

THE USE OF CHEMICAL CUES BY *SARGASSUM* SHRIMPS *LEANDER*
TENUICORNIS AND *LATREUTES FUCORUM* IN ESTABLISHING AND
MAINTAINING A SYMBIOSIS WITH *SARGASSUM* ALGAE

by

Jaime L. Frahm

A Thesis Submitted to the Faculty of
The Charles E. Schmidt College of Science
In Partial Fulfillment of the Requirements for the Degree of
Master of Science

Florida Atlantic University

Boca Raton, FL

August 2019

Copyright 2019 by Jaime L. Frahm

THE USE OF CHEMICAL CUES BY *SARGASSUM* SHRIMPS *LEANDER*
TENUICORNIS AND *LATREUTES FUCORUM* IN ESTABLISHING AND
MAINTAINING A SYMBIOSIS WITH *SARGASSUM* ALGAE

by

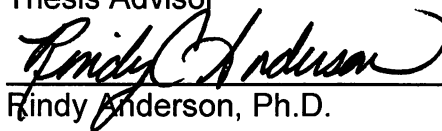
Jaime L. Frahm

This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Randy Brooks, Department of Biological Sciences, and has been approved by all members of the supervisory committee. It was submitted to the faculty of the Charles E. Schmidt College of Science and was accepted in partial fulfillment of the requirements for the degree of Master of Science.

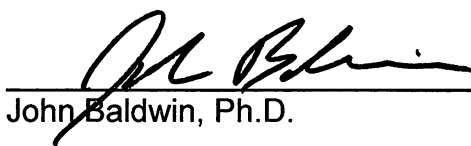
SUPERVISORY COMMITTEE:



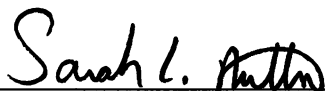
Randy Brooks, Ph.D.
Thesis Advisor



Randy Anderson, Ph.D.



John Baldwin, Ph.D.



Sarah L. Milton, Ph.D.
Interim Chair, Department of Biological
Sciences



Ata Sarajedini, Ph.D.
Dean, Charles E. Schmidt College of
Science



Robert W. Stackman Jr., Ph.D.
Dean, Graduate College

August 1, 2019
Date

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor, Dr. Randy Brooks, for his support and expertise during my time in graduate school at Florida Atlantic University. This research could not have been completed without his knowledge, advice, and time spent in the field. I would also like to thank my thesis committee members, Dr. Rindy Anderson and Dr. John Baldwin. Their suggestions and expertise on the subject matter were incredibly helpful in the process of completing my thesis. I would also like to thank my parents, Gary and Tracy Frahm, for their love and support. A final thank you to my fiancé, Robert Dennis, and my best friend, Nicky Montagna for cheering me on and providing unending support. I could not have done this without you all.

ABSTRACT

Author: Jaime L. Frahm
Title: The Use of Chemical Cues by *Sargassum* Shrimps *Leander tenuicornis* and *Latreutes fucorum* in Establishing and Maintaining a Symbiosis with *Sargassum* algae
Institution: Florida Atlantic University
Thesis Advisor: Dr. Randy Brooks
Degree: Master of Science
Year: 2019

A mutualistic symbiosis exists between the alga *Sargassum* spp. and two shrimp species, *L. tenuicornis* and *L. fucorum*. But little is known about how the shrimp locate their host alga. Both visual and chemical cues are potentially available. Visual cues would be presumably restricted at night but chemical cues are potentially available continuously. Additionally, a previous study has looked at both cue variables with results that are mixed. This current research elaborates on the previous study in an attempt to fully understand *Sargassum* shrimp chemoreception. A y-maze and four-chambered apparatus were used to test if the shrimp were able to detect *Sargassum* cues, conspecific cues, and Dimethylsulfoniopropionate (DMSP) cues. Neither shrimp species showed a strong directional response to any of the chemical cues, but the *Sargassum* and DMSP cues did cause more shrimp to exhibit searching behavior. Additionally, several differences in response between male and female shrimp were found for

each cue. A weaker dilution of DMSP was tested in an attempt to determine sensitivity of *L. fucorum* shrimp to the chemical cue. This weaker dilution also caused *L. fucorum* to exhibit searching behavior, but the sensitivity to the cue was not found and further research is needed to fully answer this question.

These results show the shrimp are able to detect chemical cues in their environment and help determine more accurately the role of chemoreception in initiating and maintaining this shrimp/algal association.

THE USE OF CHEMICAL CUES BY *SARGASSUM* SHRIMPS *LEANDER*
TENUICORNIS AND *LATREUTES FUCORUM* IN ESTABLISHING AND
MAINTAINING A SYMBIOSIS WITH *SARGASSUM* ALGAE

LIST OF FIGURES	viii
INTRODUCTION	1
OBJECTIVES	10
MATERIALS AND METHODS	11
Collection and maintenance of specimens	11
Four-chambered apparatus trials	12
Y-maze trials	14
RESULTS	17
Four-chambered apparatus trials	17
Y-maze trials	18
DISCUSSION	24
Four-chambered apparatus trials	24
Y-maze trials	25
CONCLUSION.....	30
REFERENCES.....	32

LIST OF FIGURES

Figure 1: Four chambered choice apparatus (Image from Jobe & Brooks, 2009).....	12
Figure 2: Y-maze apparatus. Blue and yellow dyes demonstrate symmetrical flow from each branch (A & B).	15
Figure 3: Average number of shrimp that moved out of the central chamber for the control, Sargassum, DMSP and conspecifics trials (one-way ANOVA, $p=0.14$).	17
Figure 4: Average number of shrimp that chose the chemical cue chamber in Sargassum, DMSP, and conspecific trials (chi-square, Sargassum $p=0.99$, DMSP $p=0.92$, Conspecifics $p=0.68$).	18
Figure 5: Percent of <i>L. fucorum</i> that moved in the y-maze for the control, Sargassum, DMSP 10-5 M, DMSP 10-9 M, and conspecific cue trials (binomial test, Sargassum $p=0.03$, DMSP 10-5 M $p=0.05$, DMSP 10-9 M $p=0.01$, conspecifics $p=0.08$).	19
Figure 6: Percent of <i>L. tenuicornis</i> that moved in the y-maze for the control, Sargassum, DMSP 10-5 M, and conspecific cue trials (binomial test, Sargassum $p=0.03$, DMSP $p=0.08$, conspecifics $p=0.01$).	20
Figure 7: Percent of <i>L. fucorum</i> males that moved in the y-maze for the control, Sargassum, DMSP 10-5 M, DMSP 10-9 M, and conspecific cue	

<p>trials (binomial test, Sargassum $p=0.01$, DMSP 10-5 M $p=0.10$, DMSP 10-9 M $p=0.08$, conspecifics $p=0.08$).</p>	21
<p>Figure 8: Percent of <i>L. fucorum</i> females that moved in the y-maze for the control, Sargassum, DMSP 10-5 M, DMSP 10-9 M, and conspecific cue trials (binomial test, Sargassum $p=0.11$, DMSP10-5 $p=0.01$, DMSP 10-9 $p=0.02$, conspecifics $p=0.11$).</p>	21
<p>Figure 9: Percent of <i>L. tenuicornis</i> females that moved in the y-maze for the control, Sargassum, DMSP, and conspecific cue trials (binomial test, Sargassum $p=0.02$, DMSP $p=0.05$, conspecifics $p=0.01$).</p>	22
<p>Figure 10: Initial choice between the region with the chemical cue vs the region with plain seawater in conspecific chemical cue trials based on sex (binomial test, males $p=0.05$, females $p=0.18$).</p>	23

INTRODUCTION

Pelagic, floating mats of primarily *Sargassum fluitans* and *Sargassum natans* algae are found in both tropical and temperate waters of the Atlantic Ocean (Coston-Clements et al., 1991; Sehein et al., 2014). These species are characterized with highly branched thalli and small air bladders (pneumatocysts) that keep the algae afloat (Coston-Clements et al., 1991). *Sargassum natans* differs from *Sargassum fluitans* with thinner blades and a spine located on the pneumatocysts (Jobe & Brooks, 2009).

These floating mats range greatly in size from small clumps less than 0.5m in horizontal diameter up to huge mats 50m in diameter (Coston-Clements et al., 1991; Marmorino et al., 2011). Several factors contribute to this broad range in sizes. For example, sustained calm conditions can allow *Sargassum* mats to form large aggregations, but harsher weather conditions such as high winds can break up large mats of *Sargassum* or create narrower windrows (Marmorino et al., 2011). As the algae approach the shoreline, wave action can break up mats into significantly smaller sizes. Boats and other watercrafts running through the mats could also cause further disruption of *Sargassum* mats structure. Wind can also push *Sargassum* on to shores in the Atlantic and Caribbean, sometimes in huge mass events (Hu et al., 2016). However, *Sargassum* can be found in the Sargasso Sea in high quantities (Coston-Clements et al., 1991; Niermann, 1986).

The Sargasso Sea is located in the western Atlantic Ocean approximately parallel in latitude to the coast of Florida and is defined by ocean currents instead of land boundaries (Coston-Clements et al., 1991; Sehein et al., 2014). This area is incredibly important because of the large amounts of *Sargassum*, and conservation groups are asking for its protection due to threats from anthropogenic impacts (e.g., pollution, ocean acidification, overfishing, and heavy shipping traffic) (Sehein et al., 2014). In the open ocean, *Sargassum* provides a natural, pelagic substrate for a highly diverse assemblage of organisms that use the mats for shelter, food, and as a nursery (Coston-Clements et al., 1991; Ballard & Rakocinski, 2012). Some of these organisms, such as the Kemp's Ridley sea turtle, are critically endangered (Coston-Clements et al., 1991). In fact, four species of sea turtles use *Sargassum* mats as a habitat during the "lost years" (the years between hatchling and adulthood that a sea turtle spends as a pelagic juvenile) (Coston-Clements et al., 1991; Witherington et al., 2012).

Many fish species also rely on the *Sargassum* habitat. For example, flying fishes (Exocoetidae) use the algae as a nursery for juveniles (Coston-Clements et al., 1991). Predatory fishes such as jacks (Carangidae), tunas (Scombridae), dolphinfish (Coryphaenidae) and swordfish (Xiphiidae) feed on organisms living in the algae (Coston-Clements et al, 1991). Additional organisms that depend on *Sargassum* mats include crabs, molluscs, polychaetes, nudibranchs, and shrimp (Coston-Clements et al, 1991). In all, there are over 100 species of invertebrates that make up the *Sargassum* community (Coston-Clements et al, 1991).

Some associated species, such as *Sargassum* shrimp, form a symbiosis with these algae and are rarely found elsewhere (Coston-Clements et al, 1991). With so many species reliant either part of or their entire lives on the *Sargassum* habitat, it has been designated as an Essential Fish Habitat (Ballard & Rakocinski, 2012). While many studies have focused on which species are found with the *Sargassum* habitat, very little is known about details of interactions among the symbionts and host (Jobe & Brooks, 2009). *Sargassum* shrimp are the most abundant macro invertebrates in *Sargassum* mats, but again, very little is known about their ecological and behavioral adaptations to facilitate this association, including how this symbiosis is initiated and maintained (Sehein et al., 2014).

There are two species of *Sargassum* shrimp, *Leander tenuicornis* and *Latreutes fucorum* (Coston-Clements et al, 1991). Both species of shrimp exhibit camouflage and coloration closely resembling the brown and yellow colors of *Sargassum* algae making it challenging for the observer to locate them within the habitat (Coston-Clements et al., 1991). These shrimp occupy positions in the algal mats based on the frond characteristics and depth of the fronds in the water column. For example, *L. tenuicornis* showed a preference for deeper floating *Sargassum* patches (10-12 cm below the surface) than shallow patches. Additionally, *L. tenuicornis* positioned itself in a parallel alignment with the fronds, thus allowing the shrimp to blend with the fronds and exhibit algal morphology mimicry (Bennice & Brooks, 2016). Such behaviors by *L. tenuicornis* and its innate camouflage maximize protection from predators such as jacks, sargassum

fish (*Histrio histrio*) and the gray triggerfish (*Balistes capriscus*), the last of which is one of the most abundant fish in *Sargassum* and feeds almost exclusively on *Sargassum* shrimp (Coston-Clements et al., 1991; Ballard & Rakocinski, 2012). Clearly, *Sargassum* shrimps are vital components of the *Sargassum* community and food chain. Therefore, this study focused on these two species of shrimp.

A mutualistic symbiosis is formed when two organisms live together and provide reciprocal benefits. In the symbiosis between *Sargassum* and these shrimp, the alga provides shelter and protection to the shrimp (Brooks et al., 2007). In return, shrimp and other animals provide nutrients to the algae (Lapointe et al., 2014). Mutual benefits to symbionts can help explain why an association occurs, but another critical aspect is understanding how the symbiosis is initiated and maintained. Typically, the smaller, more mobile symbiont is the one to seek out and form the symbiosis with the larger symbiont or host (Jobe & Brooks, 2009).

This leads to the question about how shrimp symbionts locate the *Sargassum* patches, which are temporally and spatially highly variable in abundance (Stoner, 1983; Lapointe, 1995; Wells and Rooker, 2004). Presumably, the shrimp must initially locate such patches of *Sargassum* algae. DNA analysis of *L. fucorum* showed the shrimp are highly dispersive during the planktonic larval stage (Sehein et al., 2014). Additionally, juveniles and those individuals separated from mats by biotic perturbations (e.g., disruptive feeding actions by large, pelagic species like dolphinfish, *Coryphaena hippurus*

(Dooley, 1972; Coston-Clements, 1991; Wells and Rooker, 2004; Rudershausen et al., 2010)), and abiotic perturbations (e.g., wind and waves that break up patches, or when mats are driven onto beaches, cf. Hu et al., 2016) must locate and re-establish association with new, displaced or larger algal mats.

Visual cues are mostly available during the daytime and would be potentially available at limited distances only. However, chemical cues would likely be available most of the time, and distance would be potentially less of a problem than using visual cues exclusively. My proposed research will focus on chemoreception; therefore, a brief review follows.

There is an abundance of chemical cues in the marine habitat, as every organism releases some type of chemical signal into the environment via metabolic activity (Atema, 1995). Once a chemical is released into the water, the molecules disperse by undergoing diffusion or bulk flow, which is the movement of molecules from high to low pressure (Atema, 1995). There are many marine organisms that can detect chemicals via chemoreceptors (Carr et al., 1987). Receptors must be both highly specific and diverse for organisms to identify an array of specific chemicals within the environment (Atema, 1995). These chemicals are used to locate food, avoid predators, find a mate, find a suitable habitat, homing, recognize conspecifics, and mediate social behavior (such as forming a hierarchy) (Carr et al., 1987). Specifically, chemoreceptive organs on decapods contain sensilla, which are either hair like or rod shaped (Carr et al., 1987). The sensilla are usually located on the antennules, but can also be found on parts of the mouth and legs (Carr et al., 1987).

The American lobster (*Homarus americanus*) can detect specific chemical cues to recognize individual lobsters and form dominance hierarchies (Atema, 1995). Another example of a chemoreceptive crustacean is the porcellanid crab (*Porcellana sayana*), which uses chemical cues to locate sea anemones associated with hermit crabs (Brooks & Rittschof, 1995). Yet another crustacean, the rock shrimp (*Rhynchocinetes typus*) uses chemoreception to find and select mates (Díaz & Thiel, 2004).

In some cases, an organism responds to certain chemical cues as a larva, but may lose the capability to detect the same chemical cues as an adult. Fiddler crab (*Uca* spp.) larvae can detect cues from food it typically consumes, but adults are insensitive to those same chemical cues (Weissburg & Zimmer-Faust, 1991). Those results suggest that the life history stage of an organism may influence which chemical cues they respond to based on requirements of the organism at different stages in their life history.

Dimethylsulfoniopropionate (DMSP) is a chemical cue excreted by some marine algae (e.g., dinoflagellates; Dacey & Wakeham, 1986, Hill & Dacey, 2006; benthic *Sargassum* species (Broadbent et al., 2002), that is detected by a variety of marine animals (DeBose et al., 2008, Nevitt, 1995, Kowalewsky et al., 2006). It can potentially attract zooplankton predators to minimize grazing on the phytoplankton (Hay, 1996). Potentially high DMSP concentrations are also present in areas where primary productivity and foraging activity are high, which include windrows where *Sargassum* and other potential DMSP-producing organisms aggregate to form weedlines (DeBose et al., 2010). DeBose et al.,

(2010) showed that some fish (e.g., jacks, *Caranx hippos* and *C. melampygus*) associated with *Sargassum* mats responded significantly to cues of DMSP. DMSP concentrations of 10^{-9} M in the Sargasso Sea were found, which indicate that this compound could be used as a cue for symbionts to locate *Sargassum* patches (Vila-Costa et al., 2014).

This current study is based on a study on the importance of chemical and visual cues for *Sargassum* shrimp in locating and selecting habitat (Jobe & Brooks, 2009). The questions addressed by this study were whether chemical and visual cue responses to *Sargassum* algae occurred by *L. tenuicornis* or *L. fucorum*, and if these shrimp have preferences for a different *Sargassum* species. To answer these questions they used two types of apparatus: 1) 4-chambered olfactometer (Figure 1) to test for chemical cues only, and 2) aquarium with, first, chemical cues blocked and visual cues available (by placing the algae in beakers thereby allowing only visual cues), then chemical cues and visual cues by placing algae directly in the water with the shrimp

In the first set of trials, the 4-chambered apparatus had 4 water sources that flowed into the radial chambers, then to the central chamber where the currents meet before reaching the outflow tube. The control trials had plain seawater for all 4 radial water sources, and a chemical cue was added to one of the radial water sources in treatment trials. Groups of shrimp were placed in the central chamber and left for a duration of 4 h before the location of the shrimp were recorded. Both controls and treatment results showed that shrimp did not

respond differently from random (i.e., 25% probability of going into each chamber).

In the second set of trials, an aquarium divided into three sections was used to test the response of *S. fluitans*, *S. natans* to visual only cues and then visual plus chemical cues. For visual only cues, the algae were put on each side of the aquarium in a beaker to keep chemical cues from the algae out of the water in the aquarium. A single shrimp for each trial was placed in the center and observed to see which choice it would make. For the visual plus chemical cue trials, the same procedure was used as visual only, but the different *Sargassum* types were placed directly in the water of the aquarium to allow chemical cues to also be available to the shrimp.

Although there were no significant differences detected in the “chemical cues only” trials, there was significance in the “visual only” and the “visual plus chemical” cue trials. Specifically, “visual cues only” trials showed several specific significant results: 1) *L. fucorum* chose live *Sargassum* over artificial *Sargassum* and *S. natans* over *S. fluitans*; 2) Small-sized *L. tenuicornis* chose *S. natans* over artificial *Sargassum*, and large-sized *L. tenuicornis* chose *S. fluitans* over *S. natans* and artificial *Sargassum*. However, when both visual and chemical cues were present there were additional significant similar and differing results: 1) neither shrimp species had a significant preference for a specific *Sargassum* species, and 2) small *L. tenuicornis* selected *S. fluitans* over artificial algae. Although chemical cues from *Sargassum* were unavailable in the “visual cues only” trials, they were still present in the latter set of trials (i.e., both visual

and chemical cues were available to the shrimp). While these results do not demonstrate that *Sargassum* shrimp responded to chemical cues in the 4-chambered apparatus trials, it is still possible that chemoreception was employed by the shrimp.

OBJECTIVES

This current study attempted to answer the following questions about chemoreception by these shrimp using either the y-maze and/or 4-chambered apparatus. To reduce verbiage rather than repeat the following objectives for each shrimp species the word “shrimp” refers to either *L. tenuicornis* or *L. fucorum*.

- 1. Do shrimp respond to *Sargassum* chemical cues?**
- 2. Do shrimp respond to DMSP chemical cues?**
- 3. Does the response to *Sargassum* chemical cues differ based on shrimp size (and possibly life history stage)?**
- 4. Does the response to DMSP chemical cues differ based on shrimp size (and possibly life history stage)?**
- 5. Does the response to *Sargassum* chemical cues differ based on shrimp sex?**
- 6. Does the response to DMSP chemical cues differ based on shrimp sex?**
- 7. Does the response to *Sargassum* chemical cues differ based on cue strength?**
- 8. Does the response to DMSP chemical cues differ based on cue strength?**
- 9. Do shrimp respond to conspecific chemical cues?**
- 10. Does the response to conspecific chemical cues differ based on shrimp sex?**

MATERIALS AND METHODS

Collection and maintenance of specimens

The organisms used in this study were collected via boat off the east coast of Florida near the Boca Inlet. Using a large dip net, clumps of *Sargassum* algae were collected and put into plastic coolers. The shrimps, *Latreutes fucorum* and *Leander tenuicornis*, were separated from the algae immediately on the boat. Extra algae and any other organisms collected unintentionally were returned to the water. The algae and shrimp were then transported back to Florida Atlantic University in plastic coolers. A total of 2,800 shrimp were collected for use in trials.

Organisms were placed in holding tanks with filters in the laboratory with seawater made from sea salt mix, and the salinity maintained at 32-35 ppt. Once the temperature of the water in the coolers matched the temperature of the water in the holding tanks, the shrimp were separated by size groups (e.g., shrimp less than 5mm were separated from larger shrimp to minimize potential aggression and predation). Shrimp were allowed to acclimate to their laboratory environment a minimum of 6h before being used in any experimental trials. All shrimp were tested within 3 days of collection.

Four-chambered apparatus trials

A four-chambered apparatus (Figure 1, below) was set up with equal amounts 0

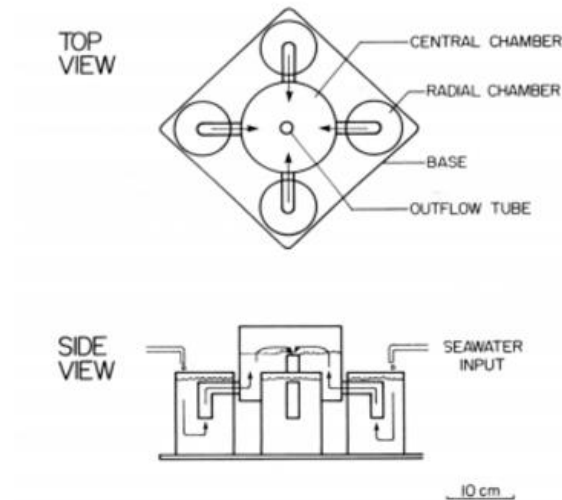


Figure 1: Four chambered choice apparatus (Image from Jobe & Brooks, 2009).

of synthetic seawater running through each radial chamber. Thus, water from the 4 radial chambers entered the central chamber simultaneously before draining out of the apparatus. Animals placed in the central chamber can enter any of the 4 radial chambers. Most light was blocked by placing a blind around the apparatus to minimize light cues and maximize the potential use of chemical cues.

For the control trials, all four radial chambers had plain seawater running through them. This allowed for examination of the shrimps' behavior in the absence of an added chemical cue. Groups of 30 shrimp were placed into the central chamber of the apparatus. Two size groups of *L. fucorum* were tested:

Group 1 ≥ 10 mm, and Group 2 < 10 mm. *L. tenuicornis* was not tested using the four-chambered apparatus due to low collection numbers. After 4 h, the location of the shrimp within the apparatus was recorded. No shrimp individuals were used more than once in any trials in this and all other experiments. Trials were replicated with new groups of 30 shrimp a minimum of 10 times. The distribution of the animals within the apparatus after 4 h was analyzed using binomial tests.

Three types of chemical cue sources were used in the choice experiments, *Sargassum*, Dimethylsulfoniopropionate (DMSP), and conspecifics. For the *Sargassum* chemical cue treatment, 2 kg of *Sargassum* was collected and placed in 37 L of synthetic seawater for 48 h to allow any chemical compounds to be released. *Sargassum* composition can vary greatly throughout the year due to many factors such as age, amount of epibiotic growth, species present, and seasonal conditions. To minimize chemical composition variability in *Sargassum* effluent between trials, 45 ml aliquots were taken from one batch and then frozen for subsequent use throughout experimentation (after Cox, 2016). Three additional batches of *Sargassum* chemical effluent were made using *Sargassum* collected at different times of the year. This was done to minimize potential variation between chemical cues from *Sargassum* batches collected throughout three years of research. For use in trials, a 100% concentration of *Sargassum* effluent solution was used. For the DMSP chemical

treatment, synthetic seawater mixture containing DMSP at a concentration of 10^{-5} was used (10^{-9} M is a concentration commonly occurring *in situ*; Vila-Costa et al. 2014 and DeBose et al. 2010). These *Sargassum* and DMSP solutions were the strongest concentrations used in trials, and were tested first to see if the shrimp responded to them.

For the *Sargassum* chemical cue trials, *Sargassum* effluent was added to one randomly selected radial chamber water source. Again, groups of *L. fucorum* were placed in the center and allowed to move between the radial chambers for a 4 h duration before the location of the shrimp was recorded. The same procedure occurred for the DMSP chemical cue trials. For conspecific chemical cues, 10 shrimp were placed alive, directly into a water source for the 4-chambered apparatus. Chi-square goodness of fit test was used to compare the number of shrimp that moved based on the two size groups, and a one-way ANOVA was used to see if the number of shrimp that moved in the control data and the three treatments were significantly different.

Y-maze trials

A Y-maze apparatus (Figure 2, below) was set up with equal amounts of synthetic seawater running through each branch (areas A & B) and draining out of the apparatus (area C).

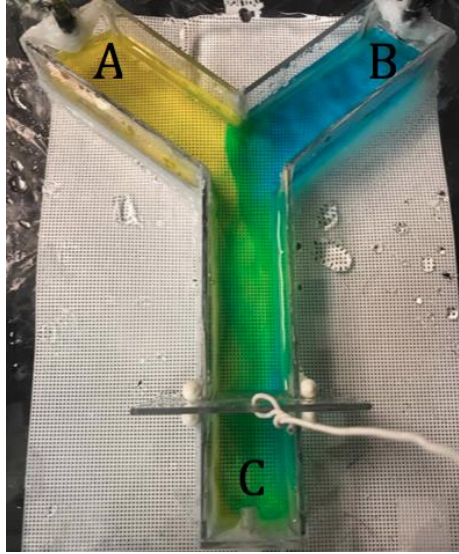


Figure 2: Y-maze apparatus. Blue and yellow dyes demonstrate symmetrical flow from each branch (A & B).

Shrimp were tested by placing an individual in area C initially. A perforated plexiglass gate was in place to keep the shrimp in the initial area C for a 10 minute acclimation period while the flow of water from both branches moved through the system. After 10 minutes, the perforated gate was raised using a pulley system, and the shrimp were free to explore the remaining areas of the Y-maze for an additional 20 min. A camera was used to monitor initial choice and subsequent movements of the shrimp. Preliminary observations showed that smaller shrimp were difficult to see in the video recordings, so only shrimp that are over 10mm in length were used in these trials. The shrimp were measured from rostrum to tail to determine total length after use in a trial to minimize observer physical contact and stress on the shrimp. The sex of each shrimp was determined after use in trials by observing the presence (male) or absence (female) of the appendix masculina on the second pleopods (Penha-

Lopes et al., 2007). Again, light levels were minimized by placing a blind around the apparatus.

For control trials, the two branches received synthetic seawater with no chemicals added. For treatment trials, the three chemical cues were tested in separate trials by randomly assigning the branches (either A or B) with the chemical cue using the same preparation and solution concentrations described for the 4-chambered apparatus trials. After the data was statistically analyzed for these concentrations, it was deemed appropriate to use a weaker DMSP solution in the *L. fucorum* trials in order to test the sensitivity of the shrimps' chemoreception to the cue. A concentration of 10^{-9} M was used, which is a concentration commonly occurring *in situ* (Vila-Costa et al. 2014 and DeBose et al. 2010). Approximately 100 replications for *L. fucorum* and 50 replications for *L. tenuicornis* were tested for the controls and three treatments.

A binomial test was used to test if significantly more shrimp move out of area C in the presence of a cue than without. Of the shrimp that moved out of area C, a binomial test was used to see if significantly more shrimp initially chose the cue side. Additionally, of the shrimp that moved out of area C, a repeated measures ANOVA was used to see if there were any differences in time spent in the arm with the cue than the arm without the cue between the control and treatment trials.

RESULTS

Four-chambered apparatus trials

Controls showed no significant difference in shrimp choice from the null hypothesis of 25% for each of the chambers (binomial test, all $p > 0.05$). This shows the system is unbiased.

A one-way ANOVA showed that the average number of shrimp that moved from the center of the apparatus did not differ significantly between the control and three treatments ($p=0.14$) (Fig. 3). When testing to see if the shrimp that moved chose the chamber with the chemical cue, a chi-square goodness of fit test showed no significant movement towards the cues (all $p > 0.05$) (Fig. 4).

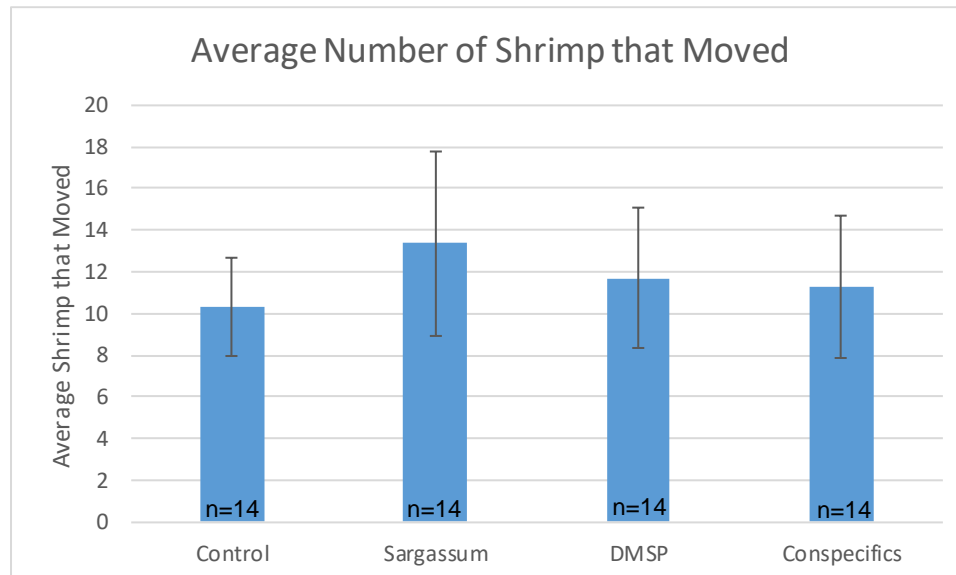


Figure 3: Average number of shrimp that moved out of the central chamber for the control, Sargassum, DMSP and conspecifics trials (one-way ANOVA, $p=0.14$).

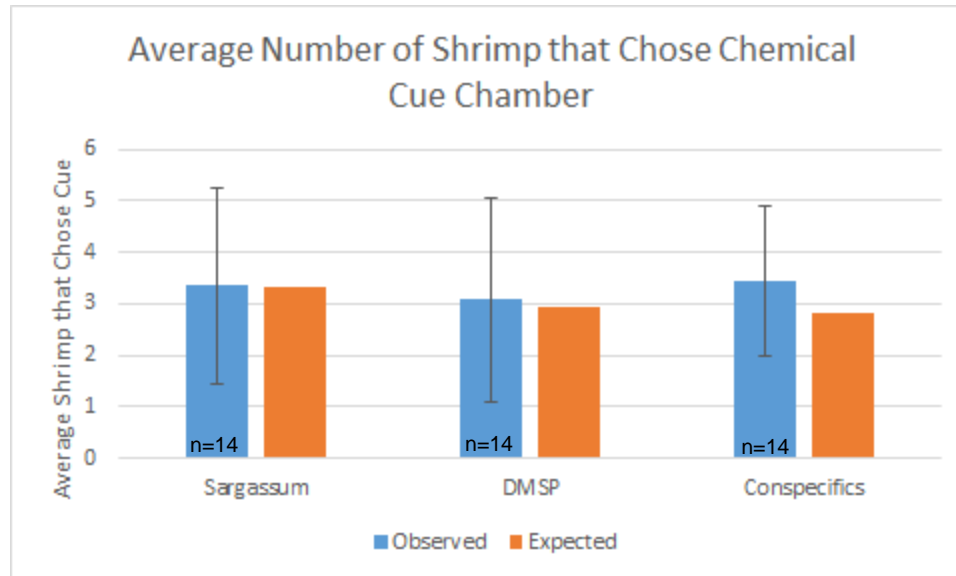


Figure 4: Average number of shrimp that chose the chemical cue chamber in Sargassum, DMSP, and conspecific trials (chi-square, Sargassum $p=0.99$, DMSP $p=0.92$, Conspecifics $p=0.68$).

Chi-square tests showed no significant difference in responses to chemical cues between the two size groups of shrimp (all $p > 0.05$).

Y-maze trials

A T-test on the control data showed no significant difference in the amount of time spent in each arm for both species of shrimp, showing the system is unbiased (*L. fucorum* $p=0.10$, *L. tenuicornis* $p=0.25$). Additionally, a chi-square analysis of the control data showed no significant difference between sex and the number of shrimp that moved out of area C (*L. fucorum* $p=0.24$, *L. tenuicornis* $p=0.53$).

Binomial tests showed that significantly fewer shrimp for both species moved in the presence of a *Sargassum* chemical cue (*L. fucorum* $p=0.03$, *L. tenuicornis* $p=0.03$) (Figs. 5 & 6). Also, significantly fewer *L. tenuicornis* moved

in the presence of conspecific chemical cues ($p=0.01$) (Fig. 6). Alternatively, significantly more *L. fucorum* moved in the presence of both DMSP chemical cue concentrations when compared with the control (DMSP 10^{-5} M $p=0.05$, DMSP 10^{-9} M $p=0.01$) (Fig. 5).

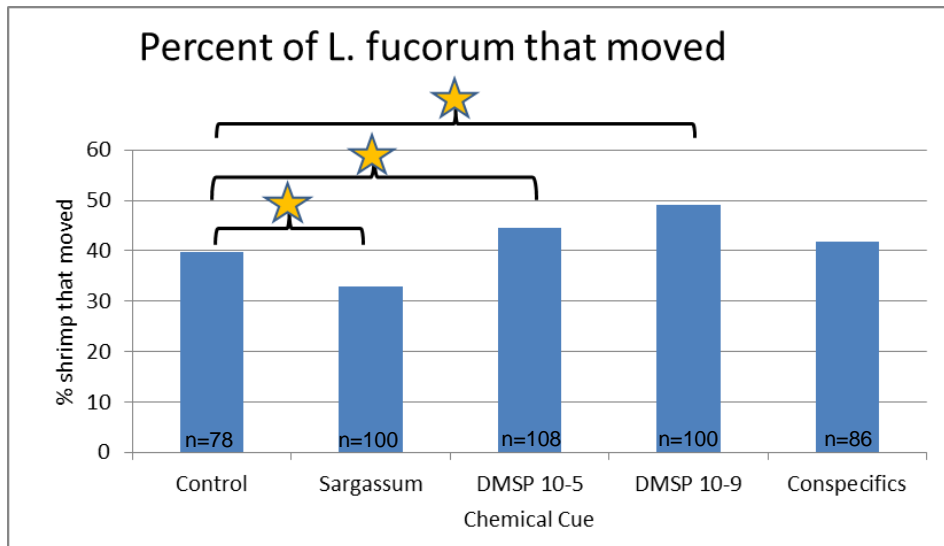


Figure 5: Percent of *L. fucorum* that moved in the y-maze for the control, Sargassum, DMSP 10^{-5} M, DMSP 10^{-9} M, and conspecific cue trials (binomial test, Sargassum $p=0.03$, DMSP 10^{-5} M $p=0.05$, DMSP 10^{-9} M $p=0.01$, conspecifics $p=0.08$).

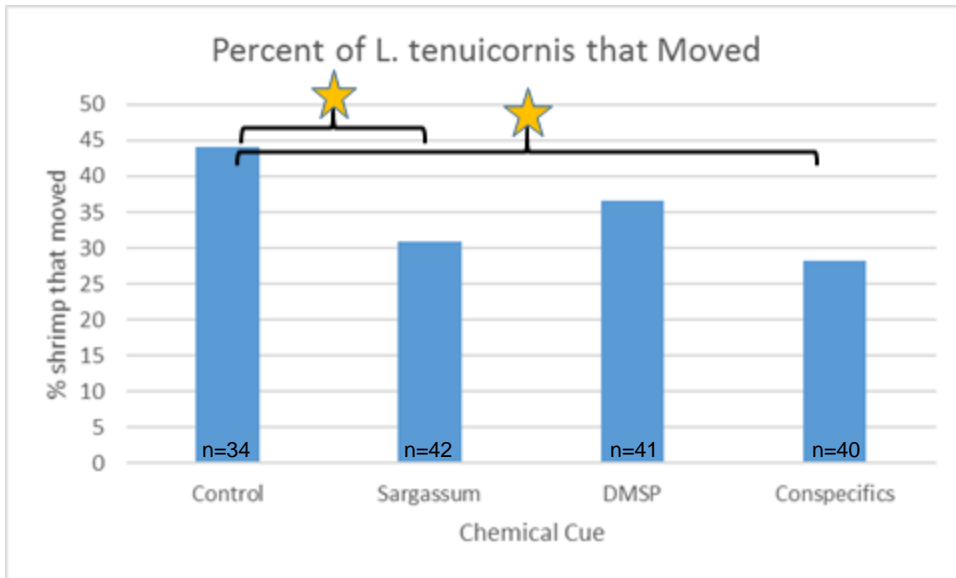


Figure 6: Percent of *L. tenuicornis* that moved in the y-maze for the control, Sargassum, DMSP 10⁻⁵ M, and conspecific cue trials (binomial test, Sargassum p=0.03, DMSP p=0.08, conspecifics p=0.01).

The same binomial tests were also done to see if parsing the data by sex had any effect on significant findings. *L. fucorum* males moved less in Sargassum cue trials when compared to the control males (p=0.01) (Fig. 7), and the females moved more in both DMSP concentrations compared to the control females (DMSP 10⁻⁵ M p=0.01, DMSP 10⁻⁹ M p=0.02) (Fig. 8). *L. tenuicornis* females moved significantly less in both the Sargassum and the conspecifics cues (Sargassum p=0.02, conspecifics p=0.01) (Fig. 9). No significance was found for *L. tenuicornis* males (all p>0.05).

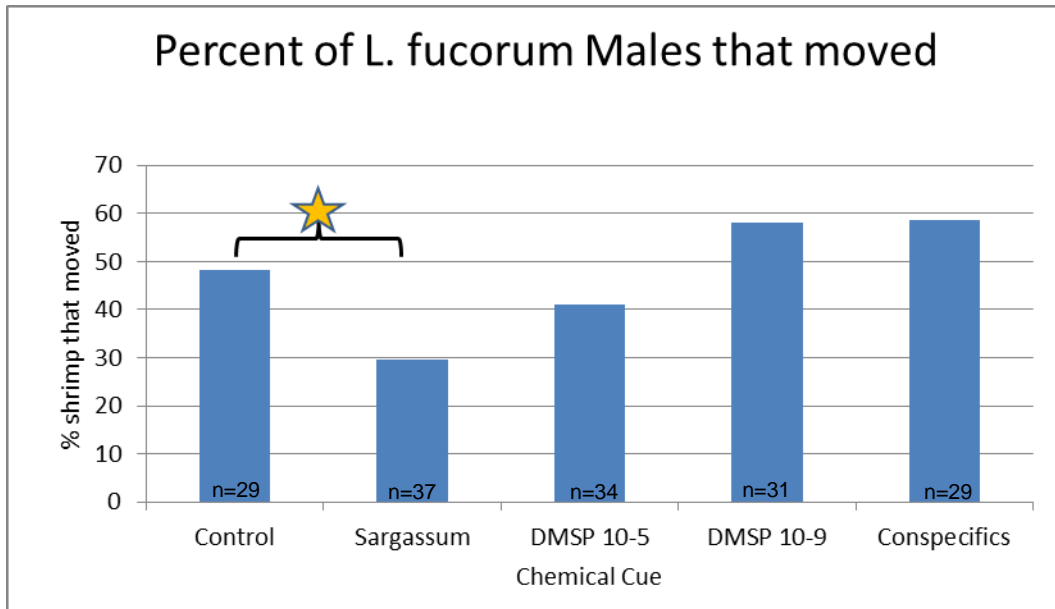


Figure 7: Percent of *L. fucorum* males that moved in the y-maze for the control, Sargassum, DMSP 10-5 M, DMSP 10-9 M, and conspecific cue trials (binomial test, Sargassum $p=0.01$, DMSP 10-5 M $p=0.10$, DMSP 10-9 M $p=0.08$, conspecifics $p=0.08$).

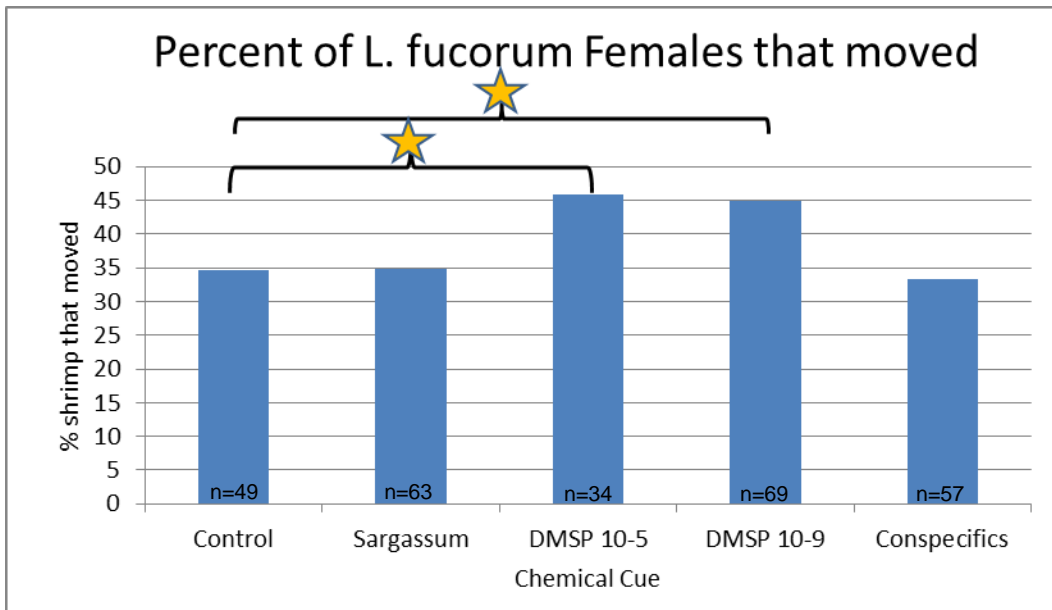


Figure 8: Percent of *L. fucorum* females that moved in the y-maze for the control, Sargassum, DMSP 10-5 M, DMSP 10-9 M, and conspecific cue trials (binomial test, Sargassum $p=0.11$, DMSP10-5 $p=0.01$, DMSP 10-9 $p=0.02$, conspecifics $p=0.11$).

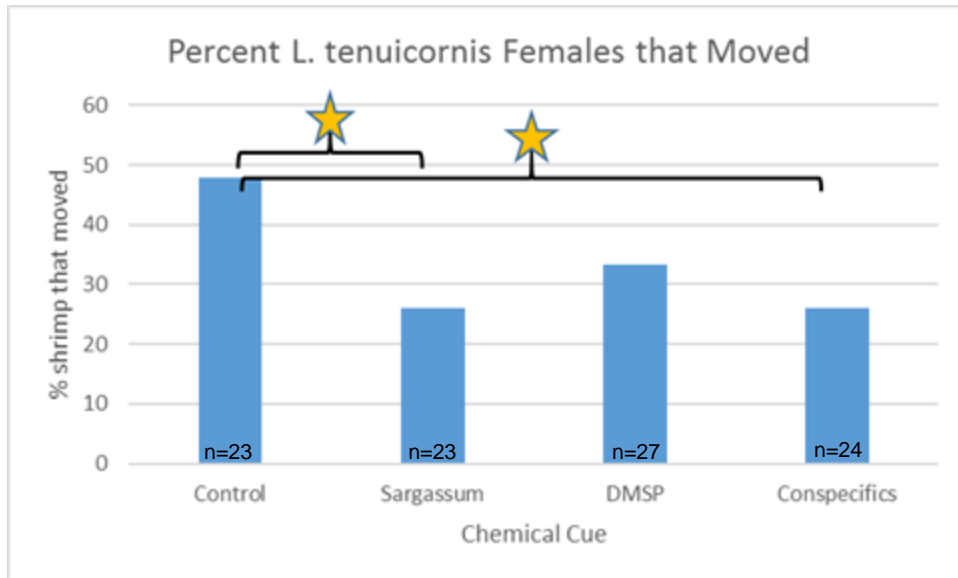


Figure 9: Percent of *L. tenuicornis* females that moved in the y-maze for the control, Sargassum, DMSP, and conspecific cue trials (binomial test, Sargassum $p=0.02$, DMSP $p=0.05$, conspecifics $p=0.01$).

Of the shrimp that moved out of area C, a binomial test was used to see if significantly more shrimp initially chose the arm of the maze with a cue over the arm of the maze without a cue. *L. fucorum* males chose the cue side over the non-cue side in the conspecifics chemical cue trials ($p=0.05$) (Fig. 10). No other significance was found for *L. fucorum* in the *Sargassum* or DMSP trials, and no significance was found for *L. tenuicornis* for any of the cues (all $p>0.05$).

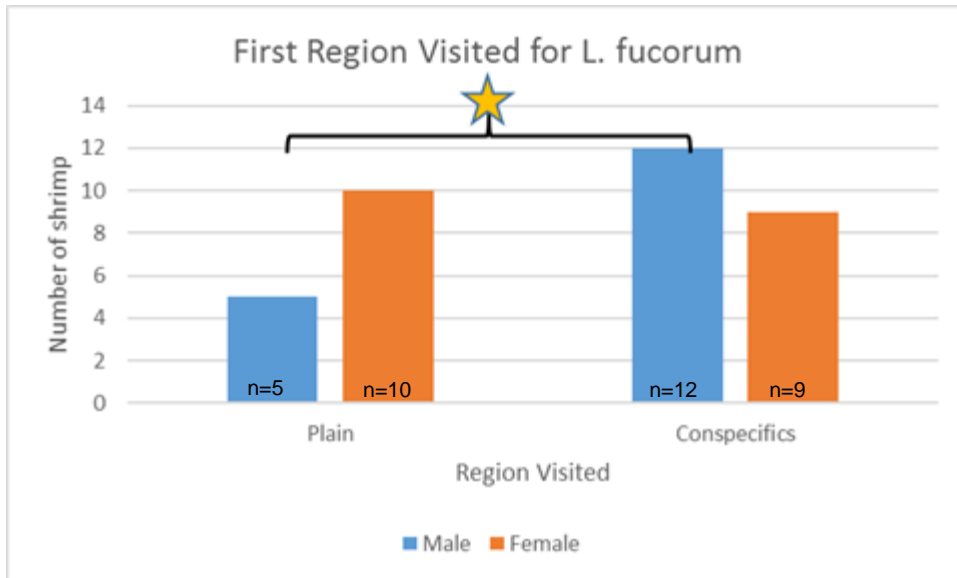


Figure 10: Initial choice between the region with the chemical cue vs the region with plain seawater in conspecific chemical cue trials based on sex (binomial test, males $p=0.05$, females $p=0.18$).

A repeated measures ANOVA done on the shrimp that moved in the maze showed no significance when comparing the time shrimp spent in the arm of the maze with the cue than without the cue, between the control, *Sargassum*, DMSP, and conspecific trials (all $p>0.05$).

DISCUSSION

Four-chambered apparatus trials

Results of the four-chambered apparatus trials show that there was no significant difference in the number of shrimp that moved in the control trials versus the treatment trials. This result agrees with the previous 2009 study, which also did not show the shrimp significantly responding to a *Sargassum* chemical cue.

The first hypothesis was that either species of shrimp would respond to a *Sargassum* chemical cue by moving toward the cue source. *L. fucorum* did not respond to the *Sargassum* chemical cue in the 4-chambered trials, and no statistical significance was found that the shrimp moved directionally towards the cue (Fig. 3 & 4). Due to these results, no weaker dilutions of *Sargassum* chemical cue were tested for either apparatus, and the first hypothesis is rejected. If the shrimp were not able to respond to the strongest dilution, they would most likely not respond to a weaker dilution. Therefore, the seventh hypothesis stating that shrimp movement would correlate to cue strength cannot be accepted or rejected.

L. fucorum did not respond significantly to DMSP or conspecific cue trials. Since groups of shrimp were placed in the central chamber, there were conspecific cues in the apparatus and not just from the radial chamber with the cue source. This fact, along with not knowing how the shrimp may disperse

based on conspecific interactions, may decrease the chance of detecting the shrimps' response to the cue source.

The third and fourth hypotheses that smaller shrimp would have a stronger response to chemical cues than larger shrimp, is rejected, and the null hypothesis is accepted. The two size groups of shrimp did not have any statistically different responses to any of the chemical cues tested (chi-square, all $p > 0.05$). While this may suggest no difference between life history stages of the shrimp, future studies should attempt to record larval shrimp responses to chemical cues, as it may be more important for the larvae to detect patches and settle into the habitat. Larvae of other species of invertebrates rely heavily on habitat chemical cues for settlement. For example, swimming larvae of the hydrozoan *Coryne uchidai* settle in response to chemical cues from *Sargassum tortile* (Kato et al., 1975). *L. fucorum* has a larval stage which lasts 18-30 days in the lab (Bailey, 1980). Such a long period of time would indicate that the larvae must have a means to locate suitable habitat.

Y-maze trials

L. fucorum males and *L. tenuicornis* females moved significantly less in the y-maze in the presence of the *Sargassum* chemical cue (Fig. 7 & 9). This data confirms that the first hypothesis cannot be fully accepted, but supports the fifth, sixth, and tenth hypotheses that the response of either shrimp species would differ based on sex. Both species of shrimp exhibiting less searching behavior in the *Sargassum* trials may have been caused by a few different

factors. For one, if there are no predator cues the shrimp may not seek shelter in the *Sargassum*. The shrimp were given a 10 min acclimation period before allowed to move throughout the apparatus. In those ten minutes, the shrimp would settle into place and remain undisturbed. An additional possibility is that the shrimp may freeze or reduce movement in order to avoid predation, since they had just been disturbed by being taken from the holding tank to the y-maze. It is well known that some organisms will reduce movement when a predator is near. A study done on a freshwater species of shrimp is one example of this occurring. This study looked at the behavioral response of the freshwater shrimp species, *Atya lanipes*, to the presence of a larger, predatory shrimp species, *Macrobrachium carcinus*. It was found that when the prey shrimp species was located in the same area as the predatory shrimp, the prey species reduced movement by 55% in an attempt to avoid detection and predation (Crowl & Covich, 1994).

The fifth, sixth, and tenth hypotheses that the response of either shrimp species would differ based on sex can all be accepted. While there does not appear to be a clear pattern of males or females having a greater response to chemical cues, there were differences between sexes for each cue used. When parsing the data by sex, *L. tenuicornis* females moved statistically less in the presence of conspecific cues (Fig. 9). Finally, *L. fucorum* females moved statistically more in the presence of both DMSP dilutions (Fig. 8). Only *L. fucorum* males moved significantly less, but it was the females that moved less for *L. tenuicornis*. With these differences in response between sexes, the

hypotheses are accepted. A limitation of this study is that the shrimp were not sexed until after use in a trial. Since the female shrimp are larger than males (Martinez-Mayen & Roman-Contreras, 2011), when choosing shrimp from the tank, it is likely that there was bias towards choosing females, especially highly visible, ovigerous ones. This bias is especially noticeable in *L. fucorum* trials since this species is small and difficult to locate. Additionally, only shrimp with a total length of 10mm or greater were used in the y-maze to ensure visibility in the videos. This bias caused fewer males to be tested than females (*L. fucorum*, 1.88:1; *L. tenuicornis*, 1.6:1). Having low replications for male shrimp decreases the power of statistical analyses, and future studies may choose to incorporate all sizes of shrimp and more male replications.

The ninth hypothesis that either species of shrimp would respond to conspecific chemical cues by moving toward the cue can be partially accepted. Of the *L. fucorum* males that moved in the y-maze, significantly more chose the conspecific cue side initially (Fig. 10). However, no other statistical significance was found using the conspecific cue besides *L. tenuicornis* moving less in the y-maze (Fig. 6). *L. tenuicornis* is the larger of the two species of shrimp, interactions observed in the lab between conspecifics have occasionally been aggressive, especially larger shrimp towards smaller ones. In general, the shrimp seemed to disperse when kept together in a small container. This may mean that the conspecific chemical cue for *L. tenuicornis* may have acted as a deterrent. Another possible explanation for the shrimp moving less in response to a conspecific chemical cue could be that the conspecific shrimp were releasing

stress chemicals. A study on the crayfish species *Procambarus clarkii* showed that the crayfish moved away from conspecifics if those conspecifics were stressed. Such crayfish released chemicals into the water which acted as a deterrent (Zulandt Schneider & Moore, 2000). Since the shrimp being used as the conspecific chemical cue had just been disturbed by being netted out of the holding tank and into the cue source, it is possible that the shrimp released stress chemicals as well.

In the previous study done by Jobe and Brooks in 2009, it was recommended that future studies try to test one shrimp at a time and with a more specific chemical cue (Jobe & Brooks, 2009). This is exactly what this current study did using the y-maze with DMSP as a chemical cue. The second hypothesis that either species of shrimp would respond to DMSP chemical cues by moving toward the cue source can be partially accepted. While no significant movement in the 4-chambered apparatus was found, both dilutions of DMSP caused *L. fucorum* to exhibit searching behavior and move significantly more in the y-maze apparatus (Fig. 5). However, directional movement was not statistically detectable, leading this hypothesis to be partially accepted. Additionally, the eighth hypothesis that the shrimps' response to the DMSP cue would correlate to cue strength cannot be accepted or rejected. *L. fucorum* did respond to both dilutions used, but the shrimp moved slightly more in the weaker dilution (Fig. 5). Further research is required in order to formally accept or reject this hypothesis.

Parsing the DMSP data by sex showed that *L. fucorum* females moved significantly more in the y-maze in the presence of this cue when compared to the controls, but the males did not, suggesting the female shrimp are more capable of detecting this chemical cue. Since *L. fucorum* responded to the DMSP chemical cue, a weaker dilution was tested to see if the shrimps' response correlates to cue strength. A slightly higher percentage of *L. fucorum* females responded significantly to the weaker DMSP cue, and further research is needed to fully understand how the chemoreceptive response to DMSP strength is correlated.

CONCLUSION

A study done on the chemical feeding cues for *Leander tenuicornis* found that only 5 out of the 28 single compounds used as cues were significantly stimulating (Johnson & Atema, 1986). This shows that chemoreception can be highly specific, and thus searching for an organism's ability to use chemoreception may require testing several cues. Future studies should conduct a chemical assay on *Sargassum* algae, potentially identifying more specific bioactive compounds that can be used to gain further insight into how this crucial mutualistic symbiosis is formed and maintained.

Sargassum is an Essential Fish Habitat (Ballard & Rakocinski, 2012), and entire food chains and marine ecosystems rely on the success of the *Sargassum* community. Even though a previous study was not able to confirm the use of chemoreception by these shrimp species, it did stress the importance of testing different cues (Jobe & Brooks, 2009). The current study was able to expand on the previous study by Jobe and Brooks and clearly show that the shrimp do respond to chemical cues in their environment, and chemoreception may be an important mechanism used by the shrimp to form and maintain this symbiosis.

It is important to note that while chemoreception may be utilized by these shrimp, that the shrimp are utilizing all senses available to them in order to maintain a symbiosis with *Sargassum* algae. Additionally, both species of *Sargassum* shrimp are brooders since they carry embryos until hatching (Bauer,

1989). Since the shrimp are hatched within the *Sargassum*, it is possible that the shrimp do not need to travel long distances to establish this symbiosis.

It is abundantly clear how important the shrimp are to the continuity of the *Sargassum* community. In addition to these shrimp species, several other *Sargassum* organisms have been studied. The *Sargassum* crab *Portunus sayi* is abundant in this community and was also found to detect *Sargassum* chemical cues (West & Brooks, 2012). When looking at both this current study and the previously mentioned studies done on this community, it is clear that chemoreception is a major mechanism being utilized by several organisms in the *Sargassum* habitat. Another study looked at habitat selection by both *L. tenuicornis* and the common fish species, *Histrion histrio*, and found that the organisms have preferred habitats based on structural complexity (Bennice & Brooks, 2016). This just shows that this community is so much more complex than just using chemoreception as a mechanism for symbiosis. Yet another study on a common *Sargassum* organism found chemoreception to be the mechanism by which the planehead filefish, *Stephanolepis hispidus*, is able to locate its habitat (Cox, 2016). All of these organisms are interconnected and play an important role in maintaining this community, and these organisms using chemoreception is one vital component to the success of the symbiosis.

REFERENCES

- Atema J. 1995. Chemical signals in the marine environment: Dispersal, detection, and temporal signal analysis. *Proc. Natl. Acad. Sci. USA*. 92: 62-66.
- Bailey DJ. 1980. The larval development of the Sargassum shrimps *Leander tenuicornis* (Say) (Palaemonidae) and *Latreutes fucorum* (Fabricius) (Hippolytidae) reared in the laboratory (Master's Thesis). Florida Atlantic University.
- Ballard SE and Rakocinski CF. 2012. Flexible feeding strategies of juvenile Gray Triggerfish (*Balistes capriscus*) and Planehead Filefish (*Stephanolepis hispidus*) within *Sargassum* habitat. *Gulf and Caribbean Research*. 24.
- Bauer R. 1989. Continuous reproduction and episodic recruitment in nine shrimp species inhabiting a tropical seagrass meadow. *J. Exp. Mar. Biol. Ecol.* 127: 175-187.
- Bennice CO and Brooks WR. 2016. Habitat selection among fishes and shrimp in the Pelagic *Sargassum* Community: The role of habitat architecture. *Gulf of Mexico Science*. 33(1): 1-13.
- Broadbent AD, Jones GB, and Jones RJ. 2002. DMSP in Corals and Benthic Algae from the Great Barrier Reef. *Estuarine, Coastal, and Shelf Science*. 55:547-555.

- Brooks WR and Rittschof D. 1995. Chemical detection and host selection by the symbiotic crab *Porcellana sayana*. *Invertebrate Biology*. 114(2):180-185.
- Brooks WR, Hutchinson KA, and Tolbert MG. 2007. Pelagic Sargassum Mediates Predation Among Symbiotic Fishes and Shrimps. *Gulf of Mexico Science*. 2:144-152
- Carr WES, Ache BW, and Gleeson RA. 1987. Chemoreceptors of Crustaceans: Similarities to Receptors for Neuroactive Substances in Internal Tissues. *Environmental Health Perspectives*. 71:31-46.
- Coston-Clements L, Settle LR, Hoss DE, and Cross FA. 1991. Utilization of the *Sargassum* habitat by marine invertebrates and vertebrates- a review. NOAA Technical Memorandum NMFS-SEFSC-296.
- Cox D. 2016. The role of chemical cues in locating pelagic *Sargassum* by the associated fish *Stephanolepis hispidus* (Master's Thesis). Florida Atlantic University.
- Crowl TA and Covich AP. 1994. Responses of a freshwater shrimp to chemical and tactile stimuli from a large decapod predator. *J. N. Am. Benthol. Soc.* 13(2):291-298.
- Dacey JWH and Wakeham SG. 1986. Oceanic dimethylsulfide: production during zooplankton grazing on phytoplankton. *Science*. 233:1314–1316.

- DeBose JL, Nevitt G A, and Dittman AH. 2010. Rapid communication: experimental evidence that juvenile pelagic jacks (Carangidae) respond behaviorally to DMSP. *Journal of chemical ecology*. 36(3): 326–328.
- DeBose J L, Lema SC, and Nevitt GA. 2008. Dimethylsulfoniopropionate as a foraging cue for reef fishes. *Science*. **319**(5868): 1356.
- Díaz ER and Thiel M. 2004. Chemical and Visual Communication During Mate Searching in Rock Shrimp. *Biol. Bull.* 206:134-143.
- Dooley JK. 1972. Fishes associated with the pelagic *Sargassum* complex, with a discussion of the *Sargassum* community. *Contrib. Mar. Sci.* 16:1-32.
- Hay ME. 1996. Marine chemical ecology: what's known and what's next? *Journal of Experimental Marine Biology and Ecology*. 200: 103–134.
- Hill RW and Dacey JWH. 2006. Metabolism of dimethylsulfoniopropionate (DMSP) by juvenile Atlantic menhaden (*Brevoortia tyrannus*). *Mar. Ecol. Prog. Ser.* 322: 239–248.
- Hu C, Murch B, Barnes BB, Wang M, Maréchal J, Franks J, Johnson D, Lapointe B, Goodwin DS, Schell JM, and Suida ANS. 2016. *Sargassum* Watch Warns of Incoming Seaweed. *EOS*. 97.
- Jobe CF, Brooks WR. 2009. Habitat selection and host selection by symbiotic shrimps associated with *Sargassum* communities: The role of chemical and visual cues. *Symbiosis*. 49:77-85.
- Johnson BR and Atema J. 1986. Chemical stimulants for a component of feeding behavior in the common gulf-weed shrimp *Leander tenuicornis* (Say). *Biol. Bull.* 170:1-10.

- Kato T, Kumanireng A, Ichinose I, Kitahara Y, Kakinuma Y, and Kato Y. 1975. Structure and synthesis of active component from a marine alga, *Sargassum tortile*, which induces the settling of swimming larvae of *Coryne uchidai*. *Chemical Society of Japan, Chemistry letters*: 335-338.
- Kowalewsky S, Dambach M, Mauck B, and Dehnhardt G. 2006. High olfactory sensitivity for dimethyl sulphide in harbour seals. *Biology letters*. 2(1): 106–109.
- Lapointe BE. 1995. A comparison of nutrient-limited productivity in *Sargassum natans* from neritic vs. oceanic waters of the western North Atlantic Ocean. *Limnol. Oceanogr.* 40(3):625-633.
- Lapointe BE, West LE, Sutton TT, Hu C. 2014. Ryther revisited: nutrient excretions by fishes enhance productivity of pelagic *Sargassum* in the western North Atlantic Ocean. *Experimental Marine Biology and Ecology*. 458:46-56.
- Marmorino GO, Miller WD, Smith GB, Bowles JH. 2011. Airborne imagery of a disintegrating *Sargassum* drift line. *Deep-Sea Research I*. 58:316-321.
- Martinez-Mayen M and Roman-Contreras R. 2011. Some reproductive aspects of *Latreutes fucorum* (Fabricius, 1978) (Decapoda, Hippolytidae) from Bahia de la Ascension, Quintana Roo, Mexico. *Crustaceana*. 84(11):1353-1365.
- Nevitt GA, Veit RR, and Karieva P. 1995. Dimethyl sulfide as a foraging cue for Antarctic procelariiform seabirds. *Nature*. 376(6542): 680-682. NOAA.
1996. Magnuson-Stevens Fishery Conservation and Management Act, as

- amended through Oct. 11, 1996. NOAA Tech. Mem. NMFS-F/SPO-23.
121 p.
- Niermann U. 1986. Distribution of *Sargassum natans* and some of its epibionts in the Sargasso Sea. *Helgoländer Meeresunters.* 40:343-353.
- Rudershausen, PJ, Buckel JA, Edwards J, Gannon DP, Butler CM, Averett TW. 2010. Feeding ecology of blue marlins, dolphinfish, yellowfin tuna, and wahoos from the North Atlantic Ocean and comparisons with other oceans. *Trans. Am. Fish. Soc.* 139:1335-1359.
- Sehein T, Siuda ANS, Shank TM, Govindarajan AE. 2014. Connectivity in the slender *Sargassum* shrimp (*Latreutes fucorum*): implications for a Sargasso Sea protected area. *Journal of Plankton Research.* 36(6):1408-1412.
- Stoner, AW. 1983. Pelagic Sargassum: evidence for a major decrease in biomass. *Deep Sea Res.* 30:469-474.
- Vila-Costa M, Rinta-Kanto JM, Poretsky RS, Sun S, Kiene RP, and Moran MA. 2014. Microbial controls on DMSP degradation and DMS formation in the Sargasso Sea. *Biogeochemistry.* 120(1-3):295-305.
- Weissburg MJ, Zimmer-Faust. 1991. Ontogeny Versus Phylogeny in Determining Patterns of Chemoreception: Initial Studies with Fiddler Crabs. *Biol. Bull.* 181:205-215.
- Wells DRJ, Rooker JR. 2004. Spatial and temporal patterns of habitat use by fishes associated with *Sargassum* mats in the northwestern Gulf of Mexico. *Bulletin of Marine Science.* 74(1): 81-99.

- West LE and Brooks WR. 2018. Habitat location and selection by the symbiotic Sargassum crab *Portunus sayi*: The role of chemical, visual, and tactile cues. *Symbiosis*. 76(2): 177-185.
- Witherington B, Hirama A, Hardy R. 2012. Young sea turtles of the pelagic Sargassum-dominated drift community: habitat use, population density, and threats. *Marine Ecology Progress Series*. 463:1-22.
- Zulandt Schneider RA and Moore PA. 2000. Urine as a source of conspecific disturbance signals in the crayfish *Procambarus clarkii*. *Journal of Experimental Biology*. 203:765-771.