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Wave-induced changes in seaweed toughness entail plastic modifications in snail traits maintaining consumption efficacy

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Summary

1. Environmental stress can influence species traits and performance considerably. Using a seaweed–snail system from NW (Nova Scotia) and NE (Helgoland) Atlantic rocky shores, we examined how physical stress (wave exposure) modulates traits in the seaweed *Fucus vesiculosus* and indirectly in its main consumer, the periwinkle *Littorina obtusata*.

2. In both regions, algal tissue toughness increased with wave exposure. Reciprocal-transplant experiments showed that tissue toughness adjusted plastically to the prevailing level of wave exposure.

3. Choice experiments tested the feeding preference of snails from sheltered, exposed and very exposed habitats for algae from such wave exposures. Snails from exposed and very exposed habitats consumed algal tissues at similar rates irrespective of the exposure of origin of the algae. However, snails from sheltered habitats consumed less algal tissues from very exposed habitats than tissues from sheltered and exposed habitats. Choice assays using reconstituted algal food (triturated during preparation) identified high thallus toughness as the explanation for the low preference of snails from sheltered habitats for algae from very exposed habitats.

4. Ultrastructural analyses of radulae indicated that rachidian teeth were longest and the number of cusps in lateral teeth (grazing-relevant traits) was highest in snails from very exposed habitats, suggesting that radulae are best suited to rupture tough algal tissues in such snails.

5. No-choice feeding experiments revealed that these radular traits were also phenotypically plastic, as they adjusted to the toughness of the algal food.

6. *Synthesis*. This study indicates that the observed plasticity in the feeding ability of snails is mediated by wave exposure through phenotypic plasticity in the tissue toughness of algae. Thus, plasticity in consumers and their resource species may reduce the potential effects of physical stress on their interaction.

Key-words: abiotic stress, alga, interspecific interaction, intertidal, periwinkle, phenotypic plasticity, plant–herbivore interactions, wave exposure

Introduction

Environmental stress exerts negative pressures on the performance of species. Yet, the ability of organisms to plastically modify their structural, physiological or behavioural traits allows them to perform well under a wider environmental range than they could with fixed traits (Sultan 2000). While many studies have investigated the evolution of phenotypic plasticity (Pigliucci 2005; Pfennig *et al.* 2010), its ecological impact has only recently attracted researchers (Miner *et al.* 2005). The ecological effects of phenotypic plasticity are most well documented for trophic interactions. Predators, for example, can cause morphological adjustments in mollusc prey (Reimer & Tedengren 1996; Leonard, Bertness & Yund

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1999; Trussell 2000), and herbivores can elicit chemical defence responses or anatomical modifications in plants (Agrawal & Konno 2009; Heil 2010; Gutbrodt *et al.* 2012; McArt *et al.* 2013) and seaweeds (Lewis, Norris & Searles 1987; Toth & Pavia 2007; Flöthe, Molis & John 2014). In turn, trait changes in resource species (species constituting food for consumers) can induce plastic changes in consumer traits, such as the development of stronger claws in crabs (Smith & Palmer 1994) or insect gut enzyme alterations to cope with plant chemical defences (Broadway 1997; Li, Schuler & Berenbaum 2002). This reciprocal plasticity has the potential to initiate co-evolutionary arms races in consumer–prey interactions (Agrawal 2001; Utsumi 2011; McGhee, Pintor & Bell 2013).

Abiotic conditions may also induce changes in the phenotype of resource species. For example, plants and seaweeds may acclimate to increased CO_2 concentration and ultraviolet radiation through biochemical changes (Pavia *et al.* 1997; Stiling *et al.* 2013). The possible influence of abiotically induced trait changes in resource species on consumer traits, however, has been less studied than the influence of biotically induced trait changes (Miner *et al.* 2005; Read & Stokes 2006; Nunes *et al.* 2014). Knowledge on stress-mediated trait changes in resource and consumer species should increase our understanding of trophic interactions and, ultimately, on how community organization and functioning could be altered in a changing world (Berg & Ellers 2010; Young, Zieger & Babanin 2011). With this goal in mind, we studied the relationship between physical stress (wave exposure), structural phenotypic plasticity in a resource species (a seaweed) and the resulting plasticity in radular traits of a herbivorous snail affecting its feeding ability.

In rocky intertidal habitats, wave exposure affects the growth and survival of species considerably (Weissburg, Helmuth & Witman 2014). It is important to know the fate of canopy-forming seaweeds because such algae provide food and shelter for many smaller organisms (Watt & Scrosati 2013). A variety of mechanisms have evolved in intertidal algae to mitigate the effects of physical stress caused by waves, including the development of flexible thalli to reduce drag (Boller & Carrington 2006) or harder thalli to resist wave impacts (Martone 2007). Algal tissues may toughen plastically in some species (Fowler-Walker, Wernberg & Connell 2006). For our study, we used the perennial canopy-forming seaweed *Fucus vesiculosus* and its main consumer, the snail *Littorina obtusata*. Both are common species on NW and NE Atlantic rocky shores, including our study sites (Scrosati & Heaven 2007; Zwerschke *et al.* 2013). First, we tested the hypothesis (i) that thalli of *F. vesiculosus* from wave-exposed habitats are more resistant to puncture (proxy for tissue toughness) than thalli from wave-sheltered habitats. Using a transplantation field experiment, we then tested the hypothesis (ii) that such a structural resistance is a phenotypically plastic trait.

The feeding ability of herbivorous snails decreases with increasing algal thallus toughness (Watson & Norton 1985, 1987; Chavanich & Harris 2002). Thus, using a laboratory assay, we tested the hypothesis (iii) that *L. obtusata* from sheltered habitats (hereafter, sheltered snails) prefer feeding on *F. vesiculosus* from sheltered habitats (hereafter, sheltered algae) than on *F. vesiculosus* from exposed habitats (hereafter, exposed algae), while snails from exposed habitats (hereafter, exposed snails) show no preference because they should have the ability to consume food of different toughness levels equally well. To assess whether differences in algal tissue toughness or chemical nature (e.g. nutritional value) explained the observed feeding preferences, we offered sheltered and exposed snails reconstituted food made with triturated exposed and sheltered algae (i.e. food with unaltered chemical traits but lacking the original toughness differences). Assuming that toughness differences would dictate snail feeding preferences, we tested the hypothesis (iv) that snails show no feeding preference between reconstituted food made of sheltered or exposed algae.

Snail feeding preferences also depend on traits of their radulae (Reid 1996). For instance, longer radular teeth are thought to be more effective than shorter teeth to rupture algal tissues (Padilla 2004), and the cusps of lateral teeth in *L. obtusata* are needed to excavate the thallus of fucoid macroalgae (Reid 1996). Through ultrastructural analyses of *L. obtusata* specimens, we tested the hypothesis (v) that central radular teeth are longer and cusps in lateral teeth more numerous in exposed snails than in sheltered snails. Littorinid snails, including *L. obtusata*, replace their radular teeth within a few weeks (Isarankura & Runham 1968), producing differently shaped teeth when exposed to different food sources (Padilla 2004). This confers snails the ability to change tooth morphology on demand. Thus, we tested the hypothesis (vi) that exclusive consumption of sheltered or exposed algae can plastically modulate the above-mentioned radular traits in *L. obtusata*.

Materials and methods

ALGAL TISSUE TOUGHNESS ACROSS WAVE EXPOSURES

To test whether thallus toughness differs between *F. vesiculosus* from exposed and sheltered habitats (hypothesis i), we used rocky intertidal habitats differing in wave exposure in Tor Bay Provincial Park and its vicinity (Nova Scotia, Canada) and on Helgoland Island (Germany). Between September 2009 and October 2010; we measured maximum water velocity (an indication of exposure) using dynamometers (see design in Bell & Denny 1994) attached to the mid-intertidal zone in six sites in Nova Scotia (between 45.10644–45.11153 N and 61.21160–61.21700 W) and in three sites in Helgoland (Bunker: 54.18806 N, 7.87436 E; Augusta Mole: 54.18931 N, 7.89972 E; and Nord-Ost Hafen, 54.18311 N, 7.88947 E). Each site spanned ca. 30 m of coastline. To prevent entanglement, we cleared all algal canopies within 50 cm around the dynamometers. In Nova Scotia, we identified three levels of wave exposure: sheltered (maximum water velocity = 1.7 ± 0.03 m s^{-1}, mean ± SE; range = 1.5–2.2 m s^{-1}; n = 48, two sites), exposed (5.0 ± 0.05 m s^{-1}; range = 4.1–5.8 m s^{-1}; n = 48, two sites) and very exposed (8.3 ± 0.08 m s^{-1}; range = 6.8–8.9 m s^{-1}; n = 48, two sites). Very exposed habitats

face the open Atlantic Ocean directly, while rocky formations in front of the sheltered and exposed habitats block incoming swell to varying degrees, thus reducing exposure. As Helgoland does not face the open ocean, fetch is lower and the range of wave exposure is smaller. We identified two exposure levels in Helgoland: sheltered (1.9 ± 0.05 m s^{-1}, mean ± SE; range = 1.3–2.5 m s^{-1}; $n = 41$, Nord-Ost Hafen) and exposed (4.7 ± 0.24 m s^{-1}; range = 2.1–8.8 m s^{-1}; $n = 54$, Bunker and Augusta Mole). Thus defined, our sheltered and exposed habitats were each comparable between Nova Scotia and Helgoland, while the very exposed habitats in Nova Scotia represented the highest exposure level in this study.

To determine algal tissue toughness, we measured the resistance-to-puncture in vegetative apical fragments of *F. vesiculosus* from each exposure level. First, we cut a 3-cm-long tip without feeding scars off each of 30 (Nova Scotia) and 15 (Helgoland) specimens collected randomly at each site during low tide and kept them for <24 h in running seawater until measuring toughness with a penetrometer. This was done on 15 May 2010 in Nova Scotia and on 16 December 2013 in Helgoland (where we used Südhafen, 54.17819 N, 7.89417 E, as a second sheltered site). In Nova Scotia, we used an industrial penetrometer (TA.Xtplus Texture Analyzer, Texture Technologies Corp., Scarsdale, NY, USA) with a stainless steel punch probe of 4 mm of diameter (TA-54), yielding measures in Newton. In Helgoland, we used a gravitational penetrometer that measured the mass of sand (to the nearest 1 mg) needed in a plastic tube on top of a 0.7-mm-diameter blunt insect needle to penetrate an algal sample (method adopted from Duffy & Hay 1991). For each algal sample, we calculated the average from three measurements, excluding midribs because of their stronger contexture. For Nova Scotia, we evaluated the effects of wave exposure (fixed factor with three levels: sheltered, exposed and very exposed) and site (random factor with two levels) nested within exposure on resistance-to-puncture with a nested ANOVA. For Helgoland, we tested the effects of wave exposure (fixed factor with two levels: sheltered and exposed) and site (random factor with two levels) nested within exposure on sand mass at penetration with a nested ANOVA.

PLASTICITY IN ALGAL TISSUE TOUGHNESS

To test whether algal tissue toughness changes plastically with wave exposure (hypothesis ii), we ran reciprocal-transplant experiments. In Nova Scotia, we randomly collected 60 *F. vesiculosus* specimens, including their holdfast, from a very exposed site on 12 June 2010. Within 3 h of collection, we transported them in a cooler to the laboratory, where we randomly removed a 3-cm-long apical fragment from each specimen to measure tissue toughness as described above. We kept all specimens under running seawater overnight. On the next day, we returned all specimens to the field and attached 30 of them to a sheltered site (transplants) and the other 30 to the original site (replants, used as procedural controls). On that day, we also collected 60 *F. vesiculosus* specimens from a sheltered site and measured their tissue toughness in the laboratory. On the next day, we transplanted 30 of such specimens to a very exposed site and replanted the other 30 at the original site. In Helgoland, we replicated this experiment starting on 13 February 2010, the only difference being that we collected 40 (instead of 60) specimens from each of an exposed and sheltered site, randomly assigning 20 of each group of 40 specimens to each of the two exposure levels on the following day. We fixed each transplanted and replanted specimen to the substrate by attaching the base of the stipe with a plastic cable tie to an eye-screw bolted into the substrate (the stipe was enclosed in a silicon hose to minimize damage).

After 149 days (Nova Scotia) and 90 days (Helgoland), we randomly removed a 3-cm-long apical fragment from each algal specimen to measure tissue toughness. We evaluated the effects of wave exposure (between-subjects, fixed factor with two levels: sheltered and exposed – Helgoland – or very exposed – Nova Scotia), transplantation type (between-subjects, fixed factor with two levels: replants and transplants) and time (within-subjects, fixed factor with two levels: start and end of experiment) on algal tissue toughness with a three-way repeated-measures ANOVA separately for Nova Scotia and Helgoland.

To test whether the attachment method affected toughness measurements, we measured tissue toughness for replanted and unmanipulated algae at the end of the experiment, using 30 (Nova Scotia) and 10 (Helgoland) specimens for each of the replanted and unmanipulated treatments. We evaluated the effects of the attachment method (fixed factor with two levels: replants and unmanipulated) and wave exposure (fixed factor with two levels: sheltered and exposed – Helgoland – or very exposed – Nova Scotia) on algal tissue toughness with a two-way ANOVA separately for Nova Scotia and Helgoland.

SNAIL FEEDING PREFERENCES (LIVE ALGAE)

To test whether the effects of algal origin on snail consumption depend on snail origin (hypothesis iii), we conducted choice feeding assays with live algae. In Nova Scotia, we collected 25 random *F. vesiculosus* specimens from each exposure level (sheltered, exposed and very exposed) and then removed nine 3-cm-long apical fragments (wet mass = 0.33 ± 0.02 g, mean ± SE) from each specimen on 12 May 2010 (675 fragments in total). In Helgoland, we removed six 3-cm-long apical fragments (wet mass = 0.35 ± 0.19 g) from each of 10 *F. vesiculosus* specimens from each of the two sites for each exposure level (sheltered and exposed) on 16 December 2013 (240 fragments in total). On both collection days, we removed macroscopic epiphytes from the apical fragments with a soft sponge. Then, we stored three (Nova Scotia) or two (Helgoland) apical fragments from each specimen at −80 °C to do feeding assays with reconstituted algal food (explained below). We used the remaining apical fragments (450 in Nova Scotia and 160 in Helgoland) for the feeding assays using live tissues. In Nova Scotia, we filled 150 plastic containers with 100 mL of seawater. In each container, we placed three tagged apical fragments, each one from a different exposure level. Then, we placed a sheltered snail in 25 of the containers, an exposed snail in 25 containers and a very exposed snail in 25 containers. This left 75 containers remaining, which did not contain snails. We paired the snail-free containers with snail-present containers and rearranged their position every 12 h to avoid confounding effects. In Helgoland, we replicated this experiment using a total of 80 containers filled with 800 mL of seawater; each container included an apical fragment from a sheltered alga and one from an exposed alga. We added a sheltered snail to 20 containers and an exposed snail to 20 additional containers. We then used 40 snail-free containers as autogenic controls. We paired snail-free containers with snail-present containers and rearranged their position every 12 h to avoid confounding effects.

We ran the assays at 10 °C with a 12:12 h light:dark cycle and simulated tides by manually alternating 6-h emersion with 6-h submersion periods, using new seawater in every submersion period to avoid accumulation of waste products. We ended the assays when ≥50% of any apical fragment in a container was consumed or after 3 days, whichever came first. At the beginning and end of assays, we measured the wet mass of each apical fragment to the nearest mg

after blotting them dry with paper towels. We calculated consumption (C) for each apical fragment as:

$$C = [S_i(A_f/A_i)] - S_f$$

where S and A were the wet mass of an apical fragment in a snail-present container and its paired autogenic control, respectively, while subscripts $_i$ and $_f$ indicate the initial and final measurement, respectively. We used the same number of autogenic controls and snail-present containers to minimize the risk of a type-I error (Roa 1992). For Nova Scotia, we evaluated the effects of algal origin (within-subjects, fixed factor with three levels: sheltered, exposed and very exposed) and snail origin (between-subjects, fixed factor with three levels: sheltered, exposed and very exposed) on consumption with a two-way repeated-measures ANOVA. For Helgoland, we used the same design except that there were two levels (sheltered and exposed) for both factors.

SNAIL FEEDING PREFERENCES (RECONSTITUTED FOOD)

To test whether feeding preferences in the assays with live algae resulted from differences in algal tissue toughness (hypothesis iv), we ran choice feeding assays using reconstituted food. We only show results from assays run in Nova Scotia because algal origin affected consumption of live algae only there (see Results). First, we individually ground the frozen apical algal fragments described above to a fine powder using a mortar and pestle. Then, we mixed 0.2 g of that powder with 1 mL of distilled water. Afterwards, we mixed that solution with molten agar (43 mg of agar in 1.2 mL of distilled water). After the agar had cooled to 55 °C, we poured this mixture onto a mosquito net (1 mm^2 of mesh size) and flattened it between two PVC panels coated with baking paper. After solidification, we cut two pellets (15 × 15 mm) off the flattened mixture, placed one pellet into a plastic container to which a snail was later added and put the other pellet into a container without snails (autogenic control). It is unlikely that hypothetical volatile substances that could potentially have been released during this procedure affected pellet palatability and data interpretation, because a study showed that *L. obtusata* feeding preferences were very similar between assays using fresh and reconstituted *F. vesiculosus* pieces (Flöthe, Molis & John 2014). In total, we placed together a pellet made from sheltered algae, one made from exposed algae, and one made from very exposed algae in each of 150 containers. Then, we added a sheltered snail in 25 of the containers, an exposed snail in 25 containers and a very exposed snail in 25 containers. This left 75 containers without snails, which we used as autogenic controls as we randomly paired them with containers with snails. We ran this feeding assay under the conditions described for live algal fragments. We evaluated the effects of algal origin (within-subjects, fixed factor with three levels: sheltered, exposed and very exposed) and snail origin (between-subjects, fixed factor with three levels: sheltered, exposed and very exposed) on consumption with a two-way repeated-measures ANOVA.

SNAIL RADULAR TRAITS ACROSS WAVE EXPOSURES

To test whether radular traits reported to affect the feeding efficiency of *L. obtusata* (reviewed in Reid 1996) differ across levels of wave exposure (hypothesis v), we collected 20 snails in Nova Scotia (13–13.5 mm long) from each exposure level (sheltered, exposed and very exposed) and stored them at −20 °C on 22 July 2010. Within 1 month, we removed all radulae and transferred them to a bleaching solution to eliminate soft tissues. We then rinsed the radulae with distilled water, submerged them for 3–4 min in solutions of increasing ethanol concentration (20%, 50%, 75% and 100%) and dried them at room temperature. To prepare the radulae for scanning electron microscopy (SEM), we mounted a fragment of the middle section of each radula with a carbon adhesive tab onto separate SEM stubs (9 mm in diameter) before coating it with gold. We used the middle radular section because teeth are fully developed there, but are still unworn and, thus, optimal for analyses. Using SEM pictures of radular fragments taken from a 30°-tilted top view, we measured with MAGNIFICATION 1.7.1 software (Workers Collection, http://www.workerscollection.com) the length of the central (rachidian) tooth to the nearest 1 μm and the number of cusps in lateral teeth (Fig. 1). For each radula, we calculated both variables as the average from four consecutive rows of teeth. We evaluated the effects of snail origin (fixed factor with three levels: sheltered, exposed and very exposed) and site (random factor with two levels) on each radular trait with two-way nested ANOVAs.

PLASTICITY IN SNAIL RADULAR TRAITS

To test whether consumption of either sheltered or very exposed algae can plastically affect radular traits (hypothesis vi), we randomly collected in Nova Scotia 40 snails (shell length = 13.1 ± 0.2 mm, mean ± SE) and 40 apical algal fragments from each of two sheltered and two very exposed sites (160 snails and 160 algal fragments) on 8 November 2010. Within 3 h of collection, we placed each algal fragment in a separate container filled with 200 mL of seawater. Then, we placed one sheltered snail together with each of 40 sheltered and 40 very exposed algal fragments, and one very exposed snail together with each of the other 40 sheltered and 40 very exposed algal fragments. We ran a no-choice feeding assay for 60 days under the laboratory conditions described for the multiple-choice assays. We replaced the algal fragments weekly by new ones collected from the same exposure level. At the end of the experiment, we prepared the radulae for SEM analysis. We evaluated the effects of snail origin (fixed factor with two levels: sheltered and very exposed), algal origin (fixed factor with two levels: sheltered and very exposed) and site (random factor with two levels) nested within snail origin on each radular trait with two-way nested ANOVAs.

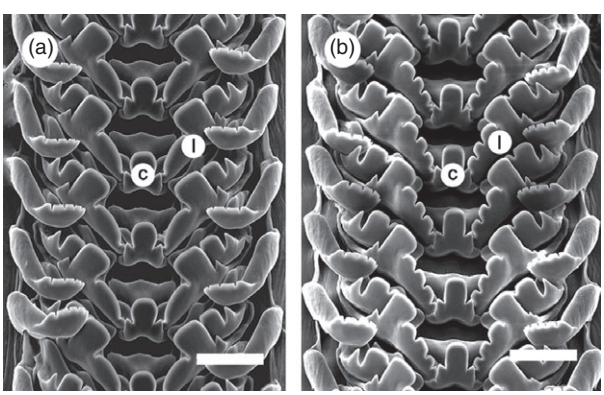

Fig. 1. Thirty degree-tilted top view of the radula of *Littorina obtusata* from (a) sheltered and (b) exposed habitats in Nova Scotia, showing (c) central (rachidian) and (l) lateral teeth. Scale bar = 50 μm.

STATISTICAL ASSUMPTIONS

We confirmed homoscedasticity with Cochran's *C*-tests and normality with normal probability plots. If necessary, data were log-transformed to meet these assumptions. According to Quinn & Keough (2002), we analysed the data from choice feeding assays with a repeated-measures ANOVA, since the sphericity assumption was supported through Mauchley's test. We used Neumann–Keuls tests to compare treatments. Significance level was 0.05 for all tests.

Results

ALGAL TISSUE TOUGHNESS ACROSS WAVE EXPOSURES

In Nova Scotia, algal tissue toughness varied significantly with wave exposure (Table 1). Tissue toughness was significantly higher for very exposed algae than for exposed and sheltered algae (23% and 97% on average, respectively) and significantly higher for exposed algae than for sheltered algae (60% on average). In Helgoland, tissue toughness was significantly higher (46% on average) for exposed algae than for sheltered algae (Table 1).

PLASTICITY IN ALGAL TISSUE TOUGHNESS

For both Nova Scotia and Helgoland, there was a significant 'time × exposure × transplantation type' interaction (Table 2). Tissue toughness of replants did not change during the experiment in either region. However, tissue toughness increased significantly (0.44% per day in Nova Scotia and 0.27% per day in Helgoland, on average) in sheltered algae transplanted to exposed habitats, while it decreased significantly (0.07% per day in Nova Scotia and 0.31% per day in Helgoland, on average) in exposed algae transplanted to sheltered habitats (Fig. 2). For both Nova Scotia and Helgoland, tissue toughness in replants did not change significantly during the experiment (Fig. 2). Furthermore, tissue toughness was not significantly different between replants and unmanipulated algae from the same exposure level (Table S1 in Supporting Information), indicating that the transplant method did not alter tissue toughness.

SNAIL FEEDING PREFERENCES

In Nova Scotia, there was a significant 'snail origin × algal origin' interaction (Table 3). Very exposed and exposed snails consumed similar amounts of algae regardless of algal origin, but sheltered snails consumed significantly less very exposed algae than sheltered and exposed algae (Fig. 3a). Snails from the three exposure levels consumed similar amounts of reconstituted food regardless of the exposure of origin of algae (Table 3, Fig. 3b). In Helgoland, snails consumed algae at similar rates regardless of the exposure level of snails and algae (Table 3).

Table 1. Nested ANOVA results on the effects of wave exposure and site on the tissue toughness of *Fucus vesiculosus* thalli in Nova Scotia and in Helgoland. Elimination of the error term 'Site (Exposure)' and recalculation of pooled residuals (Site (Exposure) + Residual) was done where $\sigma^2_{\text{Site (Exposure)}} = 0$ (not significant at $\alpha \geq 0.25$)

Source of variation	Nova Scotia				Helgoland			
	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
Exposure	2	5.29	26.01	0.013	1	22340	69.75	<0.001
Site (Exposure)	3	0.20	3.91	0.010	2	199.43	0.61	0.545
Residual	174	0.05			56	324.6		
Pooled					58	320.30		

Table 2. ANOVA results on the effects of wave exposure, transplantation type and time on the tissue toughness of *Fucus vesiculosus* thalli in Nova Scotia and Helgoland

Source of variation	Nova Scotia				Helgoland			
	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
Exposure	1	1.51	40.78	<0.001	1	1097	3.21	0.077
Transplantation type	1	0.11	3.02	0.085	1	1024	2.99	0.088
Exposure × Transplantation type	1	8.20	221.7	<0.001	1	10815	31.61	<0.001
Residual	116	0.04			76	342		
Time	1	0.58	27.57	<0.001	1	1022	5.47	0.022
Time × Exposure	1	1.06	50.57	<0.001	1	1626	8.70	0.004
Time × Transplantation type	1	0.11	5.14	0.025	1	0.5	0.01	0.960
Time × Exposure × Transplantation type	1	0.76	36.15	<0.001	1	5120	27.40	<0.001
Residual	116	0.02			76	187		

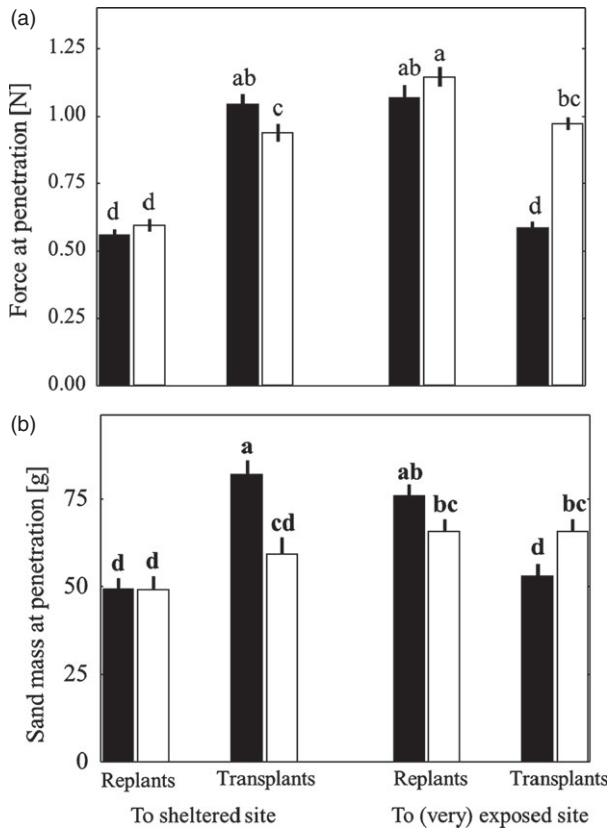

Fig. 2. Tissue toughness of *Fucus vesiculosus* (mean ± SE) at the start (black bars) and end (white bars) of the reciprocal-transplant experiment in (a) Nova Scotia and (b) Helgoland. Treatments sharing the same letter did not differ significantly from one another.

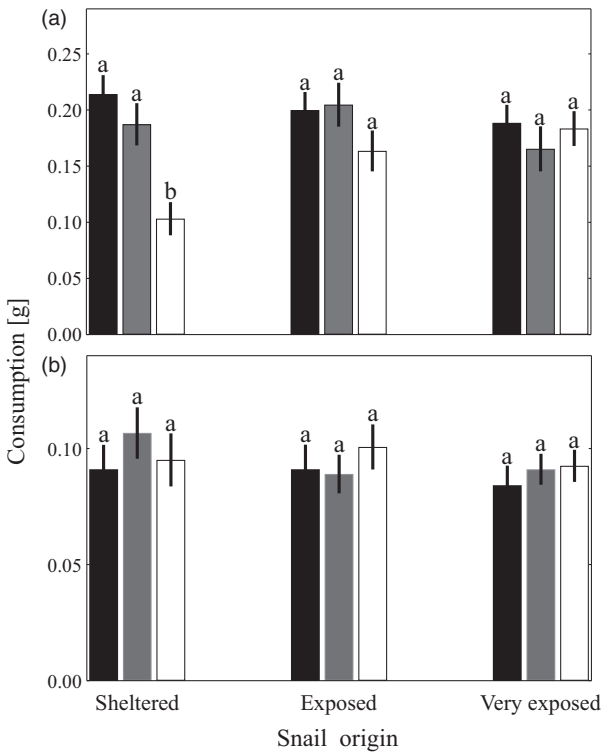

Fig. 3. *Littorina obtusata* consumption (mean ± SE) of (a) live *Fucus vesiculosus* fragments and (b) reconstituted food made of *F. vesiculosus* from a sheltered (black bars), exposed (dark grey bars) and very exposed (white bars) site in Nova Scotia. Treatments sharing the same letter did not differ significantly from one another.

SNAIL RADULAR TRAITS ACROSS WAVE EXPOSURES

The length of central radular teeth differed significantly among exposure levels (Table 4). The central teeth of sheltered snails were significantly shorter than those of exposed and very exposed snails (8% and 17% shorter, on average, respectively), while the central teeth of very exposed snails were significantly longer (10%, on average) than those of exposed snails. The number of cusps in lateral teeth also differed significantly among exposures (Table 4). Exposed snails had significantly more cusps (48%, on average) than sheltered snails, but significantly fewer cusps (17%, on average) than very exposed snails.

PLASTICITY IN SNAIL RADULAR TRAITS

A significant 'snail origin × algal origin' interaction occurred for the two radular traits (Table 5). Both radular traits were statistically unaffected by algal diet in very exposed snails, but in sheltered snails, the values of both traits increased significantly (on average, 15% for the central tooth length and 60% for the number of cusps in lateral teeth) when fed very exposed algae relative to sheltered algae (Fig. 4a,b).

Table 3. Repeated-measures ANOVA results on the effects of snail (*Littorina obtusata*) origin and algal (*Fucus vesiculosus*) origin on the consumption of live algae and reconstituted food in Nova Scotia and Helgoland

	Nova Scotia				Nova Scotia			Helgoland			
	Live algae				Reconstituted food			Live algae			
Source of variation	d.f.	MS	*F*	*P*	MS	*F*	*P*	d.f.	MS	*F*	*P*
Snail origin	2	0.008	2.20	0.118	0.001	0.94	0.395	1	0.00049	1.10	0.301
Residual	72	0.004			0.001			38	0.00045		
Algal origin	2	0.051	5.37	0.006	0.001	0.48	0.623	1	0.00062	0.59	0.448
Snail origin × Algal origin	4	0.025	2.59	0.039	0.001	0.35	0.845	1	0.00166	1.58	0.217
Residual	144	0.009			0.003			38	0.0011		

Table 4. ANOVA results on the effects of wave exposure and site on the length of central teeth and the number of cusps in lateral teeth in snail (*Littorina obtusata*) radulae in Nova Scotia. Elimination of the error term 'Site (Exposure)' and recalculation of pooled residuals (Site (Exposure) + Residual) was done for response variables having $\sigma^2_{\text{Site (Exposure)}} = 0$ (non-significant at $\alpha \geq 0.25$)

	Length of central teeth				Number of cusps in lateral teeth		
Source of variation	d.f.	MS	*F*	*P*	MS	*F*	*P*
Exposure	2	53.42	22.50	<0.001	5.04	36.91	<0.001
Site (Exposure)	3	1.64	0.68	0.568	0.04	0.28	0.836
Residual	54	2.41			0.14		
Pooled	57	2.37			0.14		

Table 5. Nested ANOVA results on the effects of snail (*Littorina obtusata*) origin, algal (*Fucus vesiculosus*) origin and site on the length of central teeth and the number of cusps in lateral teeth in snail radulae in Nova Scotia. Elimination of the error term 'Site (Snail origin) × Algal origin' and recalculation of pooled residuals (Site (Snail origin) × Algal origin + Residual) was done for response variables having $\sigma^2_{\text{Site (Snail origin) × Algal origin}} = 0$ (not significant at $\alpha \geq 0.25$)

	Length of central teeth				Number of cusps in lateral teeth		
Source of variation	d.f.	MS	*F*	*P*	MS	*F*	*P*
Snail origin	1	74.31	4.02	0.183	1.29	1.85	0.307
Algal origin	1	33.39	9.66	0.002	4.95	14.33	<0.001
Snail origin × Algal origin	1	128.0	37.02	<0.001	9.83	28.43	<0.001
Site (Snail origin)	2	18.47	5.34	0.006	0.70	2.02	0.137
Site (Snail origin) × Algal origin	2	2.01	0.58	0.562	0.23	0.67	0.515
Residual	152	3.48			0.35		
Pooled	154	3.46			0.35		

Discussion

As predicted, the tissue toughness of thalli of the seaweed *F. vesiculosus* increased with wave exposure. Also as predicted, such changes were phenotypically plastic. Because similar results occurred in Nova Scotia and Helgoland, two systems ca. 5000 km apart, these conclusions appear to be general for this seaweed. For many algal species, thallus strengthening confers survival advantages in wave-exposed environments (Dudgeon & Johnson 1992; Blanchette, Miner & Gaines 2002; Martone 2007), as strengthening may reduce the risk of detachment or damage of the thallus (Fowler-Walker, Wernberg & Connell 2006). The latter, for instance, may stimulate seaweed consumption (Molis, Enge & Karsten 2010) and may increase infestation rate by pathogens, further increasing the chances of mortality. A high thallus stiffness could be detrimental for seaweed survival (Demes *et al.* 2013), but *F. vesiculosus* thalli were always flexible under the range of tissue toughness reported in this study, suggesting that the observed toughening may indeed benefit this species under exposed conditions. The anatomical basis of the plasticity that we observed remains to be determined, but increasing wave action might lead to a higher carbon allocation to cell walls or to the addition of medullary tissue (Hurd 2000; Fowler-Walker, Wernberg & Connell 2006; Demes *et al.* 2013). Equivalent responses have been observed in vascular plants, which can plastically develop structural reinforcements under increasing wind stress (Read & Stokes 2006).

This study also supports the hypotheses on the feeding preferences of *L. obtusata*. Algal tissue toughness did not influence the feeding preference of exposed snails, as they consumed sheltered and exposed algae in similar amounts in both studied regions. The feeding assays with reconstituted food suggest that nutritional value did not differ between sheltered and exposed *F. vesiculosus*. Potential differences in, for example, C:N ratio between sheltered and exposed *F. vesiculosus* (Long *et al.* 2013) did not affect their palatability for *L. obtusata*. The feeding ability of *L. obtusata* may actually depend more on radular traits (Reid 1996). In this sense, the lack of a feeding preference in exposed snails for sheltered or exposed algae may be explained by their longer central teeth and more abundant cusps in lateral teeth than in sheltered snails. Such radular traits are considered relevant to breaking algal tissues more effectively (Reid 1996; Padilla 2004). Conversely, the lower feeding preference of sheltered *L. obtusata* for very exposed algae than for sheltered and exposed algae reveals a threshold in algal tissue toughness beyond which the feeding efficacy of sheltered snails dropped significantly. This conclusion is supported by the feeding assay with reconstituted food, which eliminated any feeding preferences in sheltered snails. The radular analyses further support this notion, as the two measured traits were less suited to breaking tough algal food in sheltered snails than in specimens from higher exposures. Overall, these results suggest that algal tissue toughness was the proximate factor, and wave exposure the ultimate factor determining the feeding preference of

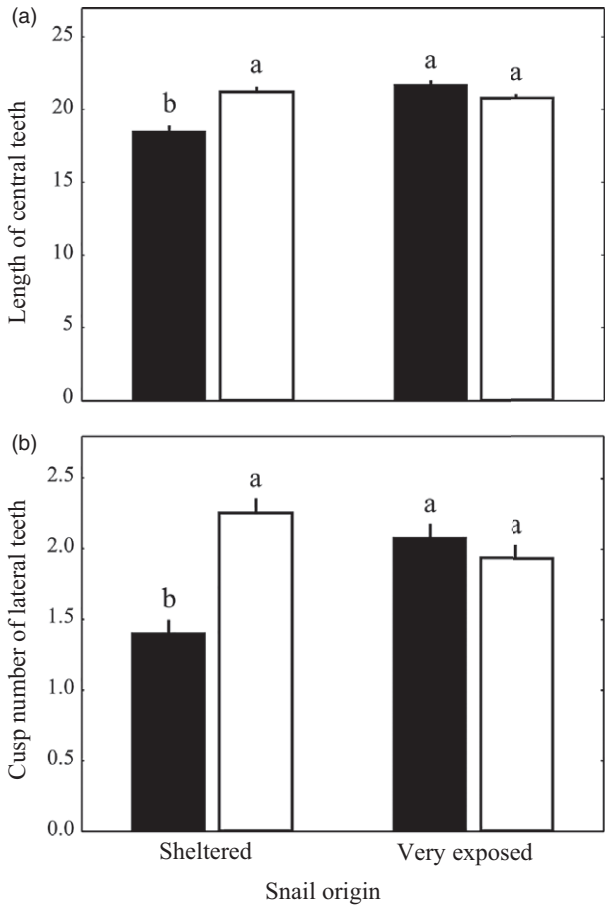

Fig. 4. (a) Length of central teeth (in μm) and (b) number of cusps in lateral teeth (mean + SE) of radulae in *Littorina obtusata* fed *Fucus vesiculosus* from sheltered (black bars) or exposed (white bars) habitats in Nova Scotia. Treatments sharing the same letter did not differ significantly from one another.

sheltered *L. obtusata*. It has been reported before that tissue toughness may confer protection against herbivores for algae (Watson & Norton 1985, 1987; Hurd 2000) and terrestrial plants (Hakes & Cronin 2011; Dimarco, Nice & Fordyce 2012; Ricklefs & Marquis 2012).

Finally, this study also supports the predicted phenotypic plasticity in snail radular traits, as the central teeth were longer and the cusps in lateral teeth were more numerous in sheltered snails fed very exposed algae relative to snails fed sheltered algae. These radular traits were thus modified by the toughness of the food as the teeth were renewed in radulae with time (Isarankura & Runham 1968). This finding also suggests that sheltered snails that may be taken to very exposed habitats by, for example, storms could likely adjust their feeding apparatus to effectively consume the tough algae occurring in such habitats. In this regard, Trussell (2002) has shown that traits related to snail mobility and attachment can also adjust plastically with wave exposure, an important capability to forage effectively on wave-swept shores (Engkvist, Malm & Nilsson 2004).

The seaweed–snail system that we studied shows how an abiotically induced trait change in a resource species can modulate feeding traits in its main consumer to maintain consumption efficacy. Thus, resource species may only be transiently protected against consumption by abiotically induced structural changes, as consumers have the ability to modify their phenotype accordingly. Evidence of bottom-up effects from abiotically induced trait changes is rare in the literature. In general, trait changes in a resource species that plastically modify consumer traits are more commonly known to be biotically induced by the consumers (Utsumi 2011).

Phenotypic plasticity is a fundamental property of organisms that allows them to adjust to changing environments to maintain performance (West-Eberhard 1989; Pigliucci 2005). To the studies that have shown plasticity directly resulting from abiotic pressures (Berg & Ellers 2010; Pfennig *et al.* 2010), this study provides an example of how abiotic variation may indirectly lead to plasticity in a consumer by promoting plastic structural changes in a resource species. The sequential nature by which physical stress directly modulated traits in the resource species and indirectly in the consumer species indicates that the effects of stress at the level of individuals may reduce effects at the level of species interactions. More knowledge on the consequences of phenotypic plasticity on species interactions is needed to better understand how ecological communities may be structured and function in our rapidly changing world.

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Data accessibility

Data are available at http://dx.doi.org/10.1594/PANGAEA.842537.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. ANOVA results on the effects of attachment method (replants and unmanipulated) and wave exposure (sheltered and exposed –Helgoland– or very exposed –Nova Scotia–) on the tissue toughness of *Fucus vesiculosus* thalli.

