



Invasion success of the seaweed *Gracilaria vermiculophylla* correlates with low palatability

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ABSTRACT: Differences with respect to anti-herbivore defense were investigated in invasive and native populations of the seaweed *Gracilaria vermiculophylla*. Specimens from 6 native populations in East Asia and from 8 populations invasive in Europe and the Mexican Pacific coast were maintained under identical conditions and offered to herbivorous snails from both the native range (*Littorina brevicula*) and Europe (*L. littorea*) in no-choice feeding assays. *L. brevicula* consumed in total significantly larger amounts of *G. vermiculophylla* tissue than did *L. littorea*. Further, both snail species least consumed the seaweed specimens originating from either non-native populations or from populations native to the Korean East Sea/Sea of Japan. The Korean East Sea/Sea of Japan had previously been identified as putative donor region of all the invasive populations of *G. vermiculophylla*. Thus, populations in the donor region as well as non-native populations in different invaded realms feature an increased capacity to resist feeding pressure. Differences in nutrient content did not account for the observed patterns of consumption, as palatability and carbon to nitrogen (C:N) ratio were not significantly correlated. Thus, mechanical or chemical defenses or the content of feeding cues influenced the behavior of the snails. We suggest that low palatability contributed to the invasion success of the species.

KEY WORDS: Biological invasion · Invasive seaweeds · Enemy release hypothesis · Novel weapons hypothesis · Herbivory · *Gracilaria vermiculophylla*

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INTRODUCTION

For terrestrial plants and also for seaweeds (Nyberg & Wallentinus 2005), grazing defense is thought to be an important biological trait that determines invasiveness. High levels of novel phytochemicals (so called 'novel weapons') that native plants, grazers, or pathogens have not previously encountered have often been predicted to favor introduced plants in new environments (Callaway & Ridenour 2004, Cappuccino & Carpenter 2005, Cappuccino & Arnason 2006, Forslund et al. 2010). Correspondingly, pres-

ence of generalist enemies in new environments might result in a selection of defensive traits in introduced plants (Blossey & Notzold 1995, Callaway & Ridenour 2004). In contrast, the 'enemy release' hypothesis (Keane & Crawley 2002) attributes the success of introduced plants to a lack of specialist enemies in the invaders' new ranges. Under such conditions, invasive plants might lose their protection against specialist herbivores, allowing a shift towards higher protection against generalist herbivores (Joshi & Vrieling 2005). However, in contrast to terrestrial plants, introduced seaweeds are often not

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released from grazers, as significantly more herbivores in the sea than on land are generalists (Hay & Steinberg 1992).

Several studies have quantified grazing on invasive seaweed species in their new range, and in most cases, grazers from the introduced range preferred native over non-native seaweeds (Weinberger et al. 2008, Monteiro et al. 2009, Cacabelos et al. 2010, Engelen et al. 2011, Nejrup et al. 2012). This low feeding pressure could explain the invasiveness of non-native algae. However, all those studies were conducted only in the invader's introduced range. Hierro et al. (2005) state the importance of studying exotic species not only in their introduced ranges, as there is a gap in knowledge about the ecology of these species in their native habitats. For testing herbivory-related invasion hypotheses, comparisons of the effects of grazers in the native and the introduced range are therefore crucial. Wikström et al. (2006) were the first to compare native and non-native populations of a seaweed species with regard to feeding pressure and grazing defense, instead of comparing invasive and co-occurring native species. Their study revealed that the rockweed *Fucus evanescens* is less exposed to herbivory in its non-native range in Sweden than in its native range in Iceland, probably due to an increased chemical defense in invasive populations. Both Iceland and Sweden belong to the same Northern European coastal ecoprovince, and the inventories of seaweed-consuming species that are present in these regions show considerable overlap (Spalding et al. 2007). Introduction into new ecoprovinces or even realms might confront the invader with more drastic changes in interspecific interactions. Our aim was therefore to expand the comparison of invasive and non-invasive macroalgal populations over a larger geographical scale, in order to cover environments with more divergent species inventories.

The red macroalga *Gracilaria vermiculophylla* (Ohmi) Papenfuss was chosen as a model organism, as its large tolerance towards environmental stress (Yokoya et al. 1999, Thomsen & McGlathery 2007, Weinberger et al. 2008, Nyberg & Wallentinus 2009) makes it suitable for common garden-like experiments that require long-distance transportation under suboptimal conditions. The native distribution range of the species is in the cold and warm temperate Northwest Pacific ecoprovinces, ranging from the Northern Sea of Japan to the East China Sea and the Central Kuroshio Current. During the last 2 decades, *G. vermiculophylla* has not only spread to the cold (Saunders 2009) and warm (Bellorin et al. 2004) tem-

perate North American west coasts, but also to most ecoprovinces of the temperate Northern Atlantic realm: cold (Saunders 2009) and warm (Freshwater et al. 2006) temperate North American east coasts, Mediterranean Sea (Sfriso et al. 2010), Lusitanian coasts (Guillemin et al. 2008), and Northern European seas, including the Baltic Sea (Schories & Selig 2006, Nyberg 2007, Thomsen et al. 2007a,b, Weinberger et al. 2008). *G. vermiculophylla* spreads rapidly, tends to change the flora and fauna of invaded habitats, and has been included among the most invasive seaweeds in Europe (Nyberg 2007). The invasion process has resulted in a considerable loss of genetic diversity in non-native populations of *G. vermiculophylla*, all of which seem to have a common genetic origin in a donor region within the Korean East Sea/Sea of Japan (Kim et al. 2010).

Local mass appearances and ecological dominance of *Gracilaria vermiculophylla* have repeatedly been observed in areas outside of its native range (Freshwater et al. 2006, Thomsen et al. 2006, 2007a, Pinon-Gimate et al. 2008, Weinberger et al. 2008). To our knowledge, mass appearances have not been reported for native populations of *G. vermiculophylla*. This discrepancy could result from many possible reasons. A more stringent control by native than by non-native feeding enemies is one of them, as *G. vermiculophylla* from non-native populations seems to be less affected by herbivores than native seaweeds from the same environment (Weinberger et al. 2008, Nejrup et al. 2012).

Unlike terrestrial plants, many algae only have a low proportion of structural material that could act as a morphological defense against grazers. Therefore they rely on other defense mechanisms, such as the use of chemical feeding deterrents (Hay & Steinberg 1992). Also, *Gracilaria vermiculophylla* is capable of defending itself from herbivory by mesograzers: if tissue is damaged—either by artificial mechanical wounding or by grazing—the alga produces oxylipins (Nylund et al. 2011, Rempt et al. 2012). These compounds deter potential grazers of *G. vermiculophylla*, such as the omnivorous isopod *Idotea balthica* (Nylund et al. 2011) or the herbivorous sea snail *Echinolittorina peruviana* (Rempt et al. 2012). Wound-activated oxylipins are also molecular messengers that mediate induced defenses of gracilarioids (Weinberger et al. 2011).

The consumption of *Gracilaria vermiculophylla* by certain mesograzers may therefore be reduced due to chemical defenses of the alga. On the other hand, herbivores depend on the ingestion of plant-derived nitrogen and are therefore assumed to prefer plants

with a high proportion of nitrogen, thus with a low carbon to nitrogen (C:N) ratio (Mattson 1980). In accordance with this, *Littorina littorea* consumed and retained more food in nutrient-enriched environments and gained a higher biomass (Diaz et al. 2012). Likewise, feeding preferences of the snail *L. sitkeana* among different seaweeds were primarily determined by the C:N ratio (Van Alstyne et al. 2009), and the amphipod *Gammarus locusta* actively chose high-quality nutrient-rich food as well (Kraufvelin et al. 2006).

The main hypotheses tested in the present study were the following: (1) *Gracilaria vermiculophylla* is less palatable to grazers from the invaded range as compared to grazers from the native range because invader and grazers in the new habitat do not share a co-evolutionary history (as predicted by the enemy release hypothesis), (2) *G. vermiculophylla* originating from non-native populations is in general less palatable than *G. vermiculophylla* from native populations because it has evolved resistance against herbivores during the invasion process (as predicted by the novel weapons hypothesis), and (3) a disposition to relatively low palatability of *G. vermiculophylla* populations from the Korean East Sea/Sea of Japan has facilitated the invasion success of specimens from this donor region (through selection of preadapted genotypes).

To test these hypotheses, *Gracilaria vermiculophylla* from 6 native and 8 invasive populations was fed in no-choice feeding experiments of common-garden type to (1) the snail *Littorina brevicula* Philippi, which has a similar distribution range in East Asia as *G. vermiculophylla* (Son & Hong 1998,

Kim et al. 2003), and (2) *L. littorea* L., a common grazer in most of the regions invaded by *G. vermiculophylla* in the North Atlantic realm. *L. littorea* is able to feed on a variety of macroalgae (Watson & Norton 1985, Diaz et al. 2012), and its Asian conspecific is a generalist herbivore as well (Molis et al. 2008). Feeding experiments with *L. brevicula* were conducted in Qingdao, China, and feeding assays with *L. littorea* were carried out in Kiel, Germany. C:N ratios of *G. vermiculophylla* specimens offered in feeding assays were analyzed in order to investigate whether differences in nutrient content explained differences in palatability. The combination of several native and non-native seaweed populations with congeneric grazers of either the native or the introduced range in a common garden was a new approach in the frame of testing herbivory-related invasion theories in marine ecology. Furthermore, it also ensured that the detected effects were not only due to local adaptation (Williams et al. 2008).

MATERIALS AND METHODS

Collection, transport, and cultivation of *Gracilaria vermiculophylla*

Between 10 February and 22 April 2010, 6 native populations of *Gracilaria vermiculophylla* were sampled along the Korean peninsula and in Shandong Province, China, and 8 non-native populations were sampled around the Danish-German peninsula of Jutland and Schleswig-Holstein, in the west of France, and on the Pacific coast of Mexico (Table 1).

Table 1. *Gracilaria vermiculophylla*. Geographic location and taxonomic reference (GenBank accession numbers are given for new *cox1* gene sequences generated in the framework of the present study) of native and non-native populations

	Population/abbreviation (sea area)	Geographic coordinates	Taxonomic reference
Native	Donghae/Dong (East Sea, South Korea)	37° 29' 27.65" N, 129° 08' 30.42" E	JQ736332
	Odo-ri/Odo (East Sea, South Korea)	36° 09' 52.40" N, 129° 24' 00.07" E	JQ736333
	Jindo/Jin (Yellow Sea, South Korea)	34° 25' 16.98" N, 126° 20' 48.21" E	Kim et al. (2010)
	Gyeokpo/Gye (Yellow Sea, South Korea)	35° 37' 05.54" N, 126° 28' 01.70" E	Kim et al. (2010)
	Daecheon/Dae (Yellow Sea, South Korea)	36° 19' 23.07" N, 126° 30' 07.81" E	Kim et al. (2010)
	Qingdao/Qing (Yellow Sea, China)	36° 03' 18.51" N, 120° 21' 57.01" E	Kim et al. (2010)
Non-native	Heiligenhafen/Hhafen (Baltic Sea, Germany)	54° 22' 48.14" N, 10° 58' 58.94" E	JQ736335
	Nyborg/Ny (Baltic Sea, Denmark)	55° 17' 54.23" N, 10° 49' 07.35" E	JQ736339
	Horsens/Hor (Baltic Sea, Denmark)	55° 51' 35.02" N, 9° 52' 35.76" E	KC731475
	Nibe/Nibe (Limfjord, Denmark)	56° 59' 03.34" N, 9° 37' 48.89" E	JQ736338
	Mandö/Man (Wadden Sea, Denmark)	55° 18' 35.59" N, 8° 38' 34.74" E	JQ736337
	Nordstrand/Nord (Wadden Sea, Germany)	54° 31' 48.01" N, 8° 52' 18.88" E	JQ736336
	St.-Pol-de-Leon/St.-Pol (Brittania, France)	48° 40' 41.09" N, 3° 58' 08.04" W	Kim et al. (2010)
	Ensenada/Ens (Baja California, Mexico)	31° 46' 28.67" N, 116° 36' 40.92" W	JQ736334

The identity of all studied populations was confirmed by DNA barcoding, either by Kim et al. (2010) or by us, using the methodology described in Kim et al. (2010) (Table 1). *G. vermiculophylla* individuals from native populations were easily distinguishable, as each plant was growing attached to hard substratum. However, *G. vermiculophylla* from non-native populations was often growing unattached, and so it cannot be ruled out that some of the used 'individuals' were in fact clonal, although both native and non-native plants were sampled in extended areas of >1000 m² to avoid taking clonal samples.

For the feeding experiments with *Littorina brevicula* and *L. littorea*, specimens from all *Gracilaria vermiculophylla* populations were transferred alive to both Qingdao (China) and Kiel (Germany), respectively. They were transported wrapped into seawater-wetted lab paper and in cooling containers (Nyberg & Wallentinus 2009). In order to exert as little stress as possible, transportation periods never exceeded 5 d, and for logistic reasons the transportation between collection site and experimental site could not always be direct. Specimens originating from native populations were transported directly to Qingdao and Kiel, while specimens originating from non-native populations were brought to Kiel first. After a regeneration period where they were kept under non-stressful conditions for at least 2 wk, one half was transported to Qingdao, while the other half remained in Kiel. Losses due to transportation stress were not observed. After transportation and prior to feeding assays, all specimens were kept under non-stressful conditions for at least 1 wk before conducting the feeding assays to allow for regeneration. In Qingdao, they were for this purpose cultivated in separate Erlenmeyer flasks at identical conditions (room temperature, i.e. approx. 20°C; indirect sunlight, i.e. maximum photosynthetically active radiation [PAR_{max}] of 20 μmol m⁻² s⁻¹; no aeration). The medium (Yellow Sea water) was exchanged daily. At Kiel, the cultivation for regeneration took place in separate indoor tanks under identical conditions (water temperature: 15 to 20°C; PAR: 30 μmol m⁻² s⁻¹ for 16 h d⁻¹; aeration). The medium was Baltic Sea water from the Kiel Fjord. It was constantly exchanged between all tanks and a central storage container, in order to maintain an identical composition in all tanks. At the exit of the central container and prior to redistribution into the tanks, the water was UV-sterilized, in order to prevent exchange of algal propagules among tanks containing material of different origin. For experiments conducted in Qingdao, seaweeds that were sampled at low salinity condi-

tions (Baltic Sea) were slowly (stepwise over several days) acclimatized to fully marine conditions, while seaweeds from fully marine conditions were slowly adapted to low salinity conditions (salinity: 15 ± 2) before using them for experiments in Kiel.

Collection of *Littorina brevicula* and *L. littorea*

Three days before the feeding experiment started, *Littorina brevicula* individuals were collected on the Yellow Sea coast in Qingdao (36° 03' 18.51" N, 120° 21' 57.01" E) at a site where *Gracilaria vermiculophylla* was present. Snails were maintained in the laboratory in an aquarium containing 5 l of Yellow Sea water (salinity: 33), which was exchanged twice every day. During this period they were allowed to graze on green algae (*Ulva* sp.). The diameter of the snails was measured prior to the experiment. Snails with a diameter between 7 and 9 mm were most common in the habitat at the time of sampling (April) and were therefore used for experiments.

Littorina littorea individuals were collected on the shore of the Kiel Fjord (54° 21.965' N, 10° 8.908' E) at a site where *Gracilaria vermiculophylla* was present. They were kept in a flow-through aquarium containing 5 l of Baltic Sea water (salinity: 15 ± 2) prior to the bioassay. During this period of 3 d, they were allowed to graze on green algae (*Ulva* sp.). For this experiment, snails with a diameter between 8 and 12 mm were used.

Feeding assays

The experiments with *Littorina brevicula* were conducted in April 2010 and experiments with *L. littorea* in May 2010. For the no-choice feeding assays, 10 algal individuals per *Gracilaria vermiculophylla* population were used. Each of the 10 replicates was cut in 2 pieces of 0.1 ± 0.005 g (blotted wet weight): One was exposed to snails and the second one was used as control of autogenic changes during the course of the experiment. At Qingdao, all algal pieces were incubated in Petri dishes (diameter: 9 cm) containing water from the Yellow Sea (salinity: 33), which was exchanged every day. The experiment lasted for 7 d, and 4 snails were used in each treatment. At Kiel, the experimental setup was identical, with 3 exceptions: the water originated from the Kiel Fjord (salinity: 15 ± 2), and only 3 snails were used per Petri dish, as they were larger than those at Qingdao. Also, *L. littorea* individuals were allowed to

consume the algae for 12 d, as the feeding rates were generally lower than for *L. brevicula*. Experiments were run in a constant temperature room (16°C) in Kiel. In Qingdao, those facilities were not available, but temperatures were in the same range (15 to 20°C).

At the end of each experiment, algae exposed and unexposed to snails were weighed again and net growth was determined. To get comparable growth data for both experiments, the daily weight change of *Gracilaria vermiculophylla* was determined and related to the respective soft body dry weight (DW) of the snails. Soft body DW was ascertained from shell diameters of the 2 snail species as follows. The diameter of snails of different sizes was measured (*Littorina brevicula*: n = 46, diameter: 4 to 10 mm; *L. littorea*: n = 61, diameter: 6 to 15 mm). The soft body was removed from the shell and dried at 50°C until weight constancy. Regressions were then calculated between shell diameters and soft body DW of both species, so that the soft body DW of the snails in each Petri dish could be assessed.

C:N analysis

Remains of every *Gracilaria vermiculophylla* individual used in the feeding assays were conserved in silica gel for C:N analysis. From both series of experiments, 5 randomly selected replicates per population were analyzed. The dried algal pieces were ground in a mixer mill (Retsch), and C:N ratios were determined in a Euro Vector EA 3000 Elemental Analyzer (Eurovector).

Statistical analysis

Prior to statistical tests, consumption data and C:N ratios were Box-Cox-transformed to achieve homogeneity of variances and normality. Homogeneity of variances could not be achieved for all factors (Levine's test, $p < 0.05$), and in these cases, results were taken in the following tests as statistically significant if the probability of error was $< 1\%$ (Underwood 1997), while other results were taken as statistically significant at a p-level of 5%. Differences in consumption rates of either *Littorina brevicula* or *L. littorea* were analyzed by nested-design ANOVA, with the factor 'population' nested within the factor 'region' (native or non-native). Differences among single populations were detected with Tukey's post hoc test. A linear regression was fitted between con-

sumption rates of *L. brevicula* and *L. littorea*, in order to find out if both snail species preferably fed on the same populations of *Gracilaria vermiculophylla*. Linear regression was also used to detect correlations among C:N ratios and feeding patterns. Box-Cox transformations and statistical tests were conducted using the STATISTICA 8 software package (Stat-Soft), while regressions were fitted using GraphPad Prism 4 software.

RESULTS

Both *Littorina brevicula* and *L. littorea* found *Gracilaria vermiculophylla* individuals originating from native populations more palatable, and consumed > 3 times as much of them compared to specimens originating from invasive populations ($p < 0.001$; Fig. 1, Table 2).

Interestingly, there was a high variability within the native populations, and the origin of native specimens influenced consumption rates of *Littorina brevicula* significantly ($p < 0.001$; Fig. 2A, Table 2). Seaweeds from Jindo (South Korea) were consumed more than all other native populations, except for those from Qingdao (China) and Gyeokpo (South Korea). Populations from Daecheon, Odo, and Donghae (South Korea) were consumed significantly less than the seaweed individuals from Jindo, and had an average (\pm SE) biomass loss of $14.7 \pm 2.9 \text{ mg d}^{-1} (\text{g DW } L. \text{ brevicula})^{-1}$. Variability within the invasive

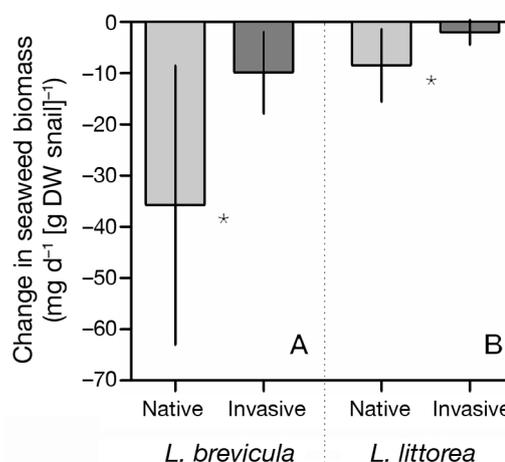


Fig. 1. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Average biomass loss of *G. vermiculophylla* from native and invasive populations caused by consumption by (A) *L. brevicula* and (B) *L. littorea* in feeding experiments. *Native and invasive populations significantly different in a nested 2-way ANOVA (see factor 'region' in Table 2; $p < 0.001$). n = 7 to 10. Whiskers: $\pm 95\%$ CI

Table 2. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Influence of the origin of *G. vermiculophylla* populations on the biomass consumed by *L. brevicula* and *L. littorea* in feeding experiments, using a nested-design ANOVA with the factor 'population' nested within the factor 'region' (either native or invasive)

	SS	df	MS	F	p
<i>L. brevicula</i>					
Population (Region)	4.50×10^{10}	12	3.75×10^9	4.41	<0.001
Region	3.02×10^{10}	1	3.02×10^{10}	35.56	<0.001
Error	1.04×10^{11}	122	8.50×10^8		
<i>L. littorea</i>					
Population (Region)	3.34×10^8	12	2.78×10^7	4.34	<0.001
Region	1.37×10^8	1	1.37×10^8	21.46	<0.001
Error	7.24×10^8	113	6.41×10^6		

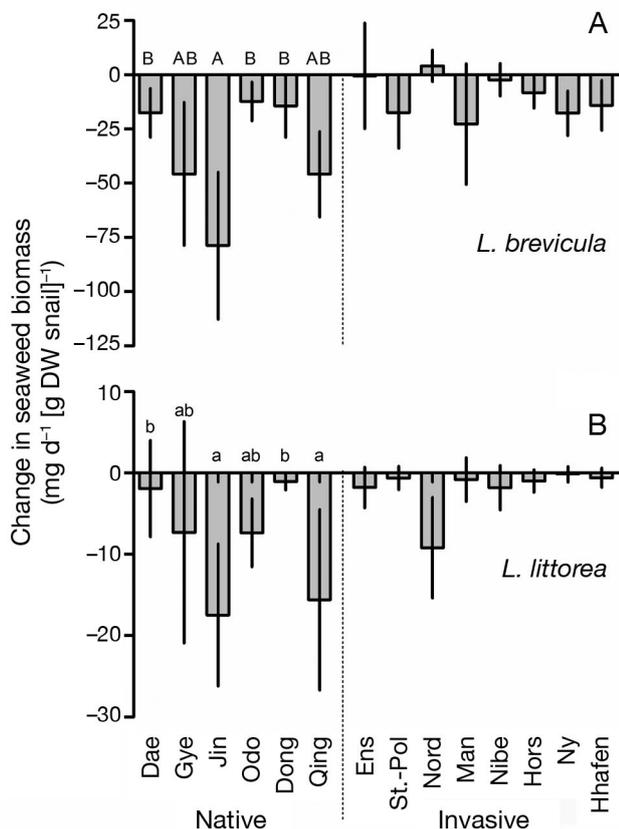


Fig. 2. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Consumption of biomass of different *G. vermiculophylla* populations by (A) *L. brevicula* and (B) *L. littorea* in feeding experiments. Different letters indicate populations that were significantly different in a Tukey test ($p < 0.01$) either within the native or within the non-native region. $n = 7$ to 10. Whiskers: $\pm 95\%$ CI. See Table 1 for population abbreviations

populations was lower than within native populations, and Tukey's post hoc test did not detect any significant differences between them (see Fig. 2A).

Average (\pm SE) biomass loss of invasive populations was 10.2 ± 2.5 mg d^{-1} (g DW *L. brevicula*) $^{-1}$, and thus similar to populations from Daecheon, Odo, and Donghae.

Consumption of *Littorina littorea* also varied significantly within native populations, but not within invasive populations ($p < 0.001$; Fig. 2B, Table 2). Specimens from Jindo (South Korea) and Qingdao (China) were consumed significantly more than algae from other native populations, with the exception of those from Gyeokpo (South Korea) and Odo (South Korea).

Material from Daecheon and Donghae (South Korea; biomass loss [\pm SE]: 1.9 ± 2.4 and 1.1 ± 0.4 mg d^{-1} [g DW *L. littorea*] $^{-1}$, respectively) was consumed significantly less than specimens from Jindo and Qingdao, and showed consumption rates in a similar range as *Gracilaria vermiculophylla* from invasive populations (average [\pm SE] biomass loss: 2.0 ± 0.5 mg d^{-1} [g DW *L. littorea*] $^{-1}$).

Littorina brevicula and *L. littorea* tended to find the same populations of *Gracilaria vermiculophylla* most palatable (Fig. 3). Two of the native populations (Jindo from South Korea, and Qingdao from China) were consumed most by both herbivores, while the consumption of 2 other native populations (Daecheon and Donghae, South Korea) was similar to that of invasive populations. Seaweeds from Gyeokpo (South Korea) were in both experiments consumed at an intermediate range. Seaweeds from Odo (South Korea), however, differed between experiments in

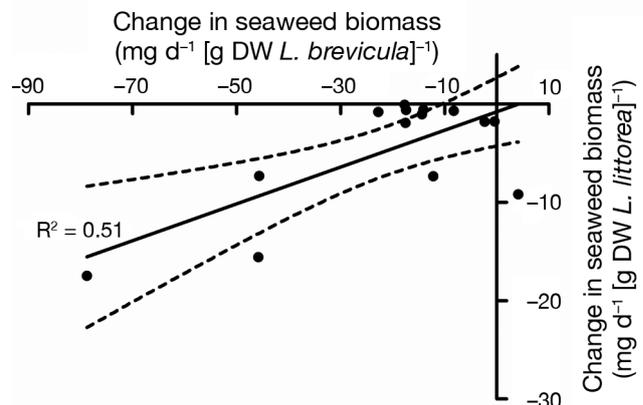


Fig. 3. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Linear regression correlating the biomass of *G. vermiculophylla* per population consumed by *L. brevicula* and *L. littorea*. Dotted lines: 95% CI

the native and the invaded range— while they were not much consumed by *L. brevicula*, the consumption by *L. littorea* was at an intermediate level.

However, the total amounts consumed were significantly higher for *Littorina brevicula*, which fed more than 5-fold the amount of *Gracilaria vermiculophylla* compared to *L. littorea* (Fig. 1). The difference becomes even more distinct when the combinations of snails and seaweeds existing in nature (*L. brevicula* and *G. vermiculophylla* from Qingdao and *L. littorea* and *G. vermiculophylla* from the German Baltic coast) are compared—*L. brevicula* consumed 90-fold the amount consumed by *L. littorea*.

At the end of both experiments, the C:N ratios differed between native and invasive populations of *Gracilaria vermiculophylla* ($p < 0.001$; Fig. 4, Table 3), with slightly higher relative nitrogen contents in the invasive species. Significant differences in C:N ratios were also detected among single populations (experiment in Qingdao: $p < 0.001$; Fig. 5A, Table 3; experiment in Kiel: $p < 0.01$; Fig. 5B, Table 3). However, there was no obvious distribution pattern among native or invasive algae, and the variability was high.

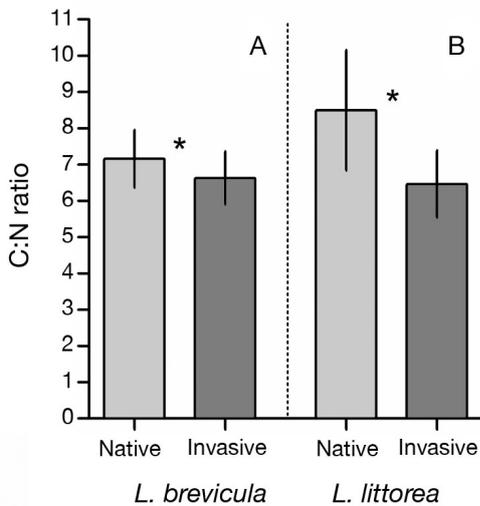


Fig. 4. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Means of C:N ratios for native and invasive *G. vermiculophylla* populations consumed by (A) *L. brevicula* and (B) *L. littorea*. *Native and invasive populations significantly different in a nested 2-way ANOVA (see factor 'region' in Table 3; $p < 0.001$). $n = 5$. Whiskers: $\pm 95\%$ CI

Table 3. *Gracilaria vermiculophylla*. Influence of the origin of *G. vermiculophylla* populations on C:N ratios of the seaweed in experiments in Qingdao and Kiel, using a nested-design ANOVA with the factor 'population' nested within the factor 'region' (either native or invasive)

	SS	df	MS	F	p
Qingdao					
Population (Region)	0.018	12	0.002	8.81	<0.001
Region	0.002	1	0.002	13.60	<0.001
Error	0.009	55	0.0002		
Kiel					
Population (Region)	0.021	11	0.002	2.80	<0.01
Region	0.020	1	0.020	28.1	<0.001
Error	0.034	49	0.0007		

We detected no significant correlations between C:N ratios and biomass consumption by *Littorina brevicula* or *L. littorea* (Fig. 6).

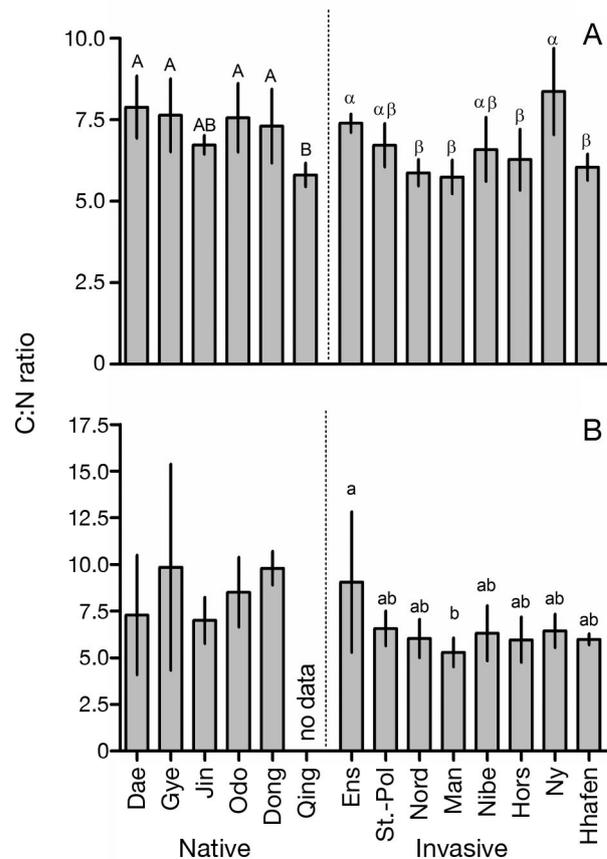


Fig. 5. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Averages of C:N ratios for single *G. vermiculophylla* populations consumed by (A) *L. brevicula* and (B) *L. littorea*. Different letters indicate populations that were significantly different in a Tukey test ($p < 0.01$) either within the native or within the non-native region. $n = 5$. Whiskers: 95% CI. See Table 1 for population abbreviations

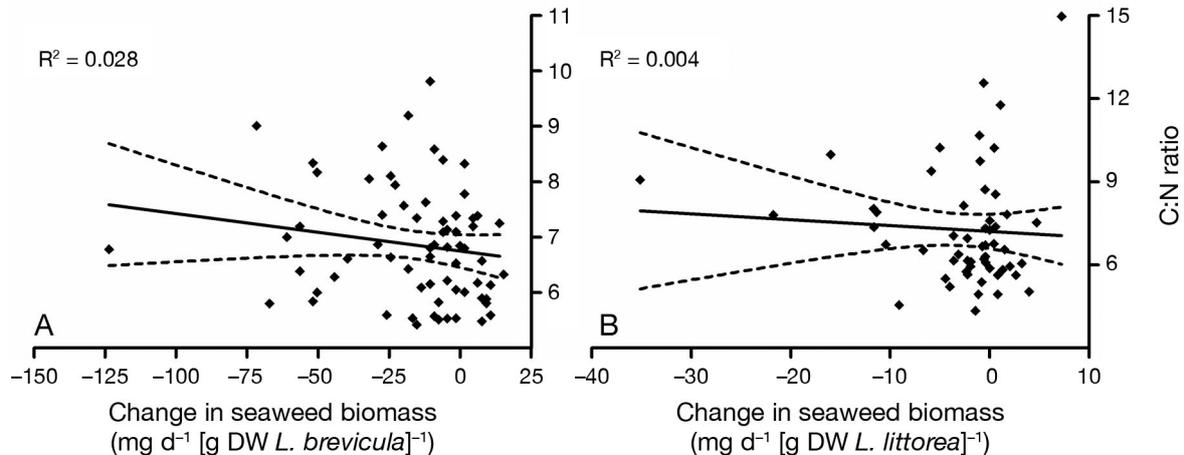


Fig. 6. *Gracilaria vermiculophylla*, *Littorina brevicula*, and *L. littorea*. Linear regression of C:N ratios of *G. vermiculophylla* biomass and biomass consumed by (A) *L. brevicula* and (B) *L. littorea*. Dotted lines indicate 95 % CI

DISCUSSION

The employment of both native and non-native populations of *Gracilaria vermiculophylla* in combination with congeneric generalist herbivores from the native range (*Littorina brevicula*) and the invaded range (*L. littorea*) allows us to answer several questions: Are non-native populations of the seaweed in general more sensitive to grazing than native populations? Do feeding rates differ among single populations? Do *L. brevicula* and *L. littorea* consume similar amounts of the seaweed or is *G. vermiculophylla* less palatable to the grazer in the new range? The comparison between the consumption rates of the 2 snail species has to be looked at with some caution, as conditions at the experimental sites were similar, but not identical. It is possible that *L. brevicula* has in general higher consumption rates than *L. littorea* or that it coped better with the laboratory conditions. However, the behavior of all snails seemed normal, and losses during the experimental period were not observed. Light and temperature regimes were in a similar range, but the salinity differed, as we used the salinity conditions that were present at the respective sampling sites of the snails to avoid stress. Nevertheless, the differences are quite remarkable: *L. brevicula* from the native range consumed in total significantly higher amounts (\pm SE) of algal tissue ($21.4 \pm 2.8 \text{ mg d}^{-1} \text{ g}^{-1}$ when averaged over all algal populations) than the phylogenetically related *L. littorea* from the non-native range ($4.3 \pm 0.8 \text{ mg d}^{-1} \text{ g}^{-1}$). The ratio was 90-fold when naturally occurring matches of seaweed and herbivore in Qingdao (China) and in the German Baltic were compared. At

Qingdao, *G. vermiculophylla* inhabits the upper eulittoral close to the supralittoral fringe. *L. brevicula* is the main character species of this habitat (Morton 1990), and the alga is frequently subject to consumption by the snail (authors' pers. obs.). In the atidal Baltic Sea at Kiel and Heiligenhafen, both *L. littorea* and *G. vermiculophylla* inhabit the upper sublittoral, and the snail is 1 of the 2 main feeding antagonists, together with the isopod *Idotea baltica* (Weinberger et al. 2008). However, both consumers preferably feed on the native brown seaweed *Fucus vesiculosus* rather than on *G. vermiculophylla* when both are offered in 2-way-choice experiments (Weinberger et al. 2008). Nejrup & Pedersen (2010) examined *in situ* grazing losses of non-native *G. vermiculophylla* in Denmark over an entire growth season, but did not find significant losses due to grazing. The feeding pressure on native populations of *G. vermiculophylla* in the field has not been determined yet. However, on the Chinese and South Korean coasts, *L. brevicula* can occur in markedly high numbers, while densities of *L. littorea* on the German and Danish coasts seem to be lower (authors' pers. obs.). At the same time, our experiments indicate that the grazer *L. littorea* from the invaded range is less attracted or more deterred by the metabolic composition of *G. vermiculophylla* than the snail from the native range.

It was most obvious that both snail species preferred to feed on the native seaweeds. Both *Littorina littorea* and *L. brevicula* consumed more than 3 times larger amounts of native than of invasive *Gracilaria vermiculophylla*. Apparently, *G. vermiculophylla* originating from the invaded range was less palatable. Consequently, the question should be asked whether

differences in C:N ratios could be responsible for the observed differences in consumption of the algal material. Similarly to the differences in consumption, C:N ratios also differed significantly among native and invasive algal specimens. Slightly higher C:N ratios were detected in native individuals. However, snails of the genus *Littorina* have been attracted to food with low rather than high C:N ratios (Van Alstyne et al. 2009). Moreover, there were no significant correlations between C:N ratios and biomass consumption by *L. brevicula* or *L. littorea*. The C:N contents of the seaweeds can therefore not explain the observed feeding patterns. In this light, it seems more likely that the snails were influenced by specific deterrents or feeding cues. *G. vermiculophylla* is able to defend itself from feeding by up-regulation of chemical compounds. After wounding, the alga produces — among other substances — arachidonic acid derivatives, which were found to deter mesograzers such as the omnivorous isopod *Idotea baltica* (Nylund et al. 2011) or the herbivorous sea snail *Echinolittorina peruviana* (Rempt et al. 2012). It seems likely that these or other substances also act as feeding deterrents against *L. brevicula* and *L. littorea*. In a study with the invasive red alga *Bonnemaisonia hamifera*, Enge et al. (2012) were able to show that low consumption in the new range of the seaweed can be directly attributed to a specific chemical defense against native generalists that did not encounter this type of chemical defense before. They conclude that novel chemical weapons against naive herbivores might provide a mechanistic explanation for algal invasion success. The lower palatability of non-native populations of *G. vermiculophylla* could similarly be due to an increased level of chemical defenses. This low palatability to one of the main grazers in invaded habitats could partly explain the invasion success of *G. vermiculophylla*. However, numerous other traits also cannot be excluded as potential factors of invasion success, as *G. vermiculophylla* can survive and spread under a wide range of abiotic conditions and is also able to survive extensive periods of desiccation and burial (Yokoya et al. 1999, Nyberg 2007, Thomsen & McGlathery 2007, Weinberger et al. 2008, Nyberg & Wallentinus 2009, Nejrup & Pedersen 2012).

The variation of palatability within native populations of *Gracilaria vermiculophylla* is high, but overall *Littorina brevicula* and *L. littorea* found the same populations most palatable. The consumption of 2 native populations (Daechon and Donghae, South Korea) by both snails was similar to that of invasive populations, while seaweeds from Odo

(South Korea) were of low palatability to *L. brevicula* only. Of all the native populations included in the present study, the Donghae population is genetically most similar to invasive populations (Kim et al. 2010), and genetic data hint at the Korean East Sea/Sea of Japan being the donor region for all invasive *G. vermiculophylla* populations (Kim et al. 2010). In this light, it is interesting that both populations from the Korean East Sea/Sea of Japan (Donghae and Odo) included in the present study were also among those native populations that have been fed on least. Possibly, low palatability is more widely spread among populations in the Korean East Sea/Sea of Japan; such pre-adapted genotypes were introduced into new habitats, and in their new environment they could settle and spread, as they were not controlled by grazers. Such a selective introduction of specific pre-adapted genotypes was also assumed for the South African ragwort *Senecio inaequidens* (Bossdorf et al. 2008).

CONCLUSIONS

Both *Littorina brevicula* and *L. littorea* consumed significantly higher amounts of native than of invasive *Gracilaria vermiculophylla*. The low palatability to *L. littorea* could thus be one reason for the invasion success of the seaweed. Furthermore, the results of our study show that it can be of great importance to compare more than only one native and one non-native population of a species to understand the mechanisms underlying an invasion process. *G. vermiculophylla* populations originating from different ecoprovinces exhibit a high variability, especially within the native populations. A simple comparison of populations from the Baltic Sea and Qingdao might have led to the assumption that native populations are in general more palatable than non-native populations. However, consumption rates of *G. vermiculophylla* from some Korean populations were just as low as those of invasive populations. Therefore, we conclude that the invasion success of this species might have been facilitated by the introduction of herbivory-resistant genotypes.

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