Effects of algal phenotype on mesograzer feeding

Edwin Cruz-Rivera[1,3,*], Michael Friedlander[2]

[1]Department of Biology, The American University in Cairo, AUC Avenue, PO Box 74, New Cairo 11835, Egypt

[2]Israel Oceanographic and Limnological Research Institute, Haifa 31080, Israel

[3]*Present address:* Environmental Science Program, Asian University for Women, 20/A M. M. Ali Road, Chittagong, Bangladesh

ABSTRACT: The consequences of intraspecific variation in prey traits for plant–herbivore interactions were tested by measuring the susceptibility of 3 phenotypes from the red alga *Gracilaria cornea* against herbivores from the coast of Israel. The 3 algal phenotypes ('fine', 'green', 'wild') differed in morphology, as well as nutritional value (organic content). When presented with the 3 *G. cornea* phenotypes simultaneously, the amphipod *Ampithoe ramondi* and the crab *Acanthonyx lunulatus* consumed significantly more of the finely branched phenotype (63 and 80% of total consumption, respectively) than of either the green or wild phenotypes. When confined with only 1 of the 3 algal phenotypes, feeding patterns of the crab changed little, consuming significantly more of the finely branched phenotype. In contrast, the amphipod responded by increasing consumption proportionally on the phenotypes with the lowest organic content. Regression analysis showed that *A. ramondi* exhibited compensatory feeding and consumed significantly more of the green phenotype than of either of the other 2 under no-choice conditions, with algal ash-free dry mass explaining approximately 83% of the variance in feeding for this mesograzer. Data suggest that feeding by crabs responded to algal morphology more, while amphipods cued on both structure and nutrient content of the algal phenotypes.

KEY WORDS: *Ampithoe ramondi* · *Acanthonyx lunulatus* · Compensatory feeding · Food choice · *Gracilaria cornea*

INTRODUCTION

Natural selection operates on the intraspecific variation in traits among individuals of a population. Under a particular selective pressure, this variation may confer an advantage to individuals demonstrating a specific form of a trait and may allow those individuals to attain higher fitness. For plant–herbivore interactions, intraspecific variations in both the prey plant and the herbivore consumer can have important consequences for the distribution and abundance of both interacting species. For example, ontogenetic, sexual, and biochemical differences among individuals in plant populations can have strong effects on their susceptibility to herbivores (Karban & Baldwin 1997, Agrawal 2005, Boege & Marquis 2005, Cornelissen & Stiling 2005, Barton & Koricheva

2010). Although fewer numerically, studies on marine and freshwater algae have found similar patterns of intraspecific variation in susceptibility as a function of algal traits and their interaction with environmental variables (Hay 1996, Paul et al. 2001, Vergés et al. 2008, Van Donk et al. 2011). Reciprocally, consumption of the same plant or algal prey species can also vary with herbivore age, feeding history, or sex (Cronin & Hay 1996, Hochuli 2001, Jormalainen et al. 2001).

Much of the work analyzing intraspecific differences in plant susceptibility to herbivory has focused on variations in defensive chemistry, particularly on the role of induction in chemical defense (Hay 1996, Karban & Baldwin 1997, Paul et al. 2001, Agrawal 2005, Amsler 2008, Van Donk et al. 2011). Less is known about how differences in nutritional quality,

structural defenses, or morphology affect herbivore feeding. In marine ecosystems, algae with complex heteromorphic life cycles may experience stage-specific differences in herbivory. In this case, changes related to the life-history of the algae some-times result in dramatically contrasting body plans that may differ in their susceptibility to natural ene-mies (Lubchenco & Cubit 1980, Slocum 1980, Vergés et al. 2008). The differences between sporophytes and gametophytes are often large enough that each stage occupies a different niche (but see Thornber et al. 2006) and, thus, will be exposed to herbivores that operate at very different scales or show different feeding modes. A similar scenario occurs for marine invertebrates that release small motile unitary larvae from adults that are large, often modular, and sessile. For these animals, pre- and post-settlement preda-tion likely involve different guilds of predators (Stoner 1990, Maldonado 2006). Yet, for many plants, algae, and sessile invertebrates, noticeable intraspe-cific variation in morphology need not be related to ontogenetic changes and can be expressed among individuals of the same sex and age as related to genotype or phenotypic plasticity resulting from environmental gradients (Agrawal 2001, Benard 2004, Miner et al. 2005).

The effectiveness of structural defenses for diverse plant and animal species has been documented (Benard 2004, Inbar & Lev-Yadun 2005, Hanley et al. 2007, Ferguson & Davis 2008). However, studies addressing the consequences of intraspecific varia-tion in structure have largely focused on induced defenses of animals (Harvell 1990, Tollrian & Harvell 1999, Benard 2004). Variation in structural suscepti-bility to herbivores has also been related to induction in plants (Harvell 1990, Hanley et al. 2007), but very few studies have addressed how constitutive struc-tural differences between conspecifics affect herbi-vore choice, distribution, and nutrient acquisition (Rudgers & Whitney 2006, Wise & Abrahamson 2008, Kawasaki et al. 2009). In part, the lack of studies in this area is related to the rarity of certain morpho-types within natural populations, which constrains the ability to experimentally quantify the susceptibil-ity of those morphologically different plants to graz-ing pressure.

Cultivated plants and algae can provide useful models for understanding the relation between intra-specific variation in prey traits and susceptibility to consumers without the need of intensive efforts find-ing, or risking overcollection of, rare phenotypes. Diverse algal strains with specific biochemical and physical traits are now available from breeding efforts on various cultured seaweeds. While many algae in aquaculture are not natural components of the environments in which they are cultivated, a number of cultured seaweeds have become invaders in these novel environments (Schaffelke et al. 2006, Schaffelke & Hewitt 2007, Williams & Smith 2007). During these events local herbivores encounter prey with which they have no evolutionary experience, and the success of an algal species establishing a viable population in this new ecosystem will likely be influenced by the phenotype that has actually escaped into the wild. Thus, measuring intraspecific susceptibility of aquacultured algae to grazers may provide insights into both fundamental ecological mechanisms and allow predictions regarding inva-sive potential.

In this study, we used 3 cultivated phenotypes (strains) from the agar-producing red seaweed *Graci-laria cornea* J. Agardh, 1852 and evaluated their sus-ceptibility against 2 herbivores from the Mediterran-ean. Clearly distinct phenotypes of *G. cornea* occur spontaneously both in the field (Ferreira et al. 2006) and in cultivation (Friedlander et al. 2001, Friedlan-der 2008), and thus correspond to natural phenotypic variation for this species. The alga has a broad distri-bution, with West Atlantic records from the United States to the coast of Brazil, the Caribbean, and east-ern Africa (Guiry & Guiry 2012). Although the herbi-vores used in this study overlap in distribution with the alga over large areas, *G. cornea* has no known established populations in the Mediterranean. Thus, herbivore responses would not likely be affected by previous experience with any of the algal pheno-types and correspond to perceived characteristics of a novel prey (e.g. an introduced species). Algae in the genus *Gracilaria* can activate the production of hydrogen peroxide against agar-degrading epiphytic bacteria (Weinberger & Friedlander 2000, Potin 2008, Weinberger et al. 2010), and 1 species is known to induce deterrent eicosanoids upon grazing (Nylund et al. 2011). However, no chemical defenses against herbivores are known to occur in *G. cornea* (Paul et al. 2001, Amsler 2008), and species in this genus tend to be preferred species for many marine consumers (e.g. Pfister & Hay 1988, Brand-Gardner et al. 1999), particularly after wounding (Diaz et al. 2006). Thus, we hypothesized that herbivore responses would be affected largely by the structural differences among the phenotypes and, potentially, by nutritional differ-ences that may exist among the conspecific algae. We tested the susceptibility of 3 algal phenotypes against 2 common and widely distributed mesograz-ers: the amphipod *Ampithoe ramondi* and the crab

Acanthonyx lunulatus. Using these organisms, the following questions were addressed: (1) Are there differences in palatability among the 3 *G. cornea* phenotypes? (2) If so, are morphological and nutritional differences among conspecific algae related to their susceptibility to herbivores? (3) Can intraspecific differences in nutrient content of the algae trigger compensatory responses in the mesograzers (e.g. compensatory feeding)?

MATERIALS AND METHODS

The gammaridean amphipod *Ampithoe ramondi* Audouin, 1826 and the majid crab *Acanthonyx lunulatus* (Risso, 1816) were collected from the common benthic algae *Jania rubens*, *Padina pavonica*, and *Ulva lactuca* around the intertidal near the Israel Oceanographic and Limnological Research Laboratory in Haifa (32° 49′ 0″ N, 34° 59′ 0″ E). *A. ramondi* is common in the Mediterranean coast of Israel (Fishelson & Haran 1987, Sorbe et al. 2002, Cruz-Rivera & Friedlander 2011) and has a worldwide distribution (Lowry et al. 2012), although reports of this amphipod may include in part misidentified congeneric or cryptic species (Myers 2012). *A. lunulatus* is a small algal-dwelling crab found on algae in the eastern Atlantic, the Azores, and the West Atlantic from Europe to South Africa, as well as on both sides of the Mediterranean (Cruz-Rivera 2001, Cruz-Rivera & Friedlander 2011, Fransen & Türkay 2012). It is known to consume a variety of temperate algae including Mediterranean species of *Gracilaria* (Cruz-Rivera 2001, Cruz-Rivera & Friedlander 2011).

The red alga *Gracilaria cornea* (=*Hydropuntia cornea* [J. Agardh] M. J. Wynne, 1989) was obtained from aquaculture tanks in which different clonal phenotypes had been grown for several years (Friedlander 2008). In these tanks, the thicker coarsely branched red wild strain ('wild' hereafter) and a spontaneously occurring thin highly branched red mutant ('fine' hereafter) grew together. A third phenotype (strain), green and moderately branching ('green' hereafter), was kept in culture separately. Descriptions of the *G. cornea* phenotypes, and their responses to culturing and environmental conditions are discussed in Levy & Friedlander (1994), Friedlander et al. (2001), and Friedlander (2008). While the terms 'wild' and 'mutant' are seldom used by ecological geneticists or evolutionary ecologists describing phenotypes, we retain the names that have been used in the works cited above (and others) to refer specifically to these *G. cornea* strains and other culti-

vated species. This allows an easier comparison with the aquaculture literature where *Gracilaria* intraspecific variation has been explicitly assessed the most.

While morphological differences among the 3 algal phenotypes were clearly noticeable and have been previously documented, potential differences in nutritional traits of the algae have not. We compared overall organic content (as a proxy for nutrient content) by quantifying ash-free dry mass (AFDM) in parts from the same algal thalli that were later used in feeding assays with amphipods and crabs (n = 10). To determine wet to dry mass ratios and percentage of ash, algal pieces were first dried with absorbent paper and weighed. These were then dried at 60°C for about 4 d, reweighed, and burned in a furnace at 450°C for at least 8 h to obtain ash masses. Most studies in animal nutrition present nutrient data as proportions of dry mass. However, animals in nature encounter foods that may contain broadly different water content. High water content in a diet can affect nutrient acquisition and assimilation for the consumer (Cruz-Rivera & Hay 2001, Lavoie & Oberhauser 2004). Therefore, ash and AFDM contents herein were analyzed as percents of dry mass (which allows comparisons with other published works) and wet mass (which is how herbivores encounter algae).

To assess the susceptibility of *Gracilaria cornea* phenotypes to marine herbivores, 2 types of feeding assays with *Ampithoe ramondi* and *Acanthonyx lunulatus* were conducted. In multiple-choice experiments, *G. cornea* pieces of all 3 phenotypes were simultaneously offered to either amphipods or crabs. In no-choice experiments, animals were confined to feeding on only 1 of the 3 phenotypes. Treatment replicates for both types of experiments were run in small plastic containers to which fresh seawater, grazers, and algae had been added. Controls for autogenic changes in algal mass (Peterson & Renaud 1989) were interspersed among treatments and had similar masses of algae in fresh seawater, but no grazers. Replicates in which animals consumed no food were eliminated from the analyses. Only healthy-looking thalli (without bleached areas that may constitute microbial infection; Friedlander 2008) were used in the assays to avoid confounding structural or nutritional differences in phenotype susceptibility with changes in palatability related to an immune response of *Gracilaria* or other activated mechanisms (Weinberger & Friedlander 2000, Potin 2008, Weinberger et al. 2010, Nylund et al. 2011).

During multiple-choice assays, pieces of the 3 algal phenotypes (ca. 80 to 120 mg for amphipods and 150 to 200 mg for crabs, as per differences in animal size)

were placed in each of the replicate containers. Three adult *Ampithoe ramondi* or a single crab were added to treatment containers (n = 10 for amphipods and n = 12 for crabs) and allowed to feed for a maximum of 3 d. The same number of containers served as controls for autogenic changes in algal mass (Peterson & Renaud 1989). Upon termination of this multiple-choice assay, animals were removed and remaining algal pieces were weighed. Consumption (mg eaten/individual consumer/day) was calculated after correcting for mass changes unrelated to consumer feeding using the formula $(H_i \times C_f / C_i) - H_f$, where H_i and H_f were the initial and final algal masses of the pieces exposed to herbivores and C_i and C_f were the initial and final masses of the paired controls for autogenic changes (Peterson & Renaud 1989, Cronin & Hay 1996).

Because herbivores may increase their feeding rates to compensate for low food quality (compensatory feeding), we also measured consumption by *Ampithoe ramondi* and *Acanthonyx lunulatus* when constrained to feed on only 1 of the 3 *Gracilaria cornea* phenotypes. Pieces of each algal phenotype (ca. 70 to 100 mg for amphipods and 150 to 200 mg for crabs, as per differences in size) were placed with either 2 adult *A. ramondi* or 1 *A. lunulatus* in single replicate containers (n = 7 for amphipods and n = 9 for crabs). Animals were allowed to feed for up to 3 d. Equal numbers of controls for autogenic changes in algal mass were run simultaneously. After allowing animals to feed, algal pieces were weighed again and consumption was calculated as explained above for the multiple-choice assays.

Differences in organic content and feeding rates from no-choice experiments were analyzed using 1-way ANOVA, followed by Tukey-Kramer post hoc tests. When *F*-tests detected heterogeneity in variances, logarithmic transformations were used. In all cases, transformations attained homoscedasticty. Because multiple-choice experiments offered all the phenotypes simultaneously to grazers, consumption of any alga was not independent of the consumption of other choices. Thus, data from these assays were analyzed using the non-parametric Friedman's test followed by pair-wise post hoc comparisons (Conover 1980). For these multiple-choice assays, we also analyzed the relative contribution of each of the 3 algal phenotypes to overall consumption for each mesograzer in order to discern differences in these patterns. This was

accomplished by calculating the percentage of the total amount consumed in each replicate that was comprised by each phenotype. These percentages (relative consumption in amphipods versus crabs) were then compared for each phenotype using unpaired *t*-tests.

To assess the possibility of compensatory feeding as a function of algal organic content, we compared the feeding rates of each herbivore, on each algal phenotype, against the %AFDM per wet mass contents of the algae. This approximation of organic content in a food is clearly a coarse measurement of food quality and does not preclude the possibility that consumers may be cueing on specific nutrients that covary with total organic mass. However, AFDM has been demonstrated to have considerable predictive power explaining feeding rates of marine crustaceans within the same families (Ampithoidae and Majidae, respectively) as those used here (Stachowicz & Hay 1999, Cruz-Rivera & Hay 2001, Sotka & Hay 2002). Regression lines and 95% confidence intervals were calculated by plotting the feeding rates in individual replicates versus the organic content of the algae used in those replicates. This was possible because AFDM values were quantified in algal subsamples that were matched to each treatment replicate.

RESULTS

Significant differences in composition (measured as water, dry mass, and ash contents) were found among the 3 *Gracilaria cornea* phenotypes (p ≤ 0.001 for all analyses, 1-way ANOVA; Table 1). This led to concomitant significant differences in AFDM when calculated as ratios of wet or dry mass (p < 0.001 for both analyses, 1-way ANOVA). The wild phenotype

Table 1. *Gracilaria cornea*. Water, dry mass, and ash content (%, ±SE) of 3 phenotypes. Some comparisons are presented both by wet (WM) and dry mass (DM). Analyses and significant groupings are from 1-way ANOVA followed by Tukey-Kramer tests (n = 10). The same superscripted letters indicate no significant difference between phenotypes for that trait. AFDM: ash-free dry mass

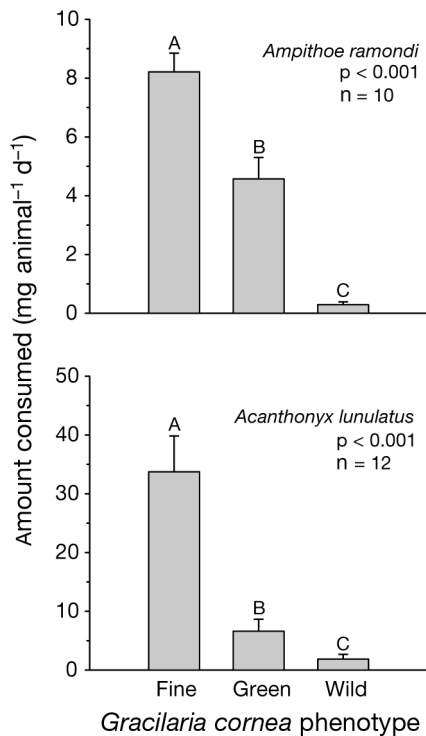|	Fine	Green	Wild	p
Water	81.41 ± 0.14[A]	88.92 ± 0.09[B]	78.38 ± 0.31[C]	<0.001
Dry mass	18.59 ± 0.14[A]	11.08 ± 0.09[B]	21.62 ± 0.31[C]	<0.001
Ash (WM)	5.41 ± 0.02[A]	5.72 ± 0.02[B]	5.32 ± 0.12[A]	0.001
Ash (DM)	29.13 ± 0.30[A]	51.65 ± 0.41[B]	24.66 ± 0.73[C]	<0.001
AFDM (WM)	13.18 ± 0.15[A]	5.36 ± 0.08[B]	16.30 ± 0.36[C]	<0.001
AFDM (DM)	70.87 ± 0.30[A]	48.35 ± 0.41[B]	75.34 ± 0.73[C]	<0.001

Fig. 1. *Gracilaria cornea*. Feeding by the amphipod *Amp-ithoe ramondi* (upper panel) and the majid crab *Acanthonyx lunulatus* (lower panel) on 3 phenotypes offered simultane-ously (food choice). Bars represent means + 1 SE. Analyses and significant groupings are from non-parametric Fried-man's tests followed by pair-wise comparisons

had the highest organic content (AFDM), followed by the fine and green phenotypes, and all were statistically different among themselves (p < 0.05, Tukey-Kramer tests). The wild phenotype contained the highest percentage of dry mass (lowest percent of water per wet mass), coupled with the lowest concentration of ash of all 3 phenotypes (Table 1). Differences in AFDM by wet mass between the 3 phenotypes were more pronounced, but the overall pattern was similar whether organic content was expressed as a percentage of dry or wet mass, with all 3 phenotypes being significantly different from each other.

When presented with pieces of all 3 *Gracilaria cornea* phenotypes, both amphipods and crabs con-sumed significantly more of the fine phenotype than of either of the other 2 phenotypes (p < 0.001, Fried-man's test; Fig. 1). Consumption of the green pheno-type was intermediate, but significantly higher than consumption of the wild phenotype for both meso-grazers. While overall patterns were similar (shown as mean mg consumed animal^{-1} d^{-1} in Fig. 1), the magnitude of the differences in relative consumption

of the 3 *G. cornea* phenotypes differed between con-sumers. When amounts eaten of each phenotype were converted to percentages of total consumption per individual replicate, the fine phenotype com-prised about 63% of overall consumption by amphi-pods and close to 80% of overall crab consumption. The green phenotype accounted for 34% of total con-sumption in amphipods, but 16% in crabs, and the wild phenotype accounted for 2 and 4% of consump-tion in amphipods and crabs, respectively. These dif-ferences in relative consumption between amphi-pods and crabs were not significant for either the fine (p = 0.079, unpaired *t*-test) or the wild (p = 0.484, unpaired *t*-test) phenotypes. However, there was a significant difference in the relative contribution of the green phenotype to overall food choice, with amphipods consuming relatively more of this pheno-type than crabs (p = 0.014, unpaired *t*-test).

In contrast to the patterns found in the multiple-choice experiments, *Ampithoe ramondi* confined with only 1 of the 3 algal phenotypes showed significantly higher feeding rates on the green than on either of the other 2 phenotypes (p < 0.001, 1-way ANOVA; Fig. 2). Post hoc analyses showed that consumption of each phenotype was significantly different from that
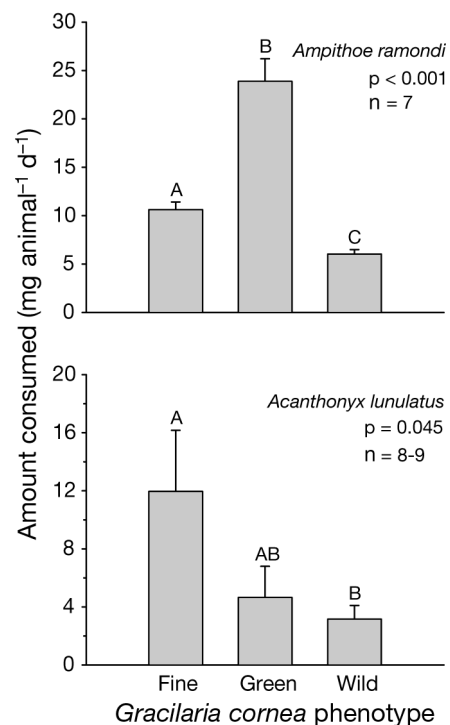

Fig. 2. *Gracilaria cornea.* Feeding by amphipods (upper panel) and crabs (lower panel) confined with 1 of 3 pheno-types (no-choice). Bars represent means + 1 SE. Analyses and significant groupings are from 1-way ANOVA followed by Tukey-Kramer tests

of the other 2 phenotypes. Under no-choice conditions, amphipod feeding rates were approximately 56% higher on the green than on the fine phenotype. Amphipods also consumed approximately 43% more of the fine phenotype than of the wild one (Fig. 2). Feeding rates under no-choice conditions for *Acanthonyx lunulatus* on the 3 algal phenotypes also showed significant differences (p = 0.045, 1-way ANOVA; Fig. 2). However, crabs consumed on average considerably more of the fine phenotype and similar amounts of both green and wild phenotypes in this experiment. Post hoc analyses revealed only a significant difference between crab feeding rates on the fine versus the wild phenotype, likely due to the high variance in consumption on both the fine and green phenotypes (Fig. 2).
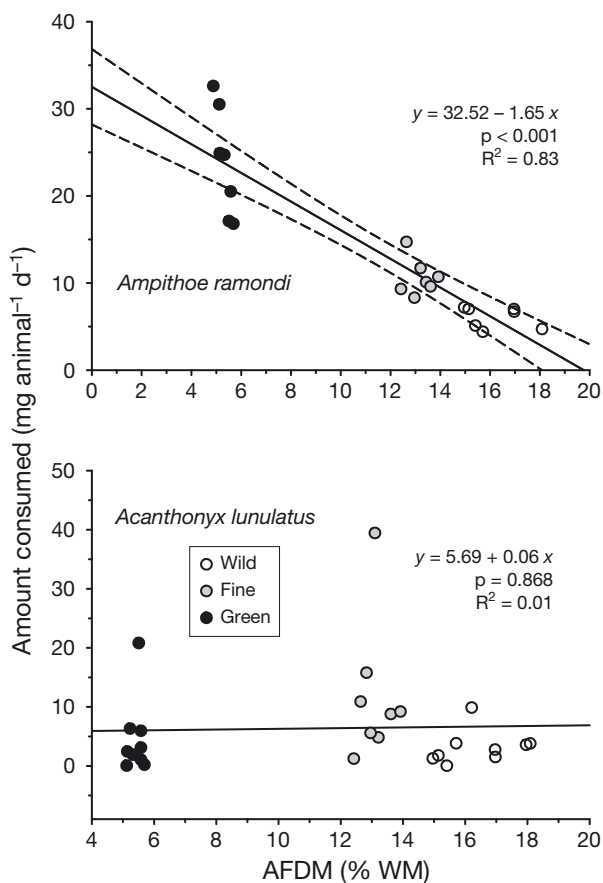

Fig. 3. *Gracilaria cornea.* Relation between the feeding rates of amphipods (upper panel) and crabs (lower panel), and the organic content (ash-free dry mass [AFDM] per wet mass [WM]) of 3 phenotypes. Feeding rates were obtained from no-choice experimental replicates (Fig. 2) and matched against the organic content of algal thalli from which the replicates were fed (see 'Materials and methods'). Because the relation between the 2 variables was statistically significant for amphipods, 95% confidence intervals are shown (dashed lines)

When feeding rates for each herbivore replicate were related to the organic content of the algal pieces they were offered, contrasting patterns emerged. Amphipod feeding rates were strongly and inversely related to the organic content of the algae per wet mass (AFDM/WM) (p < 0.001, R^2 = 0.83; Fig. 3). This relation was also found when AFDM/DM was used, but it was slightly weaker (p < 0.001, R^2 = 0.81). Thus, individuals feeding on algae with lower organic content consumed more tissue than individuals feeding on algae with higher AFDM/WM per unit of time (i.e. compensatory feeding). In contrast, crabs showed no relation between feeding rates and algal AFDM/WM (p = 0.868, R^2 = 0.01; Fig. 3) or AFDM/DM (p = 0.554, R^2 = 0.02). It should be noted that, although using organic content per dry mass might lead to significant correlations, the animals studied here would seldom encounter algae as dry items. Thus, using AFDM/WM is a more ecologically realistic approach (Cruz-Rivera & Hay 2001).

DISCUSSION

Feeding preference and feeding rates of consumers differed strongly as a function of algal phenotype (Figs. 1 & 2), thus demonstrating significant intraspecific variation in susceptibility of an algal species to herbivory. Intraspecific variation in plant and animal traits has been invoked as an important determinant of direct and indirect species interactions, community assemblage, and ecosystem-level processes including invasion dynamics (Janzen 1979, Agrawal 2001, Albert et al. 2011, Violle et al. 2012).

Although less information is available for algae than for vascular plants, studies in marine systems have shown that differences in palatability can be marked between individuals of different populations (Bolser & Hay 1996, Taylor et al. 2003) or between individuals of the same population exposed to different environmental regimes (Renaud et al. 1990, Cronin & Hay 1996), life stages or sexes of the same species (Lubchenco & Cubit 1980, Slocum 1980, Thornber et al. 2006, Vergés et al. 2008), or morphotypes of the same microalga (Long & Hay 2006). Previous history of attack can also alter susceptibility of individuals when induced chemical defenses make those plants (Karban & Baldwin 1997) or algae (Toth & Pavia 2007, Van Donk et al. 2011) less palatable to natural enemies. In fact, the majority of studies documenting intraspecific variation in plant or algal susceptibility to grazing has focused on how biotic or abiotic factors induce or constrain the expression of

chemical defenses (Hay 1996, Karban & Baldwin 1997, Paul et al. 2001, Agrawal 2005, Toth & Pavia 2007, Van Donk et al. 2011). For animals, induction of defenses has been well documented and can also lead to large differences in susceptibility to predators (Harvell 1990, Tollrian & Harvell 1999).

From this perspective, our data are uncommon in that we focused on constitutive, rather than induced traits, for a macroalga that is not known to produce defensive secondary metabolites against herbivores. Algae in this genus can produce hydrogen peroxide as an induced immune response against bacteria (Weinberger & Friedlander 2000, Potin 2008, Weinberger et al. 2010) and, in 1 species, induced eicosanoids have been shown to serve as chemical defenses against isopods (Nylund et al. 2011). However, no studies to date have addressed phenotypic variation of these activated mechanisms in *Gracilaria*; thus, their involvement in explaining our results for *G. cornea*, albeit intriguing, remain speculative.

In contrast with chemical defenses, the role of intraspecific differences in nutrient content modifying prey palatability is understood less well. The ash content of the 3 phenotypes used here, and concomitantly AFDM, differed significantly (Table 1). Previous studies have reported ash contents of *Gracilaria cornea* of between 24 and 39% dry mass (Robledo & Freile Pelegrín 1997, Orduña-Rojas et al. 2002). Our samples had ash contents of ca. 25, 29, and 52% dry mass for the wild, fine, and green phenotypes, respectively. Thus, our data overlap with previous ranges reported, but expand the intraspecific range of nutritional values for this seaweed.

Nutrient enrichment experiments on macroalgae (Boyer et al. 2004), phytoplankton (Meunier et al. 2012), and seagrasses (Goecker et al. 2005), and some studies on the effects of desiccation on intertidal algae (Renaud et al. 1990) have found that intraspecific differences in nutrient content can influence consumer food choice. In terrestrial systems intraspecific differences in foliar nutrients have also been linked to differences in herbivore choice, feeding rates, dispersal potential, and fitness (e.g. Lavoie & Oberhauser 2004, Huberty & Denno 2006, Chen et al. 2008). Studies have also linked organic content to differences in marine herbivore feeding rates when various algal species are compared (Stachowicz & Hay 1999, Cruz-Rivera & Hay 2001, Sotka & Hay 2002). However, to our knowledge, *intraspecific* differences in nutritional quality of macroalgae have not been previously shown to mediate compensatory feeding as was observed for amphipods in this study (Figs. 2 & 3). Differences in organic content of the algae explained approximately 83% of the variance in feeding rates for the amphipods, but had no relation to crab consumption rates (Fig. 3). Terrestrial herbivores such as monarch butterflies *Danaus plexippus* are known to consume more when feeding on host plants that have been manipulated for low foliar nitrogen (Lavoie & Oberhauser 2004). However, for this and many studies using live foods, the effects of intraspecific differences in nutrients versus changes in secondary chemistry are difficult to disentangle.

In our experiments, the relative susceptibility of the algal phenotypes differed strongly when animals were provided the ability to choose among them. The fine phenotype was consumed significantly more by both grazers than any of the other phenotypes (Fig. 1). Nutrients alone could not explain these differences, and it is likely that structural differences acted primarily or in concert with nutritional quality. In a previous study, Cruz-Rivera & Friedlander (2011) exposed 3 species of *Gracilaria* to the same species of amphipods and crabs used here. In choice experiments, it was found that thinner, finely branched, species (*G. lemaneiformis* and *G. conferta*) were consistently preferred to the coarse *G. cornea* wild type by both consumers. The authors hypothesized that the size of the chelae in *Acanthonyx lunulatus* hindered the manipulation of the thick fronds of *G. cornea* (wild). A similar phenomenon could be operating here, with the fine phenotype being the easiest to manipulate, and the intermediately branching green phenotype, in turn, being easier than the coarsely branched wild phenotype. In contrast, the mouth parts of the amphipods could readily slice through the thalli of different *Gracilaria* species (Cruz-Rivera & Friedlander 2011). For these amphipods, nutritional quality could be a stronger determinant of feeding behavior on algae that can be readily processed when alternatives are not present.

Nonetheless, concluding that structure was unimportant for amphipods would be misleading. Amphipods preferred significantly the finely branched phenotype in multiple-choice assays, despite the fact that this phenotype had lower AFDM than the wild type (Table 1). It is known that some amphipod species cue on the structural complexity of algae while selecting habitat (Hacker & Steneck 1990). Thus, *Ampithoe ramondi* could have been attracted to the higher complexity of the finely branching red strain, while its capacity for compensatory feeding would allow this amphipod to cope with the lower nutrient content of this phenotype. Other species in this amphipod genus are known to efficiently offset the fitness costs of feeding on lower quality diets through

compensatory feeding (Cruz-Rivera & Hay 2000, 2001).

The role of intraspecific differences in structure explaining variation in consumer–prey interactions is better understood for animals that can induce defensive structures such as spines or thicker shells upon attack (Harvell 1990, Tollrian & Harvell 1999). In some microalgae, nutrient limitation or grazing can induce morphological changes in cells and the production of thicker cell walls. These changes result in cells that are less susceptible to micrograzer digestion and cause significant losses in fitness to the consumers (reviewed in Van Donk et al. 2011). Morphology also influences ingestion of microalgae that grow as colonies or as individual cells (Long & Hay 2006, Van Donk et al. 2011). For macroalgae, reproductive tissues of *Mazzaella flaccida* that were more structurally complex were preferred by snails in food-choice assays, but when the same tissues were homogenized to maintain constant nutritional and chemical traits, this preference disappeared (Thornber et al. 2006). A few studies on plant–insect interactions have also demonstrated the importance of intraspecific differences in constitutive morphology mediating susceptibility to insects (Rudgers & Whitney 2006, Wise & Abrahamson 2008, Kawasaki et al. 2009).

Artificial selection has yielded a diversity of plant varieties and hybrids that can be readily grown and used for addressing fundamental questions in ecology. On the applied side, our findings are relevant to the selection of algae for aquaculture, such as *Gracilaria*, which is harvested for the production of agar (Buschmann et al. 2001, Friedlander 2008). Under culture conditions, small invertebrate consumers often reach high densities, resulting in losses in biomass production (Buschmann et al. 2001, Friedlander 2008). Selection of specific phenotypes that are herbivore resistant could ameliorate these problems.

From an ecological standpoint, this study adds to our knowledge of intraspecific variation in plant–herbivore interactions by focusing on phenotypic differences in nutritional quality and structure as algal traits affecting consumer feeding behavior. Our data suggest that, for communities in which these species overlap, the coarser phenotypes may be less susceptible to herbivores and could be more common than the finely branched phenotype. However, no field data on herbivory are available for *Gracilaria cornea* in its natural range, and, by focusing only on meso-grazers, the community-wide effects of larger herbivores (e.g. fishes and urchins) on populations of this alga remain untested. Given that a number of agaro-phytes, including *Gracilaria* species, have now been established as important invaders around the world (Schaffelke et al. 2006, Schaffelke & Hewitt 2007, Williams & Smith 2007), the study of relations between phenotype and grazing susceptibility for aquacultured algae increases our potential to understand and predict biological invasions.

LITERATURE CITED

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294:321–326

Agrawal AA (2005) Future directions in the study of induced plant responses to herbivory. Entomol Exp Appl 115: 97–105

Albert CH, Grassein F, Schurr FM, Vieilledent G, Violle C (2011) When and how should intraspecific variability be considered in trait-based plant ecology? Perspect Plant Ecol Evol Syst 13:217–225

Amsler CD (2008) Algal chemical ecology, 1st edn. Springer-Verlag, Berlin

Barton KE, Koricheva J (2010) The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. Am Nat 175:481–493

Benard MF (2004) Predator-induced phenotypic plasticity in organisms with complex life cycles. Annu Rev Ecol Evol Syst 35:651–673

Boege K, Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol Evol 20:441–448

Bolser RC, Hay ME (1996) Are tropical plants better defended? Palatability and defenses of temperate versus tropical seaweeds. Ecology 77:2269–2286

Boyer KE, Fong P, Armitage AR, Cohen RA (2004) Elevated nutrient content of tropical macroalgae increases rates of herbivory in coral, seagrass and mangrove habitats. Coral Reefs 23:530–538

Brand-Gardner SJ, Lanyon JM, Limpus CJ (1999) Diet selection by immature green turtles, *Chelonia mydas*, in subtropical Moreton Bay, south-east Queensland. Aust J Zool 47:181–191

Buschmann AH, Correa JA, Westermeier R, Hernandez González M, Norambuena R (2001) Red algal farming in Chile: a review. Aquaculture 194:203–220

Chen Y, Ruberson JR, Olson D (2008) Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. Entomol Exp Appl 126:244–255

Conover WJ (1980) Practical nonparametric statistics, 2nd edn. Wiley, New York, NY

Cornelissen T, Stiling P (2005) Sex-biased herbivory: a meta-analysis of the effects of gender on plant–herbivore interactions. Oikos 111:488–500

Cronin G, Hay ME (1996) Susceptibility to herbivores depends on recent history of both the plant and animal. Ecology 77:1531–1543

Cruz-Rivera E (2001) Generality and specificity in the feeding and decoration preferences of three Mediterranean crabs. J Exp Mar Biol Ecol 266:17–31

Cruz-Rivera E, Friedlander M (2011) Feeding preferences of mesograzers on aquacultured *Gracilaria* and sympatric algae. Aquaculture 322/323:218–222

Cruz-Rivera E, Hay ME (2000) Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology 81:201–219

Cruz-Rivera E, Hay ME (2001) Macroalgal traits and the feeding and fitness of an herbivorous amphipod: the roles of selectivity, mixing, and compensation. Mar Ecol Prog Ser 218:249–266

Diaz E, Guldenzoph C, Molis M, McQuaid C, Wahl M (2006) Variability in grazer-mediated defensive responses of green and red macroalgae on the south coast of South Africa. Mar Biol 149:1301–1311

Ferguson AM, Davis AR (2008) Heart of glass: spicule armament and physical defense in temperate reef sponges. Mar Ecol Prog Ser 372:77–86

Ferreira LB, Barufi JB, Plastino EM (2006) Growth of red and green strains of the tropical agarophyte *Gracilaria cornea* J. Agardh (Gracilariales, Rhodophyta) in laboratory. Rev Bras Bot 29:187–192

Fishelson L, Haran T (1987) Epifauna of algae on a rocky platform near Mikhmoret (Mediterranean Sea, Israel): composition and dynamics. Isr J Zool 34:105–123

Fransen C, Türkay M (2012) *Acanthonyx lunulatus* (Risso, 1816). World Register of Marine Species. www.marinespecies.org/aphia.php?p=taxdetails&id=107312 (accessed 29 September 2012)

Friedlander M (2008) Israeli R & D activities in seaweed cultivation. Isr J Plant Sci 56:15–28

Friedlander M, Kashman Y, Weinberger F, Dawes CJ (2001) *Gracilaria* and its epiphytes. 4. The response of two *Gracilaria* species to *Ulva lactuca* in a bacteria-limited environment. J Appl Phycol 13:501–507

Goecker ME, Heck KL Jr, Valentine JF (2005) Effects of nitrogen content in turtlegrass, *Thalassia testudinum*, on consumption by the bucktooth parrotfish, *Sparisoma radians*. Mar Ecol Prog Ser 286:239–248

Guiry MD, Guiry GM (2012) AlgaeBase. www.algaebase.org (accessed 29 September 2012)

Hacker SD, Steneck RS (1990) Habitat architecture and the abundance and body-size-dependent habitat selection of a phytal amphipod. Ecology 71:2269–2285

Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM (2007) Plant structural traits and their role in anti-herbivore defence. Perspect Plant Ecol Evol Syst 8:157–178

Harvell CD (1990) The ecology and evolution of inducible defenses. Q Rev Biol 65:323–340

Hay ME (1996) Marine chemical ecology: What's known and what's next? J Exp Mar Biol Ecol 200:103–134

Hochuli DF (2001) Insect herbivory and ontogeny: How do growth and development influence feeding behaviour, morphology and host use? Austral Ecol 26:563–570

Huberty AF, Denno RF (2006) Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. Oecologia 149:444–455

Inbar M, Lev-Yadun S (2005) Conspicuous and aposematic spines in the animal kingdom. Naturwissenschaften 92: 170–172

Janzen DH (1979) New horizons in the biology of plant defenses. In: Rosenthal GA, Janzen D H (eds) Herbivores: their interaction with secondary plant metabolites. Academic Press, New York, NY, p 331–350

Jormalainen V, Honkanen T, Hemmi A, Mäkinen A, Vesakoski O (2001) Why does herbivore sex matter? Sexual differences in utilization of *Fucus vesiculosus* by the isopod *Idotea baltica*. Oikos 93:77–86

Karban R, Baldwin IT (eds) (1997) Induced responses to herbivory. University of Chicago Press, Chicago, IL

Kawasaki N, Miyashita T, Kato Y (2009) Leaf toughness changes the effectiveness of larval aggregation in the butterfly *Byasa alcinous bradanus* (Lepidoptera: Papilionidae). Entomol Sci 12:135–140

Lavoie B, Oberhauser KS (2004) Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. Environ Entomol 33:1062–1069

Levy I, Friedlander M (1994) Seasonal growth activity of local and foreign gracilarioid strains in Israel. J Appl Phycol 6:447–454

Long JD, Hay ME (2006) When intraspecific exceeds interspecific variance: effects of phytoplankton morphology and growth phase on copepod feeding and fitness. Limnol Oceanogr 51:988–996

Lowry J, Costello M, Bellan-Santini D (2012) *Ampithoe ramondi* Audouin, 1826. In: Lowry J (ed) World Amphipoda database. World Register of Marine Species. www.marinespecies.org/aphia.php?p=taxdetails&id=102000 (accessed 29 September 2012)

Lubchenco J, Cubit J (1980) Heteromorphic life histories of certain marine algae as an adaptation to variation in herbivory. Ecology 61:676–687

Maldonado M (2006) The ecology of the sponge larva. Can J Zool 84:175–194

Meunier CL, Hantzsche FM, Cunha-Dupont AÖ, Haafke J, Oppermann B, Malzahn AM, Boersma M (2012) Intraspecific selectivity, compensatory feeding, and flexible homeostasis in the phagotrophic flagellate *Oxyrrhis marina*: three ways to handle food quality fluctuations. Hydrobiologia 680:53–62

Miner BG, Sultan SE, Morgan SG, Padilla DK, Reylea RA (2005) Ecological consequences of phenotypic plasticity. Trends Ecol Evol 20:685–692

Myers AA (2012) Amphipoda (Crustacea) from Palau, Micronesia: families Ampeliscidae, Ampithoidae, Aoridae, Colomastigidae and Cyproideidae. ZooKeys 193: 1–25

Nylund GM, Weinberger F, Rempt M, Pohnert G (2011) Metabolomic assessment of induced and activated chemical defence in the invasive red alga *Gracilaria vermiculophylla*. PLoS ONE 6:e29359

Orduña-Rojas J, Robledo D, Dawes C (2002) Studies on the tropical agarophyte *Gracilaria cornea* J. Agardh (Rhodophyta, Gracilariales) from Yucatán, Mexico. I. Seasonal physiological and biochemical responses. Bot Mar 45: 453–458

Paul VJ, Cruz-Rivera E, Thacker RW (2001) Chemical mediation of macroalgal–herbivore interactions: ecological and evolutionary perspectives. In: McClintock JB, Baker BJ (eds) Marine chemical ecology. CRC Press, Boca Raton, FL, p 227–265

Peterson CH, Renaud PE (1989) Analysis of feeding preference experiments. Oecologia 80:82–86

Pfister CA, Hay ME (1988) Associational plant refuges: convergent patterns in marine and terrestrial communities result from differing mechanisms. Oecologia 77:118–129

Potin P (2008) Oxidative burst and related responses in biotic interactions of algae. In: Amsler CD (ed) Algal chemical ecology. Springer, Berlin, p 245–271

Renaud PE, Hay ME, Schmitt TM (1990) Interactions of plant stress and herbivory: intraspecific variation in the susceptibility of a palatable versus an unpalatable seaweed to sea urchin grazing. Oecologia 82:217–226

Robledo D, Freile Pelegrín Y (1997) Chemical and mineral composition of six potentially edible seaweed species of Yucatán. Bot Mar 40:301–306

Rudgers JA, Whitney KD (2006) Interactions between insect herbivores and a plant architectural dimorphism. J Ecol 94:1249–1260

Schaffelke B, Hewitt CL (2007) Impacts of introduced seaweeds. Bot Mar 50:397–417

Schaffelke B, Smith JE, Hewitt CL (2006) Introduced macroalgae—a growing concern. J Appl Phycol 18:529–541

Slocum CJ (1980) Differential susceptibility to grazers in two phases of an intertidal alga: advantages of heteromorphic generations. J Exp Mar Biol Ecol 46:99–110

Sorbe JC, Basin A, Galil BS (2002) Contribution to the knowledge of the Amphipoda (Crustacea) of the Mediterranean coast of Israel. Isr J Zool 48:87–110

Sotka EE, Hay ME (2002) Geographic variation among herbivore populations in tolerance for a chemically rich seaweed. Ecology 83:2721–2735

Stachowicz JJ, Hay ME (1999) Reduced mobility is associated with compensatory feeding and increased diet breadth of marine crabs. Mar Ecol Prog Ser 188:169–178

Stoner DS (1990) Recruitment of a tropical colonial ascidian: relative importance of re-settlement vs. post-settlement processes. Ecology 71:1682–1690

Taylor RB, Lindquist N, Kubanek J, Hay ME (2003) Intraspecific variation in palatability and defensive chemistry of brown seaweeds: effects on herbivore fitness. Oecologia 136:412–423

Thornber C, Stachowicz JJ, Gaines S (2006) Tissue type matters: selective herbivory on different life history stages of an isomorphic alga. Ecology 87:2255–2263

Tollrian R, Harvell CD (eds) (1999) The ecology and evolution of inducible defenses. Princeton University Press, Princeton, NJ

Toth GB, Pavia H (2007) Induced herbivore resistance in seaweeds: a metaanalysis. J Ecol 95:425–434

Van Donk E, Ianora A, Vos M (2011) Induced defenses in marine and freshwater phytoplankton: a review. Hydrobiologia 668:3–19

Vergés A, Paul NA, Steinberg PD (2008) Sex and life-history stage alter herbivore responses to a chemically defended red alga. Ecology 89:1334–1343

Violle C, Enquist BJ, McGill BJ, Jiang L and others (2012) The return of the variance: intraspecific variability in community ecology. Trends Ecol Evol 27:244–252

Weinberger F, Friedlander M (2000) Response of *Gracilaria conferta* (Rhodophyta) to oligoagars results in defense against agar-degrading epiphytes. J Phycol 36:1079–1086

Weinberger F, Guillemin ML, Destombe C, Valero M and others (2010) Defense evolution in the Gracilariaceae (Rhodophyta): substrate-regulated oxidation of agar oligosaccharides is more ancient than the oligoagar-activated oxidative burst. J Phycol 46:958–968

Williams SL, Smith JE (2007) A global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annu Rev Ecol Evol Syst 38:327–359

Wise MJ, Abrahamson WG (2008) Ducking as a means of resistance to herbivory in tall goldenrod, *Solidago altissima.* Ecology 89:3275–3281