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Use of Dead Mussel Shells by Madtom Catfishes in the Green River

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Abstract

The Green River in Kentucky has high fish and macroinvertebrate diversity. As both fish and macroinvertebrates have evolved together in this system, relationships have developed between species. One type of relationship that has been observed is between madtom catfishes (*Noturus* spp.) and mussels in the Green River, where madtoms use dead mussel shells as cover when not actively foraging. In the fall of 2016 and 2017, surveys were conducted to determine if madtom catfishes use dead mussel shells more than rocks of similar size. We predicted that madtoms would select mussel shells as cover more frequently than rocks due to the natural concavity of mussel shells, which would not require excavation prior to use; rocks typically require removal of underlying substrates to create a cavity prior to use as cover. Three 12-meter by 12-meter plots were sampled at four sites along the Green River, once per year, by snorkeling in an upstream direction and searching for madtoms in dead mussel shells and under the rock substrate. Equal effort was used searching for madtoms under rocks and in mussel shells. Significantly more madtoms were found under mussel shells than under rocks of similar size. These results support our prediction and demonstrate the importance of mussel shells as cover for madtom catfishes. The decline of mussel populations, and resulting decline in available mussel shells in rivers and streams, may have negative effects on madtom populations in the future.

Keywords

Madtom catfishes, mussel shells, cover

Cover Page Footnote

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INTRODUCTION

The southeastern United States accommodates a diverse and well documented freshwater fish and macroinvertebrate fauna (Neves et al. 1997; Warren et al. 2000). Madtoms (Ictaluridae: *Noturus;* Rafinesque 1818) represent a unique component of this diversity and constitute the most specious genus of the North American catfishes (Burr et al. 2005). Madtoms are found in various bodies of water, including lakes and streams, and exploit diverse microhabitats, such as riffles and pools. These miniature catfishes are known for the toxin glands at the base of their pectoral and dorsal spines. Characteristic of the family Ictaluridae, madtoms are benthic, nocturnal hunters (Mayden et al. 1980; Burr and Mayden 1982; Starnes and Starnes 1985; Chan and Parsons 2000). As smaller fish, they are often preyed upon by large fish species, such as *Lepisosteus osseus*, Centrarchid species, Esocid species, and other Ictalurid species (Burr and Mayden 1982), as well as birds and snakes. Along with their toxic spines, madtoms utilize their small size, camouflage, and cover options to avoid predation during the daylight hours. They have been observed to utilize many cover options including rock structures, leaf packs, mussel shells, beer cans and other artificial structures, and large woody debris (Mayden et al. 1980; Burr and Mayden 1982; Mayden and Walsh 1984; Starnes and Starnes 1985). In the United States, there are 29 described species of madtoms; of which, there are multiple federally endangered and threatened species, such as *N. baileyi, N. flavipinnis,* and *N. placidus*, while other madtom species are under review for listings (Warren et al. 2000; USFWS 2011). Kentucky is home to 10 species of madtoms – six of which are found in the Green River: *N. elegans, N. eleutherus, N. gyrinus, N. miurus, N. nocturnus,* and *N. stigmosus*. The Green River in Kentucky is the study area chosen for this project due to its diversity of both madtoms and mussels.

The Green River of Kentucky is home to over 70 species of freshwater mussels throughout the main-stem of the river (Haag and Williams 2014). Haag and Williams (2014) speculate that the Green River's high heterogeneity of habitat has allowed the river to maintain large populations of these invertebrates, along with a high species diversity. Freshwater mussels, in turn, play a large role in the biotic system of the Green River and in the conservation efforts associated. Mussels have significant impacts on the health of a stream or river by filtering the water and being part of the extensive food webs of the aquatic environment (Cicerello and Schuster 2003). Freshwater mussels play an important role in the food webs of the aquatic community as omnivorous siphon-predators and as prey for fish and other vertebrates (Vaughn et al. 2008). They are also a highly imperiled group of animals with 189 out of nearly 300 species of freshwater mussels in the United States listed on the *IUCN Red List* (Lydeard et al. 2004). Not only are many species endangered or threatened, but many have already gone extinct or have been extirpated within the majority of their natural range. The loss of freshwater mussel species is attributed mainly to the impoundment and other disruptions to the natural flow of rivers and streams, as well as the introduction of invasive species into the native systems (Williams et al. 1992; Baker and Hornbach 1997; Ricciardi and Rasmussen 1999; Vaughn and Taylor 1999; Lydeard et al. 2004). However, some conservation initiatives are in place to try to save what is left of North America's mussel communities, such as the Center for Mollusk Conservation founded by the Kentucky Department of Fish and Wildlife Resources, which intends to restore and recover the populations of Kentucky's freshwater mussels. This includes the recovery and restoration of freshwater mussel populations in the Green River.

There is evidence for multiple ecological interactions between madtoms and mussels. These interactions have largely been explored in terms of mussel dispersal via use of madtoms as hosts for larval mussels (i.e., glochidia), and the use of dead mussel shells as cover for madtoms. Many catfish species in North America have been found to carry glochidia larvae of freshwater mussels that are attached to their gills (Tiemann et al. 2011). These fishes serve as mechanisms of dispersal both up- and downstream in riverine systems. Some catfish hosts are madtoms, such as *N. gyrinus* and *N. flavus* (Tiemann et al. 2011). The second relationship between madtoms and mussels is the use of dead mussel shells as cover by madtoms. It was mentioned in Tiemann et al. (2011) that small catfish and juvenile catfish have been found using dead mussel shells and mussel beds as cover options during the day time. A study by Midway et al. (2010) examined the cover preference of *N. furiosus*. The project studied the behavior of madtom individuals when given the choice of cover in aquaria between artificial structures, rock, mussel shells, and leaf packs. Artificial cover was preferred over rock structure by *N. furiosus*, while mussel shells were not utilized for cover (Midway et al. 2010).

We investigated whether madtoms prefer the cover of mussel shells to the cover of rock. The study was performed in the Green River of Kentucky, where mussel and madtom diversity and abundance are among the highest in the state. The Green River is the longest river in the state of Kentucky flowing a distance of nearly 600 km, creating high levels of habitat heterogeneity that support six species of madtoms and over 70 species of freshwater mussels. It was hypothesized that the madtoms in the Green River would prefer the cover of mussel shells to the cover of rocks. This is due to the natural concavity of the shape of mussel shells giving madtoms the ability to use shells without the need for prior excavation, compared to rocks, which typically requires removal of underlying substrates to create a cavity prior to use. To test this hypothesis, madtom cover preference was studied at four sites over two years. The statistical null hypothesis that was tested was that the use of dead mussel shells by madtoms would be less than or equal to the use of rocks as cover by madtoms.

METHODS

Study Area

The Upper Green River in Green and Hart counties of Kentucky extends from the Green River Lake to Mammoth Cave National Park. This reach of the Green River is a 6th order stream from the lake to the confluence with Russell Creek, creating a 7th order stream downstream of the confluence. Within the target section, four sites were selected based on ease of access and prior knowledge of their locations (Figure 1). Two of the four sites were on Western Kentucky University's Green River Preserve: Bush Island (37.2422, -86.0136) and Kinney Island (37.2477, -85.9853). These sites are in Hart County, Kentucky just upstream of Mammoth Cave National Park. The next site selected, Thelma Stovall Park (37.2660, -85.8890), was in Munfordville, Kentucky. The farthest upstream site, located approximately 39 km below the dam of the Green River Lake, was in Greensburg, Kentucky at the city boat ramp (37.2580, -85.5060). The Upper Green River remains nearly constant in pH of around 8, due to the Mississippian limestone bedrock of the area (US Army Corps of Engineers 2011). This limestone bedrock creates a highly karst environment within this region of Kentucky, allowing for an extensive groundwater system that feeds the Green River. The land through which the river flows mainly consists of deciduous forest and pasture land, which make up 44% and 28% of the land use, respectively (US Army Corps of Engineers 2011).

Sampling

The study used snorkel surveys to assess the number of madtoms using rocks or mussel shells as cover during the daytime. These snorkel surveys were performed from October 4-10 of 2016 and from August 28 to October 21 of 2017. At each of the four sites, three plots were selected along available riffle habitat. One of the plots was located at the downstream end of the riffle, one in the middle of the riffle and the third was located at the upstream end of the riffle. Each plot was 12 x 12 m and divided into two quadrats of equal size. The dividing line between the two quadrats followed the flow of the current. The quadrat on the left was surveyed for madtoms using rocks for cover, while the quadrat on the right was surveyed for madtoms using dead mussel shells. Each snorkel survey was 10-min long with an equivalent amount of effort. In each of the respected quadrats, the snorkeler would turn over a rock or look in/under dead mussel shells in search for madtoms. Each observed madtom was counted and recorded. Each madtom species was considered ecologically equivalent, due to similar habitat preferences (Page and Burr 2011). Therefore, there was no discrimination of madtom species when an individual was encountered. Likewise, there was no discrimination of mussel species as well for shells that were encountered. To verify that the snorkeler was inspecting cover (rocks or shells) of equal size, random sets of 30 rocks and 30 mussel shells that were inspected for madtom use were taken from the Munfordville site (which had the most available mussel shells) in 2016. The rocks and shells were measured in length between the two farthest points of the object.

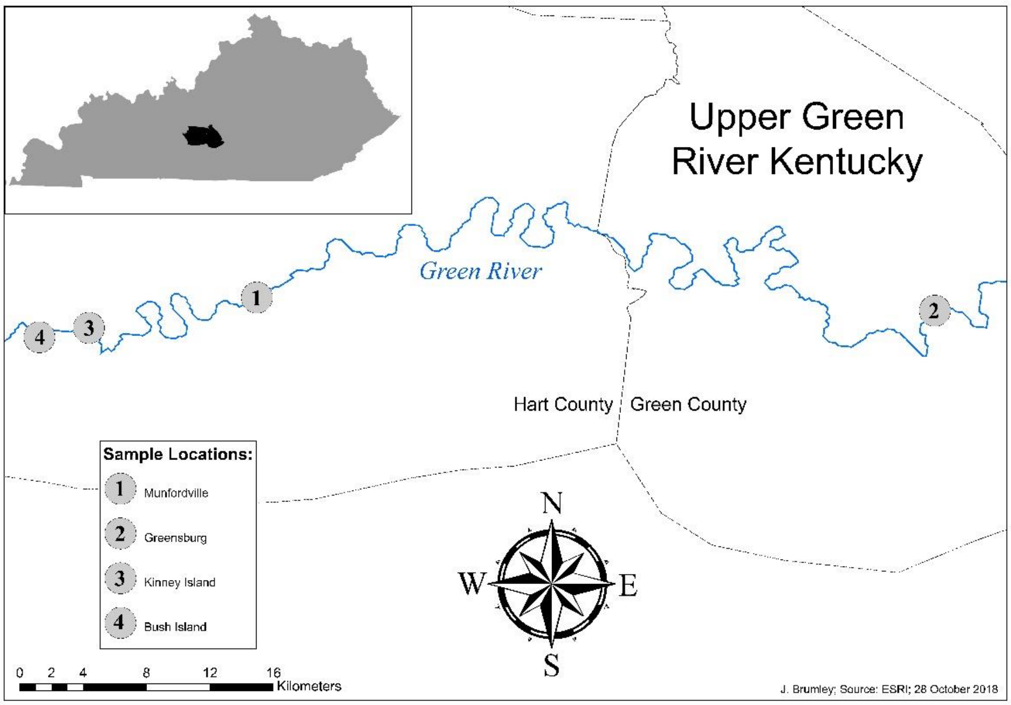

Figure 1. Map of sample locations on the Green River of Kentucky, each sampled in 2016 and 2017.

Data Analysis

All data analyses were performed using Statistica version 13 (TIBCO Software, Inc. Palo Alto, CA). A Shapiro-Wilks test was conducted to determine the normality of the data. The data were not normally distributed because they consisted of counts. Transformations of the data were conducted but failed to produce data along a normal distribution. Therefore, non-parametric analyses were used to compare the number of madtoms observed in each quadrat. To test for the effect of year (2016 vs 2017) on the data, a non-parametric Mann-Whitney *U* test was performed. Based on non-significant differences between madtoms observed in each year, the data from both years were pooled for further analyses. To test for the effect of site (Bush Island, Kinney Island, Munfordville, and Greensburg) on the number of madtoms observed, a non-parametric Kruskal-Wallis test was performed. Based on non-significant differences between madtoms observed at each site, the data were pooled by cover type for further analysis. A single-tailed Mann-Whitney *U* test was used to test the statistical null hypothesis that the use of dead mussel shells would be less than or equal to the use of rock cover.

RESULTS

Madtoms were found utilizing both rocks and mussel shells as cover. Overall, 12 individuals were observed using rocks as cover, and 33 individuals were observed using shells as cover (Table 1).

Table 1. The number of madtoms observed using each cover type (shells or rocks), organized into site, year, cover option, and plot number. Bolded data sets are the surveys with the most (Munfordville 2016) and least (Bush Island 2017) madtoms observed.

Site/Year	**Plot 1**		**Plot 2**		**Plot 3**	
	Shells	Rocks	Shells	Rocks	Shells	Rocks
Bush Island 2016	1	0	1	0	0	0
Bush Island 2017	**1**	**0**	**0**	**0**	**0**	**0**
Kinney Island 2016	1	0	3	0	1	0
Kinney Island 2017	2	0	0	0	0	0
Munfordville 2016	**2**	**0**	**8**	**2**	**6**	**1**
Munfordville 2017	1	0	2	0	2	0
Greensburg 2016	0	0	0	4	0	1
Greensburg 2017	1	2	0	1	1	1

Throughout the 48 samples, three madtom species were encountered: *N. miurus*, *N. elegans*, and *N. eleutherus*. Also, there were three mussel species observed housing madtoms: *Actinonaias ligamentina*, *Amblema plicata*, and *Tritogonia verrucosa*. The orientation of the mussel shells that housed madtom individuals ranged from single valves lying flat on the substrate to full shells cracked open and laying on one of the valves. Four ancillary species of fish [*Ictalurus punctatus*, *Percina phoxocephala*, *Etheostoma zonale*, and *Etheostoma jimmycarter*] were also found utilizing mussel shells for cover.

The size of rocks and shells examined for the presence of madtoms was compared from the Munfordville site in 2016. The size of rocks inspected for the presence of madtoms ranged from 7-15 cm long (measuring from the two farthest points) with an average of 9 cm long. The size of the shells examined ranged from 7.5-15 cm long (measuring from the two farthest points on the valve) with an average of 10.5 cm long. There was no significant difference between the sizes of mussel shells and rocks examined during the survey (*t*-test: $t = 1.67$, df = 58, $P = 0.101$).

The Shapiro-Wilks test determined that the data were not normally distributed (Shapiro-Wilks test: $W = 0.626$, $P = 8.02\times10^{-10}$). Therefore, the analyses conducted were non-parametric. Although there were fewer madtoms collected in 2017 (n = 14) than in 2016 (n = 31), there was no significant difference (Mann-Whitney *U*: $U = 250$, $P = 0.394$) in the number collected (Figure 2) so data was pooled from both years for further analysis. There also was no significant difference in the number of madtoms collected at the four sites (Kruskal-Wallis: $H = 7.619$, $P = 0.055$) with 3, 11, 21, and 10 madtoms collected at the Bush Island, Kinney Island, Munfordville, and Greensburg sites, respectively (Figure 3). Based on the lack of significant differences among sites, the data was pooled among sites for further analysis.

More madtoms were found in the quadrats where mussel shells were inspected than in the quadrats where rocks were inspected (One-tailed Mann-Whitney *U*: $U = 188.5$, $P = 0.019$; Figure 4).

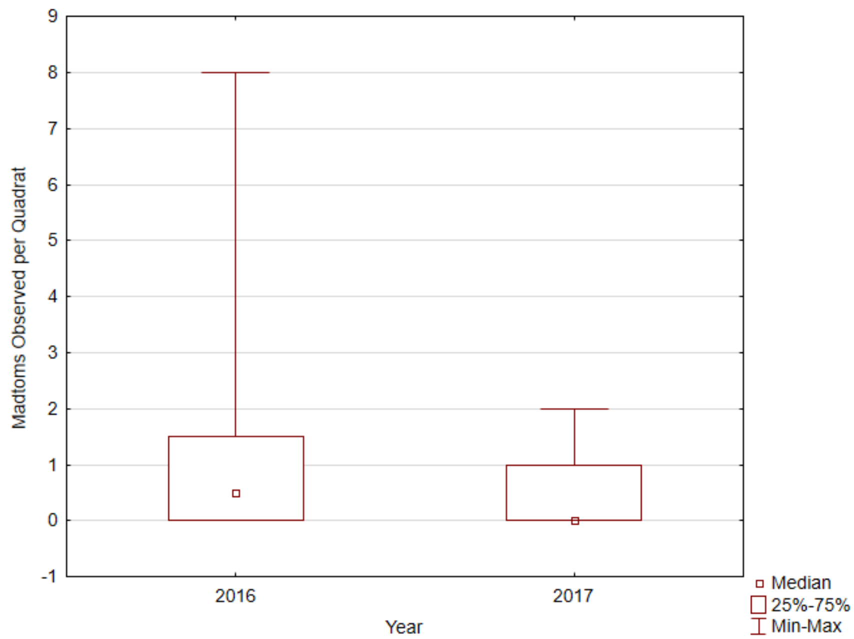

Figure 2. Boxplots of the number of madtoms observed per quadrat in 2016 and 2017.

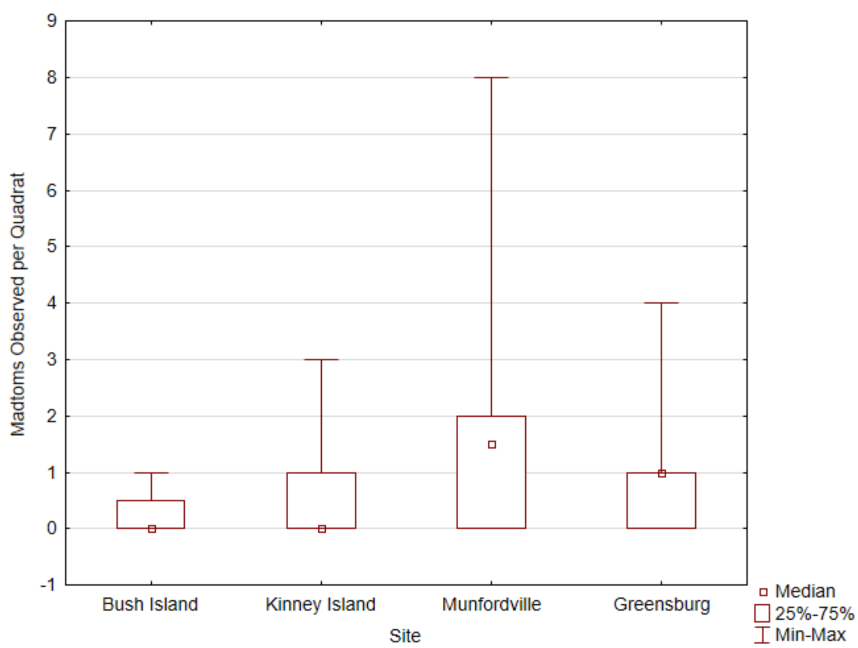

Figure 3. Boxplots of the number of madtoms observed per quadrat at 4 sites.

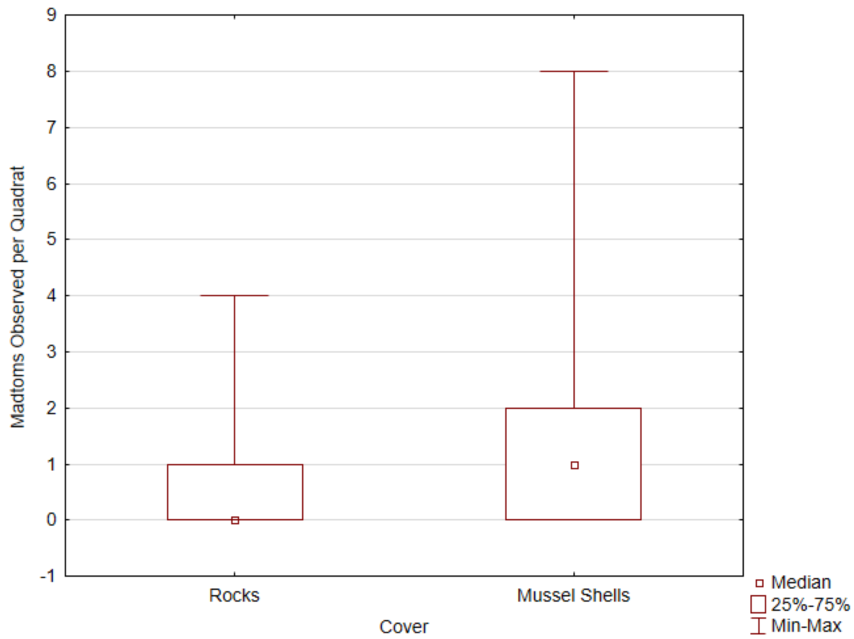

Figure 4. Boxplots of the number of madtoms observed per quadrat utilizing rocks or mussel shells for cover.

DISCUSSION

Based on the field surveys, the madtoms were found sheltering in mussel shells significantly more than under rocks in the Green River. The statistical null hypothesis that the madtom individuals would either prefer the cover of rock over cover of dead mussel shells or there would be no preference between rocks and shells was rejected in support of the biological hypothesis (the madtom individuals would prefer the cover of mussel shells over rocks) (P = 0.019). There was a clear preference of shelter at three of the sites (Bush Island, Kinney Island, and Munfordville, Table 1) with 27 of the 30 observed madtoms found inside mussel shells. Although the number of madtoms found at the Greensburg site was not different from the other three sites, more madtoms (9 of 11) choose to shelter under rocks.

The higher number of madtoms sheltering under rocks at the Greensburg site might be due to the low number of mussel shells available at this site compared to the other three. Since the Greensburg site is only 39 km from the dam of Green River Lake, the hydrological components, mainly temperature, are controlled by the output of the dam. This creates less variation and more stagnant conditions than the characteristics of the river farther downstream, along with increased sedimentation near the impoundment (Vaughn and Taylor 1999). This does not allow for the mussels to survive and reproduce in healthy populations. The harmful effects of dams on mussel shell populations has been seen across the country, an example being the Muscle Shoals incident in Alabama where 32 of 69 species in the area were extirpated due to dam construction (Lydeard et al. 2004). However, this does indicate that mussel shell availability might be a limiting factor for madtoms in the upstream sites when dealing with behavioral preference for cover. In 2016, there were only 8 mussel shells found and inspected for madtoms at Greensburg, compared to 29 at Bush Island, 83 at Munfordville, and 104 at Kinney Island. The low density of mussel shells available may create competition for the cover of mussel shells, pressing many individuals to utilize the cobble substrate as cover. Behavioral plasticity must be taken into account when examining the results of this study. Behavioral plasticity is typically defined using the context of an ever-changing environment and an individual's ability to change and adapt with the environment (Nussey et al. 2007). The preference of madtoms for mussel shells can be viewed as an extension of their behavioral plasticity that allows them to decide on cover between options. There are factors that limit this ability, such as availability of cover options and competition for these cover options. However, when analyzing further, it was found that, at Greensburg, the percentage of mussel shells occupied by madtoms was the highest among the four sites (25% Greensburg; 20.2% Munfordville; 8.4% Kinney Island; 9.7% Bush Island).

The results of this study were contrary to Midway et al. (2010) that concluded that *N. furiosus* did not use mussel shells as cover but rather preferred artificial structures and rock cover. It is not surprising that madtoms would choose to utilize the artificial structures (flowerpot saucers glued together to create a cavity) in this study, since they were designed to provide cover. However, individuals that did not use the artificial cover in the study chose rock cover over mussel shell cover. This may be explained by geographical or species-specific factors. The geography of a system impacts the river's substrate type and composition. These factors lead to diversity in availability for cover options among the substrate. For instance, the study done by Midway et al. (2010) used leaf packs as an option of cover for the madtoms. Leaf packs were not available as cover in our study, due to the discharge of the river, lack of large woody debris, and lack of fallen leaves during the autumn when we sampled. Another factor is that the study by Midway et al. (2010) focused on *N. furiosus*, while this study encountered three different madtom species (*N. miurus, N. elegans,* and *N. eleutherus*). The possibility of species-specific preference to the type of cover utilized by these different madtom species during the day should be explored in future studies.

One of the limitations to the present study was the number of sites visited and the diversity of the sites visited. The Munfordville, Kinney Island, and Bush Island sites are spatial replicates of each other and are all within the section of the upper Green River that is characterized by carbonate substrate (limestone) and high abundance and diversity of the mussel populations. Although the Greensburg site gave the study more diversity with its siliciclastic substrate and less abundance of mussel individuals, it was not replicated. The use of boats and/or canoes may allow future studies to expand the number of sites sampled.

The study was also limited temporally with surveying occurring strictly in the late summer and early fall months of both 2016 and 2017 (August – October). Some madtom species have been recorded utilizing habitat in both riffle and pool areas at differing times of year. *Noturus baileyi* is an example that has been recorded using similar slab-rock in both riffle and pool habitat; riffles are occupied from late spring to late fall, and in the colder months, pools are occupied (Dinkins and Shute 1996). Future studies of the madtom habitat preference would explore any habitat preference change during different months and seasons of the year. This would further our knowledge about their specific habitat preferences.

Further investigation and analysis in the future can be made for many of the trends observed during this study. One of these trends was that sites with high mussel shell density also had high density of madtoms using mussel shells as cover. An explanation for this trend has not yet been studied. It has been speculated, however, by the authors that interspecific competition for the cover of dead mussel shells has an impact on this trend. This came from the observation of many ancillary species, including *Ictalurus punctatus, Percina phoxocephala, Etheostoma zonale,* and various crayfish species, utilizing mussel shells as cover from predation.

Another trend that can be investigated further is how the madtoms responded when their cover was disturbed. Whereas madtoms disturbed from rocks quickly moved away to try to find cover, those in mussel shells stayed with the shells and would only abandon the shells if taken out of the water or the shell was opened to the point of vulnerability. It can be investigated to what extent madtom individuals are content within mussel shells as cover. This could also help elucidate why madtoms prefer the cover of dead mussel shells over rock.

This study revealed that madtoms in the Green River prefer to hide in mussel shells as cover rather than hiding under rocks. This habit may be important to consider in the conservation efforts to preserve both freshwater mussels and madtom populations in North America. Both madtoms and freshwater mussels are key species in the health of aquatic ecosystems in which they inhabit by creating habitat and factoring into the food web (Tiemann et al. 2011), and this study shows there is an important relationship between madtoms and freshwater mussels. In Kentucky, madtoms are factored into the Kentucky Index of Biotic Integrity (Compton et al. 2003) as intolerant species. Therefore, their presence in a fish assemblage is used as an indicator of minimally impacted habitats. The presence of freshwater mussels is also often listed as an indicator of healthy streams (Cicerello and Schuster 2003). Because of their shared sensitivity to environmental degradation and their symbiotic relationship, conservation efforts to preserve freshwater mussels will likely benefit madtoms.

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