Seawater Temperature Alters Feeding Discrimination by Cold-Temperate but not Subtropical Individuals of an Ectothermic Herbivore

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Abstract.   Seawater temperature varies across multiple spatial and temporal scales, yet the roles that such variation play in altering biotic interactions are poorly known. We assessed temperature-mediated feeding behavior exhibited by the herbivorous amphipod *Ampithoe longimana* collected from cold-temperate and subtropical estuaries (27°N and 41°N, respectively). Individuals were offered a pairwise feeding choice between lyophilized seaweeds that provide higher fitness (*Ulva intestinalis)* or lower fitness (*Halimeda tuna*, *H. opuntia*, *Amphiroa* spp., or *Stypopodium zonale*). Overall, herbivores preferentially consumed the higher quality *U. intestinalis* more than any lower quality food. However, the strength of this feeding choice was not consistent. Northern herbivores consumed proportionally more poorer quality tissue at 25 °C than at 20 °C in two assays (*H. opuntia* and *Amphiroa* sp.), consumed less poorer quality tissue at 25 °C than at 20 °C in one assay (*S. zonale*), and showed no difference in another assay (*H. tuna*). Moreover, when offered tissue coated with lipophilic extracts of *H. opuntia*, northern herbivores consumed more extract-coated tissue at 25 °C than at 20 °C. In contrast to northern herbivores, the southern herbivores did not alter their feeding choices with temperature. This study represents the first demonstration that short-term (*i.e.,* days-long) variation in ambient temperature alters feeding choices in a marine herbivore.

Introduction

The interplay of the abiotic environment and biotic interactions structures nearshore communities (Menge and Suth-

erland, 1987; Bruno *et al.,* 2003), yet we still have far more information on their independent roles than on simultaneous effects. As an example, nearshore seaweed communities are sculpted by the abiotic environment (*e.g.,* exposure to long-term desiccation, ultraviolet radiation, high wave energy, or low light attenuation; Davison and Pearson, 1996; Helmuth *et al.,* 2005; Bischof *et al.,* 2006) and by herbivory (Lubchenco and Gaines, 1981; Hay, 1991). But studies that document abiotic influence on patterns of herbivory are less common, and largely focused on changes to a seaweed's susceptibility to herbivory. For instance, upon exposure to desiccation and ultraviolet light, some palatable seaweeds appear to become less palatable, while unpalatable seaweeds become more palatable (*e.g.,* Renaud *et al.,* 1990; Cronin, 2001; Dethier *et al.,* 2005).

Moreover, there is less information on how abiotic stresses on herbivores affect local algal communities. Wave exposure limits the feeding efficiency of smaller mesograzers such as snails and isopods (*e.g.,* Nielsen, 2001; Engkvist *et al.,* 2004) but not larger consumers like urchins (Vasquez and Buschmann, 1997). Desiccation and high air temperatures restrict herbivorous molluscs to lower intertidal areas, affording palatable seaweeds an upper intertidal refuge from herbivory (Harley, 2003). Burnaford (2004) showed that the movement of the marine chiton *Katharina tunicata* under canopy-forming seaweeds lowered its exposure to temperature and desiccation stress, and as a consequence, increased feeding rates within refuges. Thus, we require studies of feeding patterns when herbivores, rather than seaweeds, are stressed.

There is mounting evidence that global increases in seawater temperature are pervasive and have already modified the dynamics of some elements of marine ecosystems (Stachowicz *et al.,* 2002; Lesser, 2004; Richardson and Schoe-

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man, 2004). For marine herbivores, seawater temperature represents an important abiotic modifier of herbivore feeding rates, as evidenced by the positive correlation of seawater temperature with feeding rate (Robertson and Lucas, 1983; Wyban *et al.,* 1995; Yee and Murray, 2004; Floeter *et al.,* 2005). Yet, because temperature alters the fundamental rates of several aspects of organismal biology (Brown *et al.,* 2004), it is likely that the effects of warming oceans on the ecology of ectothermic herbivores will be complex and context-specific. Although we have an enormous literature on the effects of ambient temperature on ectothermic terrestrial insects, recent reviews (Larsson, 1989; Stamp and Yang, 1996; Coley, 1998) concluded that direct effects of global temperature increases on insect herbivores will "differ among species, depending on their existing environments and life histories, and their ability to adapt" (p. 11 in Bale *et al.,* 2002).

Feeding choice is central to the ecological impact of marine herbivores on algal communities (*e.g.,* Lubchenco and Gaines, 1981), yet the degree to which seawater temperature may alter feeding choices has never been assessed. Here, we measure feeding choices in the presence of short-term (*i.e.,* days-long) variation in seawater temperature. A series of pairwise feeding-choice assays were offered to populations of the herbivore *Ampithoe longimana* Smith, 1873, a small (<1 cm when adult) gammaridean amphipod (Arthropoda; Crustacea; Malacostraca) found within high-saline estuaries of the northwestern Atlantic and eastern Gulf of Mexico (Nelson, 1980). *Ampithoe longimana* is a tube-dwelling brooder that lives and feeds on a variety of algae (Duffy and Hay, 1991; Sotka and Hay, 2002; McCarty and Sotka, unpubl. data). To investigate whether seawater temperature alters the ability of *A. longimana* to discriminate between seaweeds that differ in food quality, we collected two populations from very distinct thermal environments (*i.e,.* cold temperate and subtropical) and offered individuals feeding choices between chemically rich, poorer quality seaweeds and a chemically depauperate, higher quality food.

Materials and Methods

Temperature data

To assess the sea surface temperatures normally experienced by these estuarine populations in the field, we examined data from available temperature loggers in Massachusetts and Florida. Each data logger was placed <50 km from an amphipod collection location and within similar habitats (*i.e.,* high-salinity, subtidal habitats near inlets to oceanic waters). We assume that the data loggers approximate the conditions experienced by the amphipods, but it is also possible that variation in subtidal water temperatures may be found within these high-salinity estuaries. The Massachussetts data set was recorded every 10 min during 2004,

and downloaded from the NOAA National Estuarine Research Reserve System (http://nerrs.noaa.gov). The Florida data set was recorded every 10 min between October 1999 and September 2000 by the U.S. Geological Survey (Byrne and Patino, 2004).

Collection of herbivores and seaweeds

Ampithoe longimana populations were collected from across 1700 km of the geographic range of the species: Fort Pierce, Florida (27°28′N; 80°17′W), Niantic, Connecticut (41°20′N; 72°11′W), and Horseneck Beach, Massachussetts (41°30′N; 71°02′W). We are confident that these populations are indeed *A. longimana*: Connecticut, Massachussetts, and North Carolina populations readily interbreed (Sotka and Hay, 2002) and data (Sotka *et al.,* 2003; McCarty and Sotka, unpubl. data) indicate that Florida and Connecticut populations differ from each other by only about 2% at the mitochondrial locus cytochrome oxidase I. In contrast, two confamilial amphipods—*Ampithoe valida* and *Cymadusa compta*—are more than 20% divergent from any of these *A. longimana* populations (Sotka *et al.,* 2003). We will refer to the Connecticut and Massachussetts herbivores as "New England" individuals, as we grouped these assays togther for analysis when we could detect no statistical differences between populations (as is the case for all assays).

All seaweed populations were collected from the algae *Sargassum, Ectocarpus,* and *Dictyota,* and transported to the College of Charleston's Grice Marine Laboratory in Charleston, South Carolina. We estimate that populations were collected in waters between 15 and 25 °C (note: unfortunately, temperatures were not recorded), and slowly acclimated to 20 °C over several days. Populations were reared at 20 °C in separate plastic containers for several generations (two to six) before all subsequent assays of feeding preference and performance. This procedure allowed us to minimize previous environmental or maternal effects. Cultures were maintained at a practical salinity of about 30 and fed an assortment of seaweeds *(Sargassum, Ulva, Bryopsis, Polysiphonia,* and *Gracilaria).*

Seaweeds used for the feeding assays were collected in May 2005 from the Florida Keys outer reefs and in July 2005 from Radio Island, North Carolina. They were subsequently frozen at −4 °C within 12 h of collection. Seaweeds were lyophilized (*i.e.,* freeze-dried), ground to a powder, and stored at −20 °C. The seaweeds used in choice assays were three green seaweeds (*Ulva* (syn. *Enteromorpha) intestinalis, Halimeda opuntia,* and *H. tuna*); one brown seaweed *(Stypopodium zonale);* and a red seaweed (*Amphiroa* sp.). Lyophilization removes the effects of morphology but maintains concentrations of some lipophilic metabolites such as diterpenes (*e.g.,* Cronin *et al.,* 1995), and it does not substantially lessen the ability of seaweeds with known

lipophilic deterrents to lower feeding rates by consumers (*e.g.,* Bolser and Hay, 1996; Cruz-Rivera and Hay, 2001). Although we made attempts to simultaneously assay Florida and New England populations, this was not always possible. Use of lyophilized seaweeds ensured that we offered the same tissues to all amphipods.

These choice assays are used as a tool to explore the influence of temperature on feeding discrimination among populations that never use these seaweeds, either because the seaweeds do not occur at that latitude (*e.g.,* New England populations) or because the amphipods are never found on the seaweed (*e.g.,* Florida populations). Each seaweed species, with the exception of *Ulva,* is tropical in distribution (Littler and Littler, 2000), and is a low-preference food for several generalist fish, urchin, and amphipod consumers, largely because of low nutritional value, chemical deterrents, or both (*Stypopodium* and *Amphiroa:* Bolser and Hay, 1996; *Halimeda:* Paul and Van Alstyne, 1988). *Ulva* (syn. *Enteromorpha*) *intestinalis*) is a high-quality seaweed for *Ampithoe longimana* and is available to all populations. By contrast, we have never found *A. longimana* on any of the poor-quality seaweeds offered in these assays (McCarty and Sotka, unpubl. data), nor did we use any of the poor-quality seaweeds in the common-garden cultures.

Seaweed traits

We measured two nutritional traits that are known to underlie the feeding choices of *Ampithoe longimana* for all seaweed species except *Amphiroa.* Samples were combusted at 500 °C for 20 h. Mass was recorded before and after combustion. Because we depleted our original collection of tissue, we could not assess the nutritional traits of *Amphiroa* sp., and therefore we reproduce previously published data (Bolser and Hay, 1996). Protein of about 10 mg of lyophilized tissue was measured for five replicates of each seaweed. Seaweed material was extracted for about 24 h in 1 mol l^{-1} NaOH. Absorbance was measured *via* a spectrophotometer at 5- and 10-min intervals within a 1:1 solution of extract to Bradford reagent. Absorbance values were compared to a standard curve generated using bovine serum.

Juvenile performance assays

We assessed the food quality of these seaweeds by rearing 1-week-old juveniles in the laboratory for 12 days. Brooding females from our laboratory cultures from Florida and Massachussetts were isolated with lyophilized tissue of *Ulva* embedded in an agar matrix until juveniles had left the brood pouch. Brooding females from North Carolina were unavailable at the inception of this experiment. Juveniles from each mother were placed on one of four seaweeds used in feeding-preference assays. We could not assay the sur-

vivorship of amphipods on *Amphiroa* sp. because we depleted our original collection of tissue. We maintained the experiment at 20 °C, changed seawater and foods every 3–4 days, and checked survivorship every 1–3 days. A second assay was generated to attempt to detect a fitness effect of temperature (20 °C *vs.* 25 °C) on amphipod growth on the high-quality seaweed *Ulva intestinalis.* This was set up similarly to the previous assay, with the exception that we extended the assay for 20 days. Survivorship curves were analyzed *via* parametric survival-curve analysis. Growth rates and female fecundity were assessed *via* a series of nonparametric ANOVAs, where statistical significance was evaluated by comparing *F* ratios with an expected distribution generated from 1000 permutations of the data set (Anderson, 2001) using *R. Post hoc* tests were similarly generated to describe effects of temperature on growth rates within populations. The nonparametric approach is appropriate because the data are not normally distributed and could not be transformed to yield normality, and because all replicates are independent and exchangeable.

Feeding-preference assays

To quantify feeding preference, individual amphipods were offered a series of pairwise feeding choices between the green seaweed *Ulva intestinalis,* which provided relatively high survivorship for the amphipods (*i.e.,* Control), and one of four seaweeds that provided lower survivorship, contained known secondary metabolites, or both (*i.e.,* Treatment; see Results). The seaweeds were offered as ground, freeze-dried tissue embedded within agar and bound onto window screen. This procedure created a grid (6 squares × 5 squares) of either control or treatment tissue for quantification. Individual replicates were terminated and measured either after a minimum of 1/3 of an individual grid was consumed or after at least 10 squares were consumed between the two grids. We disregarded replicates in which all of one grid and at least half of another grid were consumed. The motivation for these criteria was to ensure that amphipods made choices while tissues were readily available, given that the relative abundance of available food can affect feeding behavior (Pennings *et al.,* 1993) and estimation of feeding preference (Lockwood, 1998). Assays generally lasted less than 2 days but no longer than 5 days, and were performed in darkness within upright incubators at both 20 °C and 25 °C.

Paired Student's *t*-tests were used to assess whether consumption rates of control and treatment foods differed within assays. For each replicate bowl, we calculated the proportion of poorer quality seaweed consumed using the ratio Treatment consumed × (Total consumed)$^{-1}$. We then used a nonparametric ANOVA to assess the effect of seaweed species, temperature (20 ° *vs.* 25 °C), region (northern *vs.* southern), and their interactions on relative consumption

of poorer quality seaweed. We did not assess these effects on the overall consumption rate, because assays were checked and terminated periodically (varied between 12 and 24 h) and replicates were disregarded when foods were depleted.

Extraction of Halimeda opuntia

We also assessed whether temperature-mediated shifts in feeding preference by northern amphipods were mediated by the lipophilic metabolites produced by one of these chemically rich seaweeds, *Halimeda opuntia*. One gram ($\pm$ 0.01) of lyophilized and ground tissue from *H. opuntia* was extracted three times in a total of 20 ml of 2:1 DCM:MeOH for a total of 1 h. The extract was rotary-evaporated and applied, using 4 ml of ethyl ether, to 1 g of *Ulva*. The ethyl ether was then rotary-evaporated, leaving only extract-coated *Ulva*. This extract-coated *Ulva* (treatment) and a ether-only *Ulva* (control) were offered to the Connecticut population in a choice assay at 20 ° and 25 °C. We did not have enough individuals from Floridean populations at the time of this assay. These data were analyzed *via* both paired *t*-tests and a nonparametric ANOVA.

Results

Seawater temperatures within the estuaries of subtropical Florida reached 25 °C during all months but January, whereas Massachussetts cold-temperate estuaries exceeded 25 °C during only 2 months per year and for only 4% of the recordings within those months (Fig. 1).

As a tool to assess the effects of seawater temperature on feeding discrimination in *Ampithoe longimana,* we pursued a series of pairwise feeding assays between the higher quality *Ulva* and four poorer quality seaweeds from the tropics (*Halimeda opuntia, H. tuna, Stypopodium zonale,* and *Amphiroa* sp.). Host quality was determined from a juvenile fitness assay (Fig. 2). Juvenile amphipods from Florida and Massachusetts died within 12 days when isolated on chemically rich seaweeds *Halimeda opuntia, H. tuna, and Stypopodium* (Fig. 2). In contrast, 3 of the 15 Florida juveniles (20%) and 7 of the 15 Massachusetts juveniles (47%) survived to 15 days on *Ulva*. Note that lyophilized seaweeds may not provide as high-quality food as fresh tissue, because previous work (Sotka *et al.*, 2003) demonstrated survivorship of greater than 90% for Massachusetts *A. longimana* fed on fresh *U. intestinalis,* whereas results from both assays reveal survivorship of about 50% or less. Although we did not have enough plant tissue to assess survivorship of amphipods on *Amphiroa*, it is likely that *Amphiroa* is also a low-quality plant given its relatively low organic and protein content (see Table 1), deterrent secondary metabolites (Bolser and Hay, 1996), and calcified skeleton.

During feeding choice assays, northern and southern individuals consumed significantly more of the *Ulva* than of
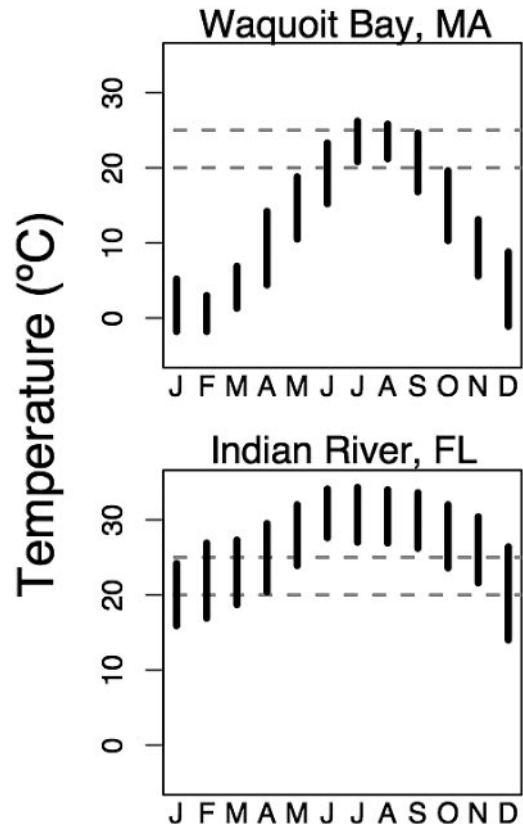

Figure 1. Estuarine water temperatures at locations close to amphipod collections. The range of temperatures within sites is shown as a single line per month. The horizontal dashed lines denote 20 °C and 25 °C. The two locations are Waquoit Bay, Massachussetts (41°34′N; 70°31′W), and south Indian River, Florida (27°12′N; 80°11′W).

the poorer quality seaweed (paired *t*-test $P < 0.001$ for each population/seaweed/temperature combination). The proportion of total consumption that was poorer quality food was always significantly less than 50% (Fig. 3). Thus, these herbivores consistently choose to eat *Ulva intestinalis*, the food that provides higher survivorship than the alternative poorer-quality food.

However, the relative strength of these feeding preferences varied with temperature, location, and the seaweed offered, as indicated by its significant interaction within a three-way ANOVA (Table 2). A *post hoc* examination of this interaction indicates that the northern herbivores changed their relative consumption of poorer quality seaweed with temperature within several assays: New England amphipods consumed relatively more *Halimeda tuna*, more *Amphiroa,* and less *Stypopodium* while at 25 °C relative to their consumption of these seaweeds while at 20 °C (Fig. 3). In contrast, southern amphipods did not significantly change their feeding preferences with temperature in any assay.

The temperature-mediated shift in feeding preference by northern amphipods was confirmed when we offered *Ulva* coated with the lipophilic extract from *Halimeda opuntia* at
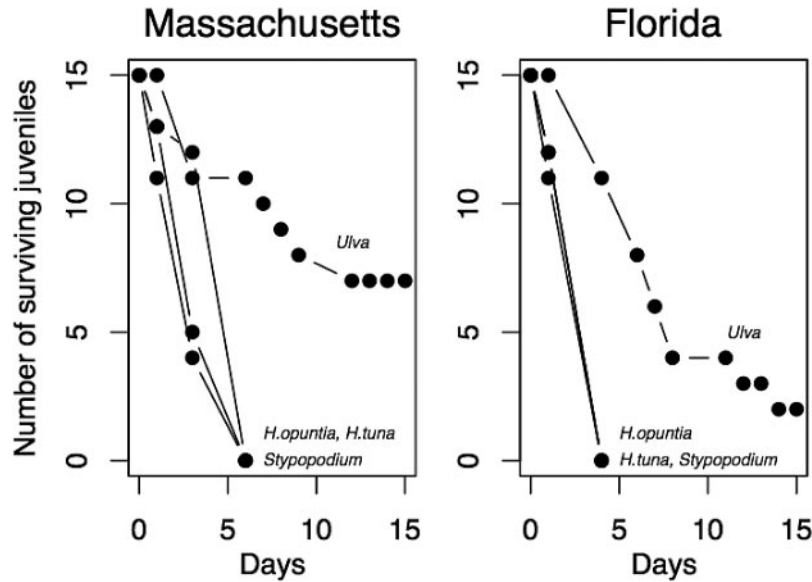
Figure 2. Survivorship of juvenile amphipods on lyophilized tissue of four seaweeds across 12 days.

natural concentrations (Fig. 4). Northern amphipods were significantly deterred by *H. opuntia* extracts at 20 °C but were not deterred at 25 °C. This difference in relative consumption rates of extract-coated tissue was confirmed by a one-way nonparametric ANOVA ($F_{1,36} = 4.94$, $P = 0.044$).

There were few performance differences between juvenile New England and Florida amphipods when reared at either 20 or 25 °C. There were no significant differences in survivorship either between or within populations (Fig. 5), with the exception that Floridean amphipods tended to die more readily at 20 °C than did New England amphipods ($P < 0.05$; parametric survival-curve analysis). Growth rates of both populations were significantly greater at 25 °C than at 20 °C, and the magnitude of this increased growth rate was equivalent between populations (see *post hoc* tests in Fig. 5). The number of eggs produced by females did not significantly differ, although Florida females at 25 °C pro-

duced an average of three eggs per female, while New England (20 °C and 25 °C) and Florida females at 20 °C produced an average of one egg per female. A proxy for absolute female fitness can be calculated by multiplying survivorship by average number of eggs produced (*e.g.,* Poore and Steinberg, 1999), although there is no test statistic that could be applied to detect significant differences. Absolute fitness of Florida females at 25 °C was three times greater than the absolute fitness of females of New England at 20 °C and 25 °C and Florida females at 20 °C (Fig. 5). Thus, inference from these tests appears to be limited by statistical power ($n \sim 15$), but these data suggest that if amphipods from New England are physiologically stressed at 25 °C (as is suggested by the feeding-choice assays), then the stress is sublethal.

Discussion

In general, individuals from northern and southern populations of the herbivorous amphipod *Ampithoe longimana* preferentially consumed the higher quality *Ulva intestinalis* more than any of the lower quality foods (Fig. 3), a result that is predicted by the fitness benefits that each seaweed affords (Fig. 2). Surprisingly, however, the strength of this feeding choice was not consistent across individuals and temperatures. In particular, northern individuals consumed proportionally more poorer quality seaweed at 25 °C than at 20 °C in two sets of assays (*Halimeda opuntia* and *Amphiroa* spp.), consumed less poorer quality seaweed at 20 °C in one assay (*Stypopodium zonale*), and showed no difference in relative consumption rate in another assay (*H. tuna*). In contrast, the southern herbivores did not alter their feeding choices at these temperatures. Moreover, the northern her-

Table 1

Nutritional traits of seaweeds

Seaweed	AFDM* (% DM)	Protein† (% DM)
Amphiroa spp.‡	19.5	1.7
Halimeda opuntia	10.6 ± 0.5	1.1 ± 0.1
H. tuna	16.8 ± 3.5	1.2 ± 0.5
Stypopodium zonale	69.5 ± 1.1	2.5 ± 0.1
Ulva intestinalis	72.6 ± 1.0	2.2 ± 0.6

* Ash-free dry mass is expressed as proportion of organic out of all material.

† Protein is expressed as mg per mg of dry mass (%).

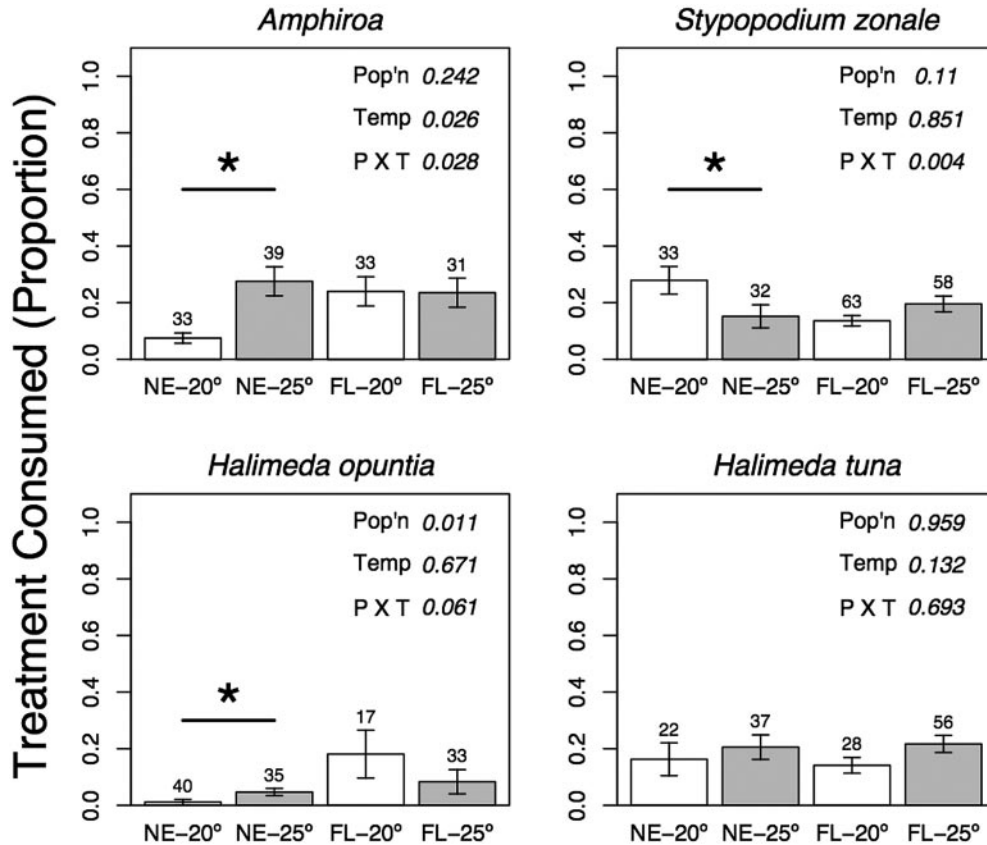‡ Data taken from Bolser and Hay (1996).

Figure 3. Relative consumption of treatment seaweeds (mean ± S.E.) by northern ("NE": Massachussetts and Connecticut) and southern ("FL": Florida) amphipods at two seawater temperatures (20 and 25 °C; white and grey bars, respectively). Lyophilized tissue from *Ulva intestinalis* (=control) and a chemically rich, nutritionally poor seaweed (=treatment) was offered to a single amphipod for up to 3 days. Feeding choice was assessed as the proportion consumed of the treatment seaweed divided by the total amount consumed. An interaction between population source, temperature, and seaweed is significant (Table 2). Asterisks above a horizontal bar indicate results of a significant ($P < 0.05$) Wilcoxan rank sum test between temperatures within populations (*e.g.,* NE-20° *vs.* NE-25°).

bivores were deterred by the lipophilic extract of *H. opuntia* when at 20 °C but not at 25 °C (Fig. 4). Thus, northern but not southern herbivores tended to alter their feeding choices with short-term variation in temperature.

Although we have no direct evidence, indirect evidence suggests that northern, but not southern, individuals are physiologically stressed at 25 °C. The geographic variation in feeding response at 25 °C reflects the geographic variation in the temperature profiles of natal estuaries: the New England estuary rarely exceeded 25 °C, but Florida estuaries regularly exceeded that temperature (Fig. 1). Across this same 1500-km coastline, southern populations of the mummichog *Fundulus heteroclitus* have higher performance than northern populations when held at warmer, more southerly temperatures (Powers and Schulte, 1998). For *A. longimana,* if northern populations are physiologically stressed at 25 °C, then the stress is sublethal: rearing Florida and New England (in this case, Connecticut) individuals at 20 °C and 25 °C on fresh *Ulva* for 3 weeks revealed no

significant differences within populations in survivorship or fecundity with temperature, though there was a significant increase in growth with temperature (Fig. 5). Interestingly, Florida individuals appeared to have higher absolute fitness at 25 °C than at 20 °C, reflecting the relative increase in reproductive output that the higher temperature affords these amphipods. By contrast, the New England individuals did not increase their reproductive output with temperature, suggesting again that these animals are stressed at the higher temperature. Ideally, we would have generated independent evidence (*e.g.,* expression of heat shock protein) to support the notion that northern individuals are experiencing physiological stress at 25 °C, but our initial attempts were unsuccessful. It is also possible that the populations differ in their responses to the rate of temperature change rather than the maximum temperature itself—a possibility that we did not assess.

If northern but not southern individuals are thermally stressed at 25 °C, then our data indicate that sublethal

Table 2

The proportion of poor-quality seaweed consumed as a function of seaweed species, temperature, and amphipod source region

Factor	d.f.	M.S.	F	P value
Seaweed	3	0.57	11.20	0.000
Temperature	1	0.19	3.77	0.053
Region	1	0.04	0.68	0.411
Seaweed × Temperature	3	0.10	1.87	0.134
Seaweed × Region	3	0.15	2.84	0.037
Temperature × Region	1	0.01	0.11	0.740
Seaweed × Temp × Region	3	0.29	5.58	0.001
Residual	574	0.05		

Each replicate contained a single amphipod, and a poor quality and palatable seaweed (*Ulva*). The underlying distribution to assess statistical significance was based on 1000 permutations following the procedure of Anderson (2001).

thermal stress is analogous to the "starvation stress" response described for the warm-temperate sea urchin *Arbacia punctulata* (Cronin and Hay, 1996). *Arbacia* is typically deterred by pachydictyol A, a diterpene alcohol produced by brown seaweeds of the genus *Dictyota*. After starving *Arbacia* for 1 to 2 days prior to a feeding-preference assay, the urchins tended to consume pachydictyol-treated foods more readily. The authors indicate that these hungry animals were less likely to discriminate because of their need to consume more quickly than when nutritionally satiated. Similarly, it is possible that northern individuals were "hungrier" at 25 °C than at 20 °C and therefore consumed more poorer quality than higher quality foods when offered a feeding choice. Note that because replicates were checked and terminated periodically (varied between 12 and 24 h), we have confidence that the relative (*i.e.,* control *vs.* treatment) but not total (*i.e.,* control plus treatment) consumption rates are reliable. Therefore, we did not analyze the prediction that overall consumption rates would be greater among amphipods at 25 °C than at 20 °C.

Alternative explanations of these data are less compelling. First, although we cannot address whether the nature or efficacy of seaweed metabolites differs when at 20 °C *versus* 25 °C, we doubt that this potential artifact can account for the geographic variation in herbivore responses, because any temperature-mediated changes in metabolites would have been experienced by all herbivores equally. There are clear effects of storage and extraction methods on the quantity of some algal diterpenes (*e.g.,* Cronin *et al.,* 1995; Cavalcanti *et al.,* 2008), which suggests that metabolites vary in their stability. However, in general, virtually nothing is known about the thermal stability of algal metabolites at the temperature ranges we tested.

Second, the geographic differences in response to temperature could hypothetically arise if there was differential exposure to field temperatures or seaweeds in the recent

history of the herbivores. However, we minimized potential effects of recent experience by rearing all animals in the laboratory for 2+ generations at 20 °C before any assays were conducted. Third, although there are genetic differences in feeding preferences among populations of *Ampithoe longimana* (Sotka and Hay, 2002; Sotka *et al.,* 2003), none of the seaweeds that we offered here host the amphipod, with the exception of *Ulva intestinalis*. This seaweed serves as an important host for both New England and Florida populations (McCarty and Sotka, unpubl. data). Finally, the notion that the amphipods appear to have altered their feeding behavior at higher temperatures because they more readily depleted the foods cannot be supported: replicates analyzed were terminated when one-third to one-half of either food was consumed.

Although thermal stress seems to be consistent with two of the four sets of assays (*i.e.,* using *H. opuntia* and *Amphiroa*), the hypothesis does not appear to explain the other two assays. When offered a choice between *U. intestinalis* and *H. tuna*, northern amphipods consumed proportionately
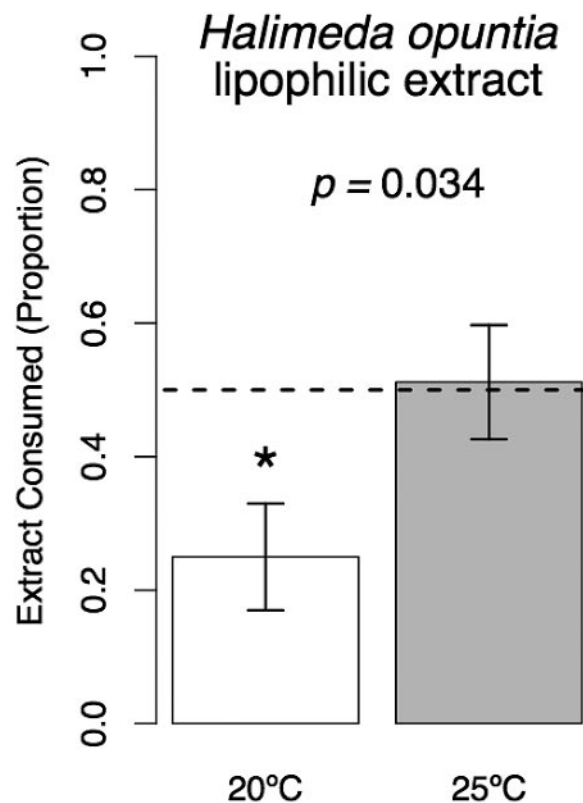

Figure 4. Relative consumption of *Ulva intestinalis* coated with lipophilic extract of the green seaweed *Halimeda opuntia* (mean ± S.E.) by northern amphipods at two temperatures (20 and 25 °C). Amphipods ate proportionately more extract-coated seaweed at 25 °C (grey bar) than at 20 °C (white bar; *P* value generated by a nonparametric ANOVA). Pairwise *t*-tests indicate that amphipods were deterred by the extract at 20 °C (as indicated by an asterisk) but not at 25 °C. The dotted line (0.5) represents equal consumption of control and extract-coated tissues.
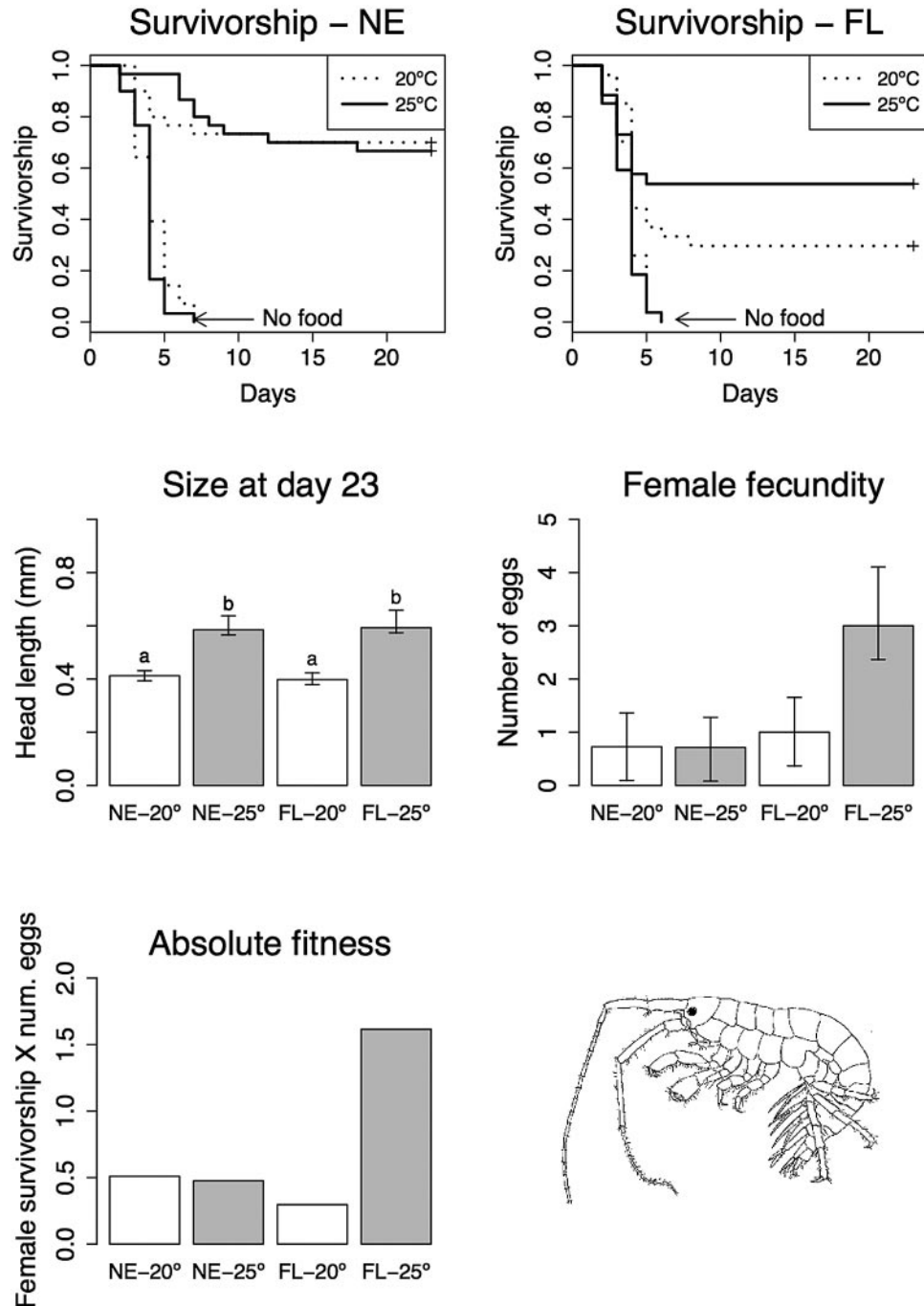
Figure 5. Survivorship, growth, female fecundity, and absoute fitness of Florida and New England populations of *Ampithoe longimana* at 20 and 25 °C when reared on fresh *Ulva intestinalis*. Letters in growth rates (*i.e.,* Size at day 23) indicate treatments that were statistically indistinguishable.

equivalent amounts of *H. tuna* at 20 and 25 °C. When offered a choice between *U. intestinalis* and *Stypopodium zonale*, the animals actually consumed more of the poorer quality *S. zonale* at 20 °C than at the higher temperature. Another inconsistency comes from the Floridean amphipods at 20 °C. That is, if the trend for lower survivorship of

Floridean amphipods at 20 °C suggests "cold stress," then this was not reflected in an alteration of feeding choices. Thus, we conclude that shifting feeding choices are partially, but not wholly, explained by physiological stressors experienced by the herbivores. This complexity in feeding response of this ectothermic herbivore is consistent with the

complexity of responses among terrestrial insects (Bale *et al.*, 2002).

We caution that we require data from more populations within northern and southern regions to confirm the patterns suggested here. We cannot reject the possibility that we have identified site-specific rather than regional differences in the degree to which temperature modifies feeding choices. Moreover, we exposed all populations to the temperatures that the northern populations likely perceive as "stressful" (25 °C) and more "typical" (20 °C). Future work should confirm our hypothesis of a "temperature-stressed" feeding response by assessing feeding behavior at "stressful" and "typical" temperatures as they are defined for local thermal environments.

Despite these caveats, our data clearly indicate that this ectothermic herbivore displays shifts in feeding preferences with short-term changes in environmental temperature. This finding has two broader implications for marine ecologists. First, our results caution researchers to ensure that laboratory-based assays of feeding behaviors replicate the temperature profiles that herbivores typically experience. For example, we would have mistakenly concluded that the northern herbivores were not deterred by a lipophilic seaweed extract (Fig. 4) if we had conducted assays only at room temperature (25 °C). Thus, several environmental forces are now known to alter laboratory-based assessment of feeding rates and preferences, including temperature (this study), recent experience (Trowbridge, 1991a; Lindquist and Hay, 1995; Thacker *et al.*, 1997), starvation state (Cronin and Hay, 1996), presence of predators (Stachowicz and Hay, 1999; Grabowski, 2004) or conspecifics (Trowbridge, 1991b), spatial arrangement of prey (Pfister and Hay, 1988), and daylight (Hay *et al.*, 1986).

Second, our results suggest that the short-term responses of subtidal marine communities to a warming ocean will be complicated by the physiological stress responses of individual herbivores. The short-term shifts in seawater temperature are similar in duration to ephemeral shifts in upwelling or downwelling conditions on open shorelines. Previous work on such sublethal temperature shifts suggests temporal changes in feeding rate (Robertson and Lucas, 1983; Wyban *et al.*, 1995; Sanford, 1999; Yee and Murray, 2004; Floeter *et al.*, 2005), but no ephemeral shifts in feeding choices have been previously addressed. Terrestrial (Stamp and Bowers, 1990) and marine (Burnaford, 2004) consumers make habitat choices that minimize exposure to thermal stresses that can have cascading effects on community structure by altering which prey are susceptible to consumers under thermally stressful conditions. In contrast to these studies, our results cautiously suggest that thermally stressed consumers who cannot or do not choose habitats that minimize elevated temperatures may tend to lose their ability to discriminate between high- and low-quality foods.

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