

Source and Specificity of Chemical Cues Mediating Shelter Preference of Caribbean Spiny Lobsters (*Panulirus argus*)

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Abstract. Caribbean spiny lobsters display a diversity of social behaviors, one of the most prevalent of which is gregarious diurnal sheltering. Previous research has demonstrated that shelter selection is chemically mediated, but the source of release and the identity of the aggregation signal are unknown. In this study, we investigated the source and specificity of the aggregation signal in Caribbean spiny lobsters, *Panulirus argus*. We developed a relatively rapid test of shelter choice in a 5000-l laboratory flume that simulated flow conditions in the spiny lobster's natural environment, and used it to examine the shelter preference of the animals in response to a variety of odorants. We found that both males and females associated preferentially with shelters emanating conspecific urine of either sex, but not with shelters emanating seawater, food odors, or the scent of a predatory octopus. These results demonstrate specificity in the cues mediating sheltering behavior and show that urine is at least one source of the aggregation signal.

Introduction

Many species of palinurid lobsters display gregarious social behaviors (Atema and Cobb, 1980). In the Caribbean spiny lobster, *Panulirus argus*, this sociality is evident in a variety of behaviors including the formation of long, single-file migratory queues, and defensive rosettes and aggregations (Herrnkind, 1969, 1970, 1980; Berrill, 1975; Atema and Cobb, 1980; Herrnkind *et al.*, 2001). However, the most

ubiquitous example of their sociality is gregarious sheltering (Childress and Herrnkind, 1997). After solitary nocturnal foraging trips, spiny lobsters often aggregate with conspecifics in dens where they remain sheltered throughout the day (Herrnkind *et al.*, 1975; Kanciruk, 1980). Both the males and females shelter gregariously (Herrnkind *et al.*, 1975, 2001), suggesting that this form of aggregation is not a sex-specific behavior (Zimmer-Faust *et al.*, 1985).

Although spiny lobsters are often aggregated in shelters, the extent of gregarious sheltering in any particular area is variable and influenced by several factors, including conspecific density, predation levels, and the number, availability, and size of suitable shelters (Eggleston *et al.*, 1990; Eggleston and Lipcius, 1992). However, multiple occupancy of a shelter occurs more often than expected by random chance (Kanciruk, 1980; Herrnkind *et al.*, 1975). The primary benefit of gregarious sheltering is believed to be a reduction in overall predation levels, which could be accomplished in several ways: through group defense or dilution effects, or *via* the guide effect, which suggests that spiny lobsters can minimize the amount of time spent searching for a shelter (thus minimizing their exposure to predators) by homing in on cues released from sheltered conspecifics (Eggleston and Lipcius, 1992; Childress and Herrnkind, 1997, 2001a,b).

An essential first step to understanding how aggregation occurs is to identify the proximal cues that attract spiny lobsters to sheltering conspecifics. Shelter choice assays conducted in both the field (Nevitt *et al.*, 2000) and laboratory (Ratchford and Eggleston, 1998, 2000) demonstrated that shelter selection by *P. argus* can be mediated by chemical signals released from conspecifics. In these studies,

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spiny lobsters sheltered in dens from which conspecific odor (water in which a conspecific was housed) was emanating significantly more often than they sheltered in unscented control dens. Conspecific attraction also facilitates shelter selection and aggregation in the California spiny lobster, *Panulirus interruptus*, a cold-water congener of *P. argus* (Zimmer-Faust *et al.*, 1985; Zimmer-Faust and Spanier, 1987), and seasonal aggregation in *Panulirus guttatus*, which is sympatric to *P. argus* (Briones-Fourzán and Lozano-Álvarez, 2005).

Although spiny lobsters are attracted to conspecifics when searching for shelter, the specific source of release and the identity of the attractive signal are currently unknown. Studies done by Ratchford and Eggleston (2000) demonstrated that although spiny lobsters are continuously receptive to the aggregation signal, the release of the signal is temporally regulated. They also showed that spiny lobsters of various sizes release the signal in a mass-dependent manner (Ratchford and Eggleston, 1998). These two release characteristics suggest that the aggregation signal may be contained within the urine.

Urine is often an important carrier of chemical information in intraspecific interactions between decapod crustaceans. Urine-borne signals mediate several aspects of courtship and mating behavior (Ryan, 1966; Christofferson, 1978; Gleeson, 1980; Bushmann and Atema, 1994, 1997, 2000; Atema, 1995; Kamio *et al.*, 2000, 2002; Hardege *et al.*, 2002; Raethke *et al.*, 2004) and also play an important role in the determination of social status and individual recognition in other species of decapods (Breithaupt and Atema, 1993, 2000; Atema, 1995; Karavanich and Atema, 1998a,b; Breithaupt *et al.*, 1999; Zulandt-Schneider *et al.*, 2001; Breithaupt and Eger, 2002).

The goals of the current study were to develop a naturalistic but relatively rapid laboratory bioassay for examining chemically mediated sheltering behavior in *P. argus*, and to use it to examine the source and specificity of the aggregation signal.

Materials and Methods

Animals

Intermolt Caribbean spiny lobsters, *Panulirus argus* (Latreille, 1804) (carapace length: mean \pm SEM = 56.9 \pm 1.0 mm, $n = 76$), were collected in the Florida Keys, shipped to Georgia State University, and held in 800-l aquaria containing aerated, recirculated, filtered artificial seawater (Instant Ocean, Aquarium Systems, Mentor, OH). For the population from which these lobsters were obtained, females as small as 57-mm carapace length can be reproductive (Bertelson and Matthews, 2001). However, none of the animals used in this study were gravid or bore a spermatophore. Judging from their size, these animals were subadults or young adults. Animals were maintained on a 12 h:12 h

light-dark cycle and fed shrimp or squid three times a week. At least 2 days before being tested in the behavioral assay, experimental animals were transported to holding aquaria (0.90 m long \times 0.58 m wide \times 0.67 m tall) at Georgia Institute of Technology, where they were maintained throughout the course of the experiments when not being tested.

Odor stimuli

Control stimulus. The control stimulus was artificial seawater taken directly from the flume before the start of trials.

Urine stimuli. Urine was collected from four subadult male (carapace length: mean \pm SEM = 64 \pm 5 mm) and four subadult female (carapace length: mean \pm SEM = 59.8 \pm 7.2 mm) *P. argus* housed singly in 40-l aquaria. Animals were catheterized using a modified version of the technique developed by Lindstrom (1991) and employed by Breithaupt and Atema (1993). Briefly, animals were immobilized on an acrylic plastic restraining device, and the area surrounding the nephropore was blotted dry. Tygon R3603 flexible tubing (inner diameter: 1.6 mm; outer diameter 3.2 mm, Saint-Gobain Performance Plastics, Akron, OH) was affixed over each nephropore using cyanoacrylate glue (Quicktite Super Glue Gel, Loctite Corp., Manco, Inc. Avon, OH, or Zap-a-Gap, Pacer Technology, Rancho Cucamonga, CA) and a catalytic accelerator (Zip Kicker, Pacer Technology). The tubes enclosing the nephropores were secured to the dorsal side of the animal and connected to a collection vial via a T-connector and an additional length of tubing. The collection vial was a 50-ml plastic centrifuge tube surrounded by a polystyrene ring, which kept the vial afloat at the water surface. As urine was released, it moved through the tubes and accumulated in the collection vial. Collection vials were emptied daily, and the collected urine was frozen and stored at -20°C . The amount of urine produced by an individual lobster varied greatly, but generally ranged from 0 to 20 ml over the course of the day.

Previous research showed that the release of the aggregation signal in *P. argus* is discontinuous (Ratchford and Eggleston, 2000), which suggests that the signal (if contained in the urine) may not necessarily be present in all urine samples. To maximize our chances of collecting some volume of the aggregation signal, the spiny lobsters were continuously catheterized, and the urine output from multiple animals collected over several days was combined into a single sample.

Collected urine for use in the experimental trials was thawed and diluted 1:10 or 1:100 in artificial seawater taken directly from the flume. Conspecific urine stimuli consisted of samples pooled from all 8 catheterized spiny lobsters (including both males and females). Sex-specific urine stimuli consisted of either pooled male urine (collected from all

4 catheterized males) or pooled female urine (collected from all 4 catheterized females).

Other odor stimuli. We also examined the sheltering behavior of spiny lobsters in response to three additional odor stimuli: shrimp extract, whole shrimp, and octopus tank water. This was done to ensure that any sheltering behavior exhibited by the lobsters in response to urine was specific to that stimulus, and not simply a generalized response to any novel odorant introduced into the flow.

Shrimp extract is a potent feeding stimulus for spiny lobsters (Carr, 1988; Derby, 2000) and was prepared by homogenizing frozen penaeid shrimp in seawater in a blender and then collecting and freezing the raw extract in 10-ml aliquots. The final concentration of the raw extract was about 300 g/l. The raw extract was diluted 1:10 (30 g/l) in artificial seawater taken directly from the flume and filtered to remove large pieces of shrimp material.

We also examined the sheltering behavior of spiny lobsters when a piece of penaeid shrimp was placed on the floor of the shelter. This was done to ensure that any lack of preference seen in the previous treatment was because shrimp is an ineffective stimulus for choosing a shelter, and not because the concentration of shrimp extract was too low to influence sheltering behavior.

Octopus odor consisted of artificial seawater taken from an 80-l aquarium in which a single small individual of *Octopus briareus* (about 30 cm from arm tip to arm tip) was living for several weeks. *P. argus* and *O. briareus* are sympatric and utilize the same types of crevice shelters, but *O. briareus* is a competitor and potential predator of *P. argus* (Berger and Butler, 2001). Previous research indicates that spiny lobsters avoid shelters that emanate octopus odors (Berger and Butler, 2001). Therefore we also expected that the lobsters would not associate with and might avoid shelters emanating the scent of a live octopus in our shelter choice assay.

Experimental setup

One experimental goal was to develop a laboratory assay that was more rapid than previous assays of sheltering behavior, which required many hours (*e.g.*, Ratchford and Eggleston, 1998, 2000), and that placed the animals in natural flow dynamics. Our shelter choice assay was performed in a 5000-l seawater flume located at Georgia Institute of Technology (Fig. 1). The flume measures 12 m long \times 0.75 m wide \times 0.35 m high with a downstream working section measuring 2 m long \times 0.75 m wide \times 0.35 m high. When in operation, the flume itself (not including the reservoir) contains over 2500 l of seawater. Details on the flow dynamics of this flume and its use in other behavioral experiments are described elsewhere (see Webster and Weissburg, 2001; Keller *et al.*, 2003; Weiss-

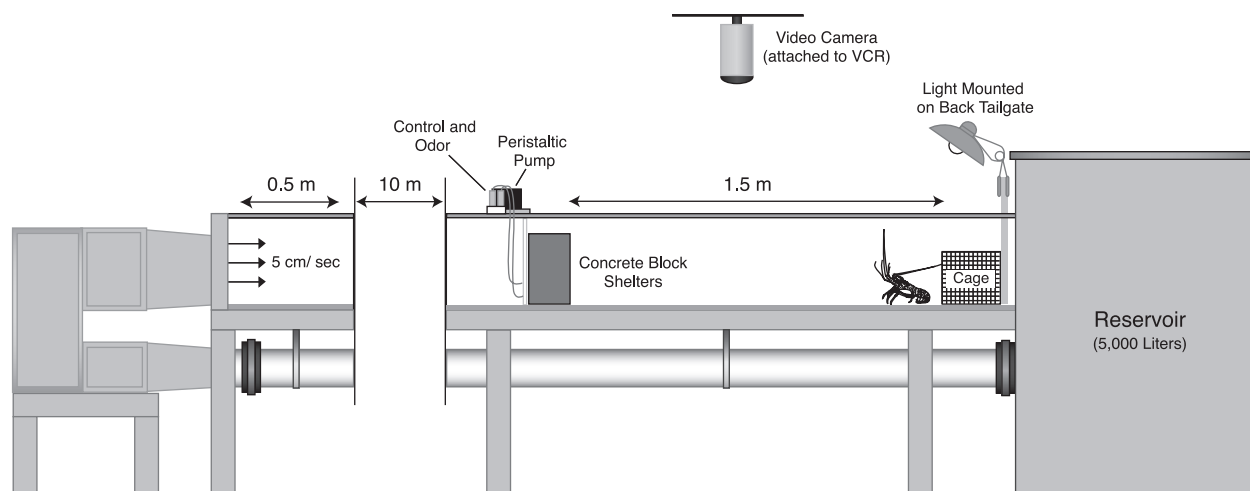
burg *et al.*, 2003; Horner *et al.*, 2004). All trials in this study were conducted with a background flow rate of 5 cm/s. The flume water was filtered through biological, particulate, activated carbon, and UV filters between trial days.

Two concrete blocks were used as shelters for the spiny lobsters (block dimensions: 39.5 cm tall \times 19.5 cm wide \times 19 cm deep, opening size: 14 cm tall \times 13 cm wide \times 19 cm deep). The blocks were placed at the upstream end of the working section, 5 cm from the wall of the flume and 26 cm apart. The area between each block and the sidewall of the flume was filled with a small section of plastic grating (1 cm \times 1 cm) to prevent the lobsters from sheltering in this area. A larger piece of plastic grating spanning the width of the flume was placed behind the blocks to prevent the lobsters from escaping the working section. We secured two handmade L-shaped plastic pipettes to the plastic grating such that the opening of each pipette was centered in the opening of each concrete block, 1 cm above the floor of the block opening. Odor stimuli were introduced into the flow by a dual-channel peristaltic pump (Masterflex, Cole Parmer Instrument Company, Vernon Hills, IL) that moved the odorants through a pair of Teflon tubes that had been threaded through each of the plastic pipettes. During control trials, artificial seawater was simultaneously pumped through both shelters. During experimental trials, the experimental odorant was pumped through one shelter while seawater was simultaneously pumped through the other shelter as a control. The experimental odor stimulus was always paired with a seawater control. We never directly tested one experimental odor against another because we were only interested in whether a particular stimulus influenced sheltering behavior, not in the relative strength of its influence. We randomly chose which shelter would release which odorant, and switched the site of odorant release between trials. Control and experimental stimuli were pumped into the flume at about 15 ml/h. (Thus with a 1:100 dilution, only about 150 μ l of urine is released into the flow over the course of 1 h.) This rate and dilution was used to conserve our odor stimuli, and because preliminary experiments indicated that these conditions were sufficient to elicit sheltering behavior.

All trials were conducted with the room lights on and with a 60-W light mounted at the downstream end of the flume to provide constant illumination in the working section. In the natural environment, spiny lobsters typically search for shelter in the early morning. However, previous research has shown that they will shelter in the presence of an aggregation signal regardless of where they are in their circadian cycle (Ratchford and Eggleston, 2000). Therefore we did not make special efforts to run the trials at specific points in the spiny lobster's natural light-dark cycle.

Trials began when a spiny lobster was placed in an acrylic and plastic grate cage (30.5 cm long \times 21 cm wide \times 20.5 cm high) 1.5 m downstream from the face of

A. Side View of the Flume and Working Section



B. Overhead View of the Working Section

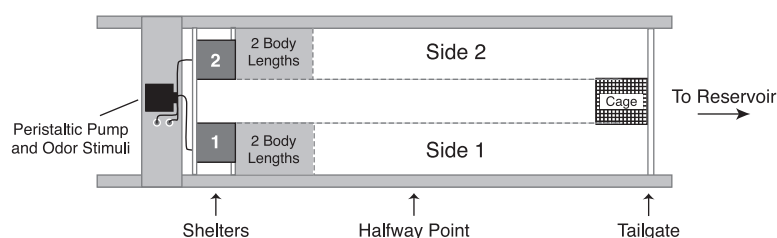


Figure 1. Condensed diagram of the seawater flume at Georgia Institute of Technology showing a side view (A) and overhead view (B) of the working section and setup for the behavioral assay.

the concrete blocks for a 5-min acclimation period. Odor stimuli were pumped into the flume during this acclimation. After 5 min, the cage was lifted and completely removed from the flume, thus forcing the lobster to explore the working section. Each trial lasted for 1 h, and the movements of the animals were recorded by a video camera mounted above the flume. Trial length was set at 1 h because we wanted to establish a short but reliable bioassay, and our preliminary experiments indicated that this was sufficient time for the spiny lobsters to establish a clear preference for one shelter over the other.

Video analysis

Videos were analyzed by an individual unaware of which shelter contained the experimental odorant. To be included in the data set, a spiny lobster had to explore both sides of the flume and walk more than halfway upstream. These criteria were set to ensure that animals had the opportunity to sample odors eluting from both shelters, and that they were healthy and motivated to explore the flume and shelters. Most spiny lobsters tested with each odorant met the

criteria to be included in the data set (Table 1). The sheltering behavior of the lobsters was quantified by recording the number of times each shelter was entered and by measuring the total time spent inside (all body parts except antennae completely within the shelter) or within 30 cm (roughly the equivalent of two body lengths of the smallest lobsters used in the trials) of each shelter. Each trial thus produced two values for total sheltering time: one for time spent in or around the control shelter, and the second for time spent in or around the experimental odor shelter. Each trial also produced two values for the number of entries into each shelter. Sheltering time was defined to include the time spent within two body lengths of each shelter because animals sometimes were outside of the shelter but behaved as if they were sheltered inside the block. For instance, in several trials, the spiny lobster approached the shelter head on, turned around, and then backed into the corner formed by the plastic grate and sidewall of the flume immediately adjacent to the shelter. In this position, the lobster's abdomen was partially protected on two sides, while its antennules were still able to sample water passing through the

Table 1*Number and percentage of spiny lobsters meeting criteria for each odor tested*

	Seawater	1:10 Urine	1:100 Urine	1:10 Shrimp	Whole Shrimp	Octopus	Female Lobsters		Male Lobsters	
							Female Urine	Male Urine	Female Urine	Male Urine
No. Lobsters Tested	10	15	36	14	10	9	14	10	10	11
No. Meeting Criteria	8	13	31	14	10	9	13	9	10	11
% Meeting Criteria	80	86	86	100	100	100	93	90	100	100

shelter. Although these animals were not inside the shelter, they still secured protection from the block.

Statistical analysis

For each of the odorants examined (seawater, conspecific urine, shrimp extract, whole shrimp, and octopus odor), we used a two-tailed Wilcoxon matched pairs test to determine whether there were statistically significant differences in the amount of time spent inside or within two body lengths of the control shelter *versus* the shelter emanating the experimental odorant. Data from all spiny lobsters that met criteria were included in the statistical analysis. The same analysis was used to examine differences in the number of entries into the control shelter *versus* the shelter emanating the experimental odorant.

We performed a different statistical analysis for the sex-specificity treatments. In this analysis, we subtracted the amount of time spent within the control shelter from the time spent within the experimental shelter. This yielded a single sheltering value for each *trial* (instead of one value for each *shelter*). We then used a Mann-Whitney *U* test to identify any statistically significant differences between males and females in the computed values for male urine trials *versus* female urine trials. If there is any sex specificity to the signal or response, then we would expect to see different patterns of sheltering behavior by male and female spiny lobsters in response to male and female urine signals.

Results

Response to seawater

Spiny lobsters generally spent most of their time exploring the flume when tested with seawater emanating from both shelters. All of the spiny lobsters tested explored both sides of the flume, and 8 out of the 10 lobsters tested walked upstream and explored the area around the shelters during the trial (Table 1). The two lobsters that did not walk upstream (and were subsequently not included in the data set) spent most of the trial walking back and forth across the flume near the back tailgate, which formed the downstream border of the working section. These results indicate that the

typical behavior of healthy spiny lobsters is to explore the working section after being placed in the flume, thus affirming our criteria for inclusion in the data set. Although most of the spiny lobsters explored the shelters at some point during the trial, only half of these lobsters actually entered the shelters (data not shown). Overall, the animals did not show a significant preference for either of the two shelters. There were no significant differences in either the number of entries into each shelter (Fig. 2B) or in the amount of time spiny lobsters spent inside or around each shelter (Fig. 2A).

Response to diluted conspecific urine

In contrast to the behavior of the spiny lobsters tested with seawater only, spiny lobsters that were given the choice between a shelter emanating seawater and a shelter emanating diluted conspecific urine showed a significant overall preference for the shelter emanating urine. The same pattern of behavior was observed regardless of whether the lobsters were tested with the 1:10 (Fig. 3A, B) or 1:100 (Fig. 3C, D) dilution of conspecific urine. About 86% of the lobsters met the criteria to be included in the data set in both the 1:10 and 1:100 dilution trials (Table 1). Animals in both sets of trials entered the shelter emanating conspecific urine significantly more often than they entered the control shelter (Fig. 3B, D) and spent significantly more time inside or within two body lengths of this shelter than in or near the control shelter (Fig. 3A, C). Thus, our 1-h assay meets our goal of being sufficiently long to reveal odor-mediated sheltering preference but also has the experimental advantage of being much shorter than previous laboratory and field assays of sheltering.

Response to male and female urine signals

In the previous section, both male and female spiny lobsters responded to dilutions of conspecific urine pooled across sexes (Fig. 3). We tested separately the response of subadult male and female spiny lobsters to subadult male and female urine signals to confirm that shelter selection in our assay was not a sex-specific behavior. There was no

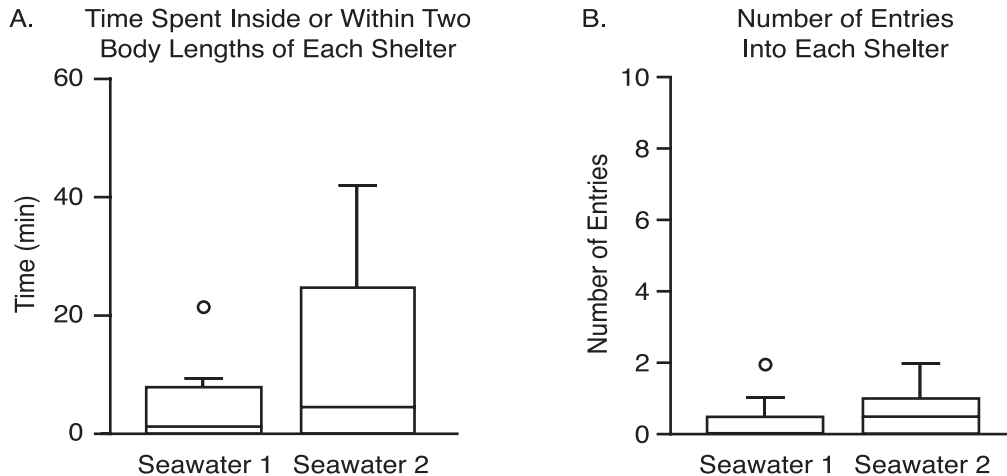


Figure 2. Sheltering behavior of spiny lobsters in response to artificial seawater. Box plots represent median (black line) and interquartile range (box length) for time spent inside or within two body lengths of each shelter (A) and number of entries into each shelter (B). Outliers (cases with values between 1.5 and 3 box lengths away from the upper or lower edge of the box) are indicated by open circles (○). All data, including outliers, were included in the statistical analysis. Sample sizes are $n = 8$ for both A and B. No statistically significant differences were observed between the responses to either shelter in any of the treatments. Wilcoxon matched pairs test, two-tailed, $P > 0.05$.

statistically significant difference in the shelter preference of females tested with male *versus* female urine (Fig. 4A). There was also no statistically significant difference in the shelter preference of males tested with male *versus* female urine (Fig. 4B). There were also no statistically significant differences between the shelter preference of male and female spiny lobsters tested with male urine, nor were there any statistically significant differences in the shelter preference of male and female spiny lobsters tested with female urine (data not shown).

Response to shrimp odor

All of the spiny lobsters tested with shrimp extract *versus* seawater met criteria to be included in the data set. The animals did not show a statistically significant preference for the shelter emanating shrimp odor over the control shelter. The number of entries into each shelter was similar (Fig. 5B), as was the amount of time spent inside or around each shelter (Fig. 5A).

Response to whole shrimp

All of the spiny lobsters tested with a whole piece of shrimp met criteria for inclusion in the data set (Table 1). Although the animals tended to show more interest in the shelter containing the piece of shrimp *versus* the control shelter, this difference was not statistically significant. A few spiny lobsters that located the shrimp stayed within the shelter after they had consumed this food. Most animals only entered the shelter to obtain the shrimp, which they often grabbed or partially consumed before exiting quickly to

resume exploration. There were no statistically significant differences between the behavioral responses to the control shelter and the shelter containing the shrimp piece (Fig. 5C, D).

Response to live octopus odor

All of the spiny lobsters tested with live octopus odor *versus* seawater met the criteria for inclusion in the data set (Table 1). Although the animals tended to show more interest in the control shelter than in the shelter emanating octopus odor, this difference was not statistically significant. There were no statistically significant differences in either the time spent in and around each shelter (Fig. 6A) or in the number of entries into each shelter (Fig. 6B). Rather than spending more time in and around the control shelter, spiny lobsters tended to avoid octopus odor by spending time at the downstream end of the working section away from both shelters.

Discussion

The aim of this study was to examine the source and specificity of the chemical signals mediating gregarious sheltering in the Caribbean spiny lobster *Panulirus argus*. We developed a relatively rapid but naturalistic bioassay and used it to show statistically significant preferences for shelters emanating conspecific urine signals, regardless of the sex of the urine donor or the sex of the responder. Spiny lobsters did not shelter preferentially with food or predator odors. These results demonstrate that dilute urine is sufficient to mediate rapid shelter selection, and strongly suggest

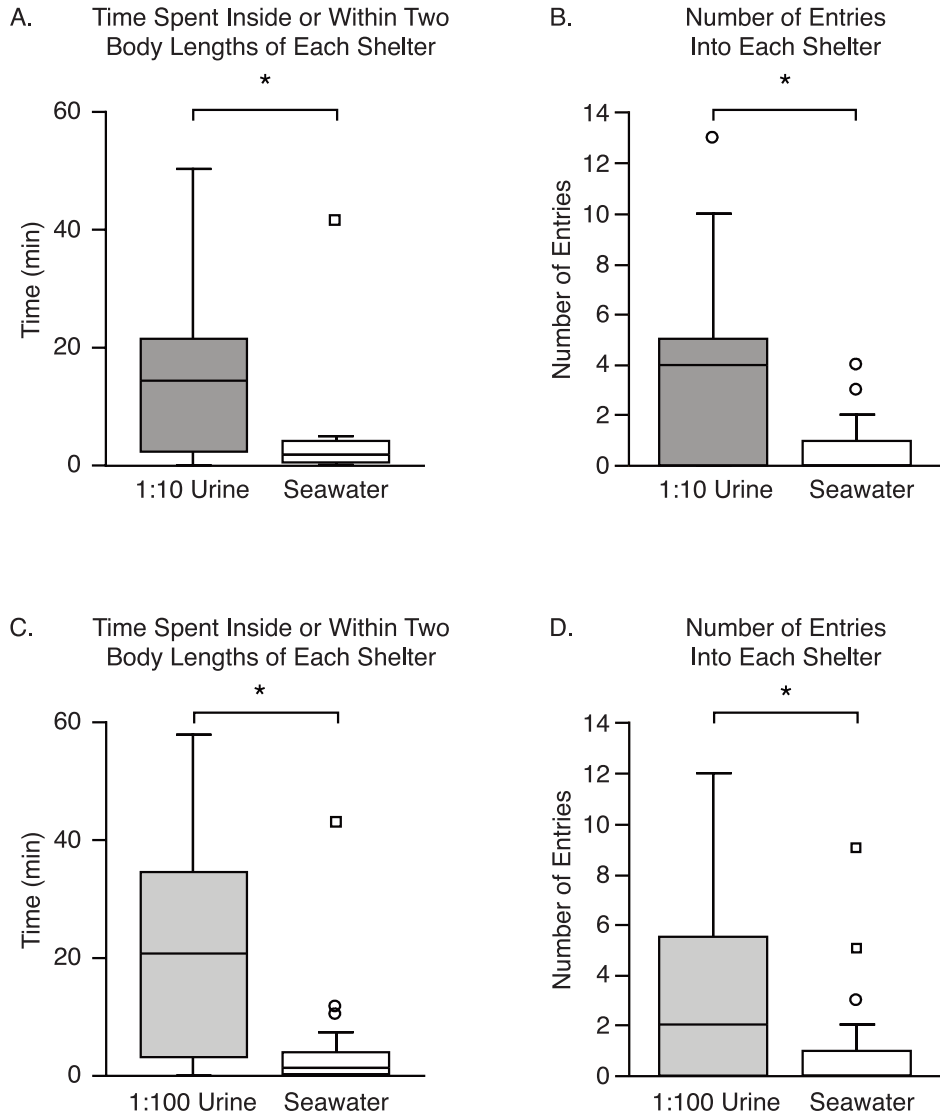


Figure 3. Sheltering behavior of spiny lobsters in response to 1:10 (A and B) and 1:100 (C and D) dilutions of conspecific urine in artificial seawater. Box plots show median and interquartile range for time spent inside or within two body lengths of each shelter (A and C) and number of entries into each shelter (B and D). Outliers are indicated by open circles (○) and extremes by open squares (□); both were included in the statistical analysis. Sample sizes are $n = 13$ for 1:10 dilution of urine in seawater, and $n = 31$ for 1:100 dilution of urine in seawater. Statistically significant differences are indicated by “*”; Wilcoxon matched pairs test, two-tailed, $P < 0.01$.

that urine is at least one source of the aggregation signal in this species.

Urine is a source of the sheltering cue

When presented with two shelters, both of which emanated seawater, spiny lobsters were not strongly motivated to shelter (*i.e.*, they did not enter or spend much time inside the shelters), and they did not display a clear preference for either refuge (*i.e.*, they spent similar amounts of time inside and around both shelters) (Fig. 2). In contrast, spiny lobsters displayed completely different behaviors when presented

with one shelter emanating seawater and another shelter emanating conspecific urine. The animals spent significantly more time inside or around the shelter emanating conspecific urine than in or around the control shelter, and they also entered this shelter significantly more often when compared to the control shelter (Fig. 3).

Even very low concentrations of conspecific urine were sufficient to mediate shelter selection. Both 1:10 and 1:100 dilutions of conspecific urine elicited the same pattern of sheltering behavior. Although it is impossible to determine when the animals detected the urine stimulus, they almost

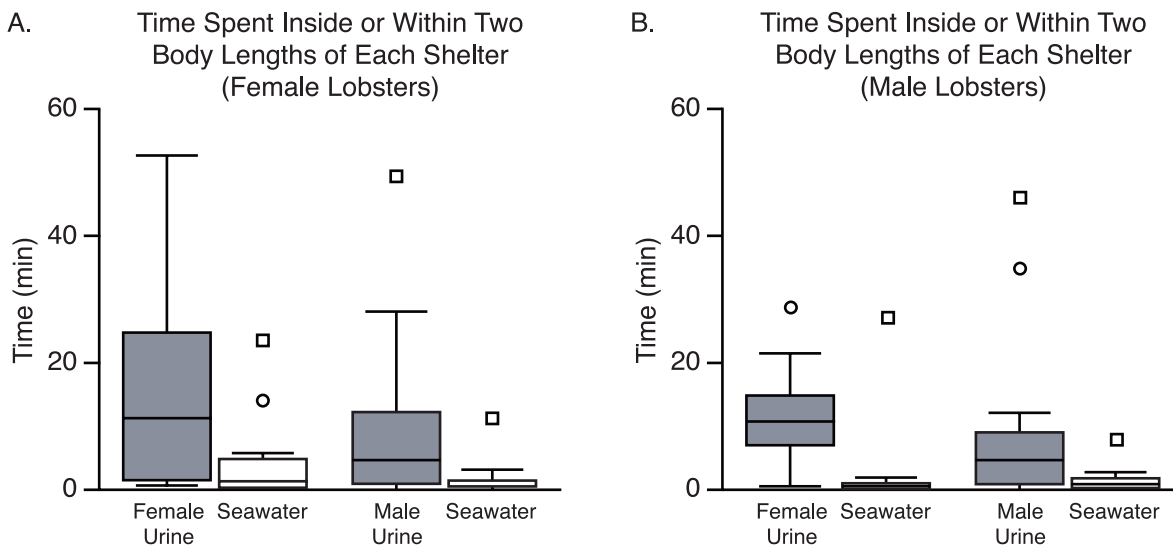


Figure 4. Sheltering behavior of female (A) and male (B) spiny lobsters in response to female and male conspecific urine. Box plots show median and interquartile range for time spent inside or within two body lengths of each shelter. Outliers are indicated by open circles (○) and extremes by open squares (□); both were included in the statistical analysis. Sample sizes are $n = 13$ female lobsters tested with female urine, $n = 9$ female lobsters tested with male urine, $n = 10$ male lobsters tested with female urine, and $n = 11$ male lobsters tested with male urine. There were no statistically significant differences in the response of female lobsters to shelters emanating male urine *versus* seawater or female urine *versus* seawater. There were also no statistically significant differences in the response of male lobsters to shelters emanating male urine *versus* seawater or female urine *versus* seawater. Mann-Whitney U test, $P > 0.05$.

certainly detected it at even lower concentrations than the initial 1:10 or 1:100 dilution. In roughly equivalent flow situations, peak odor concentrations are commonly 10% or less than the source concentration within a few tens of centimeters from the source, and they are well below 1% of the initial concentration by 1 m downstream (Webster and Weissburg, 2001). Thus, the release of even a small volume of urine by sheltering conspecifics is sufficient to attract lobsters to the den.

The sheltering cue and response are not sex specific

There does not appear to be any sex specificity to the urine signal in the context of shelter selection by the subadult or young adult spiny lobsters tested in this bioassay. Although some of the animals tested in the study were potentially of reproductive age, neither the sex nor reproductive state of either the lobster producing or responding to the urine appeared to have any effect on the behavior. Previous research has shown that spiny lobsters as small as 15 mm in carapace length are capable of producing and responding to conspecific aggregation signals, even though animals in this size class are clearly not reproductive (Ratchford and Eggleston, 1998). In our assay, animals of both sexes and of various sizes preferred shelters emanating either male or female conspecific urine over control shelters (Fig. 4). This finding mirrors the results of other laboratory

studies (Zimmer-Faust *et al.*, 1985) and observations in the field that find both male and female spiny lobsters of various sizes aggregated in a single den (Herrnkind *et al.*, 1975, 2001).

Specificity of the sheltering cue

In our bioassay, conspecific urine was the only cue that elicited a statistically significant preference for the shelter releasing it; odors from food (dead shrimp) and predators (live octopus) did not. Spiny lobsters showed no clear preference for either shelter when shrimp extract was the test stimulus in spite of the fact that this odor is a potent feeding stimulus for lobsters (Fig. 5). Even the presence of an obtainable food item—a piece of shrimp—on the floor of the shelter was not sufficient to induce significant sheltering behavior (Fig. 5). Most animals only entered the shelter to obtain the shrimp. Spiny lobsters tended to prefer the control shelter over the shelter emanating the odor of a competitor and potential predator—live *Octopus briareus*—although the difference was not statistically significant (Fig. 6). This trend to avoid octopus odor in our assay was expected since previous research showed that spiny lobsters avoid shelters scented with octopus odor (Berger and Butler, 2001).

The results of these experiments show that a strong preference for a particular shelter does not occur simply in

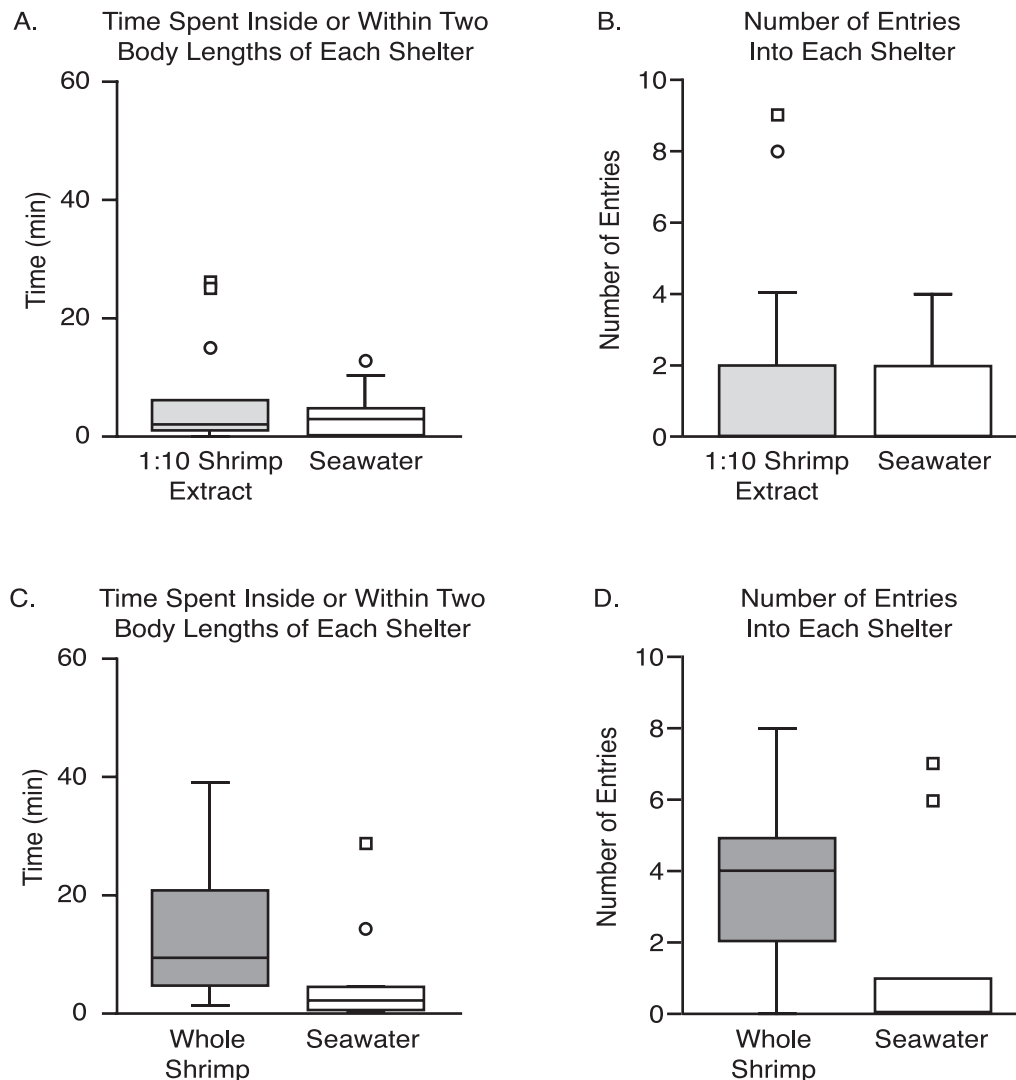


Figure 5. Sheltering behavior of spiny lobsters in response to 1:10 shrimp extract (A and B) and whole shrimp (C and D). Box plots show median and interquartile range for time spent inside or within two body lengths of each shelter (A and C) and number of entries into each shelter (B and D). Outliers are indicated by open circles (○) and extremes by open squares (□); both were included in the statistical analysis. Sample sizes are $n = 14$ for 1:10 shrimp extract, and $n = 10$ for whole shrimp. No statistically significant differences were found between the responses to either shelter in any of the treatments. Wilcoxon matched pairs test, two-tailed, $P > 0.05$.

response to any novel odorant released into the flow. The preference for one shelter over another was much more specific in our assay. Statistically significant differences in shelter preference were observed only with conspecific urine. Thus, urine appears to be at least one source of the aggregation signal in this species. An allopatric spiny lobster, *Panulirus interruptus*, and a sympatric spiny lobster, *Panulirus guttatus*, also use chemical aggregation cues (Zimmer-Faust *et al.*, 1985, Zimmer-Faust and Spanier, 1987; Briones-Fourzán and Lozano-Álvarez, 2005). Testing the species specificity of these signals and responses would be informative.

Urine as a source of conspecific cues

Facilitation of aggregation and gregarious sheltering is just one example of the importance of urine signals in decapod crustacean social interactions. Decapod crustaceans use urine signals to mediate a variety of intraspecific interactions. For example, urine-borne signals mediate many aspects of courtship and mating in species including *Homarus americanus* (Bushman and Atema, 1994, 1997, 2000; Atema, 1995), *Jasus edwardsii* (Raethke *et al.*, 2004), *Callinectes sapidus* (Gleeson, 1980), *Carcinus maenas* (Bamber and Naylor, 1997; Hardege *et al.*, 2002), *Telme-*

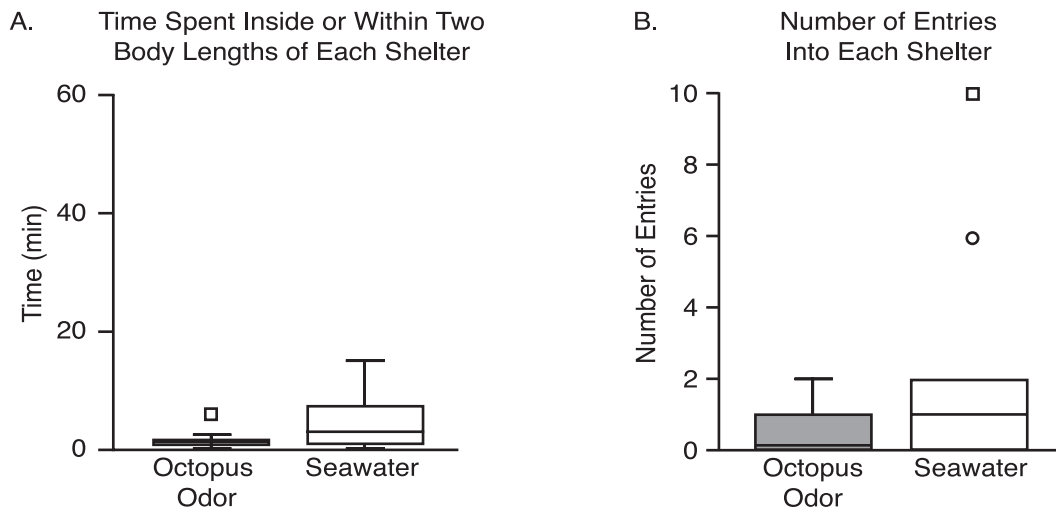


Figure 6. Sheltering behavior of spiny lobsters in response to octopus odor. Box plots show median and interquartile range for time spent inside or within two body lengths of each shelter (A) and number of entries into each shelter (B). Outliers are indicated by open circles (○) and extremes by open squares (□); both were included in the statistical analysis. Sample sizes are $n = 9$ for both A and B. No statistically significant differences were observed between the responses to either shelter in any of the treatments. Wilcoxon matched pairs test, two-tailed, $P > 0.05$.

sus cheiragonus (Kamio *et al.*, 2000, 2002), and *Portunus sanguinolentus* (Ryan, 1966; Christofferson, 1978). Urine signals also play an important role in individual recognition and the determination of social status in *H. americanus* (Breithaupt and Atema, 1993, 2000; Atema, 1995; Karavanich and Atema, 1998a, b; Breithaupt *et al.*, 1999), and they regulate the dynamics of agonistic interactions in the crayfish *Astacus leptodactylus* (Breithaupt and Eger, 2002) and *Orconectes rusticus* (Zulandt-Schneider *et al.*, 2001).

Although the results of the current study strongly suggest that urine is one source of the aggregation signal in *P. argus*, it is not necessarily the only source. Other crustaceans emit social signals concurrently in urine and other sources (Bamber and Naylor, 1997; Bushmann, 1999). There is evidence in both *Callinectes sapidus* and *Carcinus maenas* that sex pheromones are released from sources in addition to urine (Bamber and Naylor, 1997; Bushmann, 1999). It is possible that there are additional sources of the aggregation signal in the spiny lobster, as well. Ratchford (1999) found that *P. argus* is attracted to catheterized conspecifics, but the specific source of the attractant in this case is unknown. Specific non-urine conspecific odors have not yet been examined in *P. argus*.

The behavioral response to chemical signals in urine may also change depending on the context in which the urine is presented. In a different behavioral assay, Ratchford (1999) reported a potential alarm response to conspecific urine by *P. argus*. Potential alarm responses were also noted in *P. argus* when presented with conspecific urine in bioassays in small (80-1) aquaria (Shabani *et al.*, 2006). These differences in behavior are probably attributable to differences in

experimental design, and perhaps differences in the quality (*i.e.*, presence or concentration of the aggregation signal) of the collected urine. At present, we know virtually nothing about the chemical identity or the release dynamics of the aggregation cue contained within the urine. Although previous research showed that the aggregation signal is released discontinuously (Ratchford and Eggleston, 2000), the cause of this intermittency is unclear. For instance, the signal may always be present in the urine but appear discontinuous because urine release is intermittent. Alternatively, the aggregation signal itself may be present in the urine only intermittently. In addition, the release of other substances into the urine along with the aggregation signal might modify the response of receiving spiny lobsters. In any case, the manner in which the urine is collected and pooled could influence its odor quality, which in turn could affect the behavior of the spiny lobsters. Further research into the chemical identity of the specific substances within the urine that influence shelter preference, aggregations, and alarm responses will help us better understand how social behaviors are mediated in *P. argus*.

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