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Common Mudpuppy (Necturus maculosus) distribution, diet and seasonality in western New York and morphological condition in lake and stream habitats.

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Common Mudpuppy (*Necturus maculosus*) population assessment and morphological condition in habitats of western New York.

A Thesis in Biology

by

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Dedication

Yes, I finished my thesis... the new answer to the question my mother has asked seven days a week for over two years. I would like to thank all, especially my family, who have inquired about my thesis and motivated me to complete it.

I would like to thank my advisor Dr. Christopher Pennuto who took me on as a masters student, was willing to help me pursue my own questions, and study animals outside of his area of expertise. His willingness to step outside his comfort zone has put me in a better position to enter a career in herpetology. Thank you, Chris!

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My younger years were filled with fishing and looking for herpetofauna. My upbringing undoubtably led me to pursuing a job in the environmental field. I would like to thank my grandparents, parents, sister, aunts, uncles and cousins for helping mold who I am today. A special thanks to my parents, Dori and Todd Haines for… Everything.

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Thesis Abstract

Environmental context changes the behavior and morphology of organisms. The impacts of flow on sampling techniques and morphology of the Common Mudpuppy were investigated during this study. I also explored mudpuppy distribution in western New York, diet, sexual dimorphism, seasonality, and capture biases. I found rock turning to be more efficient in streams year-round and modified minnow traps to be better more efficient in cold weather months and in deeper habitats than in other seasons or habitats. During the hot weather months, mudpuppy diet consisted of invertebrates exclusively, whereas diets in cold weather months consisted of invertebrates plus vertebrate prey. Body condition reflected the change in diet, with larger body condition when large prey items were found in gut contents. Stream-captured mudpuppies were more streamlined and possessed larger digits than lake-captured mudpuppies. Mudpuppy morphological differences between habitat types indicate phenotypic plasticity as the likely mechanism of morphological change when viewed in light of other published phylogenetic work on regional haplotypes. The findings of morphological response to flow warrant more investigation with common garden experiments. Expanding the common garden experiment to encompass future changes in temperature will help inform managers on how climate change may affect mudpuppy populations.

Chapter 1

Distribution, Diet and Comparison of Capture Methods of Mudpuppies (*Necturus maculosus*) in Western New York

Abstract

The common mudpuppy (*Necturus maculosus*) is an understudied aquatic amphibian found in many major water drainages from eastern Canada to the southeastern United States. Although its range is large, we know little of its distribution at a finer scale. My study expanded the knowledge of mudpuppy distribution in the eight counties across western New York. Mudpuppies were found in all four of western New York's major watersheds in both lentic and lotic habitats. Rock-turning (RT) and trapping were used to collect mudpuppies. Rock turning in lotic habitats was more efficient than trapping when turbidity was low, and water was shallow. Trapping was a better option in deeper habitats. I also explored sex ratios and morphological metrics in relation to trapping method to analyze possible capture bias. Male-to-Female sex ratios were the same for rock turning and trapping. Four of five morphological features were not statistically different between rock turned and trapped mudpuppies. However, the largest and smallest mudpuppies were captured while rock turning, suggesting that trapping may be more size selective. Girth was significantly smaller for mudpuppies collected when rock turning, and this was attributed to seasonal differences in activity. Seasonal differences were also seen in gastric lavage samples. In summer and fall mudpuppies fed on invertebrates exclusively but during winter and spring fish and invertebrates made up the majority of their diet. Body condition reflected the change in diet; animals had a lower body condition when fish were absent from mudpuppy diet. Mudpuppy eggs were found in the stomach contents of two female mudpuppies under nest rocks, suggesting filial cannibalism. Two types of microplastics were documented in the stomach contents of mudpuppies from five different locations, both lake and stream habitats. This may be the first documentation of microplastics in adult amphibians.

Introduction

Background of Necturus maculosus

The conservation of amphibian species is complicated. Many, if not all, species exist in metapopulations (Alford and Richards 1999), meaning the same species exist in different locations separated by inhospitable habitat and/or manmade barriers but still connected by dispersal. Thus, conservation efforts that work in one area may be ineffective in another, even if the target species is the same. The conservation of amphibians relies on local stakeholders and municipalities, because each locality has unique features or stressors. Habitat preference, selection, distribution, and population dynamics must be known to develop a conservation strategy and plan. Many amphibian species, especially aquatic amphibians, are cryptic, resulting in a minimal research focus. The Common Mudpuppy, *Necturus maculosus,* is one aquatic amphibian of which little is known.

Mudpuppies are a large, purely aquatic salamander species. Their distribution covers an area from southeastern Canada to Georgia and Louisiana, representing the largest distribution of any fully aquatic salamander in North America. Mudpuppies are found throughout the Great Lakes region in lakes and streams. However, it is believed that their populations may be in decline (Mifsud 2014).

Mudpuppies, like many amphibian species, have an egg, larval, juvenile and adult life stages (Figure 1). Eggs are laid in late spring and are guarded by female mudpuppies at least until the eggs have hatched. Yolk-sac larval mudpuppies are believed to stay under the nest rock until as late as November. Once the yolk-sac is absorbed, juvenile mudpuppies leave the nest rock and have two yellow-brown stripes whereas the adults are spotted. It can take up to six years for mudpuppies to mature (Bishop 1941). Few studies have targeted larval and juvenile mudpuppies due to their cryptic nature (Gendron 1999). Adult mudpuppies can grow to lengths greater than 48 centimeters and have been documented to live upwards of 30 years. Although this species is large, long-lived and has a broad distribution, little is known about its local distribution, population dynamics and life history. Knowledge of their thermal preference is important in the temperate zone since they exhibit seasonal activity patterns.

Temperature and season play a huge role in mudpuppy activity. A lab study by Hutchison and Spriestersbach (1986) found that mudpuppies had several different activity periods in both winter and summer months when water temperature was held at 15℃. Peak activity was seen in January, followed by lower peaks in April, June, July and September. Sprint performance in mudpuppies was found to increase when water temperatures were between 5℃ and 15℃, and then declined when water temperature increased between 15℃ and 25℃ in laboratory conditions (Miller 1982). Similarly, a study conducted in natural conditions found that activity level declined above 14.1℃ (Beattie et al. 2017). In lab studies, mudpuppies have also shown a distinct preference for acclimated temperature (Hutchison and Hill 1976).

The use of baited, modified minnow traps is a common collection technique for mudpuppies, although it may not be the most efficient (Craig et al. 2015, Murphy et al. 2016). Chellman et al. (2017) found that mudpuppy trapping success was highest in spring after rain events when water temperature was ~3-6℃. Trapping susceptibility of mudpuppies declined with increasing water temperatures in both lentic and lotic environments, which further suggests that activity slows during warm weather months (Murphy et al. 2016, Beattie et al. 2017). Several recent studies have discussed the differences between and effectiveness of survey methods for common mudpuppies. In stream locations, modified Briggler traps were found to be more effective than modified minnow traps (Murphy et al. 2016). Beattie et al. (2017) suggested traps collected significantly larger mudpuppies compared to mudpuppies captured during rock turning.

Few surveys for the common mudpuppy have occurred throughout New York (Bishop 1941, Schmidt et al. 2004, Vandevark and Coleman 2010) and no known mudpuppy surveys have occurred in western New York in the last 79 years. Bishop (1941) describes distribution and life history of mudpuppies in the book <u>Salamanders of New York</u>. Schmidt et al. (2004) investigated the native or introduced status of mudpuppies in the tidal Hudson River. Vandevalk and Coleman (2010) investigated the weight-length relationship of mudpuppies in two lakes, Oneida Lake and Trout Lake, one in central and one in northern New York. By building a knowledge base about the common mudpuppy in western New York, conservation management and monitoring of populations can be initiated, including general consideration for the species when planning development projects and restoration efforts. In this study, I performed an 8-county survey for mudpuppies over a 2-year period, sampling in all seasons. During the survey, I assessed two collection methods, rock turning surveys and trapping to compare collection efficiency and size biases. Body condition was investigated using a Fulton-type index to compare seasonal changes in condition status. Lastly, I compared diets of mudpuppies by season and habitat type to determine the extent of diet specialization.

Methods

Regional distribution survey

I used modified minnow traps and rock turning/snorkel surveys to capture mudpuppies. I also spoke to anglers as often as possible and created social media posts in “Western NY Ice Fishing” forum to obtain further distribution information. Local knowledge from anglers was helpful in choosing waterbodies to sample and resulted in several new locations for mudpuppy occurrence, as well as confirming known locations. All but two mudpuppies were collected under NYSDEC License to Collect and Possess: Scientific # 2145, the two others were captured on another researchers permit.

Lentic Sampling

Eight lakes in the eight western New York counties were sampled. Lakes were found using satellite imagery on Google Maps and were chosen mainly by ease of access. Sampling in lentic environments was conducted from November thru May using modified minnow traps. Traps were approximately 43 x 23 cm in size. Traps were baited with canned pet food and deployed at sunset for an overnight set. Traps were checked within 24 hr, weather permitting. They were deployed for 1-to-3 days per location and if no mudpuppies were trapped within the first three days, traps were moved to a different location. Trapping effort was recorded as trap nights (total number of traps deployed multiplied by the total number of nights they were deployed).

Traps were deployed along a line with ~5 m between each trap and, with few exceptions, 10 traps per line. Traps were deployed from a canoe. Deploying traps through the ice proved time consuming, exhausting, and unsuccessful, as documented by Chellman et al. (2017), and therefore, was only attempted in two locations. Holes were cut in the ice with a 6” hand auger. Traps were then baited and lowered into the hole. Traps were not flagged to reduce the incidence of vandalism, but each trap had an identification tag in accordance with state regulations for minnow traps.

Lotic Sampling

For the initial selection of sites, I used surficial geology maps to identify substrates in streams and rivers that were most similar to known mudpuppy sites in the Allegheny watershed. This was successful to some extent, but few streams had the same substrate as those deemed good mudpuppy habitat. Streams with substrates outside the specific classifications were sampled when time allowed. In stream environments where pools were large enough, trap lines as described above were used. Traps were selectively placed in areas that looked to be good habitat (e.g., deep holes, areas near large rocks).

Rock-turning (RT) surveys supplemented trapping. During RT, I targeted partially buried rocks larger than 10 cm and held dipnets around the perimeter of the rock. When possible, rocks were lifted from the upstream side to allow the sediment to be washed out by the stream flow. Snorkel and masks were used to improve visibility underwater. Mudpuppies were slowly and gently corralled or lifted into the dipnets. Rock-turning was confined to depths ≤ 1.5 meters.

Mudpuppies were collected from stream environments year-round. Traps were deployed from November thru the end of May whereas RT surveys were conducted year-round with most rock-turning occurring during the late summer. Streams within the Alleghany River watershed were not sampled due to permit restrictions. One exception though was French Creek, which was sampled under another researchers permit (Robin Foster). For passive gear (traps), capture effort was recorded in the same fashion as for lake sampling (i.e., trap nights). For active collection techniques of RT, number of search hours was recorded (i.e., time spent searching).

Mudpuppy Measurements

Five measurements were taken on all mudpuppies captured: total length (TL), head width (HW), snout-vent length (SVL), body girth (Gir), and mass (Figure 2). Total length was measured from tip of the snout to tip of the tail on a fish board while SVL was measured from the tip of the snout to the anterior of the vent/cloaca. Head width was measured with a dial caliper at the widest part of the head and Gir was measured at the widest part of the body cavity. Mass was obtained with a digital field balance.

Gastric Lavage

Gastric lavage was conducted on animals whose length exceeded 18 cm. On larger mudpuppies (>24cm TL) a syringe with a tube diameter of 6.4 mm was used to flush the stomachs, whereas for animals <24 cm TL, a water bottle with a long squirt nozzle graduated from 6.4 to 3.1 mm diameter was used. Water from the collection site was used to flush contents from the stomach. Mudpuppies were restrained lightly against a fish board while gastric lavage was conducted. Contents were collected in a beaker, transferred to a sealable container, and preserved in 70% ethyl alcohol. Contents were identified to lowest possible taxonomic unit using an Olympus SZ61 microscope and counted. Identification of macroinvertebrates was done with the keys of Peckarsky et al. (1990). Gastric lavage data was used to create three sets of Costello plots: diet plots by season, by habitat, and the cumulative combined diet plot. Relative abundance was calculated as the total number of a prey item found in stomach contents of an individual divided by the total number of individual items found in all mudpuppies. Relative occurrence was calculated as the number of guts a prey item was found in divided by the number of mudpuppy guts examined.

Statistical Analyses

Differences in morphological metrics (i.e., TL, SVL, BG, HW, mass) by capture technique and sex were investigated using a series of Welch t-tests. Size distributions and observed sex ratios of captures among the different capture techniques were assessed by log-linear models (G-test). Size distributions were binned similar to Beattie et al. (2017). Survey effectiveness was calculated using number of mudpuppies captured per visit and compared using a Welch t-test. Seasonal patterns (spring, summer, fall, winter) in morphological metrics were compared using ANOVA. I designated the seasons as: winter (1 Nov – 28 Feb), spring (1 Mar – 31 May), summer (1 Jun – 31 Aug), and fall (1 Sep – 31 Oct). All statistical analyses were done in R version 3.6.3.

Body condition

Fulton's condition factor was created for each individual mudpuppy with the equation (mass/SVL^3) *100. Fulton's condition factors were split into seasons and analyzed using a Kruskal-Wallis test (package = "dplyr"). Post-hoc differences among seasons were examined using a pairwise Wilcox test following a significant Kruskal-Wallis. I plotted Fulton condition factor by Julian date and smoothed the best-fit line and 95% confidence interval to visualize changes in body condition from animals captured between 2016 and 2018.

Other miscellaneous data for future assessment

Tail Clips

A very small piece of tail tissue was snipped from the tail terminus with scissors and preserved in 70% ethyl alcohol solution. Tail clips were taken from captured animals for future genetic analysis. Scissors were disinfected with 10% bleach solution and rinsed in stream water between all uses.

Chytrid swabs

Each captured mudpuppy was swabbed for future chytrid analysis. A sterile, absorbent-tipped swab was rubbed on the ventral side of the mudpuppy, including all feet. Used swabs were placed into a sterile container, then frozen. Samples that were not stored below 0℃ within 4 hours were discarded.

Passive Integrated Transponder (PIT) Tagging

After morphological measurements were taken, most animals were PIT tagged (11 mm tags, BioSonics Inc) to obtain recapture information for population estimates. Tags were inserted under the epidermis at the anterior part of the tail (Figure 3). Mudpuppies with a length under 17 cm or a mass under 20 g were not tagged. At two locations, Case Lake and Black Rock Canal, several mudpuppies were not tagged in the beginning of sampling and populations in Chautauqua Lake and Kemptville Creek were not tagged at all.

Sampling Gear Treatment

Any gear that was deployed in different locations was washed to remove any visible debris and immersed in a 9:1 water:bleach solution for at least 10 minutes between sites (in accordance with Bio-safety protocols for Reptile and Amphibian Sampling in NYS). The canoe used was cleaned in accordance with NYSDEC Bureau of Fisheries Sampling, Survey, Boat and Equipment Protocol and Biosecurity Protocol.

Handling of Amphibians

Handling of all mudpuppies was done with bare hands that were rinsed with the water present at the sampling site. Captured individuals were housed individually in an unsealed, vented, single-use, disposable plastic bag with water from the location site until they were processed. Processing took place within ~1.5 hours of capture. Animals were removed from the holding bags and placed on a fish board for measurements, gastric lavage, tagging, chytrid swabbing and collection of DNA sample.

Results

In total, 41 different waterbodies were sampled from five different watersheds in western New York. Mudpuppies were captured in all four major watersheds (Table 1) and observed in 13 of the 41 surveyed waterbodies (31.7%) (Figure 3). Of the 41 waterbodies, nine were lentic habitats and 32 were lotic. Of the nine lakes surveyed, mudpuppies were captured in four (44.4%), and observed in 9 of the 32 lotic habitats surveyed (28.1%) (Table 1). Seven of the 20 (36.8%) waterbodies sampled within the Lake Erie watershed harbored populations of mudpuppies (Figure 3). In two of these waterbodies, mudpuppies were observed but not captured. Black Rock Canal and Buffalo outer harbor, these were combined to create a Lake Erie population for statistical analysis. The lone lentic location sampled that did not capture a mudpuppy was Lime Lake. Lime Lake is an impounded/dammed stream. I observed mudpuppies in five of the fourteen lotic waterbodies in the watershed (35.7%). In the Lake Erie watershed mudpuppies were found as far as 18 miles inland from Lake Erie.

In the Genesee River watershed, mudpuppies were only found in one of five locations sampled for an occurrence rate of 20%. No mudpuppies were found in Silver Lake, the only lake sampled in the Genesee River watershed. I only sampled waterbodies in the southern part of the Genesee River watershed due to permit restrictions. Anglers that I spoke with at the site and via forums/social media did not report any mudpuppies captured in Rushford Lake or Silver Lake. The sole mudpuppy found in the Genesee River was found approximately 70 miles from the mouth of the Genesee River.

I had very limited success finding mudpuppies in the western basin of the Lake Ontario watershed. Of the 11 streams surveyed, mudpuppies were only captured in Marsh Creek. Marsh Creek had the highest capture rate per hour of any waterbody where rock turning was conducted in western New York. However, trapping was not very successful (0.01 mp/tn). Sandy Creek had a known historical record of mudpuppies; however, none were captured during my survey. I found many reports of ice anglers capturing mudpuppies in Lake Ontario, especially the eastern basin where ice fishing is more prominent.

In the Allegheny River watershed, mudpuppies were found in two of the three lakes surveyed. Chautauqua Lake had the highest capture rate of all lakes surveyed. However, due to accessibility, it was not sampled as intensively as Case Lake or Black Rock Canal. Two sites were sampled on Chautauqua Lake, separated by ~250 m, with one of them yielding all five mudpuppies collected from the lake. Case Lake is a reservoir created by the impoundment of Gates Creek in 1970. Gates Creek and Case Lake are within the Allegheny River watershed. All mudpuppies in Case Lake were captured at the southern end of the lake, near the mouth of Gates Creek. No mudpuppies were captured at the northern end of Case Lake where the dam is located, and where the water is deeper.

Ice anglers were a particularly valuable asset during this survey of western New York. All locations where I captured mudpuppies were reported in this post. Where to capture mudpuppies in Case Lake was also specifically reported. Several anglers responding to the forum posts reported seeing and capturing mudpuppies on Lake Erie proper as well as in several tributaries. The only tributary mentioned by anglers as having mudpuppies but where none were captured in my study was Oak Orchard Creek. As many as seven previously unknown populations were found during my surveys (Figure 3).

Capture Methods

Over the study duration, 148 mudpuppies were captured; 138 were adults, eight were juveniles, and two were recaptures. Rock turning yielded mudpuppies in seven of 29 lotic environments (24.1% occurrence rate). I spent 101.5 hours searching for mudpuppies by rock turning, resulting in 59 captures for a rate of 0.60 mp/hr (Table 2). However, the maximum capture rates were higher at a few locations. In Marsh Creek, capture rate was 4.0 mp/hr, and in Cayuga Creek mudpuppies were captured at a rate of 1.2 mp/hr (Table 2). The use of traps resulted in 82 captures over 4,866 trap-nights for a total capture rate of 0.02 mudpuppies captured per trap night (mp/tn). Chautauqua Lake had the highest capture rate (0.04 mp/tn), while Case Lake and Black Rock Canal surveys had nearly the same capture rates (0.03 mp/tn) (Table 2).

Trapping in lakes was much more effective than trapping in streams (t = 8.55, df = 5, P = 0.0003). Lake trapping resulted in 84 mudpuppy capture events in 3,273 trap nights (0.03 mp/tn), whereas stream trapping resulted in five mudpuppy capture events over 1,593 trap nights (<0.01 mp/tn) (Table 2). Mudpuppies were captured in seven of 22 locations when trapping was performed for an overall mudpuppy occurrence rate of 31.8%. No juvenile mudpuppies were captured in traps in stream habitats, but six were captured in lake traps. Eight locations had both rock-turning and trapping data. Of these locations, mudpuppies were detected by both methods at the four sites and not detected by either method at four sites.

Rock turning in streams was much more effective than trapping in streams (t = 8.72, df = 3.04, P = 0.003). On average, in all streams where mudpuppies were captured with RT and trapping, RT was 16.1x as effective as trapping when assessed as mudpuppies captured per stream visit.

Mudpuppy size class distribution between rock turning and trapping were not significantly different (G_{adj} = 3.06, df = 4, P = 0.547). Of the mudpuppies captured in traps, 42 were identified as female, 38 were identified as male, two were unidentified adults, six were juveniles, and one was a recapture. Rock turning uncovered 27 females, 27 males, four unidentified adults, two juveniles, and one recapture. Unidentified individuals were excluded from the sex comparison. Sex ratios did not differ between collection methods ($G_{adj} = 0.08$, df = 1, $P = 0.776$) (Figure 4).

Capture Method Morphology

The girth of trapped mudpuppies was significantly larger than mudpuppies found while rock turning ($t = 2.81$, df = 120.82, $P = 0.006$), but there was no difference between the remaining four morphological metrics: TL, SVL, mass, and HW ($P < 0.05$)(Table 3). However, RT recovered both the largest and smallest mudpuppies in nine out of ten measurements (Figure 5), resulting in a greater coefficient of variation relative to trapping (Table 3).

Seasonal Morphology

Total length was significantly different among seasons ($P < 0.002$, Table 4). TL was significantly smaller in summer than in fall and winter ($P < 0.05$, $P < 0.05$). SVL was smaller in summer than fall, winter and spring ($P = 0.002$, 0.04, and 0.005, respectively). Summer girth was significantly smaller than girth of winter and spring captured mudpuppies ($P = 0.001$ and $P = 0.001$). Summer HW was significantly smaller than fall and spring captured mudpuppies ($P = 0.023$ and $P = 0.032$). Summer mass was smaller than fall, spring and winter mudpuppies ($P = 0.046$, 0.009, and 0.001, respectively). Additionally, Fulton-type condition factor showed strong seasonal changes ($H = 31.7$, df = 3, $P << 0.001$). Post-hoc analysis suggested difference between spring and fall, fall and winter, and summer and spring ($P < 0.05$). Fall mudpuppies had the overall lowest body condition and were significantly different than winter and spring seasons ($P < 0.05$). Summer body condition also was significantly lower than spring ($P < 0.01$). Summer and winter body conditions were not significantly different (Figure 6). A plot of the smoothed best-fit line for condition factor by date-of-capture reinforced the observed seasonal changes (Figure 7). No differences were found between male and female mudpuppy morphology at the $P < 0.05$ level of significance.

Gastric Lavage

Mudpuppy gastric lavage recovered 41 different items (Table 5). Two species of amphibians, at least three fish species, 24 species of invertebrates, mudpuppy eggs, plastics, rocks, sand, woody debris, vegetation, tapeworms, and cat food were all found during gastric lavage. Nineteen out of the 92 gastric lavage samples were empty (21%). Of those 19 samples, 15 were captured during RT (79%). Macroinvertebrates were found in 47 of the 92 total gastric lavage samples (51%), and 28 of 92 samples had pieces of fish (~30%). Ten mudpuppies had eaten some of the bait that was used to attract them. Crayfish or their body parts were found in 9 gastric lavage samples, three of which were identified as *Fraxonius propinquus*. Plastics were noted in the stomach contents of mudpuppies in five waterbodies. Tapeworms were found in six stomachs spread across five different waterbodies. Multiple mudpuppy eggs in various stages of digestion were found in two female mudpuppies and both were found under a rock with eggs attached.

Diet was examined by season and habitat and compared using Costello plots (Figure 8). Sixteen samples were collected during winter, 48 in spring, 21 in summer and 7 in fall, while 43 samples were collected in streams and 49 in lakes (Table 5). Insects appeared in at least 20% of all guts across seasons, contributing ~50% of the prey items, on average across seasons. Fish also occurred in ~20% of the guts in winter and spring, but not in summer or fall and never accounting for > 30% of the frequency. The remaining prey rarely accounted for a significant proportion of the diet total.

Miscellaneous observations

Eggs, larvae, juvenile and adult mudpuppies were observed in this study. Eight juvenile mudpuppies were captured during this study, six in minnow traps and two while rock turning. No juvenile mudpuppy morphometrics were collected in this study. Eight nests were uncovered during rock turning between May and July. The one nest found in July had newly hatched larvae, as well as eggs attached to the rock. A female mudpuppy also was captured under the rock. She was returned after morphological measurements were taken.

A single mudpuppy was observed in the upper Niagara River in early September 2018 in the beak of a gull. The gull flew up from the water surface with a MP in its beak, landed on a dock nearby, and dropped the flopping mudpuppy. Subsequently, a Great Blue Heron drove off the gull and consumed the mudpuppy.

Fifteen of 138 mudpuppies collected had bleeding gills (10.9%) and two died during processing (1.4%). Bleeding of the gills has been observed by other researchers while handling mudpuppies and in other axolotls (*Ambystoma micanum*). It is not believed to be fatal in most cases.

Discussion

Distribution

The known distribution of mudpuppies was expanded in multiple counties in western New York during my study, including the discovery of nine previously unreported populations. I suspect that some tributaries to the Genesee River and to the Great Lakes also have mudpuppies, as both contain known populations. especially streams with cold water spring inputs that may offer a refuge from higher temperatures.

Capture method comparison

Rock turning and minnow traps are both effective means of capturing mudpuppies if used correctly. There were no differences in four of the five morphological traits used to assess capture bias. I captured mudpuppies with significantly smaller girth during rock turning which may have been a result of rock turning primarily during the summer months when mudpuppies are not actively foraging and become emaciated (Figure 8). Under controlled conditions, mudpuppy activity was lowest in late August and September (Hutchinson and Spriestersbach 1986), which is when mudpuppies in my study had the lowest body condition. One mudpuppy captured on September 10, 2017 weighed 140.6 g with a girth of 25.5 mm. On February 6, 2018 she was recaptured and weighed 205.5 g with a girth of 32.7 mm. However, Hutchinson and Spriestersbach (1986) also found that activity slowed during February and March when the mudpuppies in my study had the highest body conditions. These differences may be due to a 12:12 light cycle that mudpuppies in the lab were exposed to. In western New York, changes in the natural photoperiod are not only affected by Earth’s obliquity but also by ice cover.

Although, TL, SVL, mass and HW were not significantly different by collection method, mudpuppies captured via RT had greater variability in all metrics than mudpuppies captured by trapping. HW, although not significantly different between capture methods, did have smaller variability among mudpuppies collected by trapping compared to those collected by RT. Head width was positively correlated with total length ($r = 0.93$, $df = 115$, $P < 0.0001$). Using this regression, the largest mudpuppy ever reported (TL = 48.2 cm) would have had a HW of approximately 6 cm. Thus, modified trap openings with entrances larger than 6 cm are recommended. The largest HW in my study was 5.1 cm and belonged to a mudpuppy captured by RT. The largest HW captured via trapping was 4.5 cm. The animal with the largest HW was the second longest mudpuppy captured during rock turning, both the largest and second largest were ~10 cm smaller than the largest mudpuppy ever captured. Enlarging trap entrances will likely decrease the variability observed in head width.

Beattie et al. (2017) found that total length of mudpuppies captured while rock turning was significantly smaller than those captured in traps and that distance to shore was positively correlated with mudpuppy length. My study found no difference in total length by capture method and the distance from shore was not assessed. However, Beattie et al. (2017) captured mostly juveniles whereas most of my captures were adults (TL>20 cm). I captured seven mudpuppies that measured under 20 cm in total length and of those individuals, five were identified as female, one male, and one unidentified. I differentiated juveniles by spotting pattern. As mudpuppies mature, their parallel striping pattern turns into spotting patterns.

No sex bias was observed in the two capture methods. Rock turning resulted in a 1:1 (F:M) sex ratio and trapping was 1.1:1 (F:M). The number of unidentified mudpuppies was larger while RT in the non-breeding season due to morphological changes that happen to the males' cloaca. Male mudpuppies have a swollen cloaca during the breeding season and as the breeding season slows the cloaca becomes less swollen, making the males look more similar to the females.

Lentic and lotic capture methods

Both rock turning and trapping were used in eight streams during my study. Mudpuppies were captured both by rock turning and minnow trapping in the same four streams and neither method was successful at capturing mudpuppies in the other four locations. Trapping in streams was significantly less successful than minnow trapping in lakes. Due to the different CPUE metrics (i.e., trap nights vs hours surveyed), it is difficult to quantify which technique was more efficient. However, rock turning was more effective than trapping in the stream when comparing the number of mudpuppies per visit when checking traps in the stream vs. the number of mudpuppies captured per visit while rock turning. On average, rock turning was 16.1x more effective than trapping.

Total CPUE in the lentic habitats where I captured mudpuppies was 0.03 ± 0.005 mp/tn. The three lakes with captures had differing amounts of trap nights; Lake Erie 1,919 tn, Case Lake 765 tn, and Chautauqua Lake 130 tn. However, capture rates were relatively the same in all lakes that I sampled (0.03 mp/tn and 0.04 mp/tn), similar to those found in Wolf Lake by Beattie et al. (2017) (0.04 ± 0.005 mp/tn). The capture rate in Chautauqua Lake was the closest to Wolf Lake at 0.04 mp/tn but also had the lowest number of trap nights. If capture rates are analogous to population size, Chautauqua Lake was most similar in population size to Wolf Lake. Lake Erie and Case Lake had lower capture rates than Chautauqua which may suggest lower population sizes. However, lower capture rates calculated in lotic habitats may not be reflective of lower population sizes.

In lotic habitats I had a capture rate of 0.0031 ± 0.0019 mp/tn which was lower than the capture rate I calculated for lentic habitats. Sutherland (2019) found a CPUE of 0.0076 ± 0.0014 mp/tn in the St. Clair-Detroit river system. Although they found a higher capture rate compared to my study, both rates in the lotic habitat were significantly lower than the capture rates found in lentic habitats calculated in my study and Beattie et al. (2017).

Mudpuppies are typically seen walking along the bottom of streams as opposed to swimming. When traps are set in a lotic environment, the unidirectional flow brings the scent of the bait downstream, making it likely that mudpuppies are moving against the current to find the bait. A mudpuppy would have to lift its head from the stream floor to enter the trap and would be continually exposed to the stream flow until it enters the trap. I suspect that in lotic habitats if a mudpuppy leaves the bottom it can be exposed to the force of the water and be swept downstream, contributing to lower capture rates. However, Chellman et al. (2017) reported a higher capture probability for mudpuppies in the lotic environment (< 0.04mp/tn). This may be due to better trap placement or larger population size. Chellman et al. (2017) also noted a drop in CPUE the season after TFM treatments were administered.

Rock turning was not attempted in lakes during my study due to total length biases found by Beattie et al. (2017), they also reported 0.13 captures/observer hr. In streams, my rock turning capture rate was 0.60 mp/hr. Differences in CPUE and the bias in total length of mudpuppies may be attributed to the accessibility of substrate in lotic environments compared to lentic environments. More of the habitat is accessible in a stream where the environment is shallower and more confined. Rock turning is not suggested as the sole method for studying mudpuppies in lakes unless most of the benthos can be accessed. SCUBA surveys may be a viable way to eliminate this bias.

Trapping in Chautauqua Lake was unsuccessful in one location and successful in another 250 m away. The site where mudpuppies were captured was at a point that jutted out into the flow of water moving from one end of the lake to the other. The location where traps were set and captured no mudpuppies was in a cove, created and protected by the point that jutted out. The cove had very little flow and was likely to be a depositional area. Trapping in Case Lake had a similar capture distribution. In Case Lake, no mudpuppies were captured in the deeper end of the impounded lake and all were captured on the other end at the mouth of Gates Creek. It is possible that mudpuppies are selecting the area with higher flow as opposed to depositional areas, perhaps because there is more rocky cover, more oxygen and cooler temperatures in the higher flow areas.

As mentioned above, the sole observation of a mudpuppy in the upper Niagara River was first in the beak of a gull which dropped the mudpuppy after being harassed by a Great Blue Heron. The Great Blue Heron subsequently consumed the mudpuppy. This was a rare sight not only for the theatrics, but the water temperature was high at that time in the year, when mudpuppies are expected to be inactive. Also, a gull capturing a live mudpuppy would either mean that it stole the mudpuppy from a diving bird, or the mudpuppy was near the water surface since gulls do not dive.

The most efficient way of obtaining information on mudpuppy distribution in lentic environments in New York State was by speaking to anglers and reading posts on social media. A 200-word post in an ice fishing Facebook group obtained 40 comments regarding mudpuppies, and reports of mudpuppies in 14 different waterbodies. Since physical surveys can be timely and costly, social media and crowd sourcing may be useful in contributing knowledge on the local range of mudpuppies in lake environments. Mudpuppies also are captured from shore in lotic environments, but much less often than during ice fishing. One angler report noted that mudpuppies were captured in over 170 ft of water.

Due to the expanse of my survey, not all locations were sufficiently surveyed. It is likely that some of the locations sampled for short time periods do contain mudpuppy populations. A follow-up investigation of local distributions could use eDNA to detect mudpuppies in areas where they were difficult to capture.

Future research on mudpuppy populations should examine the effects of TFM treatments. Reports of hundreds of mudpuppies dying during TFM treatment have been reported in the Great Lakes and Lake Champlain watersheds (USFWS et al. 2001). A four-year treatment schedule that is currently used, may also prevent mudpuppies from recovering after TFM treatments by preventing immature mudpuppies from reaching sexual maturity. Little is known of the effects of TFM treatments on mudpuppy populations. However, efforts such as translocation of mudpuppies prior to TFM treatments are underway (personal communications with Vermont Dept. Fish and Wildlife).

Researchers interested in searching for the salamander mussel (*Simpsonaias ambigua*) in western New York could start surveys in the areas described in this paper as well. Unfortunately, I did not survey for mudpuppy mussels or glochidia during my surveys.

Sexual Morphology

Differences in mudpuppy morphology between sexes have been documented (McDaniel et al. 2009). McDaniels et al. (2009) found that female mudpuppies were significantly larger in both length and mass. Due to these differences' sexes were split up to compare locations. I did not find differences in any of the morphological features I measured between male and female mudpuppies, therefore, I did not separate males and females to compare morphology.

Seasonal Morphology

As expected, body condition declined in warm weather months and increased during cold weather months. Mudpuppies are most trappable when water temperatures are between 3˚C and 14.1˚C (Beattie et al. 2017, Chellman et al. 2017). Anecdotally, during cold water months mudpuppies can be seen foraging and they are captured by ice anglers, but sightings and captures by anglers are rare in warm water months (Gendron 1999). Gastric lavage data from this study also shows that mudpuppies collected in fall and summer had the most empty stomachs. Stomach contents in the summer and fall were also devoid of large prey items such as fish and amphibians. As mentioned above, the only mudpuppy recapture in a stream habitat gained 66 grams and grew 1.3 cm in girth in just under five months. This mudpuppy was captured first in early September and recaptured in February the following year.

The trend in Fulton's body condition, along with gastric lavage data from my study indicates that mudpuppies are not actively foraging in the warm weather months. Therefore, mudpuppy morphological traits are clearly heavily influenced by seasonality. Comparison of body condition between populations should only be compared between similar seasons or locations with similar seasonal water temperature regimes.

McDaniels et al. (2009) compared body condition of mudpuppies captured within the Great Lakes watershed in winter using a slightly different body condition index. I transformed mudpuppies from my study using their methods to compare. I looked at body condition during winter, spring and active season (winter and spring combined). The mudpuppies analyzed in McDaniels et al. (2009) in 1995, 2002 and 2003 had higher body conditions than those in my study. I also looked at body condition of mudpuppies in lakes and streams during the active months, lake and stream body condition during the active months were not different (t = 1.71 df = 24 P = 0.09). More study of possible decreases in the body condition of mudpuppies in the Great Lakes over time is need.

Lower body condition in response to global warming has been linked to decreased fecundity in other amphibians (Reading 2007). Shortening of the mudpuppy growing season and lengthening of the inactive season may lead to decreased summer survival or lower fecundity come mating season. The drastic change in seasonal body condition of mudpuppies may be cause for concern during the Anthropocene. However, microclimates such as cold-water springs or phenotypic plasticity may help lessen the impact of climate change (Urban et al. 2013, Suggitt et al. 2018). Populations should be monitored at a local scale to better understand how mudpuppies or any amphibian will respond to climate change (Campbell Grant et al. 2016).

Gastric lavage

Several diet studies on mudpuppies have found that crayfish make up a large part of the diet (Bishop 1941, Beattie et al. 2017). This study found that crayfish make up only 3.2% and 5.7% of the abundance and occurrence in the diet of mudpuppies. Anecdotally, *Fraxonius virillus* was the most common crayfish species caught as bi-catch in Lake Erie in this study, most of which were too large for mudpuppies to consume. However, *Fraxonius propinquus*, a much smaller species, was also trapped in Lake Erie and found within mudpuppy stomachs. Invasive species such as dreissenid mussels and round gobies also were found in the stomach contents of mudpuppies. Round gobies and dreissenid mussels are distributed throughout Lake Erie and its tributaries. It may be possible that round gobies and/or dreissenids have caused a change in mudpuppy diet. Changes in diet due to round gobies have been recorded in the Lake Erie watersnake (King et al. 2006) and because mudpuppies inhabit the benthos, they are likely to be influenced by both round gobies and dreissenid mussels. More research is needed to determine the effects of non-native populations on mudpuppies, however, seasonality definitely influences mudpuppy diet.

Mudpuppy gastric lavage efforts during the summer and fall yielded mainly invertebrates at 68% and 65% relative abundance and occurrence, respectively. Summer and fall samples were all collected from stream habitats. Of the 26 gastric lavage samples taken in summer and fall, 13 were empty and three had only rocks. Of the seven gastric lavage samples taken during fall, five samples were empty. One sample had two

Megaloptera heads, and the other a tapeworm. Gastric lavage only resulted in finding a tapeworm in fall, but a leopard frog in winter. No vertebrates were found in gastric lavage samples during the summer or fall. Winter and spring gastric lavage samples from streams yielded much more and larger prey items.

During winter and spring fish were found in 28 of 64 gastric lavage samples (44%). This likely makes fish the majority of their diet by weight during winter and spring. It is not known how well mudpuppies can capture fish. One mudpuppy observed while rock turning was scavenging a fish. Bait was found in 11 lavage samples as well. This evidence suggests that scavenging may be one-way mudpuppies obtain larger food items. Two amphibians were found during processing of winter and spring gastric lavage samples as well. One was a smaller mudpuppy and the other was a leopard frog. Leopard frogs brumate in streams over winter. During this time, they are extremely lethargic or completely inactive, making them an easy prey item, but in the summer their activity increases, and they can escape mudpuppies easily. The mudpuppy found during gastric lavage was very digested, suggesting the mudpuppy captured or scavenged its prey prior to entering the trap. Only the head and front two limbs were recovered.

It appears mudpuppy diet switches from vertebrates and invertebrates in the winter and spring to exclusively invertebrates in the summer and fall. A shift in diet may be due to the increase in activity of larger prey during warm water months of summer and fall. Mudpuppies that live in stream habitats likely find a suitable place to wait out the hot months of summer and fall, getting by on whatever food crawls, swims or has been deposited under their rock.

Female mudpuppies attach eggs to the bottom of rocks during spring and guard the nest at least until the eggs are hatched. Two of three females captured under rocks with eggs attached regurgitated multiple mudpuppy eggs following gastric lavage. Pictures of the nests showed what appeared to be several embryos missing where an egg was stuck to the nest rock (Figure 9). The three nests were assumed to belong to the female mudpuppy that was under the rock because no other mudpuppies were seen under the same rock. Both females that regurgitated eggs were found under rocks that did not allow much flow and had very few points of entry. The other mudpuppy captured under a nest rock did not regurgitate eggs but did contain multiple macroinvertebrates. This nest was more open the outside environment than the other nests, suggesting that female mudpuppies eat their eggs as a source of energy, but only when other food sources aren’t readily available. During the summer, eggs were equal to amphipods for the second highest abundance (18.8%) and had the second highest occurrence (11.8%). Male hellbenders (*Cryptobranchus alleganiensis*) and Japanese Giant Salamanders (*Cryptobranchus Japonicus*) have been documented eating some of the eggs they are protecting (Okado et al. 2014, Unger and Williams 2018), making *Necturus* the second aquatic salamander family to exhibit filial cannibalism while nest guarding. DNA of the eggs was not tested to determine if the eggs eaten came from the mudpuppy guarding the nest. One nest found in July contained about twenty recently hatched mudpuppies, eggs, and a female still guarding. Therefore, nest guarding in mudpuppies may last longer than previously thought, or the female may wait until all eggs have hatched before leaving the nest.

Mudpuppies from several different locations exhibited the “death-roll” behavior when placed in plastic bags prior to processing. The death-roll behavior may serve two purposes, to rip chunks of meat off a large food item and to defend itself when swallowed. Due to the circumstances under which the death-roll was observed it is likely used as a defense mechanism. The mudpuppies that bit the plastic bags and rolled left small holes every time it was observed, so this may be an effective defense if a mudpuppy is swallowed whole by a predator. Future research into this behavior could be done with large chunk bait, in traps or with a camera trap.

Finally, at least 7 mudpuppies from five locations had plastics in their stomach contents. Plastics were found in the form of microfilms and microfibers (Figure 10). Microflakes and flakes were found entangled with amorphic stomach materials. Plastics were found in the stomachs of mudpuppies from three lakes, Erie, Chautauqua, and Case, as well as two streams, Cayuga and Smokes. The locations where plastics were found have differing levels of anthropogenic influence. Black Rock Canal, Lake Erie, is located in Buffalo, NY, New York states 2nd largest city. The mouth of Smokes Creek, where the mudpuppy was captured, is located south of Buffalo and surrounded by what was previously Lackawanna Steel Co. and later Bethlehem Steel Co. The area has a long history of industrial alterations and is still a heavy developed watershed especially in the lower reaches. Cayuga Creek is also a developed watershed especially in the lower reaches as well, however both mudpuppies that regurgitated plastic were in a more suburban area of the Cayuga Creek watershed. Case Lake has no development directly on the lake except a public park and is in a primarily agricultural watershed. The lake is heavily trafficked by fishermen due to its unique mix of game fish.

Chautauqua Lake has a very developed shoreline and is a recreation destination during both winter and summer. Due to the variety of watershed types where plastics were found in stomach contents it appears to be widespread, as would be expected, but more investigation is needed. My observations of plastics in mudpuppies are likely to be under counted due to my inexperience when I started looking at samples. These findings may be the first documentation of plastics in adult amphibians, even though microplastics have been observed in tadpoles (Hu et al. 2018).

Microplastic ingestion is known to have negative impacts such as decreased reproductive success, behavior alteration, mortality and more, on both terrestrial and aquatic organisms (Browne et al. 2013, Avio et al. 2016, Horn et al. 2020). Tadpoles show decreased body condition, function and survival when exposed to diet with microplastics compared to tadpoles that had no microplastics in their diet (Boyero et al. 2020). More research into the implications of microplastic ingestion and the effect it may have on all life stages of mudpuppies is warranted.

Juvenile mudpuppies

Very few juveniles were captured throughout this study. Two were captured during rock turning and six were captured with baited minnow traps. Juveniles captured in minnow traps were captured in mid to late May. Only two juveniles were captured in the Black Rock Canal, both captured on May 30th, 2018. One adult mudpuppy captured on May, 11th 2018 in Black Rock Canal regurgitated a smaller mudpuppy that was partially digested. The degree of digestion suggested that the adult mudpuppy had eaten the smaller mudpuppy before entering the trap, rather than eating the smaller mudpuppy as a result of being trapped. I believe juveniles may become more trappable later in the spring because they are avoiding predation by adult mudpuppies. However, during late spring, warm water predators such as smallmouth bass (*Micropterus dolomieu*) are becoming more active, as well. The four juvenile mudpuppies captured in Case Lake were also captured in May, but adults were also captured on that day and later. Mudpuppies in Case Lake on average were smaller than mudpuppies from the Black Rock Canal mudpuppies (24.1 vs 28.4 cm TL and 31.9 vs 35.1 cm TL) head width. It may be that Case Lake adult mudpuppies are gaped-limited and cannot consume juvenile mudpuppies as easily.

Processing mudpuppies

Fifteen mudpuppies showed bleeding gills during processing. This is likely a sign of stress but is not lethal. The handling of mudpuppies in previous studies showed no sign of post release mortality (Murphy et al. 2016, Beattie et al. 2017, Chellman et al. 2017). Future studies examining morphological measurements could potentially use photographs to digitally measure and reduce the stress on the captured mudpuppies. This will also reduce processing time in the field and allow for more sampling. However, mudpuppies can be hard to sex during summer months because male vents are not swollen and only a very small wrinkle can be observed to differentiate males from females.

For future morphological studies, pictures should be used to measure features due to processing time. Mudpuppies are not an easy species to gather morphological data on because they are both slippery and agitated when handled. To reduce stress on the animals, reduce processing time and increase survey time, it is suggested that photos be taken with a scale of each mudpuppy and processed later. Data collection on a single mudpuppy could take as long as 45 minutes, severely limiting how many mudpuppies could be processed while surveying. ImageJ is a free, downloadable program that was used in the measurement of digits in Chapter 2 of this study that could also have been used to measure a suite of morphological features.

Citizen science project

Mudpuppies have the characteristics necessary to be used as an environmental indicator and reveal information about changing water quality. Being a purely aquatic species, mudpuppies are intimately tied to the quality of the water they inhabit. Therefore, it is important to know how mudpuppies react to differences in the environmental context. Mudpuppies are frequently captured by ice anglers who report their findings on social media. However, there is no consolidated data forum where anglers can report mudpuppy captures. The creation of a reporting application may be useful to researchers interested in mudpuppy distribution. A citizen science initiative that focuses on monitoring incidental catches of mudpuppies could, at the very least, expand the knowledge of waterbodies they inhabit.

Mudpuppies are a unique species for several reasons. Their activity period is virtually opposite of the typical herpetofauna activity season. Poor weather and safety considerations make sampling for mudpuppies in winter difficult for state or federal agencies to undertake. However, ice anglers accumulate millions of hours of survey time every winter throughout mudpuppy ranges in New York and across the northern part of the mudpuppies range. Taking advantage of this valuable resource, at the very least, would reveal much about the distribution. If mudpuppies retain their adult spotting pattern throughout their life a population estimate may be possible. With spotting pattern recognition technology, it may be possible to obtain population estimates if the reports of mudpuppies from citizens are high.

Conclusion

This study expanded the known range of mudpuppies in western New York and discussed possible biases in capture methods based on morphological features. All capture methods were effective in western New York with minnow traps being the most effective in lake sampling during the winter and spring months. Rock turning surveys were most effective in shallow, clear streams. Only girth of mudpuppies was significantly different between trapped mudpuppies and mudpuppies captured during rock turning, which is likely affected by seasonal activity and feeding dynamics. Four out of five morphological features did not differ between capture methods; however, the largest mudpuppies may have been excluded from minnow traps by the size of the trap entrance. Rock turning is more effective in streams than minnow trapping. Gastric lavage showed a change from fish and invertebrates to exclusively invertebrates from winter and spring to summer and fall. Fulton’s condition factor reflected this change via higher body condition when fish were found in diet and lower body condition when fish were absent. Previous diet studies of mudpuppies have found that crayfish are an important diet item. However, crayfish were not found to be a common food item during this study. Future mudpuppy research in New York should focus on the effects of TFM (lampricide) on mudpuppy populations, specifically in the Great Lakes and Lake Champlain where TFM is known to be administered. Mudpuppy populations in the Great Lakes are thought to be declining and very few juvenile mudpuppies were captured throughout this study. Further research should attempt to establish population estimates for known locations of mudpuppies and the effect of dams on population persistence.

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Table 1. Waterbodies sampled for Common Mudpuppies between the November 2016 and the May 2018. Sites with mudpuppies present appear in bold. Sites with asterisks represent sites where mudpuppies were seen but not captured.

Watershed	**Lentic locations**	**Lotic locations**
Lake Erie	**Black Rock Canal, Buffalo outer harbor,** Lime Lake	**Buffalo Creek, Cayuga Creek, Little Buffalo Creek, Smokes Creek,** Cazenovia Creek*, Big Sister, Cattaraugus Creek, Chautauqua Creek, Clear Creek, Eighteen Mile Creek, Ellicott Creek, Little Sister, Tonawanda Creek, Walnut Creek
Niagara River	Hoyt Lake	Upper Niagara River*, Ellicott Creek, Tonawanda Creek, Woods Creek
Genesee River	Silver Lake	**Genesee River,** Oatka Creek, White Creek, Wiscoy Creek
Lake Ontario	Lake Ontario	**Marsh Creek**, Eighteen Mile Creek, Four Mile Creek, Fish Creek, Golden Hill Creek, Hopkins Creek, Keg Creek, Oak Orchard, Sandy Creek, Six Mile Creek, Twelve Mile Creek
Allegheny River	**Chautauqua Lake, Case Lake,** Findley Lake	**French Creek**

Table 2. Summary of mudpuppy survey efforts and success rates for habitats in western New York over a two-year duration. RT = rock turning. mp/tn = mudpuppy per trapnight. mp/hr = mudpuppy per hour. - = Not Available.

					Capture Rate	
	No. trap nights	No. hours RT	# trapped mudpuppies	# rock turned mudpuppies	mp/tn	mp/hr
Lakes						
Erie	1919	-	51	-	0.027	-
Lime	153	-	0	-	0.000	-
Hoyt	20	-	0	-	0.000	-
Ontario	100	-	0	-	0.000	-
Silver	91	-	0	-	0.000	-
Chautauqua	130	-	5	-	0.038	-
Case	765	-	21	-	0.033	-
Findley	95	-	0	-	0.000	-
Streams						
Smokes Creek	284	5.50	1	4	0.004	0.91
Buffalo Creek	288	14.00	2	8	0.007	0.57
Cayuga Creek	291	23.75	1	28	0.003	1.18
Little Buffalo	-	1.00	0	1	-	1.00
Cazenovia Creek	-	6.00	0	0	-	0.00
Clear Creek	-	1.25	0	0	-	0.00
Upper Niagara River	276	-	0	-	0.000	-
Woods Creek	28	-	0	-	0.000	-
Big Sister Creek	40	1.00	0	0	0.000	0.00
Buffalo River	120	-	0	-	0.000	-
Cattaraugus Creek	-	7.50	-	0	-	0.00
Chautauqua Creek	-	1.00	-	0	-	0.00
Eighteen Mile Creek	26	6.00	0	0	0.000	0.00
Ellicott Creek	75	0.75	0	0	0.000	0.00
Little Sister Creek	20	-	0	-	0.000	-
Walnut Creek	-	2.75	-	0	-	0.00
Tonawanda Creek	21	2.00	0	0	0.000	0.00
Genesee River	-	1.50	-	1	-	0.67
Oatka Creek	-	3.00	0	0	-	0.00
White Creek	-	1.00	0	0	-	0.00
Wiscoy Creek	-	5.00	0	0	-	0.00
Marsh Creek	96	5.50	1	15	0.010	2.73
Fish Creek	-	0.50	-	0	-	0.00
Four Mile Creek	-	1.00	-	0	-	0.00
Golden Hill Creek	-	0.50	-	0	-	0.00
Hopkins Creek	-	0.50	-	0	-	0.00
Keg Creek	-	0.5	-	0	-	0.00
Oak Orchard Creek	-	3.00	-	0	-	0.00
Six Mile Creek	-	0.5	-	0	-	0.00
Twelve Mile Creek	-	0.5	-	0	-	0.00
Sandy Creek	28	3	0	0	-	0.00
18 Mile Creek	-	1	-	0	-	0.00
French Creek	-	2	-	2	-	0.80
Total	4866	101.5	82	59	0.018	0.60

Table 3. Welch two sample t-test results of capture methods and morphometries, and coefficient of variation (CV) of metrics by collection method.

Feature	**t**	**df**	**P**	**CV**	
				RT	**trapping**
Girth	2.81	120.82	0.006	0.19	0.18
Total Length	0.48	114.30	0.598	0.17	0.15
SVL	0.09	104.89	0.927	0.18	0.14
Mass	1.30	113.46	0.197	0.51	0.42
Head Width	0.33	104.27	0.740	0.18	0.14

Table 4. AOV and Kruskal-Wallis results of morphological features by season.

Morphological Feature	**df**	**MS**	**F/H**	**P**
Total Length	3	100.76	6.19	0.0006
Error	132	16.27		
Snout-Vent Length	3	44.67	5.65	0.001
Error	132	7.91		
Head Width	3	103.90	3.69	0.014
Error	113	28.12		
Girth	3	6.78	6.78	0.0003
Error	132	40.35		
Body Condition	3	0.54	31.68	$6.1*10^{-7}$
Error	132	0.04		
Mass	3	.	18.04	0.004
Error	132	.		

Table 5: Gastric lavage result by season and by habitat type. N = number of guts flushed. n = number of a given prey item found among all the guts. Occur = number of guts a prey item was found in. Rel Abund = relative abundance (n divided by total prey items). Rel Freq = relative frequency (Occur divided by N).

	Winter (N=16)				**Spring (N=48)**			
Prey	n	Occur	Rel Abund	Rel Freq	n	Occur	Rel Abund	Rel Freq
Fish	16	11	0.21	0.69	22	17	0.12	0.35
Insect	15	7	0.19	0.38	93	20	0.49	0.42
Mollusc	0	0	0.00	0.00	9	4	0.05	0.08
Crayfish	0	0	0.00	0.00	9	9	0.05	0.19
Worms	14	2	0.18	0.06	1	1	0.01	0.02
Isopods	1	1	0.01	0.06	11	3	0.06	0.06
Amphipods	22	3	0.29	0.19	17	7	0.09	0.15
Amphibians	1	1	0.01	0.06	1	1	0.01	0.02
Eggs	0	0	0.00	0.00	0	0	0.00	0.00
Tapeworm	1	1	0.01	0.06	4	4	0.02	0.08
Plant matter	2	2	0.03	0.13	8	8	0.04	0.17
Sand/rock	1	1	0.01	0.06	3	3	0.02	0.06
Amorphic	2	2	0.03	0.13	9	9	0.05	0.19
Plastic	3	3	0.04	0.19	4	4	0.02	0.08
Total	77				191			

	Summer (N=21)				**Fall (N=7)**			
Prey	n	Occur	Rel Abund.	Rel Freq.	n	Occur	Rel Abund.	Rel Freq.
Fish	0	0	0	0	0	0	0	0
Insect	15	6	0.47	0.29	2	1	0.67	0.14
Mollusc	0	0	0	0	0	0	0	0
Crayfish	0	0	0	0	0	0	0	0
Worms	0	0	0	0	0	0	0	0
Isopods	0	0	0	0	0	0	0	0
Amphipods	6	1	0.19	0.04	0	0	0	0
Amphibians	0	0	0	0	0	0	0	0
Eggs	6	2	0.19	0.10	0	0	0	0
Tapeworm	1	1	0.03	0.05	1	1	0.33	0.14
Plant matter	2	2	0.06	0.10	0	0	0	0
Sand/rock	2	2	0.06	0.10	0	0	0	0
Amorphic	0	0	0	0	0	0	0	0
Plastic	0	0	0	0	0	0	0	0
Total	32				3			

Table 5
continued

	Lakes (N=49)				**Streams (N=43)**			
Prey	n	Occur	Rel Abund.	Rel Freq	n	Occur	Rel Abund.	Rel Freq
Fish	33	24	0.17	0.49	5	4	0.05	0.09
Insect	71	16	0.37	0.33	54	18	0.49	0.42
Mollusc	8	3	0.04	0.06	1	1	0.01	0.02
Crayfish	6	6	0.03	0.12	3	3	0.03	0.07
Worms	0	0	0	0	15	3	0.14	0.07
Isopods	11	3	0.06	0.06	1	1	0.01	0.02
Amphipods	35	9	0.18	0.18	10	2	0.09	0.05
Amphibians	1	1	0.01	0.02	1	1	0.01	0.02
Eggs	0	0	0.00	0.00	6	2	0.05	0.05
Tapeworm	3	3	0.02	0.06	4	4	0.04	0.09
Plant matter	9	9	0.05	0.18	3	3	0.03	0.07
Sand/rock	2	2	0.01	0.04	4	4	0.04	0.09
Amorphic	10	10	0.05	0.20	1	1	0.01	0.02
Plastic	4	4	0.02	0.08	3	3	0.03	0.07
Total	193				111			

	All (N=92)			
Prey	n	Occur	Rel Abund.	Rel Freq.
Fish	38	28	0.13	0.30
Insect	125	34	0.41	0.37
Mollusc	9	4	0.03	0.04
Crayfish	9	9	0.03	0.10
Worms	15	3	0.05	0.03
Isopods	12	4	0.04	0.04
Amphipods	45	11	0.15	0.12
Amphibians	2	2	0.01	0.02
Eggs	6	2	0.02	0.02
Tapeworm	7	7	0.02	0.08
Plant matter	12	12	0.04	0.13
Sand/rock	6	6	0.02	0.07
Amorphic	11	11	0.04	0.12
Plastic	7	7	0.02	0.08
Total	304			

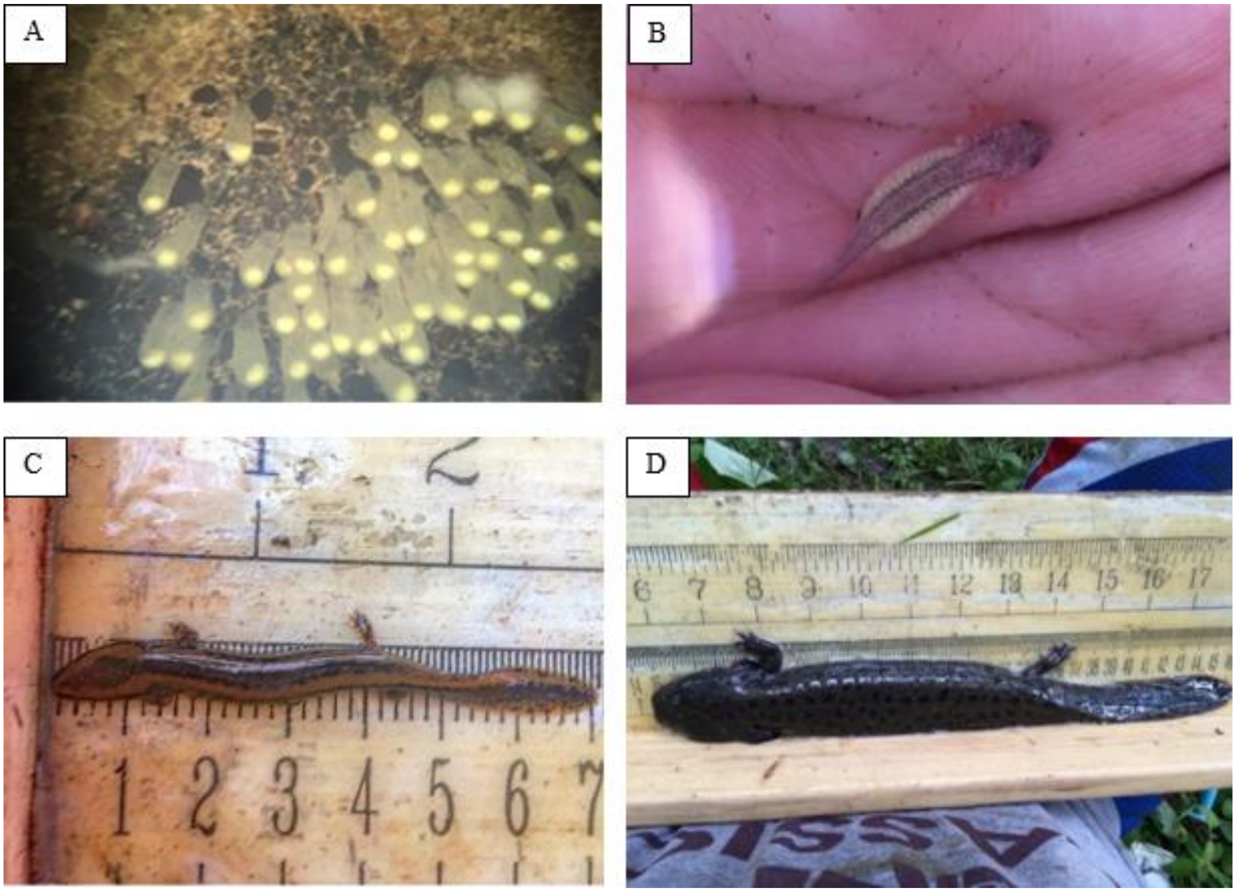

Figure 1: Life stages of *Necturus maculosus*. A. Mudpuppy eggs connected to rock B. Newly hatched larval mudpuppy. C. Juvenile mudpuppy D. Adult mudpuppy

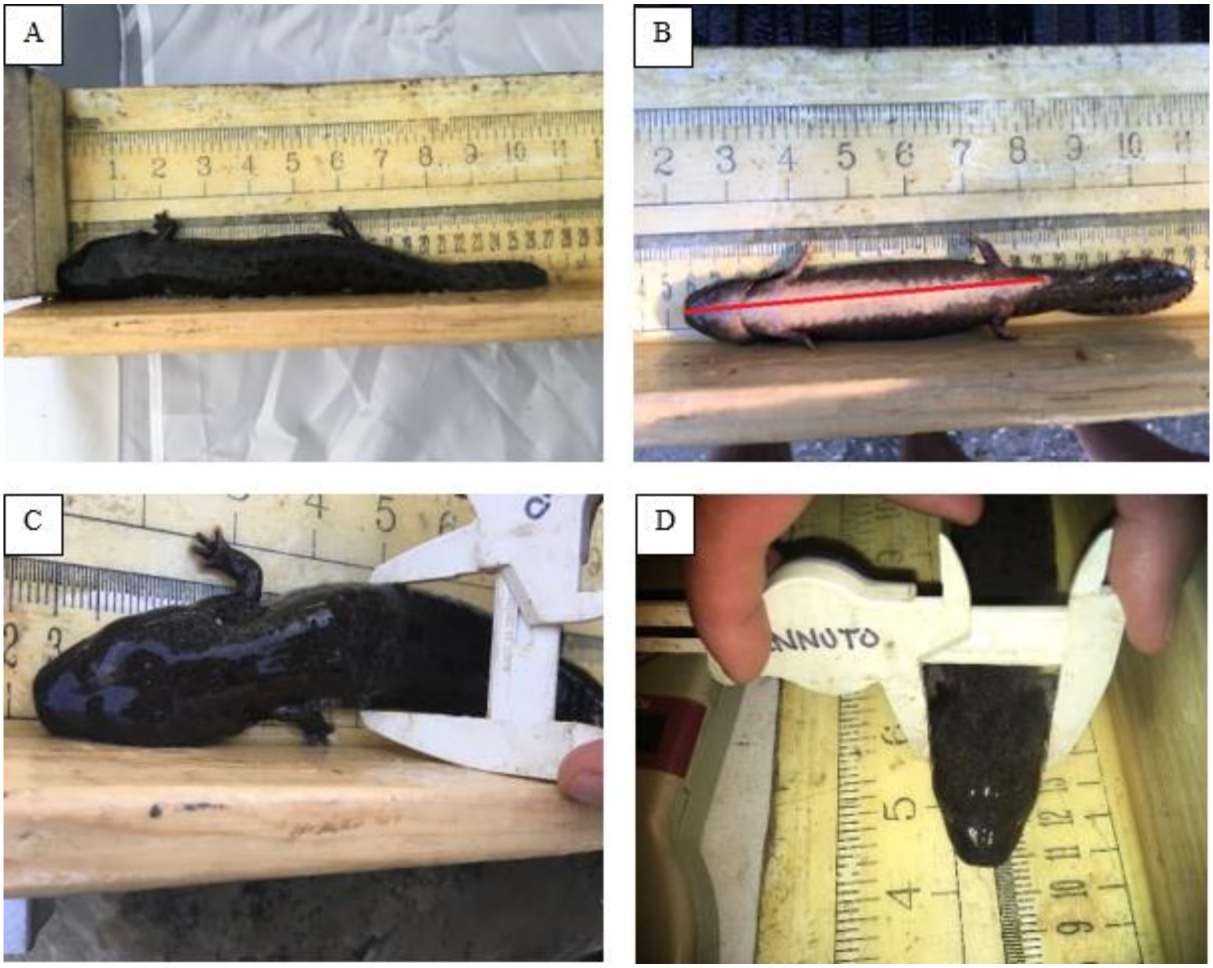

Figure 2. Morphological metrics used in analysis of capture methods. A. total length (TL), B. snout-vent-length (SVL), C. body girth (BG), D. head width (HW)

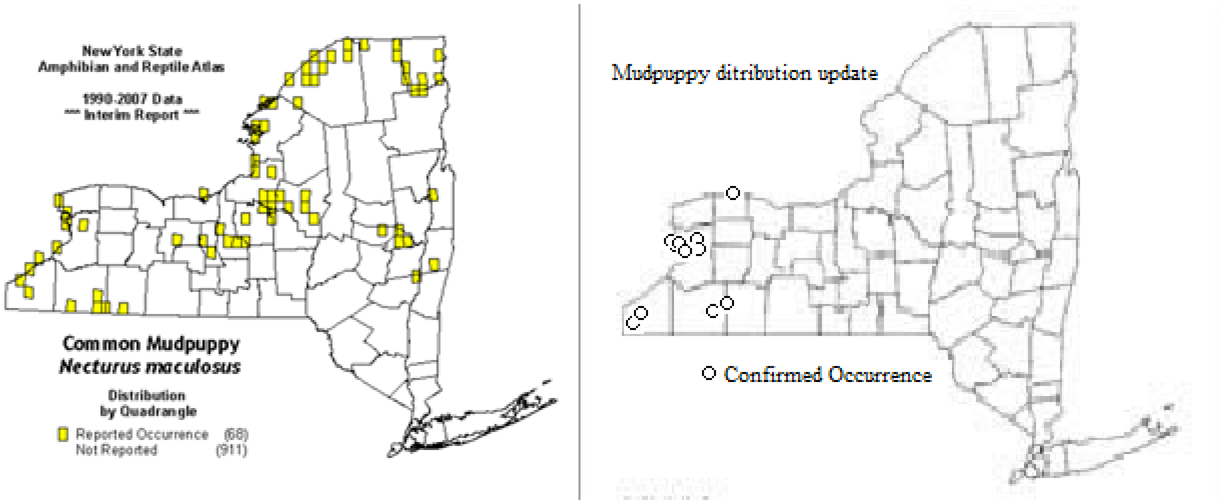

Figure 3: The image on the left shows the previous NYS distribution for mudpuppies. The results of my distribution study for western New York

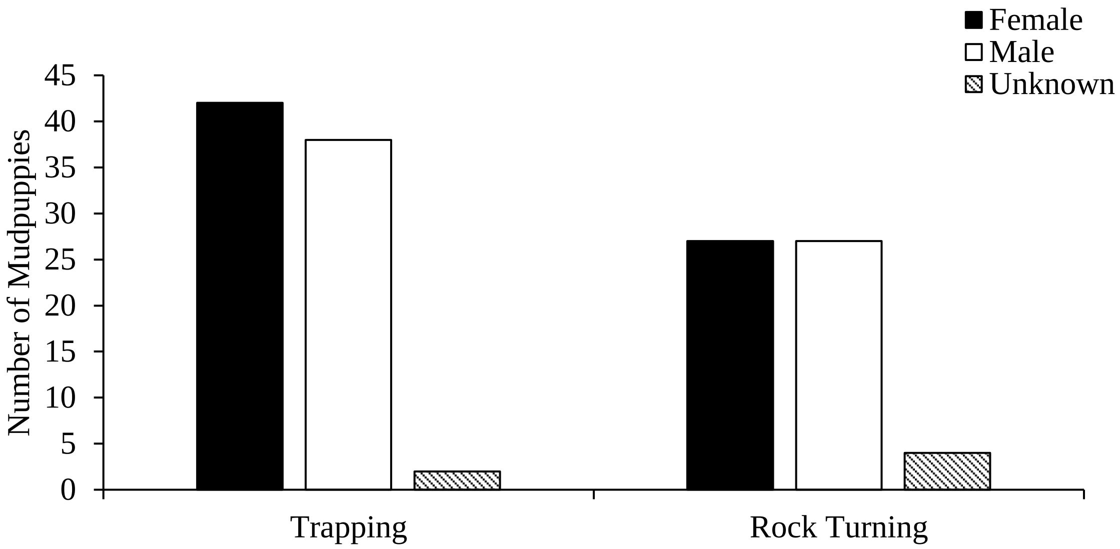

Figure 4. Total adult male, female and unknown sex mudpuppies captured in minnow traps and during rock turning surveys.

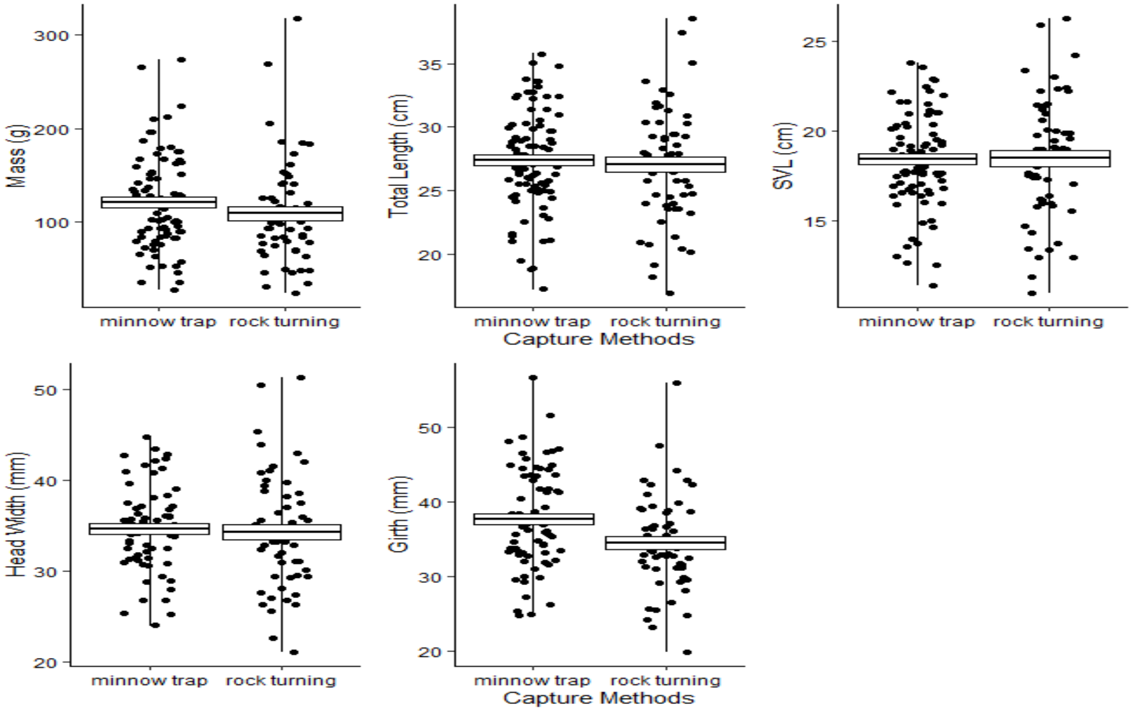

Figure 5. Morphology of mudpuppies as a function of capture method. Box plot = 95% confidence interval. Whiskers = Largest and smallest mudpuppy captured. Horizontal line = mean

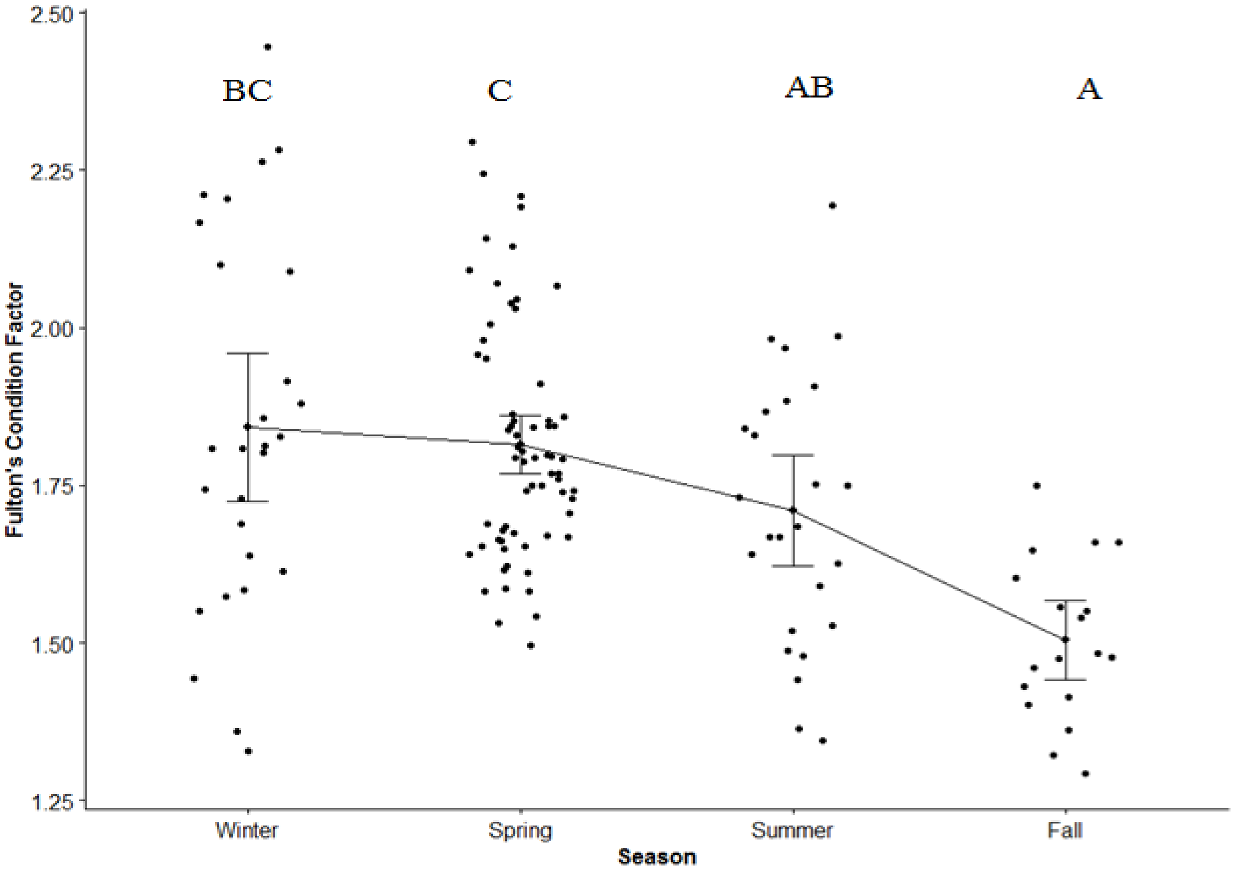

Figure 6: Fulton-type condition factor by season. Mean and 95% confidence intervals are shown. K-W analysis suggested a significant seasonal change in conditions (H = 31.684, df = 3, P = 6.1e-07). Same letters indicate seasons are not different @ $p<0.05$

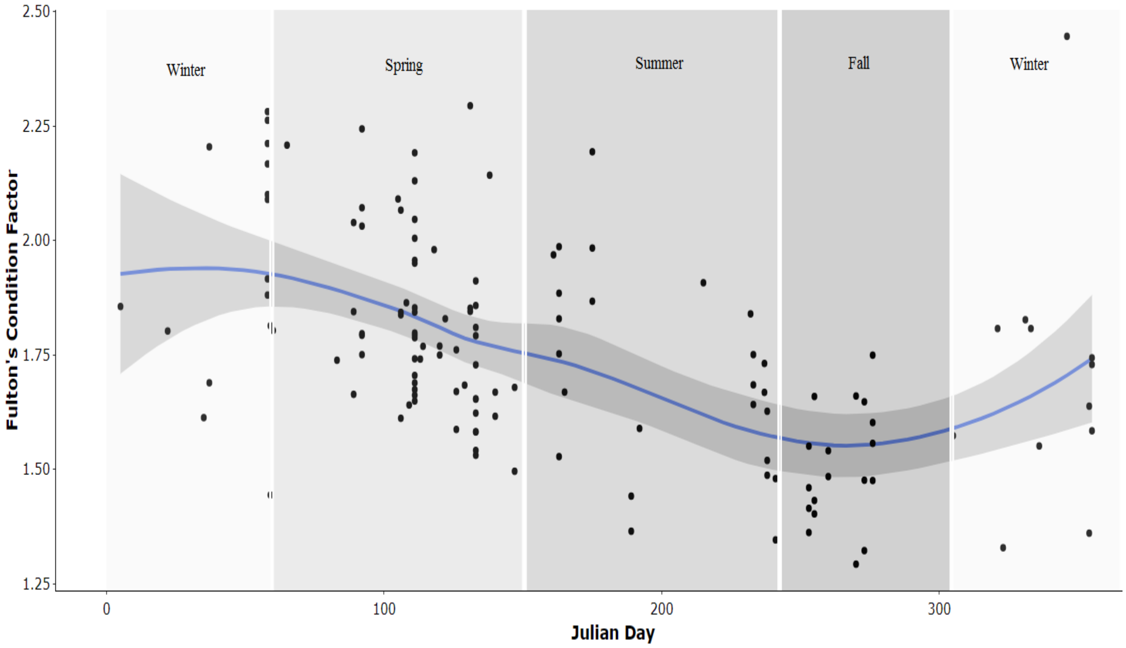

Figure 7: Smoothed, best-fit line of a Fulton-type condition index plotted against Julian Day to show the trends in mudpuppy body condition throughout the year. Shaded area around the line = 95% CI.

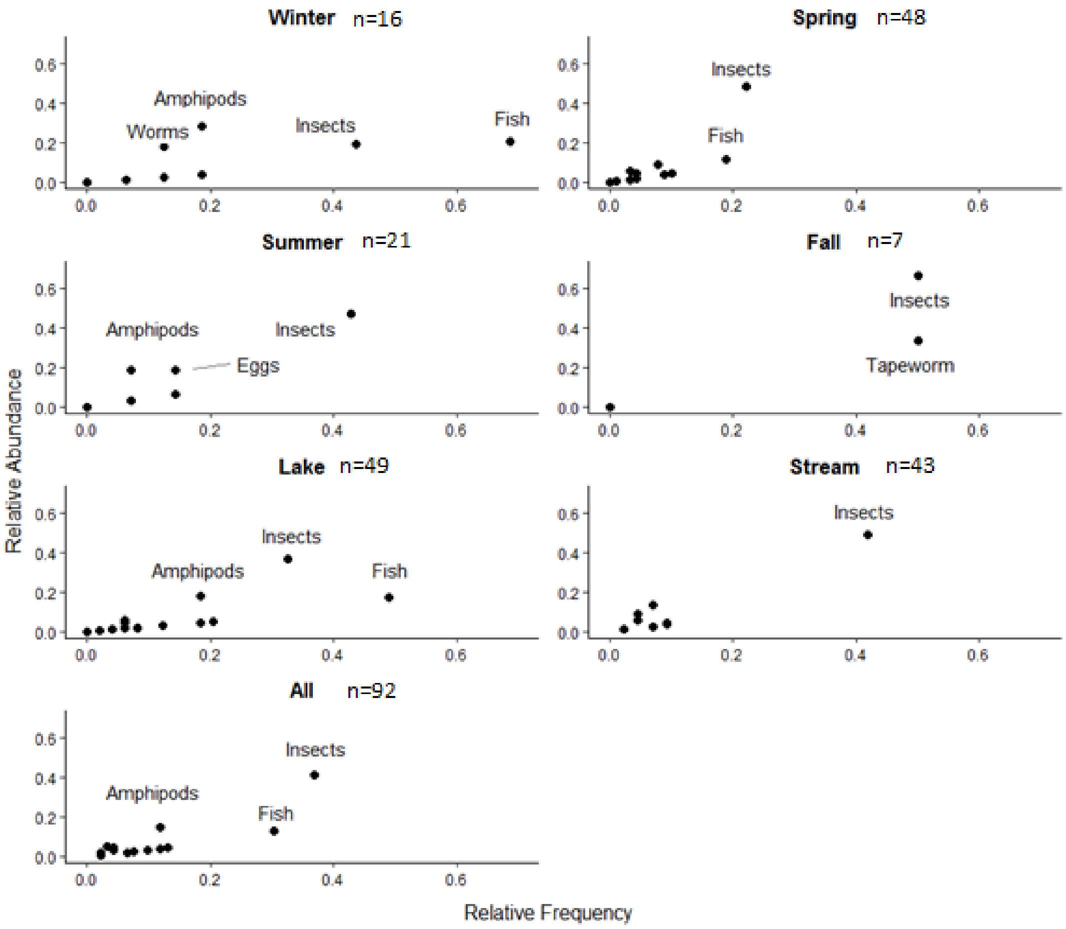

Figure 8: Costello plots of gastric lavage content divided into seasons (winter, spring, summer and fall), habitat (lake or stream) and all seasons and habitats combined

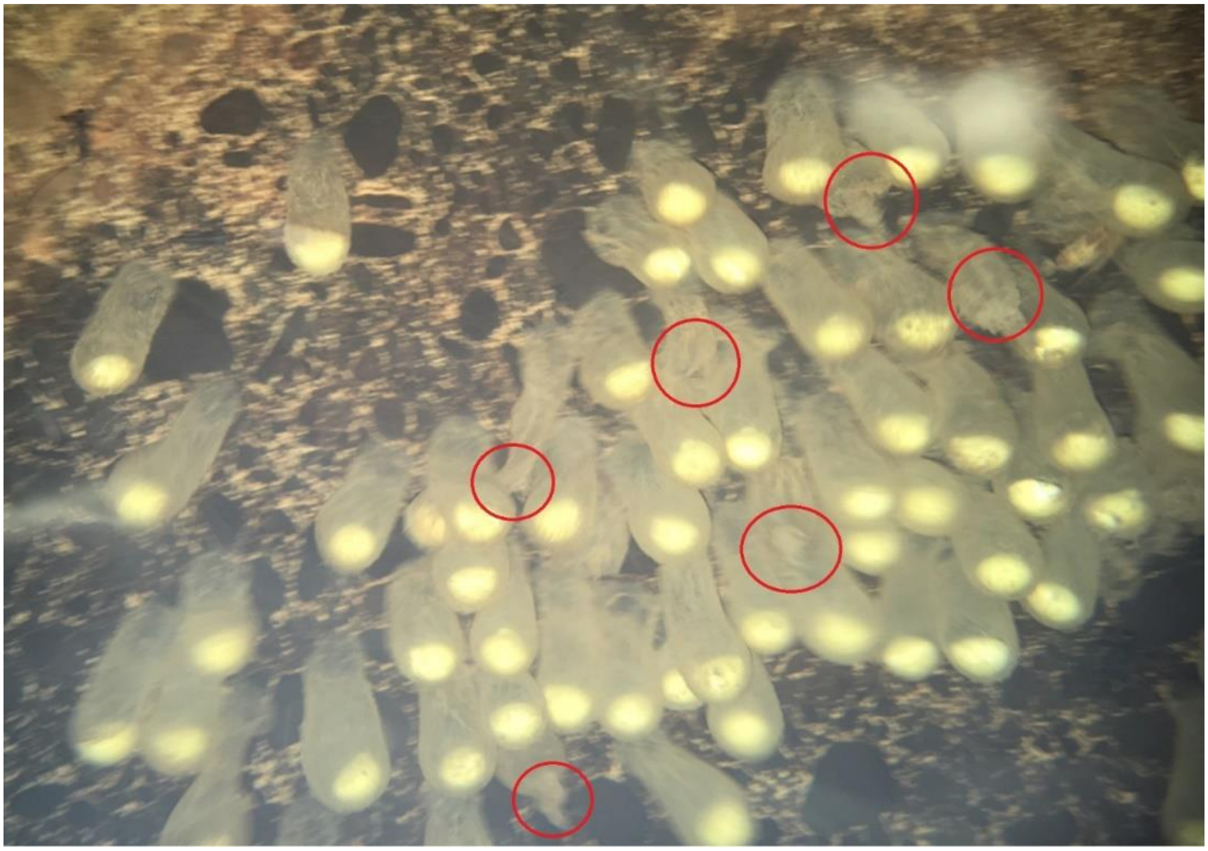

Figure 9: Mudpuppy nest with eggs connected to the nest rock. Red circles surround what appears to be missing embryos.

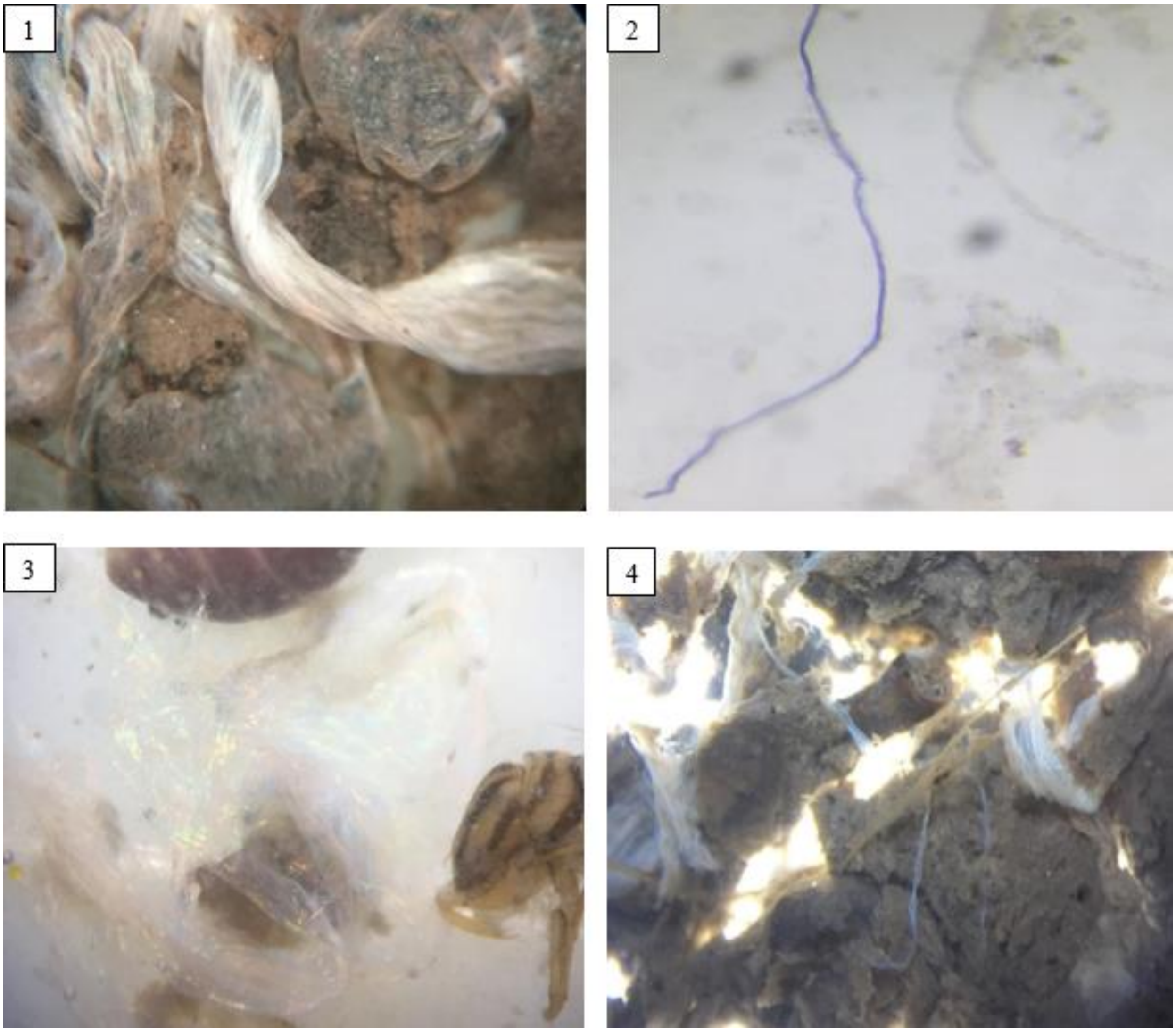

Figure 10: Images of microfilm (1,3,4) and microfibers (2) taken while inspecting mudpuppy stomach contents.

Chapter 2

Morphological Response to Environmental Context in the Common Mudpuppy, *Necturus maculosus*

Abstract

Environmental context influences the morphology of multiple plant and animal species. My study explored whether habitat type (lotic vs lentic) was associated with morphological differences of the Common Mudpuppy (*Necturus maculosus*). Mudpuppies were captured in lentic and lotic environments throughout western New York and one location in Canada, and a suite of morphological measurements first were compared by habitat type, and secondarily compared by season and sex. The habitat model was determined to be the best model. Total length and SVL were significantly different between lake and stream habitats, while girth and TDL were marginally significantly different. The differences supported the hypothesis that mudpuppies would be more hydrodynamic in stream habitats and have longer digits to grip the substrate. Mudpuppies likely have a variety of phenotypes induced by different contexts found throughout their range. However, hydrologic conditions in streams appear to influence morphology of mudpuppies. Whereas this work might suggest plasticity in Common Mudpuppies, a common garden experiment is needed to determine if the mechanism is phenotypic plasticity or selection.

Introduction

Environmental context effects on morphology

Environmental context can shape the development of physiology, behavior, morphology, and life history traits of many organisms. Changes can be induced in several different ways. Phenotypic plasticity, or the ability of a genotype to express different phenotypes under different contexts (West-Eberhard 1989), is one possible organismal response to environmental conditions. Phenotypic plasticity may be induced within a lifetime, and in many cases, during development (Relyea 2004). Over generational time frames, natural selection may favor the expression of some phenotypes in novel environmental contexts. Additionally, a combination of plasticity and natural selection has been observed (Aubret and Shine 2009) and environmental context is the driver of morphological, behavioral, and life history differences. Environmental context dependency has been observed in plants, birds, mammals, insects, fish, reptiles and amphibians around the globe (Duldley and Schmitt 1996, Reale et al. 2003, Charmantier et al. 2008, Moczek 2010, Telemeco et al. 2010, Oromi et al. 2014).

Phenotypic plasticity occurs in many forms of life. Larval fire salamanders (*Salamandra infraimmaculata*) exhibit an increase in gill area when exposed to low levels of dissolved oxygen in controlled and natural conditions (Segev et al. 2019). The tiger snake (*Notechis scutatus*), after colonizing islands for several generations, developed larger heads. The changes were driven by prey context; larger prey items on the island led to large heads relative to head sizes found on the mainland where average prey size was smaller (Aubret and Shine 2009). In plants, some species can broaden their leaves in low light conditions (Dudley and Schmitt 1996), relative to high light conditions. In response to climatic changes, the great tits (*Parus major*) in the UK have advanced their breeding and egg laying period by about two weeks since 1961 (Charmantier et al. 2008). Fish have shown very plastic morphologic changes in response to environmental conditions. In high velocity streams, Atlantic salmon (*Salmo salar*) morphology became more robust and brown trout (*Samlo trutta*) became more streamlined when compared to fish reared in low-flow environments (Pakkasmaa and Piironen 2001). Even human infants show phenotypic plasticity; infants exposed to maternal sounds and heart beats had a significantly larger auditory cortex than infants exposed to environmental noise (Webb et al. 2015).

Although plasticity can decrease survivability if it is costly or alters other life history traits, plasticity is generally thought to increase the survival of species during times of rapid biotic and/or abiotic change (Reed et al. 2011, Kelly 2019, Scheiner et al. 2020). Understanding the phenotypic plasticity of traits helps us better predict how species will react to changing climate conditions (Donelson et al. 2017). It also provides some guidance on which species warrant allocation of limited conservation resources. As of 2004, ~43% of the amphibian populations around the world were declining (Stuart et al. 2004), with current extinction rates ~200 times the background, fossil record amphibian extinction rate (McCallum 2007). Global research is needed to better understand the causes of amphibians declines and how humans can manage populations during climate change. Fine-scale research into amphibian plasticity can help conservation decision-makers as climate change continues (Seebacher et al. 2014).

Amphibians have shown an extraordinary variety of responses to different environmental contexts. In a comparison between lentic and lotic populations of the aquatic newt, *Calotriton asper*, differences in morphology, behavior and life history traits were documented (Oromi et al. 2014). The stream salamanders were larger, more robust and had keratinized warts, whereas the lake populations had smooth skin and less robust bodies. In a lab study, wood frog (*Lithobates sylvaticus*) tadpoles showed different morphological features in response to predators and competitors. When exposed to predators, wood frog tadpoles decreased activity and increased tail depth whereas exposure to competitors induced increased activity and decreased tail depth (Relyea 2004). When exposed to combinations of predators and competitors there were interactive effects, suggesting the tadpoles could sense not only predators and competitors but the risk of predation and intensity of competition. However, not all responses to environmental cues may be advantageous. In some amphibians Roundup™ herbicide induces antipredator morphological changes even in the absence of predators (Relyea 2012).

Lentic and lotic habitats differ in both biotic and abiotic conditions, providing a natural comparison for environmental context effects on organisms. Some conditions that differ between the two habitats include, but are not limited to, multidirectional vs unidirectional flow, stratified vs homogeneous water temperatures, light stratification vs no light stratification, autochthonous vs allochthonous energy inputs, large depth differences vs small depth differences, depositional vs erosional substrates, stratified oxygen content vs. well-mixed dissolved oxygen content, low vs high turbidity and low vs. high nutrient content. Lentic environments are deeper than lotic systems throughout much of the habitat. In lentic environments, such as Lake Erie, thermoclines can develop creating large differences in temperature from the top to the bottom of the system. Streams generally do not stratify due to the constant flow in a downstream direction. Lotic environments can have cold seeps where cool ground water enters the stream, cooling confined areas where the substrate meets stream water. Water in lotic environments is likely to be cooler than surface water lentic environments, but warmer than water in lakes below the thermocline.

The thermal regimes of lentic and lotic environments may have an important effect on the growth of some aquatic herpetofauna (Germano and Bury 2009, Hu et al. 2019). Thermal regimes do influence egg size and growth in salamanders, with lotic-dwelling salamanders producing larger eggs (Davenport and Summers 2010). Storms can cause streambed movement and drastic changes in flow, making them less stable than lentic systems. Flora and fauna that live in lentic and lotic environments are also different. Lentic environments tend to harbor different macroinvertebrate and fish communities than lotic environments. The flow of genetic material may also differ between lentic and lotic environments, especially for those species that are purely aquatic and become isolated by geologic or man-made barriers (Murphy 2018).

This study explored context-dependent morphological differences between wild populations of the Common Mudpuppy (*Necturus maculosus*), by comparing multiple morphometrics between lotic and lentic populations. The Common Mudpuppy is a completely aquatic salamander found throughout the Midwest and Eastern United States, as well as southern Canada.

Since lotic systems have constant, unidirectional, and higher flow velocities than lentic systems, I hypothesized that several morphological characteristics of stream mudpuppies would reflect greater streamlining. Generally, I expected lentic populations would be less streamlined and larger than the stream individuals. I hypothesized that stream populations would have smaller volume, shorter tail length, more shallow tail depth, more narrow head width, and more narrow girth relative to lake populations. These morphological differences reduce surface area in contact with the moving water. Total length (TL) and snout-vent-length (SVL) were hypothesized to be greater in stream mudpuppies because larger TL or SVL per unit mass means the animal is longer and thinner. Some morphological features, however, may increase in response to flow. For example, I expected digit length to be larger in lotic populations because I suspected that walking behavior would be more important in lotic than lentic conditions as animals avoid the main current and grip the substrate. Tail length was expected to be greater in lentic populations than lotic populations, where swimming may be more important for movement. I expected tail depth to be less in lotic environments since it would also decrease surface area. Head width in lake habitats was hypothesized to be greater than populations in streams, however head length was expected to be larger in stream populations. A thinner, longer head is more streamlined than a wide head, creating less total head surface area in stream populations. Volume and girth were hypothesized to be greater in lake populations than in stream populations (Table 1). I hypothesized that the average mudpuppy caught in lakes would be larger than the average caught in streams.

Methods

I used modified minnow traps and rock turning/snorkel surveys to capture mudpuppies. All but two mudpuppies were collected under NYSDEC License to Collect and Possess: Scientific Permit # 2145.

Mudpuppy collections

Eight lakes in the eight western New York counties were sampled. Lakes were found using satellite imagery on Google Maps and were chosen mainly by ease of access. Sampling in lentic environments was conducted from November thru May using modified minnow traps (approximately 43 x 23 cm in size). Traps were baited with canned pet food and deployed at sunset for an overnight set. Traps were deployed along a line with ~5 m between each trap and, with few exceptions, 10 traps per line. Traps were checked within 24 hr, weather permitting and were deployed for 1-to-3 days per location. If no mudpuppies were trapped within the first three days, traps were moved to a different location. Each trap had an identification tag in accordance with state regulations for minnow traps.

Thirty-four stream or river locations were sampled for mudpuppies in all seasons. In streams where pools were large enough, trap lines as described above were used for collections and were selectively placed in areas that looked to be good habitat (e.g., deep holes, areas near large rocks). Traps were used from November to May. Rock-turning (RT) surveys supplemented trapping in streams. During RT, I targeted partially buried rocks larger than 10 cm and held dipnets around the perimeter of the rock. When possible, rocks were lifted from the upstream side to allow the sediment to be washed out by the stream flow. Snorkel and masks were used to improve visibility underwater. Mudpuppies were slowly and gently corralled or lifted into the dipnets. Rock-turning was confined to depths ≤ 1.5 meters and occurred year-round, but most RT occurred in the summer months.

Body Measurements

Immediately after capture mudpuppies were placed in individual Ziplock bags, half filled with stream or lake water. All mudpuppies collected were weighed using either a Pescola scale or field balance, followed by a suite of measurements (Table 1 and Figure 1). Total length (TL) and snout-vent-length (SVL) were measured on a fish board from tip of the nose to tip of the tail (Figure 1A) and tip of the nose to anterior edge of the vent (Figure 1B), respectively. Body girth (Figure 1C) was measured at the widest point in the middle third of the body cavity using digital calipers. Tail depth (TD), head width (HW), and head length (HL) also were determined using digital calipers and were found as the maximum dorsal-to-ventral height of the tail (Figure 1D), the maximum width of the head (Figure 1E), and the distance from tip of the snout to base of the posterior gills (Figure 1F), respectively. Tail length (TaL) was found by the difference between TL and SVL. Volume was measured by displacement of water in a graduated cylinder (Figure 1G).

Digit Measurements

Each right front and rear foot were photographed with an iPhone for digit measurements. The feet were pressed against a clear acetate plate with a 1-cm scale (Figure 1H). Images were measured in ImageJ. Typically, measurements were taken from the front and rear right feet, however, if there were visible deformities of the right foot, then the left foot was measured instead. If both feet had deformities no measurements were taken. Digits were measured from the tip of the digit to where the adjacent digit met. Front, rear, and total digits lengths were recorded (Table 1).

Statistical analysis

Comparison of morphological features is difficult due to strong positive correlations between overall size (mass) and feature size. To account for the relationship between feature size and overall size, I followed procedures from Relyea (2012) to create mass-adjusted morphological measurements. First any morphological features that were not normally distributed were log-transformed. Next, I ran individual ANCOVAs with a morphological feature as the dependent variable, mass as the covariate, and habitat as the fixed effect.

I saved the residuals and estimated marginal mean from each ANCOVA using only locations where four or more mudpuppies were captured. Each individual's residual value was then added to the estimated marginal mean of the location from which it was captured. This value was substituted for the original measurement value and became the new mass-adjusted value for a given mudpuppy.

Next a mean measurement value from each location was calculated; the mean value from each location was now considered one sample to represent each location. I then used the mean location values for each feature in a MANOVA. Due to unequal sample sizes, TDL was analyzed separately from the other morphological features using an ANOVA.

Although sex and season may also influence mudpuppy morphology, my collections resulted in an unbalanced design (e.g., no summer collections from lakes) and prevented me from including sex and season in a full model with habitat. Thus, I repeated the above process to test the effects of sex and season separately on morphology. All statistical analyses were conducted in R version 3.6.3 with a $P < 0.05$ considered significant differences and a $P < 0.10$ considered to be marginally significant.

After creating habitat, season, and sex MANOVA models containing all morphological variables except TDL, I compared them to determine which created a best fit for mudpuppy morphology. I tested all three models to determine which created a better fit for mudpuppy morphology using the Akaike's Information Criterion (AIC) function ('extractAIC') in the 'stats' package in R.

The active season was defined by the first capture of a mudpuppy using passive gear sampling (minnow traps) to the last day of passive gear capture. The time between passive gear success was considered the inactive season.

Sampling Gear Treatment

Any gear that was deployed in different locations was washed to remove any visible debris and immersed in a 9:1 water:bleach solution for at least 10 minutes between sites (in accordance with Bio-safety protocols for Reptile and Amphibian Sampling in NYS). The canoe used was cleaned in accordance with NYSDEC Bureau of Fisheries Sampling, Survey, Boat and Equipment Protocol and Biosecurity Protocol.

Handling of Amphibians

Handling of all mudpuppies was done with bare hands that were rinsed with the water present at the sampling site. Captured individuals were housed individually in an unsealed, vented, single-use, disposable plastic bag with water from the location site until they were processed. Processing took place within ~1.5 hours of capture. Animals were removed from the holding bags and placed on a fish board for measurements prior to release. Mudpuppy collection and handling protocols were carried out under SUNY Buffalo State IACUC application #40 ("Assessing mudpuppy population status and habitat comparisons.").

Results

Mudpuppies were not captured in equal numbers at all locations and not all measurements were obtained at each location, creating unequal sample sizes between features (Table 2). No digit lengths were collected from Kemptville Creek due to time constraints and only two animals from Smokes Creek were usable due to foot deformities.

All mudpuppy features had a strong linear relationship with mass, with an average correlation coefficient of 0.92 across all measurements. Volume had the highest and TDL had the lowest correlation coefficient with mass at 0.99 and 0.73, respectively.

Two morphological features were significantly different between lentic and lotic habitats, TL and SVL (MANOVA: $P < 0.05$), while girth and TDL were marginally different ($P < 0.10$) (Table 3). Lotic mudpuppy features had much smaller variability than lentic mudpuppy features in all measurements (Figure 2). Adjusted lentic mudpuppy mean feature size was lower in all measurements except girth and volume.

TL, girth and TDL were significantly affected by season ($P < 0.05$). Seasonality had marginally significant effects on SVL and TaL ($P < 0.10$) (Table 4). Girth was expected to be significantly different between seasons (Figure 3). TL, SVL and TaL are all highly correlated which may be the reason for them all to be significantly and marginally significantly different. TDL was marginally significantly different between habitat types. No significant or marginally significant effects of sex on any morphological features were found.

Comparison of AIC scores suggested that the habitat model was a better fit than either the season or sex model with AIC scores of 76.5, 105.8 and 160.6, respectively.

Discussion

The significant difference of TL and SVL in lentic and lotic habitats followed the hypothesized trends. As expected, both TL and SVL were longer for mudpuppies captured in streams than in lakes. Girth and volume differences also suggested that lotic mudpuppies had smaller body cavity width and volume than lake animals. Mudpuppies in streams were longer and skinnier than in lakes. The longer, skinnier features would reduce total surface area and reduce the amount of drag a mudpuppy would experience in moving water. A mudpuppy living in a stream and exposed to higher flows may prefer moving along the stream floor and gripping the substrate. The marginal difference in

TDL supported this theory, with longer digit lengths observed in animals from the lotic habitats relative to those from lentic habitats.

Raw mass and volume were strongly correlated (r = 0.99), but they followed different trends after size adjustments. Mudpuppies in streams exhibited larger masses despite their smaller volumes and girths, than mudpuppies captured in lakes. This may be interpreted as stream mudpuppies having more muscle or more dense bones, and thus being more dense per unit size. Mudpuppies with more dense or more muscular bodies may be better adapted to living at the bottom of a stream, and physiologically more capable of remaining in place in the face of the unidirectional flow.

My results suggest that mudpuppies may be plastic in their morphology, and that the morphological response of stream mudpuppies is less variable than that of lake animals. Interpreting the results from morphological analyses of wild populations is difficult due to the number of variables, known and unknown, that impact organisms throughout all life stages. The misclassification of a lake habitat may have also contributed to the variability that I found in lake populations. The lakes that I sampled were located within two separate watersheds. Within the Allegheny watershed, I sampled both a man-made and natural lake. In 1970, Case Lake was created when Gates Creek was dammed. All mudpuppies measured from Case Lake were captured at the mouth of Gates Creek, where flow may have been influential in their morphology. The Lake Erie watershed is separated from the Allegheny watershed by hundreds of river miles. This may explain some variability in the morphology of lake animals. However, both Kemptville Creek and Marsh Creek are from watersheds with relatively distinct populations and yet the lotic populations had relatively small variability in features. The mudpuppies in the lakes that I sampled may also have an environmental context other than flow that impacts their morphology. Due to unbalanced cells, the interactions between habitat, sex and season fixed effects could not be tested together in one MANOVA. The results of the AIC suggest that the model with just habitat was the best model for mudpuppy morphology. Stream mudpuppies showed less variability in their features than lake mudpuppies, suggesting that flow played an important role in their morphology.

Morphological measurements obtained during the inactive season had more variability than measurements from the active season. Since most animals collected during the active season were obtained by trapping, it is likely that they would be of similar sizes. Previous studies have suggested that trapping is a bit size-biased as the traps preclude entry of the very largest animals and allow the escape or deter the smallest animals (Beattie et al. 2017, Haines and Pennuto (In review)). During the inactive season, animals were collected by rock-turning. The methodology allows the capture of a wider range of sizes compared to trapping.

Although TL was significantly or marginally significantly different in both habitat and season, SVL was highly significantly different (P = 0.007) only between habitats and was only marginally significantly different between seasons (P = 0.069). To better understand these differences more samples will need to be collected from different locations. Despite small samples sizes in my study fairly consistent results in the morphology of stream dwelling mudpuppies were observed.

A recent study of mudpuppy genetics throughout their range shows that genetic divergence occurred in the eastern and western portions of the mudpuppies range (Greenwald et al. 2020). However, all mudpuppies captured in this study are within the geographic distribution of the eastern haplotype. Kemptville Creek and Lake Erie were sampled by Greenwald et al. (2020) both at and near the collection locations in my study. Greenwald et al. (2020) also collected samples from the Ohio River, just outside my sites on tributaries to the Allegheny River. Thus, all of my sites lie geographically within the larger area sampled by Greenwald et al. (2020) and would presumably be genetically similar to the eastern haplotype. Therefore, it is most parsimonious that phenotypic plasticity, and not selection, is the mechanism at play in these distinct populations. However, a new species of *Necturus* was discovered in 2017 (Nelson et al. 2017).

Females were slightly larger than males in all features other than tail depth and digit length. Females also had slightly larger volumes than males. Among amphibians, it is common for females to have larger bodies than males (Shine 1988). Female-biased sexual size dimorphism is typically attributed to a fecundity advantage. However, environmental conditions can also affect male and female sizes (Angelini et al. 2015). Longer tails have been observed in female salamanders in multiple populations (Bakkegard and Rhea 2012, Oromi et al. 2016) and have been theorized to be influenced by sexual selection, respiration, predator defenses and energy storage. Longer tail lengths exhibited by females in an aquatic newt species made it easier for males to capture females for mating. Having a longer tail can also increase the amount of energy the salamander can store during periods of inactivity. Energy storage for female mudpuppies may be especially important due to nest guarding for extended periods of time and seasonal inactivity.

Male mudpuppies had slightly deeper tails than females. There could be several advantages to having a larger tail depth. Deeper tails may increase acceleration making it easier for males to capture females. Males also exhibited shorter tail lengths than females, and if tail morphology influences energy storage, males may make up for energy storage with tail depth. Males also had slightly longer digits. This may indicate that walking or gripping is more important in males than females. This result is consistent with movement studies that showed evidence of males exhibiting larger ranges and making larger movements than females (Shoop and Gunning 1967, Chellman 2017).

Conclusion

This study shows that Common Mudpuppies show plastic responses to environmental context. Specifically, mudpuppies in streams appeared to have a specific response to flowing water. This response was consistent with the expectation that mudpuppies would be more hydrodynamic. Longer digit length increases the surface area and gripping potential of mudpuppies in streams. A recent study (Greenwald et al. 2020) hints that the differences found between lake and stream habitats is unlikely to be due to genetic differences. It is difficult to determine if the morphological responses seen in these wild stream populations is driven by phenotypic plasticity or selection. A common garden experiment would help limit the number of unknown effects and determine if the results are due to plasticity or selection. Expanding the number and range of lakes and streams sampled would help us better understand how mudpuppy morphology responds to different contexts. Understanding the degree of plasticity in any target species may help resource managers and biologist make better decisions on conservation of species during a time of rapid climate change.

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Table 1: Morphological feature description and hypothesized response to environmental context. S = stream. L = lake. For each trait, the hypothesis provides an expectation that the trait is larger in stream or lake habitats.

Morphological Trait	**Description**	**Hypothesis**
TL	Tip of snout to tip of tail	S > L
SVL	Tip of snout to front of vent	S > L
Gir	Width of body at widest point	L > S
HL	Tip of snout to base of posterior gills	S > L
HW	Width of head at widest point	L > S
TD	Widest point of tail	S > L
TaL	Total length minus SVL	L > S
Vol	Maximum water displaced in graduated cylinder	L > S
TDL	Distance from tip of toes to where digits meet, all feet	S > L

Table 2: Number of feature samples taken at each location.

Location	TL	SVL	Mass	Gir	TD	TL	HW	HL	Vol	TDL
BC	10	10	10	10	10	10	10	10	8	6
CaL	20	20	20	20	20	20	20	20	20	19
CC	27	27	27	27	27	27	27	27	27	23
ChL	5	5	5	5	5	5	5	5	5	4
KC	37	37	37	37	37	37	37	37	37	0
LE	46	46	46	39	40	46	32	32	25	11
MC	11	11	11	10	11	11	11	11	11	11
SC	5	5	5	5	5	5	5	5	5	2

Table 3: Results of MANOVA and univariate ANOVA (TDL) on each mass-adjusted morphological feature investigating effects of habitat type (lakes vs streams). Bold values are significant at the 0.05 or marginally significant at the 0.10 level.

Feature	**df**	**MS**	**F**	**P**
Mass	1	0.011	0.456	0.525
Error	6	0.023		
TL	1	1.787	12.400	**0.013**
Error	6	0.144		
SVL	1	0.663	16.183	**0.007**
Error	6	0.041		
TaL	1	0.001	1.590	0.254
Error	6	0.001		
TD	1	0.858	0.260	0.629
Error	6	40.350		
Gir	1	0.001	4.283	**0.084**
Error	6	0.000		
HW	1	0.225	0.338	0.582
Error	6	0.664		
HL	1	2.818	2.049	0.202
Error	6	1.375		
Vol	1	0.000	2.909	0.139
Error	6	0.000		
TDL	1	6.780	6.572	**0.062**
Error	4	0.012		

Table 4: Results of MANOVA and ANOVA (TDL) on each mass-adjusted morphological feature investigating effects of season (active vs inactive). Bold values are significant at the 0.05 or marginally significant at the 0.10 level.

Feature	**df**	**MS**	**F**	**P**
Mass	1	0.010	0.373	0.558
Error	8	0.034		
TL	1	2.200	8.259	**0.021**
Error	8	0.253		
SVL	1	0.502	4.409	**0.069**
Error	8	0.114		
TaL	1	0.002	4.188	**0.075**
Error	8	0.001		
TD	1	2.428	0.909	0.368
Error	8	2.671		
Gir	1	0.001	7.535	**0.025**
Error	8	0.000		
HW	1	0.462	0.481	0.508
Error	8	0.961		
HL	1	0.021	0.011	0.918
Error	8	1.864		
Vol	1	0.000	0.000	0.999
Error	8	0.000		
TDL	1	0.400	24.690	**0.003**
Error	6	0.016		

Table 5: Results of MANOVA and ANOVA (TDL) of mass-adjusted features investigating effects of sex (male vs female). Bold values are significant at the 0.05 or marginally significant at the 0.10 level.

Feature	**df**	**MS**	**F**	**P**
Mass	1	0.004	0.124	0.732
Error	11	0.028		
TL	1	0.683	1.618	0.230
Error	11	0.335		
SVL	1	0.006	0.038	0.850
Error	11	0.151		
TaL	1	0.001	1.005	0.338
Error	10	0.001		
TD	1	1.806	0.548	0.475
Error	10	3.294		
Gir	1	0.000	1.237	0.290
Error	10	0.000		
HW	1	0.676	0.746	0.406
Error	10	0.906		
HL	1	0.006	0.003	0.957
Error	10	2.052		
Vol	1	0.000	0.069	0.798
Error	10	0.000		
TDL	1	0.056	1.351	0.279
Error	8	0.041		

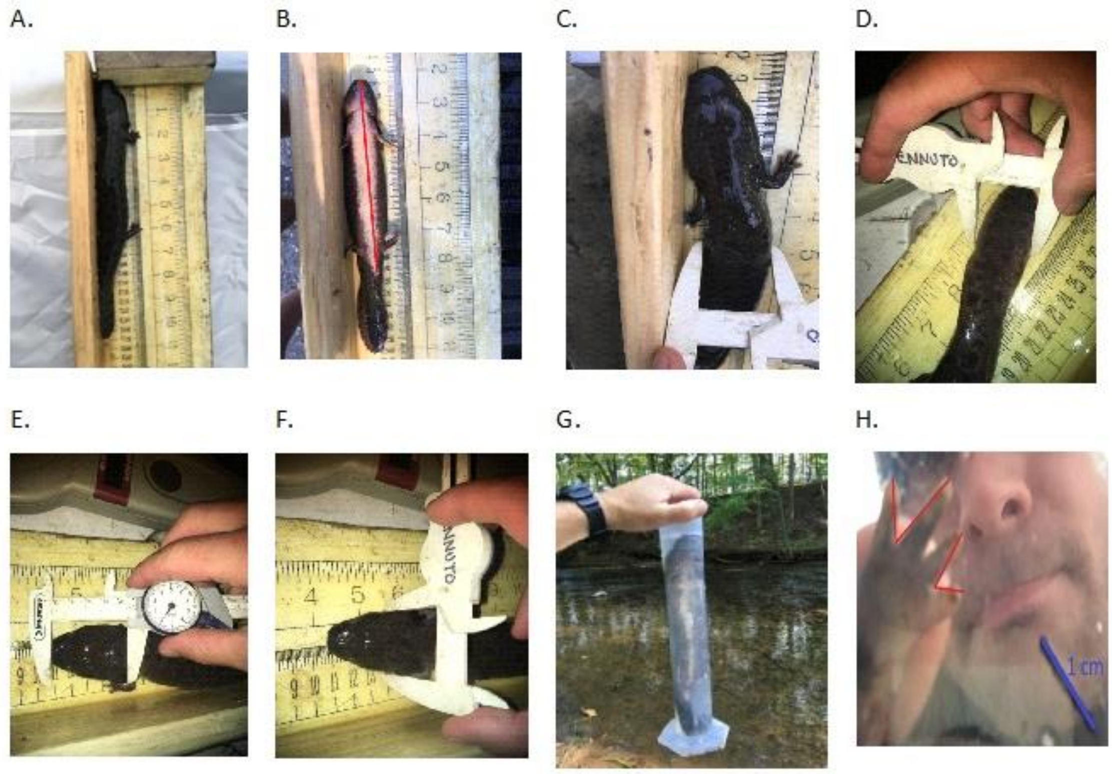

Figure 1: Visual examples of each morphological measurement of common mudpuppy. A: total length (TL), B: snout-vent-length (SVL), C: body girth (Gir), D: tail depth (TD), E: head length (HL), F: head width (HW), G: volume (Vol), H: digit length (blue line shows the 1-cm scale on acetate plate and red lines show the beginning and end of each digit).

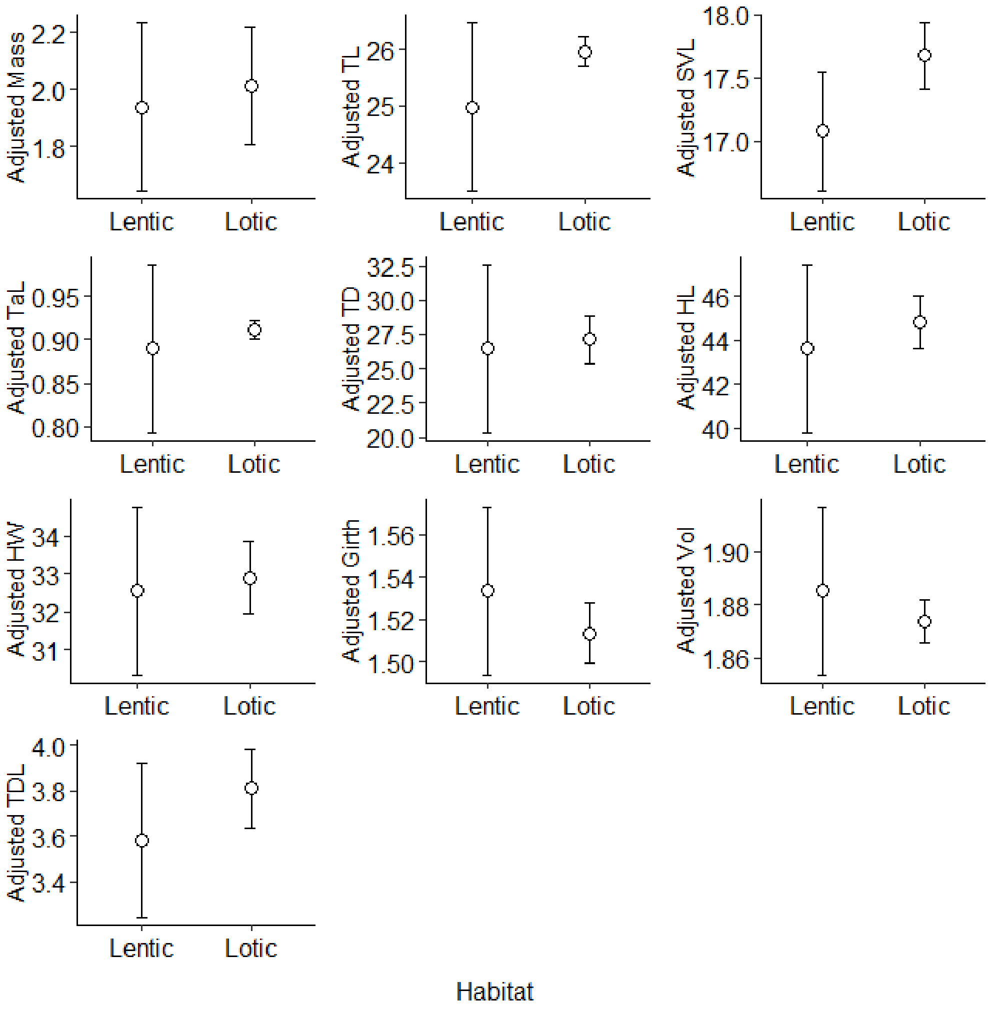

Figure 2: Mean and 95% confidence interval of mass-adjusted morphological feature size by habitat classification.

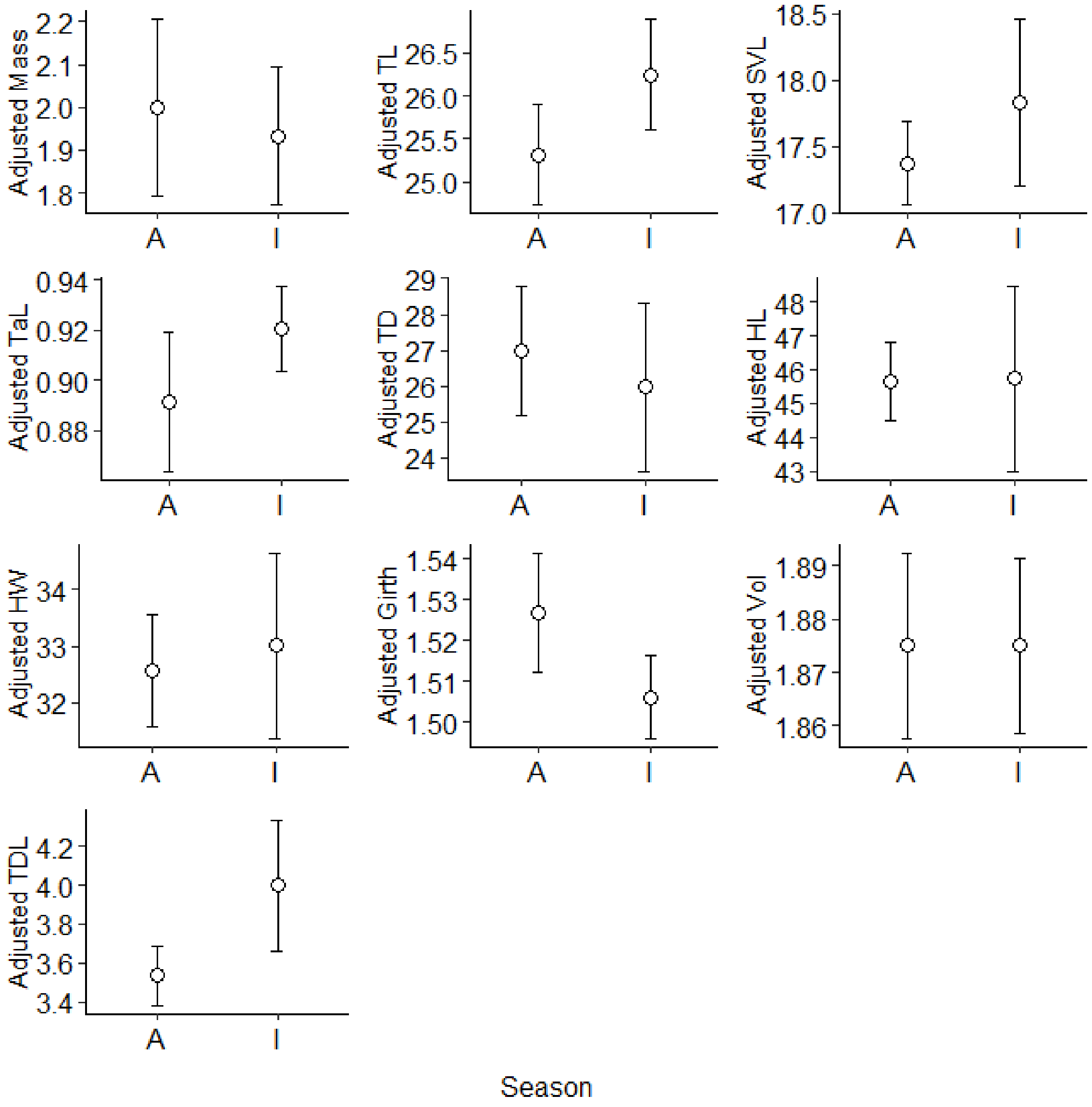

Figure 3: Mean and 95% confidence interval of mass-adjusted morphological feature size by season. Active season (A) and inactive (I) capture season are based on capture dates in passive trapping gear (see Methods).

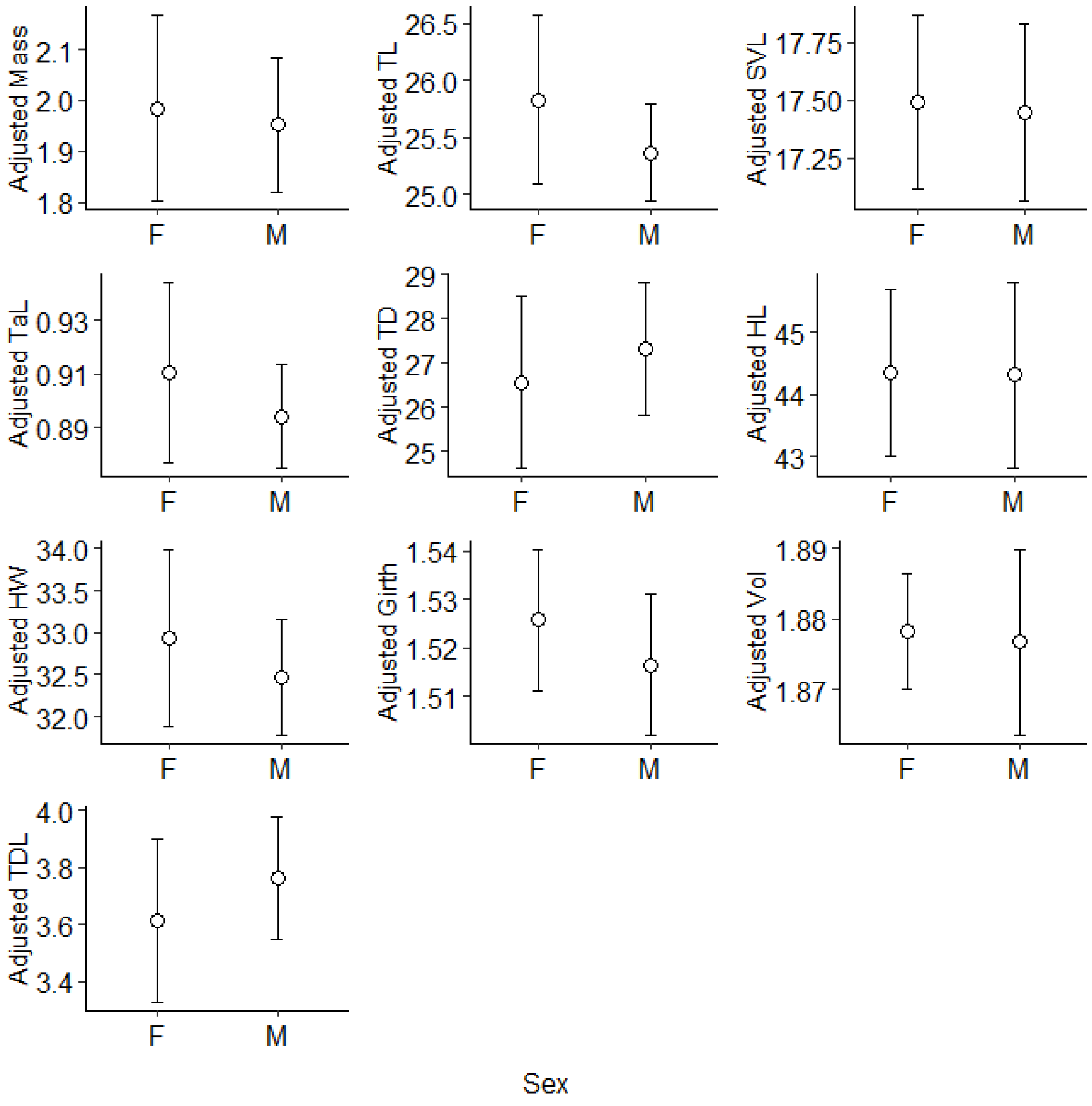

Figure 3: Mean and 95% confidence interval of mass-adjusted morphological feature size by sex. Female (F) and male (M) mudpuppies captured across all seasons and habitats.