

Grooming behaviors and fouling of the spider crab *Libinia dubia* (Decapoda: Epialtidae)

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ABSTRACT

Body fouling has been reduced by grooming behaviors. In decapods, grooming has been focused on gills, sensory structures, and jointed appendages. In this study, grooming behaviors of the spider crab, *Libinia dubia* H. Milne-Edwards, 1834, were examined; this brachyuran crab decorates and camouflages body regions by attaching materials onto hooked setae. The relationship between grooming and these camouflaged body regions was unknown. Six observational and experimental studies examined the grooming frequency, duration of grooming behaviors, body regions groomed, variance of these behaviors in the presence of another individual, and the efficiency of these grooming behaviors at removing gill fouling. Sensory and respiratory structures were groomed most frequently and for the longest duration, not body regions with decorations and hooked setae. Crabs in isolation exhibited the highest grooming time budget (5.22%). The presence of another conspecific decreased the grooming time budget (0.67%), and primary actions (e.g., fighting, displaying, mating) became priority. Ablation of a gill-grooming appendage did not impact fouling on gills. Grooming as a secondary action was supported. Reasons for not grooming body regions with hooked setae were discussed. Spider crabs had a lower time budget for grooming compared to most decapods, but similar to another brachyuran.

KEYWORDS

Decorating, setae, camouflage, crustacean, Brachyura

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INTRODUCTION

Grooming behaviors have been observed across multiple crustacean taxa (amphipods: Holmquist, 1985; anomurans: Martin and Felgenhauer, 1986; caridean shrimps: Bauer, 1975; mysids: Acosta and Poirrier, 1992; stomatopods: Wortham and Kostecka, 2019). These grooming behaviors

have been effective in removing fouling agents such as microscopic bacteria, sedimentation, algae, and epibionts that collected on body regions (Bauer, 1981). Mechanisms, morphological structures, and behaviors associated with grooming have differed greatly, but the function has remained the same (keep body regions free of fouling). Grooming behaviors in crustaceans have targeted different body regions: chemosensory antennules (Bauer, 1981; VanMaurik and Wortham, 2014), eyes (Wortham and KostECKA, 2019), gills (Wortham and Pascual, 2017), and jointed appendages (Hinsch, 1972; Videl-Gadea and Berlinger, 2009; Bauer, 2013). Bauer (1999) documented that grooming behaviors remove fouling; when grooming appendages were amputated in experiments, body fouling increased. Overall, grooming behaviors have led to body structures functioning properly (the benefit); nonetheless energy and time have been allocated towards grooming instead of other essential behaviors (the cost).

While most grooming research has focused on decapod shrimps, grooming behaviors have varied among crustacean groups such as shrimps, amphipods, and stomatopods (Bauer, 1977; 1978; 1981; 1987; 1989; Felgenhauer and Schram, 1979; Holmquist, 1985; VanMaurik and Wortham, 2011, 2014; Wortham and KostECKA, 2019). Detailed grooming behaviors of brachyurans have been reported in one study (*Callinectes sapidus* Rathbun, 1896 and *Menippe mercenaria* Say, 1818; Wortham and Pascual, 2017); differences existed in the grooming appendages, body regions groomed, fouling levels, and the grooming time budgets of these two crabs (5% vs 49%). Grooming has been unpredictable in crustaceans, even within one taxonomic group.

Molting in crustaceans has eliminated body fouling (on gills, setae, carapace, articulations etc.), but during the intermolt period, grooming behaviors have been more essential (Key *et al.*, 1997; Bauer, 2004). Individuals that have undergone a terminal molt may rely more on grooming since these “anti-fouling” events have stopped. Terminal molt individuals, that are usually larger due to age, have lost the ability to regrow hair-like setae that are involved in grooming body regions; these setae on their hard exoskeleton are replenished with each molt (Bauer, 1981; 1987). This setal loss may cause increased body fouling, resulting in gill damage, decreased locomotion, and

decreased sensory input (Holmquist, 1985; Bauer, 2002). Larger individuals, in crustacean taxa that experience a terminal molt, likely have different grooming pressures, compared with smaller, younger individuals that molt and continue to grow new setae. Many brachyuran crabs have been documented to undergo a terminal molt (*Callinectes sapidus*: Haefner and Shuster, 1964; *Chionoecetes opilio* Fabricius, 1788: Conan and Comeau, 1986; majid crabs: Hines, 1989). Grooming and a terminal molt have not been linked before; only a few researchers have even mentioned grooming in brachyuran crabs (Walker, 1974; Bauer, 1981; Sallem *et al.*, 2007), with one detailed study (Wortham and Pascual, 2017).

Study animal

Spider crabs, a group of commercially and ecologically important crustaceans, belong to the superfamily Majoidea. Larger and older individuals (including members of the family Epialtidae) have been documented to undergo a terminal molt (Hinsch, 1972; Sampedro *et al.*, 1999). These crabs have hooked setae located on the exoskeleton in body regions such as the carapace, rostrum, and walking legs (Wicksten, 1980; Ahl *et al.*, 1996; Hultgren and Stachowicz, 2011). Spider crabs have attached decorations to these hooked setae, likely concealing crabs from predators and prey (Wicksten, 1975, 1993; Stachowicz and Hay, 2000, Hultgren and Stachowicz, 2009, 2011; Wortham, 2013; Guinot and Wicksten, 2015). Nonetheless, besides molting, how (and if) spider crabs maintained these hooked setae and kept them functional for decorating was unknown.

Libinia dubia H. Milne-Edwards, 1834 (Family Epialtidae), the longnose spider crab, was used in this study; this crab, known as benthic, slow-moving, and nocturnal, has been collected along the Atlantic coast and in the Gulf of Mexico, often in close proximity to other conspecifics (Gray, 1957; Williams, 1984; Wilber and Wilber, 1991; Wicksten, 1993). No known study has documented the detailed grooming behaviors in spider crabs; the grooming setal morphology has been reported (Wortham and LaVelle, 2016). Because of the crab-like body with an enclosed gill chamber, mostly non-chelate walking legs, and a life history in the photic zone, spider crabs may be under higher environmental pressures, leading to increased fouling

levels and the subsequent requirement for increased grooming behaviors, compared with other decapod crustaceans. The known camouflaging behavior may also make grooming behaviors in spider crabs more crucial than in other crustacean species, if the hooked setae are groomed and maintained. Studying the body regions that spider crabs groom may provide insight into the relative importance of grooming (*i.e.*, general body grooming and/or cleaning hooked setae). Being able to compare past decapod research (mostly on shrimps) with the grooming behaviors of another decapod group (brachyuran crabs) has been predicted to be beneficial in studies looking at decapod phylogeny (Bauer, 1989).

Objectives and hypotheses

The objectives of this research were to determine for spider crabs the grooming behaviors, time budget for grooming, how grooming behaviors changed with the presence of a conspecific, and if areas with hooked setae were groomed more frequently or for longer durations compared to body regions not associated with decorating. Spider crabs were predicted to use the first pereopods (cheliped; P1) and third maxillipeds (3M) as their main grooming appendages (see Wortham and LaVelle, 2016 for images of structures) and groom body regions associated respiration and sensory input, not hooked setae. Due to reaching their terminal molt stage and not having an “anti-fouling” event such as molting, large crabs were expected to groom their bodies more than small individuals. Removal of a gill grooming appendage was predicted to lead to higher fouling levels on gills. The grooming time budget in these crabs was hypothesized to decrease with the presence of another individual; the spider crab time budget for grooming was projected to be higher than blue crabs and stone crabs due to spider crabs having increased dexterity in their walking legs.

MATERIAL AND METHODS

Collection and laboratory procedures

Libinia dubia were collected using push nets through mud and seagrass beds in Tampa Bay, FL (April–July, 2013) near Fort Desoto State Park at nighttime; within a 5-m push of the net, several crabs could be collected.

This species have seven or fewer spines running the length of the carapace along the median line and has a long, deeply forked rostrum (Corrington, 1927); these characteristics distinguished *L. dubia* from *L. emarginata* Leach 1815, a larger spider crab that occurred in the same areas as *L. dubia*.

Crabs were transported to the University of Tampa where the carapace width (mm) was measured using digital calipers; individuals ranged from 6.35 mm to 73.0 mm. Crabs were sexed (Hinsch, 1972) and any missing appendages were documented. Live crabs were placed into individual holding containers, with pre-drilled holes allowing water flow, and then placed in four 454-l fiberglass aquaculture tanks with filtered, continuous flowing, aerated salt water at ranges of 22–24 °C and 20–30 ppt. Individual containers decreased physical contact and agonistic interactions, ensuring that crabs' appendages and setae remained intact, as well as eliminated cannibalism (Wilber and Wilber, 1991). Containers allowed visual and pheromonal contact among individuals, as water was able to flow through the pre-drilled holes. Crabs were fed shrimp pellets and penaeid shrimp on non-testing days. Prior to observations, crabs acclimated in observational tanks for 24-h and were not used if they molted within one week. Throughout the entire study, crabs were kept on a 14-h light/10-h dark day-night cycle. A total of N=210 crabs were collected and used in the six grooming experiments: 24-h isolated, social, agonistic, ablation, bacterial fouling, and gill fouling (using scanning electron microscopy: SEM). Throughout the text and figures, body regions and appendages were abbreviated as follows starting most anterior and moving posterior: rostrum (R), left first pereopod (LC), right first pereopod (RC), first pereopod (P1), third maxilliped (3M), second maxilliped (2M), first maxilliped (1M), all maxillipeds (AM), second antennae (A2), first antennae (A1), eyes (E), gills (G), carapace ridge (RG), mid-carapace (M), abdomen (ABD), and walking legs (P2–P5).

Grooming observations

24-Hour Grooming: (N=20) The objective was to determine if grooming differed in day versus night hours. Observations were made every 30-min for 24-h (48 observations) on crabs in isolation in 38-l tanks; crabs were used only one time and each of the

48 observations lasted 15-s. Presence (1) or absence (0) of a grooming action was recorded. A red-bulb lamp was used during night observations when all other lights were turned off (Bauer, 1998). Data analyses accounted for more observations in day hours than night (14:10 light cycle).

Isolated Grooming (N=142; N=89 males, N=53 females): Objectives of these observations were to document the grooming behaviors and time budgets of genders and different sized individuals. Crabs were tested in isolation which eliminated fighting, mating, and other primary behaviors as well as encouraged grooming, a proposed secondary behavior (VanMaurik and Wortham, 2014). Each crab was used one time in the isolated grooming experiment. For each trial, a crab was put into a 38-l aquarium with black backing and natural rocky substrate. Static aquaria included aeration and continually filtered water; the water was changed frequently using water from the aquaculture water tables. Based on the results of the 24-h experiment, observations occurred during the day light cycle and all grooming behaviors were documented for 30-min using a digital recording device and later transcribed to data sheets following the methods of VanMaurik and Wortham (2011). Data collected were: 1) what appendages were used in grooming; 2) what actions or mechanisms were used in grooming (scrape, pick, brush; defined in VanMaurik and Wortham, 2011); 3) what body areas were groomed; and 4) how much time was spent grooming. Ovigerous females were observed to flap their abdomen regions, possibly providing parental care by grooming and aerating the developing eggs; these infrequent observations were not included in grooming appendage results.

Social Grooming (N=30; total of 60 crabs; 18 female–female, 12 male–male pairs): The objective was to observe how social interactions affected grooming behaviors. Individuals were observed in a grooming situation where they had visual and pheromonal contact (but not physical contact) with other individuals. These observations differed from isolated observations because those individuals had pheromonal contact through water (from aquaculture tanks) but did not have visual or other sensory input. If a crab saw another crab that it had not previously encountered, grooming behaviors should decrease because other behaviors such as fighting, defense, and mating would become

more of a priority (Jaeger, 1981). The 38-l aquaria were divided into two equal parts using a clear plastic partition that allowed water flow and visual cues. One crab was placed in each section for a total of two crabs in the test area; individuals were size matched (less than 10% difference in carapace width) and gender matched in order to control for size and intersexual interactions. Crabs were allowed 24-h acclimation time, and then the grooming behaviors were recorded. Data collection procedures were similar to the isolation observations. The grooming time budget for individuals observed in this experiment was compared to time budget of individuals in isolation.

Agonistic Interactions (N=45; total=90 crabs; 15 male–male, 15 male–female, 15 female–female pairs): The objective was to observe how agonistic interactions affected grooming behaviors and to test if grooming was a secondary behavior (VanMaurik and Wortham, 2014). Crabs had physical contact with other individuals; their grooming behaviors as well as any other behavior, such as fighting and mating were documented. Crabs were observed in a section of the 454-l fiberglass water tables; the section was 58 cm x 41 cm x 23 cm. Crabs were paired with another size-matched individual (less than 10% in body size); crabs that were previously paired in the social experiment were not paired with the same individual again. For these observations, crabs were allowed to acclimate for 30-min in the test arena without any contact and then allowed to interact for a total of 30-min with all behaviors documented. Data collection procedures were similar to the isolation observations. The agonistic grooming time budget was compared to grooming time budgets of individuals in isolation and a social setting.

Fouling experiments and observations

The objective was to determine the effectiveness of grooming on gill fouling by removing a grooming appendage. Crabs were randomly selected after being collected (mean CW=22 mm; mean mass = 53 g); only one individual used had reached sexual maturity (based on CW) and had stopped molting (Rjiba-Bahri *et al.*, 2019). Following procedures in Wortham and Pascual (2017), crabs had one grooming appendage removed; the 3M epipod has been documented to clean the ventral side of the gills in *L. dubia* (cf. Wortham and LaVelle, 2016) and was removed. Using forceps, the

epipod was dissected by reaching internally past the protopod and pulling the epipod out of the branchial chamber; the protopod was not damaged. Each crab had one 3M epipod removed (called “ablated”), the other 3M epipod on the opposite body side remained (called “intact”). The side that ablation occurred was randomly selected. Crabs were placed into their individual containers on the aquaculture tanks for three weeks and were fed daily.

Ablation Experiment (N=10, 5 males, 5 females): The objective was to determine the effectiveness of grooming on gill fouling (all types of fouling) by removal of a grooming appendage. After three weeks, the crabs were chilled, euthanized, and one gill from each body side (fifth arthrobranch, the largest gill) was viewed under a compound microscope (Swift m10L Series Microscope). Percent light transparency of gills, a measure of fouling on gills, was documented (following methods in Bauer, 1998; 1999). Three light transparency measurements were taken per gill (in Lux at 10X magnification; using Mastech Digital Light Meter Model LX-1330B); three measurements were taken for the ablated side and three measurements for intact side for each crab. The three ablated and three intact readings were averaged per crab. These values for transparency (for ablated and intact sides in all N=10 crabs) were obtained and the data was analyzed.

Bacterial fouling (N=3; 1 male, 2 females): The objective of this experiment was to determine the effectiveness of gill grooming appendages on bacterial removal. The level of bacterial fouling on the gills was compared; gills from the ablated side were compared with gills from the intact side. The same individuals used in the ablated experiment were used in this experiment; all three individuals had not reached sexual maturity and were still molting (Rjiba-Bahri *et al.*, 2019). A control was used to verify sterile methodology procedures. The dorsal sides of all seven gills were wiped with a sterile swab and then across a sterile 100 mm diameter plate (non-differential agar medium); plates were incubated for 24-h at 37 °C. While the 3M epipod mostly cleans the ventral gill side, the attached epipodal setae are long and can reach between gills; the ventral gill side was difficult to access without damaging the gills. Bacterial colony forming units were counted for each plate, and then averaged for ablated and intact grooming appendage sides.

Scanning electron microscopy and gill fouling (N=3; 1 male, 2 females): The objective of these observations was to compare and determine the effectiveness of grooming appendages on gill fouling. The same individuals from the bacterial experiment were used in these observations. One gill (fourth arthrobranch) was removed from the ablated and intact sides of crabs and viewed in a scanning electron microscope (following preparation procedures in Felgenhauer, 1987). The gill selected was based on its presence in the gill chamber, lying medially, and being the one of the largest (similar size to the fifth arthrobranch). Gills were viewed dorsally (with a central axis) and ventrally, to account for the long setae on epipods used to clean the gills (Wortham and LaVelle, 2016). The ventral side of the gills was the side mostly in contact with the 3M epipod (epipod setae can reach dorsally), which was the grooming appendage that was ablated. In the results, all three individuals were described; all three individuals were represented in the result figures to show variation between individuals.

Statistical analyses

Data were analyzed to determine if they met the criteria for parametric statistics. If normality assumptions were not met, then non-parametric statistics were used. The following statistical tests were used: chi-squared test, t-test, regression analysis, Mann Whitney, Wilcoxon Signed Rank test, and Kruskal-Wallis tests. Statistical significance was determined at $p=0.05$. When multiple statistical comparisons were conducted, the tests were based on a priori hypotheses.

Groomed areas were separated by general functionality: sensory and respiratory versus decorating. Sensory and respiratory regions included eyes, gills, first antennae (A1), and second antennae (A2), while decorating areas included rostrum, ridge, mid-carapace, and walking legs (Hultgren and Stachowicz, 2008). Mean values for the grooming time budgets were calculated for each individual. The purpose of calculating the grooming time budget was to determine how much grooming occurred in a 30-min period. For isolation, the average time budget was calculated for each individual (N=142) by averaging the total duration of grooms in each 30-min trial for all observations then divided by the trial duration (30-min or 1800-s). In social and agonistic observations, the

pairs of crabs were analyzed but the individuals were not treated as independent samples because any action performed by one individual in a pair may have affected the other individual of the pair. In order to correct for this, the total time groomed in a trial inclusive of both individuals was used (3600-s the 30-min trial of 1800-s was multiplied by two to account for the pair). Similarly, all frequencies of actions performed in social and agonistic trials were totaled, then divided by two to obtain a value for frequency per individual. Time budgets for all actions in agonistic interactions were calculated by taking the time spent performing each action and dividing by total time in trial (1800-s) and dividing by two (number of individuals in each trial).

RESULTS

Field information

The sampled population collected (N=210) consisted of 116 males and 94 females, and resulted in a sex ratio of 1.23:1 (males: females); females (36%) carried eggs on their abdomens. Females and males had equal carapace widths (average CW in mm: male=45.5; female=47.2; CW: $t=1.11$, $df=132$, $p=0.268$), but males were significantly larger in mass than females (average mass in g: male=67.1; female=54.4; mass: $t=-2.54$, $df=130$, $p=.012$). The percentage of each appendage missing in individuals was: P1=7.4%, P2=10%, P3=9.3%, P4=7.9%, and P5=5.2%. While P2 was the most frequently lost appendage and P5 was missing least often, there was no significant difference in the appendages missing ($\chi^2=7.22$, $df=4$, $p>.10$). Overall, crabs were in good condition with 57% of individuals having all ten appendages.

24-h observations

During a 24-h period, crabs groomed equally in the day and night time (day: 151 grooms; night: 94 grooms; $\chi^2=1.13$, $p>.10$). Therefore, all further grooming studies were conducted in the daytime.

Isolated grooming

Crab body size (CW) was not statistically linked with an increase in grooming frequency or grooming time (frequency: Fig. 1A: $R^2=0.003$, $y=0.092x+10.3$;

time: Fig. 1B; $R^2=0.005$, $y=1.044x+48.2$); large and small individuals were grooming equally. Examining the genders separately, small and large males and females groomed with the same frequency (Frequency: Fig. 1A; males: $R^2=0.000$, $y=0.001x+16.9$; females: $R^2=0.015$, $y=0.152x+3.28$) and duration (Time: Fig. 1B; males: $R^2=0.000$, $y=0.018x+5.64$; females: $R^2=0.039$, $y=0.245x-6.79$), regardless of body size.

Males groomed statistically higher in frequency than females (Fig. 2A; $z=-10.3$, $p<0.001$), averaging 17 grooms per observation as compared to 10 for females. With respect to grooming time, male and female crabs groomed for an average of 115 and 77 s per observation period, respectively, and have equal grooming times (Fig. 2B; $z=1.95$, $p=0.051$). Each groom a female performed (7.7 s/groom) was longer compared to males (6.7 s/groom), though this difference was not significant ($\chi^2=0.069$; $p>0.90$). For further results, grooming frequency data was separated by genders (due to the statistical difference calculated); data for duration of grooms was pooled (due to no statistical differences between male and female data).

Grooming Frequency: Areas of the body were groomed at significantly different frequencies for both males (Fig. 3A; $H=248$, $p<0.001$) and females (Fig. 3A; $H=101$, $p<0.001$); both genders groomed a smaller body region (A2) the most frequently. Males consistently groomed body regions at a higher frequency than females in all body regions, except the abdomen where ovigerous females carried eggs (Fig. 3A). With the body regions pooled and averaged for each functional group (sensory/respiratory and decorating), males groomed their sensory/respiratory structures and decorating body regions significantly more than females (Fig. 3B; sensory/respiratory: $t=-2.67$, $p=0.008$; decorating: $t=-1.97$, $p=0.049$). Males groomed their sensory/respiratory structures significantly more often than their decorating body regions, whereas in females, the difference was not statistically significant (Fig. 3B; males: $z=3.23$, $p=0.001$; females: $z=1.40$, $p=0.14$).

For pooled males and females, the 3M (inclusive of the endopod and attached epipod) was used significantly more frequently as a grooming appendage than the P1 (Fig. 4A; $z=3.39$, $p<0.001$). Males groomed with the 3M more frequently than P1 (Fig. 4B; $z=3.23$,

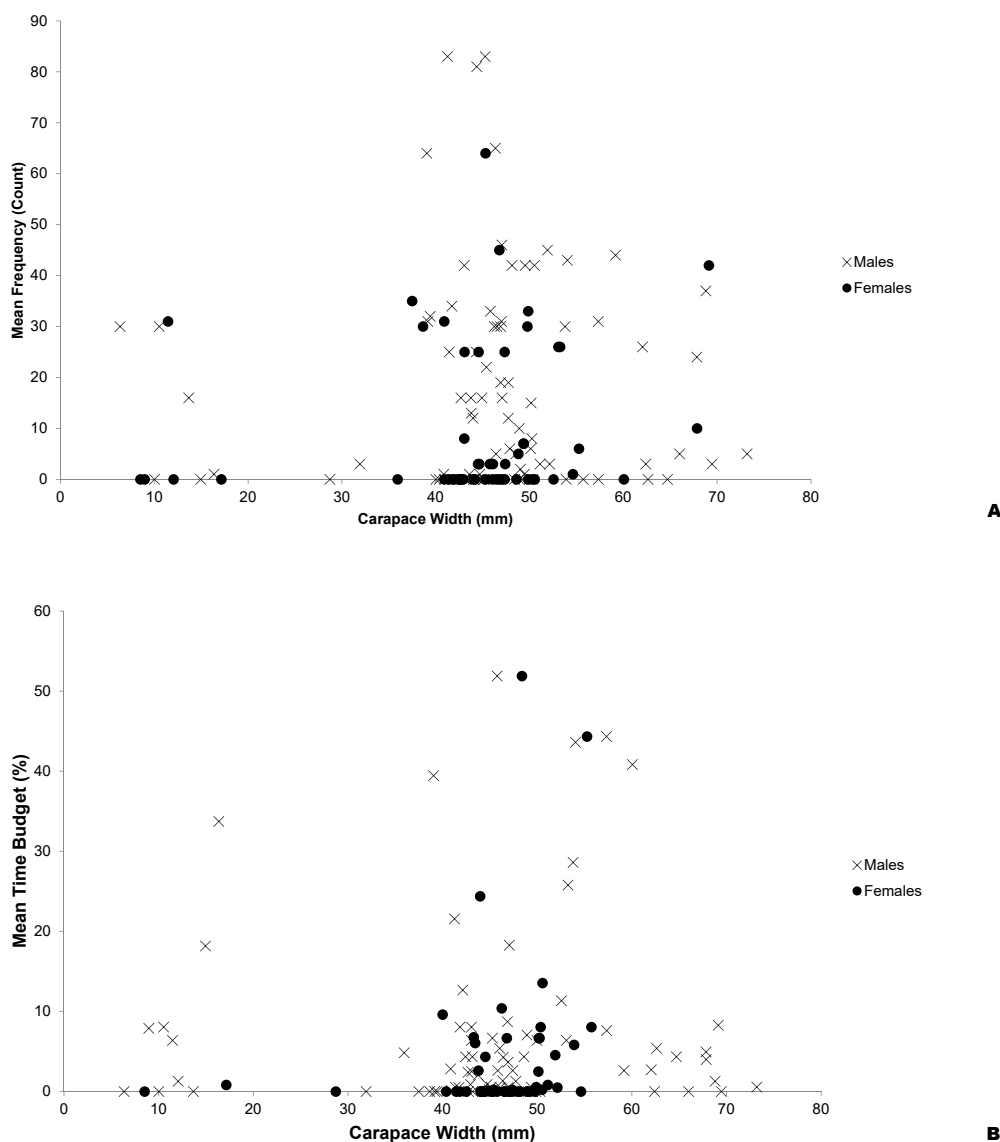


Figure 1. Relationship between grooming frequency and grooming time budget by carapace width (mm) for males and females in 30-min isolation observations ($N=142$). **A.** Mean grooming frequency by carapace width for males and females; no relationship between number of times individuals groomed and their body size ($R^2=0.003$, $y=.092x+10.3$). **B.** Mean grooming time budget by carapace width for males and females; no relationship between time spent grooming and body size ($R^2=0.005$, $y=1.044x+48.2$).

$p=0.001$) while females used the 3M and P1 equally (Fig. 4B; $z=1.40$, $p=0.140$). Comparing genders, males groomed with their 3M significantly more than females (Fig. 4B; $z=26.8$, $p<0.001$) but males and females groomed with the P1s equally (Fig. 4B; $z=-1.22$, $p=0.223$). The cheliped seems to be equally important as a grooming appendage to both genders.

In the $N=142$ trials, grooming by scraping was the most frequent action, followed by picking, flapping, and then brushing (Fig. 5). Scraping occurred when the A2 was groomed with the palp (distal segment of the 3M endopod) and these multiple actions were recorded in

quick succession, with each action lasting one second. Picking action (a longer grooming bout) was performed by the chelipeds (P1) on various regions of the body; picking occurred equally to scraping (Fig. 5; $z=1.16$, $p=0.246$). Flapping was performed by abduction of the 3M endopod or by the abdomen opening and closing. Flapping of the 3M endopod resulted in an internal groom of the gills by the attached 3M epipod moving through the gill chamber. Flapping action occurred less frequently than picking ($z=2.09$, $p=0.037$). Finally, brushing was performed by the endopods of the 3M rubbing the palps together along their medial edges

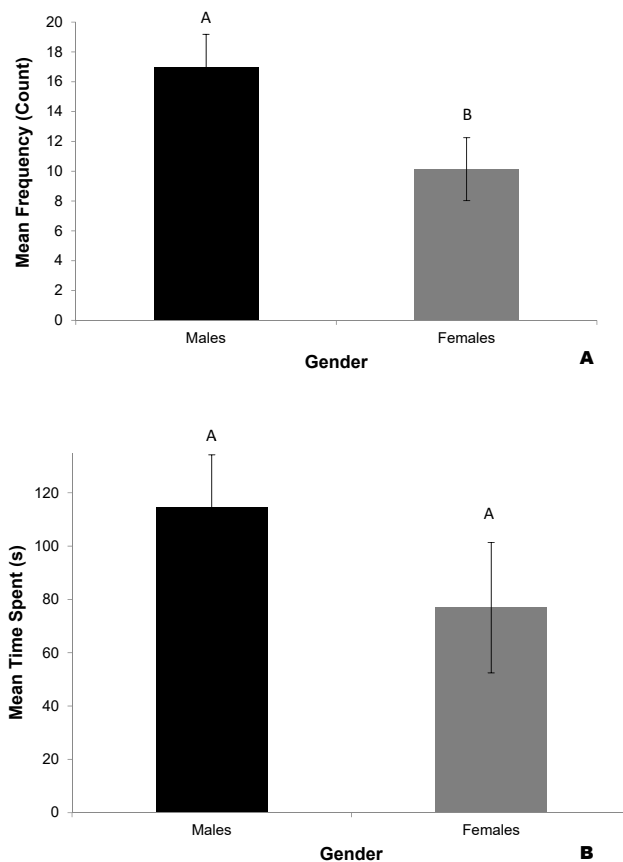


Figure 2. Grooming frequency and grooming time, with standard error bars, of males and females in 30-min isolation observations (N=142 total; N=89 males; N=53 females). **A.** Mean frequency of grooms by males and females ($z=-10.3$, $p<0.001$). **B.** Mean time spent grooming by males and females ($z=1.95$, $p=0.0507$). Note: Similar letters indicate no statistical significance ($p>0.05$) and different letters indicate a statistical significance ($p<0.05$).

and was the least frequent of any action (Fig. 5, pick: $z=4.19$, $p<0.001$, flap: $z=2.18$, $p=0.029$). Otherwise, the 3M (endopod and attached epipod) was used to scrape, flap, and brush body regions, whereas the P1 was only used to pick body regions.

Grooming Time: Spider crabs groomed specific body regions for more time than others (Fig. 6A; $H=291$, $df=14$, $p<0.001$); spider crabs groomed a small structure (A2) for the longest duration. Individuals groomed sensory and respiratory structures (14-s) for significantly longer durations compared to decorating regions (4-s; Fig. 6B; $t=9.48$, $p<0.001$).

Overall, for all N=142 individuals, spider crabs in isolation had a grooming time budget of 5.22%. The

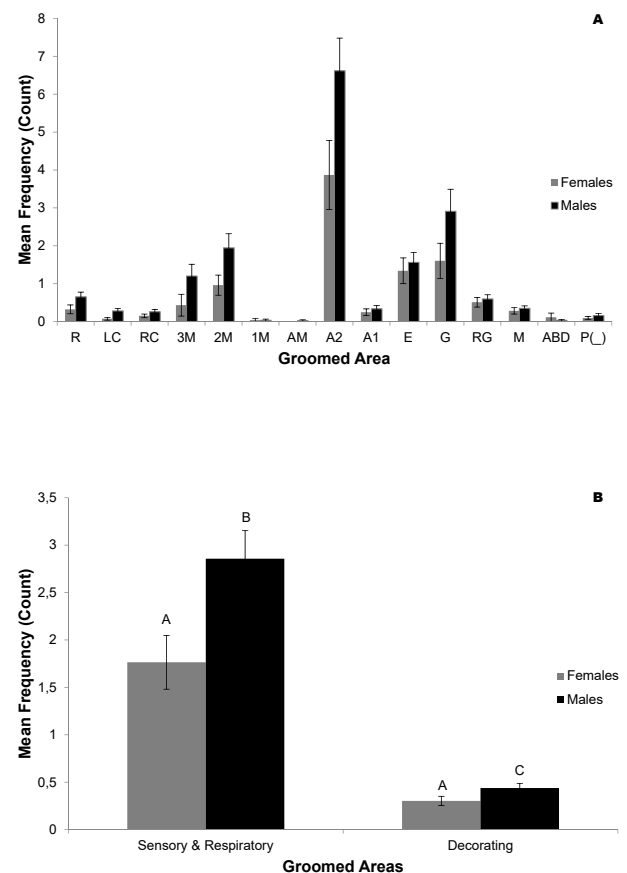


Figure 3. Frequency of grooms, with standard error bars, per body region during 30-min isolation observations (N=142; N=89 males; N=53 females). **A.** Mean frequency of grooms for males and females for body regions (males: $H=248$, $p<0.001$; females: $H=101$, $p<0.001$); x-axis organized by anterior body regions on the left to posterior body regions on the right. **B.** Grooming frequency of sensory and respiratory structures compared to body regions where decorations are attached for males and females (sensory/respiratory: N=568, $df=540$, $t=-2.67$, $p=0.008$; decorating: N=568, $df=523$, $t=-1.97$, $p=0.049$). A1, first antennae; A2, second antennae; ABD, abdomen; AM, all maxillipeds; E, eye; G, gills; M, dorsal mid-carapace; LC, left P1 cheliped; M1, first maxilliped; M2, second maxilliped; 3M, third maxilliped; P (_), pereopods 2–5 (walking legs); R, rostrum; RC, right P1 cheliped; RG, lateral ridge. Note: Sensory and respiratory body regions were A1, A2, E, G; decoration body regions were R, RG, M, P. Note: Similar letters indicate no statistical significance ($p>0.05$) and different letters indicate a statistical significance ($p<0.05$).

number of missing appendages per individual was plotted with the grooming time budget to make sure no relationship existed between missing appendages and grooming time budgets; no relationship existed ($R^2=0.00002$).

Social

In the 30-min observational period, the average frequency (count) of grooms for each pair ($N=30$; 60 individuals) was 3.13 and the average duration (seconds) of grooms for each pair was 27.3 s; the average grooming time budget for each individual was 0.76%. Most crabs were motionless, yet latched onto the screen divider and in close proximity to the other crab, but not performing grooming behaviors.

Agonistic

Behaviors recorded during the agonistic interactions were grooming, feeding, mating, fighting, and displaying. Feeding was measured when crabs picked at the substrate (with their P1) and brought P1 up to their 3M region; even though animals were not fed within 24-h of an observation, feeding behavior was still recorded. Mating was documented when the crabs were mounted. Crabs were classified as fighting when crabs pinched and grabbed the other individual, compared to displaying when the crabs spread their P1 and arched dorsally, using their back walking legs. There were significant differences in these behavioral frequencies (Fig. 7A; $H=82.6$, $df=4$, $p<0.001$), with grooming being the most frequent behavior (Fig. 7A; grooming vs. fighting $z=3.07$, $p=0.002$; grooming vs. displaying $z=3.17$, $p=0.002$), followed by feeding, and then mating being the least observed action (Fig. 7A; displaying vs. feeding $z=3.10$, $p=0.002$). Feeding and mating behaviors were not statistically different (Fig. 7A; $z=1.11$, $p=0.269$) and occurred infrequently.

The time budgets for each of these recorded behavior were significantly different (Fig. 7B; $H=75.6$, $df=4$, $p<0.001$); individuals spent significantly more time fighting (mean 56-s/observation period) than any other behavior. Feeding occurred the least amount of time (mean 1-s/observation period). All actions were significantly different from the grooming time budget except displaying ($z=-0.901$, $p=0.368$). Although grooming was the most frequent action recorded (Fig. 7A), each grooming action lasted for a short period of time (Fig. 7B). Furthermore, fighting had the second highest frequency count (Fig. 7A) and was observed for the most time (Fig. 7B). Otherwise, individuals fought often and each bout occurred for a long period. All other behaviors were different statistically from each other

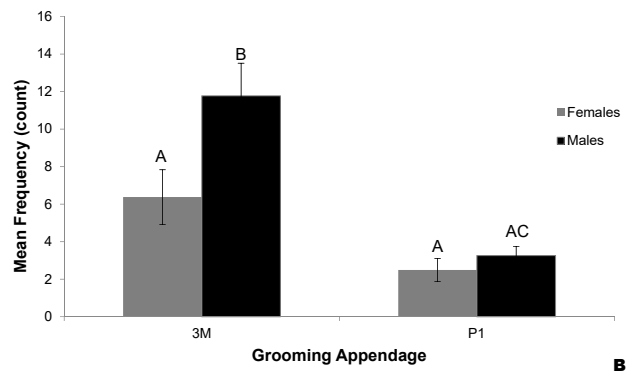
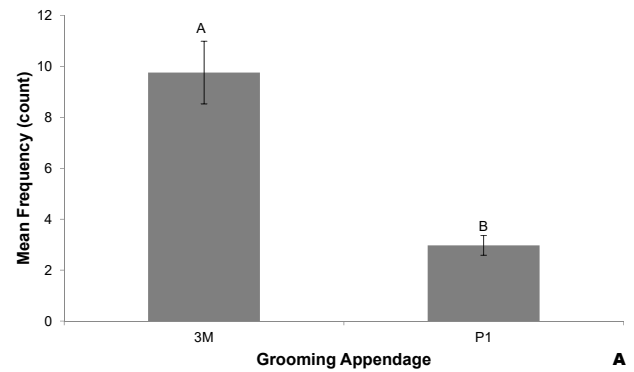


Figure 4. Mean frequency of grooms, with standard error bars, by grooming appendages in 30-min isolation observations ($N=142$). **A.** Mean frequency of grooms for each of the grooming appendages, with genders combined ($z=3.39$, $p<0.001$). **B.** Mean frequency of grooms for each of the grooming appendages, with the genders separated; males groomed with their 3M more than P1 ($z=3.23$, $p=0.001$) whereas females groomed with the 3M and P1 appendages equally ($z=1.40$, $p=0.140$). 3M, third maxilliped; P1, pereopod #1 (cheliped). Note: Similar letters indicate no statistical significance ($p>0.05$) and different letters indicate a statistical significance ($p<0.05$).

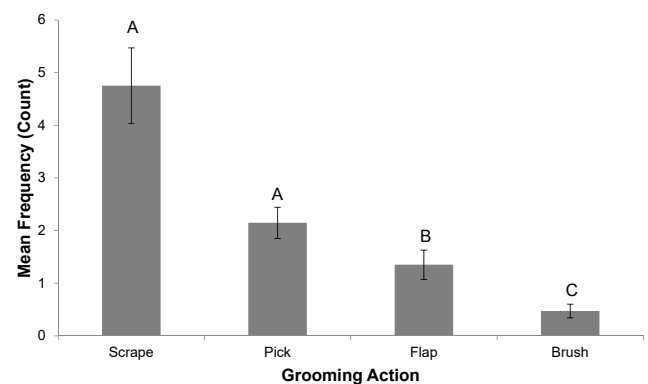


Figure 5. Mean frequency of grooming mechanism, with standard error bars, mechanisms in 30-min isolation observations ($N=142$; scrape/pick: $z=1.16$, $p=0.246$; flap/brush: $z=2.18$, $p=0.029$). Note: Similar letters indicate no statistical significance ($p>0.05$) and different letters indicate a statistical significance ($p<0.05$).

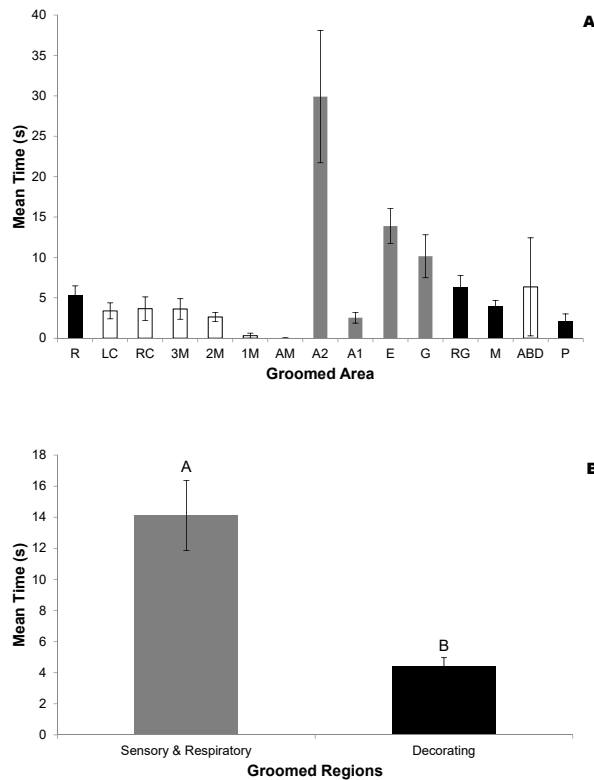


Figure 6. Mean time (s), with standard error bars, spent grooming body regions in 30-min isolation observations (N=142). **A.** Mean time grooming body regions ($H=291$, $df=14$, $p<0.001$), with x-axis organized by anterior body regions on the left to posterior body regions on the right. Sensory and respiratory structures were designated by gray bars: A1, A2, E, G; decoration body regions were designated by a black bars: R, RG, M, P. White bars are body regions not associated with sensory, respiratory, or decorations. **B.** Mean time grooming of sensory/respiratory structures (gray bars) compared to body regions where decorations (black bars) are attached (N=598, $t=9.48$, $p<0.001$). A1, first antennae; A2, second antennae; ABD, abdomen; AM, all maxillipeds; E, eye; G, gills; M, dorsal mid-carapace; LC, left P1 cheliped; M1, first maxilliped; M2, second maxilliped; 3M, third maxilliped; P, pereopods 2–5 (walking legs); R, rostrum; RC, right P1 cheliped; RG, lateral ridge. Note: Similar letters indicate no statistical significance ($p>0.05$) and different letters indicate a statistical significance ($p<0.05$).

(z ranges= -4.26 – 10.7 ; p values= <0.001). Behavioral time budget ranges for all behaviors in these trials were: grooming: 0–6.58%; fighting: 0–19.6%; displaying: 0–2.61%; feeding (left over food in tank): 0–0.67%; and mating: 0–30.8%. The pooled mean time budget of all actions collectively was 5.74%. Fighting had the highest mean time budget at 3.08%; next was mating (1.63%) followed by grooming (0.67%), displaying (0.32%), and feeding (0.035%). For the remainder of the time (approx. 94%), pairs remained stationary,

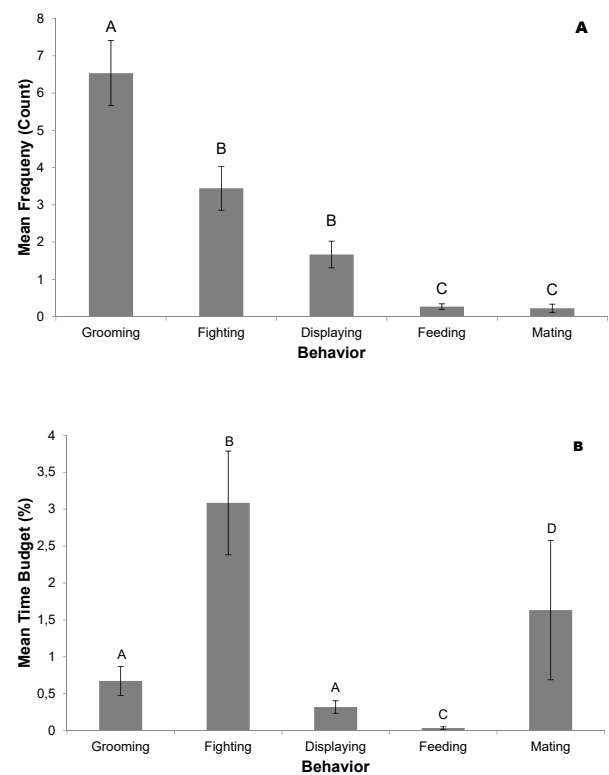


Figure 7. Mean frequency and time (s), with standard error bars, of individuals behaviors in 30-min agonistic observations (N=45). **A.** Mean frequency of behaviors ($H=82.6$, $df=4$, $p<0.001$). **B.** Mean time budget spent performing behaviors ($H=75.6$, $df=4$, $p<0.001$). Note: Similar letters indicate no statistical significance ($p>0.05$) and different letters indicate a statistical significance ($p<0.05$).

up to a body length away from each other, and not exhibiting noticeable behaviors or visibly interacting.

Time budgets

Average grooming time budgets for each experiment (isolation: 5.22%; social: 0.76%; and agonistic: 0.67%) were compared to determine how presence of another individual influenced grooming behavior. Crabs in isolation had a higher grooming time budget than in the social observations ($z=2.58$, $p<0.05$); the grooming time budgets of crabs in social and agonistic observations were similar ($z=1.07$, $p=0.286$).

Ablation and bacterial fouling experiments

There were nine gills one each side in *L. dubia*. Two podobranchs were anterior and associated with the second and third maxilliped. Five arthrobranchs existed along with two pleurobranchs, which were the two posterior gills. Gill fouling was not impacted by

the removal of the 3M epipod ($Z = -0.357$; $p = 0.724$); individuals had the same light transparency of gills on intact and ablated sides. The number of bacterial colony forming units was the same on intact and ablated sides ($Z = -1.604$; $p = 0.250$), suggesting that the epipods were not effective in removing bacteria from the gills.

SEM

Gills of all crabs were fouled; little differences in fouling existed between intact (Fig. 8A–D) and ablated sides of gills (Fig. 8E–H). Overall, spider crab gills were fouled with sediment, debris, and gooseneck

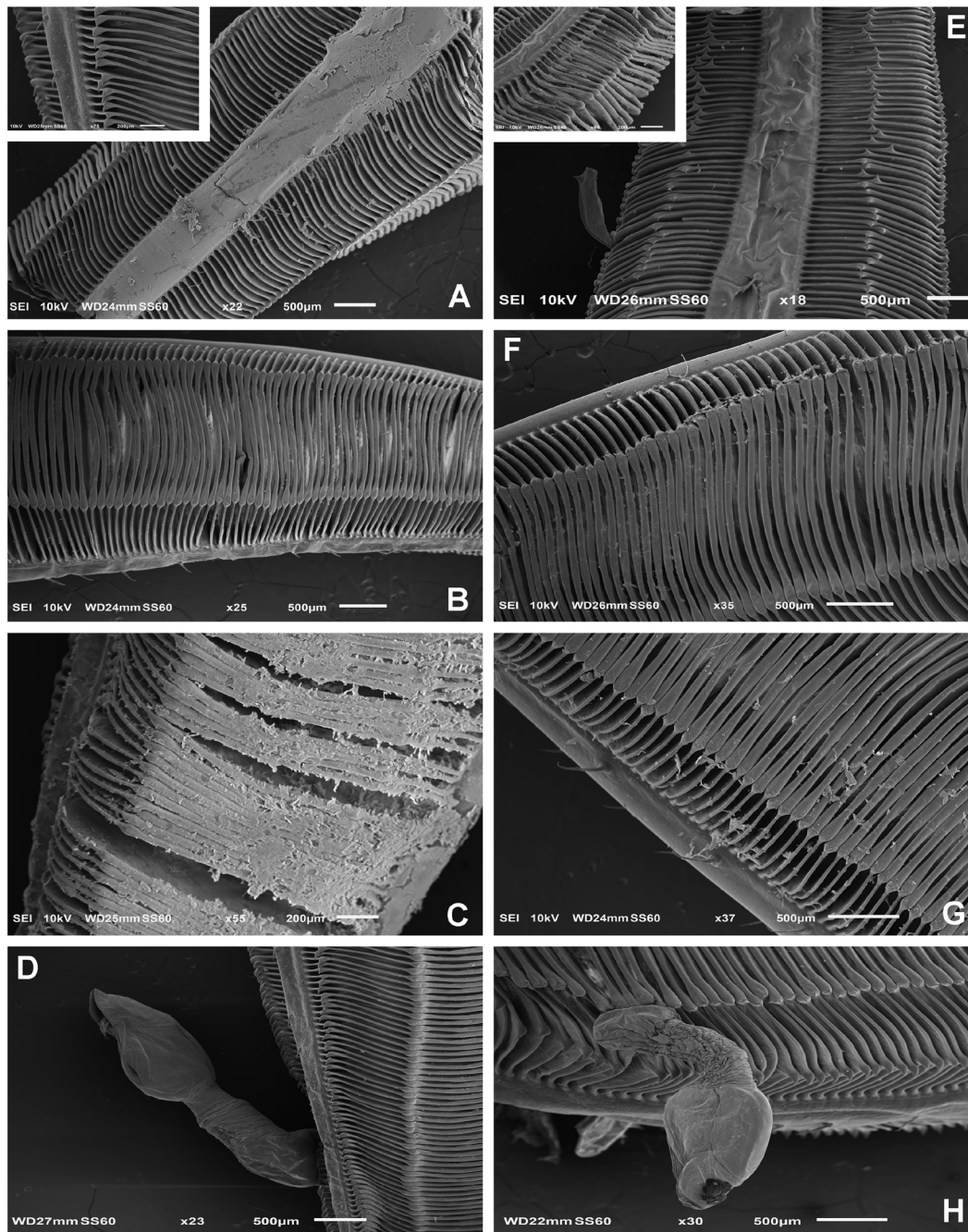


Figure 8. Gills from the ablation experiment, with images A–D from the side with the 3M epipod ablated and images E–H from the side with all gill grooming appendages intact. **A.** Dorsal view of gill, with the central axis and lamellae both fouled; inset showing “shark teeth” nodules on lamellae with minimal fouling. **B.** Ventral view of gill, with minimal fouling between lamellae. **C.** Ventral view of gill, with heavily fouled lamellae mostly of sediment. **D.** Fouling by a gooseneck barnacle attached to lamellae, with minimal. **E.** Dorsal view of gill, with the central axis and lamellae both fouled; inset showing “shark teeth” nodules on lamellae with fouling. **F.** Ventral view of gill, with fouling in between lamellae. **G.** Ventral view of gill, with fouling on gill surface and simple setae along edge. **H.** Fouling by a gooseneck barnacle attached to lamellae, with minimal fouling.

barnacles. The central axis on the dorsal gill side was fouled, with fouling near the morphological structures that resemble “shark teeth” (called nodules; Farrelly and Greenaway, 1992) on the lamellae (Fig. 8A, E). Fouling was visible between ablated and intact lamellae on both the dorsal (Fig. 8A, E) and ventral gill sides (Fig. 8C, G). Simple setae was located randomly on the gills (Fig. 8G). The dorsal (Fig. 8A, E) and ventral gills (Fig. 8B, C, F, G) were similarly fouled, going against the prediction that the ventral gill sides would be more heavily fouled on the ablated sides. One individual (on the intact side) had much fouling on the ventral gill with lamellae being stuck together (Fig. 8C). The removal of a gill grooming appendage (3M epipod, mostly in contact the ventral gill side) did not result in differences in fouling (between the dorsal/ventral gill sides or between the ablated/intact sides); both sides were fouled with barnacles (Fig. 8D, H).

DISCUSSION

Field data

From the population sample (N=210), males were larger than females in mass, while being equal in CW. Collecting in the same season but at a different location in Tampa Bay, Wortham (2013) reported exactly opposite results with males and females having equal mass but females with larger in CW. Body sizes of *Libinia dubia* have varied based on collection site.

Spider crabs were in good body condition upon collection with few body appendages missing. In terms of grooming, the chelipeds were not missing more than other walking legs, so grooming was still possible in most crabs. Because these brachyurans did not groom with their P2–P5, loss of these appendages would not affect grooming. Even though these crabs lived in close proximity to other spider crabs, their overall body condition provided evidence that they were not often engaging in competitive battles that would likely have led to loss of limbs.

Behavioral data

Grooming was a consistent behavior throughout the day and night hours. Based on how the grooming time budget decreased (from around 5% to less than 1%) when a conspecific was introduced (individual vs

social/agonistic observations), grooming appeared to be a secondary behavior that occurred when primary behaviors were not necessary, supporting other research on decapods (VanMaurik and Wortham, 2011; 2014). While grooming was secondary in the overall time budget of a crab, it occurred consistently throughout a 24-h period. Because a behavior is considered secondary, the behavior could still be important in survival; for example, in humans, eating is a primary behavior whereas grooming/cleaning is not a primary behavior. Nonetheless, grooming likely increases survival rates.

Large individuals of *Libinia* have been documented to: 1) undergo a terminal molt (Hinsch, 1972; Jones and Hartnoll, 1997); 2) not decorate often (Hultgren and Stachowicz, 2009); and 3) not have as many decorating setae as smaller crabs (Ahl *et al.*, 1996; Hultgren and Stachowicz, 2009). In this study, large individuals were predicted to groom their bodies more than smaller individual; smaller crabs, that were immature (CW 40–50 mm; Rjiba-Bahri *et al.*, 2019) and still molting, groomed the same as larger crabs that had undergone a terminal molt. Large individuals that were collected in abundance may be benefiting from increased body fouling as a survival mechanism (Bauer, 1989); a fouled carapace may enhance their camouflage and increase survival (possibly a trade-off to not molting).

Males and females varied in their grooming behaviors. While they groomed for similar time, males groomed their bodies more frequently than females. Both genders groomed their A2 the most frequently, likely related to the role in sensory reception; cleaning of the A2 has been reported in other crustaceans (Bauer, 1989; Wortham and Pascual, 2017). The P1, as the main grooming appendage, has rarely been observed in crustaceans; shrimps used P2 as a main grooming appendage whereas two brachyurans used their P4 and P5 (Bauer, 1989; Wortham and Pascual, 2017). Spider crabs have P1 that are more dexterous than blue crabs and stone crabs; grooming appendages might significantly vary in brachyurans depending on body morphology and flexibility of articulations.

Mechanisms of grooming (pick, scrape, brush) were common in other decapods (Bauer, 1989; VanMaurik and Wortham, 2014). The setal morphology of the P1 (a picking action) was sparse, with only patches of fouled pappose setae in the joints (Wortham and LaValle,

2016). The 3M palp that cleaned the A2 repetitively (by scraping) was not fouled and had many types of serrate setae in brushes (Wortham and LaVelle, 2016); these setae have been associated with grooming. The 3M endopod (brushing together) was heavily fouled with pappose setal patches (Wortham and LaVelle, 2016); this brushing action was rare and likely associated with high levels of fouling observed on the grooming appendage. Looking at these pappose setal patches that are variable in morphology, prevalence, and likely function would be an area of future focus as these setae are fouled but not frequently groomed.

Spider crabs groomed body regions associated with sensory and respiratory structures more often and for longer durations (14 s) than body regions where decorations are located (4 s). In other decapods (VanMaurik and Wortham, 2011; 2014; Wortham and Pascual, 2017), the same body regions associated with sensory and respiration were groomed at high frequencies and for much time. Being aware of predators, prey, conspecifics, food locations, environmental changes, and respiratory functions, therefore appears to be a priority in all decapods. These hooked setae could have another anti-fouling mechanism other than grooming, such as secretions, the lack of ultrastructures (*i.e.*, denticules, setules) that accumulate fouling, or being robust (not being tangled with other setae and having a low surface area).

In the agonistic observations, grooming occurred more frequently than fighting but fighting happened for longer time than grooming; this grooming behavior pattern was also reported in blue crabs and stone crabs (Wortham and Pascual, 2017). Most grooming actions were quick, lasting less than one second (VanMaurik and Wortham, 2011, 2014) and occurred frequently. Engaging in fighting behaviors happened less frequently, but once the behavior began, the individual committed to a long engagement until the contest was decided (also seen in blue crabs and stone crabs; Wortham and Pascual, 2017). So while grooming occurred frequently, the individual was not devoting large amounts of time to the behavior and hence the low time budget for grooming. Interestingly, when all primary behaviors were pooled, grooming (a secondary behavior) had a higher frequency compared with pooled primary actions; pooled primary behaviors had approximately

a 10× time budget compared to that of a secondary behavior (grooming).

In the agonistic observations, a time budget for all behaviors pooled was 5.74%; for the majority of the time (about 94%), individuals were not exhibiting any noticeable behaviors. Spider crabs live in high densities in their natural habitat, helping explain why such a low activity level was recorded. As described in the dear enemy recognition principle (Jaeger, 1981), spider crabs living in high densities (like collected in this study) may interact briefly with a conspecific, assess each other, and then not interact again unless approached which decreases time spent in competitive, energetically costly behaviors.

The importance of gill formula and gill structure has been documented as important regarding taxonomy (Martin and Abele, 1986). The gill formula reported in this study for *L. dubia* matches the gill formula reported in another species of *Libinia* (Yang and McLaughlin, 1979). The results of the ablation of a gill grooming appendage in spider crabs was similar to results in stone crabs but not blue crabs (Wortham and Pascual, 2017). Blue crab gills had more fouling when the grooming appendage was ablated compared to intact sides; this difference in stone crabs and spider crabs was not evident. In terms of setal morphologies, spider crabs have a protopod that serves as a screen and filters incoming water before entering the branchial chamber. The protopod in spider crabs was heavily fouled with broken setae in setal patches, mostly of pappose setae (Wortham and LaVelle, 2016). The gill cleaning epipods, in comparison, were clean, not fouled, with many types of intact serrate setae for grooming (Wortham and LaVelle, 2016). Overall, gill lamellae in spider crabs (ablated and intact) were not heavily fouled; the setae on the protopod and epipods in the branchial chamber appears to be efficient. While the molt stage of individuals was not known in this study, all individuals used in the SEM observations were immature, still molting, and still getting replenishment of hooked setae and “anti-fouling” episodes.

Similar to spider crabs, blue crabs and stone crabs had sedimentary fouling in between the gill lamellae. Blue crabs have double the gill area and more gill lamellae (but a similar grooming time budget) compared with *L. dubia* (Gray, 1957), yet blue crabs did not have gills with more fouling than spider crabs

(when comparing intact individuals). All brachyurans (spider crabs: present study; blue and stone crabs: Wortham and Pascual, 2017) did not have less bacterial fouling on intact sides compared with ablated sides. The setae involved in grooming the brachyuran gills might not be suited for bacterial removal, similar to crayfish (Bauer, 1998). It is possible that individuals used in ablation, fouling, and SEM experiments/observations were at different stages in molt cycles; individuals in the intermolt period would be more heavily fouled than individuals that recently molted. Using a larger sample size, documenting molt stages, and using individuals that had reached their terminal molt may be beneficial in the future for these fouling studies.

Grooming time budget

Brachyuran crabs were predicted to groom less than other crustaceans due to their morphology (Bauer, 1989; Holmquist, 1989). Specifically, spider crabs were predicted to groom irregularly (Hartnoll, 1993; Sallam *et al.*, 2007). The isolation grooming time budget of *L. dubia* (5.22%) was much lower than other crustacean grooming time budgets: *Heptacarpus pictus* Stimpson 1871: 27% (Bauer, 1977); *Macrobrachium grandimanus* Randall 1840: 25% (VanMaurik and Wortham, 2011); *Macrobrachium rosenbergii* de Man 1879: 19% (VanMaurik and Wortham, 2014); and mantis shrimps: 3% (Wortham and KostECKA, 2019). Compared to other brachyurans, grooming in *L. dubia* was similar to blue crabs (5% grooming time budget: Wortham and Pascual, 2017) but different from stone crabs (49%). Blue crabs used the P1 to groom and not the P5 (modified for swimming), which is similar to spider crabs. Stone crabs, which do not use the cumbersome P1 to groom, may have a higher grooming time budget because of the use of their P2–P5 as grooming appendages.

Spider crabs have lower dexterity in their pereopods compared to most shrimps, as well as have gills that cannot be reached by their walking legs. These morphological characteristics may be the main reasons that the grooming time budgets in crabs is lower than shrimps (Bauer, 1989). Spider crabs seem to have morphologically similar walking legs (P2–P5) as stone crabs, yet stone crabs use their P4 and P5 in grooming and spider crabs did not. The setal morphology of the P4 and P5 in stone crabs was complex with many

visible brushes, similar to the P5 brushes seen in porcelain crabs (Ferreira and TAVARES, 2018); the P2–P5 in spider crabs did not have these visible brushes. Stone crabs have a higher grooming time budget with more morphological structures, possibly because of more morphological structures/setal brushes that can be used in grooming. Presence of morphological structures such as setal brushes could be influencing grooming behaviors and the removal of fouling.

Conclusions

Regarding the hypotheses, data analyses supported several predictions. *Libinia dubia* used the first pereopods (P1) and third maxillipeds (3M) as their main grooming appendages. Groomed body regions were not those associated with locations of decorating setae, but with sensory/respiration. The grooming time budget decreased with the presence of another individual, supporting grooming as a secondary behavior. The time budget for grooming in spider crabs was lower than other shrimps, but similar to another brachyuran. A couple of predictions were not supported. Larger crabs did not groom their bodies more than smaller individuals and ablation of a grooming appendage did not visually increase fouling levels on the gills, against initial predictions. Spider crabs had a similar grooming time budget as blue crabs, but not as stone crabs.

This research illuminated additional issues in decorating spider crabs. Large crabs, with few decorations and hooked setae, were not grooming decorating body regions; larger individuals were collected in the field without this camouflage. While hiding in the benthic sediment provided some camouflage from predators, researchers have hypothesized that large spider crabs were simply too large for predators to consume (Stachowicz and Hay, 1999; Hultgren and Stachowicz, 2009). In Tampa Bay, there were many fish (red drum, sheepshead, Atlantic stingrays, cownose rays, bonnethead sharks, etc.) living in the same seagrasses where the crabs were collected (Springer and Woodburn, 1960); these large fish could feed on crustaceans the size of *L. dubia*. It is possible that larger individuals, while not heavily decorated, could be heavily fouled, providing a secondary type of camouflage. Large and small spider crabs have other setal types besides hooked setae on their bodies that

may aid in hiding from predators and prey. A type of unique setae (euphorbia pappose, function unknown) occurred on all body regions of *L. dubia* associated with decorations. This setal type could aid in camouflage (VanMaurik and Wortham, 2015; Wortham and LaVelle, 2016).

How hooked setae are maintained remains unknown. While molting renews these setae in smaller individuals, the lack of grooming body regions with hooked setae provided hooked setae body regions provided evidence that either grooming may damage the hooked setae, inadvertently remove decorations, or grooming is not needed as another antifouling mechanism existed. The structure of hooked setae has not been thoroughly investigated and there could be anti-fouling secretions that aid these setae from being fouled.

In general, crabs appear to groom their bodies much less frequently and for less time than other decapods. In crabs, body joints could become fouled leading to possible complications during molting and even limit grooming by the P1. While spider crabs groomed their bodies more similarly to blue crabs, differences exist between the grooming behaviors within brachyurans. More studies documenting the grooming behaviors of brachyuran crabs are needed to confirm this decrease in grooming time budget, as well as to document how fouling affects economically important crustaceans.

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