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Habitat Selection and Host Detection in the Salamander Mussel, *Simpsonaias ambigua*

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**HABITAT SELECTION AND HOST DETECTION IN THE SALAMANDER MUSSEL,
*SIMPSONALIAS AMBIGUA***

A Master's Thesis

Presented to

The Graduate College of
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Eric Stegmann

May 2020

HABITAT SELECTION AND HOST DETECTION IN THE SALAMANDER MUSSEL, *SIMPSONAIAS AMBIGUA*

Biology

Missouri State University, May 2020

Master of Science

Eric Stegmann

ABSTRACT

The native freshwater mussels, Order Unionida, have suffered many species extinctions and loss of abundance. Two important threats to native mussels are loss of habitat and loss of access to the vertebrate hosts of the parasitic mussel larvae. The Salamander mussel, *Simpsonaias ambigua*, is a habitat specialist, living under flat rocks. It is often found in direct association with its only known host, the common mudpuppy, *Necturus maculosus*. This association could result from movement and habitat selection by the mussels themselves. Alternatively, it might result from the deposition of juveniles by a resident host. Habitat selection and host detection by *Simpsonaias* was examined using choice arenas and choice flumes. Variables tested in arenas included taxis with respect to flow, toward vertical edges, to positions beneath clear or opaque shelters, and aggregation with other individuals. Variables tested in choice flumes included movement toward host scent and dissolved oxygen. Significantly more *Simpsonaias* were found upstream, underneath shelters, underneath dark shelters, in contact with one another, and along the edge of the arena respectively. In choice flumes, mussels showed no preference for host salamander scented water, fish scented water, or control well water. Mussels showed no taxis with respect to dissolved oxygen at 15C, while at 20C mussels were found more often on the hypoxic side, perhaps because of inhibition of locomotion by hypoxia. Overall, the results show that *Simpsonaias* prefer and actively seek darkened shelter where they come into contact with solid surfaces and with each other. The results also suggest that construction of suitable shelters could be a useful conservation tool for this species.

KEYWORDS: *Simpsonaias ambigua*, *Necturus maculosus*, habitat, scent cues, hypoxia, conservation

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May 2020

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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INTRODUCTION

Freshwater mussels of the Order Unionida are among the most endangered groups of aquatic organisms (Williams et al. 1993, Ferreira-Rodríguez et al. 2019). It has been estimated that populations of North American freshwater mussels have been decreasing for decades and possibly centuries in response to human impacts (Ricciardi and Rasmussen 1999, Peacock and Haag 2005). More than 10% of the approximately 300 North American species have become extinct and many others are on the brink (Lydeard et al. 2004). Several anthropogenic factors are affecting freshwater mussel populations. Climate change is a large threat to habitats, with changes in temperature and precipitation altering the distribution of suitable habitat for aquatic species including mussels (Pandolfo et al. 2009, Gangloff and Feminella 2007, Hastie et al. 2003). Other direct human-attributed risks to freshwater mussel populations include the construction of dams, reduction of host populations, introduction of invasive species, and water pollution (Dean et al. 2002, Ricciardi et al. 1998, Keller 1993, Randklev et al. 2013).

The loss of diversity and abundance of freshwater mussels is ecologically significant because mussels provide a variety of ecological services in their habitats. They are filter feeders, collecting microorganisms and particulates from the water column by filtering large volumes of water to obtain the food they need (Allen 1914, Riisgård and Larsen 2010). The biomass of mussel populations can be very high, so that mussels are significant links in nutrient and energy cycles in streams (Atkinson et al. 2013, Strayer 2014). Detritus, bacteria, and algae are removed from the water column and are either ingested or discarded as mucus-bound pseudofeces. Mussel consumption and excretion returns dissolved nutrients to the water that are then utilized by producers (Vaughn and Hakenkamp 2008, Atkinson et al. 2011). Mussel flesh is fed upon by

both terrestrial and aquatic predators. Mussel shells provide a physical habitat for benthic organisms including algae, bacteria and a wide variety of macroinvertebrates (Spooner and Vaughn 2008).

Mussels are vulnerable in part because of an unusual life cycle that includes larval parasitism on specific vertebrate hosts (Lefevre and Curtis 2012, Barnhart et al. 2008). Most mussel larvae, also known as glochidia, attach to the gills or fins of the host fish, where they develop during a parasitic period lasting from weeks to months, depending on species and temperature. Once this metamorphosis is complete, the juvenile mussel drops off the fish and becomes a free-living juvenile. Many mussels are highly host-specific, so that their reproduction can be limited by the availability of the host (Zale and Neves 1982, Riusech and Barnhart 2000, Barnhart et al. 2008, Reis et al. 2014).

Many species of mussels have evolved morphological features and behaviors that visually attract their host fish. These adaptations include conglutinates, which are masses of eggs that resemble host prey items, and mantle lures, which are mobile appendages that mimic prey and entice attack by host fish (Haag and Warren 1999, Barnhart et al. 2008). Attempted predation by the host frees glochidia, which then attach to the host.

Mussels may also be capable of sensing the presence of potential hosts and may alter their behavior in response. Mussels have been observed to increase the movement of their mantle lures in response to moving shadows and vibration (Welsh 1933, Kraemer 1970). There are also suggestions that mussels may chemically sense their hosts. Mussels have been observed to release glochidia in response to the presence of hosts even without direct contact, suggesting a chemical cue from the host is being sensed by the mussel (Haag and Warren 2000). Glochidia respond to body secretions of host fish by closing their valves (Arey 1921).

In addition to the problem of acquiring a suitable host, mussels also have other critical habitat requirements (Howard and Cuffey 2003, Allen and Vaughn 2010, Hinck et al. 2012). Flow rate, for example, must be sufficient to deliver food and oxygen, but with a low enough shear stress to not disrupt the substrate or to prevent juvenile settlement. Flow patterns in rivers can predict mussel distribution (Hardison and Layzer 2001, Parasiewicz et al. 2012, May and Pryor 2015).

Another important habitat requirement is dissolved oxygen (DO). Oxygen enters water from the atmosphere and from photosynthesis by algae and cyanobacteria, and is consumed by respiration. Hypoxia (low DO) is most likely to occur in areas where respiration exceeds photosynthesis, and where water flow is limited. Dark, low-flow conditions are most common in the benthos. Hypoxia can be exacerbated by excessive respiration resulting from eutrophication and by high temperatures, which reduce oxygen solubility (Diaz 2001, Vaquer-Sunyer and Duarte 2008).

Mussels are often found aggregated in areas called mussel beds. These aggregations may develop in those areas of decreased shear stress and higher DO that allow juveniles recruitment (Strayer 1999). Mussels may also create mussel beds by actively moving around until they come into contact with one another. Because mussels live for decades or even centuries, the presence of adult mussels is an indication of stable suitable habitat. Aggregation could also enhance fertilization and reproduction (Stansbery 1966, Vicentini 2005).

The Salamander Mussel, a Host- and Habitat-Specialist

Almost every freshwater mussel species utilizes a fish as its primary host, except for the Salamander mussel, *Simpsonaias ambigua* (Say 1825). A fully aquatic salamander, the common

mudpuppy *Necturus maculosus* (Rafinesque 1818), is the only documented host of this mussel species (Howard 1915, Barnhart 1998). Even though there have been few records of other species of mussel successfully metamorphosing on amphibian hosts (Watters 1997, Watters and O'Dee 1998), the dependence of *S. ambigua* on *N. maculosus* (hereafter '*Simpsonaias*' and '*Necturus*' respectively) seems to be unique among the freshwater mussels.

Necturus is found in both river and lake environments of the upper Midwest and into Canada. The diet of these salamanders varies, ranging from insects and amphipods all the way to mollusks and even fish (Davie and Welsh Jr. 2004, Beattie et al. 2017). They are mostly nocturnal, and their activity increases during the winter months, possibly due to the predators of *Necturus* being less likely to detect *Necturus* during foraging (Craig et al. 2015, Beattie et al. 2017). In general, mudpuppies have limited home ranges and exhibit site fidelity, particularly when females guard their eggs in the spring (Shoop and Gunning 1967, Sajdak 1982).

Both *Simpsonaias* and its host *Necturus* are normally found in the Upper Mississippi river basin and the Great Lakes (Clarke 1985, McKercher 2020). *Simpsonaias* and *Necturus* occupy similar habitat, typically being found under large rocks in rivers (Shimek 1888, Call 1900, Howard 1915, Frierson 1927, Clarke 1985). By occupying the same habitat, the contact that is needed for female *Simpsonaias* to transfer their glochidia to the appropriate host can be achieved. *Simpsonaias* broods its glochidia during the spring (Megan Bradley pers. comm.) which is the same time that female *Necturus* deposit their eggs in rock crevices (Gendron 1999).

Several features of *Simpsonaias* can be interpreted as adaptations to its unusual habitat and host. *Simpsonaias* is among the smallest North American mussels as an adult, only growing up to 2-5 cm in length as adults (Clarke 1985), which allows it to fit into small crevices in the same habitat with its host. Small size should also allow for slower water flow to meet food and

oxygen requirements. Another unusual feature is mobility of the adults. *Simpsonaias* is one of the most active mussels in captivity, even climbing the walls of their tanks (Megan Bradley and Isabel Hannes pers. comm.).

Conservation of both species has been difficult. *Simpsonaias* is listed as a threatened species, and *Necturus* has been gaining conservation attention due to the decrease of another salamander species occupying similar habitat, *Cryptobranchus alleganiensis* (Daudin 1803) (Bogan et al. 2017). The habitat of both species, being under large rocks in areas of flow, are difficult to access and study without destroying it. Also, many challenges are currently affecting *Necturus*, including the application of lampricides, habitat alteration, climate change, excessive harvest from scientific companies due to popularity as a dissection specimen, and even anglers killing them due to a misconception that they are poisonous (Matson 1990, Holman 2012, Beattie et al. 2017). Due to the host specificity, any threat to a host of a freshwater mussel is at least indirectly a threat to the mussel as well. Understanding the behaviors of both mussel and host and how they interact can help to create a more complete conservation plan.

Objectives

The purpose of the present study is to test possible explanations for why *Simpsonaias* tends to occur under rocks with its host. The co-occurrence of *Simpsonaias* with *Necturus* may result from the mussel's active search for physical habitat features or cues from the host itself, and this activity may be influenced by low levels of DO. I tested the following predictions: (1) *Simpsonaias* would move towards physical habitats that are dark, offer flow refuge, and allow contact with solid surfaces, (2) *Simpsonaias* might locate and orient toward its host using water-

borne chemical cues (i.e. by scent), and (3) *Simpsonaias* could actively avoid hypoxia by moving away.

METHODS

Study Animals

Research compliance for all taxa was completed by February 2019 (Appendix A). Upon review, the Missouri State IACUC committee determined that this project did was exempt from needing their approval due to no direct manipulation of living vertebrates (Appendix B).

Simpsonaias were captive cultured in May 2018 at the Genoa National Fish Hatchery (GNFH) from larvae collected from brooding females from the Chippewa River, Minnesota. Hosts were *Necturus* obtained from the Minnesota glacial lakes. Following metamorphosis, the mussels were maintained in reverse upwelling (RU) pans filled with water from the hatchery ponds. Culture techniques have been described in the GNFH 2018 Annual Report (Megan Bradley, pers. comm.). The mussels were about 13 months old at the time of the study. Additional *Simpsonaias* from this cohort were sent to Missouri State University on 26 February 2020 for hypoxia testing.

Salamanders used in scent trials were domestic, long-term captive *Necturus* from the host population that hatched at GNFH in July 2016. Salamanders were kept in a circular 177 L tank (117 cm wide x 16.5 cm deep) as part of a 1200 L recirculating system at 20 °C. Largemouth bass, *Micropterus salmoides*, (Mississippi strain) used in scent trials were cultured at GNFH and kept in a 30 L tank as part of a recirculating system at 20 °C. Fish were not fed, as they were being used as hosts for a *Lampsilis siliquoidea* inoculation. Salamanders were fed a diet of krill and blood worms. Photoperiod was 8L:16D, however the building housing the animals did allow natural sunlight.

Habitat Preference

Habitat choice was assessed in habitat arenas. Each arena consisted of a polyethylene box (43.2 cm long x 28.3 cm wide x 16.5 cm tall). Conditioned well water (aerated and 18-22 C) was recirculated lengthways through the arenas from a reservoir. Water entered each box at the upper end through a manifold and exited via a $\frac{3}{4}$ inch (1.9 cm) bulkhead fitting in the center of the opposite end (Figure 1). The manifold was a 22 cm-long piece of 0.5 cm PVC pipe with three holes drilled at equidistant points on the underside and a barbed fitting in the middle to accept a water line. A partition of open-cell urethane foam (22.2 cm long x 10.8 cm wide x 2.5 cm thick) was placed just after the manifold at the upstream end of the box to rectify the flow (Figure 1). Each habitat box was provided with 400 mL of sand (QuickCrete play sand, depth approximately 6 mm) as substrate. The boxes were inclined slightly, so that water depth was 6.35 cm at the upstream end and 6.83 cm at the downstream end.

The habitat choice boxes were provided with 'lean-to' shelters to test mussel preference for cover. The shelters were 7.6 cm x 7.6 cm tiles of either transparent glass or opaque flooring tile. At the edge of each tile, a 1 cm tall, 1.2 cm wide prop of PVC pipe was attached with aquarium sealant, so that one edge of the shelter was elevated and the other declined and rested in the substrate (Figure 2). One glass and one tile 'lean-to' structure were placed at opposite sides of the box 3.8 cm downstream of the foam partition, each with their declining edge flush against the edge of the box. The location of which side of the habitat box had glass and flooring tile structures alternated 7.6 cm downstream (Figure 2).

Flow was introduced to each box by pumping water with a MN 606 Mini Jet aquarium pump through 139.7 cm of natural rubber tubing which connected to the pipe placed over the upstream portion of the box (Figure 3). Flow was measured by collecting water exiting the

bulkhead fitting at the downstream end of the box in a beaker and timing how long it took to fill 300 mL. Flow in the habitat boxes ranged from 45-50 mL/s. Water exiting each box returned to a reservoir (Figure 3). The pumps for each box were positioned in the reservoir to create a recirculating system.

At the start of each day of testing, *Simpsonaias* were randomly selected from their RU pans, and 10 mussels were systematically placed in each of three habitat arenas. A random number generator was used to determine from which pan (1-7) each mussel would be taken from. Each pan was split into four equally sized quadrants and the quadrant a mussel would be taken from would also be randomly determined by the random number generator. Before being placed in the habitat box, each mussel's body length was measured to the nearest tenth of a centimeter with calipers. Mussel placement in the box was randomly determined between being under one of the structures or down the middle in an upstream, middle, or downstream position (Figure 2).

Mussels were placed in the arenas at 09:00 h, and trials ran for 22 hours. Overhead fluorescent lights in the mussel building remained on from 7:00-19:00 h, after which they were turned off. At 07:00 h the next morning, mussel location was assessed. Mussels were recorded as (1) upstream or downstream of the midpoint of the box, (2) in the open or under shelter (if under shelter, type described), (3) when within 1.5 cm (average body length of the mussels, hereafter considered along the edge) of the wall of the arena or declined edge of a shelter, or not, and (4) as aggregated, if they were within 1.5 cm of another individual, or not. Structures were removed one at a time to record concealed mussels, taking care not to alter mussel positions. A few mussels managed to escape the habitat arenas during trials by climbing the walls and falling through the exit bulk head fitting. Once all mussels were accounted for inside the habitat arenas, escapes were assigned to their respective arenas.

After all mussel positions were recorded, water from each box was emptied into the reservoir. Tested mussels were returned to a separate RU pan to ensure no retesting of animals. Sand from each habitat box was removed and replaced with 400 mL of clean sand. Half of the water from the reservoir was removed to remove excess waste from the previous trials and replaced with fresh conditioned well water to remove excess waste from the previous trials. Habitat arenas were then reassembled for the next round of trials.

Scent detection

Choice flumes (Figure 4) were adapted from the two-current choice flumes described in Jutfelt et al. 2016. Choice flumes were designed to produce two parallel, longitudinal, laminar flows, so that the mussels could choose scented or unscented water by choosing opposite sides of a rectangular open choice area. The choice flumes (30 cm long x 15 cm wide x 10 cm deep) were made of 0.3-cm thick darkened acrylic. A central longitudinal partition divided each flume into left and right channels. The partition was incomplete, with a 15 cm upstream section and a 5 cm downstream section, leaving a 10 cm long x 15 cm wide unpartitioned choice area between. Water entered and exited the channels through bulkhead fittings at the upstream and downstream ends. The flow in each channel was stabilized by first a section of plastic light diffuser panel (1 cm square grid of openings) and a series of 5 screens, 4 of 300 μm mesh and one of 105 μm mesh, then by blocks of open-cell filter foam (2.5 cm thick) that were fitted flush with the edges of the upstream and downstream partitions to form the boundaries of the choice area.

Water was supplied from a 380-L reservoir and was aerated and warmed to 18-20 °C using a thermostatted 1800W heater, as the well water available was too cold for testing. Water was delivered by submerged pumps in the reservoir to flow through the choice flumes, from

which it exited to floor drains (Figure 5). Flow rate was determined volumetrically as described above. One pump delivered water to both sides of each flume, with a Y-connector splitting the flow to the two sides. Flow was balanced between the two sides by clamping the rubber tubing of the side with a higher flow rate. A drop of food coloring was introduced to the upstream end of one channel and observed as it flowed through the test section. If the dye showed mixing between the parallel flows, adjustments were made to correct the imbalance.

Mussels for testing were chosen with the same methods as described for the habitat arenas. A single mussel was placed in the middle of the choice area (Figure 6) and after 5 min the position of the mussel (side of the choice area) was recorded every 5 min. Trials ran for a total of 2 h. Scent cues from mudpuppies and from largemouth bass were collected by taking 1 L of water from hatchery tanks that were holding the animals already. Well water (1 L) for trials that had a blank control was warmed to room temperature before use for testing. Scent cue (5 mL) was introduced by using a separate plastic syringe for each water type simultaneously. Fresh scent cue was introduced every two min for the duration of the experiment.

After a trial was completed, mussels were returned to the collection holding container to ensure no retesting of individuals. Screens and biofilter blocks were removed and rinsed with clean well water. Scent flumes were disconnected to drain water, remove sand, and rinse the boxes with clean well water to remove any lingering scent cue. Flumes were reassembled, and the side each type of water was on would be switched between trials.

Hypoxia

A choice flume, as described above, was attached to an oxygen stripping tower (Barnhart 1995) (Figure 7). Hypoxic water (<1 mg/L DO) from the stripping tower was gravity-fed to one

lane, while aerated water (8-10 mg/L DO) was delivered to the other side of the box. The upstream end of the box was filled with biofilter foam to ensure laminar flow, with a piece of acrylic placed on top of the foam block of the hypoxic side to reduce influence of atmospheric oxygen (Figure 8). Flow was 700mL/min on either side of the box.

Two rectangular baskets were made of acrylic (bottom and sides) and 500 μ m mesh screen (front and back sides, so that water could flow through). The baskets held substrate and could be inserted into the test area of the choice flume. The substrate in each basket was 50 mL of 1.5-2 mm glass beads spread evenly. Baskets were removed and switched in between trials. After recording the water temperature and dissolved oxygen levels, the mussel for that trial would be placed in the center of the choice area as described above. Dissolved oxygen measurements were taken with a probe (HACH HQ30d) placed at different positions in the test section.

Response to hypoxia was tested at two temperature ranges, 14-16 °C and 21-22 °C. Mussels tested at 14-16 °C had been kept at 10 °C and were warmed to 15 °C for at least 30 min before testing. Mussels tested at 21-22 °C had been acclimated to 24 °C for at least 24 hours before testing. Mussel position was recorded as described in the scent trials. Individual mussels were tested only once.

Statistics

All data were analyzed in R (version 3.6.1). Tests run from data collected from the habitat arenas used proportion of mussels per category instead of counts due to some mussels escaping the habitat boxes. Habitat tests had a Bonferroni correction assigned, as multiple tests were derived from the same group of tested mussels. Shelter and edge tests used a selection ratio

(the proportion of mussels divided by the fraction of a specific habitat category's area) to control for habitat categories of different sizes in the habitat boxes. Normality of data was assessed using Shapiro-Wilks normality tests. Stream position and type of shelter data were not normally distributed and were compared with nonparametric paired Wilcoxon tests. Presence of shelter and edge tests were normally distributed and compared with parametric paired t-tests.

A Monte Carlo simulation, based on the dimensions of the mussels and dimensions of the choice arenas, was run 1,000 times to find the number of mussels predicted to be found within 1 body length of another mussel, if distribution were random in the area of the arena. A one sample t-test was run comparing the expectation from the Monte Carlo to the average proportion of aggregated mussels in the trials.

Observations along the midline of the choice area in scent and hypoxia trials were removed from the analysis, with remaining counts converted to proportions. If any mussels had a total of 15 observations or more along the boundary layer (of 25 observations total), their other results were also removed from the statistical analysis. One mussel in the control/salamander, three mussels in the control/fish, and three mussels in the fish/salamander were excluded from scent analysis. Nine mussels from the cool water trials and five mussels from the warm water trials were excluded from hypoxia analysis. Paired Wilcoxon Tests compared data for all scent combinations as well as both cool and warm dissolved oxygen trials.

RESULTS

Habitat Preference

Habitat preferences were assessed from 21 June-1 July 2019. The average length of tested mussels was 1.4 cm with a standard deviation of 1.64. 30 trials were conducted in total. Of all 300 mussels that were tested, 17 mussels were found in the collection basin the following day. No more than two mussels escaped a single box at a time. The Bonferonni correction for habitat results was $\alpha = 0.01$. Significantly more mussels, about four times more mussels, were found in the upstream half of the boxes compared to the downstream half (Figure 9: $V = 435, p < 0.001$). About twice as many mussels were found under shelters more often than in the uncovered open area of the box, relative to the respective areas (Figure 10: $t = -4.78, p < 0.001$). Significantly more, four times more, mussels were found under dark shelters compared to light shelters (Figure 11: $V = 3, p < 0.001$). Four times more mussels were found near the edge of the habitat box or their respective shelter than away from the edge (Figure 12: $t = 18.80, p < 0.001$). Given the dimensions of the box, the Monte Carlo simulations resulted in the expected average number of mussels to aggregate was 1.74 with a standard deviation of 1.63, which was significantly less mussels found aggregated together in the trials (Figure 13: $t = 5.29, p < 0.001$).

Scent Detection

Scent trials were conducted from 8-24 July 2019. Two choice flumes were run simultaneously, with a total of six trials run per day. A total of 30 trials were conducted for each scent type combination (control/salamander, control/fish, and fish/salamander). The average length of tested mussels was 1.4 cm with a standard deviation of 1.87.

Mussels did not show a significant preference between control well water or water that contained salamander scent (Figure 14a: $V = 225.5$, $p = 0.87$). Mussels also did not show a significant preference between control well water or water that contained fish scent (Figure 14b: $V = 183$, $p = 0.89$). Mussels further did not show a significant preference between water that contained fish scent or water that contained salamander scent (Figure 14c: $V = 138.5$, $p = 0.35$).

Hypoxia

Hypoxia trials were conducted from 6-24 March 2020. Between two to four trials were conducted per day. A total of 30 trials were conducted with cooler water and 20 trials were conducted with warmer water. The average length of cool water tested mussels was 2.6 cm with a standard deviation of 0.21 while warm water tested mussels were on average 2.5 cm long with standard deviation of 0.19.

Under cool water conditions, mussels did not show a significant preference for hypoxic or normoxic water (Figure 15: $V = 107.5$, $p = 0.79$). Under warm water conditions, however, mussels did show a significantly higher tendency to occupy the hypoxic stream compared to the oxygenated stream (Figure 16: $V = 102.5$, $p = 0.02$).

DISCUSSION

Simpsonaias is an unusually active mussel, and is often observed moving even when undisturbed. Unionid mussels vary in their mobility among species and ages. Early juveniles of most species are often active, but adults of many species typically burrow into the substrate and may reside in the same general area for months or decades. However, some species still retain activity as adults, like *Simpsonaias*. Reproductive adults of *Unio crassus* and *Theliderma cylindrica* may move inshore to shallow water to disperse their glochidia (Vicentini 2005, Fobian 2007).

Mussels that are found in lotic environments are always at risk of being displaced downstream by flow. If there is no upstream recruitment, mussels would tend to be displaced downstream over time. Upstream recruitment could occur by either attaching to a mobile host that moves upstream and deposits juveniles or by the mussels crawling. Previous studies regarding mussel orientation and movement with respect to flow have yielded mixed results. In one study, captive *Lampsilis siliquoidea* tended to orient their posterior apertures upstream, which indicates potential for downstream movement. However, no pattern of orientation was detected in a field population (Perles et al. 2003). Another field study found that apertures of *Lasmigona costata* were oriented upstream, but reported no significant orientation of apertures upstream or downstream in *L. siliquoidea* (Maio and Corkum 1997). The direction of horizontal mussel movement along the substrate in field observations was often random compared to the flow direction (Schwalb and Pusch 2007, Newton et al. 2015).

The results support the hypothesis that *Simpsonaias* actively chooses habitat with characteristics associated with spaces under rocks, specifically, occupying shelter, being in

contact with solid surfaces, and being in the dark. Unlike *Simpsonaias*, most mussel species burrow into the substrate, and very few are generally found under large rocks. *Cumberlandia monodonta* is another exception, being a mussel species that occupies similar habitat to *Simpsonaias* (Stansbery 1973, Parmalee and Bogan 1998). A possible advantage to this habitat is large rocks and bedrock crevice might provide stability even in swiftly moving water. In the case of *Simpsonaias*, at least, another advantage might be increased likelihood of encountering the host that inhabits these areas. Bivalves are often negatively phototactic, either burrowing into the substrate or attempting to find areas of reduced light (Uryu et al. 1996, Toomey et al. 2002). The preference of *Simpsonaias* under shelter and particularly under opaque tiles indicates that both surfaces and darkness are important physical cues.

The tendency of mussels to form multispecies aggregations may result from patterns of recruitment in areas of stable habitat and suitable flow patterns (Strayer 1999). These processes are apparently relatively non-specific because they concentrate a wide variety of species. However, studies of several species, including *Simpsonaias*, seem to document the selective aggregation of conspecifics in nature (Shimek 1888, Stansbery 1973, Downing et al. 1993, Perles et al. 2003). Such aggregation could presumably help to increase reproductive success (Downing et al. 1993). The mechanism for such aggregation could be direct or indirect. That is, individuals could either detect and seek each other directly, or they could choose a relatively limited set of habitat conditions, and find each other indirectly by seeking that habitat. Although my results also seem to indicate direct aggregation, my test did not account for the tendency of the mussels to be concentrated under shelters and along edges, which would increase the frequency of proximity to other individuals. Further research is necessary to determine if

Simpsonaias aggregation is due to attraction to limited habitat, conspecifics, or artificial due to deposition of juveniles from the host in a limited area.

Chemical cues have been examined in bivalve behavior in attempt to try and explain activity as opposed to environmental cues being the sole factor influencing mussel movement. When exposed to predator chemical cues, blue mussels, *Mytilus edulis*, have been observed to produce stronger byssus threads as well as aggregate in denser patches (Reimer and Tedengren 1997). Zebra mussels, *Dreissena polymorpha*, had similar results of stronger byssus production when exposed to predator scent cues as well (Kobak et al. 2010). Green mussels, *Perna veridis*, will alter their shell growth patterns when exposed to predator scent cues, typically growing thicker shell lips (Cheung et al. 2004).

Few studies have examined mussel taxis toward their host. *Simpsonaias* high mobility may be an adaptation that increases the chances of getting close to *Necturus*. Although mobility may increase the probability of finding the habitat that is preferred by the host, it was not apparent that *Simpsonaias* was directing its movements based on chemical cues from the host. The concentration of scent cue used in trials may not have been representative of wild concentrations to elicit clear attraction. In the wild, a mudpuppy may be located under a rock of small surface area, meaning the low flowing water may become saturated with salamander scent and thus making it easier to detect. Salamander mussel may also be attracted to different host cues, like vibrations in the water from the mudpuppy moving around or even just direct contact.

Hypoxia is often regarded as a significant threat to aquatic life, especially for benthic species. Hypoxic conditions alter the behaviors of several bivalves, from intolerance of higher temperatures, to reduced byssus production, to stimulating premature glochidia release (Aldridge and McIvor 2003, Wang et al. 2010, Galbraith et al. 2012). Bivalve species that regularly

encounter hypoxia may be better adapted to surviving in hypoxia (Sheldon and Walker 1989). The spaces under rocks, preferred by *Simpsonaias*, could possibly present an increased danger of hypoxia, because tight spaces could restrict water flow and because both mussels and salamanders might deplete available oxygen in a confined space. My experiments, however, did not indicate any obvious tendency of the mussels to avoid hypoxia in the choice flume. No difference in position was observed at 14-16 °C, and the mussels were more likely, rather than less likely, to be found in the hypoxic stream when tested at 21-22 °C.

The respiratory rate of ectothermic animals such as mussels increases with increasing temperature (Bartsch et al. 2000). The mussels tended to stop moving if they entered the hypoxic stream, as though they were stunned. This might be interpreted as a kind of trapping effect, rather than a preference for hypoxia, and might be more severe at higher temperatures because of the lower DO and higher respiration rates at higher temperatures. If a mussel did not move out of the hypoxic water after about 25 min of continued exposure, often that mussel would stay in the hypoxic stream for the remainder of the test. If a mussel moved to the stream with oxygen, they continued to move. More mussels were able to keep moving and exit the hypoxic stream in cooler water compared to warmer water. This may be due to a decreased metabolic rate at cooler temperatures. If a mussel has a lower oxygen demand due to lower environmental temperatures, they can possibly spend a longer amount of time in hypoxic conditions and still be able to move. Once this oxygen demand increases, a mussel will deplete oxygen stores, drastically reducing its ability to move. It is also possible the mussels could sense the hypoxic conditions and stopped movement instead of moving around to avoid further depletion of oxygen. Reducing movement may allow them to outlast hypoxic conditions.

Further experiments with hypoxia might be carried out with a vertical, rather than a horizontal gradient. Interstitial hypoxia and reduced interstitial water flow have been identified as a major problem for mussels when interstitial spaces are occluded by fine sediments, a common problem resulting from sedimentation in streams (Denic and Geist 2014, Fung and Ackerman 2019). Another refinement might test the tendency of mussels to enter shelters and confined spaces in hypoxic water versus normoxic water.

Simpsonaias are able to climb up vertical surfaces, even smooth plastic. Individuals were observed scaling the walls of the habitat arenas as well as the flow-through pans for captive holding. Although other surfaces were not tested, it seems likely that mussels would also climb natural surfaces like rocks. It is not clear what the adaptive advantage of climbing would be. Perhaps in moving upstream in rocky substrate, mussels might need to traverse vertical barriers. Another hypothesis is that climbing allows more mussels to be under the same rock, effectively increasing the surface area that can be covered with mussels when densities get great enough. In one early report, salamander mussel density was over a mussel per square inch under a single, 18 in. x 16 in. rock (Shimek 1888). In captive culture, where large numbers of individuals aggregated, it was not uncommon to see some individuals climbing the walls. This could be interpreted as an attempt to avoid excessive crowding.

Understanding how *Simpsonaias* interacts with its environment and host can help inform managers on critical needs for its conservation. Both *Simpsonaias* and *Necturus* are species of conservation concern and habitat loss is a likely explanation for reduction of both species (King et al. 1997, Choquette and Jolin 2018, Jessica Pruden USFWS pers. comm.). The preferred habitat of these species, spaces under large flat rocks, is difficult to sample without destroying it. However, there appears to be an excellent opportunity to create artificial habitats. Artificial rock

habitats have been used to understand and enhance the habitat of a variety of terrestrial animals (Croak et al. 2012). Deploying shelters suitably designed for periodic observation can enhance monitoring and, if habitat is limiting, may also increase abundance. In the case of *Necturus* and *Simpsonaias*, it may even be possible to protect two species with the same stones.

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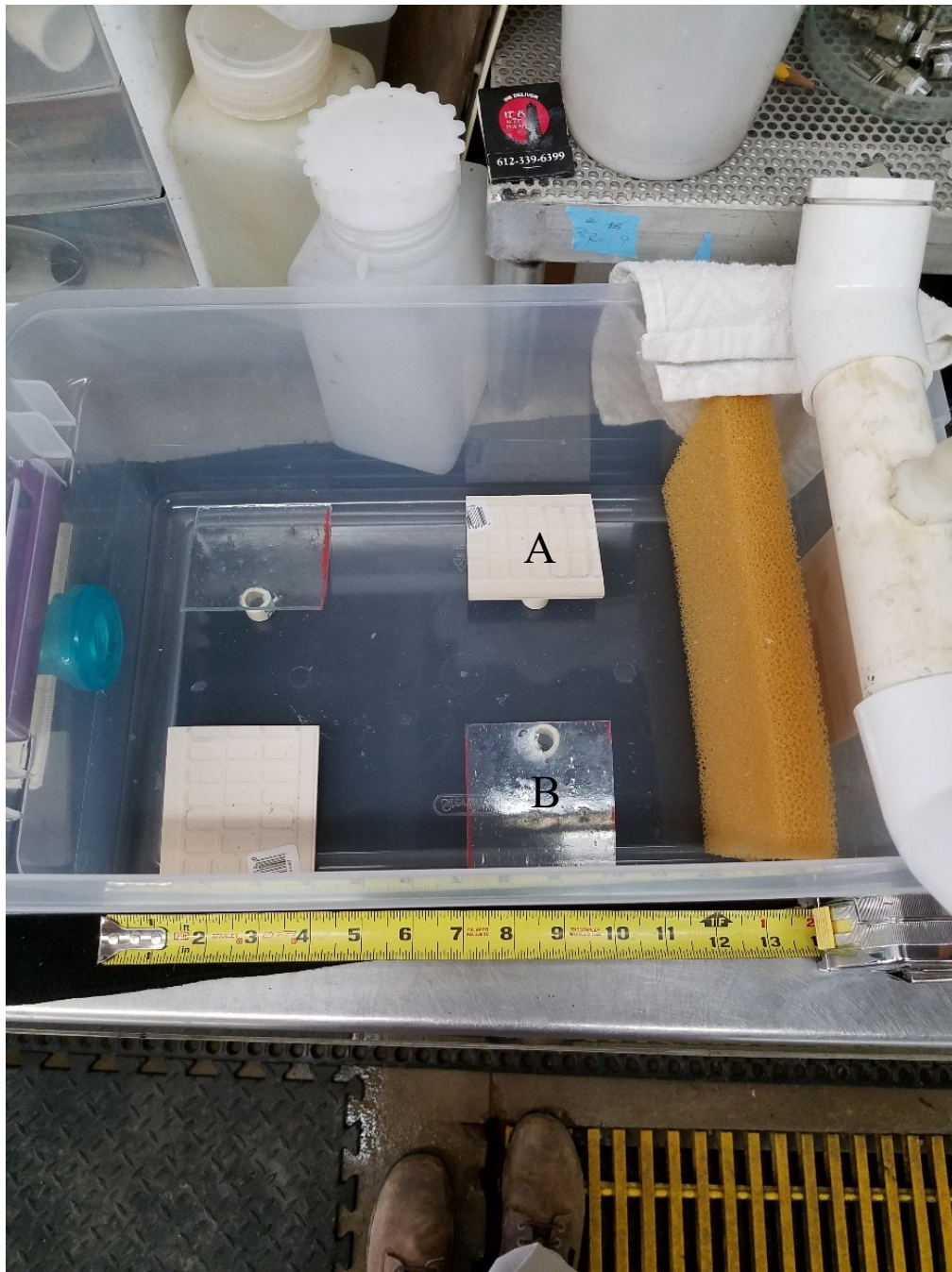


Figure 1. Habitat arena. The upstream end of the box is on the right, starting with the biofilter foam followed by a series of lean-to shelters made of either flooring tile (A) or glass (B).

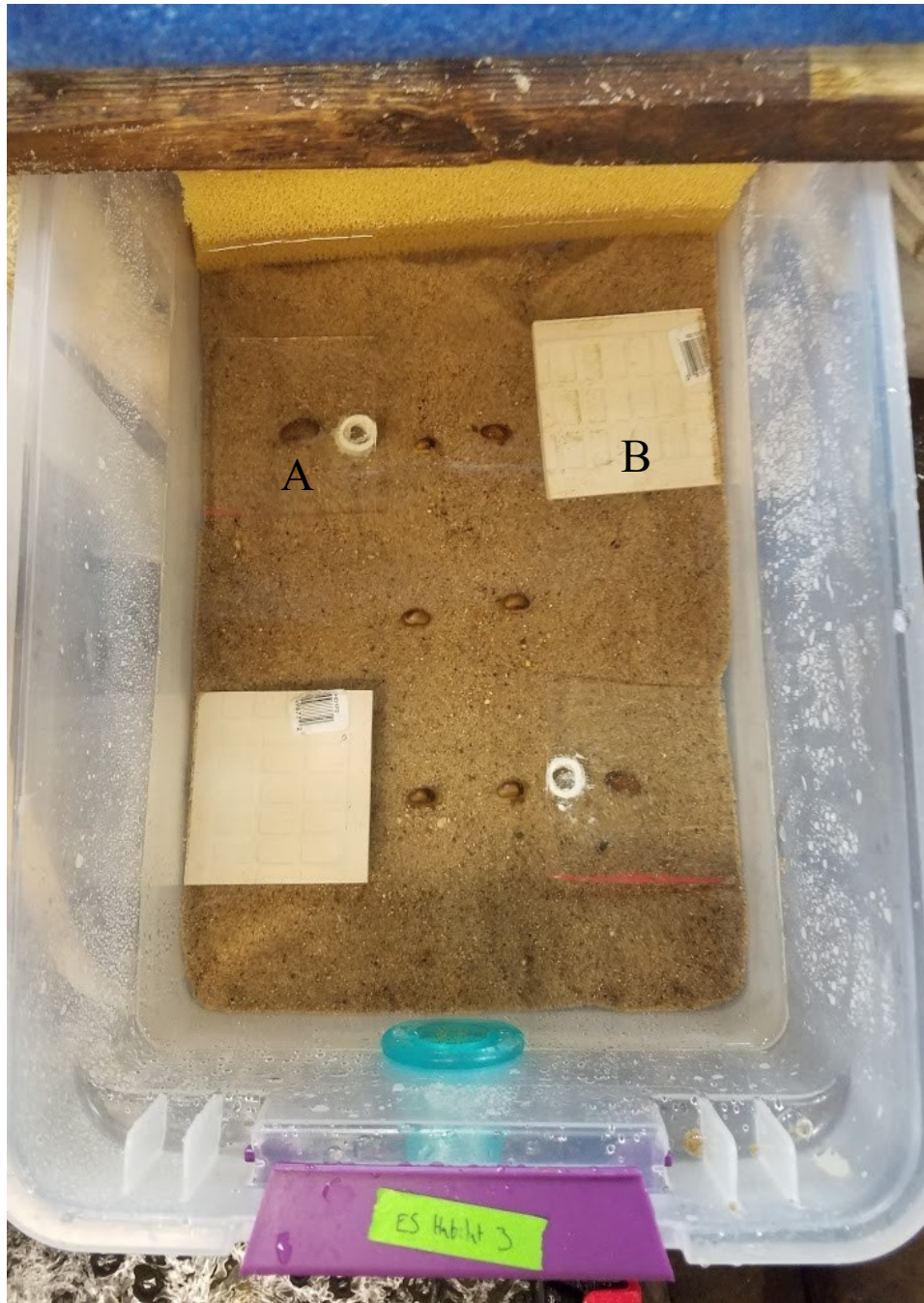


Figure 2. Habitat arena at beginning of a trial. Upstream shelters are glass on the left (A) and tile on the right (B). Downstream shelter material switches positions.



Figure 3. Habitat arenas (A) and water reservoir (B). The pumps (C) and lines for delivering water to the arenas are visible.

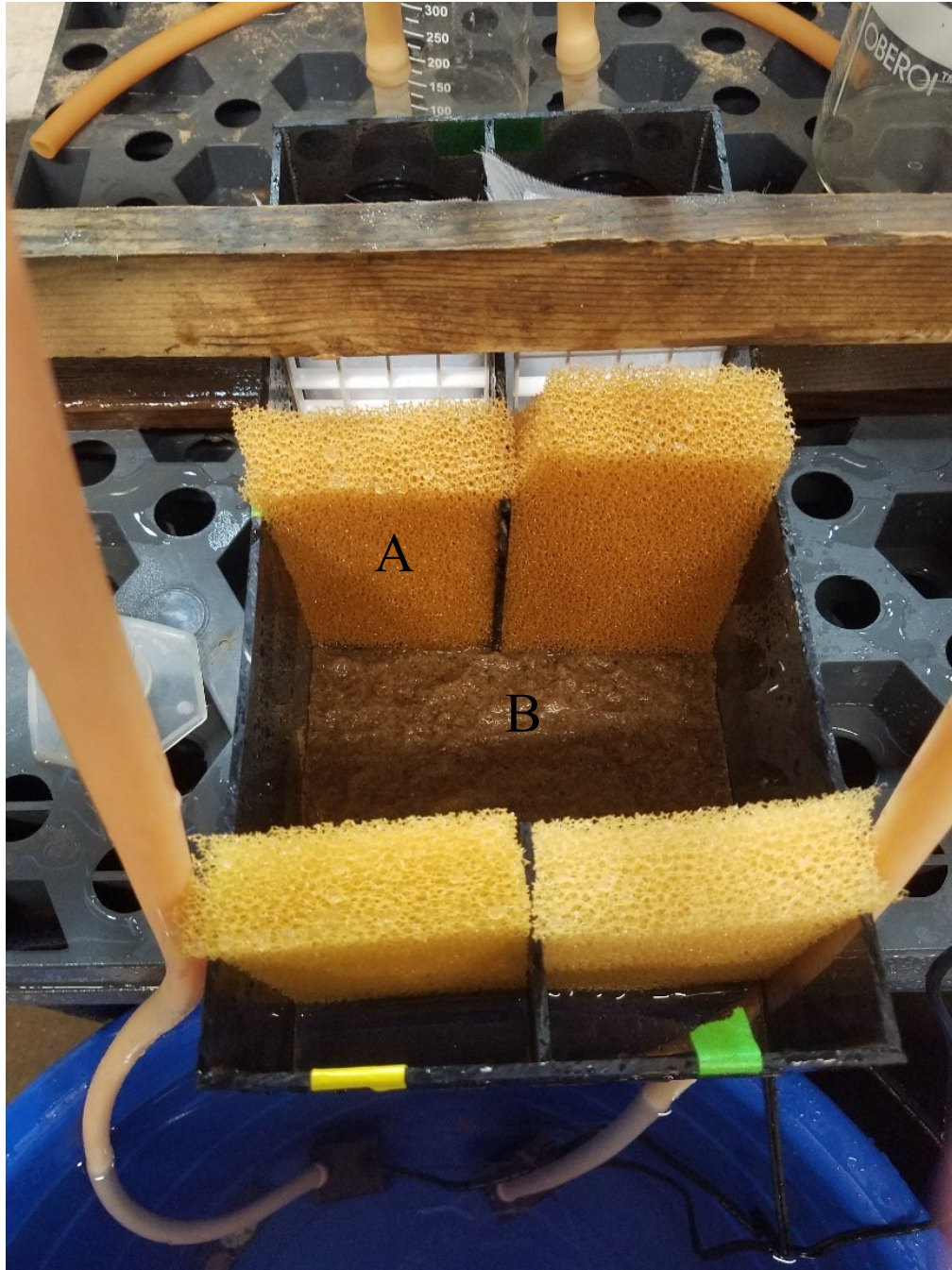


Figure 4. Choice flume for scent trials. The choice area lies between the foam blocks.



Figure 5. Choice flumes with water reservoir. The choice flumes are the boxes on the small table (A). The water reservoir with submerged pumps is the large blue box in the foreground. The large cylinders contain water being warmed to test temperature.



Figure 6. Mussel placement at beginning of scent trial. Fish scent introduced in the left stream, salamander scent introduced in the right stream.



Figure 7. Choice flume set-up for dissolved oxygen trials (A). Water from a temperature controlled reservoir (B) at right was pumped to provide both aerated water and deoxygenated water to the choice flume. Water was deoxygenated via an oxygen stripping column (C) (foil-wrapped column at left).

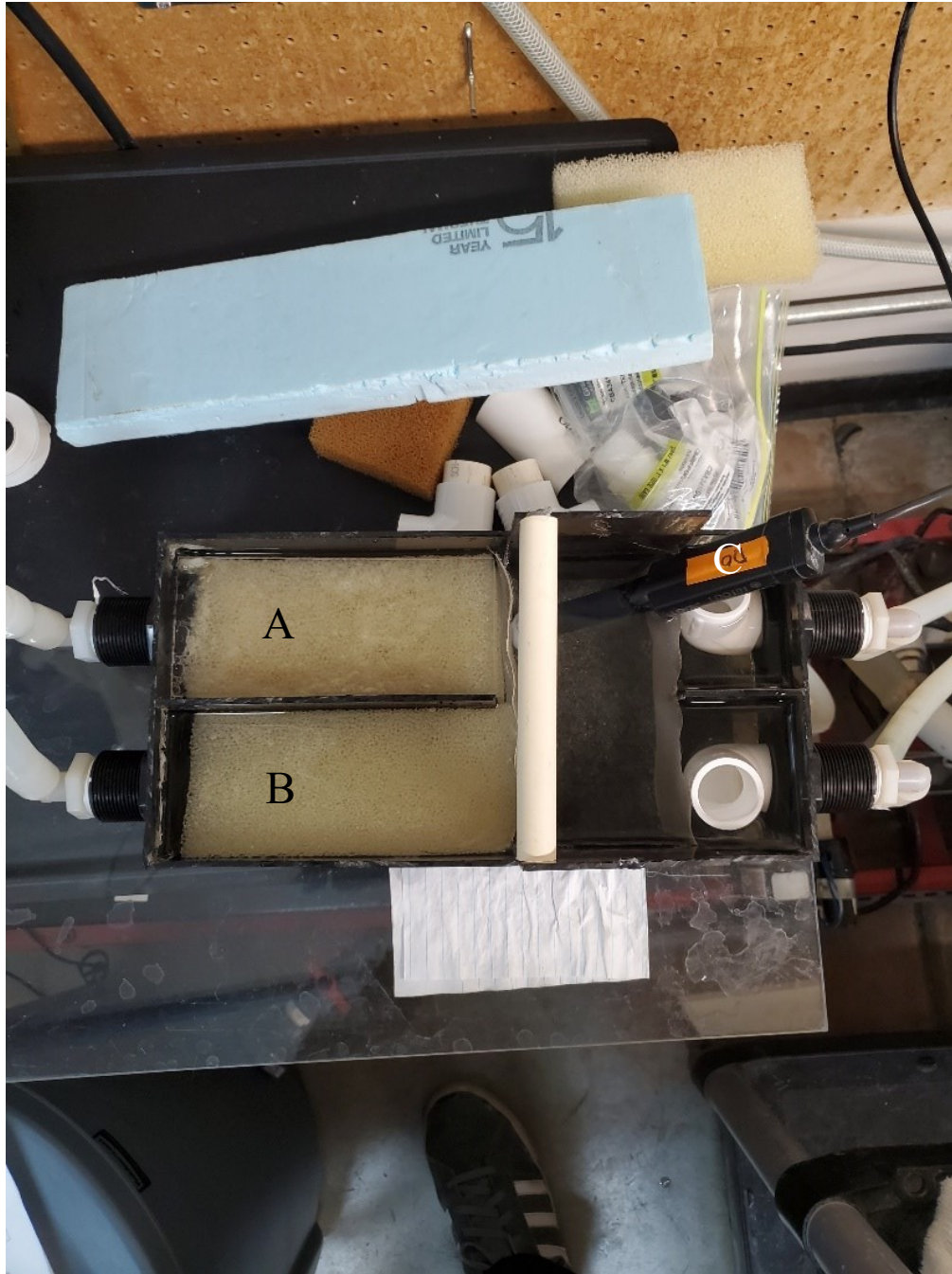


Figure 8. Choice flume for dissolved oxygen trials. Hypoxic (A) and normoxic (B) water enter the two sides of the flume from the left and pass through foam blocks that rectify flow. The two flows then pass through the test section, which was contained by a flow-through basket of fine mesh containing substrate. A DO probe (C) is visible in the test section. The water exits through standpipes at right and returns to an aerated, thermostatted reservoir.

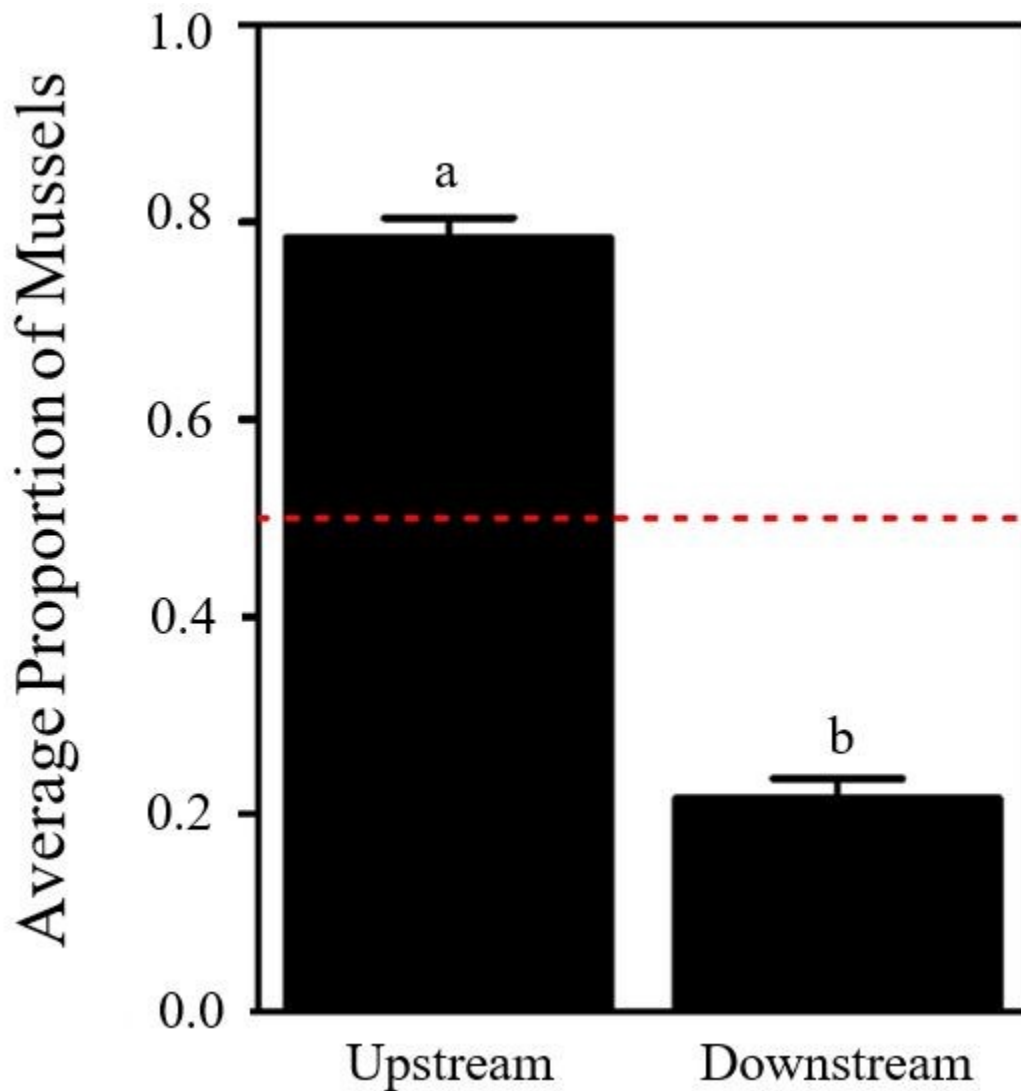


Figure 9. Average proportion of mussels found upstream versus downstream. The dashed line indicates the proportion of mussels expected given two equivalent choices. Different letters above bars indicate significant differences at $\alpha < 0.01$ (Bonferroni correction). Error bars indicate \pm standard error.

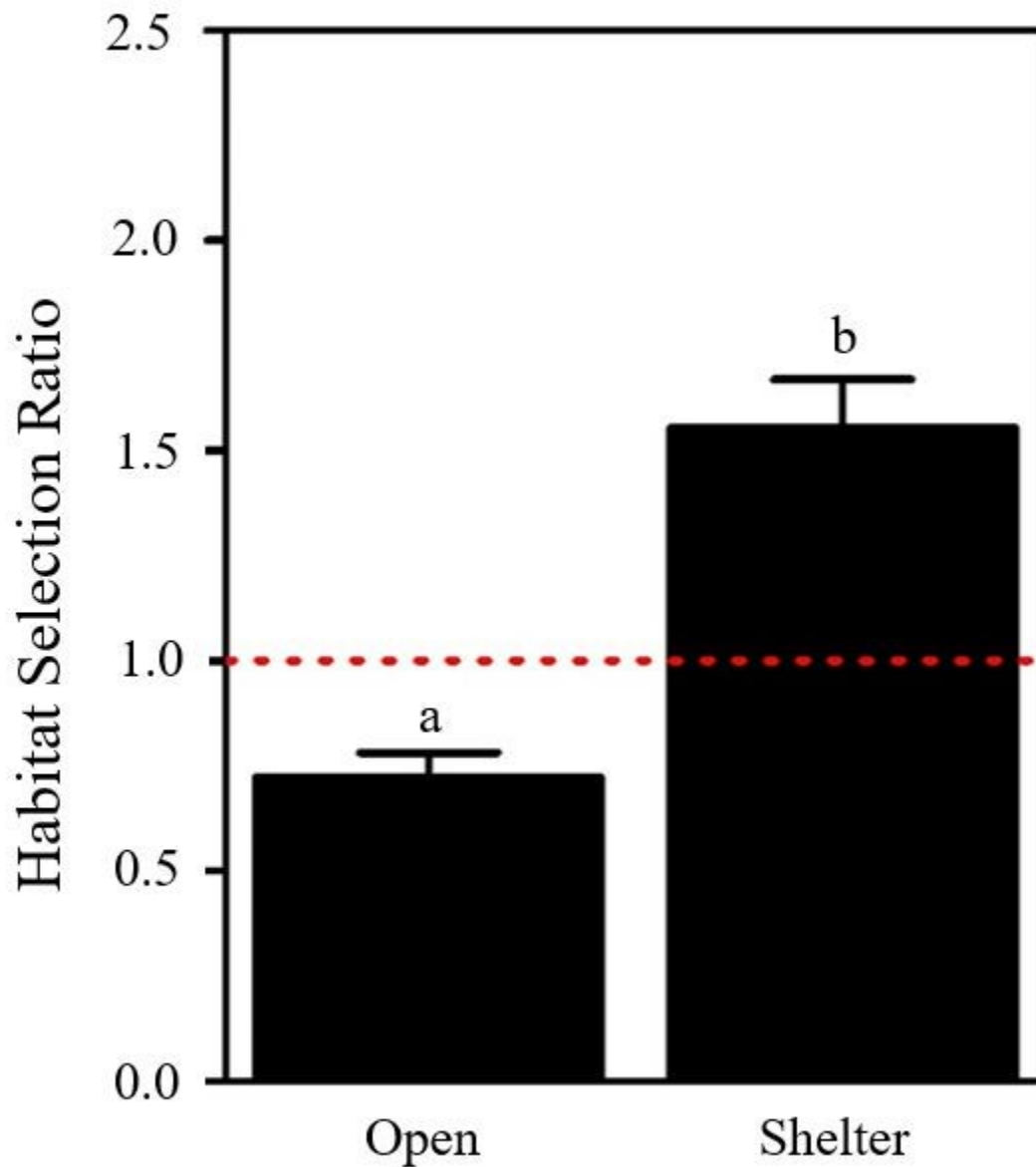


Figure 10. Position in the open versus under shelters. Habitat selection ratio estimated by dividing the proportion of mussels in the open or under shelter by the respective fraction of area in the arena. The dashed line indicates the proportion of mussels expected given relative ratios. Different letters above bars indicate significant differences at $\alpha < 0.01$ (Bonferroni correction). Error bars indicate \pm standard error.

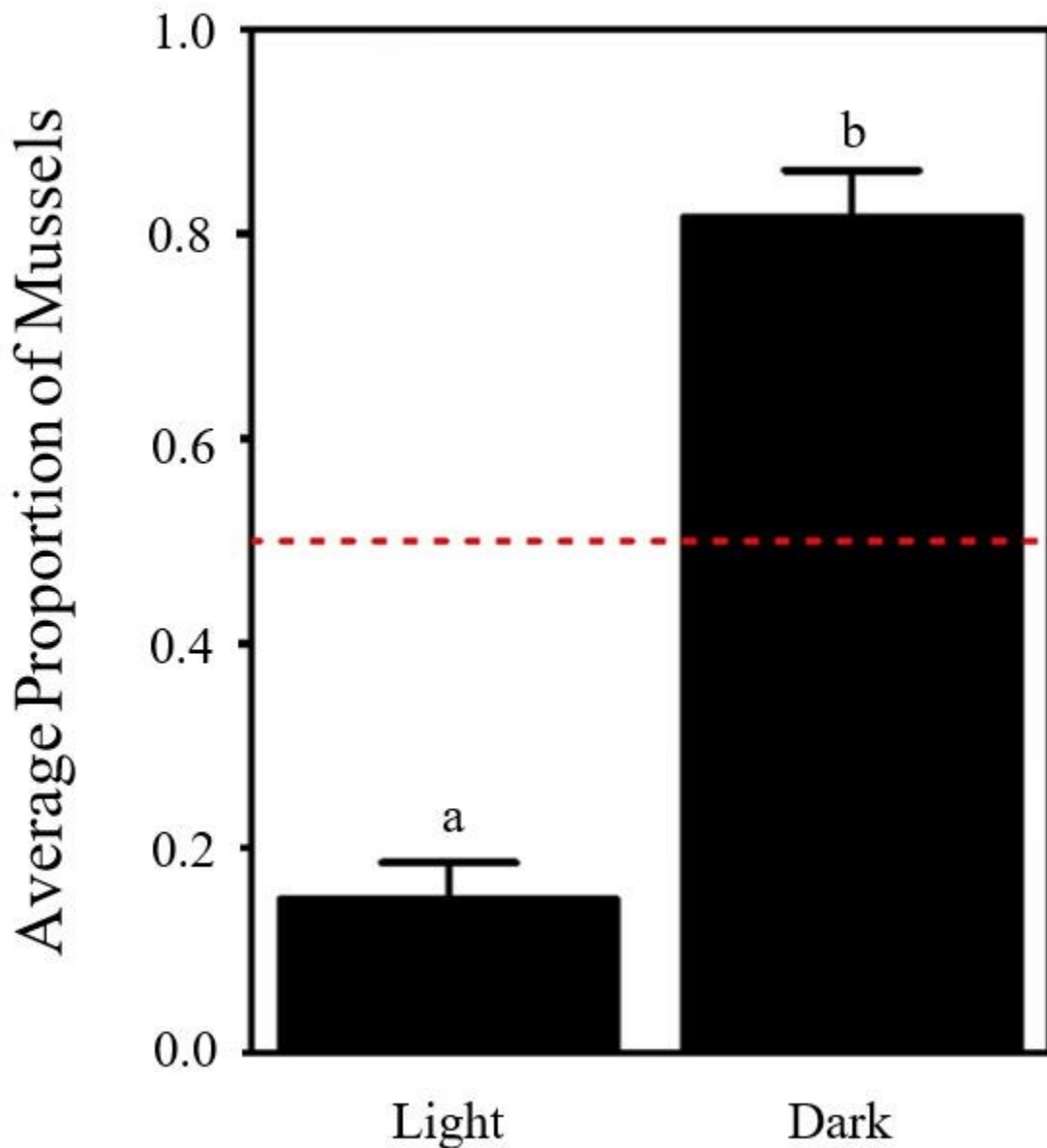


Figure 11. Average proportion of mussels under clear (light) versus opaque (dark) shelters. The dashed line indicates the proportion of mussels expected given two equivalent choices. Different letters above bars indicate significant differences at $\alpha < 0.01$ (Bonferroni correction). Error bars indicate \pm standard error.

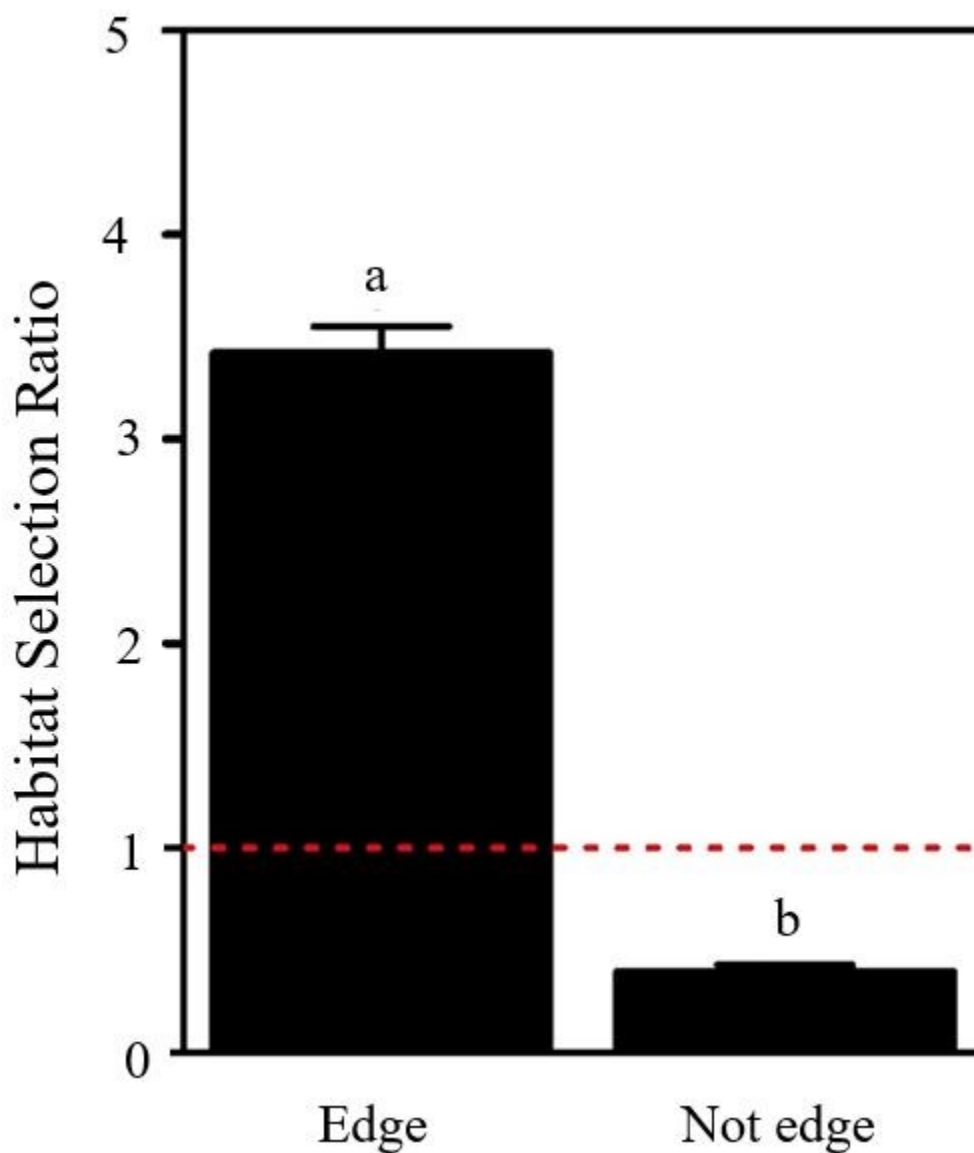


Figure 12. Mussel position at the edge (within 1.5 cm of the side of the arena) versus other areas. Habitat selection ratio estimated by dividing the proportion of mussels along the edge or not by the respective fraction of area in the box. The dashed line indicates the proportion of mussels expected given relative ratios. Different letters above bars indicate significant differences at $\alpha < 0.01$ (Bonferroni correction). Error bars indicate \pm standard error.

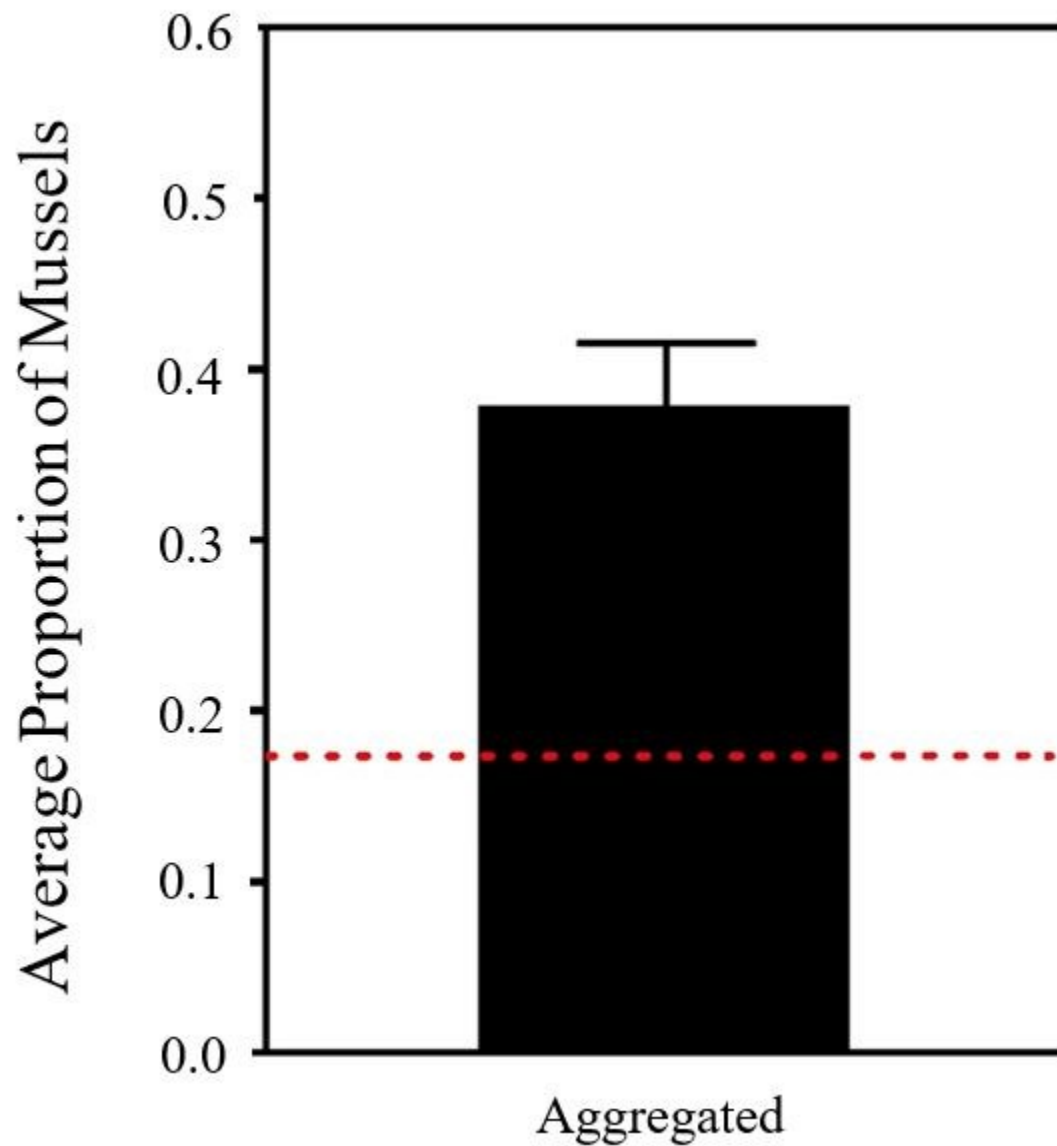


Figure 13. Average proportion of mussels aggregating (within 1.5 cm of another mussel). The expected proportion (dashed line) was found from Monte Carlo simulations accounting for how often mussels of the tested body size would encounter one another given the dimensions of the choice arenas. Error bars indicate \pm standard e

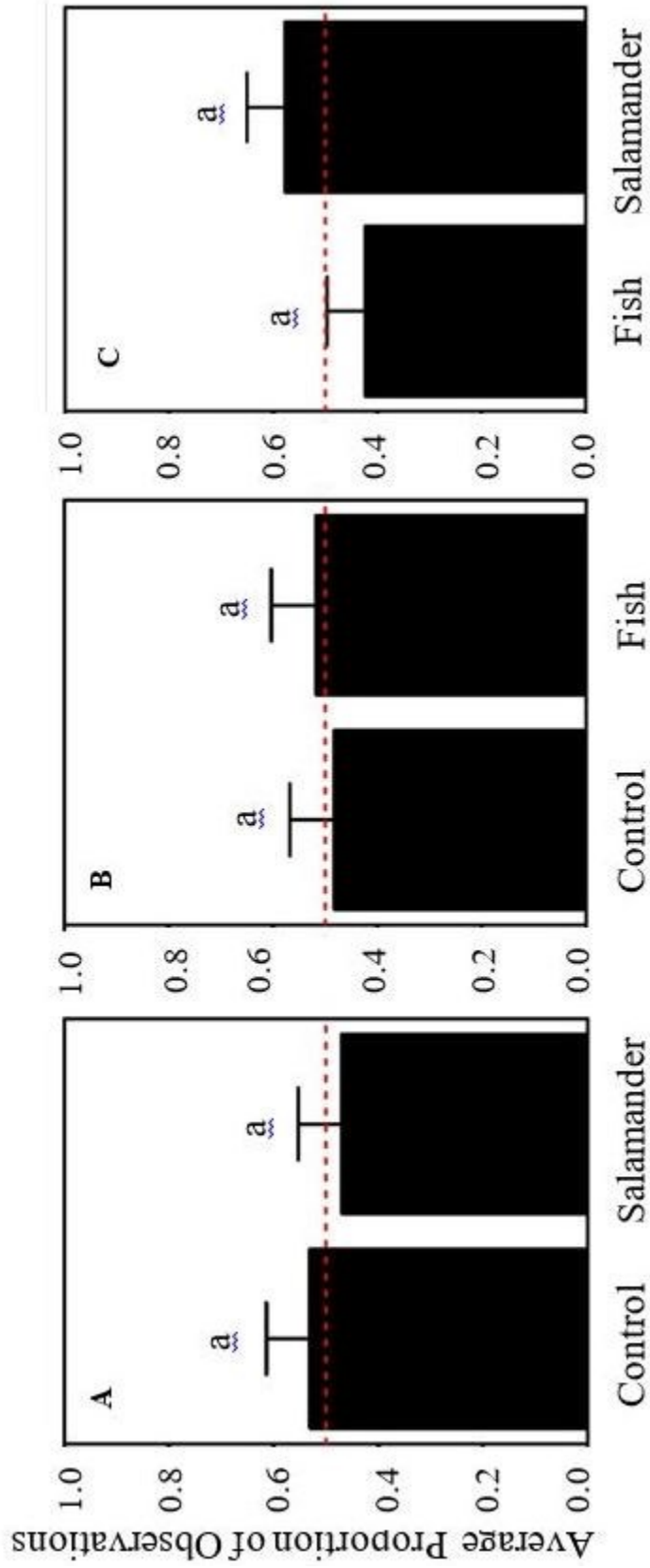


Figure 14. Average proportion of observations of mussels found in either a) control water or salamander scent cue water, b) control water or fish scent cue water, c) fish scent cue water or salamander scent cue water. The dashed line indicates the proportion of mussels expected under random circumstances. Different letters indicate statistically different results. Error bars indicate +/- standard error.

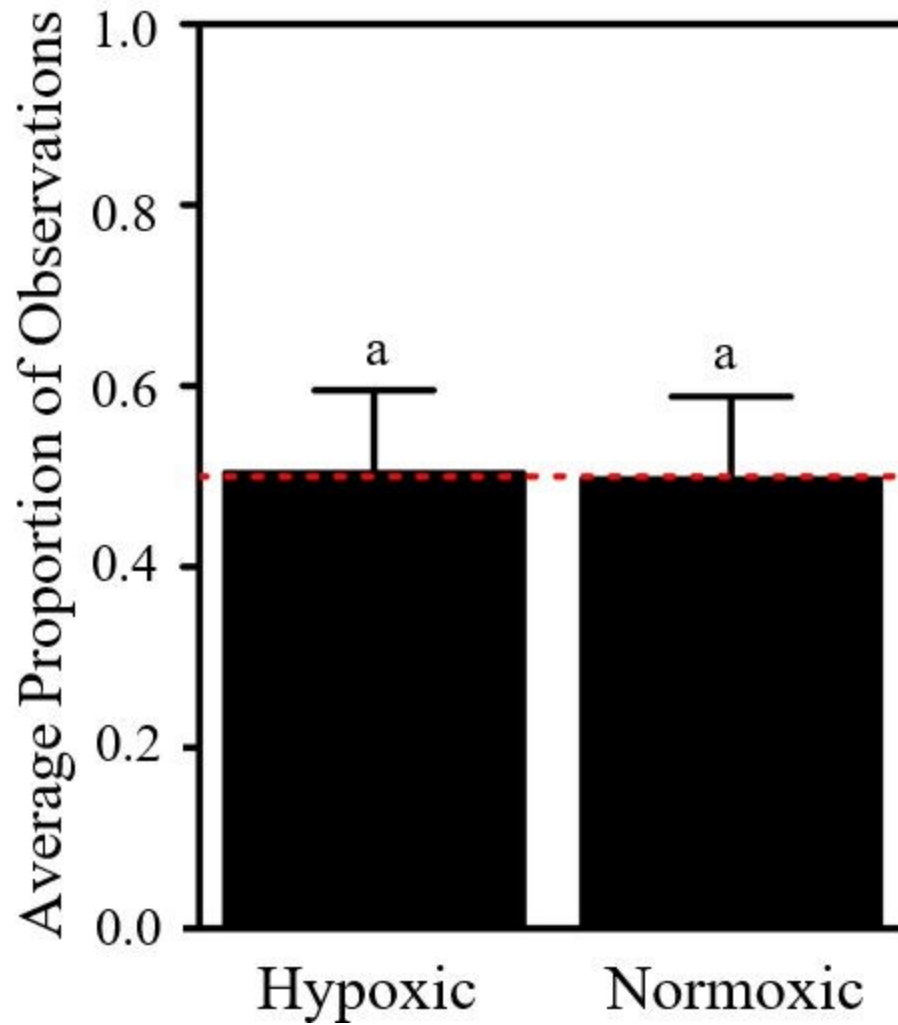


Figure 15. Average proportion of observations of mussels found in hypoxic or normoxic water at 14-16C. The dashed line indicates the proportion of mussels expected given two equivalent choices. Different letters above bars indicate significant differences. Error bars indicate +/- standard error.

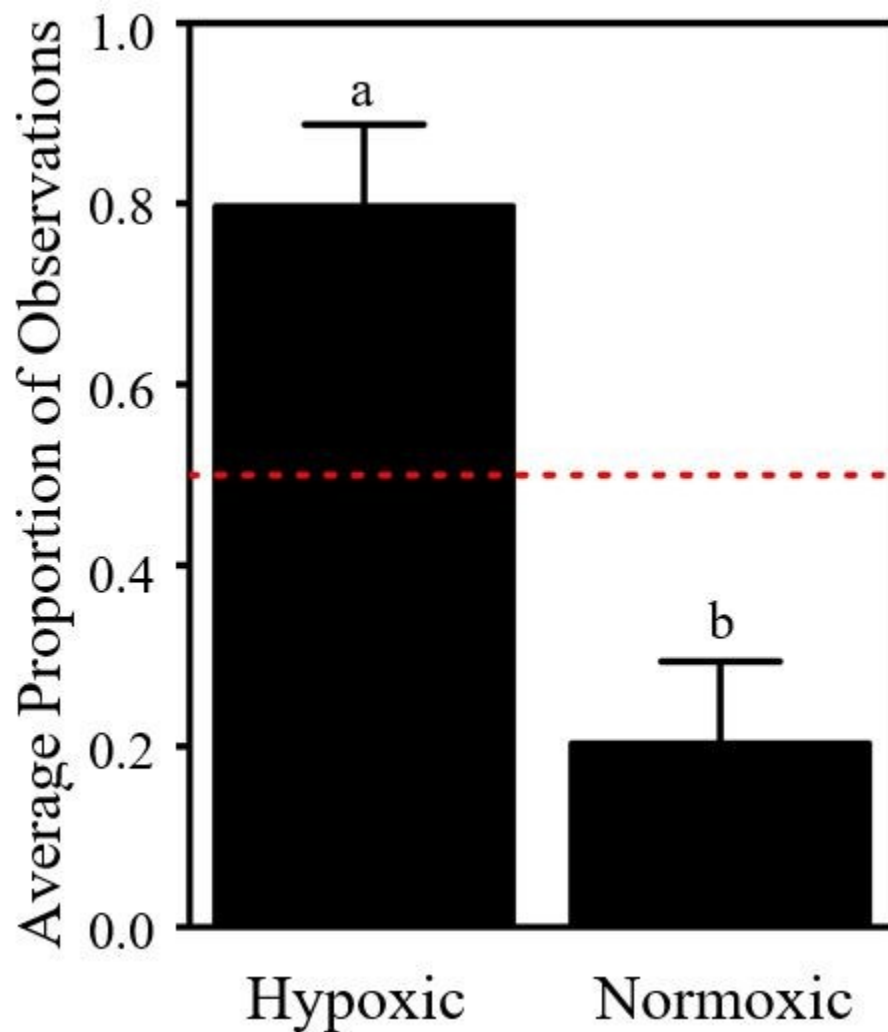


Figure 16. Average proportion of observations of mussels found in hypoxic or normoxic water at 21-22C. The dashed line indicates the proportion of mussels expected given two equivalent choices. Different letters above bars indicate significant differences. Error bars indicate \pm standard error.

APPENDICIES



Completion Date 01-Nov-2017
Expiration Date 31-Oct-2020
Record ID 24966689

This is to certify that:

Eric Stegmann

Has completed the following CITI Program course:

Animal Care and Use (Curriculum Group)
Researcher - Wildlife (Course Learner Group)
1 - Basic Course (Stage)

Not valid for renewal of certification through CME. Do not use for TransCelerate mutual recognition (see Completion Report).

Under requirements set by:

Missouri State University



Verify at www.citiprogram.org/verify/?w0c414cc3-4d5c-4c3c-82cf-34844050d1bc-24966689



Completion Date 06-Feb-2019
Expiration Date N/A
Record ID 30474749

This is to certify that:

Eric Stegmann

Has completed the following CITI Program course:

Working with Amphibians in Research Settings (Curriculum Group)
Working with Amphibians (Course Learner Group)
1 - Basic Course (Stage)

Not valid for renewal of certification through CME. Do not use for TransCelerate mutual recognition (see Completion Report).

Under requirements set by:

Missouri State University



Verify at www.citiprogram.org/verify/?wdb71b32d-7bac-4f24-af18-e08782166907-30474749



Completion Date 01-Nov-2017
Expiration Date N/A
Record ID 24966690

This is to certify that:

Eric Stegmann

Has completed the following CITI Program course:

Working With Fish in Research Settings (Curriculum Group)
Working with Fish (Course Learner Group)
1 - Lab Animal Research (Stage)

Not valid for renewal of certification through CME. Do not use for TransCelerate mutual recognition (see Completion Report).

Under requirements set by:

Missouri State University



Verify at www.citiprogram.org/verify/?wdad26815-7b87-4f99-9000-e0d2cde3bae2-24966690

Appendix A. Approved research compliance training to work with wildlife, amphibians, and fish.



DATE: Thursday, April 18, 2019

TO: Christopher Barnhart, Biology (Principal Investigator)
Eric Stegmann, Biology graduate student

FROM: Brian Greene, IACUC Chair

SUBJECT: IACUC inquiry – salamander mussel study

The IACUC has determined that your project entitled “Habitat selection and host detection in the salamander mussel, *Simpsonaias ambigua*” does not require IACUC oversight because the research does not involve manipulation of a live vertebrate animal. By documenting the IACUC’s decision this memo is intended to satisfy Graduate College requirements for IACUC approval of thesis projects involving animal research. Please keep in mind that if the project design changes to include live vertebrates, the IACUC must be notified to determine if the project changes warrant oversight.

Please contact IACUC Chair, Brian Greene (briangreene@missouristate.edu) if you have any questions.
Appendix B. IACUC waiver.