THE
BIOLOGICAL
BULLETIN

Nest-Building Behavior by the Amphipod *Peramphithoe femorata* (Krøyer) on the Kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh From Northern-Central Chile

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Abstract. Many small arthropod herbivores from terrestrial and marine environments construct tubicolous nest-like domiciles on their host plants or algae by rolling up selected portions of the leaf or blade. Nests serve as both shelter and food, which results in conflicting needs for the grazers because feeding activity continuously destroys parts of the nests. While the nesting habit of ampithoid amphipods and other peracarid crustaceans is widely known, very little is known about the nest dynamics and how the conflict between shelter and food requirements is resolved. Herein we examined the nest-building behavior and nest occupancy of the kelp-dwelling amphipod *Peramphithoe femorata* on the giant kelp *Macrocystis pyrifera*. Domiciles on the distal-most part of the blades were occupied by amphipods and were steadily advanced toward the blade base. Since the blades grow from a basal growth meristem, blades and nests develop in opposite directions. Thus, the amphipods exploit the kelp growth pattern to maintain their nests in the medium-distal part of the blades. During a 2-week-long experiment, we observed that blade elongation equaled nest advancement during the first 8 days after nest construction. Thereafter, blade growth slowed down and was surpassed by nest advancement rates, possibly forcing amphipods to seek out new blades. Nest occupancy was generally short (1–4 days), but some amphipods resided longer (>4 days) in their nests. The sophisticated nest-building behavior of *P. femorata* and other ampithoids maximizes nest persistence, offers optimal protection against predators, and promotes feeding on nutritive or, alternatively, on less defended tissues.

Introduction

The use of a host plant as food source and refuge against natural enemies is a common feature of small arthropod grazers, both terrestrial (*e.g.*, caterpillars: Sagers, 1992; Weiss *et al.*, 2003) and marine (*e.g.*, amphipods: Duffy and Hay, 1991; Poore and Steinberg, 1999). These mesoherbivores construct their nests by rolling up the selected leaf or blade into a tube (Barnard *et al.*, 1991; Fukui, 2001), which might reduce the probability of being eaten by large predators. The change of the normal leaf or blade structure can lead to a concomitant modification in some important tissue attributes of the host plant, such as growth rate or chemical and physical defensive traits (*e.g.*, tannin concentration and tissue toughness, respectively). An increase in the quality of tissues in the nest, due to reduced chemical defenses or higher levels of nitrogen, has shown positive effects on terrestrial leaf-rolling caterpillars (*e.g.*, enhanced growth: Sandberg and Berenbaum, 1989; Sagers, 1992; Fukui *et al.*, 2002).

Marine amphipods from the family Ampithoidae are conspicuous mesograzers that build nests in holdfasts, stipes, or blades of macroalgae (Poore *et al.*, 2008). The nest-building behavior of ampithoid amphipods has been documented since the first part of the 20th century (*e.g.*, Holmes, 1901; Skutch, 1926). These studies described the nest as being held together by silk threads that are secreted from glands in the pereopods. Since most ampithoids appear to consume the same algal tissues that make up the walls of their nests (Jones, 1971; Griffiths, 1979; Poore and Steinberg 1999),

their feeding activity might actually compromise the integrity of the nest (*e.g.*, Heller, 1968). How these conflicting needs within the nest (shelter and food) influence the nest dynamics and the residence times of amphipods is not known.

Nest-fidelity of amphipods is highly variable and apparently related to feeding strategies. For example, it could be expected that stipe-boring and stipe-consuming specialists stay within their nests most of the time (*e.g.*, Conlan and Chess, 1992). Generalist herbivorous amphipods, in contrast, commonly move around on their host alga, foraging on different types of available seaweeds (*e.g.*, Duffy and Hay, 1994). It has been proposed that breeding amphipod females are normally restricted to their nests, whereas "cruising males" (*sensu* Borowsky, 1983) are constantly visiting as many dwellings as possible in search of receptive mates. On the basis of this suggestion, we expected that ovigerous females of herbivorous amphipods would exhibit prolonged occupancy within the nest, which might provoke reactions by the host algae and in turn influence amphipod behavior. For example, it could be expected that blade tissues within a nest would quickly start to deteriorate due to shading or grazing (as observed in terrestrial plants—*e.g.*, Sagers, 1992), thereby shortening residence times of the amphipod.

Nest-building and grazing activities by ampithoid amphipods can also have strong impacts on seaweed performance and survival (Duffy and Hay, 2000). Herbivorous ampithoids from the genera *Ampithoe* and *Peramphithoe* often cause blade loss on brown algae from the orders Dictyotales and Laminariales (*e.g.*, Hay *et al.*, 1987; Chess, 1993; Sotka, 2007). In a recent study, Rothäusler *et al.* (2009) reported mean daily consumption of 37 mg individual^{-1} day^{-1} of fresh blade tissue of the laminarian *Macrocystis pyrifera* by *Peramphithoe femorata*. Grazer impacts may exceed mere consumption losses by causing breakage of stipes or blades and damage to growth meristems or reproductive tissues. These impacts may be exacerbated when domiciles are overpopulated (by females sharing their nests with offspring) or when these mesograzers reach very high densities. For example, Gunnill (1982) reported densities of 200–500 individuals of *P. tea* (reported as *Ampithoe tea*) on a single *Pelvetia fastigiata* sporophyte. The interactive effect of nest-building and feeding activities by kelp-curler amphipods on particular blade tissues (*e.g.*, meristems and the influence on growth rate) is not yet completely understood.

It has been suggested that amphipod host-choice is strongly influenced by seaweed chemical defense and value as refuge against predators. For example, some ampithoids use dictyotalean algae, which are efficiently defended by nonpolar secondary metabolites (*e.g.*, terpenoids) that deter large consumers such as omnivorous fishes (Duffy and Hay, 1994). Other ampithoids use hosts from the seaweed orders Fucales and Laminariales, which are poor in nonpolar chemical defensive metabolites (Macaya *et al.*, 2005; Macaya and Thiel, 2008; Poore *et al.*, 2008). The amphipods exploit the complex morphological architecture of these algae to obtain an effective protection against predators (*e.g.*, Poore and Steinberg, 2001) or abiotic stressors (*e.g.*, wave action: Sotka, 2007).

Within the ampithoids, species from the genus *Peramphithoe* can be found living on several algal types, but available reports suggest strong preferences for temperate brown seaweeds from the genera *Macrocystis* and *Sargassum* (Poore *et al.*, 2008). Reports on the mobility of nest-building ampithoids suggest variable residence times on their host algae (*e.g.*, Duffy and Hay, 1994; Poore, 2004, 2005), which could be due to the fact that nest dynamics are closely related to algal growth patterns. Within a host alga, the growth rates of blades vary, possibly affecting nest residency of the amphipods. For example, growth rates of *Macrocystis* blades decrease with distance from the apical meristem (Clendenning, 1971; Cerda *et al.*, 2009), and thus consumption of nests might exceed growth rates of blades, possibly provoking amphipods to abandon their nests.

Peramphithoe femorata (Krøyer) is a common kelp-curler from the southern temperate ocean, inhabiting the giant kelp *Macrocystis pyrifera* (Poore and Steinberg, 2001). Along the coast of Chile, *P. femorata* lives and feeds on *M. pyrifera* sporophytes (pers. obs.). The amphipods construct nests mainly on the upper blades of the frond near the sea surface, thereby avoiding benthic predators while simultaneously consuming protein-rich tissues (Wheeler and North, 1981). The objective of this study was to examine the nest-building behavior and nest occupancy by *P. femorata* on blades of *M. pyrifera* from the northern-central coast of Chile, and to relate nest advancement along the blade to the growth rates of nest-carrying blades. Specifically, we hypothesized that (i) nest-building by *P. femorata* follows a regular pattern, (ii) nest advancement is related to the growth dynamics of blades, and (iii) *P. femorata* females reside for long time periods within their nests.

Materials and Methods

Study site and organisms

Ovigerous females of *Peramphithoe femorata* (about 11–13 mm in body length) and entire sporophytes of *Macrocystis pyrifera* were collected in a shallow subtidal kelp forest at Los Vilos (31°54′S, 71°31′W) in northern-central Chile and immediately transported to the flowing seawater laboratory at Universidad Católica del Norte in Coquimbo (29°57′S, 71°20′W). We selected *Macrocystis* sporophytes with a minimum number of 15 and a maximum of 25 free blades from the apical meristem. One day after sampling, 12 sporophytes (mean length: 147.1 ± 17.4 cm) were placed

individually at normal ambient conditions (*i.e.*, shade/full sunlight), in 90-liter outdoor plastic tanks supplied with constant air and unfiltered seawater.

Nest-building behavior

To formulate qualitative descriptions of nest construction and amphipod behavior, amphipods from a culture initiated with individuals from Los Vilos (see above) were observed in the laboratory. We examined nest-building behavior by monitoring one *Peramphithoe* individual per *Macrocystis* blade at a time, noting how the amphipods started to build their nests and the frequency with which the silk was interconnected between the two sides of the blade to extend the domicile. We used a dissecting microscope to observe feeding strategies in detail, and a compound microscope to examine blade sections grazed by amphipods.

Nest advancement and blade elongation

We calculated the daily rates of nest advancement and blade elongation on individual blades. During the 14-day experiment, we monitored a total of 60 amphipods that were distributed over 12 containers, each with one sporophyte of *Macrocystis pyrifera*.

Initial nests for *P. femorata* were prepared on five subapical blades from each sporophyte. These blades were located below the first three apical blades that had separated from the apical meristem. Subapical blades were chosen for nests because field observations at two locations from the northern-central coast of Chile indicated that *P. femorata* is commonly found in the upper portions of the sporophyte (L. Gutow *et al.*, Alfred Wegener Institute for Polar and Marine Research (AWI), Germany; unpubl. data), and also because those blades were long enough to permit the construction of our artificial nests. We made initial nests by rolling the distal part of the blades and fixing it with a rubber band. We placed one amphipod in each initial nest. Preliminary experiments had shown these initial nests to be readily occupied by *P. femorata* (for details, see Cerda *et al.*, 2009).

Every 2 days for 14 days we measured blade and nest elongation on the same five nest-carrying blades of each sporophyte. In order not to harm the animals (amphipods are small and lack the hard carapace found in decapod crustaceans), they were not marked individually. Therefore, we could not identify the original nest of each individual if several amphipods were encountered outside their nests during daily inspections. To ensure that nests were constructed by a single amphipod, we used measurements of nest advancement only if an amphipod was observed inside the nest for at least 2 consecutive days. There might have been different subsequent users in a single nest, but occasional observations of aggressive behavior by resident amphipods strongly indicated that the same individual remained in the same nest on consecutive days.

Blade elongation was estimated using the hole-punch method (see, *e.g.*, Rothäusler *et al.*, 2009). A 3-mm hole was punched just above the growth meristem of the blade—that is, about 9 cm above the blade base—and the displacement distance of the hole from the blade base was measured every 2 days. The daily blade elongation rate (BER) was then quantified as the difference between the position of perforations at 2-day intervals: BER (mm day^{-1}) = (H_f–H_i)/2 days, where H_i is the initial position of the hole (9 cm), and H_f is the position of the hole at each subsequent measurement. The same perforation was used as reference to measure nest advancement. Nest advancement rate (NAR) was quantified as the difference between the distance of the hole and the most proximal silk string on the nest at 2-day intervals: NAR (mm day^{-1}) = (N_i–N_f)/2 days, where N_i is the initial distance between the hole and the nest entrance and N_f is the subsequent distance between the hole and the front edge of the nest at each measurement.

Observations of nest occupancy

Using the same amphipods and kelp blades from the nest advancement and blade elongation measurements explained above, we recorded every day (for the 14 days of the experiment) the presence or absence of amphipods in the nest on each sporophyte. To reduce disturbance due to handling, each nest was monitored under water for occupancy by amphipods. If a nest was empty, the free amphipod was recovered in the tank and carefully placed inside the nest. If the amphipod again abandoned the nest during the next minute, we replaced it with another ovigerous female of the same size from a laboratory culture. Nest occupancy was estimated by counting the total number of observed 2-day intervals. This criterion was based on our previous assumption that a nest continuously occupied for 2 consecutive days was used by a single amphipod.

Statistics

All analyses were conducted using SPSS 11.5 (SPSS Inc., 2002). To test whether blade elongation and nest advancement rates differed over time, for each sampling interval (days 2–4, 4–6, 6–8, 8–10, 10–12, and 12–14) we conducted a dependent-samples Student's *t* test comparing the advancement rates of nest and blade on each single blade (Zar, 1999). To control for a Type I error, we performed the pairwise comparisons using a Bonferroni adjustment on the confidence intervals. The number of algal replicates for each time interval was variable, since we did not always obtain data from the five potential subreplicates (*i.e.*, the five nest-carrying blades) on each sporophyte, because amphipods were outside their nests during the entire time interval. However, for each time interval we had a minimum of two subreplicates from at least 50% of the sporophytes to cal-
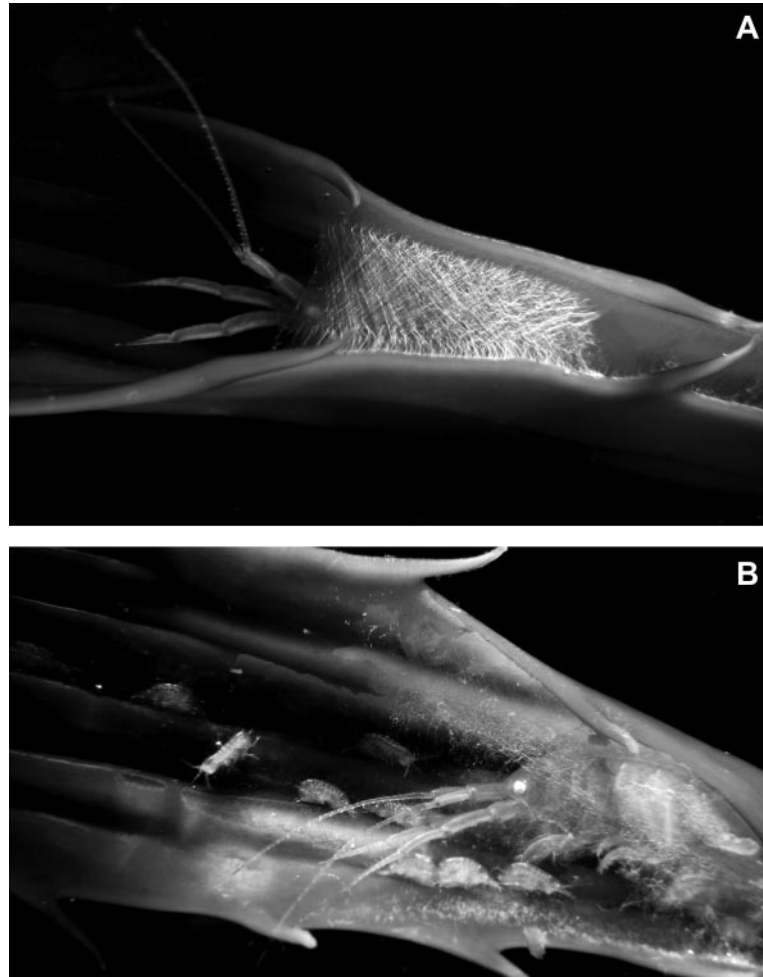
Figure 1. Tubiculous nests of *Peramphithoe femorata* on *Macrocystis pyrifera* blades. (A) Weblike amphipod-silk produced by pereopod glands. (B) Amphipod female accompanied by its juvenile offspring.

culate the mean values. Prior to the analysis we checked for parametric assumptions of normality and homoscedasticity.

To examine whether nest advancement rates depended on the total duration of occupancy, we used univariate analysis of variance (ANOVA) to compare the advancement of nests occupied at 2-day intervals, with nest occupancy as fixed factor. Since few nests were occupied for more than 8 days, we pooled the data for nests that were continuously occupied for 8, 10, and 12 days. The *F* statistic for unbalanced data with missing cells was obtained using Type IV sums of squares (Landau and Everitt, 2004). Prior to the analysis of variance we checked for normality and homoscedasticity using Shapiro-Wilks and Levene tests, respectively. When the results from Shapiro-Wilks test were close to the critical value (*i.e.*, 0.05), we examined deviations from normality using graphical evaluation of data and residuals. As no severe deviation from normality was found in our data, parametric ANOVA was conducted.

Results

Nest-building and feeding behavior

Amphipods quickly started to build a nest on the natural blades when introduced to the aquaria, and after a relatively short time (about 2 h) they had stabilized their domiciles with abundant silk (Fig. 1A, B). The silk threads were produced in glands on the third and fourth pairs of pereopods (Fig. 2A). Normally, the amphipods initiated nest construction by producing an amorphous mass of silk on the blade surface. This mass of silk served as anchorage for pereopods 5, 6, and 7 during the initial phase of nest construction. These posterior pereopods are typically oriented backward, which allows the animals to hold onto the blade firmly while producing new silk threads with pereopods 3 and 4. At the same time, the amphipods utilized their first and second pairs of pereopods (gnathopods) to
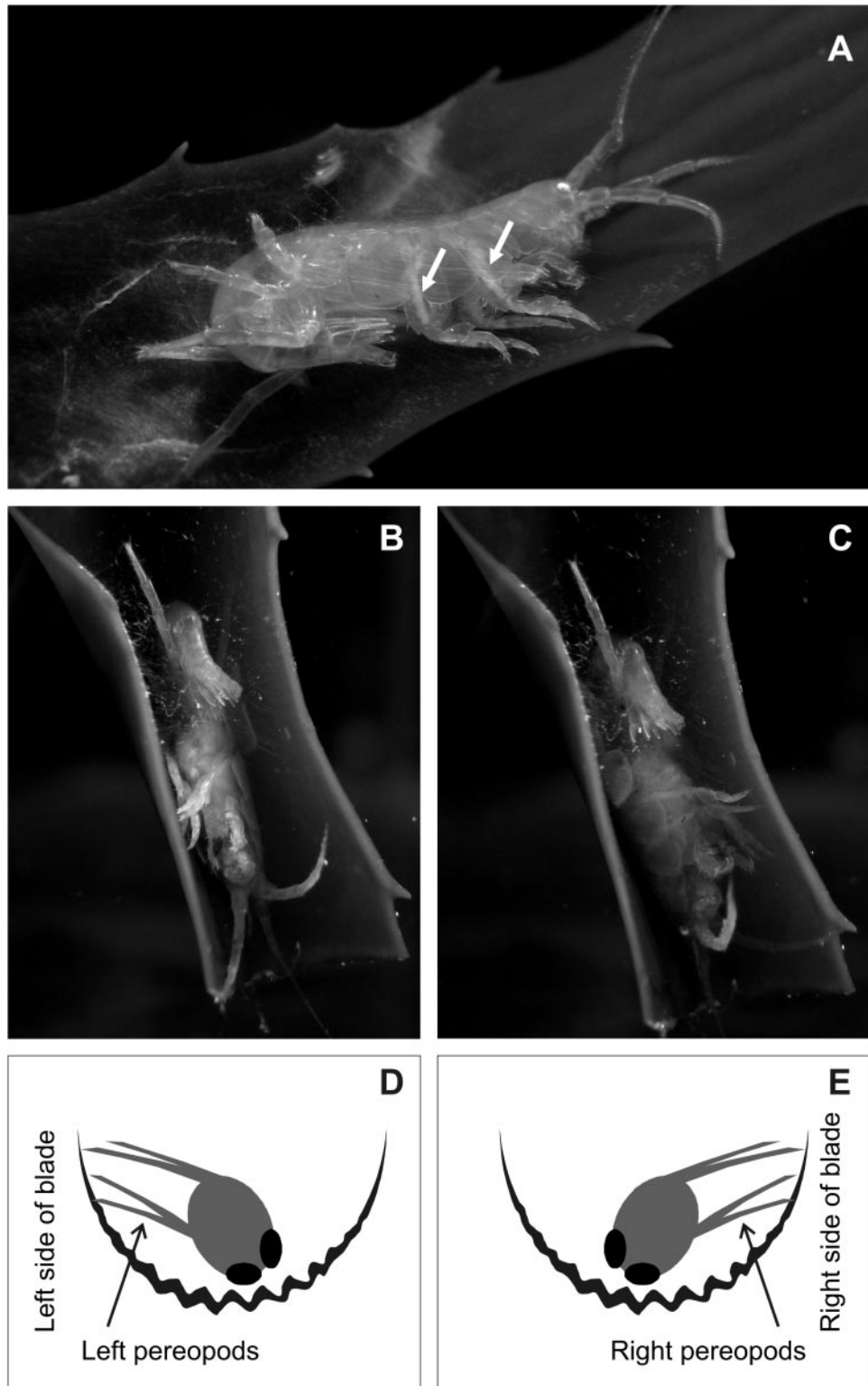
Figure 2. (A) Silk glands of *Peramphithoe femorata* at the third and fourth pairs of pereopods (arrows). (B–C) Successive positions of pereopods 3–4 while spinning the silk threads to each blade border. (D–E) Schematic overview of positioning of pereopods 3–4 during nest construction.
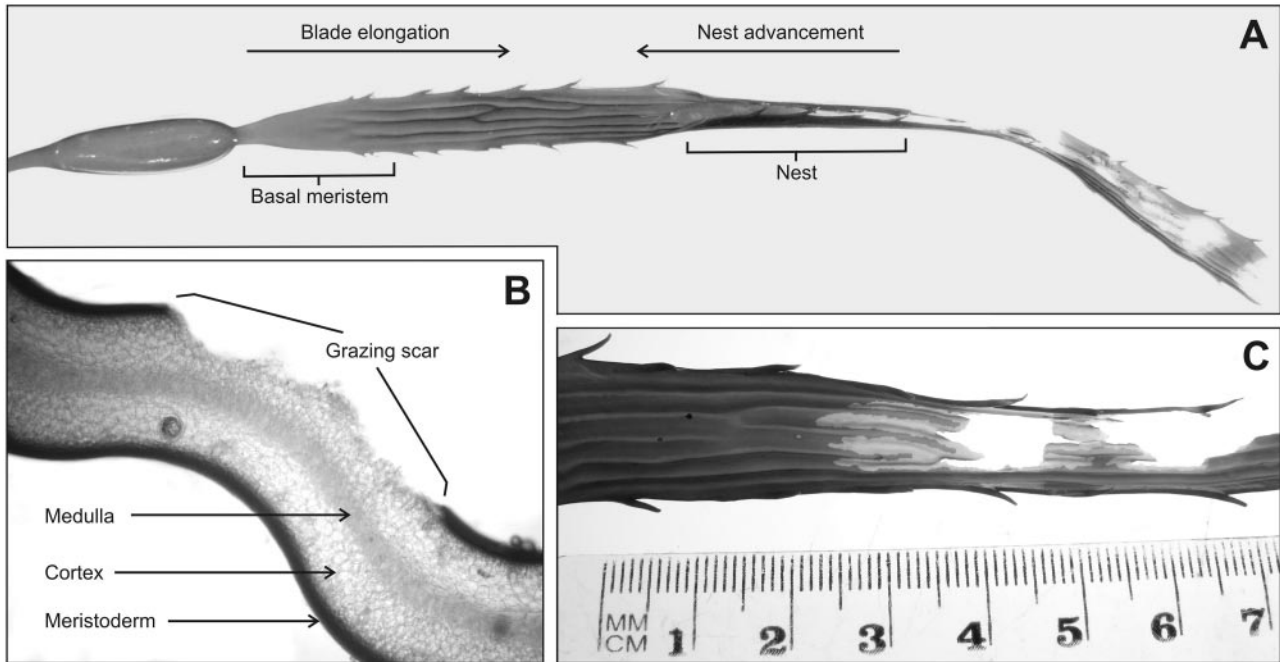
Figure 3. (A) Blade of giant kelp *Macrocystis pyrifera* showing pneumatocyst, lamina, and a nest with one *Peramphithoe femorata* individual. The direction of blade elongation and nest advancement is presented in the upper part of the figure. (B) Transverse section of a blade at the anterior part of the nest showing meristoderm, cortex, medulla, and a grazing scar (8×). (C) Opened nest showing grazed tissues by *Peramphithoe femorata* (slightly transparent tissues); the front edge of the nest was at 0 cm.

manipulate the initial silk strands, possibly pulling them tight to curl the kelp blade.

During the initial phase of nest construction, the amphipods had the side of the body toward the blade surface, and at irregular intervals they changed position from one side of the blade to the other. Once the nest length approached the body length of the amphipods, they advanced the construction while keeeping their dorsal side toward the blade surface. In this position they rhythmically moved pereopods 3–4 from one side of the blade to the other, spinning the silk threads between the blade edges (Fig. 2B, C). These movements occurred at a frequency of about 24 silk attachments per minute considering both sides of the blade—that is, 12 attachments to the left and 12 to the right side ($n = 6$ amphipods) (Fig. 2B, C). The active manipulation of the newly spun silk threads by the gnathopods (see above) persisted throughout the process.

The web of silk produced by the amphipods had an intricate crossed arrangement of the silk strings (Fig. 1A). With ventral side up, the amphipods used their four silk-producing limbs to attach the silk threads to each blade edge. In a highly coordinated manner, the amphipods moved first those pereopods that attached the silk threads to the opposite side of the blade: the four appendages placed silk on the left side of the blade, then the upper positioned right pereopods moved to the right side of the blade before the left pereopods, which were weaving below (Fig. 2B, D).

At the right side, the left pereopods were then attaching their silk threads above the right pereopods (Fig. 2C, E). The resulting terminal portion of the blades had a characteristic tubelike appearance (Fig. 3A).

Commonly, the nest entrance was not entirely glued together, since the amphipod silk usually served as top-cover. In most cases, nest construction advanced toward the base of the algal blade where new tissues are generated (Fig. 3A). Only in a very few cases did the nests advance toward the older and senescent tissue of the blade apex. Occasionally, we observed juvenile amphipods inhabiting their own domiciles outside or near the entrance of the mother's nest. Unlike the tubicolous dwelling of adults, juveniles constructed a weblike nest between blade corrugations.

The amphipods fed on algal tissues inside and outside the nest (Fig. 1B, 3A). In the anterior part or the center of the nest they normally fed on the upper meristoderm without touching the medulla (Fig. 3B). At the distal section of the nest they apparently fed on the entire remaining blade tissues, or possibly on the lower meristoderm, whereupon the medulla might be lost due to decomposition (Fig. 3C).

Nest advancement and blade elongation

Amphipods readily occupied the offered initial nests, which they rapidly extended and transformed into natural nests. The nests exhibited a constant advancement toward
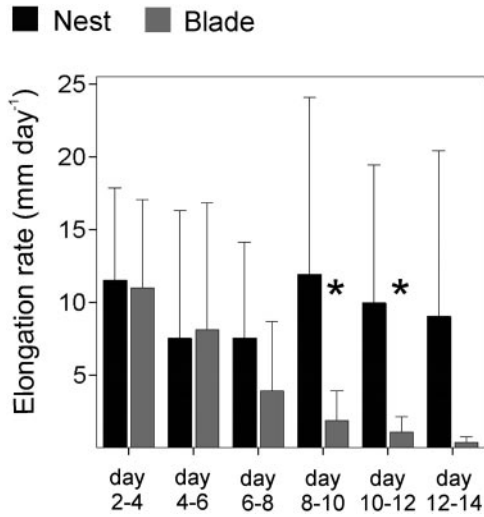
Figure 4. Elongation (advancement) rate of nests built by *Peramphithoe femorata* and elongation rate of the respective subapical blades of *Macrocystis pyrifera* during the 2 weeks of the experiment. Columns represent mean elongations, and vertical lines represent standard deviations (*n* = 12). Symbols represent statistically different groups (paired *t* test, *P* < 0.05).

the blade base throughout the 14 days of the experiment. On the other hand, the blades showed a progressive decline in their growing activity over time. While both nest and blade elongation rates were equal at the beginning of the experiment, the rate of blade elongation decreased significantly after 6 days. Pairwise comparisons revealed that most variation was due to differences in blade elongation rate (BER) and nest advancement rate (NAR) at days 8–10 (paired *t* test, *P* = 0.03) and days 10–12 (*P* = 0.011); the comparison at days 12–14 was very close to significance (*P* = 0.051) (Fig. 4). Interestingly, NAR became highly variable after 8 days, indicating variations in nest-building activity among females or temporally within individual females.

Nest occupancy

Most female amphipods changed their nests during the 14-day experiment. If another female amphipod attempted to enter an occupied nest, aggressive behavior by the resident female was observed. Consequently, females shared nests less often than expected by chance ($\chi^2_{\text{yates}(1)}$, *P* < 0.001): in 840 nest surveys conducted during the experiment (5 nests per sporophyte, 12 sporophytes, and 14 survey days) two adult females were observed together in one nest on only 6 occasions. Amphipod females inside their nests were often in a resting state, while individuals outside their nests were found crawling on the stipe, pneumatocysts, or other blades, probably consuming some tissue as indicated by the small grazing scars commonly observed on these other sporophyte parts. Amphipods that had abandoned their "assigned" nests were occasionally found constructing nests on other blades. The amphipods outside their nests that built another domicile were always found on blades at the upper portions of the kelp sporophyte, consistent with previous field observations.

From the 420 possible observations of 2-day occupancy intervals (5 blades × 12 sporophytes × 7 time intervals), amphipods were inside their domiciles for at least 2 days on 150 occasions. We recorded 64 observations for 2-day occupancy, 38 observations (*i.e.*, 2-day intervals) for 4-day occupancy, and the remaining observations were for 6 and >8 days (Fig. 5A). The longest nest occupancy observed was 12 days (*i.e.*, one nest that was occupied for six consecutive 2-day intervals). No nest was used uninterruptedly for the entire observation period. Occupancy had no influence on nest advancement rates (one-way ANOVA, df = 3, *F* = 1.217, *P* = 0.306): nests that were only briefly occupied advanced with the same rhythm as those of longer duration (Fig. 5B). Throughout the experiment, every day about 50% of the amphipods stayed in their nest while the other 50% had left their domiciles (Fig. 6). Toward the end
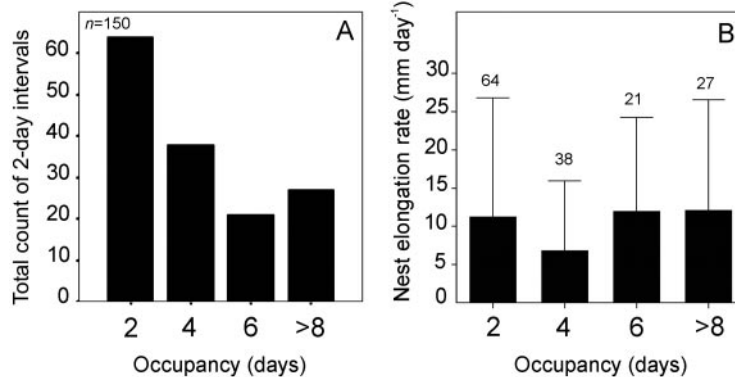
Figure 5. (A) Number of observations of nest occupancy by *Peramphithoe femorata* on *Macrocystis pyrifera* subapical blades (*n* = 150). No nest was continuously occupied by the same amphipod over the 14 days of the study. (B) Elongation rates of nests observed in Fig. 5A (mean ± sd).
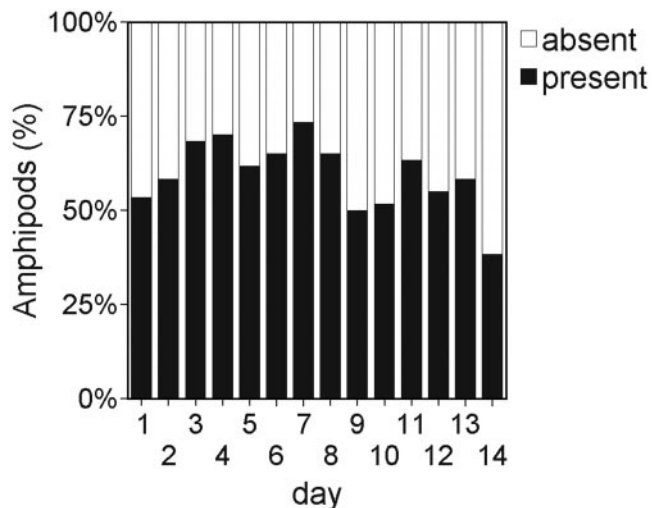
Figure 6. Total number of amphipods counted inside or outside their nests every day at each of the five subapical blades used to build an artificial nest for the kelp-curlers (*n* = 60 per day).

of the study period we observed a slight decrease in the daily percentage of residents.

Discussion

Our detailed observations on the nest-building behavior exhibited by *Peramphithoe femorata* on blades of the giant kelp *Macrocystis pyrifera* produced three principal results. First, many amphipods were highly mobile—more so than originally expected. Second, they were able to construct and advance their nests quickly. Third, they exploited the blade growth pattern to strategically construct their nests in a particular way: while the basal growth meristem of the blade constantly pushed new blade tissues away from the blade base, the nests were advanced in the opposite direction toward the blade base, that is, like an object (nest) moving against a running conveyor belt (blade). This nest-building strategy probably permits extended residence times and nest-positioning on tissue portions at relatively consistent distances (and food value) from the basal growth meristem.

Nest-building and feeding behavior

The silk glands on pereopods 3 and 4 of *Peramphithoe femorata* and other ampithoid amphipods are instrumental during nest construction (*e.g.*, Lewis and Kensley, 1982; Poore and Steinberg, 1999; Appadoo and Myers, 2003). The crossed form in which *P. femorata* spins the silk threads is similar to that reported for *Peramphithoe* sp., which was originally reported as *P. humeralis* by Griffiths (1979) but corresponds to an undescribed species according to Barnard and Karaman (1991). Silk spinning has also been observed in other herbivorous and filter-feeding amphipods, which

use a wide variety of materials to construct their domiciles (*e.g.*, Holmes, 1901; Skutch, 1926; Harris and Musko, 1999; Appadoo and Myers, 2003). Shillaker and Moore (1978) described silk-spinning by the filter-feeders *Lembos websteri* and *Corophium bonnellii* in detail. The process is described as a "knitting" activity of the pereopods, during which particles or blade pieces are attached to the nest wall. This knitting action (*sensu* Shillaker and Moore, 1978) is characterized by the continuous flexion/retraction of pereopods 3 and 4 while spinning the silk threads to different parts of the nest. Our observations on the herbivorous *P. femorata* revealed a very similar pattern of silk-spinning. The basic behavior during nest construction thus appears to be similar in most nest-building amphipods. In *P. femorata* and other ampithoids, the recently spun silk threads are additionally manipulated by pereopods 1 and 2, the gnathopods (*e.g.*, present study; Skutch, 1926; Heller, 1968), but the functional significance of this behavior is not yet fully understood. Possibly, the manipulation tautens the new silk threads and thereby curles the blades.

The ability to curl seaweed blades using silk threads secreted from the pereopods has been reported for several ampithoid species (therefore their common name: kelp-curler amphipods). For example, *Peramphithoe humeralis* (Jones, 1971) and *Cymadusa uncinata* (Barnard *et al.*, 1991) curl blades of *Macrocystis pyrifera*; south African *Peramphithoe* sp. uses blades of *Ecklonia maxima* (Griffiths, 1979); while *Sunamphitoe graxon* (Freewater and Lowry, 1994) and *Peramphithoe parmerong* (Poore and Steinberg, 1999) roll blades of *Sargassum* spp. (Table 1). Other ampithoids glue algal or seagrass pieces together without curling the blades (Holmes, 1901; Skutch, 1926; Poore and Lowry, 1997). On the basis of these observations, two principal nest types can be distinguished: those constructed by curling a single kelp blade and those made by gluing together two or more blades (Table 1). Occasionally more than one nest type has been reported for one amphipod species. Thus the nest type might be plastic, depending on the environmental conditions and the available substratum (stiff *versus* lithe foliose algae).

Nest construction and algal growth dynamics

Predation risk can affect host selection in ampithoid amphipods (Duffy and Hay, 1991, 1994). However, living in a nest that serves as both refuge and food resource produces conflicting needs, because feeding activity leads to the continuous destruction of the nest. *Peramphithoe femorata* appears to overcome this conflict by taking advantage of the intercalary growth pattern of blades of the giant kelp *Macrocystis pyrifera*. The intercalary meristem is situated at the basal portion of the blade, near the junction with the pneumatocyst, and new tissues are constantly moved along the longitudinal axis of the blade (Hoek *et al.*, 1995). The

Table 1

Summary of nest-building behavior and algal hosts of tubicolous amphipod grazers from the family Ampithoidae

Amphipod species*	Algal host	Nest category†	Nest advancement	Construction time (hours)	Nest occupancy (days)	Nest inhabitants	Feeding
Peramphithoe femorata[a]	*Macrocystis pyrifera*	C	From tip to blade base (>10 mm day^{-1})	≈ 2	Intermediate (1-10)	Adults and juveniles	Within the nest
Peramphithoe parmerong[b]	*Sargassum* spp.	C, Ga	?	≈ 1	Long (?)	Adults	Within the nest
Peramphithoe humeralis [c]	*Macrocystis pyrifera*	C	From tip to blade base	?	?	Adults and juveniles	Within the nest
Peramphithoe sp.[d]	*Ecklonia maxima*	C	From tip to blade base	?	?	Adults and juveniles	Within the nest
Peramphithoe tea[e,f]	*Egregia menziesii, Pelvetia fastigiata*	Ga	?	?	?	?	?
Ampithoe rubricata[g]	Filamentous/foliose	Ga	?	≈ 12 to 48	Long (?)	Adults	Outside the nest
Ampithoe longimana[h]	Filamentous/foliose, eel-grass	Ga	?	≈ 1/2	Short (?)	Adults	Outside the nest
Ampithoe kava[i]	*Sargassum linearifolium, Zonaria diesingiana*	C, Ga	?	?	?	?	?
Ampithoe ngana[i]	*Sargassum linearifolium, Zonaria diesingiana*	C, Ga	?	?	?	?	?
Ampithoe caddi[i]	*Sargassum linearifolium, Zonaria diesingiana*	C, Ga	?	?	?	?	?
Exampithoe kutti[i]	Brown algae from order Dictyotales	Ga	?	1-2 ‡	?	Adults and juveniles	?
Sunamphithoe graxon[j]	*Sargassum* sp., *Ulva lactuca*	C, Ga	?	?	?	Most females	?
Pseudamphithoides incurvaria[k]	Brown algae from family Dictyotaceae	Gm	?	≈ 1/2-2	Long (?)	Adults	Within and outside the nest
Cymadusa uncinata[l]	*Macrocystis pyrifera*	C	?	?	?	?	?
Cymadusa filosa[m]	*Sargassum binderi, Ulva lactuca*	Ga	17.4 ± 3.6 mm (4-6 weeks)	1-2 ‡	Long (?)	Adults	Within the nest
Peramphithoe stypotrupetes [n,o]	*Eisenia arborea, Laminaria dentigera, L. setchellii*	Stipe burrower	From the tip to the base of the stipe (67.1-11 mm month^{-1})	?	Long (223)	Adults and juveniles	Within the stipe burrow
Amphitholina cuniculus[p]	*Alaria esculenta*	Stipe burrower	?	?	?	Adults	Within the stipe burrow

* Sources for data on species: [a]present study; [b]Poore and Steinberg, 1999; [c]Jones, 1971; [d]Griffiths, 1979; [e]Sotka, 2007; [f]Gunnill, 1982; [g]Skutch, 1926; [h]Holmes, 1901; [i]Poore and Lowry, 1997; [j]Freewater and Lowry, 1994; [k]Lewis and Kensley, 1982; [l]Barnard *et al.*, 1991; [m]Appadoo and Myers, 2003; [n]Conlan and Chess, 1992; [o]Chess, 1993; [p]Myers, 1974.

† Type of nest construction: C, one blade curled into a tube; Ga, two or more blades glued together; Gm, two blades glued into a mobile bivalved domicile.

? Not available or unclear information.

‡ Juvenile performance.

amphipods thus build a "dynamic" nest, which advances in a direction opposite to the growth direction of the blades. Future studies should examine whether this behavior varies across species that inhabit algal hosts with different growth patterns—for example, those with apical meristems such as species from the genus *Sargassum*.

The exploitation of the growing pattern of macroalgae during construction of a protective nest has also been documented for the amphipod *Ericthonius brasiliensis* (Sotka *et al.*, 1999). This filter-feeding species builds its nest on apical segments of the calcified green alga *Halimeda tuna*. When new segments of these algae are produced at night, they are still noncalcified and flexible (Hay *et al.*, 1988), enabling *E. brasiliensis* to construct a tubicolous nest (also at night) by rolling up the blades while they are soft.

Reports on the tube-building of North American *Peramphithoe humeralis* living on *Macrocystis pyrifera* (Jones, 1971) and of South African *Peramphithoe* sp. inhabiting

Ecklonia maxima (Griffiths, 1979) suggest that nests are initiated on the apical part of kelp blades and then advanced toward the blade base in a way similar to that reported here for *P. femorata* (Table 1). Both *Peramphithoe* sp. (see Griffiths, 1979) and *P. femorata* also heavily consumed the blade tissues within their nests. While their feeding activity destroys nest walls, the amphipods maintain the nest intact by continuously moving it toward ungrazed blade parts. These amphipod species thus appear capable of fine-tuning the construction of their nests with the algal growth dynamics in order to extend their residence (and the associated benefits) on a single blade.

Nests as food source

Nest occupancy by *P. femorata* was surprisingly short, and the amphipods were highly mobile within the sporophytes. Most individuals of *P. femorata* constructed the nest far from the basal meristem in the distal portions of the blade. This could also be advantageous, since meristems of most brown algae have been suggested to be highly defended against herbivores (Taylor *et al.*, 2002; Pelletreau and Targett, 2008). Possibly, a trade-off between nutritional compounds and deterrent chemicals in particular blade zones of the giant kelp *M. pyrifera* influences mobility and tissue selection by the amphipods. Since the far distal parts of the blade are usually sloughed off during blade growth, *P. femorata* might position its dynamic nests in blade sections with the best balance between nutrients and antiherbivore compounds. Although grazing of *P. femorata* did not affect carbon, nitrogen, and reserve compounds on middle sections of grazed blades (Cerda *et al.*, 2009), future studies should examine variations in tissue quality along the axis of kelp blades in the presence and absence of grazers and consider whether the proposed differences are responsible for the interaction between feeding habits and nest-building behavior by *P. femorata*.

The short time occupancy of nests by *P. femorata* individuals could also be a consequence of maternal care and the nutritive requirements of females after releasing their juveniles into the nest. Once juveniles were released from the marsupium, they were also sheltering and grazing on blade tissues inside and outside the female's nest. When small juveniles take over the maternal blade, females might leave their nests and search for new blades. Maternal care for recently released offspring could also explain the high variability in nest elongation rates in this study: we occasionally observed very high nest advancement rates (NAR) between two consecutive sampling days: for example, on one blade NARs were 20.8 and 41.0 mm day^{-1} at days 6–8 and 8–10, respectively. Females that are about to release their offspring might advance their nests in such a manner that both females and juveniles could feed and take refuge in the same domicile without further need of nest expansion during the maternal care period. Females of *Cymadusa filosa* also stopped nest construction after the release of juveniles, but it is not known whether building rates of females were higher before offspring release into the nest (Appadoo and Myers, 2003). Once juveniles start to graze and occupy all parts of the nest (see also Fig. 1B), this "overpopulation" might finally trigger females to abandon these nests and move to other blades within the sporophyte. Future studies should document maternal care behavior in *P. femorata* and examine whether extreme nest advancement rates indeed coincide with the moment of offspring release into the maternal nest.

Outlook

Detailed descriptions of the nest-building behavior have revealed a high variability in the construction capabilities and techniques of the species within the family Ampithoidae (Table 1). Most previous reports described the nests simply as algal parts held together by the amphipod silk. However, our quantitative study on nest advancement rates suggests that these nests are highly dynamic structures. In the nests of *Peramphithoe femorata* on *Macrocystis pyrifera*, the amphipods have adapted to the growth patterns of the kelp. To what degree nest dynamics are governed by food value or chemical defenses of the kelp tissues (or species) is not known at present. Neither chemical defense (Poore *et al.*, 2008) nor morphological complexity (Duffy and Hay, 1991; Sotka, 2007) of the algal hosts can independently explain herbivore preferences. We suggest that inherent traits of both the seaweed (*e.g.*, growth, chemical composition, stiffness) and the amphipod (*e.g.*, feeding and nest-building behavior) interact in determining host selection and nest maintenance. Our results suggest that a careful description of the nest building behavior and the tissue-specific food preferences of nest-building amphipods may help to better understand their host selection.

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