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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 trophic 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-

chuk & Bertness 2003; see reviews by Lima & Dill 1990; Werner & Peacor 2003; Preisser, Bolnick & Benard 2005). In those instances in which the antipredator behaviours lower herbivore fitness, the predator cues suppress the growth rates of herbivore populations (Nelson, Matthews & Rosenheim 2004). Although the relative importance of these predator effects (i.e. lethal and behaviourally induced) is under current debate, both effects can increase plant biomass (Shurin *et al.* 2002; Newcombe & Taylor 2010).

Predators are also known to alter the ecological and evolutionary dynamics of plant phenotypes, including chemical and morphological defences, via their effects on herbivore grazing intensity. When herbivores escape predation, the subsequent grazing pressure may induce production of secondary metabolites and other plant defensive traits that minimize future grazing. Such induced responses are common to vascular plants and aquatic and marine algae (Hessen & van Donk

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1993; Karban & Baldwin 1997; Toth & Pavia 2007), and can themselves have cascading effects on herbivore populations (Agrawal & Rutter 1998) and communities (Denno, McClure & Ott 1995; Hay & Kubanek 2002; Long, Hamilton & Mitchell 2007). When plants across broad spatial and temporal scales are consistently grazed by herbivores that have escaped predation, evolution favours the constitutive production of plant defences to deter this greater herbivore pressure. As an example, marine seaweeds that co-occur in biogeographic areas with predatory sea otters and herbivorous urchins evolved lower levels of water soluble chemical defences relative to seaweeds from areas that are historically devoid of otters but replete with higher densities of urchins (Estes & Steinberg 1988; Steinberg, Estes & Winter 1995). Virtually all of these studies assume that the effect of predators on plant phenotype is through a consumptive mechanism and relatively few have tested non-consumptive predator effects on plant quality (but see Griffin & Thaler 2006; Kaplan & Thaler 2010).

Here we examine how predator cues and herbivore grazing intensity interactively influence the growth and food quality of a fucal brown macroalga, *Sargassum filipendula* 1824. Within an estuarine community in North Carolina (USA), *S. filipendula* is abundant year round and inhabited by a dense and diverse array of amphipod, isopod and gastropod grazers (Hay & Sutherland 1988). Amphipod abundances range from 10 to 145 individuals per gram (wet mass) on *S. filipendula*, although species composition and abundance fluctuates seasonally (Duffy 1989). The common herbivorous amphipod *A. longimana* has strong effects on algal biomass and community structure (Duffy 1989). It is also known to induce grazing resistance in several local algae including *S. filipendula* (Cronin & Hay 1996; Duffy & Hay 2000; Taylor, Sotka & Hay 2002). The pinfish *L. rhomboides* (Linnaeus 1766) is a predominant predator of amphipods in this system (Nelson 1978, 1979; Stoner 1979).

It has been hypothesized that pinfish and other common fishes in North Carolina estuaries heavily consume *A. longimana* and other mesograzers, yielding cascading effects on macroalgal composition (Duffy & Hay 2000). However, it is also possible that this trophic cascade has a non-consumptive mechanism when pinfish have strong non-consumptive effects on *A. longimana* grazing behaviour (P. Reynolds & J. Bruno, unpubl. data) in a manner similar to that seen for activity levels of other gammaridean amphipods (Wooster 1998; Wisenden, Cline & Sparkes 1999).

We used this model marine community to address whether predators alter macroalgal biomass and inducible defences via non-consumptive effects on their herbivore prey. We conducted a 6-week experiment in outdoor mesocosms to test the effects of *L. rhomboides* effluent ('cues') on *A. longimana* grazing rates (Grazing assay) and population growth, and feeding assays to assess the singular and interactive effects of fish cues and grazing on the tissue palatability of *S. filipendula* (Induction experiment). Specifically, we asked the following questions: (i) Can pinfish non-consumptively reduce amphipod grazing intensity by reducing per capita grazing rates, overall

population growth, or both?; (ii) Does lowered grazing pressure facilitate algal biomass and (iii) elevate tissue palatability of *S. filipendula* by weakening the strength of its induction of chemical defences?

Materials and methods

GRAZING ASSAY

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 $\mu\text{m m}^{-2} \text{s}^{-1}$ 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 11.4 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): $T_i(C_f/C_i) - T_f$ 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 Sotka, Taylor & Hay 2002; Taylor, Sotka & 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 4π irradiance; 23–25 °C, 34.5–36 ppt)(Taylor, Sotka & 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* ramets (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 $R = N_1 (N_0)^{-1}$ 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 offspring 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 *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 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 food squares 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 and their analyses were conducted as described in Materials and methods: 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 herbivore 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–Ciocalteu 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 M reagent with 1 M Na₂CO₃, vortexed and heated. Absorbance (read at 760 nm) was then compared to a standard curve generated with phloroglucinol (Sigma, St. Louis, MO, USA) samples. Phlorotannin concentration was calculated as a percentage of dry mass and was analysed with a two-way ANOVA ($n = 3$) given the data were normally distributed and homoscedastic.

Nitrogen is generally considered to be limiting to primary and secondary productivity (Mann 1979; Mattson 1980) and can influence plant chemical defences and herbivore performance (Yates & Peckol 1993; Koricheva *et al.* 1998; Herms 2002), potentially counteracting induced resistance (Herms 2002; Cruz-Rivera & Hay 2003; Norderhaug, Nygaard & Fredriksen 2006). We measured algal tissue nitrogen content, total nitrogen and total organic carbon (TOC, TN) as well as tissue C:N ratio by gas chromatography–mass spectrometry at UNC-Chapel Hill's Marine Sciences Department (Fry *et al.* 1992; Brand 1996) ($n = 3$). The C:N and TN data could not be transformed to meet assumptions of normality, and the sample sizes were too small to pursue nonparametric *post hoc* tests (i.e. Wilcoxon tests). We thus rank-transformed the data (Conover & Iman 1981) and pursued parametric one-way ANOVAs and *post hoc* tests. Arguably, these three traits are not independent, and so we used a sequential Bonferroni procedure to generate an appropriate alpha (cf. Rice 1989). All three results were significant by this procedure. We combined all samples within a treatment because we had low amounts of top stipe tissue, 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

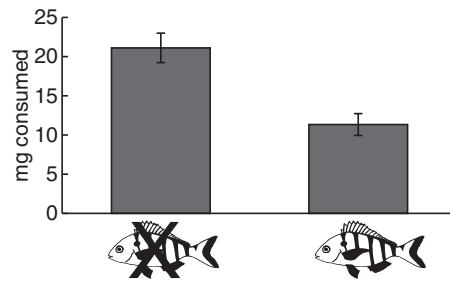


Fig. 1. Amphipod grazing rates over 7 days in the presence (+) and absence (–) of olfactory cues from fish (fish icon). ($T = -4.18$, $P < 0.001$) Mean consumption (mg) \pm 1 SE are shown.

Table 1. Results of two-factor ANOVAs on final amphipod abundance, algal wet mass and tissue phlorotannin concentration

Factor	SS	d.f.	<i>F</i>	<i>P</i>
Grazer abundance				
+ Grazers (G)	46.83	1	104.62	< 0.001
+ Fish-cue (F)	6.39	1	14.26	< 0.001
G \times F	2.00	1	4.47	0.043
Error	13.43	30		
Algal wet mass				
+ Grazers	13437.10	1	118.72	< 0.001
+ Fish-cue	923.67	1	8.16	0.008
G \times F	499.95	1	4.42	0.044
Error	3395.38	30		
Algal phlorotannins				
+ Grazers	3.03e-7	1	7.54	0.025
+ Fish-cue	4.62e-7	1	11.49	0.010
G \times F	4.40e-7	1	10.95	0.011
Error	3.22e-7	8		

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. filipendula* was significant (Table 1; Fig. 2b), indicating that grazers consistently lowered *S. filipendula* 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

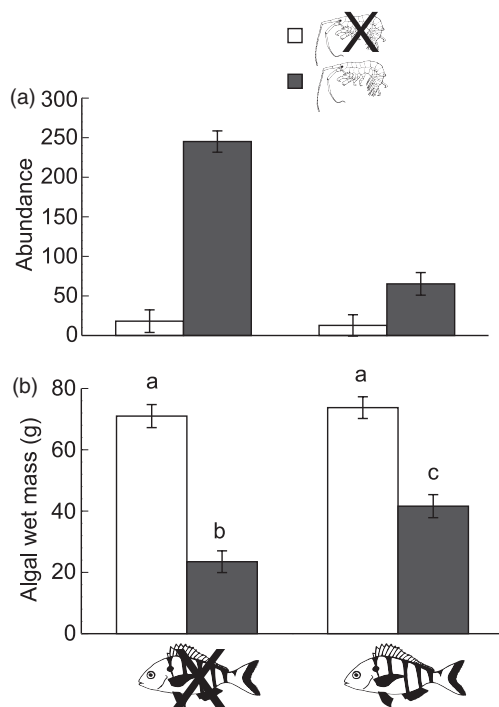


Fig. 2. Effects of grazer (dark bars) and fish cue (fish icon) treatments on final (a) grazer population size and (b) algal wet mass after 42 days. Letters represent treatments that are significantly different by Tukey's HSD. Mean \pm SE values are shown.

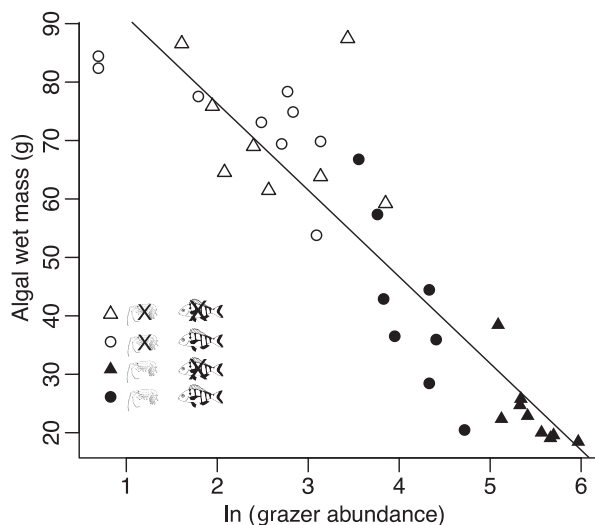


Fig. 3. Exponential decay of final algal biomass with grazer density (Biomass \times ln(grazer abundance): $r^2 = 0.827$; $P < 0.001$). Filled shapes represent replicates initially stocked with grazers, and triangles and circles represent replicates without and with olfactory cues from fish, respectively.

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

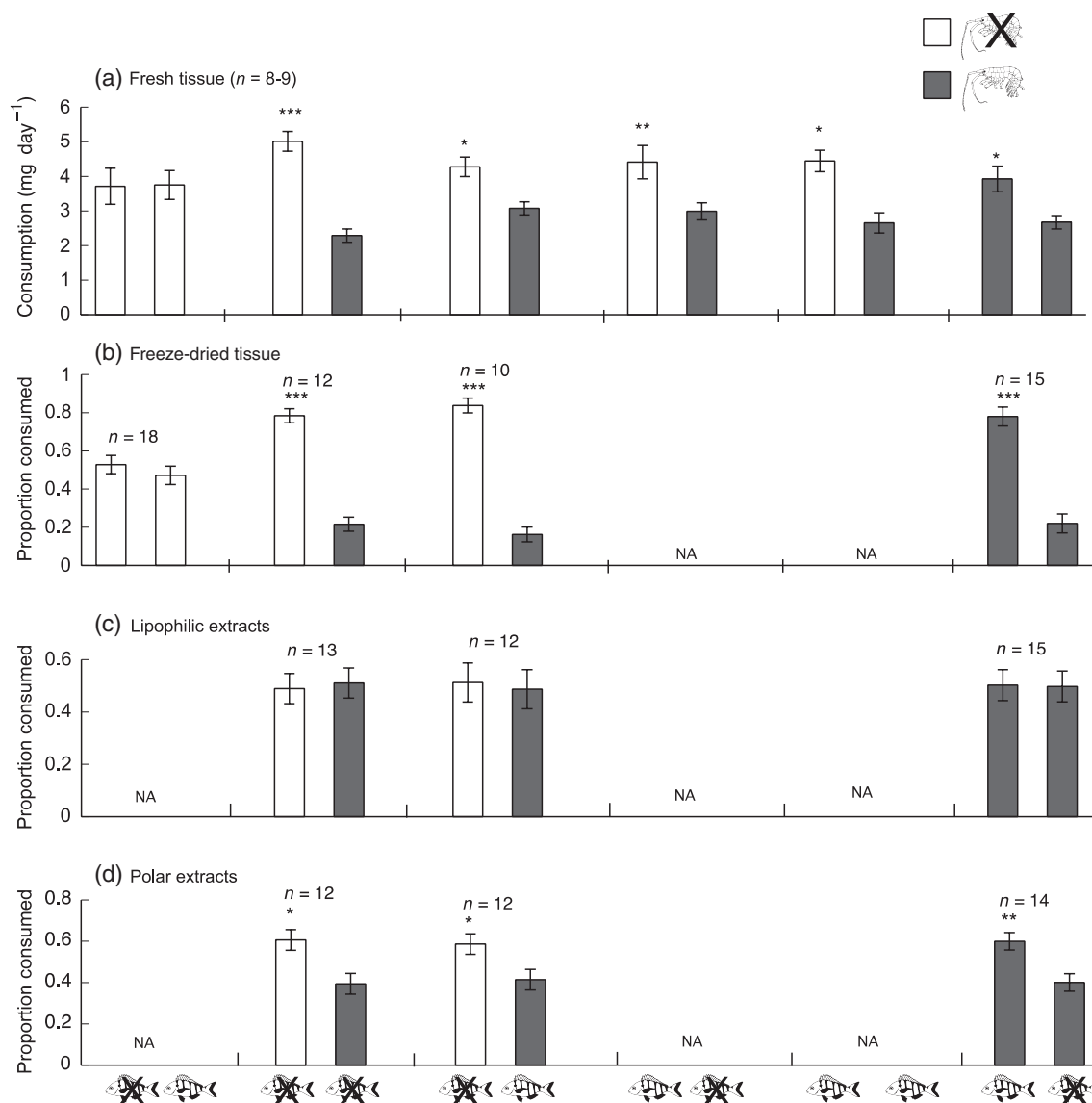


Fig. 4. Effects of grazer (dark bars) and fish cue (fish icon) treatments on algal palatability in (a) fresh tissue (statistics are for two-tailed *t*-tests of first tissue choice with null = 50% consumption; $n = 8-9$); and pooled (b) freeze-dried tissue, (c) lipophilic extracts and (d) polar extract assays (non-parametric ANOVA permutation with cup as block). Mean \pm 1 SE are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NA refers to treatments that were not conducted due to a lack of a direct fish cue effect found in (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; Korpinen, 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

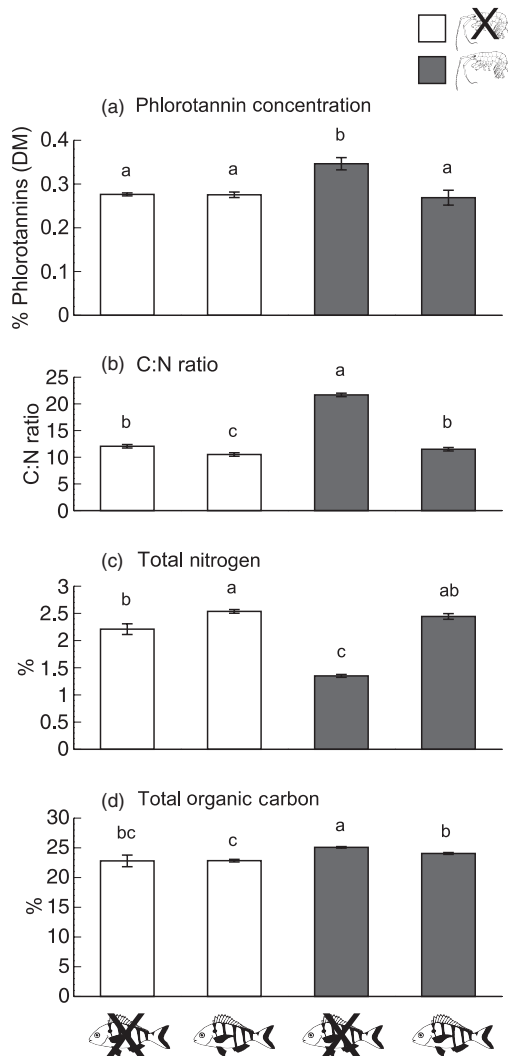


Fig. 5. Effects of grazer (dark bars) and fish cue (fish icon) treatments on pooled algal tissue (a) phlorotannin concentration, (b) C : N ratio ($F_{3,8} = 254.6$, $P < 0.001$), (c) total nitrogen ($F_{3,8} = 81.952$, $P < 0.001$) and (d) total organic carbon ($F_{3,8} = 4.570$, $P = 0.030$). ± 1 SE. Letters in represent treatments that are significantly different by Tukey's HSD. Refer to Table 1 for overall test for phlorotannins.

2005; Peckarsky *et al.* 2008; Preisser & Bolnick 2008b). This bias occurs despite the fact that predator-induced reductions in per-capita grazing rates are commonly documented within many predator-prey systems (e.g. McIntosh & Townsend 1996; Trussell, Ewanchuk & Bertness 2003; Byrnes *et al.* 2006; Nelson 2007; Steffan & Snyder 2010). Moreover, reductions in feeding rates that occur with chronic exposure to predator cues over the lifespan of an individual or across generations can generate persistent reductions prey in population size (Fig. 2) (Nelson, Matthews & Rosenheim 2004; Pangle, Peacor & Johannsson 2007) and this long-term impact magnifies the non-consumptive effect on per capita feeding. However, prey state (e.g. body condition) may modify trade-offs between foraging and predation risk, thereby regulating the expression of prey antipredator behaviour (Lima & Bednekoff 1999; Stephens, Brown & Ydenberg 2007). Starving prey are more likely to

engage in risky activities, thereby limiting the degree of NCEs in systems where prey experience persistent predation risk and/or reduced resource abundance and quality.

As a consequence of the growing recognition of non-consumptive predator 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 holbrookii*) 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 mesograzers 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 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 readily consume these and greater levels of phlorotannins (Kubaneck *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 – mesograzers 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. filpendula* 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 pinfish 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-phylum nodosum* decrease the fecundity of gastropod grazers (Toth, Langhamer & Pavia 2005). Similarly, Haavisto,

Välikangas & Jormalainen (2010) found that the isopod *Idotea balthica* 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.

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References

- Agrawal, A. & Rutter, M.T. (1998) Dynamic anti-herbivore defense in ants: the role of induced responses. *Oikos*, **83**, 227.
- van Alstyne, K.L. (1988) Herbivore grazing increases polyphenolic defenses in the intertidal brown alga *Fucus distichus*. *Ecology*, **69**, 655–663.
- van Alstyne, K. (1995) Comparison of three methods for quantifying brown algal polyphenolic compounds. *Journal of Chemical Ecology*, **21**, 45–58.
- van Alstyne, K.L., Duggins, D.O. & Dethier, M.N. (2001) Spatial patterns in macroalgal chemical defenses. *Marine Chemical Ecology* (eds J.B. McClintock & B.J. Baker), pp. 301–324. CRC Press, New York.
- Anderson, M.J. (2001) Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries & Aquatic Sciences*, **58**, 626–639.
- Begon, M., Townsend, C.R. & Harper, J.L. (2006) Ecology: from individuals to ecosystems. *Blackwell Publishing*, Oxford, UK.
- Bernays, E.A. & Wedge, P. (1987) Significance levels of inferential statistics and their interpretation – a lesson from feeding deterrent experiments. *Annals of the Entomological Society of America*, **80**, 9.
- Boettcher, A.A. & Targett, N.M. (1993) Role of polyphenolic molecular size in reduction of assimilation efficiency in *Xiphister mucosus*. *Ecology*, **74**, 891–903.
- Bracken, M.E.S. & Stachowicz, J.J. (2007) Top-down modification of bottom-up processes: selective grazing reduces macroalgal nitrogen uptake. *Marine Ecology-Progress Series*, **330**, 75–82.
- Brand, W.A. (1996) High precision isotope ratio monitoring techniques in mass spectrometry. *Journal of Mass Spectrometry*, **31**, 225–235.
- Brawley, S. (1992) Mesoherbivores. *Plant–Animal Interactions in the Marine Benthos* (eds D.M. John, S.J. Hawkins & J.H. Price), pp. 235–263. Clarendon, Oxford, UK.

- Brawley, S.H. & Fei, X.G. (1987) Studies of mesoherbivory in aquaria and in an unbarricaded mariculture farm on the Chinese coast. *Journal of Phycology*, **23**, 614–623.
- Bruno, J.F. & O'Connor, M.I. (2005) Cascading effects of predator diversity and omnivory in a marine food web. *Ecology Letters*, **8**, 1048–1056.
- Byrnes, J.E., Stachowicz, J.J., Hultgren, K.M., Hughes, A.R., Olyarnik, S.V. & Thornber, C.S. (2006) Predator diversity strengthens trophic cascades in kelp forests by modifying herbivore behaviour. *Ecology Letters*, **9**, 61–71.
- Coleman, R., Ramchunder, S., Davies, K., Moody, A. & Foggo, A. (2007) Herbivore-induced infochemicals influence foraging behaviour in two intertidal predators. *Oecologia*, **151**, 454–463.
- Conover, W.J. & Iman, R.L. (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *American Statistician*, **35**, 124–129.
- Cronin, G. & Hay, M.E. (1996) Induction of seaweed chemical defenses by amphipod grazing. *Ecology*, **77**, 2287–2301.
- Cronin, G., Paul, V.J., Hay, M.E. & Fenical, W. (1997) Are tropical herbivores more resistant than temperate herbivores to seaweed chemical defenses? Diterpenoid metabolites from *Dictyota acutiloba* as feeding deterrents for tropical versus temperate fishes and urchins. *Journal of Chemical Ecology*, **23**, 289–302.
- Cruz-Rivera, E. & Hay, M.E. (2001) Macroalgal traits and the feeding and fitness of an herbivorous amphipod: the roles of selectivity, mixing and compensation. *Marine Ecology Progress Series*, **218**, 249–266.
- Cruz-Rivera, E. & Hay, M.E. (2003) Prey nutritional quality interacts with chemical defenses to affect consumer feeding and fitness. *Ecological Monographs*, **73**, 483–506.
- Davenport, A.C. & Anderson, T.W. (2007) Positive indirect effects of reef fishes on kelp performance: the importance of mesograzers. *Ecological Applications*, **88**, 1548–1561.
- Dayton, P.K., Currie, V., Gerrodette, T., Keller, B.D., Rosenthal, R. & Tresca, D.V. (1984) Patch dynamics and stability of some California kelp communities. *Ecological Monographs*, **54**, 254–289.
- Deal, M.S., Hay, M.E., Wilson, D. & Fenical, W. (2003) Galactolipids rather than phlorotannins as herbivore deterrents in the brown seaweed *Fucus vesiculosus*. *Oecologia*, **136**, 107–114.
- Denno, R.F., McClure, M.S. & Ott, J.R. (1995) Interspecific interactions in phytophagous insects: competition re-examined and resurrected. *Annual Review of Entomology*, **40**, 297–331.
- Duffy, J. (1989) *Ecology and Evolution of Herbivory by Marine Amphipods*. Dissertation, University of North Carolina at Chapel Hill, Chapel Hill.
- Duffy, J.E. (1990) Amphipods on seaweeds: partners or pests? *Oecologia*, **83**, 267–276.
- Duffy, J.E. & Hay, M.E. (2000) Strong impacts of grazing amphipods on the organization of a benthic community. *Ecological Monographs*, **70**, 237–263.
- Estes, J.A. & Steinberg, P.D. (1988) Predation, herbivory and help evolution. *Paleobiology*, **14**, 19–36.
- Fry, B., Brand, W., Mersch, F.J., Tholke, K. & Garritt, R. (1992) Automated analysis system for coupled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements. *Analytical Chemistry*, **64**, 288–291.
- Griffin, C.A. & Thaler, J.S. (2006) Insect predators affect plant resistance via density- and trait-mediated indirect interactions. *Ecology Letters*, **9**, 335–343.
- Haavisto, F., Välikangas, T. & Jormalainen, V. (2010) Induced resistance in a brown alga: phlorotannins, genotypic variation and fitness costs for the crustacean herbivore. *Oecologia*, **162**, 685–95.
- Hairton, N.G., Smith, F.E. & Slobodkin, L.B. (1960) Community structure, population control and competition. *The American Naturalist*, **94**, 425.
- Hawlana, D. & Schmitz, O.J. (2010a) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences*, **107**, 15503–15507.
- Hawlana, D. & Schmitz, O.J. (2010b) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *The American Naturalist*, **176**, 537–556.
- Hay, M.E. & Kubanek, J. (2002) Community and ecosystem level consequences of chemical cues in the plankton. *Journal of Chemical Ecology*, **28**, 2001–2016.
- Hay, M.E. & Sutherland, J.P. (1988) The ecology of rubble structures of the south Atlantic bight: a community profile. *Biological Report* (ed U. S. D. o. t. Fish and Wildlife Service), pp. 67. National Wetlands Research Center, Washington, DC.
- Hemmi, A., Mäkinen, A., Jormalainen, V. & Honkanen, T. (2005) Responses of growth and phlorotannins in *Fucus vesiculosus* to nutrient enrichment and herbivory. *Aquatic Ecology*, **39**, 201–211.
- Herns, D.A. (2002) Effects of fertilization on insect resistance of woody ornamental plants: reassessing an entrenched paradigm. *Environmental Entomology*, **31**, 923–933.
- Hessen, D. & van Donk, E. (1993) Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. *Archiv Fur Hydrobiologie*, **127**, 129–140.
- Kaplan, I. & Thaler, J.S. (2010) Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos*, **119**, 1105–1113.
- Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. University of Chicago Press, Chicago.
- Kennelly, S.J. (1983) An experimental approach to the study of factors affecting algal colonization in a sublittoral kelp forest. *Journal of Experimental Marine Biology and Ecology*, **68**, 257–276.
- Koricheva, J., Larsson, S., Haukioja, E. & Keinänen, M. (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos*, **83**, 212–226.
- Korpinen, S., Jormalainen, V. & Honkanen, T. (2007) Bottom-up and cascading top-down control of macroalgae along a depth gradient. *Journal of Experimental Marine Biology and Ecology*, **343**, 52–63.
- Kubanek, J., Lester, S.E., Fenical, W. & Hay, M.E. (2004) Ambiguous role of phlorotannins as chemical defenses in the brown alga *Fucus vesiculosus*. *Marine Ecology Progress Series*, **277**, 79–93.
- Lima, S.L. & Dill, L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.
- Lima, S.L. & Bednekoff, P.A. (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *American Naturalist*, **153**, 649–659.
- Long, J.D., Hamilton, R.S. & Mitchell, J.L. (2007) Asymmetric competition via induced resistance: specialist herbivores indirectly suppress generalist preference and populations. *Ecology*, **88**, 1232–1240.
- Lüder, U. & Clayton, M. (2004) Induction of phlorotannins in the brown macroalga *Ecklonia radiata* (Laminariales, Phaeophyta) in response to simulated herbivory – the first microscopic study. *Planta*, **218**, 928–937.
- Mancinelli, G. & Rossi, L. (2001) Indirect, size-dependent effects of crustacean mesograzers on the growth of the Rhodophyta *Gracilaria verrucosa* (Hudson) *Papenfuss*: evidences from a short-term study in the Lesina Lagoon (Italy). *Marine Biology*, **138**, 1163–1173.
- Mann, K.H. (1979) Nitrogen limitations on the productivity of *Spartina* marshes, *Laminaria* kelp beds and higher trophic levels. *Ecological Processes on Coastal Environments* (R.L. Jefferies & A.L. Davy), pp. 363–370. Blackwell Scientific Publications, Oxford, UK.
- Mattson, W.J. (1980) Herbivory in relation to plant nitrogen content. *Annual Review of Ecology & Systematics*, **11**, 119–161.
- McIntosh, A.R. & Townsend, C.R. (1996) Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish induced changes to grazer behaviour? *Oecologia*, **108**, 174–181.
- Nelson, W.G. (1978) *The community ecology of seagrass amphipods: predation and community structure, life histories and biogeography*. Dissertation, Duke University, Durham.
- Nelson, W.G. (1979) Experimental studies of selective predation on amphipods: consequences for amphipod distribution and abundance. *Journal of Experimental Marine Biology and Ecology*, **38**, 225–245.
- Nelson, E.H. (2007) Predator avoidance behaviour in the pea aphid: costs, frequency and population consequences. *Oecologia*, **151**, 22–32.
- Nelson, E.H., Matthews, C.E. & Rosenheim, J.A. (2004) Predators reduce prey population growth by inducing changes in prey behaviour. *Ecology*, **85**, 1853–1858.
- Newcombe, E.M. & Taylor, R.B. (2010) Trophic cascade in a seaweed-epifauna-fish food chain. *Marine Ecology Progress Series*, **408**, 161–167.
- Norderhaug, K.M., Nygaard, K. & Fredriksen, S. (2006) Importance of phlorotannin content and C:N ratio of *Laminaria hyperborea* in determining its palatability as food for consumers. *Marine Biology Research*, **2**, 367–371.
- Norton, T.A. & Benson, M.R. (1983) Ecological interactions between the brown seaweed *Sargassum muticum* and its associated fauna. *Marine Biology*, **75**, 169–177.
- O'Hara, R.B. & Kotze, D.J. (2010) Do not log-transform count data. *Methods in Ecology and Evolution*, **1**, 118–122.
- Pangle, K.L., Peacor, S.D. & Johannsson, O.E. (2007) Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology*, **88**, 402–412.
- Pavia, H. & Toth, G.B. (2000) Inducible chemical resistance to herbivory in the brown seaweed *Ascophyllum nodosum*. *Ecology*, **81**, 3212–3225.
- Pavia, H., Toth, G.B. & Aberg, P. (2002) Optimal defense theory: elasticity analysis as a tool to predict intraplant variation in defenses. *Ecology*, **83**, 891–897.
- Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B., Orrock, J.L., Peacor, S.D., Preisser, E.L., Schmitz, O.J. &

- Trussell, G.C. (2008) Revisiting the classics: considering nonconsumptive effects in textbook examples of predator–prey interactions. *Ecology*, **89**, 2416–2425.
- Pereira, R.C. & Yoneshigue-Valentin, Y. (1999) The role of polyphenols from the tropical brown alga *Sargassum furcatum* on the feeding by amphipod herbivores. *Botanica Marina*, **42**, 441–448.
- Poore, A.G.B., Campbell, A.H. & Steinberg, P.D. (2009) Natural densities of mesograzers fail to limit growth of macroalgae or their epiphytes in a temperate algal bed. *Journal of Ecology*, **97**, 164–175.
- Preisser, E.L. & Bolnick, D.I. (2008) When predators don't eat their prey: non-consumptive predator effects on prey dynamics. *Ecology*, **89**, 2414–2415.
- Preisser, E.L., Bolnick, D.I. & Benard, M.E. (2005) Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology*, **86**, 501–509.
- Raubenheimer, D. & Simpson, S.J. (2009) Nutritional pharmacology: doses nutrients, toxins and medicines. *Integrative and Comparative Biology*, **49**, 329–337.
- Rice, W.R. (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.
- Ritchie, M.E., Tilman, D. & Knops, J.M.H. (1998) Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology*, **79**, 165–177.
- Rohde, S., Molis, M. & Wahl, M. (2004) Regulation of anti-herbivore defence by *Fucus vesiculosus* in response to various cues. *Journal of Ecology*, **92**, 1011–1018.
- Schmitz, O.J. (2005) Behaviour of predators and prey links with population level processes. *Ecology of Predator–Prey Interactions* (eds P. Barbosa & I. Castellanos), pp. 256–278, Oxford University Press, Oxford, UK.
- Shacklock, P.F. & Croft, G.B. (1981) Effect of grazers on *Chondrus crispus* in culture. *Aquaculture*, **22**, 331–342.
- Shurin, J.B., Borer, E.T., Seabloom, E.W., Anderson, K., Blanchette, C.A., Broitman, B., Cooper, S.D. & Halpern, B.S. (2002) A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters*, **5**, 785–791.
- Sotka, E.E. (2003) Genetic control of feeding preference in the herbivorous amphipod *Ampithoe longimana*. *Marine Ecology Progress Series*, **256**, 305–310.
- Sotka, E.E. & Reynolds, P.L. (in press) Rapid experimental shift in host use traits of a polyphagous marine herbivore reveals fitness costs on alternative hosts. *Evolutionary Ecology*.
- Sotka, E.E., Taylor, R.B. & Hay, M.E. (2002) Tissue-specific induction of resistance to herbivores in a brown seaweed: the importance of direct grazing versus waterborne signals from grazed neighbours. *Journal of Experimental Marine Biology and Ecology*, **277**, 1–12.
- Steffan, S.A. & Snyder, W.E. (2010) Cascading diversity effects transmitted exclusively by behavioural interactions. *Ecology*, **91**, 2242–2252.
- Steinberg, P.D., Estes, J.A. & Winter, F.C. (1995) Evolutionary consequences of food chain length in kelp forest communities. *Proceedings of the National Academy of Sciences*, **92**, 8145–8148.
- Steinberg, P.D. & Paul, V.J. (1990) Fish feeding and chemical defenses of tropical brown algae in Western Australia. *Marine Ecology Progress Series*, **58**, 253–259.
- Stephens, D.W., Brown, J.A. & Ydenberg, R.C. (2007) *Foraging: Behavior and Ecology*. University of Chicago Press, Chicago.
- Stoner, A.W. (1979) Species-specific predation on amphipod crustacea by the pinfish *Lagodon rhomboides*: Mediation by macrophyte standing crop. *Marine Biology*, **55**, 201–207.
- Taylor, R.B. & Rees, T.A.V. (1998) Excretory products of mobile epifauna as a nitrogen source for seaweeds. *Limnology and Oceanography*, **43**, 600–606.
- Taylor, R.B., Sotka, E. & Hay, M.E. (2002) Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. *Oecologia (Berlin)*, **132**, 68–76.
- Taylor, R.B., Lindquist, N., Kubanek, J. & Hay, M.E. (2003) Intraspecific variation in palatability and defensive chemistry of brown seaweeds: effects on herbivore fitness. *Oecologia*, **136**, 412–423.
- Toth, G., Karlsson, M. & Pavia, H. (2007) Mesoherbivores reduce net growth and induce chemical resistance in natural seaweed populations. *Oecologia*, **152**, 245–255.
- Toth, G.B., Langhamer, O. & Pavia, H. (2005) Inducible and constitutive defenses of valuable seaweed tissues: consequences for herbivore fitness. *Ecology*, **86**, 612–618.
- Toth, G.B. & Pavia, H. (2000) Water-borne cues induce chemical defense in a marine alga (*Ascophyllum nodosum*). *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 14418–14420.
- Toth, G. & Pavia, H. (2002) Lack of phlorotannin induction in the kelp *Laminaria hyperborea* in response to grazing by two gastropod herbivores. *Marine Biology*, **140**, 403–409.
- Toth, G.B. & Pavia, H. (2007) Induced herbivore resistance in seaweeds: a meta-analysis. *Journal of Ecology*, **95**, 425–434.
- Trussell, G.C., Ewanchuk, P.J. & Matassa, C.M. (2006) The fear of being eaten reduces energy transfer in a simple food chain. *Ecology*, **87**, 2979–2984.
- Trussell, G.C., Ewanchuk, P.J. & Bertness, M.D. (2003) Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, **84**, 629–640.
- Werner, E.E. & Peacor, S.D. (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.
- Wisenden, B.D., Cline, A. & Sparkes, T.C. (1999) Survival benefit to antipredator behaviour in the amphipod *Gammarus minus* (Cristacea: Amphipoda) in response to injury-released chemical cues from conspecifics and heterospecifics. *Ethology*, **105**, 407–414.
- Wooster, D.E. (1998) Amphipod (*Gammarus minus*) responses to predators and predator impact on amphipod density. *Oecologia*, **115**, 253–259.
- Yates, J.L. & Peckol, P. (1993) Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. *Ecology*, **74**, 1757–1766.

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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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