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Using Occupancy Modeling for Protocol Development and Habitat Assessment of Necturus maculosus in a Large Nagivational River

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USING OCCUPANCY MODELING FOR PROTOCOL DEVELOPMENT AND HABITAT ASSESSMENT OF *NECTURUS MACULOSUS* IN A LARGE NAVIGATIONAL RIVER

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Master of Science
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by
Alyssa Rachelle Jones
Approved by
Dr. Jayme Waldron, Committee Chairperson
Dr. Shane Welch
Dr. Anne Axel

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APPROVAL OF THESIS

We, the faculty supervising the work of Alyssa Rachelle Jones, affirm that the thesis, *Using Occupancy Modeling for Protocol Development and Habitat Assessment of Necturus Maculosus in a Large Navigational River*, meets the high academic standards for original scholarship and creative work established by the Department of Biological Sciences Graduate Program and the College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

		14 November 2022
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		November 14, 2022
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We did it…

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ABSTRACT

Determining the survey effort required to reliably detect population change can be challenging for cryptic, elusive species. The secretive nature of amphibians makes it difficult to monitor population status and gather information about their natural history, including habitat use, which is essential for amphibian monitoring programs. The goals of this study were to examine if detection probabilities were affected by bait (i.e., light and food), breeding activity, and environmental covariates in a population of fully aquatic salamanders, *Necturus maculosus*. I evaluated the effectiveness of three bait treatments (light bait, food bait, combined light and food bait) and an unbaited control. I expected detection probabilities would be affected by changes in breeding behavior and nest attendance associated with breeding phenology. As predicted, I detected heterogeneities in detection probability that were congruous with the breeding season and the timing of surveys. Variability in water temperature was a limiting factor in mudpuppy detection. Highly water temperatures negatively affected detection probabilities (β = -4.56 ± 1.2). Bait influenced mudpuppy detection probability, with baited traps yielding higher detection estimates than light and unbaited traps. Clearly, investing sampling effort early in the season, before females nest and when water temperatures are cooler, is an efficient way to improve the accuracy of parameter estimates in this species. Our findings stress the importance of establishing study designs that take into account the population and behavioral ecology of the focal species.

INTRODUCTION

Freshwater ecosystems are among the most threatened habitats supporting populations of some of the most imperiled species (Gangloff et al. 2016). In North America, projected mean future extinction rates for freshwater fauna are about five times greater than for terrestrial fauna (Pimm et al. 1995; Ricciardi and Rasmussen 1999). Declines in these systems have been attributed to combined and interacting influences of stream channelization and damming, industrial development, overexploitation, water pollution, and habitat destruction or degradation (Benke 1990; Humphries and Pauley 2005; Strayer and Dudgeon 2010). These anthropogenic stressors may interact with natural stressors such as competition, predation, resource availability, and disease to facilitate amphibian population declines (Blaustein et al. 2012). Monitoring these unprecedented population declines has become an important focus of freshwater ecology and conservation (Strayer & Dudgeon 2010).

The Common Mudpuppy, *Necturus maculosus*, has been identified by the Northeast Association of Fish and Wildlife as a Species of High Conservation Concern. However, our knowledge of mudpuppy distribution contains substantial data gaps (Terrell et al. 2016). Most mudpuppy conservation efforts and research have focused on lentic systems, high elevation mountain streams or medium-sized rivers. While mudpuppies have a relatively wide distribution, much information about their natural history, habitat preferences, home range, seasonal movements, and population structure remains largely unknown (Gendron 1999). Mudpuppies are long-lived animals, with life spans estimated at over 30 years of age (Matson 2005). Females delay breeding until 7 to 10 years of age, when they have reached minimum body size of 20 cm (Bishop 1941; Matson 2005). These characteristics make them vulnerable to human disturbance and perturbations with ecological consequences, including changes in primary production, aquatic invertebrate communities, sediment dynamics, and algal assemblages (Whiles et al. 2006).

Mudpuppies currently exist in the main stem Greenup Pool of the Ohio River, but population size, density, habitats, and other life history data are not present in the literature. Large-scale impacts to the Ohio River, particularly large dam building and stream channelization, converted a free-flowing system to a regulated waterway with greatly reduced, and modified flow. These modifications increased water depth and altered the natural seasonal variations in temperature, oxygen regimes, and the patterns in which sediments were transported and deposited (Miller et al. 1984). Sediment data collected from a previous study (Kriege 2018), using SCUBA, indicated fine sediment build-up in the lower portion of the pool (Fig. 1), eliminating the lower pool as suitable mudpuppy habitat and possibly fragmenting populations. Along with damming, chemical water pollutants, and heavy siltation from agriculture, industrial and urban practices have also contributed to degraded habitat and contributed to mudpuppy declines (Matson 2005).

Effective sampling for mudpuppies in large navigable rivers is complex due to water depth, commercial and recreational boat traffic, strong currents, and low visibility. Because large rivers pose difficulties in accessibility, no effective sampling protocol to assess occupancy has been developed. Given the lack of data for mudpuppy populations in the Ohio River, my goals were to examine how mudpuppy detection probability was affected by season and sampling approach in a large-river system. Understanding such seasonal variation in capture success is important when examining population dynamics and life history strategies (i.e., habitat preferences, seasonal movements, gene flow, and dispersal) and how these change over time (McDaniel et al. 2009).

Occupancy modeling is a tool that is well-suited to assess presence in large landscapes and allow variable occupancy and detection rates to be calculated within a habitat (MacKenzie and

Royle 2005). This model-based approach estimates the probability of species presence in an area while accounting for the imperfect detection probabilities that are inherent in most sampling methods (MacKenzie et al. 2002). Detection probability, which may vary across species, time, and space (McKelvey and Pearson 2001; MacKenzie et al. 2002), is the probability of detecting a species at a site, given the site is occupied (Donovan and Hines 2007). Presence/absence data are based on patterns of detection and non-detection that are used to estimate both site occupancy (i.e., the probability of a randomly selected site being occupied by a species) and detection probability, accounting for imperfect detection. These models incorporate variation in detection that may result from survey specific or site-specific covariates (Mackenzie et al. 2006). Because mudpuppies are often difficult to sample due to their elusive, cryptic habits and variable sampling conditions, occupancy modeling may provide more accurate depictions of species' status and a better understanding of the factors that affect them.

Information about the best trapping methods for mudpuppy sampling is lacking, although several different approaches have been used to sample the species, including turning substrate and netting while wading and snorkeling, scuba/hookah diving, nocturnal spotlighting, bow-hooks/trotlines, electrofishing, underwater camera systems, and wire mesh baited traps (Browne et al. 2011). I evaluated minnow trap baiting methods to determine which bait treatment, or combination of treatments, yielded the highest detection probabilities for *N. maculatus* in a large navigable river. I used two types of bait (light sticks and food) alone and in combination, providing three treatments (light, food, and the combination of light and food) that I compared to unbaited controls. I expected all bait treatments would yield higher detection probabilities as compared to controls. I expected light bait would increase mudpuppy detection probability, given that many aquatic amphibians are phototaxic (Hailman and Jaeger 1976; Bennett et al. 2012). For example, glow sticks increase capture rates of aquatic amphibians (Smith and Rettig 1996; Grayson and Roe 2007; Bennett et al. 2012), including several larval Ambystoma species, American bullfrog tadpoles (*Rana catesbeiana*) and eastern red-spotted newt (*Notophthalmus viridescens*). Food also increases capture rates of amphibians (Smith and Gunzburger 2009; Briggler et al. 2013), including Eastern hellbender (*Cryptobranchus allegeniensis*), Two-toed amphiuma (*Amphiuma means*), Greater siren (*Siren lacertina*), and Lesser siren (*Siren intermedia*). Therefore, I expected the combination of light and bait would maximize detection probability and thus improve occupancy estimates of mudpuppies.

In this study, I used an occupancy framework to develop a mudpuppy sampling protocol suitable for large navigational rivers, identifying biotic and abiotic covariates that affect detection probability. I used a single-species, single-season-modeling framework (MacKenzie et al. 2017) to compare method-specific detection probabilities between four minnow trap sampling methods (i.e., bait and glow stick, bait, glow stick, and un-baited) and tested a suite of models with survey and site-specific covariates that might impact occupancy and detection. I expected detection probability would be affected by changes in breeding behavior and nest attendance associated with breeding phenology, and that increasing water temperature would negatively affect detection. I hypothesized that river morphology and cover objects would be limiting factors in mudpuppy occupancy. Understanding seasonal changes in capture success is essential when comparing relative abundance across systems as well as limiting the potential for biased population estimates. The results of this study will provide valuable information for conservation and management decisions concerning large aquatic salamanders in navigational rivers and fill significant data gaps in our knowledge of their distribution.

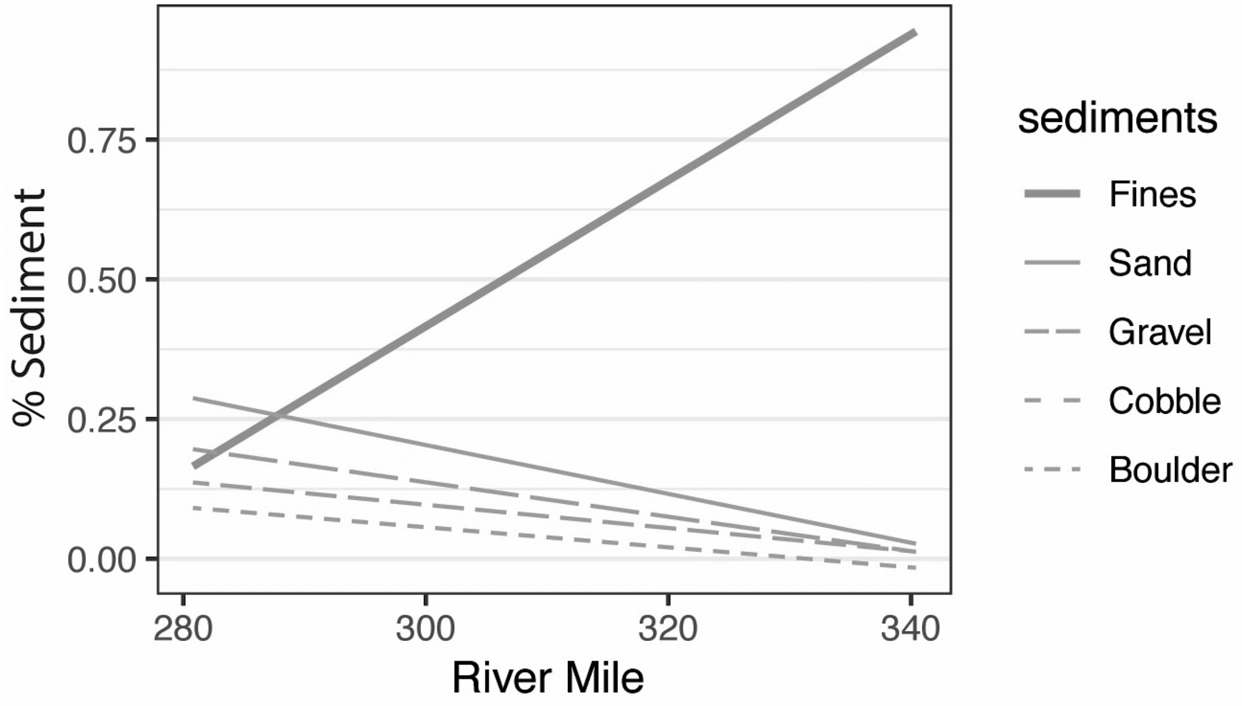

Figure 1. *Regression analysis of sediment composition***.** Changes in sediment composition by river mile in the Greenup Pool of the Ohio River, WV, USA.

METHODS

Study Area

I sampled the Greenup Pool of the Ohio River, WV, USA between August 2018 to March 2019 (Fig. 2). The Greenup Pool is formed by the RC Byrd Locks and Dam in Apple Grove, WV at river mile (RM) 279.2 and flows 61.8 miles downstream to the Greenup Locks and Dam in Kentucky at RM 341.0 (Zeto et al. 1987). The average depth is approximately 26 feet with a gradient drop of 0.4 ft/mile (ORSANCO 2011). Our study area encompassed a 36.53 km reach that extended from just downstream of the RC Byrd Dam, beginning at RM 280.1 to the confluence of the Guyandotte River with the Ohio River at RM 302.8. River substrate composition was dominated by bedrock, boulder, cobble, and gravel within approximately 500 m of the dam.

Substrate composition changed to primarily gravel, sand, silt, and scattered boulders throughout the rest of the study reach. Average width of the river in the study area was 339 m (n=22, calculated using ArcMap measure tool at each river mile).

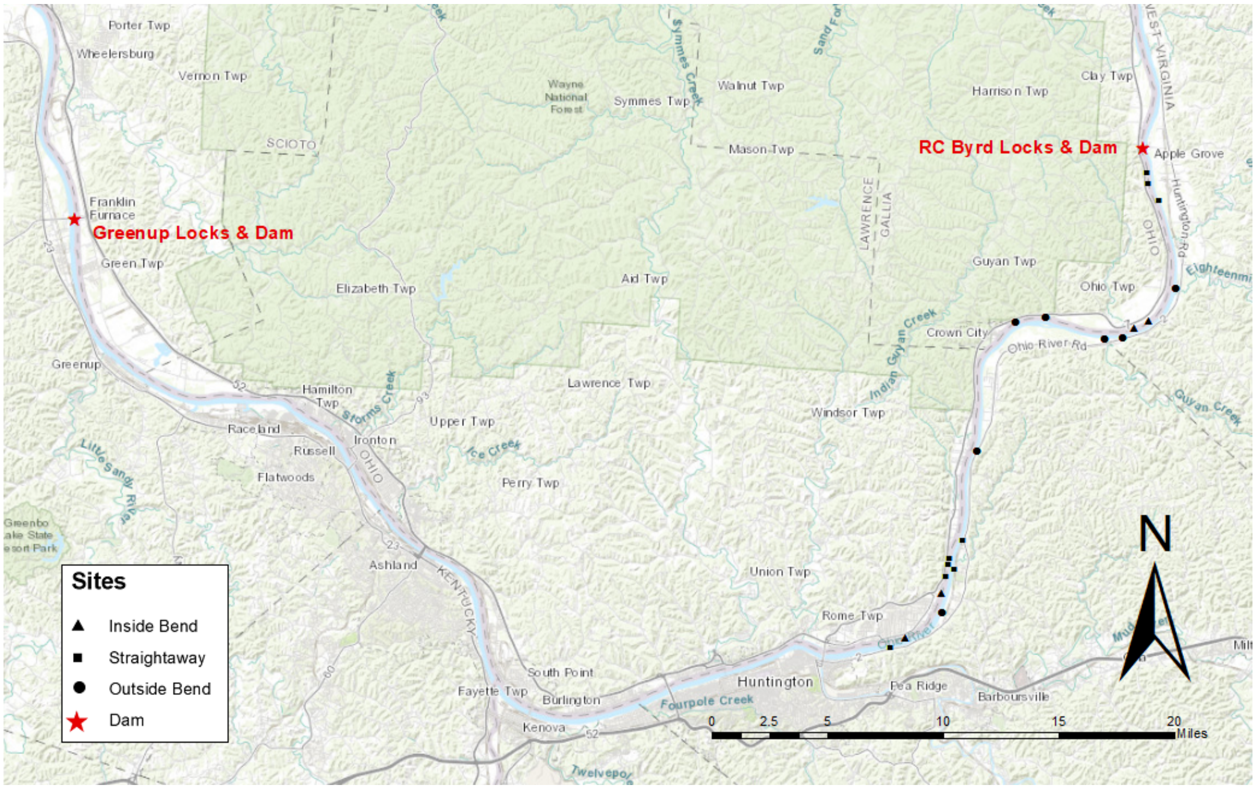

Figure 2. *Study area***.** Mudpuppy sampling sites within the Greenup Pool of the Ohio River, West Virginia, USA.

Data Collection

I sampled mudpuppies at 20 randomly selected sites in the upper portion of the Greenup Pool. I chose this area based on sediment data collected from previous research showing the most suitable habitat (Fig. 1) (Kriege 2018). I surveyed additional sites that had suitable mudpuppy habitat (high percentage of cover objects); I did not include captures from these two sites in occupancy analysis, however, because the sites were added later in the study. I sampled each site four times, and sampling periods ranged from (1) 25 August to 3 September, (2) 20 October to 5 November, (3) 25 March to 30 March, and (4) 5 April to 12 April. I chose sampling dates that encompassed all seasons; however, specific time frames were influenced by base flow conditions of the river. Significant rainfall events increase water depth and velocity and introduce large woody debris into the system creating hazards for boat operation and trap deployment and retrieval. My sampling protocol was intended to 1) develop an effective sampling technique for *N. maculatus* in large navigational rivers, 2) effectively capture *N. maculatus* in a mark-recapture framework to better allow for current and future study, 3) give managers the ability to monitor population status over time, and 4) minimize interference from recreational and commercial boaters in high-traffic navigable rivers.

I used rectangular modified minnow traps made of vinyl-dipped steel mesh with an inward-facing conical entrance on each end (Fig. 3; KUFA Corp., Blaine, WA, USA). The openings were enlarged to 6.0 cm using a Dremel tool and metal cutting wheel to allow entry of adult mudpuppies (McDaniel et al. 2009). This type of trap has been used in previous studies without any subsequent mortality or injury of mudpuppies (Gendron 1999). Four minnow traps were attached along a ten-meter line (trap array) and randomly assigned to one of three treatments or a control: (1) combination of light and food bait, (2) food bait (dog food and chicken liver), and (3) light bait (glow stick; 15 cm green military grade, 24-hour duration chemlights, Cyalume Technologies, West Springfield, MA, USA).

I used 100-m lead lines separated into 10-m intervals with stainless steel rings to randomly attach four sets of trap arrays (Fig. 4). This design was created to solve an inherent problem with navigable rivers: to avoid contact with heavy boat traffic from recreational and commercial boats, I could set 16 traps with only one buoy on the surface of the water for retrieval, minimizing the chance of accidental or intentional interference from boaters. I deployed each trap line over the front of the boat at a randomized distance between 30 and 50 meters parallel to shore. These distances were chosen from existing data showing optimal percent sediment composition and abundance of cover objects in this range for mudpuppy habitat (Kriege 2018). Distance to shore was measured using a Bushnell Yardage Pro Sport Model 450 range finder. Sites were side-scanned and georeferenced in the field using the HELIX 10 CHIRP MEGA SI GPS G2N.

Figure 3. *Mudpuppy comparison and trap array setup***.** Gravid female (top left) and male (bottom left) mudpuppy size comparison from the Greenup Pool, Ohio River, and rectangular modified minnow trap arrays (right) set up for deployment.

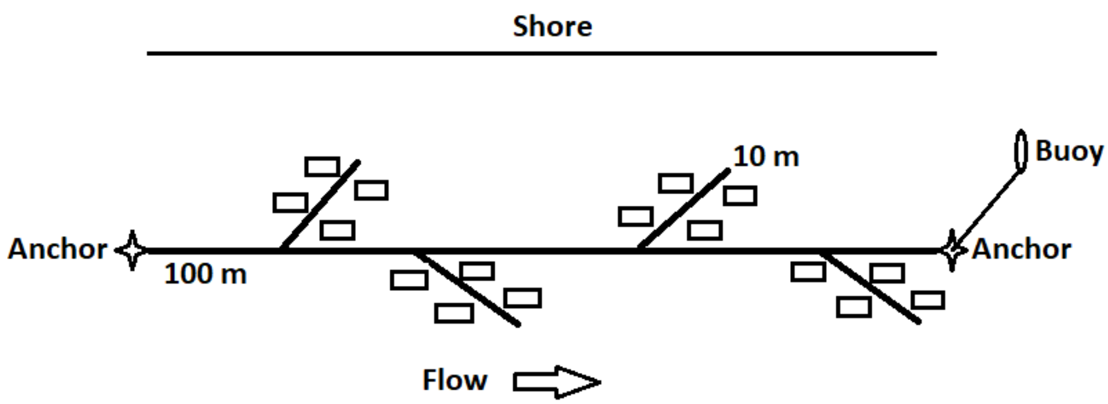

Figure 4. *Trap array design***.** Diagram of trap array design showing arrangement of trap line and direction of flow.

I used SCUBA to survey one meter on each side of the lead line and recorded percent substrate composition and all cover objects. I defined suitable cover objects as those measuring at least 30 cm (Gottlieb 1991) in diameter and not embedded. I used linear regression to test the relationship of percent fines and cover objects with river mile. I attached 4.5 kg anchors at each end of the lead line to hold the line in place in the current. A float line was attached to the downstream anchor to allow for retrieval of the traps the next day. I left traps in place overnight to capture mudpuppies during nocturnal foraging. I calculated daily catch per unit effort (CPUE) as the number of captures divided by the number of traps deployed. I compared CPUE using Kruskal-Wallis analysis.

I weighed mudpuppies to the nearest gram and secured each individual in a modified PVC pipe to measure snout-vent-length (SVL) and total length (TL) to the nearest cm. I compared TL and mass of males and females using a two-sample t-test. I used Pearson's correlation to examine the linear relationship of TL and mass of males and females. I determined adult mudpuppy sex by inspecting the cloaca. Males have a swollen cloaca that contains paired papillae at the posterior end throughout winter and early spring. Females do not possess a swollen cloaca or papillae (Bishop 1926; Gendron 1999). To uniquely mark individual mudpuppies, I implanted a Biomark 12.5mm, 134.2 kHz tag (Biomark, Boise, Idaho, USA) subcutaneously at the base of the tail on the left side. Tail clippings were collected for a separate, ongoing genetic study. I held mudpuppies briefly in aerated river water before release at their point of capture.

Predictor Variables

I used ArcMap (10.8.2) to define river morphology (i.e., straightaway, inside bend, and outside bend) for each site. I used SCUBA to visually quantify cover objects and estimate percent sediment composition by following a 100-meter lead line transect. I visually estimated substrate composition using the modified Wentworth scale: boulder (>30 cm), coble (2.5 – 30 cm), gravel (0.2 – 2.5 cm), sand (< 0.2 cm), and fines (material that could be suspended in water column) (Grossman and Ratajczak 1998). I used linear regression to determine the relationship of percent fines and abundance of cover objects by river mile.

Sampling Covariates

I collected water quality parameters using a YSI EXO2 multiparameter datasonde stationed below the RC Byrd Locks and Dam. The datasonde recorded water temperature (°C), pH, turbidity (NTU), and conductivity (μS/cm) every 15 minutes and is available as live data online as part of a Marshall University water quality and HAB monitoring program (https://v2.wqdatalive.com/public/1010). I standardized all continuous site and sample covariates to z-scores to reduce the influence of variables that had larger ranges (Donovan and Hines 2007).

Data Analysis

Occupancy models. I used correlation analysis in SAS [9.4] (Copyright 2002-2012) to examine collinearity among covariates (Tables 1 and 2) to retain the most biologically appropriate variables when $r \geq 0.7$ or $r \geq -0.7$. I used single-season, single-species occupancy models in program PRESENCE 12.7 (Hines 2006) to examine 14 candidate models (Table 3) estimating covariate effects on mudpuppy occurrence and detection probability (Tables 1 and 2). Site-specific encounter histories collected over multiple site visits enabled us to estimate occupancy, i.e., the probability that a site is occupied, while accounting for imperfect detection (MacKenzie et al. 2002; Guillera-Arroita et al. 2010; MacKenzie et al. 2017).

I examined occupancy, Ψ, as a function of site-level covariates (river morphology and cover objects; Table 1). I held detection probability constant, *p*(.), and allowed Ψ to vary as a function of site covariates. River morphology was treated as a categorical covariate: (1) inside bend, (2) outside bend, and (3) straightaway. However, I was unable to retain any models that included site covariates due to limited power. The site estimate for occupancy was 1 indicating occupancy was constant throughout our study site. Thus, I was unable to say that occupancy varied with any covariates and these models were removed from analysis (Table 1). To investigate the relationships between detection probability and environmental variables, I held the proportion of sites occupied constant, Ψ (.), and allowed *p* to vary with each sample covariate separately. To investigate whether breeding season would affect the probability of detection, I examined water temperature by sampling period (Ψ(.), *p* (water temp_season). I used analysis of variance (ANOVA) to compare detection probabilities by season using estimates derived from model (Ψ(.), *p* (water temp_season). I examined model fit using a chi-squared goodness-of-fit test, with 1000 parametric bootstraps on our most parameterized model (Ψ (River_morph + Cover_objects), *p* (Water_temp)). Lastly, I used estimated detection probabilities to assess the efficiency of our sampling design for detecting species.

Due to small sample size (n = 20), I ranked models according to AIC_c (Akaike 1973; Burnham and Anderson 2002). Models used to examine sampling-occasion-specific detection probability estimates for trapping methods were ranked using $QAIC_c$, a measure that corrects for overdispersion ($\hat{c}$ >1.0). The models were chosen a priori to compare several factors I felt were likely to affect parameter estimates. The model selected as "best" does not necessarily represent all of the environmental or biological processes that influenced the probability of occupancy or species detection probabilities (Bailey et al. 2004).

Table 1. *Site Covariate Summary***.** Summary of site covariates included in single-season, single-species occupancy models.

Variable	Data source	Definition	Unit	Abbr.
River morphology	ArcMap	Categorical predictor (straightaway, inside bend, or outside bend)	_	River_morph
Cover Objects	Field measurement	Boulders or woody debris > 30 cm	cm	Cover_ob
Fines	Field measurement	The amount of fines covering each transect, observed and recorded to the nearest 5% of fine coverage	%	fine
Gravel	Field measurement	The amount of gravel covering each transect, observed and recorded to the nearest 5% of gravel coverage	%	grav
Cobble	Field measurement	The amount of cobble covering each transect, observed and recorded to the nearest 5% of cobble coverage	%	cobl
Boulder	Field measurement	The amount of boulder covering each transect, observed and recorded to the nearest 5% of boulder coverage	%	boul

Table 2. *Sampling Covariate Summary***.** Summary of sampling covariates collected from YSI EXO2 multiparameter datasonde.

Variable	Data Definition	Unit	Abbr.
Water temperature	Water temperature retrieved from datasonde at nearest 15 minute interval to trap set	°C	Water_temp
pH	pH retrieved from datasonde at nearest 15 minute interval to trap set	-	pH
Conductivity	Conductivity retrieved from datasonde at nearest 15 minute interval to trap set	µS/cm	Cond
Turbidity	Turbidity retrieved from datasonde at nearest 15 minute interval to trap set	NTU	Turb

Table 3. *Candidate models: random sites***.** Candidate site occupancy and detection models used to examine mudpuppy occupancy and detection.

Ψ(.), *p*(.)	Occupancy and detection probabilities are constant
Ψ(.), *p*(pH)	Constant occupancy; detection probability as a function of pH
Ψ(.), *p*(Water_temp)	Constant occupancy; detection probability as a function of water temperature
Ψ(.), *p*(Cond)	Constant occupancy; detection probability as a function of conductivity
Ψ(.), *p*(Turb)	Constant occupancy; detection probability as a function of turbidity
Ψ(.), *p*(Water temp_season)	Constant occupancy; detection probability as a function of water temperature by season
Ψ(Cover_ob), *p*(.)	Occupancy as a function of # of cover objects; constant detection probability
Ψ(River_morph), *p*(.)	Occupancy as a function of river morphology; constant detection probability
Ψ(River_morph+Cover_ob), *p*(.)	Occupancy as a function of river morphology and # of cover objects; constant detection probability
Ψ(River_morph+Cover_ob), *p*(Water_temp)	Occupancy as a function of river morphology and # of cover objects; detection probability as a function of water temperature
Ψ(River_morph), *p*(Water_temp)	Occupancy as a function of river morphology; detection probability as a function of water temperature
Ψ(River_morph), *p*(Turb)	Occupancy as a function of river morphology; detection probability as a function of turbidity
Ψ(Cover_ob), *p*(Water_temp)	Occupancy as a function of # of cover objects; detection probability as a function of water temperature
Ψ(Cover_ob), *p*(Turb)	Occupancy as a function of # of cover objects; detection probability as a function of turbidity

RESULTS

I captured mudpuppies (n = 20) at 13 sites (naïve Ψ = 0.65). I captured 26 mudpuppies in this study, including six from the two sites that were not included in the occupancy analysis. Food bait yielded the most captures for both males (n=6) and females (n=11) (Table 4). Food bait in combination with light bait yielded the second highest captures for both males (n=3) and females (n=3) (Table 4). Two males were captured in unbaited traps and one male was captured in a light baited trap. I failed to capture females in unbaited and light baited traps (Table 4). The March sampling period yielded 14 females and eight males, while April yielded no females and four males.

Mudpuppy TL averaged 32 cm (males = 29 cm, SD = 3; females = 32 cm, SD = 3). Male and female mass averaged 166 g (SD = 12) and 231 g (SD = 47), respectively. Sexual dimorphism was apparent in TL and mass. Females were significantly larger ($t = 2.06$; df = 24; $p = 0.01$) and heavier ($t = 2.06$; df = 24; $p = 0.001$) than males (Fig. 5). All captured females were gravid. Pearson's correlation indicated a significant relationship between TL and mass in both males ($r^2 = 0.7463$; $p < 0.0001$) and females ($r^2 = 0.6596$; $p < 0.0001$).

Table 4. *Mudpuppy captures by treatment by sampling period*. Number of mudpuppies captured using four different treatment methods from March 25th to March 30th and April 5th to April 30th. Sampling intervals one and two (August 25th to September 3rd and October 20th to November 5th) are excluded because no mudpuppies were captured. Light bait = glow stick, food bait = dog food and chicken liver, and light/food bait = glow stick/dog food and chicken liver.

	Treatment							
	Control		Light Bait		Food Bait		Light/Food Bait	
Sampling period	Male	Female	Male	Female	Male	Female	Male	Female
March 25th – 30th	2	0	0	0	3	8	1	2
March 25th -30th *	0	0	0	0	1	3	1	1
April 5th – 12th	0	0	1	0	2	0	1	0
Totals	2	0	1	0	6	11	3	3

*denotes two additional sites that were sampled post hoc but not included in statistical analyses

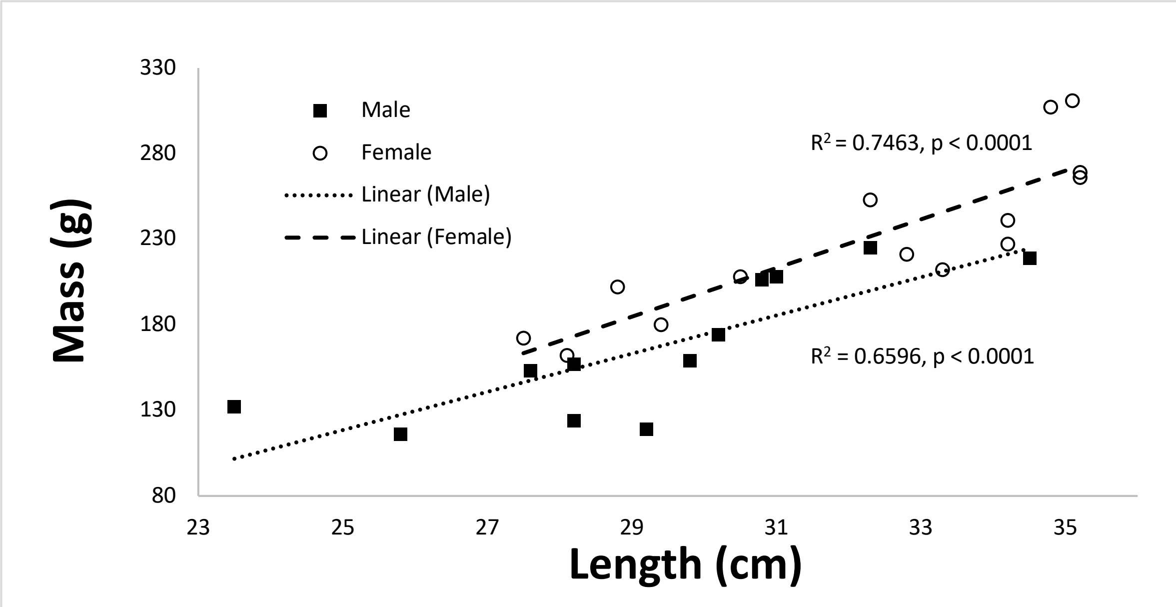

Figure 5. *Pearson's Correlation***.** Total length relative to mass of male and female mudpuppy from the Greenup Pool, Ohio River.

Occupancy analysis

The global occupancy model was underdispersed ($\hat{c}$ = 0.64). While $\hat{c} < 1$ indicates underdispersion, corrections are typically only made to overdispersion, and it is recommended to set $\hat{c} = 1$ in cases of underdispersion (Burnham and Anderson 2002; Mackenzie et al. 2017). I was unable to retain any models that included site covariates due to limited power. The site estimate for occupancy was 1 indicating occupancy was constant throughout our study site. Thus, I was unable to say that occupancy varied with any covariates and these models were removed from analysis. Environmental covariates were not correlated (i.e., $r \geq 0.7$ or $r \geq -0.7$). Our top model held occupancy constant and estimated detection probability as a function of water temperature (Table 5). Water temperature accounted for 52% of the model weights (Table 5). Detection probability was negatively associated with water temperature (model-averaged β = -4.5624 ± 1.5655; 95% CI=0.1061-3.148; Fig. 6). Water temperature by season accounted for 48% of the model weights (Table 5). Detection probability differed among seasons (F=433.12, df=3, n=80,

$p<0.0001$; Fig. 7). No mudpuppies were captured during the first two seasons and detection probabilities averaged 0.49 ± 0.11 and 0.26 ± 0.09 for season three and four, respectively. Turbidity, conductivity, and pH were poor covariates of detection, accounting for 0% of the model weights.

Table 5. *Best supported models: random sites***.** Candidate models predicting occupancy and detection probabilities ranked using second order AIC (AICc).

Model	*k*	*AICc*	*Δ AICc*	*Model Weight*
Ψ(.), *p*(Water_temp)	3	60.13	0.00	0.52
Ψ(.), *p*(Water temp_season)	4	60.13	0.16	0.48
Ψ(.), *p*(.)	2	81.92	21.79	0.00
Ψ(.), *p*(pH)	3	83.30	23.17	0.00
Ψ(.), *p*(Conductivity)	3	84.52	24.39	0.00
Ψ(.), *p*(Turbidity)	3	84.70	24.57	0.00

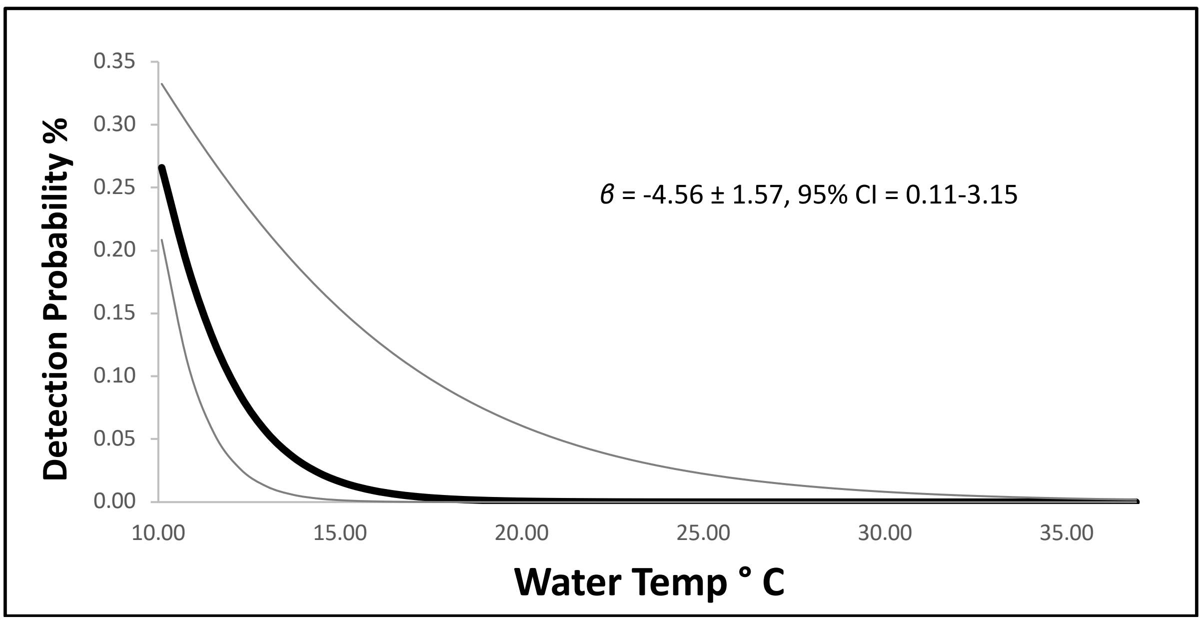

Figure 6. *Predicted detection probabilities***.** The relationship between mudpuppy detection probability and water temperature (β = -4.56 ± 1.2 derived from model (Ψ (.), *p* (Water temp)).

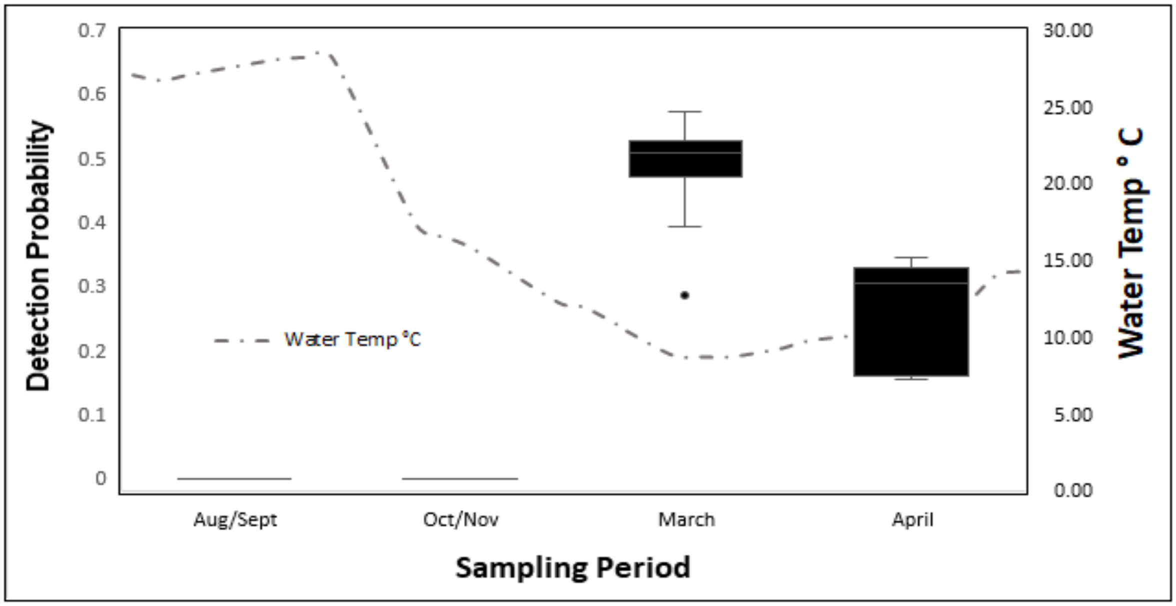

Figure 7. *Detection probability by sampling period***.** Results of ANOVA comparing detection probabilities by season derived from model Ψ(.), *p*(Water temp_season) containing presence/absence data for each season. Note: No mudpuppies were captured during the Aug./Sept. and Oct./Nov. sampling periods.

Sediment analysis

Percent fines were positively associated with river mile (r = 0.75, $t = 4.7$; df = 18; $n = 19$, $p = 0.0004$; Fig. 8) and cover objects were negatively associated with river mile (r = -0.64, $t = 3.65$; df = 18; $n = 19$, $p = 0.002$; Fig.8).

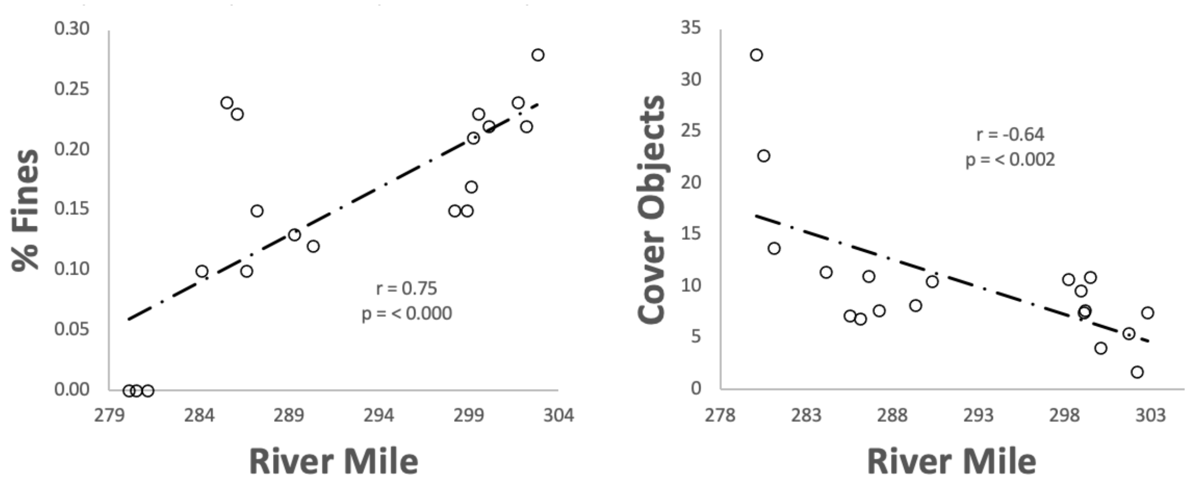

Figure 8. *Regression analysis***.** Linear relationship of percent fines and cover objects by river mile in the Greenup Pool (r = 0.75, $t = 4.7$; df = 18; $n = 19$, $p = 0.0004$; r = -0.64, $t = 3.65$; df = 18; $n = 19$, $p = 0.002$).

Treatment method analysis

I captured 2, 1, 13, and 4 mudpuppies in un-baited, light, food, and light and food, respectively. Comparison of all four treatments revealed that CPUEs differed among treatments (Kruskal-Wallis χ 2 = 11.1, df = 3, P < 0.01; Fig. 9). Traps containing food yielded the highest median CPUE, followed by light and food, no bait, and light, respectively.

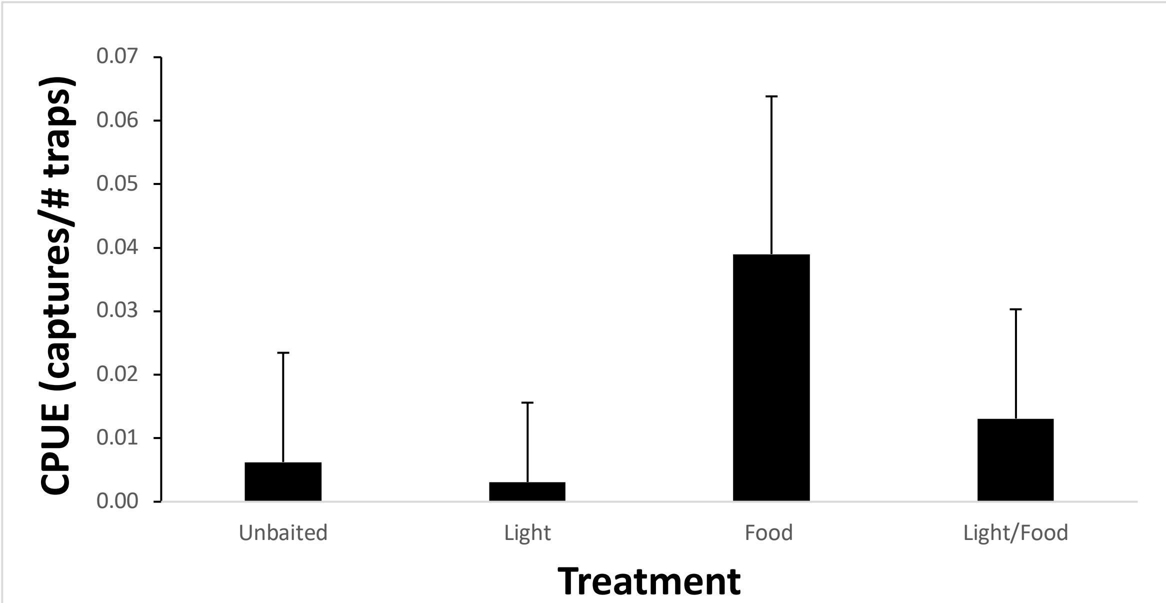

Figure 9. *Kruskal-Wallis***.** Average catch per unit effort (CPUE) for mudpuppy by treatment method.

DISCUSSION

This study provides insights into sampling methodology and the influence of environmental variables on mudpuppy detection probability in large navigable rivers. Like some other large rivers, the Ohio River is difficult to sample because of its length, depth, variable discharge, large woody debris, and recreational/commercial traffic. Most mudpuppy studies have occurred in small streams and rivers at depths that do not exceed 2 m because large rivers pose logistical difficulties and no effective occupancy protocol has been developed, though mudpuppies inhabit deeper sites in such habitats (Sajdak 1982; Chellman 2011; Craig et al. 2015). Our results indicate that: (1) high water temperature negatively influenced detection probability, such that detection probability was higher during the breeding season; (2) breeding season and timing of survey produced heterogeneities in detection probability; and (3) traps treated with food bait tended to be more successful at capturing mudpuppies. The results reported here are consistent with findings of previous work suggesting water temperature should be considered when sampling for mudpuppies, and that trapping efforts should occur within the period when mudpuppies are most active (Sadjak 1982; McDaniel et al. 2009; Craig et al. 2015).

Water temperature and season affected our ability to detect mudpuppies in the Greenup Pool. Water temperature was negatively associated with detection probability. All captures occurred during late winter and early spring when water temperatures ranged between 7 and 12° C, decreasing to 0 captures when water temperature was above 12° C. While many factors can cause detection probabilities to vary (Chellman et al. 2017), our best supported model indicated water temperature was the most important factor covariate of detection probability. Our findings are consistent with recent studies by Craig et al. (2015) and Beattie et al. (2017), who found that trapping in cooler spring months resulted in higher detection probability at lower temperatures.

Mudpuppy detection probability varied seasonally and was likely associated with breeding activity and nest attendance (Fig. 7). I did not capture mudpuppies between August and October when water temperature averaged 21° C. Detection probability was highest in March when water temperature was cooler but decreased in April (Fig. 7). Temporal variation in detection probabilities reflected the seasonal variation of mudpuppy activity in late fall through early spring (Matson 1990; Gendron 1999; Holman 2012; Chellman et al. 2017). During this portion of the year, it is likely that changes in water temperature along with breeding behavior and nest attendance are associated with changes in foraging patterns. In particular, lower detection probability in April would suggest breeding cycles within the population alter movement patterns of females at that time. Females guarding eggs may be reluctant to leave their nest unattended (Gendron 1999). The results reported here are consistent with the previous suggestion that sampling outside of optimal mudpuppy activity may underestimate their abundance or lead to failure of detection (Chellman et al. 2017). Our study also builds upon previous work that suggest conventional sampling methods for this species suffer from low detection probabilities relative to seasonal activity patterns and variable environmental conditions and are important factors to consider in sampling and monitoring programs.

Conductivity, pH, and turbidity were poor covariates of detection and not supported in the models (Table 5). Water quality can change substantially during and immediately following a precipitation event; however, our sampling occurred only during base flow conditions, likely reducing water quality variation in this study. Large rivers pose serious risks to researchers during high water events (e.g., large woody debris and extremely high water velocities) making boat navigation difficult. Difficulties associated with large river sampling is reflected on a broader scale with the overall lack of reports in the herpetological literature making conservation and management decisions difficult.

Given the limited power in my occupancy analysis, I used CPUE to examine the effectiveness of bait as a post hoc analysis. I expected the combination of light and food bait to yield the highest CPUE; however, traps baited with food only yielded the highest capture rates for both males and females (Fig. 9). The March sampling period yielded the highest number of captures for both males (n = 6) and females (n = 10). All females captured during this sampling period were gravid. Sampling in April yielded only males (n = 4). During the March and April sampling period, traps baited with food accounted for 65% of successful captures, followed by light in combination with food (20%), unbaited (10%), and light (.05%). While glow sticks can increase capture success in some aquatic amphibians and larval fishes in shallow habitats (Grayson and Roe 2007; Bennett et al. 2012; Budria et al. 2016), glow sticks were ineffective for capturing mudpuppies in this study. This suggests light, even in combination with food, dissuades mudpuppies from approaching bait and reduces capture success. Light penetration in deeper habitats of large rivers is limited, therefore, mudpuppies would not necessarily associate light with an advantage in locating prey, unlike species that inhabit shallow habitats.

Our ability to capture mudpuppies was strongly affected by sampling methodology. Minnow traps were effective at capturing large, sexually mature adults; however, it was not effective in obtaining larvae or small juveniles. Our site selection was based on previous data collected from the pool indicating the 30- to 50- meter range parallel from shore contained the highest concentration of large cover objects (boulders > 30 cm and large woody debris) (Kriege 2017; Miller 2021; personal observations). Mudpuppy larvae and juveniles are found in greater numbers in shallow water with small to medium cobble less frequently occupied by adult mudpuppies or predatory fishes (Matson 1990; personal observations). Future sampling methods should incorporate active survey techniques (i.e., scuba) to search for larvae and juvenile individuals in areas with smaller substrate size and organic debris that can be found in shallow riverine habitats. During previous studies of freshwater mussels and crayfish, we have encountered larvae and juvenile mudpuppies during surveys when lifting cover objects (personal observations). It is interesting to note that mudpuppies found in smaller streams and river do not exhibit the same anti-predator defense as those found in large rivers (personal observation). Mudpuppies observed in large rivers swim away immediately when the cover object is lifted, while individuals in small streams tend to remain where they are and seem less perturbed by the disturbance (personal observations). These anecdotal observations suggest potential future studies that might quantify these habits in mudpuppies.

Sediment analysis indicated a positive correlation between river mile and percent fines: the percentage of fines increased from upstream to downstream (Fig. 8). These results compliment those of Kriege (2017) based on data collected in the Greenup Pool (Fig. 2). Increased siltation is thought to negatively impact habitat suitability for mudpuppies, filling interstitial spaces, decreasing cover object availability used for nesting, and decreasing prey availability (Braswell and Ashton, 1985). Fluvial sediments are transported in suspension (suspended load) or by being rolled, skipped, or slip along the riverbed (bedload) (Antilla and Tobin 1978). The suspended load consists of fine sediments held in suspension by the upward components of turbulent currents and travels at the velocity of the river. As particle-fall velocity decreases, fine particles (fines) begin to settle out of the water column and accumulate on the riverbed (Antilla and Tobin 1978). This deposition of fines increases as velocity decreases in the lower portion of the pool, ultimately causing fines to become trapped by the downstream dam, reaching a meter deep in some areas (Antilla and Tobin 1978; personal observations). Dams and channelization have massively reshaped large river systems and are a key driver of biodiversity loss, with most of the remaining suitable habitats relatively small and geographically isolated (McAllister et al. 2001). Sampling mudpuppies throughout the pool in relation to hydrologic alterations may be useful in identifying impacts related to damming. Cover objects, however, were not a significant predictor of CPUE, which would suggest that silt deposition within the upper portion of the pool is within mudpuppies tolerance range.

Understanding how long-term landscape changes resulting from habitat alteration can negatively affect habitat suitability for herpetofauna is essential for effective management strategies. Further investigation into possible predictors of abundance of mudpuppies (i.e., cover objects, fine sediment deposition, dam effect) throughout the Greenup Pool and other large river settings should be considered when identifying impacts created by dams and development. This study provides a foundation for long-term studies in a mark-recapture framework needed to detect rates of population change and gain reliable estimates of demographic parameters for mudpuppies in large navigable rivers.

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APPENDIX A: IRB LETTER

MARSHALL UNIVERSITY.
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Office of Research Integrity

November 14, 2022

Alyssa Brady
938 13th Avenue, Apt. 4
Huntington, WV 25701

Dear Alyssa:

This letter is in response to the submitted thesis abstract entitled *"Using Occupancy Modeling for Protocol Development and Habitat Assessment of Necturus Maculosus in a Large Navigational River."* After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #712. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract, you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director

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APPENDIX B: SAMPLING PROTOCOL FOR THE COMMOM MUDPUPPY (NECTURUS MACULOSUS) IN LARGE NAVIGABLE RIVERS.

Large rivers present unique challenges to researchers who wish to study their inhabitants, particularly species likely to hide beneath bottom cover and others that are not easily collected using standard techniques such as netting or electroshocking. Among such species is the Common Mudpuppy (*Necturus maculosus*), a large aquatic salamander found in many freshwater habitats in midwestern and eastern North America. The following protocol for sampling a mudpuppy population in a large navigable river uses trapping and scuba technology and is based on a study conducted in the Greenup Pool of the Ohio River and should be applicable in many other large rivers.

This protocol can be used to investigate a diversity of ecological and conservation driven questions, relating to species abundance and density, animal behavior, temporal activity, and landscape-level occurrence. Wildlife researchers/managers with limited knowledge of the relative abundance and likelihood of detection can apply this general guideline for reliably estimating detection probability across mudpuppies range regardless of location. With relevant modifications, this protocol may serve for other species as well.

Site Selection

Large navigable rivers are defined by 10th of a mile delineation for commercial navigational purposes. Use a random number generator to select sites by this delineation.

To ensure independence among sampling locations, sites should be located a minimum of 136 m apart (Matson 1998).

Occupancy and detection estimates often require spatial and temporal replication; in occupancy studies this generates a trade-off in survey effort between the number of sites to sample and the number of replicates to conduct at each site (MacKenzie et al. 2002; MacKenzie et al. 2006). Our survey design consisted of 20 randomly selected sites sampled on four occasions. While the sampling occasions provided the necessary replication for use in an occupancy modeling framework, our survey design was limited by the number of sites visited and statistical analyses indicated more effort was required and requires future research.

> To optimize mudpuppy detection for protocol development, our study was confined to the upper portion of the pool, where the accumulation of fines that limit cover object availability was minimal (Kriege 2017). Future studies could account for changes in substrate composition across an entire pool to assess the impact of fine sediment accumulation created by dams on mudpuppy distribution throughout pool.

Define Suitable Habitat Across the Area of Study

Divers using scuba will use the Wentworth scale to classify substrate composition and determine the number of suitable cover objects >30 cm and not embedded.

> In our study, a detailed substrate analysis was available for the entire Greenup Pool from a recent freshwater mussel study. Substrate data might be available for other pools from similar studies, reducing the effort to quantify habitat. The substrate analysis was used to select the upper 20 miles of the river for site selection and define the range of 30 to 50 meters parallel to shore for trap array placement. This range contained the highest concentration of suitable cover objects (Kriege 2017). Based on Chellman et al. 2017, we assumed mudpuppy detection would be higher in sites dominated by cobble/boulder.

Trap Array Design

The trap array uses four modified rectangular minnow traps made of vinyl-dipped steel mesh with an inward-facing conical entrance on each end (KUFA Corp., Blaine, WA, USA). Increase the opening of the minnow traps to 6 cm using a Dremel tool to allow for adult mudpuppy entrance (McDaniel et al. 2009). Using a 100-meter lead line marked into 10-meter intervals, randomly select four of the intervals and attach the ten-meter trap arrays to the line.

Scuba Substrate Assessment

Divers using scuba should assess substrate composition and number of cover objects at each site. Lay a 100-meter lead line at the site with anchors attached at each end and a buoy attached to the downstream anchor where the diver will descend to the lead line. Begin recording substrate composition and number of cover objects for each 10-meter interval.

> As side-scan sonar, which uses high-frequency sound pulses to create an image of the river bed, becomes more economical and precise in the future, it may be possible to use this method as an alternative to scuba to assess substrates (Richter et al. 2016; Hamill et al. 2017).

Water Quality Parameters

Before each survey, use a multiparameter water quality meter to record water temperature, pH, conductivity, and turbidity. This will provide data used to determine sample covariates effect on detection probability. Surveys should take place when water temperature is below 12 degrees Celsius (Craig et al. 2015; Beattie et al. 2017; Jones 2022).

Trap Treatment

Depending on the focus of the study, treatment method could vary. To increase capture success, use traps baited with dog food and chicken liver (Jones 2022).

Trap Placement and Retrieval

Attach anchors to the beginning and end of the 100-meter lead line with trap arrays to hold line in position in the current. Attach a floatline with buoy to the anchor at the end of the line for retrieval. Leave traps overnight in order to capture mudpuppies during their nocturnal foraging. To retrieve traps, use the buoy at the surface to pull anchor attached to lead line and trap arrays into the boat.

> Our study determined that the area of river bottom 30 to 50 meters parallel to shore contained the highest concentration of suitable cover objects. This could vary from to pool to pool based on geology and topography of the river. Because our study only captured large, sexually mature adult mudpuppies, one variation of our protocol would be to lay 100-meter transects at an angle from shore to channel to survey in shallow water where nesting and larval development occur (Harding 1997, Craig et al 2015).

Animal Handling

Captured mudpuppies should be placed in a container on boat with aerated river water during processing. After processing, release mudpuppies back into river at point of capture.