presented in the lowest abundance in mixed algal diets. *Dolabella auricularia* was found to sequester secondary metabolites from an artificial diet within its digestive gland (Pennings & Paul 1993). The secondary metabolites in the natural dietary algae of this species have not been investigated.

Petalifera petalifera is a relatively small sea hare species (Figure 1.1e) found circumglobally (Rudman 2003). There is considerable confusion in regards to its taxonomy, phylogeny, and the number of related species (discussed in Martinez 1996). *Petalifera petalifera* is a member of the family Dolabriferinae which is characterized by a flattened body and almost completely fused parapodia (Martinez 1996; Rudman 2003). It has been reported to reach a maximum body length of 40mm (Rudman 2003). *Petalifera petalifera* is a mesograzer, living and feeding amongst the thalli of its host macrophyte. The limited reports of its habitat suggest geographical differences in its host macrophyte species. In the Mediterranean Sea the host species was observed to be *Posidonia oceanica*, while in the Canary Islands it was found on *Caulerpa* and *Padina* (Ortea & Martinez 1991 in Martinez 1996) and in Barbados it was found living on *Sargassum* and *Enteromorpha* (Marcus & Hughes 1974). It is currently unknown whether *P. petalifera* feeds on the biofilm of the host or grazes directly on the host macrophyte (Martinez 1996).

Stylocheilus striatus is a small elongate species found in tropical waters from the Indo-West Pacific and the Atlantic (Rudman 1999c). It is distinguished by longitudinal stripes and pink or blue coloured ocelli (Figure 1.1f). This species is a member of the Notarchinae family. The maximum length reported is 75mm and 15g (Switzer-Dunlap & Hadfield 1979; Marshall & Willan 1999). *Stylocheilus striatus* is a common species in coastal waters and is known to occur in a range of habitats that support cyanobacteria species (Switzer-Dunlap & Hadfield 1979; Marshall & Willan 1999). *Stylocheilus striatus* is considered a specialist on the cyanobacteria species *Lyngbya majuscula* (Family Oscillatoriaceae) because it actively prefers it during preference testing, settles upon it in rearing experiments and grows best when fed *L. majuscula* (Switzer-Dunlap & Hadfield 1979; Paul & Pennings 1991; Nagle *et al.* 1998). *Lyngbya majuscula* contains a range of potent biologically active secondary metabolites (Pennings *et al.* 1996). These chemicals have been shown to be a deterrent to reef fish feeding (Paul & Pennings 1991; Pennings *et al.* 1996). Sequestration of these compounds has been hypothesized to act as a defensive mechanism for *S. striatus* (Paul & Pennings 1991). However, the artificial manipulation of *L. majuscula* secondary metabolites in *S. striatus* was not found to affect the susceptibility of *S. striatus* to predation (Pennings *et al.* 2001). Thus the mechanisms underlying the origin of the specialization for chemically rich algae remain unclear.

The objective of the present study was to investigate the feeding ecology of sea hares in tropical habitats within northeastern Australia. To address this objective, four related investigations were carried out on local sea hare species. First, the variation in abundance and distribution of sea hares was monitored at four sites in the Townsville area. Secondly, the population dynamics of two sea hare species, *Bursatella leachii* and *Stylocheilus striatus*, that occurred in large numbers within the seagrass beds of one field site (Shelly Beach) were examined. Thirdly, the feeding preferences of *Bursatella leachii* were determined using laboratory experimentation. Fourthly, the interrelationships of the feeding habits and the morphology of the radula (feeding organ) were investigated and presented in Chapter 5. Finally, the main findings and overall conclusions from these studies are discussed in unison in Chapter 6.

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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).

Chapter 2: The spatial and temporal variation of sea hare populations in tropical intertidal habitats

Introduction

Distribution and Abundance

Marine ecosystems are highly heterogeneous both in space and time (Thrush 1991; Levin 1992). In shallow coastal habitats, variations in density of the resident flora and fauna are great and for the most part, poorly understood (Underwood & Chapman 1996, 1998). Variation also occurs on a hierarchy of scales. Small-scale variation is commonly low because of the similar environmental conditions experienced, while large-scale variations are generally great because of the separation in conditions (Jenkins *et al.* 2000). Documenting patterns in variation, both spatially and temporally, is the first step in formulating hypotheses about the ecology of the ecosystem (Underwood 1991; Levin 1992; Underwood *et al.* 2000; Olabarria & Chapman 2002).

Spatial and temporal changes in abundance and distribution have been attributed to factors broadly categorized as biotic and abiotic. Biotic factors that influence marine benthic organisms include competition (Underwood 1984; Fletcher & Underwood 1987), predation (Choat & Kingett 1982), food availability (Underwood 1984) and recruitment (Loosanoff 1964; Underwood & McFadyen 1983; Dayton 1984). Abiotic factors that cause spatial and temporal variation include natural stochastic disturbances such as cyclones, floods, and storms (Underwood 1999) and physical factors such as temperature, tidal flow, desiccation due to exposure, coastal oceanographic conditions, depth, and local and micro-scale topography (Bell *et al.* 1997; Martinez & Navarrete 2002; Middelboe *et al.* 2003). These variables can also interact and complicate the process of predicting the abundance and distribution of any organism.

The factors that affect the distribution and abundance of temperate benthic animals are relatively well studied (for examples see Wells 1980; Bertness & Miller 1984; Bushek 1988; Skilleter 1991; Minchinton & Scheibling 1993; Ong *et al.* 1999; Medernack *et al.* 2000). However, tropical ecosystems have not been the focus of the same research intensity and are subject to different environmental conditions. The factors controlling the dynamics of tropical ecosystems are not well understood. Seasonality occurs through different mechanisms in temperate and tropical ecosystems. In the temperate systems temperature changes are more important while in the tropics seasonality depends on factors such as rainfall (Reich 1995; Peach *et al.* 2001; Stillman 2001; Diaz-Pulido & Garzon-Ferreira 2002). Seasons in the tropics can have profound effects on the variation experienced by organisms within a system. Although these seasonal differences can be considered small in comparison to temperate systems they can still have large effects on the organisms experiencing them (see Jackson & Moltschaniwskyj 2002). These large effects may be felt because organisms within tropical systems live under extreme physical conditions and may exist at the upper limits of their physiological tolerances (Hoegh-Guldberg 1999).

A group of herbivorous molluscs, the sea hares contain both exclusively tropical or temperate species as well as cosmopolitan species (Carefoot 1987). Therefore this group contains ideal study animals for investigating the factors influencing the distribution and abundance of tropical animals in comparison to their temperate counterparts. The majority of ecological work for this group has been performed on temperate species with very few studies on tropical species. Sea hares inhabit a range of shallow water habitats worldwide, including seagrass beds, rocky reefs, and coral reefs, but are considered highly unpredictable and variable in their spatial and temporal distribution (Carefoot 1987).

Studies have shown sea hare abundance and distribution to be influenced by variation in recruitment, habitat characteristics, algal abundance, migration, mortality and predation, competition, and abiotic conditions. These will be discussed in detail as follows.

Recruitment

All marine species with obligate planktonic larval stages are believed to be subject to large variation in recruitment (Keogh & Downes 1982; Keogh 1983; Gaines & Roughgarden 1985) and sea hares are no exception. The recruitment of sea hares has been measured by the completion of the successful metamorphosis of new individuals (Sarver 1979; Gev *et al.* 1984; Pawlik 1989; Plaut *et al.* 1998). The degree of successful recruitment is affected by variation at several of the life history stages of the potential recruits. The factors affecting mating and egg laying, larval survival and dispersal in the plankton and finally, settlement and metamorphosis, all influence the supply of recruits to the population. Therefore, recruitment is affected by variation in egg supply, the oceanographic conditions affecting larval supply to the habitat, physical conditions within the plankton affecting food supply and survival, and settlement preferences for host algae. Although sea hares have been successfully cultured in the laboratory, very little is known about the processes affecting sea hare larvae before settlement in the wild. The factors that are thought to be important for other marine invertebrate species are predation, temperature, and food supply (Hoegh-Guldberg & Pearse 1995). Carefoot (1987) showed that temperature, salinity, desiccation and oxygen deprivation can have a negative impact on sea hare recruits.

In the laboratory, sea hares settle on a range of host algae although one or a few algae are preferred and post-metamorphic growth is highest on the preferred algal species (Switzer-Dunlap & Hadfield 1977; Paige 1988; Pawlik 1989; Plaut *et al.* 1995). *Aplysia californica* undergoes metamorphosis in response to a range of red, green and brown macroalgae although it only grew when fed the red algae *Plocamium cartilagineum* or *Laurencia pacifica* (Pawlik 1989). Field studies of *A. californica* made by Pennings (1991) in the shallow rocky reefs off the coast of California revealed that juvenile *A. californica* were found only on *Plocamium cartilagineum*. Other sea hares species, including *Aplysia oculifera*, *Dolabella auricularia*, *Bursatella leachii* and *Stylocheilus striatus*, preferentially consume the same algae species that promotes settlement and growth in the laboratory (Switzer-Dunlap & Hadfield 1977; Paige 1988; Plaut *et al.* 1995).

Habitat Characteristics

The presence of algae has been the most studied factor in habitat selection for sea hares and is closely linked with recruitment. Based on the earlier discussed settlement experiments, initial recruitment into an area might be based on the presence of a small number of preferred algae species. However, most sea hares are not restricted to feeding on a single alga as adults, although *A. juliana* and *Stylocheilus striatus* are exceptions (Switzer-Dunlap & Hadfield 1977; Rogers *et al.* 1995). Steneck (1982) hypothesized that small, less mobile herbivores will be more likely to be specialized than large, mobile ones. Thus, small sea hares are more likely to have specific habitat preferences than large sea hares as first suggested by Hay & Fenical (1988). A study by Pennings (1990b) found that feeding specificity was related to age with early recruits of *A. californica* only feeding on *Plocamium* sp. Juvenile *A. californica* consumed *Plocamium* sp. and *Ulva* sp. and adults consumed *Plocamium* sp., *Ulva* sp. and *Codium* sp. Interspecific differences in feeding specificity may be related to body size. Although some species that attain large sizes (eg. *A. dactylomela* and *Dolabella auricularia*) have large dietary ranges, there is little known about other species and thus generalization regarding the relationship between size and specialization is difficult (Carefoot 1987; Pennings *et al.* 1993).

Some temperate studies have found that sea hare abundance is highly correlated with the preferred algal abundance within a habitat (Rogers *et al.* 1995; Rogers *et al.* 2003). The abundance of *Aplysia parvula* was directly related to the local abundance of its preferred host macrophyte, *Delisea pulchra* (Rogers *et al.* 2003). *Aplysia juliana* is a green algal specialist consuming *Ulva* spp. and *Enteromorpha* spp. in a range of habitats all over the world (Carefoot 1970; Sarver 1979; Switzer-Dunlap & Hadfield 1979; Willan 1979). In exposed coastal habitats of New South Wales, *A. juliana* abundance is directly related to the abundance of *U. lactuca* (Rogers *et al.* 1995). For sea hares with more general feeding preferences, such as *Dolabella auricularia*, the presence of a range of dietary algae may be an important feature of a suitable habitat as demonstrated by Pennings *et al*. (1993). In addition to the food algae present in a habitat, sea hares may also select habitats based on hydrological conditions. Achituv & Susswein (1985) related the presence of *Aplysia depilans* and *A. fasciata* to their preference for Mediterranean habitats with moderate wave action.

Migration & Nocturnalism

Daily or seasonal migration may alter the spatial and temporal distribution of sea hares. Mobile species may move between habitats to exploit them for feeding, mating, and egg laying. Carefoot (1985) reported a daily localized migration between habitats for the purpose of feeding. Directed migration occurred in New Zealand (Willan 1979) but the author could not explain the behaviour as it was not correlated with food abundance or the incidence of storms. On a microhabitat scale, the small sea hare, *A. parvula*, exhibits daily vertical migrations within the host macrophyte, presumably to avoid daytime predators (Rogers *et al.* 1998). Differences in the distribution of sea hares can also vary daily. Nocturnalism has been documented in a number of species, including *A. dactylomela*, *A. oculifera*, *A. juliana*, *A. fasciata*, *A. brasiliana* and *A. vaccaria* (Carefoot & Taylor 1988; Rogers *et al.* 1998; Plaut 2000). *Aplysia dactylomela* is active at night engaged in feeding, copulation and egg laying. During daylight hours, this species is buried in the sediment, crevices or under rocks (Carefoot & Taylor 1988).

Mortality & Predation

Severe weather events are stochastic by nature although in some areas storms are mainly restricted to specific seasons. Thus mortality resulting from weather action would be concentrated during storm events. Willan (1979) attributed mortality in *A. dactylomela* to storm action and small individuals were more susceptible to storm-caused mortality (Willan 1979). After the main losses due to the storm-caused mortality Willan (1979) attributed minor losses to starfish predation. Predation by the pycnogonid, *Anoplodactylus evansi*, has also been shown to be a greater threat for small size classes of *A. parvula* (Rogers *et al.* 2000b). Large sea hares are believed to be free from the threat of predation (DiMatteo 1982; Carefoot 1987; Pennings 1990b, 1990c) although fish will consume sea hares when presented artificially in the water column (Paul & Pennings 1991; Rogers *et al.* 2002).

Competition

Variation in the population dynamics of co-occurring sea hare species can potentially affect the sea hares in complicated ways, however, very few studies have addressed competition in this group. Sea hares may be found at different places at different times because of competitive interactions with sympatric sea hare species (Achituv & Susswein 1985; Carefoot 1989). In Hawaii, *A. dactylomela* and *A. parvula* coexist although both are active at different times of the day, suggesting the possibility of habitat partitioning due to competition (Carefoot 1989). Competition for habitats with intermediate wave exposure was implicated between *A. depilans* and *A. fasciata* so that only when *A. depilans* was absent, was *A. fasciata* found in this preferred habitat type (Achituv & Susswein 1985).

Abiotic Conditions

Similar to other marine organisms, sea hares have a range of environmental tolerances dictated by their physiological make up. By monitoring blood-glucose levels, Carefoot

(1994) showed that small increases in temperature, oxygen content, air exposure, and lowered salinity cause stress in adult *A. dactylomela*.

In summary, a range of factors influences the distribution and abundance of sea hares. These can vary in importance for different species both spatially and temporally. There is little ecological data available for Australian sea hares species (Beesley 1998). The objective of this study is to identify factors that contribute to the distribution and abundance of sea hares within four sites in the Townsville and Magnetic Island area. The spatial and temporal variation of the local algal communities and environmental conditions are investigated for their role in influencing the population dynamics of local sea hares.

Materials and Methods

Site Description

Sampling was conducted at four sites in Cleveland Bay near Townsville S 19° 10', E 146° 45') and Magnetic Island (S 19° 10', E 146° 50') area. Cleveland Bay is a shallow embayment spanning 30 km protected from the prevailing winds by Magnetic Island, Cape Cleveland and the Great Barrier Reef (Lanyon & Marsh 1995). Cleveland Bay is slightly turbid as a result of freshwater runoff from local creeks, a shipping channel and resuspended sediment from wave turbulence (Walker & O'Donnell 1981). The tidal pattern in these waters is mixed semi-diurnal. Cleveland Bay is tropical but experiences large seasonal variation in rainfall and minor temperature changes between summer and winter months (Lanyon & Marsh 1995; Jackson & Moltschaniwskyj 2002).

Sites were selected based on previous observations of sea hares occurring there. The four sites are intertidal reef flats and seagrass meadows with similar tidal regimes and depth. The study sites differ in the local conditions such as aspect and distance to human habitation. Site one (Kissing Point) is a rocky reef and mangrove system near the centre of Townsville (Figure 2.1). It has an eastern-facing shoreline and a long tidal mud flat. Kissing Point had the highest tidal retention and the shortest exposure time at low tide of all four sites. Its close proximity to Townsville’s urban development means it is subject to a range of unknown disturbances. It is also a popular reef walking, collecting and fishing location for locals. Site two (Shelly Beach) is a bay covered by seagrass beds with mangroves at the northern end and a rocky point at the south end. It is located at the northern end of Rowes Bay and faces east towards Magnetic Island (Figure 2.1). Site three (Picnic Bay) is a south-facing bay on Magnetic Island (Figure 2.1) and is composed of a reef flat with shallow coral colonies and rubble, as well as macroalgae and seagrass. Picnic bay has the smallest area of the four study sites. Picnic Bay supported a busy ferry jetty until September 2003 so it would therefore have been influenced by human disturbance for most of the study period. Cockle Bay is also on Magnetic Island and is a large bay facing west

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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.

towards the mainland (Figure 2.1). Cockle Bay has mangroves and an extensive area of reef flat consisting of mostly coral rubble with some coral colonies, macroalgae and seagrass.

Spatial and Temporal Algal Variation

In October 2002, six 1m^2 permanent quadrats were set out in each of the four sites. Permanent quadrats were chosen to reduce the noise associated with small-scale spatial variability. The number of quadrats monitored was maximized for the length of time typically available during a low tide sampling period. Quadrat locations were randomly chosen within areas of the habitat consisting of a range of macroalgal species and seagrasses. In this way, quadrats were less likely to be placed in barren areas where sea hares would not be found feeding. Small PVC pipes were firmly pushed into the sediment at the corners of each quadrat to mark the location. Sampling commenced 2nd October 2002 and was completed 25th September 2003. Sampling was performed approximately one month apart for the entire sampling period with the exception of January when sampling could not be completed. In total, there were 11 sampling trips made to each of the four sites (Figure 2.2). In December 2002 and January 2003, low tides occurred at night so sampling was conducted with the aid of a torch. Sampling was also carried out on consecutive day and night low tides to assess the effect of differences between temporal sampling.

During a sampling trip to each site, the percentage cover of macroalgae and seagrass in each 1m^2 quadrat was recorded. The procedure for recording the percentage cover of algal species consisted of placing a 0.25m^2 PVC portable quadrat over each quadrant of the permanent quadrat. The portable PVC quadrat consisted of four PVC pipes, each 0.5m in length, joined at the corners, with fishing line strung across the square 10cm apart to create 25 squares of equal size. The algal species at the centre of each square was recorded. When the portable PVC quadrat was placed four times over the four quadrants of the permanent quadrat, there were 100 squares in total for which the algal species was recorded. This procedure was repeated for each of the six permanent quadrats within each site. If an alga could not be identified in the field, a small sample was bagged and taken back to the laboratory for identification. Algal identification was made using keys for local and Australian seaweed species (Fuhrer *et al.* 1981; Price & Scott 1992; Cribb 1996).

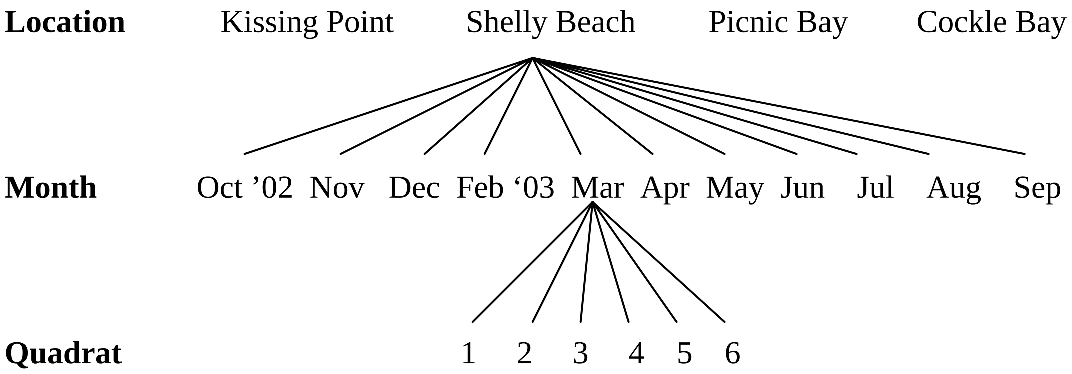

Figure 2.2: Hierarchical nested sampling design for monitoring the spatial and temporal variation in algal species diversity and abundance.

Spatial and Temporal Sea Hare Variation

During the 11 sampling trips to measure algal percentage cover, sea hare density was also measured. A different method was used to monitor the density of sea hares throughout the year, because of the possibility of low densities. During a sampling trip to each site, three replicate transects were laid down, each measuring 30 x 1m (Figure 2.3). The effect of depth gradients along the transects was not considered a

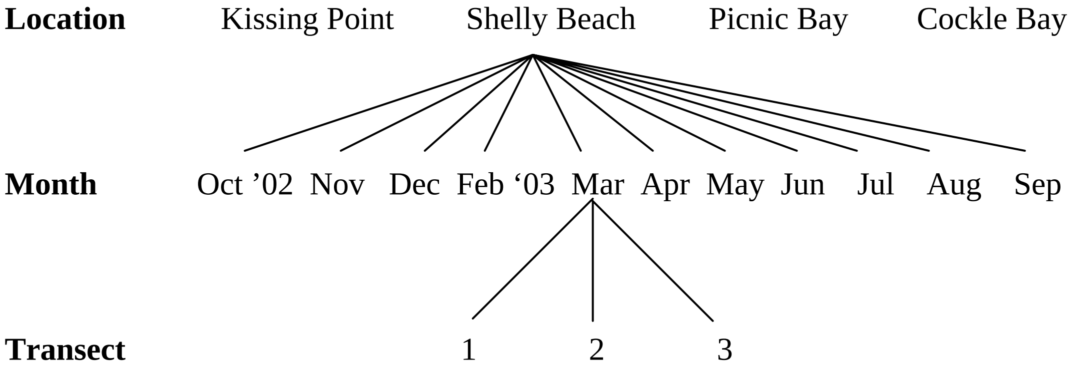

Figure 2.3: Hierarchical nested sampling design for monitoring the spatial and temporal variation in sea hare species diversity and density.

significant factor because of the long tidal flats at the study sites. The procedure used to record sea hares present within this area involved a two stage process; 1) walking in a randomly selected compass direction and looking for large, mobile sea hares while rolling out the tape, 2) walking back along the transect, searching the sediment and macrophytes more closely for smaller and less mobile sea hares and for the presence of sea hare egg masses. When a sea hare was located, the macrophyte species it was found upon and the sea hare species was recorded. The animal was then placed on top of the transect tape to measure its length from the base of the cephalic tentacles to the tip of the tail.

Environmental Data

Measurements of the environmental variables, sea surface temperature, air temperature and light intensity were provide by Janice Lough at the Australian Institute of Marine Science (AIMS). The data was collected by a Cleveland Bay weather station operated jointly by AIMS and the Great Barrier Reef Marine Park Authority. Environmental variables were recorded daily in half-hourly intervals over the entire sampling period and the daily average was utilized in the present study. Two replicate devices measured sea surface temperatures and these measurements were averaged.

Data Analysis

The mean percentage cover of the six replicate quadrats at each site, during each trip was calculated. The mean sea hare density was calculated for the three replicate transects at each site during each sampling trip. The dynamics of algal species that had >5% percentage cover during any month at the four sites was examined. Principal components analysis (PCA) with a correlation matrix was used to examine the relationships between the percentage cover of algae species and sea hare density. Correlation analysis (Pearson's correlation coefficient (r)) was used to examine possible relationships between all algae species and the mean density of sea hares. Algal species were then grouped into the four macrophytic groups, Rhodophyta (red algae), Heterokontophyta (brown algae), Chlorophyta (green algae) and the Seagrasses. Linear regression was performed on the relationship between *Aplysia dactylomela* and Rhodophyta, *Petalifera petalifera* and *Padina tenuis* because they were likely to be causatively linked based on previous study. The influence of environmental factors, air temperature, sea surface temperature and light intensity on sea hare densities was analysed with correlation analysis. The statistics package, SPSS, was used to analyse the data and Microsoft Excel was used to produce the figures.

Results

Algal Distribution and Abundance

Algal Species Variation

A total of 42 species of algae were identified within the four study sites (Table 2.1). Kissing Point was the most species rich site, with 37 algal species present, and also contained the highest number of unique species. Shelly Beach contained the lowest number of species, with only three seagrass species, *Halodule uninervis*, *Zostera capricornis,* and *Halophila ovalis*, present within the site. Rhodophyta (red algae) species dominated the algal community of all the study sites, with the exception of Shelly Beach. Kissing Point contained the highest number of Rhodophyta and Chlorophyta (green algae) species. Cockle Bay and Picnic Bay had a lower species richness of Heterokontophyta (brown algae). Kissing Point, Cockle Bay and Picnic Bay contained two seagrass species, *Halodule uninervis* and *Halophila ovalis*.

Each of the four study sites contained a very different complement of algal species. At Kissing Point, the seagrass species *Halodule uninervis* had the highest percentage cover (40.0% +/- 1.3%), followed by a large number of red, green and brown algae species with low percent cover (all < 5%) (Figure 2.4). At Shelly Beach *Halodule uninervis* had the highest mean percent cover (61.4 +/- 1.8%), followed by *Zostera capricornis* (19.22 +/- 2.1%) and *Halophila ovalis* (12.2 +/- 0.6%) (Figure 2.5). At Picnic Bay, *Halodule uninervis* covered the majority of the area (74.0 +/- 1.0%). The remaining species each covered less than 5% of the area of the study site (Figure 2.6).

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.

	Kissing Point	**Cockle Bay**	**Picnic Bay**	**Shelly Beach**
Seagrasses	*Halodule uninervis* *Halophila ovalis*	*Halodule uninervis* *Halophila ovalis*	*Halodule uninervis* *Halophila ovalis*	*Halodule uninervis* *Halophila ovalis* *Zostera capricornis*
Rhodophyta	*Giffordia mitchelliae* *Ceramium itonoi* *Gelidium australe* *Gelidium sp.* *Pterocladia pinnata* *Hypnea cervicornis* *Hypnea spinella* *Laurencia carolinensis* *Acrocystis nana* *Sarconema filiforme* *Crouania sp.* *Spyridia filamentosa* *Gelidiella acerosa* *Solieria robusta* *Ceramium sp.* *Jania adherens* *Ceramium sp.* *Hypnea pannosa* *Laurencia implicata* *Acanthophora spicifera* Red sp. 1 Red sp. 2 Red sp. 3 Red sp. 4	*Giffordia mitchelliae* *Ceramium itonoi* *Gelidium sp.* *Pterocladia pinnata* *Laurencia implicata* *Acanthophora spicifera* *Hypnea cervicornis* *Hypnea spinella* *Laurencia carolinensis* *Acrocystis nana* *Crouania sp.* *Spyridia filamentosa* *Solieria robusta* *Ceramium sp.* *Jania adherens* *Hypnea pannosa* Red sp. 1	*Pterocladia pinnata* *Laurencia implicata* *Acanthophora spicifera* *Hypnea cervicornis* *Hypnea spinella* *Laurencia carolinensis* *Acrocystis nana* *Sarconema filiforme* *Crouania sp.* *Jania adherens* *Ceramium sp.* *Hypnea pannosa*	
Heterokontophyta	*Sargassum sp.* *Padina tenuis* Brown sp. 1	*Sargassum sp.* *Padina tenuis* *Dictyota dichotoma* *Hydroclathrus clathratus*	*Sargassum sp.* *Padina tenuis* *Dictyota dichotoma* *Colpomenia sinuosa*	
Chlorophyta	*Neomeris van-bosseae* *Chlorodesmis fastigiata* *Caulerpa racemosa* *Ulva sp.* *Enteromorpha sp.* *Cladophora rugulosa* *Chordaria cladosiphon*	*Caulerpa racemosa* *Halimeda opuntia* *Codium mamillosum* *Chordaria cladosiphon*	*Caulerpa racemosa* *Halimeda opuntia* *Codium mamillosum* *Chordaria cladosiphon*	

Again at Cockle Bay, *Halodule uninervis* covered the most area (17.5 +/- 1.15%) and *Acanthophora spicifera* the next highest in coverage (5.8 +/- 0.2%). All the remaining species each covered less than 5% of the study site area (Figure 2.7).

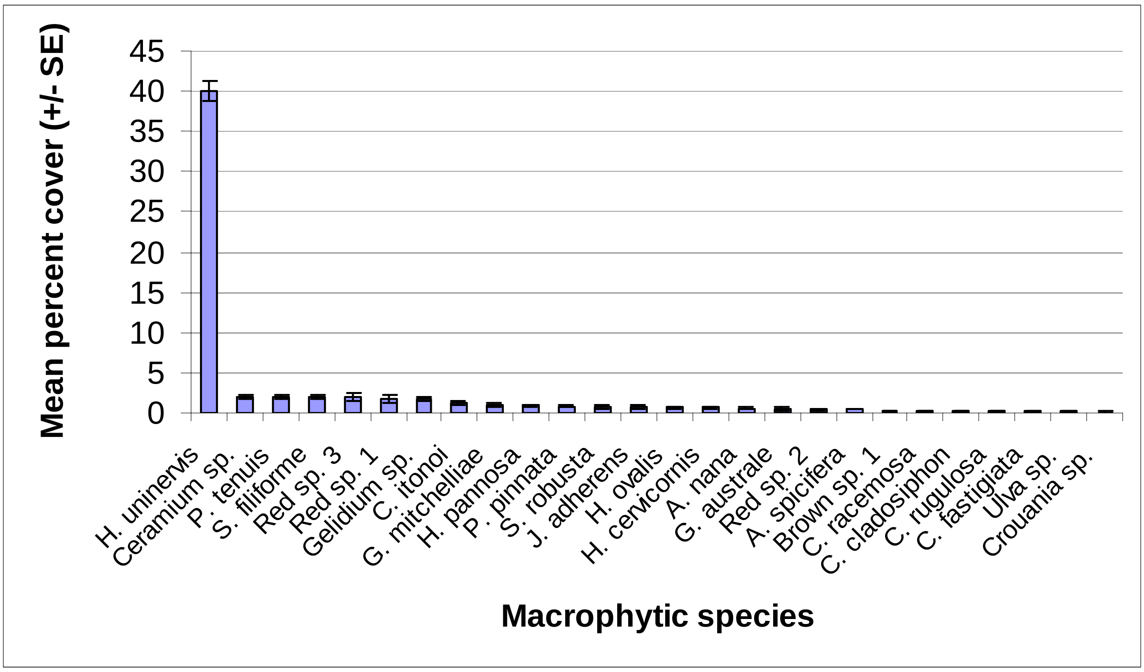

Figure 2.4: The mean percent cover of each marine macrophytic species present at Kissing Point during the October 2002-September 2003 sampling period. Only those species with a mean percent cover of greater than 0.1% are shown. For complete genus names see Table 2.1.

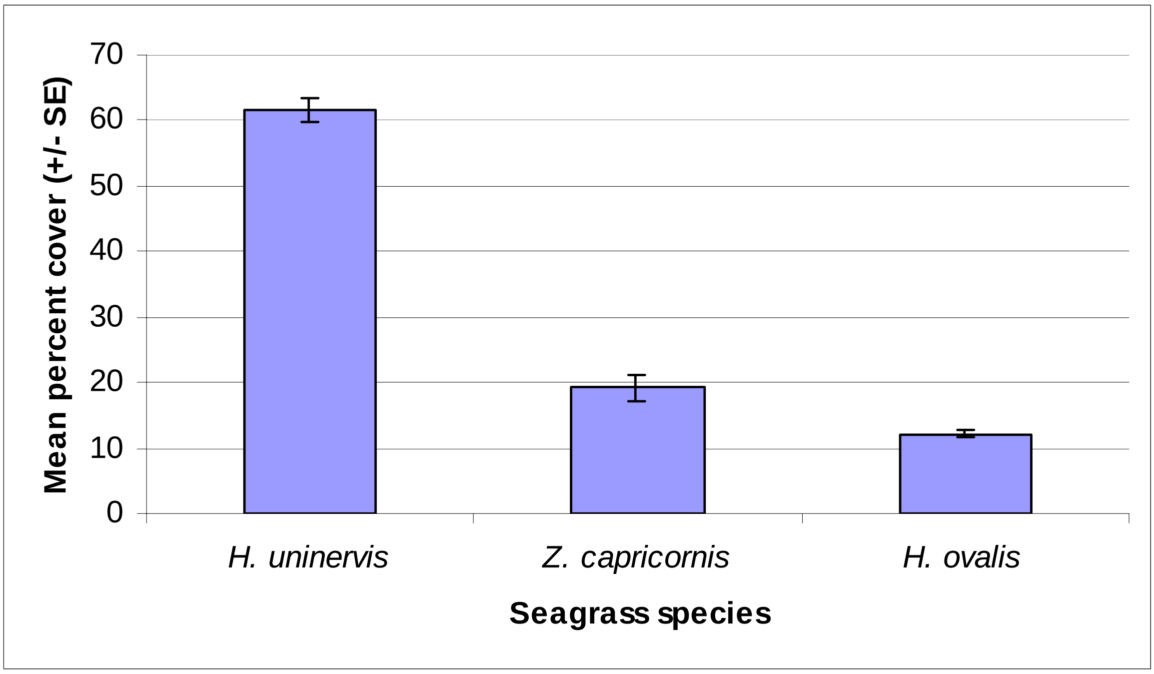

Figure 2.5: The mean percent cover (+/- standard error) of the three seagrass species present at Shelly Beach during the October 2002-September 2003 sampling period.

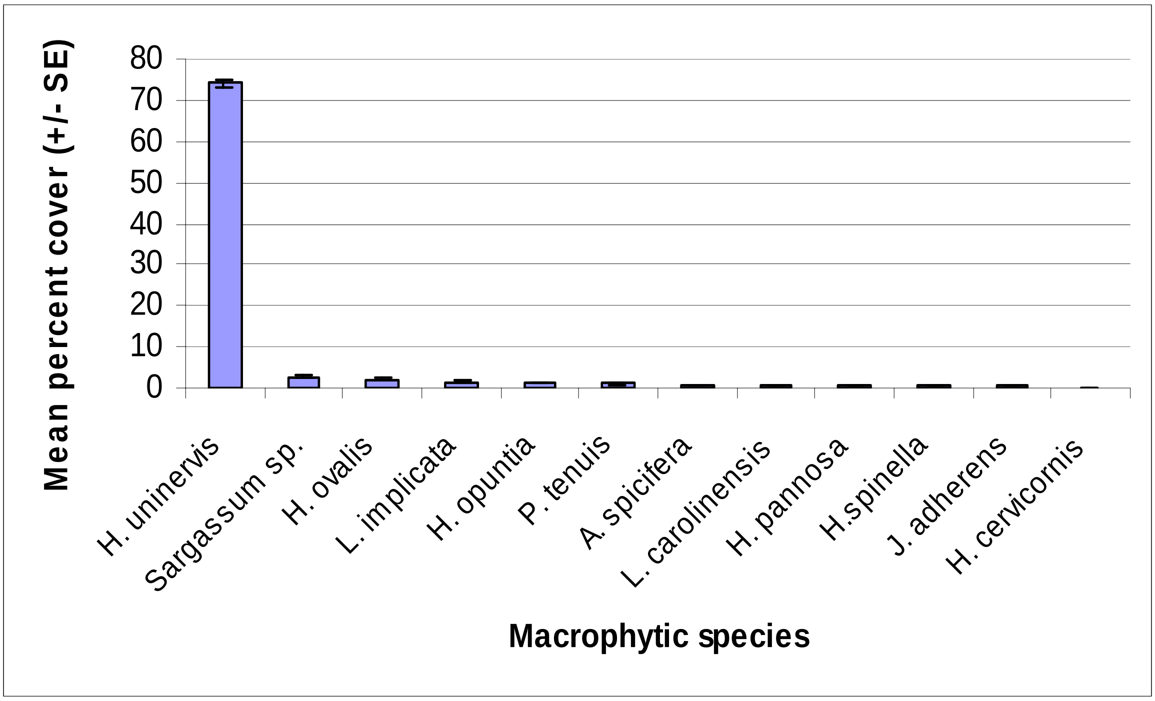

Figure 2.6: The mean percent cover (+/- standard error) of the marine macrophytes present at Picnic Bay during the October 2002-September 2003 sampling period. Only those species with a mean percent cover greater than 0.1% were included.

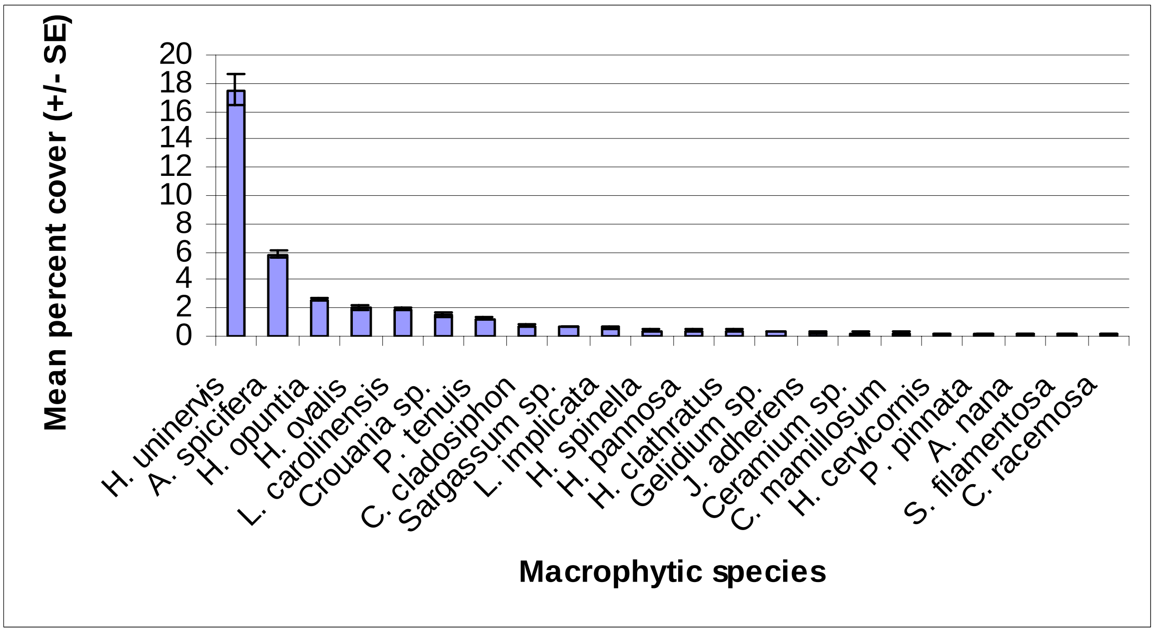

Figure 2.7: The mean percent cover (+/- standard error) of the marine macrophytes present at Cockle Bay during the October 2002-September 2003 sampling period. Only those species with a mean percent cover greater than 0.1% were included.

The percentage cover of each algal species also varied temporally over the duration of the sampling period. The algal species with a percentage cover greater than 5% at any time during the sampling year were examined. At Kissing Point, the cover of *H.*

uninervis increased from October to March and then decreased slightly until June (Figure 2.8a). The coverage peaked in July and then decreased slightly in August and September. *Padina tenuis* coverage was low throughout the entire year but exhibited a small peak in December (Figure 2.8a). Red sp. 3 was only present in the initial month of sampling and was not present during the remainder of the year (Figure 2.8a). *Gelidium* sp. was present at low percent cover with two peaks in cover in November and August (Figure 2.8b). *Sarconema filiforme* had the highest percent cover in October and declined steadily until March and remained at low cover for the rest of the year (Figure 2.8b). Red sp. 1 was only present during a single sampling month, December, when it covered close to 20% of the benthos (Figure 2.8b). *Acrocystis nana* was only present at Kissing Point in June and September, with the largest peak in June (Figure 2.8c). *Ceramium* sp. was first present in March and rapidly increased in cover to peak in May and then decreasing to zero in August, with a slight increase in September (Figure 2.8c). *Hypnea pannosa* was not present until July and peaked in August and then showed a slight decline in cover in September (Figure 2.8c).

At Shelly Beach, the mean percent cover of *Halodule uninervis* varied between 66.3 and 85.3% cover. *Halodule uninervis* cover remained high the entire sampling year although there was a slight downward trend in the last four months sampled (Figure 2.9). *Halophila ovalis* cover remained at relatively low levels for the entire period although there was a slight upward trend in coverage (Figure 2.9). *Zostera capricornis* also remained at relatively low coverage levels with a peak in February and another smaller peak in June (Figure 2.9).

At Picnic Bay, *Halophila ovalis* was present only at very low levels throughout the sampling period with a slight increase in cover between June and August (Figure 2.10). *Halodule uninervis* had the highest percent cover and peaked in December, followed by decreasing cover for the rest of the year (Figure 2.10). *Sargassum* sp. was present at low coverage levels for the duration of the sampling year and had a small peak in cover in February (Figure 2.10).

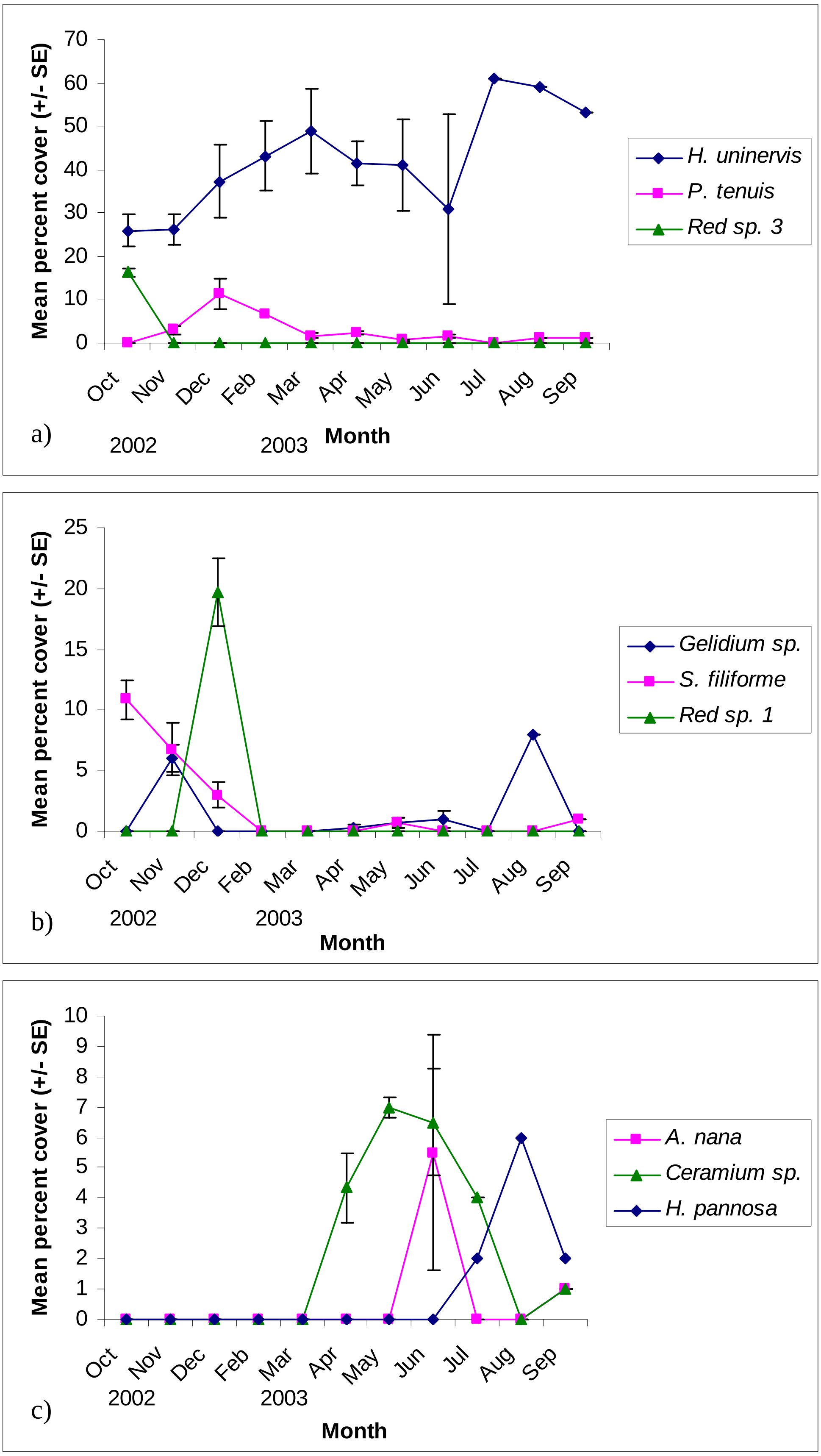

Figure 2.8: Temporal variation of marine macrophytes found at greater than 5% mean percent cover (+/- standard error) in any of the sampling months (October 2002 – September 2003) at Kissing Point, a) *Halodule uninervis*, *Padina tenuis*, and Red sp.3, b) *Gelidium* sp., *Sacronema filiforme*, and Red sp. 1, c) *Acrocystis nana, Ceramium* sp., and *Hypnea pannosa.*

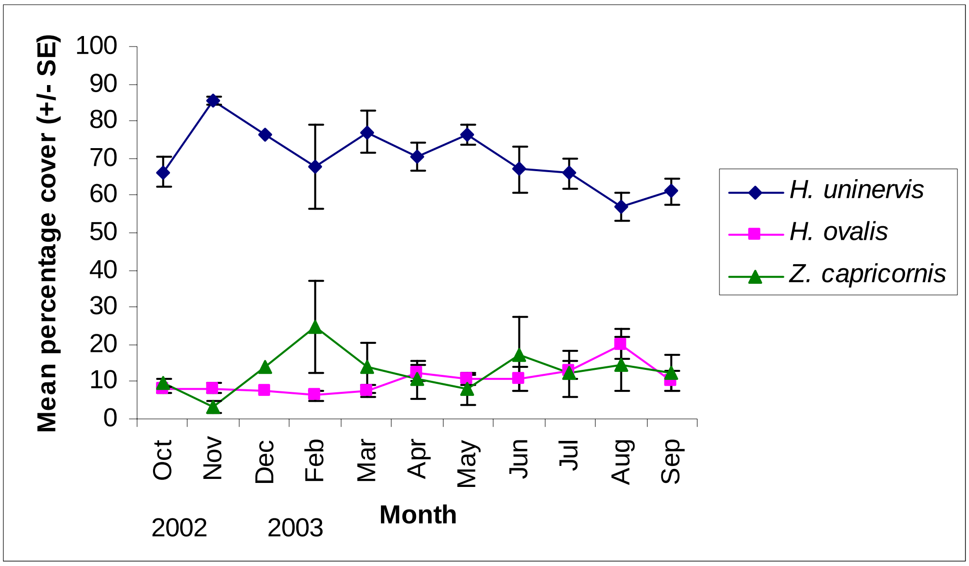

Figure 2.9: The temporal variation in mean percent cover (+/- standard error) of the three seagrass species found at Shelly Beach between October 2002 and September 2003.

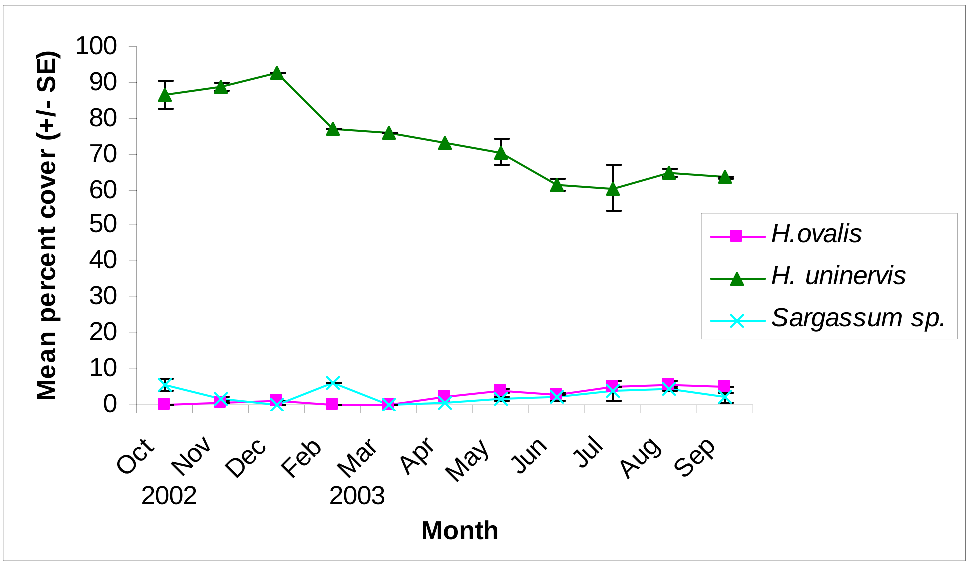

Figure 2.10: The temporal variation in mean percent cover (+/- standard error) of those species with greater than 5% cover at any time during sampling at Picnic Bay between October 2002 and September 2003.

At Cockle Bay, *Halophila ovalis* was present in October and declined to zero cover from November to April. In May its cover began to increase to a peak in July followed by a steady decrease (Figure 2.11a). *Halodule uninervis* had the highest cover at Cockle Bay with a peak in December followed by a rapid decline to low levels for the remainder of the sampling period (Figure 2.11a). *Acanthophora*

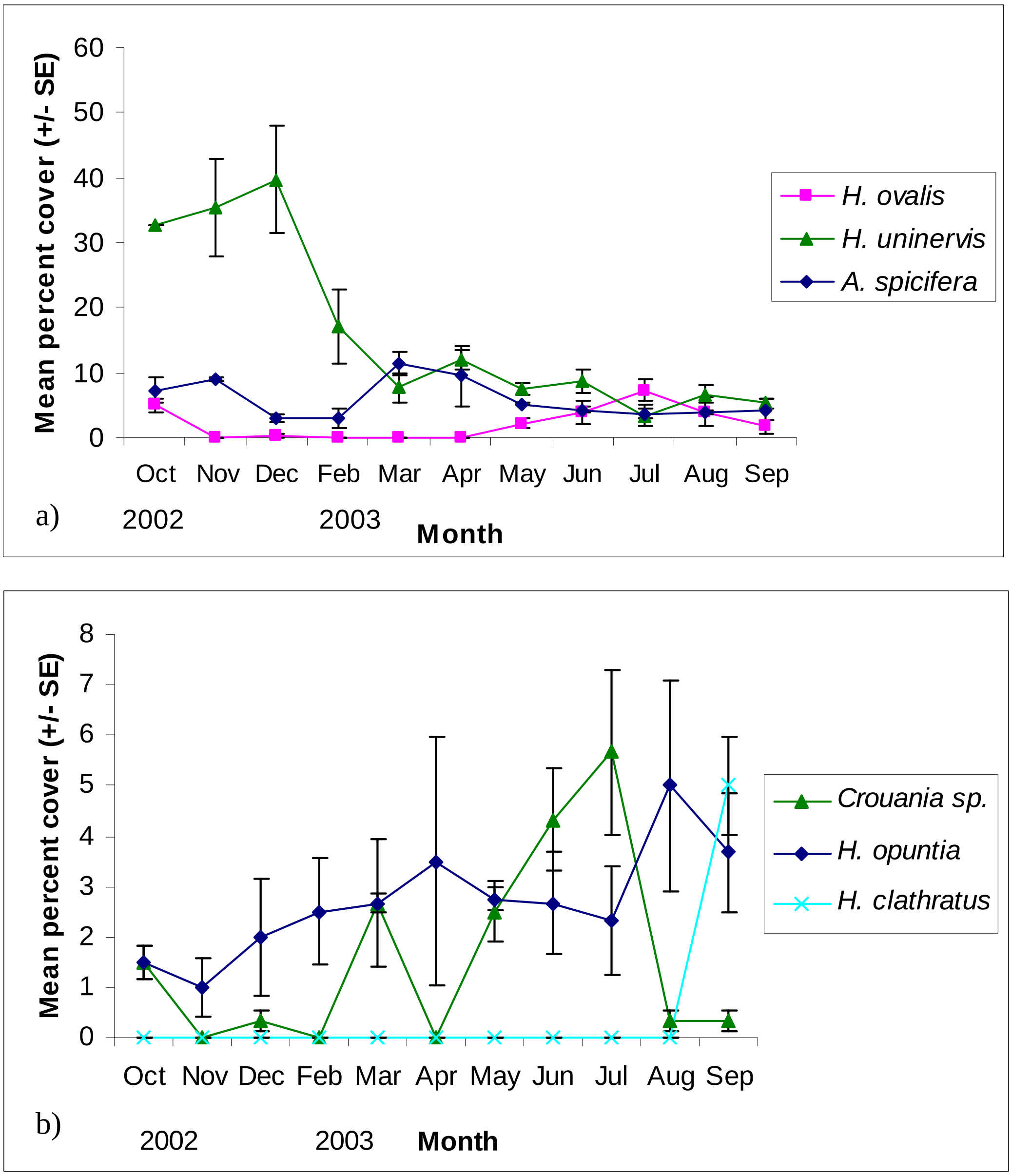

Figure 2.11: Temporal variation in mean percent cover (+/- standard error) of those species with greater than 5% cover at any time during sampling at Cockle Bay between October 2002 and September 2003. Broken into two graphs for clarity where a) *Halophila ovalis*, *Halodule uninervis*, and *Acanthophora spicifera*, b) *Crouania* sp., *Halimeda opuntia*, and *Hydroclathratus clathratus*.

spicifera varied in its coverage at low levels for the entire year of sampling (Figure 2.11a). *Crouania* sp. exhibited large fluctuations in percent cover with a small peak in October declining to zero and then a slightly larger peak in March and a large peak in July, followed by a rapid decline in August and September (Figure 2.11b). The cover of *Halimeda opuntia* showed a gradual increase during the year with a peak in August and then a small decrease in September (Figure 2.11b). *Hydroclathratus clathratus* was only present in the final sampling month, September (Figure 2.11b).

The temporal dynamics of each of the 42 species of algae were examined and there was no consistent pattern between their percentage cover at four study sites. For example, the two species that were present at all four sites are presented in Figure 2.12. *Halodule uninervis* increased in coverage at Kissing Point but was decreasing at Shelly Beach, Picnic Bay and Cockle Bay (Figure 2.12a). At Shelly Beach, *Halophila ovalis* increased in cover steadily to its peak in August and then declined in September (Figure 2.12b). In contrast, the same species at Kissing Point declined from its peak in October to zero cover in May and did not increase its cover for the rest of the year. *Halophila ovalis* at Picnic Bay and Cockle Bay show similar dynamics though this species peaks in cover in June at Picnic Bay while the peak occurs in July at Cockle Bay (Figure 2.12b).

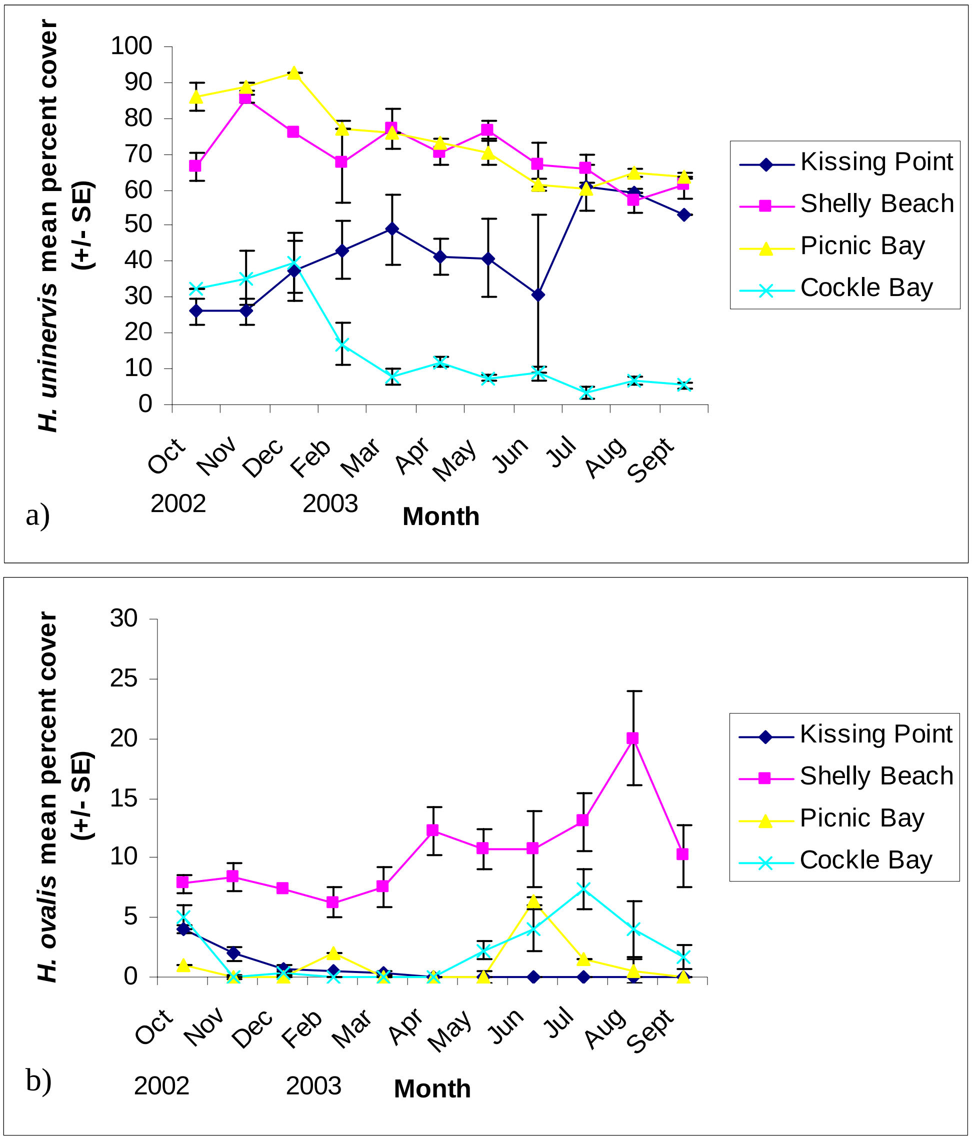

Figure 2.12: Temporal variation of mean percentage cover (+/- standard error) of a) *Halodule uninervis* and b) *Halophila ovalis* at each of the four study sites between October 2002 and September 2003.

Macrophytic Groups Variation

Overall, there were no patterns in temporal variation of the four macrophytic groups when compared between sites. Seagrass had the highest percent cover at all four study sites (Figure 2.13). At Kissing Point, Seagrass showed an increasing cover from October 2002 and peaked in July 2003 with 61.0% cover (Figure 2.13a). The Rhodophyta had the next highest cover, decreasing from its highest cover in October (29.2%) to a low of 2.0% in March and then increasing to 23.0% in August (Figure 2.13a). The Chlorophyta remained at low percent cover throughout the sampling period (Figure 2.13a). The Heterokontophyta has similarly low coverage levels with small peaks in December 2002 and June 2003 (Figure 2.13a).

At Shelly Beach, the Seagrass increased from its lowest level in October 2002 to a high plateau at 98.0% from December 2002 to March 2003 (Figure 2.13b). The percent cover then began to decline to low levels again in September 2003.

At Picnic Bay, Seagrass showed a steady decline in cover from December 2002 to September 2003 (Figure 2.13c). The other three macrophytic groups varied slightly but only at very low levels of cover. The Rhodophyta had a slight rise in cover in June and July 2003.

At Cockle Bay, the Seagrass was at its highest level in December 2002 with 40.0% cover and then there was a large decline in cover to the lowest level (7.7%) in March 2003 (Figure 2.13d). The Rhodophyta declined from 15.8% in October 2002 to a low of 5.0% in February 2003. In March, the Rhodophyta increased in cover to a high of 17.0% and then decreased slightly and remained around 15% for the remainder of the year (Figure 2.13d). The Chlorophyta covered less than 5% of the area for the entire year except for August 2003 where it reached 5.3% cover (Figure 2.13d). The Heterokontophyta varied very little at low levels of percent cover for the year but there was a slight increase in cover in September 2003 to a high of 10.0% cover (Figure 2.13d).

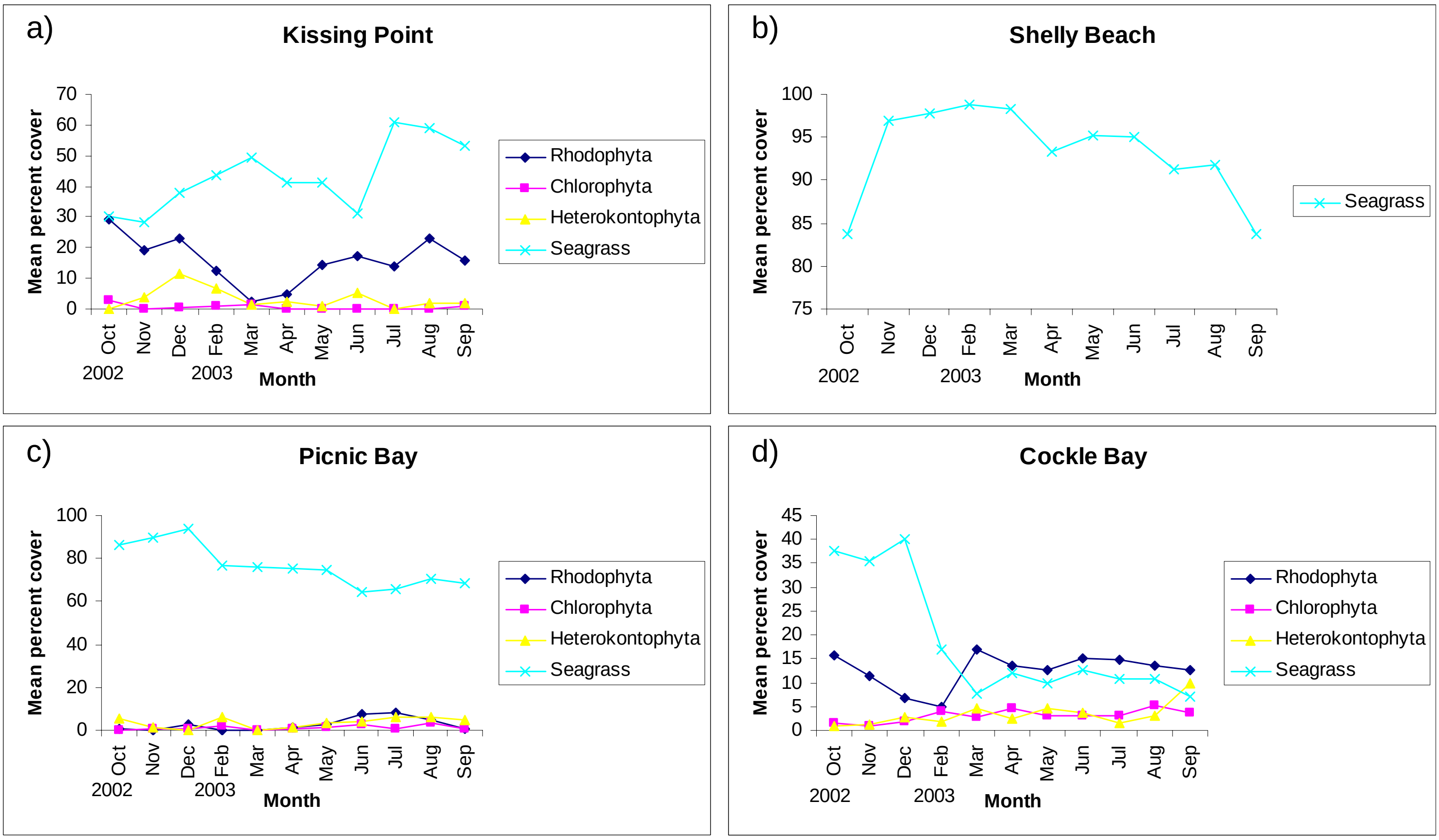

Figure 2.13: The mean density of the four macrophytic groups (Rhodophyta, Chlorophyta, Heterokontophyta, and Seagrass) at each of the four study sites where a) Kissing Point, b) Shelly Beach, c) Picnic Bay and d) Cockle Bay through time (October 2002 through September 2003).

The percentage cover of Rhodophyta, Heterokontophyta and Chlorophyta tend to vary together and were found at the same sites. The density of Rhodophyta had a weak positive correlation with the Heterokontophyta (Figure 2.14a, r = 0.435, N = 45, p=0.003) and the Chlorophyta (Figure 2.14b, R = 0.364, N=45, p=0.014). The density of the Chlorophyta and the Heterokontophyta were also weakly positively correlated (Figure 2.14c, R = 0.370, N = 45, p = 0.012), whereas Seagrass and the three other macrophytic groups were inversely related to each other. When Seagrass was in high abundance, the macrophytic groups were found in lower abundance or were not present at all (eg. Shelly Beach). Seagrass cover was strongly related to the Chlorophyta (Figure 2.14d, R = -0.695, N = 45, p < 0.0001) and exhibited a medium strength correlation with the Heterokontophyta (Figure 2.14e, R = -0.455, N = 45, p = 0.002). The relationship between Seagrasses and Rhodophyta also had a strong negative significant correlation (Figure 2.14f, R = -0.743, N = 45, p < 0.0001).

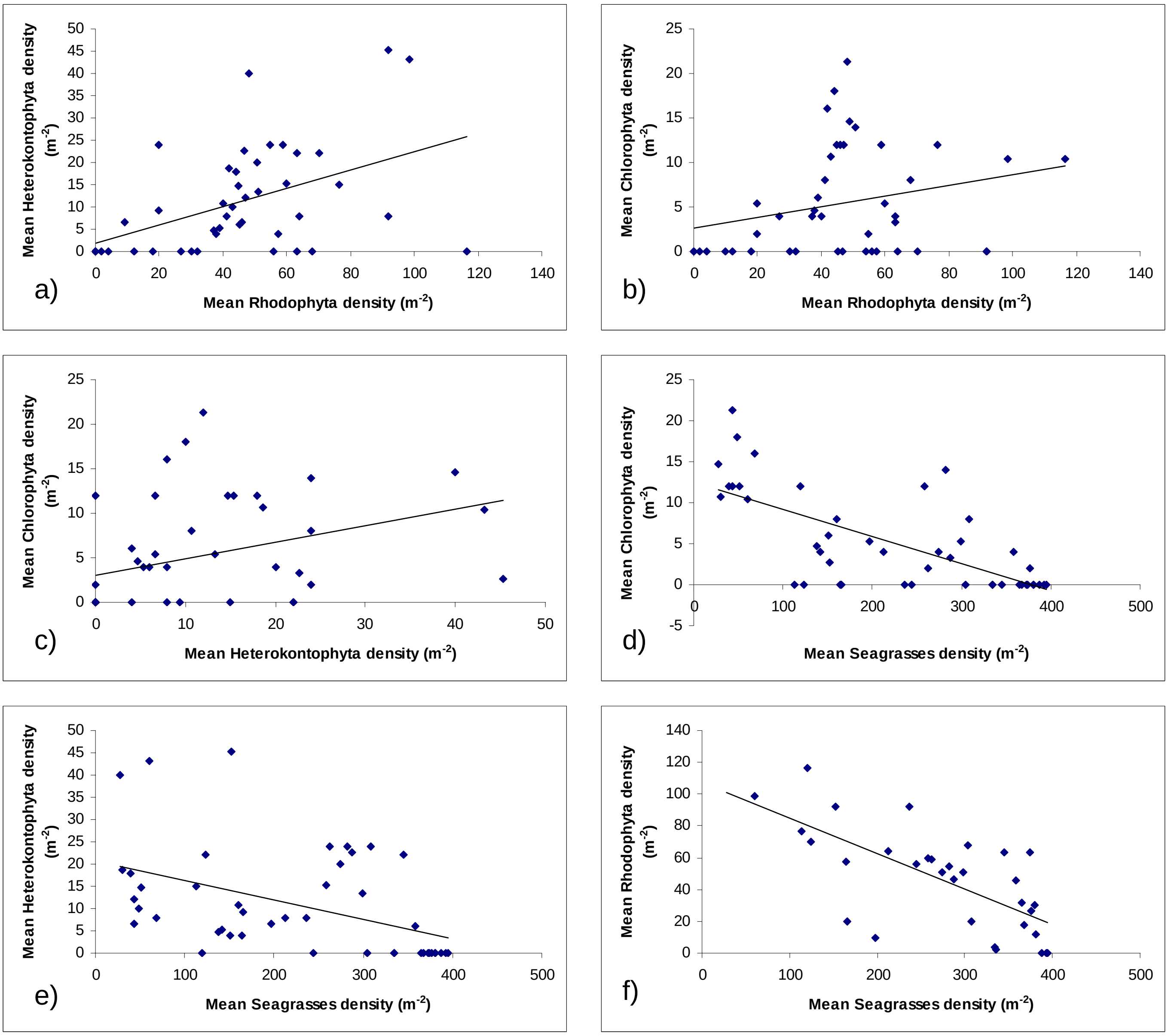

Figure 2.14: Scatter plots of the correlation between macrophytic groups where a) is Rhodophyta and Heterokontophyta, b) is Rhodophyta and Chlorophyta, c) is Heterokontophyta and Chlorophyta, d) is Seagrasses and Chlorophyta, e) is Seagrasses and Heterokontophyta and f) is Seagrasses and Rhodophyta.

Sea Hare Distribution and Abundance

Spatial variation

Sea hares were found for at least part of the year at each site. This was expected because these four sites were chosen specifically for the known presence of sea hares. However, each site was found to have a different community of sea hare species throughout the year (Table 2.2). Kissing Point and Picnic Bay were the most species rich sites, with three sea hare species. The sea hare community at Kissing Point consisted of *Stylocheilus striatus, Aplysia extraordinaria* and *A. dactylomela*. *Petalifera petalifera*, *A. dactylomela* and *A. extraordinaria* were found at Picnic Bay. The Shelly Beach community consisted of two species, *Bursatella leachii* and *Stylocheilus striatus*. Only *A. extraordinaria* was found at Cockle Bay.

In turn, each sea hare species was present only within specific sites (Table 2.1). *Petalifera petalifera* was only found at Picnic Bay and *Bursatella leachii* was only found at Shelly Beach. *Stylocheilus striatus* was found in high densities at Shelly Beach and a single occurrence at Kissing Point. *Aplysia dactylomela* was found at Picnic Bay and Kissing Point while *A. extraordinaria* was found at three sites, Kissing Point, Picnic Bay, and Cockle Bay.

Temporal variation

There were no sea hares present during any of the sampling trips from 2nd October 2002, up to, and including, 16th April 2003 (Table 2.2). Sea hares were only observed during sampling from 16th May to 25th September 2003, although *Aplysia dactylomela* and *A. extraordinaria* may have continued to be present at Kissing Point after sampling ceased. The length of time sea hares were present also differed at each of the sites. At Kissing Point, sea hares were found during all trips made from July to September 2003. At Picnic Bay, sea hares were found from May to August 2003. At Cockle Bay, sea hares were found between June and August 2003 and at Shelly Beach, sea hares were only found in June 2003.

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*.

	Kissing Point	Shelly Beach	Picnic Bay	Cockle Bay
Oct-02	0	0	0	0
Nov-02	0	0	0	0
Dec-02	0	0	0	0
Feb-03	0	0	0	0
Mar-03	0	0	0	0
Apr-03	0	0	0	0
May-03	0	0	P	0
Jun-03	E S	B S	P	E
Jul-03	E	0	P D E	E
Aug-03	D E	0	P	E
Sep-03	D E	0	0	0

Individual sea hare species were found in certain months of the year (Table 2.2). *Stylocheilus striatus* and *Bursatella leachii* were found only in June 2003. *Aplysia dactylomela* was found only in July, August and September 2003. *Aplysia extraordinaria* was found in June, July, August, and September 2003 and *Petalifera petalifera* was also found during four months of the sampling year: May, June, July, and August 2003.

At Kissing Point, *A. extraordinaria* was at low density in June and July (1.0 per 100m^{-2}), peaked in density in August at 9.0 per 100m^{-2} and then dropped back to 1.0 per 100m^{-2} in September 2003 (Figure 2.15a). *Aplysia dactylomela* increased in density from 3.0 per 100m^{-2} in August to 6.0 per 100m^{-2} in September 2003. *Stylocheilus striatus* was only found at Kissing Point in June 2003 and had a density of 2.0 per 100m^{-2}. At Shelly Beach, *S. striatus* occurred in high density in June (384.0 per 100m^{-2}) and *Bursatella leachii* had a lower density of 24.0 per 100m^{-2} (Figure 2.15b). At Picnic Bay, all three species of sea hare peaked in July 2003 (Figure 2.15c). *Petalifera petalifera* increased from 1.0per 100m^{-2} in May to a peak of 3.0 per 100m^{-2} in August

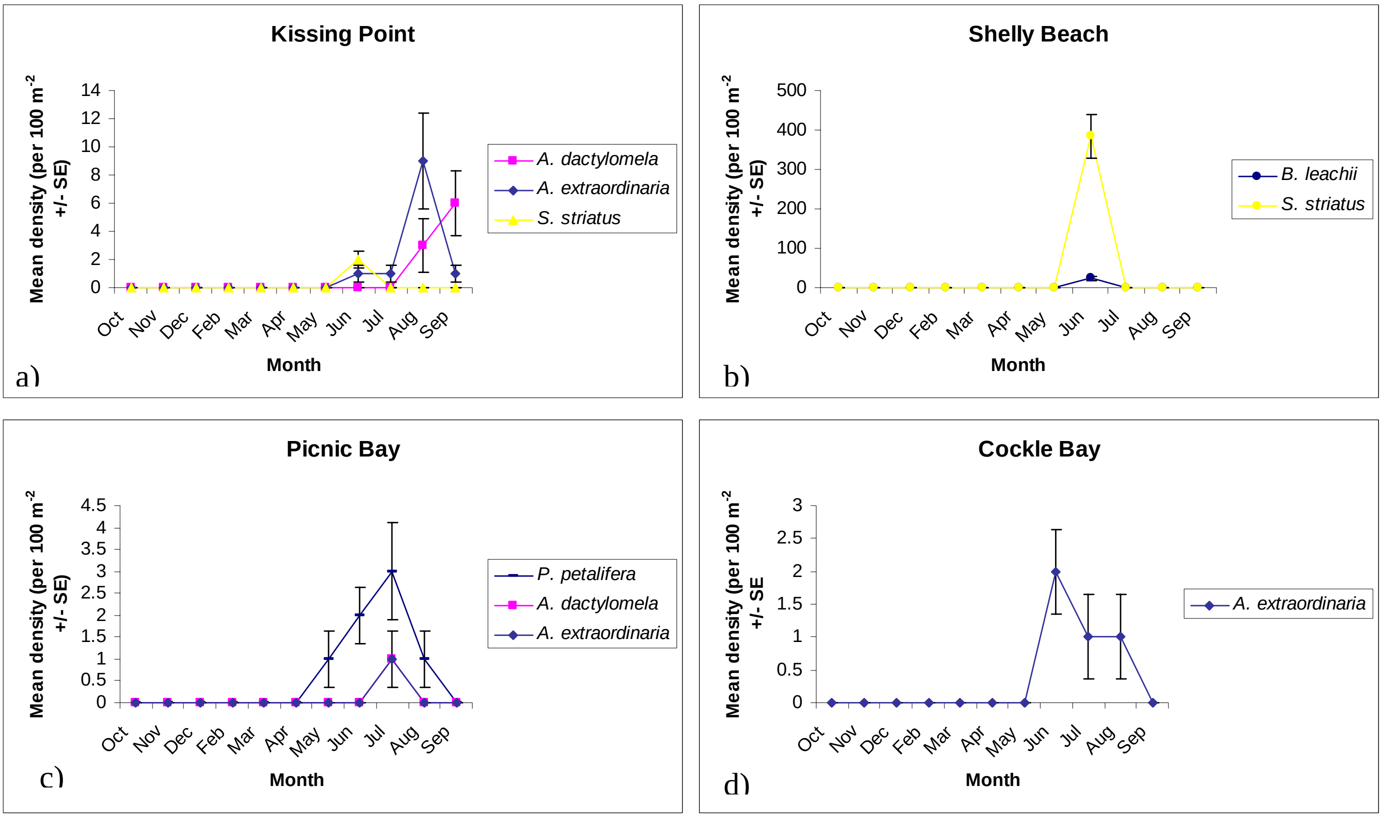

Figure 2.15: The temporal variation of the mean density (+/- standard error) of sea hare species between October 2002 and September 2003 at a) Kissing Point, b) Shelly Beach, c) Picnic Bay and d) Cockle Bay.

and then returned to zero density in September 2003. *Aplysia extraordinaria* and *A. dactylomela* had identical density variation with a small peak in density (1.0 per 100m^{-2}) in July 2003 (Figure 2.15c). At Cockle Bay, *A. extraordinaria* peaked in abundance at 2.0 per 100m^{-2} in June, dropped to 1.0 per 100m^{-2} and remained stable during July and August and then dropped back to zero in September 2003 (Figure 2.15d).

With the exception of *S. striatus,* sea hare species that occurred at more than one site exhibited different density dynamics at the different sites (Figure 2.15). *Aplysia dactylomela* increased in density from August to September at Kissing Point (maximum 6.0 per 100m^2) but only had a single, small peak (1.0 per 100m^2) in July at Picnic Bay (Figure 2.15a,c). *Aplysia extraordinaria* had the highest density in June at Cockle Bay (2.0 per 100m^2, Figure 2.15d), in July at Picnic Bay (1.0 per 100m^2, Figure 2.15c), and in August at Kissing Point (9.0 per 100m^2), Figure 2.15a). *Stylocheilus striatus* had the highest density in June 2003 at both Kissing Point (2.0 per 100m^{-2}, Figure 2.15a) and Shelly Beach (384.0 per 100m^2, Figure 2.15b).

Size Variation

Each of the five sea hare species observed during the course of this study had different size characteristics (Table 2.3). *Aplysia dactylomela* had the largest maximum length and *Petalifera petalifera* had the smallest maximum length. *Aplysia extraordinaria* had the largest range in size while *P. petalifera* had the smallest range. *Stylocheilus striatus* and *Bursatella leachii* had similar size characteristics to one another.

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.

	A. extraordinaria	***A. dactylomela***	***P. petalifera***	***S. striata***	***B. leachii***
Maximum length	137	150	16	36	30
Minimum length	12	100	8.1	10	19
Mean length	67	129	11	22	27
N	14	9	7	85	14

Aplysia extraordinaria was found at three of the four sites and there was a significant difference in the length at these sites. At Kissing Point, individuals were larger than those found at Picnic Bay and Cockle Bay (Figure 2.16). This difference was significant between Kissing Point and Cockle Bay (One-way ANOVA: F = 11.899, df = 2, p = 0.002). Picnic Bay could not be analysed formally because only a single animal of this species was found at this location however the size at Picnic Bay was similar to those animals found at Cockle Bay. The variation in length of the four other sea hare species was not examined because of the extremely low numbers observed.

Egg Mass Variation

Sea hare egg masses were observed in all sites except Cockle Bay indicating that reproductively active animals were present at Kissing Point, Shelly Beach and Picnic Bay (Figure 2.17). At Picnic Bay, egg masses were present at low densities in June and August 2003 (2.0 per $100m^2$). Egg mass density peaked at Shelly Beach in June 2003 (27 per 100 m^2), at the same time that sea hares were present there. At Kissing Point, egg masses were observed at the highest density (20.0 per 100 m^2) in August and then decreased to 6.0 per 100 m^2 in September 2003. The egg masses at Kissing Point were found in close proximity to large *A. extraordinaria* and *A. dactylomela*. Sea hare egg masses are difficult to identify to species in the field although *P. petalifera* has a characteristic spiral pattern egg mass. The spiral egg masses of *Petalifera petalifera* were observed on the thalli of *Padina tenuis* at Picnic Bay. *Petalifera petalifera* egg masses were observed during the same sampling period that individual *P. petalifera* were found (May – August 2003). However, there was no observed correlation between the densities of egg masses and adults of *P. petalifera*.

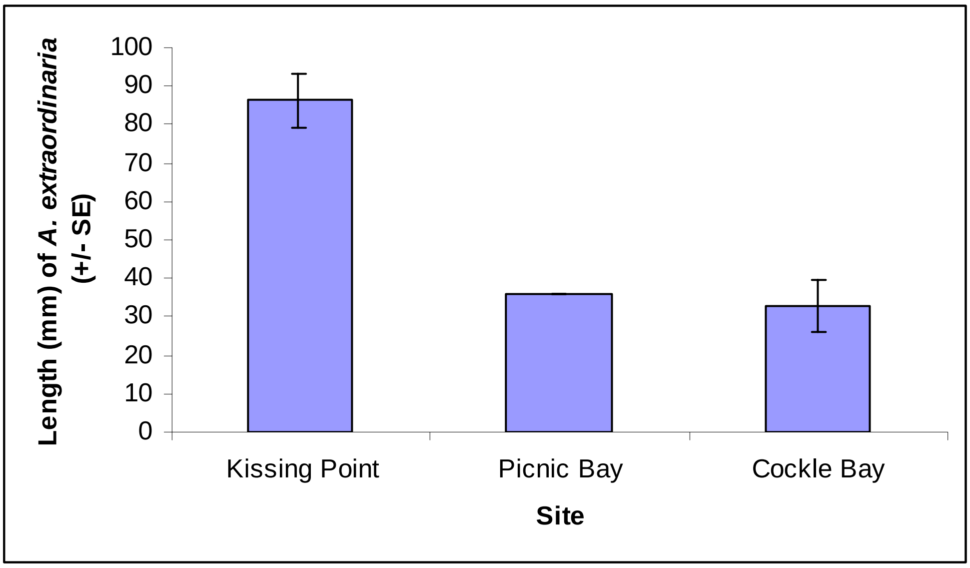

Figure 2.16: The mean length (mm, +/- standard error) of *Aplysia extraordinaria* at each of the three sites this species was found within.

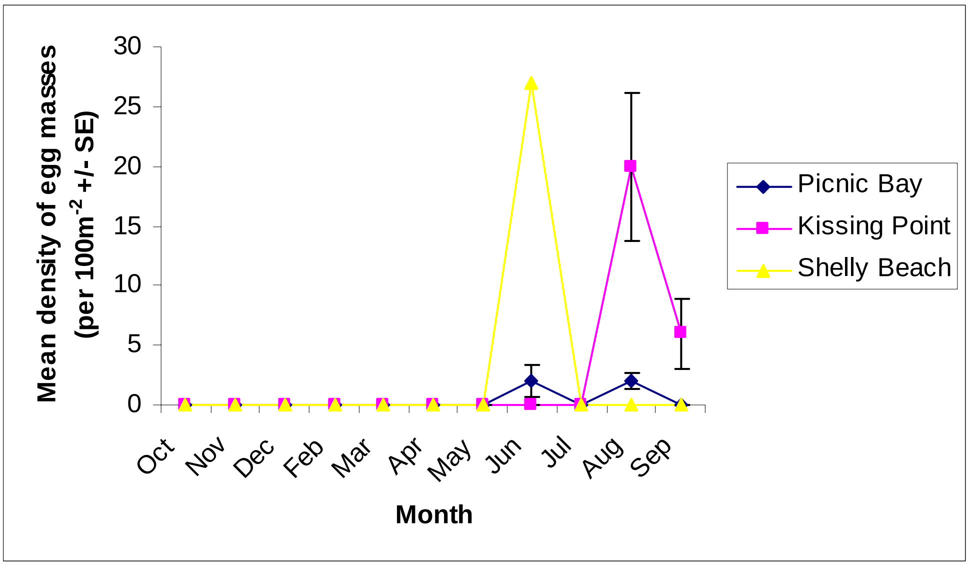

Figure 2.17: The temporal variation of egg mass density (+/- standard error) at Picnic Bay, Kissing Point and Shelly Beach between October 2002 and September 2003.

Sea Hare and Algal Relationships

Sea Hare-Algal Cover Correlations

Sea hares were not found at densities great enough to formally analyse the algae each hare was found upon. However, *Petalifera petalifera* was always found on the brown algae, *Padina tenuis* (N=7). *Aplysia extraordinaria* was found in association with a range of algae: *Acanthophora spicifera* (N=1), *Crouania* sp. (N=2), *Halimeda* sp. (N=1), *Hypnea spinella* (N=1), *Laurencia implicata* (N=1) and *L. carolinensis* (N=1). *Aplysia dactylomela* was also found feeding on *Laurencia carolinensis* (N=1). At Kissing Point, both *A. extraordinaria* and *A. dactylomela* were found amongst rocks above the low tide mark without any algae present in the immediate area.

There was no significant correlation between the overall percentage cover of algae at each of the sites (Figure 2.18, Pearson's correlation coefficients, p>0.05). At Kissing Point, however, the highest algal cover corresponded to the highest sea hare density (Figure 2.18a). Shelly Beach had a high algal cover for the entire year but sea hares were only present in June 2003 (Figure 2.18b). Picnic Bay had a fairly stable algal cover throughout the year (Figure 2.18c), remaining between 80-100% the entire sampling period, but sea hares were only recorded in the winter months. Cockle Bay exhibited the opposite trend, where a long period of low algal cover corresponded to the highest sea hare density (Figure 2.18d).

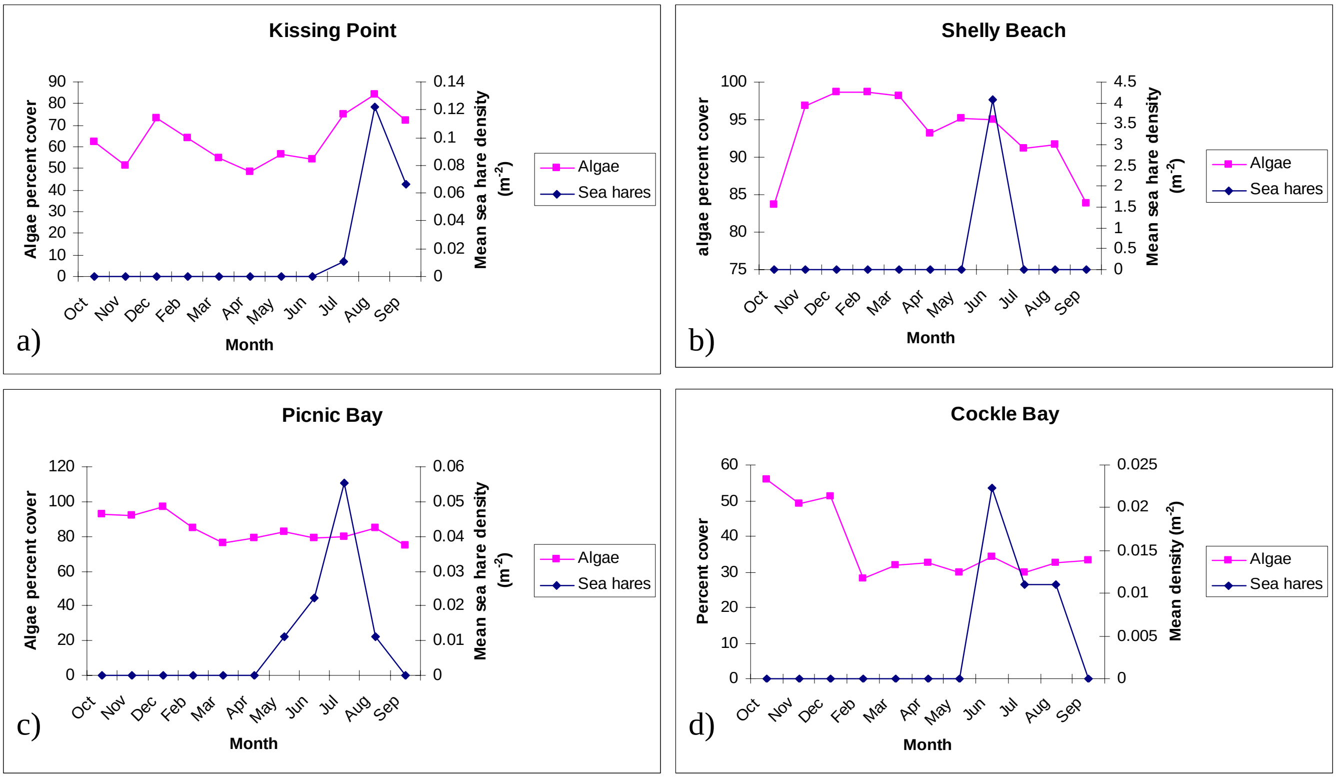

Figure 2.18: The fluctuation in the mean percentage cover of algae and sea hare density through time (October 2002 through September 2003) at a) Kissing Point, b) Shelly Beach, c) Picnic Bay and d) Cockle Bay.

Sea Hare-Macrophytic Groups Correlations

In those months when sea hares were present there were significant relationships between the algal species cover and the sea hare densities recorded. Using Principal Components Analysis, the algae and sea hare community at each site differed from one another because of a small number of factors (Figure 2.19). Plots of Principal Components 1 and 3 (PC1 and PC3) and Principal Components 2 and 3 (PC2 and PC3) were shown because they provide the most biologically relevant information. The length and direction of the loading vectors (represented by arrows in the Figures) represent correlations within the data that caused the differences between sites in the reduced space of the PCA scatter plot. Picnic Bay was characterized by the presence of the sea hare, *Petalifera petalifera* and relatively high percent cover of the

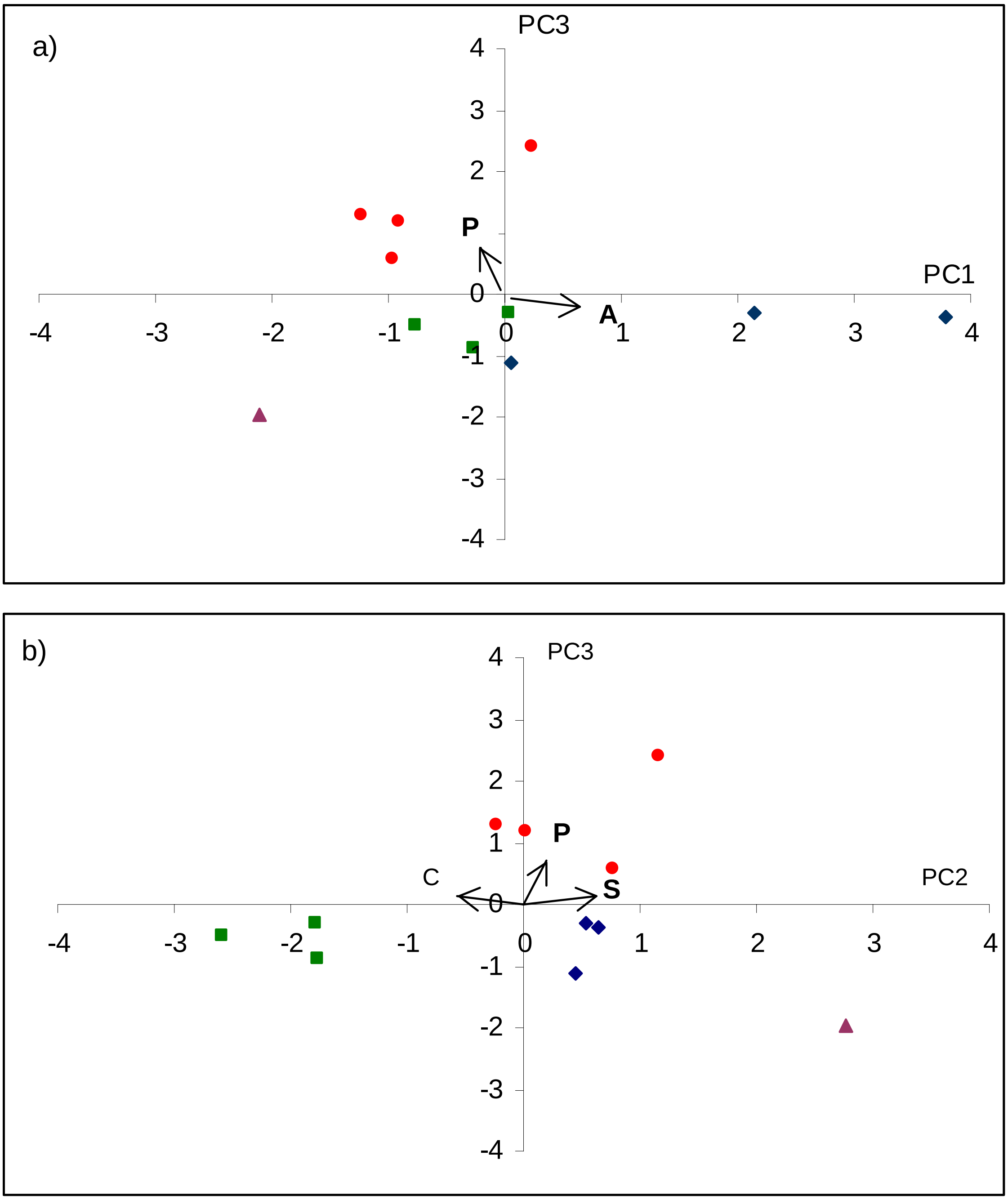

Figure 2.19: Principal components analysis of the months when sea hares were present. PC1 = 34.15% of variance, PC2 = 29.35%, PC3 = 19.78%. The arrows represent important component loadings: a) scatter plot of principal components 1 and 3, b) scatter plot of principal components 2 and 3. Legend: red 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.

Heterokontophyta (Vector P, Figure 2.19a). High densities of *Aplysia dactylomela* and *A. extraordinaria,* and its high percentage cover of Rhodophyta characterize Kissing Point (Vector A, Figure 2.19a). Cockle Bay had a high percent cover of Chlorophyta (Vector C, Figure 2.19b) and low coverage of the Seagrasses (Vector S, Figure 2.19b). The high density of Seagrasses separated Shelly Beach and Kissing Point from each other (Vector S, Figure 2.19b). Picnic Bay has intermediate densities of Chlorophyta and Seagrasses. The three principle components that were extracted represent a total of 83.29% of the variance in this dataset. The KMO statistic was 0.38 was fairly low, signifying low correlations between the variables. These plots would be best understood in three dimensions but in the present context, two plots show the same information.

Sea Hare-Alga Correlations

At Kissing Point there was a significant correlation between the density of *A. dactylomela* and the percent cover of four species of Rhodophyta algae: *Pterocladia pinnata* (Figure 2.20a, R = 0.670, p = 0.024), *Crouania* sp. (Figure 2.20b, R = 0.780, p = 0.005), *Solieria robusta* (Figure 2.20c, R = 0.822, p = 0.002) and *Hypnea pannosa* (Figure 2.20d R = 0.660, p = 0.027). The density of *A. extraordinaria* was significantly correlated to three species of Rhodophyta algae at Kissing Point: *P. pinnata* (Figure 2.20a, R = 0.803, p = 0.003), *H. pannosa* (Figure 2.20d, R = 0.932, p<0.001), *Gelidium* sp. (Figure 2.20e, R = 0.747, p = 0.008) and one species of Heterokontophyta algae, *Sargassum* sp. (Figure 2.20f, R = 0.992, p<0.001).

There were no significant correlations between the sea hare species found at Shelly Beach and the seagrass species present there (Pearson correlations p>0.05). At Picnic Bay the density of *A. dactylomela* was significantly related to the percent cover of *Hypnea spinella* (Figure 2.21a, R = 0.773, p = 0.005) and *Laurencia carolinensis* (Figure 2.21b, R = 0.960, p < 0.001). The density of *A. extraordinaria* was also significantly related to *H. spinella* (Figure 2.21a, R = 0.773, p = 0.005) and *L. carolinensis* (Figure 2.21b, R = 0.960, p < 0.001).

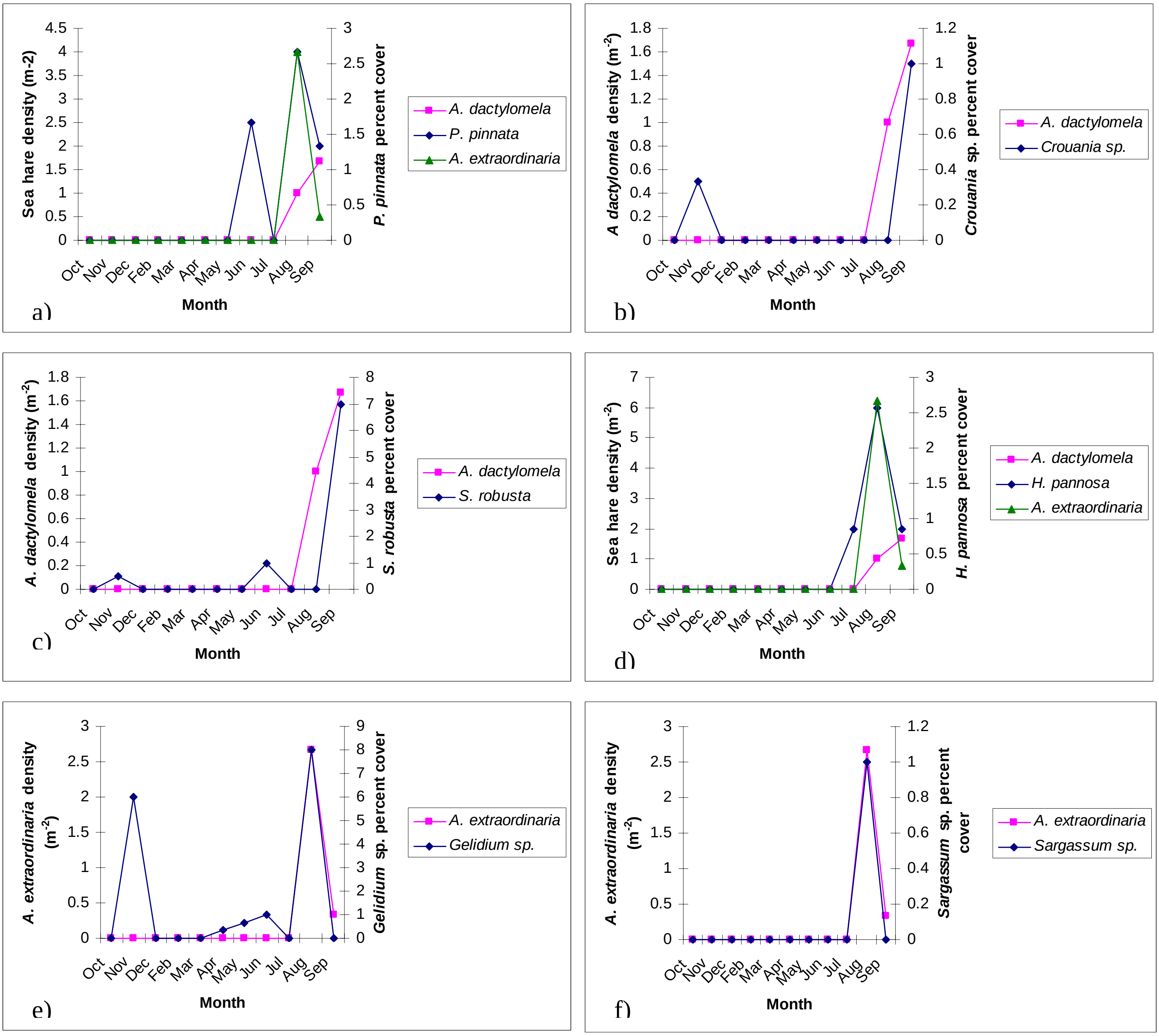

Figure 2.20: The temporal relationships between algae species and sea hare species at Kissing Point between October 2002 and September 2003 where a) *Aplysia dactylomela* and *A. extraordinaria* densities and *Pterocladia pinnata* percent cover, b) *A. dactylomela* and *Crouania* sp., c) *A. dactylomela* 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.

At Cockle Bay, the density of *A. extraordinaria* was significantly related to the percent cover of the Chlorophyte *Caulerpa racemosa* ssp. (Figure 2.22a, R’ = 0.821, p =0.001). *Aplysia extraordinaria* was negatively related to the percent cover of the Heterokontophyte *Sargassum* (Figure 2.22b, R = -0.585, p =0.046). The red alga, *Pterocladia pinnata* (Figure 2.22c, R = 0.731, p = 0.007) and *Crouania* sp. (Figure

2.22d, R = 0.635, p = 0.026) were positively related to the density of *A. extraordinaria*. There was a significant relationship between the density of *A. dactylomela* and the percent cover of the Rhodophyta (Figure 2.23). The positive relationship between *A. dactylomela* and Rhodophyta can be described by the equation √(*A. dactylomela*) = 0.0382*√(Rhodophyta) – 0.170 (One-way ANOVA: F = 11.643, df = 1. p = 0.009). Thus the percent cover of the Rhodophyta explains the majority of the variation in the density of *Aplysia dactylomela* (r value = 0.770).

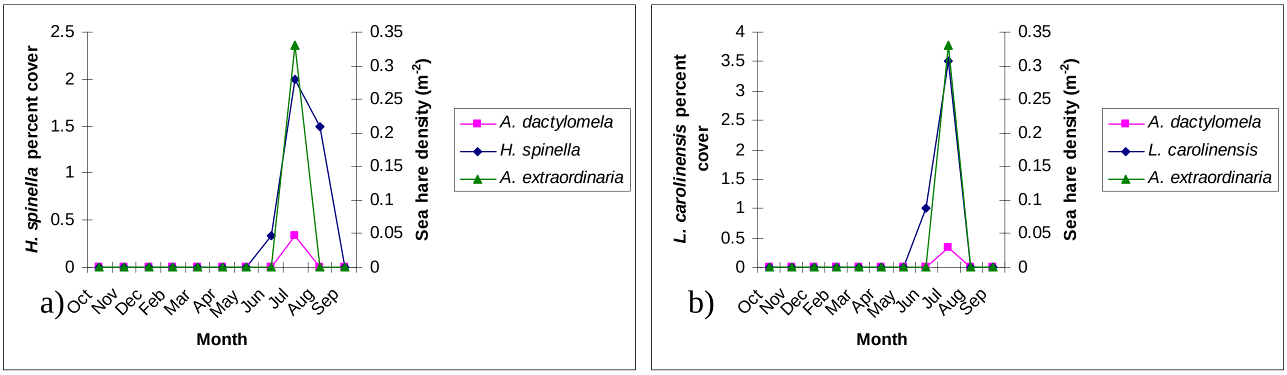

Figure 2.21: The temporal relationships between algae species and sea hare species at Picnic Bay between October 2002 and September 2003 where a) *A. dactylomela* and *A. extraordinaria* mean densities and *Hypnea spinella* mean percent cover and b) *A. dactylomela* and *A. extraordinaria* mean densities and *Laurencia carolinensis*.

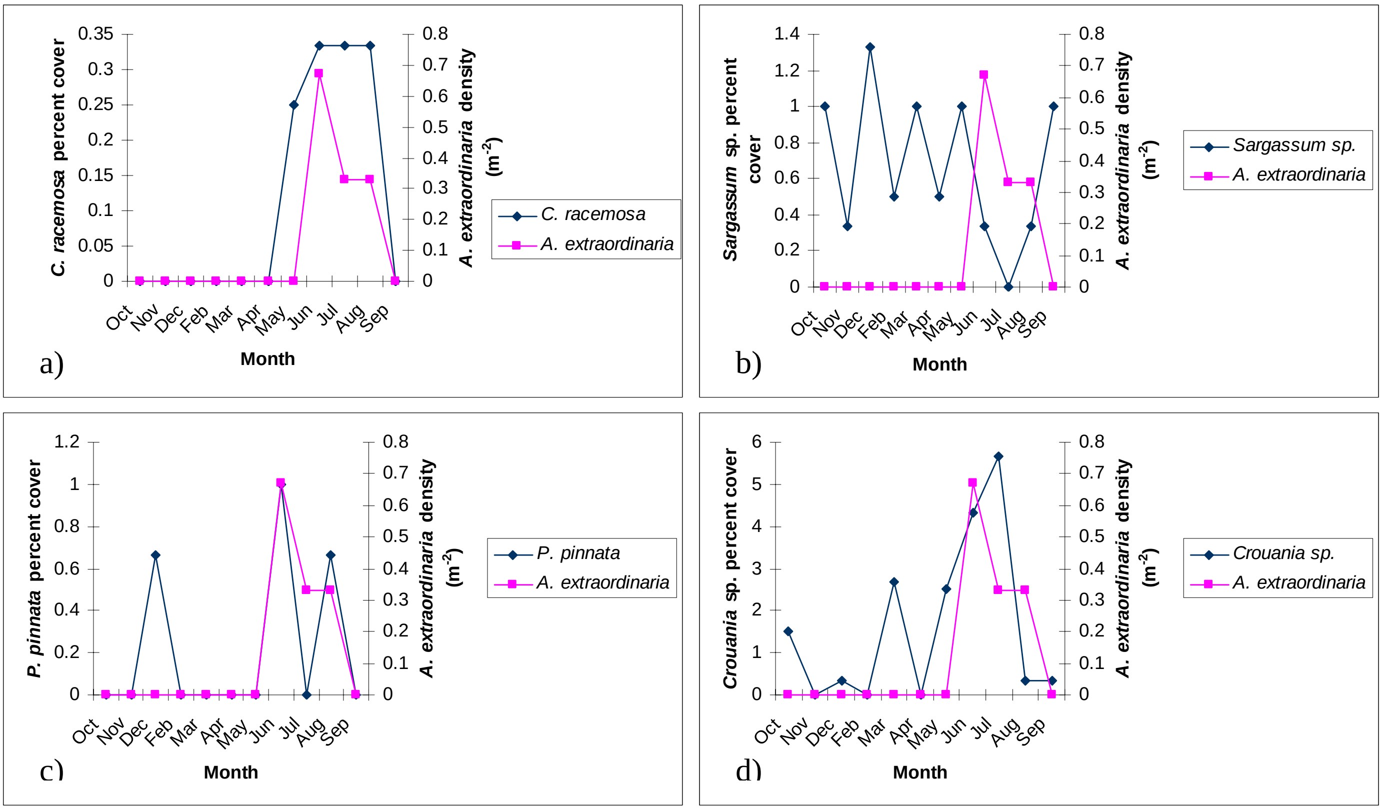

Figure 2.22: The temporal relationships between algal species and *A. extraordinaria* at Cockle Bay between October 2002 and September 2003 where a) *Caulerpa racemosa*, b) *Sargassum* sp., c) *Pterocladia pinnata* and d) *Crouania* sp.

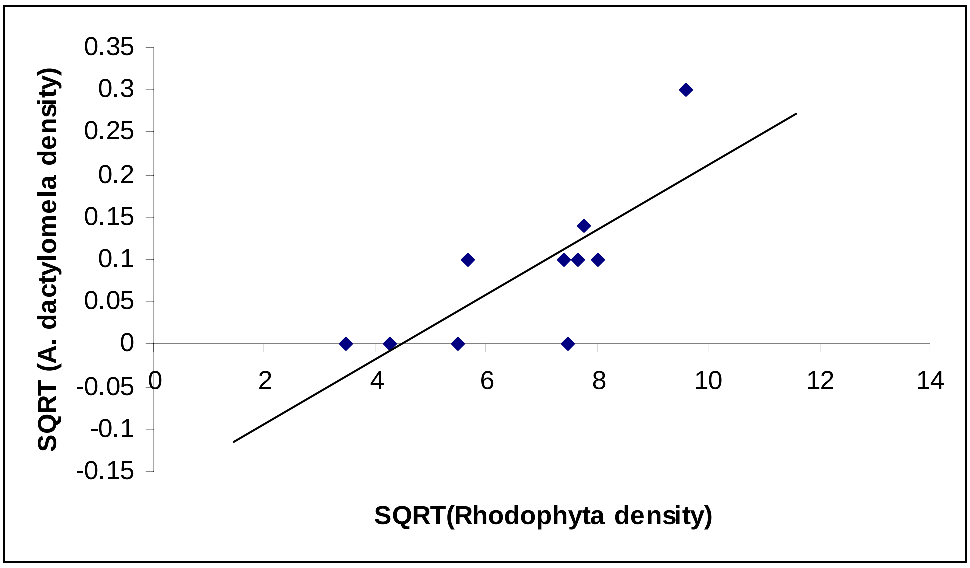

Figure 2.23: Linear regression of the square root transformed Rhodophyta percentage cover and square root transformed *Aplysia dactylomela* density. The equation of the fitted line is: $\sqrt{(A.\ dactylomela)} = 0.0382*\sqrt{(\text{Rhodophyta})} - 0.170$, r value of 0.770.

Petalifera petalifera is positively related to the percent cover of the Heterokontophyta alga, *Padina tenuis* (Figure 2.24). This significant regression can be described by the equation *P. petalifera* = 0.0032**Padina tenuis* (One-way ANOVA: F=47.701, df =1, p < 0.001). The percent cover of *Padina tenuis* explains a high amount of the variation in the density of *P. petalifera* (r-value = 0.925).

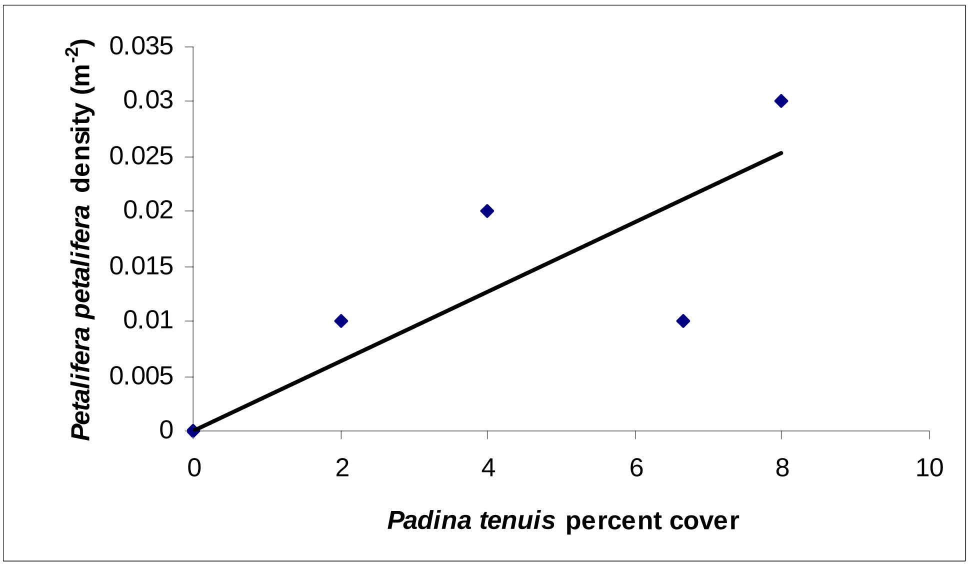

Figure 2.24: Linear regression of Heterokontophyta percentage cover and *Petalifera petalifera* density. The equation of the fitted line is *P. petalifera* = 0.001028*Heterokontophyta – 0.0058, r value = 0.7283.

Sea Hares and Abiotic Factors

Air temperature, sea surface temperature and light intensity varied throughout the sampling year (Figure 2.25). Air temperature was highest in April 2003 but remained high from December 2002 until April 2003. Air temperature was lowest in July 2003. Sea surface temperature was highest in December 2002 and lowest in July 2003. Light intensity was highest in December 2002, but was consistently high from October 2002 until March 2003. Light intensity was lowest in June 2003. As would be expected, sea surface and air temperatures were highly correlated (R = 0.914, $p < 0.0001$, N = 11).

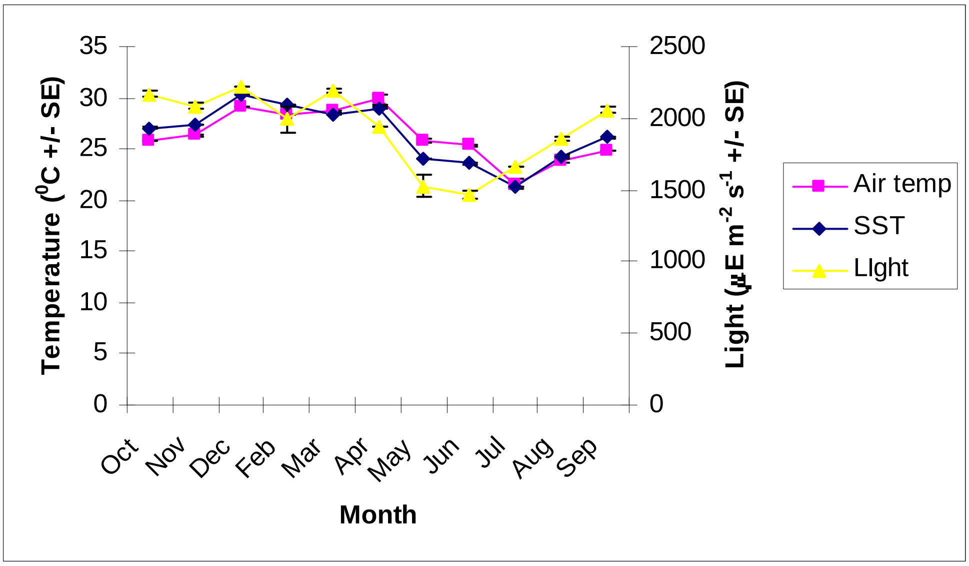

Figure 2.25: The variation in air temperature, sea surface temperature (^{o}C) and light intensity (microEinsteins m^{-2} s^{-1}) over the sampling period (October 2002 to September 2003).

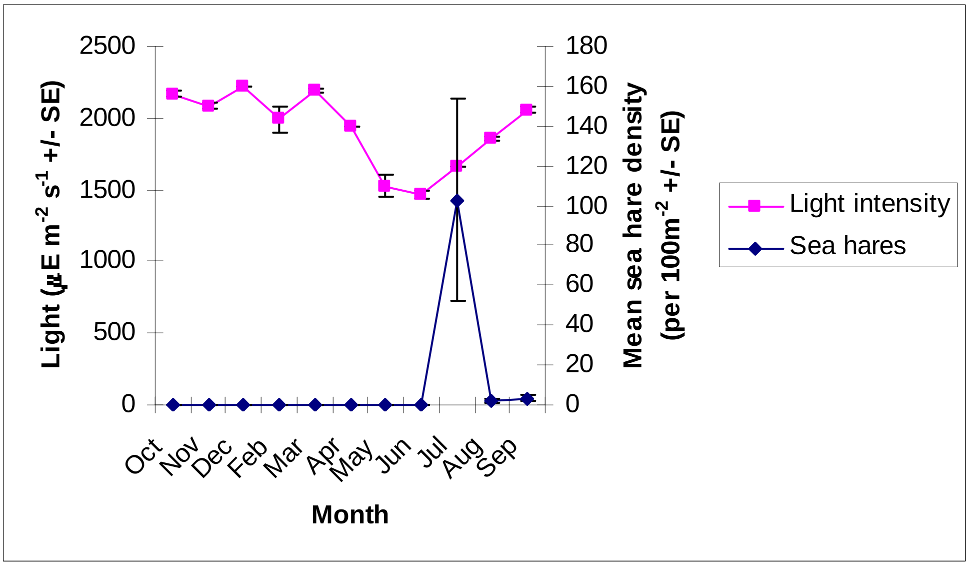

Figure 2.26: The temporal relationship between light intensity (microEinsteins m^{-2} s^{-1} +/- standard error) and mean sea hare density (+/- standard error) between October 2002 and September 2003.

More interesting however, is the correlation between the mean density of sea hares and light intensity. Sea hare density is negatively correlated with light intensity (Figure 2.26). This relationship is only moderate strength but is significant (R =-0.588, p=0.044, N=12).

Discussion

Species Specific Relationships

The factors influencing the density of tropical sea hare species in north Queensland differed for each individual species.

Petalifera petalifera

In tropical north Queensland habitats, *P. petalifera* is a specialist on *Padina tenuis. Petalifera petalifera* was highly host-specific and was only found on the calcified brown algae, *Padina tenuis*. The abundance of these two species was highly correlated. *Padina tenuis* was most abundant at Picnic Bay where *P. petalifera* was found and it was temporally abundant in the same months as *P. petalifera*. This relationship has probably resulted from specific larval settlement cues and monospecific feeding preferences of *Petalifera petalifera.* However, the ecology of *Petalifera petalifera* has not been previously examined and the diet of this species has not been investigated experimentally. This species is found circumglobally and different host macrophytes have been reported for different regions. *Petalifera petalifera* was also found to inhabit *Padina tenuis* on Orpheus Island (A. Klussmann-Kolb, pers. comm.) and at Dingo Beach (personal observation). In the Mediterranean it is associated with *Posidonia oceanica*, in the Canary Islands with *Caulerpa* spp. and *Padina tenuis* (Martinez 1996) and in Barbados with *Enteromorpha* spp. and *Sargassum* spp. (Marcus & Hughes 1974). It was speculated that *P. petalifera* feeds on a surface film of microalgae (Martinez 1996) but animals collected from Picnic Bay were observed to graze directly on the thalli of *Padina tenuis.*

Aplysia dactylomela

The density of *A. dactylomela* was positively related to the percent cover of four red algae species at Kissing Point (*Pterocladia pinnata, Crouania* sp., *Solieria robusta*, and

Hypnea pannosa) and two different red algal species at Picnic Bay (*Hypnea spinella* and *Laurencia carolinensis*). The very different algae communities at the two sites likely cause the distinct algal-sea hare relationships at each site. *Aplysia dactylomela* is known to consume a wide range of red algal species from feeding preference testing, including species of *Laurencia* (Carefoot 1970; Switzer-Dunlap & Hadfield 1979; Carefoot 1985). There was a much stronger relationship between the Rhodophyta as a group and *Aplysia dactylomela* density over all the study sites. This relationship is consistent with previous studies of the feeding preferences in this species (Carefoot 1987). Carefoot (1970; 1985) showed that this species feeds on red algae in temperate regions. Juvenile *A. dactylomela* feed on the red algae, *Acanthophora spicifera*, *Laurencia* sp., and *Spyridia filamentosa* (Switzer-Dunlap & Hadfield 1979), all of which were found in the habitats studied. The same study showed that adults feed on a greater range of red algae species as well as the green algae *Ulva* spp. The patterns observed for *A. dactylomela* could also be recruitment-driven. *A. dactylomela* settles on a range of red algae including species of the genera *Chondrococcus, Gelidium, Laurencia, Martensia, Polysiphonia,* and *Spyridia* although *Laurencia* spp. promoted the highest metamorphic success (Switzer-Dunlap & Hadfield 1977).

Aplysia extraordinaria

The wide range of algae that *Aplysia extraordinaria* was found in association with may reflect a general red algal diet, although it has not been studied previously. The coverage of a number of red algal species were related to the density of *Aplysia extraordinaria* within the three sites where it was found. *Pterocladia pinnata*, *Hypnea pannosa, Gelidium* sp. and *Sargassum* sp. were correlated with the density of *A. extraordinaria* at Kissing Point. The red algae, *Pterocladia pinnata* and *Crouania* sp. and the green algae *Caulerpa racemosa* were related to *A. extraordinaria* density at Cockle Bay. At Picnic Bay, *A. extraordinaria* was correlated with the same species as *A. dactylomela*: *Hypnea spinella* and *Laurencia carolinensis*. There was some overlap in related algae between Kissing Point and Picnic Bay and therefore animals at these two sites may therefore consume similar algae diets. The feeding preferences and settlement cues of *A. extraordinaria* have not been tested. It is therefore unknown whether the correlations found here are the result of a direct relationship between the establishment of these algae and this sea hare’s feeding preferences or caused by the settlement and metamorphosis of sea hare larvae on these particular algae.

The large size difference between the Kissing Point and Magnetic Island *Aplysia extraordinaria* has two possible causes. First, the smaller individuals at the Magnetic Island sites may be juveniles while those found at Kissing Point were adults. Histological examination of the gonads would have to be completed before differences in maturity between the two populations could be ruled out. Finally, these two populations may be different cohorts that settled at different times of year. Size at age is plastic in another species of *Aplysia*. In California, mature summer recruits of *Aplysia californica* reached 500g or more while winter recruits only attained sizes less than 1g although they both had gained reproductive maturity (Pennings 1991). The author hypothesized this was caused by the effect of temperature on growth but may also result from differences in food quality.

Stylocheilus striatus & Bursatella leachii

No relationship between the density of *S. striatus* and *B. leachii* and any of the algal groups was detected. *Stylocheilus striatus* and *B. leachii* are considered highly specialized feeders on cyanobacteria, especially *Lyngbya majuscula* (Paige 1988; Nagle *et al.* 1998) . Both species preferentially settle on species of cyanobacteria and post-metamorphic growth is best supported on cyanobacteria (Switzer-Dunlap & Hadfield 1977; Paige 1988). These two sea hare species were only present at Shelly Beach during a cyanobacterial bloom. The cyanobacterial community was not monitored during the course of this study; however, the dynamics of the cyanobacteria-*B. leachii* association will be more closely investigated in Chapter 3.

Spatial variation

The four sites showed extreme differences in sea hare and algal communities throughout the sampling year. This is not unexpected since they differ in their topography and orientation and thus face different wind, current, exposure and weather patterns affecting the supply of propagules and the intensity of settlement events within each site. The magnitude of recruitment is extremely variable between sites and locations (Connell 1985; Pennings 1991; Plaut *et al.* 1998). Therefore, it would be expected that each site would have very different communities and would exhibit differential temporal variation.

Temporal variation

Temporal variation was also an important factor determining the distribution and abundance sea hares within the sites studied. Sea hares were present in local habitats only May-September. There was total absence of sea hares in the four habitats from October 2002 through April 2003. Because of the time constraint of the present study it is unknown whether this trend is annual and whether sea hares would disappear again in October 2003. Sea hare density is also extremely variable between years. In California, *Aplysia vaccaria* was found at varying densities for an entire sampling year but was completely absent at the start of the following year (Angeloni *et al.* 1999). Gaps in sea hare occurrence have been documented for a number of populations and species (Audeskirk 1979; Gev *et al.* 1984; Achituv & Susswein 1985; Carefoot 1987; Angeloni *et al.* 1999). In the Mediterranean, *Aplysia depilans* and *A. fasciata* were only found in the spring and summer (Gev *et al.* 1984). As found in the present study, the duration of occurrence of sea hares in the Mediterranean was species specific as *Aplysia depilans* disappeared in July each year while *A. fasciata* was found until September (Gev *et al.* 1984; Achituv & Susswein 1985). Over a four year period, various habitats of *A. depilans* and *A. fasciata* were devoid of sea hares for varying amounts of time (Achituv & Susswein 1985). The cause of these disappearances was unknown as the preferred algae was present even when sea hares were absent (Achituv & Susswein 1985).

There are a number of theories that could account for the observed absence of sea hares for long periods in tropical Queensland; 1) that juveniles are difficult to see, 2) at certain times of the year populations are at such low density that they cannot be sampled, 3) sea hares migrate to habitats that were not sampled, 4) larvae delay their settlement until suitable conditions exist, 5) recruitment variation and 6) metapopulation dynamics. Their relevance to the current study system will be discussed as follows.

The first theory to address is the difficulty of finding juvenile sea hares. It is unlikely the presence of juvenile sea hares was overlooked in the seven-month sea hare absence. The techniques used for searching for sea hares were accurate enough to detect even relatively small sea hares (smallest *Petalifera* = 8.1mm). Cryptic sea hares were also detected. *Petalifera petalifera* is difficult to see when on its host macrophyte, *Padina* *tenuis*, and some species of *Aplysia* are considered cryptic against their preferred algal food (Carefoot 1987). Searching by touch and sight was used to locate the presence of small and cryptic animals in the study sites.

The low density of sea hares is the next theory to discuss. The possibility that sea hares were not sampled, even though present, could have occurred if they were at extremely low densities. At Shelly Beach, the likelihood that sea hares exist at low population levels cannot be ignored as they have been observed in other months but at extremely low density by other workers (B. Bendell pers. comm.). The other sites contained sea hares that were found at low densities even during what seemed to be the peak in density in the winter months. Therefore, it is reasonable to suppose that sea hares could have been at even lower densities in other months.

Another theory used to explain sea hare absences is migration (3). Carefoot (1987) discounts theories of long distance migration to explain the gaps in sea hare occurrence although *Aplysia californica* was shown to undergo localized movements to form breeding aggregations (Audeskirk 1979) and *A. dactylomela* uses small-scale migration in feeding (Carefoot 1985). In other cases, the reason for migration is unclear. Willan (1979) observed offshore migration in *S. striatus* even while the preferred food, *L. majuscula* remained abundant in the intertidal habitat.
Seasonal migration is possible for species that are relatively mobile and long-lived. The maximum known lifespan for a sea hare species is 18 months, for *Dolabella auricularia*, a large generalist sea hare species (Pennings *et al.* 1993). *Aplysia* spp. live for less than one year (Carefoot 1987), whereas *B. leachii* and *S. striatus* live for approximately three months (Switzer-Dunlap & Hadfield 1977; Paige 1988). *Petalifera petalifera* is unlikely to be mobile with its small size and high host specificity. These time frames are likely to reduce the selection pressure for seasonal migration in these sea hares species as the cost of moving to a new location would outweigh the benefit of increased resources. For the sea hare species found in the present study, it therefore seems unlikely that migration is the reason for the observed absences. However, because the present work was only performed in intertidal habitats there exists the possibility that sea hares migrated to deeper, subtidal habitats in the summer months.

Diel migration could also be used to explain the cause of the absence of sea hares. Sampling nocturnal animals during the day would increase the possibility of sampling error. To account for this, sampling was conducted at night in the four sites during low tides during summer months. No sea hares were recorded during these night excursions. In addition, even those species considered to be nocturnal, such as *A. dactylomela*, were found within their daytime refuges in the winter months during daylight hours. Thus diel migration is not likely to be the cause of the observed seven-month gap in sea hare presence.

The fourth theory is metamorphosis delay. Some sea hares have been shown to delay metamorphosis in the absence of appropriate settlement cues (Switzer-Dunlap & Hadfield 1977). *Bursatella leachii* is reported to be able to delay metamorphosis for more than 75 days (Paige 1988) and *A. juliana* was reported to delay for more than 200 days (Kempf 1981). However, the seven-month period where sea hares were absent from the sites in this sampling year is thus stretching the limit of the length of time that resistant sea hares species are competent to settle. In addition, the increased amount of time that these larvae would spend in the plankton would substantially increase the probability of mortality as a result of starvation, predation, or unfavourable environmental conditions. Therefore, it should be concluded that delayed metamorphosis was not the cause of the gap in sea hare occurrence.

The fifth theory is related to variation in recruitment. The gap in occurrence of sea hares may be caused by recruitment through a number of processes. The majority of temperate marine species recruit annually in pulses, linked to seasonal conditions (Pennings 1991). On the west coast of the United States, *A. californica* had the highest recruitment in summer with a smaller winter peak in some years (Pennings 1991). Tropical marine species may also respond to seasonal cues for recruitment, though these are not as pronounced as they are in temperate regions. For example, freshwater input, temperature, or ultraviolet light may serve as seasonal cues for reproduction. Appropriate settlement sites may only be available in certain months of the year. This may be true for some of the more host-specific sea hare species in this study. Many of the correlated algae serve as preferred settlement sites so that settlement may be limited to times when appropriate cues are present, ie. when preferred algae are abundant. In Israel, *A. oculifera* recruitment only occurs in the winter and the population disappears in late spring (Plaut *et al.* 1998). This recruitment pulse was linked to the presence of green macroalgae in the habitats. *Aplysia californica* settles on a wide variety of red algae but moves preferentially to *Laurencia pacifica* and *Plocamium cartilagineum* after metamorphosis (Pawlik 1989). In addition, larval supply may vary throughout the year as a result of varying physical conditions in the plankton affecting larva survival or because of seasonal variation in currents supplying larvae to these sites. Larval tows would be required to determine whether there is a continuous supply of sea hare larvae to these areas or if supply is seasonal. It therefore seems likely that recruitment plays a large role in sea hare abundance although further study would be required to determine at which scale the temporal limitation occurs in this system.

Finally, the theory of metapopulations (6) could be applied to sea hares to explain periods of absence. Marine species with a dispersive larval stage and relatively sedentary adult populations have been modeled as metapopulations (Roughgarden & Iwasa 1986). A common larval pool links these metapopulations but the adults do not mix. If metapopulations are geographically distant they may experience substantially different environmental conditions. Differences in temperature or other local conditions can affect growth rate and age at reproduction causing metapopulations to become out of phase with one another. Therefore, a mature population in one area may become the source of recruits for other metapopulations. Metapopulation dynamics were hypothesized to be responsible for the different temporal dynamics of *A. californica* on the west coast of the United States (Pennings 1991). In the present study, at least three of the species have a large geographic range and are found along the entire east coast of Australia, north to Asia, and in many other parts of the world: *A. dactylomela*, *Stylocheilus striatus* and *Bursatella leachii*. The population dynamics of these species are not known for other populations. In contrast, *Aplysia parvula* is found throughout the year in habitats near temperate Sydney but its abundance varies (Rogers *et al.* 2003). If there is a constant supply of recruits but a limited sea hare season this suggests the influence of additional environmental factors limiting recruitment to local metapopulations.

Environmental Factors

Tropical shallow water habitats are exposed to especially high levels of ultraviolet radiation and these levels increase in summer (Middelboe *et al.* 2003). The negative relationship between light intensity and sea hare density in these four sites adds evidence to the theory that ultraviolet radiation has a negative effect on sea hares. This theory was first examined by Carefoot & Taylor (1988) in relation to the evolution of nocturnalism in *Aplysia*. Light was the cue that regulated diel activity patterns in *A. californica* but whether light was merely a cue or the underlying cause is unknown. Recently, Plaut (2000) demonstrated that shaded *A. oculifera* ceased their nocturnal activity patterns while unshaded animals continued to display nocturnal activities. Carefoot *et al* (1998) investigated the effect of UV light on reproduction in *A. dactylomela*. They found that adults treated with UV light showed indications of stress as evidenced by a high rate of oxygen consumption, elevated blood-glucose levels and increased spawning behaviours. In addition, the eggs laid by UV treated adults hatched faster than those without UV light, which could be caused by increased metabolic rate. Eggs treated with UV light had decreased hatching efficiency, which suggests deleterious effects of UV light on eggs. UV-protective compounds, called mycosporine-like amino acids, have been identified in sea hare food algae and egg masses by Carefoot *et al.* (1998).

It must be considered that the food algae of sea hares are also affected by increased ultraviolet radiation in summer months. Seagrass species are affected by UV light in different ways and these effects also interact with the presence of epiphytes (Dawson & Dennison 1996). However, there was no correlation between the percentage cover of any of the macrophytic groups and light or temperature parameters. The only factor measured in the present study, macrophytic percentage cover, may not provide a good indication of the effects of environmental factors. Therefore the indirect effect of light intensity on sea hare food species cannot be ruled out.

Conclusions

All five local sea hare species were restricted to the winter months. Settlement cues, algal dynamics, low density and larval supply may be responsible for this temporal relationship. The factors important for determining sea hare density in these four sites are species-specific. The density of *Petalifera petalifera* was related to the percentage cover of its host macrophyte, *Padina tenuis.* *Aplysia dactylomela* density was related to the percentage cover of red algal species. *Stylocheilus striatus* and *Bursatella leachii* were not related to any of the factors studied in this chapter so their relationship with cyanobacteria will be further examined in Chapter 3. The nature of the sampling design does not allow extrapolation to other North Queensland habitats although it seems likely that these factors would also be important where sea hares are found.   High levels of UV light in summer months may prevent the settlement of sea hare recruits and contribute to the absence of sea hares in summer months in these sites. The single year of sampling does not allow generalization to other years but the consistent physical conditions at these sites makes it likely that these factors would be present in other years.

Chapter 3: The population dynamics of a *Bursatella leachii* population irruption at Shelly Beach

Introduction

Population Dynamics

The single unifying feature of all populations is their dynamism. Documenting natural population fluctuation has become increasingly important in recent times where the need exists to distinguish natural fluctuations in systems from those caused by anthropogenic disturbance. The majority of fluctuations in populations occur on a local scale (Smith 1996). Local populations can recruit, grow, decline or disappear due to interactions with other organisms and their environment (Krebs 2001b). But in most cases, the density of the species over its entire geographical range remains stable (Smith 1996). Population changes have been attributed to a range of factors and often more than one of these is operating on a single population, although some factors may be more important than others. Natural reasons for population change include alteration of the growth and fecundity of the organisms (Campbell 1990; Smith 1996). This may be caused by changes in their interactions with conspecific individuals, competing species, and the dynamics of predators and prey (Campbell 1990; Gotelli 1998). Changes in the rate of mortality can also affect populations and this is usually mediated by stochastic events such as variations in the physical environment (Smith 1996). Such stochastic events can have direct effects on the mortality of the population in question or indirect impacts by affecting other organisms within the system (Campbell 1990). Populations can also be affected by the activities of humans. Pollution, habitat destruction, sedimentation, global climate change and a range of other influences can disturb natural populations in various ways (Campbell 1990; Smith 1996; Gotelli 1998).

Aggregations of large numbers of animals in a single area are an extreme example of population fluctuations. These events are often referred to as population explosions or irruptions (Colgan 1987; Campbell 1990; Burla & Ribi 1998; Cote & Reynolds 1998; Williams *et al.* 2001). These aggregations are often an obvious and highly visible incident to human visitors to the site in question and can have a strong effect on the local animal and macrophyte communities. Monospecific groups can put pressure on local resources and exclude their competitors. The position of the animal within the food chain determines the effects of the irruption, with organisms further up in the chain exhibiting positive effects while those below will be negatively affected.

Population Irruption

The underlying reasons for the occurrence of population irruptions or aggregations have not been investigated in great detail. In some cases, these events have been attributed to migration from other locations for the purpose of reproduction, either in mate finding, releasing gametes, or egg-laying. Examples come from birds (Bleiweiss 1999), amphibians (Roberts 1994; Edwin & Lucas 2002), fish (Hendry *et al.* 1995; Oliveira *et al.* 1999), and invertebrates (Trowbridge 1991b; Young *et al.* 1992). Recruitment has also been suggested as a cause of aggregations in low mobility species. A large recruitment pulse can allow a large settlement of juveniles and subsequently, there is the potential for a large density of adult animals in the area (Carefoot 1987). Interactions with other species can cause cycles in population growth and decline. The 9-10 year cyclic fluctuation of the lynx and snowshoe hare of Canada is a classic examples of these cycles (Smith 1996). Boom and bust cycles are also common in the insect world. Locusts (Ibrahim 2001) and geometrid moths (Kaitaniemi *et al.* 1998) can reach plague proportions and devastate the targeted plant resource when in the boom phase of the cycle. In between abundance peaks the predator population exists in a dormant stage or at extremely low population levels (Ibrahim 2001). Similar population examples can also be found in the marine environment. In Canada, the urchin *Strongylocentrus droebachiensis* forms dense feeding aggregations that decimate kelp beds and create barren areas absent of macrophyte life (Scheibling *et al.* 1999). Crown-of-thorns starfish (*Acanthaster planci*) appear to exhibit a similar cyclic population fluctuation and during population peaks ravage the coral communities of the Great Barrier Reef (Lourey *et al.* 2000).

Anecdotal evidence suggests some sea hare species exhibit boom-and-bust cycles. There are a number of hypotheses as to why populations of sea hares exhibit this dynamic. One hypothesis is that the sea hares settle in a range of habitats and migrate to areas of highly dense conspecifics for the purpose of breeding (reviewed by Carefoot 1987). In California surf-grass beds, *Aplysia californica* form dense breeding aggregations in summer months (Audeskirk 1979). Breeding aggregations of *Bursatella leachii* during the Florida winter were observed but animals in summer aggregations were mostly immature (Lowe & Turner 1976).

The second hypothesis is that aggregation occurs where food is localized (Carefoot 1987). In habitats where food is concentrated sea hares may aggregate accidentally as a result of settlement or feeding preferences. That is, a large recruitment of sea hares may settle in an area where the preferred host alga is high in abundance. In Israel, *A. oculifera* abundance was highly correlated with the variable presence of *Ulva* and *Enteromorpha* (Plaut *et al.* 1998). *Aplysia californica* juveniles for example, are primarily found on the red algae *Plocamium cartilagineum* and *Laurencia pacifica* (Pennings 1991). However, Pawlik (1989) demonstrated that *A. californica* settles on a wider range of algae and crawls to the preferred species. Sites with greater *P. cartilageum* cover contained higher numbers of *A. californica* recruits however this characteristic did not explain the variation in recruitment intensity which may be related to larval supply to the sites (Pennings 1991). The combination of favourable larval conditions and a high abundance of a preferred algae species for competent larvae could cause a large settlement of juveniles in these areas.

The third hypothesis for the cause of aggregation is a response to hydrological conditions. Lowe & Turner (1976) hypothesized that aggregations of juvenile *B. leachii* were caused by the hydrological conditions in subtidal habitats. Additionally, the intensity of water movement in Israel intertidal habitats was negatively related to sea hare abundance (Plaut *et al.* 1998). The occurrence of sea hares in intertidal habitats may be a compromise between a preference for low-intensity wave action and their preference for intertidal algal species. In one year, Plaut *et al.* (1998) observed a rare algal bloom in deeper water and *A. oculifera* was found in greater abundance in association with this bloom than those populations in the exposed shallow-water habitats.

Stylocheilus striatus and *Bursatella leachii* are often reported to be found in dense feeding aggregations in association with blooms of cyanobacteria (Switzer-Dunlap & Hadfield 1979; Paul & Pennings 1991). The dynamics of these aggregations has never been quantitatively examined for either of these two species. The sole report, Lowe &

Turner (1976) examined aggregations of *B. leachii* at a beach in Florida but measured the densities of animals washed up on the shoreline, therefore giving no indication of thee natural density of this species. The temporal dynamics of these types of aggregations have not been previously investigated and therefore, the underlying cause of population irruption in sea hares is unknown.

Stylocheilus striatus is considered a specialist on the cyanobacteria, *Lyngbya majuscula* (Nagle *et al.* 1998). *Lyngbya majuscula* forms dense blooms that can cause a range of medical conditions in humans (Moore 1981). A number of novel toxins have been detected in this cyanobacteria, including aplysiotoxins, majusculamides and malyngamides (Pennings *et al.* 1996) although the concentration of these toxins can vary (Nagle *et al.* 1998) . The occurrence of these blooms in coastal waters has been repeatedly reported in Australia and Hawaii and may be increasing in frequency (Hallegraeff 1993; Turner & Tester 1997). These blooms potentially affect the health of humans and the tourism industry by diminishing the appeal of coastal habitats. Therefore, the population dynamics of their herbivorous consumers are of interest.

The following chapter documents and analyses the dynamics of the *Bursatella leachii* and *Stylocheilus striatus* population irruption events at Shelly Beach. Two specific questions were addressed about the characteristics of these population events: Is the population irruption a single cohort settling within the seagrass beds, which grows, matures, reproduces and then dies? Or, conversely, is the population composed of more than one cohort and supported by the addition of new recruits?

Materials & Methods

Study Site

Shelly Beach is an ideal place to closely examine the population dynamics of *S. striatus* and *B. leachii* because of the relative simplicity of the benthic habitat. The length of seagrass blades allow for easy detection of any sea hares present within the seagrass bed. The Shelly Beach seagrass beds are never completely dry because some water is retained even at extremely low tides. Furthermore, sampling can be completed intertidally when the water is low, as sea hares are not forced to migrate by dropping water levels.

Population Irruption Monitoring

Five sampling trips were made to the Shelly Beach seagrass beds on 25 June, 7 July, 21 July, 27 July, and 3 September 2002. On each trip three 25 x 0.5m belt transects were run parallel to the beach, yielding an estimate of sea hare density per 12.5m^2. Transects were run at approximately 100m from the shore and were always oriented parallel to the beach to eliminate the effect of tidal height and to keep the measurement area within the dense seagrass habitat. Within each transect the sea hare species present and the number and length of individual sea hares was recorded. The sea hare species present was identified according to Coleman (2001). The length of each sea hare was measured by placing the individual on the transect tape and allowing the animal a few seconds to start to crawl along it. Once crawling, the distance between the base of the cephalic tentacles and the tip of the tail was recorded. The number of sea hare egg masses observed within transects was also recorded.

Statistical Analysis

Descriptive length-frequency analysis was used to examine the population dynamics the sea hare populations. More formal length-frequency techniques could not be used because the full life history characteristics of these species have not been tested. The sea hare lengths were divided into 5mm classes as this provided the best resolution of the patterns of recruitment and mortality. The assumptions of heterogeneity and normality were met and one-way ANOVA was performed on the length of sea hares found during each trip.

Results

Population Irruption Characteristics

Sea hares were found in large numbers at Shelly Beach from 25 June to 27 July 2002. Two species of sea hare were present: *Stylocheilus striatus* and *Bursatella leachii*. Individuals of the two species were observed in the same areas, often in close association. A cyanobacterial bloom was also present at Shelly Beach during the sea hare population irruption. The cyanobacterium was identified as *Calothrix crustacea* (B. Bendell pers. comm.) and was present both in large tufts on the sediment and growing epiphytically on the seagrass leaves.

During the initial sampling trip, 25th June 2002, only *Bursatella leachii* was recorded in the transect area. The mean density of *B. leachii* at this time was 512.0 per 100 m^2 (Figure 3.1). The highest recorded density of *B. leachii* was observed on 7 July when it increased to 744.0 per 100 m^2 and then decreased in the subsequent sampling trips. No animals were recorded on the final sampling trip, 3 September 2002. Throughout the entire month of sampling, the predominant species remained *B. leachii* (Figure 3.1). The density of *S. striatus* was much lower than that recorded for *B. leachii* for the duration of the population irruption.

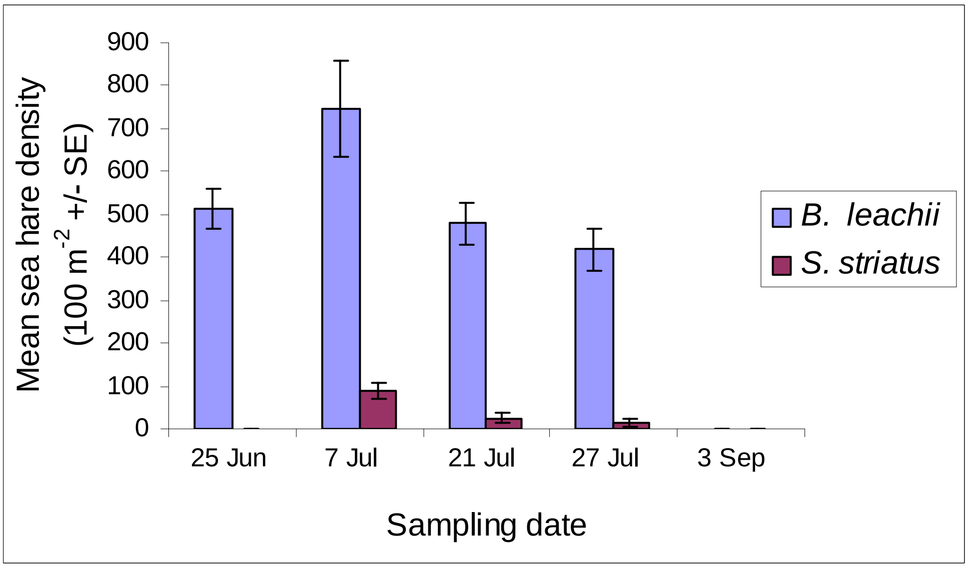

Figure 3.1: The mean density (per 100m^2) of *Bursatella leachii* and *Stylocheilus striatus* within the seagrass beds of Shelly Beach in 2002.

Stylocheilus striatus was first recorded within in the transect area on 7th July 2002. Its density peaked at 88.0 per 100 m^2 and steadily declined in the following trips until no animals were recorded on 3 September 2002. The mean density of *S. striatus* during the three trips it was present (7th, 21st, and 27th July, 2002) was 42.0 per 100 m^2 (Figure 3.1). Formal analysis of the length of *S. striatus* was performed but is not included here because it did not illuminate any useful trends for this study. This may be a result of the relatively low numbers recorded during most sampling trips.

Pulse Settlement vs. Continuous Recruitment

Evidence against a single settlement event is the mean size of each sampling trip. The sizes of *B. leachii* found during each of the four sampling trips were significantly different (One-way ANOVA: df = 3, F = 35.588, $p < 0.001$). There was a decrease in mean length of *B. leachii* between 25 June and 7 July, a small increase during 21 July and then another small decline during 27 July (Figure 3.2). The animals found on 25 June and 21 July were similar in size and those in 7 July and 27 July were similar in size (Tukey's HSD post-hoc tests: homogeneous subsets A and B, Figure 3.2). This indicates that there was more than a single settlement event occurring at Shelly Beach.

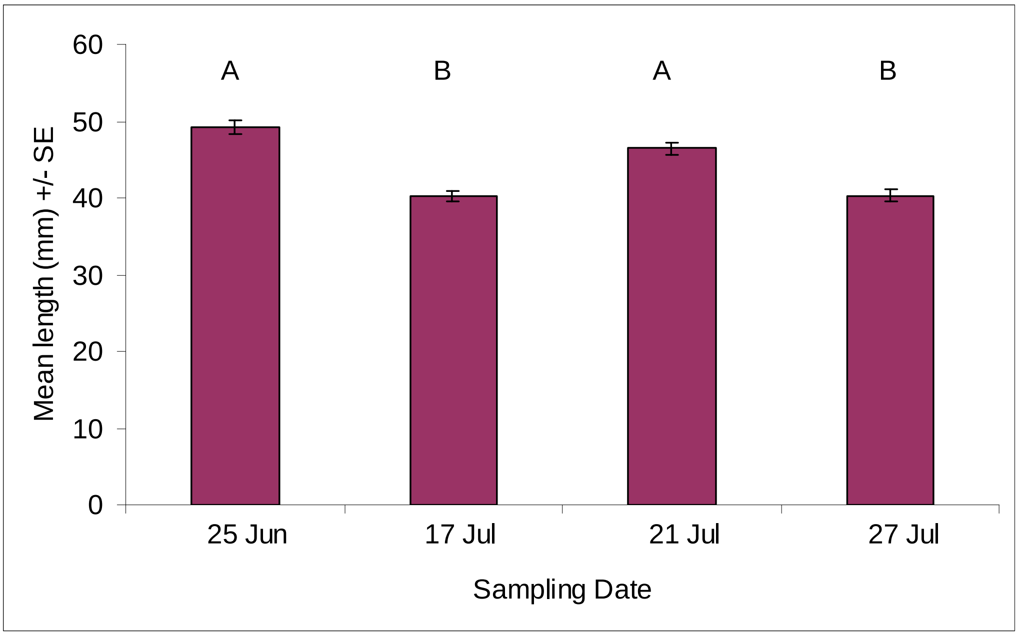

Figure 3.2: The mean length (mm +/- standard error) of *Bursatella leachii* individuals during the four sampling trips to Shelly Beach in 2002. The letters denote significantly different subgroups as determined by Tukey's HSD post-hoc tests.

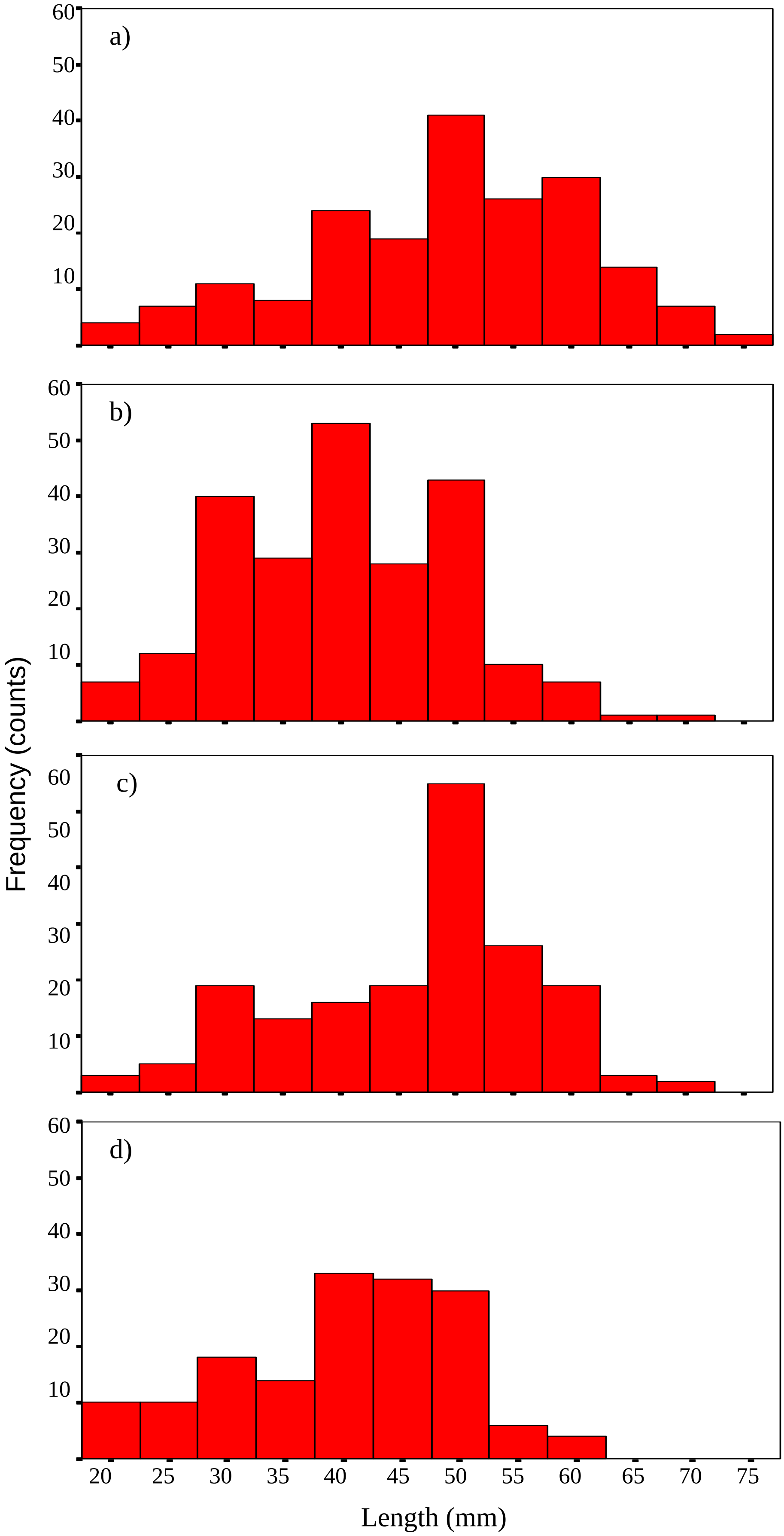

Figure 3.3: Length-frequency histograms of *Bursatella leachii* present at Shelly Beach in 2002 during each of the four sampling trips where a) 25 June, b) 7 July, c) 21 July, and d) 27 July.

Length-Frequency Analysis

There was a continual input of smaller individuals into the population at Shelly Beach during June-July 2002 (Figure 3.3). It seems that the small sea hares grew larger and moved into the larger size classes over the course of sampling in Figure 3.3. There was also a gradual decline in the abundance of larger size classes and a disappearance completely in the last sampling trip of the three largest classes. In late June 2002, the size classes were normally distributed with a peak at the 50mm length class although there were slightly more individuals in the larger size classes (Figure 3.3a). In early July, there was a peak of 40mm animals and two smaller peaks at 30 and 50mm. There were a high proportion of smaller individuals at this time (Figure 3.3b). There was an increase in the number of larger individuals sampled during the 21 July sampling trip (Figure 3.3c). At the end of July not one of the size classes dominated the distribution and again there was an increase in the number of small individuals (Figure 3.3d).

Egg Mass Variation

The presence of characteristic string-like sea hare egg masses within the sampling area indicated spawning activity and the presence of reproductively mature sea hares. However, which sea hare species had laid each mass could not be identified from the egg masses themselves in a field setting. The density of egg masses found during each sampling trip in 2002 peaked during the July 7th sampling trip and decreased in the following two trips (Figure 3.4). The egg mass density during the 2002 sea hare population irruption peaked on July 7th, which corresponds with the peak in sea hare density (see Figure 3.1 and 3.2). The density of egg masses at this time was 228.0 egg masses 100 m^{-2}, yielding a sea hare to egg mass ratio of 3.65 : 1.

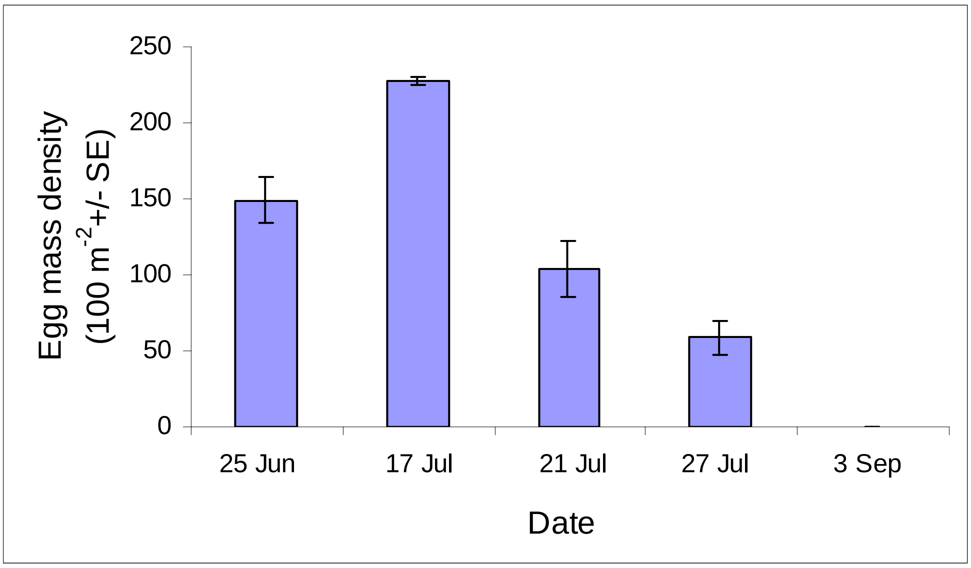

Figure 3.4: The mean density of egg masses (per 100 m^2, +/- standard error) found during each sampling trip within the seagrass beds of Shelly Beach in 2002.

Discussion

Sea Hare Density

The sea hare population at Shelly Beach reached extremely high densities during the sampling period. The highest density of *B. leachii* found at Shelly Beach was 744 per m^2. Combining the two species yields a mean density of 832.0 sea hares per 100 m^2 at the population peak. In June 2003, another population irruption event was documented at the same location (present study, Chapter 2). *Stylocheilus striatus* reached high density (384.0 per 100 m^2) while *B. leachii* was found at lower density (24.0 per 100 m^2). Therefore population density was high again in 2003 but less than that observed in 2002. *Stylocheilus striatus* has been reported in large numbers in association with blooms of *Lyngbya majuscula* in Hawaii (Switzer-Dunlap & Hadfield 1979) and Guam (Paul & Pennings 1991; Nagle *et al.* 1998) but the densities were never quantified. Lowe & Turner (1976) reported substantially higher densities of *B. leachii* in Florida. However, the density reported for subtidal populations was based on the number of animals within a single aggregation. Other sea hare species have much lower densities. Carefoot (1987) reviewed the reported densities of several species of *Aplysia* and the densities never exceeded 5.0 per m^2 with most reports less than 1 m^{-2} (Willan 1979; Carefoot 1987). The low densities of *Aplysia* species are used as evidence for the limited role of all sea hare species in the marine community (Carefoot 1987; Rogers *et al.* 2003). However, the present study indicates much higher densities for *B. leachii* than the more commonly studied *Aplysia* spp.

Because of the large transect size the measurement of sea hare density in the present study was more representative of the density of these species over the entire bay. At this location, the seagrass itself is not homogeneous; there are patches of dry sand where the seagrass has been burned away during low tide and areas of differing composition of seagrass species. Due to the sea hares' patchy and highly aggregated distribution it seems likely that densities would be much higher if only healthy, dense seagrass areas or sea hare aggregations were sampled.

Sea Hares and Cyanobacterial Blooms

The co-occurrence of *B. leachii* and *S. striatus* and the bloom of the cyanobacterium, *Calothrix crustacea* in 2002 has not been documented previously. In a subsequent year (2003, presented in Chapter 2) these sea hare species were associated with a different unidentified cyanobacteria species. *Bursatella leachii* is believed to have a more catholic diet than *S. striatus* (Wu 1980; Paige 1988). In Hong Kong, *B. leachii* consumes a range of macroalgae, preferring *Enteromorpha prolifera,* although cyanobacteria species were not tested (Wu 1980). In Florida, only species of cyanobacteria (including *Lyngbya majuscula*) were preferred in food choice tests and as metamorphic substrates (Paige 1988). In contrast, all previous reports have linked *S. striatus* with *Lyngbya,* its preferred food according to a number of authors (Paul & Pennings 1991; Pennings *et al.* 1996; Nagle *et al.* 1998).

Species Coexistence

Bursatella leachii and *S. striatus* were both found closely associated in the same seagrass habitat at Shelly Beach. The coexistence of *Stylocheilus striatus* and *Bursatella leachii* has been reported previously (Switzer-Dunlap & Hadfield 1979) and were observed in the same location in June 2003 (Chapter 2). No investigations have been made to determine the mechanisms that allow these species to exploit the same habitat. The "competitive exclusion principle" states that competitors cannot coexist under food-limiting conditions (Krebs 2001a). Both species settle and feed preferentially on cyanobacterial species (Switzer-Dunlap & Hadfield 1977; Paige 1988). These two species therefore occupy the same habitat at the same time of year and have similar feeding preferences, suggesting that competition may exist between them.

Bursatella leachii was present at consistently higher densities throughout the population irruption. In contrast, *S. striatus* was the predominant species when sampling in June 2003 at Shelly Beach (Chapter 2). Habitat partitioning was suggested for two sympatric species in the Mediterranean where *Aplysia fasciata* was restricted to calm habitats while *A. depilans* resides in highly turbulent areas. Competition was likely occurring in intermediate areas although the mechanism was not identified (Achituv & Susswein 1985). *Stylocheilus striatus* and *B. leachii* may partition the habitat by exhibiting different feeding behaviours. Both species of sea hare were observed engaged in feeding behaviours upon tufts of cyanobacteria (personal observation). However, *B. leachii* was seen grazing on cyanobacterial tufts while crawling along the sediment and the smaller, *S. striatus* was observed to crawl directly on the seagrass leaves to access epiphytic cyanobacterial tufts. An additional possibility is that these two species reduce competition by occupying the seagrass beds at slightly different times, as *Stylocheilus striatus* recruited into the area later than *B. leachii*. Temporal partitioning has been observed for *A. depilans* and *A. fasciata* where the superior competitor, *A. depilans*, entered the population early in the season (Gev *et al.* 1984). However, in 2003, no difference in recruitment timing was observed and the duration of the event was much shorter than that observed in 2002. The differences observed in the two population irruption events make speculation about possible competitive dominance and/or coexistence in this system difficult. Care should be taken when discussing the dynamics of population irruptions that contain both *S. striatus* and *B. leachii* because they may exhibit different population dynamics and differing life history traits such as rate of maturity, age at maturity, fecundity or even settlement cues, and environmental tolerances.

Population Irruption Dynamics

The length-frequency distribution of this population can be used to investigate hypotheses about its dynamics (following Gev *et al.* 1984). It is important to note that length-frequency analysis is based on the assumption that length is a reasonable proxy for age. A single study found that length and age are directly related for *B. leachii* although only a small number of animals were used in experiments (Willan 1979). Other molluscs shrink under conditions of stress or low food availability (Russell-Hunter *et al.* 1984; Russell-Hunter 1985). In the present study the concurrent cyanobacterial bloom likely provided an abundance of food, at least for the majority of the sea hare population irruption event. The hypothesis being tested was that sea hare population irruptions are the result of settlement of a single cohort on the cyanobacterial bloom. Similar studies with co-occurring *Aplysia depilans* and *A. fasciata* in Israel showed a single recruitment pulse (Achituv & Susswein 1985). If a single pulse occurred we would expect the length-frequency analysis would have detected a large cohort of animals of similar size moving through the size classes. For *B. leachii,* there was a continual addition of small recruits. In conjunction, there were also a small number of individuals in the large size classes. Thus, the size distribution of this population indicates that recruitment of individuals into the population is occurring throughout the duration of the cyanobacterial bloom.

If the sea hare population irruption was a pulse-recruitment event we would expect to see a gradual increase in size and then an abrupt end to the population. The mean length of *B. leachii* was not significantly different through the duration of the cyanobacterial bloom. Thus, the animals were similar in size or size range throughout their presence in the seagrass beds. The presence of egg masses over the entire seven-week period also suggests that the population in Shelly Beach was not the result of a single recruitment pulse. Egg masses indicate the existence of reproductively mature and active adults within the population at all times. *Aplysia* species have been reported to restrict reproductive activities to certain habitats and certain seasons (Willan 1979; Carefoot 1987). For example, Willan (1979) found that the intertidal area he studied only contained immature *Aplysia dactylomela* and therefore no egg-laying occurred. The presence of sea hares from a wide size-range and the observed egg masses indicates that this population contained both immature and reproductively mature sea hares at the same time and therefore was not the result of a pulse recruitment event.

Once it has been established that the population irruption is not a pulse recruitment event two questions remain. 1) What happens to the large adults in the population? and

2) Where do the small recruits come from? Large adults that were lost throughout the duration of the cyanobacterial bloom were either removed from the population by mortality, migration, or natural senescence. Decreasing abundance of cyanobacteria could cause starvation-induced mortality or trigger the migration of animals to other areas. *Nassarius* snails were observed feeding upon sea hare carcasses although it is unknown whether this predator caused the death of the animals or were merely scavenging. Predation is not thought to be a major cause of mortality for sea hares (Carefoot 1987; Johnson & Willows 1999; Rogers *et al.* 2000b). However, a limited number of predators are known to regularly prey upon them. These include the sea anemone *Anthopleura xanthogrammica* in California (Winkler & Tilton 1962), the pycnogonid *Anoplodactylus evansi* in temperate Australia (Rogers *et al.* 2000b), the sea star *Coscinasterias calamaria* in New Zealand (Willan 1979) and the gastropods *Navanax* and *Melo amphora* (Pennings 1990a; Johnson & Willows 1999). Many of these predators feed mainly upon juvenile sea hares (Rogers *et al.* 2000b) causing differential mortality on the various size classes. Field predation studies on *Aplysia* spp. suggested that juvenile sea hares are highly susceptible to predation at small sizes (Pennings 1990a; Johnson & Willows 1999; Rogers *et al.* 2000b) but escape this threat with large size due to the inability of predators to handle prey items above a certain size limit (Pennings 1990b, a; Johnson & Willows 1999).

A small number of omnivorous fish are able to consume *S. striatus* but these observations have been the result of artificially manipulating the animals and placing them in the water column rather than upon the benthos or amongst algae stands (Pennings *et al.* 1996). Willan (1979) observed a single predator of *B. leachii*, the sea anemone *Actinia olivacea*. It is unknown whether natural predation events are important for the population dynamics of *B. leachii* and *S. striatus* in Shelly Beach but there is no evidence supporting predation as a significant factor in this location.

The other possibility for the observed depletion of large sea hares is migration. If the animals were migrating to areas unavailable to observation then we would expect to see an abrupt appearance or disappearance of mature animals. The continual recruitment of small *B. leachii* into the population in 2002 rules out the possibility that this high-density occurrence was merely a roving population of adults. It would be expected that migration would cause an increase/decrease in abundance of all size classes, which was not observed here. There was a gradual decline of the number of large animals over time, suggesting that a mass migration was not the cause of the disappearance of sea hares in this population. This gradual decline is suggestive of a natural senescence of older sea hares, especially when it is considered that the timing of the population irruption event is comparable to the three month life span of *B. leachii* (Paige 1988).

The recruitment of animals into the population at Shelly Beach may be caused by the species existing in low abundance within various habitats and recruiting in high numbers when conditions are suitable. To examine the viability of this hypothesis we must examine the reproductive characteristics of *B. leachii*. Sea hares are known to spawn millions of eggs in each egg mass and reproduce continually once maturity is reached (Carefoot 1987). Many sea hare species have relatively long larval development, more than 30 days in the laboratory (Switzer-Dunlap & Hadfield 1977). However, *B. leachii*, from Florida, has been reported to settle after a shortened larval duration – 19 days under laboratory conditions (Paige 1988). The maximum duration that *B. leachii* larvae can remain in the plankton without a suitable settlement site is 77 days (Paige 1988). It seems probable that the massive reproductive output observed would release large numbers of larvae into the plankton to utilize settlement sites in other areas where cyanobacterial blooms are occurring.

The large amount of cyanobacteria biomass seen in Shelly Beach would provide a large area of suitable settlement sites. In Florida, *B. leachii* preferred to settle on three species of cyanobacteria, *L. majuscula, Schizothrix calciola* and *Porphyrosiphon notarisii* (Paige 1988). In Hawaii, *Lyngbya majuscula* is known to serve as a preferred site of settlement for *S. striatus,* along with a small number of red algal species (Switzer-Dunlap & Hadfield 1977). The physical conditions that promote cyanobacterial blooms may also be favourable for the influx of sea hare larvae into Shelly Beach seagrass beds. In addition, the ability of sea hare larvae to move towards chemical cues for settlement is currently unknown but if they possess a certain degree of swimming ability (as seen in reef fish larvae Fisher *et al.* 2000), these cyanobacterial blooms would send out strong signals into the water column for larvae to orient towards.

Conclusions

The sea hares *Bursatella leachii* and *Stylocheilus striatus* are found at densities far greater than those previously reported for other sea hare species. This suggests that these sea hare species may be important consumers of the sporadic cyanobacterial blooms that occur in sea grass beds and other coastal habitats. Analysis of the population dynamics revealed that the population irruption observed was not the result of a pulse recruitment event, in contrast to previous studies. Small sea hares continually recruited into the area likely from reproductively active animals existing at low population levels in other habitats. Techniques such as population genetics and larval tows may shed further light on recruitment patterns in this ecosystem. Sea hares in large size classes declined over the course of the event possibly as a result of natural senescence and death rather than migration or predation. Further sampling in the surrounding areas and predation trials would be required to rule out these factors.

Chapter 4: The feeding preferences of *Bursatella leachii* from Shelly Beach

Introduction

Feeding Preferences

The feeding preferences exhibited by a herbivore yield valuable insight into the ecology of the herbivore and the trophic relationships within an ecosystem. These feeding preferences have implications for both the primary producers community and the associated herbivores. They will influence which resources are consumed, which in turn will directly affect the animal's nutrition, growth, and reproduction (Hsiao & Franekel 1968). The species and quantity of macrophytes consumed also affects the response of the macrophytes by influencing their abundance, species composition, and distribution (Paine & Vadas 1969b; Nicotri 1977; Lubchenco 1978). To understand the feeding preferences of an animal and subsequent role within an ecosystem, the most common approach is to test the organism under controlled laboratory conditions (see Dethier 1954; Paine & Vadas 1969a; Carefoot 1973; 1987; Tsuda & Bryan 1973; Nicotri 1980; Pennings *et al.* 1993).

The term, 'feeding preference' is used widely in the literature (for examples see Carefoot 1967; Tsuda & Bryan 1973; Lubchenco 1978; Nicotri 1980; Carefoot 1987; Sotka 2003; Vaitilingon *et al.* 2003) but can have several meanings, which is not always clarified. Nicotri (1980) distinguished two aspects of food preference; edibility and attractiveness. Edibility is ideally measured by the rate of actual consumption of the food item. The relative edibility of a food item will reflect not only the rate at which the animal is physiologically satisfied, but also the ability of the animal to manipulate and physically consume the prey. However, edibility is not always correlated with attractiveness. Edibility is one of the factors that contribute to the attractiveness of the food item, but not the sole one. Attractiveness is the actual choice of the prey item by the herbivore. Attractiveness is tested when the herbivore must sense the prey items offered either by chemical cues or by direct contact with the choices offered. Ideally, both edibility and attractiveness should be tested in order to completely grasp the way in which the herbivore feeds.

Edibility

Using the above definitions of feeding preferences, we can divide the factors responsible for such preferences into two groups based on edibility and attractiveness. With regard to edibility, perhaps the most intuitive factor is the nutritional quality of the food source. Various attempts have been made to relate feeding preference to nutritional quality using several different parameters, including caloric value (Paine & Vadas 1969a), organic and nitrogenous content (Nicotri 1980; Rogers *et al.* 1995), protein, fat and carbohydrate content (Carefoot 1967; Nicotri 1980), presence of essential amino acids (Carefoot 1967, 1980, 1981), conversion and absorption efficiency (Carefoot 1967, 1970; Rogers *et al.* 1995) and others. Herbivores need to ensure that the food they ingest contains everything required to successfully grow and reproduce. Thus the ability to grow and reproduce on a specific diet should be linked to the composition of the food source, although this relationship has not always yielded the expected correlations (see Trowbridge 1991a; Rogers *et al.* 1995; Poore & Steinberg 1999).

The ease of manipulation and digestion of the food in question is a further aspect of edibility. The ability of the herbivore to effectively manipulate the food item is related to the size, structure, and texture of the macrophyte. Macrophytes utilize a range of physical defences to ward off herbivory, including leaf hairs, thickened cuticles and spines. They may also incorporate chemicals such as silica and calcium carbonate into the skeleton (Nicotri 1980; Pennings & Paul 1992). Food digestion is related to components of nutrition such as absorption and assimilation efficiency, because the amount of energy or nutrients to be gained from food ingested and processed may vary between foods (Carefoot 1970; Vadas 1978; Kennish & Williams 1997; Stead *et al.* 2003). Rate of digestion may also be important in affecting the activity budget of an animal as more time would be spent digesting and/or resting after consuming foods that are not rapidly digested (Vadas 1978; Carefoot 1989).

An additional component of edibility is the ability of the herbivore to grow and reproduce on the selected diet. Many studies have shown that the ability to grow on a food item is correlated with feeding preference (Carefoot 1967; Pennings 1990b, 1990c; Trowbridge 1991a). However, it cannot be said whether this is a causative relationship. Over evolutionary time, we would expect that a herbivore would become adapted to a food source and thus would always grow best on the food item it specialized upon (Rogers *et al.* 1995).

Attractiveness

There are several factors to consider when addressing attractiveness in feeding preference. First, the habitat characteristics of the macrophyte itself may be important; where the herbivore is small enough to use the macrophyte both as a food source and as habitat. In these cases the herbivore is termed a mesograzer (Nicotri 1980). Whether a macrophyte serves as a suitable habitat choice can be influenced by a number of macrophyte morphological characteristics. The ability of the herbivore to stay attached to the macrophyte and move within the structure can be important. In addition, the individual macrophyte may provide shelter from waves and ultraviolet radiation plus act as camouflage from potential predators (Carefoot 1987).

Another attractiveness component is the chemistry of macrophytes. The co-evolution of macrophytes and their herbivorous predators has led to the production of secondary metabolites in macrophytes. These compounds probably originated to deter herbivory. In response, selected herbivores have evolved mechanisms capable of dealing with, and in some cases, even taking advantage of these metabolites (Hay & Fenical 1988). Herbivores use macrophyte secondary metabolites in two ways. First, mesograzers gain a selective advantage by inhabiting chemically defended host macrophytes. By residing within these macrophytes the small animals avoid accidental ingestion by larger herbivores that are deterred from feeding on the chemically defended host (Bernays & Graham 1988; Hay *et al.* 1989; Brawley 1992; Pennings & Paul 1992; Trowbridge 1992; Hay 1997). Secondly, some herbivores have become specially adapted to feed on chemically rich macrophytes and can even incorporate and sequester macrophyte-derived chemicals for use in their own defence (Brower 1984; Hay *et al.* 1987; Hay & Fenical 1988; Paul & Van Alstyne 1988b; Hay *et al.* 1990; Pennings 1990c). When extracted, the chemicals present in host macrophytes cause little reduction in feeding rate and even sometimes promote feeding, by their specialized herbivores (Hay *et al.* 1989; Nagle *et al.* 1998).

A third component of attractiveness is interspecific competition (Nicotri 1980). If an animal is out-competed for the preferred food source, that food is essentially unavailable. In order to persist, the animal must utilize alternative resources. In this way, potential competition from co-occurring herbivores can promote specialization on sub-optimal resources. Specialisation thereby partitions the available resources and increases the potential diversity of the community (Pianka 1988). Competition has been shown to be an important driving factor in food preference for a variety of herbivorous gastropods in Australia (Andrew & Underwood 1989; Jones & Andrew 1990).

An additional important attractiveness factor is the abundance and predictability of the macrophyte resource both spatially and temporally. The presence and reliability of a macrophyte host species, within various herbivore habitats and throughout the lifetime of the organism, will have strong selection pressure on the herbivore (Paine & Vadas 1969b; Feeny 1976). It would be expected that the most common and widespread macrophyte species will be preferred (Steneck 1982; Pianka 1988; Thompson 1988), but this is often not the case (Carefoot 1967; Feeny 1976). Abundance and predictability are additionally complicated by the continuous selection and co-evolution of macrophyte defences and herbivore specialisations for consumption.

Sea Hare Feeding Preferences

The relative importance of any of the above factors in affecting feeding preferences can vary between ecosystems, species, populations and individuals. Sea hares are useful study organisms for investigating feeding preferences (Carefoot 1987; Rogers *et al.* 1995). Specialists seem to be relatively rare in the marine environment when compared with terrestrial systems (Lubchenco & Gaines 1981; Hay *et al.* 1989). In contrast, most sea hares are relatively specialized feeders and there is a substantial body of work addressing the feeding preferences of various species. However, differences in methodology, species and ecosystem have contributed to confusion about the factors responsible for feeding preferences within this group. When reviewing previous work on this group, the only common theme in factors responsible for feeding preferences is the lack of similarity between species.

Nutrition

Analyses of the nutritional qualities of algae tested for sea hare feeding preferences have largely failed to correlate specific qualities with feeding preferences. Carefoot

(1967) found no clear trends in the relationship between preferences and various nutritional parameters tested for *Aplysia punctata*. Similarly, Rogers *et al.* (1995) found that the nutritional value of preferred dietary algae was no higher than that of other algae found in the habitat of *Aplysia parvula* and *A. juliana.* In contrast, energy absorption and growth efficiency in *A. juliana* was highest for the preferred food, *Ulva fasciata* (Carefoot 1970). *Aplysia dactylomela,* on the other hand, had the highest absorption efficiency but not the highest growth efficiency on a diet of *Enteromorpha* sp., a rare alga in the local environment (Carefoot 1970). Sea hares may possess adaptations that allow them to exploit suboptimal food resources. For example, symbiotic bacteria in the digestive system of sea hares have been shown to increase the amount of amino acids available to the host animal and *Aplysia juliana* grew at a slower rate when treated with antibiotics (Vitalis *et al.* 1988). Additionally, the bacteria found in *A. juliana* and *A. dactylomela* were able to process carbohydrates and increase the supply of amino acids in test media (Vitalis *et al.* 1988).

Local Abundance

Foraging theory states that feeding on the most abundant and therefore readily obtainable food sources will require the least energy and thus be advantageous to the herbivore (Crawley 1983). We would thereby expect that sea hares would prefer abundant algal species. This is true for some species under certain conditions. For example, the specialist sea hare, *Aplysia juliana*, feeds on and occupies a single green algal species, *Ulva lactuca,* in the laboratory and in the field (Carefoot 1970; Rogers *et al.* 1995). This host species is abundant in *A. juliana*'s local habitat and does not possess deterrent secondary metabolites (Carefoot 1970). In contrast, *Aplysia parvula* utilizes two species of algae which are relatively rare in the local environment (Rogers *et al.* 1995). The authors hypothesized that the presence of secondary metabolites in the host algae, which deter local fish, caused *A. parvula* to prefer this algae (Rogers *et al.* 1995). An extreme example of the influence of abundance is the generalist sea hare, *Dolabella auricularia*. This species reportedly exhibits a preference for the rarest algae, or the algae that the animal was not conditioned upon (Pennings *et al.* 1993). It was hypothesized that the requirement for a mixed diet causes the flexible preference for rare algae in the natural habitat (Pennings *et al.* 1993). Therefore, for *D. auricularia* and possibly other sea hare species, the previous dietary history is of upmost importance in determining subsequent feeding preferences.

Secondary Chemistry

Numerous studies have examined the influence of algal secondary chemistry on the feeding preferences of sea hares. Investigations into the role of these chemicals have produced mixed results. The generalist herbivore, *Dolabella auricularia*, was relatively unaffected by the presence of chemical defences in the host algae (Pennings & Paul 1992). Yet, other sea hare species seem to be substantially affected by the type and concentration of macrophyte secondary metabolites. *Lyngbya majuscula* chemical extracts presented at natural concentrations deterred feeding by *S. striatus*, but had no observed effect at low concentrations. Other *L. majuscula* extracts induced feeding by *S. striatus* at concentrations equal to those found naturally (Nagle *et al.* 1998). In contrast, *Aplysia parvula* was unaffected by low concentrations of secondary metabolites from its host alga, *Portieria hornemannii*. At higher concentrations, this mesograzer was deterred from feeding by *P. hornemannii* metabolites (Ginsburg & Paul 2001). Therefore, intraspecific differences in the levels of secondary metabolites in host algae may influence feeding preferences within a single algal species, where sea hares can avoid those individuals with higher levels of secondary metabolites.

The isolation of macrophyte-derived secondary metabolites from within sea hare tissue has led to the hypothesis that these chemicals provide sea hares with chemical protection from predation (Pennings 1994; Rogers *et al.* 2000b). Chemical extracts from *Stylocheilus striatus* were found to deter feeding by predatory fish (Pennings *et al.* 1996). Diet manipulation experiments have provided further support to this hypothesis. *Aplysia californica* fed the chemically defended alga, *Plocamium* sp., were less susceptible to predation by rock wrasses than their conspecifics fed the chemically neutral, *Ulva* sp. (Pennings 1990c). *Aplysia parvula* found on the red alga *Portieria hornemannii* in Guam exhibits preference for *P. hornemannii* under laboratory conditions (Ginsburg & Paul 2001). Both whole *A. parvula* and specific body parts were rejected by reef fishes from feeding, while those sea hares found on an alternate algal host (*Acanthophora spicifera*), which does not have similar chemical compounds, were consumed by fishes (Ginsburg & Paul 2001).

Opposition of the “macrophyte-derived secondary metabolite defence” hypothesis in sea hares is based on three pieces of evidence. Firstly, the majority of secondary metabolites are sequestered in the digestive gland and are therefore not optimally placed for defence (Carefoot 1987). Secondly, at least one sea hare species is known to alter the structure of macrophyte secondary metabolites and the resultant compound is less toxic than the original. After sequestration by *Stylocheilus striatus*, the derivative of a chemical in *Lyngbya* was less toxic to general predators than the original form found in the macrophyte (Pennings *et al.* 1996). This finding suggests that the sequestration of secondary metabolites in *S. striatus* may not serve as an anti-predatory defence, and may be a detoxification process instead. Finally, it has recently been shown that the identity of the fish predator is more important in determining the palatability of *S. striatus* than the presence of secondary metabolites in the diet (Pennings *et al.* 2001).

Macrophyte Morphology

Physical characteristics of the host macrophyte have been mostly overlooked in general studies on sea hare feeding preferences and even more rarely addressed directly. One exception is the study by Pennings & Paul (1992) where the authors demonstrated that low preference is negatively correlated with macrophyte toughness and calcification in *Dolabella auricularia*. In addition, the feeding preference range of *Aplysia californica* has been found to expand with ontogenetic development (Pennings 1990c) and this trend was thought to be limited by the soft, weak mouth parts found in younger individuals.

Biotic Interactions

Biotic interactions have also rarely been examined as influences on feeding preferences in sea hares. However, an implied result of competition, niche partitioning, was suggested for two sympatric species, *Aplysia depilans* and *A. fasciata,* where the two species seemed to occupy habitats with differing hydrological conditions (Achituv & Susswein 1985). Competition for the alga, *Laurencia* sp., was also proposed for *A. parvula* and *A. dactylomela* (Willan 1979). In recent work, it has been argued that food is not sufficiently limited in abundance to cause competition to occur in the habitats where *Aplysia* spp. occur (Carefoot 1989; Rogers *et al.* 1995). Predation pressure may also affect sea hare feeding preferences. Rogers *et al.* (2000b) observed a higher abundance of *A. parvula* at sites where its predator, the pycnogonid *Anoplodactylus evansi,* was absent. However, a study of this factor by Pennings (1990a) did not support this hypothesis. In that study, a known predator of opisthobranchs, *Aglaja inermis*

(Pennings 1990a) was more common on *A. californica*'s preferred host alga, *Plocamium*, than any other algae in their habitat, leading to the conclusion that this host alga does not serve as a refuge from *Aglaja inermis*.

Bursatella leachii is often found in population irruptions with the cyanobacteria specialist, *S. striatus* (Switzer-Dunlap & Hadfield 1979; Paul & Pennings 1991; Capper 2003), but its feeding ecology has rarely been examined. Wu (1980) found that *B. leachii* in southern China preferred the green algae, *Enteromorpha* (= *Ulva*) *prolifera,* however only macroalgae species were tested. Paige (1988) found that *B. leachii* in Florida preferred a number of cyanobacteria species. Populations in tropical habitats experience different environmental conditions than their temperate counterparts. It is unknown whether the same factors that determine feeding preferences in temperate regions operate for tropical populations. The feeding preferences of *B. leachii* have not been determined for tropical populations. The present study investigates the feeding preferences of *B. leachii,* which are occasionally found in high abundance within the coastal environments of tropical North Queensland.

Materials & Methods

Collection of Sea Hares

In July 2002, 40 individual *Bursatella leachii* were collected at low tide from Shelly Beach, Townsville, Queensland, Australia. Animals were placed in fresh seawater in 1L containers and transported back to the laboratory located in the Marine Biology and Aquaculture Research Facility Unit (MARFU) aquarium system at James Cook University (JCU). All *B. leachii* were placed in a large aquarium (maintenance tank) supplied with continuously flowing filtered seawater (salinity ~33ppt, pH ~ 8.0, temperature 27-28^{o}C). Animals were held overnight to allow acclimatisation before experimentation. Both maintenance and experimental tanks were on a 12h:12h light:dark diel cycle.

Algae Collection

In total, three species of macroalgae (*Enteromorpha* sp., *Sargassum* sp., and *Pterocladia pinnata*) and one species of cyanobacterium (*Calothrix crustacea)* were tested. The latter species was the cyanobacterium found in the cyanobacterial bloom where the sea hares were collected and was an observed prey item of *B. leachii* in the field (see Chapter 3). *Calothrix crustacea* was collected from sea grass beds at Shelly Beach. The green alga, *Enteromorpha* sp., was chosen as a chemically neutral alga (Pennings *et al.* 1993) and was collected from the algal scrubbers at the JCU aquaculture facility. *Sargassum* sp. (Heterokontophyta) and *Pterocladia pinnata* (Rhodophyta) were chosen for their abundance in areas where *B. leachii* is found along the coast of Queensland, although it is not abundant at the site where the animals were collected. Red and brown algae were only present at extremely low densities in the study site so *Sargassum* sp. and *Pterocladia pinnata* were collected from Kissing Point, Rowes Bay, Townsville. Only fresh algae were used in the feeding assays.

Feeding Assays

To determine the feeding preferences of *B. leachii*, two-way choice experiments were conducted. Sixteen replicate 1.5L aquaria supplied with continuously flowing filtered seawater acted as the experimental arenas. Pre-weighed, equal amounts (approximately 2 g wet weight) of each of two species of macroalgae or cyanobacteria were placed at opposite ends of each experimental arena. For each two-way choice experiment, eight experimental tanks and eight control (no-herbivore) replicates were used. Control tanks were identical in design and set-up, except for the presence of the herbivore. The loss of algae in the control tanks was used to calculate the loss of algae due to autogenic change from natural breakdown in the experimental tanks. Experimental tanks were randomly assigned at the start of each trial to remove the possibility of any single aquaria-associated bias. At the commencement of a trial, the wet weight of the algae in each tank was recorded and a randomly chosen *B. leachii* from the maintenance tank was placed at the centre of each experimental tank. Individual sea hares were allowed to move about the experimental tank and feed for a 24-hour period. Five times over the course of the trial, the position of each individual sea hare was recorded and scored as to presence on alga 1, alga 2, or on the tank walls (Attractiveness trial). At the termination of the trial, the algae were removed from the aquaria, patted dry and re-weighed to determine percentage consumption of each alga (Edibility trial).

Statistical Analysis

The autogenic change calculated for each alga was used to correct the percentage consumption values (after Nicotri 1980; Peterson & Renaud 1989). Paired-samples t-tests were used to analyse the percentage consumption and behavioural choice data from each two-way choice trial. Where the data did not meet the assumptions of heterogeneity and normality a non-parametric Wilcoxon-signed ranks test was used to assess significance.

Results

Edibility

Bursatella leachii significantly preferred the green alga *Enteromorpha* over all other algae tested, including the cyanobacteria, *Calothrix crustacea*. The mean percentage consumption, corrected for autogenic change, of each pairwise trial is shown in Figure 4.1. Only *Enteromorpha* and *Calothrix* were consumed in amounts greater than 10%. The feeding preference hierarchy of *B. leachii* was *Enteromorpha* sp. > *Calothrix crustacea* > *Sargassum* sp. and *Pterocladia pinnata*. *Enteromorpha* was consumed significantly more than *C. crustacea* (Wilcoxon Signed ranks test: Z = -2.240, p = 0.025), *Sargassum* (Wilcoxon signed ranks test: Z = -2.100, p = 0.036) and *P. pinnata* (Wilcoxon signed ranks test: Z = -2.521, p = 0.012). *Calothrix crustacea* was consumed significantly more than *Sargassum* sp. (Wilcoxon signed ranks test: Z = -2.521, p = 0.012) and *P. pinnata* (Wilcoxon signed ranks: -2.521, p = 0.012). There was no significant difference between the consumption of *Sargassum* sp. and *Pterocladia pinnata* (Wilcoxon signed ranks test: Z = -1.400, p>0.05).

Attractiveness

The presence of individual sea hares on each algal choice yielded almost the same feeding preference hierarchy as that obtained by the direct measure of percentage consumption. *Enteromorpha* sp. > *Calothrix crustacea* > *Pterocladia pinnata* and *Sargassum* sp. The place of *Pterocladia pinnata* and *Sargassum* sp. were reversed in this data set but there was no significant difference between the two. The mean number of times a sea hare was present on each pairwise trial is shown in Figure 4.2. *B. leachii* was recorded significantly more on *Enteromorpha* than *Calothrix crustacea* (Wilcoxon signed ranks test: Z = -2.058, p = 0.040) and *Pterocladia pinnata* (Wilcoxon signed ranks: Z = -2.251, p = 0.024). *Bursatella leachii* was found significantly more on *C. crustacea* than on *P. pinnata* (Wilcoxon signed ranks: Z = -2.041, p = 0.041). Each of the four algae presented to *B. leachii* had at least one individual found present upon it at least one time period during the trials.

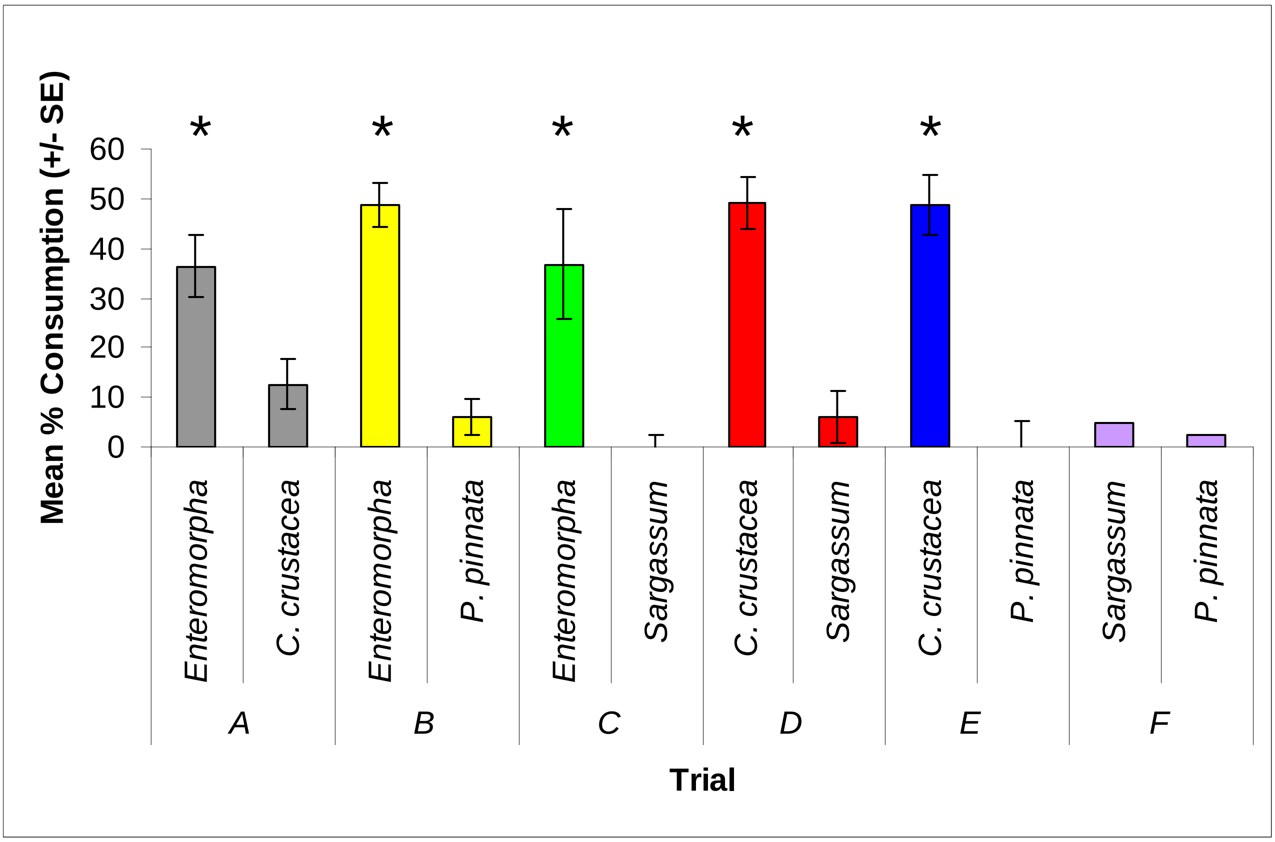

Figure 4.1: The mean percentage consumption (+/- standard error) of each pairwise choice trial by *Bursatella leachii*. Asterisks (*) indicate significant choices as evidenced by the respective statistical test. Each colour code represents a single two-way choice experiment denoted by letters A-F where 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*.

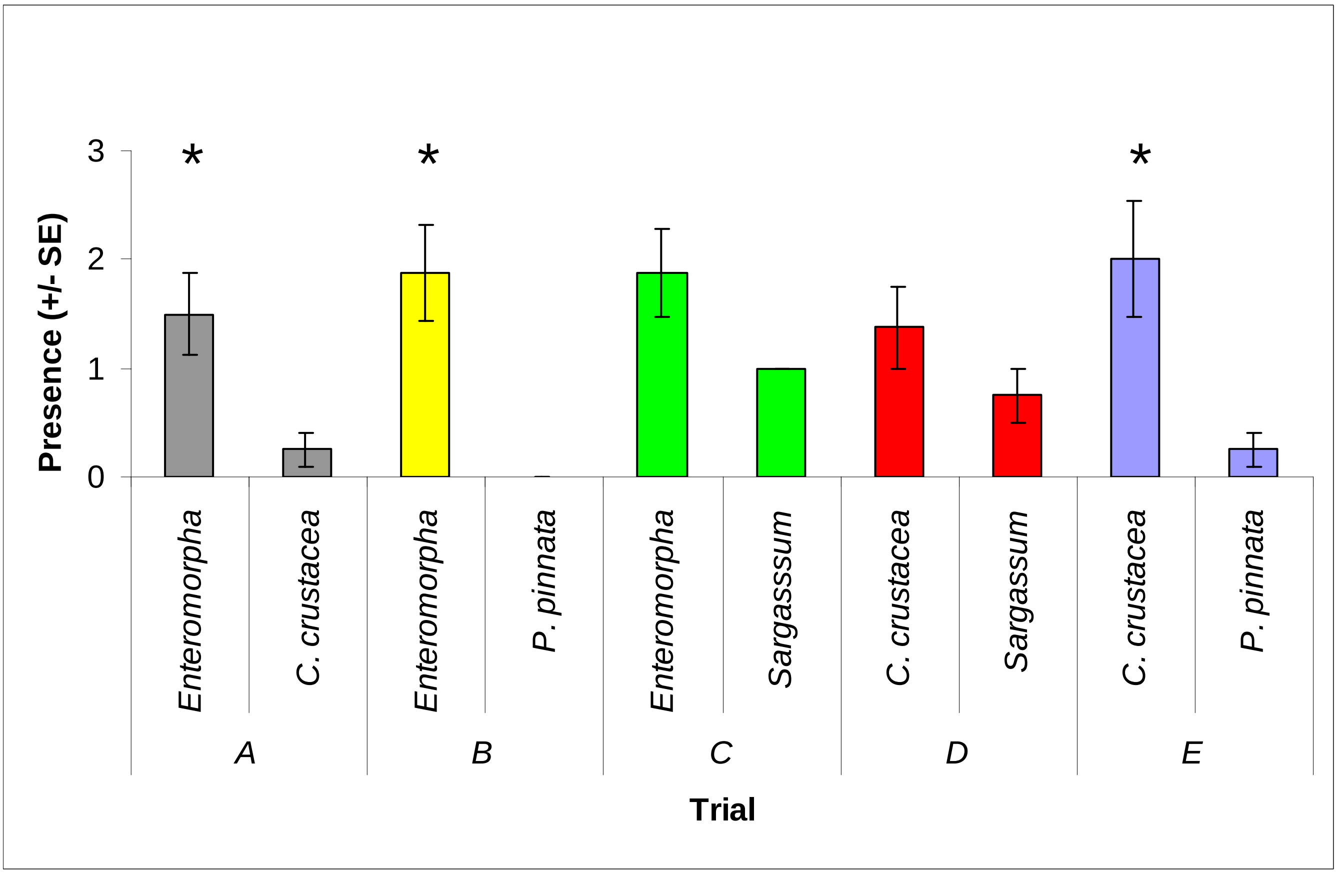

Figure 4-2: The mean number of times *Bursatella leachii* was recorded present on each alga within in each pairwise trial. Asterisks (*) indicate statistically significant differences. Each colour code represents a single two-way choice experiment denoted by letters A-F where A = *Enteromorpha* sp. vs. *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*.

Discussion

Bursatella leachii Preferences

Bursatella leachii preferred the green algae, *Enteromorpha* sp., to cyanobacterium *C. crustacea*, the brown macroalga *Sargassum* sp., and the red macroalga *Pterocladia pinnata*. *Enteromorpha* sp. was preferred even to the cyanobacterial species (*C. crustacea*) that the animals were seen to be feeding upon in the field. However, several sea hare species exhibit greater generalization of feeding preferences in the laboratory than are exhibited in the field (Saito & Nakamura 1961; Winkler & Dawson 1963; Carefoot 1987; Rogers *et al.* 2003). For example, some members of the genus *Aplysia* are often considered to specialize on red algae in the field (Carefoot 1987) and yet laboratory experiments have repeatedly shown their preference for green algae (Winkler & Dawson 1963; Rogers *et al.* 2003). Winkler & Dawson (1963) found that *Aplysia californica* were only inhabiting red algae in the field, but preferred *Enteromorpha* in laboratory testing. Additionally, Saito & Nakamura (1961) found that *Aplysia juliana* and *A. kurodai* consumed species of brown and red algae respectively in the field but both preferred *Enteromorpha* and *Ulva* in the laboratory. Rogers *et al.* (2003) found that *Aplysia parvula* consumed a variety of red, green, and brown macroalgal species at similar levels to that at which it consumed *Laurencia obtusa*, the preferred food in the field.

All sea hare species previously tested have shown the ability to consume green algae in the laboratory; these include the various *Aplysia* species (Saito & Nakamura 1961; Winkler & Dawson 1963; Carefoot 1967, 1970, 1987; Pennings 1990b, 1990c, 1994; Rogers *et al.* 2003)*, Dolabella auricularia* (Pennings *et al.* 1993)*, Stylocheilus striatus* and *Bursatella leachii* (Wu 1980). An exception is a population of *Aplysia juliana* found along the Sydney coastline. This species was found to feed exclusively upon *Ulva,* both in the field and in the laboratory (Rogers *et al.* 1995). It would be interesting to test whether the extreme specialist, *Petalifera petalifera,* which is only found on the calcified algae, *Padina tenuis*, would prefer green algae under laboratory conditions. Green algae are unavailable in most tropical marine habitats as a result of continuous, heavy grazing by herbivorous fishes (Hay 1991). Within the seagrass beds where the animals used in this study were found, *Enteromorpha* and all other green algae species are extremely rare (personal observation). Therefore, using green algae in preference tests is offering sea hares an alga that they may prefer to eat but is at such low level in their natural habitat that it is not a real option under natural conditions.

Competition

Competition was previously not considered to be a factor in the feeding preferences of sea hares (Carefoot 1987; Rogers *et al.* 1995). This assertion was based on two assumptions. First, the low density of sea hares in the field generally made it unlikely that sea hares had the ability to deplete their food source (Carefoot 1987; Rogers *et al.* 1995). However, this trend may be restricted to members of *Aplysia* because there have been many reports of large aggregations of sea hares (Switzer-Dunlap & Hadfield 1979;

Paul & Pennings 1991; Pennings 1994) and countless more anecdotal reports. *Stylocheilus striatus* was reported to occur in aggregations of thousands of animals in Hawaii (Switzer-Dunlap & Hadfield 1979) and in Guam (Paul & Pennings 1991; Pennings 1994)). *Bursatella leachii* has also been found in similar aggregations at high densities during the course of the present work (see Chapters 2 & 3). While it is true that sea hares do not reach the same densities as sea urchins or fishes, they do have a comparatively large appetite (Carefoot 1987).

The second assumption is that sea hares occur in habitats where there is a high abundance of algae and therefore food would not be limiting enough to cause competition to occur. Green algae, especially *Ulva* and *Enteromorpha*, are preferred by most marine herbivores because they are chemically undefended, possess a simple morphology and have low toughness and no calcification (Rogers *et al.* 1995). Herbivorous fishes remove the majority of algal biomass (50-100% on shallow reefs, with the exception of coralline algae, the bases of small filamentous algae, and chemically or structurally defended macroalgae (Hatcher 1981; Hay 1981; Hatcher & Larkum 1983; Hay 1984b; Carpenter 1986; Lewis 1986; Russ 1987; Morrison 1988; Steneck 1988; Klumpp & Polunin 1989). There may be a large abundance and diversity of algae in sea hare habitats but if herbivorous fish remove the preferred species there is a lowered abundance of algae available for sea hares, perhaps forcing the consumption of less-preferred algae and, over evolutionary time, a shift in feeding preferences. Therefore sea hares may have been forced to exploit chemically defended algae in the field, such as cyanobacteria or red algae species, even if green algae are preferred.

The origins of feeding preferences are complicated by additional factors other than nutrition and abundance. *Aplysia punctata* consumes *Enteromorpha* sp. preferentially in the laboratory but animals on a diet of the red algae, *Plocamium* sp., have lowered faecal production and better growth (Carefoot 1967). Monospecific diets of preferred chemically rich species have shown increased mortality for *B. leachii* and *S. striatus* (A. Capper, *pers. comm.*) and for *Aplysia parvula* (Rogers *et al.* 1995). Therefore, even preferred choices may not offer the best long-term growth and fitness. The next step in investigating the feeding preferences of *B. leachii* would be to compare the growth and reproduction of animals fed *Enteromorpha* sp. with those fed cyanobacteria.

Edibility vs. Attractiveness

The feeding preference hierarchy of *B. leachii* was identical whether obtained by testing edibility or attractiveness. Therefore the alga that individual sea hares chose (the most attractive) was also the one that is the most edible. In the attractiveness test of the present study, the only non-significant pairs involved *Sargassum* sp. and either *C. crustacea* or *Enteromorpha* sp. Test animals were observed in feeding behaviours when located on *C. crustacea* and *Enteromorpha* sp. but were not feeding while on *Sargassum* sp. *Sargassum* sp. was never consumed in any substantial amount so it is possible that *Sargassum* sp. offers *B. leachii* some other quality. It seems likely that shelter and thus, habitat characteristics of *Sargassum* sp. may be affecting the attractiveness of this alga. *Sargassum* sp. is a large, branching, leafy alga whereas *C. crustacea* and *Enteromorpha* sp. do not offer any sheltering characteristics with their simple morphology. In the present study, it is possible that *B. leachii* moved from the preferred food algae, *C. crustacea* and *Enteromorpha* sp., to *Sargassum* sp. for shelter, perhaps to allow rest and digestion in a more structurally complex environment. Habitat complexity has been shown to be related to predation efficiency in the field (Pennings 1990a) so this seems a plausible explanation for these results. Habitat and morphological characteristics can therefore affect the attractiveness of the food source to some degree.

In the present study, *Bursatella leachii* consumed only two of the algal species tested in any substantial amount (>40%); *Calothrix crustacea* and *Enteromorpha*. *Calothrix crustacea* is a member of the Cyanophyta and *Enteromorpha* is a member of the Chlorophyta. *Enteromorpha* is a chemically undefended alga (Pennings *et al.* 1993) while the chemistry of *Calothrix crustacea* is unknown. Most bloom-forming cyanobacteria are believed to contain biologically active secondary metabolites (Paerl 1988). *Calothrix* spp. are members of the family Rivulariaceae and have a basic filamentous morphology (Fay 1983). Previously, *Stylocheilus striatus* has only been reported to feed upon *Lyngbya majuscula* (Switzer-Dunlap & Hadfield 1977; Paul & Pennings 1991; Nagle *et al.* 1998). *Bursatella leachii* is reported to feed on a number of red and green algae. The reason for the greater dietary range of *B. leachii* has not been previously discussed but recent work on the way these two sea hares deal with chemically rich foods has been conducted. Capper (2003) showed that there are differences in the way *B. leachii* and *S. striatus* deal with the secondary metabolites present in *L. majuscula*. *B. leachii* excretes secondary metabolites in the ink and faecal matter while *S. striatus* stores these metabolites in the digestive gland. The strategies of these two co-occurring species thus differ; *S. striatus* has specialized to deal with the specific metabolites of *L. majuscula* while *B. leachii* excretes the chemicals and thus is more likely to be able to deal with a larger range of metabolites than *S. striatus*.

Conclusions

The difference between the preferred algae in the laboratory and the algae that *B. leachii* was feeding upon in the field reflects the complications associated with attempting to separate the various factors influencing feeding preferences in sea hares. The demonstrated ability of *B. leachii* to reach large population sizes while feeding on a sub-optimal food source highlights the flexibility of feeding in these species. The plasticity in feeding preferences is likely to enhance the ability of sea hares to successfully exploit an abundant, but often, unpredictable resource. The difficulty in synthesizing feeding preferences for sea hares is that the results of a single factor approach to testing is unrealistic because sea hares are attempting to maximize fitness based on a range of factors. The relative importance of these factors can vary between individuals, populations, and species both through time and throughout ontogeny. From this study, it seems that the most important factors dictating feeding preferences in *B. leachii* are the edibility, abundance in the natural habitat, and more than likely the secondary chemistry of the food item.

Most previous studies have concentrated on the feeding preferences of *S. striatus* while *B. leachii* has been overlooked. *B. leachii* is a more generalised feeder than *S. striatus* although very recent research in southern Queensland has shown that it still prefers *L. majuscula* to other macroalgae (A. Capper *pers. comm.*). It is unknown whether *B. leachii* would have preferred to consume *L. majuscula* to the cyanobacteria species, *Calothrix crustacea,* tested in the present study. The edibility of cyanobacterial species and *Enteromorpha* may be related to the ability of sea hares to handle, manipulate and ingest the food items. Both *C. crustacea* and *Enteromorpha* sp. have gross similarities in structure with their simple filamentous morphologies and it is possible that interaction with the radula on a microscale may dictate that these properties are essential for successful ingestion. The microstructure of the radula will be investigated further in

Chapter 5: The interaction of sea hare radula teeth morphology and feeding type

Introduction

Herbivore Mouthparts

Optimal foraging theory states that herbivore food choice may be restricted by the structure of the mouthparts rather than the animal's feeding behaviour (Hughes 1980). Though herbivores may use different sensory organs to locate and distinguish between food types, the feeding apparatus is in direct contact with the morphology of the macrophyte. Therefore, the fine detail of this interaction has important implications for the feeding ecology of the herbivore. Many members of the Phylum Mollusca possess a unique feeding organ, called the radula. The radula is situated within a radular sac where new teeth are continuously produced by specialized cells, the odontoblasts (Brusca & Brusca 1990; Ruppert *et al.* 2004). The radula itself consists of a ribbon-like membrane bearing rows of chitinous teeth. Radular muscles move the ribbon over the odontophore and the odontophore muscles evert the radula from the mouth and apply the radular teeth to the substrate (Brusca & Brusca 1990; Ruppert *et al.* 2004). In its basic function, the radula acts like a scraper to trap food particles and bring them to the mouth (Brusca & Brusca 1990). However, radulae exhibit a remarkable diversity in form and function.

The Gastropod Radula and Diet

The direct interaction between the macrophyte and the radula would suggest that there is a close relationship between radular morphology and diet (Padilla 1989, 1998).
A number of gastropod herbivores possess morphological adaptations of the feeding apparatus specifically modified for consuming their prey items (Steneck & Watling 1982). Mesogastropods, such as *Littorina littorea,* have radulae similar in form to garden rakes with a high number of contact points, which allow them to consume tough macroalgae (Steneck & Watling 1982). Eogastropods, such as limpets, have radulae adapted for excavating. Excavation is achieved using increased force on a limited number of contact points. This strategy allows limpets to consume extremely hardened

and calcified algae (Steneck & Watling 1982). Other eogasatropods, such as *Fissurella* spp., possess radulae that function like brooms in sweeping food particles into the mouth. Other molluscan groups such as the chitons possess radulae with both excavation and sweeping adaptations and can cut into tough macroalgae and brush food into the mouth simultaneously (Steneck & Watling 1982). A number of gastropod species have highly modified radulae, for example, the cell wall piercing radula teeth of the sacoglossans and the harpoon-like teeth of the carnivorous *Conus*. These adaptations potentially decrease the costs associated with food handling by increasing the efficiency of the mouthparts and thereby indirectly improving the value of the food (Hughes 1980). This type of modification allows a herbivore to specialize on foods of otherwise low value.

Phylogeny

The arrangement of teeth on the radula, their structural type and the number of each type present are commonly used as taxonomic characters in many molluscs (Ruppert *et al.* 2004). Different taxa possess different arrangements of radula teeth. According to the scheme in Brusca & Brusca (1990), the sea hares possess a rhipidoglossate radular arrangement of teeth consisting of a large central tooth (the rachidian tooth), several lateral teeth, and high numbers of marginal teeth at the periphery of the radula ribbon. The form of the radula is considered species specific. However, with advances in microscopic technology a growing body of evidence suggests that in addition to interspecific variation, there are considerable intraspecific differences (Maes 1966; Bleakney 1989, 1990; Nybakken 1990; Trowbridge 1997; Reid & Mak 1999; Roberts *et al.* 1999). Intraspecific variation in radular morphology has been documented for different sexes (Maes 1966), different ontogenetic stages (Nybakken 1990; Roberts *et al.* 1999), and different populations (Bleakney 1989, 1990; Trowbridge 1997; Padilla 1998; Reid & Mak 1999). The differences between radulae have been attributed to phylogenetic constraints, ontogenetic variation and ecological factors.

When examined on a broad scale, groups of related gastropod species were often observed to have similar radula types (Kool 1987; Taylor & Lewis 1995). For example, most species of the gastropod family Fasciolariidae possess comb-like radula teeth, although some species are sipunculid feeders while others are predators of molluscs (Taylor & Lewis 1995). Similarly, Kool (1987) found that phylogeny was the most important determinant of radular morphology in Thaidiidae, with little relationship to diet. Thus it seems that phylogenetic constraints control the basic tooth morphology in at least some gastropod groups.

Ontogeny

Radular morphology also varies with the stage of ontogenetic development (Nybakken & McDonald 1981; Nybakken 1990; Roberts *et al.* 1999). In abalone, the shape of the radula teeth and their arrangement on the radula membrane changed during post-larval growth (Roberts *et al.* 1999). Juvenile abalone (*Haliotis iris*) showed a reduction in serrations, increased spacing of radula teeth, and lengthened marginal teeth with increasing age (Roberts *et al.* 1999). Abalone radula variation was correlated with changes in juvenile diet. Similarly, within the genus *Conus*, ontogenetic variation of the radula occurred in those species that underwent diet changes throughout ontogeny (Nybakken 1990). In addition, Nybakken & McDonald (1981) found that radula structure differed between adult and juvenile nudibranchs, reflecting the differences in prey type between developmental stages.

The radulae of gastropods that feed on similar food types are often of a similar morphology. Placing species into functional groups based on their radular morphology has allowed the investigation of links between radula type and prey items (Nybakken & McDonald 1981; Steneck & Watling 1982; Vietti & Balduzzi 1991). Steneck and Watling (1982) divided gastropod and chiton radulae into four functional types, 1) brooms, 2) rakes, 3) shovels and 4) multi-purpose tools. These radula types were in turn related to the functional groups of the algae they consume (microalgae, filamentous algae, foliose algae, corticated macrophytes, leathery macrophytes, articulated calcareous algae, and crustose coralline algae). One of the findings of Steneck & Watling (1982) was that animals that possess rhipidoglossate radulae were found to predominantly consume three groups of algae: microalgae, filamentous algae, and foliose algae. However, all opisthobranchs were excluded from their analysis because of limited information about diet and the functional use of the radula in this group. In two separate studies, the relationship between radula shape and feeding preferences was highly correlated in (Opisthobranch) dorid nudibranchs (Nybakken & McDonald 1981; Vietti & Balduzzi 1991). Nudibranchs that fed on tunicates, for example, possess a single radula type while those feeding on bryozoans and hydroids possessed more variable radula types (Nybakken & McDonald 1981). Additionally, Jensen (1993) found that the fine detail and shape of the radula teeth of sacoglossan sea slugs was strongly correlated with macrophyte cell wall structure.

Based on this evidence it appears likely that the morphology of gastropod radula teeth is highly inter-connected with diet. The relationship between food type and the morphology of the sea hare radulae has never been investigated. Given the high degree of specialization on chemically and physically defended algae, it would be expected that sea hares possess morphological adaptations of the radula that correspond to feeding type or algal functional group. The aim of the following study was to document the differences in radular morphology of local sea hare species and determine if a relationship exists between radular morphology and feeding specialization.

Methods & Materials

Specimen Collection

Sea hares were collected intertidally from seven different North Queensland locations (Table 5.1). The radulae of all six sea hare species found in north Queensland habitats were investigated, with the exception of *Petalifera petalifera*. This latter species was not examined due to the small number of specimens collected and the fragile nature of the radulae encountered during the preparation procedure.

Radula Preparation

Upon collection, sea hares were preserved in 6% formaldehyde in seawater for at least four weeks before transfer to 70% alcohol. The length of the preserved animal was measured to provide a size reference. Because the fixative alters the shape of the living animal, the differences in length are considered relative. A total of 10–15 animals were dissected for each species investigated. The radula sac was removed from each individual and placed in a solution of 6% sodium hypochlorite to dissolve the surrounding tissue. The radula was then cleaned in an ultrasonic bath for ten

Table 5.1: Collecting sites and locations in Queensland, Australia where sea hare specimens were obtained.

Sea hare species	Collection site	Location
Aplysia dactylomela	Kissing Point	Pallarenda
	Nelly Bay	Magnetic Island
Aplysia extraordinaria	Kissing Point	Pallarenda
	Picnic Bay	Magnetic Island
	Cockle Bay	Magnetic Island
	Pioneer Bay	Orpheus Island
Bursatella leachii	Shelly Beach	Pallarenda
Dolabella auricularia	Pioneer Bay	Orpheus Island
Stylocheilus striatus	Shelly Beach	Pallarenda
	Pioneer Bay	Orpheus Island
	Dingo Beach	Whitsundays

minutes to remove any remaining particulate matter. Each radula was mounted on a standard SEM stud. The studs were coated with gold using a JUC-5000 Magnetron Sputtering Device and examined using a JEOL JSM-5410LV Scanning Electron Microscope. A whole mount scan of each radula was taken as well as a series of scans of the individual teeth. Close up scans were always taken from the beginning or middle of the radula ribbon, where the teeth were not exposed to scraping action and thus had little or no mechanical damage.

Results

Radula Types

The radulae of the five species of local sea hares were divided into radula types based on the morphology of the rachidian, lateral and marginal teeth. The teeth shape, number of cusps, denticles, and serrations were used to define radula types. The radulae were classified into three structural types: 1) simple, 2) bilobed, and 3) denticulate

(Table 5.2). Teeth with no denticulations and a smooth rachidian tooth with a small cusp characterize the 'simple' radula type (Rachidian: Figure 5.1d; Lateral: Figure 5.2d, Marginal: Figure 5.3d). *Dolabella auricularia* was the sole member with a simple radula type. The second group, 'bilobed', was so named because its members possessed lateral and marginal teeth divided into two lobes (Lateral: Figure 5.2 a,b; Marginal: Figure 5.3 a,b). In the bilobed type, the rachidian tooth cusp is divided into three lobes with denticulations (fine serrations) on the primary lobe (Figure 5.1a,b). The radulae of *Aplysia dactylomela* (Figure 5.1a, 5.2a, 5.3a) and *A. extraordinaria* (Figure 5.1b, 5.2b, 5.3b) were included under this bilobed type. The 'denticulate' radula type has a highly subdivided rachidian tooth, with as many as 10 lateral lobes and a sharply denticulated medial cusp (Figure 5.1c,e). The lateral teeth are also divided into at least 8 lobes and the marginal teeth have 1-3 lobes and were highly denticulated (Figure 5.2c,e). *Stylocheilus striatus* and *Bursatella leachii* were placed in this third group, 'denticulate'.

Table 5.2: The classification of each local sea hare species according to radula type.

Sea hare species	Radula type
Dolabella auricularia	Simple
Aplysia dactylomela	Bilobed
Aplysia extraordinaria	Bilobed
Bursatella leachii	Denticulate
Stylocheilus striatus	Denticulate

Radular morphology

Aplysia dactylomela

Aplysia dactylomela has a broad rachidian tooth with a large median cusp (Figure 5.1a). The median and lateral cusps are slightly serrated. The lateral teeth of this species have two lobes. The median primary lobe is slightly serrated, while the secondary lobe is smooth and pointed (Figure 5.2a). The marginal teeth of *A. dactylomela* consist of two smooth lobes of unequal length, with the medial lobe shorter than the lateral one (Figure 5.3a).

Aplysia extraordinaria

The rachidian tooth of *A. extraordinaria* has a wide base and a large median cusp that curves over to reach the base of the tooth (Figure 5.1b). The median cusp is strongly serrated and the lateral cusps are differentiated with a number of denticles of various shapes. The first lateral tooth of *A. extraordinaria* has large blunt serrations on the lateral side and sharp pointed serrations on the medial side of the primary lobe (Figure 5.2b). The secondary lobe is much smaller and pointed with no serrations. The marginal tooth in this species has a highly serrated, pointed lateral lobe (Figure 5.3b). The medial lobe is differentiated into two smaller unequal-sized denticles.

Bursatella leachii

Bursatella leachii has a wide rachidian tooth base and the median cusp curls halfway down the base of the tooth (Figure 5.1c). The median cusp is elongate with large serrations and numerous lateral cusps. The first lateral tooth is digitiform with a long primary lobe and at least four equal sized secondary lobes on either side (Figure 5.2c). The marginal teeth are simple, elongate with either a single small denticle or numerous small denticles on the median edge (Figure 5.3c).

Dolabella auricularia

The rachidian tooth of *Dolabella auricularia* is elongate, bifurcate at the base and has a narrow median cusp (Figure 5.1d). The lateral and marginal teeth are virtually identical in this species, being simple sickle-shaped teeth with no denticulations (Figure 5.2d). In most of the radulae examined the rachidian teeth were partially or completely covered by the large lateral teeth.

Stylocheilus striatus

The rachidian tooth of *Stylocheilus striatus* has a wide tooth base and the median cusp extends to the base of the tooth (Figure 5.1e). The median cusp is highly serrated and there are at least five lateral cusps. The first lateral tooth is digitiform with a blunt primary lobe and a number of smaller secondary lobes on each side, with more on the lateral edge (Figure 5.2e). The marginal teeth are elongate with a single small denticle or many small denticles on the median edge (Figure 5.3d).

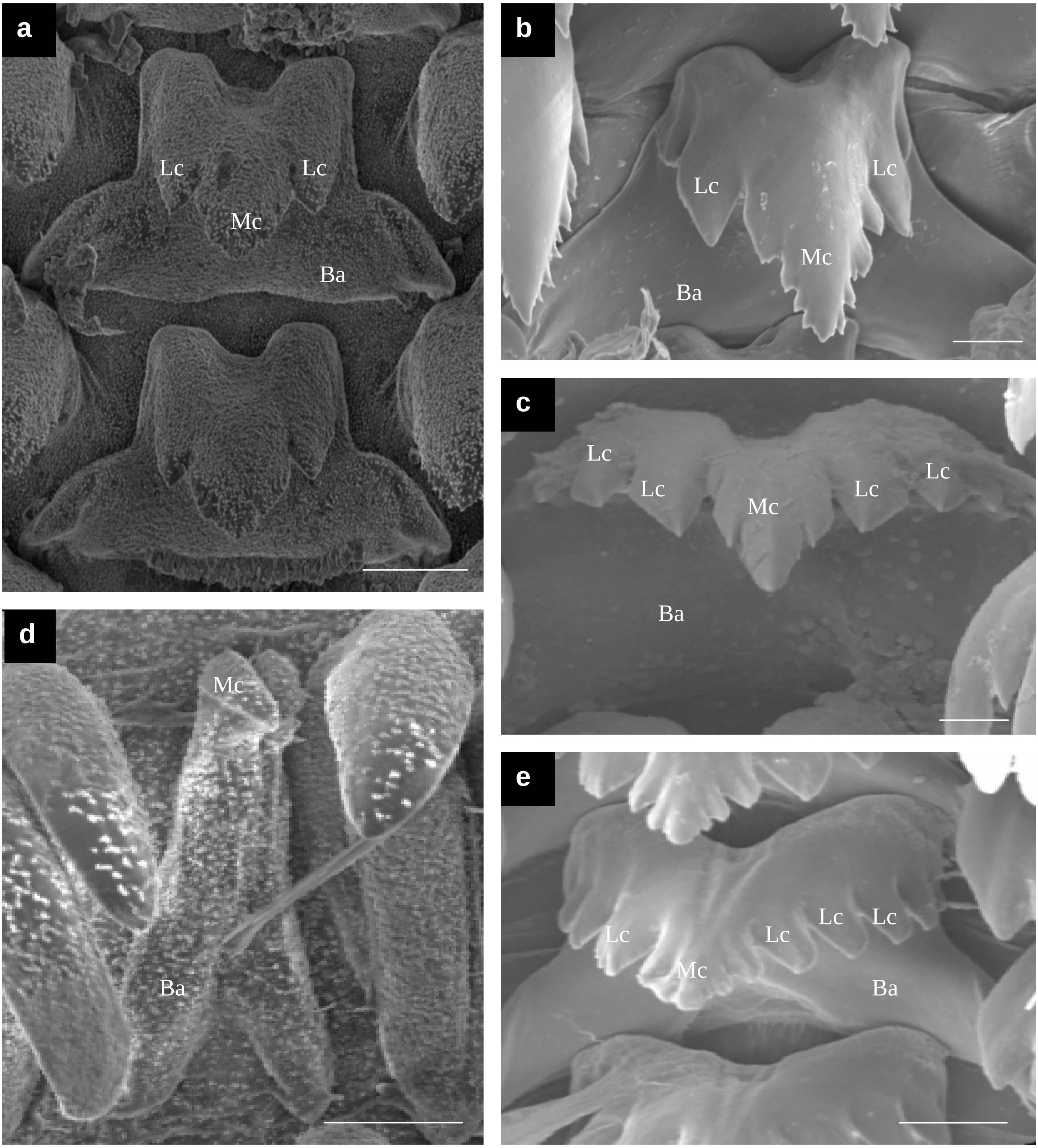

Figure 5.1: Scanning electron micrographs of the rachidian teeth of a) *Aplysia dactylomela*, scale bar = 50 µm, b) *Aplysia extraordinaria*, scale bar = 15µm, c) *Bursataella leachii*, scale bar = 10µm d) *Dolabella auricularia*, scale bar = 25 µm, e) *Stylocheilus striatus*, scale bar = 25 µm. Legend: Ba = tooth base, Lc = lateral cusp, Mc = median cusp.

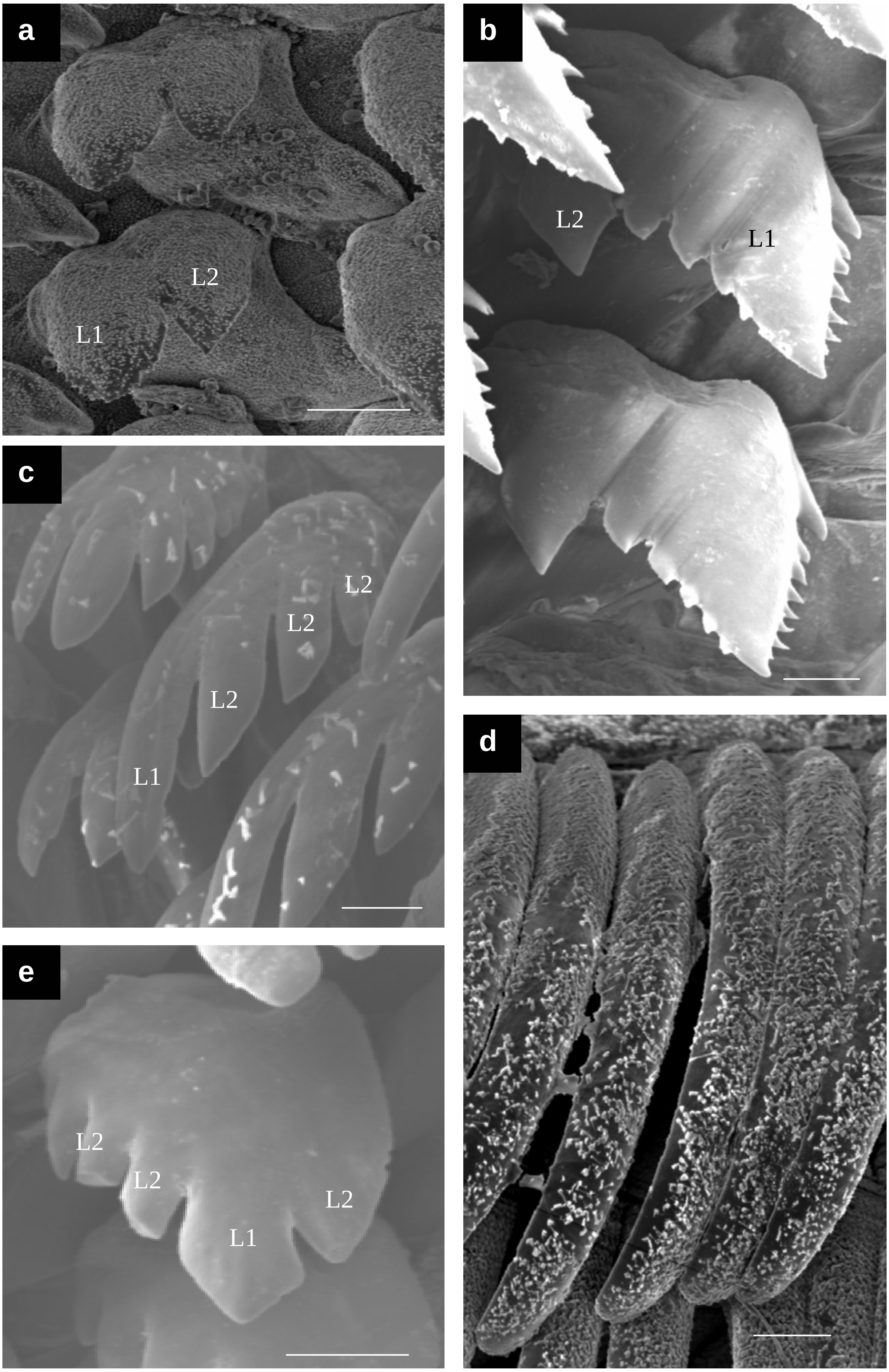

Figure 5.2: Scanning electron micrographs of the lateral teeth of a) *Aplysia dactylomela*, rachidian teeth are to left, scale bar = 50 µm b) *Aplysia extraordinaria*, rachidian are to right, scale bar = 30 µm; c) *Bursatella leachii*, rachidian are to left, scale bar = 25 µm; d) *Dolabella auricularia*, rachidian teeth are to left, scale bar = 25 µm; e) *Stylocheilus striatus*, rachidian is to right, scale bar = 25 µm. Legend: L1 = primary lobe, L2 = secondary lobes.

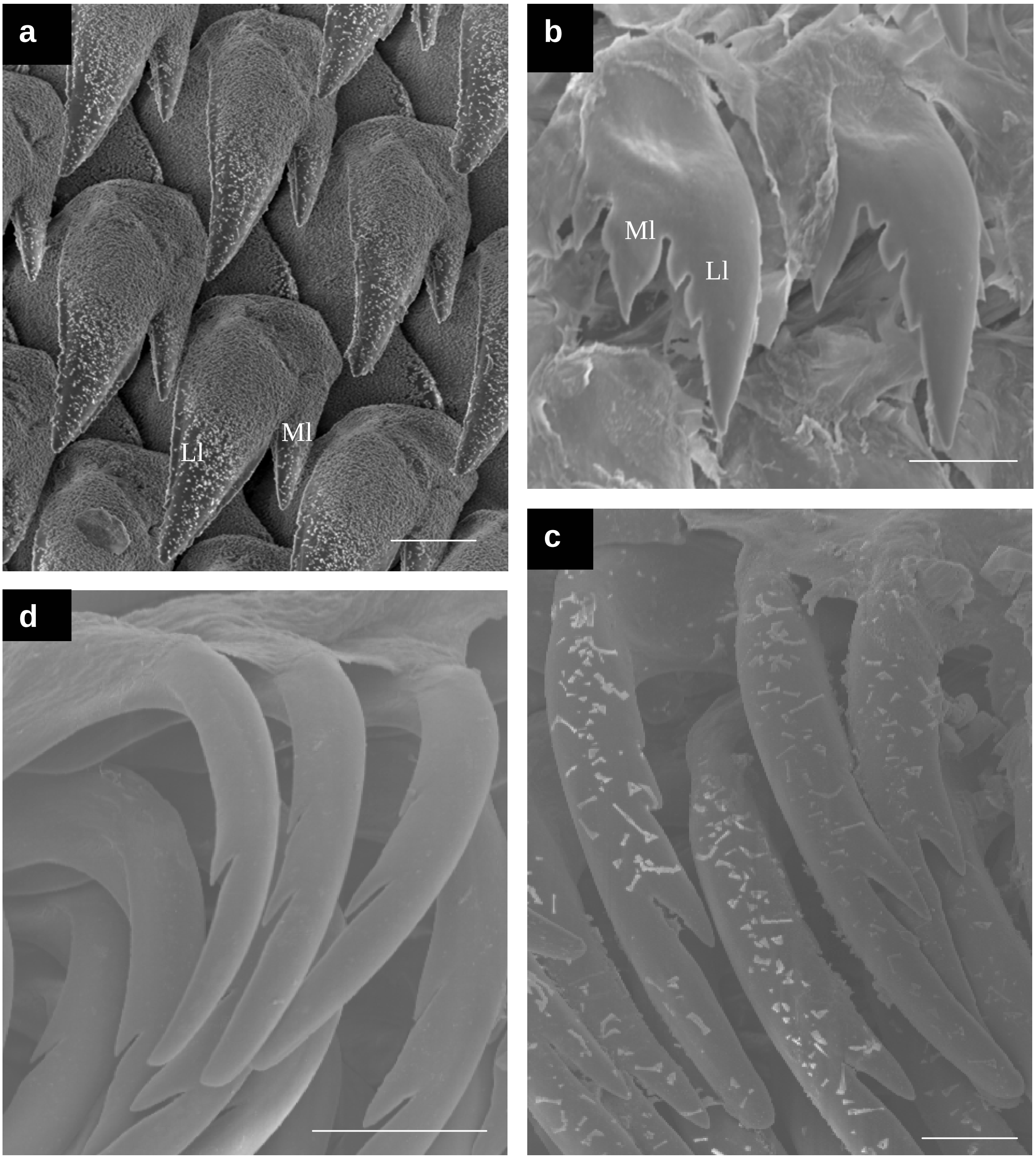

Figure 5.3: Scanning electron micrographs of the marginal teeth of a) *Aplysia dactylomela*, left edge of mounted radula, scale bar = 50µm; b) *Aplysia extraordinaria*, right edge of mounted radula, scale bar = 25 µm; c) *Bursatella leachii*, right edge of mounted radula, scale bar = 25 µm; d) S*tylocheilus striatus*, left edge of mounted radula, scale bar = 50µm. Legend: Ml = medial lobe, Ll = lateral lobe.

Discussion

Radula Types

Simple Radulae

The teeth of *Dolabella auricularia* are simple and this is considered to be the primitive state for the sea hares (Klussmann-Kolb in press). This species consumes a range of brown, red and green algae, including both filamentous and erect, branching macroalgae (Table 5.3). *Dolabella auricularia* is the only true generalist sea hare species of those species investigated (Pennings *et al.* 1993). This species is considered a true generalist because it actively selects the least abundant algae in the environment (Pennings *et al.* 1993). Pennings *et al.* (1993) found that when *D. auricularia* was offered different ratios of algae species, the animals always chose the food species present in the smallest amount and grew better on a diet of mixed algae than on single alga diets.

Bilobed Radulae

Aplysia dactylomela and *A. extraordinaria* both possess bilobed radulae. *Aplysia dactylomela* has a broad diet range, consuming a variety of red macroalgae and foliose green algae (Table 5.3). The radula of *A. extraordinaria* is slightly more complex than that of *A. dactylomela* with fine serrations on the lobes of the rachidian and lateral teeth. The diet of *A. extraordinaria* has not yet been tested although individuals were observed in association with red algae at local study sites (Chapter 2).

Denticulate Radulae

Within the denticulate radula type, *Bursatella leachii* possessed slightly less complex radula teeth than *S. striatus*. Paige (1988) found that *B. leachii* settled and preferentially fed upon a range of cyanobacteria species in Florida, while in Japan, *B. leachii* preferred a range of macroalgae (Wu 1980). Feeding preference testing conducted by the present author showed that *B. leachii* consumed the green algae, *Enteromorpha* in addition to the cyanobacterium, *Calothrix crustacea* eaten in the field (see Chapter 4). In contrast, *S. striatus* is considered an extreme specialist on the cyanobacterium, *L. majuscula*, preferring this species to a number of macroalgae and cyanobacteria species (Switzer-Dunlap & Hadfield 1977; Paul & Pennings 1991). *Stylocheilus striatus* possessed the most complicated radula of all those studied.

Algae-Herbivore Interactions

For gastropods, it has been suggested that the fine morphology of the radular teeth cusps would closely reflect the structure of the food source (Hickman 1980). Each sea hare radula type may be more effective than other types at handling and manipulating certain algal functional groups. For example, the fine denticles and serrations found in denticulate radulae provide a high number of contact points for use during feeding. Therefore, denticulate teeth would be more efficient at handling small food items or thin strands of algae. Both *B. leachii* and *S. striatus* possess denticulate radulae, and they consume cyanobacteria and filamentous green algae (Pennings *et al.* 1996; Nagle *et al.* 1998). The high number of denticles and serrations would not exert a high degree of force but would allow the radula to pull fine food items into the mouth for mechanical breakdown by the crop and gizzard (Steneck & Watling 1982). This is further supported by similar results during research on mesogastropods (Steneck & Watling 1982). The mesogastropods examined by Steneck and Watling (1982) possessed a large number of sharply pointed serrations on the radula teeth (like denticulate teeth in the present study) and were also more likely to specialize upon cyanobacteria and filamentous algae (Steneck & Watling 1982).

Radula teeth with a small number of large cusps (eg. bilobed radulae) would be more efficient at puncturing and grazing on the tougher cell walls of macroalgae. The reduced number of contact points allows more force to be exerted on the algal cell walls and thereby physically break apart the macrophyte tissues into smaller pieces for ingestion (Steneck & Watling 1982). Steneck & Watling (1982) found that blunt radula teeth with a small number of cusps were more likely to belong to gastropods that feed upon tougher macroalgae. Therefore sea hares with bilobed radulae would be expected to feed upon macroalgae.

Aplysia dactylomela possesses a bilobed radula and consumes a small number of filamentous and foliose green algae as well as a large number of red algae species (Table 5.3). However, the inclusion of the filamentous green algae in the diet does not seem to agree with the radula type. An increase in the degree of denticulation or complexity of the teeth may allow these species to exploit the preferred filamentous green algae in addition to the red macroalgae that forms the majority of the diet.

Aplysia dactylomela has fine serrations on the lateral teeth, which may allow filamentous algae to be eaten. It is likely that this radular morphology represents a compromise that allows the consumption of two food types. Small denticles allow *A. dactylomela* to consume filamentous and foliose green algae when it is available but the large lobes can be used to consume macroalgae when there are no green algae. Similar to the versatile radulae of the chitons, sea hares that possess these multifunctional radulae could sweep and excavate simultaneously or alternately to feed on different algae when presented (Steneck & Watling 1982).

As seen in the present study, *Dolabella auricularia* has neither of the adaptations required for consuming macroalgae (large cusps) nor filamentous algae (serrations or denticles) and yet it consumes both (Pennings *et al.* 1993). This may be related to the way in which the radula is applied to the food macrophyte. *Dolabella auricularia* may possess adaptations of the pharyngeal muscles or modifications to its feeding behaviour for different algae or some combination of the two. The radular muscles associated with the teeth-bearing ribbon may be able to change the angle and pressure of the teeth in response to the type of food being targeted although these possibilities have not been previously investigated. This species may also handle and approach different types of algae in different ways that allows this species to consume a large range of the available food species.

Phylogenetic Control

Phylogenetic constraints imposed by the form of the radula may affect the feeding specialization of sea hares. The family Aplysiidae have been divided into four subfamilies (Table 5.4). The three radula types defined here correspond to the phylogenetic grouping of the Aplysiidae recently performed by Klussmann-Kolb (in press). Members of the subfamily Aplysiinae examined (*A. dactylomela* and *A.*

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).

Sea hare species	Radula type	Reported algal feeding preferences	Algal functional group (Steneck & Watling 1982)	Feeding reference
Dolabella auricularia	Simple	*Enteromorpha clathrata, Dictyota cervicornis, Padina tenuis, Hydroclathrus clathratus, Sargassum polcystum, Acanthophora spicifera, Laurencia papillosa* and other species in small amounts	Filamentous algae, Foliose algae, Corticated macrophytes, Leathery macrophytes	Pennings & Paul 1992; Pennings *et al* 1993
Aplysia dactylomela	Bilobed	*Corallina, Laurencia, Ulva, Centroceras clavulatum, Cladophora, Gracililaria, Laurencia, Acanthophora spicifera, Spyridia filamentosa*	Foliose algae, Corticated macrophytes, Articulated calcarerous algae	Carefoot 1970; 1985; Switzer & Dunlap 1979; Willan 1979
Aplysia extraordinaria	Bilobed	unknown	Unknown	
Bursatella leachii	Denticulate	*Lyngbya majuscula, Calothrix crustacea, Enteromorpha, Hypnea, Ulva,* and *Graciliaria*	Microalgae, Filamentous algae, Foliose algae	Capper 2003; Wu 1980; this study: Chapter 4
Stylocheilus striatus	Denticulate	*Lyngbya majuscula*	Microalgae	Capper 2003; Wu 1980; Paul & Pennings 1991

extraordinaria) possessed bilobed radulae. The sole member of the subfamily Dolabellinae examined, *Dolabella auricularia*, possessed a simple radula. The members of the subfamily Notarchinae (*B. leachii* and *S. striatus*) possess denticulate radulae. The correspondence between radula type and phylogenetic grouping is not surprising, however, because Klussmann-Kolb (in press) used the shape of the rachidian and lateral teeth as characters in her analysis. Phylogeny may therefore be responsible for limiting the feeding preferences of sea hares by controlling the morphology of the radula teeth.

Table 5.4: The phylogeny of the sea hare species investigated in the present study, as adapted from Klussmann-Kolb (in press).

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Generalist-Specialist Continuum

There was a continuum of generalist-specialists within the sea hares that is reflected in the radular morphology described in the present study (Figure 5.4). Generalist sea hares had simplified radula teeth, as exemplified by *D. auricularia*. More specialized sea hares had more complicated tooth structures with more denticles, lobes and branches on the rachidian, lateral and marginal teeth. Within the current study, the most complex and highly denticulated radula belongs to *Stylocheilus striatus*. This extreme in complexity agrees with the diet of this species. *Stylocheilus striatus* is considered an extreme specialist on the cyanobacteria, *Lyngbya majuscula* (Paul & Pennings 1991; Nagle *et al.* 1998). Complex tooth structures may act as alga-specific tools that improve the handling efficiency of the radula-food interaction (Jensen 1993). The generalist-specialist continuum model is predictive and based on the limited number of sea hare species investigated here. Further investigation will be required to test its generality for all sea hare species.

Ecological models predict that small herbivores are more likely to be specialists than large ones (Pennings 1990b; Trowbridge 1991a). Size is interrelated with mobility; large sea hares exhibit a higher degree of mobility and therefore are more likely to be generalised feeders. Of the five sea hare species considered here, two of them would be considered large (>500g): *Dolabella auricularia* and *A. dactylomela*. These two species possess simple and bilobed radulae, respectively, but they are simplified teeth with smooth lobes with little or no serrations on them. *Dolabella auricularia* is a true generalist herbivore (Pennings *et al.* 1993) and *A. dactylomela* consumes a wide variety of red algae and a small number of green algae species (Carefoot 1987).

An increase in radula complexity with animal size, in sea hares, is consistent with the reports of ontogenetic change in feeding preferences within a single species (Pennings 1990b; Roberts *et al.* 1999). Sea hares increase their range of feeding preferences with increasing age and size. Pennings (1990b) attributed the specialization of young *A. californica* to the small, weak mouthparts of the juveniles; however the morphology of the mouthparts, including the radula, were not examined. Only adult sea hares were examined in the present work. Therefore it remains to be tested whether sea hares also exhibit changes in the radular morphology in conjunction with ontogenetic changes in diet. Other herbivorous gastropods, such as the abalone, exhibit such ontogenetic changes in the radula teeth (Roberts *et al.* 1999). Larger post-larval abalone had a reduction in serrations, increased spacing between teeth and lengthening of outer lateral teeth (Roberts *et al.* 1999).

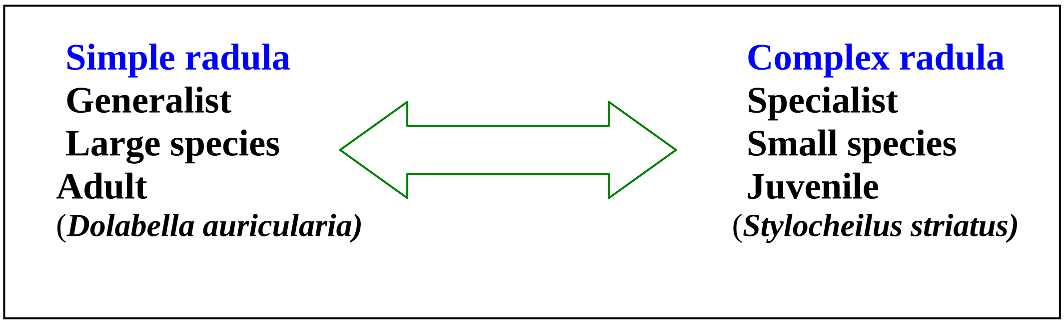

Figure 5.4: Diagram demonstrating the generalist-specialist continuum and its associated predictions. Sea hares with simple radulae are more likely to be generalist feeders, large species and/or adults. At the other end of the spectrum, sea hares with complex radulae are more likely to be specialised feeders, small species and/or juveniles. In brackets are the species that exemplify the two extremes of this relationship.

Radula-Mediated Coexistence

The slightly different radulae of co-occurring sea hares may explain their ability to coexist as seen at Kissing Point (*Aplysia dactylomela* and *A. extraordinaria*) and Shelly Beach (*Stylocheilus striatus* and *Bursatella leachii*) (see Chapters 2 & 3) and in other parts of the world (see Achituv & Susswein 1985). *Aplysia extraordinaria* has more denticles on the rachidian and lateral teeth than *A. dactylomela.* This may suggest that *A. extraordinaria* has a more specialised diet than that of *A. dactylomela*. However, the diet of the former species is currently unknown. *Stylocheilus striatus* has more cusps and denticles on the rachidian teeth than *B. leachii,* which would lead to the hypothesis that *B. leachii,* has a more generalized diet. Preference testing on these two species supports this prediction; *B. leachii* consumes both cyanobacteria and green filamentous algae (Wu 1980; Paige 1988). *Stylocheilus striatus* only consumes the cyanobacteria, *Lyngbya majuscula*. Morphological differences in gastropod mouthparts have been hypothesized to allow co-existing species to interact without competitively excluding one another (Steneck & Watling 1982). Differences in the radulae of closely related sea hare species, and therefore slightly different diets, may allow them to coexist in the same habitats.

Macrophyte Morphology Differences

Differences in the palatability of the various parts of a single macrophyte species also complicate the study of relationships between radula and food type. A herbivore may discriminate between the different parts of a single algal macrophyte (Steneck & Watling 1982). The thalli, holdfasts, and stipes of macrophytes have varying degrees of physical and chemical defences (Steneck & Watling 1982; Carefoot 1987). Often, the part of the food macrophyte that the sea hare was ingesting was not specified. Macrophyte differences add another dimension to the relationship between diet and radular morphology. For instance, a sea hare that feeds on both foliose green algae and toughened red macroalgae may seem very generalized in its diet. However, a sea hare may feed only on the softer parts of the macroalgae and thus be restricted in its diet to a greater degree than is superficially obvious.

Conclusions

There is a relationship between sea hare radular morphology and diet. With further testing of additional sea hare feeding preferences and radular morphology the extent of this relationship can be better understood. The establishment of sea hare radula types allows testable hypotheses to be formulated about the feeding preference of sea hares based on the morphology of the radula. This can be especially useful for those species that have not undergone the same research focus, for example, *A. extraordinaria* and *Petalifera petalifera.* Phylogeny seems to be an overlying force in controlling sea hare radular morphology. The influence of ontogeny is an area where future research is required. The generalist-specialist continuum provides a testable theoretical framework that can be further investigated with additional sea hare species and other marine herbivores.

Chapter 6: The role of sea hares as herbivores in tropical habitats

The main objective of the present study was to determine the role of sea hares as herbivores in tropical intertidal habitats. This objective was addressed in two ways, firstly by examining the abundance and distribution of sea hares in selected tropical habitats in north Queensland, Australia and secondly by investigating the feeding specificity of the local sea hares. The results of these studies revealed that the role of sea hares in these habitats is complex and involved several overlapping issues. Not only is the density and degree of feeding specificity different for each of the five species studied but in addition, the roles that sea hares play as herbivores depend not only on the species in question but also vary with the time of year.

What role do individual sea hare species play?

The five sea hares found in the North Queensland intertidal habitats during this study, *Aplysia dactylomela, A. extraordinaria, Bursatella leachii, Stylocheilus striatus* and *Petalifera petalifera* exhibited varying relationships with marine algae.

Red Algal Oligophagy

The abundance of *Aplysia dactylomela* was correlated with the abundance of red algae in the local habitats. This relationship agrees with the wide feeding preferences that *A. dactylomela* has for many different species within the Rhodophyta (red) macrophytic group, as reviewed by Carefoot (1987). These feeding preferences are the result of adult feeding behaviour (Carefoot 1985, 1987, 1991), larval settlement cues (Switzer-Dunlap & Hadfield 1977, 1979), and the morphology of the feeding apparatus (see Chapter 5). *Aplysia dactylomela* also possesses a bilobed radula as designated by the functional scheme developed during the course of the present study. A bilobed radula is adapted for feeding on tough macroalgae because of its blunt cusps useful in excavating. Many of the red algae species present in sea hare habitats are chemically defended algae that are low-preference food items for other herbivores in the ecosystem (Steneck & Watling 1982; Rogers *et al.* 1995). Red algae were highly abundant in the four sites examined in the current study so the distribution of *A. dactylomela* in these habitats is likely caused by this group's local abundance. Whether this relationship is the result of metamorphic settlement preferences or adult movement is currently unknown. The abundance of species of *Aplysia* in other studies were positively related to whichever algae in their habitats served as their primary food sources (Plaut *et al.* 1998; Rogers *et al.* 2003). For example, in New South Wales, Australia, *Aplysia parvula* abundance co-varied with the abundance of the red algae *Delisea pulchra* and *Laurencia obtusa* (Rogers *et al.* 2003). Similarly, the recruitment of *Aplysia oculifera* in the Red Sea occurs in concert with blooms of green algae (Plaut *et al.* 1998). Within the present study, the density of *Aplysia dactylomela* never exceeded 10 per 100m^2, even at its peak abundance. This is much lower than many of the previous reports of *Aplysia* spp. densities from other parts of the world (reviewed in Carefoot 1987). Therefore even with its feeding specificity for red algae, the low density of this species within the current study suggests that it would not exert a strong influence on its host macrophytes.

The ecology of *Aplysia extraordinaria* remains unclear. During the present study, *Aplysia extraordinaria* was found in association with both red and green algae although whether this relationship was feeding-related remains unknown. In addition, the abundance of *A. extraordinaria* was correlated with three species of red algae. However, its preferred food algae has not been previously tested and therefore a causal relationship cannot be drawn between them (Carefoot 1987). Based solely on the radular morphology *A. extraordinaria* is hypothesized to be a macroalgae feeder similar to *A. dactylomela* but it may be more specialized because of its complex radula teeth. *Aplysia extraordinaria* was the most widely distributed sea hare species examined in the current study, in that it occurred at three of the four study sites. However, its density remained below 10 per 100m^2 for the entire year sampled. The consistent low density of this species seems to rule out the possibility that this species acts as an important herbivore within the study sites although the lack of ecological information for this species calls for caution in drawing conclusions.

Aplysia extraordinaria and *A. dactylomela* co-existed at two of the study sites. Such coexistence has often been recorded for species of sea hares (see Eales 1960; Achituv & Susswein 1985; Carefoot 1987, 1989). In Israel, *Aplysia depilans* and *A. fasciata* appear to partition their shared habitat according to hydrological conditions (Achituv & Susswein 1985). While in Hawaii, *Aplysia dactylomela* and *A. parvula* coexist through differing activity patterns where *A. parvula* is diurnal and *A. dactylomela* is nocturnal (Carefoot 1989). In contrast, within the current study, both *A. dactylomela* and *A. extraordinaria* are nocturnal as these species were observed hiding in rocks during the day, often within the same microhabitat. However, their behaviour during the night is unknown so the mechanisms underlying this apparently close existence have yet to be observed. Further research into the basic ecology and feeding preferences of *A. extraordinaria* are required before the factors allowing for its coexistence with *A. dactylomela* can be determined.

Cyanobacterial Oligophagy

In contrast to the low densities of *Aplysia* spp. often cited in the literature (reviewed by Carefoot 1987), the cyanobacteria specialists, *Stylocheilus striatus* and *Bursatella leachii* were found in high densities at Shelly Beach, Townsville. In two consecutive years (June-July 2002 and 2003), blooms of two different cyanobacteria species occurred at this location and were associated with high numbers of *S. striatus* and *B. leachii*. The two years differed dramatically in their population dynamics. In 2002, *B. leachii* was the dominant species with *S. striatus* present at low density. Recruitment of *B. leachii* into the population occurred throughout the entire seven-week period that sea hares were present. The continual input of small sea hares into the area indicates a continually recruiting population rather than a pulse settlement event, the latter being what is generally believed to occur in sea hares (Gev *et al.* 1984; Carefoot 1987; Pennings 1991). In contrast, the 2003 population was dominated by *S. striatus* with only a small number of *B. leachii* present. This change clearly shows that even co-occurring sea hare species may exhibit markedly different population dynamics.

The dietary specialization of *B. leachii* and *S. striatus* on cyanobacteria leads to speculation about the ability of these sea hares to consume the troublesome outbreaks of cyanobacteria in coastal waters. This is particularly relevant since the ability to exploit cyanobacterial species is exhibited by very few marine herbivores (Pennings *et al.* 1996). It is possible that *Bursatella leachii* and *S. striatus* may be specially adapted to exploit the occurrence of cyanobacteria blooms and are therefore important consumers of cyanobacteria. However, when tested in the laboratory, *Bursatella leachii* was found to preferentially consume the chemically neutral filamentous green algae, *Enteromorpha* over the cyanobacteria it was observed to feed on in the field. Green algae were not obviously available in the habitats where these sea hares were found and was not abundant in any of the sites investigated. To build on the results of this part of the current study, experiments to monitor long-term growth are needed to determine if green algae promotes higher levels of growth and reproductive output for *B. leachii*. Several authors have found that the most preferred alga did not always provide the highest growth potential for sea hares (Switzer-Dunlap & Hadfield 1979; Carefoot 1981; Rogers *et al.* 1995). Thus for *B. leachii*, at least, there may be a higher preferred food than cyanobacteria and thus their effectiveness as cyanobacteria consumers would be lessened if cyanobacteria blooms occurred in areas with high density of preferred green algae.

Strict Monophagy

Petalifera petalifera is an extreme specialist on the calcified brown algae, *Padina tenuis*. During the present study, *P. petalifera* was only found living amongst the thalli of *Padina tenuis* and its occurrence was highly correlated with the abundance of this alga in the intertidal habitats investigated. The close relationship between *Petalifera petalifera* and its host alga suggests that this sea hare has the potential to exert a large effect on its host algae. However, *Petalifera petalifera* did not occur at high densities during any part of the year sampled. In addition, at a maximum length of 16mm, this is the smallest sea hare species encountered and it is a mesograzer on the host alga suggesting a low rate of consumption compared to the larger species (Martinez 1996). Since mesograzers are not considered to consume the entire host macrophyte (Hay *et al.* 1989; Brawley 1992; Hay 1997; Rogers *et al.* 1998) the effect of this species on the local *P. tenuis* population is not likely to be large although the effect on individual macrophytes cannot be overlooked. The mechanisms underlying macrophyte choice by *P. petalifera* may have important implications for the dynamics of this common algal species. For example, the grazing intensity of herbivores was hypothesized to cause variation in concentration of secondary metabolites between macrophytes within a population (see Lewis 1985; Paul & Fenical 1986; Paul & Van Alstyne 1988a; Hay 1997). *Padina tenuis* is a lightly calcified algal species and calcification in macrophytes has been hypothesized to act as a herbivory defense (Padilla 1989; Hay 1997). In a similar manner to grazing and secondary metabolites, the feeding action of *P. petalifera* may cause variation in the degree of calcification individual *P. tenuis* macrophytes contain as defense.

The previously observed but undocumented specialization of *Petalifera petalifera* on *Padina tenuis* in North Queensland provides an intriguing model for the study of specialization in marine habitats. Laboratory studies are required to fully understand the relationship between these two species.   For example, how much damage a single *P. petalifera* causes to its host is unknown.   Tagging of individual animals and host macrophytes may provide information about the mobility of this species and its life span. Such experiments have previously been performed for *Aplysia vaccaria* (Angeloni *et al.* 1999). Chemical ecological investigations may also be able to shed light on the relationship between the secondary chemistry and calcification of *Padina tenuis* and the ability of *P. petalifera* to exploit it as a host. Rogers *et al.* (2000a) used chemical analysis to investigate the relationships between the chemistry of the sea hares, *Aplysia parvula* and *Aplysia dactylomela,* and the secondary metabolites in their host algae.

Overall, the results of the current study have clearly shown that sea hare population dynamics are likely to be more species-specific than previously thought and care must be exercised in extrapolating information about the most commonly studied sea hares (eg. *A. californica* and *A. dactylomela*) to all sea hare species.

What role do sea hares play as herbivores?

The occurrence of sea hares, in the four habitats selected for field monitoring, appears to be highly seasonal. Sea hares were only found during five months within the year studied although this finding cannot be extrapolated inter-annually. If this trend is constant in other years, the seasonal nature of sea hare populations may be related to the constraints imposed by the harsh local environment. Sea hare abundance corresponded to the coolest months of the year, as well as the times when light intensity was at its lowest levels. This was supported by the significant negative relationship between sea hare density and light intensity found in the present study. The underlying cause of this relationship is currently unknown but UV light may affect the populations in several ways.   Ultraviolet light may be negatively affecting the larval supply by increasing mortality in the plankton or it may also prevent the settlement of juveniles. There have been no previous investigations into the effect of UV light on sea hare recruits.

Ultraviolet light may further reduce sea hare survival by negatively affecting adult growth, survival and fecundity as demonstrated by Carefoot *et al* (1998).

Alternatively, seasonality may result simply by differences in oceanographic conditions preventing larvae reaching these sites in the summer months. According to Pennings (1991), seasonality of populations of *Aplysia californica* was caused by metapopulation recruitment. There is very little information on the forces governing sea hare larvae while they develop in the plankton. For other marine invertebrate larvae, current patterns have been linked with the differences in recruitment between sites, most likely as a result of differences in larval delivery (Ebert & Russell 1988). Further research using larval tows is required to determine whether the seasonality of sea hares in these habitats is caused by larval supply, variation in larval mortality at different times of year, or early stage juvenile mortality.

The findings of the present study suggest that sea hares potentially have an increased role as herbivores in shallow, tropical intertidal habitats. In tropical North Queensland, sea hares inhabit intertidal reef flats and seagrass beds that are not available to reef fish and many other herbivores at all times (due to the shallow water and large tidal regimes). Sea hares become important herbivores as a result of their ability to remain in these shallow water habitats even at low tide. An increased risk of predation has been hypothesized as the reason that shallow water habitats are avoided by many herbivores (Hay 1991). In contrast, adult sea hares seem to have a low diversity of predators (Willan 1979; Carefoot 1987; Rogers *et al.* 2000b). During the present study many sea hares were often observed openly browsing and crawling amongst stands of algae during daylight hours when the risk of predation from diurnal predators would be highest. Sea hares possess a suite of defensive mechanisms (Johnson & Willows 1999). They are often cryptically coloured, possess toxic ink and opaline glands, and sequester distasteful secondary metabolites (Carefoot 1987; Johnson & Willows 1999). Possibly as a result of their myriad defensive systems, the seemingly reduced predation pressure that sea hares appear to be exposed to allows their exploitation of these habitats. The converse may also be true, that living in high-risk intertidal habitats has caused the evolution of the defensive systems that sea hares now exhibit.

Sea hares consume algae that many other marine herbivores pass over (Paul & Pennings 1991; Hay 1992, 1997) and small marine herbivores often exhibit feeding specialization on algae that are chemically defended (Hay *et al.* 1987; Hay & Fenical 1988; Hay *et al.* 1989). However, according to several studies at least eight species of *Aplysia* prefer to consume green algae during laboratory testing (Saito & Nakamura 1961; Winkler & Dawson 1963; Carefoot 1970; Sarver 1979; Switzer-Dunlap & Hadfield 1979; Achituv & Susswein 1985; Carefoot 1987). The present study also supported this result, showing that *B. leachii* prefers green algae in preference testing. However, this outcome may be an artefact of the testing procedure resulting from the removal of influential pressures, such as the threat of predation or inter- and intra-competition, under artificial conditions in the laboratory. Nevertheless, the low density of green algae in most natural habitats because of its removal by herbivorous fish (Hay 1981, 1984a; Lewis 1985), leads to the supposition that sea hares have developed the ability to consume non-preferred algal groups in response to competition from herbivorous fish. Such competition between herbivorous fishes and urchins was shown to be very important within Caribbean marine ecosystems (Williams 1981; Hay 1984a; Hay & Taylor 1985; Carpenter 1986). Therefore, sea hares are likely to play an important role in controlling those members of the algal community that are not generally palatable to other herbivores. In spite of this, the low density of most sea hare species studied (*B. leachii* and *S. striatus* being exceptions) even in areas with seemingly abundant preferred algae, suggests that other factors limit the abundance of sea hares in addition to food availability.

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