PREY NUTRITIONAL QUALITY INTERACTS WITH CHEMICAL DEFENSES TO AFFECT CONSUMER FEEDING AND FITNESS

EDWIN CRUZ-RIVERA[1,3] AND MARK E. HAY[2,4]

[1]*University of North Carolina at Chapel Hill, Institute of Marine Sciences,*
3431 Arendell Street, Morehead City, North Carolina 28557 USA
[2]*School of Biology, Georgia Institute of Technology, Atlanta, Georgia 30332-0230 USA*

Abstract. Numerous studies have assessed the individual effects of prey nutritional quality or chemical defenses on consumer feeding behavior. However, little is known about how these traits interact to affect consumer feeding and performance. We tested the separate and interactive effects of prey chemical defenses and nutritional quality on the feeding behavior and fitness of six sympatric crustacean mesograzers. Natural concentrations of diterpene alcohols (dictyols) from the brown alga *Dictyota menstrualis* were incorporated, or not incorporated, into lower quality and higher quality foods to create artificial diets mimicking prey of variable value and defense. Five amphipods (*Ampithoe longimana*, *A. valida*, *Cymadusa compta*, *Gammarus mucronatus*, and *Elasmopus levis*) and one isopod (*Paracerceis caudata*), representing a continuum of closely to distantly related organisms, were fed intact algae or lower and higher quality diets containing or lacking dictyols. All six mesograzers preferred the green alga *Enteromorpha intestinalis* to the dictyol producing alga *Dictyota menstrualis*. In assays allowing consumers to choose between simultaneously available foods, dictyols deterred feeding by all five amphipods, but not the isopod; this occurred for both lower and higher quality foods. In no-choice assays, where consumers were confined with only one of our four treatment diets, effects on feeding became more complex. Nutritional quality alone affected feeding by five of the six species. These grazers compensated for lower quality by increasing consumption. Dictyols suppressed feeding for four of the six species. More interestingly, there were significant dictyol × quality interactions for three species. Dictyols decreased feeding more when placed in lower quality foods than higher quality foods. Two amphipods deterred by dictyols in the choice assays readily consumed dictyol-containing foods in no-choice situations and suffered few negative effects of doing so. Although all amphipods were deterred by dictyols in choice assays, dictyols decreased fitness (survivorship, growth, or reproduction) for only four of the five species. These effects included large and immediate decreases in survivorship, dramatic effects on reproduction, and modest effects on female growth. Dictyols enhanced survivorship of the isopod. Thus, the effects of secondary metabolites on feeding in choice situations vs. fitness in long-term assays were inconsistent. For three amphipods, certain effects of food quality, dictyols, or their interaction were detected only for females. In general, negative effects of dictyols on fitness were greater in lower than in higher quality foods, suggesting that prey nutritional value may counteract the effects of defensive metabolites. For example, when *G. mucronatus* consumed dictyols in lower quality foods, mortality was >80% by day 5; for dictyols in higher quality foods, 80% mortality took 28 days to develop. Lower quality foods alone significantly decreased growth for the isopod, three of the amphipods, and the females of a fourth amphipod, concomitantly reducing fecundity for four of the five amphipods studied. The effects of both chemical defenses and nutritional quality were unrelated to consumer phylogeny; responses of congeners varied as much, or more, than responses of more distantly related consumers. Understanding mechanisms and consequences of food selection requires that the interactive effects of both chemical defenses and prey nutritional characteristics be considered explicitly.

Key words: amphipods; Ampithoe; *chemical defenses and consumer food choice; compensatory feeding;* Cymadusa; Dictyota; Elasmopus; Gammarus; *isopods; nutritional interactions with chemical defenses;* Paracerceis; *prey defense and value affect consumer fitness.*

INTRODUCTION

Nutritional quality and chemical defenses of prey are both important determinants of consumer feeding choices (Slansky and Rodriguez 1987, Rosenthal and Berenbaum 1992, White 1993, McClintock and Baker 2001), but these traits have seldom been investigated simultaneously. Low nutritional quality has been pro-

posed as an important barrier constraining the evolution of herbivory (Southwood 1973, Mattson 1980, Strong et al. 1984) and as an important ecological factor determining herbivore food choice (Neighbors and Horn 1991, White 1993, Augner 1995). Similarly, the role of secondary metabolites as chemical defenses against consumers has been well established in both terrestrial and aquatic environments (Fritz and Simms 1992, Paul 1992, Rosenthal and Berenbaum 1992, McClintock and Baker 2001). Prey secondary chemistry also has been proposed as a major evolutionary force influencing the speciation of herbivores (Ehrlich and Raven 1964, Strong et al. 1984, Hay and Fenical 1996).

Despite the studies cited above, few investigations have assessed the interactive roles of nutritional value and chemical defenses in determining consumer choice, and this is especially true for marine systems. Because all potential foods are more than just their nutrient content or their defensive chemistry, the occurrence of synergistic or additive effects among multiple prey traits could be an important aspect of consumer–prey interactions (Slansky 1992, 1993, Hay 1996). A few previous investigations have documented interactions between chemical deterrents and prey nutritional quality (Duffy and Paul 1992, Hay et al. 1994, Pennings et al. 1994) or between multiple defensive traits (Berenbaum and Neal 1985, Hay et al. 1994). However, there is little information on the frequency of such phenomena or how they may influence consumer fitness (Hay 1996).

Chemical defenses occur in a wide array of plants, algae, and animals (Paul 1992, Rosenthal and Berenbaum 1992, Berenbaum 1995, McClintock and Baker 2001). Therefore, interactions between nutritional quality and secondary chemistry potentially occur across a wide spectrum of prey nutritional values under natural conditions. In contrast with terrestrial systems where plants constitute the dominant hosts, small marine consumers (mesograzers) regularly encounter potential hosts that are sessile, benthic, and belong to different kingdoms (e.g., cyanobacteria, seaweeds, seagrasses, sponges, hydroids, corals). Algal and animal tissues differ considerably in nutritional value. Protein content in marine organisms, for example, ranges between 0.8% for some algae to 51% for some animals, a 64-fold difference (reviewed in Duffy and Paul 1992). Differences in nitrogen among algae alone range from 1% (Neighbors and Horn 1991) to 6.9% (Kaehler and Kennish 1996), a difference of almost sevenfold, and carbon varies from 15% to 47.1% (Atkinson and Smith 1983, Kaehler and Kennish 1996), a difference of over threefold. While these comparisons only account for highest and lowest values, comparisons among sympatric algae have generally found two- to fivefold differences in nitrogen or carbon (Nicotri 1980, Atkinson and Smith 1983, Neighbors and Horn 1991, Kaehler and Kennish 1996, Wakefield and Murray 1998, Poore and Steinberg 1999, Cruz-Rivera and Hay 2001). Dif-

ferences will be higher if algae are compared to benthic invertebrates.

Assessing how prey defenses and nutritional quality affect consumer fitness is important for understanding the evolution of feeding preferences among consumers, and of defensive strategies among prey. Although feeding choices are commonly assumed to be adaptive, few data are available to rigorously assess this assumption. For example, many studies show that chemical defenses deter feeding or attack by natural enemies, but few investigations show how these chemicals affect consumer fitness. For strong toxins or chemicals with fast modes of action, consumption of prey containing such substances could result in immediate death or severe physiological stress of the consumer (e.g., Huntley et al. 1986, Kvitek and Beitler 1988, Kvitek et al. 1991, Lindquist and Hay 1995). Under such conditions, consumers that avoid these foods would have a clear selective advantage.

However, many secondary metabolites do not appear to be highly toxic (Hay and Fenical 1988). Thus, consumer avoidance of prey containing these metabolites is more difficult to explain (Cottee et al. 1988, Bernays 1991, Bernays and Cornelius 1992, Hay 1996, Paul et al. 2001). Also, extrinsic factors such as temperature (Stamp and Yang 1996, Stamp et al. 1997), ultraviolet radiation (Thor Arnason et al. 1992, Cronin and Hay 1996a), or nutritional stress of the consumer (Anderson and Kappas 1991, Yang et al. 1992, Cronin and Hay 1996a) can significantly alter prey deterrency or the physiological cost of consuming prey defensive metabolites. This argues for a higher degree of complexity in consumer–prey interactions than is generally appreciated. Finally, very few studies compare multiple and related consumers. This makes it difficult to assess potential phylogenetic constraints to the evolution of observed feeding behaviors. Because theories regarding the evolution of chemical defenses (e.g., plant apparency theory) have often assumed particular modes of action for different groups of compounds (Feeny 1976, Coley et al. 1985, Hay and Steinberg 1992), it is necessary that the links between prey defenses, consumer feeding behavior, and consumer fitness be assessed more broadly.

In this study, we address how prey chemical defenses and overall nutritional quality (measured as protein, nitrogen, and organic carbon content) affect the feeding behavior and fitness of a guild of marine mesograzers. Our prey is the common temperate brown alga *Dictyota menstrualis*, which produces diterpene alcohols (dictyols) that have been demonstrated to deter feeding by several species of marine consumers (Hay et al. 1987, 1988a, Duffy and Hay 1994, Cronin and Hay 1996b, Stachowicz and Hay 1999a).

Despite *Dictyota*'s general deterrency, this alga is a preferred food for North Carolina populations of the amphipod *Ampithoe longimana* (Duffy and Hay 1994, Cruz-Rivera and Hay 2001, Sotka and Hay 2002). The

amphipod lives in mucilaginous tubes it constructs on *Dictyota*, and can occur at densities of >4 individuals per gram of *D. menstrualis* (Cronin and Hay 1996*b*). It not only survives, grows, and reproduces on monospecific diets of this alga (Duffy and Hay 1991, Cruz-Rivera and Hay 2001, Sotka and Hay 2002), but it, and other herbivores, also gain a refuge from omnivorous fishes by living with this chemically defended host (Duffy and Hay 1994, Stachowicz and Hay 1999*a*). Other species of co-occurring amphipods do not feed on, or associate with, *Dictyota* and, in the two cases tested, are deterred by its chemical defenses (Duffy and Hay 1994). We take advantage of these contrasting patterns of *Dictyota* use by sympatric mesograzers by comparing the effects of chemically defended diets on a guild (six species) of consumers ranging from more closely (*Ampithoe* spp.) to more distantly related species (amphipods in different genera, different families, and amphipods vs. an isopod).

By incorporating *Dictyota* secondary metabolites into foods of differing nutritional quality we were able to determine the effects of secondary metabolites, of food nutritional quality, and of their interaction on the feeding responses and fitness (survivorship, growth, and reproduction) of five amphipods and one isopod. In this study we asked: (1) How do *D. menstrualis* chemical defenses (dictyols) affect the feeding choices of six consumers that co-occur with the alga? (2) Do the effects of dictyols vary with the nutritional quality of the prey? (3) Do consumer feeding choices mirror the effects of the foods on consumer fitness? and (4) Are the effects of diet more similar among more closely related consumers?

METHODS

The organisms

Addressing dietary effects on behavior and fitness of marine organisms is often difficult under natural settings because: (1) animals can rarely be confined to specific experimental foods in the field, (2) animals are cryptic or have long or complex life cycles that constrain adequate monitoring, and (3) factors such as predation risk and competition can alter behavior, thus confounding dietary effects with other ecological effects. However, within these constraints, amphipods and isopods provide excellent models for studying relationships among diet, feeding behavior, and fitness because: (1) they have short generation times, (2) they can be cultured on artificial diets that can be reproduced and manipulated, (3) they can be closely monitored under controlled conditions in the laboratory, and (4) they are direct developers, thus allowing fitness-related parameters to be quantified accurately.

The amphipod and isopod species used in our assays are widely distributed along the Atlantic coast of North America (Bousfield 1973, Kensley and Schotte 1989), and all co-occur in association with algae in coastal

North Carolina. Because predators often limit the densities of mesoconsumers in the field (Nelson 1979*a*, Duffy and Hay 1991, 1994), we cultured animals for our assays by placing undefaunated algae in outdoor flow-through tanks where, in the absence of predators, these species attained large numbers and provided a constant supply of animals for our experiments. Some of the amphipod species were more abundant on particular species of algae, and individual cultures were biased toward those algae in order to obtain a larger number of the species we wanted to study. However, none of the cultures contained only one algal or animal species alone.

Every 1–2 weeks, we added additional undefaunated algae (mixtures of green [*Enteromorpha* spp., *Ulva* spp.], red [*Gracilaria tikvahiae*, *Hypnea musciformis*, *Spyridia clavata*], and brown [*Dictyota menstrualis*, *Fucus vesiculosus*, *Sargassum filipendula*] seaweeds) to provide food for the mesograzers and to continually introduce animals from the field so that our culture populations would not diverge from field populations.

The six consumer species used (*Ampithoe longimana*, *Ampithoe valida*, *Cymadusa compta*, *Gammarus mucronatus*, *Elasmopus levis*, and *Paracerceis caudata*) represent a taxonomic range of marine mesoconsumers (Bousfield 1973, Kensley and Schotte 1989). *Ampithoe longimana*, *A. valida*, and *Cymadusa compta* belong to the family Ampithoidae. Species in this family of tube-building amphipods commonly consume algae (Brawley 1992, Poore and Steinberg 1999, Cruz-Rivera and Hay 2000*b*). *Ampithoe longimana* is a relatively sedentary amphipod that lives, feeds, and can reach maturity, on a variety of green, red, and brown macroalgae (Duffy and Hay 1991, 1994, Cruz-Rivera and Hay 2001), but it will also consume detritus and vascular plant material (Nelson 1979*b*). *A. longimana* prefers to consume the brown alga *Dictyota menstrualis*, a chemically defended seaweed common on mudflats and hard bottoms along the North Carolina coast (Schneider and Searles 1991). The dictyols produced by *D. menstrualis* minimally affect feeding by *A. longimana*, but strongly deter feeding by fishes and sea urchins (Hay et al. 1987, 1988*a*, *c*, Duffy and Hay 1994, Cronin and Hay 1996*b*). The preferential association of this amphipod with *Dictyota* provides *A. longimana* with a chemically defended refuge against omnivorous fishes that are deterred by the chemical defenses of the alga (Duffy and Hay 1994). However, *A. longimana* feeding on *D. menstrualis* can induce higher concentrations of dictyols, which then reduces the palatability of the alga to the amphipod (Cronin and Hay 1996*b*).

Ampithoe valida also inhabits, feeds readily on, and reaches maturity on, various green, brown, and red algae (Nicotri 1980, Duffy and Hay 1994, Alonso et al. 1995, Cruz-Rivera and Hay 2000*b*). In North Carolina, *A. valida* is commonly found on the fucalean brown algae *Fucus vesiculosus* and *Sargassum filipendula*

(Deal 1997, Cruz-Rivera and Hay 2000*b*), but it is also found on green seaweeds (Duffy and Hay 1994). Unlike *A. longimana*, this species is more strongly deterred by the chemical defenses of *Dictyota menstrualis* (Duffy and Hay 1994). *Cymadusa compta* consumes algae, detritus, and some vascular plant tissue (Nelson 1979*b*, Zimmerman et al. 1979, Hauxwell et al. 1998), and it can reach maturity feeding on various species of macroalgae alone, although its fitness on monospecific diets of *Dictyota* has not been assessed (Cruz-Rivera and Hay 2000*b*). This amphipod can be abundant in seagrass beds (Nelson 1979*a*, *b*, Stoner 1979) and among green filamentous macroalgae (Hauxwell et al. 1998). In the field, we commonly collected *C. compta* from green and red macroalgae (e.g., *Enteromorpha* spp., *Hypnea musciformis*) growing on shallow mud flats.

Gammarus mucronatus belongs to the family Gammaridae. This amphipod appears to be omnivorous, consuming green, red, and brown macroalgae, detritus, and probably animal matter (Zimmerman et al. 1979, Fredette and Diaz 1986, Duffy and Hay 1994, Cruz-Rivera and Hay 2000*b*). *G. mucronatus* can reach maturity feeding on either algal or animal tissue, but its fitness is enhanced by providing it with multiple algal species, or with mixtures of algal and animal tissues (Cruz-Rivera and Hay 2000*b*). In the laboratory, *G. mucronatus* prefers the green macroalga *Ulva* (Duffy and Hay 1994), but *G. mucronatus* appears to be less dependent on algae than are some other amphipods; individuals feeding on algal-only diets experience decreases in fitness compared to conspecifics on more nutrient-rich diets (Cruz-Rivera and Hay 2000*a*). This amphipod is strongly deterred by the chemical defenses of *D. menstrualis* (Duffy and Hay 1994).

Elasmopus levis is in the family Melitidae. Feeding by *E. levis* has rarely been studied, but *E. levis* appears to be an omnivore (Nelson 1979*b*). It is found associated with invertebrate egg cases, seagrasses, and macroalgae (Nelson 1979*a*, *b*, Stoner 1979, Fox 1980, Duffy 1990). Long-term experiments generally found decreases in fitness when this amphipod was confined to algal diets, as compared to individuals fed diets containing some animal material (Cruz-Rivera and Hay 2000*a*). The scarce data available on gut contents for this species (Nelson 1979*b*) indicated that copepods and detritus were common dietary items.

In addition to the five amphipod species, we also studied one sphaeromatid isopod that is common along the North Carolina coast. *Paracerceis caudata* is commonly found among macroalgae including *Dictyota* (Kensley and Schotte 1989). To our knowledge, no data are available on its feeding behavior. Other species in this genus are known to consume coralline algae (Shuster and Guthrie 1999). Our range of consumers thus included an amphipod that prefers *Dictyota menstrualis*, a congener, one noncongeneric species within the same family, two other amphipod species from two

different families (but within the suborder Gammaridea), and one isopod, representing a different order of crustaceans.

The algal compounds we incorporated into some treatment diets were diterpene alcohols from the brown seaweed *Dictyota menstrualis*. This alga occurs from southern Virginia (USA) throughout the Caribbean (Schneider and Searles 1991). For our experiments, algae were collected from mudflats and seagrass beds at Mitchell Village, near Morehead City, North Carolina, USA (34°42′ N, 76°41′ W). *D. menstrualis* from these habitats can contain higher concentrations of dictyols than other populations that are nearby (Cronin and Hay 1996*b*). *Dictyota menstrualis* produces three closely related terpenoid secondary metabolites, the dictyols pachydictyol A, dictyol E, and dictyodial (Cronin et al. 1995, Cronin and Hay 1996*b*). Pachydictyol A and dictyol E occur at concentrations of 0.007–0.01% and 0.02–0.045% of algal wet mass, respectively, and can deter feeding by fishes, sea urchins, and some mesograzers (Hay et al. 1987, 1988*a*, *c*, Duffy and Hay 1994, Cronin and Hay 1996*b*). The third compound, dictyodial, is unstable (Cronin et al. 1995), thus limiting our ability to use it in long-term bioassays. Indirect evidence suggests that dictyodial might deter grazing by urchins, but appears ineffective against *A. longimana* or fish (Cronin and Hay 1996*a*).

Extraction of dictyols and preparation of artificial foods

Secondary metabolites from *Dictyota menstrualis* were obtained by modifying the methods of Cronin et al. (1995). Algae were brought from the field, spun in a salad spinner to remove excess water, and freeze-dried. A known dry mass of *D. menstrualis* was extracted in 2:1 dichloromethane/ethanol, within a week of collection. Solvents were eliminated using rotary evaporation, and the crude extract was partitioned using 1:1 water/dichloromethane. The dictyols are hydrophobic, so the water-soluble extract was discarded. The lipophylic crude extract was fractionated on a silica flash column, using 1:0, 3:7, 2:3, 1:1, and 0:1 hexanes: ether as eluents. The collected fractions were tested for the presence of pachydictyol A and dictyol E against a standard of purified dictyols using thin layer chromatography (TLC). Fractions containing pachydictyol A and dictyol E were combined, and separated again on silica thick plates (1000 μm layer thickness). Compounds form bands on the plates that can be compared with similarly developed TLC plates containing a dictyols standard. Bands containing pachydictyol A and dictyol E were scraped from the plate and extracted with ether. This final fractionation through thick plate chromatography provided the dictyols we used in all our experiments. Because dictyodial decomposes during freeze-drying (Cronin et al. 1995, Cronin and Hay 1996*a*, *b*), we targeted our extractions toward pachydictyol A and dictyol E. These metabolites remain sta-

ble over prolonged periods, thus allowing us to conduct bioassays and to store the compounds for months to years without noticeable degradation. The integrity of the compounds was tested through time using TLC. TLC is not quantitative, but should detect degradation products if they occur.

To assess the effects of food quality and secondary metabolites on consumers, we incorporated the dictyols at natural concentrations (by dry mass) into two types of basic foods: freeze-dried fish food (Wardley's Total Marine Fish Food Flakes, Hartz Mountain Corporation, Secaucus, New Jersey, USA), and freeze-dried algae (equal masses of *Enteromorpha* sp., *Gracilaria tikvahiae*, *Ulva* sp., and *Hypnea musciformis*). These foods were chosen as diets because previous experiments had shown that they could be used to mimic nutritionally distinct foods, and because we could successfully feed and raise different amphipod species using these two basic foods (Cruz-Rivera and Hay 2000*a*). The fish food was 21 times higher in protein, three times higher in nitrogen, and two times higher in total organic carbon (TOC) than the algal mixture (Cruz-Rivera and Hay 2000*a*). Although these differences in food quality were marked, they fell well within the natural variation in these nutrients that occurs among potential foods within natural marine systems (see *Introduction*).

Treatment foods were prepared by dissolving the dictyols in enough ethyl ether to totally wet the food, and then removing the solvent by rotary evaporation (Hay et al. 1994, 1998). Control foods were treated similarly, but with ethyl ether alone. With this methodology, we created four experimental diets: low quality food (algae), low quality food with chemical defenses (algae + dictyols), high quality food (fish food), and high quality food with chemical defenses (fish food + dictyols).

The foods were offered to the mesograzers as agar-based food strips (methods in Hay et al. 1994, 1998). This technique has been successfully used to test diverse foods, and feeding deterrents in laboratory assays (reviewed in Hay et al. 1998). In this method, the powdered freeze-dried food (algae or fish food here) is evenly mixed with heated agar and water, and poured on strips of plastic screen mesh. The agar-based food adheres to the screen mesh, which is then cut to desired sizes and offered to the experimental animals. Feeding rates are measured by counting the number of squares consumed from the screen mesh.

Food choice and feeding rates

We tested the feeding responses of mesograzers to different foods by running a series of feeding choice and no-choice assays. Adult animals for all experiments were collected from the mesocosms described previously and were never starved before beginning the assays. All choice and no-choice experiments were run in replicate ~230 mL containers. Food choice was tested using both whole algae and artificial diets, in pairwise choice experiments. Whole alga assays were conducted by offering the animals pieces of *D. menstrualis* and the green alga *Enteromorpha intestinalis*, which is not known to contain defensive chemistry. Pieces of each seaweed (50–100 mg) were placed together in replicate containers ($N = 40$ for each mesograzer species). For each mesoconsumer, 4–6 individuals were added to each of 20 of the containers with algae, and allowed to feed for a maximum of two days. The remaining 20 containers received no grazers and served as controls for autogenic changes in algal mass. These controls were interspersed among the feeding replicates. After the feeding period, algal pieces were removed and weighed, and the eaten mass calculated after correcting for autogenic changes (Peterson and Renaud 1989, Cronin and Hay 1996*b*).

Mating behavior of *Gammarus mucronatus* complicated feeding assays for this species. *G. mucronatus* forms precopulatory pairs in which the male grabs a female and swims with her until she molts; insemination occurs at molting (Borowsky 1984). When individuals were collected for these assays, they immediately paired. We assumed that this behavior might affect feeding, but avoided this potential problem by placing only females in half of our replicates and only males in the remaining half.

To test if the effectiveness of *Dictyota* chemical defenses varied with prey nutritional quality, pairwise feeding choice experiments were performed using artificial diets of higher or lower nutritional quality. For each mesograzer species, several individuals were placed in each of 20–30 replicate 230-mL containers and offered a choice of algal-based foods with or without dictyols, or of fish food-based foods with or without dictyols. These experiments tested the effectiveness of the secondary metabolites when contained at natural concentrations in low and high quality foods, respectively. Animals were allowed to feed until ~50% of the food had been consumed from one of the artificial food strips, or for 3 d (to minimize degradation of the foods). Because the compounds were lipid-soluble and stable, they were not lost to the water and did not decompose. Feeding in these assays was quantified by counting the number of squares cleared of food on each strip. Replicates in which no food was eaten were not included in the analysis because they offered no information on feeding preferences.

For the low quality food (depending on the size of the individuals), 4–7 animals of each species were used in each replicate container, and we segregated *G. mucronatus* by sex. Preliminary results showed that our mesograzers fed at slower rates when given higher quality foods (see also Cruz-Rivera and Hay 2000*a*, 2001). Because we needed to minimize microbial degradation of the foods, while maximizing the number of replicates in which feeding occurred, we used 5–10 individuals in the containers holding high quality diets. Although differences in consumer density could potentially confound contrasts between our high and low

quality trials, our results from both assays show similar patterns and suggest that density-dependent effects did not alter the overall patterns of food preference (see *Results*). However, to further minimize any confounding effects of density, we analyzed these assays by quantifying consumption on a per capita basis.

We measured how each of the four food types (low and high quality, with or without dictyols) affected feeding of each of the six mesograzers using no-choice assays. For these assays, replicate groups of each species ($n = 19$ for *A. valida*, $n = 17$ for *C. compta*, and $n = 20$ for all other species) were confined to feeding on each diet alone. The four treatments were interspersed and run simultaneously for each species. Before the experiments, consumers in each replicate container were allowed to feed for 24 h on the food that would be used in the assay. After this period, a new food strip of the same type was placed inside the container, and the experiment was begun. This reduced the number of replicates in which no food was consumed by allowing a short period of acclimation to the artificial foods (E. Cruz-Rivera, *personal observation*). Animals in these no-choice experiments were allowed to feed for a maximum of 48 h. Thus, for all consumers, total time of exposure to our artificial diets was 3 d, as was the case for our choice assays. By measuring feeding rates in this fashion, we were able to assess whether these animals exhibited compensatory feeding on lower quality foods, and how this compensation may be affected by the presence of algal chemical defenses.

Diet consequences on fitness

The effects of food quality and *D. menstrualis* secondary metabolites on consumer fitness were evaluated by raising each mesoconsumer on the four artificial diets. The amphipods we used are direct developers, making it possible to dispense juveniles from the same mother among each of our treatments. Ovigerous females of each amphipod species were individually placed in 16×20 mm petri dishes, and allowed to hatch their young over a one-week period. Females were fed on *Enteromorpha* and fish food during this period. Five newborns were collected from each female, and one was randomly assigned to each of the four diets and to a starvation treatment receiving no food. To determine if the agar we used as a matrix for our artificial diets could sustain any of the amphipods, we also conducted assays growing each amphipod species on our agar matrix alone or on no food. Although the isopod is also a direct developer, eggs are brooded internally (Kensley and Schotte 1989) and ovigerous females are difficult to determine, so we used young individuals collected from our cultures. We measured each of these juveniles from head to telson, and randomly assigned them to one of our four fed treatments or to our starvation treatment. We did not measure the initial size of amphipods before our experiments. However, with a microscopic resolution of 20 μm, differ-ences in size among siblings of these amphipods, as well as among families, are small after one week of growth (E. Cruz-Rivera, *unpublished data*). Because the newborns were assigned randomly, variance among families would contribute equally to each of the experimental treatments, making it reasonable to assume that each of our treatments started with amphipods of equivalent size. Numbers of replicate individuals per treatment were: *A. longimana* = 60, *A. valida* = 32, *C. compta* = 40, *G. mucronatus* = 37, *E. levis* = 60, and *P. caudata* = 40. Sample size varied among species due to differences in availability of ovigerous females from which to collect young amphipods.

Food and water were replaced daily, and the petri dishes were scrubbed every two days to eliminate fecal pellets, and reduce the growth of microbes that could serve as alternative food sources or potential pathogens. We measured survivorship, growth, fecundity, and associated reproductive traits (e.g., female size at ovulation, egg volume) for all the amphipod species, but only survivorship and growth for the isopods. Survivorship (in days) was measured as the number of individuals alive until the first female became ovigerous in any of the treatments (in those species that ovulated) or until the experiment was terminated. This was necessary because females were sacrificed at ovulation in order to quantify fecundity. However, for those species that ovulated, we also analyzed survivorship of the males alone; this should have allowed treatment effects to become apparent even if they took longer to develop.

Growth (in micrometers per day) was calculated by measuring individuals at the time of death (subtracting the initial length in the case of the isopods), and dividing by the time lived in the experiments. Amphipods were measured from the rostrum to the last large coxa, and isopods were measured from head to telson. A few individuals died early in our assays and could not be measured because they were deformed from being trapped in the surface tension following molting or because of microbial degradation. These individuals were excluded from the analysis.

Ovigerous amphipods in our long-term assays were fixed in formalin, measured, and decapitated to extract the eggs from the brood pouch. For species that produced eggs, we measured female growth (micrometers per day), size at ovulation (micrometers), days to ovulation, clutch size, and we calculated individual egg volumes (in microliters) by measuring the length and width of eggs, and applying the formula for the volume of a spheroid. Individual egg volumes were summed to obtain total clutch volumes. For *G. mucronatus*, which did not produce eggs during our experiments, we assessed reproductive potential by calculating the area of gonads visible on each side of the female body and dividing by two (square micrometers per female side). Additionally, for some amphipod species in which sexual dimorphism was clearly noticeable, we

were able to measure the percentage of females that successfully ovulated in each treatment during our experiments. This also allowed us to calculate growth in all females (rather than just ovulating females) within those experimental populations.

The long-term assays were terminated when: (1) all discernible females had produced eggs (*A. longimana* and *C. compta*), (2) when individuals of one treatment had reached adulthood disproportionately earlier than the rest (*A. valida* and *E. levis*), or (3) when most individuals in our experimental population had developed obvious adult characters (*G. mucronatus* and *P. caudata*). These criteria were necessary because growth rate decreases with size, and dietary effects on growth could have been obscured by extending the culturing period without measuring the animals. Upon termination of the experiments, all individuals remaining alive were killed, measured, and, when possible, sexed.

Statistical analyses

Survivorship data were analyzed using chi-square analyses for main effects and groupings. Because females were killed for measurement following ovulation, we analyzed survivorship until the first reproductive female became ovigerous in any of the treatments for that species. Survivorship of the males alone also was analyzed in a similar fashion in hopes of detecting longer term effects. This expanded the time period over which we could evaluate survivorship, especially for species whose females ovulated rapidly.

Homogeneity of variances was tested using F_{max} tests. Pairwise comparisons of feeding preferences were performed using two-tailed, paired-sample t tests. When data were not normally distributed, and transformations failed to correct for this, we used Wilcoxon paired-sample tests. Because both our experimental factors were fixed, data on feeding rates (no-choice experiments), growth, length and growth of females, days to ovulation, clutch size, egg volume, and clutch volume were analyzed by two-way Model I ANOVA. Post hoc comparisons were made using Tukey's honestly significant difference tests (hsd), adjusted for unequal sample sizes by using the Kramer modification (Day and Quinn 1989). If heterogeneity among variances could not be corrected through transformations, we used Welch's tests (Day and Quinn 1989).

We used Type I procedures for two-way ANOVA when sample sizes were balanced. However, we followed the recommendations of Shaw and Mitchell-Olds (1993), and used Type III ANOVA procedures for dealing with unbalanced samples. Although the multiway analysis of unbalanced sample sizes has limitations, particularly in detecting interactions between factors, it has several advantages over the traditional approaches for analyzing unbalanced data sets (Shaw and Mitchell-Olds 1993). Cases in which only two of the four treatments could be compared (due to few replicates in the other treatments) were analyzed with unpaired t tests. For those consumer species in which we could differentiate sexes, the frequencies of females ovulating in each treatment were analyzed with chi-square analyses.

RESULTS

Effects of food quality and secondary chemistry on consumer feeding

When offered a choice between the algae *Dictyota menstrualis* and *Enteromorpha intestinalis*, all six mesograzer species strongly preferred *Enteromorpha* ($P < 0.001$ for all six species, paired-sample t tests; Fig. 1). However, the relative consumption of the two algae varied among consumer species. *Ampithoe longimana* consumed 49% more *Enteromorpha* than *Dictyota*, whereas the other five consumers ate >80% more *Enteromorpha*.

When offered a choice of food with vs. without natural concentrations of dictyols, all five species of amphipods avoided the dictyol containing foods whether they were made with less nutritional algal tissues ($P \leq 0.007$; Fig. 2) or with more nutritional fish foods ($P \leq 0.048$; Fig. 3). Despite the isopod *Paracerceis caudata* having a preference for whole algae that paralleled the patterns shown by all five amphipod species (Fig. 1), feeding by the isopod was not affected by adding dictyols to either low or high quality foods ($P = 0.892$ and $P = 0.695$, paired-sample t tests; Figs. 2 and 3). Thus, when a choice between foods was available, dictyols significantly deterred feeding by the five amphipods, but did not affect feeding by the isopod. Although different numbers of animals were used for assays with low or high quality foods (4–7 vs. 5–10 individuals/replicate, respectively), the almost identical patterns of preference for each of the mesograzers when compared between these two assays suggested that density-dependent effects did not alter the overall patterns of food preference for any of the consumers.

When animals were confined to one of each of our four diets (i.e., with no choice of other foods), food quality significantly affected feeding for five of the six consumers (Fig. 4). Consumers usually compensated for low quality by consuming more total food. For all species but *E. levis*, feeding on the low quality food without dictyols was a significant 50–70% higher than feeding on the high quality food without dictyols.

In these same assays, dictyols significantly affected feeding rates for four of the six species assayed, with significant interactions between food quality and dictyols ($Q \times D$) occurring for three species (Fig. 4). For all of the species demonstrating interactions, dictyols tended to be more deterrent in the lower quality, algal-based food than in the higher quality food. The addition of dictyols to the low quality food caused a 23% reduction in *A. longimana* feeding compared to the same food without the dictyols. This effect did not occur in the high quality diet, accounting for the significant
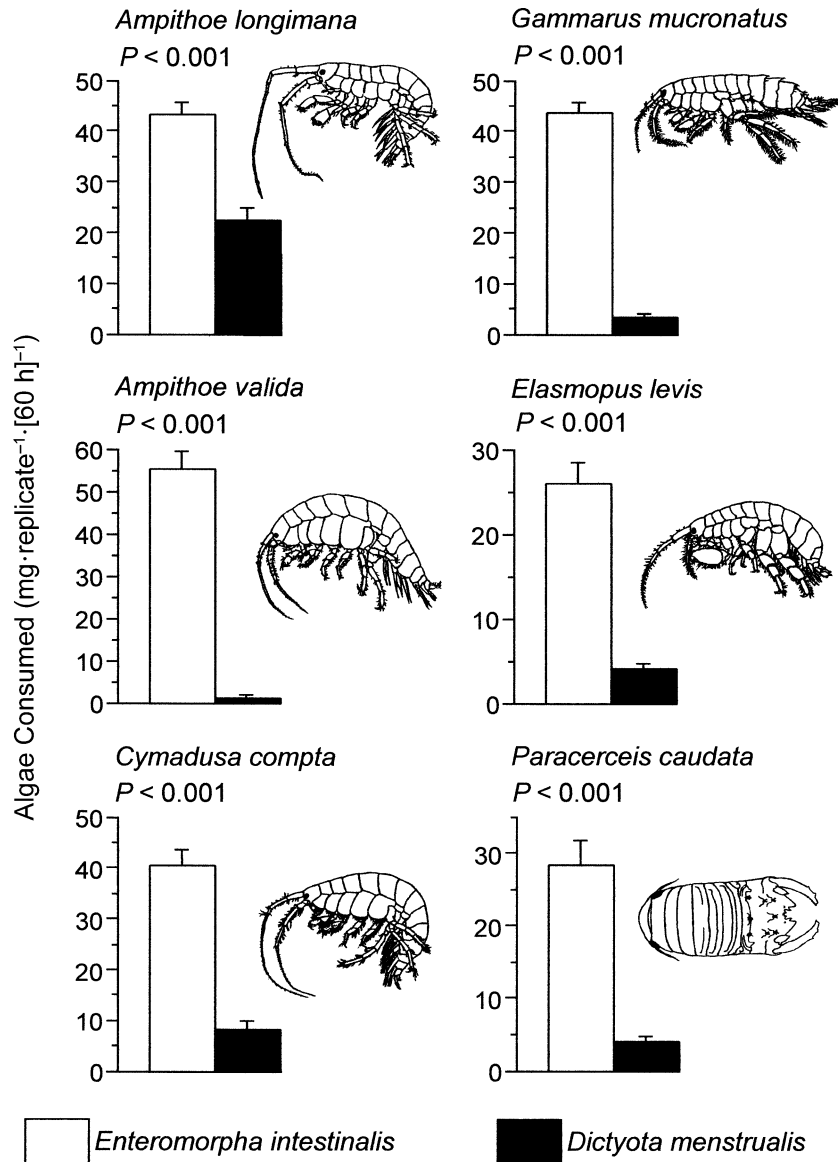
Fig. 1. Feeding by six species of North Carolina mesograzers when simultaneously offered two common macroalgae (mean + 1 SE). $N = 20$ for each species, with each container holding 4–6 individual mesograzers. Analyses were by paired-sample t tests.

quality × dictyols (Q × D, $P = 0.019$, two-way ANOVA; Fig. 4) interaction. In spite of these differences in feeding rates, post hoc tests could not detect significant differences between the low quality treatments with vs. without dictyols. For *G. mucronatus*, *E. levis*, and *P. caudata*, interactions of dictyols and food quality were more consistent. Dictyols significantly decreased feeding on the lower quality, algal-based food, but not on the more nutritious food (Fig. 4). In fact, for *G. mucronatus* and *E. levis*, the pattern suggesting compensatory feeding on low quality foods (i.e., eating more low quality than high quality food when dictyols were lacking) was reversed by the presence of the dictyols.

When compared to feeding on low quality foods, feeding by *A. valida* was diminished on both types of high quality foods; feeding was also suppressed on foods containing dictyols (Quality, $P < 0.001$; Dictyols, $P < 0.001$; Q × D, $P = 0.249$; Fig. 4). For this species, overall consumption of low quality foods was 70% higher than of high quality foods, and 55% higher for foods without dictyols than for dictyol-containing foods. Dictyols decreased feeding in a similar way on both the higher quality and lower quality foods (Fig. 4). This species thus compensated for lower quality by consuming more food, but addition of the dictyols to the lower quality food limited their ability to compensate. Feeding by *C. compta* was strongly affected by
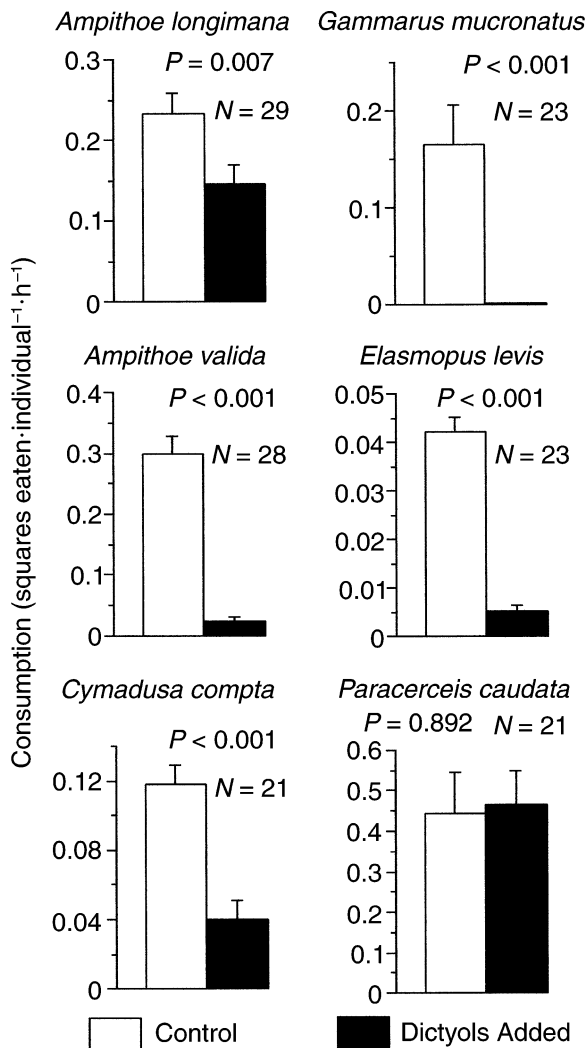
Fig. 2. Effects of *Dictyota menstrualis* chemical defenses (the dictyols pachydictyol A and dictyol E) on the feeding choices of six sympatric consumers when natural concentrations of these metabolites were incorporated into artificial foods of low nutritional quality (palatable algae). Animals had simultaneous access to both foods. Analyses were by paired-sample *t* tests or Wilcoxon paired-sample tests. Bars represent means + 1 SE.

nutritional quality, but not dictyols (Quality, $P < 0.001$; Dictyols, $P = 0.348$; Q × D, $P = 0.565$; Fig. 4). *Cymadusa* consumed 64% more of the low quality compared to the high quality foods, and dictyols did not significantly affect this pattern of compensatory feeding. In contrast to the assays when a choice of foods was available (Figs. 2 and 3), feeding by the isopod *P. caudata* was affected not only by food quality ($P = 0.004$), but also by dictyols ($P = 0.031$). The interaction was not significant (Q × D, $P = 0.090$; Fig. 4). Post hoc comparisons demonstrated that dictyols strongly decreased feeding rates on the low quality food, but not on the high quality food (Fig. 4).

Diet effects on fitness

We determined the effects of food quality and dictyols on survivorship, growth, and fecundity by culturing each of the six crustaceans on our four artificial diets and on a starvation control. Because these experiments were conducted over the span of approximately a year, we periodically tested for the presence of the dictyols, or potential degradation products, in our artificial foods by using thin-layer chromatography (TLC). The compounds were always present, and no obvious breakdown products were detected.

The agar matrix in which our diets were imbedded was not of nutritional benefit to any of the amphipods we assayed; survivorship of the starved amphipods never differed from survivorship of those with access to agar alone (Fig. 5). In our long-term assays, all
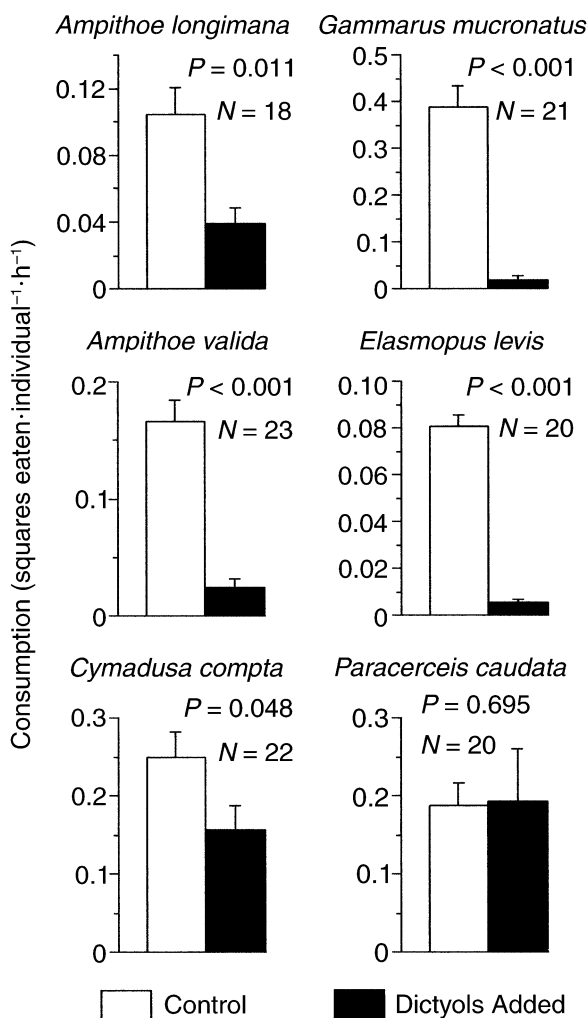

Fig. 3. Effects of *Dictyota menstrualis* chemical defenses (the dictyols pachydictyol A and dictyol E) on the feeding choices of six sympatric consumers when natural concentrations of these metabolites were incorporated into artificial foods of high nutritional quality (commercial fish food). Symbols and analyses are as in Fig. 2.
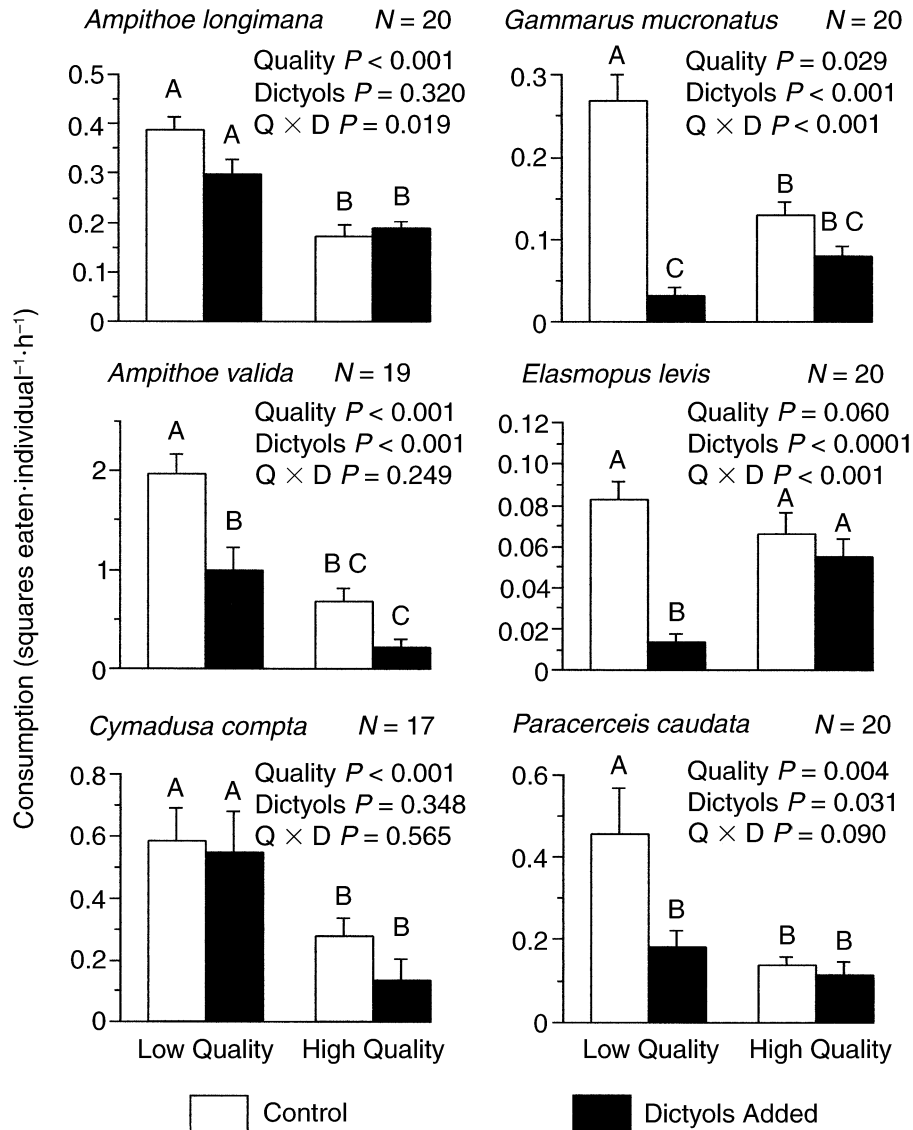
FIG. 4. Effects of natural concentrations of dictyols (secondary metabolites from *Dictyota menstrualis*) and of food nutritional quality on the feeding of six sympatric mesograzers (mean + 1 SE). Individuals were confined to feeding on one food type and thus had no choice among foods. Analyses were by two-way ANOVA followed by Tukey's hsd or Welch's tests for post hoc comparisons. Letters above bars indicate significant groupings of treatments.

starved amphipods died within 3–7 days (Fig. 6), but the majority of the starved isopods lived for ~30 days.

Survivorship as a function of diet varied considerably among species (Fig. 6). *Ampithoe longimana*, which readily consumes dictyol containing foods when given no choice (Fig. 4), survived similarly, and well, on all four diets. Because females of *A. longimana* began ovulating after only 10 days (necessitating that we sacrifice them to measure egg size and number), we were concerned that this limited duration compromised our ability to detect treatment effects on survivorship. We therefore analyzed survivorship of the males alone, because they fed on the diets for a duration of 23 days.

Survivorship of the males paralleled survivorship of the entire experimental population; no differences in survivorship were found among fed treatments. Survivorship of *Cymadusa compta* also did not differ among fed treatments, although survivorship on the low quality food with dictyols tended to be lower (Fig. 6). However, for this species, survivorship on all treatments was <50%, suggesting that our culture methods may have been stressful for this species. Analyzing only the males of this species revealed a similar pattern; overall mortality was high and differences among treatments were nonsignificant. Thus, dictyols did not affect survivorship of the two species whose feeding was not
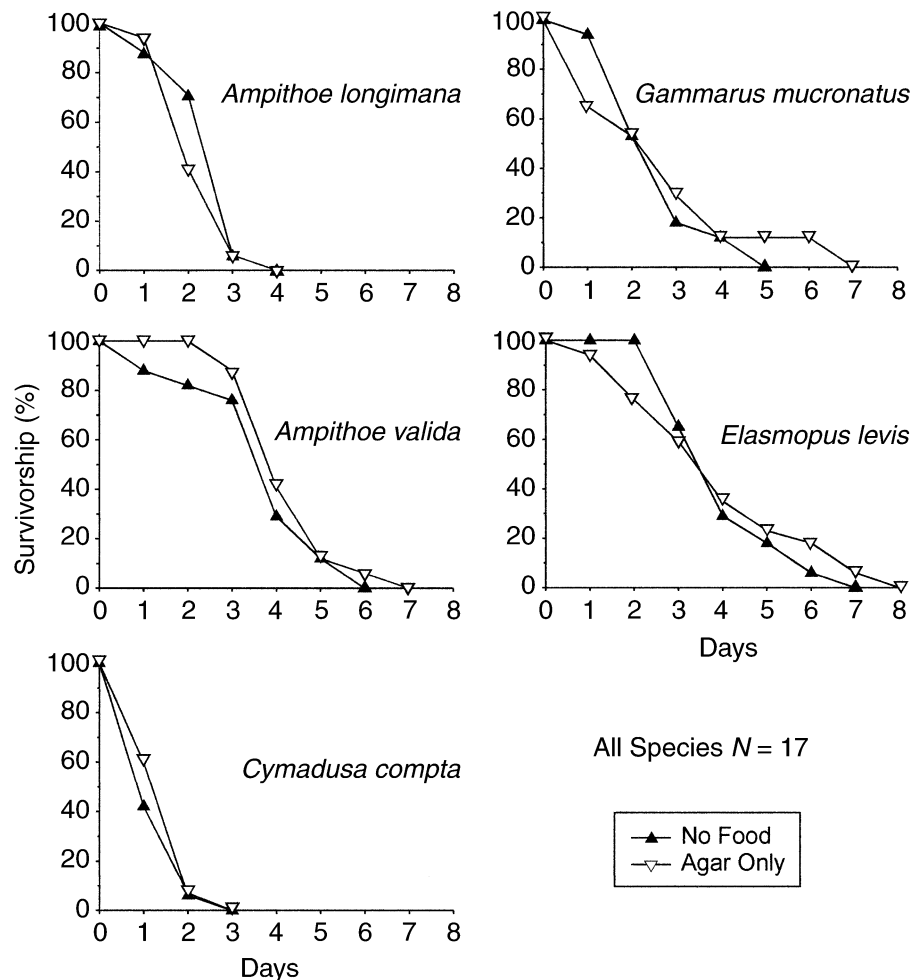
FIG. 5.   Survivorship of amphipods when raised either on agar alone or without any potential food.

significantly affected by dictyols in our multifactorial feeding assay (Fig. 4).

In contrast to *Ampithoe longimana*, survivorship of *A. valida* was significantly decreased by dictyols, regardless of nutritional quality of the diets. Food quality alone had no effect on survivorship for *A. valida* (Fig. 6). Effects of diet on survivorship of *Gammarus mucronatus* were ultimately similar to those of *A. valida*, but there were fundamental differences in the temporal development of these patterns. Nutritional quality of the food alone did not affect survivorship; however, food quality interacted with the dictyols to decrease survivorship at different rates. In the low quality food containing dictyols, mortality was 85% during the first week, paralleling the mortality observed in starvation controls. By day 8, the difference in survivorship between the low quality food with dictyols treatment and the other three treatments was highly significant ($P <$ 0.001, chi-square test). In contrast, it took 28 days for dictyols placed in the high quality food to produce an equivalent decrease in survivorship. The negative ef-

fects of dictyols on survivorship were clearly delayed by the higher quality diet.

For *Elasmopus levis*, survivorship was greatest on high quality foods regardless of dictyol presence or absence, but survivorship was lowered significantly if dictyols were added to the low quality food (Fig. 6). Survivorship on low quality food without dictyols was intermediate.

For the isopod *Paracerceis caudata*, diets containing dictyols tended to enhance survivorship (Fig. 6). The highest survivorship occurred in the low quality diet with dictyols. Survivorship was intermediate for the high quality food with dictyols, and was lowest for the two no-dictyols diets.

Consistent with the patterns for survivorship, individual growth rates for the whole experimental populations of *A. longimana* and *C. compta* were not affected by either food quality or dictyols (Fig. 7). In contrast, nutritional quality strongly affected growth of *A. valida*, *G. mucronatus*, *E. levis*, and *P. caudata* (Quality $P \leq$ 0.004, two-way ANOVA, Fig. 7). For
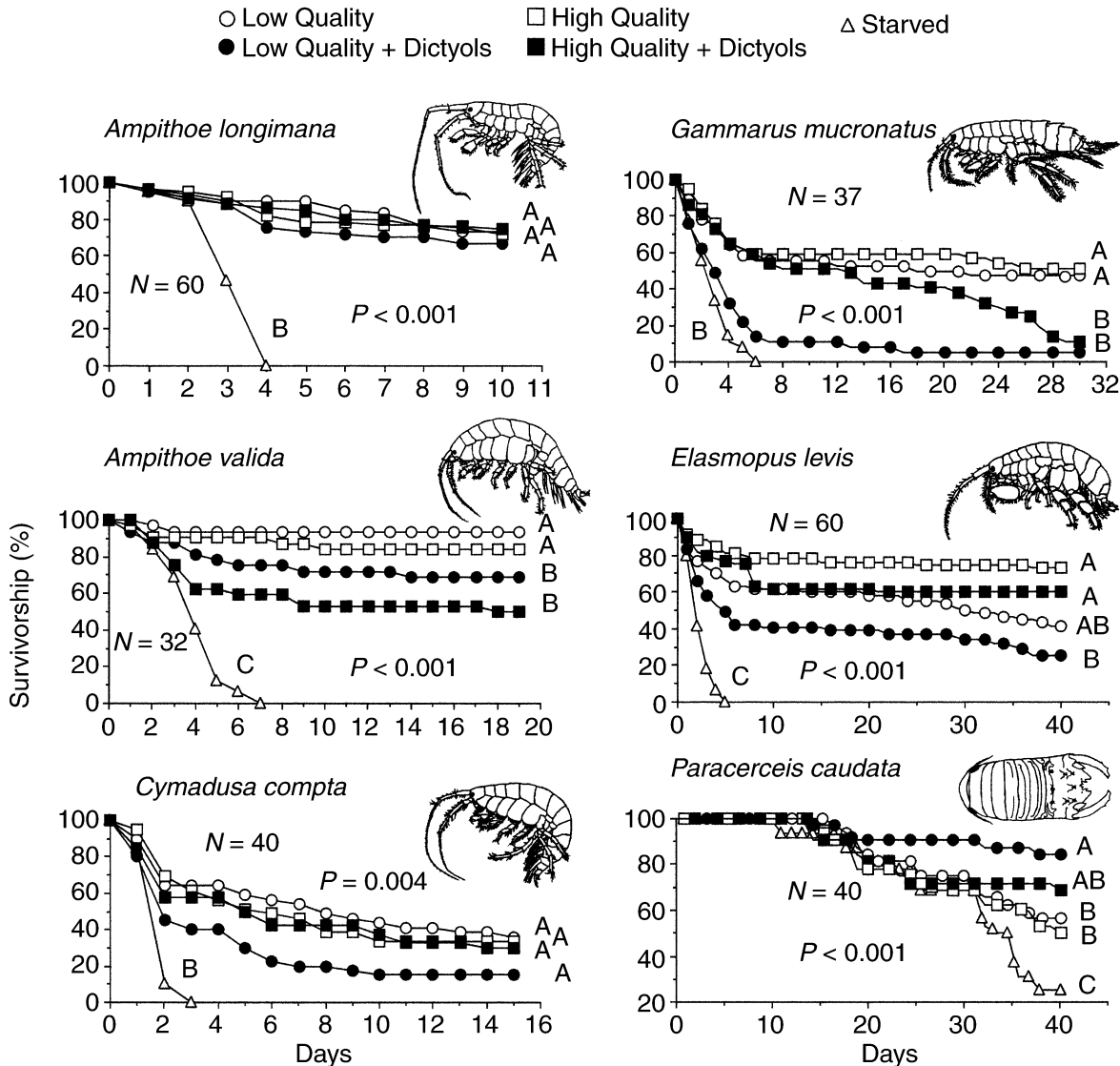
FIG. 6. Survivorship of amphipods and an isopod raised on one of four experimental diets or starved. Chi-square analyses were used to test for differences among treatments. Letters on the right indicate significant groupings among treatments. Analyses account for surviving individuals either on the day the first female ovulated in any of the treatments or when the experiment was terminated (see *Methods*).

each of these four species, growth was reduced by 20–40% when fed on the lower quality foods. The dictyols significantly reduced growth of *A. valida* and *E. levis*, but had no effects on growth of any of the other species when the entire experimental populations were considered. In *A. valida*, the dictyols reduced growth by 12% whereas low food quality reduced growth by ~40%. For *E. levis*, dictyols and low nutritional quality caused more similar decreases in growth (14–20%). For mean growth, we could not detect any interactions between diet quality and dictyols for any of the consumers tested (Fig. 7). Although food quality had a significant effect on the growth of *P. caudata*, post hoc comparisons

using the less powerful Welch's test failed to detect significant differences among any of the means (Fig. 7).

Four of the five amphipod species ovulated during our long-term experiments, allowing us to quantify the effects of diet on reproductive traits. *Gammarus mucronatus* never ovulated, but we were able to measure reproductive potential by quantifying the size of the gonads. We were unable to quantify fecundity in the isopod because it did not produce visible eggs.

All *Ampithoe longimana* females in each treatment ovulated during the course of our experiments. Although growth of *A. longimana* was similar across all
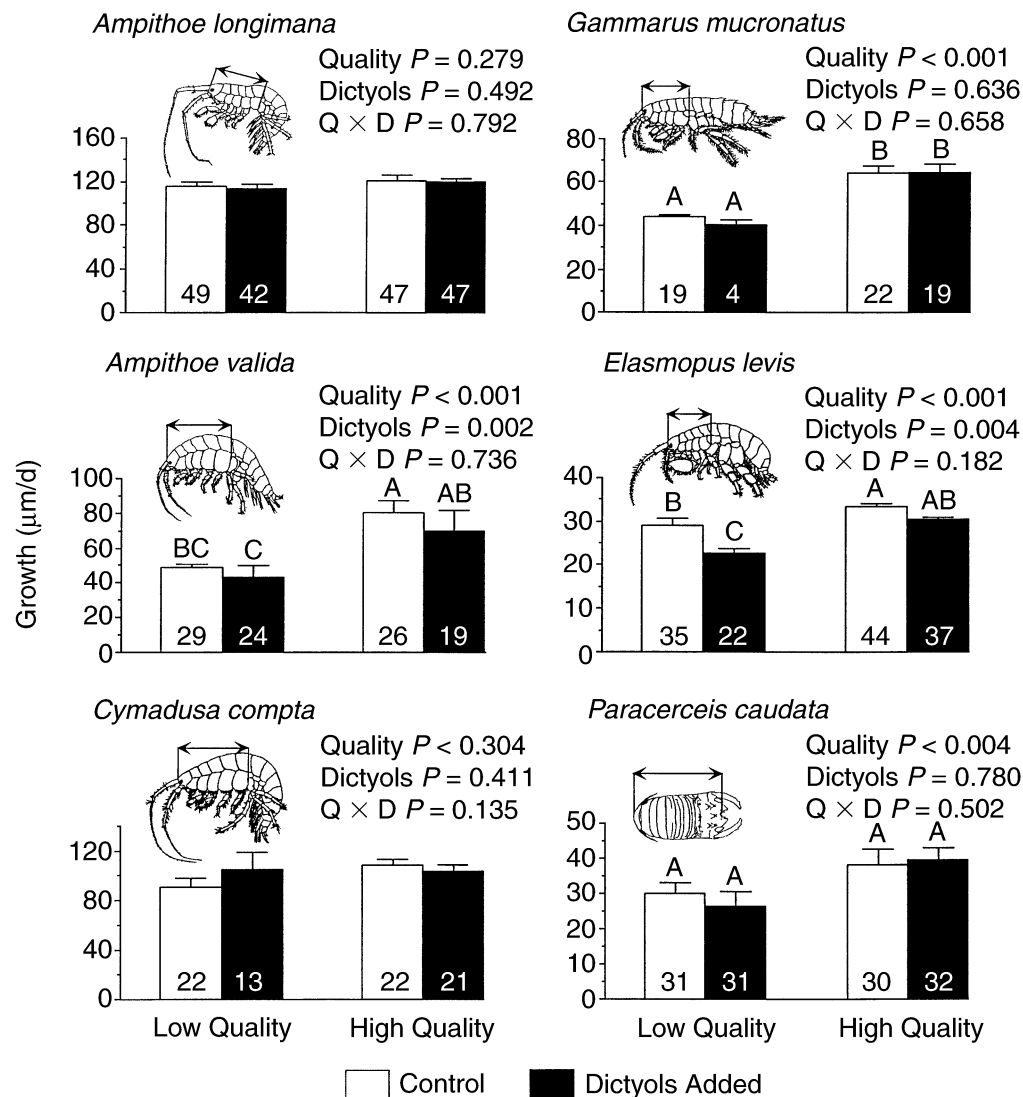
FIG. 7. Effects of each of the four experimental diets on the growth of six species of mesograzers (mean + 1 SE). Arrows on the animals indicate how length measures were made for each species. The number of measured replicates per treatment is given at the base of each bar. Analyses and groupings are from two-way ANOVA followed by Tukey's hsd.

experimental foods when the entire experimental population was considered (Fig. 7), the dictyols (but not food quality) caused a significant reduction (~10%) in the growth of the females (Dictyols, $P = 0.006$, two-way ANOVA; Fig. 8). Dictyols, however, did not significantly affect any of the other fitness parameters we measured. Food quality, in contrast, caused a small (~8%), but significant, reduction in the volume of individual eggs (Quality, $P = 0.036$, two-way ANOVA, Fig. 8), which, when added with non significant trends in numbers of eggs/female, caused a significant decrease of ~30% in total clutch volume (Quality, $P = 0.024$, two-way ANOVA). Food quality had no significant effect on any of the other reproductive traits measured. Although for *A. longimana* both egg volume and

clutch volume were significantly affected by food nutritional quality, post hoc tests could not detect differences among treatments. There were no nutritional quality × dictyols (Q × D) interactions for any of the reproductive parameters measured for *Ampithoe longimana*.

In contrast to the minimal effects of dictyols on *Ampithoe longimana*, *A. valida* experienced dramatic reductions in fecundity when feeding on dictyol-containing foods (Fig. 9). When raised on the low quality or high quality foods with dictyols, 0% and 22% of the females ovulated, respectively. In contrast, 71% and 100% of the females ovulated when feeding on low quality or high quality food without dictyols. Thus, the number of females ovulating was significantly lower
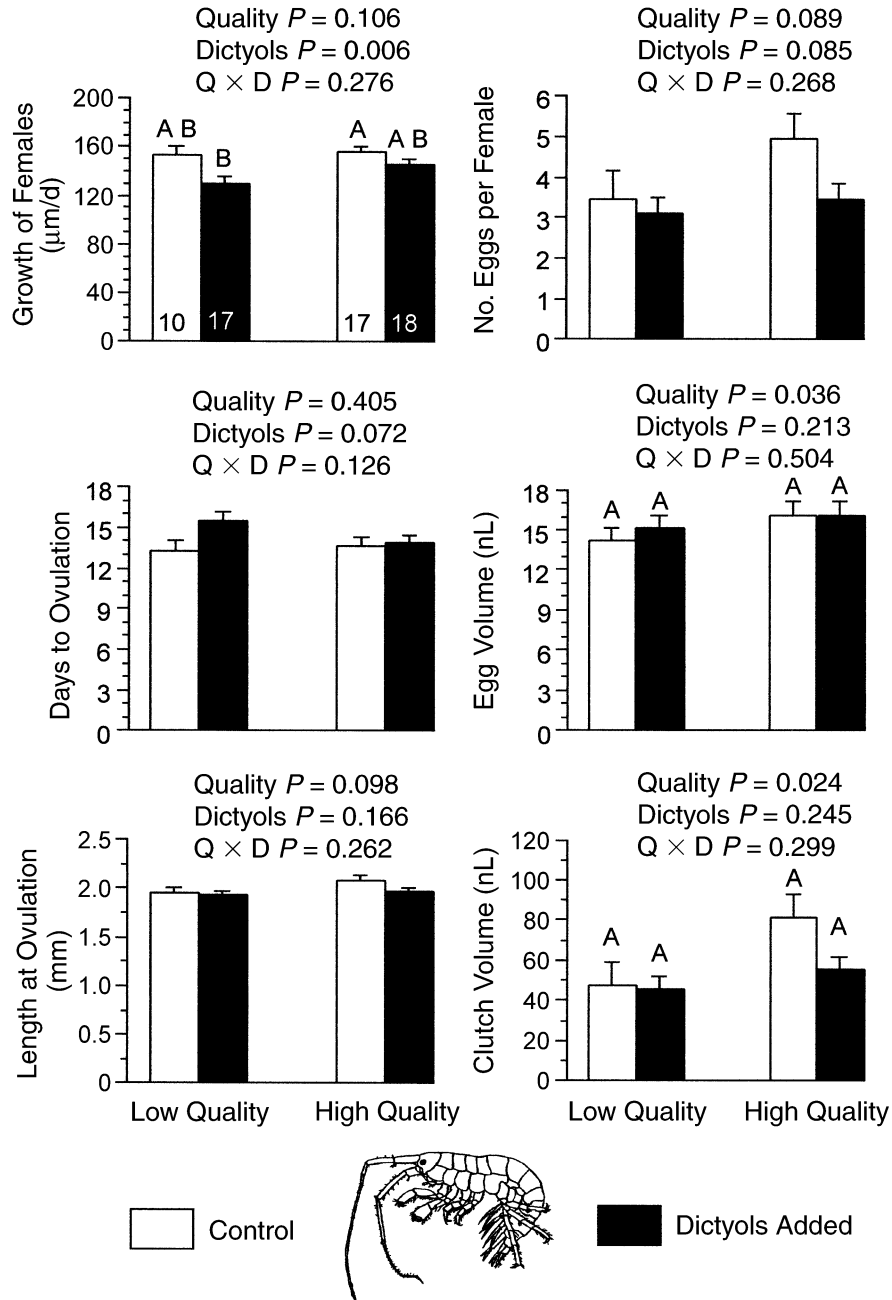
FIG. 8. Selected fitness parameters measured on *Ampithoe longimana* females raised on low or high quality foods, with or without dictyols. Bars indicate means + 1 SE. All females in these experiments produced eggs. The number of females per treatment is given at the base of the bars on the first graph. Analyses and groupings are from two-way ANOVA and Tukey's hsd.

on the foods containing dictyols ($P < 0.001$, chi-square; Fig. 9). In the absence of dictyols, lower food quality also suppressed ovulation. Although no interactions between nutritional quality and dictyols were detected for *A. valida* growth when the whole experimental population was considered (Fig. 7), if only females were considered, food quality and dictyols in-

teracted to affect growth ($Q \times D$, $P < 0.001$, two-way ANOVA; Fig. 9). Post hoc tests showed that growth of the females was significantly reduced both by low quality of the food, and by the presence of the dictyols in the food, but that the effect of the dictyols on female growth was more pronounced on the high, than on the low, quality diet. Dictyols decreased female growth by
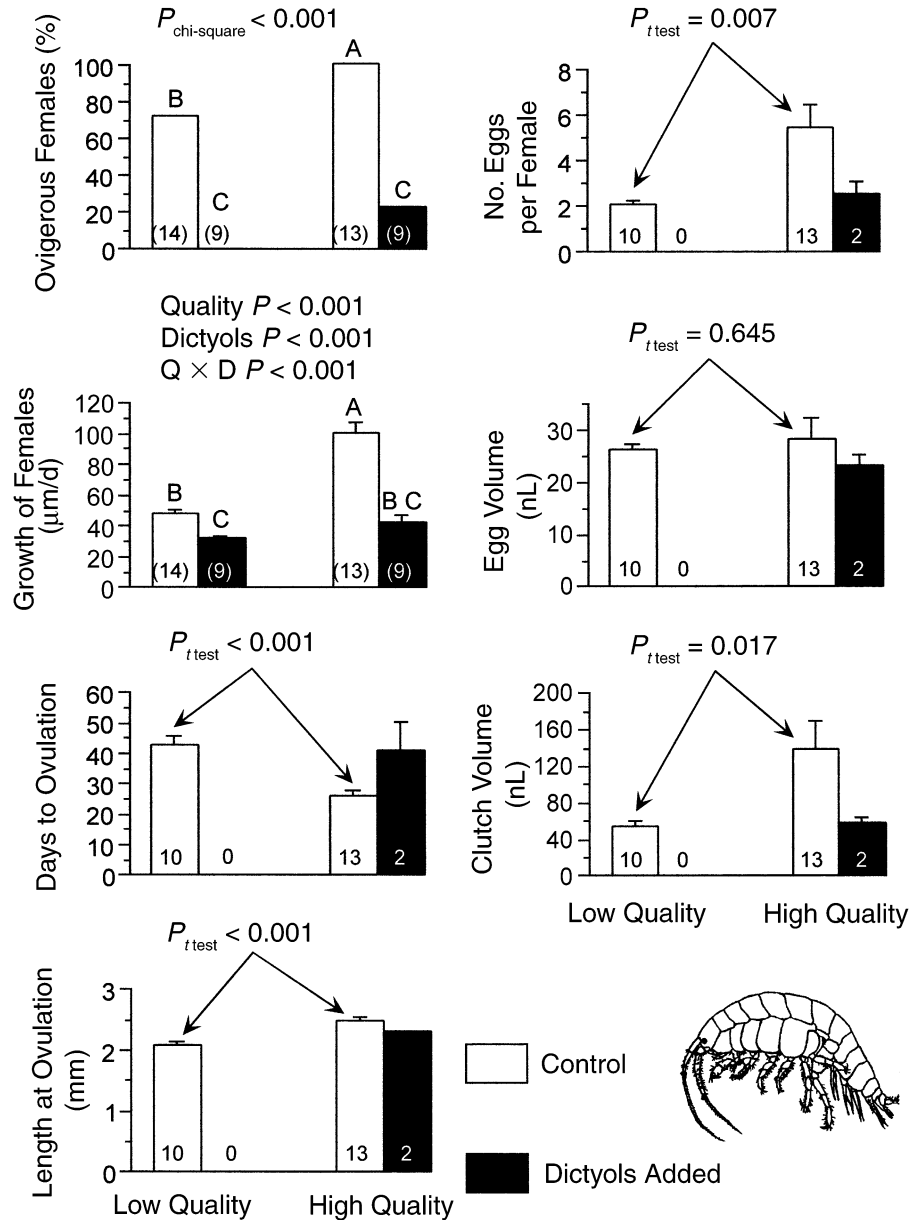
Fig. 9. Selected fitness parameters measured on *Ampithoe valida* females. Significance level and groupings from the number of ovigerous females in each treatment (first graph) are from chi-square analyses. Numbers in parentheses at the base of the bars indicate the total number of females present in each treatment. Numbers not in parentheses at the base of bars or above the *x*-axes indicate the number of females that produced eggs in each treatment. Bars represent means ($+1$ SE with the exception of the first graph). Female growth was analyzed by two-way ANOVA followed by Tukey's hsd (second graph, left column). Other fitness parameters were analyzed with *t* tests, contrasting only the two no-dictyols treatments (pointed by the arrows). The extremely low numbers of females ovulating when fed on dictyols (0 and 2) precluded contrasts with these treatments. Data from the dictyols treatments are shown only for comparison.

58% when placed in the high quality diet, compared to 33% when placed in the low quality food. Growth on the high quality food with dictyols did not differ from growth on the low quality food with or without the compounds (Fig. 9).

Dictyols decreased ovulation so dramatically in *A. valida* that the effects of dictyols on most other repro-

ductive traits could not be reliably analyzed (due to the low number of ovulating females in the treatments). For these other traits, we statistically evaluated only the effects of food nutritional quality by comparing the low and high quality diets without dictyols. When feeding on the lower quality food, females ovulated significantly later ($P < 0.001$, unpaired *t* test), were small-
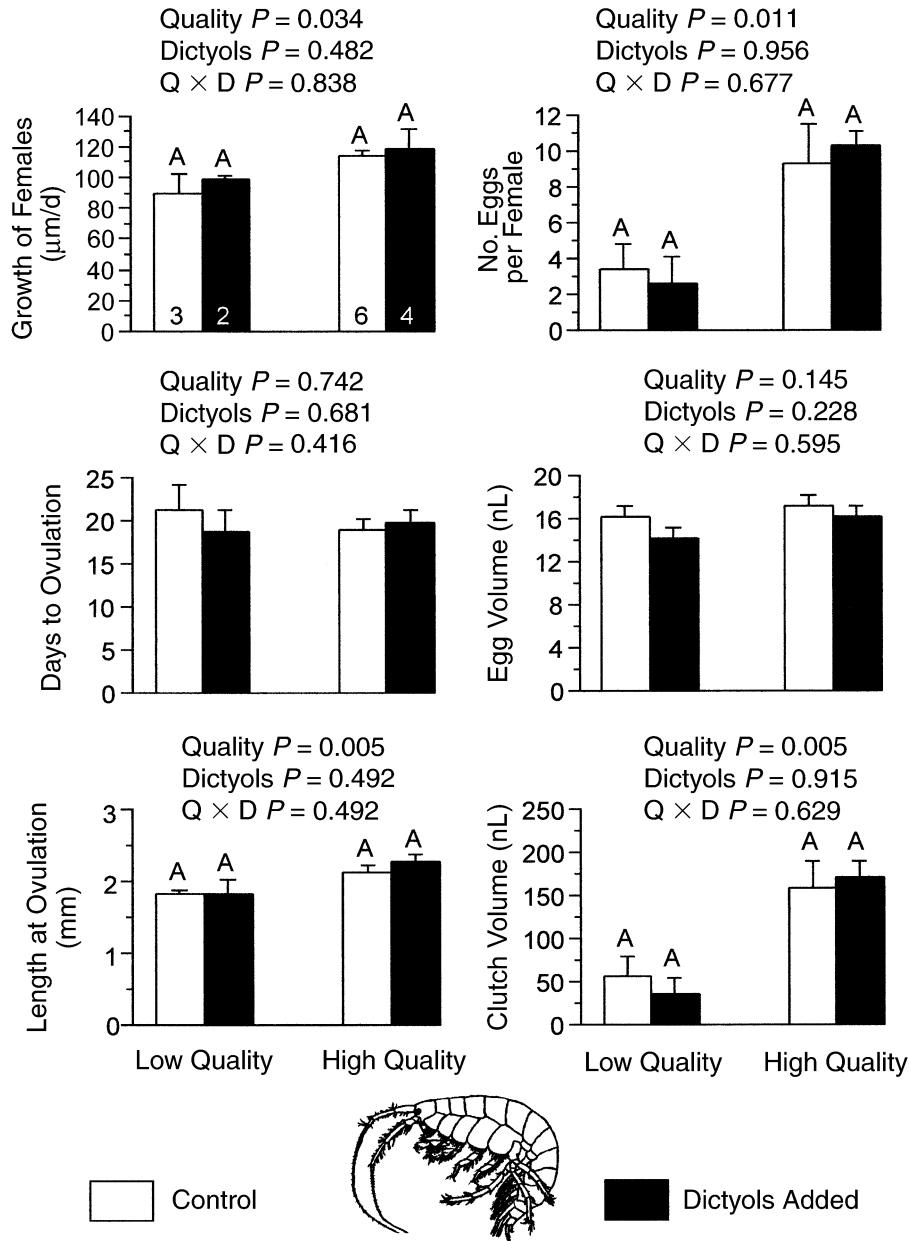
Fig. 10. Selected fitness parameters for *Cymadusa compta* females. All females in the experiment produced eggs. Symbols and analyses are as in Fig. 7. The low number of surviving females (2–6) constrained the power of some of these statistical analyses (see *Results*).

er at ovulation ($P = 0.001$, unpaired t test), produced fewer eggs ($P = 0.007$, unpaired t test), and had smaller clutch volumes ($P = 0.017$, unpaired t test) than females feeding on the higher quality food without dictyols (Fig. 9). The volume of individual eggs was not affected by diet quality ($P = 0.645$, unpaired t test).

Because mortality of *Cymadusa compta* was high among all treatments (Fig. 6), the number of females from which we could gather reproductive information was small. This constrained statistical power of our ANOVA. Possibly because of this, none of the post hoc

comparisons could detect differences among treatments in those cases where significant main effects were detectable. All surviving *Cymadusa* females produced eggs, with lower quality foods significantly decreasing female growth, length at ovulation, number of eggs produced, and total clutch volume (Fig. 10). Age at ovulation and the volume of individual eggs were unaffected by either food quality or dictyols, and no significant Q × D interactions were found for any of the reproductive parameters measured. Dictyols had no detectable effects on any reproductive trait for *Cymadusa*

compta (Fig. 10). While sample size constrained our analyses, effect size increased statistical power in those cases where significant effects were found. For example, our power to detect food quality effects was lowest (0.60) for female growth, but it was between 0.80 and 0.90 for length at ovulation, number of eggs, and clutch volume. Nevertheless, power was <0.40 for most other analyses and results from those analyses should be interpreted cautiously.

High mortality of *Gammarus mucronatus* in the dictyol treatments (Fig. 6) prevented us from determining the effects of dictyols on reproductive traits. We did not observe gonad development in any of the few survivors from the dictyol treatments. Therefore, we analyzed only the effects of food quality on females by comparing the low and high quality foods without dictyols. Low food quality reduced female size ($P <$ 0.001, unpaired *t* test), and caused a large reduction (76%) in gonad size ($P = 0.004$, unpaired *t* test; Fig. 11).

For *Elasmopus levis*, 0% of the females consuming low quality foods ovulated (Fig. 12). In contrast, 74% and 79% of females consuming high quality food with or without dictyols ovulated. Therefore, low food quality completely suppressed ovulation in this species ($P <$ 0.001, chi-square test; Fig. 12). Both food quality and dictyols affected female growth (Quality, $P < 0.001$; Dictyols, $P = 0.022$; two-way ANOVA; Fig. 12). Female length at ovulation was suppressed by dictyols in the high quality food ($P = 0.010$, unpaired *t* test). Dictyols in the high quality diet had no effects on female age at ovulation, clutch size, volume of individual eggs, or total volume of the clutch (Fig. 12).

DISCUSSION

Small marine herbivores can dramatically reduce their susceptibility to predators by associating with, or specializing on, chemically defended hosts (Hay and Steinberg 1992, Hay 1996, Paul et al. 2001). One of our test species, *Ampithoe longimana*, achieves this escape from consumers by selectively living on *Dictyota* and being able to retain high fitness while feeding on this dictyol-rich diet (Hay et al. 1987, Duffy and Hay 1991, 1994, Cruz-Rivera and Hay 2001). The other sympatric mesograzers we studied do not form such associations with *Dictyota*. Our assays evaluated the potential roles of behavior-based vs. physiologically based reasons for these differences by comparing the effects of food quality and chemical defenses on these species *as if* they were constrained to behave like *A. longimana*. Our results show a complex scenario in which prey chemical defenses and nutritional quality can operate independently, or via interactive effects, to determine the effects of diet on consumer feeding behavior and fitness.

Effects of food quality and chemical defenses on consumer feeding behavior

When given a choice between *Dictyota* and *Enteromorpha*, all six consumers preferred *Enteromorpha*
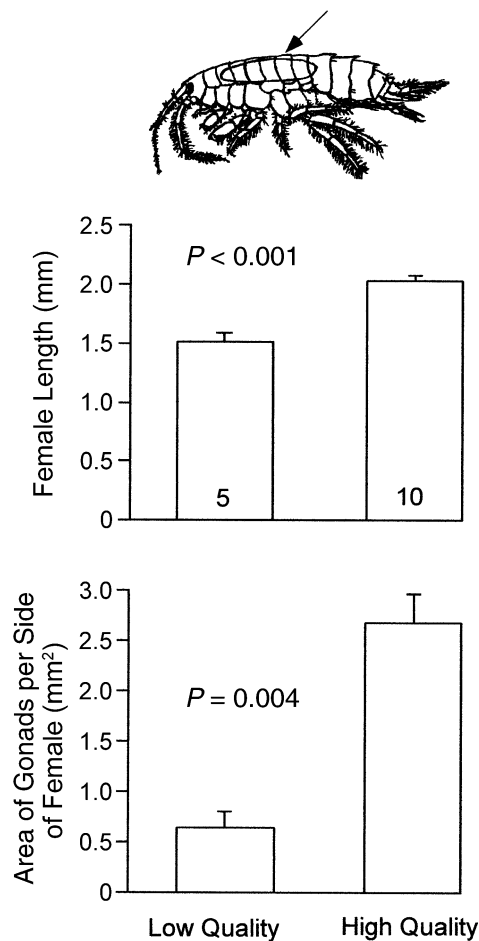

FIG. 11. Fitness parameters measured on *Gammarus mucronatus* females. No females survived in the dictyols treatments, thus preventing an analysis that included effects of the dictyols on these parameters. Area of gonads (pointed by the arrow on the animal) was used as a proxy for reproductive potential. Numbers at the base of bars indicate the total females per treatment. *P* values are from unpaired *t* tests. Bars represent means + 1 SE.

(Fig. 1). The similar responses from all six mesograzers suggest that *Dictyota* is generally deterrent, *Enteromorpha* is generally attractive, or both. In support of *Dictyota* being deterrent, all five amphipods were deterred when given a choice between low quality (Fig. 2) or high quality (Fig. 3) foods with or without dictyols incorporated at natural concentrations. In contrast, but in support of *Enteromorpha* being more attractive than *Dictyota*, the isopod *Paracerceis caudata* was not deterred by the dictyols regardless of food quality (Figs. 2 and 3, but see Fig. 4).

Although *A. longimana* prefers *Dictyota*, grazing on this alga can induce higher concentrations of dictyols. At those levels, dictyols can become deterrent (Cronin and Hay 1996*b*). The algae we used came from populations in which higher dictyol concentrations have been detected previously (Cronin and Hay 1996*b*), pos-
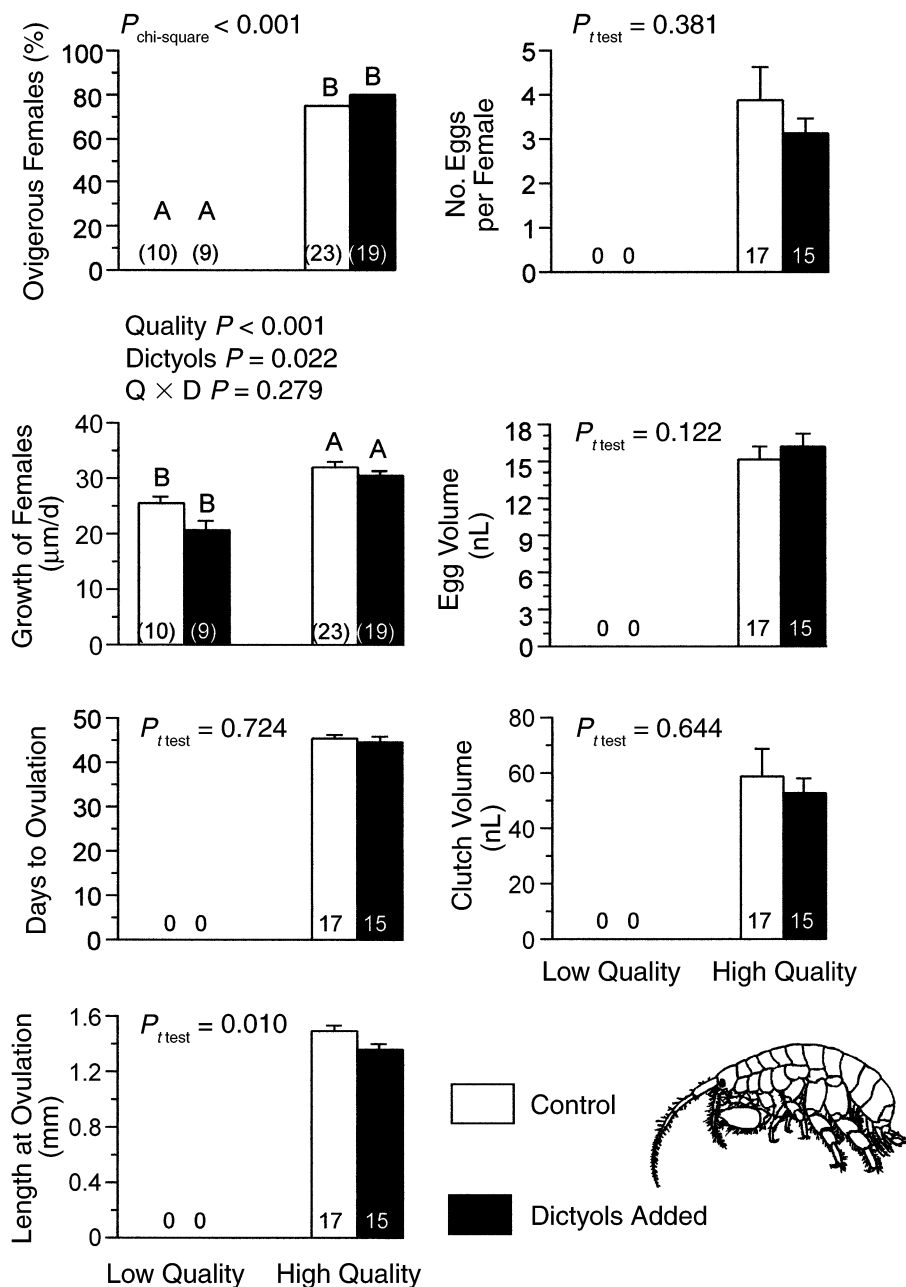
FIG. 12. Selected fitness parameters measured on *Elasmopus levis* females. Symbols and analyses are as in Fig. 8. Because no females produced eggs in the low quality treatments, some analyses (unpaired *t* tests) were performed only on the data from the two high quality food treatments.

sibly explaining why this amphipod preferred *Enteromorpha* and was deterred by the concentrations of dictyols in our artificial foods.

In no-choice assays (Fig. 4), assessing how mesograzers responded to variance in nutritional value in the presence and absence of chemical defenses, we often noted that consumers tried to compensate for low quality by consuming more food; for several species, this response was constrained by the presence of secondary metabolites from *Dictyota*. A variety of marine

and terrestrial consumers employ compensatory feeding when confronted with low quality foods (Simpson and Simpson 1990, Rueda et al. 1991, Slansky 1993, Stachowicz and Hay 1996, 1999*b*, Cruz-Rivera and Hay 2000*a*, 2001). This strategy can reduce the detrimental effects of low quality foods when access to preferred diets is constrained by natural enemies, environmental factors, or low mobility of the consumers (Simpson and Simpson 1990, Slansky 1993, Stachowicz and Hay 1999*b*, Cruz-Rivera and Hay 2000*a*).

However, compensation can be constrained by chemical defenses because increasing consumption to acquire sufficient nutrients will necessitate increased ingestion of secondary metabolites, possibly increasing dosages to toxic or lethal levels (Slansky 1992, 1993, Slansky and Wheeler 1992).

In our assays, we observed compensatory feeding for five of our six mesograzers ($P \leq 0.029$); all species except *Elasmopus levis* ($P = 0.060$) significantly increased consumption when confined with low quality relative to high quality diets without dictyols (Fig. 4). Dictyols commonly interacted with food quality to affect mesograzer feeding behavior, and did so in a way that constrained compensatory feeding for three of the six mesograzers. For *Gammarus mucronatus* and *Elasmopus levis*, and *Ampithoe longimana*, compensatory feeding on low quality foods was constrained or prevented by the addition of dictyols. For the first two species, the pattern of compensatory feeding was almost reversed when dictyols were present in their foods (Fig. 4). Dictyols also suppressed feeding by *Ampithoe valida* and *Paracerceis caudata*, but for these species, dictyols did not interact significantly with food quality. Overall, dictyols significantly reduced feeding when incorporated into low quality algal foods for four of the six mesograzers, but consumption of the high quality diets with and without dictyols was not affected significantly for any of the species we assayed. Thus, in contrast with our choice assays (Figs. 1 and 2), high nutritional quality largely ameliorated the effects of dictyols under no-choice conditions.

A few studies have documented interactions between prey food quality and chemical defenses for feeding behavior (Duffy and Paul 1992, Hay et al. 1994, Pennings et al. 1994), but the consequences for consumer fitness have not been addressed (but see Slansky and Wheeler 1992, Simpson and Raubenheimer 2001). As examples of the former, Duffy and Paul (1992) and Pennings et al. (1994) demonstrated that similar concentrations of secondary metabolites from marine algae and invertebrates were less effective in deterring predators when incorporated into high quality foods than when placed in low quality foods. Our results support this in that they show dictyols to be generally more deterrent in low quality foods than in high quality foods when herbivores are confined with only one food type. In choice assays, dictyols tended to affect feeding on both foods suggesting that mesograzer species might use different feeding strategies depending on their ability to find alternative foods when faced with chemically defended prey. For *Ampithoe valida*, *Gammarus mucronatus*, and *Elasmopus levis*, dictyols suppressed feeding under choice and no-choice conditions, suggesting a potential for adverse effects if dictyols were consumed. In contrast, *Paracerceis caudata* was not deterred by dictyols in choice assays, but showed a strong reduction in consumption when confined with low quality diets containing dictyols in no-choice assays (Figs. 2–4). Two possibilities could explain the contrasting results for *P. caudata*. Isopods in our choice assays could have been diluting the negative effects of dictyols by consuming equivalent amounts of foods containing and lacking dictyols. Similar behaviors have been proposed for herbivores mixing foods containing secondary metabolites (Freeland and Janzen 1974, Hägele and Rowell-Rahier 1999). Under no-choice conditions, dictyols were deterrent enough to inhibit feeding on low, but not high quality foods. Alternatively, *P. caudata* could obtain some nutrition by metabolizing dictyols, thus decreasing the need for compensatory feeding on the low quality food. Data from long-term assays (Figs. 6 and 7) provided limited support for this hypothesis.

Both *Ampithoe longimana* and *Cymadusa compta* preferred foods without dictyols when given a choice (Figs. 1–3). However, when alternative foods were not available, *Cymadusa* feeding was unaffected by dictyols (Fig. 4). Although *Ampithoe* showed a significant quality × dictyols interaction, it also readily consumed dictyols-containing foods under no-choice conditions; post hoc tests could not detect differences in consumption between diets of the same nutritional quality, with or without dictyols (Fig. 4). Thus, both species were relatively tolerant of *Dictyota* chemical defenses. When predation constrains searching for preferred algae, a high tolerance for chemically defended foods can benefit mesograzers that associate with algae noxious to predators. This has been demonstrated for *A. longimana* (Hay et al. 1987, Duffy and Hay 1994) and for other crustaceans associating with noxious algae (Hay et al. 1990, Sotka et al. 1999, Stachowicz and Hay 1999a), but has not been addressed for *Cymadusa*.

Effects of food quality and chemical defenses on consumer fitness

Feeding avoidance of prey that produce secondary metabolites is generally assumed to be adaptive. We tested this assumption by culturing consumers on diets containing or lacking dictyols, and measured how these effects were modified by prey nutrient content. Although dictyols at natural concentrations decreased feeding by five of six species in choice assays and by four of six species in no-choice assays (Figs. 2–4), strong negative effects of these chemicals on consumer survivorship, growth, or reproduction could be documented only for *Ampithoe valida*, *Gammarus mucronatus*, and *Elasmopus levis*. Dictyols had only mild effects on *A. longimana* (reducing female growth but not reproductive output), no detectable effects on *Cymadusa compta*, and positive effects on *Paracerceis caudata* survivorship (Figs. 6–12). Thus, the relationship between feeding deterrence and the fitness costs of consuming chemically defended prey was variable.

Numerous terrestrial studies show that rejection of defensive metabolites is often related to their negative physiological effects on particular consumers (e.g.,

Bernays et al. 1989, Guglielmo et al. 1996, Ayres et al. 1997, Dearing 1997, Dearing et al. 2002). Studies for aquatic systems are fewer, but also suggest that consuming deterrent metabolites may negatively affect some consumers, while not affecting others (reviewed in Hay 1996, Paul et al. 2001). However, inconsistencies in the effects of chemical defenses often do not conform with predictions from influential models regarding plant defense (Feeny 1976, Coley et al. 1985) as incongruities have been found for both qualitative (Cottee et al. 1988, Bernays 1991, Bernays and Cornelius 1992) and quantitative (Bernays et al. 1989, Steinberg and van Altena 1992, Boettcher and Targett 1993, Dearing 1997) chemical defenses. Most models (e.g., plant apparency) would consider dictyols as qualitative defenses, yet they did not show the toxicity often assumed for such defenses, and their effects were commonly modified by food quality.

Interactions between nutritional quality and dictyols affected fitness parameters for three of the five amphipods we investigated. For *G. mucronatus*, survivorship declined rapidly when individuals fed on low quality food with dictyols, but this lowered survivorship took more than 20 additional days to be expressed if the dictyols were in higher quality food (Fig. 6). Similarly, a decrease in survivorship of *E. levis* was observed only in the low quality food with dictyols; we could not detect an effect on survivorship when dictyols occurred in the high quality food (Fig. 6). For *A. valida*, dictyols significantly lowered survivorship, growth, and fecundity regardless of food value (Figs. 6, 7, and 9). For this species, there also was a significant quality × dictyols interaction affecting female growth, but this interaction was not detected when both males and females were analyzed together (Figs. 7 and 9).

Dictyols increased survivorship of *P. caudata*, and did this most obviously for individuals confined to the lower quality diet (Fig. 6). This isopod was not deterred by dictyols in choice assays (Figs. 2 and 3), although feeding was decreased on the low quality diet with dictyols in no-choice assays (Fig. 4). The increased survivorship of isopods on diets with dictyols could have occurred if compounds indirectly benefited isopods by inhibiting deleterious microorganisms or parasites either in the foods or in the isopods (Krischik et al. 1988, Schultz et al. 1990, Hunter and Schultz 1993). Isopods could also have the ability to use dictyols as assimilable products (see Slansky 1992 for examples in insects), which could explain the decrease in compensatory feeding observed (Fig. 4), as well as the increased survivorship, on dictyols. However, the low total concentration of dictyols (<1% of algal dry mass [Cronin et al. 1995]) suggests that the latter explanation is unlikely.

Although all consumers except *Elasmopus levis* showed significant compensatory feeding (Fig. 4), mesograzers varied in their ability to circumvent the negative effects of low food quality on fitness, even when

dictyols were not present in the foods. For example, *Ampithoe longimana* and *Cymadusa compta* appeared to successfully compensate for nutritional quality by consuming more of the low quality foods without suffering decreases in survivorship and growth (Figs. 6 and 7). However, *A. longimana* was more effective at this than was *C. compta*, as evidenced by the reductions in growth and fecundity of *C. compta* females on low quality foods (Fig. 10). For *Ampithoe valida*, *Gammarus mucronatus* and *Paracerceis caudata* compensation decreased the effects of low quality food on survivorship (Fig. 6), but not on growth or fecundity (Figs. 7, 9, and 11). For *E. levis*, the effects of food quality on reproductive output were substantially larger than the effects of dictyols (Fig. 12). Although low food quality significantly decreased egg and clutch volumes for *A. longimana* (Fig. 8), the magnitude of these effects was considerably less than for any of the other species. Therefore, *A. longimana* was the most effective mesograzer at offsetting differences in food quality through compensatory feeding. This agrees with previous findings using both artificial and natural foods (Cruz-Rivera and Hay 2000a, 2001, Sotka and Hay 2002). Thus, *A. longimana* can use compensation to live on low quality foods. The other species may use compensation to survive during stressful periods, but they cannot maintain high fitness via this mechanism alone.

Phylogenetic relation among consumers was a poor predictor of the effects of prey food quality, defensive chemistry, and their interaction on consumers. For example, although *Ampithoe longimana* and *A. valida* are congeners, their behavioral (Fig. 4) and physiological (Figs. 6–9) responses to prey nutritional value and chemical defenses varied dramatically. Responses of *A. longimana* were more similar to those of *Cymadusa compta* than to those of *A. valida* (Figs. 4, 6–10). Patterns of feeding and dietary effects on fitness were similar for *Gammarus mucronatus* and *Elasmopus levis*, despite these species being from different families. For both species, the feeding patterns on low vs. high nutritional quality foods were reversed when dictyols were present in the diets and a choice among foods was not available (Fig. 4), survivorship was affected by dictyols more strongly on low than on high quality foods (Fig. 6), and low quality diets significantly decreased growth and fecundity (Figs. 7, 11, and 12). Both these species appear to be omnivorous and less dependent on algal material for their nourishment (Nelson 1979b, Cruz-Rivera and Hay 2000a, b).

The broader context of nutritional quality–chemical defense interactions

Food items often represent a combination of multiple attracting (e.g., nutrients) and deterring (e.g., defensive metabolites) cues to consumers. Although this is generally recognized among ecologists (Rosenthal and Berenbaum 1992, Hay 1996, Paul et al. 2001), exper-

imental manipulations testing the interactive roles of nutritional quality and secondary chemistry are rare (e.g., Duffy and Paul 1992, Slansky and Wheeler 1992, Hay et al. 1994, Pennings et al. 1994, Simpson and Raubenheimer 2001). Basal species such as plants and algae are dynamic systems known to vary in both nutrient content and defensive chemistry as a function of season, geographic region, recent history of physical or biological stress, or part within the individual (Slansky 1992, Hay 1996, Karban and Baldwin 1997, Paul et al. 2001). If both prey nutrients and chemical defenses are determinants of consumer choice and performance, and consumers are an important selective force on prey, then the interactions between defensive chemistry and prey nutritional value could have important consequences for the ecology and evolution of defensive strategies (Slansky 1992, Paul et al. 2001).

High food quality could increase a chemically defended prey's susceptibility to attack by masking the stimuli that elicit avoidance, by enhancing detoxification abilities of the consumer, or by minimizing postingestive toxicity when nutrients bind to deterrent metabolites or compete for enzyme binding sites against deterrent metabolites (Duffy and Paul 1992, Slansky 1992, Simpson and Raubenheimer 2001). We found that dictyols strongly deterred amphipod feeding in choice situations regardless of food nutrient content (Figs. 2 and 3), but often had greater deterrent effects in low quality diets than in high quality diets when a choice between foods was not available (Fig. 4). Similarly, it was on the low quality, dictyol-rich diet that decreases in fitness were more pronounced for some species (Figs. 6 and 7, but see *Paracerceis*). Although negative postingestive effects of the dictyols could have been important for some of these consumers, the patterns observed suggest that dictyols reduce fitness in part by diminishing feeding rates of potential herbivores, a strategy that could clearly benefit *Dictyota*.

The contrasting effects of dictyols across a range of food quality has ecological relevance because dictyols defend not only seaweeds (Hay and Fenical 1988, Hay et al. 1988*a*, *c*, Cronin and Hay 1996*b*), but also animals such as gastropods that physiologically sequester dictyols from their algal diet (Faulkner 1992), and crustaceans that deter consumers by covering their bodies with dictyol-producing algae (Hay et al. 1990, Stachowicz and Hay 1999*a*, Cruz-Rivera 2001). A diverse mix of both terrestrial and marine invertebrates sequester chemicals from food plants and algae and use them for their own defense (Duffey 1980, Rowell-Rahier and Pasteels 1992, Hay and Fenical 1996, Paul et al. 2001), so the same secondary metabolites can be used as defenses in both lower quality plant (or algal), and higher quality animal, tissues.

The higher nutrient content of animal tissue compared to plant or algal material could constrain the types or concentrations of compounds that can effectively serve as sequestered defenses for animals (Duffy

and Paul 1992, Hay et al. 1994, Pennings et al. 1994). When nutritional value of a potential prey is high and the prey is predictably exposed to consumers, selection should favor the production of larger amounts of, or more potent types of, chemical defenses. Alternatively, if chemical defenses are costly and low nutritional quality enhances the effectiveness of secondary metabolites, then selection for lower nutrient content could be favored (Duffy and Paul 1992, White 1993). Whether more nutritious species contain more potent or higher levels of deterrents is an intriguing question that has rarely been studied. Chanas and Pawlik (1995) demonstrated that this was not the case for marine sponges, but the sponges they assessed appeared to differ in their natural levels of exposure to consumers, thus complicating this contrast.

Several investigations suggest that interactions of chemical defenses with prey nutritional value may be important and more widespread than is currently appreciated (Bernays et al. 1989, Slansky 1992, Paul et al. 2001, Simpson and Raubenheimer 2001). For example, in both marine algae and terrestrial plants, younger, more nutritious tissues often contain larger amounts of defensive metabolites, and these levels often decline, along with nutrient content, as the individual tissue ages (Hay et al. 1988*b*, Slansky 1992, Paul et al. 2001). It has also been suggested that polyphenolics may be deterrent not because of their negative effects on consumers, but because they provide cues associated with tissues of low nutritional quality (Bernays et al. 1989). In our study, the effects of low nutritional quality on consumer feeding and fitness were of equal or greater magnitude than the effects of the dictyols, although interactions between these traits were common and also strongly affected consumer feeding and performance (e.g., *Gammarus mucronatus* and *Elasmopus levis*). The strong individual and interactive effects of dictyols and nutritional quality for several of our mesograzers illustrate the need to simultaneously consider food value and chemical defenses when assessing traits affecting food choices and consumer fitness.

Such considerations may be further complicated by the need to understand that physiological performance is not the only, or even primary, factor affecting diet selection. As an example appropriate to this study, although *Ampithoe longimana* prefers to avoid dictyols if given a choice (Figs. 1–3), and consuming these compounds significantly decreases female growth (Fig. 8), this species selectively lives on, and feeds from, *Dictyota* in the field (Duffy and Hay 1991, 1994). Through this association, *A. longimana*, as well as some other mesograzers, significantly decrease their probability of being consumed by fishes (Hay et al. 1990, Duffy and Hay 1994, Stachowicz and Hay 1999*a*). When populations of *A. longimana* occur in geographic regions where *Dictyota* is absent, they cannot use *Dictyota* as a spatial escape from predation. In

contrast to the *A. longimana* population studies here, these displaced populations of *A. longimana* prefer to avoid *Dictyota* as a food, are more strongly deterred by the dictyols, and their fitness is lowered dramatically when they are forced to consume *Dictyota* (Sotka and Hay 2002). This suggests that some of the feeding and fitness patterns shown in this study are shaped by ecological constraints such as predator escape, rather than by physiological needs alone.

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