Non-consumptive predator effects indirectly influence marine plant biomass and palatability

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Summary

1. Predators can reduce herbivory and increase plant biomass by consuming herbivores, lowering individual herbivore feeding rates, or both. We tested whether the presence of predators increases plant quality by non-consumptively reducing grazing pressure and thereby weakening the strength of the induced response in plant chemical defences.

2. We performed a 42-day outdoor mesocosm experiment in which the herbivorous amphipod Ampithoe longimana was cultured on the brown seaweed Sargassum filipendula in the presence and absence of olfactory cues of its principal fish predator, the pinfish Lagodon rhomboides. The presence of fish cues reduced per capita rates of amphipod grazing by nearly 50%. Over the span of the mesocosm experiment, this per capita reduction in feeding rate yielded at least a 40% lower growth rate of amphipod populations (i.e. \( r \) reduced from 1.01 to 0.61). The lower rates of amphipod grazing (overall or per capita) correlated with higher algal biomass.

3. We pursued a series of laboratory-based feeding choice assays with naïve amphipods to determine tissue palatability and the plant traits that mediate feeding choices. Tissue from tanks without grazers was more palatable than tissue from tanks with grazers, a pattern of induced plant defences that has been documented previously. Surprisingly, however, plant tissue from tanks with grazers and fish cues was more palatable than tissue from tanks with grazers but without fish cues. All changes in algal palatability were mediated by polar, but not lipophilic metabolites. These results suggest that the non-consumptive effects of fish predators increases the food quality of Sargassum by weakening the strength of its induced chemical defences.

4. Synthesis. The smell of predators has the potential to regulate herbivore populations and affect the ecological dynamics of plant biomass and chemical defences.

Key-words: algal induction, amphipod, behaviour, herbivory, inducible defence, macroalgae, phenotypic plasticity, plant–herbivore interactions, predator–prey interaction

Introduction

Predators often regulate herbivore densities and thereby facilitate plant growth (Hairston, Smith & Slobodkin 1960). Historically, ecologists assumed that lethal, or consumptive effects of predators largely mediated these tritrophic cascades by reducing herbivore densities. However, more recent evidence suggests that predator-induced changes in prey grazing behaviour (a non-consumptive effect) may commonly underlie tritrophic cascades (Peckarsky et al. 2008). The presence of predators often lowers per capita grazing rates and alters herbivore host use and feeding preferences of their prey (e.g. Trussell, Ewan-

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To assess a potential mechanism for observed changes in grazer population growth and algal biomass (see Results), assays were begun to test the short-term effects of predator cues on prey grazing in outdoor water tables on 10 August 2009. Temperature, light and salinity in these assays were within the range of conditions experienced throughout the tidal cycle in the field (24 °C, c. 400 µm m−2 s−1 µA, 35 ppt; P. Reynolds & J. Bruno unpubl. data). We placed one female A. longimana in a plastic 9 mL cup with 50 mg of freshly collected S. filipendula. Paired cups without A. longimana were placed with A. longimana addition cups in a 1:1.4 L ‘predator tub’ provided with flow-through filtered seawater (Fig. S1a). Three juvenile pinfish were added to half of the predator tubs (n = 9 for a total of 36 cups). Predators could swim freely around the cups but could not directly consume the amphipods. The opaque cups were weighed with small pebbles to provide additional habitat, and had holes that allowed transfer of olfactory cues from the surrounding water. To determine grazing rates of A. longimana on S. filipendula across predator and cue treatments, we first compensated for autogenic changes in the control cups according to Sotka (2003): Ti/C/Ci–IT for grazer presence T and absence C, and initial i and final f macroalgal wet mass. The experiment ran for 7 days and replicates were excluded if the grazer died. Results were analysed with a two-tailed t-test.

INDUCTION EXPERIMENT

To examine how predator olfactory cues affect algal biomass and quality, we conducted experiments in outdoor mesocosms at the Institute of Marine Sciences in Morehead City, NC, USA (see Appendix S1 in Supporting Information). Replicates were established in two rows of tanks (replicate tank volume = 11.7 L) for a total of 36 top and bottom tanks (see Sokta, Taylor & Hay 2002; Taylor, Sokta & Hay 2002 for description of tank setup). Tanks were covered with window screen and a layer of 0.5 mm diameter Vexar plastic; light, temperature and salinity approximated field conditions (Li-100, measuring 4× irradiance; 23–25 °C, 34.5–36 ppt)(Taylor, Sokta & Hay 2002). The top tanks received filtered seawater (at 0.08 L s−1) from the adjacent sound which then flowed into the bottom tanks in one direction. Top tanks were supplied with air stones. Filter bags (200 µm mesh) reduced natural colonization from the water system, and screens prevented emigration from experimental tanks.

Algae and amphipods were collected on 19 June 2009 from the shallow subtidal at Radio Island, NC, USA (34° 42′N, 76° 41′W) at 1–0.5 m below low tide. All bottom tanks received two 200- to 300-mm-long S. filipendula fronds (9.5 ± 0.1 g each) culled from two individual plants. Half of the bottom tanks then received 12 fecund female A. longimana. The next day, half of the top tanks received four juvenile pinfish L. rhomboides (1.53 ± 0.06 g, 37 ± 0.4 mm each fish) per tank. The bottom tanks therefore represent one of four treatments (n = 9): fish cue only (+G + F), grazers only (+G − F), grazers and fish cues (+G + F) or control (+G − F).

All outflow screens were cleaned and the algae lightly disturbed daily to remove sediment buildup. After 21 days, half the water within each tank was exchanged with fresh seawater in order to

remove buildup of sediment and potential benthic microalgae at the bottom of the tanks; seawater was drained through a 500 μm mesh to retain all amphipods. Throughout the experiment, fish were fed a slurry of crushed gammaridean amphipods supplemented with frozen brine shrimp. Dead or sick fish were immediately replaced. Fish grew throughout the experiment; fish density was reduced to three fish per tank after 12 days and to two fish per tank after 24 days to maintain similar initial and final fish biomass.

Potential increases in nitrogen concentration due to bacterial degradation of fish excrement or food were low and likely transient in our flow-through tank system (levels for all tanks: nitrite c.0.25 p.p.m., nitrate c.10 p.p.m.). Incidental grazer immigration was low in grazer control tanks (–G + F; see Results), and thus, this treatment serves as a proxy for the direct effect of fish cues on algal growth and palatability.

Algae were exposed to treatments for 42 days to allow adequate time for induced resistance to develop (Sotka, Taylor & Hay 2002; Taylor, Sotka & Hay 2002). At the end of the experiment bottom tanks were drained and all algae and grazers removed. Algal wet mass was determined after 60 revolutions in a salad spinner. Final algal wet mass could not be transformed to meet assumptions of normality and was analysed with a two-way nonparametric ANOVA. Significance was evaluated by comparing observed F-ratios with a distribution generated from 1000 permutations of the data set (Anderson 2001) using a custom R script (http://cran.r-project.org).

All grazers were live counted. Final grazer abundances were natural log-transformed for normality and analysed with a two-way ANOVA. We pursued log-transformation despite its known limitations (O’Hara & Kotze 2010), which are especially pronounced when means are relatively low and raw data include zeros. In our case, means were relatively high (>10 animals per replicate) and no tank had zero animals. We calculated the fundamental net reproductive rate (R or λ) using the equation \[ R = N_1 \left( T_0 \right)^{-1}. \] and the intrinsic rate of natural increase (r) using the equation \[ r = \ln R / T^{-1}. \] (Begon, Townsend & Harper 2006). This calculation was used as we found no evidence for density dependence (see Fig. 3), and overlapping generations are implicit in the equation. We assumed generation time was equivalent between fish cue treatments (mean age of females at off-spring birth = 14 days; Sotka & Reynolds, in press).

An undamaged portion of the uppermost (top stipe) tissue from one plant in each tank was retained for fresh tissue feeding assays. Inducible responses are known to occur primarily in apical tissue in one plant in each tank was retained for fresh tissue feeding assays. On 2 August 2009 one S. filipendula Inducible responses are known to occur primarily in apical tissue in one plant in each tank was retained for fresh tissue feeding assays. Fish grew throughout the experiment; fish density was reduced to three fish per tank after 12 days and to two fish per tank after 24 days to maintain similar initial and final fish biomass.

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An undamaged portion of the uppermost (top stipe) tissue from one plant in each tank was retained for fresh tissue feeding assays. Inducible responses are known to occur primarily in apical tissue in S. filipendula (Taylor, Sotka & Hay 2002) and other brown seaweeds (Rohde, Molis & Wahl 2004; Hemmi et al. 2005). Meristems in S. filipendula are apical, where the youngest and potentially more valuable tissue is found at the tips of the plant, and the oldest tissues at the holdfast. Due to a lack of sufficient tissue per replicate, all remaining healthy top stipe tissue was combined by treatment and immediately frozen for future assays. Frozen tissues were later freeze-dried and ground as in Taylor, Sotka & Hay (2002) to create an algal powder for reconstituted tissue and extract feeding assays, as well as phlorotannin and C:N analysis. Two tanks were excluded from all analyses due to inconsistent water flow.

**CHOICE ASSAY – FRESH TISSUE**

Feeding choice assays, in which algal tissues from different treatments were simultaneously offered, were conducted as in Sotka, Taylor & Hay (2002) to assess grazer feeding preferences. Grazers were given pairwise choices of fresh S. filipendula top stipes from all six combinations of the four treatments. On 2 August 2009 one freshly collected A. longimana was added to dishes containing two 30 mg (blotted wet mass) pieces of tissue separated by a plastic rod. Because there was no natural or experimental pairing of replicates across treatments, four pieces of tissue (genets) per replicate were randomly and independently paired with four other genets from different replicates of the compared treatment for a total of 36 pairwise comparisons per choice assay. Tissue without grazers was retained in separate dishes to control for autogenic changes in plant mass. After 2 days all tissues were reweighed and the amount of algae consumed calculated as described above in Materials and methods: Grazing assay. In order to assure that grazers made a choice, replicates were dropped if < 3 mg was consumed of either piece of tissue (<4 replicates per experimental pairing). To reduce potential pseudoreplication from the use of multiple genets per replicate, the proportion consumed of each tissue was averaged without error for each replicate and then compared to a null of 0.5 using two-tailed t-tests.

**CHOICE ASSAY – FREEZE-DRIED TISSUE**

To examine the influence of structural characteristics on palatability, we conducted feeding choice assays using reconstituted top stipe tissue from the four treatments. Top stipes were lyophilized (i.e. freeze-dried) and ground to a fine powder using a Wiley mill. We created reconstituted foods by adding 1.5 g of ground tissue to 6 mL of water, dissolving 0.36 g of agar in 10 mL of heated water and combining both mixtures. This cooled mixture was then poured onto a window screen, covered on both sides with wax paper, and pressed. We cut several 5 × 6-cell feeding grids, which were then offered to amphipods as described for the fresh-tissue assays. Choice assay comparisons were conducted on 3 November 2009 using freshly caught amphipods in all of the above pairings except fish cue vs. grazed and fish cue vs. grazed + fish cue because tissues from control and fish cue treatments were equally palatable in the fresh-tissue assay (see Results). A small clip was made in the corner of squares made of one tissue type per pairing for identification purposes. The trial ended when at least half of either treatment (<9 cells of one treatment or no more than 45 total) was consumed, typically after 3 days. We calculated consumption per treatment as a percentage of the total number of cells cleared for both squares, where a value of 50% represents no choice (following Bernays & Wedge 1987). Data were assessed statistically using blocked one-way permutation ANOVAs (analogous to a paired t-test) because data were non-normally distributed and could not be transformed to yield normality. Significance from a permuted distribution was generated as described within Materials and methods: Induction experiment.

**CHOICE ASSAY – MACROALGAL EXTRACTS**

The palatability of crude water-soluble (polar) and lipophilic (non-polar) extracts were assayed to assess the potential role of chemical defences in determining feeding preferences observed in the fresh and reconstituted tissue assays. Extractions were modified from Taylor et al. (2003) and Long, Hamilton & Mitchell (2007), and conducted at the College of Charleston’s Grice Marine Laboratory. Freeze-dried algal tissue was extracted three times in 2:1 ethyl acetate:methanol and in 70:30 methanol:water. Organic solvents were removed by rotary evaporation and partitioned between ethyl acetate and water. Extracts were incorporated at natural concentrations by dry mass into 2 g freeze-dried powdered Ectocarpus sp. (a highly palatable, filamentous brown alga), which was used to make reconstituted foods similar to those described in Materials and methods: choice assays – freeze-dried tissue. Lipophilic extracts were
added to powdered *Ectocarpus* after dissolution in ethyl acetate, and tissue was rotary evaporated to remove the solvent. Water-soluble extracts were dissolved in distilled water and added to the powdered *Ectocarpus* when creating foods. Control foods were treated similarly but did not contain extracts. Choice assays – freeze-dried tissue. Choice assays began on 1 March 2010 for lipophilic extracts, and on 8 May 2010 for water-soluble extracts.

**MACROALGAL TRAITS**

We measured several macroalgal traits that may correlate with herbi-vore feeding choices. Phlorotannins are polyphenolics produced by many brown seaweeds including *S. filipendula* (Cronin *et al.* 1997) that can deter marine grazers and may be induced by prior grazing (van Alstyne 1988; Pavia & Toth 2000; Toth & Pavia 2000; Pavia, Toth & Aberg 2002; Lüder & Clayton 2004; but see Toth & Pavia 2002; Deal *et al.* 2003; Kubanek *et al.* 2004; Long, Hamilton & Mitchell 2007). Phlorotannin analysis was conducted at Grice using the Folin–Cioiculatu method following van Alstyne (1995) and Long, Hamilton & Mitchell (2007). Freeze-dried tissue was extracted with 8:2 MeOH:H2O and chilled for 4 h. Extracted supernatant was then filtered to remove particulates and added to 2 μl reagent with 1 μl Na2CO3, vortexed and heated. Absorbance (read at 760 nm) was measured and thus, statistical estimates of variation in algal traits largely reflect measurement error.

**Results**

The smell of fish reduced *A. longimana* grazing by 46% during the 1-week grazing assay (two-tailed *t*-test, *T* = −4.18; *P* < 0.001, Fig. 1). Fish cues also significantly reduced the population growth rate of *A. longimana* during the 42-day induction experiment (Table 1; Fig. 2a). Grazer populations exposed to fish cues averaged c. 65 amphipods by the end of the 42-day experiment (*r* c. 0.61), while populations lacking fish cues averaged c.245 amphipods (*r* c. 1.01). This represents a

40% decline in population growth rate in the presence of fish cues. It is possible that the mean generation time differed among grazer populations across treatments, which could affect our estimates of *r*. Given that fish cues reduced grazer feeding and likely reduced individual growth rates, our estimated decline in grazer population growth in the presence of fish cues is likely conservative and the true decline is likely greater.

There was incidental immigration of grazers into tanks that were not initially seeded (i.e. ‘+grazer’ treatments), but they averaged fewer than 16 amphipods per tank. It is unlikely that predator-induced reductions are due to emigration because fine-mesh screens at the outflow of all tanks effectively impeded movement of *A. longimana* and because direct assays indicated that *A. longimana* reduces its movement in the presence of pinfish (P. Reynolds & J. Bruno, unpubl. data). Our final densities ranged from <1 to 10.4 per g algal wet weight, and were within levels measured in the field (Duffy 1989), suggesting that grazing rates were ecologically realistic.

The interactive effect of grazer and fish cue treatments on final biomass of the alga *S. filipedula* was significant (Table 1; Fig. 2b), indicating that grazers consistently lowered *S. filipedula* biomass, but the effect was greater when fish cues were absent. The negative relationship between algal biomass and grazer density was reflected across treatment means (Fig. 2) as
well as replicate tanks (Fig. 3). Overall, our manipulations effectively created three levels of grazing intensity: lower (‘−grazer−fish-cue’ and ‘−grazer + fish-cue’ treatments), intermediate (‘+grazer + fish-cue’) and higher (‘+grazer − fish-cue’).

To test whether these levels of prior grazing altered the palatability of algal tissue, we offered fresh tissue to naïve amphipods in a pairwise series of feeding choice assays (Fig. 4a). Culturing water with fish cues in the absence of amphipods did not alter the palatability of plant tissues to amphipods (−G − F vs. −G + F), indicating that plant tissue quality did not respond to fish cues directly. Tissues from tanks without grazers were more palatable than those from tanks with grazers (−G−F vs. + G − F), a pattern of induction that was documented previously (Sotka, Taylor & Hay 2002; Taylor, Sotka & Hay 2002). Plant tissue from tanks with amphipods and fish cues was more palatable than tissue from tanks with amphipods but without fish cues (+ G + F vs. + G − F).

Summarizing all feeding assays, plant palatability grouped into three levels: higher (both treatments without amphipods; i.e. ‘−grazer’), intermediate (‘+ grazer + fish-cue’) and lower (‘+ grazer − fish-cue’) palatability. Thus, grazing intensity, as determined by grazer densities and behavioural responses to fish cues, negatively correlated with plant palatability.

We used several approaches to identify the plant traits mediating observed shifts in palatability. Firstly, feeding choice assays using freeze-dried and reconstituted algal tissue replicated the ranking of palatability seen in fresh-plant assays without exception (compare Fig. 4a,b). Because freeze-drying tissue removes the effects of morphological differences, these results indicate that observed patterns of algal palatability were mediated by secondary metabolites, nutritional traits or both. Secondly, feeding choice assays using the lipophilic extracts of S. filipendula revealed no differences in palatability among treatment types (Fig. 4c). The feeding responses toward polar extracts (Fig. 4d) did replicate the freeze-dried and fresh-tissue assays, suggesting one or more unknown polar compounds were responsible.

Finally, we measured candidate plant traits that have been shown previously to affect herbivore feeding behaviours. Tissues differed significantly in all algal traits (Table 1; Fig. 5), but these differences were largely explained by the plants that were most intensively grazed (+ G − F treatment). Phlorotannin concentration increased from 0.27% to 0.34% (by dry mass) between control (G − F) and grazed tissues (+ G− F; Fig. 5a), which represents a 25% increase. In contrast, phlorotannin concentration from control (−G + F; fish cue) (−G + F) and ‘+ grazer + fish-cue’ (−G + F) tissues were similar (0.27–0.28%). Grazed tissues also had 38% less TN than did control tissue (control vs. grazed: 2.16% vs. 1.35%) and 10% more total carbon (22.8% vs. 25.1%). This yielded a far lower C:N ratio within control than grazed tissues (12.1 vs. 21.7), signifying that grazers would gain nearly twice as much nitrogen (standardized by carbon intake) while consuming control versus grazed tissue. There were much smaller differences in TN (2.21–2.54%), total carbon (22.8–24.1%) and C:N ratios (10.5–12.1) among control, fish cue and ‘+ grazer + fish-cue’ treatments (Figs 5b–d).

**Discussion**

Cascading impacts of non-consumptive effects appear to be strong within North Carolina fish–epifauna–seaweed interactions. Olfactory cues from the pinfish L. rhomboides yielded a
46% reduction in per capita grazing rates in *A. longimana*, 40% reduction in its population growth rate, and a 74% reduction in overall population size after 42 days (or at least 2–3 overlapping generations). Consistent with a trophic cascade, these artificial and predator-induced reductions in amphipod population size correlated with increasing *S. filipendula* biomass (Figs 2 and 3) and palatability (Figs 4 and 5). Thus, non-lethal effects of predators can have cascading effects on both plant quantity and quality in nearshore marine environments.

**NON-CONSUMPTIVE EFFECT OF FISH PREDATORS ON PLANT BIOMASS**

Small herbivorous species that live on seagrasses and macroalgae (termed mesograzers, Brawley 1992) can lower macroalgal growth rates (Norton & Benson 1983) and biomass (Shacklock & Croft 1981; Duffy & Hay 2000; Bruno & O’Connor 2005), as well as alter macroalgal community composition (Duffy 1990; Bruno & O’Connor 2005). A negative effect of mesograzers on macrophyte biomass is not ubiquitous (Poore, Campbell & Steinberg 2009) in part because some mesograzers can also reduce epiphytic biomass (Brawley & Fei 1987; Duffy 1990; Mancinelli & Rossi 2001), which could alternatively promote plants via competitive release. In many habitats, fishes facilitate macrophytes by reducing densities of these mesograzers (Kennelly 1983; Dayton *et al.* 1984; Davenport & Anderson 2007; Korpipää, Jormalainen & Honkanen 2007; Newcombe & Taylor 2010). Historically, such trophic cascades were thought to be largely mediated by consumption rates rather than non-consumptive, behavioural effects (Schmitz...
Fig. 5. Effects of grazer (dark bars) and fish cue (fish icon) treatments on pooled algal tissue (a) phlorotannin concentration, (b) C : N ratio ($F_{3,8} = 254.6, P < 0.001$), (c) total nitrogen ($F_{3,8} = 81.952, P < 0.001$) and (d) total organic carbon ($F_{3,8} = 4.570, P = 0.030$), ±1 SE. Letters in represent treatments that are significantly different by Tukey’s HSD. Refer to Table 1 for overall test for phlorotannins.

As a consequence of the growing recognition of non-consumptive effects, published studies that demonstrated trophic cascades are being re-assessed (Peckarsky et al. 2008). As an example, Duffy & Hay (2000) found relatively weak evidence for a trophic cascade involving spottail pinfish (Diplodus holbrooki) and A. longimana in experimental algal communities. In their 22-week experiment, outdoor mesocosms stocked with algae and amphipods were divided in half with a mesh barrier and predators were added to one side. Surprisingly, spottail pinfish did not significantly reduce A. longimana densities nor set in motion a trophic cascade effect on algal biomass. Assuming amphipods are able to detect the presence of fish predators, our data suggest that the lack of a trophic cascade in Duffy & Hay (2000) may have reflected the presence of fish cues, which would have non-consumptively inhibited A. longimana grazing and population growth rates on both sides of the mesocosm including in the ‘no fish’ treatments. Thus, the real effect of A. longimana on seaweed communities may be even greater than quantified by Duffy and Hay. In a similar vein, field studies that use cages (e.g. Davenport & Anderson 2007) or chemical means (Poore, Campbell & Steinberg 2009) to remove predatory fishes and increase mesograzers densities may underestimate the true effect of mesograzers on algal communities because olfactory cues from fish will continue to lower grazing rates. For these scenarios to be true, the spatial scale of the experimental manipulation must be similarly sized or smaller relative to the spatial scale across which fish cues are effective. If the spatial scale of manipulation is greater than the scale at which fish cues are effective, then the ensuing heterogeneity will complicate interpretations of observed responses by plants and mesograzers.

**NON-CONSUMPTIVE EFFECT OF FISH PREDATORS ON PLANT QUALITY**

As with numerous plant and algal species (Toth, Karlsson & Pavia 2007; Toth & Pavia 2007), *S. filipendula* responds to grazing by *A. longimana* by becoming less palatable (Taylor, Sotka & Hay 2002). We demonstrate that the strength of this induced resistance is graded with the degree of mesograzer herbivory (Fig. 4). In particular, the non-consumptive effect of pinfish moderated *A. longimana* grazing pressure and increased *S. filipendula* food quality by lowering its induced response. Although induced resistance after prior grazing in *S. filipendula* was previously documented by Taylor, Sotka & Hay (2002) and Sotka, Taylor & Hay (2002), the seaweed traits responsible have yet to be explored. We show here that shifts in seaweed palatability with grazing pressure were mediated by polar, and not lipophilic, algal tissue extracts (Fig. 4) and likely reflect an increase in the production of secondary metabolites, a decrease in nutritional content, or both. We do not believe that water-soluble phlorotannins explain observed tissue palatability patterns because the concentration of phlorotannins was very low (<0.5%) relative to other secondary metabolites.
to other brown seaweeds (van Alstyne, Duggins & Dethier 2001), but is consistent with previous observations from other tropical regions (e.g. Steinberg & Paul 1990; Pereira & Yoneshigue-Valentin 1999), and A. longimana appears to increase these and greater levels of phlorotannins (Kubanek et al. 2004). Moreover, definitive proof that phlorotannins play a role requires that herbivores are offered the isolated compounds in a feeding choice assay (e.g. Boettcher & Targett 1993). Regardless, it seems likely that a non-phenolic water-soluble deterrent was induced by amphipod grazing, as has been suggested for other brown seaweed – mesograzer interactions (Deal et al. 2003; Long, Hamilton & Mitchell 2007).

Nutritional traits or the interaction of nutritional traits with secondary metabolites may be responsible for shifts in tissue palatability. Plant nutritional traits can shift with herbivory pressure and may be adaptive in some cases (Ritchie, Tilman & Knops 1998; Norderhaug, Nygaard & Fredriksen 2006; Bracken & Stachowicz 2007). We found that S. filenphila tissues that were least preferred (i.e. exposed to prior grazing) exhibited lower nitrogen, greater carbon and a substantially greater C:N ratio compared to more preferred tissues exposed to less grazing pressure (Fig. 5). In theory, herbivores are nitrogen-limited (Mann 1979; Mattson 1980), and many herbivores attempt to maximize nitrogen relative to carbon intake (or protein to carbohydrate, Raubenheimer & Simpson 2009). However, when in the presence of predators, stressed grazers may selectively seek carbohydrate-rich foods and consume higher C:N plant tissue (Hawlena & Schmitz 2010a), with consequences for plant tissue composition, grazer nutrient assimilation and nutrient cycling (Trussell, Ewanchuck & Matassa 2006; Hawlena & Schmitz 2010b). Unfortunately, we did not generate ash-free dry mass values relative to wet mass, which is known to negatively correlate with no-choice feeding rates exhibited by A. longimana across algal species, although strong variation in this parameter was unlikely within our single, focal algal species (Cruz-Rivera & Hay 2001).

We do not believe that predator cues alone or direct grazer excretions strongly affected algal traits. Fish cues in the absence of herbivores (−G+F) had neither effect on algal palatability (Fig. 4) nor on algal biomass (Fig. 3). Although finfish effluent may have increased ammonia concentration in the water, promoting N uptake and leading to decreased algal tissue C:N in the absence of grazers, the magnitude of these changes was relatively slight (Fig. 5). Similarly, although it is possible that increased herbivore density can lead to elevated local deposits of nutrient-rich herbivore excretions (Taylor & Rees 1998), we found lower nitrogen within tissues that were exposed to greater grazer densities (Fig. 5).

The non-consumptive effect of predators on plant biomass is positive, but counter-intuitively, predator effects on plant quality may result in higher grazing rates over time. This is because when predators weaken algal-induced responses and increase food quality, the growth rates and fecundity of herbivores can increase. Indeed, grazer-induced defences of Asco-phyllum nodosum decrease the fecundity of gastropod grazers (Toth, Langhammer & Pavia 2005). Similarly, Haavisto, Välkkangas & Jormalainen (2010) found that the isopod Idotea balitica exhibited decreased egg production when fed defended Fucus vesiculosus compared to ungrazed algae. On the other hand, recent evidence suggests that waterborne cues of grazed macroalgae attract predators (Coleman et al. 2007), which raises the possibility that grazer-induced tissue will lower herbivore fitness by both increasing predator pressure and lowering food quality. Clearly, the chemical mediation of tritrophic interactions between predators, mesograzers and macrophytes deserve increased attention.

SUMMARY

Marine herbivores can alter algal community dynamics and their phenotype, including morphology and defensive chemistry (Cronin & Hay 1996). Here, we demonstrate that predator cues can ameliorate the effects of grazers on algal growth and improve algal food quality by weakening the strength of induction. The behaviourally mediated trophic cascade among pinfish, amphipods and Sargassum raises the untested possibility that non-consumptive effects of predators are as large as their consumptive effects, especially in regions of low predation pressure where predators scare more herbivores than they consume.

Acknowledgements

We thank John Bruno, the University of North Carolina’s Institute of Marine Sciences, and Grec Marine Lab for logistical support, Chris Nicolini for field assistance, and Chris Martens and Howard Mendlovitz for stable isotope analysis. This manuscript was greatly improved by comments from G. Trussell, J. Stachowicz, and an anonymous reviewer. Funding was provided by the National Science Foundation (OCE-0550245 to J. Bruno; OCE-0550245 and DEB-0919064 to E. Sotka) and a UNC Wilson award to P. Reynolds for field research.

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Received 21 October 2010; accepted 22 February 2011

Handling Editor: Richard Bardgett

Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Supplemental figures.

Figure S1. Experimental images.

Figure S2. Design of algal induction experiment.

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