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No Effects and No Control of Epibionts in Two Species of Temperate Pycnogonids

STEVEN J. LANE[1,*], CAITLIN M. SHISHIDO[2], AMY L. MORAN[2], BRET W. TOBALSKE[1], AND H. ARTHUR WOODS[1]

[1]*Division of Biological Sciences, University of Montana, Missoula, Montana 59812; and* [2]*Department of Biology, University of Hawai'i at Mānoa, Honolulu, Hawaii 96822*

Abstract. Essentially all surfaces of marine plants and animals host epibionts. These organisms may harm their hosts in a number of ways, including impeding gas exchange or increasing the costs of locomotion. Epibionts can also be beneficial. For example, they may camouflage their hosts, and photosynthetic epibionts can produce oxygen. In general, however, the costs of epibionts appear to outweigh their benefits. Many organisms, therefore, shed epibionts by grooming, molting, or preventing them from initially attaching, using surface waxes and cuticular structures. In this study, we examined how epibionts affect local oxygen supply to temperate species of pycnogonids (sea spiders). We also tested the effectiveness of different methods that pycnogonids may use to control epibionts (grooming, cuticle wettability, and cuticular waxes). In two temperate species: *Achelia chelata* and *Achelia gracilipes*, epibionts consisted primarily of algae and diatoms, formed layers approximately 0.25-mm thick, and they colonized at least 75% of available surface area. We used microelectrodes to measure oxygen levels in and under the layers of epibionts. In bright light, these organisms produced high levels of oxygen; in the dark, they had no negative effect on local oxygen supply. We tested mechanisms of control of epibionts by pycnogonids in three ways: disabling their ovigers to prevent grooming, extracting wax layers from the cuticle, and measuring the wettability of the cuticle; however, none of these experiments affected epibiont coverage. These findings indicate that in temperate environments, epibionts are not costly to pycnogonids and, in some circumstances, they may be beneficial.

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* To whom correspondence should be addressed. E-mail: steven.lane@umontana.edu

Introduction

Virtually all marine plants and animals host communities of other organisms on their surfaces (Wahl, 1989; Wahl and Lafargue, 1990). These external organisms are known as epibionts, and they can be roughly classified into two groups: the microepibionts and the macroepibionts. Microepibionts include diatoms, protozoa, and biofilms, and communities of these components form biofilms (Davis *et al.*, 1989; Wahl, 1989). Macroepibionts include larger multicellular organisms, such as macroalgae, fungi, and sessile invertebrates (Richmond and Seed, 1991; Satuito *et al.*, 1997). Micro- and macroepibionts can occur together, and they influence one another (Richmond and Seed, 1991; Wahl *et al.,* 2012).

Epibionts can harm their hosts, for example, by limiting gas exchange in egg masses (Cohen and Strathmann, 1996) and across invertebrate gills (Burnett *et al*., 2006; Scholnick *et al.*, 2006), which magnifies the effects of other environmental stressors. Epibionts can also increase the costs of locomotion by increasing drag and decreasing the flexibility of hosts, which may render them more susceptible to predators (Witman and Suchanek 1984; Shine *et al.*, 2010; Key *et al.*, 2013). In other cases, epibionts appear to help their hosts. Photosynthetic epibiotic algae can raise local levels of oxygen (Cohen and Strathmann, 1996; Larkum *et al.*, 2003; Roberts *et al.*, 2007; Woods and Podolsky, 2007). Some arthropods promote the growth of microepibionts that can reduce or prevent infection by toxic fungi (Gil-Turnes *et al.*, 1989; Armstrong *et al.*, 2000), and others use macroepibionts to conceal themselves from predators (Wicksten, 1980; Feifarek, 1987; Dougherty and Russell, 2005). Such camouflage is also used by decorator crabs (Wicksten, 1980).

Negative effects often appear to outweigh positive effects, and many host organisms have evolved ways to remove epibionts, or to prevent them from attaching in the first place. Most crustaceans and nematodes shed their external cuticles regularly (*i.e.,* molt), removing any existing epibionts (Dyrynda, 1986; Thomas *et al.*, 1999; Smirnov, 2014). Crustaceans and echinoderms also groom their gills and external surfaces (Bauer, 1981, 2013; Russell, 1984; Dyrynda, 1986). Crustaceans secrete waxes onto their cuticles, reducing cuticular wettability and possibly making it more difficult for epibionts to adhere (Becker *et al.*, 2000; Callow *et al.,* 2002). Slow-moving or sessile organisms, such as ascidians and gastropod molluscs, produce antifouling chemicals that prevent micro- and macroepibionts from attaching (Wahl and Banaigs, 1991; Wahl and Sönnichsen, 1992). Finally, the microtopography of the cuticle can prevent colonization or growth (Callow *et al.,* 2002; Bers and Wahl, 2004). For instance, surface ridges on the mussel *Mytilus galloprovincialis* prevented barnacle larvae from attaching, likely by decreasing the area available for attachment (Scardino *et al.*, 2003).

Although host organisms often appear to remove or control epibionts, the effects of epibionts on gas exchange have not been rigorously tested. Here we examine the effects of epibionts on gas exchange by pycnogonids (phylum Arthropoda), and test for the presence of active and passive mechanisms for epibiont control. Pycnogonids are ideal for this study because they are often found harboring epibionts (Pipe, 1982; Key *et al*., 2013), and they lack a true respiratory system (Arnaud and Bamber, 1987), relying instead on the diffusion of oxygen directly across their thin cuticles or through pores piercing the cuticle (Davenport *et al.*, 1987; Lane *et al.,* unpublished data). Therefore, epibionts are likely to disrupt gas exchange by (i) increasing the thickness of the unstirred layers through which oxygen must diffuse, and (ii) directly consuming oxygen themselves. Both processes depress oxygen levels at the outer cuticular surface of the pycnogonids. We tested for these effects by measuring oxygen levels, using microelectrodes.

Pycnogonids, like crustaceans, have been observed grooming their legs with specialized appendages. These appendages are called ovigers, and they are also used by some species to carry egg masses (Arnaud and Bamber, 1987; Bamber, 2007). Previous work has reported that males carrying eggs have higher epibiont loads, suggesting a trade-off between egg-carrying and grooming (King, 1973). However, the role of ovigers in grooming has not been experimentally tested, and is suggested by only a few observations. We tested whether two species: *Achelia chelata* (Hilton, 1939) and *Achelia gracilipes* (Cole, 1904), use their ovigers to control epibionts. Additionally, we tested whether the structure and composition of the cuticle play a role in reducing colonization.

Materials and Methods

We collected pycnogonids from hydroids (Family Haleciidae; 1–30 m deep) by scuba-diving in and around Friday Harbor, Washington (48° 32′ N, 123° 00′ W), in June, 2015. Seawater temperatures in this area averaged approximately 10 °C. Individuals were kept in seawater tables (12–15 °C) at the University of Washington's Friday Harbor Laboratories (FHL) until their use in experiments. No animal was held in the seawater tables for more than two weeks before being used in an experiment. We used only adult *Achelia chelata* and *Achelia gracilipes* with leg spans between 8–17 mm. Individuals were identified to species based on Kozloff and Price (1996) and Carlton (2007). The majority of individuals collected were *A. chelata* ($n = 27$ of 40 total); patterns of epibiont coverage and control, use of ovigers, and cuticular structure appeared similar between the two species. Both species were collected off of the same hydroids, and it was difficult to identify the individuals to species until after they were killed, after the experiments were finished. Merging the two datasets was justified, because epibiont coverage and species composition did not vary, and the individuals were similar in size, morphology, and habitat.

Oxygen profiles through epibionts

Oxygen concentrations were measured using a Clark-style O_2 microelectrode (50-m tip; Unisense, Aarhus, Denmark) connected to a picoammeter (PA2000; Unisense). Measurements were made at 10.5 ± 0.2 °C (mean ± SEM), to approximate the average temperature of the pycnogonids' natural habitat, using a water-jacketed glass cell connected to a recirculating water bath. The electrode was calibrated at the experimental temperature in air- or N_2-bubbled seawater. The electrode was mounted in a micromanipulator. Data were recorded once per second by a Sable Systems (North Las Vegas, NV) UI-2 acquisition interface controlled by ExpeData (v1.8.4; Sable Systems).

We measured oxygen levels in the bulk seawater 1 cm from the pycnogonid, and at the surface of the cuticle of individuals with or without heavy epibiont coverage ($n = 8$ per group). Most of the individuals that we collected from the wild had low epibiont coverage (less than 10%); so the heavily fouled individuals (with greater than 75% epibiont coverage) from the Manipulation of Grooming Assay (see *Manipulation of Grooming* below) were used in this assay. We chose individuals with high epibiont loads to estimate how severely epibionts alter patterns of gas exchange. Pycnogonids were held between two glass coverslips connected at the corners by modeling clay. Air was bubbled gently into the chamber to maintain air-saturated conditions. In pycnogonids that were visually free of epibionts, oxygen

measurements were made at three randomly chosen locations along the legs and one on the trunk. On fouled individuals, or on those that were visually hosting epibionts, the same number of measurements were taken, but sampling locations that had thick epibiont layers were chosen.

Pycnogonids that were free of visible macroepibionts were illuminated, using a Fiber-Lite 180 illuminator (Dolan-Jenner Industries, Inc., Boxborough, MA) equipped with light guides featuring an EKE 21 Volt, 150-watt lamp (Ushio America, Cypress, CA) with a nominal color temperature of 3250 K, which is slightly redder than sunlight (5800 K). To standardize light intensity, the light guides were positioned 10 cm away from each pycnogonid, and the power on the illuminator was set at maximum. To determine whether epibionts could produce significant amounts of photosynthetic oxygen, we measured oxygen levels under epibionts in both well-lighted (as mentioned above) and dark conditions (no light; covered by black paper). Oxygen levels at the cuticle surfaces were given 1–2 min to stabilize between light treatments. Because the duration of each measurement varied greatly (40 s–5 min), oxygen concentrations were averaged over the final 30–40 s only. The variation in oxygen measurements was due to the thickness of the epibionts in the patches being measured; thicker epibiont layers tended to produce more oxygen over a longer time interval.

Manipulation of grooming

To test whether *Achelia* spp. use their ovigers to control epibionts, we haphazardly selected 16 animals and separated them into two groups, restricted ovigers and controls (unrestricted ovigers). Pycnogonids from both groups were placed individually in chambers with approximately 2 g of hydroids as a food source. The chambers were designed to prevent pycnogonids from escaping while allowing for adequate water flow. Three holes (2–3-cm diameter) were cut into 60-ml round plastic containers with snap-top lips (Ziploc) fitted with nylon mesh (mesh openings approximately 1 mm^2). The chambers were suspended from the docks at Friday Harbor Labs, and submerged at a depth of 0.5–1 m for two weeks.

In the experimental group, ovigers were restricted by applying Loctite marine epoxy (Henkel Corp., Düsseldorf, Germany) to their distal ends, thereby gluing them together. Twenty-four hours later, ovigers were examined and, if the glue and/or epoxy was gone, another attempt was made. To avoid potential adverse effects of epoxy handling, individuals requiring more than two applications of epoxy were excluded from the experiment. At the end of the experiment, all individuals survived and the ovigers remained disabled. However, one pycnogonid from the experimental group lost a single oviger. Because this individual seemed healthy otherwise, and its epibiont coverage was not different from the others in the group, it was included in the analysis.

Percent cover by macroepibionts (estimating coverage by microepibionts was impossible *via* standard microscopy) was estimated from photographs taken before and after the experiment. Images (dorsal view) were taken under a stereomicroscope fitted with a Nikon D7100 digital camera and stereomicroscope adapter. Images were analyzed using ImageJ software (v1.49) (Rasband, 2014). Individual patches of epibionts were outlined, summed, and divided by the total area of the pycnogonid (Chung *et al.*, 2007). Change in percent cover was then calculated for each individual by subtracting the percent cover at the beginning of the experiment from the percent cover at the end. The individual animals that we collected from the wild were not completely free of epibionts at the beginning of the experiment. By estimating the change in cover, we corrected for some of this initial variation, and better estimated how much colonization and growth occurred.

Cuticular wax extraction

We tested the effect of cuticular waxes on epibiont cover by cutting each of 16 pycnogonids longitudinally down the center of its trunk and separating the halves into two groups: an experimental group, in which waxes were removed, and a control group, in which the waxes were left intact. Only individuals visibly free of epibionts were used in this assay. Cuticle pieces in the experimental group were soaked in a methanol:chloroform (1:1; a solvent used for general lipid extraction) treatment (30 min at 21 °C), then glued with Loctite marine epoxy (at the trunk) to a microscope slide, with legs suspended freely. The slides were then placed in individually marked, 50-ml Falcon tubes. Two 2 × 4 cm windows were cut on opposite sides of the tubes and fitted with 1-mm^2 nylon mesh. These windows permitted water to flow across the samples, but prevented access by larger organisms. The tubes were then hung off of the FHL dock, submerged to a depth of 0.5–1 m for two weeks. After recovery, percent epibiont cover was estimated, using the methods described above in *Manipulation of grooming*.

Cuticle wettability

We estimated wettability by measuring contact angles of small water droplets placed on the cuticle. Higher contact angles indicate hydrophobic surfaces (*i.e.,* low wettability) and low-contact angles denote hydrophilic surfaces (*i.e.,* high wettability) (Yuan and Lee, 2013). Live sea spiders were gently centrifuged for 1 s at low speeds in a 1.0-ml microcentrifuge tube, packed with a small piece of KimWipe (Kimberly-Clark Corp., Irving, TX), which removed excess water from the cuticle. We used only individuals that had no visible epibionts. It is possible that bacterial biofilms were present on the cuticle, which would

affect our estimate of cuticle wettability, although it is not clear how (Thomas and Muirhead (2009) found that biofilms increased wettability; Epstein *et al*. (2011) found that they decreased it). However, the individuals likely had, at most, sparsely distributed individual bacteria (not biofilms), as revealed by images (using scanning electron microscopy, SEM) of areas of clean cuticle from individuals in the group with restricted ovigers (images not shown).

To test whether wettability varied across the cuticle, droplets (approximately 50 nl) of fresh tap water were placed on cuticle using a Drummond Nanoject nanoliter injector (Drummond Scientific Co., Broomall, PA), and the droplets were immediately imaged from the side by a stereomicroscope fitted with a digital camera (Nikon D7100). Images were analyzed in ImageJ. Contact angles were obtained from the femur, first tibia, second tibia, and trunk of eight individuals.

We also tested whether there was an association between local wettability and the presence of epibionts on different parts of the cuticle. We measured the percent cover of epibionts on the legs *versus* the trunk of the eight control individuals from the oviger restriction experiment. Percent cover was estimated as described above in *Manipulation of grooming*.

Scanning electron microscopy and maximum epibiont thickness

Individuals from the grooming experiment were fixed in 2% cacodylate-buffered glutaraldehyde at 4 °C for 24 h, then washed with cacodylate buffer (pH 7.0) for 5 min and rinsed in deionized water. Samples were dehydrated in a graded ethanol series (50%, 70%, 90%, 95%, 100% (×2)) for 10 min each, followed by 20 min in a 1:1 mixture of hexamethyldisilazane (HMDS) and ethanol. Samples were moved to 100% HMDS and left to air-dry in a fume hood overnight. Samples were sputter coated with gold (Denton Desk-V sputtercoater; Denton Vacuum, Inc., Moorestown, NJ), then viewed with an S-4700 Hitachi-model scanning electron microscope at the University of Montana.

To estimate thickness of the epibiont layers on the most heavily fouled individuals, we focused on the areas of greatest colonization from SEM images (*e.g.,* on the femur in Fig. 1D). Thickness was measured from the surface of the cuticle to the top of the epibionts. Images were analyzed in ImageJ (v1.49) (Rasband, 2014). Maximum epibiont thickness was used as an estimate of maximum possible fouling.

Statistical analyses

Mean values (± standard error) were calculated for each of the above-mentioned variables. Unless otherwise stated, Type I error was set at 0.05; all statistical analyses were performed in R (v3.0.2) (R Core Team, 2013).

Oxygen concentration at the bottom of the epibionts in the light cycle was compared against the dark cycle, using a paired *t*-test. These data were log-transformed to normality, which was confirmed using an Anderson-Darling test.

Oxygen concentrations between the bulk water, control group, and epibionts in the dark cycle were assessed by Kruskal-Wallis test, because the data were non-normal and had unequal variances among groups. We chose to compare differences between the dark epibiont group, bulk water, and control group because there was no apparent photosynthesis on the pycnogonid cuticle in the control group.

To assess the role of ovigers in epibiont control, a Wilcoxon signed-rank test was conducted; this test was chosen because the data were not normal and had an upper bound of 100%. These parameters violate the assumptions of a parametric test, and the values could not be normalized by transforming them. Similarly, the function of cuticle waxes in epibiont control and the concentration of epibionts on the trunk *versus* the legs were evaluated with a related-samples Wilcoxon signed-rank test (the observations were paired). These data also were not normal, and they had an upper bound of 100%. In the cuticle wax assay, however, the Q-Q plots were not definitive in detecting nonnormality. Therefore, we also transformed the original data using arcsin, a process typical for percentage data (Sokal and Rolf, 1981), and analyzed them, using a paired *t*-test in addition to a related-samples Wilcoxon signed-rank test.

Variation in cuticle wettability across body location was assessed using repeated measures analysis of variance followed by a pairwise *t*-test for multiple comparisons, with *P*-values adjusted using a Bonferroni correction. Normality of the data was confirmed with an Anderson-Darling test.

Results

Epibiont oxygen assay

Oxygen concentrations at the surface of the cuticle and in the bulk water did not differ significantly in the presence or absence of epibionts ($\chi^2 = 5.099$, d.f. = 2, $P = 0.078$) (Figs. 1 and 2). However, exposure to light in the presence of epibionts caused oxygen concentrations to rise significantly ($t = 3.937$, d.f. = 7, $P = 0.006$) (Figs. 2 and 3). In the experimental group, epibionts consisted primarily of algae and diatoms, forming layers approximately 0.25-mm thick (Fig. 1). Maximum epibiont thickness averaged 240 ± 42 μm ($n = 9$), with a peak thickness of 565 μm seen on one individual.

Grooming analysis

Change in percent epibiont cover did not vary significantly with experimental (restricted ovigers) and control (unrestricted ovigers) pycnogonids ($Z = 37$, d.f. = 15, $P = 0.645$) (Fig. 4). Change in percent cover in the unrestricted ovigers group averaged 81.9% ± 3.6% compared to 74.8% ± 9.1% in the restricted ovigers group.

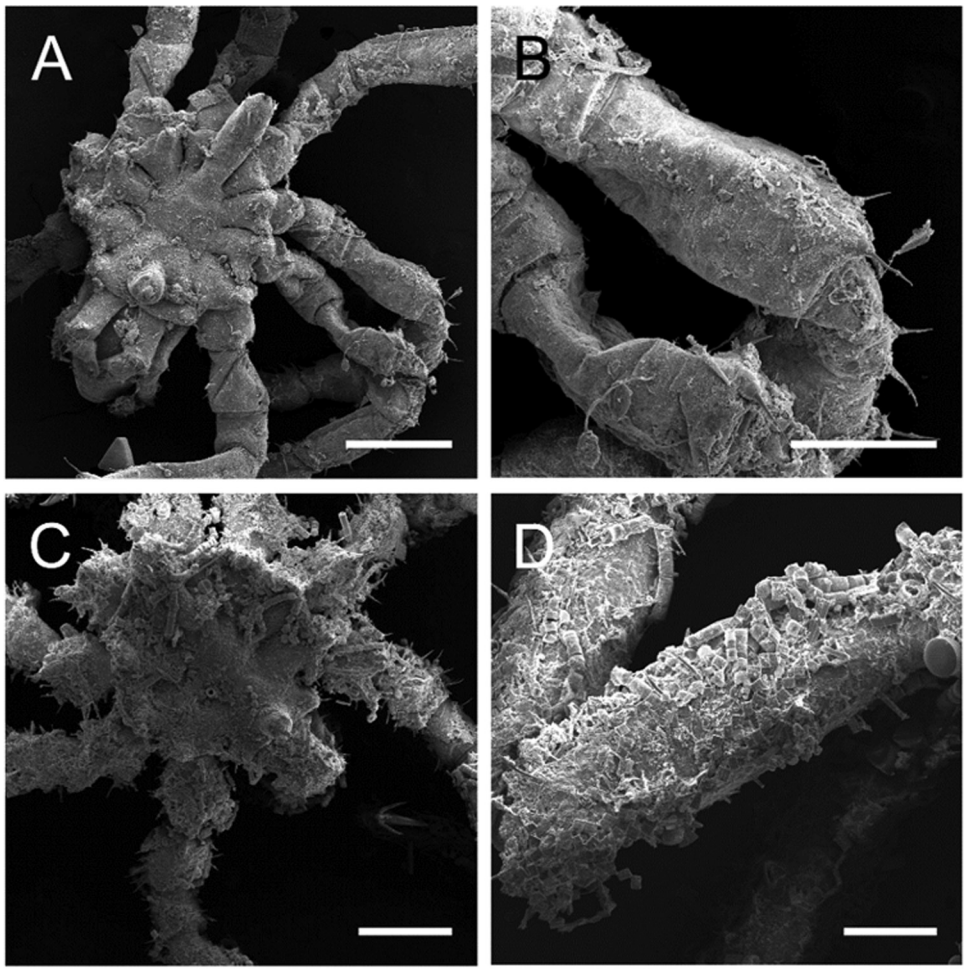

Figure 1. Scanning electron micrographs of *Achelia* spp. (A) Trunk of a pycnogonid with relatively few epibionts (scale bar = 500 μm; total magnification = 40×); (B) higher magnification view of femur from (A) (scale bar = 250 μm; total magnification = 110×); (C) trunk of substantially fouled individual from the oviger-use experiment (scale bar = 500 μm; total magnification = 35×); (D) higher magnification view of femur from (C) (scale bar = 250 μm; total magnification = 70×).

Wettability

Contact angles varied depending on where on the body they were measured, but all of these angles were low, indicating that the cuticle was fairly wettable ($F = 25.8$, d.f. = 24, $P < 0.001$) (Fig. 5). The contact angles of the femur and first and second tibias averaged 65.9 ± 2.0, 68.7 ± 2.9, and 64.8 ± 1.4 degrees, respectively. The trunk cuticle had higher wettability, as its contact angle was 45.8 ± 1.6 degrees. Pairwise comparisons indicated there were no differences in the contact angles among the leg segments, but each segment differed from that of the trunk. Although contact angles differed between the trunk and legs, epibiont percent cover did not differ significantly at these locations: 78.9% ± 5.5% and 75.7% ± 7.0%, respectively ($Z = 11$, d.f. = 7, $P = 0.383$) (data not shown).

Cuticular wax analysis

The change in epibiont percent cover after wax extraction averaged 35.0% ± 7.3%, and percent cover averaged 18.7% ± 3.0% in the intact wax group (Fig. 4). A related-samples Wilcoxon rank-sum test indicated no significant difference between the groups ($Z = 35$, d.f. = 31, $P = 0.093$). However, our paired *t*-test, using arcsin-transformed data, showed that the wax-extracted group had a significantly higher percent coverage of epibionts than the intact wax group ($t = -2.34$, d.f. = 15, $P = 0.033$).

Discussion

In bright light, epibionts on pycnogonids produced high levels of oxygen locally. In the dark, however, epibionts had no negative effect on local oxygen supply (Figs. 2 and 3).

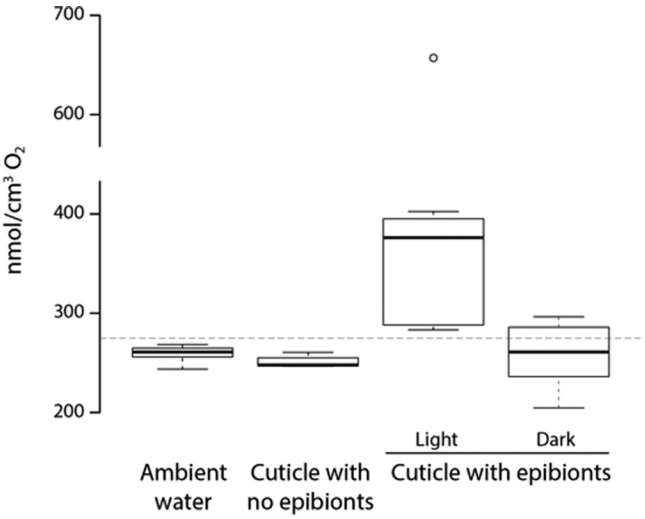

Figure 2. Oxygen concentrations in ambient water, at the surface of the cuticle in unfouled pycnogonids (see Fig. 1A, C), and at the surface of the cuticle under a layer of epibionts in light and dark treatments (see Fig. 1B, D) ($n = 8$ per group). The dashed line represents the theoretical oxygen concentration in air-saturated seawater at the experimental temperature (10 °C). Bold lines indicate medians, boxes indicate quartiles, and lines represent minimum (min) and maximum (max) extremes.

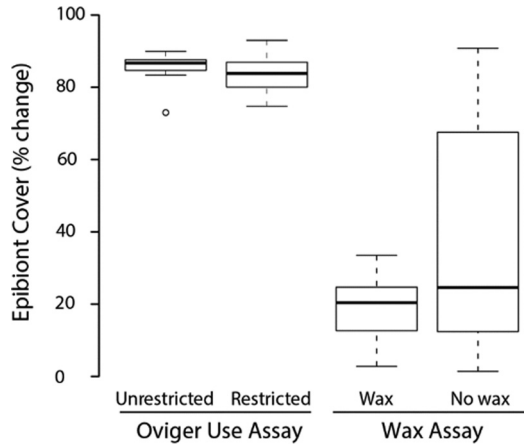

Figure 4. Change in percent cover of epibionts on pycnogonids from the oviger use assay ($n = 8$ per group) and from the wax assay ($n = 16$ per group). Changes occurred over two weeks. Bold lines indicate the median, boxes indicate quartiles, and lines represent minimum (min) and maximum (max) extremes.

This result differed from the findings of Cohen and Strathmann (1996) and Woods and Podolsky (2007), who studied oxygen profiles in the gelatinous egg masses of gastropods. Those masses often contained dense populations of microalgae and cyanobacteria, which produced substantial photosynthetic oxygen when illuminated. In the dark, oxygen levels fell almost to zero in central parts of the gel matrix due to respiration by the eggs, and possibly also by the unicells. In our study, the relatively high levels of oxygen in darkness may have reflected the location and arrangement of epibionts on the cuticle, where they were exposed directly to moving water. They may also have reflected the low metabolic rate of the epibionts on the pycnogonids, or the pycnogonids themselves relative to the gastropod egg masses.

How important is oxygen production by epibionts in nature? The answer depends on available levels of light, which, in our study, varied significantly across collection sites. We collected some pycnogonids at depths of 20–30 m and on the undersides of submerged concrete blocks—sites that likely received very little light and would support very

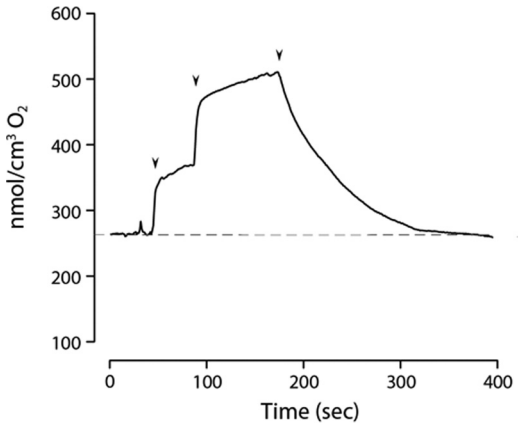

Figure 3. Representative trace of oxygen concentration through epibionts. From the origin, the first two arrowheads indicate measurements made above and below epibionts during the light treatment, and the third arrowhead marks the transition to the dark treatment. The first arrowhead marks the oxygen level at the top of the epibiont layer; the second arrowhead marks the oxygen level at bottom of epibionts. The dashed line indicates baseline oxygen level of ambient water.

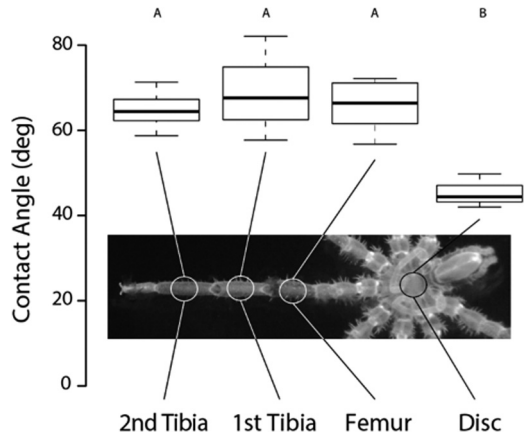

Figure 5. Cuticle wettability for different leg segments and body. Groups that do not share a letter are significantly different ($n = 8$ per group), repeated-measures analysis of variance. Bold lines indicate the median, boxes indicate quartiles, and lines represent minimum (min) and maximum (max) extremes.

low levels of photosynthesis. For example, at 25 m, red (700 nm) is completely attenuated, blue (420 nm) is 90% of value at the surface, and ultraviolet (326 nm) is about 50% of surface level (Denny, 1993). We collected other pycnogonids at just 1–2-m depth, which were well-lighted during daylight hours. At these depths, blue and ultraviolet are virtually unchanged, and red is attenuated to approximately 60% to 70% of its value in air (Denny, 1993). These are the locations in which photosynthesis could play a significant role in altering local levels of oxygen.

Several species of pycnogonids have been reported to use their ovigers to groom their cuticles (King, 1973; Arnaud and Bamber, 1987; Davenport *et al.*, 1987; Bamber, 2007). Our study, however, is the first to experimentally test the role of the ovigers in grooming. Here two species of *Achelia* showed no increase in epibiont coverage after two weeks when their ovigers were disabled (Fig. 4). Pycnogonid ovigers can be classified into two types: those with compound spines and those without; compound spines appear to be necessary for grooming (Bamber, 2007). Species within the genus *Achelia* lack compound spines on their ovigers. Our results, therefore, are consistent with the hypothesis that pycnogonids lacking compound spines do not groom (Bamber, 2007). A more powerful test of this hypothesis would experimentally test the effectiveness of grooming in species that do possess compound spines.

In many marine invertebrates, waxes alter cuticular wettability by rendering the cuticle more hydrophobic, which has been hypothesized to make it more difficult for epibionts to attach (Finlay *et al.*, 2002; Dahlström *et al.*, 2004; Sun *et al.*, 2012). The attachment hypothesis leads to several specific predictions, namely, cuticle wettability should in general be low, epibiont coverage should vary positively with wettability, and experimental increases in wettability should stimulate the growth of epibionts. Our results largely do not support these predictions. In *Achelia*, cuticle wettability was high (contact angles $< 90°$; Fig. 5) and, although it varied between legs and trunk, the densities of epibionts did not. Similarly, removing waxes gave inconclusive results. There was some evidence that waxes (or chemicals present in those waxes; see Fahrenbach, 1994, and Melzer *et al.*, 1996) on pycnogonids prevent epibiont attachment (Fig. 4; paired *t*-test), but our nonparametric analysis did not support this conclusion. Furthermore, like pycnogonids, decapod crustaceans had low contact angles and no covariation between contact angle and density of epibionts (Becker *et al.,* 2000). Together, these results suggest that the wettability-attachment hypothesis should be discarded, at least for marine arthropods.

This is the first study to assess how macroepibionts affect gas exchange in adult marine invertebrates. Using two species of temperate pycnogonids, we have shown that macroepibionts do not restrict local oxygen availability, and, in some cases, they may raise local oxygen levels. However, epibionts may incur other negative effects not explored here. For example, encrusting epibionts can restrict movement of their hosts, which may make them more prone to predation; these effects have been suggested to occur in pycnogonids (Key *et al.*, 2013). Indeed, heavily fouled individuals in our study appeared less mobile due to algae wrapped around their legs. Increased weight and drag could also make the host more likely to be dislodged from the hydroids on which it lives. Furthermore, epibionts growing on the pycnogonids' eyes, palps, or sensory setae could interfere with finding food and mates, or sensing predators. In our study, many of the pycnogonids were only lightly fouled when they were initially brought into the lab.

Therefore, these animals must in some way prevent epibiont growth and attachment. Future experiments will explore other potential mechanisms of control, including molting (Thomas *et al.*, 1999), taking advantage of strong currents to remove loosely attached epibionts (Wolff, 1959), and nocturnality, which can reduce the growth of algae (Becker and Wahl, 1996). Future studies should also determine whether pycnogonids structure their cuticles to recruit epibionts—for camouflage or to control infections (Armstrong *et al.*, 2000; Dougherty and Russell, 2005). Perhaps pycnogonids frequently support epibionts because the positive effects derived from them are stronger than previously suspected.

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