

Interaction between the canopy dwelling echinoid *Holopneustes purpureescens* and its host kelp *Ecklonia radiata*

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ABSTRACT: I examined the interaction between the unusual, canopy dwelling echinoid *Holopneustes purpureescens* and its main host plant, the kelp *Ecklonia radiata*. During a 4 yr study at Cape Banks, New South Wales, Australia, *H. purpureescens* reached densities as high as 1 ind. per thallus of *E. radiata* and $>17 \text{ m}^{-2}$, with densities declining strongly in the latter years of the study. These sea urchins also occurred, although at lower densities, on the dictyotalean alga *Homoeostrichus sinclairii*. *H. purpureescens* consumed laminae of *E. radiata* in the field at the rate of $\sim 1 \text{ g large ind.}^{-1} (\text{diameter } >40 \text{ mm}) \text{ d}^{-1}$. Consumption by the sea urchins was not affected by variation in phlorotannin levels among laminae. The impact of feeding by *H. purpureescens* on *E. radiata*, measured as (1) changes in the biomass of the kelps and (2) changes in thallus elongation rates, was examined in field experiments done in 2 seasons in which different numbers and sizes of sea urchins were caged with individual *E. radiata*. In spring, all densities and sizes of *H. purpureescens* caused significant damage (biomass) to *E. radiata* after 4 wk, and higher densities (2 per kelp thallus) of large sea urchins resulted in kelp mortality. No measurable damage occurred in autumn, with all kelps losing large amounts of biomass. No significant effect on thallus elongation rates was seen in either season after 4 wk. However, elongation rates varied among treatments after 2 wk in autumn. Grazing by *H. purpureescens* did not result in compensatory growth by *E. radiata*, nor did levels of phlorotannins in the kelps increase in response to grazing (chemical induction).

KEY WORDS: *Holopneustes purpureescens* · Herbivory · Kelp · Echinoids · Mesograzers · Inducible defenses

INTRODUCTION

Marine herbivores have a major impact on populations and communities of benthic macroalgae (Lubchenco & Gaines 1981, Hawkins & Hartnoll 1983, John et al. 1992). In temperate sublittoral algal communities, probably the most ecologically important plant/herbivore interaction occurs between regular echinoids, or sea urchins, and brown algae in the Orders Laminariales (kelps) and Fucales. The extensive literature on the effects of sea urchins in temperate kelp or furoid forests (reviews by Lawrence 1975, Lawrence & Sam-

marco 1982, Harrold & Pearse 1987, Andrew 1988) has repeatedly shown that grazing by sea urchins can completely change the physical and biological structure of these algal communities. In extreme cases sea urchins can denude the substratum of extensive areas of kelp, creating so-called 'barren' or (more properly) 'deforested' areas which then become dominated by encrusting coralline and small turfing algae (Lawrence 1975, Schiel & Foster 1986, Harrold & Pearse 1987, Andrew 1988). The striking dichotomy in habitat types represented by kelp forests vs deforested areas has prompted some authors (e.g. Harrold & Pearse 1987) to consider sea urchins as 'all or nothing' herbivores. That is, they either eliminate most large macroalgae from the habitat or have relatively little effect, surviving primarily on drift algae (Harrold & Reed 1985, Andrew & Stocker 1986).

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Contrasting with sea urchins — and a variety of other large marine grazers such as fishes or some gastropods — is the taxonomically diverse group of marine herbivores known as 'mesograzers' or 'meso-herbivores' (Fauchald & Jumars 1979, Hay et al. 1987, Brawley 1992, Hay 1992). These smaller herbivores (~1 mm to 2 cm in size), which include amphipods, crabs, other arthropods, polychaetes and small gastropods such as ascoglossans, have a number of features which distinguish them from larger herbivorous sea urchins. Firstly, mesograzers typically live within an alga, using it as habitat as well as food (Hacker & Steneck 1990, Duffy & Hay 1991a, Brawley 1992). Secondly, mesograzers rarely denude substrata of established stands of macroalgae, and in general the effects of mesograzers on macroalgae are thought to be much weaker (Carpenter 1986, Hay & Steinberg 1992) than the effects of larger herbivores such as sea urchins (although see Brawley 1992, Poore 1994). Thirdly, although feeding specialization is rare among marine herbivores (Hay 1992, Hay & Steinberg 1992), those marine specialists which do exist are frequently mesograzers. Herbivorous sea urchins, in contrast, are typically very generalist feeders (Lawrence 1975, Harrold & Pearse 1987). Fourthly, mesograzers are often resistant or tolerant to algal secondary metabolites which deter feeding or inhibit growth of large generalist herbivores such as sea urchins or fishes (Hay et al. 1987, Hay 1992, Hay & Steinberg 1992).

In this paper I examine aspects of the ecology of an unusual herbivorous sea urchin, *Holopneustes purpureus* (Family Temnopleuridae), that exhibits characteristics of both mesograzers and more typical herbivorous sea urchins. Although comparable in size (test diameter ≥ 50 mm) to many other sea urchins which live and forage directly on the sea floor, *H. purpureus* lives largely or exclusively off the substratum, enmeshed in the fronds of seaweeds such as the kelp *Ecklonia radiata* (Dakin 1980). Because of its use of seaweeds as habitat as well as food, its interaction with its macroalgal hosts is likely to be quite different from that of other sea urchins. Here I explore 5 aspects of the interaction between *H. purpureus* and its major host plant, *E. radiata*. Firstly, I ask: What is the distribution and abundance of *H. purpureus* in and around *E. radiata* forests? Secondly: At what rate does *H. purpureus* consume *E. radiata*? Thirdly, because both algal dwelling mesograzers (Hay & Steinberg 1992) and large invertebrate herbivores in Australia (Steinberg & van Altena 1992) are often resistant to algal chemical defenses, I ask: Is consumption of *E. radiata* by *H. purpureus* affected by variation in phlorotannin content — the major secondary metabolite of *E. radiata* — of the kelps? Fourthly, I examine the impact of *H. purpureus* herbivory on *E. radiata*.

Finally, I investigate whether *E. radiata* responds to damage caused by *H. purpureus* via either (1) compensatory growth or (2) induction of elevated levels of phlorotannins.

MATERIALS AND METHODS

Abundance, size distribution, and occurrence of *Holopneustes purpureus* on host plants. This study was done in sublittoral habitats at the Cape Banks Marine Research Reserve near Sydney, New South Wales, Australia (described in Fletcher 1987, Andrew 1993) between 1986 and 1990 (except for 1 short-term feeding experiment done in 1993). This area is comprised of a mosaic of forests of the kelp *Ecklonia radiata* interspersed with deforested areas covered in encrusting coralline or small turfing algae.

The abundance of *Holopneustes purpureus* in and around forests of *Ecklonia radiata* was measured in 2 ways. Firstly, in 1986 and 1987 a preliminary survey was done by marking out a 5 × 5 m grid in a forest of *E. radiata* (designated as Forest 2) at 6 m depth at Cape Banks. Within this large quadrat 100 large (stage 3 of Kirkman 1984) *E. radiata* were randomly selected, tagged with Dymo tape and cable ties, and the number and sizes of *H. purpureus* on these 100 plants measured in October and December 1986 and March 1987. Secondly, in April (autumn) 1988, a monitoring program was begun in which sizes and densities of *H. purpureus* were measured in 3 kelp forests (designated Forests 1, 2, and 3) approximately seasonally for 2½ yr until autumn 1990 (monitoring in Forest 3 was begun in winter 1988). These kelp forests were at 5 to 7 m depth at Cape Banks, with Forest 1 the most inshore and protected and Forest 3 the most seaward and exposed. At each kelp forest at each sampling time, a grid was established using 30 m transect tapes and twelve 0.25 m² quadrats placed randomly within the forest. In each quadrat all large (stage 3) *E. radiata* were counted, each kelp and all understory algae searched for *H. purpureus*, and the number and size of *H. purpureus* in each plant (kelp or otherwise) noted. Sizes of *H. purpureus* were measured as the maximum oral-aboral diameter, as these sea urchins tend to elongate along the oral-aboral axis as they get large (pers. obs.).

Beginning in winter 1988, beds of the dictyotalean algae *Homoeostrichus sinclairii*, which occurred in nearly monospecific patches on the margins of these *Ecklonia radiata* forests, were also sampled for *Holopneustes purpureus*. At each sampling time for each kelp bed, twelve 0.25 m² quadrats with at least 75% cover of *H. sinclairii* were searched for *H. purpureus*. Numbers and sizes of *H. purpureus* were measured as above.

Consumption of *Ecklonia radiata* by *Holopneustes purpureus*. Gut contents of 10 *H. purpureus* collected from *E. radiata* in October 1986 contained obvious fragments of *E. radiata*. In order to estimate consumption rates of *H. purpureus* when eating kelp, and to determine preferences by the sea urchins for different parts of the kelp, 3 consumption experiments were done. In the first experiment, 22 large (oral-aboral diameter >40 mm) *H. purpureus* were individually caged in plastic prawn crates bolted to the substratum at 4 m depth (Steinberg & van Altena 1992). Each individual was fed 1 piece each of pre-weighed (to 0.1 g) secondary and primary laminae of *E. radiata*, each massing ~15 g. Five pieces of laminae were individually caged without sea urchins to control for autogenic tissue loss. After 4 d the algae were removed from all cages and reweighed. In addition, prior to adding the pieces of *E. radiata* to the cages, samples of approximately 1 g were removed from each laminae. These samples were brought back to the laboratory, extracted in aqueous methanol, and their phlorotannin content measured using the Folin-Denis assay (Ragan & Jensen 1977, Steinberg 1989).

In the second and third experiments, one done in 1989 and one in 1993, 21 large *Holopneustes purpureus* were caged with individual pieces of secondary laminae, each massing ~20 g. Controls for autogenic losses were run concurrently as above. The algae were removed from the cages after 5 (1989) or 6 (1993) days and reweighed. Between 2 and 4 individuals died or developed abraded patches on their tests in each experiment, and data from these cages were excluded from subsequent analyses. Phlorotannin levels of the laminae were measured at the start of the experiment as above.

Effects of *Holopneustes purpureus* on *Ecklonia radiata*. The effects of *H. purpureus* on *E. radiata* were assessed by varying the numbers and sizes of *H. purpureus* enclosed within plastic cages surrounding individual kelp plants. Cages consisted of flexible plastic garden fencing with a mesh size of 20 mm. The fencing was formed into tubes approximately 0.5 m in diameter using plastic cable ties, and placed around each kelp. The tube cages were attached to the substratum using eye bolts bolted into the rock, with the tops of the cages extending 25 to 30 cm above the distal tip of the enclosed kelp. The cages moved with the current to some extent, minimizing abrasion on the kelp.

These sea urchin/kelp enclosure experiments were carried out in Forest 1 (5 m depth) on 2 occasions, autumn/early winter (April to July, henceforth designated 'autumn'), and late winter/spring (August to November, designated 'spring') 1988. Each experiment lasted for ~14 wk. Treatments in the experiments

consisted initially of uncaged kelp plants (Controls), and individual plants caged with either 0, 1 medium (1M), 1 large (1L), 2 medium (2M), or 2 large (2L; spring only) sea urchins (as described below under 'Responses of *Ecklonia radiata* to herbivory', some treatments were modified 4 wk into each experiment by removal of sea urchins from some cages). There were 5 kelp per treatment. Size classes of the sea urchins were arbitrarily defined as medium, with oral-aboral diameters 28 to 40 mm, and large, with oral-aboral diameters >40 mm.

Effects of the sea urchins on the kelp were determined by measuring changes in (1) biomass and (2) elongation of the individual plants. Changes in biomass were measured as follows: prior to caging, each plant was carefully chipped off the substratum at the base of the holdfast. The holdfast was then attached to a triangular piece of stiff plastic mesh (mesh size 5 mm) using cable ties. Three dynabolts were fixed into the substratum, spaced to accommodate the plastic mesh, and the plastic mesh, with attached plant, was then attached to the bolts using stainless steel wingnuts. The assembly was then placed back in the plant's original position. The entire procedure was done underwater.

This setup allowed the plants to be removed from the water, weighed, and returned to their position/cage. On Day 0 of each experiment (autumn and spring) plants were removed from the water in groups of 4 to 5, quickly weighed to the nearest 1 g on a portable top loading balance, and placed back in position within their cages. The kelp were kept wet as long as possible during this procedure, with excess water removed by shaking the plants just prior to weighing. When all plants had been weighed, the appropriate number and size of *Holopneustes purpureus* were placed on each caged plant. Plants were weighed again after 2, 4, 8 and 14 wk in the autumn experiment, and 2, 4, 9 and 13.5 wk in the spring experiment. This technique both enabled accurate measurements of the change in mass of the plants and, importantly, allowed changes in biomass and elongation rates (below) to be scaled to the initial size of the plants (since presumably the initial size of the plants will have a significant effect on growth). Measuring changes in biomass in this fashion incorporates positive changes due to growth of the plants and loss of tissue due to herbivory, sloughing, etc.

Elongation of the plants was measured by a standard technique for measuring growth in kelp, in which holes are punched in the primary laminae above the intercalary meristematic region and movement of the holes towards the distal end of the plant monitored (Mann 1972, Mann & Kirkman 1981).

Responses of *Ecklonia radiata* to herbivory. Does damage by *Holopneustes purpureus* stimulate compensatory growth in *E. radiata*? In order to inves-

tigate the extent to which *E. radiata* recovers from grazing by *H. purpureus* and whether grazing stimulates an increase in growth by the kelps, responses of the kelps following grazing were examined in both the autumn and spring enclosure experiments. In autumn, all sea urchins were removed from caged plants 4 wk after the start of the experiment. Plants were weighed 4 and 10 wk later (8 and 14 wk into the experiment, respectively) so as to compare differences in growth among treatments after the removal of sea urchins.

In spring, after 4 wk of the experiment, sea urchins were removed from 3 of the 5 plants in each treatment containing sea urchins, leaving urchins on the remaining 2 plants in each treatment. This generated 'sea urchin removed' and 'sea urchin remaining' treatments, with $n = 3$ or 2 (respectively) kelps per treat-

ment. The new treatments were designated as '-' or '+' for each relevant combination of size and density of *H. purpureus*. Thus (for example) 2M- indicates a cage from which the 2 medium sea urchins in the cage were removed after 4 wk, 2M+ a cage in which the sea urchins were left in place. Plants in spring were weighed again at 9 and 13.5 wk into the experiment (5 and 9.5 wk after urchin removal).

Does grazing by Holopneustes purpureus induce elevated levels of phlorotannins in Ecklonia radiata? Phlorotannin content of the *E. radiata* in the sea urchin/kelp enclosure experiments was measured at the beginning of the experiment and again after 4 wk in order to test for induction of phlorotannins due to grazing. This was done by removing approximately 1 g of tissue from a secondary lamina midway up each plant and subsequently analyzing phlorotannin con-

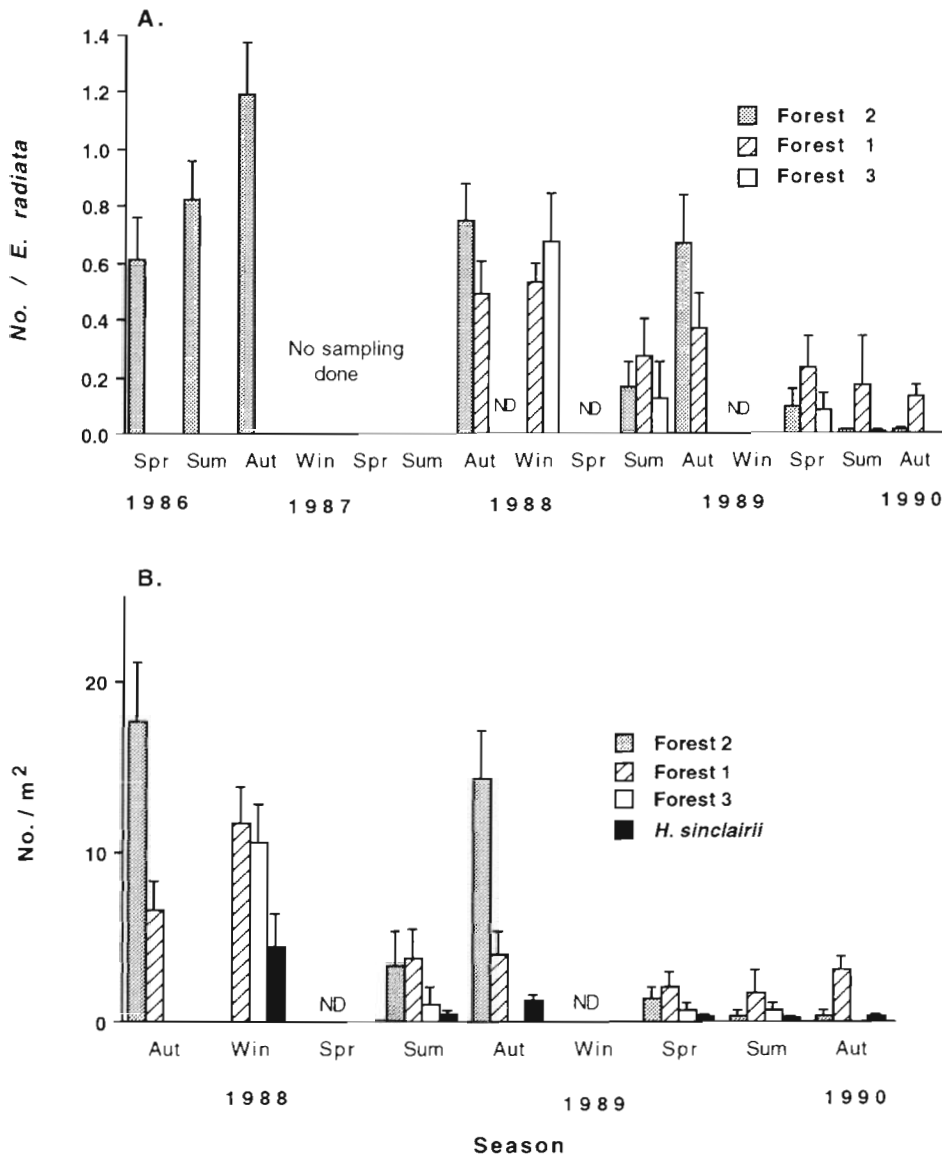


Fig. 1 *Holopneustes purpureus*. Number of urchins (A) per *Ecklonia radiata* and (B) per m² in 3 kelp forests at Cape Banks, New South Wales, Australia, from 1986 to 1990. Data given as means + SE. Data for abundance on *Homoeostrichus sinclairii* combined for all forests at each season. ND: no data collected for a given season (in 1988, no data were collected for Forest 2 in winter). No *H. purpureus* were found in Forest 3 during autumn 1989 and 1990. Note that data collection began in (A) 1986 and (B) 1988

tent using the Folin-Denis technique as described above. As removal of up to 25% of the mass of *E. radiata* by artificial means (clipping the laminae) has no significant effect on phlorotannin levels (Steinberg 1994), I assumed that clipping 1 g of tissue from plants which weigh >200 g would have little effect on the plants. Measurement of phlorotannins consistently from a specified part of the thallus is important as the

phlorotannin content varies significantly among different parts of the thallus (Steinberg 1989). Secondary laminae were used because *H. purpureus* primarily consumes secondary laminae and phlorotannin levels in *E. radiata* are highest in this part of the thallus (Steinberg 1989, Andrew & Jones 1990).

By analyzing phlorotannin content at the beginning of the experiment and after 4 wk, changes in phlorotannin content in individual kelps could be calculated. To test for induction of phlorotannins, changes in phlorotannin levels of the kelp were compared among treatments after 4 wk, and were also regressed against changes in biomass over the same period.

RESULTS

Abundance of *Holopneustes purpureus* in and around *Ecklonia radiata* forests

Maximum mean densities of *Holopneustes purpureus* at Cape Banks during the study were 1.16 ± 0.22 (SE) per *Ecklonia radiata* plant (Fig. 1A) and 17.6 ± 3.1 m⁻² (Fig. 1B). The maximum number of sea urchins observed on a single plant during the study was 6. In 1986 to 1988, densities of >0.5 sea urchins per plant and >10 individuals m⁻² were common. In the latter years of the study (1989 to 1990), the abundance of *Holopneustes* substantially declined, with densities typically below 0.4 plant⁻¹ and 3 m⁻². No *H. purpureus* were found at Forest 3 on several sampling occasions in 1989 and 1990. Densities of *H. purpureus* currently remain low at these sites at Cape Banks (pers. obs., summer 1994). All *H. purpureus* sampled in the kelp beds were found on *E. radiata* with the exception of a single individual enmeshed in the understory alga *Sargassum vestitum*. No *H. purpureus* were found on the substratum.

Size distributions for *Holopneustes purpureus* are shown in Fig. 2, with urchin sizes arbitrarily divided into 3 categories: small (0 to 28 mm oral-aboral diameter), medium (28 to 40 mm diameter), and large (>40 mm). Large and medium urchins were common at all sites in the early years of the study (Fig. 2), but the average size of *H. purpureus* decreased substantially in 1989 and 1990, particularly at Forests 2 and 3 (Fig. 2). No large urchins were found in the last several samples at these sites.

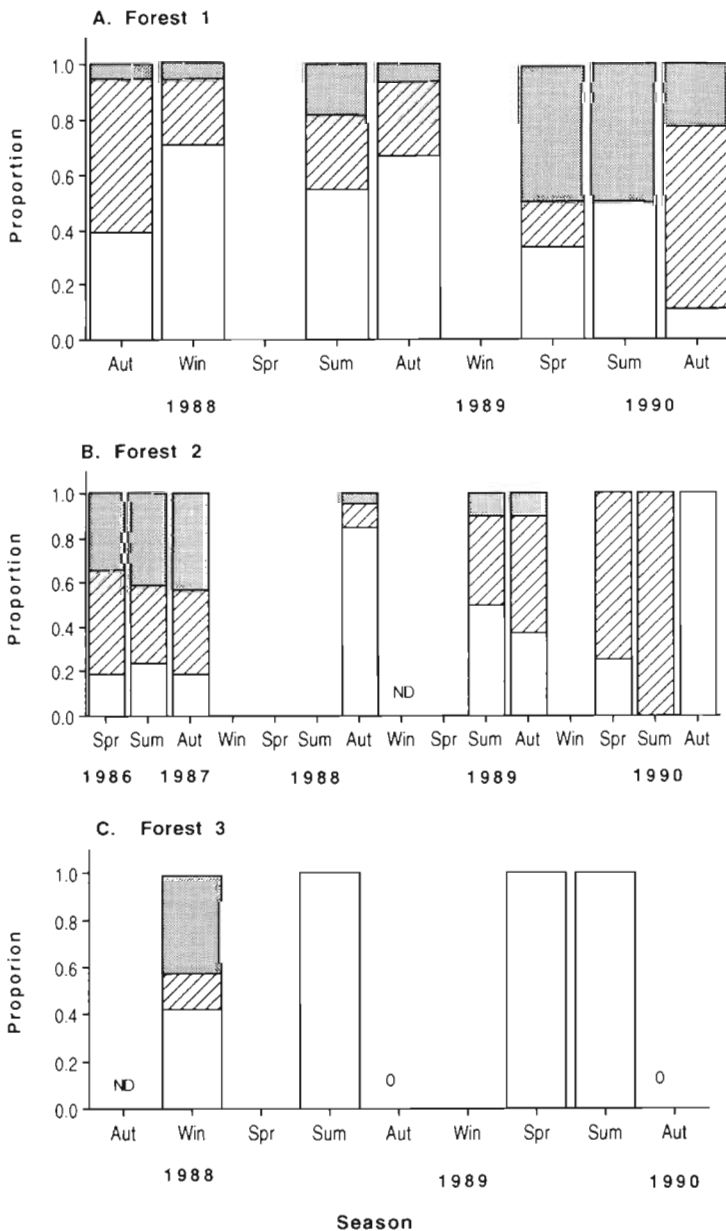


Fig. 2. *Holopneustes purpureus*. Proportion of each of 3 size classes in 3 kelp forests at Cape Banks from 1988 to 1990 (1986 to 1990 for Forest 2). Data from all quadrats at each forest at each time have been combined. Size classes are large (oral-aboral diameter >40 mm; shaded bars), medium (diameter 28 to 40 mm; striped bars) and small (diameter <28 mm; open bars). 0: no urchins found at that forest at that time

Holopneustes purpureescens also occurred in beds of *Homoeostrichus sinclairii* on the margins of the forests of *Ecklonia radiata*. However, both densities (Fig. 1B) and mean sizes (Fig. 3) of the sea urchins were typically much less in beds of *H. sinclairii* than in the adjacent kelp forests. Except for the winter sample of 1988, densities of *H. purpureescens* in beds of *H. sinclairii* were always $<1.2 \text{ m}^{-2}$. This winter 1988 sample contained an unusually large number of sea urchins on *H. sinclairii* at Forest 3 and also contained all the large ($>40 \text{ mm}$) *H. purpureescens* urchins found on *H. sinclairii* during the study.

Consumption of laminae of *Ecklonia radiata* by *Holopneustes purpureescens* and its correlation with phlorotannin content

In field consumption experiments, *Holopneustes purpureescens* consumed an average of $\sim 1.0 \text{ g}$ of *Ecklonia radiata* d^{-1} and consumed secondary laminae (mean \pm SE consumption = $0.74 \pm 0.21 \text{ g d}^{-1}$) preferentially to primary laminae ($0.23 \pm 0.14 \text{ g d}^{-1}$). The mean consumption of secondary laminae was significantly greater, as determined by a paired *t*-test ($\text{df} = 18$, $t = 3.90$, $p < 0.001$). Variation in phlorotannins among pieces of laminae was not correlated with variation in consumption, neither for consumption of secondary laminae (phlorotannin content = $6.54 \pm 0.87\%$ dry mass) vs primary laminae (phlorotannin content = $1.82 \pm 0.35\%$) nor for consumption of different secondary laminae which varied in phlorotannin content (Fig. 4A, B) between 3 and 9% dry mass.

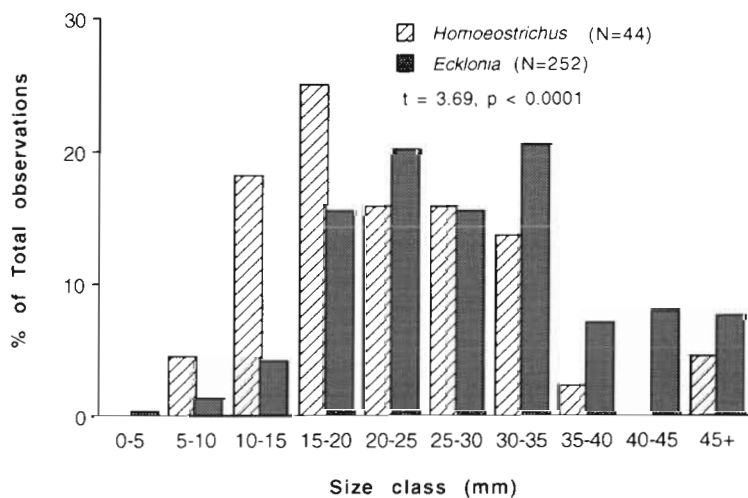


Fig. 3. *Holopneustes purpureescens*. Size frequency distributions in *Ecklonia radiata* forests and fringing beds of *Homeostrichus sinclairii*. Data from all forests and sampling dates from 1988 through 1990 have been combined

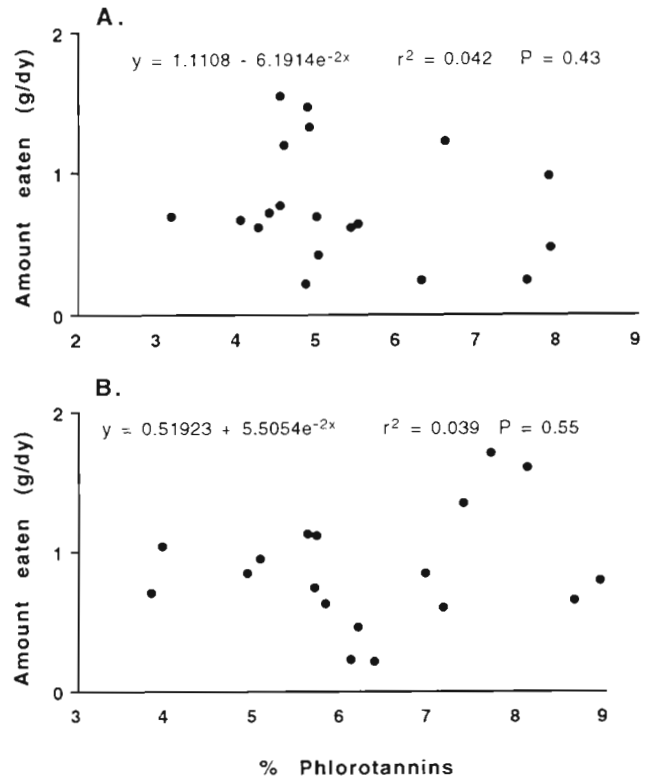


Fig. 4. *Holopneustes purpureescens*. Consumption of individual secondary laminae of *Ecklonia radiata* vs phlorotannin content of the laminae. Results of regression analyses are shown for 2 separate experiments, (A) 1989 and (B) 1993. Neither regression has a slope significantly different from zero

Effects of *Holopneustes purpureescens* on *Ecklonia radiata*

The effects of *Holopneustes purpureescens* on changes in biomass of *Ecklonia radiata* varied strongly with size, density, length of time of the sea urchins on the kelp, and with season. In autumn, there was no effect of *H. purpureescens* on *E. radiata*, with plants in all treatments losing substantial (25 to 40% of initial size) amounts of biomass after 4 wk (Fig. 5A, B).

In spring, however, the sea urchins caused substantial damage to the kelps (Fig. 5C, D). The amount of damage caused by the urchins varied predictably, with increases in the size, density, or length of time of *Holopneustes purpureescens* on the plants reducing biomass in an additive fashion (Fig. 5C, D). After 4 wk, grazing by 2 (medium or large) *H. purpureescens* had removed all the new

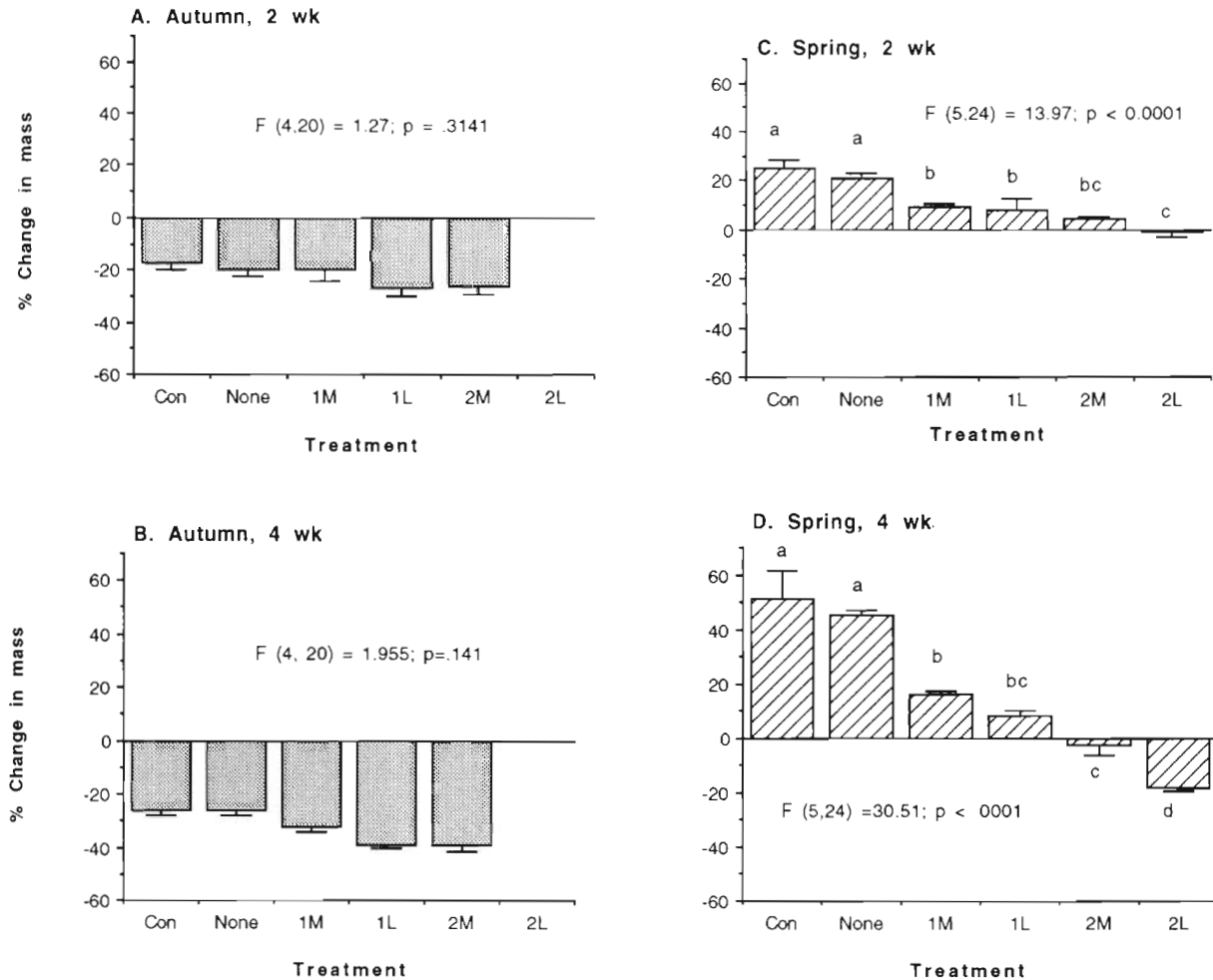


Fig. 5. *Ecklonia radiata*. Change in biomass of kelps caged with different sizes (M: medium; L: large) and densities (None, 1, or 2) of *Holopneustes purpureescens*. Con: control plants lacking cages or sea urchins. Data given as mean + SE of percent change in mass (relative to the initial mass of each plant). Analyses for each time/season are single factor ANOVAs on $\ln(x+1)$ of the proportional change in mass, followed by Tukey's simultaneous multiple range test (where the ANOVA was significant). Means sharing a letter are not significantly different. $n = 5$. Autumn experiment after (A) 2 and (B) 4 wk. Spring experiment after (C) 2 and (D) 4 wk. The 2L treatment was not done in the autumn

production of the enclosed plants, resulting in a net weight loss (Fig. 5D). One plant in the 2L treatment developed a large hole in the meristematic region after 2 wk, apparently due to the feeding behavior of *H. purpureescens*, and ultimately died (6 wk into the experiment; an additional plant in this treatment died after 8 wk). However, in most instances, primary laminae and meristems of the kelps were undamaged, indicating that the sea urchins mostly consumed secondary laminae.

In contrast to the effects on kelp biomass, *Holopneustes purpureescens* had no effect on elongation of *Ecklonia radiata* after 4 wk in either season (Fig. 6). There was statistically significant variation (ANOVA, $p = 0.036$) in rates of elongation among treatments in autumn after 2 wk (Fig. 6A), but the pattern of varia-

tion was not consistent with any simple effect of the sea urchins on the kelps.

Longer-term effects and compensatory growth

In autumn, removal of all *Holopneustes purpureescens* from the cages after 4 wk did not result in a significant increase in biomass of the *Ecklonia radiata* plants over the subsequent 10 wk (Fig. 7; paired *t*-test between plant sizes at Week 4 vs Week 14 for all plants was not significant), nor were there differences in biomass among treatments at Week 14 (ANOVA). Comparison of the final masses of the autumnal plants and the mean initial mass of the plants in the spring experiment also indicated that little growth of the kelp had

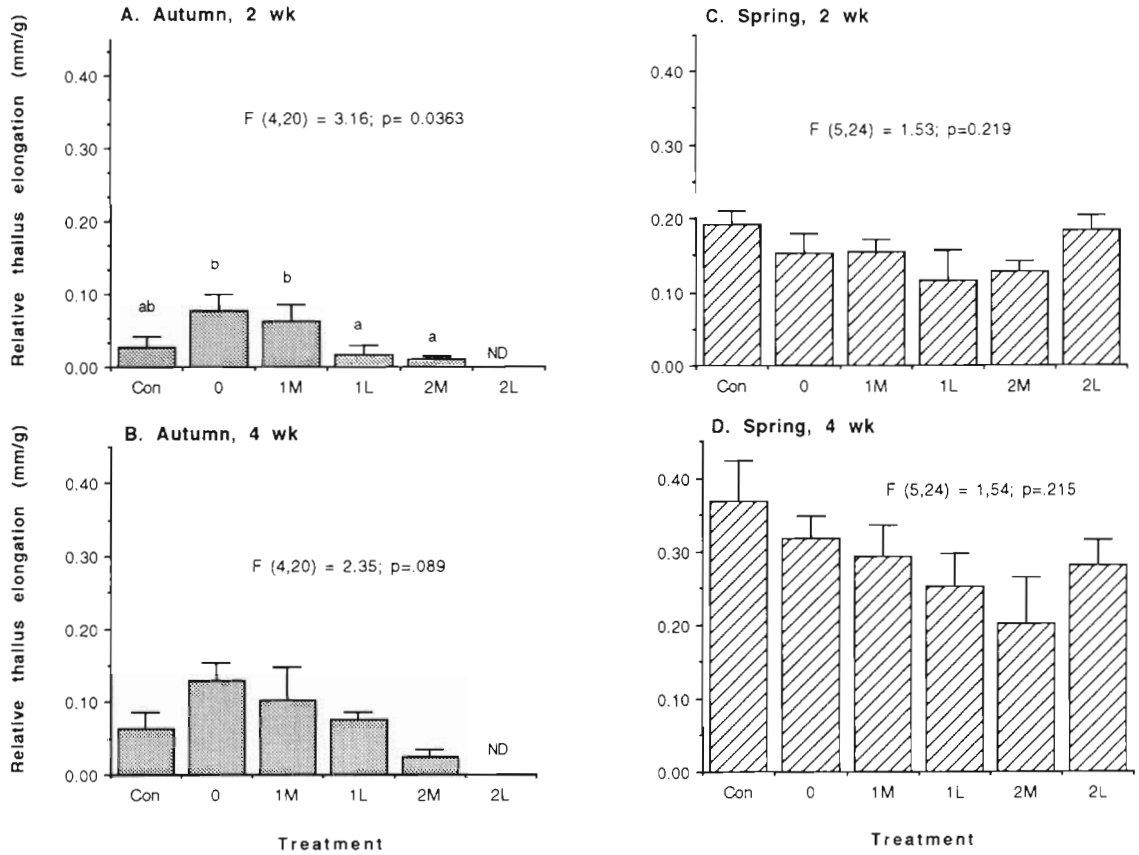


Fig. 6. *Ecklonia radiata*. Elongation of plants caged with different sizes and densities (as per Fig. 5) of *Holopneustes purpureus*. Data given as mean + SE of mass specific elongation rates (total length elongated in a given period scaled to the plant's initial mass). Analyses are single factor ANOVA on $\ln(x+1)$ transformed data, followed by Tukey's test where appropriate. n = 5. Elongation after (A) 2 and (B) 4 wk in autumn and after (C) 2 and (D) 4 wk in spring

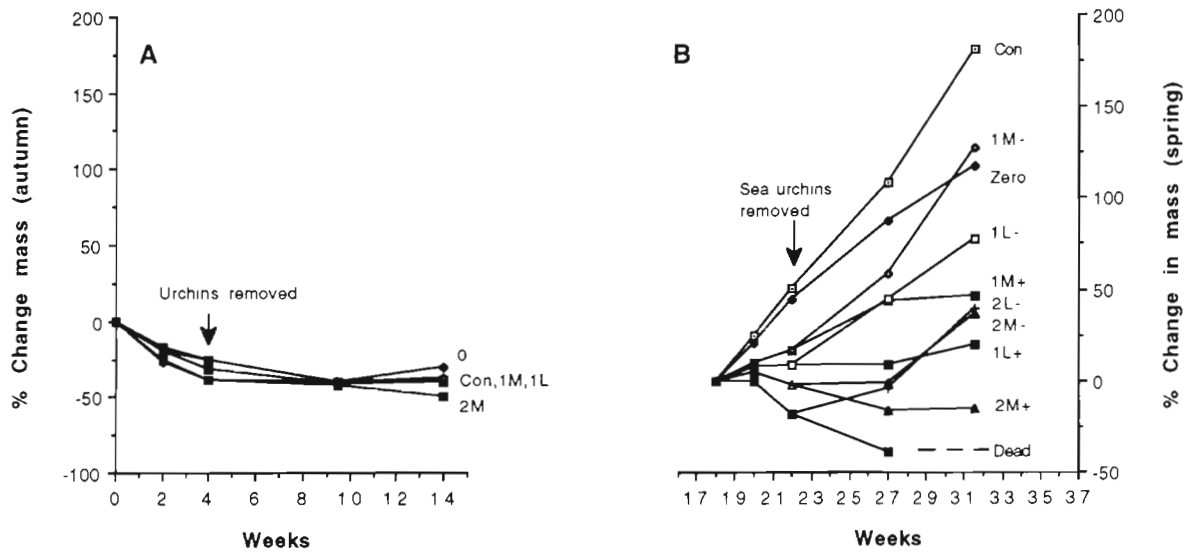


Fig. 7. *Ecklonia radiata*. Percent change in biomass for plants in (A) autumn and (B) spring caging experiments for the entire course of these experiments. Data are means per treatment, n = 2 to 5 (see 'Materials and methods'). Error bars omitted for clarity. Treatments indicate the number of urchins per kelp (0, 1 or 2), their sizes (S: small; M: medium; L: large) and whether, after 4 wk in the spring experiment, sea urchins were removed or left on the plants (-, + respectively). x-axis: number of weeks since the start of the autumn experiment

occurred in the 4 wk period between the 2 experiments.

In spring, however, 4 wk of herbivory by *Holopneustes purpureascens* had long-term effects on *Ecklonia radiata*, even when the sea urchins were removed from the plants (Fig. 7). This conclusion is based on a comparison of the proportional change in biomass among treatments after 9 and 13.5 wk in the spring experiment (single-factor ANOVA followed by SNK tests), with treatments in the experiment now consisting of (a) the original Zero and Control, (b) 'urchin removed' (e.g. 1M-, 1L-, 2M-, 2L-), and (c) 'urchin remaining' (1M+, 1L+, 2M+, 2L+) treatments. Because these divided treatments resulted in small sample sizes ($n = 2$ to 3 per treatment), differences were assessed using SNK tests with α set at both 0.05 and 0.10. After 9 wk, the treatments differed significantly (Fig. 7; single-factor ANOVA, $F_{8,19} = 9.17$, $p < 0.001$; the 2L+ treatment was excluded from the analy-

sis as only 1 plant survived to 9 wk in this treatment) with change in biomass of the plants in the Control and Zero treatments greater than that in the 2M-, 2L-, and 2M+ treatments at $\alpha = 0.05$ and greater than that in the 1L+, 1M+, 2M-, 2L-, 2M+, and 1L- treatments at $\alpha = 0.10$. After 13.5 wk ($F_{7,15} = 9.176$, $p = 0.0002$; 2L+ and 1M+ treatments now excluded because of 0 and 1 plant remaining, respectively) the Control and Zero treatments differed significantly (SNK tests) from the 1L+ and 2M+ treatments at $\alpha = 0.05$ and differed significantly from the 2L-, 2M-, 1L-, 1L+, and 2M+ treatments at $\alpha = 0.10$. These results indicate that *E. radiata* subjected to short bursts (4 wk) of grazing by *H. purpureascens* in the spring did not in general 'catch up' to ungrazed plants even after urchins had been removed for 2½ mo.

Does grazing by *Holopneustes purpureascens* result in increased levels of phlorotannins in *Ecklonia radiata*?

There was no significant difference in change in phlorotannin levels among treatments for either the autumn or spring experiment (Fig. 8A, B), indicating that grazing by *Holopneustes purpureascens* does not induce increased levels of phlorotannins in *Ecklonia radiata*. Moreover, if grazing does result in elevated levels of phlorotannins, then we would predict a negative relationship between changes in phlorotannins and changes in biomass for individual kelps (since changes in the biomass of *E. radiata* are negatively correlated with the damage done by the sea urchins). Regression analyses of changes in phlorotannin levels vs change in mass showed no such relationship for either spring or autumn ($r^2 < 0.02$, $p > 0.55$ for both analyses).

DISCUSSION

Holopneustes purpureascens: a 'mega' mesograzer

In recent years there has been an attempt to categorize marine herbivores by both taxon and ecological 'type', with a certain amount of concordance between the two. One 'type' includes larger herbivores such as herbivorous fishes, sea urchins, and large gastropods which are not restricted to individual host plants, are relatively large in comparison to most seaweeds, are typically generalist feeders (Hay et al. 1988, Hay 1992, Hay & Steinberg 1992), and are generally thought to have the greatest ecological and evolutionary effects on seaweeds (Carpenter 1986, Hay & Steinberg 1992).

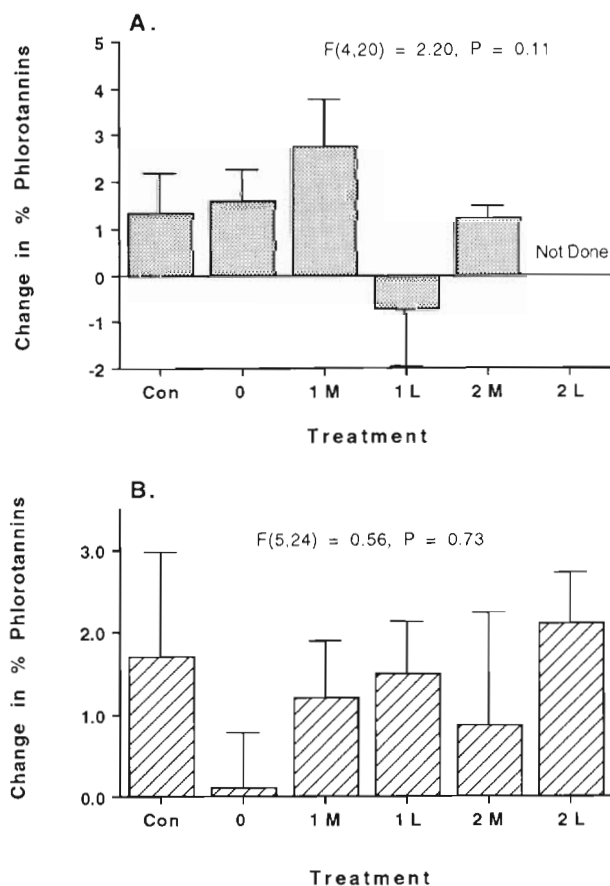


Fig. 8. *Ecklonia radiata*. Change in % phlorotannins by dry mass after 4 wk (calculated as phlorotannin levels after 4 wk - initial levels) for plants in various treatments in the (A) autumn and (B) spring *Holopneustes purpureascens* enclosure experiments. Data given as mean + SE, $n = 5$. Statistical results from single-factor ANOVA

In contrast to these larger grazers is the taxonomically diverse group of smaller herbivores termed 'mesograzers', which include arthropods, polychaetes, and smaller gastropods (Hay et al. 1987, Hay 1992, Brawley 1992). Mesograzers have smaller body sizes, use individual seaweed thalli as habitats, typically consume only part of a seaweed thallus rather than the whole plant, and are thought to have less ecological and evolutionary impact on seaweeds than large mobile herbivores (Hay & Steinberg 1992). In addition, marine herbivores with specialized diets are often mesograzers (Hay 1992, Hay & Steinberg 1992).

Holopneustes purpureescens represents a mixture of these 2 different categories of herbivores. The sea urchins are large herbivores (≥ 50 mm test diameter), comparable in size to many other bottom dwelling sea urchins, and can have a significant impact on macroalgae. However, they are relatively sedentary, apparently restricted to moving within and among algal thalli (I never saw an individual moving across the substratum), and like smaller, more typical mesograzers, use their seaweed hosts as food and habitat. The sea urchins have a limited range of host plants and, in this study, were only found on 2 species of algae, *Ecklonia radiata* and *Homoeostrichus sinclairii*. Observations of *H. purpureescens* at other sites near Sydney (pers. obs.) indicate that they can occur on other species of algae (usually other large phaeophytes), but such individuals are generally small (ca < 25 mm test diameter), as was the case for individuals found on *H. sinclairii* at Cape Banks. Given the animals' size as adults, they are probably ultimately restricted to using only the largest local seaweeds as host plants. These observations are consistent with the notion (Hay & Steinberg 1992) that the use of plants as habitat as well as for food facilitates restricted host plant range for marine herbivores.

The mesograzing strategy of using seaweeds as both food and habitat by *Holopneustes purpureescens* has resulted in a foraging behavior very different from that of most other herbivorous sea urchins. Most herbivorous sea urchins typically adopt either a sit and wait foraging strategy, in which they feed primarily on drift algae which washes by, or they actively move about the substratum, grazing on living algae or other organisms (Lawrence & Sammarco 1982, Harrold & Pearse 1987). Many species of sea urchins in fact exhibit both kinds of behavior, with temporal shifts between the 2 types of foraging mode resulting from a complex interaction of food availability, abundance of refuges, phylogenetic history, etc. (Harrold & Reed 1985, Andrew & Stocker 1986). Most herbivorous sea urchins are also very generalized feeders, unlike *H. purpureescens*.

A variety of other marine herbivores also share the mixture of mesograzing and large herbivore character-

istics exhibited by *Holopneustes purpureescens*. A good example are the so-called 'kelp limpets', which live obligately on the thalli of a number of species of Laminariales (kelps) throughout the world (Branch 1981). Like *H. purpureescens* these limpets can be relatively large, use individual plants as habitats, have restricted (often monospecific) host plant ranges, and can cause significant damage to their host plants (Black 1976). Some kelp limpets also have distinct shell shapes (Black 1976, Branch 1981) which facilitate the use of a kelp thallus as a habitat. The morphology of *H. purpureescens* should also facilitate the use of kelp as a habitat. The animals have very short spines, which presumably allows them to wrap laminae close to the test, minimizing the chance of being dislodged from the kelp.

Some mesograzers, particularly amphipods and other crustaceans, brood their offspring, rather than producing planktonic larvae. Such a life history may facilitate dietary/habitat specialization since producing short-lived or brooded larvae would increase the probability that juveniles recruit into the adult habitat (Hay & Steinberg 1992). Interestingly, *Holopneustes purpureescens*, unlike the majority of regular sea urchins (Emlet et al. 1987), produces a large floating egg (Mortensen 1915) which develops into a short-lived (2 to 4 d) lecithotrophic larva (J. Crystal pers. comm.). However, the generalist, bottom dwelling Australian sea urchin *Heliocidaris erythrogramma* also has a short-lived lecithotrophic larva (Mortensen 1915, Wray & Raff 1991); thus, any connection between life history and dietary or habitat specialization in these sea urchins is unlikely to be a simple one.

Effects of *Holopneustes purpureescens* on *Ecklonia radiata*

The extent to which mesograzers have significant effects on macroalgae — particularly non-epiphytes — is the subject of current debate (Bell 1991, Duffy & Hay 1991b, Brawley 1992, Poore 1994). One common view is that mesograzers generally have little direct effect on larger seaweeds, in contrast to large grazers such as sea urchins which can clear entire kelp forests. The effect of *Holopneustes purpureescens* on *Ecklonia radiata* is intermediate between these extremes. The sea urchins consume the kelp, removing significant amounts of biomass, and higher densities of large individuals can kill kelps. These effects vary predictably with season, size and density of the sea urchins, and length of time the sea urchins are on a kelp. However, unlike grazing by many other temperate sea urchins (reviews by Schiel & Foster 1986, Harrold & Pearse 1987), consumption of *E. radiata* by *H. purpureescens*

does not usually result in the death of the kelp. This non-lethal effect is due both to the rapid growth of *E. radiata* in spring and to the sea urchin's pattern of feeding on the kelp — *H. purpurescens* consumes secondary laminae preferentially to the more valuable (to the kelp) primary laminae or meristem. Johnson & Mann (1988) describe a similar pattern for consumption of kelp by herbivorous gastropods.

The lack of an effect of *Holopneustes purpurescens* on *Ecklonia radiata* in autumn has 2 likely explanations. Firstly, in autumn and early winter *E. radiata* loses biomass naturally at a rapid rate (Novaczek 1984a, b, Larkum 1986, this study), and the sea urchins may simply be feeding on tissue that is sloughing from the plant, which would be lost even in the absence of grazing. Alternatively, feeding rates of *H. purpurescens* may be significantly lower in autumn/winter than in spring due to lower water temperatures.

A surprising aspect of the interaction was that, although the magnitude and seasonal pattern of elongation by *Ecklonia radiata* observed in this study was comparable to previous research (Kirkman 1984, Novaczek 1984a, b, Larkum 1986), *Holopneustes purpurescens* had no apparent effect on elongation rates of *E. radiata*. Even though the sea urchins removed as much as 50% of the biomass from some thalli in spring, the kelp continued to elongate at a seasonally dependent rate, even if consumption ultimately resulted in death (as in the 2L treatment). Loss of substantial amounts of photosynthetic tissue did not result in slowed rates of elongation, indicating that growth (as measured by elongation) is not a simple function of the biomass present at any given time. The rapid growth of *E. radiata* in spring is probably due in part to stored reserves, as for other kelps (Chapman & Craigie 1977).

Compensatory growth in response to consumption is known for a number of terrestrial plants (McNaughton 1983), but did not occur in *Ecklonia radiata* in this study. This lack of a compensatory growth response, and the fact that even short bursts (e.g. 2 to 4 wk) of grazing by *Holopneustes purpurescens* can have significant and lasting effects on *E. radiata*, is important to their interaction because it suggests that even an intermittent regime of grazing by the sea urchins will still probably have an impact on the kelp.

Effects of phlorotannins on the interaction

The role of algal secondary metabolites as defenses against marine herbivores has received considerable recent attention (reviews by Hay & Fenical 1988, Hay & Steinberg 1992). In temperate brown algae, the major putative chemical defenses are phlorotannins (Ragan & Glombitza 1986). Although species of the

genus *Ecklonia*, and *E. radiata* in particular, typically contain high levels of phlorotannins (Steinberg 1989, Tugwell & Branch 1989, Steinberg & van Altena 1992), variation in phlorotannin levels in laminae of *E. radiata* was not correlated with variation in consumption rates by *Holopneustes purpurescens*. Tolerance to phlorotannins occurs in other large invertebrate herbivores in temperate Australasia (Steinberg & van Altena 1992), and mesograzers are also often resistant to a host's secondary metabolites (Hay et al. 1987, 1988, Hay 1992). In this respect the characteristics of mesograzers and large herbivores in Australia appear to have converged.

Induction of increased levels of phlorotannins following grazing or artificial damage has been shown for *Fucus* spp. from North America (Van Alstyne 1988, Yates & Peckol 1993). However, artificial clipping of laminae of *Ecklonia radiata* and *Sargassum vestitum* (Steinberg 1994) did not induce elevated levels of phlorotannins, and the results presented here confirm these earlier observations for a natural herbivore of *E. radiata* for a range of levels of damage (e.g. Fig. 8). This result may be explained by a lack of effect of phlorotannins on *Holopneustes purpurescens*. However, other herbivores feed on *E. radiata* (Schiel 1982, Andrew & Jones 1990) and induction of phlorotannins could potentially be beneficial as a general response to damage if other herbivores or natural enemies (e.g. pathogens) are deterred by *E. radiata* phlorotannins. In the only other study to date on induction of phlorotannins in kelps (Order Laminariales), Pfister (1992) found no evidence of induction in *Alaria nana*.

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