

2019

Assessment of mudpuppy (*Necturus maculosus*) presence along the St. Clair-Detroit River System using environmental DNA and occupancy modeling

Jenny L. Sutherland

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Assessment of Mudpuppy (*Necturus maculosus*) Presence along the St. Clair-Detroit River
System using Environmental DNA and Occupancy Modeling

by

Jenny L. Sutherland

Thesis

Submitted to the Department of Biology

Eastern Michigan University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Biology

Ecology and Organismal Biology

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March 15, 2019

Ypsilanti, Michigan

Abstract

The mudpuppy (*Necturus maculosus*) is cryptic, fully aquatic salamander within the Great Lakes region. Once abundant throughout its range, evidence now suggests that there have been declines due to habitat loss and lampricide use. Information on the status of mudpuppies along the St. Clair-Detroit River System (SCDRS) is lacking, and since they are important bio-indicators, they could be a gauge for restoration success. Environmental DNA (eDNA) and occupancy modeling were used to determine best detection practices for this cryptic species. Mudpuppy eDNA was detected at all known mudpuppy sites with the addition of one site. Occupancy was highest at shoreline restoration sites, while reef restoration did not affect mudpuppy occupancy. Additionally, eDNA resulted in the highest detection probability. Restoration efforts have shown to be successful by increasing the occupancy of this indicator species; therefore, these efforts should be used as a template for other restoration practices.

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CHAPTER 1

INTRODUCTION AND BACKGROUND

Issues with Detection in Conservation

Vertebrate populations have declined globally by 58% between the years 1970 and 2012. The cause of this decline is due to the loss of available resources because of a growing human population (World Wildlife Fund 2016). With this rapid loss it is necessary that the status of species be monitored often and rapidly. Effective conservation relies on the ability to predict how species will respond to environmental change; this can sometimes be difficult because some species are cryptic and inhabit ecosystems that are not easily accessible (McKinney 1999). Many of these species are understudied, and the result of this lack of information could be imperilment or extinction if problems are not detected until it is too late.

The assessment of the distribution and abundance of a species is very important when studying biodiversity, ecology, and conservation biology. To successfully accomplish effective wildlife management, accurate detection of a species needs to be met, and appropriate survey and analysis methods that minimize false negatives are necessary (Cossel et al. 2012). Cryptic species are difficult to survey using traditional methods. This could be due to where they are found, how rare they are, how small they are, when they are active, or their sensitivity to being caught. Better survey methods that have high levels of certainty, lower costs, and less stress for the animals are needed for sampling these species. If detection probability is low, inferences about occupancy are reduced, leading to inaccurate assumptions about the distribution and status of the species (Pilliod et al. 2013).

Data deficiency is a common problem for cryptic species. Difficulty detecting these species makes surveying time and money intensive. Data deficiency also leads to difficulty managing for the species. The sun bear *Helarctos malayanus* is classified on the International Union for Conservation of Nature Red List as Data Deficient (IUCN), a likely result of their cryptic nature and has led to management deficiencies (IUCN 2018). Detection probability modeled from camera trap surveys only yielded a detection of 0.1848–0.3768 at three study locations, meaning they were only seen in 18 to 37% of photos (Linkie et al. 2007). Occupancy also indicated that sun bears might be using degraded forest more than expected, showing the importance of management of a habitat not previously known to require conservation (Linkie et al. 2007). Necessary studies such as this one illustrate that the accurate definition of habitat requirements is a crucial component for management and conservation of cryptic species.

Species in freshwater ecosystems, particularly lotic ones, are especially understudied and the data that are available suggest that their population declines and extinction rates greatly exceed those of terrestrial species (Ricciardi and Rasmussen 1999; Dudgeon et al. 2006; Collen et al. 2014; WWF 2016). Freshwater species are jeopardized because of the rapid degradation, alteration, and destruction of their ecosystem. Rivers and streams are impacted by direct destruction, such as dredging for channelization and damming, as well as land use changes of the terrestrial ecosystems within their watersheds (Collen et al. 2014). Freshwater systems also have a very high level of connectivity, intensifying the effects of fragmentation, pollution, invasive species, and disease (Darwall et al. 2009). The species that live in these ecosystems are greatly affected by this fragmentation and studying these affects are essential for management and conservation. Achieving this for cryptic species is difficult because short term, small scale

studies are highly insufficient. Fragmentation and dredging have made over 85% of sturgeon species threatened or endangered, which makes them one of the world's most imperiled vertebrate groups (IUCN 2018). These fish are cryptic because of their elusive life history, migratory behavior, and habitat requirements, making detection and subsequent management and conservation difficult. Monitoring where lake sturgeon migrate requires large scale efforts that are time intensive but necessary to protect their habitat. Successful detection of sturgeon species has been accomplished using eDNA making management more successful by better understanding habitat use (Farrington and Lance 2014; Pflieger et al. 2016). Due to the necessity to increase the detectability of sensitive, cryptic species, novel methods are being used.

Advances in technology, such as eDNA and occupancy modeling, have allowed more reliable monitoring of cryptic species, particularly in aquatic habitats. The Roanoke logperch, *Percina rex*, an endangered darter, is a cryptic species that occupies rivers in Virginia and North Carolina (Strickland and Roberts 2018). This species has patchy occurrence and lives in a wide range of habitats. These features make sampling for the Roanoke logperch difficult, particularly with traditional methods such as electrofishing and snorkeling. Environmental DNA and occupancy modeling were used and revealed that eDNA had a higher detection rate compared to the traditional sampling methods and logperch were found at 11 of 12 sites (Strickland and Roberts 2018). These sites can now be properly managed for this endangered species.

Amphibians are particularly difficult to obtain distribution and abundance information for because of how cryptic they are. This problem is highlighted in the Global Amphibian Assessment (GAA), which points out that 22.5% of approximately 6,000 species of amphibians

have insufficient population data, and 32.5% of amphibians that do have sufficient data are listed as threatened (Stuart et al. 2004). Many organizations are encouraging long-term, large-scale studies to monitor amphibian populations because of these statistics (e.g., North American Amphibian Monitoring Program, Amphibian Research and Monitoring Initiative, Declining Amphibian Populations Task Force, Partners in Amphibian and Reptile Conservation, and U.S. state and federal agencies). Fully aquatic amphibians can be even more difficult to survey than terrestrial amphibians, and they face their own risks including destruction of their habitat, water contamination, and alteration of riparian zones (WWF 2016; Collins 2017). The eastern hellbender (*Cryptobranchus a. alleganiensis*), a fully aquatic and extremely cryptic giant salamander native to North America, has been classified as nearly threatened by the IUCN and therefore has been made a priority for management in several states (Freake and DePerno 2017; IUCN 2018). This management priority has been difficult due to the extremely low detection probability of the eastern hellbender. Surveying eastern hellbenders requires extremely high survey effort because of their secretive behavior, occupying cool, clear streams where they spend most of their life cycle hidden under large rocks (Peterson et al. 1988). Locating sites they still occupy is difficult and sometimes insurmountable due to the high surveying efforts required causing subsequent management issues. Using eDNA has become common for locating still existing populations of eastern hellbenders (Olson et al. 2012; Santas et al. 2013; Spear et al. 2015; Franklin 2016; Pitt et al. 2017; Takahashi et al. 2018). Fully aquatic amphibians have an important role in the ecosystem, serve as indicator species, and are at risk, all of which make reliable sampling techniques vital (Welsh and Ollivier 1998). Determining the abundance of cryptic species with any degree of accuracy can be difficult although such information is critical

for developing management and conservation strategies (Price and Endo 1989; Piggott and Taylor 2003).

Environmental DNA

Environmental DNA (eDNA) is genetic material from an organism that can be collected from that organisms' environment, such as, soil, water, or air (Barnes and Turner 2015). Environmental DNA can be introduced into the environment from the organism through shedding, waste, decomposition, or reproduction (Fig. 1). Environmental DNA has made the detection of organisms easier where the collection of the whole organism can be difficult, harmful, or inaccurate (Jerde et al. 2011; Jerde et al. 2013). Common targets for eDNA studies have been with threatened and endangered species, invasive species, and for bioassessment (Barnes and Turner 2015). A few unique studies that have used eDNA include using saliva from browsed twigs to identify ungulates species in Sweden (Nichols et al. 2012) and collecting carrion flies to study local mammalian biodiversity in Madagascar (Calvignac-Spencer et al. 2013).

Environmental DNA is a new technique and there are still concerns when using this type of sampling in an aquatic river habitat. These concerns have to do with the origin, degradation, and transportation of the DNA. The first concern is how the origin of the eDNA will affect the final concentration of eDNA in the sample. If the origin of the eDNA is from reproduction, it is possible that there would be a higher concentration, therefore higher detectability, of the eDNA versus getting the eDNA from a different source (i.e., decomposition). The second question has to do with degradation of the eDNA itself. Factors that affect how long eDNA will last in a

system fall into three categories: DNA characteristics (i.e., length, conformation), biotic environment (i.e., microbial community, enzymes), and abiotic environment (i.e., pH, salinity, oxygen, etc.; Barnes and Turner 2015). The last concern is considering how far the eDNA can travel before it degrades. This is important when studying a river system because it needs to be known how far away from an actual organism you can still get a positive eDNA sample. It is also possible to get a false positive eDNA sample if old sedimentary eDNA is stirred up and captured (Turner et al. 2015). There are also many limitations to using eDNA as a tool to monitor organisms. These included not knowing whether the organism being detected is dead or alive, not knowing how many organisms are being detected, and not knowing specifically where the organism is located due to the eDNA transportation concern.

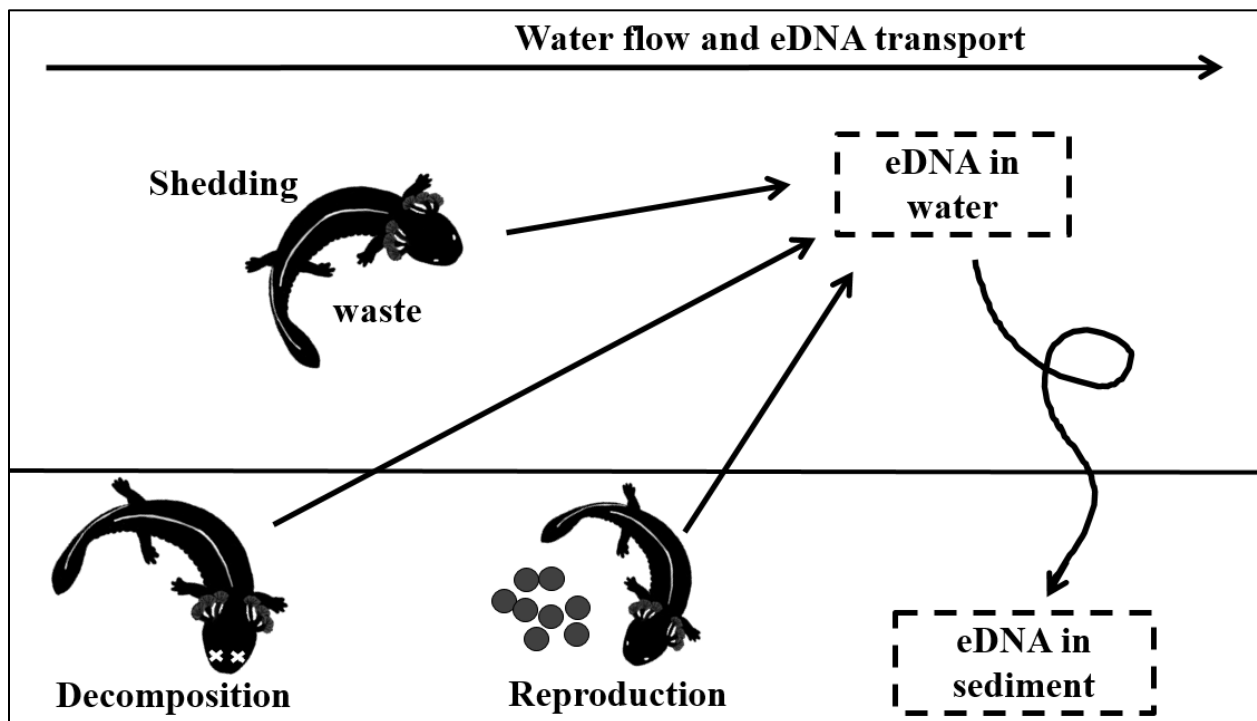


Figure 1: How mudpuppy eDNA is introduced and transported through an aquatic system.

There have been several studies looking at how long eDNA can last in a system (Fig. 1). A study in a zoo pen found that eDNA could be detected in soil samples six years after the animal had been removed (Andersen et al. 2012). Similar studies conducted in aquatic ecosystems have found eDNA not lasting as long. A laboratory study found that after four days there was less than a 5% chance of detecting common carp eDNA in an aquarium (Barnes et al. 2014). Environmental DNA is also more detectable in sediments versus water samples (Andersen et al. 2012; Thomsen et al. 2012; Barnes et al. 2014; Turner et al. 2015). It was found that bighead carp eDNA had a sediment concentration of 8-1800 times more per gram than per milliliter of water and that eDNA was detectable in the sediment for 132 days after the carp was removed, which was five times longer than the eDNA in water (Turner et al. 2015). There have also been studies that look at the persistence of eDNA in streams. The movement of artificial DNA tracers downstream was studied, and it was found that there was a large decrease in DNA abundance as is moved downstream but that DNA remained detectable at the furthest downstream sampling site (1192 m; Foppen et al. 2011).

Environmental DNA (eDNA) has also been used in aquatic ecosystems to determine location of aquatic species (Fig. 1; Ficetola et al. 2008; Lodge et al. 2012; Deiner and Altermatt 2014). The most widely applied use of eDNA in the Great Lakes region is for detection of bighead (*Hypophthalmichthys nobilis*) and silver (*Hypophthalmichthys molitrix*) carp, both of which are the invasive species collectively known as Asian carp (Jerde et al. 2013). Between 2009 and 2013, there have been 2,822 eDNA samples have been collected in the Great Lakes basin to determine the extent of the Asian carp invasion. It was suggested that eDNA continue to be used as a monitoring tool for Asian carp after their DNA was detected in the Chicago Area

Waterway System and the western basin of Lake Erie, each of which are suspected locations of Asian carp entry into the Great Lakes (Jerde et al. 2013).

Environmental DNA sampling has also been used for detecting the eastern hellbender (Olson et al. 2012; Santas et al. 2013; Spear et al. 2015; Franklin 2016; Pitt et al. 2017; Takahashi et al. 2018). In North Carolina, hellbenders were successfully detected at 33 of 61 sites (Spear et al. 2015). Quantitative polymerase chain reaction (qPCR) can be used to try to determine abundance, which is done by relating the concentration of eDNA detected at sites to the abundance of hellbenders. Many studies have tried to achieve this result with mixed outcomes (Takahara et al., 2012; Thomsen et al. 2012; Pilliod et al. 2013; Spear et al. 2015).

Occupancy Modeling

Population surveys typically use individual counts to determine abundance and number of sites where a species was observed as an estimate of the proportion of area occupied. These studies assume that each individual is detected and that the probability of detection is equal for each sampling event, location, and sampling method (Boulinier et al. 1998). When conducting a population survey using trapping alone, it is important to remember that not detecting individuals at a site does not mean that the species isn't there, and it is unlikely that all individuals will be detected. Calculating detection probability and occupancy of a species using occupancy modeling accounts for this problem (Pollock et al. 2002).

It is also difficult to compare results between different studies because of different sampling techniques with different detectability rates and because the detection of a species at a given site can vary widely. If population surveys are inaccurate due to these assumptions, it can

lead to improper management of the species. Using occupancy modeling to account for this inaccuracy is therefore very important. A method has been developed to account for the detection probability being less than one using the program PRESENCE (MacKenzie et al. 2002). This program uses capture data (or detection/non-detection data) to determine the status of a species. The method involves visiting sites multiple times within a season where the target species is either detected or not detected. The goal is to estimate the proportion of sites that are occupied, ψ , knowing the species is not always detected, even when present.

Environmental DNA Occupancy Modeling

Environmental DNA is a powerful method to detect a species at a site because of its potentially high sensitivity and period of persistence in water (~2 weeks; Dejean et al. 2011). Though eDNA has a higher detection than trapping, studies show that it does still not have perfect detection (Ficetola et al. 2008; Hyman and Collins 2012; Thomsen et al. 2012). Because various sources of error with eDNA (e.g., sequencing errors, species identification, imperfect detection) site occupancy models can also be used to analyze eDNA survey data (MacKenzie et al. 2002; Yoccoz et al. 2012). The use of occupancy modeling based on eDNA data would give an estimate of detection probability which could be used to determine the number of replicates needed to confirm that a species is absent from a site (Kery and Schmidt 2008). Site occupancy modelling can be used to analyze detection/non-detection data from surveys based on eDNA.

Mudpuppies

Mudpuppies (*Necturus maculosus*) are an elusive, fully aquatic salamander that occupy streams, lakes, and ponds in eastern North America. Many life history traits about the mudpuppy are still unknown because of their cryptic nature such as dispersal, population structure, and seasonal movements (Murphy et al. 2016). The mudpuppy has a long lifespan (~30 years) and females can delay breeding until they are 7 to 10 years of age, traits that make this species particularly vulnerable to population declines (Bonin et al. 1995). This species usually can be found under large flat rocks or logs where egg deposition also occurs in the spring and summer (Petranka 1998; Matson 2005). The mudpuppy is a carnivore that feeds on crayfish, fish, and mollusks, which give it an important role in ecosystems it occupies (VanDeValk and Coleman 2010). The mudpuppy is also a critical host for the salamander mussel *Simpsonaias ambigua*, which is listed as vulnerable on the IUCN Red List and is a federally endangered species in Canada (Congdon et al. 1994; McDaniel et al. 2009; IUCN 2018). The larval salamander mussel (glochidia) attach to the gills of a mudpuppy and are encysted there for 19 to 30 days where they will then drop off as sedentary juveniles (Watson et al. 2001). Siltation and mudpuppy declines are the primary threats to the salamander mussel in the Great Lakes watershed, and given the status of the salamander mussel and their dependency on mudpuppies, recent state efforts have focused on locating mudpuppy populations (Watson et al. 2001).

Mudpuppies (along with other amphibians) are good habitat quality indicators due to their sensitivity to the environment (Welsh and Ollivier 1998). Though the mudpuppy was once abundant throughout its range, recent data suggests that they have been experiencing widespread

population declines (Pfungsten and White 1989; Harding 1997). Over 42% of amphibians are listed as threatened, and mudpuppies are considered a Species of Special Concern in Michigan and a Species of High Conservation Concern by the Northeast Partners in Amphibian and Reptile Conservation (NEPARC 2010; Harding and Mifsud 2017). The common methods for determining mudpuppy presence are minnow trapping, electroshocking, manual surveys, and seining. Unfortunately, because mudpuppies are cryptic, use of any of these survey methods can produce low success (Murphy et al. 2016).

Threats to mudpuppies in the Great Lakes region include direct human-caused mortality, lampricide use, habitat modification, and pollution. Negative pressures to mudpuppy populations are caused by humans collecting mudpuppies for educational use and medical research. There is also a great deal of misinformation about mudpuppies. Mortality due to anglers believing they are predators to (or competitors with) game fish often occurs (Petranka 1998). Anglers also sometimes believe mudpuppies are poisonous and will kill them when caught (Harding 1997).

Another threat to mudpuppy conservation in the Great Lakes is the use of lampricide. Lampricide (3-trifluoromethyl-4-nitrophenol, or TFM) is a chemical that has been used since the 1960's to combat the invasive sea lamprey (*Petromyzon marinus*). Unfortunately, TFM is toxic to many other species as well, including the mudpuppy (Boogaard et al. 2003; Kilmer 2010). Though research is limited on the extent of lampricide reductions to mudpuppy populations, in 1980 it was reported that dead mudpuppies were found in 32% of tributaries to Lake Superior and 36% of tributaries to Lake Michigan since lampricide treatments began (Gilderhus and Johnson 1980). There have been mass die-offs in the Detroit River, Lake St. Clair, and Lake Erie

suspected to be the result of lampricide applications (Matson 2005; Craig et al. 2015). Alternatives to TFM are being studied (Twohey et al. 2003; Imre et al. 2014), but it is still used as the main defense against the invasive sea lamprey. Mudpuppies are also sensitive to other environmental contaminants, such as pesticides, polychlorinated biphenyl (PCBs), and mercury (Gendron et al. 1997; Bonin et al. 1995).

Long-term data on mudpuppies are necessary to detect declines in populations, especially with the current threats, including the use of TFM and habitat degradation. New surveying methods are also needed to obtain more accurate data on mudpuppy population structure.

St. Clair-Detroit River System

One location where more research on mudpuppies would be beneficial, due to their ability to reveal information about ecosystem health, is the St. Clair-Detroit River System (SCDRS). The SCDRS is the connecting channel between the southern tip of Lake Huron and the western basin of Lake Erie, and it is part of the boundary between Canada and the United States (Fig. 2). Since 1874 this corridor has undergone many habitat modifications due to the need for urban development and shipping channels (Bennion and Manny 2011). Some of the habitat modifications include the loss of coastal wetlands, construction of sea walls, dredging, channelization, and industrialization (Benion and Manny 2011; Haponski and Stepien 2014). The St. Clair and Detroit Rivers were designated as Areas of Concern (AOC) in 1987 under the Great Lakes Water Quality Agreement (U.S. Environmental Protection Agency 2018). These rivers were included as an AOC due to habitat degradation and because of the many sources of pollution that enter the system. Sources of pollution included landfills, atmospheric deposition,

urban and agriculture runoff, waste disposal, and unregulated discharge from industrial plants (chemical manufacturers, petroleum refineries, paper mills, electric power plants, and salt producers). Because of these sources, mercury, polychlorinated biphenyl (PCBs), phosphorus, and heavy metals are all put into the river system (USEPA 2018).

The St. Clair River (the northern portion of the SCDRS) runs 40 miles from Lake Huron to Lake St. Clair. At its mouth into Lake St. Clair, the river branches into several channels, which create a wide delta of wetlands. The USEPA created a list of impairments that needed to be met to remove the St. Clair River as an AOC. These requirements are called Beneficial Use Impairments (BUIs). Some of the BUIs were restrictions on fish and wildlife consumption, beach closings, bird or animal deformities or reproduction problems, and the loss of fish and wildlife habitat (USEPA 2018). A report came out in 2012 that outlined eleven habitat restoration projects along the St. Clair River, that if completed would be able to complete the BUI for loss of fish and wildlife habitat (Public Advisory Commission for the St. Clair River 2012; Great Lakes Architect-Engineer Services 2014). Since 1987 the St. Clair River has reduced its present beneficial use impairments from ten down to four. The BUI “loss of fish and wildlife habitat” was removed in 2017 (USEPA 2018).

The Detroit River runs 32 miles between Lake St. Clair and the western basin of Lake Erie. The Detroit River contains the same list of BUIs as the St. Clair River and was listed as an AOC due to urban and industrial development, sewer overflows, municipal and industrial discharge, and storm water and tributary runoff. These sources of pollution have input bacteria,

PCBs, polycyclic aromatic hydrocarbons (PAHs), metals, oils, and greases into the system (USEPA 2018).

To monitor continued restoration success and habitat conditions, monitoring of fish and wildlife populations are necessary. The mudpuppy is a sensitive species that can serve as an effective biological indicator that will reflect the success of current restoration efforts and habitat conditions along the St. Clair-Detroit River System.

Objective of Study

The objectives of this study are to determine the efficacy of using eDNA and occupancy modeling for monitoring mudpuppies along the St. Clair-Detroit River System. More specifically, the goal is to determine the presence/absence of mudpuppies at restored and unrestored sites along the St. Clair-Detroit River System using eDNA, trapping, and occupancy modeling.

We predict that (1) mudpuppy catch-per-unit-effort and occupancy will be higher at restored versus unrestored sites, (2) detection probability will be highest for trapping using set lines as opposed to minnow traps, (3) mudpuppy eDNA will be detected at sites where mudpuppies were also trapped, and (4) detection probability will be higher for eDNA sampling versus traditional trapping methods.

CHAPTER 2

RESEARCH DESIGN AND METHODOLOGY

Study Area

This study took place at 29 restored and unrestored sites along the St. Clair-Detroit River System in Michigan (Fig. 2; Table 1). These sites have sufficient mudpuppy trapping data available to provide a basis for comparison to eDNA results. The trapping was conducted by Herpetological Resource and Management (HRM), USGS Great Lakes Science Center, and US Fish and Wildlife Service.

Restoration sites along the St. Clair River included Cottrellville, Marysville, and the Blue Water River Walk. The restoration at Cottrellville occurred in 2015 and was called “Cottrellville Township Shoreline Preservation and Restorations.” This included restoration of 425 feet of shoreline (Fig. 3A). The seawall was removed, breakwaters were installed, cobble and boulders were placed throughout the shallow shelf, and trees were planted (Michigan Department of Environmental Quality 2017). The restoration at the Marysville site was completed in 2013 and was titled “Marysville St. Clair River Living Shoreline Restoration” (Fig. 3B). At this site the seawall was removed, the shoreline was graded, rock riprap was installed, and native emergent and submergent wetland vegetation was planted (MDEQ 2017). “The Blue Water River Walk Restoration” project was completed in 2012; it is the largest restoration project on the St. Clair River and included restoration of 4,300 feet of shoreline (Fig. 3C). The site is a former train yard and the site restoration began with the removal of 3,250 tons of debris. At this site, using rock

and native vegetation, 0.75 acres of fish spawning habitat and 2.25 acres of nursery habitat was created, and 14 mussel and mudpuppy structures were installed (MDEQ 2017).

The restoration projects on Lake St. Clair were less extensive than those on the St. Clair River, but included the addition of rocks suitable for mudpuppy habitat. At the DNR Fairhaven Boat Launch rocks were placed along the entire shoreline of the site and extend down into the water. At Lake St. Clair Metropark and Lake Erie Metropark, concrete slabs were submerged along the shoreline to create habitat for mudpuppies (Fig. 3D).

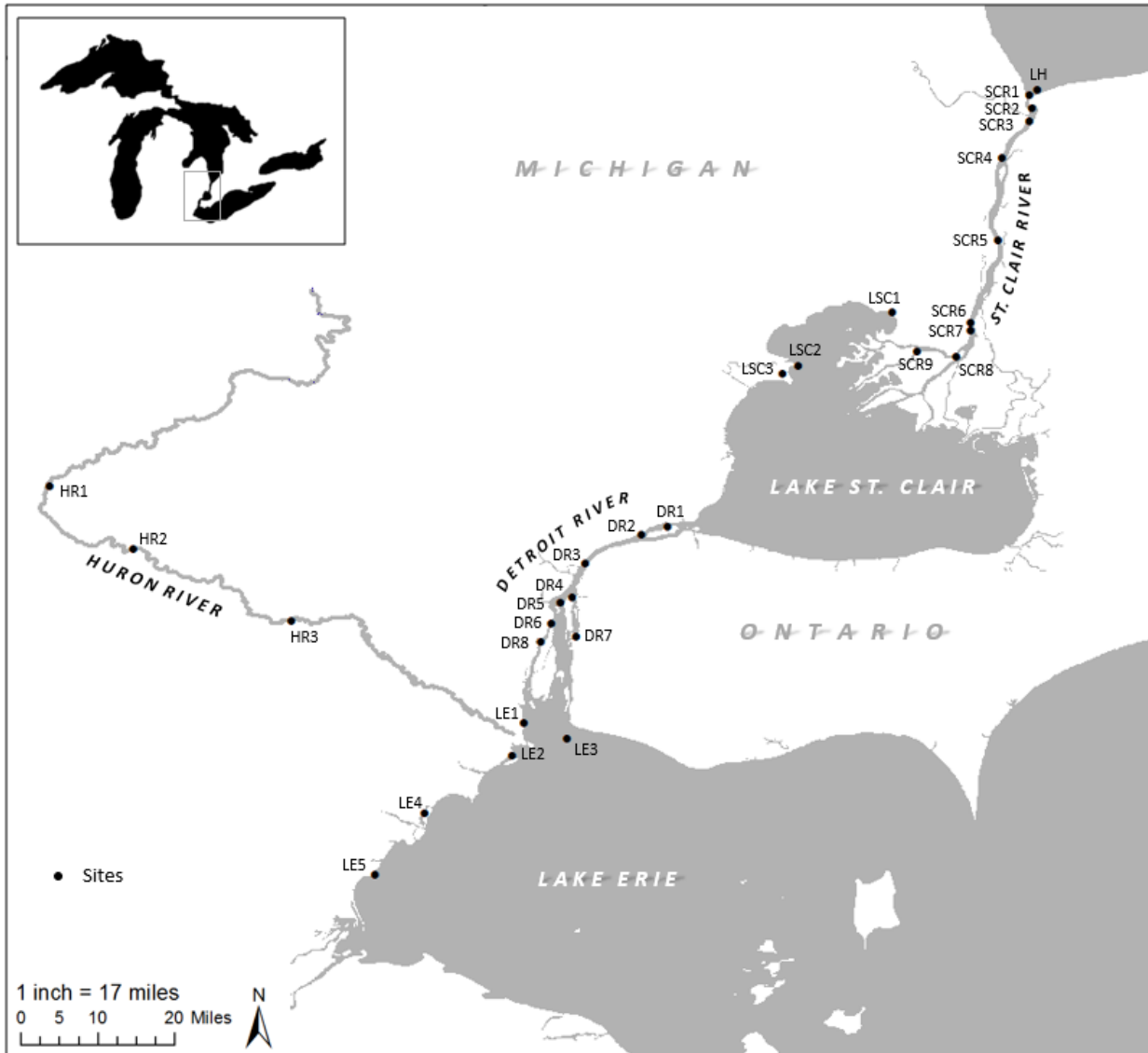


Figure 2: Location of 29 sampling sites along the St. Clair-Detroit River System used for trapping and eDNA data.

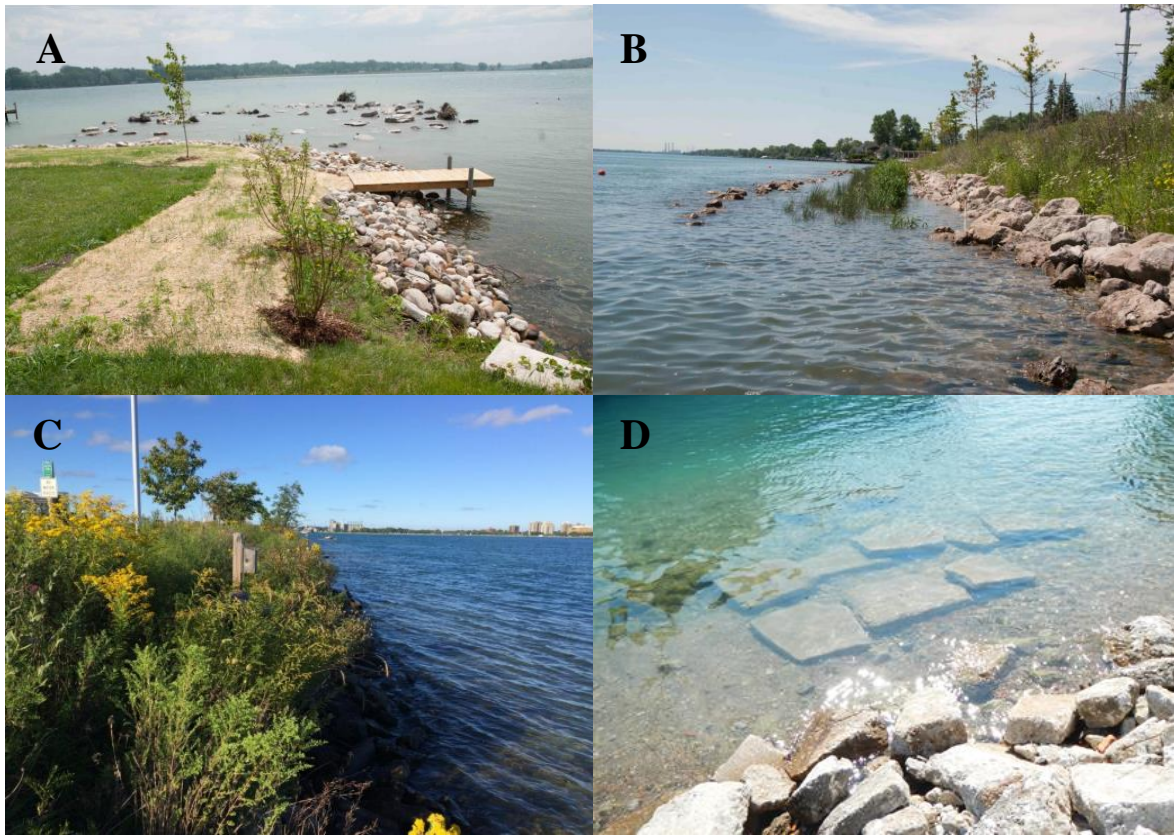


Figure 3: Restoration sites that were sampled for mudpuppy eDNA: (A) Cottrellville Township Shoreline Preservation and Restorations (MDEQ 2017), (B) Marysville St. Clair River Living Shoreline Restoration (MDEQ 2017), (C) Blue Water River Walk (MDEQ 2017), and (D) Lake St. Clair Metropark (HRM).

Trapping

A multi-agency approach was used, obtaining setline and minnow trap records from Herpetological Resource Management (HRM), U.S. Fish and Wildlife Service (USFWS), and U.S. Geological Survey Great Lake Science Center (USGS GLSC) for the years 2014-2016 (Table 1). Setline bycatch data were from USFWS, while minnow trap bycatch data were from USFWS and USGS. Herpetological Resource and Management conducted minnow trapping specifically targeting mudpuppies. Setlines are set along the bottom of the river and have small (1/0) and large hooks (9/0) baited with dead round goby, along with three attached minnow traps baited with cheese cubes. Setlines were checked and re-set every 24 hours by USFWS (Craig et al. 2015). Shoreline minnow traps baited with cheese cubes and/or raw chicken were also set every 24 hours by USGS and HRM. Shoreline and spawning reef restoration sites were targeted along with presumed unoccupied sites as controls. Setline and minnow trap data were used to calculate catch-per-unit-effort for each site, gear type, and year. Catch-per-unit-effort was calculated for the months of April and May when mudpuppy detection was higher. Difference between catch-per-unit-effort values were calculated using two-sample t-tests.

Environmental DNA

Sites were selected for eDNA sampling based on whether they have sufficient trapping data and whether or not restoration had occurred. Environmental DNA was only collected at shoreline sites (Table 1). These sites were located on the St. Clair River (four sites), Lake St. Clair (two sites), and the Huron River (four sites). Three of the sites on the St. Clair River (Cottrellville, Marysville, and Blue Water River Walk) were restored and both sites on

Lake St. Clair (Fairhaven Boat Launch and Lake St. Clair Metropark) had rock additions. To help verify the status (presence/absence) of mudpuppies at sites along the St. Clair-Detroit River System, trapping data conducted by Herpetological Resource Management (HRM), U.S. Fish and Wildlife Service (USFWS), and U.S. Geological Survey (USGS) were analyzed and compared to eDNA results.

Table 1: Sites where minnow trap (MT) and setline (SL) data were collected from U.S. Fish and Wildlife Service, U.S. Geological Survey, and Herpetological Resource and Management.

	Map Labels		eDNA	U.S. Fish and Wildlife Service			U.S. Geological Survey		Herpetological Resource and Management		
				2014	2015	2016	2015	2016	2014	2015	2016
Lake Huron	LH	St. Clair River Headwaters		SL	MT/SL	SL					
St. Clair River	SCR1	Blue Water Bridge*							MT	MT	MT
	SCR2	Keifer Park*					MT			MT	MT
	SCR3	Blue Water River Walk*	x				MT	MT	MT	MT	MT
	SCR4	Marysville*	x					MT		MT	MT
	SCR5	East China		MT/SL	MT						
	SCR6	Cottrellville*	x				MT	MT		MT	MT
	SCR7	Algonac State Park	x						MT	MT	MT
	SCR8	Russel Island		MT	MT						
	SCR9	Middle Channel**		MT	MT						
	SCR10	Pearl Beach				MT					
Lake St. Clair	LSC1	Fair Haven Boat Launch	x						MT	MT	MT
	LSC2	Lake St. Clair Fisheries and Research Station							MT	MT	MT
	LSC3	Lake St. Clair Metropark***	x						MT	MT	MT
Detroit River	DR1	Belle Isle* **		MT					MT		MT
	DR2	Milliken State Park									MT
	DR3	Delray Public Access Boat Ramp		MT	MT	MT					
	DR4	Fighting Island**		MT/SL	MT/SL	MT/SL					
	DR5	Grassy Island		MT/SL	MT/SL	MT/SL					
	DR6	Hennepin Point (Grosse Ile)		MT/SL	MT/SL	MT/SL					
	DR7	Turkey Island		MT/SL	MT						
	DR9	Bridge to Grosse Ile		MT/SL	MT/SL	MT/SL					
	HR	Huron River	x							MT	MT
Lake Erie	LE1	Lake Erie Metropark***									MT
	LE2	Pt. Mouilee									MT
	LE3	Mouth of Lake Erie		SL							MT
	LE4	Sterling State Park									MT
	LE5	Toledo Beach Marina									MT

* indicates shoreline restoration, ** indicates fish spawning reef *** indicates shoreline concrete slab additions specifically placed for mudpuppies.

Collection, filtration, and isolation of mudpuppy eDNA, and qPCR was conducted using methods developed by Stephen Spear (Spear et al. 2015). Environmental DNA was collected from the shore at these sites by filling one to four one-liter containers depending on site size. To prevent contamination, this was done without entering the water and while wearing gloves. To preserve DNA, 1 ml of 10% Benzalkonium chloride (BAC) at a final concentration of 0.01% was added to each one-liter container after collection of each sample before transport back to the lab for filtration (Yamanaka et al. 2016). After the 1 L samples were brought back to the lab, they were run through a 0.45 μm cellulose nitrate filter (Whatman International, Ltd.) in a filter cup inserted into a one-liter vacuum flask to catch the DNA in the sample. After filtering, and to prevent contamination between samples, the filter paper was removed with forceps that were treated with DNA AwayTM (Molecular Bioproducts). The filter paper was put into 95% ethanol in a centrifuge tube and frozen until it could be processed. To test for contamination, deionized water was also filtered every time samples were filtered in the lab.

Extraction of DNA from the filters was done using methods described in Spear et al. (2015). Quantitative PCR (qPCR) and a primer and probe set were used to analyze the mudpuppy eDNA in the samples (Spear et al. 2015). The primer sequence is general and amplifies DNA from many species, while the probe is specific to mudpuppy DNA (Table 2). The primer/probe combination amplifies a 149 bp region.

Table 2: Mudpuppy primer and probe sequences

Name	Sequence
NemaForward	AGCAACAGCCTTTGTAGGGTA
NemaReverse	TCGCCTTATCGACGGAGAATC
NemaProbe (Quasar 670)	CGTACTACCATGAGGCCAAATATCCTTC

Quantitative PCR reactions of 15 μL were run which included 2.85 μL water, 7.5 μL QuantiTect Multiples PCR Mix (Qiagen, Inc.), 0.4 μM primer, 0.2 μM probe, 0.6 μL TaqMan® Exogenous Internal Positive Control 10X Exo IPC Mix (Applied Biosystems), 0.3 μL of TaqMan® Exogenous Internal Positive Control 50X Exo IPC DNA (Applied Biosystems), and 3 μL of sample. The qPCR cycle was as follows: initial denaturation at 95 °C for 10 minutes, 50 cycles of 94 °C for 60 seconds for denaturation, and annealing at 60 °C for 45 seconds. Each sample had a replication of three and was run with positive controls from tail tip tissue extraction and negative controls. Samples from tail tip tissue extraction had a concentration of DNA which was estimated using a NanoDrop™ fluorospectrometer (Thermo Scientific). These positive controls were diluted to include four different concentrations which covered the range of DNA concentration typically seen with eDNA extractions: 10^{-3} ng/ μl , 10^{-4} ng/ μl , 10^{-5} ng/ μl , and 10^{-6} ng/ μl . A positive control sample from an indoor tank containing adult mudpuppies was also used.

To determine if sites were positive for mudpuppy eDNA, the following protocol was used based on Spear et al. (2015). If two out of the three replicates from a single site were positive for mudpuppy eDNA, a positive detection of a mudpuppy at that site was concluded. These three replicates were also rerun to confirm the results. If only one out of three replicates from a site

was positive, the sample was also rerun. If the next three replicates were negative for mudpuppy eDNA, the site was considered negative for eDNA. If one or more of the three replicates was positive during the second run, the site was considered positive for mudpuppies.

Status of Mudpuppies Using Occupancy Modeling

Occupancy modeling was used to determine the detection probability and occupancy of mudpuppies along the St. Clair-Detroit River System using trapping data. Calculations for this model were carried out by the program PRESENCE, which estimates detection probability and the proportion of sites occupied when the detection of the species is less than one (MacKenzie et al. 2002). The assumptions for this model are (1) the population is closed to immigration and colonization, and emigration and extinction; (2) the species is identified correctly; and (3) detecting mudpuppies at one site is independent from detecting mudpuppies at all other sites. To carry out the field methods for this model, sites must be surveyed (detection/no detection) at least two times per sampling season. Parameters include ψ_i , the probability that a mudpuppy is present at site i , and p_{it} , the probability that a species is detected at site i at time t , assuming it is present. The method involves visiting sites multiple times within a season where the target species is either detected, with probability p , or not detected. The intent is to estimate the proportion of sites that are occupied, ψ , knowing the species is not always detected, even when present. On any given sampling occasion, the species is either detected, which requires occupancy, $\psi \times p$, or not detected, which arises when either the species is present but not detected, $\psi \times (1 - p)$, or when it is not present, $(1 - \psi)$.

To be entered into PRESENCE, the data were set up as shown in Fig. 4. Rows were sites, and columns were dates written as “sampling season-sampling event.” Sites were sampled for three separate seasons. Each cell needed a designation whether mudpuppies were caught on that sampling event (1), not caught on that event (0), or no sampling occurred (-).

rows	cols	#srvy/season	#meth/srvy	#site covs	#samp covs
14	49	19,14,16	1	0	0

data	1-1	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	1-10	1-11	1-12	1-13	1-14	1-15	1-16	1-17	1-18	1-19	2-1	2-2	2-3	2-4	2-5	2-6	2-7
site1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
site2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
site3	-	-	-	-	-	0	0	-	-	-	-	-	-	0	-	-	-	-	1	1	1	1	0	0	1	-
site4	-	-	-	-	-	-	-	0	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
site5	-	0	0	0	0	0	0	-	-	-	-	-	-	0	-	-	-	-	1	0	1	1	1	1	0	-
site6	-	-	-	-	-	-	-	0	0	0	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
site7	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
site8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	1	-	-	-	-	-	-	-	-
site9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
site10	-	-	-	-	-	1	1	-	-	-	-	-	-	0	-	-	-	-	1	-	-	-	-	-	-	-
site11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
site12	1	1	1	1	1	0	-	-	-	-	-	-	-	0	-	-	-	-	1	1	1	1	1	1	1	1
site13	-	-	-	-	-	1	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-
site14	-	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0

Figure 4: Data entry form for the program PRESENCE. (-) = no sampling occurred on sampling occasion, (1) = mudpuppies detected on given sampling occasion, (0) = sampling occurred, but no mudpuppies were detected on given sampling occasion.

Data from 14 setline sites and 50 minnow trap sites were used to estimate ψ and p for mudpuppies. With this analysis, we assumed that species occupancy was constant across the three-season period due to mudpuppies being long lived and relatively sedentary. We varied detection probability across the three-year sampling period, and we examined the effects of survey method on occupancy (ψ) and detectability (p).

Each sampling event represented one week. Sites were eliminated if they were only sampled during one week. Sampling covariates included temperature and restoration status (shoreline restoration, shoreline control, artificial reef, and artificial reef control). Temperature covariates were z-transformed. Shoreline restoration was defined by the addition of rocks while control sites did not have rocky substrates. Artificial reefs were the addition of rocks to the bottom of the river for the benefit of fish that use broadcast spawning as their primary mode of reproduction; these sites could also benefit mudpuppies that use the rocks as habitat and for reproduction. We tested for differences between covariate occupancy and detection probability using two-sample t-tests and ANOVAs with post-hoc Tuckey tests. Spearman's Rho test was also used to determine the correlation between detection probability using minnow traps and setlines and temperature.

Status of Mudpuppies Using eDNA Occupancy Modeling

Calculations for this model were carried out by the program PRESENCE (MacKenzie 2002). Estimable parameters include ψ_i , the probability that mudpuppy eDNA is present at site i , and p_{it} , the probability that a species eDNA is detected at site i at time t , assuming it is present. Sampling events were the replicates that were taken at each site.

CHAPTER 3

RESULTS

Trapping and CPUE

Over the three field seasons (2014-2016), 372 mudpuppies were caught using setlines and minnow traps. Minnow traps caught 200 individuals while setlines caught 172. Setlines had a total of 49 sampling events at 14 sites, and minnow trapping occurred on 561 occasions at 50 sites. For setlines, there were 19 sampling events in 2014, 14 sampling events in 2015, and 16 sampling events in 2016. For minnow traps, there were 159 sampling events in 2014, 181 sampling events in 2015, and 221 sampling events in 2016. Mudpuppies were caught at five sites along the St. Clair River, one site on Lake St. Clair, and ten sites on the Detroit River (Fig. 5). Catch-per-unit-effort data were calculated using the months of April and May when mudpuppy detection was highest.

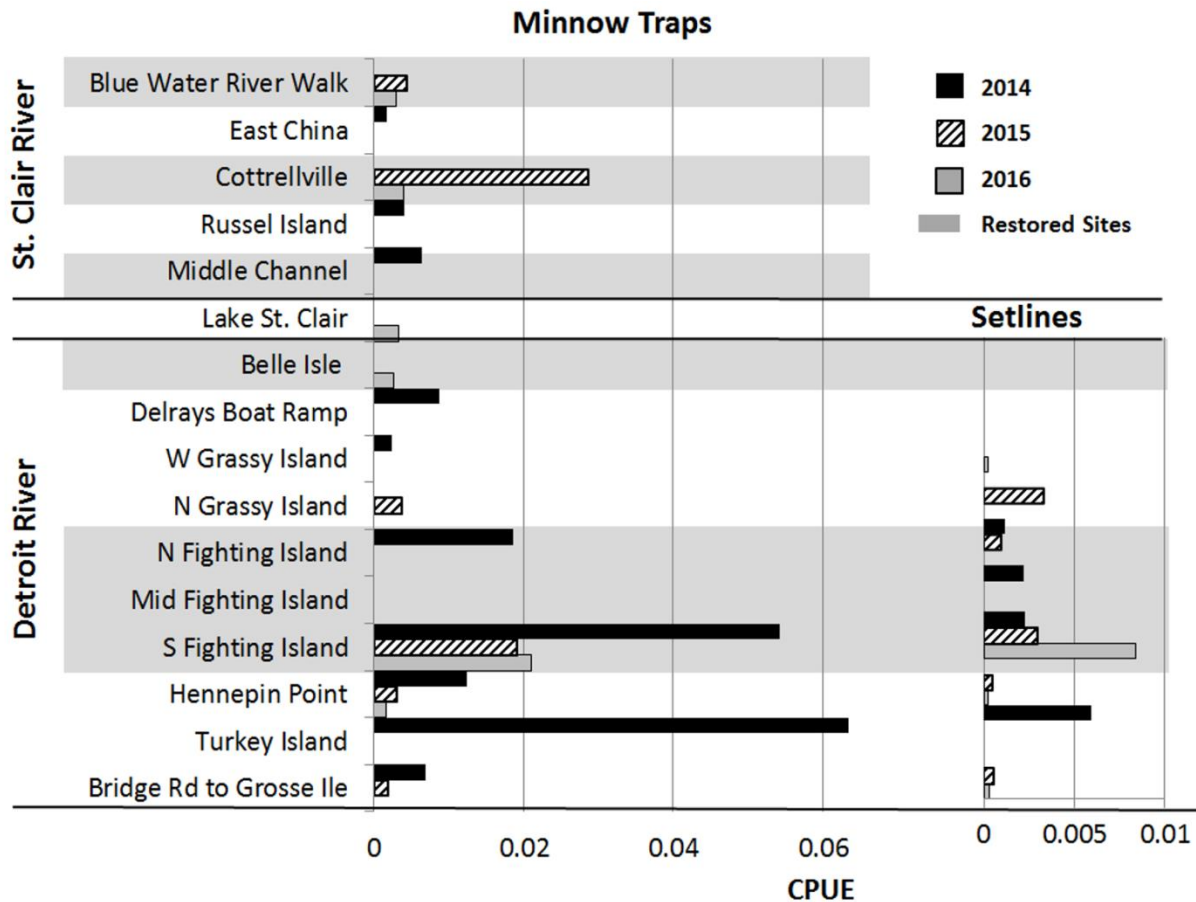


Figure 5: Catch-per-unit-effort (CPUE) calculated for minnow traps and setlines during the months of April and May. Shaded sites indicate restorations which includes any sort of rock additions (fish spawning reefs or shoreline). Sites with no successful captures have been excluded from the figure.

Minnow traps had a CPUE of 0.0076 ± 0.0014 and setlines had a CPUE of 0.0014 ± 0.0022 and were not significantly different ($P = 0.154$; Fig. 6A; Unpaired t -test $t = 1.4405$, $P = 0.1536$). Restoration sites where there were rock additions had a CPUE of 0.0074 ± 0.013 for minnow traps and 0.0022 ± 0.0027 for setlines, while control sites had a CPUE of 0.0031 ± 0.011 for minnow traps and 0.00069 ± 0.0016 for setlines (Fig. 6B). Differences between rock

additions and controls were not different for minnow traps ($P = 0.1921$; Unpaired t -test $t = 1.3204$) or setlines ($P = 0.0894$; Unpaired t -test $t = 1.7770$).

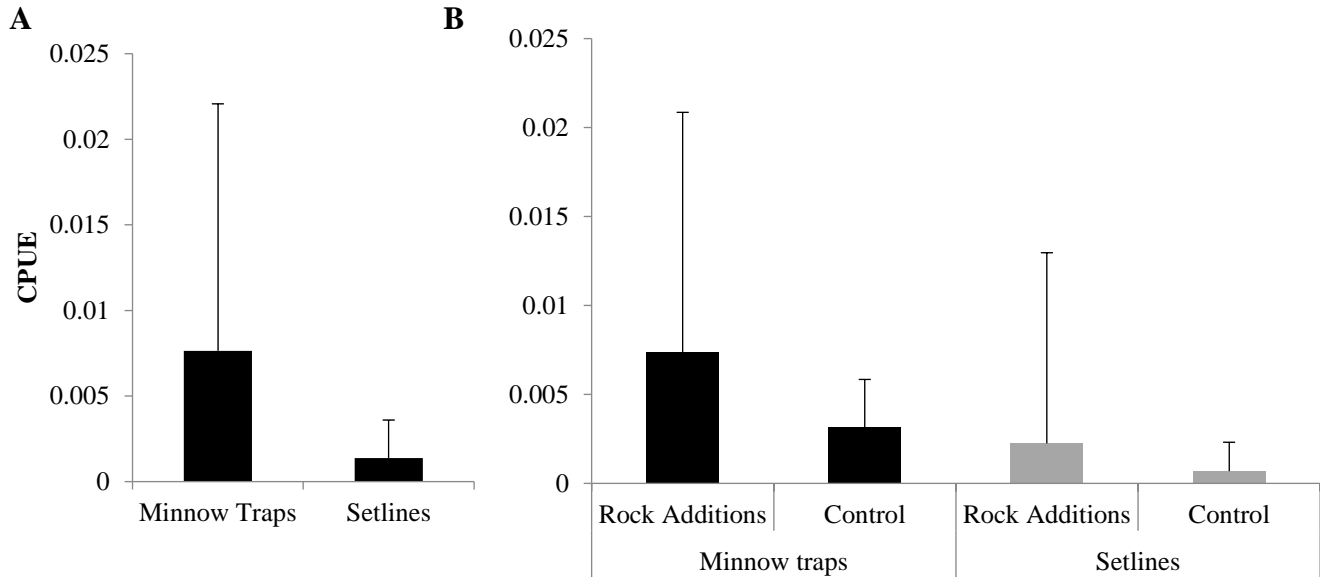


Figure 6: Review of catch-per-unit-effort for setlines and minnow traps during the months of April and May: (A) sites with rocks added (spawning reefs/shoreline restorations) and (B) control sites without rock additions.

Environmental DNA

Mudpuppy eDNA was detected in water samples from nine sites and was not detected at five sites (Table 3). All sites where mudpuppies have been detected through trapping had positive eDNA results. Mudpuppies have never been detected at the five sites where mudpuppy eDNA was not detected. The only “mismatch” was at Fairhaven Boat Launch, which had a positive detection of eDNA but mudpuppies have not been detected there by trapping (Fig. 7D). There were no instances of sites with positive trapping records having negative eDNA results. Examples of positive qPCR results for mudpuppy eDNA are shown in Fig. 7.

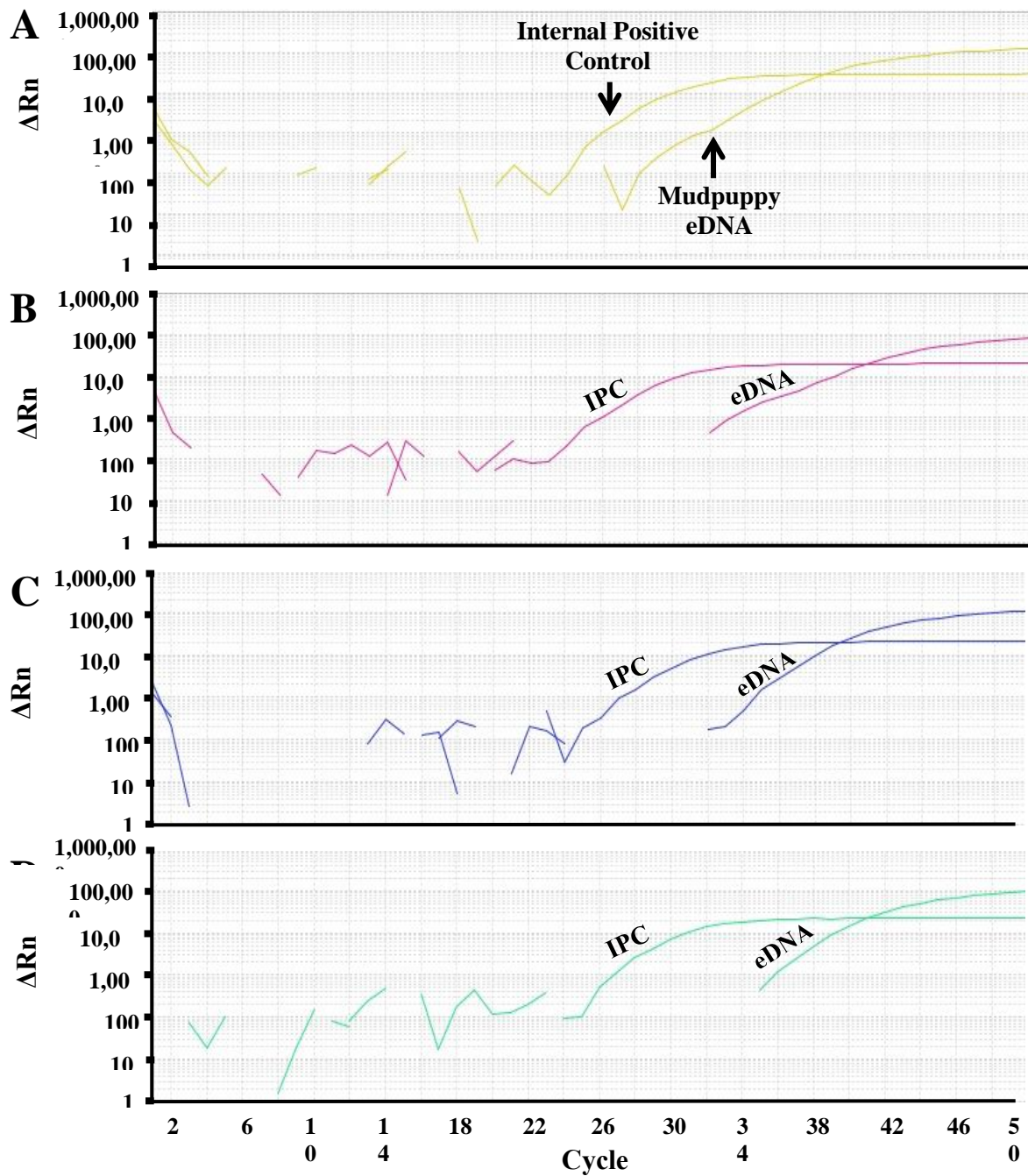


Figure 7: Examples of sites that were positive for mudpuppy eDNA showing amplification of both mudpuppy eDNA within the sample and of the internal positive control: (A) Blue Water River Walk, (B) Cottrellville, (C) Marysville Living Shoreline, and (D) Fairhaven Boat Launch.

Table 3: Environmental DNA results compared to detection by trapping

	Restoration	Trapping detection	eDNA detection	eDNA detection 1 st run	eDNA detection 2 nd run
Huron River (1)		-	-	0/3	0/3
Huron River (8)		-	-	0/3	
Huron River (10)		-	-	0/3	
Huron River (15)		-	-	0/3	0/3
Lake St. Clair Metropark	x	-	*	0/3*	1/2*
Cottrellville Up	x	+	+	3/3	
Cottrellville Middle			+	3/3	
Cottrellville Down			+	2/3	
Blue Water River Walk (Sugar)	x	+	+	3/3	
Blue Water River Walk (Gray Fox)	x	+	+	3/3	
Blue Water River Walk (Kramer)	x	+	+	3/3	
Algonac State Park Up		+	+	0/3	
Algonac State Park Middle				2/3	
Algonac State Park Down				2/3	
Marysville (1)	x	+	+	2/3	
Marysville (2)		+	+	3/3	
Fairhaven Boat Launch Up	x	-	+	2/3	
Fairhaven Boat Launch Middle			+	1/3	1/3
Fairhaven Boat Launch Down			-	1/3	0/3
Belle Isle Tank A		+	+	3/3	
Belle Isle Tank B			+	3/3	

*Inhibition with the internal positive controls during the qPCR run, potentially due to silt within the sample.

Occupancy Modeling

The estimate of occupancy (ψ) was the same for minnow traps ($\psi = 0.87 \pm 0.084$), setlines ($\psi = 0.86 \pm 0.133$), and eDNA ($\psi = 0.86 \pm 0.132$; Fig. 4A; ANOVA $F = 0.0028$, $P = 0.9972$). Detection probability (p) for the three-year period was $p = 0.185 \pm 0.019$ for minnow traps, $p = 0.479 \pm 0.058$ for setlines and $p = 0.81 \pm 0.099$ for eDNA (Fig. 8B). Detection probability differed between the three sampling methods (ANOVA $F = 32.3436$, $P < 0.00001$)

and a post-hoc Tuckey test showed that eDNA had a higher detection probability compared to minnow traps ($P < 0.00001$) and setlines ($P = 0.0078$). A post-hoc Tuckey test also showed that setlines had a higher detection probability compared to minnow traps ($P < 0.00001$).

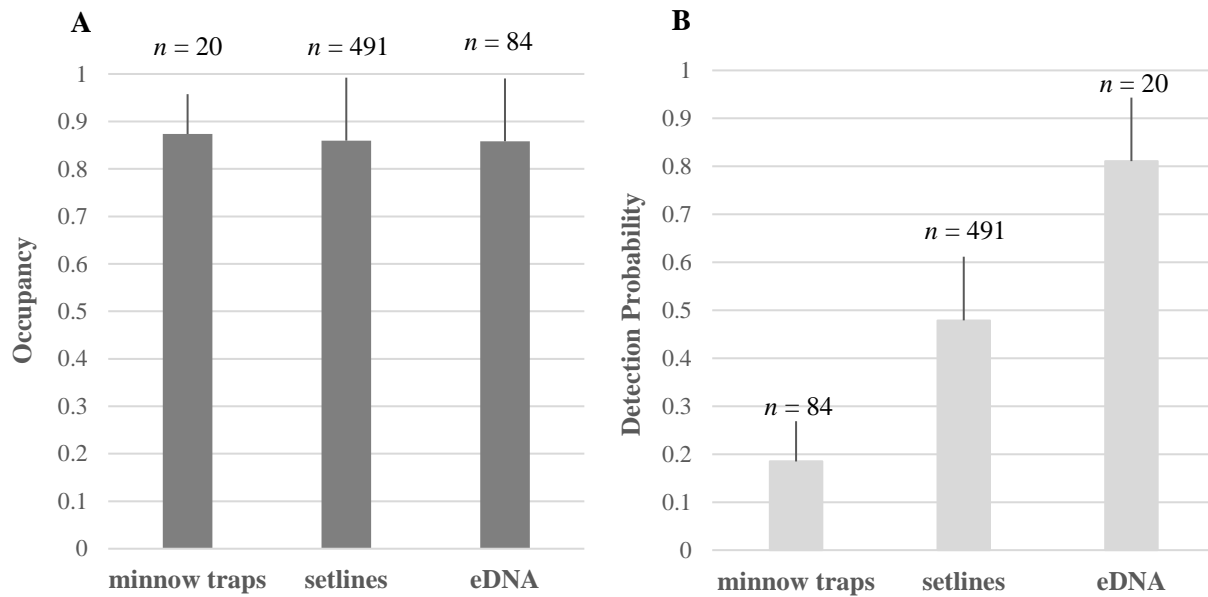


Figure 8: Occupancy (A) and detection probability (B) for mudpuppies along the St. Clair-Detroit River System using the program PRESENCE and three different sampling methods (minnow traps, setlines, and eDNA).

At shoreline restoration sites (minnow traps) there was a higher occupancy ($\psi = 0.87 \pm 0.11$) than at shoreline sites without restoration (“shoreline control,” $\psi = 0.49 \pm 0.21$; Fig. 9A; two-sample t -test, $t = 20.4726$, $df = 283$, $P < 0.0001$). Reef restoration sites did not have a higher occupancy than sites without a reef for both minnow traps (“reef” $\psi = 0.77 \pm 0.11$, “control” $\psi = 0.79 \pm 0.12$; Fig. 9A; two-sample t -test, $t = 1.1890$, $df = 207$, $P = 0.2398$) and setlines (“reef” $\psi =$

0.80 ± 0.17, “control” $\psi = 0.79 \pm 0.12$; Fig. 9B; two-sample *t*-test $t = 0.7783$, $df = 207$, $P = 0.4373$).

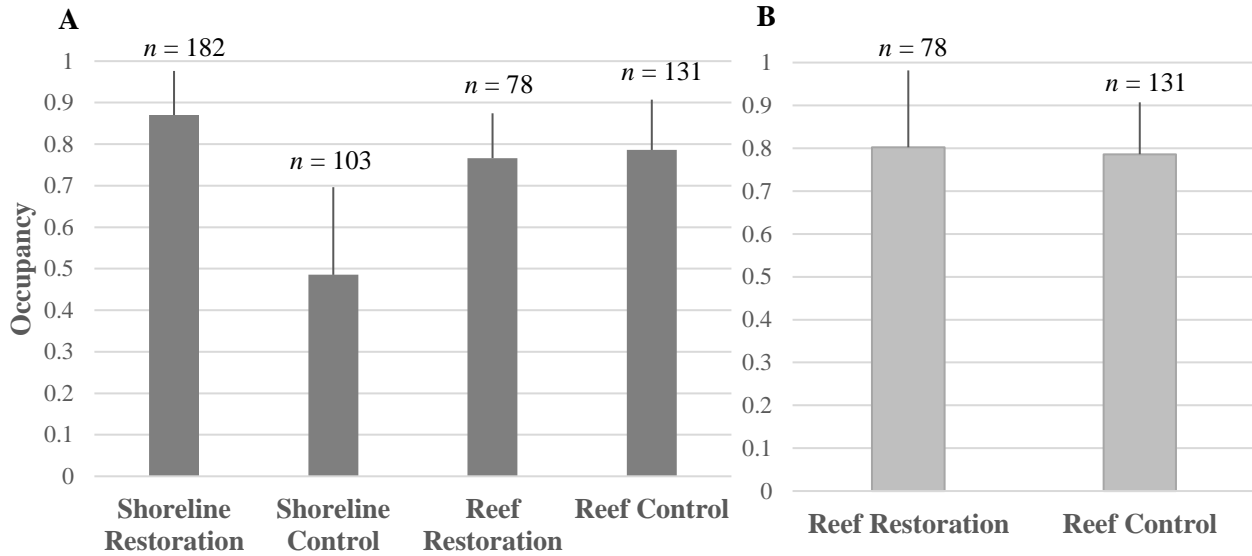


Figure 9: Occupancy of mudpuppies at restoration and control sites using minnow traps (A) and setlines (B). Mudpuppies had a higher occupancy at shoreline sites when there was restoration, but not a higher occupancy at sites where there was reef restoration for minnow traps and setlines.

For minnow trapping, the sampling season was from April 13 to December 14 in 2014, April 12 to December 13 in 2015, and March 13 to December 4 in 2016 (Fig. 10). The highest detection probability was on the last day of sampling (December 14, $p=0.37$) in 2014 when the surface water temperature (stated as “water temperature” going forward) was 3.03 °C. The highest detection probability in 2015 was $p= 0.27$ and occurred on December 13 (also the last day of sampling) when the water temperature was 6.5 °C. In 2016, the highest detection

probability was $p = 0.39$, and occurred on April 3 when the water temperature was $2.22\text{ }^{\circ}\text{C}$, which was the coldest (water) sampling day of the year.

For setlines the sampling season was from April 13 to November 2 in 2014, April 12 to June 7 in 2015, and March 20 to June 19 in 2016 (Fig. 10). The dates with the highest detection probability occurred on the first day of sampling each year: April 13 2014, when the water temperature was $7.15\text{ }^{\circ}\text{C}$ ($p = 0.68$); April 12 2015, when the water temperature was $7.16\text{ }^{\circ}\text{C}$ ($p = 0.70$); and March 20 2016, when the water temperature was $4.05\text{ }^{\circ}\text{C}$ ($p = 0.93$). In all three years, the highest detection probability occurred on the coldest (water temperature) sampling day of the year.

The detection probability was lowest on the hottest (water temperature) sampling days of the year in 2014 and 2015, in 2016 it was the second hottest sampling day of the year by $0.3\text{ }^{\circ}\text{C}$ (Fig. 10). In 2014, the lowest detection probability of the year for minnow traps was on June 29 ($p = 0.037$) with a water temperature of $22.67\text{ }^{\circ}\text{C}$, August 16 ($p = 0.04$) in 2015, with a temperature of $22.25\text{ }^{\circ}\text{C}$, and July 31 ($p = 0.031$) in 2016 with a water temperature of $24.43\text{ }^{\circ}\text{C}$. Spearman's Rho rank correlation coefficient was $r_s = -0.96541$ for minnow trap detection probability and temperature (Fig. 11). The detection probability was lowest for setlines during all three sampling seasons on the hottest (water temperature) sampling day of the season. In 2014, it was on July 20 ($p = 0.0002$) when the temperature was $22.78\text{ }^{\circ}\text{C}$, May 10 ($p = 0.081$) when the water temperature was $12.76\text{ }^{\circ}\text{C}$, and June 19 ($p = 0.011$) when the water temperature was $16.27\text{ }^{\circ}\text{C}$. Spearman's Rho rank correlation coefficient was $r_s = -0.9808$ for setline detection probability and temperature (Fig. 11).

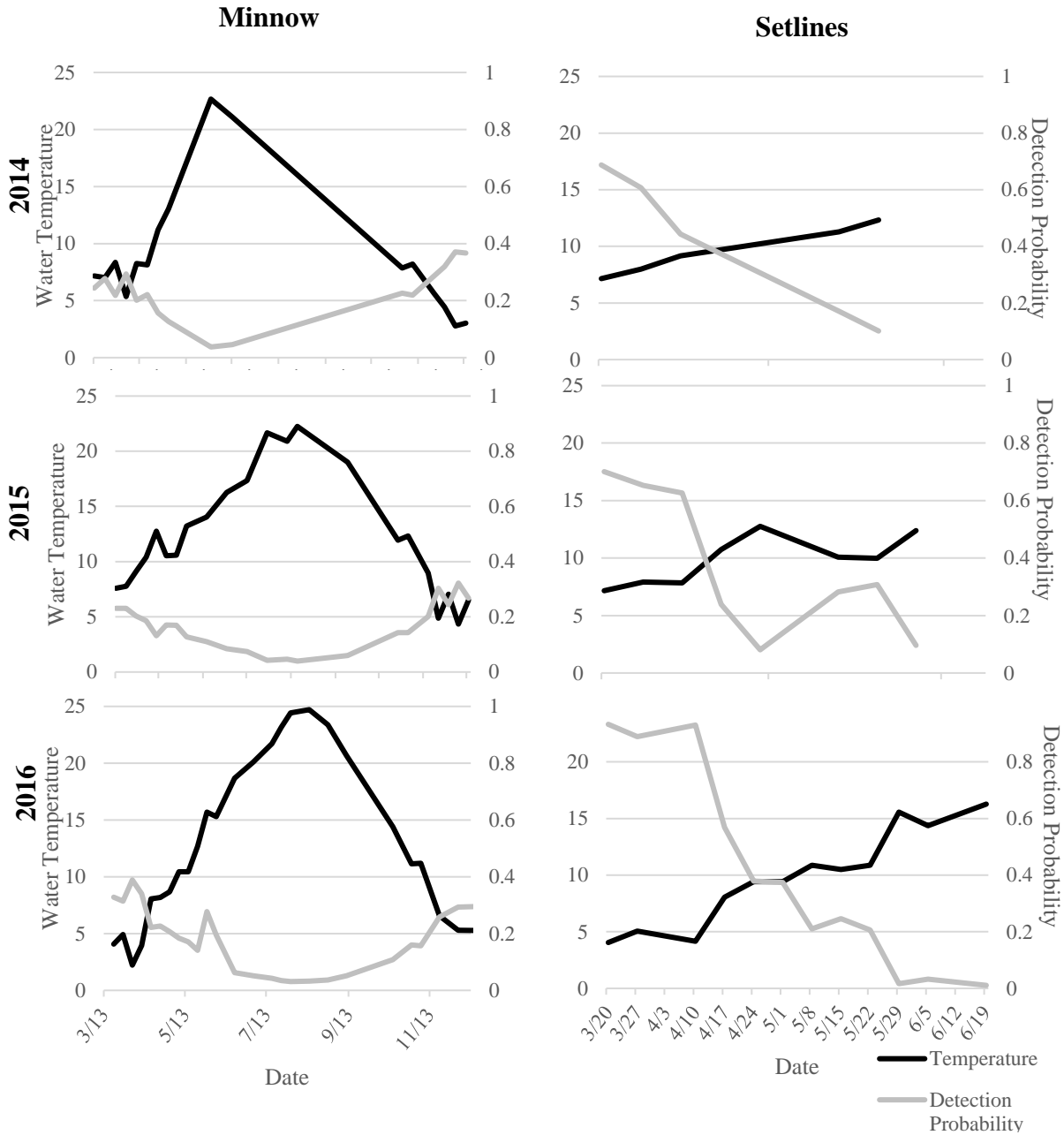


Figure 10: Detection probability and water temperature across the entire sampling period for setlines and minnow traps during each sampling year (2014, 2015, and 2016). Detection probability was lowest during the warmest parts of the year. Note that minnow traps are sampled for a longer time during the year than setlines.

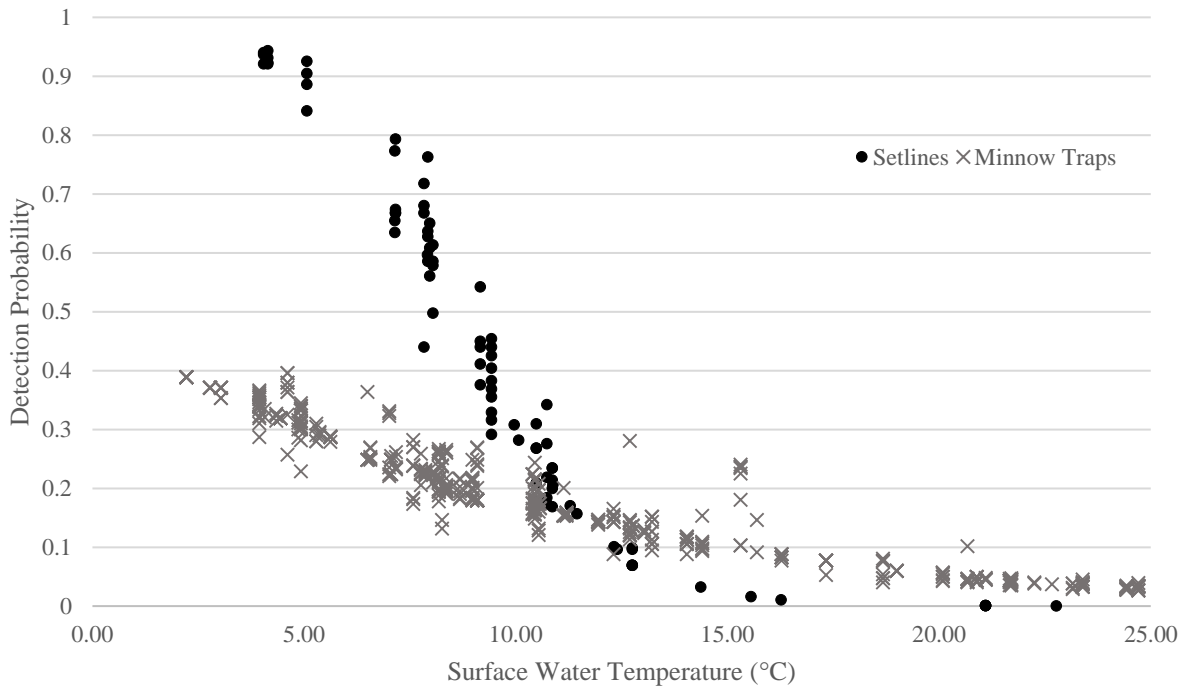


Figure 11: Regression for minnow traps and setlines displaying detection probability trends with temperature. Spearman's Rho rank correlation $r_s = -0.96541$ for minnow traps and $r_s = -0.9808$ for setlines.

CHAPTER 4

DISCUSSION

To accomplish effective wildlife management, detection of a species must be accurate, and survey and analysis methods that minimize false negatives are necessary. Studying cryptic species and obtaining these data is challenging, but with the use of eDNA and occupancy modeling population status of a species can be acquired. We used occupancy modeling and eDNA to determine mudpuppy presence at restored and unrestored locations along the St. Clair-Detroit River System. Shoreline sites had a higher occupancy than unrestored sites, which followed our prediction, though we did not find a higher catch-per-unit-effort at shoreline restoration sites (Fig. 6B). Reef restoration sites did not show the same pattern and did not have higher occupancy than at sites without a reef for both minnow traps and setlines. All sites where mudpuppies had been trapped had positive eDNA results (Table 3), and eDNA had the highest detection probability compared to the traditional trapping methods (minnow traps and setlines).

Our results indicate that shoreline restoration is the best practice for increasing mudpuppy occurrence. Mudpuppies occur at a higher proportion at restoration sites ($\psi = 0.87 \pm 0.11$) compared to control sites ($\psi = 0.49 \pm 0.211$; Fig. 9A) based on our analysis of minnow trap data. The shoreline sites that were sampled for mudpuppies had extensive restoration events that included the addition of large rocks known to be suitable as mudpuppy habitat and reproduction (Fig. 3). These sites also included the addition of terrestrial and aquatic plants and the removal of seawalls. Future management along the St. Clair-Detroit River System for mudpuppies should continue to improve shoreline habitat. This study shows that the removal of seawalls, along with

the addition of large rocks and vegetation increases the occupancy of mudpuppies at those locations. Additional restoration sites, one of which was sampled for eDNA, had less extensive restoration events and only included the addition of concrete slabs specifically placed for mudpuppy habitat (Lake St. Clair Metropark; Fig. 3D). Trapping indicated that these sites do not currently have resident mudpuppies. Even though one of these sites was included in the eDNA survey, the data could not be used because of failure of internal positive controls to amplify during qPCR. This is possibly due to silt at the site causing inhibition. Future eDNA studies could resample these locations.

Restoration events designed to increase fish spawning habitat were not indicated to increase the presence of mudpuppies with this study. Our results show the occurrence of mudpuppies at reef restoration sites to not be higher than sites without a reef by both minnow traps (reef - $\psi = 0.77 \pm 0.11$, control - $\psi = 0.79 \pm 0.12$; Fig. 9A) and setlines (reef - $\psi = 0.80 \pm 0.18$, control - $\psi = 0.79 \pm 0.12$; Fig. 9B). This result may be because setlines and minnow traps that were not placed directly on a reef and were just within the vicinity of the reef were included as a “reef restoration” site in this analysis. Mudpuppy studies typically do not exceed a depth of 2.0 m, but they have been shown to inhabit deeper water and even lay eggs on artificial structures in deep water along the Detroit River (Hacker 1957; Sajdak 1982; Chellman 2011; Craig et al. 2015). Future studies may want to split the sites we used as reef restoration sites further.

Setlines had a higher detection probability compared to minnow traps likely due to the number of hooks on a setline, which increase the surface area that a line can cover. There was not a difference for catch-per-unit-effort (CPUE) between setlines and minnow traps. The same

result was found when CPUE was calculated on the same dataset for the years 2003-2013 (Craig et al. 2015). Since setlines and minnow traps are a passive fishing gear, the more hooks or traps there are, the higher the probability of an individual of encountering the hook or minnow trap (Rudstam et al. 1984). Catch-per-unit-effort has been found to be higher for setlines during other studies. When comparing setlines to gill nets and angling when capturing white sturgeon, setlines were 1.24 times the catch rate of gillnets and 1.78 times the catch rate of angling (Skalski et al. 1992). Minnow traps are typically used to detect mudpuppies (Bonin et al. 1995; McDaniel et al. 2009; Hoffman et al. 2014). Since this study shows that mudpuppies are difficult to detect using minnow traps compared to setlines and eDNA, future studies may want to use gear that covers a wider surface area or increase the amount of minnow traps on a line.

Environmental DNA (eDNA) sampling was found to increase the detection of mudpuppies along the St. Clair-Detroit River System. Environmental DNA was detected at all shoreline sites where mudpuppies have been previously captured using minnow traps (Table 3). Our eDNA results also indicated the addition of one site (Fairhaven Boat Launch) where mudpuppies may be occurring due to a positive mudpuppy eDNA result and where they have not been detected via trapping (Table 3). This site does contain large rocks suitable for mudpuppy habitat and reproduction. Another mudpuppy eDNA study in Ohio determined the presence of mudpuppies at six out of ten site locations, but only detected them via trapping at one site (Collins 2017). Environmental DNA has also been used to locate eastern hellbenders located in the Midwestern United States whose populations are much lower compared to the mudpuppy. In Pennsylvania, positive eDNA results were found at ten of 24 historical hellbender locations, five of which were known positive sites (Pitt et al. 2017). At another Pennsylvanian location, four

tributaries known to contain hellbenders continuously had eDNA detections along with two of four other tributaries demonstrating positive results where they had not been detected using traditional methods (Takahashi et al. 2018). In Ohio, eastern hellbenders were detected by traditional field surveys at nine sites where eDNA was also detected with the addition of 24 other sites that had a positive eDNA collection without known occurrences (Spear et al. 2015). This shows that our results are not unusual and my help to document an additional mudpuppy location on the SCDRS along with an additional tool to locate mudpuppies and monitor habitat quality.

Environmental DNA had the highest detection probability ($p = 0.81 \pm 0.99$) of the three surveying methods (minnow traps, setline, and eDNA; Fig. 8B). All sampling techniques showed equal occupancy (Fig. 8), which suggests that studies trying to determine the occupancy of mudpuppies in a system could use any of these methods (minnow traps, setlines, and eDNA). Other studies have also found that eDNA reveals the highest detection probability compared to traditional field surveys. When locating Idaho giant salamanders, eDNA surveys resulted in higher detection rates compared to traditional kick-net surveys across 13 streams and identified two formally unknown locations (Pilliod et al. 2013). The use of eDNA occupancy modeling would give an estimate of detection probability and could therefore be used to determine the number of replicates needed to confirm that mudpuppies are absent from a site. Environmental DNA can be used with the addition of trapping methods to locate mudpuppies. Future studies should utilize eDNA as a tool to determine whether mudpuppies are present/absent at a site.

There was a strong relationship between water temperature and detection probability for both setlines ($r_s = -0.96541$) and minnow traps ($r_s = -0.9808$; Fig. 11). Detection probability was the lowest during the warmest sampling days of the year and highest during the coldest sampling

days of the year (Figs. 10, 11). This relationship was also seen on Wolf Lake, a former estuarine wetland complex to Lake Michigan, where overall mudpuppy trapping success declined quickly at water temperatures above 14.1 °C (Beattie et al. 2017). Other studies only sample for mudpuppies when the water temperature is at or below 5 °C because of a decrease in detection (McDaniel et al. 2009). Future sample for mudpuppies should continue to be conducted when water temperatures are colder.

Recommendations for Future Research

It is presumed that eDNA sampling is cheaper than traditional sampling methods in both cost and time. It may be useful to calculate the actual cost of these sampling methods for researchers trying to decide about which method to use. It is suggested that if researchers are trying to determine larger ranges of the location of a species, they use eDNA as baseline data and potentially using trapping as a source for more specific site locations and demography data.

Other studies may want to look at detection probability of mudpuppies during colder sampling days and compare it to eDNA detection probabilities to increase detection. It would be interesting to know whether detection of mudpuppies when only sampled for during colder months would compete with the detection of them using eDNA.

It may be interesting to look at the detection probability of mudpuppies at shoreline and reef sites during different points in the season. It is presumed that mudpuppies move to deeper water during warmer months, indicating that occupancy may be higher at deeper locations during these months and lower at shoreline sites and may also be an explanation for low detection at warmer water temperatures (Figs. 10, 11). Studies have shown that mudpuppies prefer water depths between 0.2-1.0 m, but they can be found in depths up to 30 m (Hacker 1957; Sajdak

1982). It has been noted that mudpuppies will seek out cooler depths in lakes during warmer months in New York (Bogert 1952). With ectotherms, movement is closely associated with thermoregulation; this is especially true in aquatic environments where organisms must move further to gain a change in temperature compared to terrestrial environments (Hutchison and Spriestersbach 1986). Mudpuppies have shown to choose colder water during winter months, while in the summer they choose colder temperatures during the day and warmer temperatures during the night (Hutchison and Spriestersbach 1986). Whether this translates to seasonal movement to deeper waters in the Great Lakes is unknown.

Summary and Conclusions

Future restoration along the SCDRS for mudpuppies should include shoreline seawall removal and the addition of large rocks and vegetation because our study showed mudpuppy occurrence was higher at shoreline restoration sites compared to sites without these restoration practices. Mudpuppy eDNA was successfully detected at every site where they had been detected via trapping, and it had the highest detection probability of the three methods while setlines had a higher detection probability than minnow traps. This indicates eDNA is a useful tool for locating mudpuppies. There was a strong relationship between temperature and detection probability, with detection being highest at colder water temperatures; therefore, monitoring mudpuppy populations will be more successful if done during colder times of the year.

Though a lot about mudpuppy life history is still unknown, there are already many reasons to protect and continue to monitor them. Mudpuppies have an important role in the ecosystem because they are top predators. Mudpuppies are also obligate hosts for the mudpuppy

mussel (*Simpsonaias ambigua*). This endangered species of mussel is completely reliant on the mudpuppy to complete their lifecycle (McDaniel et al. 2009). Another reason to protect and increase mudpuppy populations is that they are known to eat invasive species such as the round goby (*Neogobius melanostomus*) and zebra mussels (*Dreissena* spp.), both very successful invasive species in the Laurentian Great Lakes (Beattie et al. 2017). Mudpuppies, along with other amphibians, are also great biological indicators because of their sensitivity to environmental stressors (Davic and Welsh 2004). This species can be used to determine the health of an environment and to gauge whether restoration practices are successful. Finding mudpuppies, along with other species, at restoration sites along the St. Clair River was able to fulfill the Beneficial Use Impairment for “loss of fish and wildlife habitat” and successfully bring the river a step closer to not being labeled as an Area of Concern (USEPA 2018). Mudpuppy monitoring can aid the same result for other systems, including continued monitoring on the Detroit River to remove the same BUI and gauge restoration practices.

Mudpuppy populations should continue to be monitored because of suspected population declines, because they are an ecologically important species, and because they are essential indicators of quality habitat and restoration success. Our study shows that to accomplish this the use of eDNA and occupancy modeling should be utilized to implement good management and conservation practices with mudpuppies and other cryptic taxa. With worldwide evidence of declining amphibian populations and evidence that they are crucial indicators of quality habitat, it is necessary to accurately monitor long-term amphibian population trends (Hyde and Simons 2001). Effective monitoring and conservation of amphibians and other cryptic species has been hindered by the low detection probability of sampling methods; with the use of eDNA and

occupancy modeling, detecting, monitoring, and managing for these species may not be so insurmountable.

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