

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

Charles W. Martin¹

¹Department of Oceanography and Coastal Sciences; 1229 Energy Coast and Environment Building; Louisiana State University; Baton Rouge, LA 70806

Email: martin.charles.w@gmail.com

Fax: (225)578-6326

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.

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

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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 (Dixon 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, Dixon 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 $p \leq 0.05$.

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 ($t = 8.33$, $p \leq 0.001$) 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 ($t = 14.94$, $p \leq 0.001$) preferred hydrilla, spending roughly 90% of time in the flow downstream of the hyacinth tank. Likewise, sailfin mollies (arcsin square root transformed, $t = 6.54$, $p \leq 0.001$) 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). Dixon 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 (Dixon 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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Literature Cited

- Atema, J., Kingsford, M. J., & Gerlach, G. (2002). Larval reef fish could use odour for detection, retention and orientation to reefs. *Marine Ecology Progress Series*, 241, 151-160.
- Beck, M. W., Heck Jr, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., ... & Weinstein, M. P. (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: A better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. *Bioscience*, 51(8), 633-641.
- Beck, M. W., Brumbaugh, R. D., Airoidi, L., Carranza, A., Coen, L. D., Crawford, C., ... & Guo, X. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience*, 61(2), 107-116.
- Benfield, M. C., & Aldrich, D. V. (1992). Attraction of postlarval *Penaeus aztecus* Ives and *P. setiferus* (L.)(Crustacea: Decapoda: Penaeidae) to estuarine water in a laminar-flow choice chamber. *Journal of Experimental Marine Biology and Ecology*, 156(1), 39-52.
- Deegan, L. A., Johnson, D. S., Warren, R. S., Peterson, B. J., Fleeger, J. W., Fagherazzi, S., & Wollheim, W. M. (2012). Coastal eutrophication as a driver of salt marsh loss. *Nature*, 490(7420), 388-392.
- Dittman, A., & Quinn, T. (1996). Homing in Pacific salmon: mechanisms and ecological basis. *The Journal of Experimental Biology*, 199(1), 83-91.

- 232 Dixon, D. L., Jones, G. P., Munday, P. L., Planes, S., Pratchett, M. S., Srinivasan, M., ... &
233 Thorrold, S. R. (2008). Coral reef fish smell leaves to find island homes. *Proceedings of*
234 *the Royal Society of London B: Biological Sciences*, 275(1653), 2831-2839.
- 235 Dixon, D. L., Munday, P. L., & Jones, G. P. (2010). Ocean acidification disrupts the innate
236 ability of fish to detect predator olfactory cues. *Ecology Letters*, 13(1), 68-75.
- 237 Ferrari, M. C., Wisenden, B. D., & Chivers, D. P. (2010). Chemical ecology of predator-prey
238 interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of*
239 *Zoology*, 88(7), 698-724.
- 240 Forward, R., Tankersley, R., Smith, K., & Welch, J. (2003). Effects of chemical cues on
241 orientation of blue crab, *Callinectes sapidus*, megalopae in flow: implications for location
242 of nursery areas. *Marine Biology*, 142(4), 747-756.
- 243 Gerlach, G., Atema, J., Kingsford, M. J., Black, K. P., & Miller-Sims, V. (2007). Smelling home
244 can prevent dispersal of reef fish larvae. *Proceedings of the National Academy of*
245 *Sciences*, 104(3), 858-863.
- 246 Heck, K. L., Hays, G., & Orth, R. J. (2003). Critical evaluation of the nursery role hypothesis for
247 seagrass meadows. *Marine Ecology Progress Series*, 253, 123-136.
- 248 Hinkle-Conn, C., Fleeger, J. W., Gregg, J. C., Carman, K. R. (1998). Effects of sediment-bound
249 polycyclic aromatic hydrocarbons on feeding behavior in juvenile spot (*Leiostomus*
250 *xanthurus* Lacépède: Pisces). *J. Exp. Mar. Biol. Ecol.* 227, 113-132.

- 251 Huijbers, C. M., Nagelkerken, I., Lössbroek, P. A., Schulten, I. E., Siegenthaler, A., Holderied,
252 M. W., & Simpson, S. D. (2012). A test of the senses: Fish select novel habitats by
253 responding to multiple cues. *Ecology*, 93(1), 46-55.
- 254 Lecchini, D., Planes, S., & Galzin, R. (2005). Experimental assessment of sensory modalities of
255 coral-reef fish larvae in the recognition of their settlement habitat. *Behavioral Ecology*
256 *and Sociobiology*, 58(1), 18-26.
- 257 Lecchini, D., Waqalevu, V. P., Parmentier, E., Radford, C. A., & Banaigs, B. (2013). Fish larvae
258 prefer coral over algal water cues: implications of coral reef degradation. *Marine Ecology*
259 *Progress Series*, 475, 303-307.
- 260 Little, E. E., Archeski, R. D., Flerov, B. A., & Kozlovskaya, V. I. (1990). Behavioral indicators
261 of sublethal toxicity in rainbow trout. *Archives of Environmental Contamination and*
262 *Toxicology*, 19(3), 380-385.
- 263 Lohmann, K. J., Lohmann, C. M., & Endres, C. S. (2008). The sensory ecology of ocean
264 navigation. *Journal of Experimental Biology*, 211(11), 1719-1728.
- 265 Mann, K. D., Turnell, E. R., Atema, J., & Gerlach, G. (2003). Kin recognition in juvenile
266 zebrafish (*Danio rerio*) based on olfactory cues. *The Biological Bulletin*, 205(2), 224-
267 225.
- 268 Martin, C. W., Hollis, L. O., & Turner, R. E. (2015). Effects of Oil-Contaminated Sediments on
269 Submerged Vegetation: An Experimental Assessment of *Ruppia maritima*. *PloS one*,
270 10(10), e0138797.

- 271 Martin, C. W., & Valentine, J. F. (2014). Sexual and asexual reproductive strategies of invasive
272 Eurasian milfoil (*Myriophyllum spicatum*) in estuarine environments. *Hydrobiologia*,
273 727(1), 177-184.
- 274 Martin, C. W. (2014). Naïve prey exhibit reduced antipredator behavior and survivorship. *PeerJ*,
275 2, e665.
- 276 Martin, C. W., & Valentine, J. F. (2012). Eurasian milfoil invasion in estuaries: physical
277 disturbance can reduce the proliferation of an aquatic nuisance species. *Marine Ecology*
278 *Progress Series*, 449, 109-119.
- 279 Martin, C. W., Fodrie, F. J., Heck Jr, K. L., & Mattila, J. (2010). Differential habitat use and
280 antipredator response of juvenile roach (*Rutilus rutilus*) to olfactory and visual cues from
281 multiple predators. *Oecologia*, 162(4), 893-902.
- 282 Mercado-Silva, N. (2005). Condition index of the eastern oyster, *Crassostrea virginica* (Gmelin,
283 1791) in Sapelo Island Georgia-Effects of site, position on bed and pea crab parasitism.
284 *Journal of Shellfish Research*, 24(1), 121-126.
- 285 Munday, P. L., Dixon, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V., &
286 Døving, K. B. (2009). Ocean acidification impairs olfactory discrimination and homing
287 ability of a marine fish. *Proceedings of the National Academy of Sciences*, 106(6), 1848-
288 1852.
- 289 Pennings, S. C., Carefoot, T. H., Siska, E. L., Chase, M. E., & Page, T. A. (1998). Feeding
290 preferences of a generalist salt-marsh crab: relative importance of multiple plant traits.
291 *Ecology*, 79(6), 1968-1979.

- 292 Peterson, C. H., & Renaud, P. E. (1989). Analysis of feeding preference experiments. *Oecologia*,
293 80(1), 82-86.
- 294 Rooker, J. R., Secor, D. H., DeMetrio, G., Kaufman, A. J., Ríos, A. B., & Ticina, V. (2008).
295 Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable
296 isotopes in otoliths. *Marine Ecology Progress Series*, 368, 231-239.
- 297 Rozas, L. P., Martin, C. W., & Valentine, J. F. (2013). Effects of reduced hydrological
298 connectivity on the nursery use of shallow estuarine habitats within a river delta. *Marine*
299 *Ecology Progress Series*, 492, 9-20.
- 300 Ryer, C. H. (1988). Pipefish foraging: effects of fish size, prey size and altered habitat
301 complexity. *Marine Ecology Progress Series*, 48(1), 37-45.
- 302 Schumacker, E. J., Dumbauld, B. R., & Kauffman, B. E. (1998). Investigations using oyster
303 condition index to monitor the aquatic environment of Willapa Bay Washington. *Journal*
304 *of Shellfish Research*, 17(1), 338-339.
- 305 Stoner, A. W. (1982). The influence of benthic macrophytes on the foraging behavior of pinfish,
306 *Lagodon rhomboides* (Linnaeus). *Journal of Experimental Marine Biology and Ecology*,
307 58(2), 271-284.
- 308 Thorrold, S. R., Latkoczy, C., Swart, P. K., & Jones, C. M. (2001). Natal homing in a marine fish
309 metapopulation. *Science*, 291(5502), 297-299.
- 310 Ward, A. J., & Hart, P. J. (2003). The effects of kin and familiarity on interactions between fish.
311 *Fish and Fisheries*, 4(4), 348-358.

- 312 Waycott, M., Duarte, C. M., Carruthers, T. J., Orth, R. J., Dennison, W. C., Olyarnik, S., ... &
313 Williams, S. L. (2009). Accelerating loss of seagrasses across the globe threatens coastal
314 ecosystems. *Proceedings of the National Academy of Sciences*, 106(30), 12377-12381.
- 315 Weber, J. C., & Epifanio, C. E. (1996). Response of mud crab (*Panopeus herbstii*) megalopae to
316 cues from adult habitat. *Marine Biology*, 126(4), 655-661.
- 317 Zhou, T., & Weis, J. S. (1998). Swimming behavior and predator avoidance in three populations
318 of *Fundulus heteroclitus* larvae after embryonic and/or larval exposure to methylmercury.
319 *Aquatic Toxicology*, 43(2), 131-148.
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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 $p \leq 0.05$.

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 $p \leq 0.05$.

Figure 1.

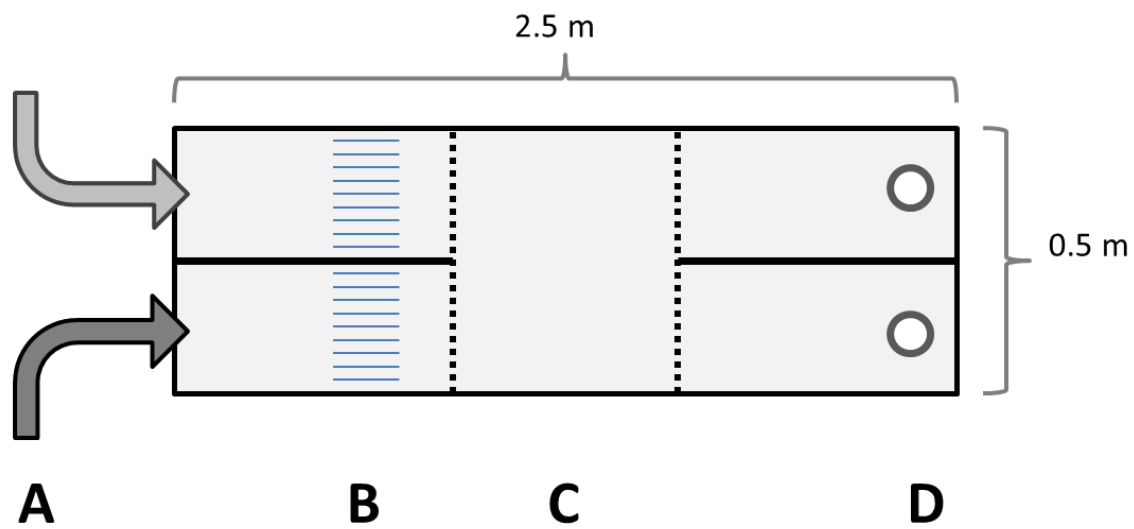


Figure 2.

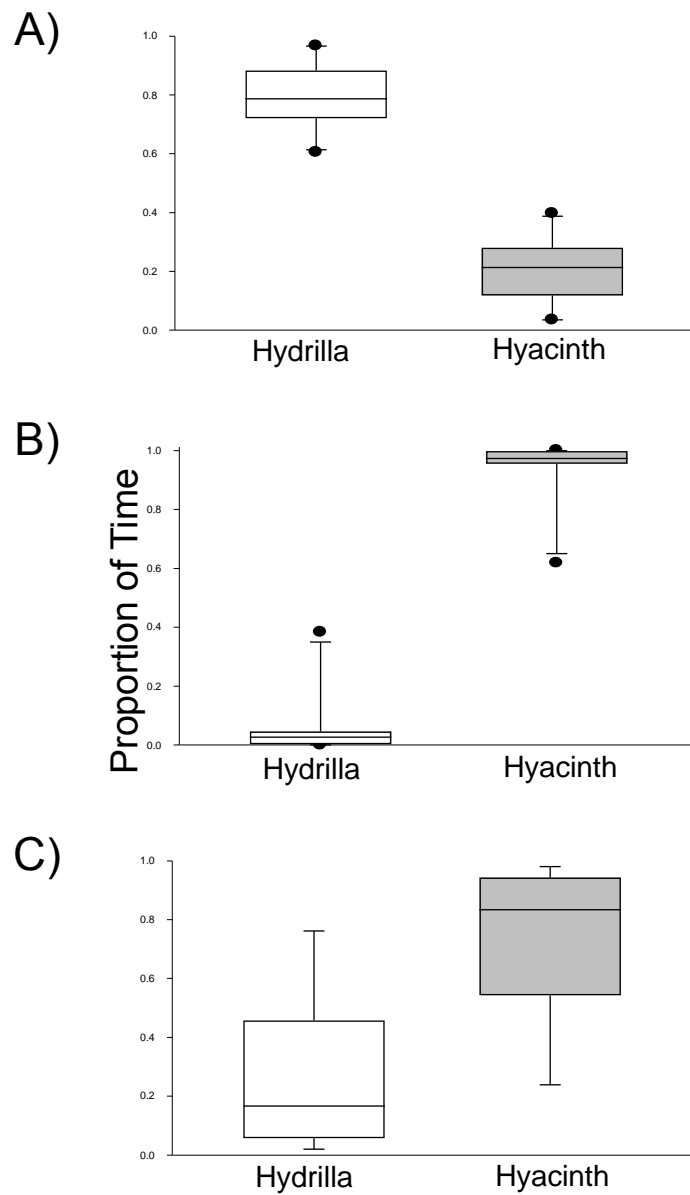


Figure 3.

