Experimental evidence that habitat-specific chemical cues influence fish preference patterns

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Running title: Olfactory recognition of habitats
Abstract

Vegetated habitats provide numerous benefits to nekton, including structural refuge from predators and food sources. However, the sensory mechanisms by which fishes locate these habitats remain unclear for many species, especially when environmental conditions (such as increased turbidity) are unfavorable for visual identification of habitats. Here, a series of laboratory experiments test whether three species of adult fish (golden topminnow Fundulus chrysotus Günther 1866, sailfin molly Poecilia latipinna Lesueur 1821, and western mosquitofish Gambusia affinis Baird and Girard 1853) use plant chemical cues to orient to one of two habitats (hydrilla Hydrilla verticillata (L.f.) Royle or water hyacinth Eichhornia crassipes (Mart.) Solms). First, experiments in aquaria were conducted offering fish a choice of the two habitats to determine preference patterns. Next, a two-channel flume, with each side containing flow originating in one of the two habitats, was used to determine if preferences were still exhibited when fish could only detect habitats through olfactory means. While patterns among the three fish species tested here were variable, results did indicate consistent habitat preferences despite the lack of cues other than olfactory, suggesting that these organisms are capable of discriminating habitats via chemical exudates from plants. As such, olfactory mechanisms likely provide vital information about the surrounding environment and future work should be directed at determining how anthropogenic inputs such as eutrophication and sediment runoff affect the physiology of these sensory capabilities.

Keywords: olfactory, flume, choice, Hydrilla, Eichhornia, Fundulus, Poecilia, Gambusia
Introduction

Structurally-complex foundation species form important habitats for many aquatic organisms, such as fishes, providing protection from predators and foraging opportunities (Beck et al. 2001, Heck et al. 2003). As a result, these habitats are important for maintaining populations of many commercially and recreationally important fishes, as well as smaller forage fishes. Moreover, biogenic habitats, such as submerged aquatic vegetation or seagrasses, often provide a variety of ecosystem services including nutrient cycling, production of dissolved oxygen, and stabilization of sediments. Anthropogenic activities, however, have led to a reduction in the areal coverage of many of these habitats, leading to a decrease in water quality in many aquatic ecosystems (Beck et al. 2011, Deegan et al. 2012, Waycott et al. 2009).

Moreover, excess nutrients triggering plankton blooms, increased runoff, and sedimentation from agricultural and human development may also function to increase turbidity and decrease water clarity. Despite reduced coverage of habitats and changing conditions, fishes still must navigate and orient to suitable nursery and foraging grounds appropriately. In the absence of visual cues, it is possible that alternate mechanisms of habitat detection exist through chemical exudates originating from these habitats and released into the water column.

Numerous instances of organisms using olfactory cues to orient to different habitats have been described, most notably with larval stages. Early life stages of many species often possess an innate ability to recognize adult habitat through olfactory cues. Larvae of blue (Forward et al. 2003) and mud crabs (Weber and Epifanio 1996), as well as penaeid shrimps (Benfield and Aldrich 1992), are known to have strong preferences for water originating from areas of appropriate habitats. Likewise, larvae of many coral reef fishes have well-documented olfactory preference patterns (Atema et al. 2002, Lecchini et al. 2005). Experiments have demonstrated
these larval fishes often prefer water from habitats near adult grounds as opposed to offshore or from other habitats (Atema et al. 2002; Lecchini et al. 2013). Other experiments have demonstrated that reef-dwelling clownfish prefer water from islands with coral reefs over that of water from offshore or islands with no coral reefs (Dixson et al. 2008).

While the orientation and navigation of larval fishes in response to habitat-related olfactory cues is well documented, the ability of adult fishes to respond to these same cues is not as well understood. A number of different cues, including olfactory, are thought play a role in adult fishes returning to natal areas, including salmonids (Dittman and Quinn 1996), weakfish (Thorrold et al. 2001), and bluefin tuna (Rooker et al. 2008). It is equally plausible that a number of other adult fishes utilize similar habitat-derived cues to find preferable habitats at smaller spatial scales.

Here, we test the notion that fish can recognize different habitats via olfactory mechanisms using three adult fishes common to the wetlands of Louisiana in the United States of America: golden topminnow (*Fundulus chrysotus*), sailfin molly (*Poecilia latipinna*), and western mosquitofish (*Gambusia affinis*). First, we tested the preference patterns for these three species of fishes for either hydrilla (*Hydrilla verticillata*) or water hyacinth (*Eichhornia crassipes*) using tanks stocked with each habitat. Next, we used a two-channel flume to test preference patterns for water originating from one of these two habitats.

**Methods**

**Study Organisms**

Plants and fishes were collected from various aquatic habitats in East Baton Rouge Parish, Louisiana using dipnets. All fishes were held in 38-L aquaria containing filtered,
dechlorinated tap water with an airstone until use in trials, fed daily with fish food, and 10% of the water was changed daily. No fish was used more than once in trials. All fishes used were similar in size (golden topminnow: 30-43 mm; sailfin molly: 41-60 mm; mosquitofish: 31-45 mm). Approval for this study was granted by the state of Louisiana (permit #2580) and Louisiana State University (IACUC #14057) and all fishes released into the wild after experiments were completed.

Two common, yet invasive, habitats were used in this study and the amount of plant material used was standardized to the amount of water displaced (Schumacker et al. 1998, Mercado-Silva 2005). All plants were rinsed with tap water and housed outdoors under natural lighting with an airstone until use in experiments. New plants were used in each trial. Prior to use in trials, plants were shaken vigorously and spun in a salad spinner for approximately one minute to remove epiphytic algae and associated invertebrates (Martin and Valentine 2012, Martin et al. 2015).

**Aquaria Experiment**

Habitat preferences of each fish species were tested using 38-L aquaria containing equal areal coverage of each plant (1-L of displaced water) placed on randomized tank sides (right or left) with an airstone in the middle (temperature: 27.0-28.2°C). During trials, one fish was placed in the center, allowed to acclimate for 30 mins, then its movements between the two habitats recorded using a digital video camera at 1080HD for 30 mins to prevent observer bias. The proportion of time spent in each habitat was calculated and preference trials were replicated 10 times for each species of fish.

**Flume Choice Experiment**
To test whether fish exhibit similar preference patterns to chemical cues from habitats, a 127-L two-channel flume (Figure 1) was used. Flume design was based on previous studies (Atema et al. 2002, Gerlach et al. 2007, Dixson et al. 2008, Munday et al. 2009) and incorporated a gravity-driven flow of water that was recirculated throughout the experiment at a rate of 0.5-L per second. Two 55-L head tanks, containing one of the two randomized plants at equal displacements (5-L), flowed into separated channels, through a collimator constructed of drinking straws to homogenize turbulence, and into a 0.5 x 0.5 m experimental arena separated by 0.8 mm mesh barriers before re-entering separated channels and departing into two 55-L sump tanks via standpipes. Total water capacity of the system is 347-L. Prior to experiments, the flume was tested by placing dye into the water in one channel, which remained separated throughout the length of the flume indicating little mixing of the two water sources occurred (sensu Huijbers et al. 2012). Water was drained and refilled after the dye test and prior to each trial.

During trials, one fish was placed in the center of the experimental arena, allowed to acclimate for 5 mins, then recorded as before for a period of 15 mins (temperature: 26.0-31.8°C). This length of time is within the range of those used in previous studies (Munday et al. 2009, Lecchini et al. 2013). The proportion of time spent in each side was then calculated for comparison. Trials were replicated 10 times for each fish species.

**Statistical Analyses**

Assumptions (normality and homogeneity of variance) were tested prior to analysis, and data were transformed if assumptions were not satisfied. In one comparison (tank experiment, sailfin molly), transformed data still violated the assumption of normality and a nonparametric alternative (Wilcoxon sign rank test) was used. In both experiments, the proportion of time spent
in each habitat was analyzed using a one sample t-test to determine whether the difference was statistically different from zero (Peterson and Renaud 1989, Pennings et al. 1998, Martin 2014). All results were considered significant at p≤ 0.05.

Results

Aquaria Experiment

Fishes used in this experiment exhibited clear and significant trends in their preference patterns (Figure 2). This preference however, varied based on species. Golden topminnows preferred hydrilla (t= 8.33, p≤ 0.001) spending approximately 80% of the trial duration in the side of the tank containing hydrilla. Sailfin mollies (Wilcoxon sign rank test, p= 0.006) and mosquitofish (t= -3.14, p= 0.012), however, preferred hyacinth as habitat over hydrilla, spending approximately 90% and 75% of time in hyacinth, respectively.

Flume Choice Experiment

When fishes were given a choice between water originating in one the two plants, fishes again exhibited significant and consistent tendencies (Figure 3), albeit variable among the different species of fish. As in tank trials, golden topminnows (t= 14.94, p≤ 0.001) preferred hydrilla, spending roughly 90% of time in the flow downstream of the hyacinth tank. Likewise, sailfin mollies (arcsin square root transformed, t= 6.54, p≤ 0.001) and mosquitofish (t= -2.97, p= 0.016) preferred flow originating in hyacinth. Mollies spent around 80% of the trial period in the hyacinth flow and mosquitofish occupied the hyacinth flow for approximately 70% of the trial duration.

Discussion
A number of studies have noted the utility of olfactory cues as an information source for fishes, including predator detection (Ferrari et al. 2010, Martin et al. 2010), kin recognition (Mann et al. 2003, Ward and Hart 2003), detection of toxic compounds (Hinkle-Conn et al. 1998), and, as demonstrated here, habitats. Vegetated habitats, such as the floating and submerged vegetation used here, often contain elevated densities of nekton (Rozas et al. 2013) providing opportunities for herbivory or foraging on other associated fauna, as well as protection from predators. Cues relaying information about the presence of such areas, therefore, are likely vital for the continued fitness of shelter-seeking aquatic organisms.

Interestingly, we found species-specific preference patterns to exist among the fishes tested here (e.g., golden topminnow preferred hydrilla, while mosquitofish and mollies preferred water hyacinth). These preferences may be the result of learned behavior in the wild, for example the repeated capture of prey items that may have preference for these habitats. In addition, these preferences may be due to the size of the interstitial spaces within the leaves (in the case of hydrilla) and roots (in the case of water hyacinth). Studies measuring foraging success have found that habitat morphology and identity can play a large role in determining prey survival (Stoner 1982, Ryer 1988). Therefore, the size of the residing fauna can be an important determinant of their habitat preference (i.e., a fish with size matching the interstitial spaces may provide the most protection). In this study, the deeper-bodied sailfin molly may have preference for floating water hyacinth because of the larger gaps between root structures. Likewise, mosquitofish may find more protection from water hyacinth because of their tendency to use the upper portion of the water column (personal observation).

In many instances, multiple biotic and abiotic environmental cues are used by organisms to orient and navigate to appropriate habitats (Huijbers et al. 2012). Dixson et al. (2008), for
example found that clownfish from coral reefs demonstrated a preference for water exposed to anemones or terrestrial leaves from nearby rainforests as a surrogate for coral reefs. Similarly, a number of additional cues emanating from the submerged vegetation used here could also serve as indicators of the habitat, such as benthic and epiphytic algae, diatoms, etc. Moreover, cues from conspecifics or the scent of prey may also attract individuals to these habitats. Other cues that may influence behavior include water quality, light, sound, and magnetic fields (Lohmann et al. 2008). However, these covariates were eliminated in our experiments, thus the only cues present were those from the macrophytes themselves indicating these fish can recognize these habitat-specific cues.

We acknowledge the potential for experimental artifacts in the current study. The behavior of these fish under controlled, laboratory conditions may not represent natural behavior in the field. For example, the stress involved in handling and being under novel aquaria conditions may result in fish opting to use more complex habitats. This is likely not the case, however, as the different fish species used here had variable preference patterns and not a general predilection for the more complex submerged macrophyte. Even if true, however, a preference for a particular habitat under stressful conditions would not disqualify the main finding of this study that fishes do use olfactory cues to identify specific habitats. Additional experiments need to be conducted to verify the validity of these experimental findings under field conditions.

Future research needs to be directed at determining the reliability of information contained in these cues in the prevailing physical and chemical landscape. Physical forces may affect cues through currents that may misdirect or dilute cues and result in plumes distributed distant from the source. Chemical additions to the water column may hinder an organism’s
ability to recognize and respond to the information contained in the cue. Anthropogenic
alterations to the environment have the potential to significantly disrupt olfactory cognition of
habitats. Elevated carbon dioxide and lower pH, for example, interfered with the identification of
adult habitats (Munday et al. 2009) and predators (Dixson et al. 2010) by larvae of clownfish.
Likewise, fish from areas with environmental contaminants such as heavy metals often
demonstrate an impaired ability to detect predators, thereby decreasing survival (Little et al.
1990, Zhou and Weis 1998), and this disruption to the sensory organs may also deter other uses
of the olfactory system, such habitat detection.

Chemical signals and olfactory cues are an important source of information for aquatic
organisms. Results presented here suggest that fish use water-borne cues to identify habitats at
small spatial scales, and this may be important for orienting and navigating in uncertain
environments, such as when other sources of information (such as visual cues) are decreased or
absent. This study joins a growing list of contributions highlighting the extensive role that
olfactory cues can play in the day-to-day functioning of aquatic organisms.

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**Figure Legends**

**Figure 1.** Overview of two channel flume, indicating gravity-driven water entry from two elevated source tanks (A), collimators within separated section of tank (B), 0.5-m x 0.5-m experimental arena sectioned off by 0.8-mm barriers at either end (C), and water outflow via standpipes (D).

**Figure 2.** Boxplots illustrating proportion of time (+ 1 SD) for paired habitat preference trials for golden topminnow (A), sailfin molly (B), and western mosquitofish (C). All comparisons were statistically significant at $p < 0.05$.

**Figure 3.** Boxplots illustrating proportion of time (+ 1 SD) for flume choice experiments for golden topminnow (A), sailfin molly (B), and western mosquitofish (C). All comparisons were statistically significant at $p < 0.05$. 
Figure 1.
Figure 2.

A) 

B) 

C)
Figure 3.