THE ROLE OF CHEMICAL CUES IN LOCATING PELAGIC *SARGASSUM* BY THE ASSOCIATED FISH *STEPHANOLEPIS HISPIDUS*

by

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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 the members of his 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.

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ABSTRACT

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The ecosystem created by pelagic *Sargassum* is important in the life histories of a number of economically and ecologically important associated organisms. Fishes play a vital role in this food web and nutrient flow within these systems, but it is unknown how they locate these floating habitats. This study examined the role of natural chemical cues from *Sargassum* patches and the synthetic chemical Dimethylsulfonionpropionate (DMSP) for an associated fish, the planehead filefish (*Stephanolepis hispidus*) and a control fish species not associated with *Sargassum*, the masked goby (*Coryphopterus personatus*). Choice trials with a Y-maze apparatus determined that *S. hispidus* responded significantly to chemical cues from *Sargassum* while *C. personatus* did not. DMSP cues did not result in any significant behavioral responses for either fish. Demonstrating that *S. hispidus* can respond to chemical cues from *Sargassum* helps further our understanding of this unique floating algal reef and how fishes may locate it.

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INTRODUCTION

Sargassum is a brown macroalga (Class Phaeophyceae; commonly called gulfweed) with thalli characterized by long, branching stipes with numerous blades and spherical, gas-filled bladders (pneumatocysts) that assist in floatation. While many congenerics are often observed floating at the surface, only two species are truly holopelagic: *Sargassum fluitans* (Børgsen) and *Sargassum natans* (Linnaeus). Both holopelagic species reproduce only asexually and never attach to the sea floor during their life cycle (Costen-Clements et al. 1991, Lapointe 1995, Wells & Rooker 2004). These pelagic species, typically found in the western North Atlantic Ocean, Gulf of Mexico, and Caribbean Sea are similar in appearance; both varying in color from orange to yellow to brown (Hacker & Madin 1991, Brooks et al. 2007). Distinguishing features include broader blades on *S. fluitans* and pneumatocysts tipped with spine-like projections on *S. natans*.

Sargassum survives in the Sargasso Sea, despite characteristically low primary productivity in this oligotrophic gyre (Ryther & Menzel 1960, Lapointe 1995) and thrives in neritic regions where nutrients are more abundant (Lapointe 2014). These floating mats can account for up to 60% of the total primary productivity in the upper meter of water (Carpenter & Cox 1974, Wells & Rooker 2004). Major currents, such as the Loop Current and Florida Current carry *Sargassum* from the Gulf of Mexico around the southern edge of Florida and into the Gulf Stream where it is carried up the east coast of

the United States and into the Sargasso Sea (Wells & Rooker 2004, Casazza & Ross 2008). From there *Sargassum* can make its way into the Caribbean waters. Other physical factors, such as gyres, eddies, winds, and storms can also influence *Sargassum* distribution and abundance, causing very high variability along coastal waters (Dooley 1972). This high variability creates a system that is difficult to study due to a wide range of issues including consistent sampling and continuous field observations.

Mats of *Sargassum* commonly accumulate in large windrows due to Langmuir circulation (Ryther 1956). These aggregations are commonly referred to as "weedlines" and align parallel with the wind direction (Fig. 1) (Ryther 1956, Lapointe 1995, Wells & Rooker 2004). Floating materials in the ocean, like *Sargassum*, can attract and concentrate fauna by providing substrate and structure for many relatively small organisms (Hunter & Mitchell 1968, Kingsford 1995, Lapointe 1995, Ingólfsson 1998, Casazza & Ross 2008). Colonization typically begins with epiphytic cyanobacteria, hydroids, and bryozoans that serve as food that subsequently attract a large variety of invertebrates, fishes, birds, and sea turtles (Dooley 1972, Bortone et al. 1977, Fedoryako 1989, Wells & Rooker 2004, Casazza & Ross 2008). Approximately 100 species of invertebrates can be found among these mats (Dooley 1972) and consist of, in addition to those organisms mentioned previously, anthozoans, turbellarians, polychaetes, gastropods, nudibranchs, pycnogonids, isopods, amphipods, copepods, and decapod crustaceans (Butler et al. 1983, Johnson & Atema 1986).

These algal patches serve as important habitat for many vertebrates as well. This includes 80 species of fishes from 28 families. Many of these fish are important commercially and recreationally such as billfish, jacks, and dolphin fish. Additionally,

33 of these fishes inhabiting *Sargassum* patches are not found in surrounding open water (Wells & Rooker 2004, Casazza & Ross 2008). *Sargassum* also serves as an important habitat for 23 species of sea bird including white-tailed tropicbirds, masked boobies, and bridled terns (Haney 1986) along 4 of the 7 species of sea turtle: loggerhead, green, hawksbill, and kemp's ridley (Carr 1987, Manzella & Williams 1991, Witherington et al. 2012).

Many of these organisms associated with *Sargassum* are endemic, including 10 species of invertebrates; e.g., the Sargassum sea slug (*Scyllaea pelagica*) and the Sargassum swimming crab (*Portunus sayi*) (Dooley 1972, Costen-Clements et al. 1991). Obligatory fishes also exist, such as the sargassum fish (*Histrio histrio*) and the sargassum pipefish (*Sygnathus pelagicus*) (Wells & Rooker 2004). Each of these endemic organisms shows unique morphological adaptations to living within *Sargassum*.

With such a large diversity of organisms utilizing these mats, it is easy to understand why pelagic *Sargassum* is commonly referred to as a "floating reef." However, these algal patches serve other functions as well, such as providing temporary shelter and protection when organisms are swept away from previous habitats by currents or storms and left stranded in open water (Lapointe 1986; Brooks et al. 2007). Additionally, these mats act as a nursery for juvenile fishes, providing food resources and protection from predators (Lenanton et al. 1982, Lenanton & Caputi 1989, Wells & Rooker 2004). Among known fish nursery ecosystems, the *Sargassum* community has been studied the least, despite evidence that it may be critical during the larval and juvenile stages of development for many commercially and recreationally important fish

species such as dolphin fish, tuna, ballyhoo, jacks, sailfish, swordfish, and marlin (Costen-Clements 1991; Wells & Rooker 2004; Rudershausen et al. 2010).

Because of this high level of importance as habitat for so many organisms, including endemic and ecologically or economically important species, pelagic *Sargassum* mats have been designated as Essential Fish Habitat (EFH) by the National Marine Fisheries Service (NMFS) (NOAA 1996). *Sargassum* is also a vital feature in energy flow and community food webs of coastal ecosystems because it provides nutrients that support terrestrial flora and fauna when washed up on shore (Polis & Hurd 1995, Spiller et al. 2010, Williams & Feagin 2010) as well as the naturally accruing wrack of accumulated *Sargassum* helping to minimize beach erosion by wind and waves and stabilize dunes (Williams & Feagin 2010, Gheskiere et al. 2006).

While typical amounts will typically aid coastal communities, unusually large amounts of *Sargassum* washing up on shores can be considered harmful algal blooms (HABs) that can have negative impacts on communities such as dead zones and fish dying from excessive hydrogen sulfide being produced (Hu et al. 2016). These HABs can affect tourism negatively as well, causing potential issues in many areas with economies that rely heavily on these vacationers.

Fishes

Sargassum mats are important habitat for the survival of many species of fish. Some fishes rely on these patches temporarily, while others remain permanently. Similarly, several species have developed obligatory relationships with *Sargassum* while others have a facultative association (Wells & Rooker 2004). These differences can often

be seen in the unique adaptations that certain species possess to survive in this environment such as unique coloration and patterns to mimic the algae and provide camouflage seen in the Sargassum fish, *Histrio histrio* (Fig 2).

One fish that is extremely abundant (especially as juveniles) in the *Sargassum* community is the planehead filefish, *Stephanolepis hispidus* (Family Monacanthidae) (Casazza & Ross 2008) (Fig. 3). As a facultative associate of these floating reefs, *S. hispidus* uses *Sargassum* for a portion of its early life, feeding mainly on hydroids (Stachowicz & Lindquist 1997, Brooks et al. 2007) and secondarily on other small invertebrates such as Sargassum Shrimp (Dooley 1972, Brooks et al. 2007). This diet has been shown to vary depending on age and presence of other species of fish such as gray triggerfish (Ballard & Rakocinski 2012). Similar to *H. histrio*, its coloration is also adapted to blend in with the macroalgae; however, *S. hispidus* is a much more mobile species, preferring to swim underneath the mat unless threatened, when it moves up into the algal fronds (Dooley 1972). Once juvenile filefish reach between 50 and 100 mm in length they begin migrating to reef habitat (Berry & Vogele 1961).

Mancera-Rodriguez & Castro-Hernandez (2015) found that *S. hispidus* reaches sexual maturity around 2 years of age and at an average length of 13.9 and 14.9 cm for females and males, respectively. At this stage they begin to reproduce with an average fecundity of 55,000 eggs, and increasing numbers with fish length. Spawning peaks in July and August when juveniles will congregate amongst *Sargassum* patches that also happen to be at their peak for the year.

A recent study by Lapointe et al. (2014) suggests that this relationship between *S. hispidus* and *Sargassum* goes beyond commensalism and is perhaps mutualistic. In

addition to planehead filefish utilizing the algal patches for food and shelter, they also provide nutrients from their waste in the forms of nitrogen and phosphorous, enhancing growth and productivity. That could be the reason *Sargassum* is able to survive in nutrient deficient regions like the oligotrophic Sargasso Sea. This mutualistic relationship strengthens the necessity of maintaining large fish populations among pelagic *Sargassum,* especially those that occur in such high numbers as *S. hisipidus*.

Another fish not commonly found in *Sargassum* patches, but that was utilized in this study as a control species is the masked goby, *Coryphopterus personatus* (Family Gobiidae) (Fig 4). This species is also common in the Caribbean and off the coast of South Florida, but is benthic and usually found on coral reefs. *C. personatus* is an ideal control species for this experiment because it is similar in size to juvenile *S. hispidus*, allowing it to be used in the same experimental setup, but is not associated with *Sargassum* patches.

Chemoreception use in locating habitat

Currents, gyres, winds, and storms cause high variability in the distribution and abundance of *Sargassum* along coastal waters (Dooley 1972, Wells & Rooker 2004). Along with these mats constantly drifting, Stoner (1984) also showed seasonal variability in the patch size and abundance. These factors, along with wave action, large predators, and occasional storms breaking up *Sargassum* mats, can potentially cause mat inhabitants to be separated from their patch and subsequently have difficulty relocating the mat or finding a replacement.

Chemical cues could be critical for these organisms to locate *Sargassum* patches, especially when visual cues are limited or unavailable. Visibility in the open ocean and nearshore environments is highly variable and dependent on a number of factors, including levels of dissolved organic matter, wave action, phytoplankton abundance, and sediment runoff. Low visibility and the potential to be separated by relatively large distances from *Sargassum* mats (e.g., during storms) could result in a shift to relying more on chemical rather than visual cues to locate these patches.

Chemoreception is used commonly by many organisms to facilitate or initiate a variety of activities from feeding, to habitat location, to mate choices (Pawlik 1992, Hay 1996, Huijbers et al. 2012). There are three ways fishes process these chemical cues: olfaction, gustation, and solitary chemosensory cells.

Olfaction occurs as water containing chemical cues flows across either of two nares, generally located on the dorsal side of the head in teleost fishes. As the water flows into the anterior naris using the current produced by swimming or ciliary action within the olfactory chamber, it crosses sensory cells that detect the dissolved chemical compounds in the water and then exits via the posterior naris. These cells then send a signal to the olfactory nerve and to the brain for processing (Hara 1994).

Gustation occurs in cells that can be located in a variety of places along a fish, depending on the species, but are generally concentrated around the head in areas such as the mouth, pharynx, gill arches and skin, and in barbels. Chemical compounds detected by these cells are sent to the facial, glossopharyngeal, and vagal nerves of the brain for processing (Hara 1994).

Solitary chemosensory cells are similar to the cells used for gustation, but are located relatively equally along the body and are more specific in their responsiveness to stimuli. Facial or spinal nerves send these signals to the brain for processing (Peters et al. 1991, Kotrschal 1991).

Among fishes, Pacific salmon can use chemical cues to locate small streams from which they were born after migrating to the ocean (Scholz et al. 1976). Other species, such as sticklebacks, use odors to determine kinship (Mehlis et al. 2008), while swordtail fish utilize chemical cues for mate recognition (Wong et al 2005). Additionally, fishes can use odors to detect distressed conspecifics and predators (Höglund et al. 2005, Nelson et al. 2013), and to locate food (Debose & Nevitt 2008). Using chemical cues to locate habitat or initiate settlement occurs in several reef fishes, both in the lab (Lecchini et al. 2005, Munday et al. 2009, Dixson et al. 2011) and in the field (Paris et al. 2013).

Fishes can detect a variety of compounds, many of which could also be used to locate *Sargassum* patches. There are 7 main classes of chemical stimuli that fishes have been demonstrated to detect and which can influence fish activity. These include amino acids, amines, nucleotides, bile acids, aminosterols, sex steroids, and prostaglandins (Derby & Sorenson 2008). While any of these cues may be used to locate habitat, secondary metabolites from *Sargassum* and its epiphytes contain amino acids and fatty acids that could potentially be detected by the target fish within the marine environment (Wong & Cheung 2001, Turner & Rooker 2006).

Dimethylsulfoniopropionate (DMSP) is a common chemical cue detected by many marine organisms (Debose et al. 2008, Nevitt 1995, Kowalewsky et al. 2006). DMSP is excreted by marine algae such as dinoflagellate *Prorocentrum micans* when

attacked by zooplankton or other predators (Dacey & Wakeham 1986, Hill & Dacey 2006), possibly to attract organisms that prey on the zooplankton and thereby lessen the grazing impact on the phytoplankton patch (Hay 2009). DMSP can have cascading effects throughout the food chain, including being utilized by reef fishes (DeBose et al. 2008), sea birds (Nevitt 1995), harbor seals (Kowalewsky et al. 2006), and potentially whale sharks (Martin 2007). High DMSP concentrations are often found in areas of high primary productivity and foraging activity, such as along frontal zones where *Sargassum* often forms weedlines (Debose et al. 2010). Recently, Debose et al. (2010) showed that certain jack species (*Caranx hippos* and *C. melampygus*) associated with *Sargassum* mats showed a behavioral response to the presence of DMSP and suggest that this chemical is used as a chemosensory cue. Concentration of DMSP in the Sargasso Sea was found to be in the order of magnitude of 10^{-9} M (Vila-Costa et al. 2014) and could likely be used by jacks and other species to locate areas with high probabilities of containing *Sargassum*.

Sensitivity to stimuli varies from species to species, but several cues are thought to be learned as individuals age (Brown & Smith 1998). In contrast, some larval species reared in the lab were able to distinguish between predator and non-predator odors, providing support that odors can also be innately identifiable (Dixson et al. 2010). In addition to being learned or innate, reactions to chemical cues can have an ontogenetic shift in response throughout the life cycle of a species (Harvey & Brown 2004, Golub & Brown 2003, Lecchini et al. 2007). Certain cues may be utilized during the larval or juvenile stages, but then later in development of the fish can elicit a different response or be neglected in favor of another sense. For this reason, it is also important to look at

differences in ages (often correlated with size) of fish that could potentially be using these chemical cues to locate habitat.

Objectives

In this study using a species of fish found commonly in *Sargassum* patches, *Stephanolepis hispidus*, and a control species not associated with these patches, *C. personatus*, the following questions were addressed:

1. Do *S. hispidus* and *C. personatus* respond to chemical cues from *Sargassum* patches?

   H_{01}: There is no response to chemical cues from *Sargassum* patches by these fishes.

   H_{a1}: There is a response to chemical cues from *Sargassum* patches by these fishes.

2. Do *S. hispidus* and *C. personatus* respond to Dimethylsulfoniopropionate (DMSP) in seawater?

   H_{02}: There is no response to DMSP in seawater by these fishes.

   H_{a2}: There is a response to DMSP in seawater by these fishes.

3. Does *S. hispidus* show a shift in response to chemical cues from *Sargassum* patches or DMSP in the size range commonly found inhabiting *Sargassum*?

   H_{03}: There is no ontogenetic shift in response to chemical cues from *Sargassum* patches or DMSP in the size range commonly found inhabiting *Sargassum*.

H_{a3}: There is an ontogenetic shift in response to chemical cues from *Sargassum* patches or DMSP in the size range commonly found inhabiting *Sargassum*.

MATERIALS AND METHODS

Collection and maintenance of specimens

All specimens were collected using a fine mesh dip net over the side of a boat from 1-12 km offshore from the Boca Raton Inlet in southeast Florida. The net was placed fully in the water while the boat slowly drifted by the floating *Sargassum* mat in an effort to collect *S. hispidus* and *Sargassum* spp. Collected samples were then shaken and rinsed in seawater and visually inspected afterwards to ensure all possible macroscopic organisms had been removed and sorted quickly by hand. Animals not being used for experimentation were released back into the *in situ* patches. Fish and algae to be used for trials were transferred into insulated plastic coolers containing seawater with portable aerators until they could be transported back to the laboratory in the Biological Sciences building at Florida Atlantic University.

Once in the laboratory, the filefish were placed into a 75 L aquarium with synthetic seawater mixed to a specific gravity matching natural seawater (1.023 - 1.025). Larger fish were kept separated from smaller ones to ensure cannibalism or aggressive behavior did not take place. Tanks were exposed to a 12L:12D photoperiod with aeration systems and water filters to maintain appropriate living conditions for the organisms. Water level and parameters were checked daily including pH, salinity, ammonia, and nitrate. Visual checks and feeding of frozen brine shrimp or flakes also took place each day. Fishes were given at least 24 h to acclimate to their new environment prior to being used in a trial. For the *Sargassum* chemical cue treatment, 1 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 ensure that there was no chemical composition variability in *Sargassum* effluent between trials, 45 ml aliquots were taken from one batch and then frozen for subsequent use throughout experimentation. *Sargassum* was not used in trials more than 72 h after collection due to the alga's rapid deterioration in aquaria (Jobe & Brooks 2009).

Experimental Procedure

Chemoreception trials took place in laboratories at the Florida Atlantic University Boca Raton Campus. Prior to trials, all fish standard lengths and wet-body weights were recorded. The test apparatus consisted of a modified Y-maze made from clear acrylic, and measuring 10 cm in depth, 10 cm wide, and two 28 cm long arms (Fig. 5). The Y-maze was composed of three regions, A, B, and C. Before trials started, the Y-maze was filled with 6 L of synthetic seawater. During trials, each arm (A and B) received a constant flow of synthetic seawater at 60 ml/min via two 5 mm diameter tubes connected to two source water buckets. This flow of water from the two source buckets eventually caused water to exit the tank via an overflow tube at the base of region C, thereby maintaining a constant depth in the aquarium throughout the experiment. Black plastic surrounded the Y-maze to minimize the use of any visual cues throughout the trials. Control tests were run with colored dyes from both sources to ensure that the cues are not crossing over between regions A and B (Fig. 6).

Source cues for A and B were decided randomly prior to each trial by coin toss. The fish was placed and contained (by a perforated divider) in the base region C to acclimate for 10 min. or until they appeared calm, whichever came second. After acclimation, flow of water from each source was initiated through the tank simultaneously for 5 min, exposing the fish to potential chemical cues prior to its release from the contained area in region C. The perforated divider was then removed to allow the fish access to the entire tank for 10 min. Each trial was recorded using a digital camera to allow for review of behavior and time spent by the fish in each region of the tank.

The control setup for each species was run as a control and had unaltered synthetic seawater from both sources flowing into regions A and B. This allowed examination of any behaviors by these fishes without chemical effluents. The second treatment had unaltered synthetic seawater flowing into the apparatus from one source, and from the other synthetic seawater that had a 45 ml addition of *Sargassum* effluent seawater. This treatment examined whether chemical cues from *Sargassum* are being detected and utilized by these fishes. The third treatment used synthetic seawater from one source and synthetic seawater containing DMSP at a concentration found commonly *in situ* of 10^{-9} M (from Vila-Costa et al. 2014 and Debose et al. 2010) from the other.

At the end of each trial, the Y-maze and water sources were emptied and cleaned to remove any residual chemical cues before beginning the next trial. 25 replicates were performed for each experiment and species, while each fish was used only once.

Statistical analysis

Analysis of the fish response consisted of several variables including the first choice of regions, the number of visits to each region, and the total time spent in each region. The fish was only considered to have left or entered a region once the entire body of the fish had crossed into or out of the region. Results were analyzed using: 1) the binomial test of significance for region choices, 2) t-tests for number of visits to each region as well as total time spent in each region, and 3) logistic regression to analyze the effect of length and mass of *S. hispidus* on first region entered and 4) linear regression to determine mass and length effect on time spent in each region and number of times each region was visited. Data on length and mass of *C. personatus* were not recorded due to all being similar size and weight, and presumably adults.

RESULTS

Sargassum *cue trials*

S. hispidus showed no significant preference for the region containing *Sargassum* chemical cues by choice of region first entered (p=1.000) (Fig. 7) or by number of visits to that region (p=0.862) (Fig. 8) with both metrics being almost equal. However, *S. hispidus* did show a significant response towards the *Sargassum* cues in the amount of time spent in each region, spending an average of almost twice the amount of time in the region containing the algal effluent (165 s) as the region without (86.8 s) (p=0.045) (Fig. 9). Size of fishes (i.e., mass and length) did not have a significant effect on their response for any of the three previously mentioned variables (all p>0.050).

C. personatus also showed no significant response to the region containing *Sargassum* cues in the first two measured behaviors including initial region entered (p=0.560) (Fig. 7) and number of visits (p=1.000) (Fig. 8). Results from the trials regarding total time spent by the masked gobies in each region also were insignificant (p=0.084) (Fig. 9).

DMSP cue trials

During the trials using DMSP cues, *S. hispidus* and*C. personatus* showed no significant preference for regions containing DMSP concentrations in either first region entered (p=0.166, p=0.782) (Fig. 10), number of visits to each region (p=0.532, p=0.285) (Fig. 11), or time spent in either region (p=0.066, p=0.378) (Fig. 12). Size of the filefish

also did not have a significant impact on any preference variables for DMSP cues (all $p>0.05$).

DISCUSSION

Sargassum cue trials

S. hispidus can detect *Sargassum* chemical cues as seen in the significant difference in amount of time spent in each region, averaging almost twice the amount of time in the *Sargassum* region of the Y- maze than in the plain seawater region (Fig. 9). Whether they utilize these cues as a primary means of locating these floating habitats is not as clear. Initial choice of regions did not indicate a preference, which supports the conclusion that chemical cues may be utilized secondarily to visual cues or perhaps for longer distance navigation. Some organisms that inhabit these *Sargassum* mats such as the shrimps *Leander tenuicornis* and *L. fucorum* were found to not utilize chemical cues in the absence of visual cues (Jobe and Brooks 2009). It is probable that a variety of cues are utilized simultaneously including both olfactory and visual cues to locate habitat (Huijbers et al. 2012).

Future studies may show as *S. hispidus* reaches adult stages there exists an ontogenetic shift in response to chemical cues as seen in other species such as Yellow Perch (*Perca flavescens)* and Green Sunfish (*Lepomis cyanellus*) (Harvey and Brown 2004, Golub and Brown 2003) all of which avoid *Sargassum* chemical cues and redirect their responses to benthic habitat chemical cues. There was no significant difference within the size range (21-69 mm, 0.27-9.34 g) collected for this study. All the filefish sampled were juveniles as they still inhabited *Sargassum* patches and had not yet made

the transition to adults which generally occurs after leaving the algal mats at around 1 year of age and maturing around 2 years and at a length of around 13.9 and 14.9 cm for females and males, respectively (Mancera-Rodriguez and Castro-Hernandez 2015)

C. personatus did not show a preference for the *Sargassum* chemical cues, as predicted since it is a reef dwelling fish and not found in *Sargassum* patches. The amount of visits to each region and first region visited were very similar and, while not by a significant amount, it did appear to preferentially avoid the *Sargassum* cues, spending over three times the amount of time in the plain seawater region compared to the *Sargassum* region.

Other olfactory cues that were not looked at in this study such as cues from conspecifics or heterospecifics could also have potential impact when locating habitat. A combination of chemical cues may be utilized to find *Sargassum* patches or perhaps other cues in addition to those given off by *Sargassum* may illicit a stronger response than the habitat cues alone.

Concentration of compounds given off by *Sargassum* patches was not looked at in this study, but could impact the response of these fishes as well. Molecules potentially used by fishes to locate habitat are more likely to be encountered closer to patches or weedlines, meaning the distance from the source and size of the *Sargassum* patch could affect the response of the fish. This study aimed to investigate whether there was any response to cues from *Sargassum* and therefore did not measure any precise concentrations or identify specific compounds present.

DMSP cue trials

Contrary to other findings for reef fishes associated with *Sargassum* patches as juveniles such as Jacks (Debose et al. 2010), *S. hispidus* and *C. personatus* did not have any significant responses in behavior when exposed to DMSP. While none of the data were significant, there were several notable trends during the DMSP trials. For instance, *S. hispidus* initially visited the region containing DMSP more than twice as often as not (9 versus 4 times) and stayed in that region an average of more than three times as long as the region without cues (138 s versus 45s. Similarly, *C. personatus* visited the DMSP region an average of about half as many times as the plain region (0.28 times versus 0.52 times), but spent almost double the amount of time in the DMSP portion as opposed to the portion without DMSP cues (105.9 s vs 63 s).

Given that gobies are generally benthic and would likely never swim away from the reef, they may utilize chemical cues such as DMSP less than other species that travel greater distances. Another possibility is that masked gobies utilize DMSP in their larval stages, but as they become adults tend to rely less on chemical cues for locating habitat. That all the gobies used in these trials were adults could then potentially explain the lack of significant response.

One possibility that was not investigated during this experiment was that chemical cues may be utilized more when visual cues are limited or unavailable. Visual cues were kept to a minimum during this experiment, but lights were used so that fish positions and behavior could be monitored and evaluated. Future studies should consider the option of eliminating visual cues completely by performing the trials in darkness and utilizing infrared cameras to see if responses differ when forced to rely on olfactory cues alone.

Conclusions

The means by which fish locate habitat are important in understanding the ecology of any system, but especially so for habitats that are not stationary. *Sargassum* mats are essential for such a large number of species and knowledge on these complex ecosystems is vital for their management and conservation. This study examined the possibility that one fish common to these algal patches, *S. hispidus*, and one species not associated with *Sargassum, C. personatus,* can locate this habitat through the use of general chemical cues as well as a specific cue, DMSP. *S. hispidus* did respond significantly to the presence of general chemical cues released from *Sargassum* patches and *C. personatus* did not. Neither fish responded significantly to DMSP chemical cues. The ability to locate these mats quickly through any sensory input available is an important factor for many fishes as they are more vulnerable to predation when isolated from this protective habitat. Not only is this important for fish survival, but if this relationship is mutualistic (cf. Lapointe, 2014) then the *Sargassum* mats located in oligotrophic environments also rely on large numbers of fishes, especially planehead filefish which comprise the most common and abundant fish associated with these patches (Wells & Rooker 2004), locating this habitat to provide such nutrients. If *S. hispidus* can detect these chemical cues from *Sargassum* mats and utilize them to locate essential habitat then it is likely other fish species found in these weedlines can do so, too. Further work to gain insight on these interactions is vital to achieving a more comprehensive understanding of this unique ecosystem.

APPENDIX

Figure 1. *Sargassum* aggregation forming a “weedline”. Photo source: http://www.usm.edu/gcrl/sargassum/. This image is fair use.

Figure 2. The Sargassum fish (*Histrio histrio*). Photo credit http://www.hayrettindagci.com/ansiklopedi/index.php?main_page=product_info&products_id=6. This image is fair use.

Figure 3. The planehead filefish (*Stephanolepis hispidus)*. Photo credit: http://oceanexplorer.noaa.gov/explorations/03edge/background/sargassum/media/filefish.html. This image is fair use.

Figure 4. The masked goby (*Coryphopterus personatus*). Photo credit: Ocean, Reefs, and Aquariums (ORA) LLC. This image is fair use.

Figure 5. Y-maze with chemical cues coming in from both sources with choice regions A and B. Region C is where the fish will begin the experiment. The dotted line indicates where the perforated divider will be placed during acclimation. Dark arrows represent flow of water through the apparatus, starting at each end of regions A and B and exiting at the base of region C.

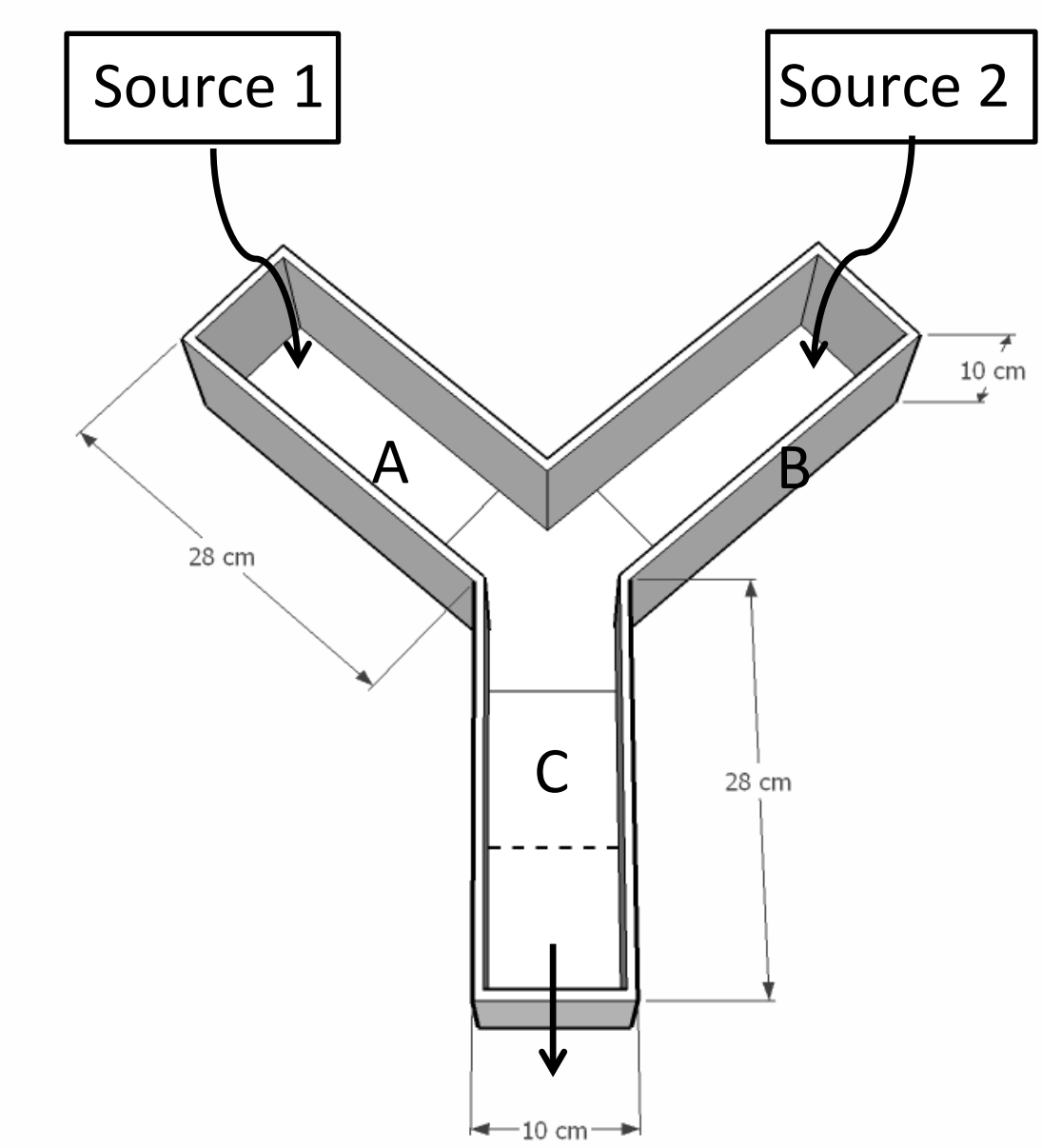


Figure 6. Experimental setup with sources for A and B flowing in from the orange buckets, through the tank, and out the overflow tube. Dye shows separation of chemical cues until reaching Region C. Camera mounted on tripod over Y-maze to record trials and black plastic eliminates visual cues.

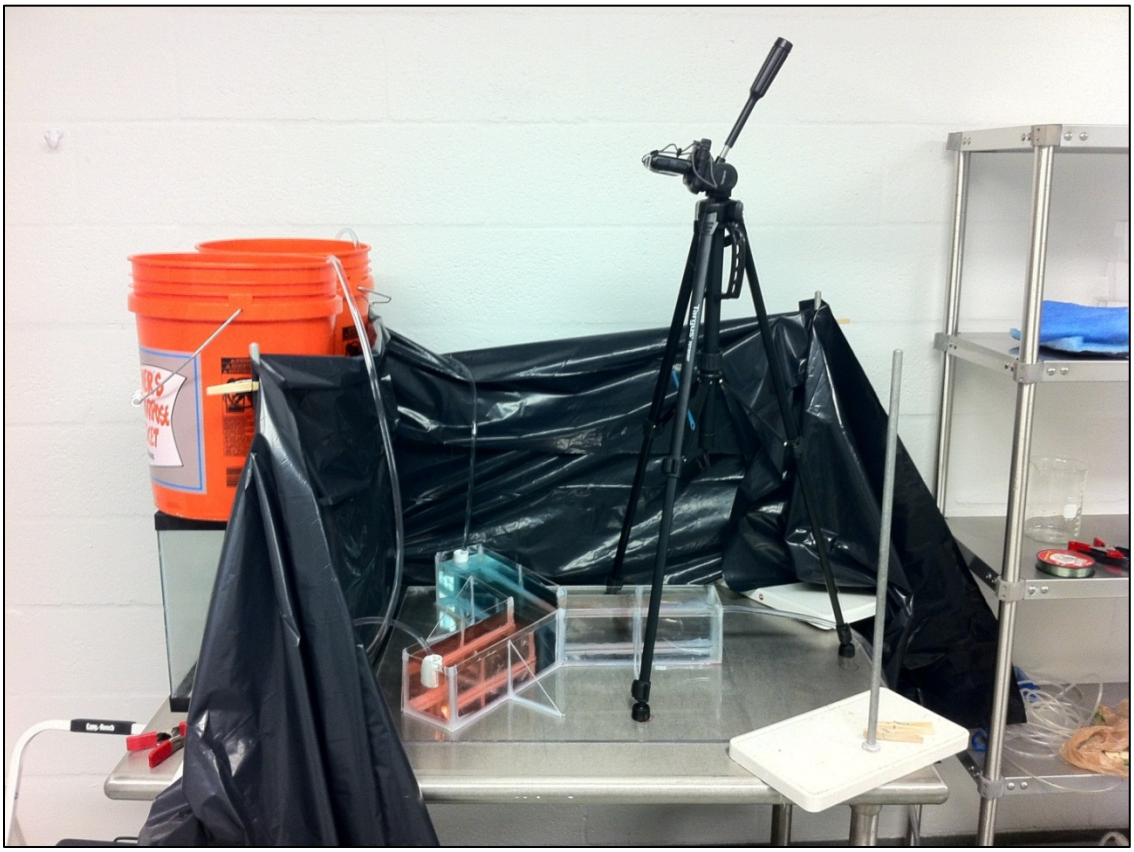


Figure 7. First choice of region visited during *Sargassum* chemical cue trials (binomial test, *S. hispidus* p=1, *C. personatus* p=0.56).

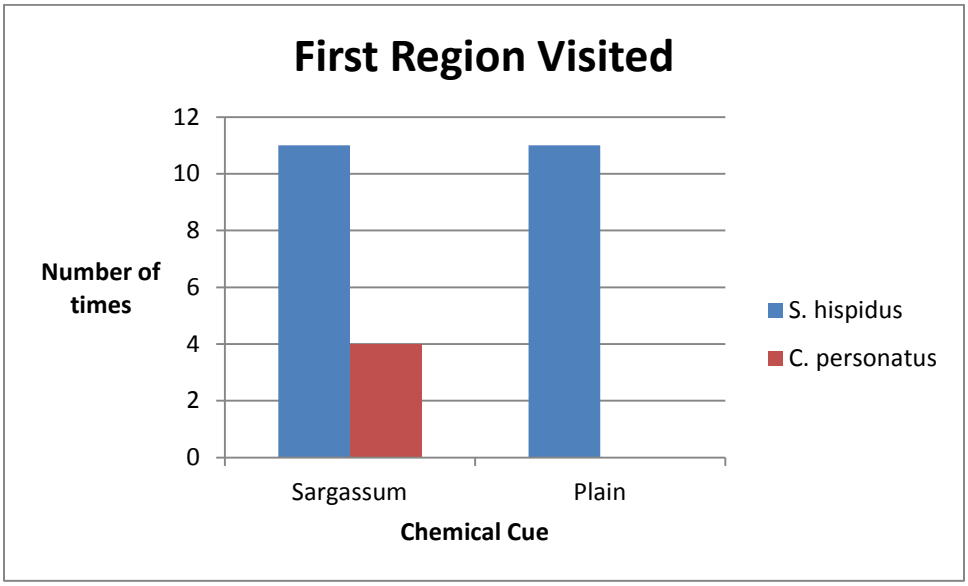


Figure 8. Number of visits to each region during *Sargassum* chemical cue trials (t-test, *S. hispidus* p=0.8627, *C. personatus* p=1).

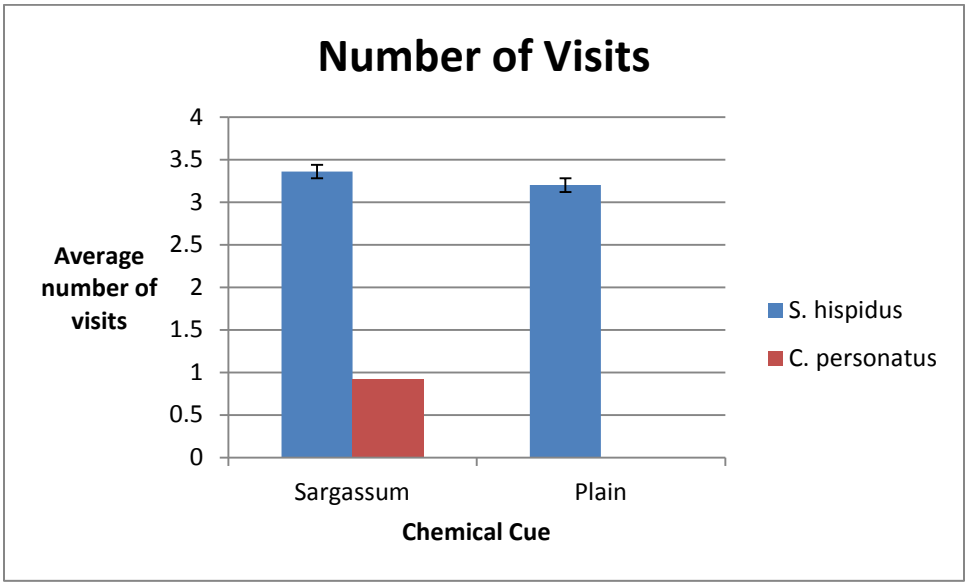


Figure 9. Time spent in each region during *Sargassum* chemical cue trials (t-test, *S. hispidus* p=0.045, *C. personatus* p=0.084)

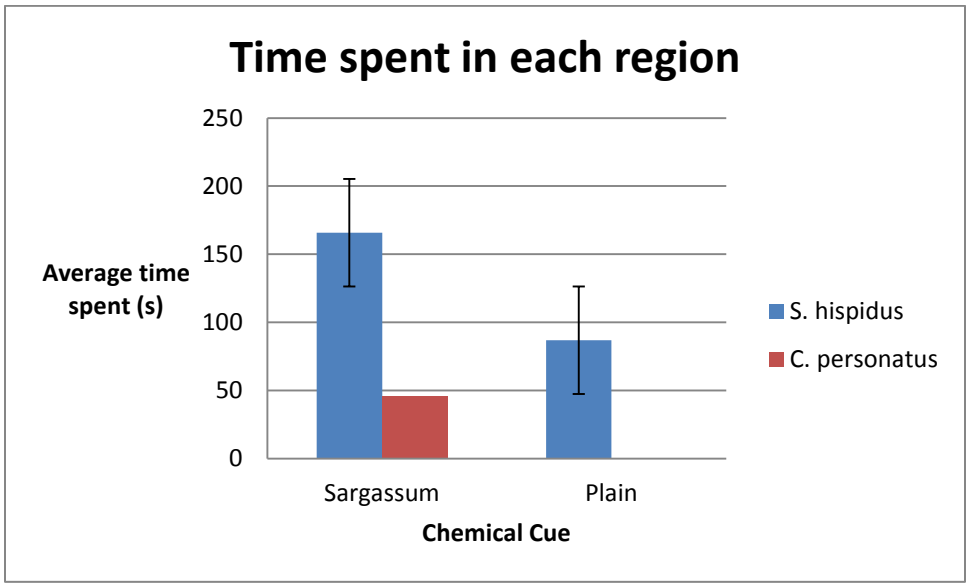


Figure 10. First choice of region visited during DMSP chemical cue trials (binomial test, *S. hispidus* p=0.166, *C. personatus* p=0.782).

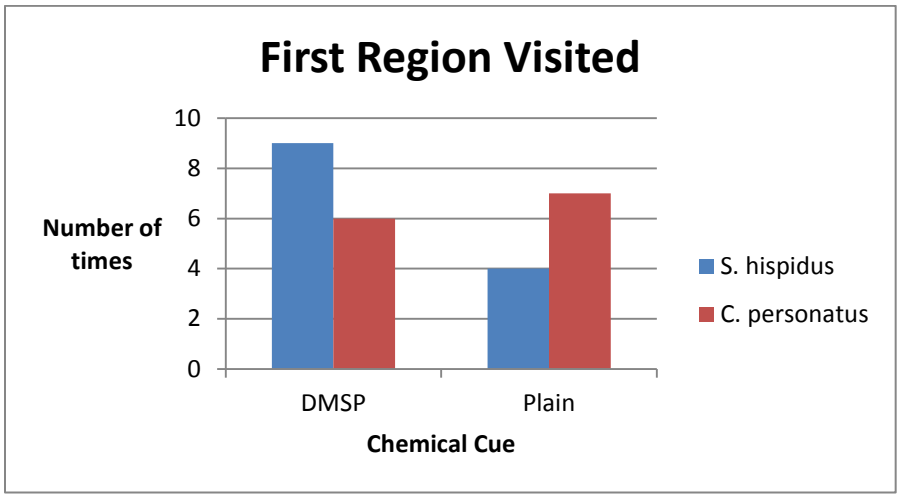


Figure 11. Number of visits to each region during DMSP chemical cue trials (t-test, *S. hispidus* p=0.523, *C. personatus* p=0.285).

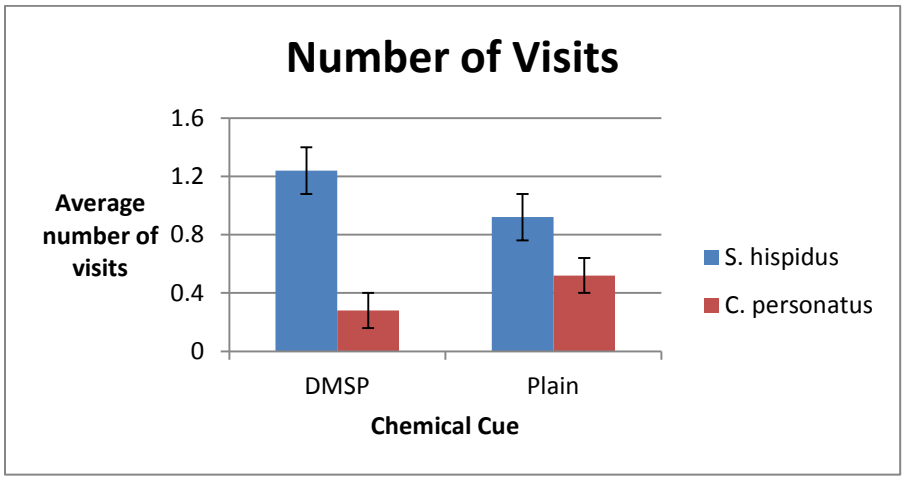
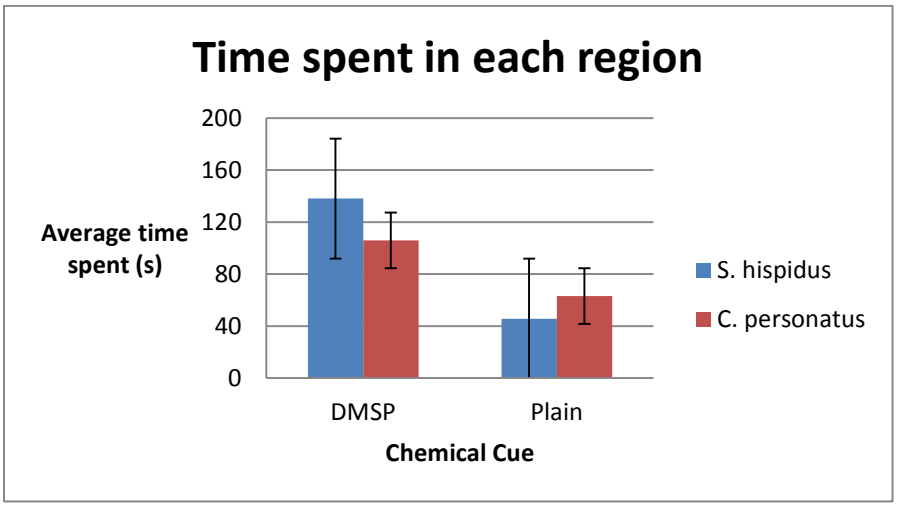


Figure 12. Time spent in each region during *Sargassum* chemical cue trials (t-test, *S. hispidus* p=0.066, *C. personatus* p=0.378)



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