GRAZING IMPACT OF, AND INDIRECT INTERACTIONS BETWEEN MESOGRAZERS ASSOCIATED WITH KELP (*LAMINARIA DIGITATA*)¹

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Little is known about the indirect effects of nonlethal grazing impacts in mesograzer-seaweed interactions. Using laboratory experiments, the effect of grazing by the seasonally abundant kelp-associated gastropod Lacuna vincta on subsequent kelp consumption by one kelp-associated (Idotea granulosa) and one nonassociated species of isopod (I. emarginata) was determined. Measurements of the toughness and elemental composition of different parts of the sporophyte of Laminaria digitata (Huds.) J. V. Lamour., as well as grazer-induced changes in the palatability of the blade, were conducted to explore possible mechanisms of indirect effects. In situ grazing pressure was the highest between July and September, with the blade being the preferred part of the kelp sporophyte, despite missing differences in the elemental composition among kelp parts. The laboratory experiments supported our hypotheses in that kelp consumption by both species of isopods was lower on intact than on L. vinctadamaged areas of the blade. This pattern was not caused by grazing-induced changes in blade palatability. Instead, the observed increase in isopod consumption following grazing by L. vincta resulted more likely from the combined effects of a reduction in the toughness of L. vincta-damaged kelp blades and some unknown gastropod cue(s). These results suggest that kelp-associated and nonassociated mesograzers may benefit from the nonlethal grazing impact of L. vincta due to changes in physical traits of the seaweed. Thus, the nonlethal grazing impact by one species of mesograzer can positively modify the trophic interactions between kelp and other potential competitors, suggesting that the interactions among mesograzers might be more complex than previously assumed.

Key index words: facilitation; indirect interaction; interaction modification; Lacuna vincta; Laminaria digitata; seasonal patterns; seaweed; trophic interaction

Abbreviations: AC, actual consumption; EU, experimental unit

Herbivores can strongly alter the fitness and performance of algae and plants. There are numerous studies documenting, for instance, the impact of grazers on plant biomass and community structure (Cyr and Pace 1993, McQuaid 1996, Korpinen et al. 2008), feeding preferences of herbivores (Toth and Pavia 2002), or plant responses to herbivory (Cronin 2001). However, most studies assessed the magnitude of lethal effects between one species of grazer and plant. Studies on pair-wise plant-animal interactions reveal useful information on, for example, the feeding biology of grazers. However, they lack realism as they are decoupled from the underlying community context, thereby truncating the possibility to study such phenomena as indirect effects in species interactions (Hay et al. 2004). Models and empirical work on indirect effects among one basal and multiple consumer species suggest a prevalence of negative indirect effects among consumers (Menge 1995, Long et al. 2007), but less is known about seaweed-mediated positive indirect effects (Viejo and Arrontes 1992).

Experiments using multiple species are required to assess whether the mechanism, magnitude, and direction in species interactions will be different from the conclusions drawn from studies on pairwise interactions. In this context, trophic interactions between seaweeds and mesograzers may serve for at least two reasons as useful model systems. First, seaweeds host species-rich assemblages with a high density of associated herbivores. For instance, >40 species of mobile macrofauna, of which about one-third are herbivorous, were found inhabiting the brown seaweeds Laminaria digitata and L. hyperborea in different NE Atlantic regions at sometimes very high densities (Schultze et al. 1990, Christie et al. 2003). Such associations at close range make seaweed-mediated indirect interactions among grazers very likely. Second, many trophic interactions between macroalgae and grazers from

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temperate habitats are nonlethal as most herbivores in this region belong to the group of relatively small-sized mesograzers (<2.5 cm), like gastropods and isopods (sensu Brawley 1992). The partial consumption of macroalgae by mesograzers allows the former to change physical and/or chemical traits in response to grazing. For instance, previous grazing by isopods facilitated the consumption of algal biomass for subsequently grazing amphipods (Viejo and Arrontes 1992). Furthermore, many species of seaweeds induce chemical antiherbivory defenses in response to direct grazing that repel conspecific grazers (Toth and Pavia 2007). Grazer-associated cues can trigger the induction of antiherbivory responses in some interactions. For instance, an enzyme in periwinkle (Littorina obtusata) saliva caused lower consumption rates of the brown seaweed Ascophyllum nodosum (Coleman et al. 2007b). Changes in seaweed traits are known to be ecologically important. Besides grazing-induced direct changes in trophic interactions, altered seaweed traits can indirectly affect the interactions of associated or neighboring organisms in several ways. First, changes in seaweed traits can alter patterns of interspecific competition. For instance, grazing by the periwinkle L. obtusata lowered the palatability of F. vesiculosus for the congener L. littorea but not vice versa, causing asymmetry in the ability to use resources (Long et al. 2007). Second, predator-prey interactions can be influenced by seaweeds. The study by Coleman et al. (2007a) revealed that waterborne chemicals released by periwinkle-grazed seaweeds increased the susceptibility of the grazer to its predators. Last but not least, defense levels in seaweeds may rise in response to water-borne signals received from grazed conspecifics (Toth and Pavia 2000, Macaya et al. 2005).

Kelp species of the genus Laminaria provide habitat and food for a large number of herbivores, thereby playing an important role on temperate rocky shores, especially in the Northern Hemisphere (reviewed in Bartsch et al. 2008). Grazers like the banded chink snail (L. vincta) cause superficial damage on the tough kelp blades, which may be beneficial for kelp consumption by other mesograzers that cannot penetrate these tissues themselves. In particular, the strong dependency of L. vincta on L. digitata for successful recruitment (Johnson and Mann 1986) may provide predictable cues to a permanently associated (e.g., Idotea granulosa) herbivore in tailoring its food demands to the life cycle of L. vincta. However, if snail grazing is more generally beneficial for kelp consumption by mesograzers, we also expect positive indirect effects of L. vincta grazing to mesograzers that sporadically visit and consume kelp (e.g., I. emarginata). Thus, indirect effects between snails and other species of mesograzers may occur in that subsequent kelp consumption by the latter may be facilitated. The aim of this study was to assess whether seaweedmediated indirect effects occur between kelp-associated mesograzers and whether indirect effects were dependent on trait quality and residency status of grazers. Using manipulative laboratory experiments, we tested four hypotheses: (1) Previous grazing by L. vincta increases the consumption of the kelp L. digitata by the isopod I. granulosa relative to undamaged kelp blades due to reduction in kelp toughness. (2) To differentiate whether purely mechanical or grazer-associated cues were responsible for changes in kelp consumption by isopods, grazing rates between artificially damaged and L. vincta-grazed pieces of L. digitata were compared. (3) If grazing by L. vincta is generally beneficial for isopods, the consumption of kelp biomass after L. vincta attacks should be comparable between associated species (i.e., I. granulosa) and nonassociated species (i.e., I. emarginata) of isopods. (4) Grazing by L. vincta or by isopods will not induce antiherbivory defenses for subsequently grazing conspecifics or heterospecifics.

MATERIALS AND METHODS

Study site. Mesoherbivores and the perennial brown seaweed L. digitata were collected during spring low tides at Augusta Mole (54°10.70′ N, 7°53.52′ E), Helgoland, NE Atlantic. Average tidal range at the wave-exposed study site was 2.3 m. During the study period, surface seawater temperature at Helgoland ranged from 2.1°C (March) to 19.6°C (August) with a salinity from 29.4‰ to 34.2‰ (M. Molis, personal observation). Average (±SD) maximum water velocity (an index of wave exposure), measured five times with three dynamometers (Bell and Denny 1994), was 2.45 (±0.42) m · s⁻¹. This was 1.8 times higher than inside a sheltered harbor on Helgoland Island (M. Molis, unpublished data).

At Augusta Mole, L. digitata occurs from the mean low water level down to ~ 4 m water depth. At 3 to 4 m water depth, L. digitata exists in mixed stands with L. hyperborea, with the latter prevailing at greater depth, while dense stands of Fucus serratus occur above the L. digitata zone. The understory communities in the sub- and intertidal zone are composed of a large number of species of invertebrates and macroalgae (Bartsch and Tittley 2004, Reichert and Buchholz 2006). The sporophyte of L. digitata hosts a species-rich assemblage of >70 species of invertebrates, including the banded chink snail L. vincta and the isopod I. granulosa (Schultze et al. 1990). The second species of isopod used in this study, I. emarginata, prefers decaying seaweeds on the seafloor as a habitat (Franke et al. 2007) but is an active swimmer (Ingolfsson and Agnarsson 2003) and can move between its preferred habitat and the Laminaria zone.

Estimating ambient levels of grazing pressure. To quantify ambient levels of grazing pressure on L. digitata at the study site, the proportion of feeding scars from total thallus area (without holdfast) was determined. Using SCUBA, eight randomly selected L. digitata were removed at eight times between January and September 2006 from the study site and transported to the laboratory to quantify the area of feedingrelated damage. Due to low replication, the March sample was excluded from the statistical analysis. Algae were divided into stipe, meristem, lower, and upper blade. The border between upper and lower blade was defined as half of the maximum blade length, to reflect any age-specific differences in grazing pressure between younger basal and older apical parts of the blade. The meristem was defined as the slightly brighter colored area between the lower blade and the stipe.

From each of the four parts of the same kelp specimen, the area of feeding scars was estimated (n = 8). Feeding-related damage of algal tissues could be distinguished from nonfeeding-related (e.g., wave-induced) damage. The latter caused stripes of homogenous shape and size, while the former resulted in either irregular-shaped trails with radular markings on the surface (gastropod grazing) or complete penetrations with irregular-shaped margins (isopod and amphipod grazing). Although feeding scars differed in size, form, and depth, no species-specific patterns could be identified, and thus only total damage was determined. To quantify the area of damaged tissue, we defined seven size classes: (i) <0.01, (ii) 0.01 to <0.04, (iii) 0.04 to <0.25, (iv) 0.25 to <1, (v) 1 to <2.25, (vi) 2.25 to <4, and (vii) ≥ 4 cm². Each feeding scar was assigned to one size class, and all records summed per thallus part to get the total area of feeding damage. Total thallus area was determined with different methods for the blade and stipe. Blade area was calculated from digital pictures by the program WinFOLIA 5.0 (WinfoliaTM 2001a; Régent Instruments Inc., Québec, QC, Canada). Stipe area A was calculated using the formula

$$A = \left(\frac{1}{2}p_{\mathrm{u}} \times \frac{1}{2}l_{\mathrm{s}}\right) + \left(p_{\mathrm{m}} \times \frac{1}{2}l_{\mathrm{s}}\right) + \left(\frac{1}{2}p_{\mathrm{l}} \times \frac{1}{2}l_{\mathrm{s}}\right) \tag{1}$$

where l_s = stipe length, and p_u , p_m , and p_1 represent upper-, middle-, and lower-stipe perimeter, respectively.

Toughness of L. digitata thallus surface. Thallus toughness was measured among kelp parts and between damaged and intact blade surfaces to approximate tissue specificity and damage-related morphological resistance to herbivore attack in L. digitata. Using SCUBA, seven L. digitata were collected at Augusta Mole on 25 August 2006 and immediately transferred to the laboratory. Thallus toughness of the meristem and lower and upper blade was measured with a penetrometer (Wagner Force DialTM FDN 100; Wagner Instruments, Greenwich, CT, USA) using the flat attachment (Ø 0.6 cm). For each measurement, the attachment was placed vertically on the kelp surface, and pressure was constantly increased until the tissue was penetrated. For each of the three kelp parts of each specimen, five random measurements were taken from the intact surface, and the mean calculated (n = 7). The number of random measurements for damaged kelp surfaces was ≤5 because superficial grazing damage of at least 0.3 cm² was needed to use the penetrometer, but damage of this size did not occur on all sampled kelp parts.

Elemental composition of thallus parts. The elemental composition of four different thallus parts was determined to test for differences in nutritious value among thallus parts. In November 2005, seven pieces (7–9 mg wet mass) each of the stipe, meristem, blade, and sorus of each of four *L. digitata* (n = 4) were cut, weighed, and put into aluminium tins for elemental analysis (Elemental Analyzer EA 1108; Fisons, Elementar Analyse Systeme GmbH, Hanau, Germany). Acetanilid was used as a calibration standard for C, H, and N with a relative mass of 71.09% C and 10.36% N.

Design and setup of the multigrazer experiment. Two laboratory experiments were conducted each in 2006 and in 2007 to assess the grazing effect of the snail *L. vincta* on the consumption of *L. digitata* by subsequently feeding isopods. Experiments using *I. granulosa* started on 25 March 2006 and 17 April 2007, and those with *I. emarginata* on 1 April 2006 and 12 March 2007. The day the experiments started, 10 *L. digitata*, 50 *L. vincta* (3–6 mm shell height), and 40 *I. granulosa* (12–20 mm body length without antennae) were collected at the study site and immediately transferred to the laboratory. Forty *I. emarginata* (12–20 mm body length without antennae) were taken from a culture at the Biologische Anstalt Helgoland. Due to problems in one aquarium containing *I. emarginata* in 2007, the sample size of this experiment was reduced to nine, while the sample

size was 10 in the remaining three experiments. From each L. digitata, five undamaged $5 \text{ cm} \times 5 \text{ cm}$ pieces (2–3.5 g) were cut from the lower blade, and all visible epibionts were removed with a soft sponge. Afterward, each piece was placed over a quadratic 9 cm² central opening between two layers of green plastic mesh (18.5×16 cm, mesh size 1 mm) that were held by a 1 cm wide PVC frame. This arrangement was vertically inserted in the middle of a transparent plastic aquarium $(32 \times 19 \times 17 \text{ cm}, 8.1 \pm 0.4 \text{ L volume})$, dividing the aquarium into one upstream and one downstream compartment of equal size. Each of the 50 aquaria used per experiment was supplied with a unidirectional flow $(1.4 \text{ L} \cdot \text{h}^{-1})$ of filtered (sand and cotton) and aerated seawater at ambient temperature coming from the North Sea next to the laboratory. Each aquarium (experimental unit = EU) was irradiated by fluorescent lamps (TL-D de Luxe 36W/950; Philips, Amsterdam, the Netherlands) emitting, on average, $45 \pm 4 \mu mol$ photons \cdot $s^{-1} \cdot m^{-2}$ to the bottom of the aquaria. This irradiance simulated ambient PAR levels, ranging at 0.5 m water depth from 39 ± 1 to 89 ± 2 µmol photons $\cdot s^{-1} \cdot m^{-2}$ between January and May, respectively. Measurements of light regimes in the laboratory and the field were done with a cosine quanta sensor (LI-COR UWQ 6534), connected to a LI-1400 data logger (both LI-COR Biosciences, Lincoln, NE, USA).

Each experiment was divided into two phases. During the 4 d long precondition phase, four of the five algal pieces originating from the same L. digitata individual were randomly allocated to one of the four treatments: First, five specimens of L. vincta were added to the upstream compartment of a single EU and allowed to graze on the accessible surface of L. digitata for 4 d (Lacuna damage). Second, the accessible surface of Laminaria pieces was superficially removed with a scalpel, simulating *Lacuna* grazing (artificial damage). Laminaria surface tissues were removed at the same daily rate in the artificial treatment as in the Lacuna treatment by snails. Third, no treatment was applied during the first 4 d (intact surface). Fourth, in the absence of L. vincta, the algal piece did not cover the entire central opening so that the margin of the piece was accessible to grazers in the subsequent experimental phase (accessible margin). No isopods were added during the precondition phase. The second, 8 d long, grazing phase started at day 5 of the experiments by removing all L. vincta from the setup and adding one isopod to the upstream compartment of each of the four EUs containing pieces of replicate kelp specimens. Isopods were offered a mixed diet of Fucus spp., Mastocarpus stellatus, and Ascophyllum nodosum during the precondition phase to avoid unnatural high consumption during the grazing phase of the experiment. The fifth piece of each Laminaria specimen remained during both phases without treatment in the EU and was used to calculate nonfeeding-related autogenic changes in wet mass during the grazing phase. The wet mass of each algal piece was measured to the nearest 0.001 g at the beginning and end of the grazing phase after spinning each piece 10 times in a salad spinner. Actual consumption (AC) was then calculated by using the formula of Peterson and Renaud (1989)

$$AC = LI_b \times (C_e \times C_b^{-1}) - LI_e$$
⁽²⁾

where LI and C indicate the wet mass of an *L. digitata* piece exposed to isopods during the grazing phase and of the autogenic control, respectively, and the subscripts b and e indicate the beginning and end of the grazing phase, respectively. Actual consumption of each replicate piece was calculated with a separate piece of autogenic controls to include the variation in growth rates of kelp pieces (Roa 1992). To normalize consumption rates among EUs having different isopod biomasses, the wet mass of each isopod was recorded after blotting animals dry with paper towel and dividing consumed algal biomass by isopod biomass to express algal consumption per gram isopod biomass.

Design and setup of the induction experiment. To assess the possibility that inducible seaweed responses caused the observed patterns in the multigrazer experiment, an induction experiment was conducted with I. emarginata and L. vincta as well as with I. granulosa and L. vincta between 13 and 29 April 2007 and between 9 and 25 April 2008, respectively. The induction experiments tested the hypothesis that the palatability of previously L. vincta-grazed pieces of L. digitata was significantly different from ungrazed controls for subsequently feeding grazers. Twelve L. digitata were collected at the study site and transferred within 2 h to the laboratory. Twelve pieces were cut from the blade of each specimen (2-4 g) after removing all visible epiphytes with a sponge but without damaging the tissues of L. digitata. Pieces originating from the same specimen (i.e., being genetically identical) were randomly allocated in triplets to four plastic aquaria (Fig. 1, see multigrazer experiment for aquarium specifications), resulting in a total of 48 aquaria (n = 12). During the subsequent 4 d long acclimation phase, all pieces of L. digitata were maintained without grazers at the same irradiance level and water flow rate as in the multigrazer experiment. The 8 d long induction phase started on day 5 of the experiments by adding six L. vincta to one aquarium and six isopods to a second aquarium (=inducers) of the four aquaria containing genetically identical pieces of L. digitata. No grazers (control) were added to the remaining two aquaria (Fig. 1). This was performed with all replicate L. digitata, resulting in a total of 12 aquaria containing either snails or isopods and 24 grazerfree aquaria. At the end of the induction phase, all grazers were removed from the aquaria, and all algal pieces weighed. Subsequently, the relative palatability of previously grazed and ungrazed control algae was compared in 3 d long choicefeeding assays, using either one naïve L. vincta or one isopod (=consumer). Choice-feeding assays are regarded as the appropriate way to determine food preferences (Roa 1992, Taplin 2007), while no-choice assays do not allow an assessment of feeding preference because the consumer has no choice (Peterson and Renaud 1989, Roa 1992). Four different types of feeding assays were set up, testing for changes in algal palatability when inducer and consumer were either conspecifics or heterospecifics (Fig. 1). Two algal pieces were transferred from each aquarium to different feeding arenas made of glass petri dishes (filled with 2 L seawater), containing either L. vincta or isopods (see Fig. 1 for allocation pattern). The third algal piece from each aquarium was transferred to a feeding arena without grazers to assess nonfeeding-related changes in algal wet mass (autogenic control). As we calculated consumption rates of algal pieces in feeding arenas with separate pieces of autogenic control, rather than using a correction constant, we did not suppress the variance in autogenic controls (Peterson and Renaud 1989, Roa 1992). Consequently, error variance was not underestimated, and statistical significance not inflated. Furthermore, autogenic controls shared the entire treatment history with those pieces used in feeding assays; that is, kelp pieces used as autogenic controls during feeding assays were exposed to the same level of grazing as pieces that were used in the feeding assay. In this way, we minimized any effects of differential stimulation of physiological or ecological changes between grazed and nongrazed algae (Peterson and Renaud 1989). To minimize the effects of waste products on grazer activity, the water in feeding arenas were exchanged every 12 h. At the end of feeding assays, all grazers were removed from feeding arenas, and algal wet mass was measured. The actual consumption of algal biomass during feeding assays was calculated by the same formula provided above. Data were omitted from the statistical analysis if grazers did not feed on pieces of L. digitata during the feeding assay, which resulted in different sample sizes among assays. Pieces of L. digitata showed neither at the end of the multigrazer nor the induction experiments any visible sign of deterioration. Furthermore, the use of autogenic controls minimized confounding effects of nonvisible changes in algal biomass that might have been caused by the way the experiments were conducted.

Statistical analysis. One-factor analyses of variance (ANOVA) compared differences in (i) total in situ feeding damage among months (seven levels, fixed), (ii) tissue toughness among intact thallus parts (three levels, fixed), (iii) the elemental content and (iv) C:N ratio among thallus parts (four levels, fixed), and (v) consumption rates among the four fixed treatments from the multigrazer experiment. Using two-factor repeated-measures ANOVA, we assessed (i) thallus part



FIG. 1. Setup of induction experiments for a single replicate of *Laminaria digitata* showing the allocation of algal pieces (octagons) to different aquaria (rectangles) and feeding arenas (circles) during the experiment. Stippled and solid lines indicate containers with and without grazers, respectively. Numbers and letters denote grazer-exposed and control pieces of *L. digitata*, respectively. Feeding assays in which inducer and consumer species were identical are marked "conspecifics," whereas "heterospecifics" indicates feeding assays where inducer and consumer were different species.

specificity in feeding damage over the study period by comparing differences in the area of feeding damage of different thallus parts (four levels, fixed) among monthly samples (seven levels, fixed). Furthermore, two-factor ANOVA was used to assess tissue toughness between different surface conditions (two levels, fixed) among thallus parts (two levels, fixed). All ANOVAs were performed after confirming homogeneity of variances with Cochran's test. If applicable, data were log transformed to meet the assumptions. All one-factorial data sets with heterogeneous variances after transformation were analyzed by the nonparametric Kruskal-Wallis test. To allow testing for interactive effects in two-factor data sets with heterogeneous variances, the level of significance was set at $\alpha = 0.001$. For post hoc comparisons, Tukey's honest significant difference test was used to detect significant differences among treatments.

Using paired *t*-tests after confirming normality of differences with the Kolmogorov–Smirnov test, we determined whether previous consumption by *L. vincta* or *I. emarginata* as well as by *L. vincta* or *I. granulosa* altered the level of palatability in *L. digitata* for subsequent grazing by conspecifics or heterospecifics (induction experiment).

We used the program Gpower V3.0 (http://www.psycho. uni-duesseldorf.de/aap/projects/gpower) to estimate the percent probability of detecting differences (Faul et al. 2007) for those assays of the multigrazer experiment that showed no significant effect.

RESULTS

Estimates of ambient grazing pressure. The average cover of feeding scars on *L. digitata* ranged from 0.5% in June to 7.9% in August. The area covered with feeding scars changed significantly over the study period (one-factor ANOVA: $F_{6,49} = 9.23$, P < 0.001), being, on average, 3.5 to15 times higher in August than between April and June (Fig. 2). Furthermore, a significant 3–12 times greater area was covered with feeding scars in September than between April and June. Finally, feeding scar cover was up to 10 times higher in July than in May and June (Fig. 2).

There were also significant differences in the area of damage among thallus parts (repeated-measures two-factor ANOVA: $F_{3,147} = 10.99$, P < 0.0001). The upper blade (7.66 ± 5.6% [mean ± SD] consump-



FIG. 2. Mean (+SEM) total percent cover of feeding scars on individual *Laminaria digitata* at eight different sampling dates in 2006 (n = 8). Data for March are shown but excluded from analysis due to low replication (n = 4). Months sharing a letter were not significantly different.

tion of this thallus part) was, on average, a significant 4.6, 3.6, and 2.3 times more damaged than the stipe $(1.66 \pm 1.09\%)$, meristem $(2.13 \pm 3.9\%)$, and the lower blade $(3.33 \pm 3.6\%)$, respectively. A significant thallus part × time interaction (repeated-measures two-factor ANOVA: $F_{18,147} = 3.01$, P = 0.0001) indicates that significant differences in the area of feeding-related damage among thallus parts was dependent on the time of the year. In July but not at other times of the study period, the upper blade was more damaged than the other thallus parts.

Toughness. Tissue toughness was significantly different among intact thallus parts (one-factor ANOVA: $F_{2,27} = 197.64$, P < 0.001). The meristem was on average 1.5 and 2.7 times tougher than the lower and upper blade, respectively. In addition, the lower blade was a significant 1.8 times tougher than the upper blade (Fig. 3). Damaged blade tissues were a significant 13% lower in toughness than undamaged parts (two-factor ANOVA: $F_{1,22} = 5.43$, P = 0.029). Yet, a significant interaction with thallus parts (two-factor ANOVA: $F_{1.22} = 4.58$, P = 0.044) indicated that damage-related changes in toughness were dependent on the thallus part that was damaged. While damage reduced toughness significantly by 20% in the lower blade, no such effect was apparent in the upper blade (Fig. 3).

Elemental composition of thallus parts. There were only small (<8%) nonsignificant differences in carbon (average ±SD of 29.28 ± 1.4% of algal wet mass), nitrogen (1.39 ± 0.19%), and hydrogen contents (4.43 ± 0.39%) among the four tested kelp parts (one-factor ANOVA: all $F_{3,28} < 1.02$, P > 0.40). Moreover, the C:N ratio (mean ± SD of 21.46 ± 2.41) was a nonsignificant <4% different among the four kelp parts (one-way ANOVA: $F_{3,28} = 0.18$, P = 0.912).

Multigrazer experiment. I. granulosa: In 2006, the consumption of *L. digitata* by *I. granulosa* was not significantly different among blade treatments (one-factor ANOVA: $F_{3,36} = 0.64$, P = 0.596, Fig. 4A). However, the power for detecting significant



FIG. 3. Mean (+SEM) toughness of undamaged (black) and natural grazer-damaged (white) areas of the meristem, upper blade, and lower blade (n = 7).



FIG. 4. Mean (±SEM) daily consumption of lower *Laminaria digitata* blade per gram grazer wet mass by *Idotea granulosa* in 2006 (A) and 2007 (B) and by *I. emarginata* in 2006 (C) and 2007 (D) among four treatments (undamaged = intact surface; *L. vincta* damaged = surface damaged by grazing of the gastropod *Lacuna vincta*; artificially damaged = surface damaged with a scalpel; accessible margin = intact surface with accessible blade margin). Sample size was 10 and nine in 2006 and 2007, respectively. Note different scales of abscissa for both species of isopods. Stippled lines indicate the level of no consumption. Treatments sharing a letter were not significantly different.

differences was very low (17%). In 2007, previous grazing by *L. vincta* and artificial damage of blades significantly increased consumption rates of *I. granulosa* (one-factor ANOVA: $F_{3,32} = 3.39$, P = 0.030, Fig. 4B).

I. emarginata: In 2006, manipulation of blade surfaces significantly changed the consumption of *L. digitata* by *I. emarginata* (Kruskal–Wallis test: $H_{3,40} = 14.37$, P = 0.002). Isopods consumed significantly more biomass from *L. digitata* pieces that were previously grazed by *L. vincta* and when blade margins were accessible than from undamaged pieces. Artificially damaged blades were of intermediate attractiveness (Fig. 4C). In 2007, *I. emarginata* consumed undamaged blades significantly less than blades that were previously grazed by *L. vincta* or artificially damaged, while blades with accessible margins were consumed at intermediate levels (onefactor ANOVA: $F_{3,32} = 4.35$, P = 0.011, Fig. 4D).

Induction experiment. The palatability of ungrazed pieces of *L. digitata* was never significantly different than the palatability of pieces that were previously grazed by *I. granulosa*, *I. emarginata*, or *L. vincta*, regardless of whether conspecifics or heterospecifics were used as consumers in feeding assays (paired *t*-tests: all t < 2.75, all P > 0.05 with degrees of freedom ranging between 4 and 11, Fig. 5). The relative feeding preference of *L. vincta* for *L. digitata* pieces that were previously grazed by conspecifics and

ungrazed controls was not significantly different between both years (Student's *t*-test: $t_{13} = -1.17$, P = 0.263).

DISCUSSION

In situ grazing impact on *L. digitata* was the strongest from July to September and decreased within the sporophyte monotonically from apical (blade) to basal (stipe) parts. Blade preference was unrelated to the elemental composition of kelp parts. In the laboratory, feeding scars caused by *L. vincta* stimulated consumption by isopods. A reduction in the toughness of *L. vincta*-grazed kelp blades explained this indirect effect better than grazing induced changes in kelp palatability.

Both isopod species strongly consumed more biomass from previously *L. vincta*-grazed than undamaged kelp. Thus, the nonlethal grazing impact by *L. vincta* facilitated kelp consumption by another associated mesograzer (i.e., *I. granulosa*), as well as by a nonassociated species (i.e., *I. emarginata*). Viejo and Arrontes (1992) also reported positive indirect interactions between macroalgae-associated mesograzers with grazing by the isopod *Dynamene bidentata* enhancing the consumption of the brown seaweed *Fucus vesiculosus* by the amphipod *Hyale nilssoni*. The authors speculated that superficial damage of the alga by grazing isopods provided



FIG. 5. Mean relative preference (\pm SEM) during 3 d choice feeding assays of previously *Idotea emarginata* (IE)–, *I. granulosa* (IG)–, or *Lacuna vincta* (LV)–grazed pieces of *Laminaria digitata* as a percentage of total consumption [consumption grazed alga/(consumption grazed + control alga) × 100] for different grazer combinations. The first is the inducer, and the second is the consumer. The stippled line indicates the null hypothesis (i.e., no preference of grazed and control alga). Bold numbers refer to sample size. Black = assays conducted in 2007; white = assays conducted in 2008.

suitable areas for amphipod grazing but did not further investigate the underlying mechanism. Alternatively, a grazing-induced stimulation of seaweed consumption, as suggested by Hay (1996), could explain higher consumption rates of isopods on L. vincta grazed than on undamaged kelp blades. Empirical work by Diaz et al. (2006) confirmed for two species of red macroalgae that previous grazing can stimulate consumption by subsequently grazing conspecifics. However, the induction experiments of our study did not support that grazing by L. vincta induced traits in kelp that increased the palatability of L. digitata for any of the isopod species significantly. Thus, it seems more likely that L. vincta grazing enhanced the accessibility rather than the palatability of kelp for isopods.

Several results of this study support the statement that an enhanced accessibility of kelp blades could be the mechanism by which L. vincta facilitates kelp consumption by isopods. First, the observation that isopods preferably position themselves along the margin of kelp blades, in combination with the measured intermediate increase in kelp consumption by isopods that had access to the margin of kelp blades, indicates that successful grazing of isopods may depend to some degree on the ability to attach to kelp blades. Although we made no direct measurements, L. vincta-damaged kelp blades probably increase attachment abilities of isopods by disrupting the smooth and slippery surface of kelp blades, especially in wave-exposed conditions like at our study site. This statement is indirectly corroborated by Schultze et al. (1990), who recorded minimal densities of kelp-associated animals on the blades, and this pattern was even stronger for kelps collected at a wave-exposed site. Yet, other reasons for lower densities on blades, like predator avoidance, should also affect isopod distribution along kelp sporophytes.

Second, direct measurements of the toughness of L. vincta-grazed and undamaged blades indicate that previous L. vincta grazing lowered blade toughness, suggesting that surface layers are harder than interior kelp tissues and may serve as a physical antiherbivory defense. In kelps, epidermal cells are small and tightly packed, whereas cortical cells (those below the surface cell layer) are increasingly larger toward the interior of the thallus (Chung et al. 1987, Scrosati 1993). Thus, the concentration of cell-wall material (per unit volume) is higher in surface layers than in deeper (cortical) layers. It should be most effective to position the toughest tissues at the blade surface because this is where grazers will encounter them first. Similarly, Tugwell and Branch (1989) reported about near-surface positioning of chemical antiherbivory defenses in several species of kelps. Furthermore, work by Padilla (2001) indicates that kelps can induce changes in the feeding apparatus of mesograzers that enhance their feeding abilities. She showed that changes from blunt to pointed teeth in two Lacuna species occurred when their diet changed to kelp, suggesting that the toughness of the kelp sporophyte may be a selective trait for optimizing kelp consumption by mesograzers.

Third, comparable amounts of consumed kelp biomass of artificially damaged and *L. vincta*-damaged kelp blades (at least in 2007) indicate that changes in the physical properties of kelp were more important in the facilitation of isopod grazing than *L. vincta*-associated cues.

Fourth, the observed spatiotemporal patterns of in situ grazing pressure indirectly support the statement that L. vincta grazing enhanced the accessibility of kelp biomass for isopods by lowering the kelp toughness. In situ grazing impact was highest on the upper blade, which was also the softest part of the kelp sporophyte. Measurements of the elemental composition suggest that differences in grazing pressure may not be based on the nutritive quality of kelp parts. Yet, upper blade parts were only preferred in July, while feeding impact was of comparable magnitude among kelp parts later in summer. Probably, consumption by L. vincta during July when this snail reaches maximum densities on L. digitata (Janke 1986, Reichert 2002) increased the accessibility of biomass of lower parts of L. digitata blades for isopods and other associated mesograzers that were hardly able to consume this part of the kelp sporophyte when it was less damaged (i.e., before July).

The effect of L. vincta grazing on the consumption rate of both species of isopods was comparable in direction and magnitude and, at least for I. emarginata, reproducible in time. This finding suggests that the indirect positive effect of L. vincta grazing may be persistently beneficial for mesograzers that are not associated with L. digitata. I. emarginata is classified as a good swimmer (Ingolfsson and Agnarsson 2003) that is able to move between its preferred habitat-that is, decaying kelp on the seafloor (Franke et al. 2007)—and attached specimens of kelp. Probably, I. emarginata visits attached L. digitata to broaden its diet. Although not tested in this study, amphipods, which occur in extremely high abundance in the maze of the L. digitata holdfast (Chrapkowski 2005), where they find maximum shelter but least accessible food, may experience even stronger benefit from the grazing activity of L. vincta, as they are smaller in size and may be less able to penetrate the tough surface of L. digitata than the larger isopods. Work by Viejo and Arrontes (1992) confirms positive indirect effects of grazing trails by other species of grazers on the feeding rate and growth of amphipods.

The beneficial effects of *L. vincta*–feeding damage on the consumption rate of isopods should be negligible for *L. digitata*, which yearly replaces the blade, thereby losing potentially more biomass than by the here-reported extra consumption of isopods. However, from the perspective of the isopods, the availability of *L. vincta*–damaged *L. digitata* should be for several reasons advantageous. First, food supply is maximized during summer (i.e., the period of strongest breeding activity, Salemaa 1986). Second, under the assumption that the visibility of isopods to their predators is higher at the margin than in more basal parts of the kelp sporophyte, gastropod feeding scars on kelp blades may increase isopod survival by avoiding microhabitats of higher predation risk. For instance, the study by Jormalainen et al. (2001) revealed the existence of a differential predation risk for isopods between apical and basal parts of the brown seaweed *F. vesiculosus*. Finally, it could be speculated that internal kelp tissues may pose a higher food quality for isopods than epidermal tissues.

In contrast to terrestrial research, few marine studies addressed the role of changing morphological or chemical species traits in indirectly mediating consumer interactions among multiple species (Long et al. 2007). To our knowledge, only Viejo and Arrontes (1992) demonstrated for another important species of seaweed (i.e., Fucus vesiculosus) that grazing-related changes in its morphology facilitated consumption by other species of grazers. The study by Raimondi et al. (2000) revealed that morphological trait change can result in trait-mediated indirect effects that affect community structure. Thus, future models predicting the structure of marine communities need to consider besides the lethal trophic interactions also nonlethal effects to fully understand the mechanisms and consequences of consumption in shaping species interactions and ultimately ecological communities.

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