



A Rhizocephalan Parasite Induces Pervasive Effects on Its Shrimp Host

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Abstract

*Rhizocephalan barnacles are parasites of crustaceans that are known for dramatic effects on hosts, including parasitic castration, feminization, molt inhibition, and the facilitation of epibiosis. Most research on rhizocephalans has focused on carcinized hosts, with relatively little research directed to shrimp hosts that may experience distinct consequences of infection. Here, we describe a high-prevalence rhizocephalan-shrimp system in which multiple host changes are associated with infection: the dock shrimp *Pandalus danae* infected by the rhizocephalan *Sylon hippolytes*. In field-collected *P. danae*, infection by *Sylon* was associated with development of female sex characters at a smaller size and greater probability of epibiosis. Standardized video observations showed that infected *P. danae* performed grooming activities at higher rates than uninfected shrimp, suggesting that inhibited molting rather than direct behavioral modification is a likely mechanism for higher epibiosis rates. There was no difference in the composition of grooming behavior types or in general activity between infected and uninfected shrimp. Fatty acid compositions differed with infection, but total lipid concentrations did not, suggesting that parasite-driven shifts in host resource allocation were compensated or redirected from unmeasured tissues. Our results show that *Sylon* alters its host's role by provisioning an epibiotic substrate and also that it influences host physiology, resulting in feminization and fatty acid shifts. This study lays the groundwork for expanding rhizocephalan-shrimp research and encourages recognition of oft-ignored roles of parasitism in ecological communities.*

Introduction

Evolving from a filter-feeding ancestor, rhizocephalan barnacles infect crustaceans, most commonly decapods (Høeg, 1995; Glenner and Hebsgaard, 2006; Ewers-Saucedo *et al.*, 2019; Høeg *et al.*, 2020). They are adapted to a parasitic lifestyle and have lost typical adult barnacle features, with a body plan consisting of a rootlike, trophic internal structure (*interna*) and a bulbous, reproductive external structure (*externa*; Høeg, 1995). Rhizocephalans are particularly fascinating in their adaptations to parasitism and their well-known capacity to affect their ecosystems by influencing host abundances and traits (Bishop and Cannon, 1979; Høeg, 1995; Torchin *et al.*, 2001; Mouritsen and Jensen,

2006). Unlike epicaridean isopods, another parasite of crustaceans with substantial host effects, rhizocephalans have a direct life cycle; larvae released by the *externa* go on to infect the same host species (Høeg, 1995; Høeg and Lützen, 1995; Williams and Bunkley-Williams, 2019). As parasitic castrators, rhizocephalans may regulate host populations by limiting reproduction (Kuris and Lafferty, 1992; Torchin *et al.*, 2001; Innocenti and Galil, 2011a) and alter host ecological function through behavioral and physiological changes (Smith, 1911; Bishop and Cannon, 1979; Mouritsen and Jensen, 2006). The long legacy of rhizocephalan research has contributed to the growing recognition of parasites as influential members of their local communities

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Abbreviations: AICc, corrected Akaike's information criterion; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl ester; FHL, Friday Harbor Laboratories; nMDS, nonmetric multidimensional scaling; PERMANOVA, permutational multivariate analysis of variance.

Online enhancements: supplemental material.

(see Lafferty *et al.*, 2006; Kuris *et al.*, 2008; Preston *et al.*, 2016; Buck, 2019; Gehman *et al.*, 2019; Morton and Lafferty, 2022).

A central strategy of rhizocephalan infection is the co-opting of host reproductive investment for parasite growth and reproduction (Høeg, 1995). By targeting resources that would be otherwise “wasted,” from its perspective, the rhizocephalan can leave other host functions intact, thereby facilitating both host and parasite longevity at the expense of host reproduction (Baudoin, 1975; Lafferty and Kuris, 2009). Parasitic castration is employed across diverse parasitic taxa, including trematode worms, bopyrid isopods, and various protists (Baudoin, 1975; Lafferty and Kuris, 2009). Hosts infected by rhizocephalans may have degenerated gonad and/or gametes, reflecting the diversion of resources from their reproduction (*e.g.*, Lützen, 1981; Bower and Boutillier, 1990a; Høeg, 1995; Fazhan *et al.*, 2020). In addition, resource location and mobilization can be affected. Glucose, glycogen, and fat content in the hepatopancreas, muscle, and hemolymph can differ between infected and uninfected hosts (Smith, 1911; Reinhard and Von Brand, 1944; Abdelsater *et al.*, 2015; Hsiao *et al.*, 2016), with a greater relative hepatopancreas size in infected ones (Fazhan *et al.*, 2020).

Another common host consequence of rhizocephalan infection is the cessation of molting, which may prevent the dislodging of the externa during the molt and the unnecessary energetic expense of host growth (Høeg, 1995; Høeg and Lützen, 1995). In addition to facilitating growth in crustaceans (Hartnoll, 1983), molting is a defense against epibiotic fouling, as accumulated epibionts are shed with the molted exoskeleton (Carman and Dobbs, 1997; Bauer, 2013). Thus, increased epibiosis is frequently associated with rhizocephalan infection (Phillips and Cannon, 1978; Gaddes and Sumpton, 2004; Innocenti and Galil, 2011b). Increased epibiosis can also arise from parasite-altered behaviors, particularly burrowing (Mouritsen and Jensen, 2006; Innocenti and Galil, 2011a). Green crabs *Carcinus maenas* (Linnaeus) infected by *Sacculina carcini* Thompson burrow less than uninfected conspecifics (Mouritsen and Jensen, 2006). Burrowing scours away epibiotic organisms on the crabs’ surface, and consequently an infected crab becomes a more available hard substrate for sessile organisms in an otherwise soft-sediment habitat (Mouritsen and Jensen, 2006).

Rhizocephalan parasites of brachyuran and anomuran crabs are documented to induce female morphological features and physiological changes in infected male crabs (Høeg, 1995). For example, wide pleons are secondary sex characters of female crabs, and infected male crabs often show a broadening of the pleon relative to their uninfected male counterparts (Høeg, 1995; Høeg and Lützen, 1995; Innocenti and Galil, 2011a; Kristensen *et al.*, 2012). The broad female pleon is protective of the parasite’s externa, which is responsible for reproduction and emerges in a location similar to the host’s brood (Lützen, 1984; Kristensen

et al., 2012). Rhizocephalans can induce behaviors typical of ovigerous female crabs, resulting in host care of the parasite’s externa (Phillips and Cannon, 1978; Ritchie and Høeg, 1981; Høeg and Lützen, 1995). Feminization may also manifest in the mobilization of host energetic resources: in *Carcinus maenas* and *Inachus* sp. Weber, parasitism by *Drepanorhynchus* (Fraisse) was associated with the development of fat in the hepatopancreas and blood typical for female crabs with maturing ovaries (Smith, 1911).

Most research on rhizocephalan effects on hosts focuses on crab-like brachyuran and anomuran hosts, with relatively little study on those parasitizing shrimp hosts. Through histological studies, Lützen (1981) and Bower and Boutillier (1990a, b) described sterilization in the shrimps *Spirontocaris lilljeborgii* (Danielssen) and *Pandalus platyceros* J.F. Brandt, respectively, with both finding impacted oocyte development and altered secondary sex characters. In terms of feminization, Lützen (1981) observed two male *S. lilljeborgii* shrimp infected by *Sylon hippolytes* M. Sars with morphologies of pleopod 1 between those of male and female shrimp; in one, the gonad appeared to hold both spermatogonia and oocytes. Nagler *et al.* (2017) compared parasite-host volume ratios of *S. hippolytes* infecting *Pandalina brevisrostris* (Rathke) to those of *Peltogaster* spp. Rathke infecting hermit crabs; a larger ratio for *Sylon* was attributed to the single-brood reproductive strategy of *Sylon* relative to the multiple-brood strategy of *Peltogaster*. Additional effects, such as those relating to epibiosis and nutrient reallocation, are, to our knowledge, not yet described in shrimp hosts, and evidence of feminization remains limited.

Relative to “carcinized” or crab-like decapod body forms, fouling may be more detrimental for shrimps or shrimplike forms. Shrimplike decapods often rely on swimming or a tail-flip escape for locomotion and defense, which could be impaired by epibiont-produced drag (Bauer, 1978, 1981). This difference is suggested to explain why specialized grooming behaviors are more common in shrimps than crabs (Bauer, 1975, 1981, 1989), although crabs may rely on other antifouling defenses such as burrowing (Bauer, 1989, 2013). If rhizocephalans affect fouling in shrimps, they in turn could affect other organisms by providing additional substrate for epibionts and, through impairing escape responses, by increasing the predation success on the shrimps.

Spirontocaris lilljeborgii, a shrimp for which feminization may occur based on two specimens (Lützen, 1981), is gonochoric (Pike, 1954). While most decapod crustaceans are gonochoric, some shrimps, particularly of the genus *Pandalus*, are protandrous hermaphrodites, developing first into males and transitioning to females through development (Berkeley, 1929; Bergström, 2000; Chiba, 2007). This raises the question of whether feminization may occur in shrimps already destined to become female.

Previous studies on rhizocephalans have identified various host biochemical properties influenced by parasitism,

such as glucose and fat content of hemolymph and hepatopancreas (Smith, 1911; Reinhard and Von Brand, 1944; Abdelsater *et al.*, 2015; Hsiao *et al.*, 2016). Fatty acids offer another potential measure. As key lipid components, fatty acids constitute and influence the properties of fats and membranes in biological tissues, act as energy sources, and can serve as precursors for other biomolecules (Parrish, 2009; De Carvalho and Caramujo, 2018). Some fatty acids, especially certain ω -3 polyunsaturated fatty acids, are particularly important for growth, reproduction, and signaling (Parrish, 2009; Winder *et al.*, 2017; De Carvalho and Caramujo, 2018). For most animal consumers, necessary fatty acids must come from their diets, with limited (though important) endogenous biosynthesis or modification, which leads to fatty acids' use as trophic biomarkers (Dalsgaard *et al.*, 2003; Budge *et al.*, 2006; Kelly and Scheibling, 2012; Galloway and Budge, 2020). In this context, assessing the fatty acids of rhizocephalans and hosts should reveal a reallocation of host resources; fatty acids accumulated in the parasite would be expected to be host derived, with a corresponding reduction or compensation of those fatty acids in host tissues.

Shrimps of the genus *Pandalus* are widespread and ecologically important (Bergström, 2000). In the northeastern Pacific Ocean, they have a variety of predators, including rockfish, cod, octopus, and dogfish (Butler, 1980; Albers and Anderson, 1985; Bergström, 2000; Turner *et al.*, 2017). Species such as *P. platyceros*, *P. jordani* (Balss), *P. eous* Makarov, and *P. danae* Stimpson are caught commercially and/or recreationally (Bergström, 2000). The rhizo-

cephalan *S. hippolytes* infects these and other shrimp species in the region and can reach high prevalences (over 32%; in Bower and Boutillier, 1990b). Globally, pandalid shrimps are only second to penaeid shrimps in terms of their commercial importance (Holthuis, 1980). With the ecological and economic importance of these shrimps, it is especially valuable to understand the potential effects of *Sylon* on pandalid shrimps.

We investigated the effects of parasitism by *S. hippolytes* in the dock shrimp *P. danae* (Fig. 1) in a high-prevalence population found at Friday Harbor Laboratories (FHL; University of Washington, Friday Harbor). We surveyed the population to quantify *Sylon* prevalence and the association of epibiotic fouling and parasitism. Using video recordings of shrimp, we compared grooming behaviors and general activities (foraging and locomotion) between infected and uninfected shrimp. We hypothesized that epibiosis rates would be positively related to parasitism, based on patterns observed in crab hosts (*e.g.*, Mouritsen and Jensen, 2006; Innocenti and Galil, 2011b). Because grooming plays a particularly important role for shrimp (Bauer, 1975, 1981, 1989), we hypothesized that increased epibiosis rates would correspond to reduced grooming behaviors in infected shrimp and a release of that time toward other general activities. Using the same survey data, we assessed whether *Sylon* induced feminization in *P. danae* by evaluating the combined influences of size, as a proxy for age, and infection status on host sex. We hypothesized that infected shrimp would be transitional or female at smaller

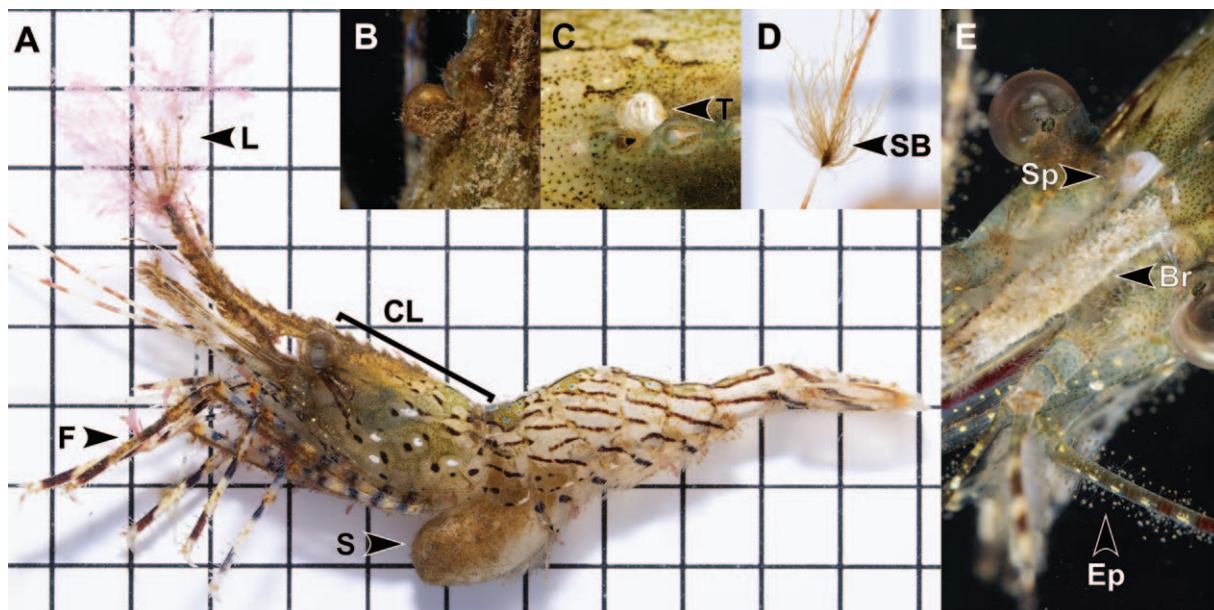


Figure 1. Shrimp host *Pandalus danae*, parasite *Sylon hippolytes*, and observed epibionts of *P. danae*. (A) *Pandalus danae* infected by *Sylon* with visible epibionts. Infection at this stage is apparent with a conspicuous externa (S) emerging from the pleon of the shrimp. Postorbital carapace length (CL) was measured from the orbit to the dorsal edge of the carapace. Among other epibionts, lacy (L) and flat (F) red algae are visible on rostrum and pereopods. Various epibionts colonizing infected or scarred shrimp: heavy epibiotic fouling including presumed diatom growth over eye (B); thoracican barnacle (T) attached to carapace (C); stringy brown (SB) algae growing on an antenna (D); spirorbid worm (Sp) and bryozoan (Br) colonizing the rostrum and *Ephelota* (Ep) colonizing an antenna (E). Grid size in (A) = 5 mm. Some exposure and contrast adjustments are applied to images for clarity.

sizes. Finally, we measured lipid concentrations and fatty acid compositions in infected and uninfected *P. danae*, inferring that differences would reflect *Sylon*-driven changes in host resource allocation. Altogether, we aimed to better characterize the *Pandalus-Sylon* relationship and highlight potential ecological implications of parasitism.

Materials and Methods

Study location and specimen collections

We collected *Pandalus danae* Stimpson, 1857 from the docks at FHL in July and August 2020 under the general FHL scientific take permit, which is overseen by the FHL director in accordance with local regulations. We used a custom dip net (opening: 30 cm × 30 cm; depth: 45 cm; bottom: 15 cm × 15 cm) made of ~150- μ m bolting cloth to catch shrimp from pilings. A flexible semicircular edge on one side of the net allowed us to more completely capture shrimp and avoid bias in collection. Shrimp were held in flow-through seawater tables at FHL.

Sylon prevalence and epibiosis

We surveyed 575 shrimp through daily collections on July 14–20 and July 22, 2020. To prevent duplicate counting of shrimp, we held all measured shrimp in a separate seawater table until measurements ended on July 22, after which all measured shrimp were released to their catch location. To facilitate handling, we slowly cooled shrimp in a bag of seawater set in a cooler of ice or ice packs. We monitored shrimp scaphognathites (bailers) as a proxy for respiration to avoid euthanizing shrimp (incidental mortality was 2.6%). For each shrimp, we measured the postorbital carapace length (hereafter, “size”) as a proxy for age using digital calipers (Fig. 1A). The sex of each shrimp (juvenile, male, transitional, and female) was scored using the first pleopod’s endopod shape and the presence of sternal spines (Hansen and Aschan, 2001). No shrimp were observed with eggs, and external determinations of head roe presence were inconclusive, which prevented direct analysis of potential sterilization by *Sylon* in this study. We recorded the presence or absence of any macroscopically visible epibionts: the suctorian ciliate *Ephelota* sp., algae (separated into morphological groups), spirorbid worms, bryozoans, and hydroids (Fig. 1). The provisional identification of *Ephelota* was based on outward appearance and its previous detection as an abundant epibiont of *P. danae* in the same location (Bauer, 1975). Organisms that were macroscopically indistinguishable or whose presence was ambiguous (e.g., diatoms) were not recorded.

We scored the presence of *Sylon* presence and infection stage visually and nonlethally by observing through the transparent ventral cuticle of the pleon (Fig. S1 [Supplemental Material is available online]). Stages were determined as (1) uninfected, showing no signs of *Sylon*; (2) interna only, showing milky, weblike growths under the pleon but no

other signs of *Sylon*; (3) primordial externa, a nucleus or developing externa that has not emerged from the pleon; (4) an emerged externa; and (5) scar, where the externa has fallen away, leaving a melanized scar. For shrimp with externa(e), we also measured the externa width. One shrimp was mistakenly recorded with an externa width despite no *Sylon* identified as being present; it was corrected.

We modeled the association of size and infection stage on epibiosis (presence or absence) for *Ephelota*, small red algae, and lacy red algae, using logistic regression models ($n = 575$ shrimp) for each epibiont type. Size was included as a potential predictor because larger shrimps were expected to have longer intermolt periods (Daoud *et al.*, 2010) and provide a larger settlement surface for epibionts, increasing the probability of epibiosis. We did not model other epibionts, which had very low prevalences (<10%), because patterns of these rare events were likely not captured accurately with our sample size. Within each epibiont type, we compared models using corrected Akaike’s information criteria (AICc) and AICc weights.

Identification of epibiotic algae

To facilitate later identification of epibiotic algae, we sampled algae from several shrimp and mounted them on slides using glycerol jelly (Dioni, 2003). This preserved much of the original color and morphology. The slides were examined with compound (Leitz Laborlux D) and stereo (Olympus SZ61) light microscopes from 4 to 45 magnification and were photographed with a Nikon COOLPIX W300 camera. The sizes of blades, cells, and reproductive structures were measured with a reticle. Many, but not all, of the specimens had reproductive structures that assisted in identification. Species identification was accomplished using a variety of references and taxonomic keys, including current publications and original descriptions of the species in question (Harvey, 1862; Hollenberg, 1942; Wollaston, 1971; Lindstrom and Gabrielson, 1989; Guiry and Guiry, 2023) and other frequently consulted references (Kylin, 1925; Drew, 1951; Scagel, 1966, 1967; Abbott *et al.*, 1976; Fritsch, 1977; Druehl and Clarkston, 2016; Gabrielson and Lindstrom, 2018). K. A. Miller, curator at Herbarium at University of California, Berkeley, assisted in the identification of *Antithamionella* sp.

Infection and host feminization

We investigated whether shrimp sex was associated with infection by modeling sex predicted by size and *Sylon* presence, fitting the aforementioned survey data by using continuation ratio models with the R package VGAM (Yee, 2021). Infection stage data were recoded as presence (interna only, primordial externa, emerged externa, or scar) or absence (uninfected). For this analysis, we considered scar stage within the infected class for biological and statistical reasons. First, scarred shrimp still retain the remnants (e.g., epibionts and melanization) of infection and

might not be expected to revert sexually due to parasite loss, having not yet molted to clear those signs. Second, while scarred shrimp may be epidemiologically considered a recovered or potentially a newly susceptible class, treating them as such would unnecessarily complicate statistical analysis by adding a third class to infection status (*e.g.*, uninfected, infected, and recovered) with a relatively small representation. We omitted juvenile shrimp from this analysis because no infected juveniles were observed. Two shrimp were accidentally moved into a communal postmeasurement tank before scoring sex, so those individuals lack sex data and were omitted from analysis ($n = 564$ shrimp). Proportional-odds ordinal logistic regression was deemed inappropriate because of violations of the parallel-lines assumption (McCullagh, 1980; Harrell, 2015). We compared models using AICc and AICc weights.

Grooming behaviors and activity

We caught 20 each of infected and uninfected shrimp on July 30 and August 1. We chose infected shrimp with relatively large, well-developed externae and then selected uninfected shrimp of similar sizes to limit variation in size and sex across treatments. We held shrimp for at least 24 h to acclimate them to the laboratory before video recording their behaviors. We recorded shrimp one at a time in random order in two 1.6-L plastic pet tanks (Lee's Kritter Keeper, Lee's Aquarium and Pet Products, San Marcos, CA), each with four sides externally painted black (back, left, right, and bottom) and plumbed with running seawater. We added gravel collected from the shoreline at FHL to both tanks to provide a substrate (~1- to 2-cm depth). An overhead 1600-lumen daylight CFL bulb provided lighting, and the rest of the room was darkened to limit visual disturbance to the shrimp. We allowed shrimp to acclimate in tanks for 40 min before recording 18-min videos and exchanged tanks between each recording. We recorded video using a 50-mm prime lens (Nikon Nikkor) attached to a digital single-lens reflex camera (Nikon D7100) set to 1/50-s shutter speed, f/8 aperture, ISO 800 sensitivity, 30-p frame rate, and 1080 resolution.

We scored grooming behaviors of shrimp from videos by using the program BORIS (Friard and Gamba, 2016) and based our scored behaviors on those identified by Bauer (1975) on *P. danae* in the same location. One author (SB) scored all videos to maintain consistency; blind scoring was not possible because externae are visible in videos when a shrimp is infected. Recorded behaviors were classified based on body parts that were cleaned: antennal flagella, antennular flagella, general body (carapace and abdomen surfaces), maxillipeds, pleopods, and pereopods. We compared grooming behaviors between uninfected and infected shrimp by using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) with 9999 permutations. Counts were analyzed to compare the number of grooming behaviors between uninfected and infected shrimp

(*i.e.*, do infected shrimp groom less than uninfected shrimp?), and percentages were analyzed to compare the distribution of behaviors between infection statuses (*i.e.*, do infected shrimp focus on different behaviors relative to uninfected shrimp?).

While grooming behaviors were recorded as point events (no duration associated with data), they could represent a time trade-off for other activities. Accordingly, we separately scored the same videos by broad categories of shrimp activity as state events: stationary (no locomotion), walking (locomotion using pereopods across substrate), swimming (locomotion using pleopods above substrate), and foraging (picking and feeding upon substrate with or without locomotion). We modeled the proportion of video time spent in each activity using Dirichlet regression (package DirichletReg; Maier, 2021) and compared the null model and the model including infection status using AICc and AICc weights.

Fatty acids of infected shrimp

We collected 22 each of infected and uninfected shrimp on July 28 and 29 and August 2 and 3, 2020. We chose infected shrimp with relatively large, well-developed externae and then selected uninfected shrimp of similar sizes. We held shrimp in sea tables without feeding for at least 24 h before preparing them for fatty acid sampling. Shrimp were euthanized by slowly cooling them as described above and then transferring to a -20 °C freezer until shrimp were completely unresponsive and scaphognathite movement ceased. We did not use chemical anesthetics (especially clove oil) for euthanasia because of the potential to contaminate samples.

We sampled 12 infected and 12 uninfected whole shrimp. We removed the pereopods, third maxillipeds, and antennal flagella to reduce debris in the later lipid extraction process, before dipping shrimp in fresh tap water and freezing at -80 °C. The freshwater dip removed excess salt that can interfere with later weighing steps prior to lipid extraction.

We dissected 10 each of infected and uninfected shrimp. For both, we dissected and stored abdominal muscle tissue and the hepatopancreas. For infected shrimp, we also removed and stored the externae. Dissected tissues were frozen at -80 °C.

We extracted lipids from frozen samples following modified methods of Taipale *et al.* (2013, 2016). Briefly, we freeze-dried and ground the samples before adding 2:1 chloroform:methanol to a weighed amount of ground sample. We then added an internal C19:0 standard and 0.9% aqueous NaCl. The samples were vortexed and sonicated, after which the chloroform layer was removed as the lipid extract. We added additional chloroform and repeated the vortexing and sonicating with the remaining layer, pooling the two chloroform lipid extracts within samples. We measured lipid content gravimetrically from the lipid extract.

We transesterified lipid extracts to derive fatty acid methyl esters (FAMES) prior to analysis, using a QP2020 Shimadzu (Columbia, MD) gas chromatographer–mass spectrometer (GC-MS). For transesterification, we evaporated chloroform from lipid extracts and redissolved lipids in toluene. We added 1% sulfuric acid in methanol and incubated the samples at 90 °C for 90 min. The sulfuric acid was neutralized with 2% KHCO₃, and hexanes were added. We vortexed and centrifuged the samples and then removed and stored the hexane layer containing FAMES, repeating hexane extraction of FAMES once. All lipid extraction and transesterification steps were performed under a nitrogen gas atmosphere. The GC-MS analysis and fatty acid identification were as in Yoshioka *et al.* (2019) and Taipale *et al.* (2013, 2016).

We visualized the multivariate fatty acid data using non-metric multidimensional scaling (nMDS) and compared fatty acid profiles between tissues and infection status, using PERMANOVA with 9999 permutations. For dissected tissues, we omitted externae and compared the remaining dissected tissues. We accounted for tissues belonging to the same shrimp by using shrimp identity as a stratum. We then compared infection status within tissues as a *post hoc* test, penalizing alpha by dividing it by 2 (=0.025), the number of additional comparisons. Multivariate analyses were performed in the vegan package (Oksanen *et al.*, 2018) with Euclidean distances. We limited analyses to fatty acids that composed at least 0.5% of the total identified fatty acids in any *Sylon*-tissue combination on average. We used PERMDISP2 (Anderson, 2006) to infer whether significant PERMANOVA results were due to differences in dispersion or location. We compared lipid content between infected and uninfected shrimp for each tissue type using Wilcoxon rank-sum tests.

We performed all data analyses in R version 4.2.3 (R Core Team, 2023). All work was conducted under institutional approval and guidelines for COVID-19 safe research practice.

Results

Sylon prevalence and epibiosis

We measured 575 shrimp, which ranged in size (carapace length) from 5.52 to 26.17 mm (mean ± SE, 13.62 ± 0.11 mm). We scored 9 (1.6%) shrimp as juvenile, 404 (70.3%) as male, 54 (9.4%) as transitional, and 106 (18.4%) as female.

Sylon prevalence was 42.8% overall (246 shrimp), with 10.4% (60) shrimp with only an interna, 15.1% (87) with a primordial externa, 13.2% (76) with an emerged externa, and 4.0% (23) with scars.

Ephelota was the most common epibiont, found on 397 (69.0%) of all shrimp. Small red algae were found on 257 (44.7%) shrimp, lacy red algae on 93 (16.2%) shrimp, flat red algae on 21 (3.7%), filamentous red algae on 8 (1.4%), and stringy brown algae on 20 (3.5%). Animal epibionts were rare but included spirorbid worms (two shrimp, *Spirorbis* sp. Daudin), bryozoans (two, unknown species), and hydroids (one, possibly *Obelia* sp. Péron & Lesueur). Thoracican barnacles were not observed in our survey described here, but one, likely *Balanus crenatus* Bruguière, was found on a single infected shrimp in 2019 (Fig. 1B; RMY, unpubl. data). Algal morphological groups represented several algal species (Table 1). One species, *Scagelia occidentalis* (Kylin) Wollaston, was also found as an epiphyte on brown and red algae collected from the FHL docks.

For *Ephelota*, small red algae, and lacy red algae rates of epibiosis increased with infection stage (Fig. 2). The two logistic regression models with infection stage only and shrimp size + infection stage as predictors had the lowest AICc values and had comparable weights for *Ephelota* (Fig. 2A, C; Table 2). For small red algae, the model with shrimp size + infection stage had the lowest AICc (Fig. 2E; Table 2), with a weight 11 times the second-lowest AICc model. The infection stage-only model had the lowest AICc for lacy red algae, but the size + infection stage model also had some support (Fig. 2C, F; Table 2). The influence of size on fouling probability was not consistent between epibionts, being positive for *Ephelota* (Fig. 2D), strongly negative for small red algae (Fig. 2E), and weakly negative for lacy red algae (Fig. 2F).

Infection and host feminization

We observed nine juvenile shrimp, none of which were infected. Uninfected shrimp were 68.4% (225 shrimp) male, 6.7% (22) transitional, and 22.2% (73) female. Infected shrimp were 72.8% (179 shrimp) male, 13.0% (32) transitional, and 13.4% (33) female. The two unsexed shrimp were both infected.

Infection status and size both influenced shrimp sex. The continuation ratio model predicting sex including size,

Table 1

Algal morphological groups assessed in survey and corresponding species identified using sampled, slide-mounted epibionts

Morphological group	Description	Species
Small red algae	Minute red algae with no macroscopically visible features apart from color and presence	Small individuals of other species
Lacy red algae	Branching and feather-like red algae	<i>Antithamnion kylinii</i> N.L. Gardner, 1927; <i>Callithamnion acutum</i> Kylin, 1925; <i>Scagelia occidentalis</i> (Kylin) Wollaston, 1972
Flat red algae	Foliose or blade-like red algae	<i>Nitophyllum hollenbergii</i> (Kylin) I.A. Abbott, 1969
Filamentous red algae	Small filiform red algae with no to few branches	<i>Polyostea robusta</i> (N.L. Gardner) Savoie & GW Saunders, 2016
Stringy brown algae	Olive-brown filamentous algae	<i>Ectocarpus commensalis</i> Setchell & N.L. Gardner, 1922

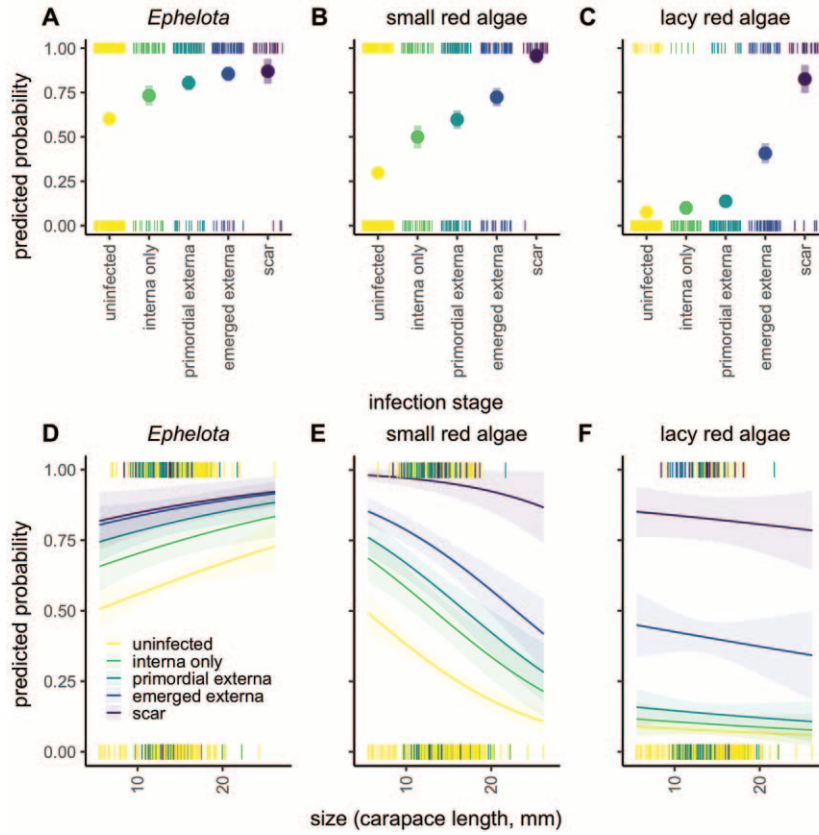


Figure 2. Epibiosis of *Pandalus danae* and infection stage of *Sylon*. Predictions of logistic regression models of the probability of epibionts by *Sylon* infection stage (A–C) and by infection stage and size (D–F). Epibionts are *Ephelota* sp. (A, D), small red algae (B, E), and lacy red algae (C, F). In increasing stage, shrimp may be uninfected, have an infection by *Sylon* with the interna only, have a primordial (still beneath cuticle) or emerged *Sylon* externa, or have a scar after the externa has been lost. Rug plots at 0 and 1 are recorded absence and presence, respectively, of the epibiont on surveyed shrimp. Points are model predictions with ± 1 SE. Curves show model predictions with ± 1 SE as shading.

Table 2

Model selection for logistic regression models of epibiotic fouling of *Pandalus danae* shrimp based on size (postorbital carapace length) and/or infection stage of *Sylon*

Model	df	Log likelihood	AICc	Δ AICc	w
<i>Ephelota</i>					
<i>Ephelota</i> ~ <i>Sylon</i>	5	−339.27	688.65	0	0.48
<i>Ephelota</i> ~ size + <i>Sylon</i>	6	−338.31	688.77	0.11	0.46
<i>Ephelota</i> ~ size + <i>Sylon</i> + size: <i>Sylon</i>	10	−336.22	692.84	4.19	0.06
<i>Ephelota</i> ~ 1 (null)	1	−355.78	713.57	24.92	0.00
<i>Ephelota</i> ~ size	2	−355.45	714.92	26.27	0.00
Small red algae					
Small red algae ~ size + <i>Sylon</i>	6	−345.14	702.43	0	0.89
Small red algae ~ size + <i>Sylon</i> + size: <i>Sylon</i>	10	−343.48	707.36	4.92	0.08
Small red algae ~ <i>Sylon</i>	5	−349.51	709.12	6.69	0.03
Small red algae ~ size	2	−389.25	782.51	80.08	0.00
Small red algae ~ 1 (null)	1	−395.32	792.64	90.21	0.00
Lacy red algae					
Lacy red algae ~ <i>Sylon</i>	5	−204.87	419.85	0	0.65
Lacy red algae ~ size + <i>Sylon</i>	6	−204.78	421.71	1.86	0.25
Lacy red algae ~ size + <i>Sylon</i> + size: <i>Sylon</i>	10	−201.6	423.58	3.73	0.10
Lacy red algae ~ 1 (null)	1	−254.46	510.93	91.08	0.00
Lacy red algae ~ size	2	−254.3	512.62	92.77	0.00

Note: df, degrees of freedom; AICc, corrected Akaike’s information criterion; Δ AICc, difference from lowest AICc model; w, AICc weight.

Table 3

Model selection for continuation ratio models of *Pandalus danae* shrimp sex based on size (carapace length) and presence of *Sylon*

Model	df	Log			w
		likelihood	AICc	Δ AICc	
Sex ~ size + <i>Sylon</i> + size: <i>Sylon</i>	8	-268.35	552.96	0	1.00
Sex ~ size + <i>Sylon</i>	6	-278.63	569.41	16.45	0.00
Sex ~ size	4	-282.96	573.99	21.03	0.00
Sex ~ <i>Sylon</i>	4	-432.51	873.1	320.13	0.00
Sex ~ 1 (null)	2	-438.67	881.36	328.4	0.00

Note: df, degrees of freedom; AICc, corrected Akaike's information criterion; Δ AICc, difference from lowest AICc model; w, AICc weight.

infection status, and their interaction had the lowest AICc values of the relevant models (Table 3). While infected shrimp were predicted to have a greater probability of being transitional or female at smaller sizes compared to uninfected shrimp, they also had a greater probability of being transitional or male at larger sizes. In other words, the transition period from male to female is predicted to occur over a greater range of sizes for infected shrimp (Fig. 3). Recoding the unsexed shrimp as either both male or both female did not affect results (see R script, available online).

Grooming behaviors and activity

On average, shrimp performed 90 ± 7 (mean \pm SE) grooming behaviors over the recording duration, with infected shrimp performing 109 ± 10 grooming behaviors and uninfected shrimp performing 71 ± 6 . The counts of grooming behaviors across types were significantly greater for infected shrimp compared to uninfected ones (PERMANOVA, $R^2 = 0.12$, pseudo- $F = 4.94$, $P = 0.0058$; Fig. 4; Table S1), but the compositions (percentages) of the grooming behavior types did not differ (PERMANOVA, $P > 0.05$; Fig. 4; Table S1). Maxillipeds were the most frequently groomed body part for both infected and uninfected shrimp, followed by pereopods, antennular flagella, and antennal flagella. Grooming of the general body and pleopods were observed uncommonly and only in infected shrimp, one of which appeared to interact with its externa. One shrimp did not fully perform maxilliped grooming and accordingly scored 0 for that behavior. Its omission did not affect results (Table S1). Shrimp did not generally appear to feed on groomed material. In a single instance, a shrimp groomed its maxillipeds with its second pereopod (cheliped) and brought the pereopod to its mouth. We observed some ambiguous instances when feeding coincided with or shortly followed grooming, which in most cases involved a first pereopod being swept through the maxillipeds and then being brought to the mouth. These cases were rare and occurred in both infected and uninfected shrimp, with most observed feeding occurring during general foraging activities.

Infected and uninfected shrimp did not differ in time spent performing general activities such as being stationary,

walking, swimming, or foraging (Dirichlet regression vs. null, Δ AICc = 7.64, $w = 0.02$; Fig. S2; Table S2).

Fatty acids of infected shrimp

We identified 57 fatty acids in our samples, of which 35 were present at 0.5% of total identified fatty acids (TFA) or greater (Table S3). Seven fatty acids were present at 5% TFA or greater: palmitic acid (16:0), palmitoleic acid (16:1 ω -7), stearic acid (18:0), cis-vaccenic acid (18:1 ω -7), oleic acid (18:1 ω -9), eicosapentaenoic acid (EPA; 20:5 ω -3), and docosahexaenoic acid (DHA; 22:6 ω -3). Fatty acid profiles were distinct between infected and uninfected whole shrimp (PERMANOVA, $R^2 = 0.52$, pseudo- $F = 23.86$, $P < 0.001$; Table S4; Fig. 5B). In dissected shrimp, fatty acid profiles differed between infected shrimp and uninfected shrimp and by tissue, but their interaction was not significant (PERMANOVA, infection status: $R^2 = 0.02$, pseudo- $F = 1.73$, $P < 0.001$; tissue: $R^2 = 0.52$, pseudo- $F = 41.01$, $P < 0.001$; interaction: $P > 0.05$; Table S4; Fig. 5C, D). Within tissues, however, differences between infected and uninfected tissues were nonsignificant (PERMANOVA, $P > 0.05$; Table S4). None of the PERMDISP2 analyses were significant.

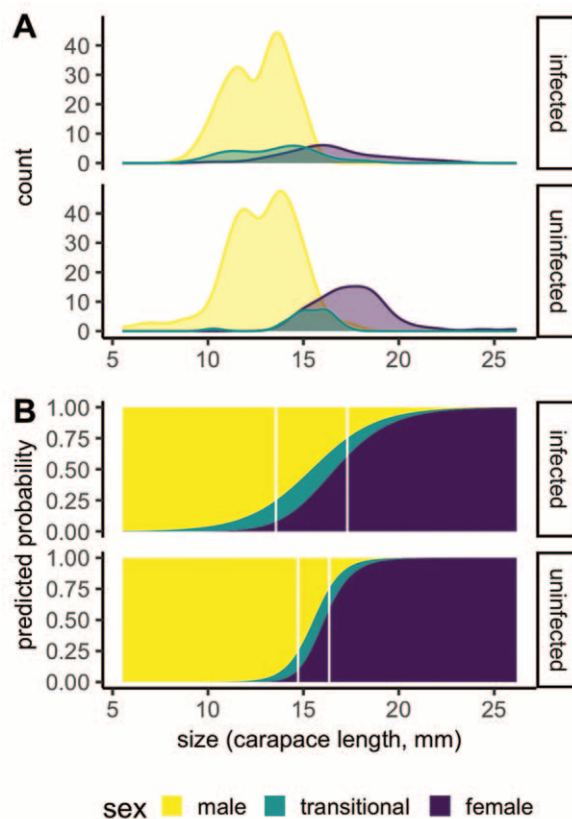


Figure 3. *Pandalus danae* shrimp size, sex, and infection status. Observed size distributions (A) and continuation ratio model predictions (B) for shrimp sex by *Sylon* infection status. Stacked, shaded areas in (B) show the predicted probabilities of a shrimp at a given size being male, transitional, or female according to the morphology of pleopod 1 and sternal spines. White vertical lines indicate size thresholds at which 25% and 75% of the shrimp are either transitional or female based on model predictions.

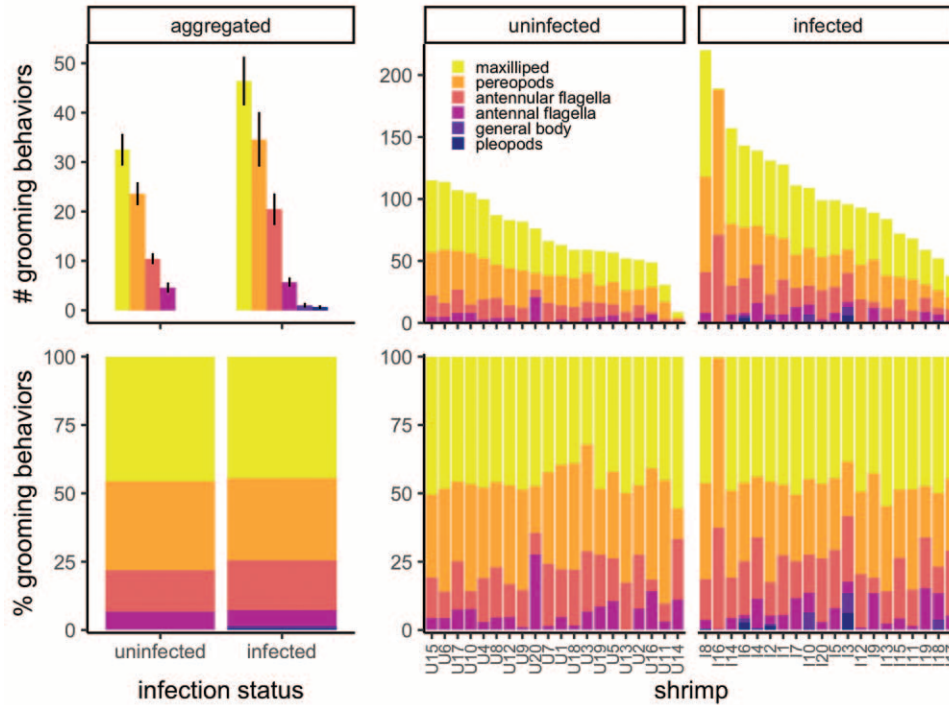


Figure 4. Counts and percentages of grooming behaviors of *Pandalus danae* shrimp, aggregated (mean) and individually, by *Sylon* infection status. Individual shrimp are ordered within infection status by descending total grooming behavior counts. Grooming behaviors are ordered by descending average count. Error bars are ± 1 SE.

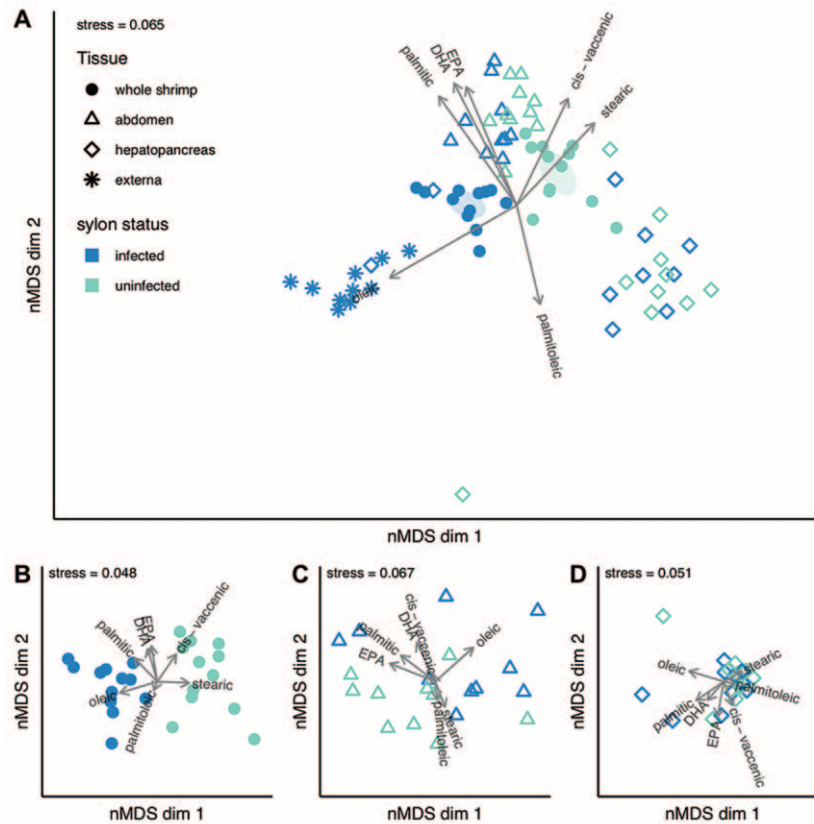


Figure 5. Nonmetric multidimensional scaling (nMDS) ordinations of fatty acid profiles of infected and uninfected *Pandalus danae* shrimp. (A) All samples; (B) whole *P. danae* shrimp; (C) dissected abdomens; (D) dissected hepatopancreases. Ellipses in (A) show standard errors around the centroids of whole uninfected and *Sylon* infected shrimp. Vectors show relative correlations of fatty acids composing at least 5% of the total identified fatty acids of any *Sylon*-tissue combination with nMDS dimensions (16:0, palmitic; 18:0, stearic; 16:1 ω -7, palmitoleic; 18:1 ω -7, cis-vaccenic; 18:1 ω -9, oleic; 20:5 ω -3, eicosapentaenoic acid [EPA]; 22:6 ω -3, docosahexaenoic acid [DHA]). Note that nMDS dimensions are not equivalent between plot panels.

In whole shrimp, EPA and palmitic acid were the most abundant fatty acids in both infected and uninfected whole shrimp (mean \pm SD, EPA: $21.0\% \pm 1.3\%$ and $20.7\% \pm 1.9\%$, respectively; palmitic: $15.7\% \pm 0.5\%$ and $14.9\% \pm 0.7\%$, respectively). Oleic acid was the third-most abundant fatty acid in whole infected shrimp ($14.9\% \pm 1.3\%$ vs. $9.2\% \pm 1.3\%$ in uninfected shrimp), whereas DHA was the third-most abundant in uninfected ones ($12.6\% \pm 1.6\%$ vs. $13.1\% \pm 1.1\%$ in infected shrimp). In both infected and uninfected hepatopancreases, EPA ($17.0\% \pm 3.9\%$ and $15.1\% \pm 3.8\%$, respectively), palmitic acid ($13.0\% \pm 1.8\%$ and $12.5\% \pm 0.9\%$, respectively), and oleic acid ($10.0\% \pm 4.8\%$ and $8.5\% \pm 5.0\%$, respectively) were the most abundant fatty acids. In infected and uninfected abdomens, the most abundant fatty acids were EPA ($20.9\% \pm 1.8\%$ and $21.5\% \pm 1.3\%$, respectively), palmitic acid ($18.8\% \pm 1.3\%$ and $18.7\% \pm 0.7\%$, respectively), and DHA ($15.9\% \pm 1.0\%$ and $15.7\% \pm 1.1\%$, respectively). Externae were dominated by oleic acid ($22.6\% \pm 2.0\%$), followed by EPA ($19.7\% \pm 1.8\%$) and palmitic acid ($16.6\% \pm 0.9\%$). Lipid content did not differ between infected and uninfected shrimp for either whole shrimp or dissected tissues (Wilcoxon rank-sum, $P > 0.05$; Table S5).

Discussion

In our study, infected shrimp had greater rates of epibiosis, an onset of female sex characters at a smaller size, increased frequency of grooming behaviors, and altered fatty acids relative to uninfected shrimp. Altogether, these changes to shrimp driven by *Sylon* suggest a substantial impact by the parasite, beyond sterilization (Lützen, 1981; Bower and Boutillier, 1990a), and potentially shifted ecological roles as a result of increased epibiosis.

Higher rates of epibiosis with infection stage are aligned with the expectation that the impacts of *Sylon* would increase with its progression of infection. Duration of infection is likely an important factor, because the longer a parasite interferes with antifouling defenses, the more epibionts are likely to accumulate. A common effect of rhizocephalans on their hosts is the cessation of molting or an increase in the intermolt period, though this is not consistent between different rhizocephalan taxa (Høeg, 1995). Molting would otherwise clear epibionts from the host surface, and the interference of molting is considered a key connection between rhizocephalan infection and epibiosis in various crabs species (Gaddes and Sumpton, 2004; Mouritsen and Jensen, 2006; Innocenti and Galil, 2011b). Altered molting is thus a likely cause for the patterns observed with *Sylon* and *Pandalus danae*. It should be noted, too, that the transition between a primordial externa and an emerged externa requires a molt (Lützen, 1981). This suggests that the intermolt period during which an emerged externa is present must be long enough to reaccumulate epibionts that were lost during the transitional molt.

The changes in epibiosis we found in shrimp might affect the susceptibility of an infected shrimp as prey.

Epibiosis can both increase and decrease predation rates, depending on the epibiont and the basibiont or host (Wahl and Hay, 1995; Wahl *et al.*, 1997; Laudien and Wahl, 2004). The shrimp epibionts, which look qualitatively similar to fouling organisms found on nearby substrate (RMY, pers. obs.), may make shrimp more cryptic, camouflaging them from predators. However, epibionts can increase predation by interfering with sensory organs and increasing hydrodynamic drag (Wahl, 1996). The shrimp observed in our study were sometimes severely fouled on their eyes and antennae, and this species relies on a swimming tail-flip escape response from predators (Daniel and Meyhöfer, 1989; Nemeth, 1997). Future research should verify the potential influence of *Sylon* on predation rates. For example, Gehman and Byers (2017) found that rhizocephalan-infected crab were more susceptible to predation in both mesocosm and tethering experiments, and the efficacy of tethering studies has already been demonstrated for *Pandalus* shrimp and their predators (Frid *et al.*, 2012; Yates *et al.*, 2020).

Contrary to expectations, infected shrimp had greater, not lower, rates of grooming behaviors relative to uninfected shrimp yet had increased epibiosis rates. A potential explanation is that grooming behaviors, while important for limiting epibionts (Bauer, 1975), are secondary to molting's more complete disruption to epibiosis. *Sylon*-induced molt impairment likely facilitated higher epibiosis rates in infected shrimp, which then increased grooming rates to compensate. The overall increase in the counts of grooming behaviors in infected shrimp without a change in their relative proportions is consistent with this explanation. Molting removes epibionts from the entire body surface, while behavior changes directly influenced by *Sylon* might be expected to shift the composition of grooming behaviors, for example, by focusing on the externa or proximal areas. Curiously, pleopod grooming (at an area near the externa) was observed only in infected shrimp, pointing to a potential minor direct effect, and one shrimp appeared to interact with its externa while grooming. General body grooming was also observed only in infected shrimp. It is possible, though, that these rare grooming behaviors were detected mainly because of the overall elevated grooming rate in infected shrimp.

Despite the increase in grooming behaviors in infected shrimp, there was no corresponding difference in overall activities (stationary vs. walking vs. swimming vs. foraging) performed by the shrimp. A difference may have indicated a time trade-off of grooming behaviors (while stationary) against locomotion or foraging. It may be that grooming is brief enough not to be a substantial time cost. In rare instances, grooming was coincident with feeding, which, despite their overlap being infrequent and of likely little dietary consequence, shows that the time trade-off between grooming and other activities may be minimal. Still, our observations were performed in simple captive conditions, and activity shifts or cost of grooming may be different when there are opportunities for food or threats of predation. For example,

Belgrad and Griffen (2015) observed reduced activity and slower reaction to food in infected crab. Brothers and Blakeslee (2021) found that infected crab used complex habitat differently from uninfected conspecifics, becoming more vulnerable to predation.

In most cases, host feminization by rhizocephalans has been studied in hosts that exhibit gonochoric sexual development. *Pandalus danae*, on the other hand, is a protandrous hermaphrodite, starting life as a juvenile, developing into a male, and then transitioning to a female (Berkeley, 1929; Bergström, 2000). Using size as a proxy for age, we found that infected *P. danae* develop female sexual characters earlier than uninfected shrimp, though conversely, the respective model also showed that infected shrimp completed the transition later (Fig. 3). This broadening of the transitional stage suggests that *Sylon* may disrupt or add to the variation of typical male-to-female transition. More thorough investigations into feminization effects should include measurements over time and internal observations of shrimp reproductive organs. Indeed, histological investigations have shown more specific disruptions in shrimp gonads in infected *Pandalus platyceros* and *Spirontocaris lijeborgii* (Lützen, 1981; Bower and Boutillier, 1990a). It is unlikely that the observed feminization effect would produce substantial reproductive impacts at the population level, especially as sterilization, the most impactful reproductive effect, may itself be incomplete and temporary. Of the infected *P. platyceros* surveyed by Bower and Boutillier (1990a), 20% did not have substantial impacts to gonad or secondary sex characters. Upon the loss of the externa and development of the scar, Lützen (1981) found no replacement externae, and Bower and Boutillier (1990b) noted a vigorous immune response against the remaining interna. In our study, the snapshot nature of our survey meant that we were unable to ascertain the persistence of infection during or past the scarred condition, for example, through the clearing of the scar or interna upon a subsequent molt. We also did not observe any infected or uninfected shrimp with eggs or externally visible gonad (head roe), which could have informed sterilization impacts in this study. Still, such observations would not have been conclusive without histological or longer-term study, as both Lützen (1981) and Bower and Boutillier (1990b) had observed some infected shrimp with developed gonad or eggs.

Sterilization is associated with a substantial reallocation of host resources, so we expected lipid measures to differ by infection status. For example, crab infected by *Drepanorhynchis* had consistently lipid-rich hepatopancreases, indicating parasite-driven changes to lipid metabolism (Smith, 1911). Interestingly, lipid content did not differ between whole shrimp or dissected tissues of *P. danae*, depending on infection status. We originally expected that lipids in the hepatopancreas could have been diverted to externa in infected shrimp, but there was no corresponding difference in lipid content, and fatty acid profiles showed considerable overlap in nMDS. While Smith (1911) found lipid-rich

hepatopancreases in infected crab, Reinhard and Von Brand (1944) found lower hepatopancreas lipid content in infected hermit crab *Pagurus pubescens* Krøyer. The crab *Metopograpus thukuhar* (Owen) infected by *Polyascus planus* (Boschma) had greater triacylglycerol concentrations in the hepatopancreas relative to uninfected ones but the opposite pattern for hemolymph (Hsiao et al., 2016). In contrast, *Actaeodes hirsutissimus* (Rüppell) infected by *Sacculina ignorata* Boschma had elevated hemolymph glucose and triacylglycerol concentrations (Abdelsater et al., 2015). Clearly, patterns of resource allocation are variable between systems. In this study, lipids or resources from other unmeasured tissues, such as developing gonads, may have been diverted to the hepatopancreas and externa, with a net neutral effect on shrimp lipid content.

Nonetheless, fatty acid profiles of whole shrimp differed between infected and uninfected shrimp. The difference seems to be mostly driven by greater proportions of oleic acid (18:1 ω -9) in infected shrimp relative to the uninfected ones. The externa was especially rich in oleic acid, and whole infected shrimp had nearly 5% TFA more oleic acid than uninfected ones. The role of the externa in driving fatty acid differences is supported by the small effect size ($R^2 = 0.02$) of *Sylon* infection in dissected tissues, for which the externa was omitted from analysis. Oleic acid, which is a common monounsaturated fatty acid, was the third-most abundant fatty acid in infected shrimp. In contrast, DHA, a very nutritious, highly unsaturated fatty acid, was the third-most abundant fatty acid in uninfected shrimp, though infected shrimp still had similar DHA amounts. Another highly unsaturated fatty acid, EPA, was the most abundant fatty acid in both infected and uninfected shrimp. The implications of oleic acid's dominance in the externae of *Sylon* is uncertain; in the ovaries of the barnacle *Balanus rostratus* Hoek, oleic acid is a substantial but not predominant fatty acid (Yamada et al., 2019). Oleic acid may serve as energy storage for developing eggs and larvae in the externa, because in the prawn *Macrobrachium borellii* (Nobili) oleic acid was a major component of storage lipids and was preferentially catabolized (Pasquevich et al., 2011). In the lobster *Homarus gammarus* (L.), oleic acid was among the most utilized fatty acids through embryogenesis (Rosa et al., 2005).

While much research on predator outcomes in the context of parasitism and fatty acids focuses on the consumption of free-living parasite stages (e.g., Gerphagnon et al., 2019; McKee et al., 2019), concomitant predation of parasites (i.e., with the host) is common and can be significant, especially when parasites are large relative to host tissues (Johnson et al., 2010). The similar lipid contents and proportions of valuable fatty acids EPA and DHA of uninfected and infected shrimp suggest, though, that their predators would not be substantially affected. Still, the clear differences in fatty acid profiles may complicate their interpretation as trophic biomarkers for predators of shrimp; for fishes, Timi and Poulin (2020) argue that

researchers should not ignore parasite-induced shifts when interpreting stable isotopes, another common trophic biomarker.

We found that *Sylon* produces multiple and diverse effects on its shrimp host. Despite relatively little research done on how rhizocephalan parasites impact shrimps, we observed comparable effects in this system relative to crab-rhizocephalan ones, including epibiosis and feminization. These similar impacts of *Sylon* on *P. danae* may have distinct implications compared to those of carcinized crustaceans, considering the shrimp as a protandrous hermaphrodite and a natant organism. Understanding the influence of *Sylon* in a naturally transitioning host may provide insight into feminization induced by other rhizocephalans, and protandrous development in pandalids remains an active research topic (Jónsdóttir *et al.*, 2018; Levy *et al.*, 2020). The influence of epibiosis on hydrodynamics can inform our understanding of energetics and predation risk, which are especially important because pandalid shrimp are valuable fisheries and important prey items (Butler, 1980; Holthuis, 1980; Bergström, 2000; Turner *et al.*, 2017). Last, we found that fatty acid profiles of infected shrimp differed significantly from those of uninfected shrimp, the consequences of which require further study. This study lays the groundwork for future research on a question-rich rhizocephalan system and underscores the importance of recognizing parasite roles in their hosts' behavior and life histories, as well as their interactions with other species (Lafferty *et al.*, 2006; Preston *et al.*, 2016; Buck, 2019; Gehman *et al.*, 2019).

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Data and Code Availability

Data and code used in this article are available in the Supplemental Material (available online).

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