Are tropical herbivores more tolerant of chemically rich seaweeds than are temperate herbivores? A test of seaweed-herbivore coevolution

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Abstract. There is a greater quantity and diversity of lipophilic secondary metabolites produced by seaweeds within tropical relative to temperate regions. Coevolutionary arms-race theory predicts that tropical herbivores should more readily tolerate consuming chemically-rich seaweeds than do temperate herbivores, however, tests of this prediction are rare. We assessed the willingness to consume freeze-dried tissue from 10 species of chemically-rich seaweeds exhibited by multiple populations of the herbivorous amphipod *Ampithoe longimana* collected from cold-temperate, warm-temperate and subtropical estuaries along the east and Gulf coasts of North America. In contrast with the coevolutionary predictions, geographic patterns of consumption of chemically-rich seaweeds were not correlated with latitude. Rather, populations in North Carolina (34ºN) consumed more tissue from the diterpene-producing seaweed genus *Dictyota* than did New England (41ºN) or Florida (27ºN) populations. Amphipod populations did not differ in their feeding responses to tissue from chemically-rich seaweeds in the genera *Amphiroa, Halimeda, Padina, Penicillus,* nor *Udotea*. Thus, Floridean *A. longimana* have not locally evolved a feeding tolerance for chemical defenses produced by tropical seaweeds, as would be predicted by an arms-race hypothesis. Rather, the geographic patterns in feeding behaviors reflect the relative importance of these seaweeds to the ecology of local populations.

Keywords. Herbivore offense, seaweed chemical defense, coevolutionary arms-race, biogeography

Introduction

Marine herbivores face profound challenges when feeding on seaweeds. Seaweeds and vascular plants generally contain low levels of nitrogen relative to herbivore tissues (Horn 1989; Choat and Clements 1998), which require herbivores to consume large quantities of plant material to maintain themselves. In addition, seaweeds have evolved structures and morphologies (e.g., crusts and calcification) that can make the algae tougher and less profitable to consume (Littler and Littler 1980; Steneck 1986; Schupp and Paul 1994). Finally, seaweeds contain an arsenal of secondary metabolites, including terpenoids, aromatics, and acetogenins, which deter herbivores (Paul 1992) and require biochemical manipulation (Targett and Arnold 2001; Sotka and Whalen 2008).

There is emerging evidence that these feeding challenges can be more profound for tropical herbivores relative to their temperate counterparts. Specifically, the concentration and diversity of lipophilic secondary metabolites within tropical seaweeds are considerably higher than those within temperate seaweeds (Hay 1991; Paul 1992; Van Alstyne et al. 2001; Pereira and de Gama 2008). In one of the few direct tests of a tropical-temperate gradient in seaweed defenses, Bolser

and Hay (1996) conducted feeding assays with two urchin species (*Lytechinus variegatus* and *Arbacia punctulata*) to demonstrate that freeze-dried tissue from temperate, North Carolina seaweeds were consumed at approximately twice the rates of tissue from closely-related tropical, Bahamian seaweeds. The authors also found that the higher palatability of temperate seaweeds was mediated by either the quality or quantity of secondary metabolites within the lipophilic extract. In contrast, variation in palatability among tropical and temperate seaweeds was not mediated by water-soluble compounds or the content of protein and ash-free organic matter.

There is general agreement that this greater deterrence of tropical seaweeds and their lipophilic metabolites relative to temperate seaweeds represents a macroevolutionary response by tropical seaweeds to an intensification of herbivory rates (Hay 1991; Paul et al. 2001; Van Alstyne et al. 2001). The greater herbivory pressures in the tropics are due in part to dramatically greater diversity and abundance of herbivorous fishes in the tropics relative to temperate regions (Gaines and Lubchenco 1982; Floeter et al. 2004). However, a neglected explanation of latitudinal trends in herbivory is the notion that tropical herbivores have a greater

feeding tolerance for chemically-defended tropical seaweeds than temperate herbivores. This geographic difference in feeding tolerance would evolve if tropical herbivores have responded to the greater levels of chemical defense of their prey via a diffuse evolutionary arms-race (cf. Vermeij 1994).

One of the few tests of an arms-race hypothesis for marine plant-herbivore interactions is Cronin et al. (1997). The authors found that diterpenoid metabolites from the tropical Pacific seaweed *Dictyota acutiloba* deterred North Carolina pinfish *Lagodon rhomboides* and the purple sea urchin *Arbacia punctulata* at concentrations that did not deter fish (*Scarus shlegeli*, *Scarus sordidus*, *Naso lituratus*, and *Naso unicornis*) and an urchin *Diadema savignyi* from tropical reefs of Guam. A second example comes from the combined results of Targett et al. (1995) and Boettcher and Targett (1993), which reveal that high concentrations of polyphenolics (~10% of tissue dry weight) lowered the assimilation efficiency (AE) of the temperate fish *Xiphaster mucosus*, but did not alter the AE of two tropical fishes (*Sparisoma radians* and *S. chrysopterum*) and a tropical crab (*Mithrax sculptus*). Because the temperate fish had a far more acidic gut (pH 2-3) relative to the tropical herbivores (pH 5-9), it was suggested that hydrogen bonding among polyphenolics and proteins is enhanced within acidic guts (Targett et al. 1995).

These studies tested the arms-race hypothesis using multiple herbivore species from temperate vs. tropical regions, but another approach is to compare geographically-isolated populations within a single herbivore species. Here, we assessed the relative feeding preferences for 10 species of chemically-rich seaweeds exhibited by the herbivorous amphipod *Ampithoe longimana* collected from cold-temperate (New England; 41ºN), warm-temperate (North Carolina; 34ºN) and subtropical (Florida; 27ºN) estuaries along the east coast of North America. The small (<1cm adults) gammaridean amphipod (Arthropoda; Crustacea; Malacostraca) is a tube-dwelling brooder that lives and feeds on a variety of algae (Duffy and Hay 1991; Sotka and Hay 2002; McCarty 2008). Our arms-race prediction is that subtropical populations of *A. longimana* should more readily consume tropical, chemically-rich seaweeds relative to amphipod populations from more temperate regions.

Materials and Methods

Specimen Collection and Storage

Ampithoe longimana specimens were collected at low tide from along 1,700 kilometers across the Atlantic coast of the United States in three regions: New England (Westport, Massachusetts (41º31'N, 71º04'W), Jamestown, Rhode Island (41º29'N, 71º23'W), and Niantic, Connecticut (41º18'N, 72º10'W)); North Carolina (Harker's Island (34º43'N,76º35'W),

Morehead City (34º42'N,76º40'W), and Wrightsville Beach (34º13N, 77º48'W)); and Florida ((Tampa Bay 27º45'N, 82º37'W) and Fort Pierce (27º27'N, 80º19'W)) between May of 2006 and August of 2007. We are confident that these populations are indeed *A. longimana*: Connecticut, Massachussetts and North Carolina populations readily interbreed (Sotka 2003) and published data (Sotka et al. 2003; McCarty 2008) indicate that Florida and Connecticut populations differ from each other by ~2% at a mitochondrial locus.

Amphipods were collected by hand from a variety of seaweeds and transported to Grice Marine Laboratory. Amphipods were cultured in ~30 ppt filtered seawater obtained from Grice Cove in Charleston, South Carolina at 20ºC. Water was changed every 5-7 days, and the containers were aerated constantly. Amphipods in the cultures were fed fresh *Ulva* spp*., Ectocarpus* spp*., Sargassum* spp*., Bryopsis* spp*., Gracilaria* spp*.,* and *Hypnea* spp. collected from throughout North and South Carolina. All seaweeds were rinsed at least twice in freshwater for 30-45 seconds to remove local biota prior to being placed in the cultures. Because amphipods used in our feeding assays represent at least the 2nd generation in these 'common garden' cultures, population-level patterns in feeding behaviors likely have a genetic basis.

Seaweeds used for the feeding assays were collected in May 2005 from the upper Florida Keys (25º07'N, 80º25'W; *Halimeda* incrassata, *H. opuntia*, *H. tuna, Penicillus dumentosus, Udotea flabellum*, an unidentified *Dictyota* sp. and an unidentified *Amphiroa* sp.) and in July 2005 from near Morehead City, NC (*Dictyota cilolata*, *D. menstrualis*, *Padina gymnospora*) and in February-March 2005 from Charleston Harbor, SC (34º45'N, 79º54'W; *Ulva (syn. Enteromorpha) intestinalis*). Seaweeds were subsequently frozen at -4°C within 12 hours of collection, lyophilized, ground to a powder, and stored at -20°C for long-term storage.

Feeding Assays

To assess feeding preferences, a series of choice assays were performed using lyophilized tissue. Offering lyophilized tissue permitted experiments at different times without changing tissue quality. Lyophilization removes morphological traits but largely maintains biochemical traits that might mediate feeding choices. The process of lyophilization reduces the quantity of some secondary metabolites (Cronin et al. 1995). However, it is unlikely this potential artifact biases our conclusions because we are primarily focused on the relative differences in feeding behavior between populations exposed to the same foods.

Frozen, lyophilized algae were ground to a fine powder using a Wiley mini-mill with a 180 mm mesh sieve or ground by hand using a mortar and pestle. Food was prepared using the following recipe: 0.5 g freeze-dried seaweed mixed with 2mL H_2O added to 0.9

g of agar that was mixed and heated with 2.5 mL H_2O. The recipe was doubled, tripled or quadrupled to make sufficient food for each assay. This agar and seaweed mixture was applied to plastic screen mesh (i.e. window screen) and pressed between two sheets of wax paper for ~ less than one hour. The mesh was then cut into feeding grids measuring 5 x 6 mesh squares.

A single replicate assay consisted of one 200 mL plastic cup with ~75 mL of seawater, and a single feeding grid each of the 'Control' (*Ulva (syn. Enteromorpha) intestinalis*) and 'Treatment' food (*D. menstrualis, D. ciliolata,* etc.). Each 'Treatment' seaweed species is tropical in distribution, and is a low-preference food for several generalist fish, urchin and amphipod consumers largely because of their low nutritional value, chemical deterrents, and/or morphological defenses (Paul 1992; Bolser and Hay 1996; Pereira and de Gama 2008). *U. intestinalis* was chosen as a 'Control' because it is locally available to temperate and tropical populations of the amphipod, is readily consumed by *A. longimana*, and there are no population differences in fitness when fed *U. intestinalis* (Sotka et al. 2003).

One amphipod was placed into each replicate with the two feeding grids. Replicates were placed in covered containers in either a 20ºC or 25ºC incubator in complete darkness for the duration of the assay. Replicates were checked twice daily and stopped when five days had lapsed, or when the amphipod had consumed either 9 squares from one feeding square or a combination of 10 squares from both feeding squares, whichever was first. Replicates with fewer than 9 squares, or more than 35 squares, were excluded from analysis. Most replicates were completed within 2-3 days, but this is sufficient time to allow soluble materials to leach, including proteins, amino acids, carbohydrates and any phlorotannins. Lipophilic compounds should, however, maintain themselves on the artificial foods.

Extremes in local temperatures alter herbivore feeding behavior (Sotka and Giddens 2009). Thus, because New England populations rarely experience 25ºC, Floridean populations rarely experience 20ºC, and North Carolina populations experience both temperatures annually (Sotka and Giddens 2009), we present assay results from New England amphipods at 20ºC and North Carolina and Florida populations at 25ºC.

Paired t-tests were used to assess whether consumption rates of the two foods differed (i.e., Control vs. Treatment). To assess whether populations differed in their feeding choices, we converted the data into a proportion of treatment tissue consumed (e.g., amount of Treatment consumed divided by total amount consumed in a replicate) and analyzed this proportion using a nonparametric ANOVA using a distribution generated from a permutation method (Anderson 2001). If we had data from multiple populations within a region, then we combined all replicates for that region for analysis.

Results

During feeding choice assays, cold-temperate (New England), warm-temperate (North Carolina) and subtropical (Florida) populations consumed significantly more *Ulva* than any of the green seaweeds (*Halimeda* incrassata, *H. opuntia, H. tuna, Penicillus dumentosus* and *Udotea flabellum*), the red seaweed *Amphiroa* and the brown unidentified *Dictyota* species (Paired t-test $p < 0.05$ for each population / seaweed combination; Table 1). In contrast, amphipod populations tended to consume statistically equivalent amounts of *Ulva* and the brown seaweeds *Padina gymnospora, D. ciliolata* and *D. menstrualis.* However, Florida amphipods ate significantly more *Ulva* than *D. ciliolata* while North Carolina amphipods ate significantly more *D. menstrualis* than *Ulva.*

Table 1. Relative consumption of 10 chemically-rich tropical seaweeds by three populations of the herbivorous amphipod *Ampithoe longimana*. The proportion of chemically-rich seaweed consumed, sample size (n) and *p*-values are indicated for each assay. Grey boxes highlight assays where the consumption of treatment foods was significantly different (i.e., less or more) than of control foods.

Seaweed	Florida (27ºN)			North Carolina (34ºN)			New England (41ºN)		
	Proportion	n	*P-value*	Proportion	n	*P-value*	Proportion	n	*P-value*
Amphiroa	0.24	31	<0.001	0.15	36	<0.001	0.07	33	<0.001
D. ciliolata	0.24	36	<0.001	0.51	38	0.203	0.43	33	0.364
D. menstrualis	0.44	40	0.521	0.64	29	0.018	0.42	15	0.578
Dictyota sp.	0.29	35	<0.001	0.35	36	0.038	0.21	37	<0.001
Halimeda incrassata	0.07	36	<0.001	0.02	21	<0.001	0.08	37	<0.001
H. opuntia	0.08	33	<0.001	0.07	9	<0.001	0.01	40	<0.001
H. tuna	0.11	32	<0.001	0.19	28	<0.001	0.16	22	<0.001
Padina	0.36	38	0.161	0.37	29	0.436	0.43	24	0.583
Penicillus	0.21	35	<0.001	0.26	35	<0.001	0.28	38	<0.001
Udotea	0.24	35	<0.001	0.18	27	<0.001	0.12	36	<0.001

A. All seaweeds

NE b
NC a
FL b

0.0 0.2 0.4 0.6 0.8 1.0

B. *Dictyota* only

NE b
NC a
FL b

0.0 0.2 0.4 0.6 0.8 1.0

C. All other seaweeds

NE
NC $p = 0.148$
FL

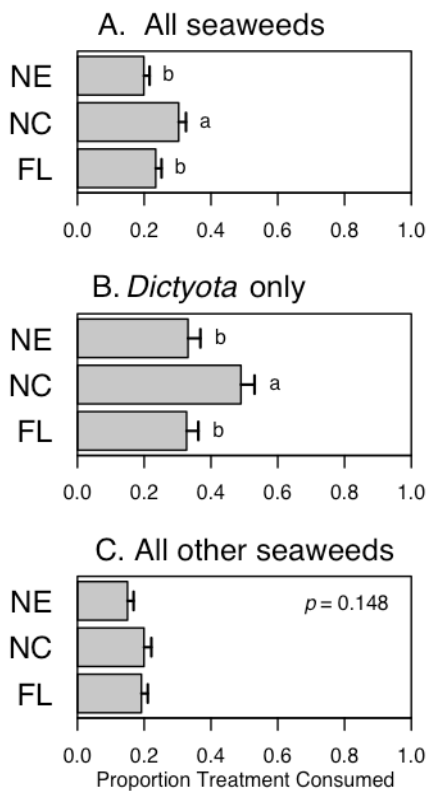0.0 0.2 0.4 0.6 0.8 1.0
Proportion Treatment Consumed

Figure 1. The average (+ S.E.) proportion of chemically-rich tropical seaweed consumed. Letters indicate groups that were significantly different.

We explicitly tested whether there are significant differences between populations in the proportion of chemically-rich tissue consumed. When all replicates are pooled, North Carolina individuals consumed significantly more of the chemically-rich tissue than did either Florida or New England populations (Figure 1A). However, as is suggested from Table 1, the regional differences in feeding preferences are driven largely by geographic variation in consumption of *Dictyota menstrualis and D. ciliolata*. Moreover, previous work has indicated that North Carolina animals use *D. menstrualis* and *D. ciliolata* as a host (Duffy and Hay 1991; Sotka and Hay 2002; Sotka et al. 2003). When we re-analyzed the feeding responses of the amphipods toward the genus *Dictyota* vs. the feeding response to the remaining seaweeds, North Carolina individuals consumed significantly more *Dictyota* tissue than did New England or Florida individuals (Figure 1B). When *Dictyota* seaweeds are removed from analysis, then there is no significant difference between the populations in the propensity to consume the other species of chemically-rich seaweeds (Figure 1C).

Discussion

Our results indicate that subtropical amphipod populations do not exhibit greater feeding preference for tropical chemically-rich seaweeds than do more temperate amphipods (Table 1; Figure 1). Rather, the warm temperate North Carolina amphipods were more

likely than either the cold temperate New England or Florida populations to consume species in the genus *Dictyota*. This result does not support the notion that Florida amphipods have responded evolutionarily to chemically-rich tropical seaweeds, as would be predicted by an arms-race hypothesis.

Instead, local host use is central to understanding the evolution of these feeding behaviors (McCarty 2008). North Carolina amphipods feed readily on *Dictyota* and are readily found on *Dictyota* (Duffy and Hay 1991; McCarty 2008). This is because the *Dictyota* produces diterpene alcohols that deter larger omnivorous pinfishes that dominate the local benthos. By associating with this chemically-defended seaweed, the amphipods gain a measure of protection from their fish consumers. Neither New England nor Florida amphipods are found on *Dicytota* because *Dictyota* is not available in their local habitats (McCarty 2008). New England populations are 100's of kilometers north of the northern endpoint of *Dictyota*. In Florida estuaries, *A. longimana* are abundant where *Dictyota* appears to be rare, while on Florida coral reefs, *Dictyota* is abundant while *A. longimana* have never been found. Thus, the greater preference for *Dictyota* seen among North Carolina individuals reflects the importance of this seaweed in its local ecology.

Similarly, the lack of a regional response in feeding behavior toward the other chemically-rich seaweeds (e.g., *Amphiroa*, *Udotea*, etc.) reflects the fact that the amphipod is rarely found naturally on these seaweeds (McCarty 2008). The Florida *A. longimana* are found within estuarine seagrass beds where chemically-rich seaweeds are rare and where there are abundant seaweeds that lack chemical, morphological or mineral defenses (Hay 1984; McCarty 2008). Thus, local ecological usage of host plants clearly predicts the evolution of regional patterns in feeding behaviors.

We suggest that future tests of the latitudinal gradient in herbivore feeding tolerance utilize tropical herbivores that are regularly exposed to chemically-rich seaweeds (e.g., Cronin et al. 1997), such as urchins, crabs and fishes found on coral reefs (Hay 1984; Paul 1992). It is these reef herbivores that are most likely to be responding coevolutionarily to the chemical defenses of the local seaweeds. Our results also suggest that it may be fruitful to test whether the lower relative abundance of chemically-rich seaweeds within estuaries and seagrass beds relative to their frequencies on coral reefs (Hay 1984) might be directly reflected in the feeding tolerances of herbivores found in estuaries versus coral reefs.

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