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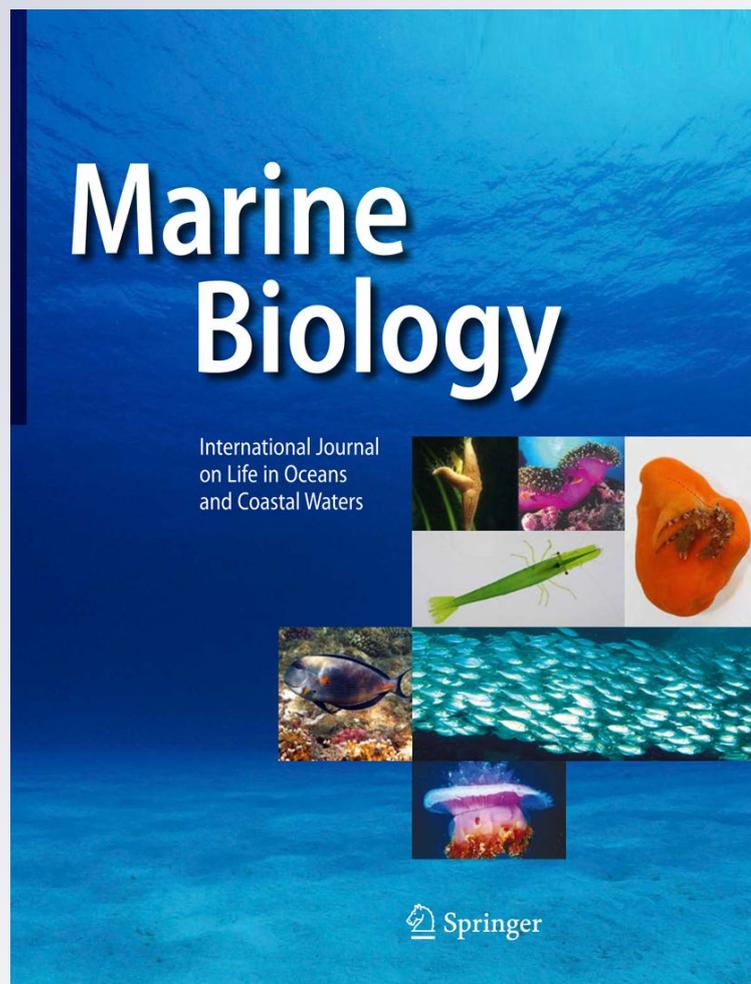
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Herbivorous amphipods inhabit protective microhabitats within thalli of giant kelp *Macrocystis pyrifera*

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Abstract Many small marine herbivores utilize specific algal hosts, but the ultimate factors that shape host selection are not well understood. For example, the use of particular microhabitats within algal hosts and the functional role of these microhabitats have received little attention, especially in large algae such as kelps. We studied microhabitat use of the herbivorous amphipod *Peramphithoe femorata* that inhabits nest-like domiciles on the blades of giant kelp *Macrocystis pyrifera*. The vertical position of nest-bearing blades along the stipe of the algal thallus and the position of the nests within the lateral blades of *M. pyrifera* were surveyed in two kelp forests in northern-central Chile. Additionally, we conducted laboratory and field experiments to unravel the mechanisms driving the observed distributions. *Peramphithoe femorata* nests were predominantly built on the distal blade tips in apical sections of the stipes. Within-blade and within-stipe feeding preferences of *P. femorata* did not explain the amphipod distribution. Amphipods did not consistently

select distal over proximal blade sections in habitat choice experiments. Mortality of tethered amphipods without nests was higher at the seafloor than at the sea surface in the field. Nests mitigated mortality of tethered amphipods, especially at the seafloor. Thus, protective microhabitats within thalli of large kelp species can substantially enhance survival of small marine herbivores. Our results suggest that differential survival from predation might be more important than food preferences in determining the microhabitat distribution of these herbivores.

Introduction

Given that many macroalgal species and tissues vary greatly with respect to food quality and predator refuge, the distribution and abundance of small marine herbivores (i.e., mesoherbivores) are determined largely through a combination of complex habitat selection decisions (e.g., food value vs. shelter) and post-selection processes (e.g., predation; Duffy and Hay 1991; Pérez-Matus and Shima 2010). Most studies have emphasized the factors (e.g., tissue palatability, herbivore performance, protection from predation) determining mesoherbivore distribution in the presence of multiple algal species (Hay et al. 1988; Buschmann 1990; Duffy and Hay 1991; McDonald and Bingham 2010). In contrast, the factors shaping the within-plant distribution of mesoherbivores are less understood. This is surprising given that mesoherbivores often inhabit hosts containing microhabitats that vary in both palatability (Poore 1994; Cronin and Hay 1996) and the protection they provide from predators and abiotic stress (Sotka 2007). Within-thallus heterogeneity is particularly relevant for small herbivores with narrow host spectra that live on large algal hosts. These herbivores are bound to specific host

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species and forage on small spatial scales. Accordingly, habitat selection decisions and post-selection processes might have similar effects on microhabitat use of small herbivores within larger, more differentiated hosts as they do on habitat use between several, less-differentiated host species.

For example, kelp-associated organisms are expected to have a heterogeneous distribution because they live on large, well-differentiated hosts with tissues that span a range of morphological complexity, palatability, and shelter from predators (Merilaita and Jormalainen 2000; Sotka 2007; Pansch et al. 2008). In support of this hypothesis, the abundance of some amphipod species on giant kelp *Macrocystis pyrifera* increased from the holdfast toward the apical thallus sections. For example, the nest-building, herbivorous amphipod *Peramphithoe plea* was more abundant on apical than on basal blades of giant kelp (Coyer 1984).

Some mobile mesograzers frequently switch within the thallus of their host algae between nutrient-rich and less palatable but protective microhabitats (Salemaa 1987; Merilaita and Jormalainen 1997, 2000). In contrast, more sedentary mesoherbivores cannot move quickly between high-quality food algae (or algal parts) and algae (or parts thereof) that provide efficient protection, and they might be confronted with a trade-off between food and shelter quality. Previous studies have indicated that predation, predator avoidance, and abiotic stress (temperature, salinity, wave, and emergence) largely determine the within-thallus distribution of sedentary mesoherbivores. For example, Coyer (1984) reported that fish predators shape the vertical distribution of the nest-building amphipod *P. plea* in a kelp forest of *M. pyrifera*. Similarly, amphipods may choose algal habitats based on the protection they provide from predation (Duffy and Hay 1991; Vesakoski et al. 2008) or wave stress (Sotka 2007).

Based on the above observations, we tested the relative importance of shelter and feeding preference in shaping the distribution of a sedentary marine mesoherbivore within thalli of a large and complex kelp species. The herbivorous amphipod *Peramphithoe femorata* is a kelp specialist that constructs nest-like domiciles on blades of *M. pyrifera* (Cerda et al. 2010). *Peramphithoe femorata* build nests by connecting the lateral blade edges with amphipod silk that is produced in glands at the third and fourth pair of pereopods. Nests are commonly occupied by single individuals. Conspecifics are aggressively repelled when trying to enter occupied nests (see also Holmes 1901). Cerda et al. (2010) also observed that nest occupancy is relatively short, and most amphipods left their nest after 1–4 days. Outside the nests, free amphipods have been found to crawl over all parts of the host thallus, feeding on various algal tissues as indicated by small grazing marks. Free *P. femorata* quickly (within about 2 h) constructed new, stable

nests on blades of *M. pyrifera* that have been offered in laboratory tanks (Cerda et al. 2010). The giant kelp, *M. pyrifera*, is the major host of *P. femorata* along the Chilean coast, but amphipods have also been found associated with young individuals of the kelp *Lessonia nigrescens* (MT pers. obs.). Amphipods can be locally common and are easily maintained in the laboratory, thus being an ideal model organism to examine the factors shaping the distribution of sedentary mesograzers.

Herein, we studied the microhabitat distribution, selection, and survival of *P. femorata* in northern-central Chile. In their natural environment, we surveyed the distribution of amphipods within stipes and blades of *M. pyrifera*. In laboratory choice assays, we tested whether *P. femorata* showed feeding preferences for particular tissue types within thalli of *M. pyrifera*. In microhabitat choice experiments, we investigated whether *P. femorata* preferentially associated with particular thallus sections of *M. pyrifera*. The mechanisms underlying any preferences were not the focus of the current study. Microhabitat-specific mortality of *P. femorata* was measured in natural kelp forests by tethering amphipods to different microhabitats within thalli of *M. pyrifera*. To test the generality of our results, the field surveys and experiments were conducted in two large kelp forests separated by ~400 km. Also, the laboratory assays were done with organisms from these two study sites.

Materials and methods

The study was conducted in two nearshore (2–5 m deep) kelp forests in northern-central Chile: Los Vilos (31°54'S, 71°31'W) and Playa Blanca (28°10'S, 71°10'W) (Fig. 1a). Both kelp forests are dominated by *M. pyrifera*, but other large kelps (e.g., *Lessonia trabeculata* and *L. nigrescens*) and diverse understory algae are also common.

Distribution of amphipod nests within thalli

Individual stipes were sampled to examine the distribution of amphipods *P. femorata* within *M. pyrifera* thalli ($n = 103$ in Los Vilos and $n = 93$ in Playa Blanca). Randomly selected stipes were cut off directly above the holdfast and carefully transferred into individual mesh bags (80 × 35 cm; mesh size = 1 mm). Stipe lengths ranged from 27 to 435 cm (135 ± 85 cm; mean ± SD) in Los Vilos and from 65 to 444 cm (170 ± 73 cm; mean ± SD) in Playa Blanca. Immediately after transferring the mesh bags to the shore, each stipe was surveyed for the presence of *P. femorata* and the position of their nests.

The specific thallus morphology of *M. pyrifera* allows determining the vertical position of nest-bearing blades along the central stipe (hereafter, within-stipe distribution)

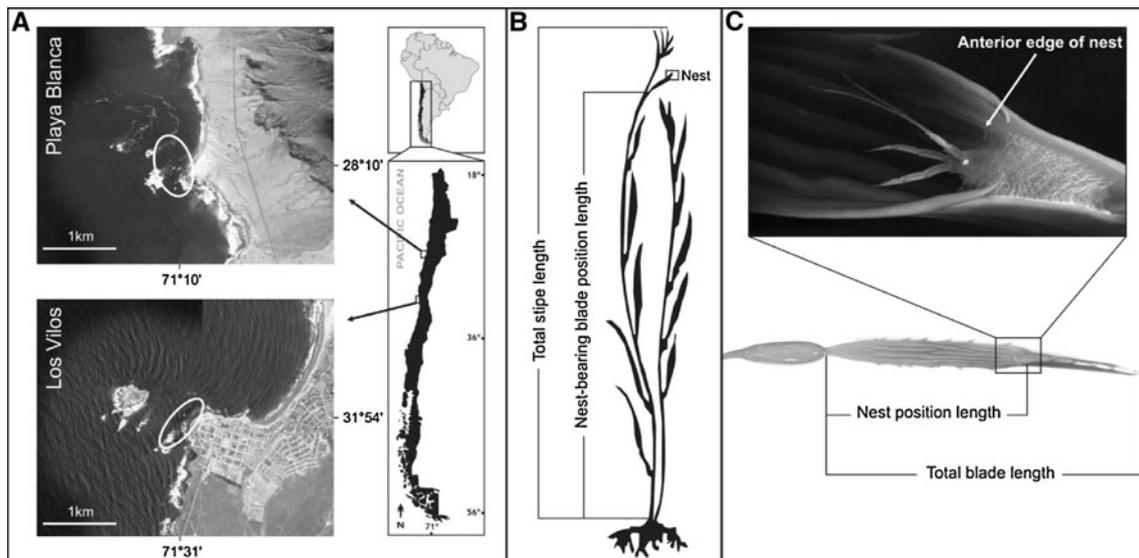


Fig. 1 **a** Map of Chile with the locations of the two kelp forests of Los Vilos and Playa Blanca. Measurements made on **b** stipes and **c** blades of giant kelp *Macrocystis pyrifera* to determine within-

thallus positions of nests of *Peramphithoe femorata*. Magnification shows an amphipod *P. femorata* inside its nest

and the position of the nests along the lateral blades (hereafter, within-blade distribution). For the within-stipe distribution, the distance from the basal end of the stipe (i.e., where the stipe was cut above the holdfast) to the node of each nest-bearing blade was measured (Fig. 1b). We also measured the total length of each stipe from the basal end to the apical growth meristem. We tested for a correlation of the stipe length and the vertical nest position length to examine whether the within-stipe nest position is primarily related to the stipe length or whether the amphipods have a preference to build nests at a certain height above the seafloor. A positive correlation would suggest that the amphipods preferentially build nests in apical sections of the thalli. If a stipe contained more than one nest, we used a conservative approach and considered only the lowermost nest for the analysis. To standardize within-stipe distributions for stipes of different lengths, each stipe was subdivided into five equal sections, each representing 20% of the total stipe length. Then, the total number of nests in each stipe section was counted.

For the within-blade distribution, we measured the distance from the blade's proximal growth meristem to the anterior edge of the nest that we defined as the most proximal silk string holding the nest together (Fig. 1c). The total blade length from the proximal growth meristem to the distal blade tip was also measured. Each nest was surveyed for occupancy by amphipods and for grazing marks. Most blades had single nests, but at each sampling site, one blade was found with two nests. In this case, the position of only one randomly chosen nest was considered for the analysis. At Los Vilos, one nest was laterally open so it was excluded from the analysis. We encountered 54

empty nests in Los Vilos and 121 in Playa Blanca. Grazing marks in all nests except one from Playa Blanca confirmed recent occupancy by amphipods. In most cases where nests were empty, the corresponding free *P. femorata* was recovered in the mesh bags, indicating that the amphipods abandoned their nests during transport from the sampling site to the shore. For 26 out of 99 nests (i.e., 26.3%) in Los Vilos and for 42 of 154 nests (i.e., 27.3%) in Playa Blanca, no amphipods were recovered in the mesh bags. To examine the within-blade distribution of nests, each blade was subdivided into five sections, each representing 20% of the total length of the blade. The total number of nests (using all nests) in each respective blade section was counted. The distribution of nests within stipes and blades was tested for deviation from randomness using chi-square analysis.

Choice feeding assays

To examine the role of amphipod feeding preferences on nest location, both within-stipe and within-blade, we conducted choice feeding assays. These experiments were conducted in mesh bags that were suspended at sea outside the seawater laboratory of the Universidad Católica del Norte in Coquimbo, Chile (29°59'S, 71°22'W). After collection at the respective field sites, amphipods were brought to the laboratory where they were maintained separately in indoor flow-through tanks until being used in the experiments.

For the feeding assays, two blade pieces [~ 0.5 g Wet Weight (WW)] and a single *P. femorata* were added to small mesh bags (17 × 9 cm; mesh size = 1 mm).

Amphipods from the two kelp forests were only offered blade pieces from their home site. Grazer-free controls were set up to correct for autogenic changes in kelp WW. The mesh bags were suspended in the field ~0.5 m below the water surface of Bahía La Herradura. After 42 h, the final WW of the blade pieces was recorded.

To examine within-stipe feeding preferences, one blade from the basal region and one blade from the apical region of the same stipe of *M. pyrifera* were offered. We selected blades separated by at least three other blades. Only one pair of blades was used from each stipe so that all pairs of blades originated from different stipes. From each blade, we cut a single piece from the distal blade part. We only used blades ≥ 1 cm wide that lacked nests and grazing marks. This assay was conducted for Los Vilos amphipods ($N = 22$) only.

To examine within-blade feeding preferences, pieces from the proximal and distal parts of the same blade (Los Vilos, $N = 28$; Playa Blanca, $N = 32$) were offered to the amphipods. From each blade, we cut one 10-cm-long piece from the proximal part of the blade that is slightly distal to the growth meristem and one 10-cm-long piece from the distal part 1 cm proximal to the undamaged blade tip. Only blades from apical stipe sections were used that were ≥ 2 cm wide and lacked nests and grazing marks.

Weight-specific consumption rates were calculated using the equation $C = [T_i \times (C_f/C_i) - T_f]/G$ where C is the consumption rate of the grazer, T_i and T_f represent the WW of the kelp tissue offered to the grazer, C_i and C_f represent the WW of control tissue at the beginning (i) and at the end (f) of the assay, and G represents the amphipod body mass (WW). To account for the mutual dependency of the amphipod feeding rates on the two simultaneously offered algal pieces, choice feeding assays were analyzed by paired t tests. Two replicates from each site were excluded from the analysis of the within-blade choice feeding assay because the amphipods molted or died during the experiment.

Microhabitat choice experiments

Our initial observations suggested that most nests were built at the distal portions of blades. To test whether the amphipods prefer the distal blade sections, individual amphipods (52 and 48 from Los Vilos and Playa Blanca, respectively) were offered a choice of proximal and distal portions of blades from local kelp populations. Each blade piece was about 3×10 cm. The pieces were placed on opposite sides of a small container ($18 \times 13 \times 9$ cm; volume: 0.75 L) that was half filled with seawater. At the start of each assay (evening), one adult *P. femorata* was added to each container. Ten hours later (morning), we scored the location of each amphipod as either distal or

proximal blade piece. In Los Vilos, one amphipod molted during the experiment and was excluded from the analysis. Six (i.e., 11.8%) and five (i.e., 10.4%) amphipods from Los Vilos and Playa Blanca, respectively, did not make a clear choice for either blade piece and were, thus, also excluded from the analysis. For each site, chi-square analysis was used to determine whether the amphipod distribution was random. Subsequently, we contrasted the distributions of amphipods from the two sites with each other using chi-square analysis to test whether amphipod distribution depended on site.

Field experiments on amphipod survival

To test whether the within-stipe distribution of *P. femorata* in the field was controlled by differential amphipod mortality, free individual amphipods without nests (hereafter, leashed amphipods) and blades with individual amphipods in nests (hereafter, nest amphipods) were tethered to *M. pyrifera* in the field. The experiments were conducted in accessible patches of the kelp forests in Los Vilos and Playa Blanca.

Tethers, consisting of 25 cm segments of nylon fishing lines (0.3 mm diameter), were fixed to the dorsal surfaces of living amphipods with superglue (Loctite Super Bonder). The free end of the tether was tied to a metal clip. Tethered amphipods were then clipped to haphazardly selected stipes separated by at least 1 m. Each stipe received a single amphipod that was either clipped to the basal part of the stipe near the seafloor (~10 cm above the holdfast) or to the apical part of the stipe near the sea surface (between the 2nd and 5th free blade below the apical growth meristem of the stipe). Three out of 30 tethers (i.e., 10.0%) of leashed amphipods were lost in Los Vilos and could not be recovered at the end of the experiments. These tethers were excluded from the analysis. In Playa Blanca, 11 out of 61 (18.0%) tethers of leashed amphipods were lost and, thus, not included in the analysis.

Entire blades with occupied nests were detached from their original stipes in the field. While in the field, these nest-bearing blades with their amphipod inhabitants were then cable tied to clips that were attached to nearby stipes either at the sea surface or the seafloor. After attachment, we carefully reconfirmed the presence of amphipods in each nest. To aid in relocation, stipes with tethers were marked with buoys and all clips and cable ties were marked with fluorescent pink flagging tape. Nest amphipods were tethered simultaneously in an adjacent area of the kelp forest. Four out of 30 (i.e., 13.3%) and six out of 31 (i.e., 19.4%) tethered nests were lost in Los Vilos and Playa Blanca, respectively. These replicates were excluded from the analysis.

After 24 h, we recovered tethers and recorded the presence/absence of live amphipods. One leashed

amphipod from Playa Blanca was dead at the end of the experiment but still tethered to the kelp. This amphipod was considered as not recovered. All other recovered amphipods were alive and showed no obvious impairments—some of the leashed amphipods were maintained after the experiments for several days in large holding tanks where they built nests in the same way as free amphipods. The experiments were analyzed separately for leashed amphipods and for nest amphipods by a $2 \times 2 \times 2$ contingency table (Sokal and Rohlf 1969) with the criteria being “Site” (Los Vilos vs. Playa Blanca), “Position” (seafloor vs. surface), and “Recovery” (amphipod recovered vs. amphipod not recovered).

Results

Distribution of amphipod nests within thalli

At both sites, nests of *P. femorata* were not randomly distributed within the stipes ($\chi^2 = 16.50, p = 0.002$ for Los Vilos; $\chi^2 = 26.10, p < 0.001$ for Playa Blanca). The majority of the nest-bearing stipes (~90%) had nests in the top 40% of the stipes near the sea surface (Fig. 2). In

the kelp forest of Los Vilos, the number of nests decreased steadily from the apical toward the basal stipe sections while in Playa Blanca, 6% of the nests were also found in the lowermost stipe section. Accordingly, the within-stipe distribution of the nests differed significantly between the two kelp forests ($\chi^2 = 11.43, p = 0.022$). At both sites, the vertical position of nest-bearing blades was correlated with the stipe length ($p < 0.001$ for both sites; see inserts in Fig. 2). Correlation coefficients R^2 were 0.87 for Los Vilos and 0.46 for Playa Blanca.

Similarly, nests of *P. femorata* were not randomly distributed along the blades of *M. pyrifera* ($\chi^2 = 69.64, p < 0.001$ for Los Vilos; $\chi^2 = 87.92, p < 0.001$ for Playa Blanca). At both sites, the amphipods predominantly built nests in the distal region of the blades—opposite from the growth meristem (Fig. 3). The within-blade distribution of the nests was similar in both kelp forests ($\chi^2 = 3.15, p = 0.534$).

Choice feeding assays

At Los Vilos, *P. femorata* did not feed preferentially on either apical or basal blades (Fig. 4a; $p = 0.602$). Amphipods from both sites consumed more tissue from proximal

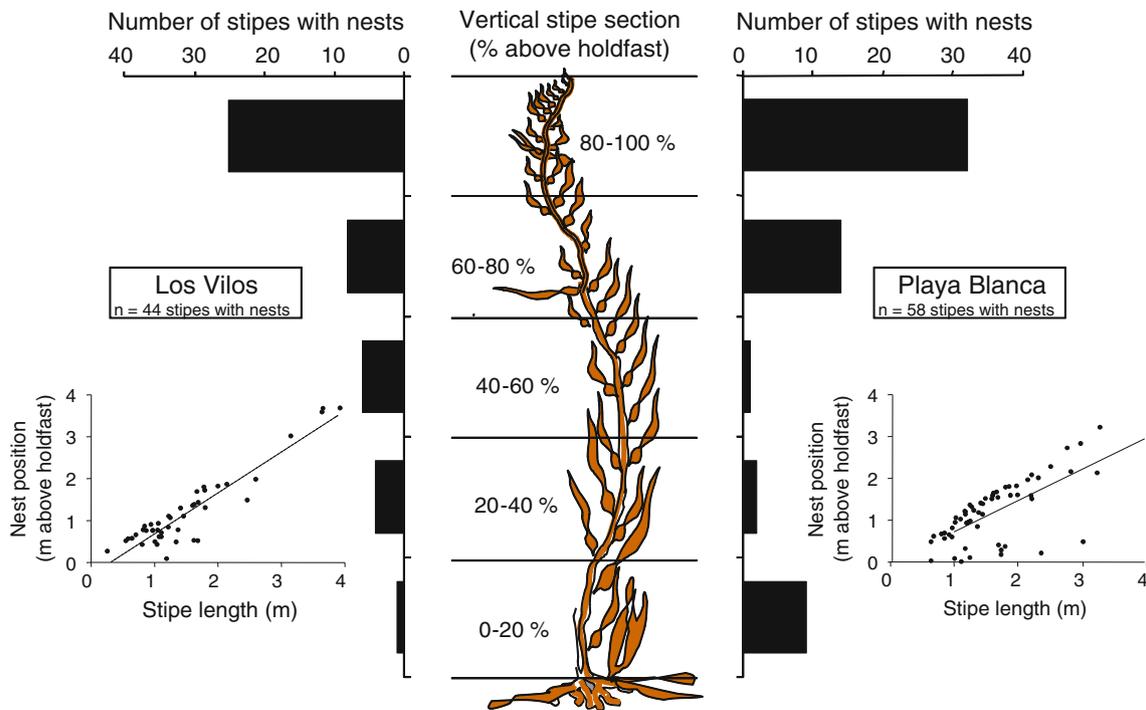


Fig. 2 Vertical within-stipe distribution of nests of *Peramphithoe femorata* on *Macrocystis pyrifera* in kelp forests at Los Vilos and Playa Blanca. For each nest-bearing stipe, only one nest position was considered. If a stipe carried more than one nest, only the lowermost nest was considered. *p* values from chi-square analysis (Los Vilos: $p = 0.002$; Playa Blanca: $p < 0.001$) indicate non-random within-stipe distributions of amphipods at both sites. Inserts: Correlation of

stipe length of *M. pyrifera* and the vertical position of blades that bear nests of *P. femorata* at both sites. Only the lowermost nest was considered if a stipe carried more than one nest. Correlation coefficients are $R^2 = 0.86$ for Los Vilos and $R^2 = 0.46$ for Playa Blanca. The correlation is significant for both sites (Los Vilos and Playa Blanca: $p < 0.001$)

blade pieces than from distal blade pieces (Fig. 4b). However, this was statistically significant only for Playa Blanca ($p = 0.012$) but not for Los Vilos ($p = 0.317$). The feeding preference for proximal blade pieces was in clear opposition to the dominant position of amphipod nests on the distal blade tips.

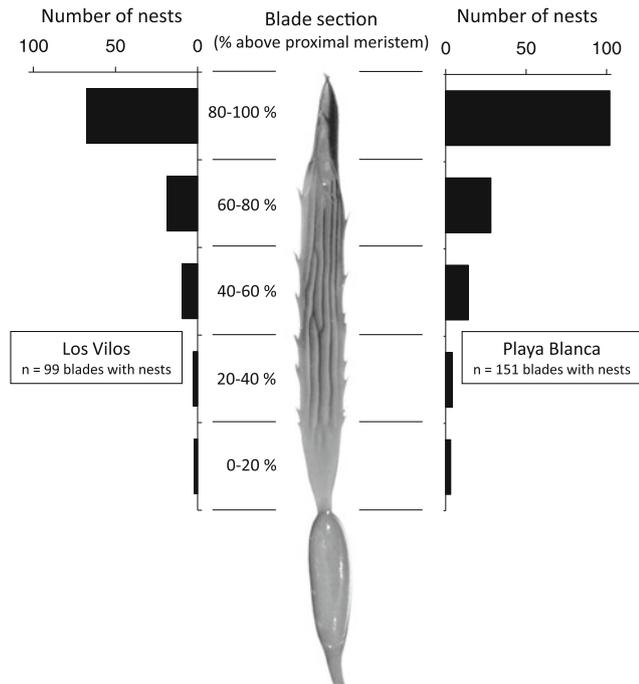
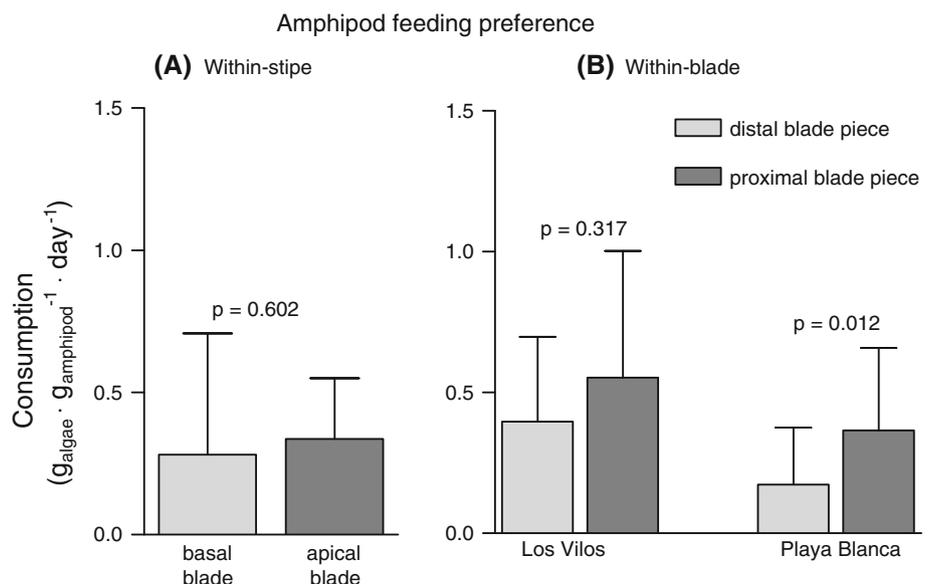


Fig. 3 Within-blade distribution of nests of *Peramphithoe femorata* on *Macrocytis pyrifera* in kelp forests at Los Vilos and Playa Blanca. For blades with more than one nest (i.e. one blade each in Los Vilos and Playa Blanca) only one randomly chosen nest was considered. p values from chi-square analysis indicate non-random within-blade distributions of amphipods at both sites (Los Vilos and Playa Blanca: $p < 0.001$)

Fig. 4 a Consumption rates (mean \pm SD) of *Peramphithoe femorata* from Los Vilos ($N = 22$) on blades from the apical and the basal region of stipes of *Macrocytis pyrifera* in choice assays. The result from the paired t test indicates no within-stipe feeding preferences of the amphipods ($p = 0.602$). **b** Consumption rates (mean \pm SD) of *P. femorata* from Los Vilos ($N = 28$) and Playa Blanca ($N = 32$) on distal and proximal pieces of blades of *M. pyrifera* in choice assays. The results from the paired t test indicate feeding preferences for the proximal blade pieces in amphipods from Playa Blanca ($p = 0.012$) but not from Los Vilos ($p = 0.317$)



Microhabitat choice experiments

In the microhabitat choice experiments, the choice of *P. femorata* was random at both sites, indicating no microhabitat preferences of the amphipods. In Los Vilos, 31 of 45 amphipods (68.9%) chose the distal part of the blades ($\chi^2 = 3.30$, $p = 0.070$; Fig. 5). In Playa Blanca, only 15 of 43 individuals (34.9%) chose the distal part of the blade while 28 (65.1%) chose the proximal part ($\chi^2 = 1.99$, $p = 0.159$). Thus, microhabitat choice of *P. femorata* differed between the two sites ($\chi^2 = 10.19$, $p = 0.001$).

Field experiments on amphipod survival

For leashed amphipods, the three criteria “Site,” “Position,” and “Recovery” were not jointly independent ($G = 186.30$, $p < 0.001$). The recovery rate of leashed amphipods was similar in both kelp forests and, thus, independent of the site ($G = 2.87$, $p > 0.05$; Fig. 6). The experiments revealed higher survival of leashed amphipods in the upper part of *M. pyrifera* stipes. For both sites, more leashed amphipods were recovered from the sea surface (26.7 and 62.5% for Los Vilos and Playa Blanca, respectively) than from the seafloor (0.0 and 3.9% for Los Vilos and Playa Blanca, respectively). Accordingly, the recovery rates for the leashed amphipods depended on the vertical position of the tethers within the stipes of *M. pyrifera* ($G = 24.92$, $p < 0.001$).

When the amphipods were in nests, the overall recovery rate increased at both sites, especially in the nests from the seafloor. The three criteria “Site,” “Position,” and “Recovery” were not jointly independent ($G = 173.85$, $p < 0.001$). The recovery rates of nest amphipods were independent of the position of the tether ($G = 0.315$,

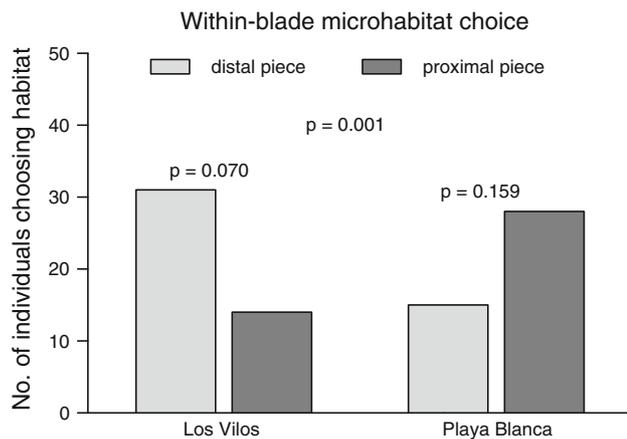


Fig. 5 Habitat preference of *Peramphithoe femorata* from kelp forests in Los Vilos and Playa Blanca when offered pieces from the distal or the proximal parts of blades of *Macrocystis pyrifera*, respectively. The results from the chi-square analysis indicate random habitat choice for amphipods from both sites ($p = 0.070$ and $p = 0.159$ for Los Vilos and Playa Blanca, respectively) but different habitat choice between amphipods from Los Vilos and Playa Blanca ($p = 0.001$)

$p > 0.5$) and, thus, did not differ between the sea surface and the seafloor (Fig. 6). Overall, recovery of nest amphipods was higher in Los Vilos than in Playa Blanca ($G = 11.05$, $p < 0.001$).

Discussion

Our field survey confirmed that *Peramphithoe femorata* occupy specific microhabitats within thalli of *Macrocystis pyrifera*. Amphipod nests were mainly found on the distal tips of blades in apical sections of the stipes. This specific within-thallus distribution of *P. femorata* was evident in both studied kelp forests. In contrast to this distinct distribution pattern, amphipods showed no clear feeding preferences for the respective thallus parts. Similarly, *P. femorata* did not make a clear choice for a particular blade section as microhabitat that would explain the observed within-blade distribution (mostly on distal tips) in the field. The only factor that was related to the natural distribution and was consistent between both sites was differential microhabitat-specific mortality. Our results thus indicate that nest-building *P. femorata* predominantly occupy microhabitats within thalli of *M. pyrifera* that maximize survival of small herbivores.

Feeding preferences and microhabitat choice of amphipods

Peramphithoe femorata did not distinguish between blades from basal and apical sections of the stipes in the choice

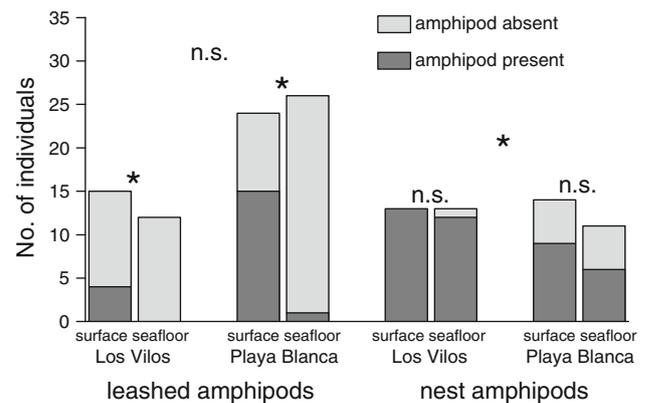


Fig. 6 Recovery of leashed amphipods and nest amphipods after field deployments in kelp forests in Los Vilos and Playa Blanca. The results from $2 \times 2 \times 2$ contingency tables indicate higher recovery of leashed amphipods at the surface than near the seafloor ($p < 0.001$). The recovery of leashed amphipods was independent of the site ($p > 0.05$). Recovery of nest amphipods was independent of the position of the tethers ($p > 0.5$) but dependent on site ($p < 0.001$)

feeding assays although previous studies have indicated clear chemical and structural within-thallus variations in *M. pyrifera* (Wheeler and North 1981; Pansch et al. 2008). This suggests that factors known to differ between apical and basal blades are unrelated to palatability for *P. femorata*. Alternatively, blade traits at our field site may have been independent of the within-stipe blade position.

The feeding preference of *P. femorata* for proximal blade sections clearly contrasts with the dominant nest position on the distal tips of the blades. The food choice of the amphipods did not match with the observed distribution of amphipod nests. Thus, herbivore feeding preferences do not appear to influence the microhabitat distribution of *P. femorata* within the thalli of *M. pyrifera*.

Habitat choice of marine mesoherbivores can be influenced by algal properties that are not related to tissue palatability. For example, some phytal amphipods tolerate nutritional deficiencies and choose hosts that allow for best offspring performance (Poore and Steinberg 1999; Sotka 2007). The results of our experiments showed that microhabitat choice of *P. femorata* clearly differed between Los Vilos and Playa Blanca. Differential microhabitat choice of amphipods from the two sites could suggest environmental differences or geographic variation in seaweeds, amphipods, or both. Herbivore populations may vary in their responses to seaweeds, perhaps as a result of local adaptation. For example, Sotka and Hay (2002) showed that geographically isolated populations of the amphipod *Ampithoe longimana* differed in their tolerance for grazer deterrent diterpene alcohols, and that this was related to the historical overlap of herbivore and seaweed ranges. Seaweed traits that influence food choice of herbivores can

vary from small (within plants to 10s of km; Honkanen et al. 2002) to large scales (100s to 1,000s of km; Sotka et al. 2003, Long and Trussell 2007). However, despite the differences in microhabitat choice among *P. femorata* from the two sites (separated by 400 km), the distribution of amphipod nests within blades of *M. pyrifera* was consistent in both kelp forests. This indicates that at both sites, a common selective factor, which is not related to intrinsic algal properties, promotes the within-blade distribution of *P. femorata* in the field.

Microhabitat-specific differences in mortality

Our results indicate that the apical blades of *M. pyrifera* provide better refuge from predation than the basal blades. The enhanced survival of the amphipods on the apical blades is in agreement with the observation that in northern-central Chilean kelp forests, fishes forage preferentially in understory algae (Palma and Ojeda 2002). Accordingly, we observed large fishes only near the seafloor but not at the sea surface in the kelp forests of Los Vilos and Playa Blanca (JDL pers. obs.). Common carnivorous fish species in Chilean kelp forests prey intensively on benthic invertebrates and they abound in habitats with high prey densities (Núñez and Vásquez 1987). For some fish species, kelp dwelling amphipods constitute a major fraction of the food spectrum (Palma and Ojeda 2002).

Small pelagic invertebrates suffer severe predation near the seafloor but are relatively safe from benthic predators a few meters above the seafloor (Allen and McAlister 2007). Similarly, marsh snails and scallops occupy above bottom microhabitats within macrophytes where they are inaccessible to most benthic predators (Warren 1985; Pohle et al. 1991; Ambrose and Irlandi 1992). We suggest that the apical sections of thalli of *M. pyrifera* provide similar vertical refuges for *P. femorata* where the amphipods are less exposed to benthic predators. Reduced predation on small invertebrates in the dense surface canopy of kelp forests is confirmed by an enhanced proportion of large individuals of gammarid amphipods and other small crustaceans in the apical thallus sections of *M. pyrifera* in California (Coyer 1984).

Recovery of tethered *P. femorata* was substantially enhanced when the amphipods were inside their nests. The protective function of nests and leaf shelters for small herbivores has been described in previous studies (Nelson 1979). The marine amphipod *Erichthonius brasiliensis* is protected from attacks by predatory fishes inside domiciles on curled terminal segments of the tropical seaweed *Halimeda tuna* (Sotka et al. 1999). Similarly, insect larvae suffer less predation in shelters consisting of tied leaves (Damman 1987). In our study, the protective function of nests is confirmed by the high

survival of nest-dwelling *P. femorata* on both apical and basal blades of *M. pyrifera*.

The protective function of the nests might explain the within-blade distribution of the amphipods. The amphipods forage intensively on the blade tissue inside their nests (Cerda et al. 2009; Rothäusler et al. 2009). Feeding on the meristematic tissue at the proximal blade base would severely affect the entire blade. By foraging on the distal tips, the amphipods minimize blade destruction and the risk of total blade loss, thereby also reducing the necessity for migrations that are periods of intense predation for unprotected mesoherbivores (Hay et al. 1989). In the case of *P. femorata*, the persistence of nest-bearing blades is enhanced by a specific nest-building behavior of the amphipods, which has been interpreted as a strategy of the amphipods to reduce the necessity of abandoning the protective nests (Cerda et al. 2010).

A considerable number of amphipod nests were found on basal blades of *M. pyrifera* in both kelp forests. Together with the results from our choice feeding assay, this substantial fraction of amphipod nests on basal sections of the stipes indicates that the observed within-stipe distribution of *P. femorata* is probably not the result of an active choice for apical blades but rather of differential mortality of randomly distributed amphipods in apical and basal microhabitats. Differential amphipod mortality between apical and basal blades of *M. pyrifera* can only occur when the amphipods spend a substantial amount of time outside the nests because mortality was enhanced in basal stipe sections only for tethered amphipods that were not protected by nests. If the observed distribution is the outcome of differential mortality, the amphipods either perform relatively frequent movements outside the nests or experience substantial mortality during periods of nest construction when the nest is still incomplete and does not provide full protection. Indeed, Cerda et al. (2010) estimated that most *P. femorata* remain inside their nests for only 1–4 days (incidentally also corresponding to about 25% of nests without amphipods found in our study), indicating relatively high movement activity of the amphipods. Intense predation on frequently moving amphipods, especially in basal kelp sections, could thus explain the observed vertical distribution pattern of *P. femorata* within the thalli of *M. pyrifera*.

Conclusions

Most studies on the habitat use of marine herbivores have focused on the choice of grazers among different host species while only very few studies have addressed the use of particular microhabitats within algal hosts. Accordingly, little is known about the evolutionary processes that

promote the use of specific microhabitats. Our results indicate that extrinsic lethal factors determine the distribution of mesoherbivores not only among but also within algal hosts in kelp forests where grazers experience high mortality, e.g., from intense predation. In contrast, intrinsic factors of the grazer and/or the algal host might have stronger influence on microhabitat use of mesoherbivores (1) in habitats with less extrinsic constraints, (2) on less-differentiated algae, or (3) when mesoherbivores have broad, generalist diets. These internal and external factors have to be considered carefully in future studies on the choice of small marine herbivores for habitats among and within algal hosts.

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References

- Allen JD, McAlister JS (2007) Testing rates of planktonic versus benthic predation in the field. *J Exp Mar Biol Ecol* 347:77–87
- Ambrose WG, Irlandi EA (1992) Height of attachment on seagrass leads to trade-off between growth and survival in the bay scallop *Argopecten irradians*. *Mar Ecol Prog Ser* 90:45–51
- Buschmann AH (1990) Intertidal macroalgae as refuge and food for Amphipoda in central Chile. *Aquat Bot* 36:237–245
- Cerda O, Karsten U, Rothäusler E, Tala F, Thiel M (2009) Compensatory growth of the kelp *Macrocystis integrifolia* (Phaeophyceae, Laminariales) against grazing of *Peramphithoe femorata* (Amphipoda, Ampithoidae) in northern-central Chile. *J Exp Mar Biol Ecol* 377:61–67
- Cerda O, Hinojosa IA, Thiel M (2010) Nest-building behavior by the amphipod *Peramphithoe femorata* (Krøyer) on the kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh from northern-central Chile. *Biol Bull* 218:248–258
- Coyer JA (1984) The invertebrate assemblage associated with the giant kelp, *Macrocystis pyrifera*, at Santa Catalina Island, California: a general description with emphasis on amphipods, copepods, mysids, and shrimps. *Fish Bull* 82:55–66
- Cronin G, Hay ME (1996) Within-plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth-differentiation balance hypothesis. *Oecologia* 105:361–368
- Damman H (1987) Leaf quality and enemy avoidance in the larvae of a pyralid moth. *Ecology* 68:88–97
- Duffy JE, Hay ME (1991) Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology* 72:1286–1298
- Hay ME, Duffy JE, Fenical W, Gustafson K (1988) Chemical defense in the seaweed *Dictyopteris delicatula*: differential effects against reef fishes and amphipods. *Mar Ecol Prog Ser* 48:185–192
- Hay ME, Pawlik JR, Duffy JE, Fenical W (1989) Seaweed-herbivore-predator interactions: host-plant specialization reduces predation on small herbivores. *Oecologia* 81:418–427
- Holmes SJ (1901) Observations on the habits and natural history of *Amphithoe longimana* Smith. *Biol Bull* 2:165–193
- Honkanen T, Jormalainen V, Hemmi A, Mäkinen A, Heikkilä N (2002) Feeding and growth of the isopod *Idotea baltica* on the brown alga *Fucus vesiculosus*: roles of inter-population and within-plant variation in plant quality. *Écoscience* 9:332–338
- Long JD, Trussell GC (2007) Geographic variation in seaweed induced responses to herbivory. *Mar Ecol Prog Ser* 333:75–80
- McDonald PS, Bingham BL (2010) Comparing macroalgal food and habitat choice in sympatric, tube-building amphipods, *Ampithoe lacertosa* and *Peramphithoe humeralis*. *Mar Biol* 157:1513–1524
- Merilaita S, Jormalainen V (1997) Evolution of sex differences in microhabitat choice and colour polymorphism in *Idotea baltica*. *Anim Behav* 54:769–778
- Merilaita S, Jormalainen V (2000) Different roles of feeding and protection in diel microhabitat choice of sexes in *Idotea baltica*. *Oecologia* 122:445–451
- Nelson WG (1979) Experimental studies of selective predation on amphipods: consequences for amphipod distribution and abundance. *J Exp Mar Biol Ecol* 38:225–245
- Núñez LN, Vásquez JA (1987) Observaciones tróficas y de distribución especial de peces asociados a un bosque submareal de *Lessonia trabeculata*. *Estud Oceanol (Chile)* 6:79–85
- Palma ÁT, Ojeda FP (2002) Abundance, distribution and feeding patterns of a temperate reef fish in subtidal environments of the Chilean coast: the importance of understory turf. *Rev Chil Hist Nat* 75:189–200
- Pansch C, Gómez I, Rothäusler E, Veliz K, Thiel M (2008) Species-specific defense strategies of vegetative versus reproductive blades of the Pacific kelps *Lessonia nigrescens* and *Macrocystis integrifolia*. *Mar Biol* 155:51–62
- Pérez-Matus A, Shima JS (2010) Density- and trait-mediated effects of fish predators on amphipod grazers: potential indirect benefits for the giant kelp *Macrocystis pyrifera*. *Mar Ecol Prog Ser* 417:151–158
- Pohle DG, Bricelj VM, García-Esquivel Z (1991) The eelgrass canopy: an above-bottom refuge from benthic predators for juvenile bay scallops *Argopecten irradians*. *Mar Ecol Prog Ser* 74:47–59
- Poore AGB (1994) Selective herbivory by amphipods inhabiting the brown alga *Zonaria angustata*. *Mar Ecol Prog Ser* 107:113–123
- Poore AGB, Steinberg PD (1999) Preference-performance relationships and effects of host plant choice in an herbivorous marine amphipod. *Ecol Monogr* 69:443–464
- Rothäusler E, Gómez I, Hinojosa IA, Karsten U, Tala F, Thiel M (2009) Effect of temperature and grazing on growth and reproduction of floating *Macrocystis* spp (Phaeophyceae) along a latitudinal gradient. *J Phycol* 45:547–559
- Salemaa H (1987) Herbivory and microhabitat preferences of *Idotea* spp (Isopoda) in the northern Baltic Sea. *Ophelia* 27:1–15
- Sokal RR, Rohlf FJ (1969) *Biometry*. Freeman and Company, San Francisco, USA
- Sotka EE (2007) Restricted host use by the herbivorous amphipod *Peramphithoe tea* is motivated by food quality and abiotic refuge. *Mar Biol* 151:1831–1838
- Sotka EE, Hay ME (2002) Geographic variation among herbivore populations in tolerance for a chemically rich seaweed. *Ecology* 83:2721–2735
- Sotka EE, Hay ME, Thomas JD (1999) Host-plant specialization by a non-herbivorous amphipod: advantages for the amphipod and costs for the seaweed. *Oecologia* 118:471–482
- Sotka EE, Wares JP, Hay ME (2003) Geographic and genetic variation in feeding preference for chemically defended seaweeds. *Evolution* 57:2262–2276
- Vesakoski O, Boström C, Ramsay T, Jormalainen V (2008) Sexual and local divergence in host exploitation in the marine herbivore *Idotea baltica* (Isopoda). *J Exp Mar Biol Ecol* 367:118–126
- Warren JH (1985) Climbing as an avoidance behavior in the salt marsh periwinkle, *Littorina irrorata* (Say). *J Exp Mar Biol Ecol* 89:11–28
- Wheeler PA, North WJ (1981) Nitrogen supply, tissue composition and frond growth rates for *Macrocystis pyrifera* off the coast of southern California. *Mar Biol* 64:59–69