



# Host location and selection by the symbiotic sargassum crab *Portunus sayi*: the role of chemical, visual and tactile cues

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## Abstract

The *Sargassum* community consists of a unique and diverse assemblage of symbiotic fauna critical to pelagic food chains. Associated symbionts presumably have adaptations to assist in finding *Sargassum*. In situ scattered *Sargassum* patches accumulate as they are pushed toward the shoreline (via wind, waves, currents or tides) and are frequently less than 1 m apart and in depths of 10 cm or less as the patches approach the shoreline. Crabs, and other symbiotic fauna, must relocate to another patch that is seaward in direction or likely perish as their current patch will likely become beached. This study investigated sensory cues used for host location and selection by the Sargassum crab, *Portunus sayi*. Chemical detection trials were conducted with a two-chamber choice apparatus with *Sargassum* spp. and *Thalassia testudinum* as habitat source odors. Visual detection trials (devoid of chemical cues) and habitat selection trials were conducted in which crabs were given a choice between hosts. Results showed that *P. sayi* responded to chemicals from *Sargassum* spp. Crabs visually located host habitats but did not visually distinguish between different hosts. In host selection trials, crabs selected *Sargassum* spp. over artificial *Sargassum* and *T. testudinum*. These results suggest that crabs isolated from *Sargassum* likely use chemoreception; within visual proximity of a potential patch, crabs likely use both chemical and visual information.

**Keywords** *Sargassum* · *Portunus sayi* · Symbiosis · Hosts · Habitat selection · Cues

## 1 Introduction

Two of the most critical aspects of symbioses are 1) the impacts of the association on the symbionts (i.e., cost/benefit ratios), and 2) how one or both symbionts locate each other. Pelagic *Sargassum* (mostly two species, *Sargassum fluitans* and *Sargassum natans* [Parr 1939]) forms a critical marine habitat and symbiotic host species to numerous endemic symbionts, many of which exhibit symbiotic aegism (i.e., potentially afforded protection without harming the host) (cf., Brooks et al. 2007). Additionally, within this holobiont there is evidence that some of these symbionts (e.g., fishes) provide return benefits to the alga in the form of essential nutrients (Lapointe et al. 2014). Thus, commensalisms and mutualisms are potentially common outcomes of these associations. The variety of organisms living in the *Sargassum* community, or “weedlines,” is vast, including 100’s of invertebrates and

fishes, and also sea turtles, sea birds and marine mammals (Parr 1939; Weis 1968; Dooley 1972; Ryland 1974; Butler et al. 1983; Stoner and Greening 1984; Kingsford and Choat 1985; Haney 1986; Carr 1987; Manzella and Williams 1991; Wells and Rooker 2004; Casazza and Ross 2008). As a result of this diversity *Sargassum* mats are designated as Essential Fish Habitat (EFH) by the National Marine Fisheries Service (NMFS) (NOAA 1996).

Regarding how these symbionts locate each other, clearly the faunal symbionts play key roles. Although faunal diversity within *Sargassum* mats is typically very high, abundance of *Sargassum* is temporally and spatially highly variable (Stoner 1983; Lapointe 1995; Wells and Rooker 2004). This variation in the abundance and presence of *Sargassum* mats raises the question about how initial recruitment and re-establishment of separated faunal symbionts occurs. Both juveniles and those individuals separated from mats by biotic events (e.g., disruptive feeding actions by large, pelagic species like dolphinfish, *Coryphaena hippurus* (Dooley 1972; Costen-Clements et al. 1991; Wells and Rooker 2004; Rudershausen et al. 2010), and abiotic events (e.g., wind and waves that break up patch sizes, or when mats are driven onto beaches, cf. Hu et al. 2016) must locate a new mat. Visual cues during the daytime would be

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potentially available at certain distances. However, chemical cues would likely be available at all times, and distance would be potentially less of a problem than using visual cues exclusively. Molecules released by *Sargassum* spp. and other brown algae have been detected in the water column (Hanson 1977; Arnold and Targett 2000; Zimmer and Butman 2000; Wong and Cheung 2001; Turner and Rooker 2006; van Ginneken et al. 2011).

Decapod crustaceans can detect numerous waterborne compounds (Rittschof 1992; Markowska et al. 2008) and are known to use chemical signaling processes for mate and symbiont detection, habitat identification and selection, metamorphosis, predator avoidance, prey location, and conspecific communication (Gleeson 1980; Brooks 1991; Zimmer and Butman 2000; Krinsky and Epifanio 2008; Anderson and Epifanio 2010).

Arthropods, including decapod crustaceans, are also unique in that they possess moveable, stalked compound eyes, permitting broader fields of view and an increased binocular spread (Cronin 1986). Adult decapods use vision for orientation in the water column, vertical migration, avoidance of predators, navigation and orientation, prey recognition and capture, habitat selection, mate selection, and communication (Forward Jr. 1976, 1977; Forward Jr. et al. 1984; Cronin 1986; Vannini 1987; Diaz et al. 1995; Cronin and Jinks 2001; Detto 2007).

In this study we focused on the question of host (note: host and habitat are used interchangeably herein as the latter is a living system “host” to the symbiotic crab, and others; i.e., holobiont) location and re-establishment by studying the Sargassum crab, *Portunus sayi* (Gibbes 1850), one of the most abundant decapod crustaceans observed within *Sargassum* mats. Specifically, the following questions related to host location and selection by the Sargassum crab, *P. sayi*, were addressed: Does *P. sayi* respond to chemical cues from *S. fluitans*, *S. natans*, and *Thalassia testudinum* (which is a seagrass sometimes intermixed within *Sargassum* mats)? Does *P. sayi* respond to visual cues from *S. fluitans*, *S. natans*, artificial *Sargassum*, and *T. testudinum*?

## 2 Methods

### 2.1 Collection and maintenance of specimens

All specimens were collected via boat 4–12 km offshore from the Boca Raton Inlet in southeast Florida from April 2011 through September 2011. *S. natans*, *S. fluitans*, *T. testudinum*, and *P. sayi* were collected using a fine mesh dip net and transported in Styrofoam™ containers with portable aerators to the laboratory in the Biological Sciences building at Florida Atlantic University.

In the laboratory, *Sargassum* spp. and *T. testudinum* were separated and placed in aquaria. Sargassum crabs were placed in individual aquaria and acclimated for 24 h prior to experimental trials. All aquaria were set up with seawater obtained from Gumbo Limbo Nature Center in Boca Raton Florida, where saltwater is pumped directly from the Atlantic Ocean. Salinity within all aquaria was maintained between 32 and 35 ppt and specimens were exposed to a 12 L:12D photoperiod. All crabs were fed brine shrimp daily.

*P. sayi* is a portunid crab with modified pereopods for both walking and swimming. Patches of *Sargassum* that are approaching shorelines may be at shallow depths allowing the crab to use either walking or swimming (or both) modalities. The following choice experiments allowed either type of locomotion, but walking occurred most often. Crab size was determined by measuring the carapace width. Individual crab sex was distinguished by morphological differences in males vs. females (Feldmann 1998) and equal numbers of male and female crabs were used for all experiments.

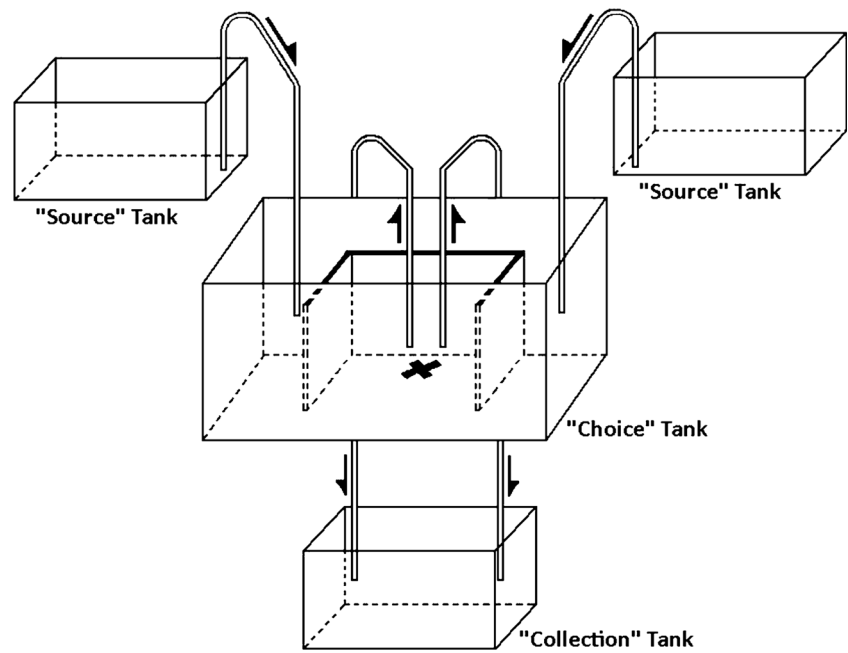
### 2.2 Chemoreception experiments

Chemical cues trials were conducted to determine if chemicals were used by *P. sayi* for host location and selection. Chemoreception trials were conducted in a test apparatus adapted from Waas and Colgan (1992) and consisted of four tanks with gravity fed seawater flow (Fig. 1). The two top “stimulus” tanks (41x20x25 cm) fed water through plastic tubes (6 mm in diameter) into the lower “choice” tank (41x20x25 cm) at a rate of 10 ml/min (after Reeves and Brooks 2001). One “stimulus” tank contained seawater with chemical cues, while the second “stimulus” tank contained only seawater. Both “stimulus” tanks were out of view from *P. sayi*, to eliminate visual cues.

The “choice” tank contained two vertical, opaque partitions sealed to the bottom and the back side of the tank, to create three semi-enclosed compartments. Each side compartment had an 8-cm wide opening for the crabs to pass through from the central compartment. A second pair of outflow tubes (secured along the backside of the center compartment) allowed seawater to flow at a rate of 10 ml/min into a 4th “collection” tank placed below the “choice” tank. All positioning of tubing was measured and placed at equal distances to ensure that flow patterns within each compartment were identical. Additionally, 2 different colored dyes were used before experiments to color seawater (one for each “stimulus” tank) to verify that the flow patterns were identical within both side compartments and that there was minimal mixing in the central compartment.

During each trial, tubing from the “stimulus” tank released seawater containing one of the three chemical cue sources: 1) *S. natans*, 2) *S. fluitans*, and 3) *T. testudinum*, into one side of the “choice” tank. The other “stimulus” tank contained and

**Fig. 1** Four tank experimental apparatus used for chemoreception trials. Opaque partitions in the choice tank create three semi-enclosed compartments. Arrows indicate water flow and “X” marks the spot where *Portunus sayi* was placed at the beginning of an experiment

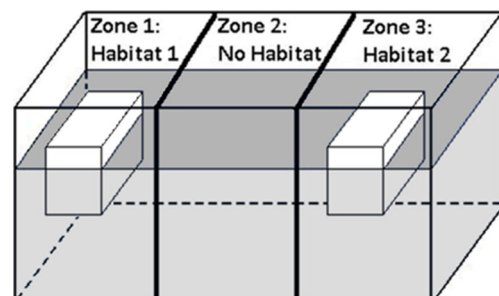


released seawater devoid of added chemical cues into the other side of the “choice” tank. The chemical cue “stimulus” source was obtained from clumps of *Sargassum* and *T. testudinum* weighing  $30 \pm 0.1$  g, adapted from Jobe and Brooks (2009). Clumps of *Sargassum* spp. and *T. testudinum* were used only once to establish odor water for the “stimulus” tank. Prior to the start of each trial the clump was randomly placed in one “stimulus” tank and after 20 min all tubing was unclamped. After 60 s, one crab was placed in front of the outflow tubes in the center compartment with a dip-net. The observer recorded the following data: 1) activity and movements of the crab; 2) the compartment that the crab first entered; 3) elapsed time before the crab left the central compartment. Tests ended when the crab chose a side compartment by leaving the central compartment and crossing the partition (adapted from Waas and Colgan 1992).

Preliminary trials showed that a majority of crabs chose a side compartment within the first 5 min and remained there ( $n = 20$ ). Therefore, trials lasted no longer than 30 min. At the end of each trial, all tanks and tubing were emptied and rinsed to remove potential lingering odors before the beginning of the next trial. Twenty replicates were conducted for each of the experimental conditions and each individual crab was used only once for one treatment. Trials were also conducted to determine if *P. sayi* could chemically distinguish between both species of *Sargassum* simultaneously. Thus, instead of keeping one “stimulus” tank devoid of chemical cues, *S. fluitans* was in one “stimulus” tank while *S. natans* was in the other. Controls in which both “stimulus” tanks contained seawater were conducted to determine how crabs responded in the absence of added chemical cues.

### 2.3 Visual reception experiments

Visual cues trials were conducted to determine if vision was used by *P. sayi* for host location and selection. Each crab was placed individually in aquaria with a choice between two habitats, each of which was placed inside a clear plastic container (9x16x11 cm) (Fig. 2). Each container was placed at opposite ends within aquaria (41x20x25 cm high). The specific visual reception treatments conducted were as follows: 1) *S. fluitans* vs. *S. natans*; 2) *S. fluitans* vs. artificial *Sargassum* (a plastic mimic custom made by Bio Models Co. used to display the visual features of *Sargassum*); 3) *S. natans* vs. artificial *Sargassum*; 4) *S. fluitans* vs. *T. testudinum*; 5) *S. natans* vs. *T. testudinum*. Control tests were also conducted to determine the response of crabs in the presence of only one habitat (e.g., one clear container was empty), which included the following trials: 1) *S. fluitans* vs. control; 2) *S. natans* vs. control; 3) artificial *Sargassum* vs. control; 4) *T. testudinum* vs. control.



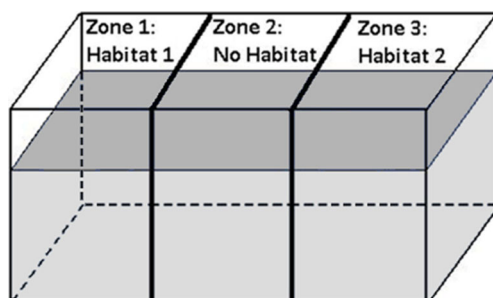
**Fig. 2** Visual reception diagram showing the visually divided zones. Plastic containers remained secured above the water level to prevent interference of chemical cues from the habitats. *Portunus sayi* was placed in the center of zone 2 at the beginning of each experiment

Preliminary trials showed that crabs had no zone preferences within aquaria when both containers were empty ( $n = 20$ ). Each clump of *Sargassum* (live and artificial) and *T. testudinum* was 9x16x7 cm within its respective container and weighed  $30 \pm 0.1$  g. *P. sayi* was placed in the center of the tank after the two habitat patches were arranged within the plastic containers. To avoid interference of chemical and tactile cues, the opening of the plastic containers remained above the water level in the aquarium.

For recording purposes, the aquaria were visually divided into three zones by drawing lines on the upper rim of the aquarium not visible to the crab (adapted from Jobe and Brooks 2009). The visual selection of the crab was recorded based on the zone in which the crab was located after a 2 h trial. Preliminary trials showed that crabs might switch back and forth between hosts for about 30 min before choosing and remaining in a habitat zone ( $n = 20$ ). Therefore, the crab's location within the aquaria was monitored and recorded initially and every 30 min.

## 2.4 Host selection experiments

Experiments were conducted for host selection by *P. sayi*. Each crab was placed in aquaria individually with the option of choosing one of the two habitats provided. Host selection treatments conducted were as follows: 1) *S. fluitans* vs. *S. natans*; 2) *S. fluitans* vs. artificial *Sargassum*; 3) *S. natans* vs. artificial *Sargassum*; 4) *S. fluitans* vs. *T. testudinum*; 5) *S. natans* vs. *T. testudinum*. The two patches of habitat were placed in aquaria (41x20x25 cm high) floating at opposite ends. Patches of *Sargassum* (live and artificial) and *T. testudinum* were spread out to be approximately 11x16x6 cm and weighed  $30 \pm 0.1$  g. Despite morphological differences between the more robust *Sargassum* spp. and the flat blades of *T. testudinum* (and using the same weight), similar habitat size was achieved by arranging the blades in various three dimensional directions as they would be found naturally, instead of flat on top of one another. Similar to the visual cues trials, aquaria were visually divided into three zones by drawing lines on the top of the aquarium rim (Fig. 3). Once the two habitat patches were arranged, each



**Fig. 3** Habitat selection diagram showing the visually divided zones. *Portunus sayi* was placed in zone 2 at the beginning of the experiment

trial began with the placement of one crab in the center of zone 2 in the aquarium. Host selection was recorded based on the zone in which the crab was located after the 2-h trial. The crab's location within the aquarium was monitored and recorded initially and every 30 min.

Control trials were conducted to determine the response of crabs when only one habitat was available (e.g., one side of the aquaria was empty) and included the following trials: 1) *S. fluitans* vs. control; 2) *S. natans* vs. control; 3) artificial *Sargassum* vs. control; 4) *T. testudinum* vs. control. Preliminary trials were conducted in which all zones were empty to show that there were no zone preferences within aquaria ( $n = 20$ ).

## 2.5 Statistical analysis

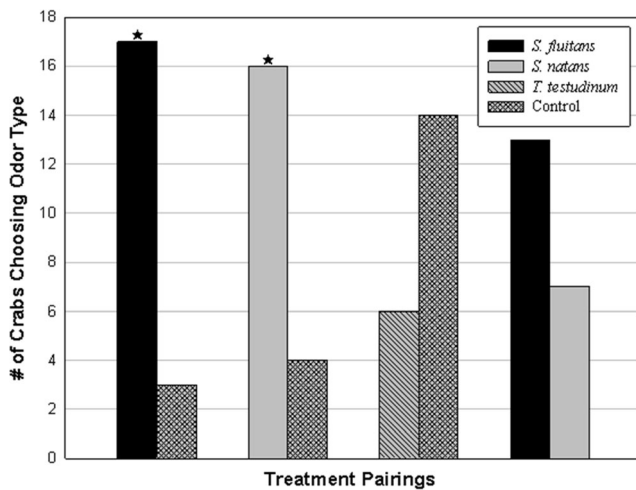
Each crab was used only once for one trial in one treatment, and a total of 20 replicates were conducted for each treatment for all experiments. Results from all experiments were analyzed using the binomial ( $Z$ ) test of significance by comparing the number of crabs in each compartment/zone to the expected probability of 50% (Brooks and Rittschof 1995; Reeves and Brooks 2001; Jobe and Brooks 2009). Fisher's Exact test was used to determine any differences in selections between males and females. To determine effects of size and weight on selections made by *P. sayi*, a logistic regression was used.

## 3 Results

### 3.1 Chemoreception experiments

*P. sayi* showed a significant ability to respond to odors in two of the three chemoreception trials (Fig. 4). *P. sayi* chose *S. fluitans* odors more than the control odors (17 out of 20 or 85%;  $Z = 3.131$ ;  $P = 0.002$ ) and *S. natans* odors more than the control (16 out of 20 or 80%;  $Z = 2.683$ ;  $P = 0.007$ ). The difference between *T. testudinum* odors and the control selection was not significant; however, numerically the control was chosen more by *P. sayi* than the *T. testudinum* odor (14 out of 20 or 70%;  $Z = 1.789$ ;  $P = 0.07$ ). *P. sayi* had no significant preference for *S. fluitans* vs. *S. natans* odors (13 vs. 7;  $Z = 1.342$ ;  $P = 0.18$ ). The results from the control trials also showed no significant difference between the right and left choice chambers (11 vs. 9;  $Z = 0.447$ ;  $P = 0.65$ ). There was no significant difference in selection between male and female crabs for all chemoreception trials (Fisher's Exact test,  $P > 0.05$ ). The size and weight of *P. sayi* ranged from 7.0–36.7 mm and 0.087–4.711 g, respectively, and had no significant effect on any chemical odor selections (Logistic regression,  $P > 0.05$  for both).





**Fig. 4** Chemoreception by *Portunus sayi* for all four treatments (black = *Sargassum fluitans*, grey = *Sargassum natans*, gridded = control, backward hatched = *Thalassia testudinum*). A sample size of 20 was used for each treatment. A significant odor selection was shown (★) for two of the four chemical odor treatments ( $P=0.002$ ,  $P=0.007$ ,  $P=.07$ ,  $P=0.18$ , respectively)

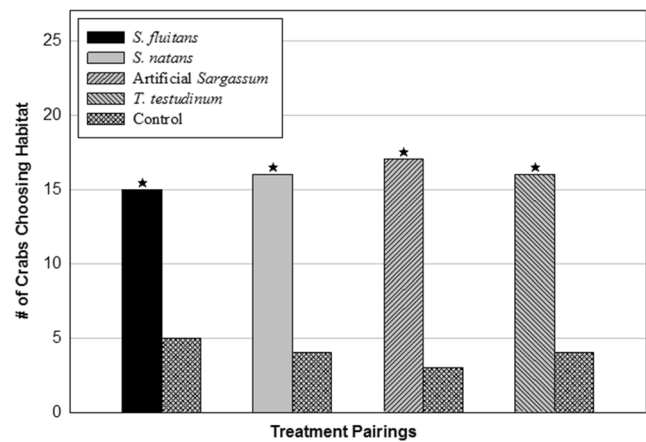
### 3.2 Visual reception experiments

*P. sayi* did not significantly choose between different habitats for any of the five visual reception treatments ( $P>0.05$ ). Although the resulting trials were not significant, *P. sayi* initially chose *S. natans* more than *T. testudinum* (15 out of 20 or 75%) and *S. fluitans* was initially chosen more than *T. testudinum* (14 out of 20 or 70%) when the trials first began. There was no significant difference between male and female choices for visual selection trials (Fisher's Exact test,  $P>0.05$ ). The size and weights of *P. sayi* ranged from 6.0–39.0 mm and 0.085–4.711 g, respectively, and had no significant effect on choices for visual selection trials (Logistic regression,  $P>0.05$ ).

Control trials showed that *P. sayi* significantly selected containers with habitats over empty containers for all four treatments (Fig. 5). Specifically, *P. sayi* chose *S. fluitans* more than the control (15 out of 20 or 75%;  $Z=2.236$ ;  $P=0.025$ ), *S. natans* more than the control (16 out of 20 or 80%;  $Z=2.683$ ;  $P=0.007$ ), *T. testudinum* more than the control (16 out of 20 or 80%;  $Z=2.683$ ;  $P=0.007$ ), and artificial *Sargassum* more than the control (17 out of 20 or 85%;  $Z=3.131$ ;  $P=0.002$ ). There were no significant differences in choices between male and female crabs or between size and weight for the visual location trials (Fisher's Exact test,  $P>0.05$ ; Logistic regression,  $P>0.05$  for both size and weight).

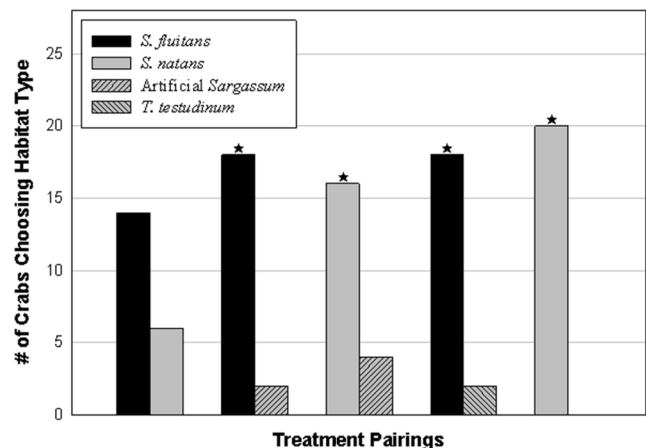
### 3.3 Host selection experiments

*P. sayi* had significant host selections in 4 out of 5 habitat pairings (Fig. 6). *P. sayi* chose *S. fluitans* more than artificial *Sargassum* (18 out of 20 or 90%;  $Z=3.578$ ;  $P<0.001$ ) and *S. natans* more than artificial *Sargassum* (16 out of 20 or 80%;



**Fig. 5** Visual reception control trials selected by *Portunus sayi* for all four pairings (black = *Sargassum fluitans*, grey = *Sargassum natans*, backward hatched = *Thalassia testudinum*, forward hatched = artificial *Sargassum*). A sample size of 20 was used for each treatment. A significant visual selection was shown (★) for all four visual reception controls ( $P=0.025$ ,  $P=0.007$ ,  $P=0.007$ ,  $P=0.002$ , respectively)

$Z=2.683$ ;  $P=0.007$ ). Differences in responses between both species of *Sargassum* and *T. testudinum* were also significant. Specifically, *P. sayi* chose *S. fluitans* more than *T. testudinum* (18 out of 20 or 90%;  $Z=3.578$ ;  $P<0.001$ ) and *S. natans* more than *T. testudinum* (20 out of 20 or 100%;  $Z=4.472$ ;  $P<0.001$ ). There was no significant selection differences between *S. fluitans* and *S. natans* by *P. sayi*, although *S. fluitans* was chosen more than *S. natans* (14 out of 20 or 70%;  $Z=1.789$ ;  $P=0.07$ ). Comparisons between male and female crabs showed no significant difference in habitat selection (Fisher's Exact test,  $P>0.05$ ). Crab sizes and weights ranged from 6.0–33.0 mm and 0.021–4.027 g, respectively, and had



**Fig. 6** Habitat selection by *Portunus sayi* for all five habitat pairings (black = *Sargassum fluitans*, grey = *Sargassum natans*, forward hatched = artificial *Sargassum*, backward hatched = *Thalassia testudinum*). A sample size of 20 was used for each habitat pairing. A significant habitat selection was shown (★) for four of the five habitat pairings ( $P=0.07$ ,  $P<0.001$ ,  $P=0.007$ ,  $P<0.001$ ,  $P<0.001$ , respectively). For the treatment pairing of *S. natans* and *T. testudinum* (on far right), *P. sayi* chose *S. natans* for each of the 20 trials

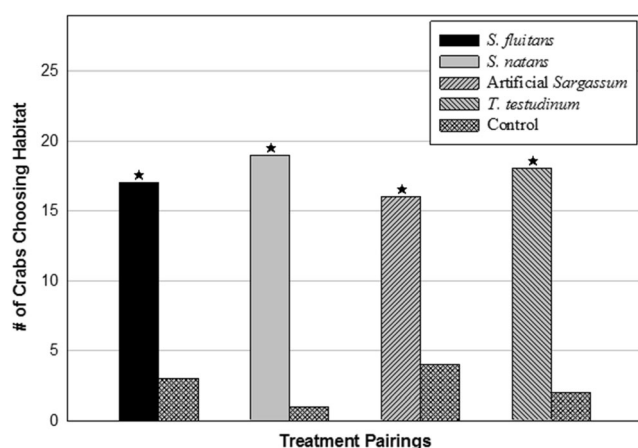
no significant effect on habitat preferences (Logistic regression,  $P > 0.05$  for both).

Control trials showed that *P. sayi* significantly selected zones with habitats over empty zones for all four treatments (Fig. 7). Specifically, *P. sayi* chose *S. fluitans* more than the control (17 out of 20 or 85%;  $Z = 3.131$ ;  $P = 0.002$ ), *S. natans* more than the control (19 out of 20 or 95%;  $Z = 3.859$ ;  $P < 0.001$ ), artificial *Sargassum* more than the control (16 out of 20 or 80%;  $Z = 2.683$ ;  $P = 0.007$ ), and *T. testudinum* more than the control (18 out of 20 or 90%;  $Z = 3.578$ ;  $P < 0.001$ ). There were no significant differences in choices between male and female crabs or between size and weight of crabs for the habitat selection control trials (Fisher's Exact test,  $P > 0.05$ ; Logistic regression,  $P > 0.05$  for both size and weight).

## 4 Discussion

### 4.1 Chemoreception experiments

Results of this study show that the symbiotic *Sargassum* crab, *Portunus sayi*, could use chemical cues alone (i.e., in the absence of visual and direct tactile cues) to distinguish and select host habitats composed of *Sargassum* spp. at short distances of 20–30 cm. These results are consistent with other studies with decapods. For example, Krimsky and Epifanio (2008) reported that megalopae of the Florida stone crab, *Menippe mercenaria* (Say 1818), respond to chemical cues associated with *S. fluitans* and that these cues induce megalopae metamorphosis. This study did not address the specific molecule types involved in this system; however, *Sargassum* spp. release potentially bioactive molecules such as amino acids and fatty acids,



**Fig. 7** Habitat selection control trials selected by *Portunus sayi* for all four habitat pairings (black = *Sargassum fluitans*, grey = *Sargassum natans*, forward hatched = artificial *Sargassum*, backward hatched = *Thalassia testudinum*). A sample size of 20 was used for each habitat. A significant habitat selection was shown (★) for all four habitat controls ( $P = 0.002$ ,  $P < 0.001$ ,  $P = 0.007$ ,  $P < 0.001$ , respectively)

phlorotannins, and photosynthate (Hanson 1977; Arnold and Targett 2000; Wong and Cheung 2001; Turner and Rooker 2006; van Ginneken et al. 2011). Specifically, Hanson (1977) showed *Sargassum* releases up to 55% of its photosynthate and Arnold and Targett (2000) showed *Sargassum* has high metabolic rates in which 100% turnover of phlorotannins occurs in 17 days. Although phlorotannins are chemical defense compounds found in brown algae, there is little evidence that they consistently function as deterrents in herbivores and have been shown to promote metamorphosis in ascidians (Tsukamoto et al. 1994). Additionally, some decapods are known to chemically detect numerous waterborne, organic compounds, including amino acids (Rittschof 1992; Markowska et al. 2008). Clearly, the *Sargassum* crab is detecting molecules exuded from *Sargassum*. Additional studies should investigate the specific nature of these bioactive compounds and at various distances to determine scale of response.

The results also showed that although the crab could chemically detect both species of host alga, there was no significant preference for either species based on chemoreception selection studies. The two species of *Sargassum* used in our study are commonly found intermingled in clumps; thus, there may be little need for *P. sayi* to distinguish between the two species. It is also possible that the chemical composition of *S. fluitans* and *S. natans* are too similar for the *Sargassum* crab to distinguish differences. Turner and Rooker (2006) found that the fatty acid composition of *S. fluitans* and *S. natans* are nearly identical.

In trials involving odors from *T. testudinum*, there was no significant selection by *P. sayi* for this seagrass even though *T. testudinum* contains compounds such as amino acids and proteins (Burkholder et al. 1959) that decapods are capable of detecting. As crabs were not attracted or repelled by odors associated with this seagrass, it is unlikely that *P. sayi* uses cues from *T. testudinum* as a potential indicator of an approaching shoreline. However, this does not preclude the possibility of crabs using *T. testudinum* seagrass beds as a habitat when *Sargassum* is unavailable. Similarly, Krimsky and Epifanio (2008) demonstrated that Florida stone crab megalopae do not respond to chemical cues associated with *T. testudinum*, even though this crab is commonly found in seagrass habitats.

While the identity and concentrations of chemicals influencing *P. sayi* behavior in this study were not identified, the results clearly demonstrate that molecules were detected. Other factors that might influence crab behavior include additional chemical cue sources within the *Sargassum* mats, such as cues from conspecifics and heterospecifics, and other sensory cues such as visual and tactile cues. Interestingly, chemical cues in the absence of visual cues were not used by the *Sargassum* shrimps, *Leander tenuicornis* (Say 1818) and *Latreutes fucorum* (Fabricius 1798), to locate *Sargassum* mats (Jobe and Brooks 2009). Clearly, habitat location by fauna inhabiting these algal mats involves multiple strategies

beyond chemoreception exclusively. Although molecules are available for detection, crabs may be unable to locate a chemical source if the direct flow of odors from currents is unavailable. Future studies on these additional cues and flow patterns would help clarify the role of chemical-based responses by *P. sayi* in establishing and maintaining symbiotic association with *Sargassum* mats.

## 4.2 Visual reception experiments

Results from these trials demonstrate that *P. sayi* can use vision alone (i.e., in the absence of chemical and direct tactile cues) to locate a habitat, but did not visually distinguish between *S. fluitans*, *S. natans*, and artificial *Sargassum* hosts. In contrast, Jobe and Brooks (2009) showed that *Sargassum* shrimps, *L. fucorum* and *L. tenuicornis*, can visually distinguish between *S. fluitans* and *S. natans*, and these selection patterns are due to morphological differences. Specifically, the more slender *L. fucorum* is better concealed within the thinner blades of *S. natans*, while the larger *L. tenuicornis* is better concealed within the thicker blades of *S. fluitans*. *P. sayi* may make distinguishing between these two algal species a lower priority than simply finding a habitat, particularly if *P. sayi* can easily be camouflaged within either species of pelagic *Sargassum*.

In trials involving *Sargassum* paired with *T. testudinum*, there were no resulting significant selection differences. However, *S. natans* and *S. fluitans* were initially chosen more than *T. testudinum* by *P. sayi*, but later in trials crabs moved numerous times between the two choices before eventually selecting one. Crabs presented with visual cues from their normal habitat but in the absence of chemical and tactile cues were likely confused and therefore continued exploring. *Sargassum* has a yellow-brown coloration and more robust structure compared to the flat green blades of *T. testudinum*. Therefore, habitat coloration and structure may have played a role in the initial habitat selections of *P. sayi*. Color discrimination has been shown in several crab species including the blue crab, *Callinectes sapidus* (Rathbun 1896; Bursey 1984; Baldwin and Johnsen 2012), portunid crabs of the genus *Carcinus* (von Buddenbrock and Friedrich 1933; Horridge 1967), and in fiddler crabs of the genus *Uca* (Hyatt 1975; Detto 2007).

## 4.3 Host selection experiments

*P. sayi* was given direct access (i.e., chemical, visual, and tactile cues were all available) to habitat patches in these pairwise choice trials. Specifically, *Sargassum* spp. were chosen significantly more than artificial *Sargassum* and *T. testudinum* hosts. These results differ from the visual reception trials where crabs did not distinguish between live and artificial *Sargassum* and *T. testudinum*. Again, in the current trials chemical, visual, and tactile cues were all available, suggesting that the crabs use multiple sensory cues in host location.

However, based on the results of the visual selection trials where distinguishing specific species was rather obscure, chemical cues are likely involved in precision decision making by the crabs. A similar study involving the use of stimulus cues used by juvenile blue crabs, *C. sapidus*, showed that they orientate toward the direction of the stimulus and that a hierarchy in responses to odors over visual cues exists (Diaz et al. 2003). In this study, tactile cues were not separated out of the host selection trials, and previous studies have shown the importance of tactile cues used by decapods for homing, predator avoidance, and prey location and therefore may play a role in habitat selection by *P. sayi*. (Wehner 1992; Crowl and Covich 1994; Dittel et al. 1996). Habitat architecture (Hacker and Steneck 1990) might also influence host selection by these crabs as *Sargassum* spp. have more fronds that could better conceal them from potential predators compared to the more open spaces between the *T. testudinum* blades. However, this influence may be minor based on the results from the chemical cues experiments in which crabs responded to *Sargassum* odors but not to *T. testudinum*.

The results from the host selection trials also showed there was no preference by *P. sayi* between *Sargassum* spp. when all cues were available. This underscores the point made previously that *P. sayi* may have little need to select one species of pelagic *Sargassum* over another as the two species are commonly found intermingled. Similarly, Jobe and Brooks (2009) found that two species of *Sargassum* shrimp showed no selection preference for either of the aforementioned *Sargassum* spp.

During the control trials for the host selection experiments, crabs significantly selected habitats over the control (e.g. no habitat). These results show the importance of simply finding a habitat. Although crabs did not significantly respond to odors from *T. testudinum* in the chemoreception trials, crabs significantly selected *T. testudinum* habitats when alternative habitats were unavailable. This shows clearly that *P. sayi* may inhabit seagrass beds when *Sargassum* is unavailable.

## 5 Conclusions

Overall, the ability of marine animals in pelagic waters to locate a suitable habitat, and in this case a symbiotic host, represents a significant undertaking and challenge. Presumably, the methods to do so include a variety of sensory cues. This study examined the sensory modalities used by the symbiotic *Sargassum* crab *P. sayi* in locating and selecting host *Sargassum* habitats. First, *P. sayi* has morphological adaptations (e.g., color patterns mimicking *Sargassum* fronds) for living within the *Sargassum* community, so understandably it would have behavioral adaptations for locating its symbiotic host. Based on the results of our study, the following model is proposed for host or habitat location and selection for



adult crabs that have been isolated from their *Sargassum* algal host patches.

Given that isolation from patches can occur at anytime, including night, chemical, waterborne cues that potentially disperse from algae could signal to the crabs (even in low light conditions) the general presence of an algal mat composed at least partially of *Sargassum* spp. While *T. testudinum* may also be present, data from our study show no significant chemical response by the crabs to this aquatic organism. Although our study investigated relatively short distances for detection, chemoreception could potentially serve as a longer distance means of initiating search behavior for specific patches compared to visual cues. Once in the visual proximity of a potential patch, the isolated and still highly vulnerable crab will likely move quickly towards a habitat, continuing to use chemical cues but now supplemented with visual cues. In situ scattered patches accumulate as they approach the shoreline and are frequently less than 1 m apart. They can also be eventually in waters of less than 1 m as their inhabited patch physically is pushed onto the shoreline by wind, waves, current or tides. The tendency for crabs in our study to walk versus swim might replicate crabs stranded in “beached” patches. In any case, crabs will likely choose the locomotory modality most efficient to reach the next available patch just offshore (walking versus swimming involves different physiological and energetic requirements; cf. Hardy et al. 2010), and might continue this directional offshore movement until either the waves, winds, currents or tides shift pushing *Sargassum* patches offshore.

In the absence of a chemical source, crabs could still potentially locate a habitat at short distances (e.g., 2–3 m) using visual cues. Once actual contact has been made with the new habitat patch, all three sensory cue modalities (including direct tactile information) can be used to confirm its likely preference of a patch composed of either species (or both) of *Sargassum*. Although our study was limited to larger, thus presumably adult crabs, it would be interesting to investigate and compare host location and selection by larval stages of this crab, and other fauna associated with pelagic *Sargassum* mats, too.

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