

Local adaptation in adult feeding preference and juvenile performance in the generalist herbivore *Idotea balthica*

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Abstract Populations can respond to environmental heterogeneity by genetic adaptation to local conditions. Evidence for local adaptation in herbivores with relatively broad host breadth is scarce, either because generalists rarely locally adapt or because fewer studies have tested for local adaptation. The marine isopod *Idotea balthica*, a small (<3 cm) generalist herbivore common to estuaries of the northwestern Atlantic, is found on multiple macroalgae and sea grasses north of 42°N, while more southerly populations utilize sea grass-dominated and macroalgal-poor habitats. Feeding preference assays revealed a latitudinal shift in preference hierarchy that mirrors this geographic variation in host availability. Northern populations have higher feeding preference for fresh and freeze-dried tissue of the brown macroalga *Fucus vesiculosus* and consumed more of its water-soluble and lipophilic extracts relative to southern populations. In contrast, southern populations have a relatively higher preference for the green macroalga *Ulva linza* and sea grass *Zostera marina*. The rank of hosts in feeding assays exhibited by northern adults (*Fucus* = *Ulva* > *Zostera*) and southern adults (*Ulva* > *Fucus* > *Zostera*) closely mirrored ranking of juvenile growth rates, suggesting that preference and performance are strongly correlated across these macrophytes. Several of our assays included

isopods that had parents reared under uniform laboratory conditions, indicating that geographic differences are genetically mediated and unlikely to reflect phenotypic plasticity or maternal effects. Local adaptation in host use traits may be common in broadly distributed, generalist herbivores in marine and terrestrial systems, and will manifest itself as local shifts in the preference ranking of hosts.

Keywords Environmental heterogeneity · *Fucus vesiculosus* · Host use · Plant herbivore interactions · Plant chemical defense · *Zostera marina*

Introduction

A species' range is a diverse landscape of biotic and abiotic conditions. Patchy distributions of environmental conditions or habitats across a species' range lead to variation in the intensity and direction of natural selection experienced by local populations. Where selective pressure is strong enough to overpower the homogenizing effects of gene flow or where gene flow is restricted, populations locally adapt to particular features of their environment (Endler 1977). This creates a situation where traits that have an increased fitness advantage for one population in one environment may be maladaptive in others (Thompson 2005; Marshall et al. 2010).

Local adaptation commonly occurs in freshwater and terrestrial systems (Coyne and Orr 2004; Hereford 2009). Although this process has been observed in marine systems, it has been less frequently tested and thus remains poorly understood (Conover 1998; Sotka 2005; Sanford and Kelly 2011). Historically, marine populations have often been thought to consist of demographically open populations with little opportunity for restricted dispersal and

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thus little opportunity for population differentiation and local adaptation (Grosberg and Cunningham 2001; Palumbi 2004). However, recent work has demonstrated that gene flow is more restricted than we expected and, consequently, local adaptation may occur across a smaller spatial scale than previously assumed (reviewed by Sotka 2005; Sanford and Kelly 2011).

The intimate interaction between an herbivore and its host plant lends itself well to tests of local adaptation. Among terrestrial herbivores with broad geographic ranges, particularly insects, local populations commonly adapt to secondary metabolites, structural defenses, and nutrient availability of local host plants (Thompson 2005; Singer 2008). However, in marine herbivores, local adaptation for host plants/algae has rarely been tested (but see Poore and Steinberg 2001; Sotka et al. 2003; Jormalainen et al. 2008; Vesakoski et al. 2009). It remains unclear whether this rarity reflects a general lack of research attention in marine systems or fundamental differences in the propensity to locally adapt among marine herbivores (Sotka 2005). Marine herbivores tend to have high dispersal potential and are much more likely to feed on multiple species than are their terrestrial invertebrate counterparts (Hay and Steinberg 1992; Poore et al. 2008), and both traits reduce the likelihood of local adaptation.

The coastline of the northwestern Atlantic offers a rich environment in which to study local adaptation in marine plant–herbivore interactions. An assemblage of large macroalgal species covers the high-energy rocky coastlines in the north (i.e., >42°N; north of Long Island Sound), while soft bottom substrates in the south are dominated by sea grass species (Bertness 2007; Jenkins et al. 2008). For an herbivore species whose range spans this dramatic transition in habitats and food resources, it is likely that localized preferences and adaptation to local plants' structural or chemical defenses will occur. As an example, the herbivorous amphipod *Ampithoe longimana* in southern areas has greater preferences for and fitness on a co-occurring tropical macroalga *Dictyota* relative to amphipod populations from northern regions that do not co-occur with *Dictyota* (Sotka et al. 2003).

The herbivorous isopod, *Idotea balthica*, is distributed throughout the North Atlantic Ocean and Baltic Sea, where it is found predominantly on large macroalgal species and in sea grass beds. It also spans the east coast of the USA across the dramatic transition from hard- to soft-bottom habitats. *Idotea* populations in New England are exposed to relatively sheltered eelgrass beds and high energy shores dominated by large macroalgal species. Southern populations are found almost exclusively in eelgrass beds (e.g., Chesapeake Bay). *I. balthica*'s rafting dispersal strategy suggests that dispersal has the potential to occur over large geographic distances (Wares and Cunningham 2001). How-

ever, it appears that *I. balthica* rarely moves along the east coast of the USA, where there is a strong genetic break at a mitochondrial locus (COI) between northern and southern populations (Wares 2001; Bell 2009). Given the possibility that these two sets of populations (north vs. south of Long Island Sound) have limited gene flow and occur in regions with locally distinct habitats and flora, it is likely that they may have evolved locally adaptive characteristics. Such shifts are known to occur in *I. balthica* populations from the Baltic Sea, where isopods have slightly greater preference for and juvenile performance on sympatric hosts (Vesakoski et al. 2008, 2009) at relatively small spatial scales (~10s of kilometers). Here, we will address whether northwestern Atlantic populations of *I. balthica* from across 100s of kilometers have greater feeding preference for and ability to utilize local hosts.

Materials and methods

Isopod collections

I. balthica has a discontinuous distribution along the western North Atlantic coast, where it is usually found in one of three habitat types: sea grass beds, drifting algal mats, or attached algae along rocky shorelines. Adult isopods were collected in the summer of 2008, 2009, and 2010 from the following locations that are dominated by either sea grass or drift algae: Cape Charles (VA), Goodwin Island (VA), Ship Bottom (NJ), Jamestown (RI), Nahant (MA), Wellfleet (MA), and Magnolia (MA). Since many of these isopod populations are ephemeral and not consistently available, isopods from all locations were not used in all of the following described experiments (see online resource 1 for a description of population coordinates and experimental use). Isopods were collected using a mesh net and then transported in aerated coolers either to the Marine Science Center (Northeastern University in Nahant, MA; 2008) or to the Grice Marine Laboratory (College of Charleston in Charleston, SC; 2009 and 2010). Upon arrival, isopods were allowed to acclimate to laboratory conditions for a minimum of 7 days before use in any feeding assay.

Field survey

In order to compare differences in host use along *I. balthica*'s geographic range, a field survey was conducted where the abundance of isopods on commonly preferred hosts was observed. We conducted this survey in the summer of 2010 at the following locations: Magnolia (MA), Nahant (MA), Wellfleet (MA), Jamestown (RI), Ship Bottom (NJ), and Cape Charles (VA). From each site, we haphazardly

collected approximately 12 L (by volume; split into three 4-L bags) of the four most highly preferred species, as previously determined by multiple choice assays conducted in 2008 (see next section). All adult *I. balthica* were removed and counted. Abundance of isopods per wet mass of algae was then compared within and between sites (online resource 2). Given our relatively low replication and highly non-normal distributions, we opted to not statistically assess the data. Thus, these data provide a qualitative pattern of host use across locations.

Fresh tissue feeding assays

Fresh tissue feeding assays (conducted in Nahant, MA, in 2008) were used to initially characterize *I. balthica*'s feeding behavior. Isopods were collected from Cape Charles and Goodwin Island (VA), and Nahant and Magnolia (MA) where they were fed *Ulva linza* during a 7-day pre-experiment acclimation period. All host species chosen for this study are either species on which isopods commonly reside in the field or species which European populations are known to consume (Nicotri 1980; Jormalainen et al. 2001; Vandendriessche et al. 2006; Vesakoski et al. 2009). These species are *Ascophyllum nodosum*, *Fucus vesiculosus*, *Ulva lactuca*, *U. linza*, *Polysiphonia harveyi*, *Gracilaria vermiculophylla*, *Chondrus crispus*, and one seagrass, *Z. marina*. All host species co-occur with isopod populations north of Long Island Sound (e.g., Massachusetts) except for *Gracilaria* which is rare. South of Long Island Sound (e.g., Virginia) isopods co-occur with all host species except *Chondrus* and *Ascophyllum*. Although *Fucus* does occur south of Long Island Sound it is less abundant and rare. After all epiphytes were removed from the hosts, they were blotted dry, and cut into 45 mg fragments. Algal fragments were separately placed into small submersible floating containers (4-oz plastic containers, sealed with small-gauge nylon mesh).

Fresh tissue: no-choice assay

Thirty adult males from each population were each isolated with one type of algae. Because of variation in body size among populations, isopods were weighed prior to the assay. To account for autogenic changes in mass during the time course of the assay, three 45-mg pieces of algae were cut from a single plant, one was offered to each herbivore population, and one was isolated without any herbivores. A total of 948 isopods out of 960 survived the assay period leaving no less than 25 replicates per host species per population. After 48 h, or when at least half the algae had been consumed, all algal fragments were collected from containers, blotted dry, and reweighed. Relative consumption of algal tissue was calculated according to Sotka and Hay

(2002); these values were then divided by the mass of the isopod.

In order to test population differences in feeding rate on each host species, we generated a two-way ANOVA with host species and population as independent factors. Prior to conducting this and all other ANOVAs mentioned in later sections, data were transformed to adjust for assumptions of unequal variances and normality. All statistical analyses both here and in subsequent sections were performed using JMP (v.8; SAS Institute, Cary, NC, USA, 1989–2008). In addition, we calculated the relative effect size (magnitude of effects ω^2 ; Graham and Edwards 2001) for each significant ANOVA term.

Fresh tissue: choice assay

A fresh tissue choice assay was conducted using methods described above for the no-choice assay with two exceptions: we offered each isopod all eight host species simultaneously and we chose isopods of similar body size from each region to account for effects of body mass on consumption rate. Acclimated isopods were starved for 24 h prior to the experiment. Treatment containers were prepared with 25 mg of each host and randomly assigned one isopod from one of four populations from Magnolia (MA), Nahant (MA), Goodwin Island (VA), or Cape Charles (VA). Forty control containers with only the eight host species were used to control for any autogenic change in the host during the assay. Individuals were allowed to feed for 48 h or until at least half of one of the hosts had been consumed. A total of 155 individuals were recovered from the assay.

Preference was measured by calculating overall consumption of each host using the equation from Sotka and Hay (2002) and then by calculating relative preference of each host (the total mass of each individual host divided by the total mass consumed across all host species). Differences in consumption of host species across populations were assessed using Hotelling's multivariate T^2 test on the proportional consumption data corrected by autogenic changes. In addition, we also estimated effect size based on estimates of η^2 for each significant MANOVA term.

Freeze-dried tissue feeding assays

No-choice and choice assays

The results of our fresh tissue feeding assays indicated that three species (*U. linza*, *F. vesiculosus*, and *Z. marina*) were differentially preferred across populations. To assess feeding rates on these three host species, we performed a no-choice and a choice feeding assay using freeze-dried tissue offered to two laboratory-reared common garden populations from

Jamestown (RI) and Goodwin Island (VA). *Z. marina*, *F. vesiculosus*, and *U. linza* were freeze-dried and ground into a fine powder. Two grams of this algal powder was then mixed with melted agar (0.5 g) and water (12 mL) that was pressed onto window screen (see Hay et al. 1994). The window screen coated in the solidified alga and agar mixture was then cut into equal square pieces. For the no-choice assay, 90 adult male isopods of equal size from each population were individually isolated into seawater filled 100-mm-square plastic containers. Each isopod (30 replicates per host species; 90 per population) was given a piece of window screen coated in one of the three host species. Isopods were allowed to consume food squares for 24 h or until half the squares from a single food were consumed. In the choice assays, we followed all of the above methods except that 30 replicates per population (60 isopods in total) were used and each isopod was given all three host species.

Feeding rate was measured by the number of squares consumed within each piece of window screen. For the no-choice assay, we used a two-way ANOVA to assess population level-differences in feeding rate on these three host species. While for the choice-assay, we used Hotelling's multivariate T^2 test to test for differences among populations in relative consumption, followed by pairwise Wilcoxon signed-rank tests.

Latitudinal preference assay

The third choice assay examined the response of six latitudinally distributed populations from Magnolia (MA), Nahant (MA), Jamestown (RI), Shipbottom (NJ), Goodwin Island (VA), and Cape Charles (VA) of isopods to a choice of *U. linza* and *F. vesiculosus*. Each field-collected isopod (30 from each location) was offered two square pieces of window screen coated in either *F. vesiculosus* or *U. linza* (see above for preparation methods and assay conditions). We performed a one-way ANOVA to test for population differences in consumption of *Fucus*. A post hoc Tukey test was used to assess the magnitude of these differences. To test how *Fucus* consumption compared to that of *Ulva* (the control), we conducted a series of pairwise Wilcoxon signed-rank tests.

Feeding responses to *Fucus* chemical extracts

We tested whether population-level differences in feeding responses toward *F. vesiculosus* are mediated by the macroalgal secondary metabolites. We extracted metabolites from *Fucus* using a protocol modified from Taylor et al. (2002). Eight grams of finely ground freeze-dried *Fucus* was treated with a 2:1 mixture of ethyl acetate and methanol in order to extract lipophilic compounds. For extraction of

water-soluble compounds, an additional 8 g of finely ground freeze-dried *Fucus* was treated with a 70:30 mixture of methanol and water. Ethyl acetate and methanol were removed from both extracts using rotary evaporation. Water-soluble extracts were added to *U. linza* at natural concentrations and then embedded in melted agar, spread onto a piece of window screen. We added 8 g of *U. linza* to *Fucus*-derived lipophilic extracts re-dissolved in ethyl acetate. The ethyl acetate was then removed from the *U. linza* using rotary evaporation. We created a control food by adding an equivalent amount of ethyl acetate to 8 g of *U. linza* and subsequently removed via rotary evaporation in order to ensure that solvent addition and removal did not confound the preference results. These foods were then embedded in agar, spread onto window screen, and offered to individual isopods as described above. Thirty isopods from common garden populations from Jamestown (RI) and Goodwin Island (VA) were offered a pairwise choice between *U. linza* with no extract (control) and freeze-dried *U. linza* coated in either water-soluble or lipophilic extracts from *F. vesiculosus*. Deterrence by a particular extract was determined by the number of window screen squares consumed. Population differences in preference for different chemical extracts were determined using a one-way ANOVA and paired Wilcoxon signed-rank tests.

Juvenile performance assay

To see whether population-level differences in feeding patterns were reflected in differences in isopod survivorship and growth, we reared naïve juvenile isopods from two populations from Goodwin Island (VA) and Jamestown (RI) for 8 weeks. Twenty field-collected gravid females from each population were isolated into separate containers. Ten to twenty juveniles (starting size: 2 mm; ~5 days old) were collected from each of the 20 mothers, pooled, and then randomly assigned to a host species. Totals of 270 juveniles from each population were raised in groups of 3 on one of three freeze-dried host species (*F. vesiculosus*, *U. linza*, or *Z. marina*) in 4.5-oz (c.120-mL) cups of seawater stored at 18 °C. Water was changed every 2 days and food was added as needed. Body length was measured as a straight-line distance from the very end of the head to the end of the longest spine on the telson via analysis (using IMAGEJ software; Abramoff et al. 2004) of weekly photographs. Average body length after the 8-week period was compared across populations and foods using an ANOVA and Tukey's test. In addition, we calculated the relative effect size (magnitude of effects ω^2 ; Graham and Edwards 2001) for each significant ANOVA term. Survivorship data was compared across populations and foods using survivorship curve analyses (log-rank X^2 test).

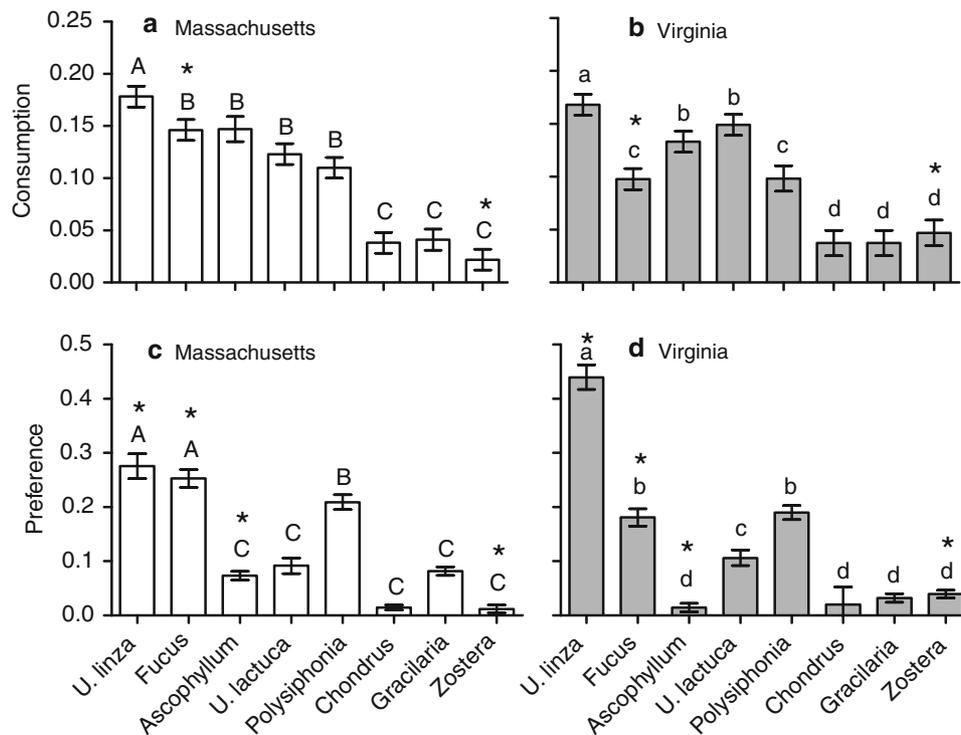


Fig. 1 Feeding preference assays for fresh tissue of eight algae by isopods from Massachusetts northern populations (white bars) and Virginia southern populations (gray bars). **a, b** Consumption (mean mg host species consumed per mg isopod \pm SE, $n = 948$) across isopods in a no-choice assay. Letters indicate algae that were consumed at statistically equivalent rates (upper- and lowercase letters for assays within Massachusetts and Virginia, respectively). Asterisks indicate significant differences between regions for particular algae based on ANOVA

and Tukey HSD (*Fucus* $P < 0.0001$, *Zostera* $P = 0.004$). **c, d** Relative preference (mean \pm SE, $n = 155$) in a choice assay based on Hotelling's T^2 and Wilcoxon signed-rank (*U. linza* $P < 0.001$, *Zostera* $P = 0.005$, *Fucus* $P < 0.001$, *Ascophyllum* $P = 0.002$). We summarized feeding rates across regions because post-hoc analyses indicated that feeding responses of populations within a region were statistically indistinguishable (see "Results" and online resource 3A–B for statistical details)

Results

Field survey

Northern populations (those north of Long Island Sound) were most common on *Chondrus*, *Fucus*, detached mixed algae, and *Ulva linza*, while southern populations (those south of Long Island Sound) most commonly occurred on *Zostera*, *Ruppia*, and detached mixed algae (online resource 2). Although northern populations occurred on attached *Fucus* in intertidal habitats, this does not appear to be as highly preferred as in European populations (Schaffelke et al. 1995; Jormalainen et al. 2001). *Fucus* was often present in the detached (i.e., drift) mixed algal assemblage at northern locations.

Regional and population-level differences in feeding response

When field-collected isopods from Massachusetts and Virginia were offered fresh tissue from a single host species (i.e., a no-choice assay), they fed most readily on *Ulva*

linza, *Fucus*, *Ascophyllum*, and *Ulva lactuca* (Fig. 1a). *Chondrus*, *Gracilaria*, and *Zostera* were consumed at the lowest rates [the effect of host: $P < 0.001$, effect size (ω^2) = 0.949]. There was also a strong interaction between host species and population in feeding rate [$P < 0.001$, effect size (ω^2) = 0.022] (online resource 3A). The feeding rates of all eight host species did not differ significantly between Virginia populations or between Massachusetts populations in post-hoc analyses. Figure 1 therefore represents the averaged feeding rates for both populations within Virginia and within Massachusetts. Although there were no differences within Massachusetts and Virginia, there were differences between these two regions for two host species: *Fucus* and *Zostera*. Post-hoc analysis (Tukey's HSD) revealed Virginia isopods consumed significantly more *Zostera* than did Massachusetts isopods ($P = 0.004$), while Massachusetts isopods consumed significantly more *Fucus* than did Virginia isopods ($P < 0.001$) (Fig. 1a).

When isopods were offered eight host species simultaneously (i.e., fresh tissue choice assay), feeding preferences mostly mirrored no-choice feeding rates. Again, there were no significant differences in consumption of the eight host

species between the two populations within Virginia or within Massachusetts in post-hoc analyses. Thus, consumption of populations within Virginia and within Massachusetts were averaged together for Fig. 1. There was a strong preference for *U. linza* across Massachusetts and Virginia populations (Fig. 1b), while *Chondrus*, and *Zostera* were the least preferred [effect of host $P < 0.001$ effect size ($\eta^2 = 0.498$)]. There were also subtle shifts in the relative rank of preference in these generalist consumers leading to an interaction between population and host ($P < 0.001$, $\eta^2 = 0.122$; online resource 3B). Virginia isopods consumed significantly more *U. linza* and *Zostera* than did Massachusetts isopods ($P < 0.001$; $P = 0.005$, respectively) (Fig. 1b) while Massachusetts isopods consumed significantly more *Fucus* and *Ascophyllum* than those from Virginia ($P < 0.001$; $P = 0.002$, respectively). Given the strong differences in feeding response toward *U. linza*, *Fucus*, and *Zostera* revealed in the fresh tissue feeding assays and the difference in the importance of these hosts among populations (online resource 3B), we focused the rest of our efforts on these macroalgae in subsequent assays.

When common garden isopods (i.e., individuals from cultures that were maintained in the laboratory in similar conditions for 6 months) were offered freeze-dried tissue of each host singly (no-choice), both Virginia and Rhode Island populations consumed *Fucus* and *U. linza* at a significantly higher rate than *Zostera*, and there was no significant population by host species interaction (Fig. 2a; online resource 3C; $P = 0.331$). When these tissues were offered in a choice assay, isopods from Rhode Island consumed *Fucus* and *Ulva* at equal rates ($P = 0.708$), while Virginia isopods preferred *Ulva* over *Fucus* ($P < 0.001$; Fig. 3b). A statistical comparison of relative feeding preferences indicated that Rhode Island isopods consumed relatively more *Fucus* than did Virginia isopods ($P < 0.001$); however, Virginia isopods consumed relatively more *Zostera* ($P = 0.004$) and *U. linza* ($P = 0.022$) than did Rhode Island isopods.

Latitudinal patterns in preference

We tested whether population-level patterns in feeding preference toward *Fucus* and *U. linza* reflects patterns in *Fucus* abundance by collecting and assaying six populations along a latitudinal transect. Populations with higher abundance of *Fucus* (from New Jersey to Massachusetts) mostly preferred *Fucus* in pairwise choice assays. Populations in Virginia (where *Fucus* is rare) preferred *Ulva* (Fig. 3 inset; $P < 0.001$). For the population from Nahant, MA, there was equal preference for *Fucus* and *Ulva* ($P = 0.571$), while isopods from Magnolia, MA, Rhode Island and New Jersey consumed more *Fucus* than *Ulva* ($F > U$: Magnolia ($P < 0.001$), Rhode Island ($P = 0.038$), NJ ($P = 0.024$)).

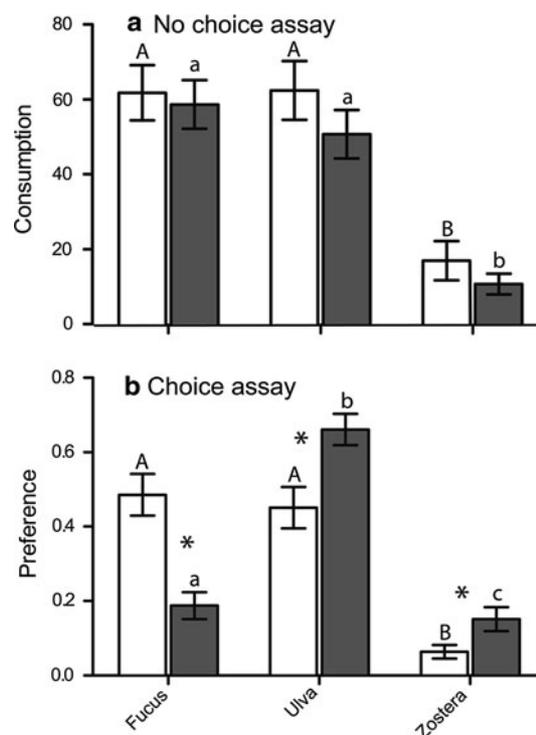


Fig. 2 Feeding preference assays for freeze-dried tissue from *Fucus*, *Ulva*, or *Zostera* by isopods from Jamestown, Rhode Island (white bars) and Goodwin Island, Virginia (gray bars). **a** Consumption (mean mg of host species consumed per mg isopod \pm SE, $n = 180$) across populations (RI and VA) in a no-choice assay based on an ANOVA. **b** Relative preference (mean \pm SE, $n = 120$) in a choice assay based on Hotelling's T^2 test and Wilcoxon signed-rank test (*Fucus* $P < 0.001$, *Ulva* $P = 0.022$, *Zostera* $P = 0.004$). Statistical details are given in online resource 3C–3D. Asterisks and letters are as defined in Fig. 1

Feeding responses to *Fucus* chemical extracts

Feeding response to lipophilic extracts exhibited high similarity to that from whole tissue, where Rhode Island isopods as well as those from Virginia consumed the same amount of food coated in lipophilic extracts from *Fucus* as they did freeze-dried *Fucus* [lipophilic extract vs. whole tissue: RI ($P = 0.850$); VA ($P = 0.950$); Fig. 4]. The Rhode Island population consumed foods coated with lipophilic extract more readily than control foods ($P = 0.038$), while isopods from Virginia were deterred by lipophilic extracts relative to the control ($P < 0.001$). Natural concentrations of water-soluble extracts were consumed significantly less than control foods [water soluble vs. control: RI ($P < 0.001$); VA ($P < 0.001$); Fig. 4], and deterrence by these extracts was stronger than lipophilic extracts for both populations. Direct statistical tests of the proportion of extract consumed revealed that Rhode Island isopods more readily consumed water-soluble ($P = 0.025$; Wilcoxon signed-rank test) and lipophilic extracts ($P = 0.022$) than did Virginia populations.

Fig. 3 Feeding preference assays for *Fucus vesiculosus* (mean relative preference \pm SE, $n = 180$, 30 per population) by six populations of isopods from northern to southern latitude. Letters indicate populations that consumed *Fucus* at statistically equivalent rates based on ANOVA and Tukey HSD. Asterisks indicate those populations that exhibit significantly stronger preference for *Fucus* over the control (*Ulva*) based on a Wilcoxon signed-rank test (Magnolia $P < 0.001$, Jamestown $P = 0.038$, Ship Bottom $P = 0.024$, and both Virginia populations $P < 0.001$). The coastal gray line represents current geographic distribution of *Fucus*, where the solid line represents a continuous distribution and the dashed line represents a patchy distribution

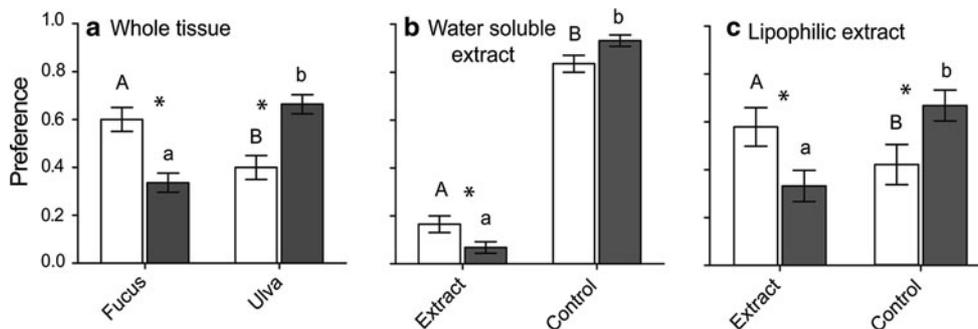
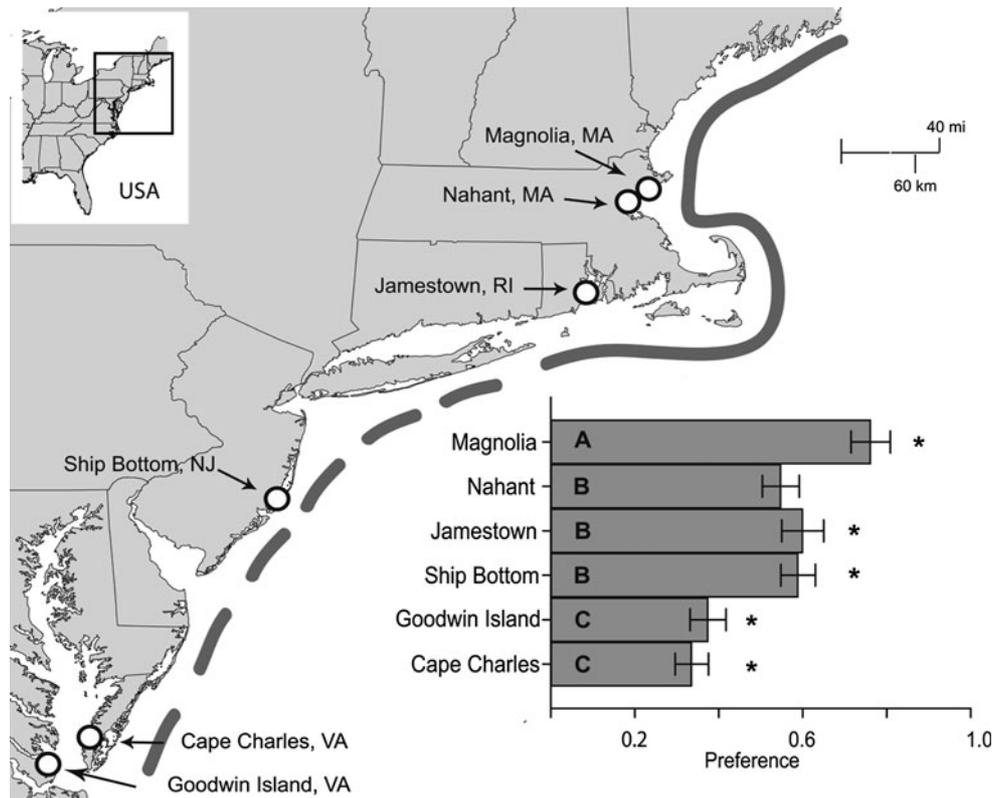


Fig. 4 Feeding preference by isopods from Jamestown, Rhode Island (white bars) and Goodwin Island, Virginia (gray bars) for **a** *Fucus vesiculosus* versus *Ulva linza* whole tissue (mean \pm SE, $n = 60$) and **b**, **c** extract-coated versus control food (mean \pm SE, $n = 60$ for both lipophilic and water soluble extracts). Uppercase letters indicate results of post-hoc analyses for feeding differences among food options based on ANOVA and Wilcoxon signed-rank tests (**a** for RI *Fucus* > *Ulva* $P = 0.038$, for VA *Ulva* > *Fucus* $P < 0.001$; **b** water soluble extract

consumption by RI control > extract $P < 0.001$, by VA control > extract $P < 0.001$; **c** lipophilic extract consumption by RI extract > control $P = 0.038$, by VA extract > control $P < 0.001$). Asterisks indicate significant differences based on ANOVA and Wilcoxon signed-rank tests between Rhode Island and Virginia populations (**a** *Fucus* $P = 0.022$, *Ulva* $P < 0.0001$; **b** extract $P = 0.025$; **c** extract $P = 0.022$, control $P < 0.001$)

Juvenile performance

When isopods were raised on each of the three foods, host species as well as the interaction of host species and population of origin had significant effects on final body length (online resource 3E). *Ulva* was a high quality food source for *I. balthica* from both Virginia and Rhode Island. Isopods raised on *Ulva* for 8 weeks were equal in size across populations and were some of the largest animals across

treatments ($P = 0.749$; Fig. 5). Isopods from Rhode Island grew equally well on *Ulva* and *Fucus* ($P = 0.962$), while those from Virginia had a reduced growth rate when reared on *Fucus* rather than on *Ulva* ($P = 0.007$). *Zostera* ($P = 0.038$) was an equally poor quality food for both populations ($P = 0.481$; Fig. 5). Isopods from both populations survived the longest when reared on *Ulva* with no significant difference in survivorship between populations ($P = 0.067$) and the shortest on *Zostera* (Fig. 5). The Rhode

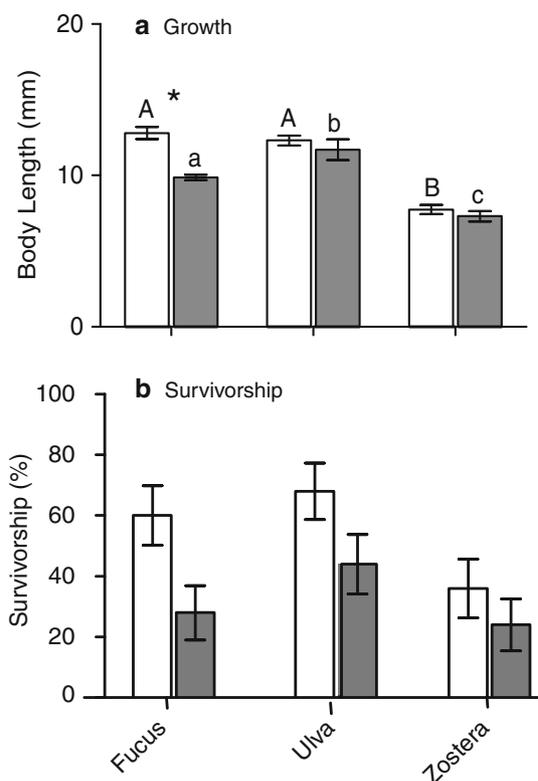


Fig. 5 **a** Growth rate (represented as final body length), and **b** juvenile survivorship of isopods from Rhode Island (white bars) and Virginia (gray bars) after 8 weeks of feeding on either *Fucus*, *Ulva*, or *Zostera*, mean \pm SE, $n = 270$. Asterisk denotes significant differences between populations, based on ANOVA and Tukey HSD, *Fucus* $P = 0.007$. Post-hoc analyses of significant body length differences among hosts within Rhode Island (uppercase letters) and Virginia (lowercase letters) populations are shown. There were no significant differences detected in survivorship from a log-rank X^2 test. Statistical details of performance analyses are presented in online resource 3E

Island animals had higher survivorship on *Fucus* than those from Virginia, but this was not significant after correction for multiple comparisons ($\alpha = 0.003$; $P = 0.006$; online resource 4).

Discussion

Marine mesograzers are analogous to terrestrial herbivorous insects in their small size, their intimate interactions with their host plants (for food and shelter), and their critical roles in their community as both prey and proficient consumers (Hay et al. 1987; Duffy 1990; Duffy et al. 2001; Jaschinski and Sommer 2008). Terrestrial insects readily evolve population-level differences in host use traits (Thompson 2005), suggesting that marine mesograzers should similarly local adapt. Surprisingly, however, we have relatively few such examples among marine mesograzers (Sotka 2005). This lack of examples may arise because

marine mesograzers (especially crustaceans) tend to have a broader host range (Poore et al. 2008), and it is relatively rare to find local genetic differences in host use traits among highly polyphagous species (Poore and Steinberg 2001; Singer 2008). Alternatively, there are other fundamental differences between plant–herbivore systems of marine versus terrestrial systems (Hay and Steinberg 1992) that may reduce the likelihood of local adaptation. It is also possible that we simply have too few tests of local adaptation in marine herbivores to assess these hypotheses.

Here, we show that populations of the generalist marine herbivore *Idotea balthica* differ in feeding preference for, as well as performance on, local food resources. Specifically, while all *I. balthica* consume most foods offered, populations shift the rankings of their preferred diets. Since two of our assays (freeze-dried tissue assays with three hosts and *Fucus* extract assays) utilize *I. balthica* from laboratory-reared, common garden cultures, distinct differences we observed in feeding preference and performance have a genetic component.

Link between preference and performance

The correlation between adult preference and juvenile performance is common among terrestrial insect herbivores and appears to be an important force in driving the evolution of their host use (Rausher and Papaj 1983; Thompson 1988; Craig et al. 1989), but this correlation is inconsistently observed in marine mesograzers (Taylor and Brown 2006). In *I. balthica*, the rank of hosts in feeding choice assays exhibited by northern animals ($F = U > Z$) and southern animals ($U > F > Z$) were closely mirrored by the host rank of juvenile growth rates, and suggests that preference and performance across the three host species are strongly correlated in western North Atlantic *I. balthica*. Interestingly, choice feeding assays were more indicative of juvenile performance than were no-choice feeding assays. This leads us to suspect that a correlation is inconsistently observed because no-choice assays are used as a proxy for preference (Nicotri 1980; Poore and Steinberg 1999; Taylor and Brown 2006). One explanation for this is that marine mesograzers are capable of compensatory feeding (Cruz-Rivera and Hay 2001) and consume macroalgae of poor nutritional quality at high rates (i.e., compensate) when given no alternative. Such compensatory feeding may minimize but not eliminate the fitness cost associated with poor-quality foods.

Geographic responses to *Fucus* versus *Zostera*

The differences in the preference hierarchies among populations when offered the macroalgae *Ulva linza* and *Fucus vesiculosus* and the seagrass *Zostera marina* reflect

geographic differences in the relative importance of these hosts. *Fucus vesiculosus* dominates northern habitats, is currently absent from most southern habitats, and was more readily consumed by northern populations than by southern populations. This geographic variation in feeding behavior was mediated by secondary metabolites (see below). Similarly, southern *I. balthica* populations occur in habitats that are dominated by sea grasses and *U. linza*, and both hosts were consumed significantly more by these individuals than by northern animals. These results are broadly consistent with previous work on Baltic Sea populations of *I. balthica* in which field-collected individuals from *Z. marina* and *F. vesiculosus* showed higher preference for, and juvenile performance on, local hosts (Jormalainen et al. 2001; Vesakoski et al. 2008, 2009). The present work expands on previous work by showing that these differences in host use traits also occur over broader spatial areas (100s of kilometers) and by supporting previous work showing that population-level differences in adult feeding behaviors traits in *I. balthica* have a genetic basis (Vesakoski et al. 2009).

We found it surprising that southern individuals exhibited a stronger preference for *F. vesiculosus* (an alga they rarely encounter) than the more commonly used *Zostera*. There are three possible and non-exclusive explanations why southern and northern animals consume *Fucus* more readily than *Zostera*. First, *I. balthica* regularly lives on and consumes *Zostera* in the field, but it primarily may graze the epiphytes off of the surface of the sea grass blades. Consumption of *Zostera* occurs when population sizes are large and the available food resources are low (Duffy et al. 2001). Thus, *Zostera* may be a consistently poor food for all populations and the selection pressure to consume it may be relatively low. Second, it is possible that *Fucus* was historically an important host in southern regions, but it is rare now because of the increase in water temperatures along the east coast (Jones et al. 2010). Finally, because *Fucus* is an important host for most *I. balthica* populations throughout its range (Nicotri 1980; Salemaa 1987; Jormalainen et al. 2001), it may be that the high preference for *Fucus* reflects evolutionary ‘baggage’ that was inherited by these more peripheral southern populations.

Geographic responses to *Fucus* metabolites

Secondary metabolites of macroalgae can have strong influence on herbivore preferences (Paul et al. 2001; Sotka et al. 2009), and the same is true for *Fucus* metabolites. Both populations of *I. balthica* were strongly deterred by water-soluble extracts. *Fucus* produces water-soluble phlorotannins (Targett and Arnold 1998) and likely constitute a portion of our water-soluble extracts, although we did not measure their presence. Like their terrestrial analogue, tannins, phlorotannins have many functions, but can deter herbivores (Van

Alstyne 1988; Steinberg and VanAltena 1992; Arnold and Targett 2003) and interfere with the digestion of proteins (Stern et al. 1996).

The strong deterrence of water-soluble extracts to northwestern *I. balthica* contrasts with *I. balthica* populations from Baltic waters in laboratory assays, which strongly prefers *Fucus* and readily consume its polar extracts (Jormalainen et al. 2001; but see Jormalainen and Ramsay 2009). It is not clear whether this apparent difference among northwestern versus Baltic populations reflects herbivore-specific differences in feeding behavior, macroalgal-specific differences in metabolite production, laboratory extraction techniques, the food quality of the control food, or temperature, each of which can alter responses to extracts (e.g., Duffy and Paul 1992; Cronin et al. 1995; Sotka and Giddens 2009).

Interestingly, feeding responses toward lipophilic metabolites perfectly matched feeding responses toward freeze-dried tissue. Specifically, southern *I. balthica* individuals were significantly deterred by lipophilic extracts, while northern *I. balthica* individuals consumed more lipophilic extract than control foods (although not significantly so). The perfect mirror of between choice assays using freeze-dried tissue and lipophilic extracts from *Fucus* suggests that lipophilic extracts and not water-soluble extracts mediate the feeding behavioral response of *I. balthica* in North American populations. The lipophilic extracts of *F. vesiculosus* also deterred the North Carolina amphipods *Ampithoe valida* and *A. longimana* (Kubaneck et al. 2004) but not the urchin *Arbacia punctulata* (Deal et al. 2003).

Summary

Adaptive differences in feeding preference in North American populations of *I. balthica* occur at a local scale and these distinct preferences correlate with regional differences in the distribution of algal species. Adaptive differences in feeding preference and performance also strongly correlate with phylogeographic patterns of genetic divergence (at mtCOI) between northern and southern populations described in *I. balthica* by Wares (2001) and Bell (2009). Thus, it is possible that algal biogeography has played a role in driving or maintaining the evolutionary trajectory of these populations.

This represents one of a growing number of examples of generalist herbivores in both marine (Sotka et al. 2003; Vesakoski et al. 2009) and terrestrial systems (Singer 2008; Thompson 1993, 2005) that undergo population-level shifts in the ranking of feeding preferences across their often expansive geographic ranges. This evolutionary solution contrasts with the more commonly accepted notion that polyphagous herbivores are composed of cryptic specialists at the population- or individual-level (Fox and Morrow

1981; Sword and Dopman 1999; Bolnick et al. 2003). We propose that these microevolutionary shifts are common because they allow generalist herbivores to minimize any fitness or ecological trade-offs that are incurred (Jormalainen et al. 2001; Sotka and Reynolds 2011), while maintaining the benefits of marine polyphagy (Hay and Steinberg 1992; Stachowicz et al. 2007).

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