Specificity of inducible seaweed anti-herbivory defences depends on identity of macroalgae and herbivores

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ABSTRACT: Meso-herbivores can strongly affect biomass accrual and macrophytobenthic community structure. To counter grazing effects, constitutive and induced anti-herbivory responses evolved in seaweeds. Feeding-assayed induction experiments were conducted in the laboratory to investigate whether 14 d grazing periods by either the periwinkle *Littorina brevicula* or the abalone *Haliotis discus* induced anti-herbivory defences in the green alga *Ulva pertusa* and the brown alga *Laminaria japonica*. Where appropriate, assays were repeated with artificial food containing lipophilic algal extracts, using dichloromethane (DCM) as a solvent, to test whether or not snails induced chemical defences. While exposure to periwinkles reduced palatability of fresh *L. japonica* pieces for naïve conspecific grazers, no such effect was displayed by abalone-exposed *L. japonica* pieces. Patterns of grazer specificity in the palatability of fresh algae were not confirmed when using artificial food containing algal DCM extracts. This suggests that *L. japonica* either induced morphological defences or periwinkle-deterrent substances originated from the non-lipophilic fraction of defensive chemicals. Grazer-specific induced responses were not apparent in *U. pertusa* assays. This study revealed that the induction of chemical anti-herbivory responses depends not only on the type of grazer but also on the type of alga on which the grazer feeds.

KEY WORDS: Plant−animal interaction · Consumption · Macroalgae · Chemical extracts · Republic of Korea

INTRODUCTION

Herbivores strongly affect biomass accrual of terrestrial and marine plants (Cyr & Pace 1993) and were experimentally shown to modify the directionality of biodiversity–productivity relationships in marine habitats (Worm et al. 1999, 2002, Hillebrand et al. 2000). Past research on benthic trophic interactions focused on the effects of macro-herbivores (e.g. fishes and urchins) on macroalgae, while knowledge on the ecological effect of the inconspicuous meso-herbivores (e.g. snails, isopods, and amphipods) has only recently emerged (Hay 1996, Duffy & Hay 2000, Toth et al. 2005, Long et al. 2007, Toth & Pavia 2007). In contrast to larger grazers, meso-herbivores use macroalgae as habitat as well as for food (Duffy & Hay 1991), suggesting persistent interactions between meso-herbivores and their prey. In this context, phenotypic plasticity in seaweed responses may represent an important trait in facilitating longer-lasting stable interactions, as macroalgae may tolerate low consumption levels but induce anti-herbivory defences to protect themselves under higher grazing pressure (Karban et al. 1999).

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In contrast to terrestrial systems, where inducible anti-herbivory defences were documented in >100 plants (Karban & Baldwin 1997), this response was detected in <20 seaweed species (Toth & Pavia 2007). The discrepancy between these 2 systems may result from a research bias. For instance, Toth & Pavia (2007) documented an exponential increase in the number of macroalgae with inducible defences over the last decade, indicating that these may be more commonly employed in seaweeds than previously suggested. Utilisation of induced defences represents for several reasons a selective advantage for its user over constitutively expressed defences. First, under the assumption that the production of anti-herbivory defences incurs a metabolic cost, permanent expression of anti-herbivory defences is detrimental under variable grazing regimes because resources were also allocated to defences at times when they were not needed (Agrawal & Karban 1999). Second, inducible anti-herbivory defences may lower the risk of self-toxicity as harmful compounds were only intermittently present in plant tissues (Agrawal & Karban 1999). Third, temporarily expressed defences can enhance algal performance by increasing feeding dispersal and reducing average meal size of meso-herbivores (Borell et al. 2004). Less predictable food quality due to inducible defences may be an important driver in the evolution of plant–animal interactions (Hay 1996).

Induction of anti-herbivory defences in macroalgae may depend on the identity of meso-grazers (Amsler 2001). Enzymes from the saliva of the periwinkle *Littorina obtusata* induced anti-herbivory responses in the fucoid *Ascophyllum nodosum* (Coleman et al. 2007). Such grazer-associated cues may trigger the evolution of grazer specificity in seaweed responses, but experimental evidence for macroalgae is extremely scarce and controversial. *L. obtusata*, but not the isopod *Idotea granulosa*, induced anti-herbivory defences in a European population of the rockweed *A. nodosum* (Pavia & Toth 2000), but neither induced anti-herbivory defences in a North American population of *A. nodosum* (Long & Trussell 2007). Moreover, since the isopod *Idotea baltica* and the periwinkle *Littorina littorea* both induced defences in the brown seaweed *Fucus vesiculosus* (Rohde et al. 2004), different types of feeding modes (radular vs. mandible) cannot fully explain grazer-specific plasticity in seaweed responses. This assumption was indirectly corroborated by Molis et al. (2006), showing grazer specificity in the induction of anti-herbivory responses in the brown seaweed *Ecklonia cava* for 2 grazer species with similar feeding modes: consumption by the periwinkle *Littorina brevicula*, but not by the abalone *Haliotis discus*, reduced the palatability of *Ecklonia cava*. To date, grazer specificity in the induction of anti-herbivory

defences has only been reported for different grazer species on single species of macroalgae. Testing for constancy in grazer-specific patterns among different seaweed species will give more insight into generality and relevance of grazer identity as a driver of trophic interactions between macroalgae and meso-herbivores. The present study features the first formal test on constancy in grazer specificity of induced seaweed responses by asking (1) whether meso-herbivores with similar feeding modes differ in their ability to induce anti-herbivory defences in different seaweed species, (2) whether induced algal responses originate from lipophilic algal substances, and (3) whether induced macroalgae can reduce defences after grazing has ceased.

MATERIALS AND METHODS

Study organisms. *Laminaria japonica* Areschoug was introduced to South Korea in the late 1970s. Because this alga does not recruit at Sangju Bay, i.e. the site where grazers were collected, algal specimens used in this study originated from aquaculture facilities at Sangju (34° 42' N, 127° 59' W) and Wan Island (34° 10' N, 126° 37' W). However, *Littorina brevicula* commonly co-occurs with *L. japonica* at other sites along the Korean shore (Son & Hong 1998). The green macroalga *Ulva pertusa* Kjellman is highly abundant, accumulating in massive mats during summer months on the shore at Sangju, from where it was collected. Pilot studies revealed that 2 snails, the periwinkle *L. brevicula* Philippi and the abalone *Haliotis discus* Hannai Ino, readily consumed *L. japonica* and *U. pertusa*. Both *U. pertusa* and periwinkles of 8 to 12 mm diameter were collected in the upper sublittoral at Sangju Bay. Due to intense harvesting, abalone is rare in the field, so 1 yr old 30 mm long individuals were obtained from the aquaculture facilities of the National Fisheries Research and Development Institute (NFRDI).

Experimental design and setup. Consumer identity (*Littorina brevicula* and *Haliotis discus*) and consumption (grazed and ungrazed control) were manipulated in a 2-factorial design to test the ability of grazers to induce anti-herbivory defences in each seaweed species.

An indoor experiment was conducted at the NFRDI, Sangju Bay. Twenty-eight transparent plastic aquaria (vol. 2 l) were placed in each of 2 adjacent (<1 m distance) containers (2.5 × 1.5 × 0.5 m). In each container, *Laminaria japonica* and *Ulva pertusa* pieces were randomly allocated to 14 aquaria each (experimental units = EUs) (Fig. 1A). In the first container, 7 of the 14 EUs containing either of the algal species were ran-
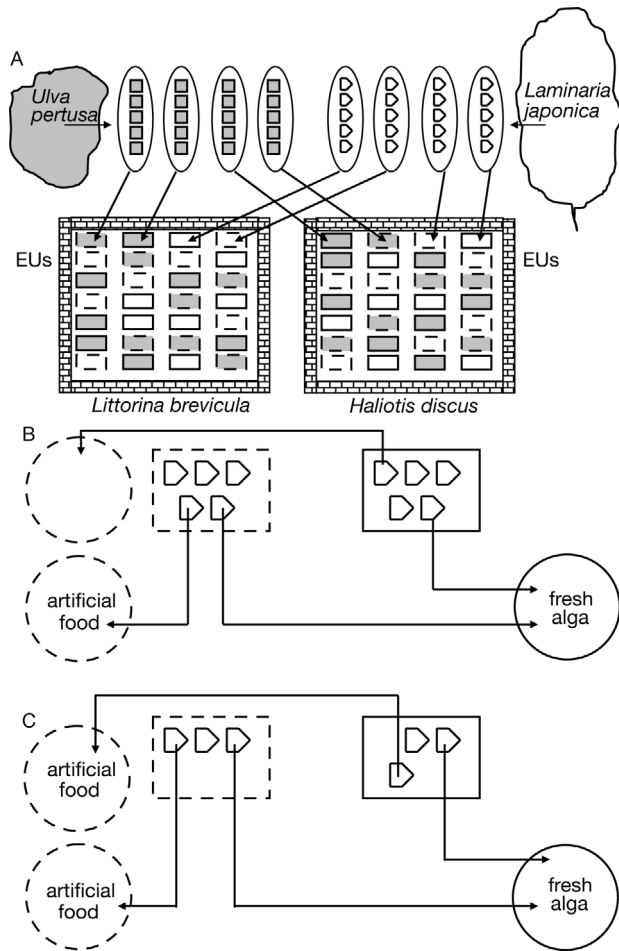
Fig. 1. Experimental setup. (A) Allocation of 20 algal pieces cut from a single specimen of each species of macroalgae *Ulva pertusa* and *Laminaria japonica* to experimental units (EUs) in both containers (bricked bars) at the beginning of the experiment. Ovals group algal pieces that were allocated to single EUs. For clarity, allocation of algal pieces to EUs is shown for only 1 of the 7 specimens of each algal species. (B) Principle allocation pattern of algal pieces (only shown for *L. japonica* pieces and 1 grazer species) for choice-feeding assays using fresh algae or no-choice feeding assays using artificial food at the end of the treatment phase. One of the 3 remaining fresh algal pieces in EUs was used as autogenic control to assess non-feeding related changes in wet mass at the end of the treatment phase. (C) Allocation of algal pieces for feeding assays at the end of the recovery phase. The remaining piece in EUs was used as autogenic control. Use of different algal pieces after the treatment and recovery phase kept data from different phases independent. EUs with solid line = control, EUs with stippled line = with grazers during treatment phase, large solid and stippled circle = feeding arena with choice and no-choice assays, respectively

domly incubated with periwinkles, while the remaining EUs were used as controls. This setup was repeated in the second container, but using *Haliotis discus*. The non-random arrangement of consumer

species in this setup could have affected the feeding behaviour of both snail species. To minimise this possibility, we controlled for temperature, light regime, and water flow, and kept these identical in both containers. The similarity of abiotic conditions in both containers was also reflected by a nonsignificant (<1%) difference in growth rates of control algae from both containers (t-test — *Laminaria*: $t = 0.37$, $p = 0.713$; *Ulva*: $t = 0.68$, $p = 0.519$). Filtered (1 µm, Micropore) seawater from Sangju Bay flowed unidirectionally (40 ml min^{-1}) at a temperature of 18 to 20°C through single EUs. Aerated EUs were exposed to ambient irradiance regimes matching nearly ambient photosynthetically active radiation (PAR) intensity at 1 m water depth (at bottom of aquaria: 85.02 ± 1.8 µmol s^{-1} m^{-2} [mean ± SD], at 1 m depth in Sangju Bay: 94.98 ± 3.9 µmol s^{-1} m^{-2}; Li-Cor underwater quantum sensor LI-192SA).

On 16 June 2003, 7 *Laminaria japonica* and 7 *Ulva pertusa* specimens were collected, their macroscopic epibionts removed with a soft sponge, and the algae spun dry in a salad spinner. The same day, 20 pieces were cut from each specimen and evenly allocated to 4 EUs (Fig. 1A). In this way, 5 genetically identical plant pieces (wet mass 3.0 ± 0.5 g) were placed in each of 2 EUs of each container (5 pieces per EU × 2 grazing treatments × 2 grazer species = 20). Multiple plant pieces per aquarium were necessary as multiple feeding assays were performed at different times during the 42 d experiment. Subsequently, plant pieces were cultivated for 14 d without consumers in the EUs, to allow adaptation to experimental conditions (acclimation phase). Afterwards, half of the EUs with *L. japonica* and with *U. pertusa* in each container were randomly selected and incubated for 14 d with 10 *Littorina brevicula* or 3 *Haliotis discus* specimens (induction treatment), respectively (Fig. 1A). The second half of the aquaria was left without herbivores (control). At the end of the treatment phase, 2 plant pieces were used from each EU in feeding assays (Fig. 1B) and all grazers were removed. The following recovery phase (14 d) was intended to test for reduction of induced defences after grazing ceased. At the end of the recovery phase, 2 of the remaining 3 algal pieces from each EU were used in feeding assays (Fig. 1C).

Feeding assays. To assess algal palatability at the end of the treatment phase, 1 fresh algal piece was withdrawn from a control EU and offered together with a genetically identical piece withdrawn from a treatment EU to naïve conspecific snails in 72 h choice-feeding assays (Fig. 1B, *Laminaria japonica*: n = 7; *Ulva pertusa*: n = 5, reduction in replication due to 2 unhealthy algae). Algal pieces were discarded after feeding assays. When significant effects with fresh algae were detected, non-polar compounds were extracted from the second withdrawn algal

piece of each EU, incorporated into artificial food (see 'Preparation of agar-based food'), and used in no-choice feeding assays (Fig. 1B). The procedure was repeated with different algal pieces withdrawn from EUs after the recovery phase (Fig. 1C). All feeding assays were performed with 10 *Littorina brevicula* or 3 *Haliotis discus*, contained within 300 ml seawater in feeding arenas made of transparent plastic Petri dishes (∅ 15 cm, 3 cm height). Feeding assays were run at the NFRDI in a constant-temperature room set to 19°C. Water in feeding arenas was exchanged every 12 h. No-choice assays with artificial food items were necessary to allocate non-grazing-related dislodgement of agar pieces unambiguously to original food items. To obtain the total mass of artificial feed at the end of feeding assays, seawater containing dislodged food parts was poured onto paper filters, retained food weighed to the nearest mg, and this mass added to that of the food item remaining in the feeding arena at the end of the feeding assay. Consumption was measured to determine algal palatability by weighing food items at the beginning and end of feeding assays. Additional pieces of fresh and artificial food were transferred into feeding arenas without grazers to assess autogenic mass changes during feeding assays (Peterson & Renaud 1989). Autogenic control and assayed alga stemmed from the same EU, i.e. they had identical experimental grazing histories and genomes. Actual consumption during feeding assays was calculated as:

$$F_b \times (C_e \times C_b^{-1}) - F_e$$

where F and C indicate wet mass of the food item and the autogenic control, respectively, and the subscripts b and e indicate measurements at the beginning and end of feeding assays, respectively.

Preparation of agar-based food. Non-polar crude extracts were obtained by submerging individual algal pieces in 10 ml dichloromethane (DCM). After a 24 h extraction period at room temperature, extracts were poured onto 3 g of powdered *Ulva lactuca* to standardise artificial feed quality (Deal et al. 2003). During the next 12 h, evaporation of DCM was accomplished at room temperature under a hood, prior to embedding extract-coated *Ulva lactuca* into agar. Two feeds were processed from single algal pieces, according to the methods of Hay et al. (1994) and were used as the assayed food and its autogenic control.

Statistical analysis. Consumption rates of fresh algae (choice assays) after treatment and recovery phases were separately analysed for each phase and algal species by 2-factorial repeated-measures ANOVA with consumption of treated versus control piece from 1 feeding assay as the repeated measure (2 levels, fixed) and consumer species as the grouping factor

(2 levels, fixed). Thus, algal pieces that were used after the treatment and recovery phase lacked independence as they originated from the same EU. Because there were only 2 treatments, testing for sphericity was not relevant (Quinn & Keough 2002). Due to ambiguous selection of an appropriate error term for post-hoc tests involving within-subject by between-group interactions (Winer et al. 1991), 1-tailed paired *t*-tests were performed as alternative post-hoc tests for each grazer species separately, after confirming normality of differences by the Kolmogorov-Smirnov test. Consumption rates of artificial food (no-choice assays) after treatment and recovery phases were separately analysed for each phase and algal species by a 2 × 2-factorial ANOVA with grazer species (2 levels, fixed) and consumption (2 levels, fixed) as main factors. Homogeneity of variances was tested with Levene's test and, where appropriate, data were log-transformed to meet the assumptions. Tukey's HSD was used as a post-hoc test.

RESULTS

Fresh algae

Laminaria japonica

Non-feeding related changes in *Laminaria japonica* wet mass indicated minimal biomass losses of 1.5 ± 0.023% (mean ± SD) during the entire experiment, with highest and lowest reductions during the acclimation phase (3 ± 0.034%) and recovery phase (0.1 ± 0.003%), respectively.

At the end of the treatment phase, changes in *Laminaria japonica* consumption following snail exposure were grazer-specific, as indicated by a significant consumer × grazing interaction (Table 1). Exposure to grazers reduced consumption rates on *L. japonica* relative to controls significantly when *Littorina brevicula* was used (paired *t*-test: $t_6 = 4.21$, p = 0.006) but not with *Haliotis discus* (paired *t*-test: $t_6 = 1.79$, p = 0.125). Control *L. japonica* were consumed on average 5.7 times more heavily than *L. brevicula*-exposed conspecifics (Fig. 2A). This effect was strong enough to produce a significant overall consumption effect (Table 1), in which control specimens of *L. japonica* were consumed on average (when pooled from *L. brevicula* and *H. discus* assays) 2-fold more than grazed conspecifics (Fig. 2A).

At the end of the recovery phase, no significant differences in consumption were detected (Table 1). However, in *Haliotis discus* assays, control algae were a non-significant 6 times more preferred than *H. discus*-exposed *Laminaria japonica* (paired *t*-test: $t_6 =$

1.65, p = 0.075), while mean difference in consumption between control specimens and *Littorina brevicula*-exposed *L. japonica* was < 20% (Fig. 2B).

Ulva pertusa

A non-significant consumer × grazing interaction in assays with fresh *Ulva pertusa* specimens indicated that the amount of consumed *U. pertusa* biomass was independent of grazer identity (Table 1). In contrast to the results with *Laminaria japonica*, *Littorina brevicula*-exposed *U. pertusa* pieces tended to stimulate consumption of *U. pertusa* by naïve conspecific snails (Fig. 3). Interestingly, the negative consumption values of *Haliotis discus*-grazed *U. pertusa* pieces indicate that *U. pertusa* pieces were gaining more biomass in the presence of grazers than did conspecifics in grazer-free EUs. Due to an absence of induction of anti-herbivory defences in *U. pertusa*, no assays were conducted at the end of its recovery phase.

Algal extracts
(only *Laminaria japonica*)

At the end of the treatment phase, consumption of agar-based food containing non-polar *Laminaria japonica* extracts (Fig. 4A,B) was on average more than 5 times higher than in assays using fresh algae (Fig. 2A,B). At the end of the treatment phase, no significant main or interactive effects were detected (Table 2), although trends between assays using *L.*
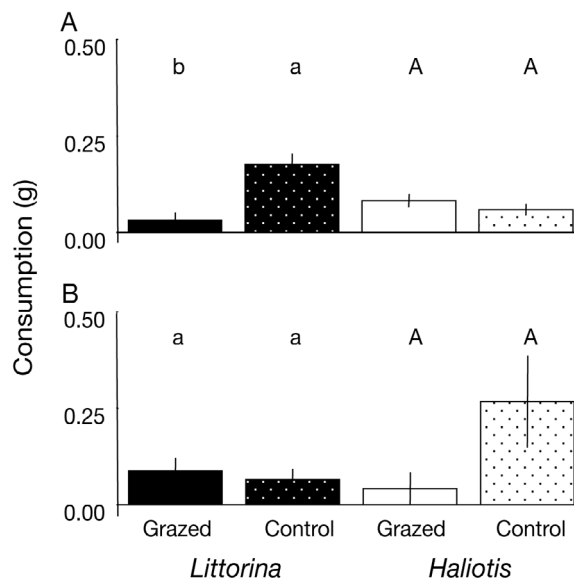

Fig. 2. Fresh *Laminaria japonica*. Mean (±SE) consumption of periwinkles *Littorina brevicula* and abalone *Haliotis discus* determined in 72 h choice-feeding assays at the end of the (A) treatment and (B) recovery phase. Treatments sharing lowercase (*L. brevicula* assays) or uppercase (*H. discus* assays) letters are not significantly different, as tested with paired *t*-tests (n = 7). Grazed: snail exposure during the treatment phase; Control: absence of snails during the treatment phase

japonica extracts and fresh conspecifics were remarkably similar (Figs. 2A vs. 4A & 2B vs. 4B).

At the end of the recovery phase, no significant differences were detected by the overall analysis (Table 2). Consumption rates on artificial food from control plants were almost identical in magnitude between the treatment and the recovery phase, indicating that the palatability of non-polar extracts of control specimens of *Laminaria japonica* was nearly constant over the course of the experiment.

DISCUSSION

The present study revealed that grazing by meso-herbivores changed the level of palatability in algae but that induced responses were grazer-specific and also dependent on the type of alga. Missing effects in assays using non-polar *Laminaria japonica* extracts suggest that the grazer-induced regulation of palatability in this seaweed species was not triggered by lipophilic compounds.
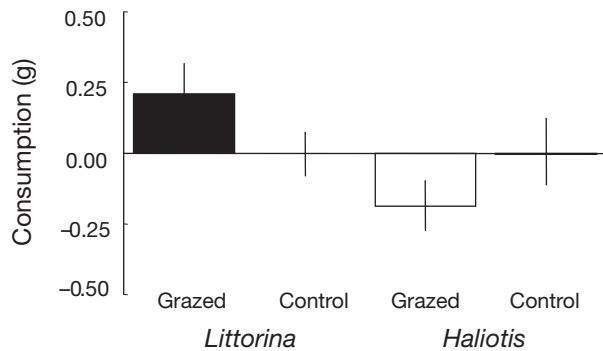
Table 1. Fresh *Laminaria japonica* (n = 7) and *Ulva pertusa* (n = 5). Results of repeated-measures ANOVA, comparing the effects of herbivore species (*Littorina brevicula* and *Haliotis discus*) and grazing (exposed to grazers and controls) on consumption of intact seaweeds immediately (treatment phase) and 14 d after grazing ceased (recovery phase, only *L. japonica*). Consumption was assessed during 72 h choice-feeding assays using either 10 or 3 individuals of *L. brevicula* or *H. discus*, respectively

Source	— *Laminaria japonica* —				— *Ulva pertusa* —			
	df	MS	F	p	df	MS	F	p
Treatment phase								
Consumer (C)	1	0.008	2.09	0.174	1	0.199	5.06	0.055
Residual	12	0.004			8	0.039		
Grazing (G)	1	0.026	10.87	0.006	1	0.001	0.02	0.900
C × G	1	0.050	20.90	0.001	1	0.191	2.82	0.132
Residual	12	0.002			8	0.068		
Recovery phase								
Consumer (C)	1	0.042	1.69	0.219				
Residual	12	0.025						
Grazing (G)	1	0.073	1.99	0.184				
C × G	1	0.106	2.90	0.115				
Residual	12	0.037						

Fig. 3. Fresh *Ulva pertusa*. Mean (±SE) consumption of periwinkles *Littorina brevicula* and abalone *Haliotis discus* at the end of the treatment phase (n = 5). Assay conditions, analysis, symbols, and their interpretation as in Fig. 2
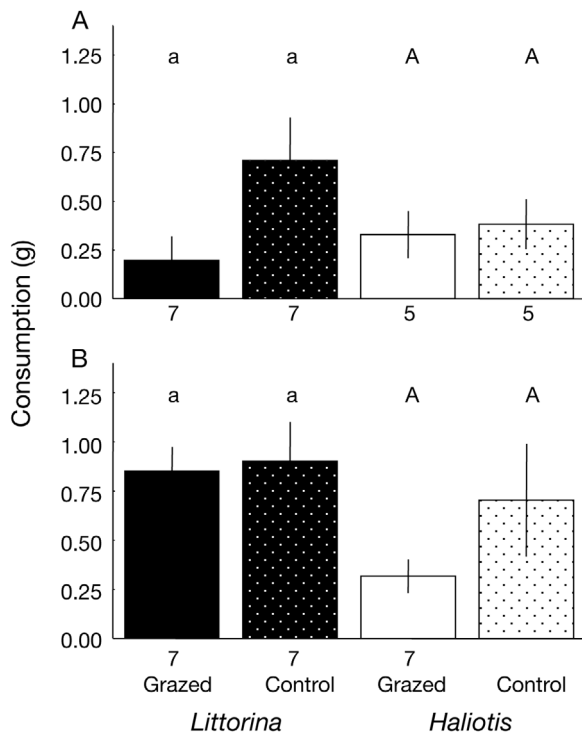
Table 2. *Laminaria japonica*. Artificial food containing nonpolar extracts of *L. japonica*. Results of 2×2 ANOVA, comparing the effects of the herbivores *Littorina brevicula* (n = 7) and *Haliotis discus* (n = 5 and 7 after treatment and recovery phase, respectively) and grazing (exposed to grazers and controls) on consumption of agar-based food containing nonpolar extracts of *L. japonica* immediately (treatment phase) and 14 d after exposure to grazers ceased (recovery phase). Consumption was assessed during 72 h no-choice feeding assays using either 10 or 3 individuals of *L. brevicula* or *H. discus*, respectively

Source	df	MS	F	p
Treatment phase				
Consumer (C)	1	0.057	0.35	0.562
Grazing (G)	1	0.469	2.88	0.106
C × G	1	0.307	1.88	0.185
Residual	20	0.163		
Recovery phase				
Consumer (C)	1	0.944	3.75	0.065
Grazing (G)	1	0.334	1.33	0.260
C × G	1	0.199	0.79	0.383
Residual	24	0.251		

Fig. 4. *Laminaria japonica*. Agar-based food containing nonpolar *L. japonica* extracts. Mean (±SE) consumption of periwinkles *Littorina brevicula* and abalone *Haliotis discus* determined in 72 h no-choice feeding assays at the end of the (A) treatment and (B) recovery phase. Numbers under bars indicate sample sizes. Treatments sharing lowercase (*L. brevicula* assays) or uppercase (*H. discus* assays) letters are not significantly different, as tested with 2×2 ANOVA

Induction of chemical anti-herbivory defences

The observed lower palatability of previously *Littorina brevicula*-attacked fresh *Laminaria japonica* pieces compared to ungrazed (control) conspecifics, and the disappearance of differences in palatability after grazing ceased, may result from either preferential feeding of littorinid snails or, alternatively, from an induction of anti-herbivory defences. Preferential consumption should have removed the most palatable *L. japonica* tissues during the treatment phase in grazer-exposed but not in control EUs. Consequently, control pieces would have been more consumed than simultaneously offered grazer-exposed *L. japonica* pieces. In the same sense, diminishing differences in palatability between control and periwinkle-exposed *L. japonica* pieces after grazing ceased, i.e. at the end of the recovery phase, could be explained by new growth of palatable tissues in previously grazed algae during recovery. However, wet mass measurements of autogenic control pieces failed to show such a growth response. The detected slight decrease in *L. japonica* biomass during the recovery phase indicates that preferential consumption cannot explain observed changes in *L. japonica* palatability during the experiment. Alternatively, the observed changes in palatability could have resulted from an induction of anti-herbivory defences. This conclusion is supported by the fact that littorinid snails consumed <15% of *L. japonica* biomass during the treatment phase. This value corresponds well with other observations of the effect of meso-grazing on algal biomass (Klumpp & Pulfrich 1989) and suggests that sufficient palatable tissue was available to meso-grazers throughout feeding assays after the treatment and recovery phase. Furthermore, feeding scars were scattered over the entire area of the *L. japonica* pieces. Preferential consumption, however, would more likely result in localised

rather than dispersed feeding activities across the algal thallus. Finally, feeding assays using agar-based feed of lipophilic *L. japonica* extracts produced similar patterns as detected with fresh algae. However, missing main and interactive effects between grazing treatments and consumer species indicate that grazing did not induce the production of repulsive lipophilic substances in either of the 2 tested species of macroalgae. To our knowledge, no study has characterised and identified induced chemicals that deter herbivores, while a few experiments have tested grazer deterrent effects of crude lipophilic algal extracts (Weidner et al. 2004, Ceh et al. 2005, Macaya et al. 2005, Rothäusler et al. 2005, Diaz et al. 2006). Only in the brown alga *Sargassum asperifolium* was an observed induction of anti-herbivory defence confirmed when lipophilic extracts were used (Ceh et al. 2005). Thus, induced defences in macroalgae most likely originate from either the non-lipophilic fraction of macroalgal secondary substances or from morphological changes (Van Alstyne 1989).

Grazer specificity of induced anti-herbivory responses

Grazing periwinkles, but not abalone, induced anti-herbivory defences in *Laminaria japonica*. The absence of an induced response by *Haliotis discus* possibly resulted from its higher tolerance against grazing-deterrent compounds compared to *Littorina brevicula*. Alternatively, anti-herbivory defences against *H. discus* grazing were employed constitutively. To obtain a more complete picture of the potential inductive role of abalone, abalone-exposed macroalgae should be offered in future experiments to *L. brevicula*, similar to the experiments conducted by Long et al. (2007). Missing effects on *L. brevicula* grazing in these assays would give additional evidence that abalone may not induce anti-herbivory defences.

Existing knowledge on grazer-specific induced anti-herbivory defences in macroalgae is extremely scarce. Pavia & Toth (2000) reported induced anti-herbivore defences in *Ascophyllum nodosum* against grazing periwinkles *Littorina obtusata* but not against the isopod *Idotea granulosa*. The authors suggested that differences in grazing damage may put *L. obtusata*-exposed *A. nodosum* individuals at a higher risk of losing more biomass than *I. granulosa*-grazed conspecifics, due to the more localised feeding mode of periwinkles, perhaps due to the slow movement of snails. Thus, for macroalgae, snail-associated traits could be highly predictable cues to strong grazing effect. If radular grazing caused different mechanical cues than mandible grazing, *A. nodosum* could possibly distinguish between snails and isopods, and thus determine the potential risk of losing biomass. Under this assumption, macroalgae should display similar anti-herbivory responses when exposed to grazers with similar feeding modes, which is clearly not the case in the present study. However, little is known about the ecological consequences of different radular morphologies (Padilla 2004). Differences in radular morphology between *Littorina brevicula* and *Haliotis discus* were possibly large enough to provoke a differential defensive response in *Laminaria japonica*. Alternatively, the induction of anti-herbivory defences in algae may not be cued by mechanical stimuli of the feeding apparatus alone. This possibility is corroborated by Rohde et al. (2004), who showed that 2 grazer species with different feeding apparatus both induced anti-herbivory defences in the fucoid *Fucus vesiculosus*. Moreover, simulated grazing (clipping treatments) did not induce anti-herbivory defences in *F. vesiculosus* (Rohde et al. 2004), demonstrating the ineffectiveness of solely mechanically damaged macroalgal tissues to induce anti-herbivory defences. Coleman et al. (2007) gave the first experimental evidence that chemical grazer cues induced anti-herbivory defences. Thus, a combination of mechanical and chemical cues might be required to induce anti-herbivory defences in *L. japonica*. Combining feeding-related mechanical and chemical grazing cues may be one way of matching deterrent responses with actual herbivore load and thus optimising defences. For instance, mechanical cues alone bear a risk of confounding non-grazing-related biomass loss in wave-battered seaweeds with attacking herbivores (Amsler 2001). Future experiments aiming to tease apart the role of separate or combined mechanical and chemical grazer cues may be important for a better understanding of grazer specificity in induced seaweed responses. Preferably, these experiments should also be conducted in the field to include additional environmental factors that may affect the outcome of species interactions.

Grazer specificity depends on seaweed identity

Grazer specificity of induced chemical anti-herbivory defences could be documented in *Laminaria japonica*, but not in *Ulva pertusa*. This suggests that besides grazer identity, seaweed-specific traits also determine whether or not repulsive responses are induced. Grazing by abalone stimulated growth in *U. pertusa*. Therefore, *U. pertusa* used an opposite mechanism to cope with herbivory than *L. japonica*. The latter reduced the effect of herbivores, while *U. pertusa* showed compensatory biomass accrual. However, Toth (2007) reported the presence of

inducible anti-herbivory defences in *Ulva lactuca* after exposure to the isopod *Idotea granulosa*. Consequently, different life history traits among *Laminaria* and *Ulva* may explain opposing responses to herbivory only to a certain extent. Tolerance to herbivory has evolved as one survival strategy in macroalgae with sheet-like thallus forms (Littler & Litter 1980), e.g. *Ulva*. Besides thallus structure, growth has been suggested as an additional algal trait for differential responses to herbivores (Steneck & Dethier 1994). Fast-growing macroalgae, e.g. *Ulva* spp., have been reported to escape consumption, while slower-growing seaweeds, e.g. *L. japonica* in the present study, may optimise their fitness by deterring grazers (Cronin 2001).

Molis et al. (2006) documented grazer-specific inducible anti-herbivory responses in the brown seaweed *Ecklonia cava* that matched those shown in the present study for *Laminaria japonica*. The consistency of patterns in both kelp species compared to the contrasting pattern in *Ulva pertusa* shows that grazer specificity in macroalgal defences may be linked to seaweed taxonomy. Evaluating grazer specificity in macroalgae over a range from closely to remotely related species of macroalgae may improve the predictability of grazer specificity in induced responses and may allow more insight into the variability of algal traits that have potential ecological implications on the structure of food chains (Agrawal 2001, Long et al. 2007) and the level of herbivore fitness (Toth et al. 2005).

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