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Mariah Slaughter

Western Kentucky University, mariah.slaughter228@topper.wku.edu

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AN INVESTIGATION ON THE COVER PREFERENCE OF THE MOUNTAIN

MADTOM (*NOTURUS ELEUTHERUS*)

A Capstone Project Presented in Partial Fulfillment
of the Requirements for the Degree Bachelor of Science
with Mahurin Honors College Graduate Distinction at
Western Kentucky University

By

Mariah Slaughter

May 2020

CE/T Committee:

Dr. Philip Lienesch, Chair

Dr. Michael Stokes

Ms. Cheryl Kirby-Stokes

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ABSTRACT

Madtom catfish, members of the genus *Noturus*, are common in the waters of the Southeastern US. A previous study observed that madtoms in the Green River, Kentucky, preferred to shelter within old mussel shells compared to under or next to rocks. A laboratory study on the Carolina Madtom (*Noturus furiosus*), found that they did not utilize mussel shells and preferred rocks as cover. I conducted a similar laboratory study to determine which cover options the Mountain Madtoms (*Noturus eleutherus*) prefer. Cover preference was determined by offering the madtoms shelter options (rocks or mussel shells) in 10-gallon aquaria. After the animal had acclimated to the tank for 24 hours the tank was inspected, and the animal's shelter choice recorded. I found that Mountain Madtoms selected to use the mussel shells over the rocks. Based on these results I conducted a second experiment to see if shell orientation impacted selection. I found that Mountain Madtoms preferred shell orientations with dorsal coverage to those without. Freshwater mussels are one of the most endangered taxa and are currently declining throughout their range. If madtoms rely on mussel shells for cover, the loss of freshwater mussels may cause a decrease in madtom populations within Kentucky waterways, negatively impacting the overall ecosystem.

ACKNOWLEDGEMENTS

I would like to thank Dr. Lienesch for his leadership, advice, and assistance throughout this project. Dr. Stokes for his input and help with all my statistics questions. Ms. Kirby-Stokes for her insightful comments while reviewing my work. Cole Clark and Nate Mattingly for their efforts in collecting the fish used in this project. The Beta Beta Beta National Biological Honors Society and the Mahurin Honors College for the financial assistance. Lastly, I would like to thank my parents, who supported me throughout this project as they have in all of my educational endeavors.

VITA

EDUCATION

Western Kentucky University, Bowling Green, KY May 2020
B.S. in Biology – Mahurin Honors College Graduate
Honors Capstone: *An Investigation on the Cover Preference of the Mountain
Madtom (Noturus eleutherus)*

Lafayette Senior High School, Lexington, KY May 2016

PROFESSIONAL EXPERIENCE

Student Support Services, WKU Sept 2018-
Tutor and Student Worker Present

Eastland Animal Clinic LLC, Lexington, KY May 2017-
Veterinary Assistant Aug 2018

AWARDS & HONORS

Biology Department Outstanding Biodiversity Student Award, WKU, Spring 2020
Mahurin Honors College Honors Development Grant, 2019
Herbert Boschung Student Travel Award, 2019
Dr. Thomas Alan Yungbluth Travel Abroad Scholarship, Summer 2018
Beta Beta Beta Research Grant, 2018

PROFESSIONAL MEMBERSHIPS

Beta Beta Beta Biological Honors Society
Association of Southeastern Biologists
Southeastern Fishes Council
Mahurin Honors College

INTERNATIONAL EXPERIENCE

Hoedspruit, South Africa June 2018
Faculty-led Study Abroad

PRESENTATIONS

Slaughter, M. and Philip Lienesch. An Investigation on the Cover Preference of the Mountain Madtom (*Noturus eleutherus*). Southeastern Fishes Council Annual Meeting. 2019.

Slaughter, M. Determination of Madtom Shelter Preference. Beta Beta Beta Southeastern Regional Conference. 2019.

Slaughter, M. Determination of Madtom Shelter Preference. Western Kentucky University Student Research Conference. 2019.

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INTRODUCTION

All living organisms have physiological needs that must be met for continued survival. From the energy required to complete cellular respiration to the micronutrients needed for growth, most components required to fulfill a need are found in the environment. These components can vary greatly among species. A Table Mountain pine (*Pinus pungens*) needs light for photosynthesis to meet its energetic demands (Welch et al., 2000) while an eastern mole (*Scalopus aquaticus*) eats non-insect arthropods to meet comparable requirements (Feldhamer et al., 2003). While specific nutritional requirements exist, the surrounding environment, as defined by biotic and abiotic factors, plays a larger role in success of organisms. Even when all physiological needs have been met, these larger considerations may prevent a population from taking root. Some common biotic factors, those resulting from other organisms, like interspecies competition and predation can prevent the establishment of a population (Jackson, Peres-Neto, & Olden, 2001).

The distribution of a species is also affected by abiotic variables in the environment, those variables independent of the other organisms (Law & Dickman, 1998). For example, seeds of *P. pungens* will not be released from their cones unless a fire disturbance occurs, so individual distribution within the larger system is dependent on periodic fires (Welch et al., 2000). Climatic abiotic factors, like temperature range or relative humidity, can also impact population establishment and are often discussed as aspects of habitat. Habitat requirements, as used in some scientific literature, refer to the

abiotic factors necessary for the persistence of an individual or population (Rosenfeld, 2003). These analyses do not consider interspecies interactions, like competition and predation, shifting the focus to the physical environment.

Habitat requirements go far beyond temperature and relative humidity, they can also include the geography of an area, soil composition, or annual weather patterns (Law & Dickman, 1998). These physical features are often uniform across a larger area, the macrohabitat, but will change as the observer's perspective narrows (Rosenfeld, 2003). Therefore, the defined habitat requirements are dependent upon the scale of measurement. For example, if studying a population of stream fish, the required habitat is described as stream features and niches (Gorman & Karr, 1978; Moyle & Baltz, 1985; Rosenfeld, 2003). Papers describing life history focus on physical, abiotic features like water temperature, dissolved oxygen, and stream velocity (Chan & Parsons, 2000; Jackson et al., 2001). Studying individuals within that population requires a different scale of focus. The presence of certain microhabitats, the characteristics within a smaller area of the stream, like a riffle or pool (Grossman & Freeman, 1987), may also be required for certain species to be present. When considering individuals, this is often conceptualized using habitat preference which looks at positive and negative selection of microhabitat characteristics (Rosenfeld, 2003), rather than the requirements for survival. Characteristics, for example cover, with greater positive selection may result in a higher individual fitness, but overall survivorship of the population is not impacted by either selection (Sogard, 1994).

Order Siluriformes includes the catfishes and is among the most diverse orders of extant fishes, with 3,093 distinct species (Ferraris 2007). Initially named for their

diagnostic barbels, which look like cat whiskers, these fish are found in marine, brackish, and freshwater habitats of all continents except Antarctica (Nelson et. al, 2016). Most catfishes are omnivorous and, unlike most freshwater fish, rely on senses other than sight as they are nocturnal (Nelson et. al, 2016). Electroreceptive systems and chemoreceptive or tactile barbels are common in these fish, enabling them to navigate in the absence of light. North American freshwater catfish are members of the family Ictaluridae, with a geographic distribution from Southern Canada to Guatemala (Nelson et. al, 2006). They can be identified by the lack of scales and the presence of eight distinctive barbels.

Madtoms, members of the genus *Noturus*, are the smallest North American catfish (Etnier, 1993). There are 26 described species of madtoms based on a combination of morphological characters and mitochondrial DNA sequencing (Egge & Simons, 2006). Madtoms are characterized by having the adipose fin fused to the caudal fin and members of the largest species grow up to 125 mm (Etnier and Starnes, 1993). Like most catfish, madtoms are nocturnal and have been observed using rock crevices and interstitial substrate space to hide during the day (Starnes and Starnes, 1985). Some species, like the Carolina Madtom (*Noturus furiosus*), are associated with specific physical features within the microhabitat. In the case of *N. furiosus*, they are cover-associated (Midway et al., 2010a), meaning they show differential occupancy, seeking out cover options and demonstrating positive selection (Chan & Parsons, 2000). Cover-seeking behavior is present in other species of madtoms (Mayden et al., 1984; Tiemann et al., 2011; Starnes and Starnes, 1985) and has been documented in the Green River in Kentucky (Brumley, 2018).

Although there is evidence for a strong cover-association in the genus *Noturus*, the habitat requirements of the species are not universal. Average sizes range from 36.6 mm to 100 mm as full-grown adults, suggesting that different species do not share specific cover preferences (Bennett et al., 2009; Midway et al., 2010a). This speculation is brought to light in Midway et al. (2010a) who dispute anecdotal evidence of *N. furiosus* using dead mussel shells as cover in a laboratory behavioral study. *Noturus furiosus* is one of the larger madtom species (Midway 2008). If on average mussel shells are 117 mm long, like the ones used in this study, they would not provide adequate cover for a *N. furiosus* individual as a previous study by Midway (2008) found breeding bulls with a total length of 101 mm. A snorkel survey in the Green River, KY, found most madtoms sheltering in the shells of dead mussels (Brumley, 2018). The majority of observed madtoms were Brindled Madtoms (*Noturus miurus*), but Mountain Madtoms (*Noturus eleutherus*) and Elegant Madtoms (*Noturus elegans*) were also observed. All three of those species are, as full-grown adults, smaller than *N. furiosus* (Etiner, 1993; Midway, 2008). The Brumley (2018) field study does not agree with the results of the only laboratory study on the madtoms shelter preference (Midway et al., 2010a).

This study sought to determine the cover preference of the Mountain Madtom (*N. eleutherus*) when given the option of an empty mussel shell or a large rock of similar shape and size. The experimental design is based on the Midway et al. (2010a) study, but the species in question is small enough to utilize empty mussel shells. They have been observed by WKU students using dead mussel shells in the Green River, the waterway in Kentucky with the greatest population and diversity of mussels and madtoms (Brumley 2018). Based on those observations it was hypothesized that *N. eleutherus* individuals

would use the mussel shells more frequently than rocks. The results of the first study demonstrated a preference for mussel shells, so I then tested whether *N. eleutherus* individuals prefer mussel shells with a specific orientation. Three mussel shell orientations were used, two which provided dorsal coverage, and the third which did not. Based on the cover-seeking tendencies of madtoms I hypothesized that there would be a preferred orientation, likely the orientations that provided dorsal coverage.

METHODS AND MATERIALS

Collection

Mountain Madtoms (*Noturus eleutherus*) were collected in the Green River at the Western Kentucky Biology Preserve during September 2018 and August 2019. Fish were located by examining rocks and mussel shells while snorkeling and then captured with small nets. Following collection, specimens were acclimated to the laboratory in a 55-gallon freshwater tank with a fine gravel substrate (0.5-2.0 mm) and cover options of small rocks and empty mussel shells wedged open to allow internal access.

Cover Selection Experiment

This study design was based on the procedures used in Midway et al. (2010a). Five, 10-gallon aquaria were established with fine gravel substrate (0.2-0.5 mm) and dechlorinated tap water. Each tank was seeded with some water from the 55-gallon acclimation tank to aid in the establishment of a healthy microbiome. Activated charcoal pump filters were set up in the middle of each tank so the water flow would be similar on both ends of the tank. Each aquarium was wrapped in black plastic to create an isolated environment with limited visual input. Lights were fixed above the five tanks, set to a 12-hour photoperiod beginning at 5 AM local time.



Figure 1 This image shows the experimental set up used in both experiments.

During the first experiment, a rock and mussel shell were placed at the opposite ends of each experimental tank and their locations were randomized prior to each trial. The cover object (rock or shell) was placed equidistant from the sides of the tank (i.e. not against the glass wall). The rock was always placed with the same side down. Mussel shells were always positioned laying horizontally with a small opening between the valves to allow the madtom access inside the shell. A madtom was selected, at random, from the large 55-gallon holding tank and placed in the tank for approximately 24 hours. At the conclusion of 24 hours, the cover selection was observed and recorded, then the specimen was moved to the next experimental tank. After each fish was tested in each experimental tank with its unique rock/ mussel shell combination, it was placed into a secondary holding tank with cover options and large pebbled substrate. This procedure was completed for all 15 specimens, seven in the Fall of 2018 and eight in the Fall of 2019.



Figure 2 An example of the "horizontal" mussel shell position used in both experiments

Mussel Shell Orientation Experiment

Based on the results of the first experiment showing that mountain madtoms selected mussel shells over most rocks, a secondary experiment was conducted. It tested the hypothesis that specimens would have no preference among three mussel shell orientations.

For this, I selected three shell orientations relative to the substrate: laying horizontally on the substrate (horizontal; Figure 2), with the ventral margin buried in the substrate (tented; Figure 3), and with the hinge buried in the substrate (open; Figure 4). The first and second orientations offer dorsal coverage while the third does not. Three of the five 10-gallon tanks were used for this experiment, and shells of similar dimensions (± 5.0 mm long) were paired for comparisons. The first tank compared the tented and horizontal orientations, the second compared the horizontal and open orientations, and the third compared the tented and open orientations. Placement and orientation of the shells within each tank was randomized before each specimen was placed in the tank.



Figure 3 An example of the "tented" orientation used in this experiment



Figure 4 An example of the "open" orientation used in this experiment

This experiment used 16 individuals, eight in the Fall of 2018 and eight in the Fall of 2019. Each fish was given approximately 24 hours to acclimate to the tank, and then its cover selection was observed and recorded. Once a fish had been tested in all three experimental tanks, it was placed into a secondary holding tank until the conclusion of the experiment. All fish were then euthanized by IACUC standards and added to the Western Kentucky University Ichthyology Collection.

Statistical Methods

The data collected during this study were counts, a type of nominal data, so I used non-parametric tests to analyze my data (Zar, 2009).

Since the data were collected over the course of two collection seasons, I first created two General Linear Models (GLM) to compare the data between years. The first

GLM compared the results of the Cover Selection Experiment between collection seasons. The second GLM compared the results of the Mussel Shell Orientation Experiment between collection seasons. Neither of these GLMs were significant so I proceeded to analyze the data as previously determined.

In order to analyze the results of the first experiment, which tested cover selection, I created a General Linear Model (GLM) which is a more flexible version of a linear regression, allowing for non-normal distributions. I used the GLM to determine if the different experimental tanks impacted cover selection. Although the experimental design compared two cover options, rock and mussel shell, there were four instances of no cover selection, wherein the madtom swam around the tank. These were included in the GLM, creating three cover options since the possibility was not discussed *a priori*. I constructed the GLM using RStudio.

As the GLM was not significant I failed to reject my null hypothesis that difference in selection is impacted by experimental tank. I then randomly selected one of the five experimental tanks using a random number generator to avoid pseudo replication. Following tank selection, I performed a Binomial Test to determine the probability of random cover selection (Zar, 2009). For this test I only used the selections of either cover option, ignoring any freely swimming data points, as defined *a priori*. This test was run using RStudio.

Once the binomial test was complete, I elected to perform a G-test of Goodness of Fit to illustrate the preference for mussel shells. Due to the experimental design, this is pseudo replication, so I did not draw any conclusions from the analysis. However, I felt

the inclusion of this test suggests that the result seen in the binomial test would be the norm if this experiment were repeated.

In order to analyze the results of the second experiment, which tested mussel shell orientation, I conducted Binomial Tests for each tank. This allowed us to calculate the exact probability that cover selection was random when given the option of different mussel shell orientations (Zar, 2009). I conducted these tests using RStudio.

RESULTS

Cover Selection Experiment

Madtoms were observed using both cover options throughout the experiment. Additionally, there were four occurrences where neither cover option was used and the madtom was swimming around the tank at time of observation (Table 1). Based on result of the first GLM comparing the data collected between years ($p=0.7947$) I proceed to analyze the rest of the results.

Table 1 *Madtom cover selection as seen in the first laboratory experiment comparing rocks to dead mussel shells. Throughout the whole experiment there were four occurrences where a fish chose neither cover option (freely swimming).*

Tank	Selection of Rock	Selection of Shell	Freely Swimming
1	0	12	3
2	0	14	1
3	4	11	0
4	7	8	0
5	5	9	0
Total	16	53	4

Although the mussel shell was selected with a higher rate of occurrence, the differences between experimental tanks was larger than anticipated. In order to determine if experimental tank had an impact on selection, I created a General Linear Model (GLM) using RStudio. The model resulted in an F-statistic of 0.6596 on 1 and 72 degrees of freedom, with a p-value of 0.4194. With this result I fail to reject my null hypothesis that there is a difference in selection based on experimental tank.

After establishing no difference in selection based on experimental tank, I randomly selected one tank to analyze using the Binomial Test. Eliminating the four

other tanks prevented pseudo-replication, as the same fish were used throughout the experiment. Successes within the Binomial Test were defined as the shell cover selections and the probability of selection was 0.5. This test was significant with a p-value of 0.00012.

I then performed a G-test of goodness-of-fit to illustrate the frequency of selection for all trials combined. Using only the two defined cover options, mussel shell and rock, I found a significant p-value of less than 0.0001.

Mussel Shell Orientation Experiment

Once I confirmed that Mountain Madtoms demonstrated a cover preference, I conducted the second experiment to determine if mussel shell orientation impacts cover selection. This tested the hypothesis that shell orientation impacted usage of mussel shells as cover. Prior to analyzing the data of that experiment, I created the second GLM comparing data collected between the years ($p=0.16$), and then proceeded to analyze the data. I used a Binomial Test again to determine the probability that cover selection was chosen randomly (Table 2).

Table 2 Cover selection by 16 Mountain Madtoms in an experiment comparing mussel shell orientations. These orientation combinations were tented vs horizontal, horizontal vs open, and tented vs open. The calculated Binomial Probabilities are included for each tank which show the exact probability of random cover selection.

Tank (Var. 1 vs Var. 2)	Var. 1 Selection	Var. 2 Selection	Binomial Probability
1 (Tented vs. horizontal)	8	8	1.0
2 (Horizontal vs. open)	14	2	0.00418
3 (Tented vs. open)	15	1	0.00052

Two of the three tanks demonstrated a highly significant probability of non-random cover selection. Tank 2, which paired the horizontal and open orientations, had a

binomial probability of 0.00418 and Tank 3, which paired the tented and open orientations, had a binomial probability of 0.00052. In comparison, Tank 1 had equal selection of the tented and the horizontal orientations.

DISCUSSION

Based on this behavioral study, Mountain Madtoms will utilize dead mussel shells as cover more often than rocks, demonstrating differential occupancy. The null hypothesis that there would be no cover preference was rejected in favor of the alternative hypothesis, that there would be a cover preference. This is the first test of cover preference in Mountain Madtoms in the laboratory setting. My data supports the conclusion of Brumley (2018) that Mountain Madtoms selectively use mussel shells for shelter during the daytime. In addition, I found that the orientation of the shells had an influence on shell preference. Mountain Madtoms selectively used mussel shells that provided dorsal coverage. With these data, I rejected my second null hypothesis that shell orientation did not impact cover selection in favor of the alternative hypothesis, that mussel shell orientation does impact cover selection.

Brumley (2018) sought to determine if Madtom catfish preferred to shelter in mussel shells or rocks in the Green River of Kentucky. He conducted snorkel surveys in the fall of 2016 and 2017 at four different sites along the main upper Green River channel. At each site he set up three plots within the riffle microhabitat, which has been commonly documented as preferred microhabitat of Madtoms (Mayden et al., 1980; Wells, 2019). He found that the observed Brindled, Elegant, and Mountain Madtoms all used the mussel shells as cover more frequently than the rocks. This study directly tested Brumley's results with greater control of other environmental characteristics, limiting the factors that could have impacted the results.

Prior to this study, the only laboratory cover preference studies with Madtoms were by Midway et al. (2008, 2010a) on endangered Carolina Madtoms (*Noturus furiosus*). In laboratory experiments, Carolina Madtoms did not utilize mussel shells as cover (Midway 2008, 2010a), but instead choose artificial shelter, rocks, and leafpacks. One possible explanation for the difference in behavior between Carolina Madtoms and Mountain Madtoms is the size of the individuals. The Carolina Madtom is one of the larger species in the genus and can be up to two times the length of the Mountain Madtoms and most other species of *Noturus* (Etiner, 1993; Midway, 2008). In my experiment, I used large shells which are readily available in the Green River, Hart County, KY. Due to the size of the available shells in the Green River, even the largest adult Mountain Madtoms captured easily fit inside the shell cavity, or under the edge of the shells oriented horizontally on the bottom. The average size of adult Carolina Madtoms is 100 mm (Midway, 2008) so it would be harder for them to fit inside of one of the shells used in my experiment which were on average 117.2 mm long.

Although my experiment affirmed the cover-seeking tendency of Mountain Madtoms, the ratios of cover selection between the experimental tanks varied and I was concerned that aspects of each cover option may have caused a difference in selection. During the setup of this experiment I had prioritized using rocks and mussel collected from local rivers, which left me with a limited selection of cover options. To the best of my ability I matched the cover options based on size but, two rocks were larger than the other cover options. Using a General Linear Model, I found no significant difference in cover selection between tanks. This result allowed me to treat all experimental tanks as equal in selection probability and randomly select tanks for statistical analysis. I then

randomly selected the second experimental tank to perform a Binomial Test using shell cover selection as a ‘success’. As the result of this test was significant, I concluded that cover selection was not random. Based on this test I elected to perform a G-test of Goodness of Fit to look at overall trends. I am not using the results of this analysis to draw conclusions, only to illustrate the degree of cover selection preference. These results were also significant, which I interpreted to support my rejection of the null hypothesis.

Prior to the Brumley (2018) study, Mountain Madtom selection for empty mussel shells was never noted in the literature, only the crevice-seeking tendencies (Midway et al., 2010b). Individuals interested in Madtom populations in rivers like the Green River of Kentucky can add selective dip netting to their sampling techniques to collect individuals utilizing mussel shells. Other sampling techniques, like seining or electroshocking will often leave these sheltered individuals undetected (Wagner et al., 2019). Nocturnal fish with cover-seeking tendencies can be overlooked during daytime samplings, resulting in an underestimated population, so the identification of ideal cover will make practices like snorkeling surveys more effective (Gibbs, Miller, Throneberry, Cook, & Kulp, 2014). These results also emphasize how important freshwater mussels are to their ecosystems. Freshwater mussels are one of the most endangered freshwater taxa globally and are the focus of many conservation efforts (Strayer et al., 2004). Many freshwater mussels are known to require fish to complete their lifecycle- using parasitic larvae that attach to gills to facilitate greater distribution throughout the stream system (Tiemann, McMurray, Barnhart, & Watters, 2011). This study simply illustrates a previously undocumented facet to that relationship.

The second experiment, which tested the effect mussel shell orientation has on cover selection, established a preference for dorsal coverage. This aspect of the study, comparing shell orientations, has never been published. Although it seems self-explanatory that individuals with known crevice seeking behaviors selectively use dorsal protecting cover, it was previously undocumented. The results of this study can be used as a starting point for others looking to observe similar tendencies in lab studies.

The results of the binomial tests I performed on the three orientation comparisons imply that orientation plays a role in cover preference. The p-values of the binomial tests in Tanks 2 and 3 were highly significant, indicating that there was a strong cover preference. In comparison, when fish were provided two cover options that adequately protected the dorsal surface, as in Tank 1, there was no preference between the two cover options. With these results I reject my null hypothesis that mussel shell orientation has no impact on cover selection in favor of the alternative hypothesis.

One of the limitations to this study was the experimental design of the first experiment. Repeated use of the same madtom through all five experimental tanks created a pseudoreplicated dataset (Hurlbert, 1984), effectively establishing fifteen test subjects with five repeated tests of cover preference for each. This design made statistical analysis difficult, as many of the collected data could not be used. Replicating this study using only one individual per experimental tank and a larger number of individual madtoms would effectively remove the pseudoreplication and enable statistical analysis with greater power.

Another problem of this study was the possible collection period. The original intent was to collect more madtoms during the Fall of 2018 and include them in my

experiment. Rainfall in the region was greater than anticipated and the river rose above safe sampling height in September. It remained there until late in the Summer of 2019. Due to this unforeseen circumstance I had a smaller data set than planned and the experiment was continued into the Fall of 2019. Although it would have been preferable to conduct all the tests at once, preliminary tests for differences between the fall 2018 and fall 2019 datasets indicated that time had no significant influence on madtom behavior.

Through these experiments I established a differential occupancy with a positive selection for mussel shells as cover, but there are more questions that could be asked. Not all cover options are equal, some provide more accessible or complete coverage. Within this study I observed two rocks that were utilized as cover more frequently than the others. These were larger and had at least one side with greater accessibility to the substrate. One of the rocks had a protrusion half as thick as the rest of the rock, and fish were observed nestled beneath the overhang it created. Another rock had a decrease in thickness at one end, allowing madtoms to burrow underneath the rock with greater ease. Based on these and other observations, a study could be designed to determine if Mountain Madtoms exhibit a positive differential occupancy for certain rock characteristics. This could be further expanded to see if rock composition impacts differential occupancy. Within the Green River of Kentucky multiple rock types are present, primarily limestone, sandstone, and shale (Hess, Wells, Quinlan, & White, 1989). They are present in different watersheds and, if differential occupancy is impacted by stone composition, may be able to indicate ideal Mountain Madtom microhabitat locations.

Another aspect of this study that I did not explore was specimen age, as all my Mountain Madtoms were adults. It has been documented within the literature that stream fish microhabitat preference can shift based on age; as they grow, different features provide ideal cover (Grossman & Freeman, 1987). A study could be designed to test differential occupancy throughout the different life stages of a madtom. Within the madtom literature there is easily accessible information on nest sites and the common microhabitats used for breeding, but nothing on juvenile microhabitat selection. In addition to age, this study did not address the cover preferences of other madtoms endemic to the Green River. In the future, this study could be expanded, eliminating the pseudoreplication and expanding the species observed. The work of Brumley (2018) established there are two other madtoms present at my sampling site, the Brindled Madtom and the Elegant Madtom. An expansion of this study could determine if the differential occupancy observed in the fish used in this study is a specific to the species or more common among the genus *Noturus*.

In this paper, I have demonstrated that *Noturus eleutherus* from the Green River in Kentucky have a differential occupancy with respect to cover options. Individuals in this experiment selected empty mussel shells more often than rocks, and when provided the option, selected empty mussel shells with dorsal coverage. This knowledge of cover preference will help guide fish biologists when selecting sampling techniques to monitor Mountain Madtom populations. Without targeted sample locations, some common sampling techniques, like electroshocking and seining, will often miss fish under cover items or buried in the substrate (Wagner, Schumann, & Smith, 2019). Identifying ideal cover options within the microhabitat will allow samplers to target madtom populations if

needed. With this establishment of a clear positive selection to features, e.g. dorsal coverage, common in both the horizontal and tented orientations of mussel shells, individuals working in the field can prioritize cover options which match those criteria. This information illustrates another aspect of freshwater mussel interaction with other members of the community. It expands the scientific community's understanding of how freshwater mussels are at the foundation of many aquatic ecosystems, further emphasizing the need for mussel conservation.

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