

K. L. Van Alstyne · S. L. Whitman · J. M. Ehlig

Differences in herbivore preferences, phlorotannin production, and nutritional quality between juvenile and adult tissues from marine brown algae

Received: 10 April 2000 / Accepted: 19 November 2000

Abstract Juvenile and adult marine organisms differ in their morphology, chemistry, physiology, behavior, and ecology. Because juvenile algae are thinner, smaller, and have more delicate tissues than adults, they are often assumed to be more susceptible to grazers. We examined within-species food preferences of four common generalist herbivores for juvenile and adult tissues of eight common brown algae in two-choice laboratory food-preference experiments. Our results showed that juvenile algae did not tend to be a preferred food of herbivores. Juvenile tissues were significantly preferred over adult tissues in only four of the 32 combinations of algae and herbivores tested. In 12 experiments, adult tissues were preferred over juvenile tissues, and no choice occurred in the remaining 16 experiments. When sea urchins exhibited a preference, it was always for adult tissues. The other three herbivores, an isopod and two snails, were more variable in their choices, sometimes preferring juveniles, sometimes adults, and sometimes having no preference. We measured nitrogen and phlorotannin concentrations in adult and juvenile seaweeds to see whether these parameters were correlated with herbivore food preferences. Nitrogen levels were similar in juveniles and adults of three algal species and were higher in juveniles of two. Phlorotannin levels were higher in juveniles of four species and lower in juveniles of one. The other three species showed no differences in phlorotannin levels. Phlorotannin concentrations decreased with increasing juvenile size in three species and

increased with increasing size in one species. Neither nitrogen nor phlorotannin concentrations explained overall herbivore food preferences for algae of different stages. Our results suggest that preferences of certain grazers for juvenile algae are not as strong as previously assumed and are dependent on herbivore species. Preferences between juveniles and adults are likely to be determined by a combination of morphological and chemical features of the tissues and the unique responses of herbivore species to those features.

Introduction

Survival to a reproductive size in algae is often dependent upon juvenile traits (see reviews by Hawkins and Hartnoll 1983; Chapman 1986; Santelices 1990; Vadas et al. 1992). Most marine macroalgae release large numbers of spores, but only a few are likely to survive and become reproductive adults (Chapman 1984; DeWreede and Klinger 1988). In intertidal algae, high rates of mortality occur in early post-settlement stages. Factors contributing to mortality include intrinsic factors involved with fertilization, germination, attachment, and growth and extrinsic factors, such as competition, desiccation, scouring, and grazing (see review by Vadas et al. 1992). In many systems, herbivory is thought to be the major factor limiting survival through juvenile stages (e.g., Hawkins 1981, 1983; Lubchenco 1983; Harris et al. 1984; Keser and Larson 1984; Gunnill 1986; Dean et al. 1988; Chapman 1990).

Juvenile marine organisms are physically and chemically different from adults (e.g., Bernard 1967; Paul and Van Alstyne 1988; Keesing and Halford 1992; Gosselin and Qian 1997). Consequently, the ecology of juveniles, particularly their interactions with other organisms, may change as they age. For example, among-species food preferences of herbivores for rockweeds and kelps have been shown to differ between juveniles and adults (Barker and Chapman 1990; Van Alstyne et al. 1999a); species that are high-preference foods during juvenile

Communicated by: M. Horn, Fullerton/O. Kinne, Oldendorf/Luhe

K. L. Van Alstyne (✉) · S. L. Whitman · J. M. Ehlig
Department of Zoology, Oregon State University,
Corvallis, OR 97331, USA

K. L. Van Alstyne,
Shannon Point Marine Center, 1900 Shannon Point Road,
Anacortes, WA 98221, USA

e-mail: kathyva@cc.wvu.edu,
Fax: +1-360-2931083

stages are not necessarily high-preference foods during adult stages. Likewise, herbivores have distinct preferences among life-history stages of algae; in laboratory feeding experiments, adult *Ulva lactuca* were preferred to juvenile *U. lactuca* by *Littorina littorea*, but juvenile *Fucus serratus* were preferred to adults (Watson and Norton 1985).

Only a few studies have examined how macroalgae change as they grow with respect to physical and chemical traits that affect resistance to herbivory. Juveniles are smaller, thinner, and have more delicate tissues than adults. Juveniles of the siphonous green alga *Halimeda macroloba* are significantly less calcified and contain higher concentrations of the diterpenoid feeding deterrent halimedatriol than adult tissues (Paul and Van Alstyne 1988). Lipid-soluble extracts from juveniles are more of a deterrent to herbivorous fishes than extracts from adults (Paul and Van Alstyne 1988). In the rock-weeds, *Fucus vesiculosus* and *F. evanesens*, concentrations of phlorotannins are lower in juveniles than adults, but phlorotannin levels in juvenile and adult *F. spiralis* are similar (Denton et al. 1990).

The goal of this study was to examine food preferences of several grazers between juvenile and adult brown-algal tissues. We expected that the morphological differences between juvenile and adult algae would result in juveniles being a preferred food. We also expected that there might be differences in food choice among grazers (purple sea urchins, *Strongylocentrotus purpuratus*, black turban snails, *Tegula funebris*, isopods, *Idotea wosnesenskii*, and snails, *Lacuna porrecta*) because of differences in their sizes and feeding apparatuses. Urchins are the largest of the four herbivores examined in this study and thus are the least likely to be deterred from feeding on plants by physical characteristics of the tissue (algae will be referred to as plants here even though, technically, they are protists). Consequently, their choices may be more influenced by the chemical composition of the foods. The two smaller, less mobile grazers, *I. wosnesenskii* and *L. porrecta*, are mesoherbivores (*sensu* Brawley and Fei, 1987). Mesoherbivores are thought to have evolved different preferences for algae than macroherbivores because their food also serves as a habitat (Duffy and Hay 1994). They have been hypothesized to be unaffected by host plant defenses whereas larger grazers are expected to be more susceptible to the effects of these compounds (Hay et al. 1989a, b, 1990a, b). Thus, the relative importance of structural and chemical characteristics of foods may be different for mesoherbivores and generalist macroherbivores, such as urchins and turban snails.

We also examined differences in the nutritional quality, as measured by nitrogen levels, and phlorotannin concentrations of juvenile and adult tissues, to see whether these correlated with food preferences. Herbivores have been suggested to be nitrogen-limited (Mattson 1980) and might feed preferentially on foods with higher nitrogen concentrations. Phlorotannins are multifunction compounds (Ragan and Glombitza 1986;

Targett and Arnold 1998) that have concentration-dependent antifeeding activity towards many herbivores (Geiselman and McConnell 1981; Steinberg 1985, 1988; Steinberg and van Altena 1992; Winter and Estes 1992; Steinberg et al. 1995; Pavia and Toth 2000), though not all of them. Isolated phlorotannins are deterrent to two of the herbivore species used in our feeding experiments, *S. purpuratus* and *T. funebris* (Steinberg et al. 1995); they have not been tested as deterrents to the other two species (*I. wosnesenskii* and *L. porrecta*).

Materials and methods

Collection sites and algal life histories

The four herbivorous invertebrates used in the feeding experiments were collected from Boiler Bay, Oregon, USA, ~5 km north of the town of Depoe Bay, Oregon, and from the Boiler Bay State Park, ~3 km north of Depoe Bay, Oregon. Sea urchins, *Strongylocentrotus purpuratus*, were collected from low- to mid-intertidal pools at Boiler Bay. These pools were near many of the algae used in this study, including *Alaria marginata*, *Costaria costata*, *Egregia menziesii*, *Hedophyllum sessile*, and *Nereocystis luetkeana*. The snails *Lacuna porrecta* and *Tegula funebris* were collected from sandstone benches in the mid-intertidal zone of the south bench at Boiler Bay. Brown algae found on those benches and included in this study were *E. menziesii*, *Fucus gardneri*, *F. spiralis*, and *H. sessile*. Isopods (*Idotea wosnesenskii*) were collected from a mid-intertidal boulder field at the north end of Boiler Bay. *I. wosnesenskii* is often found living on intertidal and high subtidal algae such as *F. gardneri* and *A. marginata*. All animals were maintained in flow-through seawater tables at the Hatfield Marine Science Center (HMSC) in Newport, Oregon. They were periodically fed a mixture of macrophytes that included species used in the feeding experiments.

The eight species of brown algae used in these experiments were collected from Boiler Bay, Oregon. *F. gardneri* (juveniles: ~2–3 cm long, adults: > 15 cm long) and *F. spiralis* (juveniles: ~2–3 cm long, adults: > 15 cm long) were collected from boulders or rock benches in the high-intertidal to mid-intertidal zone of the protected end of the main (northern) bench in Boiler Bay. *A. marginata* (juveniles: ~6–10 cm long, adults: > 0.5 m long) and *N. luetkeana* (juveniles: ~10–15 cm long, adults: > 5 m long) were collected from low-intertidal pools at the protected end of the most exposed third of the main bench. *Lessoniopsis littoralis* (juveniles: ~4–6 cm long, adults: > 25 cm long) was collected from the low-intertidal zone at the most exposed point of the main bench. *E. menziesii* (juveniles: ~5–10 cm long, adults: > 0.5 m long), *H. sessile* (juveniles: ~4–6 cm long, adults: > 30 cm long), and *C. costata* (juveniles: ~4–7 cm long, adults: > 25 cm long) were collected from the low-intertidal zone in a moderately exposed area at the south set of benches in Boiler Bay.

The six species of kelp (*A. marginata*, *N. luetkeana*, *L. littoralis*, *H. sessile*, *E. menziesii*, and *C. costata*) and two species of rockweed (*F. gardneri* and *F. spiralis*) examined in this study are common on exposed Washington and Oregon, USA, coasts. Kelps, which dominate the flora of many temperate benthic habitats along the coasts of Washington and Oregon, have a complex life history in which a macroscopic diploid sporophyte stage alternates with a microscopic haploid gametophyte stage (Bold and Wynne 1985). Eggs of gametophytes are fertilized and remain attached to the oogonial wall where they develop into the sporophyte stage. Because adult sporophytes grow in the same location as female gametophytes, the ability of gametophytes to survive and reproduce will affect the location and development of kelp beds. Little is known, however, about the ecology of the microscopic gametophyte stage, partly because this stage has rarely been found in

nature. Similarly, little is known about the ecology of juvenile sporophytes, a stage that also is likely to be susceptible to the effects of grazers and physical stresses.

Fucoid brown algae or rockweeds have a simpler haplobiontic life history (Bold and Wynne 1985). Species can be either monoecious or dioecious. They release eggs and motile sperm into seawater where fertilization occurs. In some species, gamete release is triggered by a combination of dissolved inorganic-carbon depletion during periods of low water movement and daylight (Pearson et al. 1998). Fertilization occurs within a few hours (Pearson et al. 1998) and propagules settle within a short distance of adults (Johnson and Brawley 1998). Zygotes develop directly into diploid adults.

Feeding-preference assays

Algae were collected from Boiler Bay, Oregon, and were transported on ice to HMSC where they were used in feeding-preference experiments. Pairwise feeding-preference assays were used to determine whether herbivores significantly preferred juveniles to adults of the same algal species. In each experiment, consumers were offered pairs of similar-sized pieces of juvenile and adult algae taken from non-reproductive, non-meristematic areas of the center of the blades. Even though the pieces were selected from similar areas of juvenile and adult algae, the differences in sizes between juveniles and adults made it impossible to entirely avoid having some confounding effects due to intra-plant variation in the physical and chemical properties of the tissues. The sizes of the pieces depended on the herbivore being used and the amount of juvenile algal tissue available at the time the experiment was run. The pieces offered to *S. purpuratus* had average masses ranging from 0.1 g (*A. marginata*) to 0.5 g (*E. menziesii*). The pieces offered to *T. funebris* had average masses ranging from 0.04 g (*A. marginata*) to 0.12 g (*H. sessile*). Average masses of pieces offered to *I. wosnesenskii* ranged from 0.07 g (*A. marginata*) to 0.20 g (*F. gardneri*), and to *L. porrecta*, ranged from 0.04 g (*L. littoralis*) to 0.07 g (*F. gardneri*).

Experiments with all herbivores except *L. porrecta* were conducted in 5-cm-diameter plastic Rubbermaid containers (*T. funebris* and *I. wosnesenskii*) or similar 15-cm-diameter containers (*S. purpuratus*) which had been modified by removing pieces from the bottoms and lids and replacing them with 1-mm-mesh fiberglass screening to allow water flow through the containers. Experiments with *L. porrecta* were conducted in 40-mm-diameter plastic Petri dishes which had been modified by cutting 25-mm-diameter holes in both parts of the dish and covering them with 1-mm-mesh fiberglass screening. All experiments were conducted in outdoor flow-through seawater tables at HMSC. Animals were starved for 2–4 days prior to the feeding-preference experiments in order to ensure they would consume a measurable amount of algae before it grew or deteriorated.

Ten replicate experimental containers (arenas) for each experiment held a herbivore and one piece each of juvenile and adult algae of the same species. An equal number of control arenas contained only algae and were used to assess autogenic changes in the algae that occurred during the experiments. Algal pieces were blotted dry and weighed before the start of the experiments. Containers were checked periodically, and herbivores were removed from the containers when it appeared that at least half of all the algae in the experimental containers had been consumed, usually after herbivores had fed for 1–2 days. At the conclusion of the experiment, control and experimental algae were blotted dry and weighed to determine mass changes during the experiments.

Data were analyzed according to Peterson and Renaud (1989). For each algal piece, the final mass was subtracted from the initial mass to determine the change in mass. The change in mass from one piece of tissue was then subtracted from the change in mass from the other piece in the arena to give a single number for each arena. The values for control arenas were then compared to the values from the experimental arenas for each combination of algal species and herbivore with a Student's *t*-test ($\alpha = 0.05$). Because herbivores often consumed tissues more rapidly than expected and because our visual assessments of the amounts of material con-

sumed could be inaccurate, experiments were not always ended when half the algae were consumed. If only small amounts of material were consumed, differences in consumption rates may reflect which tissue was encountered first rather than a food preference. If large amounts were consumed, strengths of preferences may be underestimated because grazers have one food left to choose from. For these reasons, we did not include in the analyses replicates in which grazers consumed less than 25% or greater than 75% of all the algae present in the containers.

Measurement of tissue characteristics

To see whether plant characteristics at different developmental stages were correlated with herbivore food preferences, we measured the nitrogen content, carbon to nitrogen ratios, and the concentration of phlorotannin in juvenile and adult tissues from most of the kelps and rockweeds used in the feeding-preference assays. For measurements of carbon and nitrogen concentrations, tissues of juvenile and adult algae were collected from Boiler Bay. Pieces of tissue were excised from non-reproductive, non-meristematic areas in the center of the blades and dried in an oven for 48 h at 60 °C. The dried tissues were ground by hand to a fine powder with a mortar and pestle and carbon and nitrogen measurements were made with a Control Equipment CEC 440 Analyzer.

To measure phlorotannin concentrations, juvenile and adult tissues were collected from Boiler Bay, Oregon, during a single low tide. Pieces of tissues were excised from the non-reproductive, non-meristematic blade portions of the plants, frozen to –4 °C, and transported on dry ice to the Shannon Point Marine Center in Anacortes, Washington. The length of each individual was measured, then the algae were frozen in liquid nitrogen and lyophilized. The dried tissues were ground by hand with a mortar and pestle to a fine powder then extracted in 80% aqueous methanol. Phlorotannin concentrations were measured using the methods described in Van Alstyne (1995). Phloroglucinol-dihydrate was used as a standard. Angularly transformed phlorotannin concentrations in juveniles and adults were compared with a Student's *t*-test ($\alpha = 0.05$). A simple linear regression was used to see whether there was a linear relationship between the log of plant length and the angularly transformed phlorotannin concentrations in juveniles for each of the eight species.

Results

Feeding preferences of herbivores

Relative food preferences between juvenile and adult kelps differed among species and among herbivores. *Strongylocentrotus purpuratus* were the most likely to exhibit significant preferences ($P < 0.05$) for adult tissues over juvenile tissues, and in some cases the preference was a very strong one (Fig. 1). For example, urchins consumed nearly 14 times more adult than juvenile tissue of *Egregia menziesii*.

Tegula funebris were less likely than urchins to show preferences for one tissue type over another, but when significant preferences existed ($P < 0.05$), they were also for adult tissues, with the exception of a preference for juvenile *Fucus spiralis* (Fig. 2).

Idotea wosnesenskii and *Lacuna porrecta* occasionally exhibited preferences between adult and juvenile algal tissues, but the patterns of preferences were different from the patterns for *S. purpuratus* and *T. funebris*.

Fig. 1 Mean decreases in mass ($\text{mg} \pm 1 \text{ SE}$) of juvenile and adult brown algae in pairwise feeding-preference experiments with the purple urchin *Strongylocentrotus purpuratus*. *Dark bars* represent mean mass changes of adult algae in experimental arenas with urchins. *Open bars* represent mean mass changes of juvenile algae in experimental arenas with urchins. *Shaded bars* represent mean mass changes of adult algae in control arenas used to assess autogenic changes in algal masses. *Hatched bars* represent mean mass changes of juvenile algae in control arenas. *P* values from *t*-tests comparing juvenile and adult tissues are given below each set of bars. $n = 6-10$

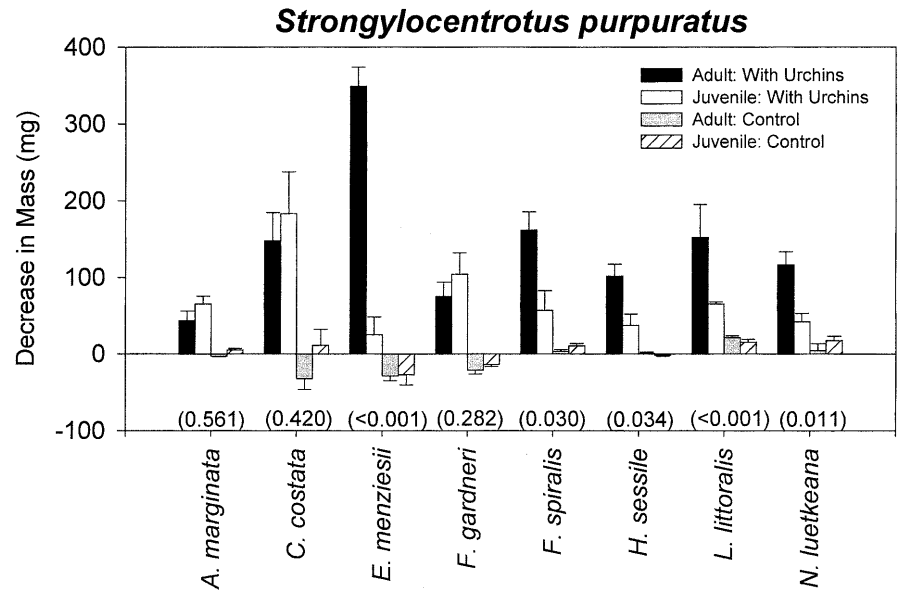
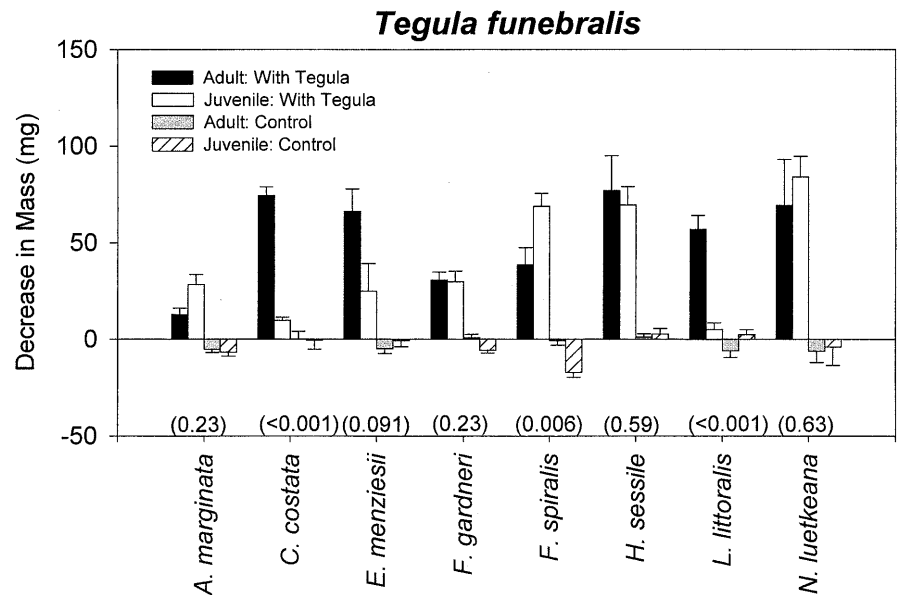


Fig. 2 Mean decreases in mass ($\text{mg} \pm 1 \text{ SE}$) of juvenile and adult brown algae in pairwise feeding-preference experiments with the turban snail *Tegula funebris*. Bars are as described in Fig. 1. *P* values from *t*-tests comparing juvenile and adult tissues are given below each set of bars. $n = 5-10$



I. wosnesenskii had significant preferences ($P < 0.05$) for juvenile tissues of *H. sessile* and *F. spiralis* (Fig. 3); however, they also exhibited significant preferences ($P < 0.05$) for adult *Nereocystis luetkeana* and *Lessoniopsis littoralis* tissues. *L. porrecta* significantly preferred ($P < 0.05$) adult *F. gardneri*, *L. littoralis*, and *N. luetkeana*, but juvenile *F. spiralis* (Fig. 4).

Preferences of herbivores were rarely consistent for a given alga species (Table 1). All herbivore species exhibited no preferences between juvenile and adult *Alaria marginata*, but significantly preferred adult *L. littoralis*. All of the smaller grazers (*T. funebris*, *I. wosnesenskii*, and *L. porrecta*) preferred juvenile *F. spiralis*, but the larger urchin grazers preferred adult *F. spiralis*. Adult *N. luetkeana* were preferred by *S. purpuratus*, *I. wosnesenskii*, and *L. porrecta*, but *T. funebris* showed no preferences between juvenile and adult *N. luetkeana*.

Chemical characteristics of tissues

Tissue-nitrogen concentrations differed between juvenile and adult tissues in only two out of the five species in which they were measured. In *A. marginata* and *H. sessile*, juveniles had higher nitrogen levels (Fig. 5). However, in the other three species, *F. gardneri*, *L. littoralis*, and *N. luetkeana*, tissue-nitrogen concentrations did not differ significantly between juveniles and adults ($P > 0.05$, Student's *t*-test). Similarly, carbon to nitrogen ratios differed only in the two species that exhibited stage-specific differences in nitrogen concentrations. Carbon to nitrogen ratios were 33% lower in juvenile *A. marginata* than in adults and 17% lower in juvenile *H. sessile* (Fig. 5). Carbon to nitrogen ratios in *F. gardneri*, *L. littoralis*, and *N. luetkeana* did not differ significantly between juveniles and adults ($P > 0.05$).

Fig. 3 Mean decreases in mass ($\text{mg} \pm 1 \text{ SE}$) of juvenile and adult brown algae in pairwise feeding-preference experiments with the isopod *Idotea wosnesenskii*. Bars are as described in Fig. 1. *P* values from *t*-tests comparing juvenile and adult tissues are given below each set of bars. $n = 6-9$

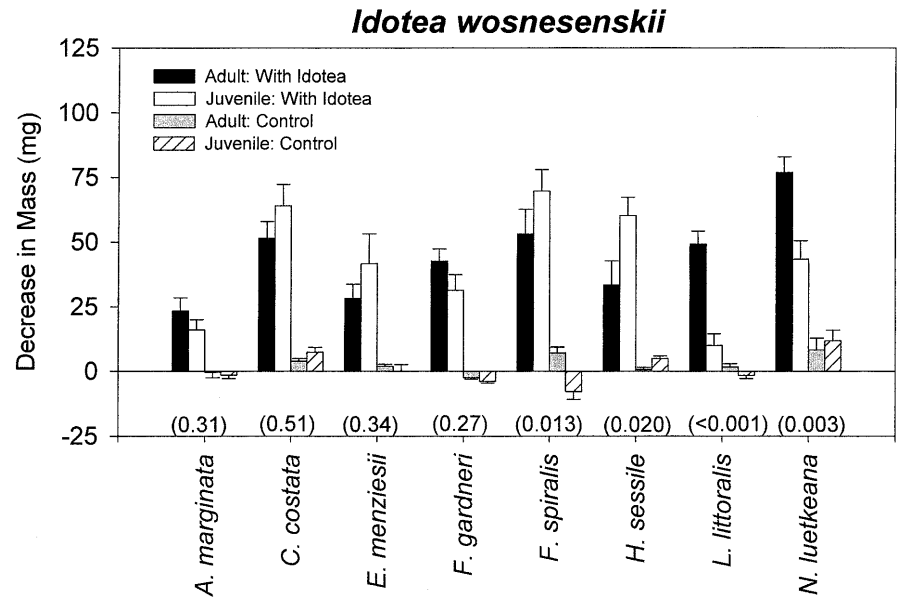
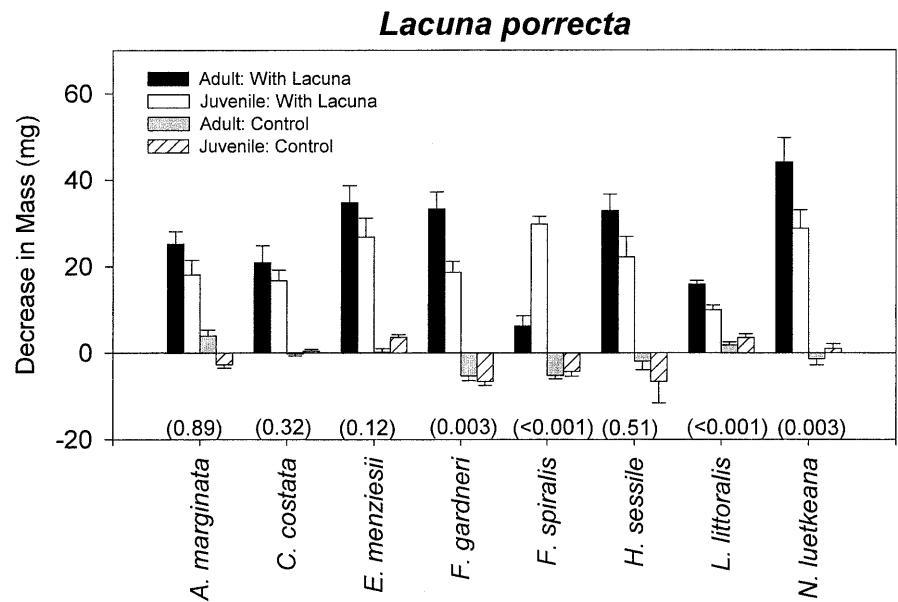


Fig. 4 Mean decreases in mass ($\text{mg} \pm 1 \text{ SE}$) of juvenile and adult brown algae in pairwise feeding-preference experiments with the snail *Lacuna porrecta*. Bars are as described in Fig. 1. *P* values from *t*-tests comparing juvenile and adult tissues are given below each set of bars. $n = 7-10$



When phlorotannins differed significantly between juveniles and adults, levels were usually higher in juveniles (Fig. 5). Phlorotannin concentrations were higher in juvenile *E. menziesii*, *F. gardneri*, *H. sessile*, and *L. littoralis*, relative to adult concentrations. In *N. luetkeana*, phlorotannin concentrations were higher in adults than in juveniles. In juveniles, phlorotannin concentrations significantly decreased with plant size in *A. marginata*, *F. gardneri*, and *H. sessile*, but significantly increased with plant length in *N. luetkeana* (Fig. 6).

Discussion

Contrary to our expectations, grazers did not uniformly prefer juvenile to adult tissues. Urchins were most likely

to prefer adult tissues (Table 1), although no preference between juvenile and adult tissues was exhibited for three of eight algal species. The three smaller herbivores were less likely than urchins to exhibit a preference between juveniles and adults and, when a preference did exist, it was not always for adult tissues. Both *Tegula funebris* and *Lacuna porrecta* preferred juvenile tissues in three of the eight trials and adult tissues in one of eight trials. *Idotea wosnesenskii* preferred juvenile tissues in two of eight trials and adult tissues in two of eight trials. These results suggest that juvenile plants may be more susceptible to attack by smaller grazers when choices between juvenile and adult plants are available. They also point out that the outcome of these interactions is highly dependent on the algal species and herbivore species involved.

It should be noted that all of these experiments involved small pieces of tissues taken from juvenile and adult plants. Although algae are not highly differentiated, particularly at juvenile stages, there are chemical and morphological differences between tissues within individual kelps and rockweeds (Steinberg 1984; Johnson and Mann 1986; Tugwell and Branch 1989; Tuomi et al. 1989; Van Alstyne et al. 1999b, in press). We attempted to standardize our assays by selecting non-reproductive, non-meristematic tissues from the center of the blades. Nonetheless, intra-plant differences in the chemical and morphological characteristics of tissues could have affected the outcome of these experiments. The hunger level of the herbivores could also have affected the experiments. In order to get measurable rates of consumption before the algal tissues deteriorated, we had to starve the herbivores before the beginning of the experiments. Cronin and Hay (1996) have shown that starved animals can be less sensitive to the presence of secondary metabolites in foods. If these patterns hold for the species used in these experiments with pieces of algal tissue, then the grazers may have been less likely to show a preference when one existed.

Because animals use proteins as a structural component, whereas plants use carbohydrates, animals typically contain much higher quantities of nitrogen than plants do (Mattson 1980). Consequently, herbivore diets are thought to be nitrogen-limited. One method herbivores can use to increase their nitrogen uptake is to preferentially consume plants or plant tissues with higher nitrogen concentrations. If tissue-nitrogen concentration were an important determinant of food choice in the herbivores we examined in our study, then the tissues that were preferentially consumed should have contained significantly higher nitrogen concentrations.

Our data, however, suggest that nitrogen concentration was not a factor determining food choice between juvenile and adult algal tissues. Tissue-nitrogen con-

centrations were higher in the juvenile tissues of two species, *Alaria marginata* and *Hedophyllum sessile*, but were not significantly different from adult tissue-nitrogen concentrations in three others, *Costaria costata*, *Fucus gardneri*, and *Lessoniopsis littoralis*. If herbivores preferentially consume high-nitrogen tissues, then juvenile *A. marginata* and *H. sessile* should have been preferred over adult tissues. However, herbivores exhibited no preferences between juvenile and adult *A. marginata* tissues, and juvenile tissues of *H. sessile* were only preferred by one herbivore. This suggests that tissue-nitrogen concentration did not play a role in food selection by these herbivores between juvenile and adult algal tissues.

Phlorotannins are another chemical component of brown algae that have been suggested to play a role in determining herbivore food preferences (Geiselman and McConnell 1981; Steinberg 1985, 1988; Steinberg and van Altena 1992; Winter and Estes 1992; Steinberg et al.

Table 1 Summary of preferences of four herbivores (the four right-hand columns) for juvenile and adult kelp and rockweed tissues (left-hand column). A = significant ($P < 0.05$) preference for adults, J = significant ($P < 0.05$) preference for juveniles, — = no significant preference

| | <i>Strongylo-</i> <i>centrotus</i> <i>purpuratus</i> | <i>Tegula</i> <i>funnebralis</i> | <i>Idotea</i> <i>wosnesenskii</i> | <i>Lacuna</i> <i>porrecta</i> |
|--|--|-------------------------------------|--------------------------------------|----------------------------------|
| <i>Alaria marginata</i> | — | — | — | — |
| <i>Costaria costata</i> | — | A | — | — |
| <i>Egredia menziesii</i> | A | A* | — | — |
| <i>Fucus gardneri</i> | — | — | — | A |
| <i>Fucus spiralis</i> | A | J | J | J |
| <i>Hedophyllum</i> <i>sessile</i> | A | — | J | — |
| <i>Lessoniopsis</i> <i>littoralis</i> | A | A | A | A |
| <i>Nereocystis</i> <i>luetkeana</i> | A | — | A | A |

* $0.05 < P < 0.10$

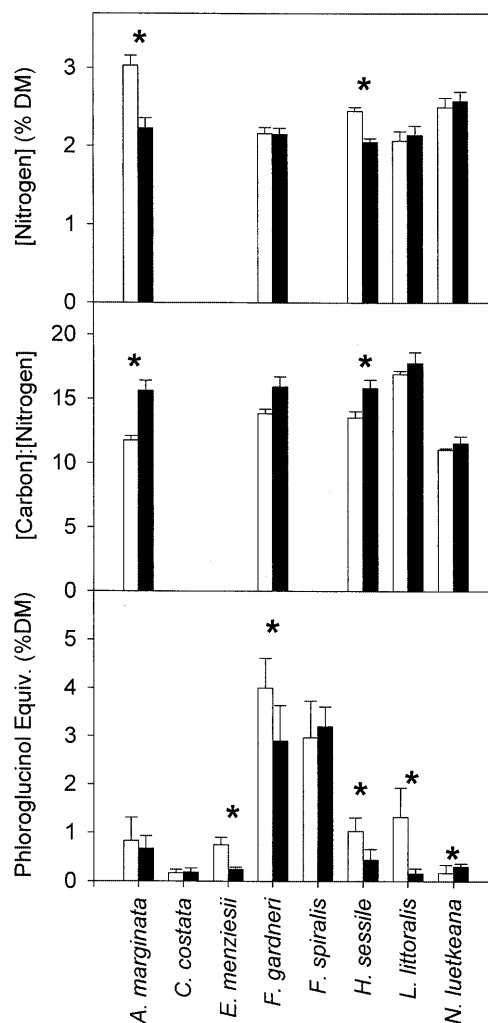
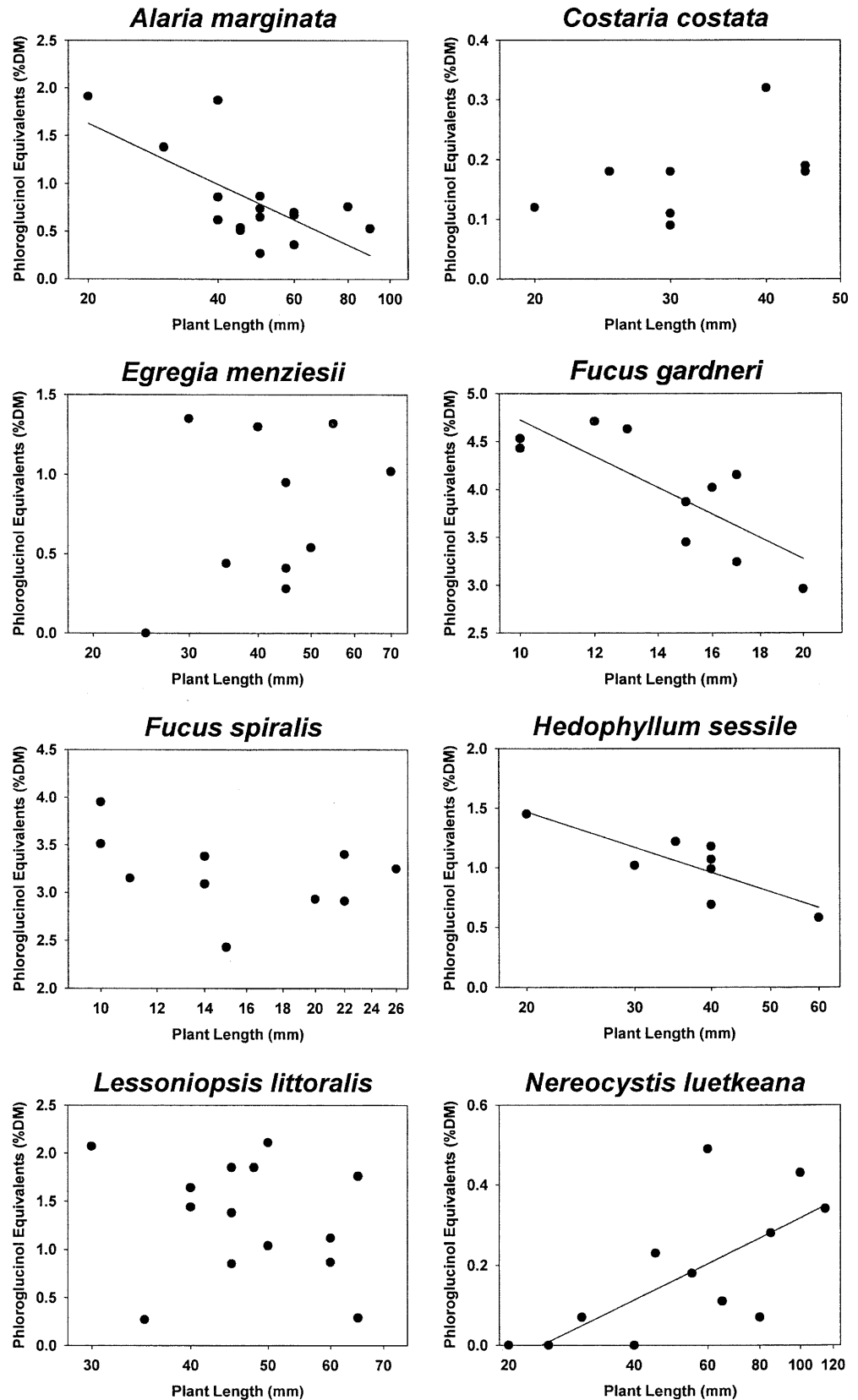


Fig. 5 Mean (± 1 SD) nitrogen concentrations (as % DM), carbon to nitrogen ratios, and phlorotannin concentrations (as % DM). Dark bars represent adult algal tissues. Open bars represent juvenile algal tissues. Asterisks indicate measurements in which juvenile values differ significantly from adult values (Student's *t*-test, $P < 0.05$)

Fig. 6 Phlorotannin concentrations as a function of plant length in juvenile kelps and rockweeds. Lines represent best-fit linear regressions of phlorotannin concentrations on the log of plant length. *Alaria marginata* ($R^2 = 45.8\%$, $P = 0.004$), *Fucus gardneri* ($R^2 = 62.9\%$, $P = 0.006$), *Hedophyllum sessile* ($R^2 = 65.7\%$, $P = 0.015$), *Nereocystis luetkeana* ($R^2 = 48.5\%$, $P = 0.008$). No line indicates there was not a significant relationship between length and phlorotannin concentration (linear regression: $P > 0.05$)



1995; Pavia and Toth 2000). Brown algae that are high-preference foods for *Tegula funebris* generally had lower phlorotannin concentrations (0.83% dry mass,

DM, on average) than low-preference species (4.53% DM on average; Steinberg 1985). Brown-algae phlorotannins incorporated in agar-based diets have been

shown to deter feeding at concentrations as low as 1% DM (Geiselman and McConnell 1981).

The phlorotannin concentrations of adult tissues examined in this study were generally low. Except for *Fucus gardneri* and *F. spiralis*, mean phlorotannin levels of adult algal tissues were less than 1% DM. These values contrast with measurements taken from the same species at the same site in May 1993 (Van Alstyne et al. 1999c). In 1993, concentrations of phlorotannins in *A. marginata*, *C. costata*, *Egrecia menziesii*, and *H. sessile* were 80–600% higher than in this study in 1998, and 20–60% higher in *F. gardneri*, *F. spiralis* and *H. sessile*. These differences reflect year-to-year variation in phlorotannin levels that likely result from changes in environmental conditions (e.g., sea temperatures, light levels, or nutrient concentrations) that occur across the entire site and that lower the phlorotannin levels of many species of algae simultaneously.

Differences in phlorotannin concentrations between juvenile and adult brown-algal tissues did not have obvious effects on herbivore food preferences. Phlorotannin concentrations of juveniles were 213% higher in *E. menziesii*, 38% higher in *F. gardneri*, 134% higher in *H. sessile*, and 725% higher in *L. littoralis*, but 43% lower in juvenile *Nereocystis luetkeana*, relative to adult concentrations. Phlorotannin concentrations did not differ significantly between juvenile and adult tissues of *A. marginata*, *C. costata*, or *F. spiralis*. Herbivore food preferences for juvenile versus adult algal tissues were not correlated with differences in phlorotannin concentrations for any of the four herbivores. Only in *L. littoralis*, the alga with the highest relative differences in phlorotannin concentrations between juveniles and adults, were preferences of all four herbivores in accordance with the preferences that would be expected if phlorotannin concentrations were determining herbivore food preferences. Thus, it appears that factors other than or in addition to phlorotannins were affecting food choice.

Changes in phlorotannin concentrations that occurred in some of the juvenile algae we examined are consistent with patterns predicted by resource-allocation models. Because phlorotannins presumably incur a metabolic cost to the alga (Steinberg 1995; Pavia et al. 1999), there should be a trade-off between phlorotannin production and growth. If phlorotannins serve a defensive function, they should be preferentially allocated to tissues most at risk from attack (Rhoades 1979). Otherwise, the algae should preferentially allocate their resources to growth. Smaller algae are generally considered to be more at risk from herbivores than larger individuals because larger plants are thought to “escape in size” upon reaching a certain size threshold. Laboratory feeding experiments have shown that littorinid snails preferred 1- to 3-cm juvenile *Fucus serratus* to individuals that were 5–7 cm long (Lubchenco 1983). In field experiments, the major cause of mortality in *Lessonia nigrescens* gametophytes < 15 μ m long was herbivory; in larger sporophytes, wave action was the

primary source of mortality (Martinez and Santelices 1998). There are two strategies that might be used by algae to survive these vulnerable stages: (1) allocate large amounts of resources to growth and few resources to defense to try to reach a large size as quickly as possible; or (2) allocate more resources to defense in order to minimize damage from grazers at a cost of growing more slowly.

It appears that some of the algae that we studied, such as *C. costata*, *E. menziesii*, and *N. luetkeana*, may be using the first strategy and producing very low concentrations of phlorotannins during juvenile stages. Others, such as *A. marginata*, *F. gardneri*, and *H. sessile*, may be using the second strategy. In these algae, phlorotannin concentrations are highest in the smaller juveniles and decrease as the algae elongate, at a time when they are presumably less vulnerable to mortality caused by herbivory (Lubchenco 1983). Interestingly, at very small juvenile stages, less than 0.5 mm in length, *F. gardneri* produces very low concentrations (< 0.5% DM) of phlorotannins when grown in laboratory cultures (Van Alstyne and Pelletreau 2000). This suggests that these plants allocate very few resources to phlorotannins during the earliest stages of growth, a size at which being sampled by an herbivore would be fatal.

In summary, we found that juvenile tissues of many kelps and rockweeds were not always a preferred food of grazers. Our data indicate that herbivore food choices between juvenile and adult tissues are likely to be determined by a complex combination of chemical and morphological features. Furthermore, the features that determine food choice may differ widely among herbivore species. Tissue nitrogen, which is frequently considered an important determinant of food preference in herbivores, did not appear to determine food choice between juvenile and adult tissues for the grazers we examined. Phlorotannin levels were higher in the juvenile tissues of several species and might account for some, but certainly not all, of the food preferences. The results of this study provide additional evidence that juvenile marine organisms can be ecologically and biochemically different from adults and reinforce Gosselin's (1997) contention that we not regard juveniles as simply “miniature adults”.

Acknowledgements We are grateful to J. Lubchenco and B. Menge for hosting K.L.V. during her stay at Oregon State University and providing access to the Boiler Bay field site. J. Stecher assisted with the carbon and nitrogen measurements and K. Pelletreau helped with the phlorotannin measurements. D. Padilla identified *Lacuna porrecta*. L. Weber of the Hatfield Marine Science Center graciously allowed us to use his laboratory and HMSC facilities. M. Horn and several anonymous reviewers made many helpful comments on the manuscript. This work was funded by an NSF grant, IBN 98–96012 to K.L.V.

References

- Barker KM, Chapman ARO (1990) Feeding preferences of periwinkles among four species of *Fucus*. *Mar Biol* 106: 113–118

- Bernard FR (1967) Studies on the biology of the naticid clam drill *Polinices lewisi* (Gould) [Gastropoda Prosobranchiata]. Fish Res Bd Can Tech Rep No. 42
- Bold HC, Wynne MJ (1985) Introduction to the algae. Prentice-Hall, Englewood Cliffs
- Brawley SH, Fei XG (1987) Studies of mesoherbivory in aquaria and in an unbarricaded mariculture farm on the Chinese coast. *J Phycol* 23: 614–623
- Chapman ARO (1984) Reproduction, recruitment and mortality in two species of *Laminaria* in southwest Nova Scotia. *J Exp Mar Biol Ecol* 78: 99–109
- Chapman ARO (1986) Population and community ecology of seaweeds. *Adv Mar Biol* 23: 1–161
- Chapman ARO (1990) Effects of grazing, canopy cover and substratum type on the abundances of common species of seaweeds inhabiting littoral fringe tidepools. *Bot Mar* 33: 319–326
- Cronin G, Hay ME (1996) Susceptibility to herbivores depends on recent history of both the plant and animal. *Ecology* 77: 1531–1543
- Dean TA, Jacobsen FR, Thies K, Lagos SL (1988) Differential effects of grazing by white sea urchins on recruitment of brown algae. *Mar Ecol Prog Ser* 48: 99–102
- Denton A, Chapman ARO, Markham J (1990) Size-specific concentrations of phlorotannins (anti-herbivore compounds) in three species of *Fucus*. *Mar Ecol Prog Ser* 65: 103–104
- DeWreede RE, Klinger T (1988) Reproductive strategies in algae. In: Doust JL, Doust LL (eds) *Plant reproductive ecology: patterns and strategies*. Oxford University Press, New York, pp 267–284
- Duffy JE, Hay ME (1994) Herbivore resistance to seaweed chemical defense: the roles of mobility and predation risk. *Ecology* 75: 1304–1319
- Geiselman JA, McConnell OJ (1981) Polyphenols in brown algae *Fucus vesiculosus* and *Ascophyllum nodosum*: chemical defenses against the marine herbivorous snail, *Littorina littorea*. *J Chem Ecol* 7: 1115–1133
- Gosselin LA (1997) An ecological transition during juvenile life in a marine snail. *Mar Ecol Prog Ser* 157: 185–194
- Gosselin LA, Qian PY (1997) Juvenile mortality in benthic marine invertebrates. *Mar Ecol Prog Ser* 146: 265–282
- Gunnill FC (1986) Demography of *Cystoseira osmundacea* and *Halidrys dioica* (Phaeophyta, Cystoseraceae) at La Jolla, California, USA. *Bot Mar* 29: 137–146
- Harris LG, Ebeling AW, Laur DR, Rowley RJ (1984) Community recovery after storm damage: a case of facilitation in primary succession. *Science* 224: 1336–1338
- Hawkins SJ (1981) The influence of *Patella* grazing on the fucoid barnacle mosaic on moderately exposed rocky shores. *Kiel Meeresforsch Sonderh* 5: 537–543
- Hawkins SJ (1983) Interactions of *Patella* and macroalgae with settling *Semibalanus balanoides* (L.). *J Exp Mar Biol Ecol* 71: 55–72
- Hawkins SJ, Hartnoll RG (1983) Grazing of intertidal algae by marine invertebrates. *Oceanogr Mar Biol Annu Rev* 21: 195–282
- Hay ME, Duffy JE, Fenical W (1989a) Host-plant specialization decreases predation on a marine amphipod: an herbivore in plant's clothing. *Ecology* 71: 733–743
- Hay ME, Pawlik JR, Duffy JE, Fenical W (1989b) Seaweed-herbivore-predator interactions: host-plant specialization reduces predation on small herbivores. *Oecologia* 75: 246–252
- Hay ME, Duffy JE, Pfister CA, Fenical W (1990a) Chemical defense against different marine herbivores: are amphipods insect equivalents? *Ecology* 68: 1567–1580
- Hay ME, Duffy JE, Paul VJ, Renaud PE, Fenical W (1990b) Specialist herbivores reduce their susceptibility to predation by feeding on the chemically defended seaweed *Avrainvillea longicaulis*. *Limnol Oceanogr* 35: 1734–1743
- Johnson LE, Brawley SH (1998) Dispersal and recruitment of a canopy-forming intertidal alga: the relative roles of propagule availability and post-settlement processes. *Oecologia* 117: 517–526
- Johnson CR, Mann KH (1986) The importance of plant defence abilities to the structure of subtidal seaweed communities: the kelp *Laminaria longicruris* de la Pylaie survives grazing by the snail *Lacuna vineta* (Montagu) at high population densities. *J Exp Mar Biol Ecol* 97: 231–267
- Keesing JK, Halford AR (1992) Field measurements of survival rates of juvenile *Acanthaster planci*: techniques and preliminary results. *Mar Ecol Prog Ser* 85: 107–114
- Keser M, Larson BR (1984) Colonization and growth dynamics of three species of *Fucus*. *Mar Ecol Prog Ser* 15: 125–134
- Lubchenco J (1983) *Littorina* and *Fucus*: effects of herbivores, substrate heterogeneity, and plant escapes during succession. *Ecology* 64: 1116–1123
- Martinez EA, Santelices B (1998) Selective mortality of haploid and diploid microscopic stages of *Lessonia nigrescens* Bory (Phaeophyta, Laminariales). *J Exp Mar Biol Ecol* 229: 219–239
- Mattson WJ (1980) Herbivory in relation to plant nitrogen content. *Annu Rev Ecol Syst* 11: 119–161
- Paul VJ, Van Alstyne KL (1988) Chemical defense and chemical variation in some tropical Pacific species of *Halimeda* (Halimedaceae; Chlorophyta). *Coral Reefs* 6: 263–269
- Pavia H, Toth G (2000) Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology* 81: 3212–3225
- Pavia H, Toth G, Åberg P (1999) Trade-offs between phlorotannin production and annual growth in natural populations of the brown seaweed *Ascophyllum nodosum*. *J Ecol* 7: 761–771.
- Pearson GA, Serrao EA, Brawley SH (1998) Control of gamete release in fucoid algae: sensing hydrodynamic conditions via carbon acquisition. *Ecology* 79: 1725–1739
- Peterson CH, Renaud PE (1989) Analysis of feeding preference experiments. *Oecologia* 80: 82–86
- Ragan MA, Glombitza K (1986) Phlorotannins, brown algal polyphenols. In: Round FE, Chapman DJ (eds) *Progress in phycological research*, vol 4. Biopress, Bristol, pp 129–241
- Rhoades D (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal G, Janzen D (eds) *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, pp 1–54
- Santelices B (1990) Patterns of reproduction and recruitment in seaweeds. *Oceanogr Mar Biol Annu Rev* 28: 177–276
- Steinberg PD (1984) Algal chemical defenses against herbivores: allocation of phenolic compounds in the kelp *Alaria marginata*. *Science* 223: 405–407
- Steinberg PD (1985) Feeding preferences of *Tegula funebris* and chemical defenses of marine brown algae. *Ecol Monogr* 55: 333–349
- Steinberg PD (1988) The effects of quantitative and qualitative variation in phenolic compounds on feeding in three species of marine invertebrate herbivores. *J Exp Mar Biol Ecol* 120: 221–237
- Steinberg PD (1995) Seasonal variation in the relationship between growth rate and phlorotannin production. *Oecologia* 102: 169–173
- Steinberg PD, van Altena I (1992) Tolerance of marine invertebrate herbivores to brown algal phlorotannins in temperate Australia. *Ecol Monogr* 62: 189–222
- Steinberg PD, Estes JA, Winter FC (1995) Evolutionary consequences of food chain length in kelp forest communities. *Proc Natl Acad Sci* 92: 8145–8148
- Targett NM, Arnold TM (1998) Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *J Phycol* 34: 195–205
- Tugwell S, Branch GM (1989) Differential polyphenolic distribution among tissues in the kelps *Ecklonia maxima*, *Laminaria pallida*, and *Macrocystis angustifolia* in relation to plant-defence theory. *J Exp Mar Biol Ecol* 129: 219–230
- Tuomi J, Niemala P, Siren S, Jormalainen V (1989) Within-plant variation in phenolic content and toughness of the brown alga *Fucus vesiculosus*. *Bot Mar* 32: 505–509

- Vadas RL Sr, Johnson S, Norton TA (1992) Recruitment and mortality of early post-settlement stages of benthic algae. *Br Phycol J* 27: 331–351
- Van Alstyne KL (1995) A comparison of three methods for quantifying brown algal polyphenolic compounds. *J Chem Ecol* 21: 45–58
- Van Alstyne KL, Pelletreau KN (2000) The effects of nutrient enrichment on growth and phlorotannin production in *Fucus gardneri* embryos. *Mar Ecol Prog Ser* 206: 33–43
- Van Alstyne KL, Ehlig JM, Whitman SL (1999a) Feeding preferences for juvenile and adult algae depend on algal stage and herbivore species. *Mar Ecol Prog Ser* 180: 179–185
- Van Alstyne KL, McCarthy JJ III, Hustead CL, Kearns LJ (1999b) Phlorotannin allocation among tissues of northeastern Pacific kelps and rockweeds. *J Phycol* 35: 483–492
- Van Alstyne KL, McCarthy JJ III, Hustead CL, Duggins DO (1999c) Geographic variation in polyphenolic levels of north-eastern Pacific kelps and rockweeds. *Mar Biol* 133: 371–379
- Van Alstyne KL, Dethier MN, Duggins DO (in press) Spatial variation in algal chemical defenses. In: McClintock J, Baker W (eds) *Marine chemical ecology* CRC press
- Watson DC, Norton TA (1985) Dietary preferences of the common periwinkle *Littorina littorea* (L.). *J Exp Mar Biol Ecol* 88: 193–211
- Winter FC, Estes JA (1992) Experimental evidence for the effects of polyphenolic compounds from *Dictyoneurum californicum* Ruprecht (Phaeophyta: Laminariales) on feeding rate and growth in the red abalone *Haliotis rufescens* Swainson. *J Exp Mar Biol Ecol* 155: 263–277