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Ephemeral macroalgae display spatial variation in relative palatability

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ABSTRACT

Although geographic variation in algal–herbivore interactions should be common, our appreciation of such variation remains incomplete. For example, feeding choice assays examining variation in palatability of algae typically compare multiple algal species or genera from a single location or a single alga from multiple locations. Unfortunately, most studies have not compared the interaction of herbivores with multiple algae from multiple locations, thus tests of variation of relative palatability are lacking. To examine spatial variation in algal palatability we determined the feeding preferences of the periwinkle snail *Littorina littorea* when offered multiple algal genera at the same time from two regions in choice assays. Multi-choice experiments were repeated throughout the year to examine temporal variation in feeding preferences. Consistent with previous studies, *L. littorea* preferred to feed on ephemeral algae compared to perennial species. Importantly, the relative palatability of the two ephemeral genera, *Ulva* and *Porphyra*, varied greatly between regions and seasons. Under certain conditions, *Ulva* only represented 24% of consumed algal wet mass in choice assays, while under other conditions, *Ulva* was eaten almost exclusively. Preferences for *Ulva* or *Porphyra* were unrelated to differences in morphology. Variation in the relative palatability of algae could influence consumer behavior leading to shifts in algal communities across broad geographic scales.

1. Introduction

Herbivore interactions with plants and algae are spatially dynamic, with variation arising from differences in species composition (Gaines and Lubchenco, 1982), local adaptation (Salgado and Pennings, 2005; Sotka and Hay, 2002), and environmentally-induced plasticity in traits (Bolser and Hay, 1996; Long et al., 2007; Renaud et al., 1990). Such variation can strongly alter the outcomes of plant-herbivore and algal–herbivore interactions, especially when traits influencing palatability change across broad geographic scales. Unfortunately, we lack a complete understanding of this variation because previous studies typically compared the palatability of 1) multiple species from a single location (Jormalainen et al., 2001; Kennish and Williams, 1997; Lubchenco, 1978; Steinberg, 1985) or 2) a single species from multiple locations (Bolser and Hay, 1996; Pennings et al., 2009). Missing are experiments that simultaneously compare the palatability of multiple algae relative to one another from multiple locations or times (Lubchenco and Gaines, 1981). This bias prevents a thorough understanding of the dynamics of herbivore–algae interactions and limits the usefulness of prey palatability categories derived from studies of algae from a single place or time.

The herbivorous snail, *Littorina littorea*, is a dominant structural architect of rocky shores along northern Atlantic coastlines (Lubchenco, 1978). *L. littorea* strongly prefers to feed upon ephemeral algae that typically lack chemical and morphological defenses (e.g. *Ulva* spp. and *Porphyra* spp.) compared to perennials (e.g. *Fucus vesiculosus* and *Ascophyllum nodosum*; Lubchenco, 1978; Watson and Norton, 1985). However, previous studies have overlooked variation within these broad categories based on life history (e.g. the relative palatability of ephemeral algae). Indeed, the relative palatability of ephemeral algae might be site- or region-specific because environmental conditions display strong heterogeneity across the range of these species and the performance of these ephemeral algae can be sensitive to these environmental factors (Gaines and Lubchenco, 1982).

In this study, we examined feeding preferences of *L. littorea* for algae collected from two regions at three different time periods in the Gulf of Maine to investigate how algal palatability changes in space and time. We focused on changes in the relative palatability of ephemeral algae within the genera *Ulva* and *Porphyra*. We also assessed palatability of homogenized algae to examine if observed patterns in palatability remained after removing morphological differences.

2. Materials and methods

2.1. Study sites and organisms

To examine spatial variation in the relative palatability of multiple algal genera, we offered *L. littorea* (hereafter *Littorina*) a choice of multiple co-occurring algae collected from two sites separated by 460 km — either Nahant, Massachusetts (42°25′27.34″ N, 70°54′52.16″ W) or Dipper Harbour, New Brunswick, Canada (hereafter DH; 45°16′24.35″ N, 66° 3′50.87″ W) during three collection periods

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(August 2007, March 2008, June 2008). In August 2007, we offered snails a choice of *F. vesiculosus*, *A. nodosum*, *Chondrus crispus* (Nahant assays)/*Mastocarpus stellatus* (DH assays), *Ulva* spp., and *Porphyra* spp. (hereafter *Fucus*, *Ascophyllum*, *Chondrus*, *Mastocarpus*, *Ulva*, and *Porphyra*; respectively). Because *Chondrus* was rare in DH, we used *Mastocarpus* in place of *Chondrus* in DH assays. *Chondrus* and *Mastocarpus* are both low intertidal red turf algae that are closely related (both belong to the order Gigartinales) and morphologically similar (Villalard-Bohnsack, 2003).

We collected algae from 2 to 3 sites within each region, pooled collections, and transported them in chilled coolers to Nahant. All algae were kept in coolers for 8 h prior to use. Multi-choice assays were conducted the following day by offering Nahant *Littorina* a choice of the five genera collected from a single region. The most striking result of the August assays was a regional difference in the relative palatability of *Ulva* and *Porphyra* (see Results section). Thus, trials performed in March and June (2008) tested differences in palatability between *Ulva* and *Porphyra* only.

2.2. Live algae multi-choice feeding assays

For all assays, we measured the decrease in algal wet mass in grazer replicates ($n=15–30$) after correcting for autogenic growth in grazer-free controls ($n=10$). Prior to assays, algae were blotted dry, cut to 0.5 g pieces, and placed in perforated replicate containers ($10\times10\times9$ cm). Replicate containers were placed in outdoor, unshaded tanks with flow-through seawater pumped from an adjacent marine cove. Grazer replicates contained 2–4 *Littorina* each (constant number per experiment). Final blotted, wet masses of algae were recorded 2–3 days later. We calculated consumption using the formula $T_i(C_f/C_i)-T_f$, where T_i and T_f represent algae exposed to grazing, and C_i and C_f represent the control algae before (i) and after (f) the trial period (Sotka et al., 2002). Because grazing rates can vary with *Littorina* size, we divided consumption by the final wet *Littorina* mass of all individuals within the corresponding replicate. We converted these to consumption rates by dividing per snail consumption by assay length ($\text{consumption}=\text{mg algae}\times\text{g snail}^{-1}\times\text{day}^{-1}$).

To correct consumption rates for changes in autogenic growth, we used a single correction factor (C_f/C_i, as above; hereafter control ratio) that averaged autogenic mass changes across control replicates ($n=10$). We adopted this approach to maximize the number of replicates that contained snails feeding on algae. Such an approach has been criticized because of concerns about artificial suppression of experimental variance (Peterson and Renaud, 1989). However, such a problem "disappears" as the variance among controls decreases (Peterson and Renaud, 1989). Although we saw very little variability in our controls (see Appendix 1), we performed a statistical analysis to examine whether our results would have changed had we calculated consumption with paired grazer and grazer-free replicates. First, we generated new control ratios by randomly resampling our measured control ratios with replacement via bootstrapping in SYSTAT 12 (version 12.02.00). Second, we paired single control ratios with experimental replicates and recalculated consumption rates. Finally, we compared 1) the original measured control ratios to the bootstrapped control ratios and 2) the original measured consumption rates to newly the calculated consumption rates, using two-sample t-tests. No differences were found between our approach and this resampling approach (see Appendix 2).

Mean consumption rates of each algae within each region (multi-choice assay) were compared using the nonparametric Friedman's rank test (>2 choices, August 2007 multi-species assays; Zar, 1999) or paired t-tests (2 choice assays, March and June 2008 assays; SYSTAT 12, version 12.02.00). Post-hoc analyses of consumption rates were conducted with nonparametric multiple comparisons (>2 choices). We used a sequential Bonferroni correction (corrected $\alpha=1-(1-\alpha_{0.05})^{1/k}$) to account for the number of comparisons in the March and June 2008 assays ($k=4$, where k represents the number of comparisons made during the analysis; Rice, 1990). *P*-values were only considered significant if less than the corrected α. Consumption rates of the least preferred algae [*Fucus*, *Ascophyllum*, and *Chondrus* (Nahant)/*Mastocarpus* (DH)] during the August 2007 multi-species trials were summed prior to comparison because they were grazed at low rates, and we were primarily interested in comparing their combined palatability to the palatability of the two most preferred genera, *Ulva* and *Porphyra*.

To examine changes in the relative palatability of *Ulva* and *Porphyra* between sites and time points, we calculated the proportion of total consumption represented by *Ulva* by dividing *Ulva* consumption by the combined consumption of *Ulva* and *Porphyra* for each assay. Thus, this proportion increases with an increasing preference for *Ulva*. If consumption of either *Ulva* or *Porphyra* was negative, it was assumed that it was completely avoided relative to the other genera, and was corrected to 0 before calculating this proportion. From all feeding assays, six replicates out of 144 total replicates displayed negative consumption of all choices. These replicates were excluded from the calculation of proportional consumption. Proportions were compared with a two factor, fixed model ANOVA (SYSTAT 12, version 12.02.00) to test for the effects of region, month, and their interaction on *Littorina* feeding preferences.

2.3. Artificial food multi-choice feeding assays

To examine the relative palatability of *Ulva* and *Porphyra* during August 2007 in the absence of algal morphology, we measured grazing on artificial foods containing reconstituted algae using methods adopted from Lindquist and Hay (1996). Artificial foods were prepared by homogenizing freeze-dried, finely-ground algae (5.6% weight: volume) with agar (2.0% w:v). This procedure removes morphological traits while maintaining chemical traits. The algae-agar solution was molded into cubes ($1\text{ cm}\times1\text{ cm}\times0.2$ cm) on fiberglass window screen, and allowed to set for 2–5 min. To avoid desiccation, we immediately placed the samples in seawater until feeding assays began. Preferences were determined using the methods described above in live plant feeding assays, by measuring the mass change of artificial foods (snail treatments, $n=25–29$; controls, $n=10$). Briefly, we 1) offered Nahant *Littorina* a choice of artificial foods made of either *Ulva* or *Porphyra* from the same region, 2) measured grazing after 2 days, and 3) compared final consumption rates of each algal species within each region using paired t-tests.

3. Results

3.1. Spatial variation in algal palatability

Consistent with previous studies (Lubchenco, 1978; Watson and Norton, 1985), *Littorina* preferred ephemeral algae (*Ulva* and *Porphyra*) to perennial algae (*Fucus*, *Ascophyllum*, and *Chondrus*/*Mastocarpus*) in multi-choice assays (August 2007), regardless of population (Fig. 1; Nahant: $x_r^2=38.067$, $p<0.001$, DH: $x_r^2=23.931$, $p<0.001$). Interestingly, the relative palatability of *Ulva* and *Porphyra* changed with algal source. *Littorina* consumed two times more *Ulva* than *Porphyra* from Nahant, but this difference was not significant ($p>0.05$, Fig. 1A). In contrast, *Littorina* consumed three times more *Porphyra* than *Ulva* from DH ($p<0.05$, Fig. 1B). Furthermore, consumption patterns persisted after morphological traits were removed using artificial foods. *Littorina* showed similar preferences for Nahant *Ulva* and *Porphyra* (Fig. 2; $t_{(24)}=-0.628$, $p=0.536$), while DH *Porphyra* was preferred over *Ulva* (Fig. 2; $t_{(28)}=-3.872$, $p=0.001$).

3.2. Temporal variation in algal palatability

The relative palatability of *Ulva* and *Porphyra* varied temporally at both Nahant and DH. For Nahant algal populations, consumption of