HERPETOLOGICAL RESOURCE & MANAGEMENT

EASTERN MICHIGAN UNIVERSITY

Mudpuppy (*Necturus maculosus*) Assessment Along the St. Clair-Detroit River System

Prepared by Herpetological Resource & Management, LLC

Acknowledgements

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Executive Summary

In 2014, a Great Lakes Fish and Wildlife Restoration Act (GLFWRA) grant was awarded to Herpetological Resource and Management, LLC (HRM) and Eastern Michigan University (EMU) to conduct an assessment of Northern Mudpuppy (*Necturus maculosus maculosus)* populations along the St. Clair-Detroit River System (SCDRS) and portions of the Huron River. Administered through the U.S. Fish and Wildlife Service (USFWS), funds from GLFWRA are directly applied to the conservation, management, and restoration of Great Lakes ecosystems and their associated fish and wildlife inhabitants. Mudpuppies serve important roles in their ecosystems as indicators of environmental health and the sole host organism to the State Endangered salamander mussel (Simpsonais ambigua). Populations of Mudpuppies in Michigan are known to be declining and the species was recently elevated to Special Concern, affording it protection under the Michigan Department of Natural Resources (MDNR). This multi-year study evaluated Mudpuppy distribution, health, and genetic structure along the SCDRS and Huron River including effects of habitat fragmentation and success of habitat restoration.

The major objectives of this project included:

1. Determine the spatial distribution, abundance, health, and population genetic structure along the assessment area.
2. Assess whether physical barriers or other factors impede Mudpuppy dispersal.
3. Evaluate the efficacy of environmental DNA (eDNA) as a non-invasive method to detect Mudpuppies.
4. Evaluate success of ongoing restoration and implement new restoration projects to boost Mudpuppy populations.
5. Increase public awareness about the importance of Mudpuppies as indicators of environmental health and encourage submission of observations to the Michigan Herp Atlas.

Between 2014 and 2017, HRM conducted Mudpuppy assessments at 39 locations spanning the SCDRS and Huron River. Nearly 24,000 trap hours were recorded with a total capture of 44 Mudpuppies including 9 males, 22 females, 12 juveniles, and 1 recapture. The presence of Mudpuppies through shoreline trapping by HRM was recorded at 11 sites between the St. Clair River, Lake St. Clair, Detroit River, and Huron River headwaters. The largest numbers of individuals were captured from the St. Clair River and Detroit River, respectively while no Mudpuppies were collected from the south west basin of Lake Erie or the main branch of Huron River sampled. Additional distributional data gathered was submitted through bycatch data from project partners MDNR Fisheries, USFWS, and United States Geological Survey (USGS).

Significant efforts from this work were focused on both conducting restoration monitoring and implementing new habitat restoration within the SCDRS. Long-term monitoring included sites located on the St. Clair River and Detroit

River. New Mudpuppy habitat was constructed at the Lake St. Clair Metropark and Lake Erie Metropark. A majority of individuals captured by HRM during the study were collected from previously restored locations where habitat structures had been placed. Creation of this critical habitat and positive results from the monitoring efforts were among contributing factors that led to the removal of the loss of fish and wildlife habitat Beneficial Use Impairment (BUI) on the St. Clair River AOC, moving it one step closer to delisting. No Mudpuppies were captured during sampling at newly restored locations within the Metroparks that were created as part of this project. Additional monitoring may likely result in detection and use by this species of these created habitats as seen elsewhere along the corridor.

Over 700 tissue samples were used to conduct numerous genetic analyses, which have resulted in a better understanding of diversity of Mudpuppy populations within the project area, within the Great Lakes region, and across the species range. Phylogeographic analyses showed a deep evolutionary split on a range wide scale between eastern and western populations assessed as part of this study. Genetic analyses on the local level are still ongoing due to technical difficulties early on, but initial results indicate a relatively high level of connectivity among sites within the SCDRS. Utilizing novel sampling techniques revealed a strong positive correlation between trapping data and eDNA analysis; every site with positive trapping records also returned positive eDNA screens.

Public engagement efforts were effective at spreading awareness for the Mudpuppy as a bioindicator of environmental health. Encouraging community members to submit observations to the Michigan Herp Atlas resulted in valuable data that has provided further insight into the species status within the Great Lakes. The increase in reported Mudpuppy sightings has been paired with a decrease in reports of persecution of the species, indicating overall outreach activities were successful. An educational video was produced to help inform and educate the public about the importance of Mudpuppies.

Major take-away points from this work:

- Additional targeted assessments are warranted for the Huron River and Lake Erie sampling areas to determine potential factors inhibiting Mudpuppy presence as well as for the newly implemented restoration sites to better evaluate target species response.
- A range of survey techniques within varying habitat types are likely necessary to accurately depict the true status of Great Lakes Mudpuppy populations and age structure. eDNA appears to be a rapid and reliable indicator of Mudpuppy presence.
- Successful restoration sites created through this project can serve as examples to which future restoration activities can be matched.
- Further genetic analyses are warranted to better determine diversity among SCDRS populations, but preliminary results do not indicate any severe disruption to population connectivity.

This novel study was successful in evaluating a critical Great Lakes bioindicator species utilizing a multi-faceted, collaborative approach. As a result, previously unknown data is now available for Mudpuppies along significant stretches of the Michigan shoreline of the SCDRS. A better understanding of species status, spatial distribution, population health, and genetic structure is essential for best management this declining salamander as well as the dependent salamander mussel. Understanding Mudpuppy populations in the Great Lakes will provide insight into areas within this watershed that likely support diverse aquatic organisms and healthy aquatic habitats. Monitoring of Mudpuppy health in correlation to surrounding land uses may be a useful tool for identifying major threats in the region. Furthermore, continued decline in Mudpuppy populations will reduce the potential for recovery of salamander mussels as the Mudpuppy is functionally critical habitat for this unique mussel species. The support obtained through this project is a testament to the value and power of scientific collaboration and the need for a greater understanding and conservation of Mudpuppies in the Great Lakes.

1. Introduction

The Laurentian Great Lakes region supports some of the world's most expansive waterways including a range of complex and sensitive aquatic ecosystems. Both economically and ecologically significant, these areas provide commercial navigation, recreational opportunities, and drinking water for millions as well as critical wildlife habitat to a diversity of organisms, many of which are considered rare and imperiled. Measuring the health of these critical ecosystems can be challenging due to their large scale and complex nature. One method for determining the condition of a natural community is the evaluation of key bioindicators, organisms that act as gauges of environmental health due to their high sensitivity to environmental pollutants and habitat disturbances (Welsh Jr. and Ollivier 1998; Barrett and Guyer 2008). Herpetofauna (amphibians and reptiles) are often used as indicator species based on their natural history, habitat requirements, and response to habitat disturbances. The Great Lakes support a number of herpetofauna bioindicator species including the Northern Mudpuppy (*Necturus maculosus maculosus*), Michigan's largest salamander and the only extant fully aquatic species. Though this species has lungs, it relies mainly on external gills for respiration. The Mudpuppy is a long-lived, slow growing species, which does not typically reach sexual maturity until 7-10 years and can live over 30 years (Lannoo 2005). Their entirely aquatic nature and long life spans make them an ideal study organism for measuring water quality and overall ecosystem health. Populations of this sensitive amphibian can serve as early warning systems for environmental problems that affect the health of fish and other wildlife (Bonin, DesGranges et al. 1995; Bishop and Gendron 1998) and have been used as an indicator for decades in some parts of their range (Environment Canada 2005).

NASA

The Great Lakes provide significant ecological and economical value to Michigan residents.

Mudpuppies can be identified by their bushy external gills as seen here.

Mudpuppies spend a majority of their time hidden beneath rocks and other surfaces.

Mudpuppies are known to consume the invasive round goby, making them an important species for biological pest control in the Great Lakes.

The Mudpuppy is cold water adapted and inhabits permanent water bodies including rivers, reservoirs, inland lakes, and Great Lakes bays and shallows (Harding and Mifsud 2017). Historically common and relatively abundant throughout the Great Lakes region, Mudpuppy populations have experienced steady and significant declines in recent years. In general, their seasonality, habitat use, and population structure have been poorly understood (McDaniel, Martin et al. 2009). Studies suggest they prefer water depth ranges of 21-35 cm and 86-95 cm depending on the season but have been found as deep as 30 m (Lembcke 2005). Their selection of water depth is believed to be seasonal, typically occupying shallower water from late fall through early spring when water temperatures are coolest. During this period of activity in shallow water a majority of the mating, nesting, and larval development occurs (Holman 2012; Harding and Mifsud 2017). As the near shore water becomes warmer in late spring, they move to deeper, cooler water. Mudpuppies are primarily nocturnal and spend most of their time hidden under large rocks, logs, and other objects (Petranka 1998; Holman 2012; Harding and Mifsud 2017). They have a varied diet consisting of fish, amphibians, insects, crustaceans, worms, and snails (Petranka 1998; Holman, 2012; Lagler, 1941). Recent studies have suggested that the species diet shifts as they reach different age classes (Beattie, Whiles et al. 2017). This species will also readily consume carrion which it locates using its well-developed sense of smell (Harding and Mifsud, 2017). Like other scavengers, the Mudpuppy provides a critical ecosystem service by helping maintain good water quality. Mudpuppies have been observed to actively feed on the invasive round goby (*Neogobius melanostomus*), zebra mussels (*Dreissena polymorpha*), and rusty crayfish (*Orconectes rusticus*) (Beattie, Whiles et al. 2017). Maintaining healthy, viable Mudpuppy populations may be an effective long-term tool in the biological control of pest species that affect Great Lakes ecosystems.

In addition to acting as bioindicators, Mudpuppies play a critical role as the obligate host organism for the larvae of the Salamander Mussel (*Simpsonais ambigua*), which relies entirely on the aquatic salamander to complete its life cycle. It has been suggested that Mudpuppy not merely be considered a host but rather be viewed as critical habitat for larval Salamander Mussels. *S. ambigua* are the only known mussel species to utilize a non-fish organism for development of young. Due to this extreme host specificity, its survival is closely tied to the persistence of Mudpuppies (Roe 2003). Studies conducted in Ontario, Canada have shown that where

Salamander Mussel populations are healthy, Mudpuppies are also abundant (McDaniel, Martin et al. 2009; Ho 2011). North America supports the highest diversity of freshwater mussels in the world, with the Midwest historically supporting the largest number of species. Currently in this region, more than half of the 78 known mussels are listed as Special Concern or greater (U.S. Fish and Wildlife Service 2017). Salamander mussels have disappeared from at least 60% of their historical sites and are listed as Endangered or Threatened in most parts of the range (McDaniel, Martin et al. 2009; Lambert, Hinz Jr. et al. 2016) (Table 1). The species has not been collected in over 30 years from Illinois and only a single population remains in Canada (Bogan, Woolnough et al. 2017). Within Michigan, the mussel was historically known from several sites in southeastern portions of the state; however since the 1930's only spent (deceased) shells have been found (Carman 2002). Its population is expected to continue declining, especially given the known decline of the Mudpuppy and its preferred habitat (Bogan, Woolnough et al. 2017).

David Dortman

Mudpuppies washed ashore Lake St. Clair following a die off event.

Michigan Sea Grant

Tricia Brockman

Factors leading to Mudpuppy declines include persecution by anglers when salamanders are left on the ice to die (A) and collection from the wild by biological supply companies for use in dissection (B).

Historically relatively abundant throughout the Great Lakes region, Mudpuppies have experienced steady declines in recent years including mass die-off events in portions of the Detroit River, Lake St. Clair, and Lake Erie (King, Oldham et al. 1997; Faisal 2006; Francis 2006; Millsap 2006). More recent die-off events are believed to have been caused by the effects of Hurricane Sandy in 2012 (Kalish 2012). Management practices including the application of lampricide chemicals are known to cause large scale mortality among local Mudpuppy populations (Matson 1998; Christie 2000; Boogaard, Bills et al. 2003; Minnesota Department of Natural Resources 2006). Other factors that contribute to the decline of this species include habitat degradation and loss, and the alteration of aquatic communities by invasive species and toxic algal blooms from excessive nutrient loading (Holman, 2012; Lanoo, 2005). Additionally, Mudpuppy populations experience negative pressure due to persecution and collection (Harding and Mifsud, 2017; Holman, 2012). Anglers will often kill Mudpuppies caught on baited hooks because of the erroneous beliefs that they are poisonous or excessively consume game fish and fish eggs (Ruthven, Thompson et al. 1912; Bishop 1941). In parts of the Great Lakes, this species is collected from the wild by the hundreds (and historically by the millions) for biological supply houses which distribute the specimens to academic institutions for dissections (Casper 1998; Minnesota Department of Natural Resources 2006; Holman 2012).

The Mudpuppy is listed as Threatened in Illinois, Special Concern in Wisconsin and Minnesota, and Species of Greatest Conservation Need in Vermont (Mankowski 2010; Minnesota Department of Natural Resources 2013; Wisconsin Department of Natural Resources 2014; Vermont Wildlife Action Plan Team 2015). In Michigan the species was elevated to Special Concern in 2016, affording it protection under the MDNR Fisheries Directors Order (Derosier, Hanshue et al. 2015; Michigan Department of Natural Resources 2016). Recent evidence of Mudpuppy declines in the region has facilitated an upcoming assessment of the species by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2015). Current data on populations in Michigan is very limited and their overall health and abundance is unknown. A significant proportion of Mudpuppy locality data within Michigan is represented by previous mortality data from lampricide application analyzed and compiled as part of a technical review for Michigan herpetofauna (Herpetological Resource and Management 2014). Information surrounding their genetic structure and diversity is also largely unknown (Chellman 2011). The recently updated Michigan Wildlife Action Plan identifies the Mudpuppy as a focal species of the St. Clair-Detroit River System. The goal of the Wildlife Action Plan is to provide a strategic framework to coordinate conservation of Michigan's wildlife and their habitats. Focal species were selected for key habitats and issues in order to help prioritize limited resources available for conservation efforts. The updated document calls for assessments of Mudpuppy distribution and abundance within the SCDRS to develop a better understanding of its life history and habitat needs and general health of the system (Derosier, Hanshue et al. 2015).

Common Name	Scientific Name	Great Lakes Mudpuppy and Salamander Mussel Status				
		Range				
		Michigan	Wisconsin	Minnesota	Illinois	Canada
Mudpuppy	Necturus maculosus	SC	SC	SC	T	NL
Salamander Mussel	Simpsonaias ambigua	E	T	E	E	E

The Conservation status of Mudpuppy and salamander mussel within Great Lakes range states. (SC = Special Concern, T = Threatened, E = Endangered, NL = Not Listed).

In 2014, Herpetological Resource and Management, LLC (HRM) in partnership with Eastern Michigan University (EMU) initiated a project to evaluate Mudpuppies and assess their current status along the SCDRS. This multifaceted study was developed to help address the significant gaps in data on Mudpuppy populations from this region in order to utilize this species as an indicator for current ecosystem health. Funding for the work was provided by the U.S. Fish and Wildlife Service (USFWS) through the Great Lakes Fish and Wildlife

Restoration Act (GLFWRA). It included baseline field assessments habitat restoration, genetic analyses, and outreach and education. Funds from the GLFWRA are directly applied to the conservation, management, and restoration of Great Lakes ecosystems and their associated fish and wildlife inhabitants.

Objectives of the study were to:

1. Determine Mudpuppy spatial distribution, abundance, health, and population genetic structure along assessment area.
2. Assess whether physical barriers or other factors impede Mudpuppy dispersal.
3. Evaluate the efficacy of the use of environmental DNA (eDNA) as a noninvasive method to detect Mudpuppies.
4. Generate predictions of population viability for Mudpuppies along assessment areas.
5. Evaluate success of ongoing restoration and implement new restoration projects to boost Mudpuppy populations.
6. Increase public awareness about the importance of Mudpuppies as indicators and encourage submission of observations to the Michigan Herp Atlas.

In addition to the partnership between HRM and EMU that was formed to develop and initiate this project, extensive collaboration was achieved through working with several other organizations, agencies, and individuals. These contributors included the Huron-Clinton Metropolitan Authority, Michigan Department of Natural Resources, Michigan Sea Grant, Michigan State University, United States Fish and Wildlife Service, United States Geological Survey, University of Michigan, and the former curator of the Belle Isle Aquarium.

Agencies and organizations included in the partnerships developed as part of this project.

2. Project Area and Sample Sites

The SCDRS has been signficanly modified from its natural conditions by industry including oil refineries like this one located in Sarnia, Ontario, Canada.

Project Area

The project was located along the St. Clair-Detroit River System (SCDRS), previously known as the Huron to Erie Corridor (HEC), extending from the mouth of Lake Huron as it feeds into the St. Clair River, through Lake St. Clair, the Detroit River, and south to the Ohio border along Lake Erie (Maps 1-2). The corridor consists of multiple linked waterways that create a significant connection between the Great Lakes regions. The SCDRS historically contained abundant habitat suitable for Mudpuppies including fractured limestone shelves and other rocky structures, and large logs and woody debris; however, the area has undergone dramatic changes due to development, channel modification, shoreline hardening, contamination, and establishment of invasive species (Manny 2003). The SCDRS includes several Environmental Protection Agency (EPA) designated Areas of Concern (AOC), some of which lie directly within the project area. In recent years, extensive remediation work including habitat restoration has been conducted in order to delist these AOCs. Several projects located within the St. Clair and Detroit Rivers included the installation of Mudpuppy habitat structures.

Mudpuppy habitat work was also conducted in the Huron River, which extends 126 miles inland from its mouth at Lake Erie. The Huron River is segregated by 100 dams throughout the system, which fragments habitat and blocks the natural movement of fish and wildlife (Vaughn and Taylor 1999). For this project, work was focused on a portion of the river known as the middle Huron located within northern and central Washtenaw County. The middle Huron watershed contains a mix of land uses ranging from agricultural practices to heavily urbanized population centers.

Northernmost sample location at the mouth of the St. Clair River with exposed near shore habitat.

St. Clair River shoreline adjacent to the Algonac State Park sample location.

Sample Sites

Sampling locations within the project area are described below by region and waterbody. Sampling was conducted where appropriate habitat was present and included previously restored sites as well as those selected for restoration as part of this project. A list of the following sites, shoreline condition, and approximate number of traps used at each is listed in the tables below.

St. Clair River

The northernmost three sample sites of the project were located in the city of Port Huron, St. Clair County. The first site was located near the Blue Water Bridge at the mouth of Lake Huron as it feeds into the St. Clair River. The shoreline in this area was modified with very large concrete blocks that extend into the water to provide shoreline erosion protection. The fast flowing water was consistently clear during each sampling event. Continuing south along the St. Clair River, the next two locations were part of larger scale, separate, multi-phase restoration projects, taking place along the river. Kiefer Park included a small two acre public park with a steep bank that previously supported little valuable wildlife habitat. Restoration included several large, partly submerged log and rock structures placed within near shore areas, as well as a buffer of native vegetation along the shoreline. The Blue Water River Walk restoration site was located directly south of the Great Lakes Maritime Center. Restoration of this heavily disturbed site began in 2013 and included the removal of invasive vegetation, planting with native species, the addition of riprap shoreline, breakwalls, and the creation of several habitat structures, including designs targeting Mudpuppies. The next sampling site was located in Marysville, along Chrysler Beach, which was approximately five miles south of the Blue Water River Walk. This point included a boat launch that recently underwent renovation to widen the launch and replace the seawall with a more natural shoreline. The shoreline was variable along the entire area and included riprap, sand, and seawall. Trapping was conducted adjacent to the boat launch before and after construction, and just downriver from the launch. Heading further south, another recent habitat restoration project was located in Cottrellville Township. In 2015, cobble was placed in the shoreline and larger limestone boulders were installed along the shore and used to help hold down sections of submerged tree trunks with intact root balls. Additionally, this site was supplemented with upland native tree and shrub plantings. Just south of Cottrellville, the southernmost sampling site on the St. Clair River was located directly east of Algonac State Park. Here a portion of the shoreline was modified with gabion baskets (wire mesh structures filled with rock and soil) and remaining habitat included mowed lawn transitioning to sand cobble beach with sparse woody vegetation along the entire site.

Lake St. Clair shoreline near the MDNR Fisheries Research Station.

Lake St. Clair

Sampling was conducted in the northern portion of Lake St. Clair, including Anchor Bay. Continuing south from the mouth of the St. Clair River, the first site on the lake included a MDNR boat launch site located in Fair Haven. A riprap breakwater on the west side of the site created a small protected bay with the boat ramp located to the east. Also sampled was the Lake St. Clair Fisheries Research Station in Macomb County. Heavily used by the public for fishing and boat access, this site also included a research station of Michigan DNR's Fisheries Division. To the north, the mouth of the Clinton River fed directly into Lake St. Clair. With the exception of the boat launch area, a majority of this site included riprap shoreline with sparse woody vegetation. Directly south of the fisheries station site and separated by Campau Bay is Lake St. Clair Metropark, the southernmost sample site on Lake St. Clair. The northern portion of the park is adjacent to Black Creek, which flows southeast between the park and residential properties and through two marinas before reaching Lake St. Clair. A majority of the lake shoreline included riprap with portions modified by seawall and watercraft access points. Black Creek included primarily emergent and submergent marsh habitat with portions of riprap dominated shoreline where a boat launch and areas for shoreline fishing was located. Sampling was also conducted within the marina that connected to Black Creek, near the northern portion of the park.

Detroit River

The northernmost sampling location in the Detroit River was Belle Isle in Wayne County, located near the connection between Lake St. Clair and the Detroit River. The park contained a range of natural communities including interconnected lakes and canals. Sample points on Belle Isle included the Blue Heron Lagoon, North Fishing Pier, South Fishing Pier, Yacht Club, and the Loop Canal situated by the entrance to the park. These points were located directly on the Detroit River shoreline as well as interior lake habitat and included previous restoration sites. The next sample location downstream of Belle Isle was the William G. Milliken State Park and Harbor. The park included a harbor, public recreation areas, wetlands, and paved trails. The park shoreline along the Detroit River was dominated by seawall but the harbor where traps were placed was primarily riprap. Continuing south down the Detroit River, the next sample location was in Trenton at the Meyer Elias Memorial Park.

Sample location near the North Fishing Pier on Belle Isle in the Detroit River.

This area supported large segments of mowed lawn, which continued right up to the river. The substrate was dominated by small cobble and rocky material. A small sample site was located just south of Meyer Elias Park and was accessed directly off Truax Street. This area included a shoreline with woody vegetation and concrete riprap. Elizabeth Park represented the southernmost sample location on the Detroit River. Within the city of Trenton, this county-owned park was situated on the western shore of the river. The island park was separated from the mainland by a narrow canal. Sample points at this site were located where the canal meets the Detroit River and from within the canal itself along the rocky riprap shoreline.

Milliken State Park marina in the Detroit River including riprap that extends into the nearshore habitat.

Lake Erie shoreline located at the Lake Erie Metropark.

Lake Erie

From north to south, sampling in this region began at Lake Erie Metropark located adjacent to Lake Erie, with the mouth of the Detroit River to the north, and the mouth of the Huron River on the southern border. The park was located in Wayne County and included Great Lakes coastal marsh with limestone and concrete riprap dominating portions of the shoreline. Although Mudpuppy habitat was present in numerous areas of the park, suitable trapping locations were limited to portions adjacent to the boat, canoe, and kayak launch on the north end of the property and near the boat entrance to the marina on the south end. Directly south, the next sampling area was Pointe Mouillee, which was located at the northernmost corner of Monroe County. The site includes a state game area containing over 4,000 acres of coastal wetland near the mouth of the Huron River. One of the largest fresh water marsh restoration projects in the world contributed to this property's complex of wetlands, diked marshes, and river bayous. Sample points here were focused along the riprap dikes of the marsh complex as well as near the DNR headquarters just north of the marsh and separated by the Huron River. Sterling State Park, located in Monroe County, was the next sample site on Lake Erie. This property included the southern portion of the Detroit River International Wildlife Refuge and covered roughly two square miles including Great Lakes coastal marsh habitats. A smaller connected water body was situated within the park, protected from the major body of Lake Erie by the park's walking trails and camping areas. This inland water body was where the majority of sample points were located. The next sample location, south of the Sterling State Park, was the Toledo Beach Marina located in La Salle. This full-service marina was situated in a naturally

protected harbor off of Lake Erie and included steep shorelines with large concrete riprap. The next sampling site was located directly on Lake Erie near the city of Luna Pier. The public land was accessed from Erie Road near the JR Whiting Plant, a recently decommissioned coal power plant owned by Consumers Energy. This site supported natural, sandy beach shorelines with woody debris throughout the near shore aquatic habitats. The final sample location, and the furthest south in the project area, was located at a boat ramp off Algonquin Street in the city of Erie. The site was in a small bay protected from the rest of Lake Erie and dominated by emergent and submergent marsh habitat.

Huron River

Within this region of the project area, sampling began in the headwaters of the Huron River watershed near the Pinckney State Recreation Area. Several headwater lakes were sampled in this region including Portage Lake, Silver Lake, Patterson Lake, Halfmoon Lake, Joslin Lake, South Lake, Sullivan Lake, and North Lake. A majority of these deep-water lakes contained a sandy substrate shoreline which supported a mixed edge of emergent marsh, scrub-shrub wetland vegetation, mowed residential lawn, and/or vertical seawalls. Following the headwater region, sampling continued throughout the Cities of Ann Arbor and Ypsilanti along the river. On this stretch of the river, sampling was concentrated in city parks and near dam locations. Ann Arbor parks sampled included Barton Nature Area, Bandemer Park, Argo Park, Nichols Arboretum, and Gallup Park. Ann Arbor dams sampled included Barton Dam and Argo Dam. Additionally within Ann Arbor, sampling was conducted at various locations of easily accessible and suitable habitat off of W. Huron River Drive. Ypsilanti parks sampled included Hydro Park, Ford Lake Park, North Bay Park, Riverside Park, and Peninsular Park. A dam was located at Hydro Park, and Peninsular Park contained a large run-of-river hydroelectric structure. Unlike traditional dams, run-of-river power plants have limited or no water storage; however, like traditional dams these structures act a potential barrier to wildlife dispersal within a river. Additionally, within Ypsilanti, one of the sampling points was located at the MDNR Belleville boat launch. Throughout Ann Arbor and Ypsilanti, the river varied from sand and gravel to predominantly cobble and broken concrete substrates. The flow rate varied between sites and was generally moderate to fast moving. Where natural habitat was present, a majority of the shoreline included scrub-shrub and other woody vegetation.

Sample location downstream of the Barton Dam in Ann Arbor.

Sample point at the Nichols Arboretum along the Huron River supporting natural conditions and suitable refugia in the near shore habitat.

SCDRS Mudpuppy Assessment Sample Locations

Water Body	*Site Name*	*Shoreline Types*	*Number of Traps*	*Additional Notes*
St. Clair River	Blue Water Bridge	Large concrete blocks	4-5	
	Kiefer Park	Natural and artificial rip rap	2-3	Restoration Site
	Blue Water River Walk	Natural and artificial rip rap	13	Restoration Site
	Marysville	Riprap, sand, and seawall	1-2	
	Cottrellville	Sandy cobble beach	5	Restoration site
	Algonac State Park	Sandy cobble beach	5	
Lake St. Clair	Fair Haven Boat Launch	Riprap breakwater	3-4	
	MDNR Fisheries Research Station	Riprap with sparse vegetation	3	
	Lake St. Clair Metropark	Riprap, seawall, and emergent/submergent marsh	3-5	Restoration Site

Blue Water River Walk

Fair Haven Boat Launch

SCDRS Mudpuppy Assessment Sample Locations				
Water Body	*Site Name*	*Shoreline Types*	*Number of Traps*	*Additional Notes*
Detroit River	Belle Isle	Riprap, cobble, and emergent marsh	18	Restoration Site
	Milliken State Park	Riprap	5	
	Meyer Ellias Park	Sandy cobble beach	3	
	Truax Street Access	Riprap and woody vegetation	2	
	Elizabeth Park	Riprap and sandy cobble	3	
Lake Erie	Lake Erie Metropark	Riprap and seawall	7	Restoration Site
	Pointe Mouillee	Riprap and submergent marsh	2-5	
	Sterling State Park	Emergent/submergent marsh and riprap	4	
	Toledo Beach Marina	Riprap	3-5	
	Erie Road Public Beach Access	Sand beach	3	
	Algonquin Street Boat Ramp	Emergent and submergent marsh	3	

Elizabeth Park

Sterling State Park

SCDRS Mudpuppy Assessment Sample Locations				
Water Body	*Site Name*	*Shoreline Types*	*Number of Traps*	*Additional Notes*
Huron River	Barton Nature Area	Varied from sand, gravel, cobble, riprap and scrub-shrub marsh.	5	Barton Dam
	Bandemer Park		2	
	Argo Park		3	Argo Dam
	Nichols Arboretum		2	
	Gallup Park		2	
	Belleville Boat Launch		2	
	Hydro Park		2	Hydro Dam
	Ford Lake Park		4	
	North Bay Park		4	
	Riverside Park		3	
	Peninsular Park		5	Peninsular Dam

Hydro Park

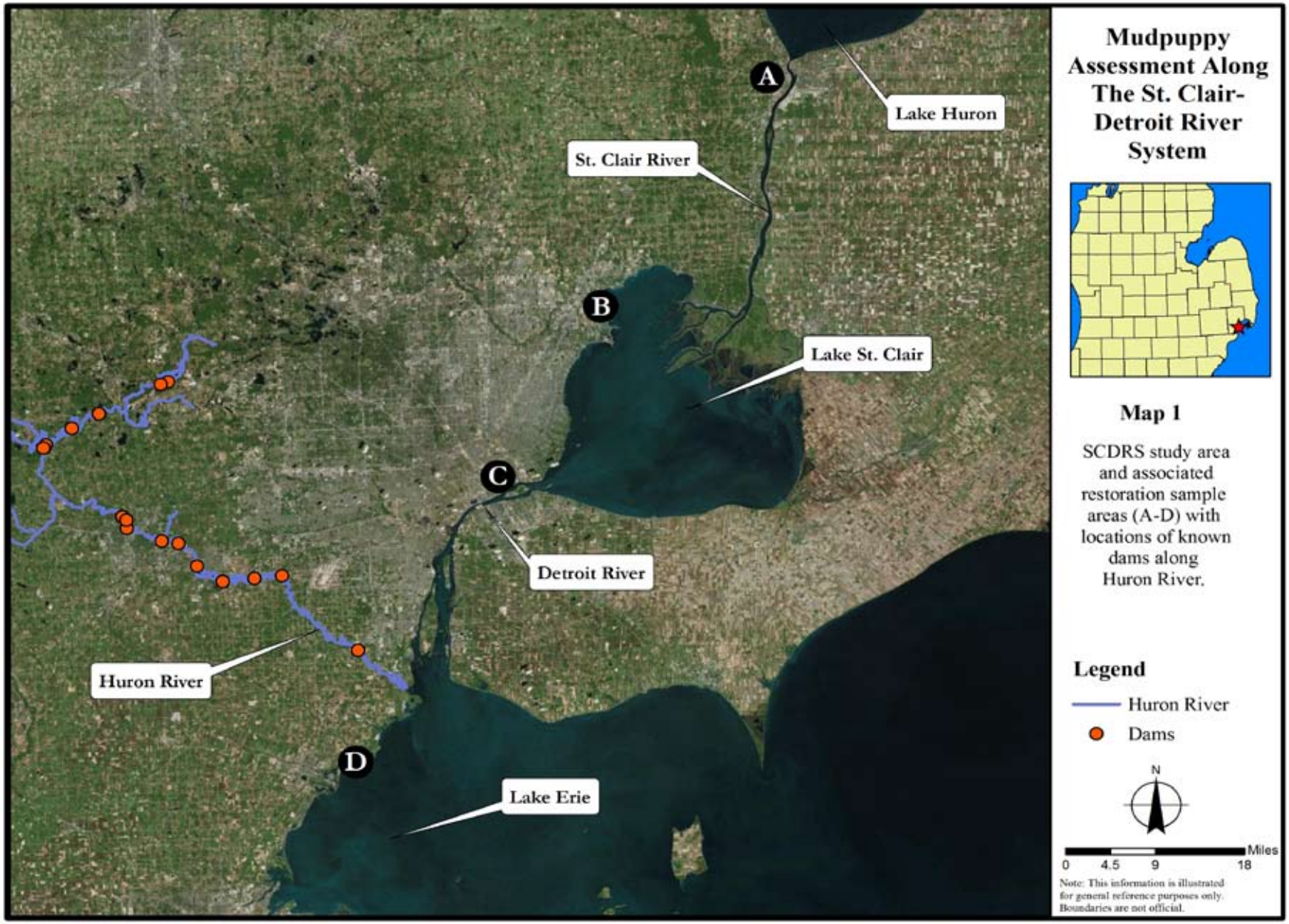
Peninsular Park Hyrdoelectric Structure

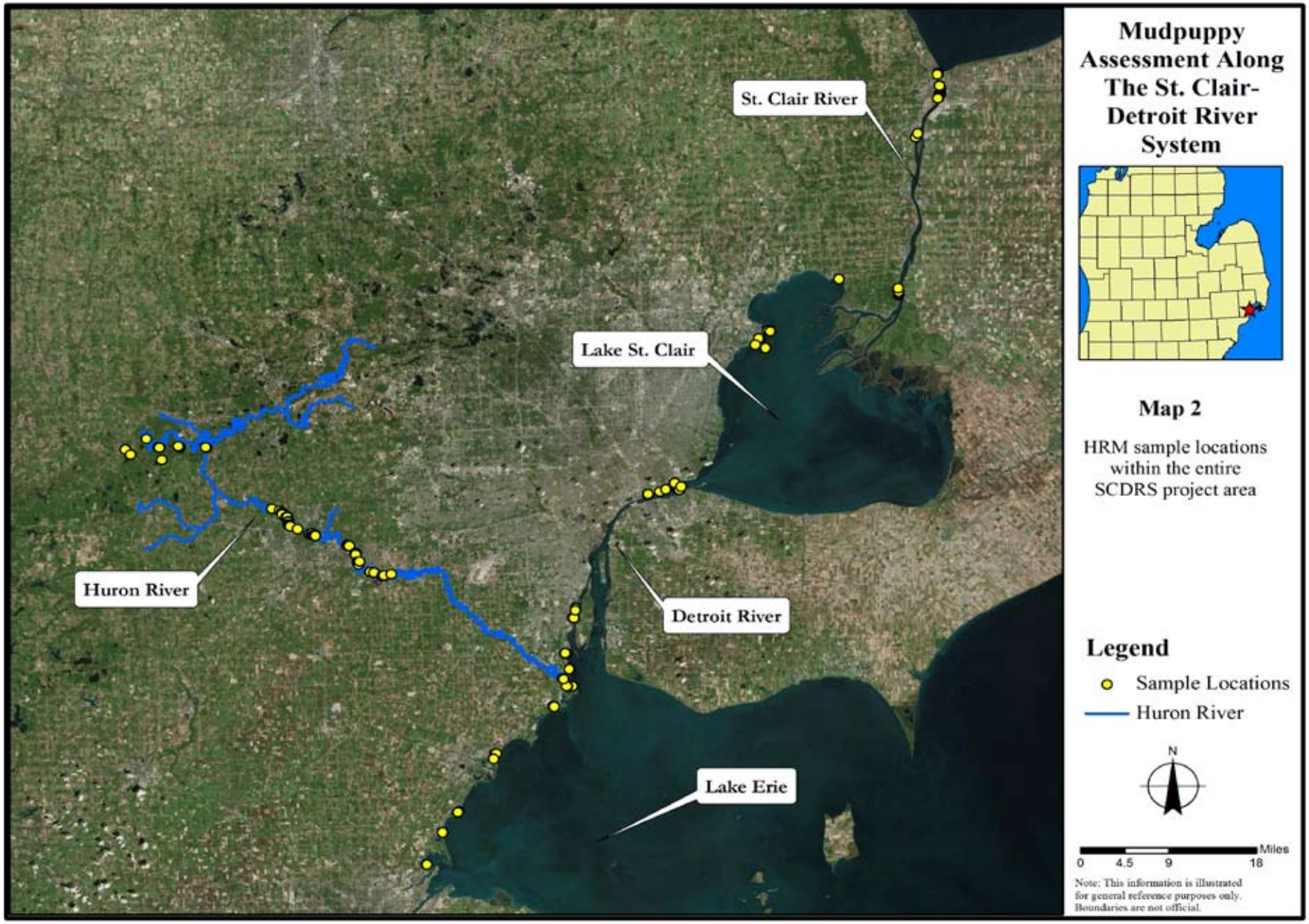
SCDRS Mudpuppy Assessment Sample Locations				
Water Body	*Site Name*	*Shoreline Types*	*Number of Traps*	*Additional Notes*
Huron River Headwaters	Portage Lake	Varied between maintained lawns, emergent, and scrub-shrub marsh.	3	
	Silver Lake		5	
	Patterson Lake		2	
	Halfmoon Lake		4	
	Joslin Lake		1	
	South Lake		2	
	Sullivan Lake		1	
	North Lake		2	

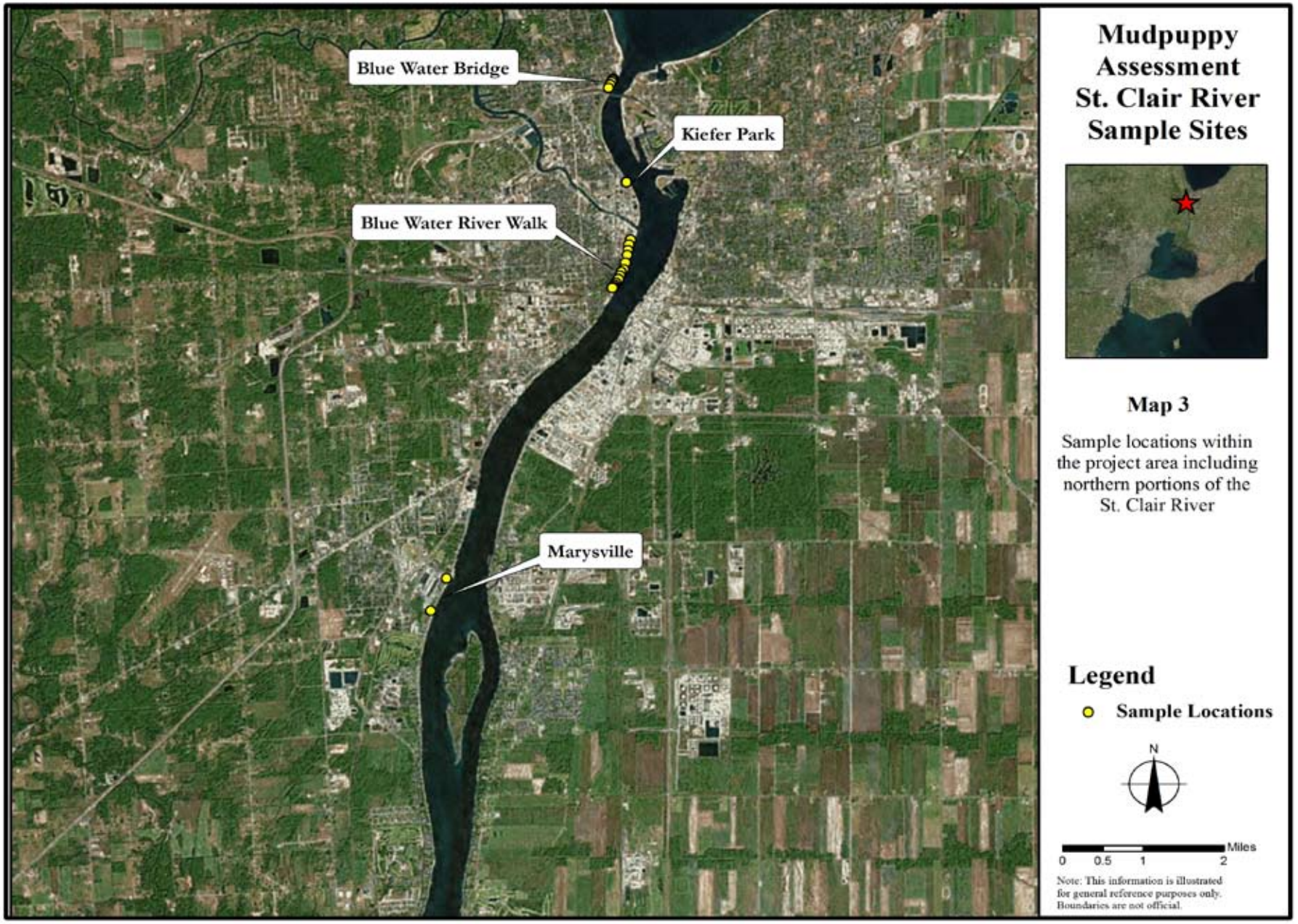
North Lake

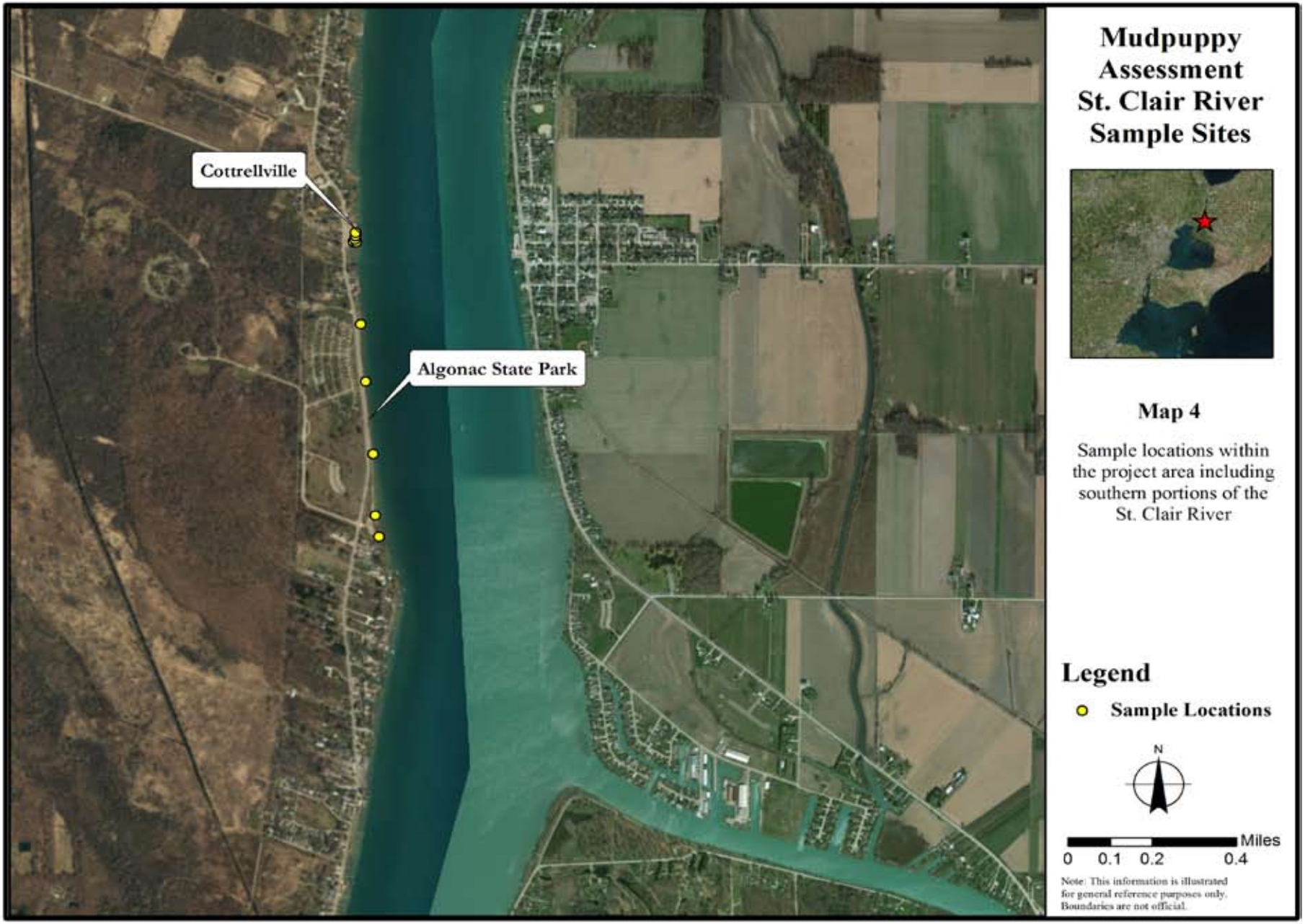
Patterson Lake

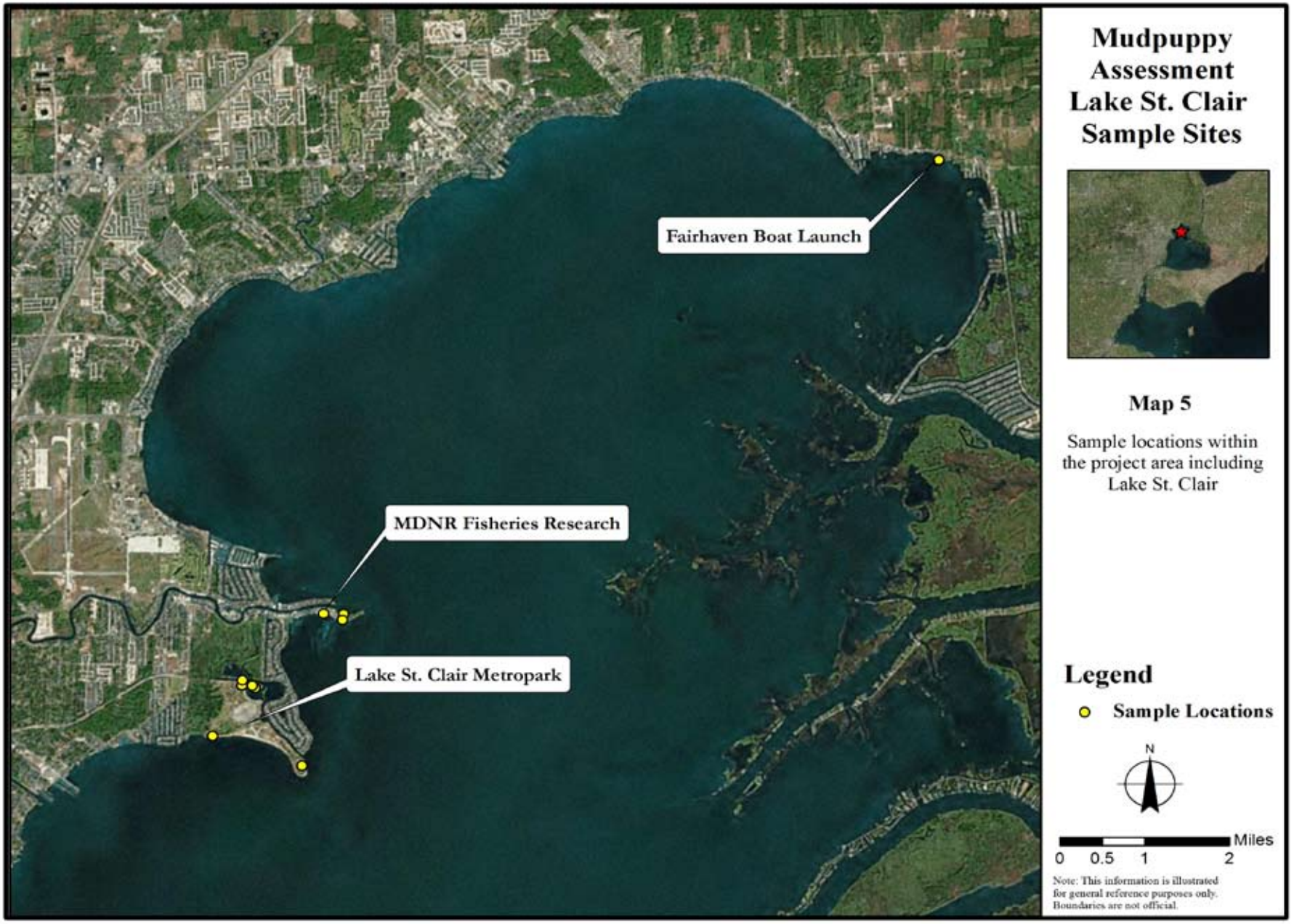
Appendix 2.1 Maps



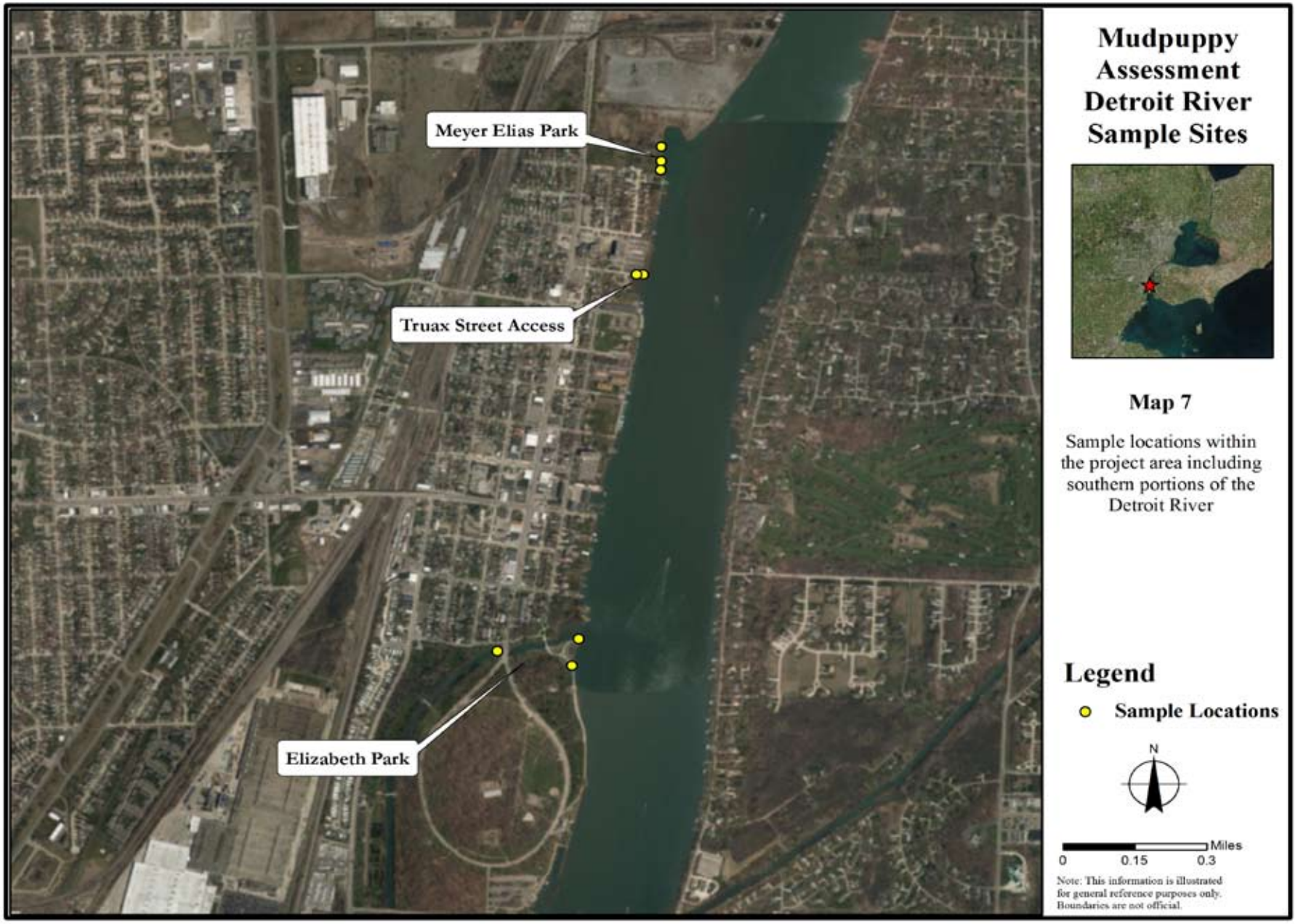

St. Clair River
Lake St. Clair
Huron River
Detroit River
Lake Erie
Mudpuppy Assessment Along The St. Clair-Detroit River System
Map 2
HRM sample locations within the entire SCDRS project area
Legend
Sample Locations
Huron River
N
0 4.5 9 18 Miles
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Blue Water Bridge
Kiefer Park
Blue Water River Walk
Marysville
Mudpuppy Assessment St. Clair River Sample Sites
Map 3
Sample locations within the project area including northern portions of the St. Clair River
Legend
Sample Locations
N
Miles
0 0.5 1 2
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

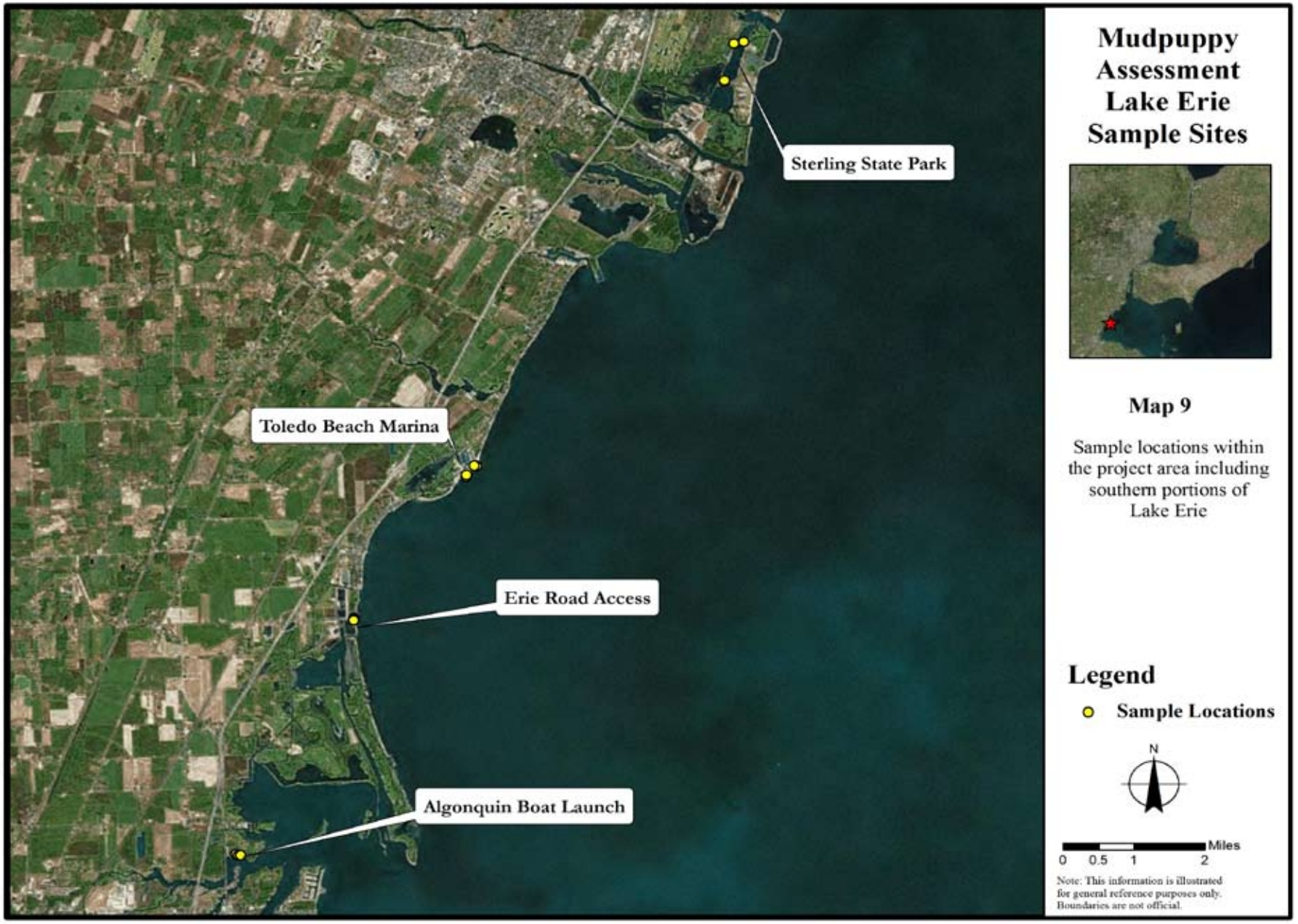

Cottrellville
Algonac State Park
Mudpuppy Assessment St. Clair River Sample Sites
Map 4
Sample locations within the project area including southern portions of the St. Clair River
Legend
Sample Locations
N
Miles
0 0.1 0.2 0.4
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

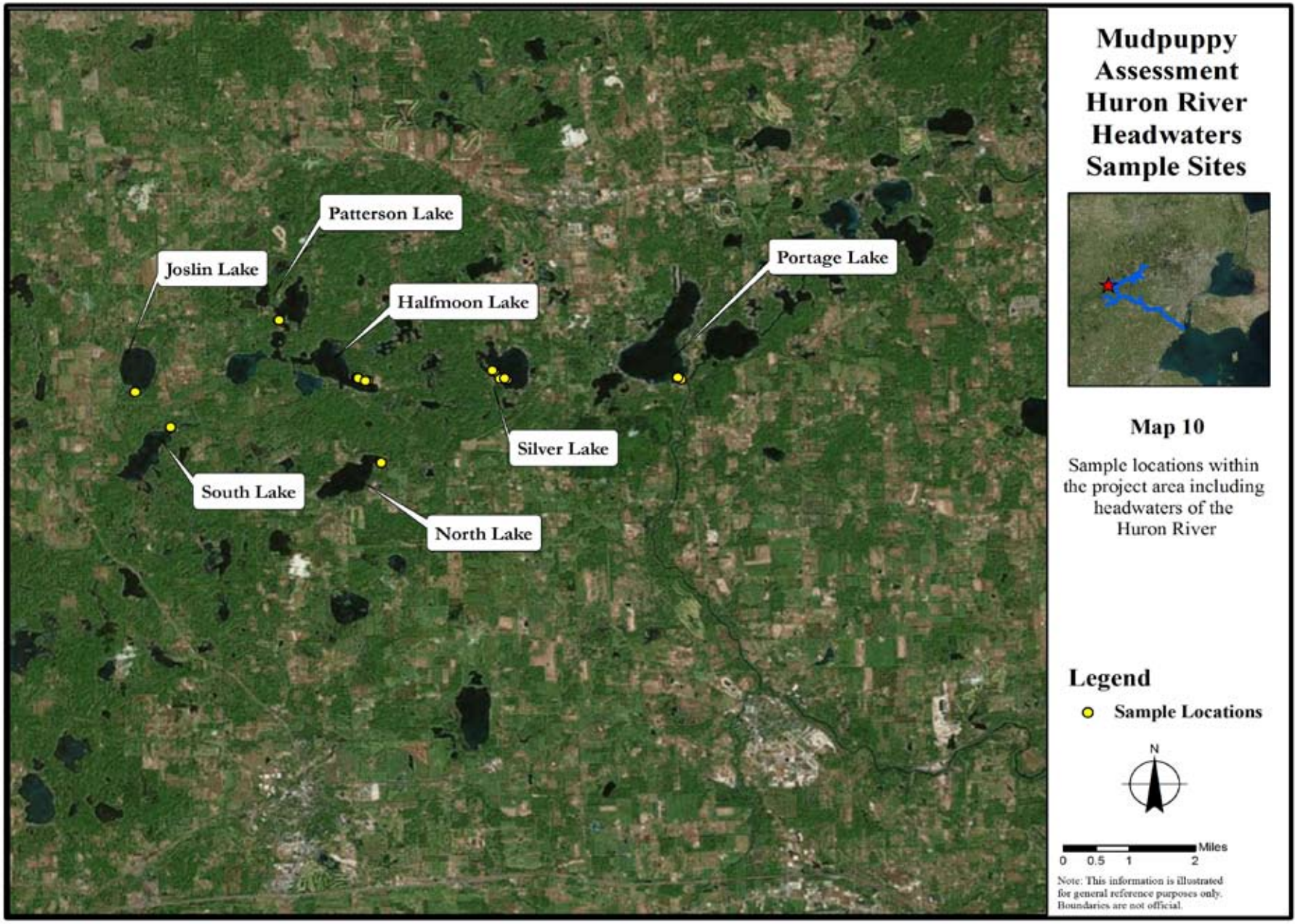

Fairhaven Boat Launch
MDNR Fisheries Research
Lake St. Clair Metropark
Mudpuppy Assessment Lake St. Clair Sample Sites
Map 5
Sample locations within the project area including Lake St. Clair
Legend
Sample Locations
N
Miles
0 0.5 1 2
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

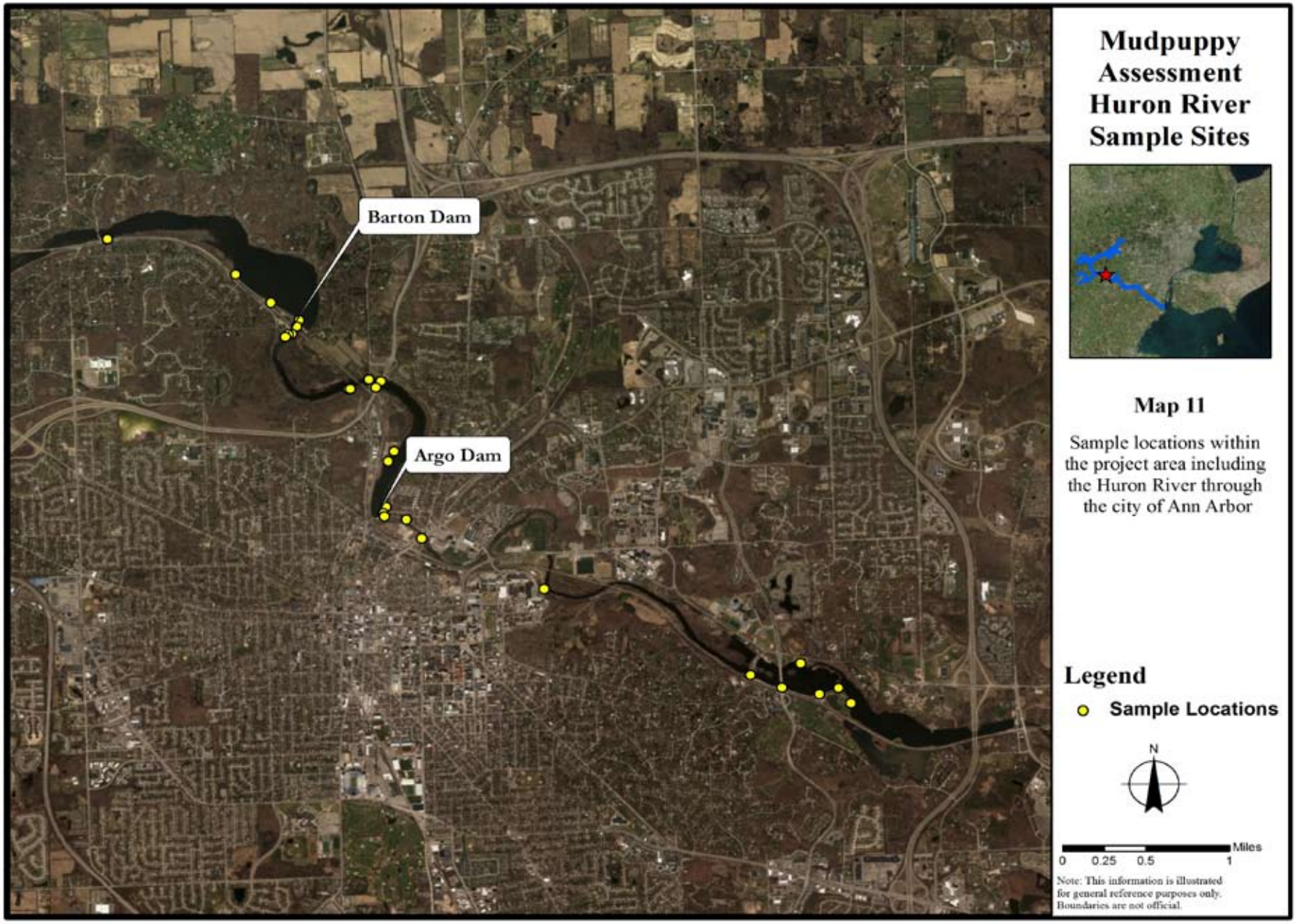

Yacht Club
Blue Heron Lagoon
North Fishing Pier
South Fishing Pier
Loop Canal
Milliken State Park
Mudpuppy Assessment Detroit River Sample Sites
Map 6
Sample locations within the project area including northern portions of the Detroit River and Belle Isle
Legend
Sample Locations
N
Miles
0
0.25
0.5
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

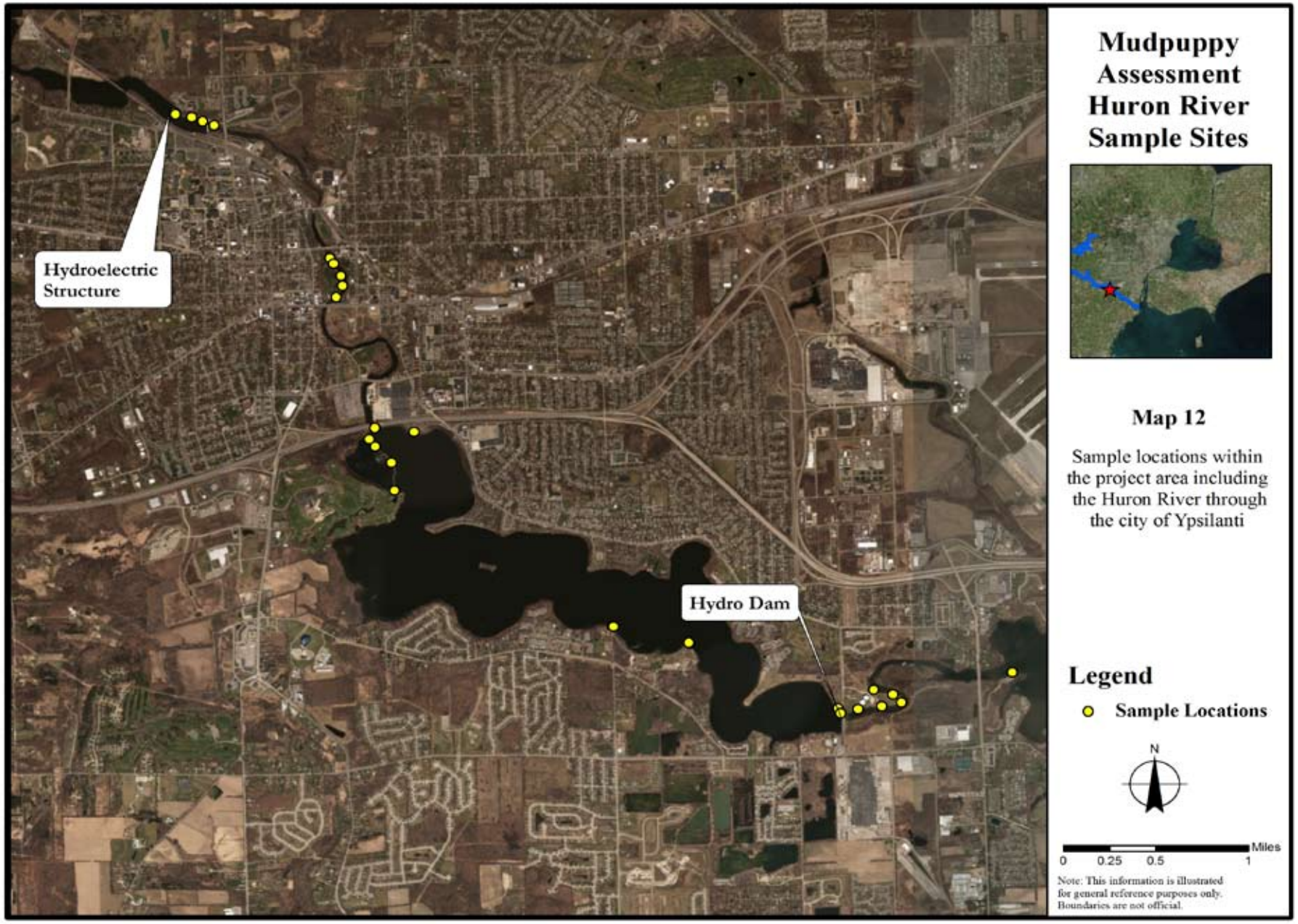

Meyer Elias Park
Truax Street Access
Elizabeth Park
Mudpuppy Assessment Detroit River Sample Sites
Map 7
Sample locations within the project area including southern portions of the Detroit River
Legend
Sample Locations
N
Miles
0
0.15
0.3
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Lake Erie Metropark
Point Mouillee
DNR Headquarters
Pointe Mouillee
Point Mouillee
Mudpuppy
Assessment
Lake Erie
Sample Sites
Map 8
Sample locations within the project area including northern portions of Lake Erie
Legend
Sample Locations
N
Miles
0 0.25 0.5 1
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Sterling State Park
Toledo Beach Marina
Erie Road Access
Algonquin Boat Launch
Mudpuppy Assessment Lake Erie Sample Sites
Map 9
Sample locations within the project area including southern portions of Lake Erie
Legend
Sample Locations
N
Miles
0 0.5 1 2
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Patterson Lake
Joslin Lake
Halfmoon Lake
Portage Lake
Silver Lake
South Lake
North Lake
Mudpuppy Assessment Huron River Headwaters Sample Sites
Map 10
Sample locations within the project area including headwaters of the Huron River
Legend
Sample Locations
N
Miles
0 0.5 1 2
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Barton Dam
Argo Dam
Mudpuppy Assessment Huron River Sample Sites
Map 11
Sample locations within the project area including the Huron River through the city of Ann Arbor
Legend
Sample Locations
N
Miles
0 0.25 0.5 1
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Hydroelectric Structure
Hydro Dam
Mudpuppy Assessment Huron River Sample Sites
Map 12
Sample locations within the project area including the Huron River through the city of Ypsilanti
Legend
Sample Locations
N
Miles
0 0.25 0.5 1
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

Appendix 2.2 Additional Site Photos

St. Clair River

Northernmost sampling location at the Blue Water Bridge.

Kiefer Park shoreline following restoration efforts.

Blue Water River Walk restoration site in Port Huron.

Marysville sampling site with the newly constructed dock.

St. Clair River

Marysville sample site, slightly downriver from the dock.

Cottrellville restoration site near Algonac State Park.

St. Clair River shoreline adjacent to Algonac State Park with gabion baskets.

St. Clair River shoreline adjancent to Algonac State Park.

Riprap dyke located at the Fairhaven boat launch.

Lake St. Clair shoreline located at the MDNR Fisheries Research station.

Shoreline along the Black Creek at the Lake St. Clair Metopark.

Seawall and watercraft access point on Lake St. Clair at the Lake St. Clair Metropark.

Detoit River

Sample point near the Yacht Club on Belle Isle.

Blue Heron Lagoon on Belle Isle where Mudpuppy habitat was restored in 2013.

Portion of the Loop Canal near the entrance to Belle Isle.

Meyer Elias Park sample site located in Trenton.

Lake Erie

Lake Erie Metropark sampling point near the northern boat launch.

Lake Erie Metropark sampling point near the marina on the southern end of the park.

Limestone riprap dikes at Pointe Mouillee State Game Area.

Pointe Mouillee MDNR headquarters north of the marsh, separated by the Huron River.

Lake Erie

Sterling State Park sampling point with rocky shoreline habitat.

Toledo Beach Marina sample location with large concrete riprap.

The Erie Road public beach access sample point including natural sandy shorelines.

The Algonquin boat launch and southernmost sample location in the project area.

Sample location on Portage Lake.

Silver Lake sample point including natural wetland vegetation.

Sample site location on Halfmoon Lake.

Sample site located on Sullivan Lake.

Huron River

Sample site located in Barton Nature Area in Ann Arbor.

Huron River shoreline at Bandamer Park in Ann Arbor.

Rocky shoreline habitat located along Gallup Park in Ann Arbor.

Sample point upstream of the Argo Dam in Ann Arbor.

Huron River

Sample point on the Huron River off W. Huron River Drive in Ann Arbor.

Overview of North Bay Park sample site on the Huron River in Ypsilanti.

Sample point location at Ford Lake Park on the Huron River in Ypsilanti.

Sample point at Riverside Park on the Huron River in Ypsilanti.

3. Field Research

Objectives

A significant portion of this project was aimed at collecting critical baseline data to better understand Mudpuppy population spatial distribution, relative abundance, and overall health in the Great Lakes region. As part of the field sampling, physical barriers including dam structures were assessed to determine if they affect Mudpuppy dispersal.

Mesh minnow traps prepped for conducting targeted Mudpuppy trapping.

HRM securing a baited minnow trap in near shore aquatic habitat.

Methods

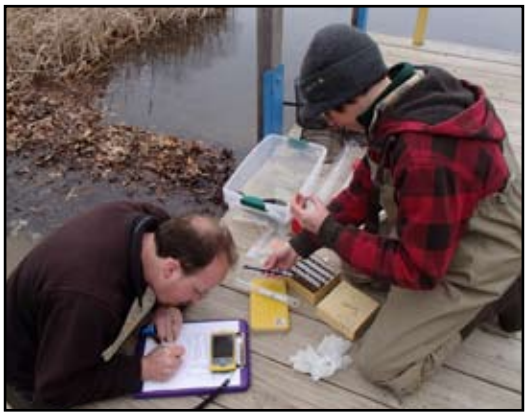
Preliminary sample locations were determined prior to field assessments based on desktop analysis of aerial photography, historical observations, and correspondence with project partners. During initial surveys, sites were reviewed more thoroughly for habitat suitability and trap locations were adjusted as needed. Trapping utilized two styles of minnow traps which included collapsible mesh and metal, each containing plastic cards describing their usage for ecological studies. Promar mesh traps measuring two feet by one foot (0.6 meters by 0.3 meters)or three feet by one foot (0.9 meters by 0.3 meters) were initially used. The minimum diameter of the opening at the ends was one inch (2.54 centimeter). Frabill vinyl-coated metal traps were approximately 1.5 feet in length (0.45 meters) with a width at the center of 8.75 inches (22.2 centimeters) in diameter and seven inches (17.8 centimeters) at the end. The opening is 1.0 inch (2.54 centimeter). These were placed in near shore aquatic habitats approximately two to three feet (0.5-1 meters) in depth. Traps were secured with military grade paracord rope and figure 8 knots with two inch carabiners on both ends of the rope. Ropes were tied on wood stakes ranging between four and six feet in length that were driven into the ground with a mallet. Traps were also tied off strongly rooted vegetation or other secure structures along the shoreline when interference by the public was not an issue. In areas where strong wave action was present, rocks were used to weigh down the traps. The bait included a combination of dead round gobies (*Neogobius melanostomus*), Colby

Jack cheese, and chicken gizzards, livers, and hearts that were placed in small thin mesh bags within the traps. In order to effectively capture Mudpuppies, which are known to feed nocturnally, traps were left in place overnight and checked daily. Other techniques utilized opportunistically to detect the species included the turning of submerged rocks and other cover materials, dip-netting, and investigating refugia habitat with a fiber optic scope.

Searching for Mudpupuppies by turning over submerged rocks in near shore areas.

Dipnetting was performed opportunisitically to capture Mudpuppies in addition to targeted trapping.

Physical data was recorded at each location on each sample event. HRM recorded weather conditions at the beginning and end of each survey day. Water temperature was taken during trap deployment and while subsequently checking traps for target species (Tables 1-4). For each Mudpuppy captured, biological data was recorded using pre-formatted data forms (Figure 1). Information recorded included snout-vent length, total length, weight, gender, abnormalities or injuries (if any), location including GPS coordinates, and habitat conditions. Snake restraining tubes were used to observe and measure the animals' length ranging in size to account for variability in Mudpuppies. Weights were taken using Pesola® spring scales. Juveniles and smaller individuals were weighed with a scale measuring up to 50 grams with increments of 1 gram and adults were weighed with a 1000 gram scale with increments of 10 grams. Tissue samples were collected for genetic analyses by taking a small clip (~3-5mm) of the tail tip with a razor blade or surgical knife. Tail clips were performed on a flat surface and all equipment was sterilized before and after use between each Mudpuppy to reduce harm from pathogens and maintain purity in genetic samples. Tissues were stored in individual vials with 95% ethanol. Each sample vial contained a unique number using University of Michigan Museum of Zoology tags for later identification and inclusion in the museum archives. As part of this work, a piloted effort at PIT tagging Mudpuppies was performed on a limited number of individuals. All survey activities were in accordance with HRM's Scientific Collector's and Threatened and Endangered Species permits issued by the State of Michigan Department of Natural Resources. Locality data was recorded using Juno SB GPS units, which record the location to U.S. Environmental Protection Agency (EPA) Tier II National Geospatial Data Spatial Standards, and was mapped using ArcMap®.



Collecting biological data on a Mudpuppy captured through near shore sampling.

Sampling was conducted by HRM during appropriate weather conditions (Tables 1-4.) Assessments were conducted by teams of two to five biologists trained in the sampling of aquatic salamanders. Surveys were conducted by HRM from 2014 to 2017 and took place during late fall and early spring with the majority of sampling occurring between March and May of each sampling year.

During the first phase of field assessments in 2014, sampling procedures were tested, evaluated and modified to establish a more

Due to heavy wave action causing damage to several traps (A), survey methodlogies were modified and incorporated sturdier, metal traps (B).

efficient and effective trap deployment system and animal processing protocols. This included moving from collapsible mesh style traps to more rigid metal minnow traps. In locations that were deemed unsafe, sampling points were relocated to a more suitable location or eliminated and noted. Baseline surveys were also utilized to finalize habitat restoration details after thoroughly assessing the site conditions and finalizing trap locations. Tissue sample collection protocols were modified to make the process more efficient as well as safe for the animals, e.g., cloth towels, slightly dampened with water from the sample site were used to restrain Mudpuppies while directly handling them to perform tail clips. This helped to better minimize risk of harm while handling or error while cutting the tissue.

In addition to HRM's shoreline sampling, the relative abundance, spatial distribution, and population health of Mudpuppies in the project area were further evaluated through data contributed by project partners. Partners included the following agencies: United States Fish and Wildlife Service (USFWS), United States Geological Survey (USGS), and Michigan Department of Natural Resources (MDNR) Fisheries Division. These institutions provided data on Mudpuppy bycatch which was encountered during fish sampling within the SCDRS using primarily setlines and minnow traps. Project partners also contributed institutional knowledge of the region, which assisted HRM in selecting sample locations and methods.

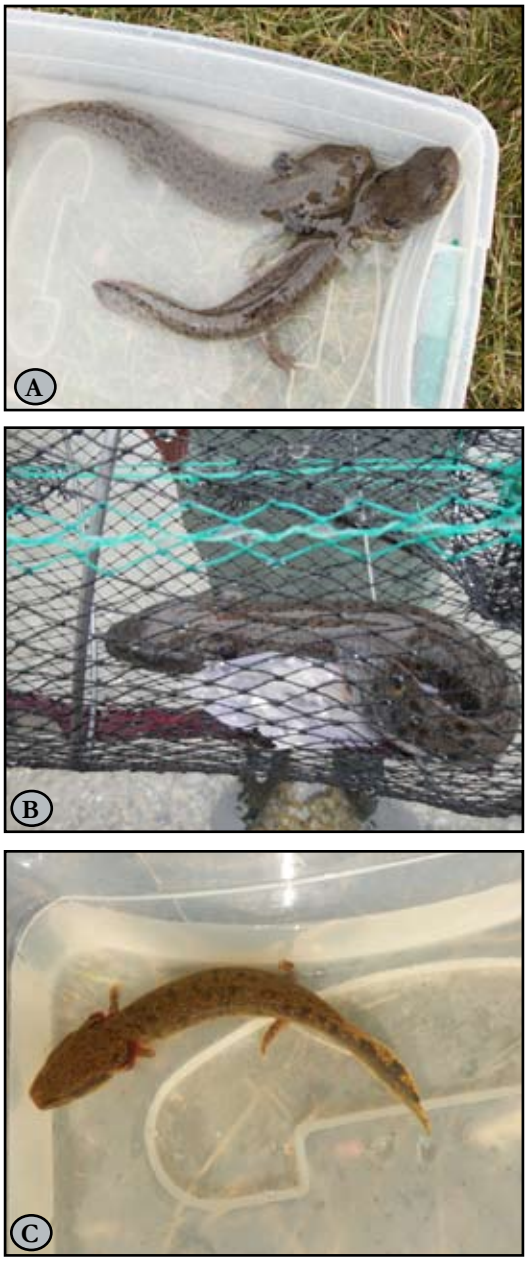
U.S. Fish and Wildlife Service

Mudpuppies captured, processed, and released by project partners during fish sampling activities.

Results

Sampling efforts conducted by HRM between 2014 and 2017 resulted in the capture of 44 Mudpuppies including nine males, 22 females, 12 juveniles, and one recapture. HRM documented species presence at ten separate sites within the project area (Table 5, Map 1) and several of these sites had multiple trap locations where Mudpuppies were found. Overall, HRM conducted surveys 65 days with nearly 24,000 trap hours recorded. Tables 6-9 provide a breakdown of the results by each sampling year.

In 2014, HRM deployed a total of 141 traps at 12 separate sample locations along the SCDRS. Traps were deployed for a maximum of 24 hours; trap hours for each point averaged approximately 20 hours with a total of nearly 3,000 hours recorded. During this effort, ten



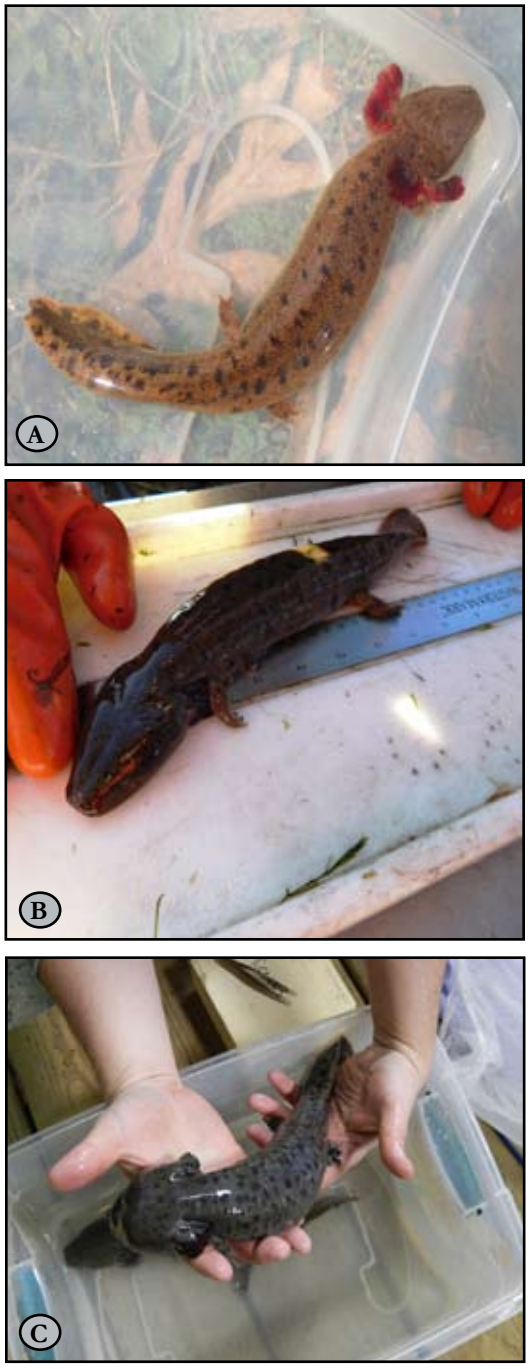
Adult Mudpuppies captured by HRM from the St. Clair River in 2014 (A) and 2015 (B). A juvenile captured from the Detroit River in 2016 (C).

Mudpuppies were captured including eight females and two males (Table 6, Map 2). Captures were recorded at three sites and included two individuals from Blue Water River Walk, two from Lake St. Clair MDNR Fisheries Research Station and six from Algonac State Park.

In 2015, HRM expanded the survey effort and deployed a total of 145 traps at 41 separate sample locations along the Huron River and SCDRS. Traps were deployed for a maximum of 72 hours, and trap hours for sample points in 2015 averaged approximately 57 hours with a total of nearly 8,000 trap hours recorded. During these efforts, 11 Mudpuppies were captured including four females, five males, and two juveniles (Table 7, Maps 3-5). Mudpuppies were observed at five sites including two individuals from Blue Water River Walk, seven from Cottrellville, one from the Belle Isle North Fishing Pier, and two headwater lakes of the Huron River (one from North Lake and one from Patterson Lake). One individual from Cottrellville was recaptured from the previous day. The observation was noted, but additional tissue sample and biological data were not taken.

In 2016, a total of 188 traps were deployed by HRM at 31 separate locations along the Huron River and St. Clair-Detroit River System. Traps were deployed for a maximum of 72 hours, averaging approximately 52 hours for a total of over 9,000 trap hours recorded. A total of 15 Mudpuppies were captured including eight females, one male, and six juveniles (Table 8, Maps 6-7). Individuals were captured at five sites including four from Blue Water River Walk, one from Lake St. Clair MDNR Fisheries Research Station, two from Cottrellville, six from Belle Isle (one from Blue Heron Lagoon, two from North Fishing Pier, two from the loop canal, and one from Yacht Club), and two from Milliken State Park. In May 2016, one dead Mudpuppy was observed washed up along the riprap dike at the Fair Haven Boat Launch in Lake St. Clair and in July 2016, another deceased individual was found washed ashore at the Trenton Boat Launch, located near Elizabeth Park in the Detroit River. Due to the rate of decomposition on both of the deceased individuals, no tissue samples were collected.

In 2017, surveys were more focused on the southern portion of the corridor, with sampling largely restricted to the Detroit River and Lake Erie. One survey was conducted on the St. Clair River at the Blue Water River Walk. HRM deployed a total of 95 traps at 16 separate locations. Traps were deployed for a maximum of 49 hours and averaged approximately 44 hours in the water for a total of over 3,000 trap hours for the sampling season. Seven Mudpuppies were captured including three adult females and four juveniles (Table 9, Maps 8-9). Successful captures were recorded from four locations on the Detroit River including one individual from the Belle Isle Loop Canal, one from Milliken State Park, three from Meyer Elias Park, and one from Elizabeth Park. One Mudpuppy was captured from the Blue Water River Walk on the St. Clair River.



U.S. Fish and Wildlife Service

Juvenile Mudpuppy captured by HRM from the Detroit River in 2017 (A). Mudpuppy undergoing measurements following capture by U.S. Fish and Wildlife Service (B). Measurements being taken on an adult Mudpuppy captured by HRM at the MDNR Fisheries Research Station on Lake St. Clair (C).

Mudpuppy bycatch data from project partners within the SCDRS was collected each year of the study (Tables 10-11, Maps 10-11). MDNR data was limited to within the St. Clair River and Lake St. Clair where most of the agency's fisheries research is based. A total of 18 Mudpuppies were recorded as bycatch from setlines, minnow traps, and trap nets. In 2015, four individuals were captured from the St. Clair River within the delta adjacent to Lake St. Clair and two individuals were captured from Anchor Bay within the northern portion of Lake St. Clair. In 2016, MDNR recorded one Mudpuppy from the St. Clair River delta and in 2017 eleven were captured including one at the Fisheries Research Station and ten from Anchor Bay in Lake St. Clair.

Research conducted by the USFWS extended through a majority of SCDRS project area. Sampling extended from the head of the St. Clair River at Lake Huron to the river delta, within the entire length of the Detroit River to just south of Pointe Mouillee, and along the southwestern basin of Lake Erie near Maumee Bay. Between 2014 and 2017 the agency processed a total of 381 Mudpuppies as bycatch from setlines, minnow traps, and gill nets. A majority of Mudpuppies were captured from setlines (288) compared to the minnow traps (91), and gill nets (2). In fall 2014, the USFWS recorded 35 Mudpuppies with 12 located in the St. Clair River delta, one from the Detroit River adjacent to Belle Isle, and 22 from the mouth of the Detroit River. In 2015, 93 Mudpuppies were documented including one from the St. Clair River delta, one from the Detroit River between Belle Isle and Grosse Ile and 91 from the Detroit River near Grosse Ile and Fighting Island. A total of 93 Mudpuppies were recorded in 2016 from the Detroit River near Grosse Ile and Fighting Island. In 2017, 159 Mudpuppies were captured including 54 from the mouth of the Detroit River and 105 from the Detroit River near Grosse Ile and Fighting Island.

Research conducted by USGS extended from the head of the St. Clair River near Lake Huron through the St. Clair River delta and within the Detroit River from near Belle Isle downstream until near Grosse Ile (Map 12). Between 2014 and 2016 a total of 65 individuals were documented. In 2014, 18 Mudpuppies were collected with five recorded from the St. Clair River. One individual was captured in between Marysville and Cottrellville and the remaining four were located in the river delta. Thirteen Mudpuppies were recorded from the Detroit River with three located near Belle Isle and the remaining ten captured near Grosse Ile and Fighting Island. In 2015, USGS recorded 26 Mudpuppies. Twenty were

documented from the St. Clair River with nine found in the upper St. Clair River (eight from Blue Water River Walk and one from Kiefer Park), one from mid St. Clair River near Marysville, and ten from the lower St. Clair River (nine from Cottrellville and one from the mouth of the river at the delta). The other six Mudpuppies documented in 2015 were found in the Detroit River near Grosse Ile. In 2016, USGS documented a total of 19 Mudpuppies, from the St. Clair River and Lake St. Clair. Three were found in the upper St. Clair River at the Blue Water River Walk, two were found in the mid St. Clair River near Marysville, and nine were found in the lower St. Clair River (eight from Cottrellville and one from slightly upriver near Marine City). One Mudpuppy was found in Lake St. Clair on the eastern shoreline of Anchor Bay near the St. Clair River delta. The agency did not record any Mudpuppies in 2017.

Adult Mudpuppy captured from the Blue Water River Walk in December 2017 following its release.

Discussion

Results of field assessments conducted from 2014 through 2017 provided important insight into the distribution and abundance of the species within the project area. Some regions appear to support healthy populations, while others appear to have lower density and detection rates that warrant additional targeted assessments to better assess population health and overall viability.

The St. Clair River provided the highest number of Mudpuppy captures by HRM, with 23 individuals recorded between 2014 and 2016. The most successful sites for this stretch included the Blue River Water Walk, Cottrellville, and Algonac State Park. The distribution of these successful sampling sites indicates that Mudpuppies are present throughout the St. Clair River corridor and potentially in larger numbers compared to other regions of the SCDRS. Two of these sites represent recent restoration locations where prior to restoration Mudpuppies were not detected at these sites. These results strongly suggest that efforts to create and improve Mudpuppy habitat were successful.

Observing a juvenile Mudpuppy captured from Belle Isle.

The Detroit River supported the second largest number of Mudpuppies captured by HRM, with 15 Mudpuppies documented between 2015 and 2017. Most of the salamanders were caught on Belle Isle, and each of the five sampling locations around the island resulted in the detection of least one individual. A significant number of juveniles were observed here including the smallest individual trapped by HRM within the study period, indicating that Belle Isle likely supports healthy breeding populations and important refugia. Despite significant sampling efforts placed on the southern portion of the Detroit River, Mudpuppies were not found in that region until 2017 when two juveniles were captured at Meyer Elias Park and one juvenile was captured at Elizabeth Park.

Captures from Lake St. Clair were limited with three Mudpuppies recorded between 2014 and 2016. This was likely due in part to the smaller number of public sample locations on the lake compared to the other regions. Few suitable publically accessible locations were available for near shore sampling due to large stretches of private property and significant portions of the shoreline dominated by seawalls and similar structures. When considering the number of captured Mudpuppies observed within the St. Clair River and Detroit River on either end of Lake St. Clair, it is likely that this area does support populations. Research conducted within Ontario suggests the species is stable within Canadian tributaries of Lake St. Clair including the Syndenham River where the only known Canadian population of salamander mussels remain (McDaniel, Martin et al. 2009). Conducting additional targeted assessments, in both shallow and deep areas of the SCDRS within Michigan is needed before their health and status in this area can be determined.

Juvenile Mudpuppy captured from the Detroit River.

Detroit News

Harmful algal blooms as seen here in western Lake Erie may be a factor in limited Mudpuppy numbers.

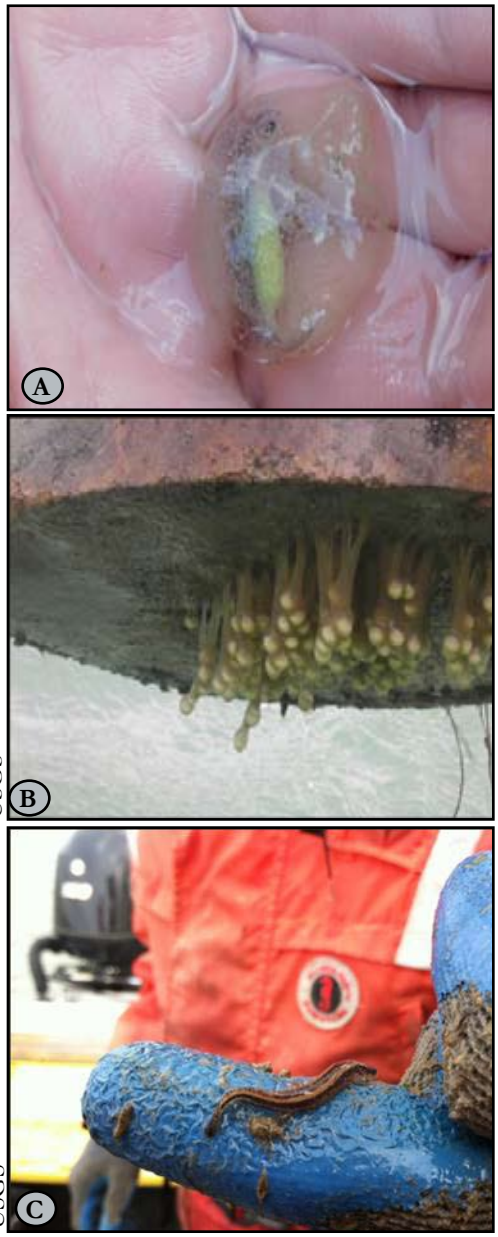
No Mudpuppies were recorded by HRM from the Lake Erie project sampling area during the four year sampling period. Desktop analyses indicate that prior to this study, one Mudpuppy occurrence was reported by anecdotal data in 2010 near Sterling State Park. In July 2016 there were two reports of dead Mudpuppies washed up along the Pointe Mouillee wetland dikes. The U.S. Fish and Wildlife Service provided 21 capture records and tissue samples from offshore sampling near Pointe Mouillee, which represent the southernmost points of positive Mudpuppy detection in the project area. The relatively few captures from this area and complete lack of detection further south along Lake Erie may be a result of sample techniques or low population density. Alternatively, this may indicate that Mudpuppies are not utilizing shallow habitats in Lake Erie as frequently as observed further north in the SCDRS and could be a function of poor habitat availability. However, if habitat was the limiting factor, nearshore restoration created as part of this project should have attracted Mudpuppies as observed in other project areas. The low capture rate, despite nearby populations detected in the Detroit River, suggests that Mudpuppy relative abundance may be significantly lower in this region. No substantial physical barriers appear to be present in the area that may prevent dispersal of Mudpuppies from the Detroit River to these habitats. Water quality and presence of contaminants may be a contributing factor, especially given the issues that the Lake Erie basin has faced in recent decades (Myers, Thomas et al. 2000; Pearsall, Carton de Grammont et al. 2012). The western basin of Lake Erie comprises the shallowest part of the lake and the nearshore habitat here has seen significant historic impacts. Nutrient pollution and the resulting harmful algal blooms (HABs) have become one of the leading re-emerging issues for this system (Watson, Miller et al. 2016). HABs are known to cause ecological impairments that may significantly affect fish and wildlife including tissue damage, growth inhibition, and poor food quality (Lopez, Jewett et al. 2008). These risks may be affecting Mudpuppies in this region and preventing healthy, sustainable populations from establishing and additional assessment to determine the effects of these stressors on Mudpuppies is warranted.

Several large dams along the Huron River function as a likely barrier to Mudpuppy dispersal.

In addition to the SCDRS corridor, widespread efforts were placed on the Huron River. The headwater region of the watershed was the only location where captures were recorded in two separate inland lakes. Survey effort across these inland lakes was limited compared to other portions of the watershed and it is likely that the species is present in additional lakes in the area as well. The low capture rate observed suggests that Mudpuppy density may be very low or the species is potentially absent from portions of the Huron River particularly in urban areas. The desktop analyses of historic and recent Mudpuppy occurrences in the region revealed the species was last reported through the Michigan Herp Atlas in 2012 from a small tributary stream located near the Huron River channel. Within the river itself, the most recent records of Mudpuppies were based on anecdotal data and they were last reported in 2010 with occurrences limited to upstream of Gallup Park in Ann Arbor. The presence of nearly 100 dams along the Huron River may be a large contributing factor to the low detection of Mudpuppies in the river. Although HRM documented suitable habitat for Mudpuppies within the river and there were historic occurrences of Mudpuppies in sampled area, the system may be too fragmented to support viable populations. Comparatively, the St. Clair and Detroit rivers do not contain dams or other water control structures, leaving these systems open for dispersal and movement of Mudpuppies and other aquatic organisms throughout the system.

Poor water quality within the Huron River may be another contributing factor to a lack of detection here. In previous years, portions of the Huron River were listed as impaired due to the presence of excessive phosphorus loading which resulted in algal blooms; this led to the development of a phosphorus total maximum daily loading (TMDL) management plan (Middle Huron Initiative 2011). A significant level of nutrient pollution was determined to originate from point sources including discharge from industrial and municipal facilities. Between 2010 and 2011 the Ann Arbor Waste Water Treatment alone was identified as producing up to 96% of the total point source phosphorus load (Middle Huron Initiative 2011). Since implementation of the TMDL management plan, extensive efforts have been put into place on reducing nutrient loading and improving conditions of the Huron River. The Middle Huron Water Quality Monitoring Programs was initiated in 2002 to track the long term health and functionality of the system. Overall total phosphorus concentrations were observed to decrease through 2009, however a large increase was observed from 2010-2013 (Lawson and Burns 2014). Disproportionate levels of this nutrient can lead to extensive growth of algae and other aquatic vegetation and result in a depletion of dissolved oxygen. These conditions are known to adversely affect aquatic wildlife, including Mudpuppies, and may potentially be a factor in their limited detection during this study. This may be supported by the fact that no records of Mudpuppies are present downstream of Gallup Park, which is directly upstream from the wastewater treatment plant known to be a leading input of nutrient pollution on the system.

Partnerships with various agencies and organizations resulted in valuable assistance and spatial distribution data throughout this multi-year study. Fish sampling methods that resulted in Mudpuppy bycatch included the use of setlines, minnow traps, gill nets, and egg mats. Data were submitted from the northern extent of the project area in Port Huron through the corridor to the mouth of the Detroit River at Lake Erie. Unique



Unique observations by partner agencies such as eggs found in deep water habitats (A) including telemetry anchors (B) and larval individuals found seeking refugia in fish egg mats (C).

observations from the corridor included larval and juvenile Mudpuppies captured from fish egg mats that were being utilized by the salamanders as refugia. USGS also documented Mudpuppy eggs attached to the bottom of telemetry anchors at depths not previously known for this species. These observations, including the collection of eggs from water depths undocumented previously, have led to a collaborative publication (Craig, Mifsud et al. 2015). In addition to providing observations of Mudpuppies from deep water habitats, another region not sampled by HRM that resulted in positive detections by each of the partner agencies was the St. Clair River delta where it feeds into Lake St. Clair. Observations submitted by USFWS and USGS were concentrated in the Detroit River . The most southern points of positive detection for Mudpuppies were submitted by USFWS within the mouth of the Detroit River as it transitions to Lake Erie between the northern portion Pointe Mouillee and the southern coastline of Essex, Ontario, Canada. The absence of Mudpuppy bycatch in the south western Lake Erie basin, despite a number of setlines and minnow traps placed in that area warrants concern and greater attention given the success of these techniques in upstream portions of the corridor (Map 13). Coupled with HRM's absence of detection in this region, this data supports that Mudpuppies may currently be present in very limited numbers. Additional targeted investigation of Mudpuppies in this region is worthwhile and may better depict species spatial distribution, population health, and potential threats and opportunities for recovery.

The combined data from HRM and partner sampling efforts support the value of integrating multiple monitoring techniques to more accurately reflect the spatial distribution and demography of Mudpuppy populations. Recent studies have suggested that targeted trapping is most effective for sexually mature adults, but is not as effective for juveniles (Beattie, Whiles et al. 2017). HRM's trapping data supports this conclusion, with a total of 31 adults detected compared to 12 juveniles. Furthermore, it has been shown that trapping is biased towards females, likely due to higher energy requirements for egg development leading females to more actively forage compared to males (Beattie, Whiles et al. 2017). HRM's data reflects this as well with nine males captured compared to 22 females. Partner agencies were highly successful in capturing a number of Mudpuppies on setlines during deep water fish sampling. The observation of larval and juvenile age classes seeking refugia in fish egg mats demonstrates the value in utilizing different approaches when sampling for this species so that all life stages are targeted. Focusing additional Mudpuppy surveys in these habitats, including alternative techniques, will provide more refined information and potentially lead to a better understanding of Mudpuppy habitat use and dispersal patterns within the SCDRS. Ultimately these data can lead to stronger conservation measures aimed at the imperiled Salamander Mussel as the species presence is positively correlated to the distribution and density of Mudpuppy populations (McDaniel, Martin et al. 2009; Ho 2011).

Appendix 3.1 Data Tables

2014 Survey Conditions							
Date	**Start Time**	**Average Air Temp (°F)**	**Average Water Temp (°F)**	**Cloud Cover**	**Average Wind Speed (mph)**	**Average Humidity (%)**	**End Time**
11/24/2014	11:35am	46	42	Cloudy	12	78	6:09pm
11/25/2014	8:00am	35	NA	Cloudy	4.8	71	1:25pm
12/2/2014	10:20am	33	40	Cloudy	2.8	78	4:46pm
12/3/2014	7:53am	37	NA	Cloudy	3.9	75	3:31pm
12/11/2014	10:09am	35	37	Overcast	3.7	37	5:15pm
12/12/2014	8:01am	36	NA	Overcast	1.6	72	2:25pm
12/15/2014	10:15am	45	37	Overcast	2.7	83	4:15pm
12/16/2014	8:13am	44	38	Overcast, Rain	2.8	94	4:15pm
12/17/2014	10:20am	34	NA	Cloudy	2.7	74	11:31am
12/18/2014	11:03am	35	34	Cloudy	1.4	76	4:45pm
12/19/2014	8:29am	32	NA	Cloudy	3.1	66	12:31pm

Table 1. Weather conditions dring field surveys conducted in 2014.

2015 Survey Conditions							
Date	**Start Time**	**Average Air Temp (°F)**	**Average Water Temp (°F)**	**Cloud Cover**	**Average Wind Speed (mph)**	**Average Humidity (%)**	**End Time**
3/24/2015	10:15am	36	36	Sunny	1.5	51	4:27pm
3/25/2015	11:41am	50	39	Overcast > Sunny	1.3	70	5:19pm
3/26/2015	11:17am	41	41	Cloudy	1.8	61	2:12pm
3/27/2015	12:30pm	31	37	NA	3.1	62	3:51pm
3/31/2015	10:49am	43	39	Partly Cloudy	2.1	73	5:24pm
4/1/2015	11:06am	55	42	Sunny	1.7	56	3:24pm
4/2/2015	10:48am	54	45	Overcast, rain	2.4	78	2:46pm
4/3/2015	10:43am	55	45	Cloudy	1.8	60	3:11pm
4/6/2015	10:48am	49	48	Cloudy	2.6	55	4:47pm
4/7/2015	10:40am	48	46	Cloudy	1.3	71	2:42pm
4/8/2015	9:31am	44	42	Cloudy	1.1	75	12:23pm
4/9/2015	11:38am	46	43	Light Rain	1.5	90	4:05pm
4/28/2015	1:28pm	58	54	Sunny	1.3	49	7:50pm
4/28/2015	1:15pm	51	50	Sunny	3.5	55	8:36pm
4/29/2015	9:30am	56	54	Sunny	1.7	65	2:44pm
4/29/2015	9:01am	53	49	Sunny > Cloudy	0.8	54	5:20pm
4/30/2015	8:16am	48	50	Rain	1.2	88	12:00pm
4/30/2015	7:28am	47	45	Cloudy	1.6	65	1:49pm
5/7/2015	10:50am	75	NA	Sunny	1.3	62	12:40pm

Table 2. Weather conditions during field surveys conducted in 2015.

2016 Survey Conditions							
Date	**Start Time**	**Average Air Temp (°F)**	**Average Water Temp (°F)**	**Cloud Cover**	**Average Wind Speed (mph)**	**Average Humidity (%)**	**End Time**
3/15/2016	10:41am	43	44	Cloudy	5	76	2:01pm
3/17/2016	10:09am	50	44	Sunny	10.2	53	12:12pm
3/18/2016	10:02am	48	NA	Sunny > Overcast	1.6	50	12:13pm
3/22/2016	9:29am	50	44	Cloudy	1.7	53	1:45pm
3/23/2016	9:21am	46	NA	Cloudy	2.2	74	11:39am
3/24/2016	8:57am	46	NA	Cloudy > Light Rain	0.7	84	11:40am
3/25/2016	9:41am	34	42	Cloudy	1.5	73	12:34pm
3/29/2016	10:10am	43	46	Sunny	2.8	62	4:30pm
3/30/2016	10:21am	55	NA	Partly Cloudy > Cloudy	3.0	55	4:35pm
3/31/2016	8:44am	55		Rain	6.2	98	12:39pm
4/5/2016	10:35am	32	36	Sunny	2.8	49	1:45pm
4/6/2016	8:58am	35.5	36	Overcast	9	60	12:37pm
4/7/2016	8:52am	38	NA	Cloudy > Snow	5.5	78	2:54pm
4/8/2016	7:15am	28	39	Clear	2.3	58	9:20am
4/13/2016	10:00am	49	42	Cloudy	0.8	54	1:35pm
4/14/2016	8:43am	45	NA	Clear, Sunny	4.6	56	1:16pm
4/15/2016	8:11am	45	45	Sunny	1.7	56	10:19am
5/2/2016	11:25am	49	47	Overcast > Sunny	3.9	78	6:40pm
5/3/2016	9:20am	51	NA	Sunny	1.6	68	11:24am
5/4/2016	9:40am	55	NA	Cloudy	3.5	74	3:56pm
5/10/2016	10:33am	55	53	Cloudy > Light Rain	3.4	69	12:50pm
5/11/2016	10:24am	60	52	Cloudy > Overcast	2.1	75	12:40pm

Table 3. Weather conditions during field surveys conducted in 2016.

2017 Survey Conditions							
Date	**Start Time**	**Average Air Temp (°F)**	**Average Water Temp (°F)**	**Cloud Cover**	**Average Wind Speed (mph)**	**Average Humidity (%)**	**End Time**
3/7/17	10:25am	55.5	NA	Light Rain	5.7	99	12:57pm
3/8/17	10:37am	50.5	40	Sunny	8	39	12:11pm
3/9/17	9:53am	42	37	Cloudy	2.1	49	11:46pm
4/10/17	10:15am	73	46	Sunny	2.8	50	3:45pm
4/11/2017	10:20am	63	47	Cloudy	2.6	70	3:41pm
4/12/2017	8:18am	55	NA	Cloudy > Sunny	1.4	55	12:26pm
4/25/2017	10:08am	60	53	Cloudy > Mostly Sunny	3	72	4:37pm
4/26/2017	10:11am	73	NA	Sunny	4	61	1:43pm
4/27/2017	8:37am	67	NA	Sunny > Rain	2.1	62	12:43pm
12/11/2017	3:27pm	28	NA	Cloudy	2.7	82	5:00pm
12/12/2017	8:10am	18	31	Cloudy > Sunny	5.6	60	9:35am

Table 4. Weather conditions during field surveys conducted in 2017.

Mudpuppy Captures by Region						
Water Body	**Site Name**	**2014**	**2015**	**2016**	**2017**	**Total**
St. Clair River	Blue Water Bridge	0	0	0	1	1
	Kiefer Park	0	0	0	X	0
	Blue Water River Walk	2	2	4	X	8
	Marysville	0	0	0	X	0
	Cottrellville	X	7	2	X	9
	Algonac State Park	6	0	0	X	6
	Total	8	9	6	X	24
Lake St. Clair	Fair Haven Boat Launch	0	0	0	X	0
	MDNR Fisheries Research Station	2	0	1	X	3
	Lake St. Clair Metropark	0	0	0	X	0
	Total	2	0	1	X	3
Detroit River	Belle Isle	0	1	6	1	8
	Milliken State Park	X	X	2	1	3
	Meyer Ellias Park	X	X	X	3	3
	Elizabeth Park	0	0	0	1	1
	Total	X	1	8	6	15
Lake Erie	Lake Erie Metropark	0	0	0	0	0
	Pointe Mouillee	0	0	0	0	0
	Sterling State Park	0	0	0	0	0
	Toledo Beach Marina	X	0	0	0	0
	Erie Road Public Beach Access	X	X	X	0	0
	Algonquin Street Boat Ramp	X	X	X	0	0
	Total	0	0	0	0	0
Huron River	Barton Nature Area	X	0	0	X	0
	Bandemer Park	X	0	0	X	0
	Argo Park	X	0	0	X	0
	Nichols Arboretum	X	0	0	X	0
	Gallup Park	X	0	0	X	0
	Belleville Boat Launch	X	0	0	X	0
	Hydro Park	X	0	0	X	0
	Ford Lake Park	X	0	0	X	0
	North Bay Park	X	0	0	X	0
	Riverside Park	X	0	0	X	0
	Peninsular Park	X	0	0	X	0
	Total	X	0	0	X	0
Huron River Headwaters	Portage Lake	X	0	X	X	0
	Silver Lake	X	0	X	X	0
	Patterson Lake	X	1	X	X	1
	Halfmoon Lake	X	0	X	X	0
	Joslin Lake	X	0	X	X	0
	South Lake	X	0	X	X	0
	Sullivan Lake	X	0	X	X	0
	North Lake	X	1	X	X	1
	Total	X	2	X	X	2

Table 5. Mudpuppies captured at each of the sampling locations by HRM between 2014 and 2017. Sites not sampled in a given year are represented by an X

2014 HRM Captured Mudpuppy Data								
Date	**Site**	**Water Temp (°F)**	**Sex**	**Weight (g)**	**Snout-Vent Length (cm)**	**Total Length (cm)**	**Total Trap Hours**	**Abnormalities/Injuries**
12/3/14	Algonac	38	F	>50	22	33	21hr 35min	None
			F	>50	24	30	21hr 35min	Right eye injured/cloudy
			F	>50	26	38	22hr 51min	None
	Lake St. Clair Fisheries Research Station	53	M	312.2	23	34	24hr 48min	Minor old scarring
			F	490.6	28.5	42	24hr 48min	Old scarring
12/12/14	Blue River Walk	34	F	155	19.5	29.5	17hr 16min	None
			F	220	20.5	30.4	17hr 16min	Minor old scarring across the back. Slight permanent curve in spine
	Algonac	36	F	325	25.5	37.6	21hr 49min	Toe on hind foot curved in toward body
			M	340	27.0	38.0	22hr 02min	None
12/16/14	Algonac	38	F	410	27.0	40.5	22hr 30min	Polydactyl

Table 6. Information for individual mudpuppies captured by HRM in 2014, including site conditions, animal description, site, and date.

2015 HRM Captured Mudpuppy Data								
Date	**Site**	**Water Temp (°F)**	**Sex**	**Weight (g)**	**Snout-Vent Length (cm)**	**Total Length (cm)**	**Total Trap Hours**	**Abnormalities/Injuries**
4/7/15	North Lake	46	F	275	21.7	32.0	21hr 27min	Notch in tail tip
4/9/15	Patterson Lake	43	M	160	17.5	27.0	71hr 30min	Small notch in top of tail fin
4/29/15	Blue River Walk	45	Unk	75	13	20.4	17 hr 31 min	Minor scratches on tail
	Cottrellville	50	F	85	14.5	22.0	20 hr 8 min	None
			M	150	16.5	25.2	20 hr 8 min	None
			M	160	18.7	28.0	20 hr 12 min	None
			M	110	16.5	24.6	20 hr 12 min	None
			F	180	17.5	26.0	20 hr 12 min	None
			F	180	20.5	31.0	20 hr 12 min	Slight discoloration on head, likely from old scarring
4/30/15	Blue River Walk	45	Unk	40	10.7	16.0	38 hr 57 min	None
	Cottrellville*						40 hr 34 min	
5/7/15	Belle Isle: N. Fishing Pier	NA	M	440	24	35	NA	Minor scarring. Cloaca inflamed and bleeding.

Table 7. Information for individual mudpuppies captured by HRM in 2015, including site conditions, animal description, site, and date.
*Represents a recent recapture and specific data/tissue sample were not collected.

2016 HRM Captured Mudpuppy Data								
Date	**Site**	**Water Temp (°F)**	**Sex**	**Weight (g)**	**Snout-Vent Length (cm)**	**Total Length (cm)**	**Total Trap Hours**	**Abnormalities/Injuries**
3/17/16	Belle Isle : Blue Heron Lagoon	46	F	150	19	27.5	47 hr 14 min	Small cut on tail
	Belle Isle: N. Fishing Pier	39	Unk	60	13.5	21	46 hr 50 min	Scrapes near anterior part of tail
	Belle Isle: Loop Canal	48	Unk	30	9.5	15	46 hr 21 min	Scrape on chin
		48	Unk	50	11.5	16.4	46 hr 21 min	None
3/18/16	Belle Isle: Yacht Club	39	F	80	15.5	22.5	70 hr 43 min	None
3/30/16	Milliken State Park	43	F	100	16	24	24 hr 17 min	None
		42	F	130	17	25	23 hr 54 min	None
4/6/16	Blue River Walk	36	F	135	16	23.5	25 hr 51 min	None
	Lake St. Clair Fisheries Research Station	39	F	130	15	26	17 hr 49 min	None
4/8/16	Cottrellville	39	F	96	14	21	68 hr 3 min	None
5/4/16	Blue River Walk	47	F	150	17.5	29	42 hr 10 min	Slight nick on tail
		46	M	270	20.5	31.5	43 hr 14 min	Significant scoliosis
		44.5	Unk	100	15.5	22.5	44 hr 5 min	None
	Cottrellville	40	Unk	70	13.5	20.2	46 hr 56 min	None
5/11/16	Belle Isle: S Fishing Pier	51	Unk	11	8	13	24 hr 15 min	None

Table 8. Information for individual mudpuppies captured by HRM in 2016, including site conditions, animal description, site, and date.

2016 HRM Captured Mudpuppy Data								
Date	**Site**	**Water Temp (°F)**	**Sex**	**Weight (g)**	**Snout-Vent Length (cm)**	**Total Length (cm)**	**Total Trap Hours**	**Abnormalities/Injuries**
3/8/17	Belle Isle: Loop Canal	41	Unk	40	12	18.5	22 hr 51 min	None
4/11/17	Milliken Park	47	Unk	100	15.5	24	24 hr 3 min	None
	Meyer Elias Park	41	F	135	16.5	23.5	23 hr 52 min	None
	Elizabeth Park	42	Unk	65	11.5	17.5	23 hr 51 min	None
4/12/17	Meyer Elias Park	42	Unk	24	10.5	14	46 hr 7 min	None
4/27/17		45	Unk	17	10	14.5	37 hr 59 min	None

Table 9. Information for individual mudpuppies captured by HRM in 2017, including site conditions, animal description, site, and date.

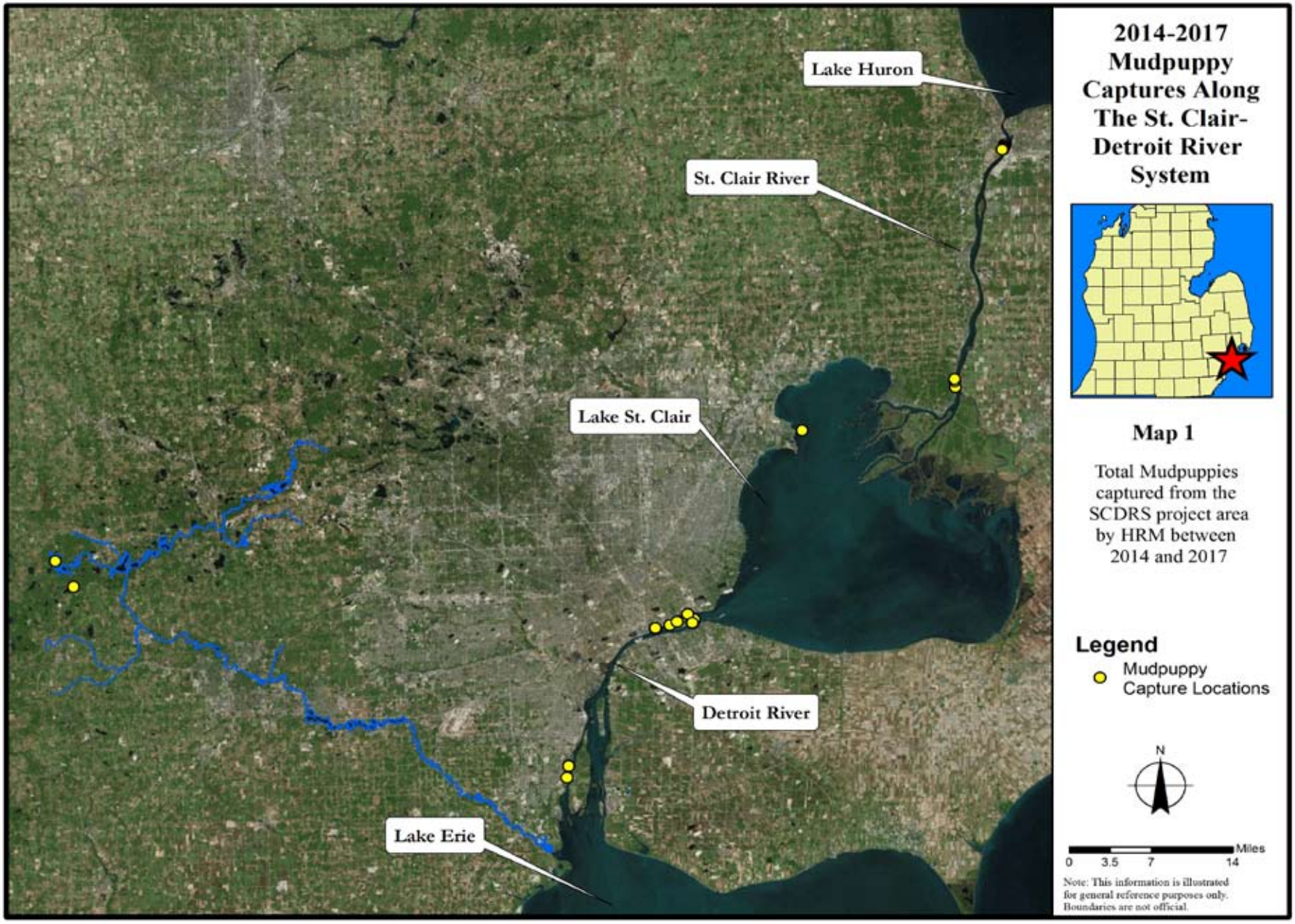
Mudpuppy Bycatch Data by Region						
Water Body	*Agency*	*2014*	*2015*	*2016*	*2017*	*Total*
St. Clair River	MDNR	0	4	0	0	4
	USFWS	12	1	0	0	13
	USGS	5	20	14	0	39
	Total	17	25	14	0	56
Lake St. Clair	MDNR	0	2	1	11	14
	USFWS	0	0	0	0	0
	USGS	1	0	1	0	2
	Total	1	2	2	11	16
Detroit River	USFWS	23	92	93	159	367
	USGS	14	6	0	0	20
	Total	37	98	93	159	387
Lake Erie	USFWS	0	0	0	0	0
	Total	0	0	0	0	0

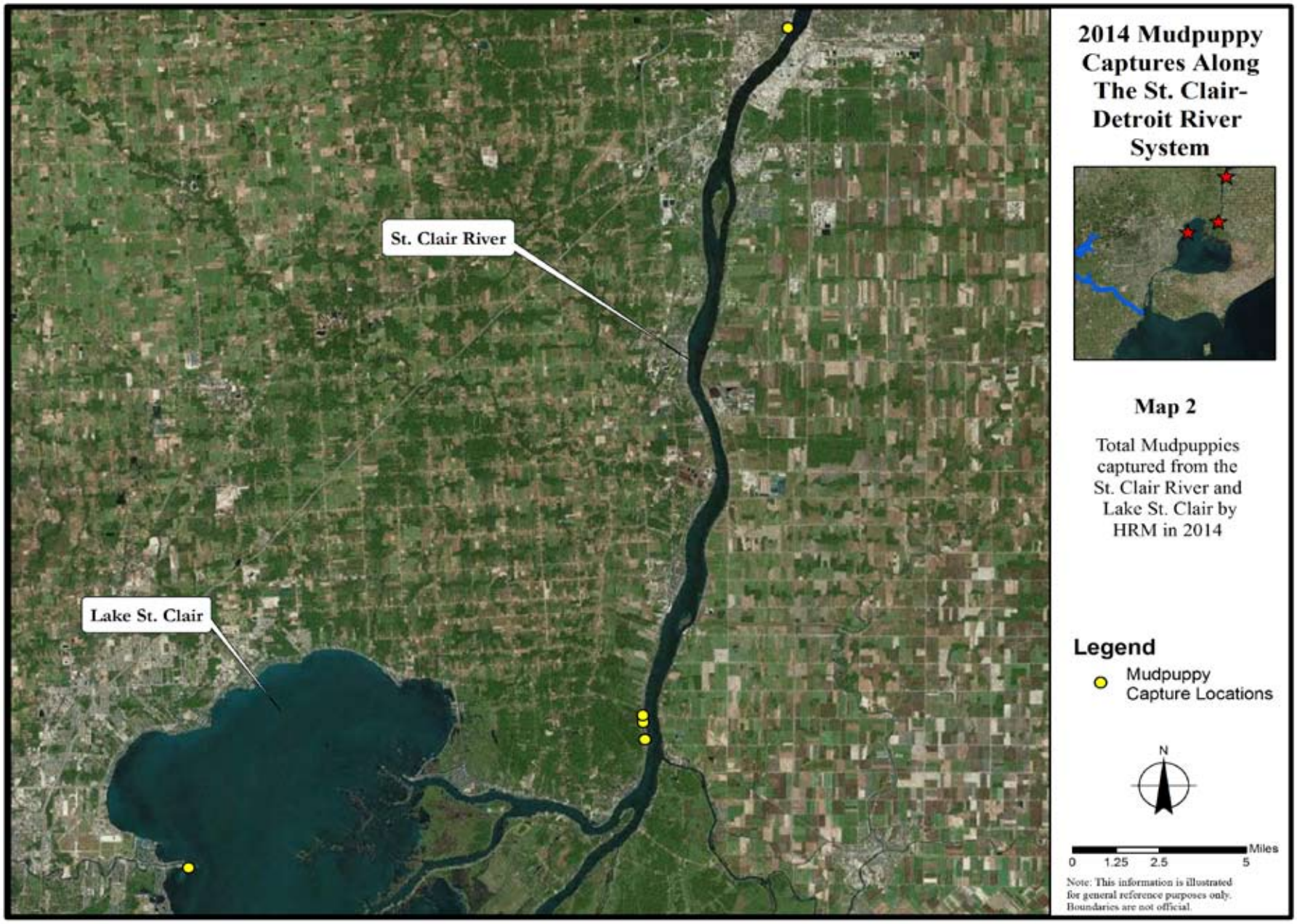
Table 10. Mudpuppies captured as bycatch by project partners between 2014 and 2017, broken down by sampling region.

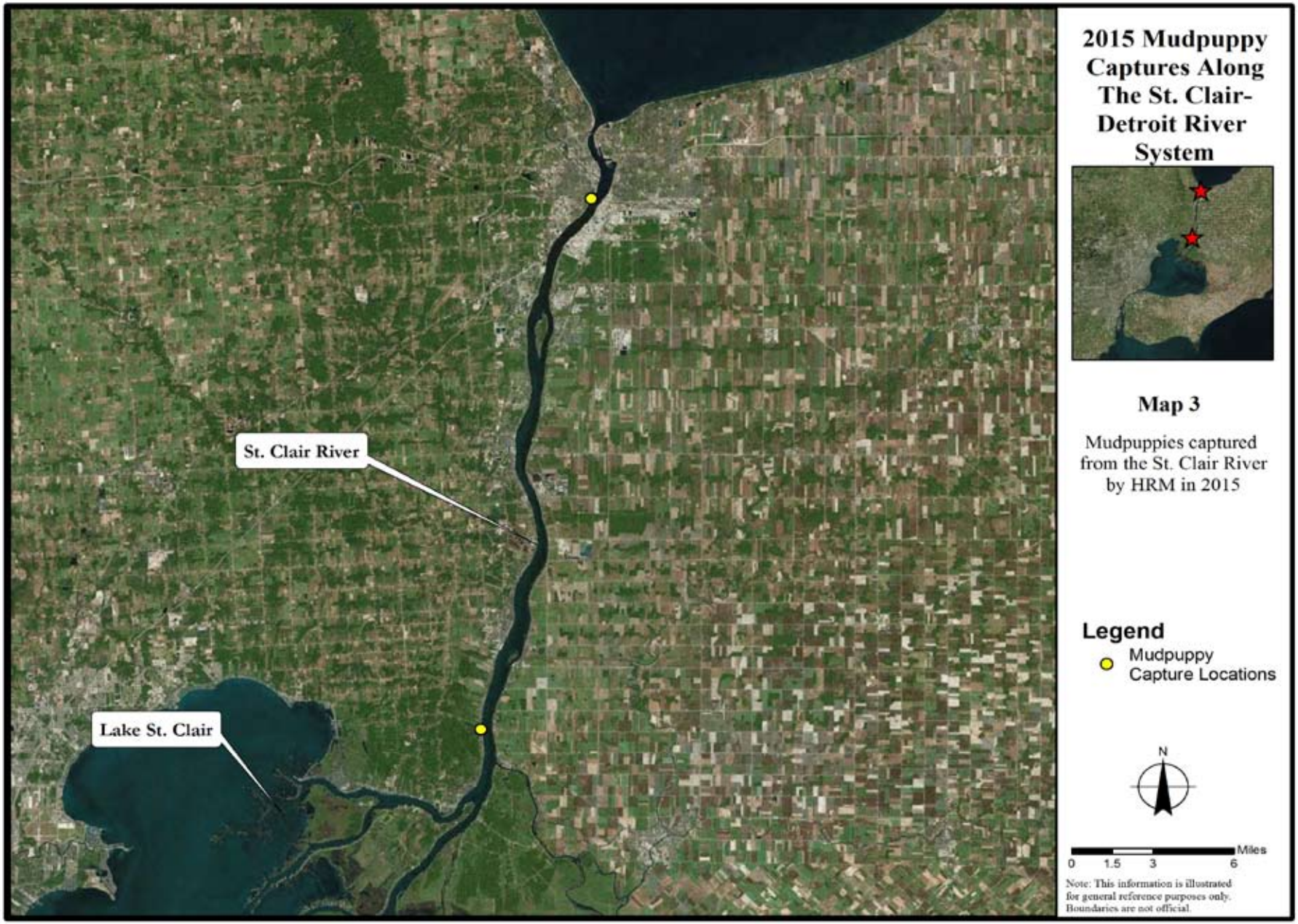
Mudpuppy Bycatch by Agency					
Agency	*Year*	*St. Clair River*	*Lake St. Clair*	*Detroit River*	*Lake Erie*
MDNR	2015	4	2	X	X
	2016	0	1	X	X
	2017	0	11	X	X
	Total	4	14	X	X
USFWS	2014	12	0	23	0
	2015	1	0	92	0
	2016	0	0	93	0
	2017	0	0	159	0
	Total	13	0	367	0
USGS	2014	5	1	14	X
	2015	20	0	6	X
	2016	14	1	0	X
	2017	0	0	0	X
	Total	39	2	20	X

Table 11. Mudpuppies captured as bycatch by project partners between 2014 and 2017, broken down by each government agency. X reprerents sites not sampled.

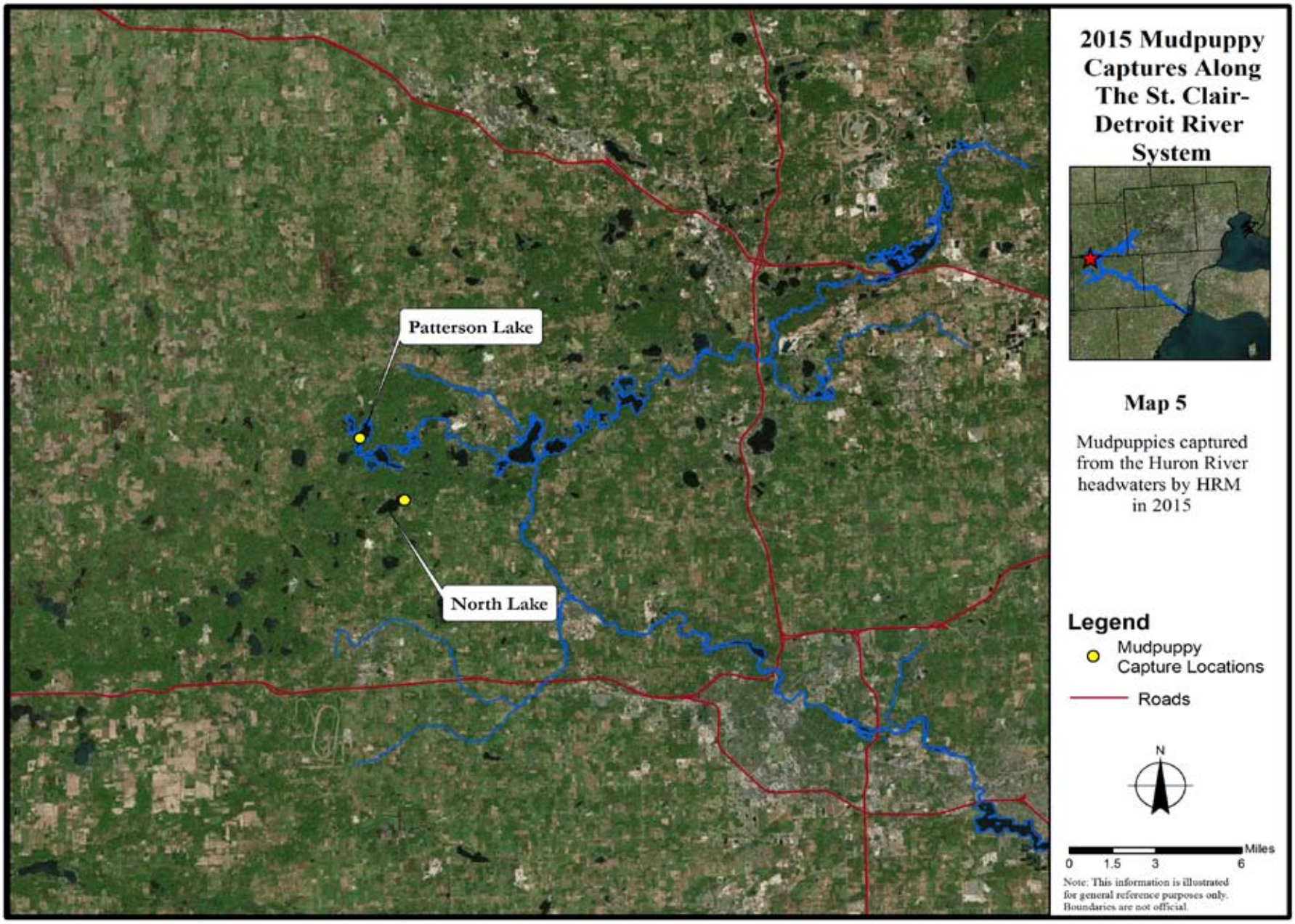
Appendix 3.2 Maps

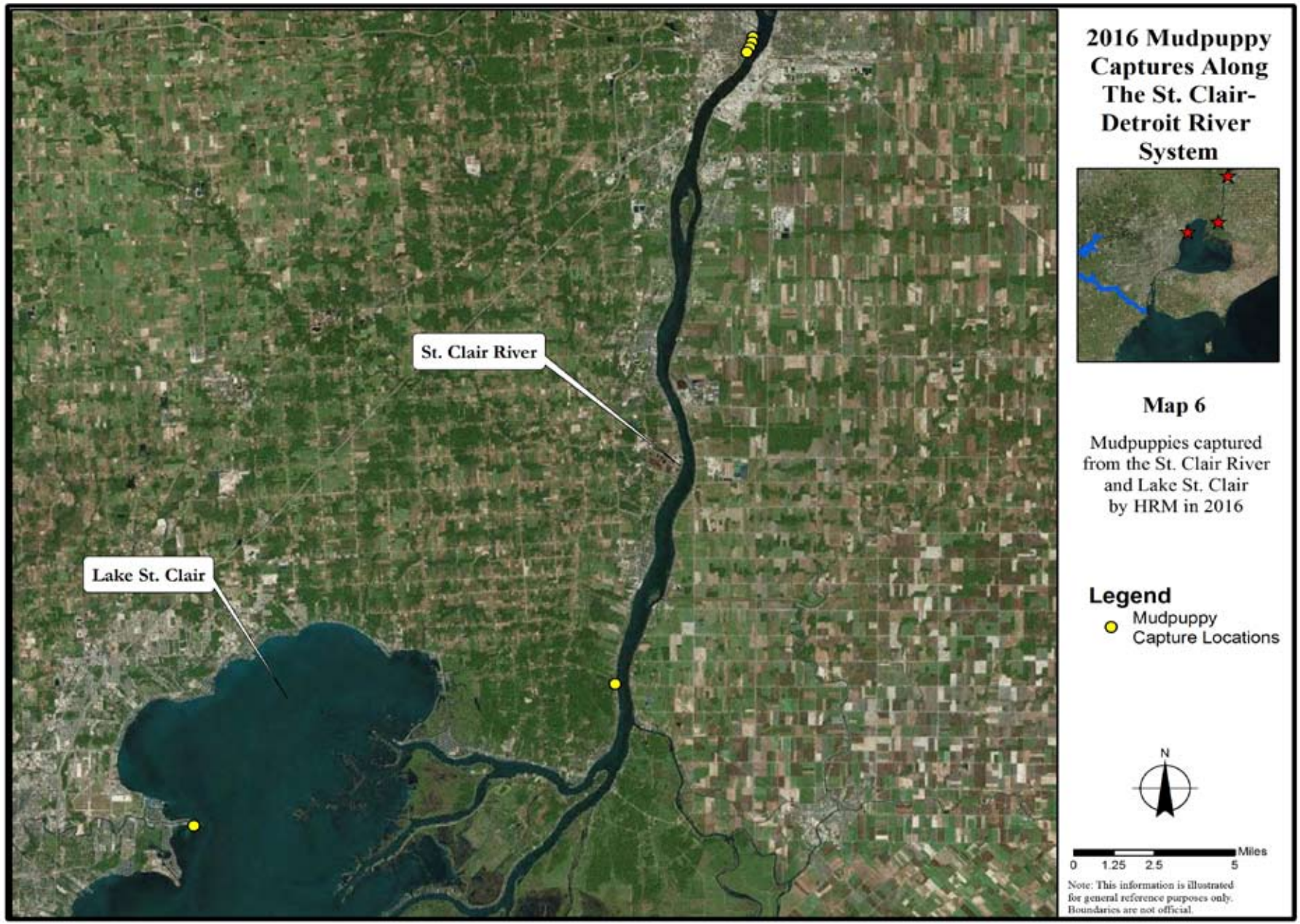


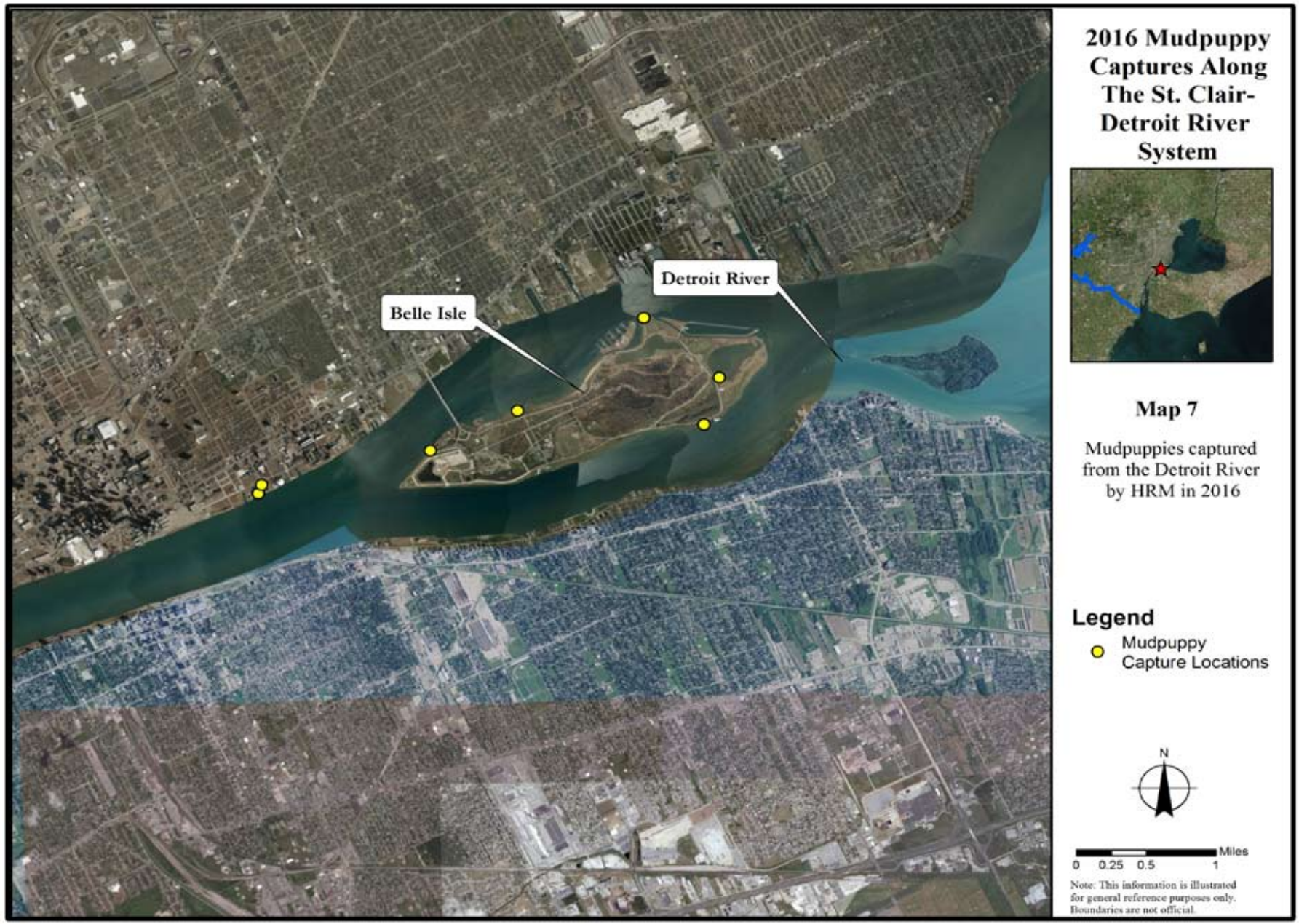

St. Clair River
Lake St. Clair
2014 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 2
Total Mudpuppies captured from the St. Clair River and Lake St. Clair by HRM in 2014
Legend
Mudpuppy Capture Locations
N
Miles
0 1.25 2.5 5
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

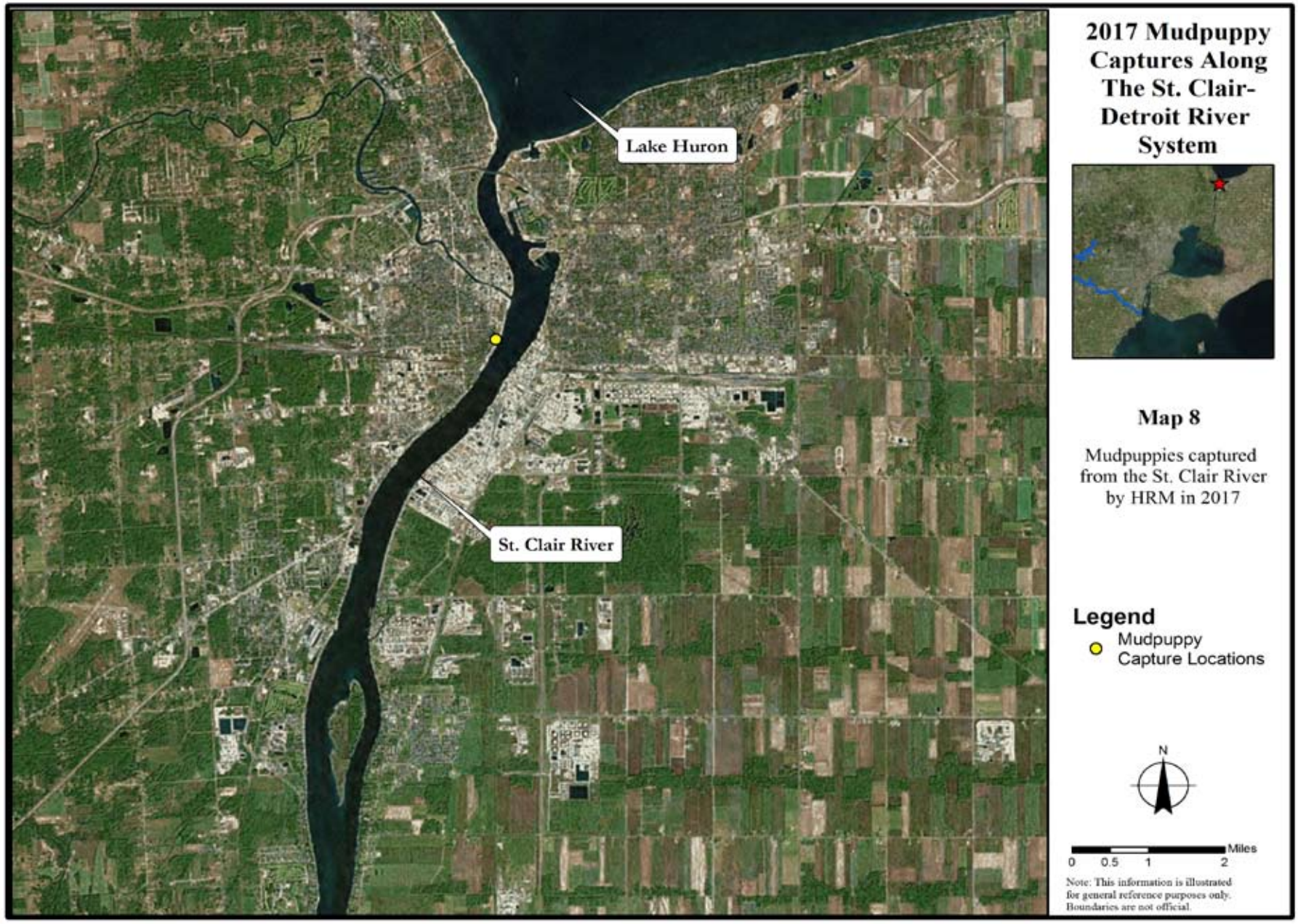

St. Clair River
Lake St. Clair
2015 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 3
Mudpuppies captured from the St. Clair River by HRM in 2015
Legend
Mudpuppy Capture Locations
N
Miles
0 1.5 3 6
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

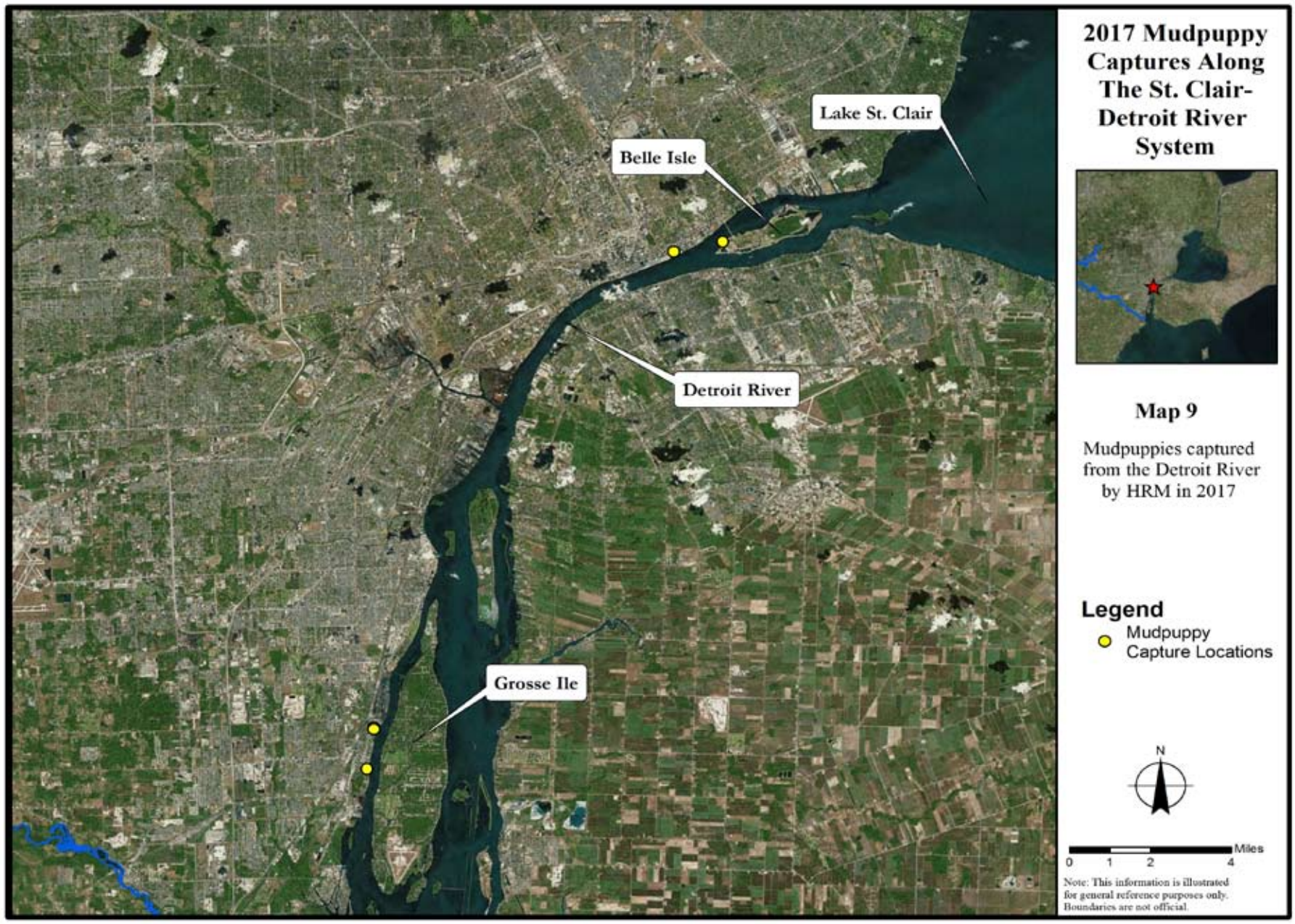

Belle Isle
Detroit River
2015 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 4
Mudpuppies captured from the Detroit River by HRM in 2015
Legend
Mudpuppy Capture Locations
N
Miles
0 0.125 0.25 0.5
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

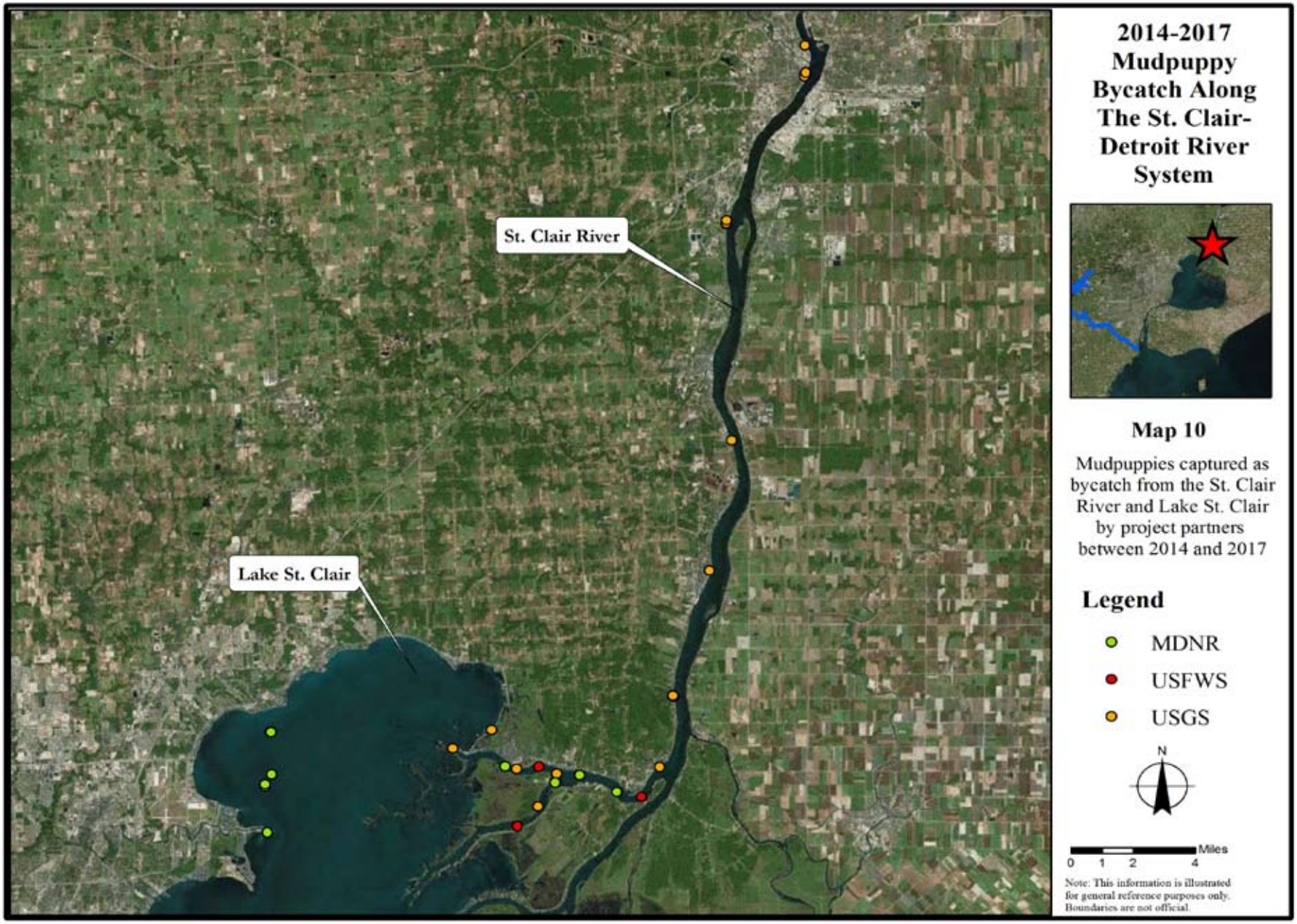

Patterson Lake
North Lake
2015 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 5
Mudpuppies captured from the Huron River headwaters by HRM in 2015
Legend
Mudpuppy Capture Locations
Roads
N
Miles
0 1.5 3 6
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

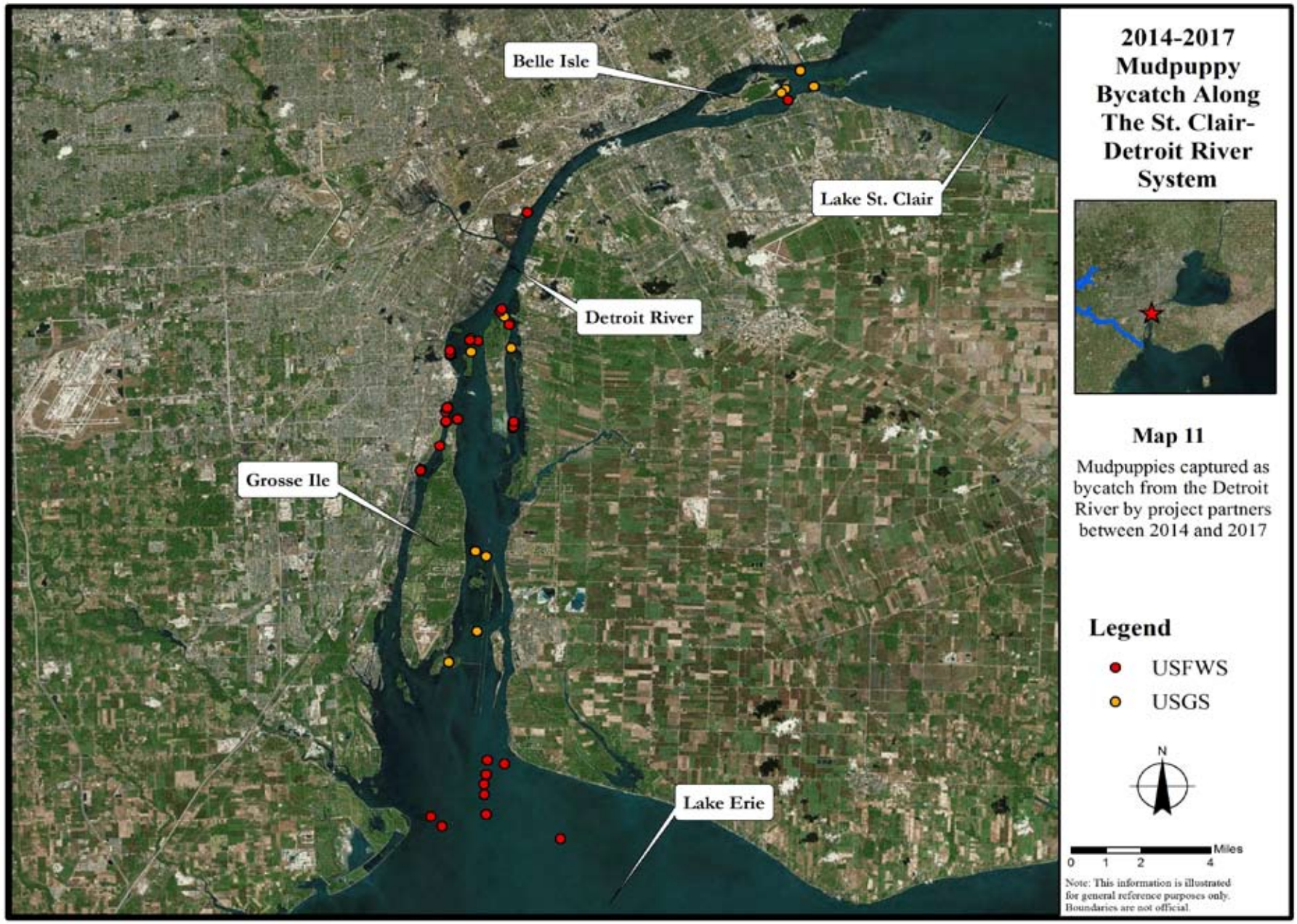

St. Clair River
Lake St. Clair
2016 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 6
Mudpuppies captured from the St. Clair River and Lake St. Clair by HRM in 2016
Legend
Mudpuppy Capture Locations
N
Miles
0 1.25 2.5 5
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

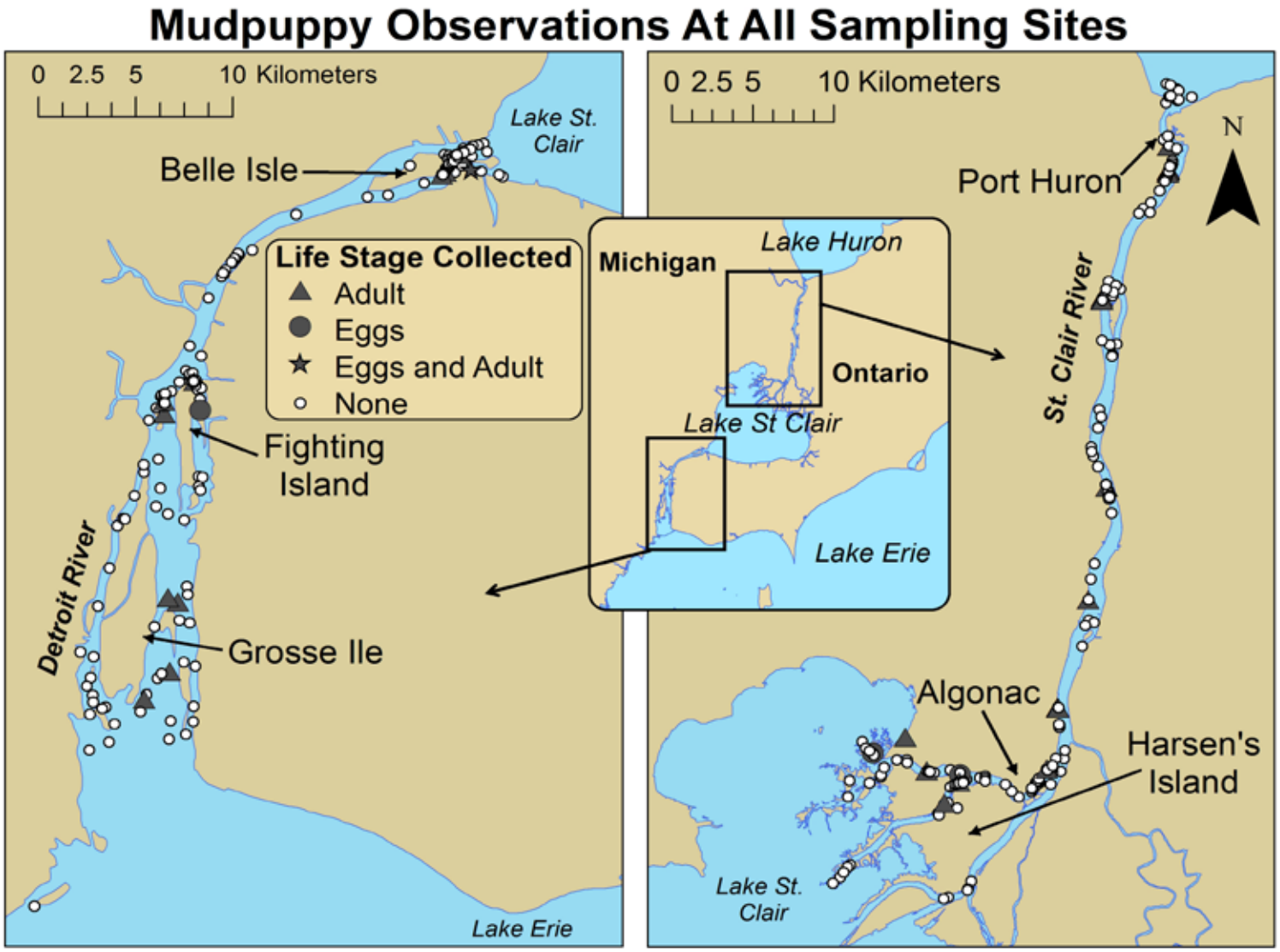

Belle Isle
Detroit River
2016 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 7
Mudpuppies captured from the Detroit River by HRM in 2016
Legend
Mudpuppy Capture Locations
N
Miles
0 0.25 0.5 1
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


Lake Huron
St. Clair River
2017 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 8
Mudpuppies captured from the St. Clair River by HRM in 2017
Legend
Mudpuppy Capture Locations
N
Miles
0 0.5 1 2
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

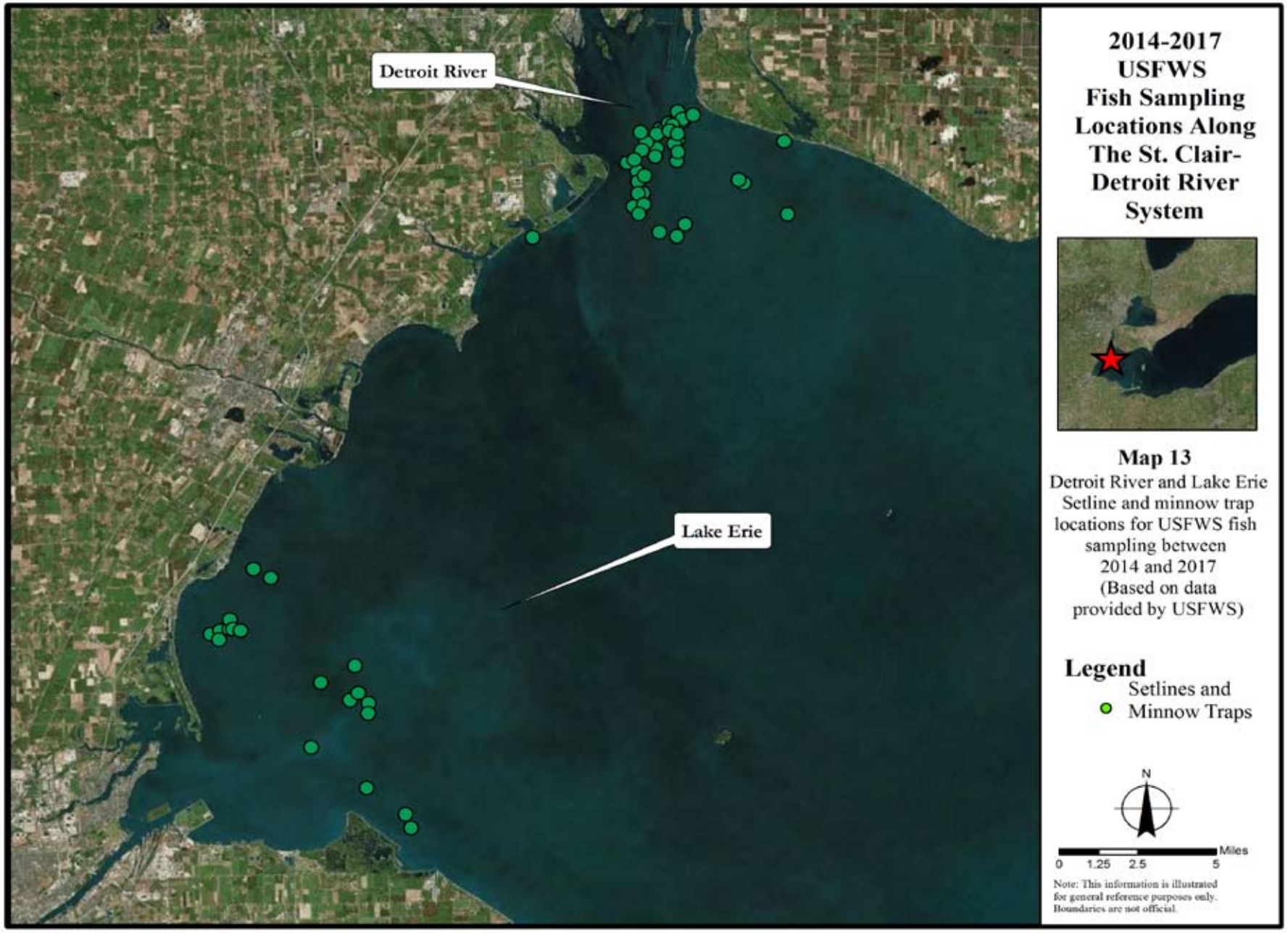

Lake St. Clair
Belle Isle
Detroit River
Grosse Ile
2017 Mudpuppy Captures Along The St. Clair-Detroit River System
Map 9
Mudpuppies captured from the Detroit River by HRM in 2017
Legend
Mudpuppy Capture Locations
N
Miles
0 1 2 4
Note: This information is illustrated for general reference purposes only. Boundaries are not official.


St. Clair River
Lake St. Clair
2014-2017 Mudpuppy Bycatch Along The St. Clair-Detroit River System
Map 10
Mudpuppies captured as bycatch from the St. Clair River and Lake St. Clair by project partners between 2014 and 2017
Legend
MDNR
USFWS
USGS
N
Miles
0 1 2 4
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

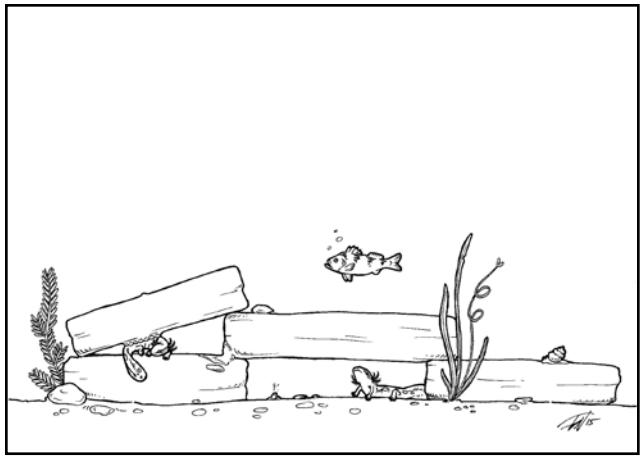

Belle Isle
Lake St. Clair
Detroit River
Grosse Ile
Lake Erie
2014-2017 Mudpuppy Bycatch Along The St. Clair-Detroit River System
Map 11
Mudpuppies captured as bycatch from the Detroit River by project partners between 2014 and 2017
Legend
USFWS
USGS
N
Miles
0 1 2 4
Note: This information is illustrated for general reference purposes only. Boundaries are not official.



Map 12. Various age classes of Mudpuppies captured as bycatch from the SCDRS project area by USGS between 2014 and 2017 (Map provided by USGS).


Detroit River
Lake Erie
2014-2017
USFWS
Fish Sampling
Locations Along
The St. Clair-
Detroit River
System
Map 13
Detroit River and Lake Erie
Setline and minnow trap
locations for USFWS fish
sampling between
2014 and 2017
(Based on data
provided by USFWS)
Legend
Setlines and
Minnow Traps
N
Miles
0 1.25 2.5 5
Note: This information is illustrated
for general reference purposes only.
Boundaries are not official.

4. Restoration



Conceptual designs of Mudpuppy habitat that were incorporated into the Lake St. Clair and Lake Erie Metropark restoration as well as several other projects along the SCDRS.

Objectives

The primary goal of this component of the project was to help address the widespread loss of aquatic habitat for Mudpuppies within the SCDRS by evaluating and testing pilot habitat restoration methods. Objectives for this study included implementing two habitat enhancement and restoration projects targeting Mudpuppies and continuing ongoing monitoring as part of restoration projects previously completed or initiated at the time of this study. In addition, HRM assisted with and provided consultation for additional restoration efforts along the project corridor scheduled to be constructed after completion of this project. Several of the habitat restoration projects evaluated by HRM for presence of Mudpuppies and functionality along the SCDRS corridor were associated with AOC delisting requirements. Objectives of these projects were to address the Beneficial Use Impairment (BUI) of loss of fish and wildlife habitat in the corridor. The Mudpuppy habitat restoration and post-restoration monitoring efforts were conducted concurrently with baseline population and spatial distribution field assessments for the duration of this project.

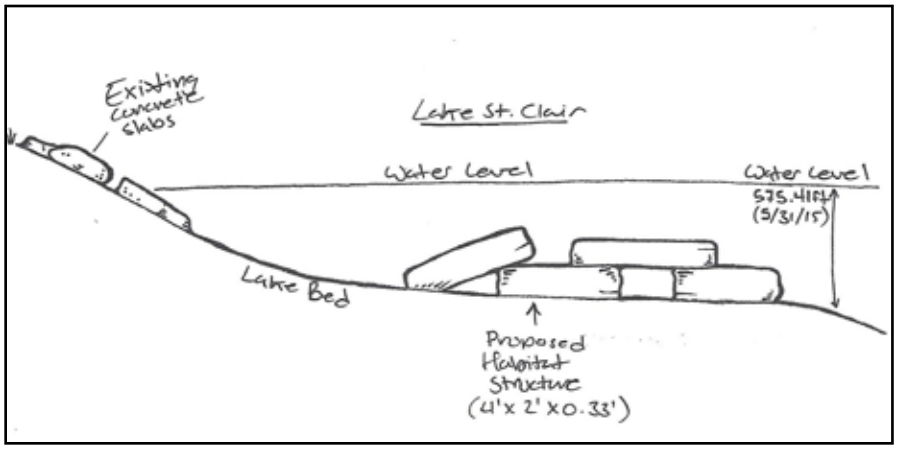
Methods

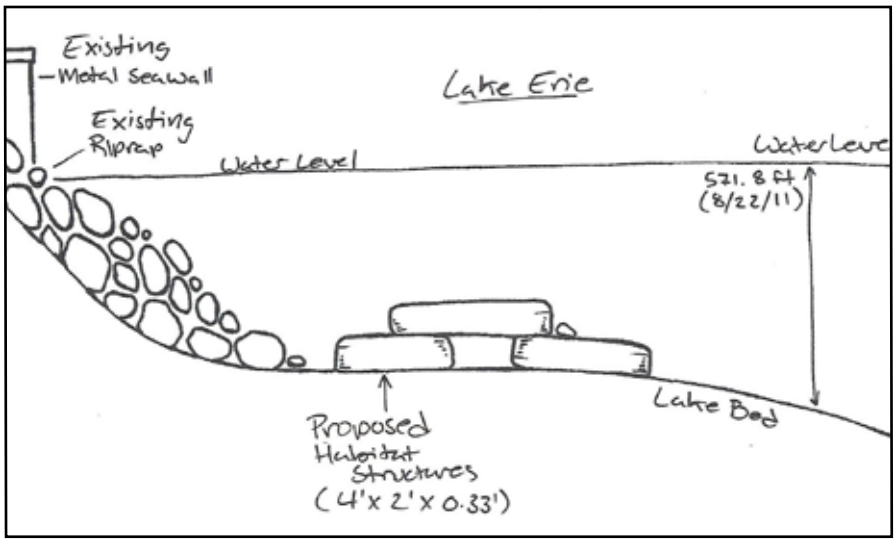
Mudpuppy sampling at each restoration location followed the standard techniques used throughout this project as previously described (See Chapter 3: Field Research). Protocols utilized the deployment of baited traps and opportunistic turning of cover material and dip netting.

Selection of the sites restored directly as part of this project was based on a combination of air photo interpretation, preliminary site assessments and field condition, historical data review, baseline surveys and feasibility. In 2014, Lake St. Clair Metropark and Lake Erie Metropark of the Huron-Clinton Metropolitan Authority (HCMA) were identified as suitable sites for creating new Mudpuppy habitat (Maps 1-2). Sites chosen within the Metroparks were located in aquatic areas where Mudpuppies were historically observed. Evaluation

and selection were also based on suitable conditions for supporting Mudpuppies, but lacked suitable natural or artificial benthic refugia under which Mudpuppies could hide, feed, and nest. Authorization and review were conducted internally within the HCMA Natural Resources and Engineering departments. All necessary permits were obtained in summer 2015 from the Michigan Department of Environmental Quality (MDEQ) and U.S. Army Corps of Engineers (ACOE) for wetland impacts. Sample drawings and proposed depths were provided to better depict restoration objectives (Figures 1-2). Artificial habitat structures measured approximately four feet by two feet by four inches and were made of flat limestone and/or repurposed concrete. These structures were placed in near shore habitat using a combination of backhoe and hand placement and were intended to provide refugia locations were placed along approximately 5,000 linear feet of aquatic habitat split between the two parks. To minimize potential disturbance to Mudpuppies in the area during the active season, restoration activities occurred in late fall when the species occupies cooler, deeper waters. The Lake St. Clair Metropark restoration was conducted in fall 2015 and the Lake Erie Metropark restoration was completed in fall 2016.

Conceptual drawings of proposed Mudpuppy habitat structures in place at Lake St. Clair Metropark and Lake Erie Metropark submitted as part of permit applications to Michigan Departmetn of Environmental Quality and U.S. Army Corps of Engineers.





Mudpuppy habitat structure being placed at the Lake St. Clair Metropark restoration site.

Huron-Clinton Metropolitan Authority

Two locations where restoration was initiated or completed prior to this project and incorporated Mudpuppy habitat structures were also monitored to assess long-term response of the target species. These sites were used as benchmarks to compare initial results from the new restoration sites to help guide additional Mudpuppy restoration elsewhere. The north half of Blue Water River Walk in Port Huron on the St. Clair River and the Blue Heron Lagoon on Belle Isle in the Detroit River were both restored just prior to the start of this project in 2013. The Blue Water River Walk restoration was conducted in multiple phases with some additional Mudpuppy habitat structures placed in 2015. Restoration efforts included the addition of concrete and limestone slabs within near shore habitat throughout approximately 4,300 linear feet of St. Clair River shoreline and 800 linear feet within Blue Heron Lagoon. Based on HRM's previous work, the Blue Heron Lagoon restoration included several hundred feet of existing broken concrete nearshore habitat structure which was left undisturbed. This area was previously proposed for removal and would have likely negatively impacted Mudpuppies in that area. HRM conducted the pre-restoration and initial post-restoration monitoring for both these sites (Herpetological Resource and Management 2013; Mifsud 2014).

The Blue Water River Walk restoration site including Mudpuppy habitat created in 2013.

The Blue Heron Lagoon on Belle Isle with created Mudpuppy habitat. Structures are exposed due to unusually low water levels at time photo was taken in 2013.

Additional restoration sites were also incorporated into HRM's monitoring after this project was initiated. The Kiefer Park and Cottrellville sample locations on the St. Clair River were restored in 2015 and monitoring began the same year following construction. The South Fishing Pier on Belle Isle, which was restored concurrently with the Blue Heron Lagoon, was also included in the sampling efforts. HRM had surveyed adjacent habitats previously and was familiar with these locations prior to restoration. Similar habitat design methods used at the Blue Water River Walk and Blue Heron Lagoon were incorporated at these other sites. Large woody debris including root balls and whole trees, large boulders, and gravel shorelines were incorporated. When feasible, submergent aquatic vegetation was planted to target a wide range of aquatic wildlife. Building on the success of Mudpuppy habitat restoration at other locations, additional restoration projects targeting Mudpuppy habitat within the SCDRS have recently been completed or will begin construction in 2018. These projects specifically target creation of Mudpuppy habitat and include areas on two islands in the Detroit River. The restoration of Stony Island was initiated in late summer 2016 and work on Celeron Island is proposed for summer 2018. These projects include the addition of large, flat rock structures within the near shore habitat and around newly constructed shoals.

The Cottrellville restoration site including Mudpuppy habitat created in 2015.

Results

HRM recorded the presence of Mudpuppies at several restoration sites including Blue Water River Walk, Blue Heron Lagoon, and Cottrellville. Prior to restoration, Mudpuppies were not observed at the Blue Water River Walk. Following the addition of cover habitat, the site yielded multiple captures during each year it was surveyed with two Mudpuppies recorded in 2014, two in 2015, and four in 2016. Sampling at this site was limited to one event in 2017, and one individual was captured. Abundant pre-restoration data were available for Belle Isle from work conducted by both HRM and the Belle Isle Aquarium. The species was documented from the Blue Heron Lagoon previous to restoration through 2012 and HRM recorded captures immediately following the installation of habitat structures in 2013, as well as in 2016. Mudpuppies had not been detected by HRM prior to restoring the South Fishing Pier; following restoration, one individual was captured from that location in 2016. Between 2015 and 2017, Mudpuppies were also recorded at the three remaining locations surrounding Belle Isle.

Juvenile Mudpuppy captured from the Blue Water River Walk by HRM in 2012.

Group of Mudpuppies captured from a single sample event at Cottrellville following restoration and addition of habitat structures.

The Cottrellville location on the St. Clair River produced the most captures at any one site, with a total of nine Mudpuppies collected in 2015 and 2016. HRM did not conduct monitoring prior to restoration in 2015, but did sample nearby. During the first sampling event after the addition of habitat structures, seven Mudpuppies were captured in a single event. In 2016, an additional two Mudpuppies were captured. This site was not assessed in 2017 so that greater focus could be applied to southern sampling sites that had more limited data. Despite the habitat at both Lake St. Clair Metropark and Lake Erie Metropark being constructed similarly to those above, no Mudpuppies were captured from either site following their restoration in neither 2015 nor 2016. The Kiefer Park restoration location also resulted in a non-detection of Mudpuppies by HRM both prior to and following restoration in 2015. One individual was recorded though by USGS in November 2015. The park represented one of the smallest sample sites in the project. While it included suitable aquatic habitat structures, Mudpuppies were not a focal species of the restoration design compared to other locations and this may have contributed to the low rate of detection.

Discussion

Once abundant along the SCDRS, suitable Mudpuppy habitat in the form of submerged logs and fractured limestone shelves has been greatly reduced though heavy anthropogenic activities such as shoreline hardening, dredging, sedimentation, and filling of wetlands. Restoration completed as part of this project created critical shelter, nesting, and nursery habitat in areas where it was absent or limited. Monitoring these and other restoration projects will provide Mudpuppy distribution data that can be used to track the species' health over time in the SCDRS. In addition to maintaining healthy and sustainable Mudpuppy populations, restoring and creating habitat will benefit the endangered Salamander Mussel through protection of their host organism.

Belle Isle's North Fishing Pier before (A) and after (B) restoration and removal of riprap. A noticeable decrease in Muduppies was observed following removal of rocky habitat.

Aquatic structures also provide habitat and refugia for a wide variety of nongame and game fish species, as well as other aquatic wildlife including reptiles and invertebrates.

The addition of fish and wildlife habitat previously absent in the SCDRS has generally improved the overall ecosystem health and functionality of the system. However, some restoration activities had the opposite unintended effect. The removal of riprap shorelines included in some of the shoreline restoration activities may result in a loss of aquatic habitat for some species including Mudpuppies. While riprap may not be considered aesthetically pleasing, it provides critical habitat structure for a variety of aquatic organisms and should be preserved when possible. During HRM's sampling efforts, a noticeable decrease in Mudpuppy captures was observed in locations where near shore riprap aquatic habitat was removed. This included the North Fishing Pier at Belle Isle, where high densities of Mudpuppies were recorded previous to this project. Following the restoration, detection rate was lower with one individual captured at this location by HRM in 2016. Within the Blue Heron Lagoon, rocky habitat was proposed for removal to create deeper water habitat but left onsite following recommendations from HRM and other Mudpuppy researchers. This area now continues to support juvenile Mudpuppies and likely acts as critical nursery habitat supporting the value of maintaining rocky near shore structures. Whenever possible this habitat type should remain on site or replaced with similar materials that is more athletically pleasing but provide similar function such as limestone.

A majority of the Mudpuppies captured by HRM during this project were recorded from restoration sites. Some locations were documented to support Mudpuppies shortly following the addition of habitat structures. At the southern portion of the Blue Water River Walk, Mudpuppies were captured within 24 hours of habitat structures being set in place in 2015. Substantial increases in Mudpuppy spatial distribution and age classes present have been observed at this location since completion of the initial restoration in 2013. During the first survey of Cottrellville following restoration, HRM recorded the highest number of Mudpuppies captured from one location during a single sampling event. Prior to restoration of Cottrellville, numerous Mudpuppies were captured at the adjacent Algonac State Park, an area that lacked substantial nearshore habitat. Following the addition of habitat structures at Cottrellville, Mudpuppy detection at Algonac declined while increasing at Cottrellville. This shift likely suggests

Portion of the Blue Water River Walk site whrere Muduppy density and distribution increased following restoration.

that the restoration efforts were successful and increasing nearshore habitat at Algonac could have an appreciable value to Mudpuppies and other nearshore aquatic species. These locations may serve as tangible examples on the importance of nearshore habitat and the relatively simple method to replicate lost habitat at other restoration sites within the SCDRS region, Great Lakes basin, and potentially throughout the Muduppy range.

Lake St. Clair Metropark (A) and Lake Erie Metropark (B) restoration sites where no Mudpuppy have been observed following addition of habitat structures.

Unique habitat use was observed by USGS including larval and juvenile Mudpuppies found seeking refugia in fish egg mats and eggs deposited on deep water cement anchors. These observations demonstrate the need for habitat that meets the required conditions for egg and larval development within the project area and reveal the potential value of incorporating non-traditional structures into future restoration projects. Few studies exist that examine variability in habitat use of different Mudpuppy age classes. Recent work on the Eastern Hellbender (*Cryptobranchus alleganiensis*), a species similar to Mudpuppy have shown positive results with incorporating artificial spawning structures in their natural habitat (Briggler and Ackerson 2012). Incorporating similar techniques into future restoration projects in the SCDRS will likely provide valuable data on Mudpuppy behavior and habitat preferences as well as provide critical habitat for young age classes that may be lacking in parts of this region.

Despite intensive, multi-year sampling efforts, no Mudpuppies were observed during pre- or post-restoration monitoring at Lake St. Clair or Lake Erie Metroparks. At Lake St. Clair Metropark, the species was recorded within the park as recently as 2012, including several dead individuals observed by HRM while conducting comprehensive herpetofauna surveys of the park in 2011. The Mudpuppies were found within the Black Creek near the marina where restoration occurred for this project. The species is likely present, given recent observations and the park's proximity to other Mudpuppy capture locations from this project. Weather conditions potentially played a significant role in sampling success at this location. Sampling during winter months was inhibited on several occasions by thick ice covering the lake and during periods of no ice coverage, wave action was severe. During several sampling events, wave action was so extreme that traps were destroyed or thrown on shore. These variable conditions could have influenced how Mudpuppies used nearshore habitat in this area. It is possible that severe wave action in the shallows may cause Mudpuppies to move to deeper waters where conditions are calmer, but where sufficient habitat for cover is lacking. This pattern may limit Mudpuppy occupancy of the near shore habitats when ice cover is not present and ultimately result in seasonal avoidance of the area. The Lake Erie Metropark surveys also did not result in any Mudpuppy observations and the species' presence and population health in this region is not clear. Continued monitoring of both of these sites to better understand potential factors inhibiting Mudpuppy colonization is strongly encouraged. It is important to note that despite the absence of detection of target species, juvenile native fish and crayfish were captured indicating the functional use of this newly available habitat and the likely potential for Mudpuppies.

Appendix 4.1 Maps


Restoration Area
Restoration Area
Lake Erie
Lake Erie Metropark Mudpuppy Habitat Restoration
Map 2
Portion of the Lake Erie Metropark where Mudpuppy habitat was restored in 2016
N
Feet
0 125 250 500
Note: This information is illustrated for general reference purposes only. Boundaries are not official.

5. Genetic Research

Overall Objectives

Similar to our understanding of their spatial distribution, little is known about the genetic diversity of Mudpuppy populations within the Great Lakes region. Through the funding provided by GLFWRA, three graduate student research projects were incorporated into this study. The goals of these projects were (1) to determine the genetic diversity of Mudpuppy populations within the SCDRS compared to that of other populations throughout the species' range (Amber Stedman); (2) to assess differentiation in populations within the project area and throughout Michigan to determine if gene flow is occurring within the SCDRS (Dana Leigh); and (3) to test the efficacy of environmental DNA (eDNA) as a non-invasive sampling technique for determining Mudpuppy presence on the landscape (Jenny Sutherland).

Methods

Over 700 tissue samples were collected during field assessments by HRM and project collaborators (See Chapter 5 Appendix A). Samples were preserved in 95% ethanol and transported to the Greenwald lab at Eastern Michigan University for analysis. Tissues from live Mudpuppy were taken via tail clips and various organs including liver and kidneys, eggs, gill operculum, and whole individuals were taken from dead specimens. Samples from nine States (Iowa, Kentucky, Massachusetts, Michigan, Minnesota, Missouri, New York, Ohio, and Vermont) and two Canadian Provinces (Ontario and Quebec) were used for comparison and analysis (Appendix 1). Samples from Michigan were collected from the SCDRS project area as well as from one individual from a Michigan inland lake in the central Lower Peninsula. QIAGEN DNeasy® Blood and Tissue Kits were used to extract DNA, after which standard polymerase chain reaction (PCR) procedures were used to amplify regions of interest. Detailed methodologies and results for each research objective are described below.

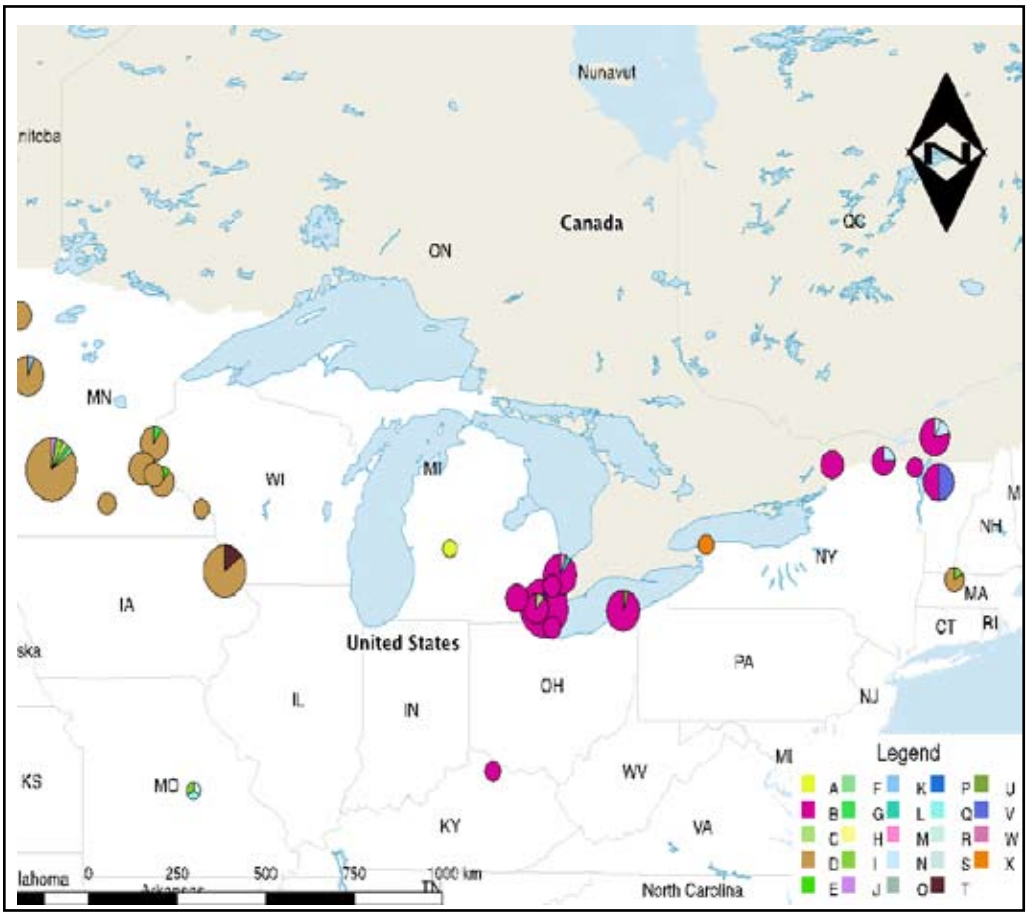
EMU graduate student Amber Stedman with a Mudpuppy captured from the SCDRS project area.

Phylogeographic Analysis

Methods

Phylogeographic analyses were conducted by amplifying a 742 bp segment of the cytochrome b (cyt b) region of the mitochondrial genome (Moritz, Schneider et al. 1992). Each reaction contained 4µl of MyTaqTM Red Mix, 1 µM of each primer, 50–100 ng of genomic DNA, and distilled H2O for a total volume of 10 µl. The PCR program consisted of an initial denaturation of 2 minutes at 95°C followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 1 minute, and extension at 72°C for 2 minutes. A final extension cycle followed for 5 minutes at 72°C. Successful amplification was confirmed using a 1% agarose gel. Successful PCR products were sequenced at the University of Michigan DNA Sequencing Core. Samples from the northeastern U.S. were processed and sequenced using slightly different protocols; see Chellman (2011) for further details.

Forward and reverse sequences were edited using Geneious ver. 6.1.8 (Kearse, Moir et al. 2012). A haplotype tree was generated using TCS in Popart ver. 1.7 using a minimal spanning network and Mega ver 7.0.16 to create a phylogenetic tree using maximum likelihood (Kumar, Stecher et al. 2016). The tree followed the Hasegawa-Kishino-Yano model of evolution, which was deemed most appropriate by Mega given the data. A cyt b sequence from a different Mudpuppy species, the Gulf Coast Waterdog (*Necturus beyeri*), was included as an outgroup from Genbank (Accession Number: GQ368658.1). DNAsp ver. 5 was used to measure molecular diversity and Arlequin ver. 3.5 was used to measure population level diversity (Librado and Rozas 2009; Excoffier



Cyt b haplotypes documented in this study. The geographic range of the mudpuppy is shown in green (REF). The most common haplotypes were D (yellow) and B (blue). The intermediate haplotype (I; light orange) is found in Massachusetts, Minnesota, and Missouri.

Table 1. Pairwise Fst comparisons of cyt b sequences collected from seven watersheds. GL = Great Lakes; MS = Mississippi River; RR = Red River; OH = Ohio River; MO = Missouri River; CT = Connecticut River; LC = Lake Champlain. Asterisks indicate significant values.

	GL	MS	RR	OH	MO	CT	LC
GL	_						
MS	0.83*	_					
RR	0.86*	0.0077	_				
OH	-0.13	0.82*	0.90*	_			
MO	0.77*	0.71*	0.78*	0.67*	_		
CT	0.83*	-0.047	0.20	0.89*	0.55*	_	
LC	0.45*	0.83*	0.85*	0.30	0.66*	0.77*	_

and Lischer 2010). For Arlequin analysis, populations were grouped by watershed with samples submitted from five different watersheds including Great Lakes, Mississippi River, Red River, Missouri River, and Ohio River. A Mantel test was used to determine whether increasing geographic distance correlates with increasing genetic distance. Genetic distances were supplied by pairwise Fst values obtained from Arlequin and geographic distances were obtained by measuring the pairwise distances in kilometers between the midpoints of sampling areas within each watershed.

Results

Phylogeographic analyses included a total of 305 cyt b sequences measuring 742 bp from 27 sampling locations across nine U.S. states and 2 Canadian provinces. The cyt b sequences contained 24 unique haplotypes. The most prevalent haplotype of the cyt b sequences was haplotype D, which was found in Iowa, Minnesota, and Massachusetts. Haplotype B was the second most prevalent haplotype and was found exclusively in eastern states and provinces (OH, NY, MI, KY, ON, QC, and VT). The phylogenetic tree of cyt b sequences shows a separation between eastern and western haplotypes (Figure 1). Haplotype I is shown to be the intermediate haplotype linking the two most common haplotypes (B and D; Figure 2). Haplotype B is one of the most ancestral haplotypes and has differentiated into many eastern and western haplotypes. Measures of sequence diversity revealed that cyt b has a haplotype gene diversity (Hd) of 0.619 ± 0.017 (SD). Nucleotide diversity (π) was 0.00175, and the number of mutations per site (θ) was 0.00545. The average number of nucleotide differences between samples (k) was 1.296. There was a high degree of population differentiation. The Fst value across all populations for cyt b analysis was 0.79. Variation among populations at this locus accounted for 79.68% of the total variation while within population variation accounted for 20.32% of the total variation (Table 1). The Mantel test does not support a correlation between increasing geographic distance and increased genetic distance ($R^2=0.006$, $p>0.05$; Figure 3). However, this result is driven by the presence of a common western haplotype (D) in a likely introduced population in Massachusetts (see Discussion). When this population is removed from the analysis, there is a significant isolation-by-distance relationship ($R^2=0.40$, $p<0.05$).

Discussion

The results of this study provide the most geographically widespread analysis of Mudpuppy genetics to date. The previous research that provided framework for this study found nine unique haplotypes from the northeastern United States (Chellman 2011). The analyses conducted as part of this project identified 21 unique haplotypes. Similar to the Chellman research, the data indicate significant population structure across the analyzed region as demonstrated by the high Fst values. When gene flow becomes increasingly unlikely due to large distances, high Fst values are typical and not unexpected for this data given the distance of hundreds of miles between some groups.

The idea that the increasing geographic separation between populations correlates with increased genetic distance between populations was supported by the results of the Mantel test. Most of the genetic variation was found among populations rather than within them. This may be a result of reduced gene flow between populations or differing selective forces in varying environments. Given that some populations in this study have been separated by hundreds of miles and thousands of years, these results are not surprising. Overall sequence diversity was low, which may be partially influenced by the relatively large body size of Mudpuppies compared to other salamanders (Mezhzherin 2002). Eight of ten pairwise Fst comparisons showed significant genetic differentiation between watersheds (Fst>0.25), a result likely influenced by low gene flow and different selective pressures.

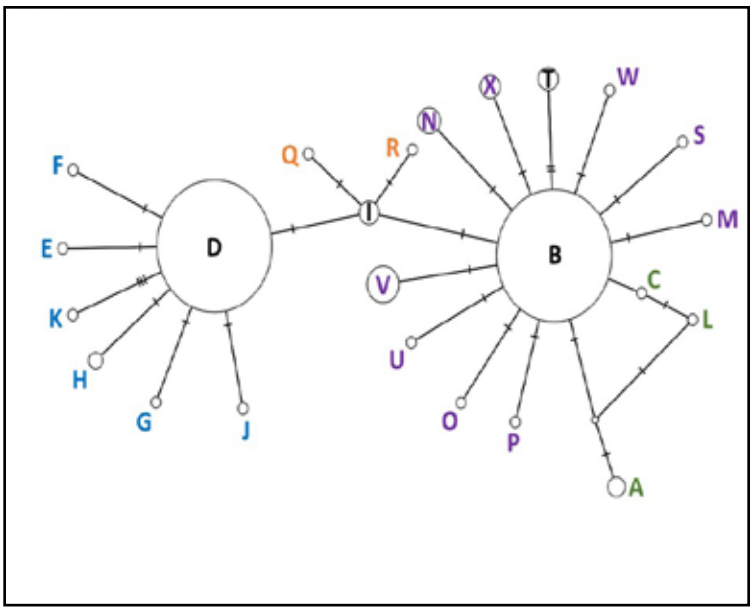


Figure 3. Haplotype network of cyt b haplotypes. Colors refer to haplotype clusters as in Figure 2 (Blue = MN; Orange = MO; Green = MI; Purple = Northeast).

An understanding of the population history of the Mudpuppy and its current levels of genetic diversity can aide in more effective management of this species. Phylogeographic analysis may identify genetically distinct populations. This can warrant separate management to maximize evolutionary potential by avoiding the effects of outbreeding depression, which occurs when the offspring from two genetically separate individuals exhibit lower fitness compared to offspring from more closely related individuals (Allendorf and Luikart 2009; Sabatino and Routman 2009). Based on historic population structure, it may be possible to organize populations into evolutionary significant units (ESUs) or management units (MUs), which may be used to guide future management (Palsbøll, Berube et al. 2007; Pan, Wang et al. 2014).

The phylogeographic tree of cyt b sequences resulting from this work indicates a deep evolutionary split between the eastern and western haplotypes. The divergence between them suggests there is a significant genetic separation between these two broad regions. This may be explained by the route that Mudpuppies took to colonize the Great Lakes after the region was no longer glaciated (Hecht and Walters 1955). Before their colonization, Mudpuppies persisted in refugia either east or west of the Great Lakes region. A study comparing current Mudpuppy distribution to ancient lake levels in Ontario suggests the species established in the Great Lakes during the Nippissing phase (4,000-5,000 BP) (Mills and Hill 2016). Given the deep separation between eastern and western groups, based on the phylogenetic tree and Fst analyses, all watersheds with significantly high Fst values should be managed as separate entities to avoid outbreeding depression. Separate management of these watersheds will help preserve local adaptations needed for survival. As a general rule, organisms removed from their environment should be returned as closely as possible to their original location especially since much remains unknown about the fine-scale genetic structure within watersheds.

Population Genetic Structure along the SCDRS and Lake Erie

Methods

For this study, to determine fine-scale population genetic analyses of Mudpuppy populations within Michigan, novel protocols for microsatellites and PCR conditions were developed. Microsatellite markers specific to the Mudpuppy genome were developed by the Savannah River Ecology Laboratory (SREL) and additional primers developed for the Gulf Coast Waterdog (*N. beyeri*) were added (Table 1). These microsatellite loci were amplified using PCR and genotyped across 312 Mudpuppy samples from the SCDRS project area and portions of eastern Lake Erie (Figure 1).

Locus	Primer Sequence 5' --> 3'	Repeat Motif	Size (bp)
Nema 9	F*: CAATTTCTTTAAGTTGTTTCAATCGC	AAAG	189-229
	R: AGACCTTGAGTCACCGTCCC		
Nema 12	F*: ACACTGCTAGAACATATTGAACCC	ATGG	215-287
	R: AGGGATCTGATGACACGTCC		
Nema 36	F*:AGCGCCTCCAGAGATTGC	ATCT	330-350
	R: GGAGGTCTTATTGCACCAACG		
Nema 2	F*: CCTTTGCATTGGACTCAGGG	ATGG	394-406
	R: AACAATGCACCAAGCAATCC		
Nema 30	F*: CTTGCAAATCACAAGGGAGC	ATCT	272-284
	R: GAACGAGTCAAGAACTTGGTCC		
Nema 43	F*: ACCAGTCTCTCTATTATGGTCCAGC	AAAG	169-197
	R: TGACTGAAGGAAGTGTTCGGC		

*Table 1. Microsatellite loci developed by the Savannah River Ecological Lab for Mudpuppies. * indicates the fluorescently labeled primer.*

At the time of this report, statistical analyses for determining the population genetic structure within the SCDRS were not fully completed. The following statistical analyses are currently underway. GENEPOP v 4.0.1 will be used to estimate observed and expected heterozygosity (HO and HE, respectively), and to evaluate the fit of an isolation by distance model in the SCDRS and eastern Lake Erie sample area (Raymond and Rousset 1995). Hardy-Weinberg Equilibrium (HWE) will be used to test for deviations, which are anticipated assuming that dams, contamination, or distance serves as a deterrent to dispersal and gene flow. This statistic will therefore be used to compare genetic and geographic distances across sampling locations. In addition, FST, which is a measure of the degree of population genetic differentiation across loci, will be used to determine the partitioning of genetic diversity within and among the sampling sites and will be used to determine subpopulations using FSTAT v 2.9.3 (Goudet 1995).

Bayesian clustering will be used to estimate the number of subpopulations of Mudpuppies sampled along the SCDRS and Lake Erie in the program STRUCTURE 3.2.3 (Pritchard, Stephens et al. 2000; Hubisz, Falush et al. 2009). K values (estimated number of subpopulations based on genetic similarities) ranging between one and the total number of sampled sites will be evaluated. Structure Harvester ver. 0.6.8 will then be used to determine the most likely number of Mudpuppy subpopulations based on the resultant delta K statistic (Evanno, Regnaut et al. 2005; Earl and VonHoldt 2011). Bayesian Analysis of Population Structure (BAPS) will also be used to estimate the number of subpopulations of Mudpuppies (Latch, Dharmarajan et al. 2006). Estimates will be compared across STRUCTURE and BAPS.

Contemporary effective population size estimates will be used to determine the influence of habitat fragmentation on genetic connectivity. Estimates using linkage disequilibrium will be generated using the program LDNE (Waples and Do 2006). Forces that alter gene frequency, such as natural selection and mutation, also influence linkage disequilibrium. Therefore, linkage disequilibrium is an important indicator of the forces at work within a system and is thus an appropriate parameter for estimates of contemporary population size (Slatkin 2008). However, effective population size will also be analyzed using a Bayesian computation model in ONESAMP (Tallmon, Koyuk et al. 2008). Both estimates will be included in a final data report and should ideally be similar.

Collectively, the influence of habitat fragmentation by damming and the potential disruption of dispersal and gene flow by water contaminants will be evaluated to provide a Population Viability Analysis (PVA). Site-specific hypotheses will be evaluated to determine the influence of restoration, contamination, and habitat fragmentation on PVA. Models will be produced using demographic and genetic data for local populations of Mudpuppies. The probability of Mudpuppy persistence along the SCDRS and Lake Erie will be estimated using the program VORTEX (Lacy 1993; Lacy 2000).

Results

Three of the microsatellite loci that were successfully amplified have been genotyped across the entire sample set (Nema 9, Nema 12, and Nema 36). The results described here include only data from these three microsatellite loci to prevent a misinterpretation of the data.

FST values for the sampling locations revealed a correlation between geographic distance and genetic differentiation. Values of the five westernmost

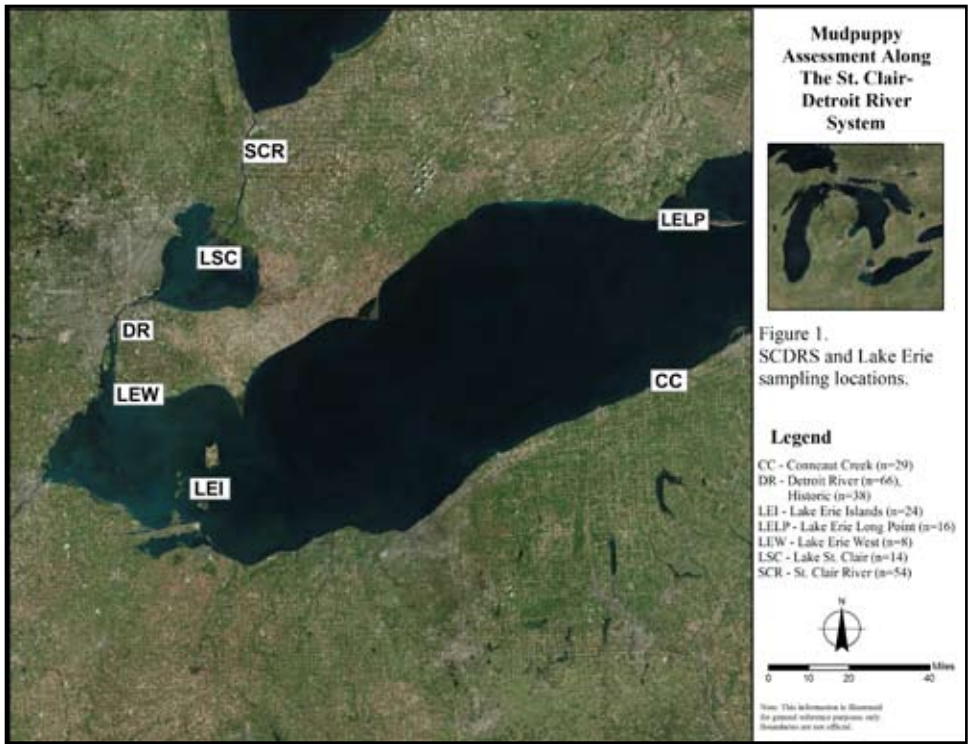


Figure 1. SCDRS and Lake Erie sampling locations.

locations (St. Clair River, Lake St. Clair, Detroit River, Lake Erie West, and Lake Erie Island) showed no significant differentiation. In contrast, the Lake Erie Long Point and Conneaut Creek locations both differed significantly from many of the western locations. Based on STRUCTURE 3.2.3 and Structure Harvester ver. 0.6.8, the samples are divided into two subpopulations (Figure 3&4). When plotted against the potential number of populations ranging between one and eight (total number of sample sites), the log-likelihood of assorting into two subpopulations is significantly higher than for any other number (Figure 3). The STRUCTURE plot for K=2 reveals the degree to which each individual assorts into one of the two subpopulations and shows a much clearer genetic differentiation into subpopulations than the K=8 plot (Figure 4).

Discussion

Microsatellites are highly variable, short segments of DNA consisting of tandem repeats of 1-6 nucleotides. As noncoding DNA, microsatellites have a much higher mutation rate than other DNA regions, providing a method of assessing fine-scale population genetics (Selkoe and Toonen 2006). This is especially important when dealing with small or recently bottlenecked populations as other genetic markers with lower mutation

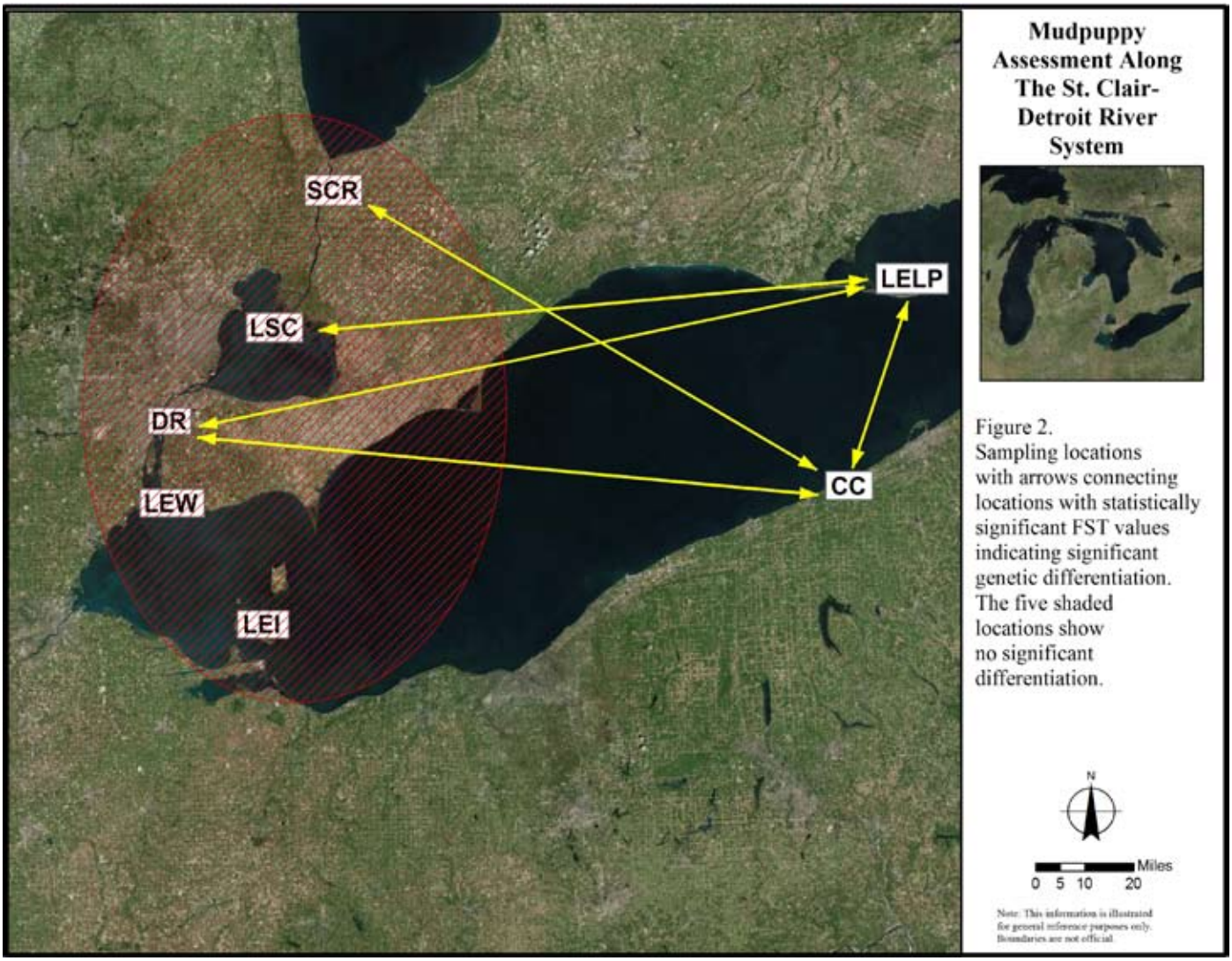
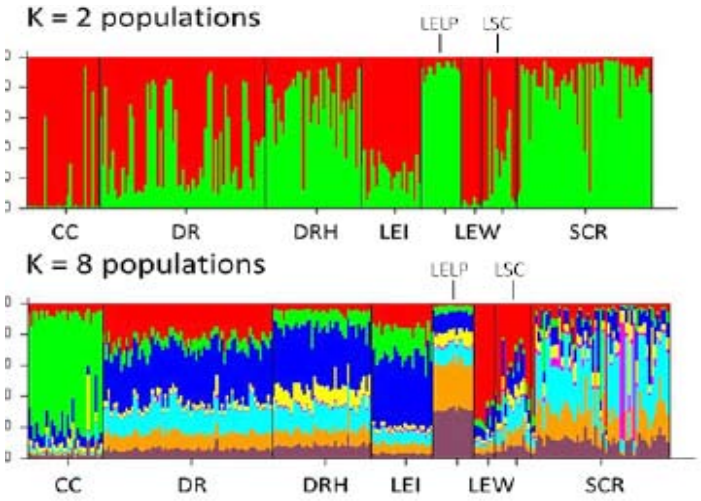


Figure 2. Sampling locations with arrows connecting locations with statistically significant FST values indicating significant genetic differentiation. The five shaded locations show no significant differentiation.



STRUCTURE plots showing the population assignment of each individual sample when divided into K=2 and K=8.

rates may not reflect genetic shifts due to these events, whereas microsatellites will accumulate variation (Selkoe and Toonen, 2006). Therefore, microsatellites are appropriate genetic markers for evaluating the genetic structure of Mudpuppy populations along the SCDRS and eastern Lake Erie. Analyzing additional molecular markers may reveal significant differentiation between various watersheds, which will assist in future management decisions.

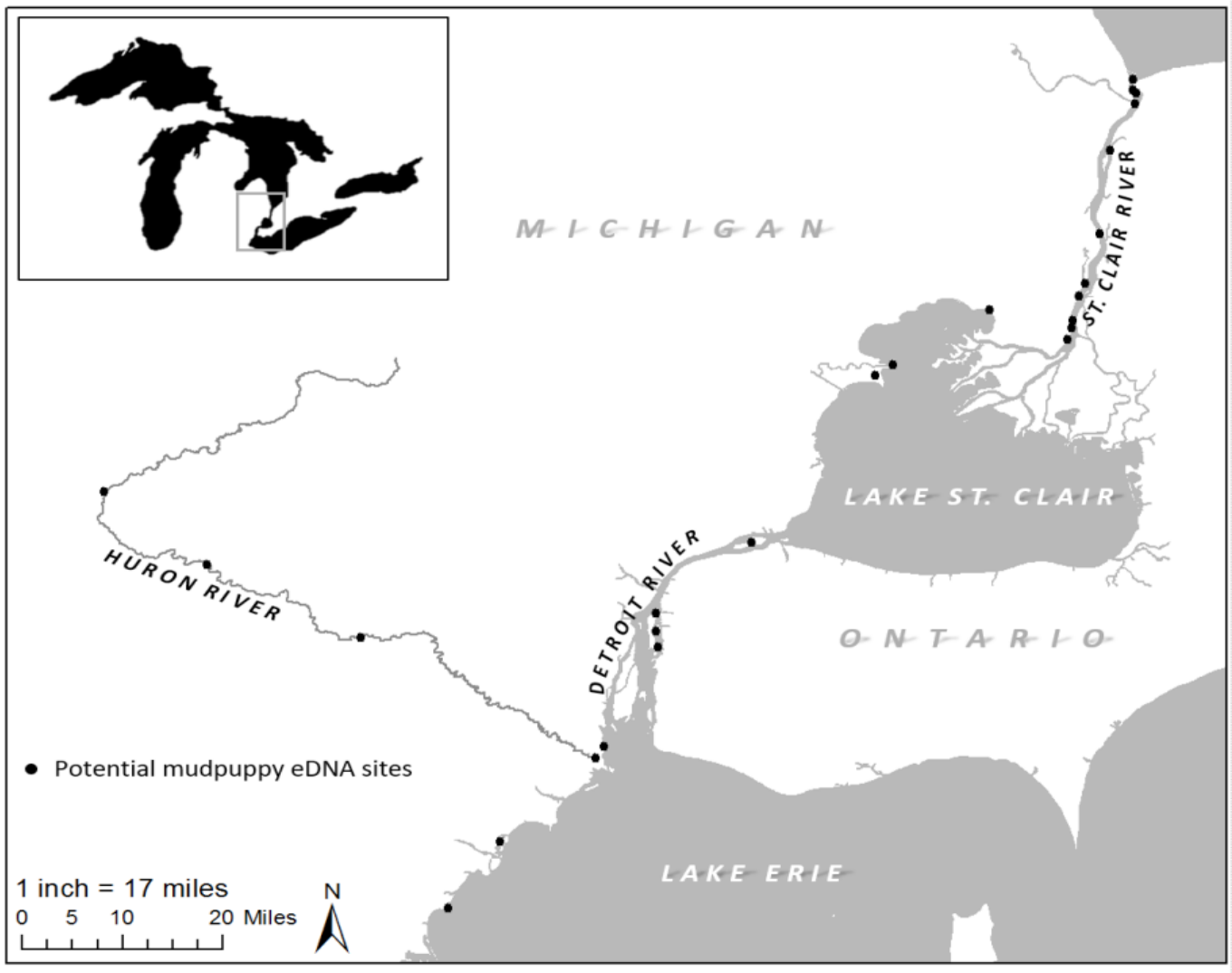
The interpretation of this data is currently limited due to the small number of loci analyzed; it is important to note that it may change once the full dataset is incorporated in spring 2018. It should also be noted that the Lake Erie West tissue samples were collected from near the mouth of the Detroit River as it feeds directly into Lake Erie. No samples were available from the southern Lake Erie basin due to a lack of detection. Based on the STRUCTURE analysis, the sampling locations for this project likely fall into two subpopulations (Figures 3 and 4). With eight geographically separated locations included in the sample set, it is implied that gene flow does occurs between them. When comparing Fst values across the locations, a genetic differentiation appears to increase with distance, separating the western and eastern locations (Figure 2). No statistically significant genetic differentiation was present between the westernmost locations, indicating there is gene flow within this region. The majority of genetic differentiation was observed across western and eastern sample sites, implying isolation-by-distance. However, the two easternmost locations, Lake Erie Long Point and Conneaut Creek, also had significantly different Fst values and fall into different subpopulations based on the STRUCTURE plot as well. Other barriers to gene flow, including contamination of waterways, should be considered upon reanalyzing the full dataset.

Considering these preliminary results, it can be expected that significant gene flow will be observed across the five westernmost locations, which all lie within the SCDRS project area. If these patterns remain following the final analyses, it may indicate that environmental conditions are present within the project area that can be used as a target baseline for other projects and locations. Reducing water contamination, limiting barriers including dams, shortening distances between suitable habitat, and increased presence of artificially restored habitat may be future options for improving gene flow and population viability of Mudpuppy populations in other parts of its range.

Occupancy Modeling and Environmental DNA

Methods

In addition to data collected by HRM through shoreline sampling, data for occupancy modeling was also obtained from setline and minnow trap records from USFWS and USGS. Setline data was from USFWS, while trap data was from HRM and USGS. Setline and minnow trap data was gathered for years 2014 through 2016. USFWS used setline surveys that were set and pulled every 24 hours along the bottom of the river with small and large hooks baited with dead round goby with attached minnow traps baited with cheese cubes (Craig, Mifsud et al. 2015). Shoreline minnow traps were pulled every 24 hours by USGS and HRM and baited with cheese cubes, worms, or a combination of cheese cubes, round gobies, and chicken organs. Shoreline and spawning reef restoration sites were targeted along with presumed unoccupied sites. Catch per unit effort was calculated for each site, gear type, and year.



Location of 22 eDNA sampling sites along the SCDRS project area.

Courtesy of Jenny Sutherland

EMU graduate student Jenny Sutherland collecting water samples for eDNA analyses

Based on initial results of genetic analyses, the feasibility of using environmental DNA (eDNA) was confirmed after testing for issues with filtration methods, contamination, primer specificity, primer binding, and PCR conditions (Stedman 2016). Collection, filtration, isolation of Mudpuppy DNA, and qPCR were conducted using methods developed by Stephen Spears (Spears, Groves et al. 2015). One to four one-liter containers were used to collect water samples from each site to extract eDNA. To prevent contamination, samples were collected 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 sample collection and before transporting back to the lab for filtration (Yamanaka, Minamoto et al. 2016). The samples were run through a Whatman International, Ltd. 0.45 µm cellulose nitrate filter. Filter papers were stored in a centrifuge tube with 95% ethanol and frozen until ready for processing. To avoid contamination between samples, Molecular Bioproducts DNA AwayTM was used to handle the filter papers. Deionized water was filtered concurrently with samples in the lab to test for contamination.

Mudpuppy eDNA within the water samples was analyzed using quantitative PCR (qPCR). A general primer sequence that amplifies DNA from many species was used along with a probe specific to Mudpuppy DNA. This primer/probe combination amplifies a 214 bp region. The qPCR reactions of 15 µL run 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 following cycle was utilized to conduct qPCR; 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. 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. Positive controls were obtained from tissue samples as well as an indoor tank containing adult mudpuppies. The concentration of DNA in the tissue extractions was estimated using a Thermo Scientific NanoDropTM fluorospectrometer.

Courtesy of Jenny Sutherland

EMU graduate student Jenny Sutherland performing eDNA anlysis with SCDRS water samples.

Mudpuppy Trapping Data		USFWS			USGS			HRM		
		2014	2015	2016	2014	2015	2016	2014	2015	2016
Lake Huron	St. Clair River Headwaters	SL	MT/ SL	SL						
St. Clair River	Blue Water Bridge*							MT	MT	MT
	Keifer Park*					MT			MT	MT
	Blue Water River Walk*					MT	MT	MT	MT	MT
	Marysville*						MT	MT	MT	MT
	East China	MT/ SL	MT							
	Cottrellville*					MT	MT		MT	MT
	Algonac State Park							MT	MT	MT
	Russel Island	MT	MT							
	Middle Channel**	MT	MT							
	Pearl Beach		MT							
Lake St. Clair	Fair Haven Boat Launch							MT	MT	MT
	MDNR Fisheries and Research Station							MT	MT	MT
	Lake St. Clair Metropark							MT	MT	MT
Detroit River	Belle Isle* **	MT						MT		MT
	Milliken State Park									MT
	Delray Public Access Boat Ramp	MT	MT	MT						
	Fighting Island**	MT/ SL	MT/ SL	MT/ SL						
	Grassy Island	MT/ SL	MT/ SL	MT/ SL						
	Hennepin Point (Grosse Ile)	MT/ SL	MT/ SL	MT/ SL						
	Turkey Island	MT/ SL	MT							
	Elizabeth Park									MT
	Bridge to Grosse Ile	MT/ SL	MT/ SL	MT/ SL						
	Huron River								MT	MT
Lake Erie	Lake Erie Metropark***									MT
	Pointe Mouilee									MT
	Mouth of Lake Erie	SL								MT
	Sterling State Park									MT
	Toledo Beach Marina									MT

Table 1. Minnow trap (MT) and setline (SL) data from USFWS, USFS, and HRM.

** indicates shoreline restoration, ** indicates fish spawning reefs *** shoreline rock additions for mudpuppies.*

Each sample was run with three replications in addition to positive and negative controls. If all three replications were positive, the samples were not re-run. If two out of three or one out of three replications were positive, the three replications were repeated once more. When two out of the three initial replicates were positive for Mudpuppy eDNA and the same results were reached after repeating the replicates, a positive detection of Mudpuppies was concluded. If only one out three initial replicates from a site were positive and negative results continued after the sample was rerun, the site was considered negative for eDNA.

Occupancy modeling was conducted using the program PRESENCE, which estimates the detection probability and the proportion of sites occupied when the detection of the species is less than one (Mackenzie, Nichols et al. 2002). The assumptions for this model are (1) the Mudpuppy 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/nondetection) at least two times per sampling season. Estimable parameters include ψi, the probability that a Mudpuppy is present at site i, and pit, the probability that a species is detected at site i at time t, assuming it is present. Data from 14 setline sites and 50 minnow trap sites were used to estimate ψ and p for Mudpuppies. With this analysis, it was assumed that species occupancy is constant across the three-season period due to Mudpuppies being long lived and relatively sedentary. Sampling was conducted during periods of time to avoid the seasonal transition of Mudpuppies between shallow water and deep water habitats. Detection probability was varied across the three-year sampling period and the effects of survey method on occupancy (ψ) and detectability (p) were examined.

Results

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 the first season (2014), 14 sampling events in the second season, and 16 sampling events in the third season. For minnow traps, there were 159 sampling events in 2014, 181 sampling events in 2015, and 221 sampling events in 2015. Five sites caught Mudpuppies along the St. Clair River, one site on Lake St. Clair, and ten sites on the Detroit River (Figure 2). Minnow traps had a CPUE of 0.0076 ± 0.0014 and setlines had a CPUE of 0.0014 ± 0.0022 (Figure 3A). 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 where there were not rock additions had a CPUE of 0.0031 ± 0.011 for minnow traps and 0.00069 ± 0.0016 for setlines (Figure 2B). Occupancy (ψ) was slightly higher for minnow traps (0.69 ± 0.17) than setlines (0.54 ± 0.11; Figure 4). Detection probability (p) for the three-year period was 0.21 for minnow traps and 0.60 for setlines (Figure 4).

Mudpuppy DNA was detected in water samples from nine sites from the St. Clair River and Detroit River. One site from the St. Clair River, at the Fairhaven Boat Launch, did not have Mudpuppies detected by trapping during this study (Table 2). However, a dead Mudpuppy was observed washed ashore at this site during HRM's sampling efforts. All sites where Mudpuppies have been trapped had positive eDNA results.

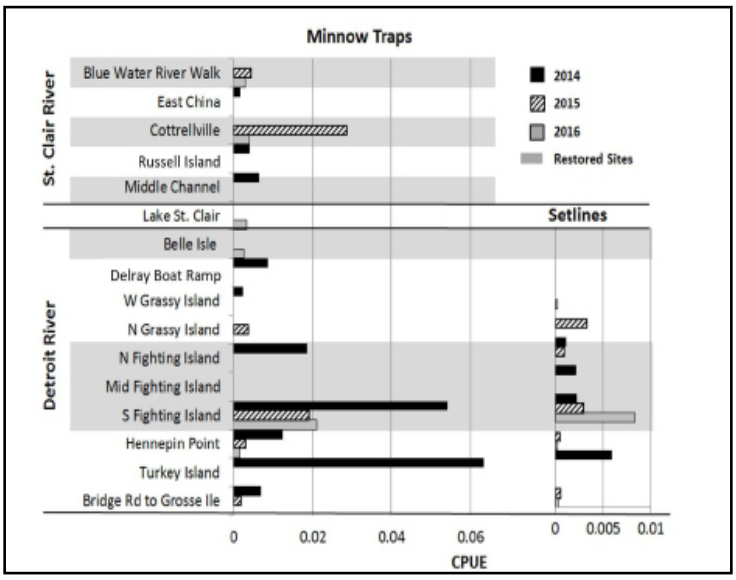


Figure 2. Catch per unit effort (CPUE) for minnow traps and setlines during the months of April and May. Figure does not include sites where mudpuppies were not captured. Shaded sites indicate restorations (fish spawning reefs or shoreline restorations).

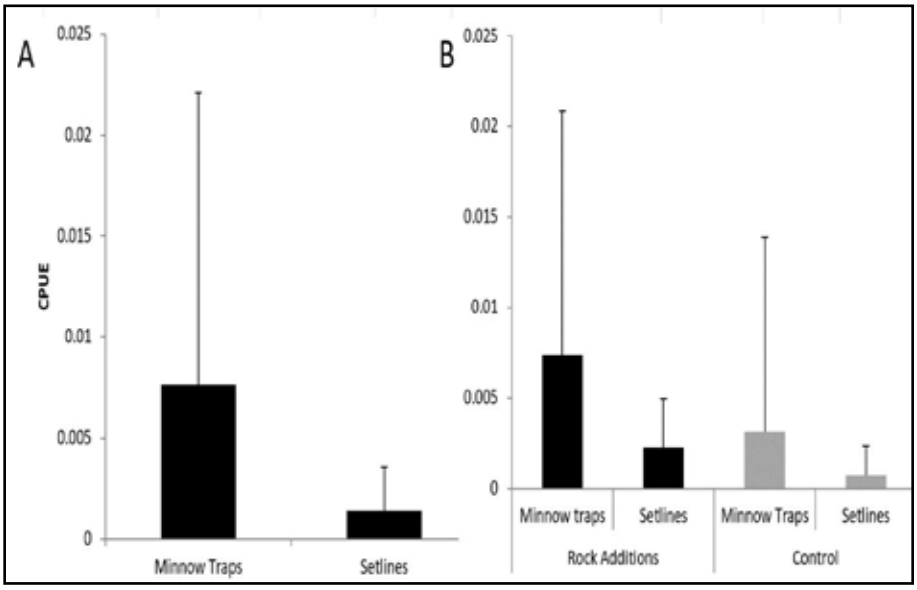


Figure 3: Review of catch per unit effort for setlines and minnow traps during the months of April and May (A) and sites with rocks added (spawning reefs/shoreline restorations) compared to control sites without rock additions (B).

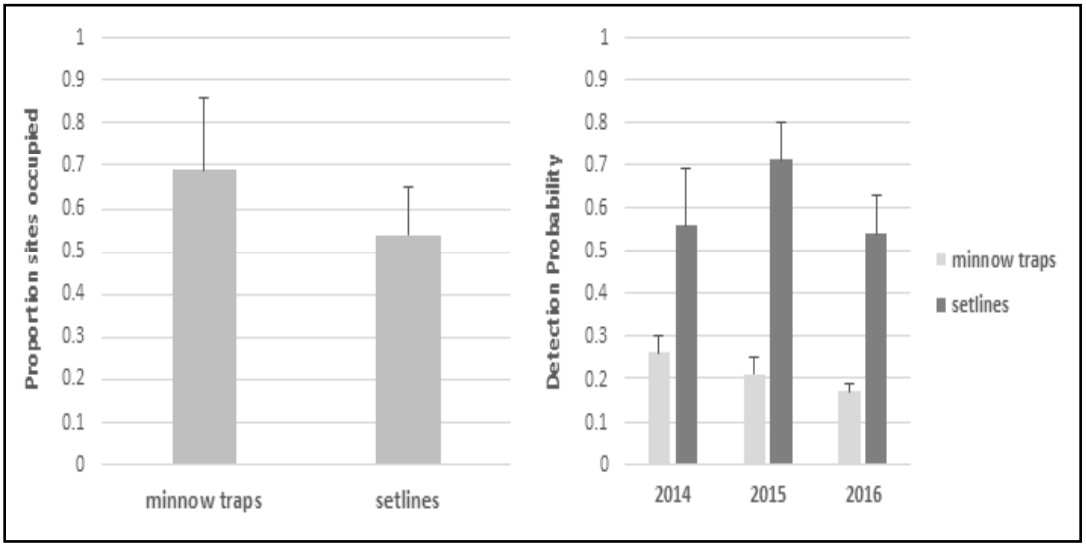


Figure 4. Occupancy and detection probability for mudpuppies along the St. Clair-Detroit River System using the program PRESENCE. Occupancy was 0.69 for minnow traps and 0.54 for setlines.

Discussion

	Trapping Detection	eDNA Detection
Huron River (1)	-	-
Huron River (8)	-	-
Huron River (10)	-	-
Huron River (15)	-	-
Lake St. Clair Metropark	-	Inconclusive
Cottrellville	+	+
Blue Water River Walk (Sugar)	+	+
Blue Water River Walk (Gray Fox)	+	+
Blue Water River Walk (Kramer)	+	+
Algonac State Park	+	+
Marysville (1)	+	+
Marysville (2)	+	+
Fairhaven Boat Launch	-	+
Belle Isle Nature Center Aquarium	+	+

Table 2: Preliminary eDNA results compared to trapping.

The methods tested as part of this research component can be important tools in the management of cryptic and hard to study species such as the Mudpuppy. Minnow traps are typically the technique used for targeting Mudpuppies and were utilized for a majority of the sampling during this project. Occupancy was determined to be slightly higher from minnow traps compared to setlines. These results likely reflect the higher concentration of minnow traps used compared to setlines for the analysis. Minnow traps were calculated to have a low probability of detection, which increased nearly threefold when setlines were utilized. The total capture numbers support these analyses with 200 Mudpuppies captured from 561 minnow trap sampling events compared to 172 Mudpuppy captures from only 49 setline sampling events. While setline surveys appear to have higher degree of success in Mudpuppy detection, this technique requires higher sampling effort, results in temporary injuries to animals, and is not commonly used for targeted Mudpuppy surveys. These results support the need for occupancy modeling to more accurately reflect species abundance, particularly when minnow traps are used. However, results should be interpreted with care as occupancy models assume the populations are closed to immigration and emigration, which is likely not the case for Mudpuppies in the SCDRS.

Results from the eDNA analysis are still preliminary; however, it appears that Mudpuppy presence can be successfully determined through eDNA along the SCDRS. All of the sampling locations known to have Mudpuppies present through conventional sampling also had positive eDNA results as well. Additional follow up studies will be required to verify the utility of eDNA sampling for Mudpuppies. However, if these positive results remain consistent at sites where Mudpuppies are known to be present from trapping, the use of eDNA can be considered as an optional tool for assessing Mudpuppy occupancy in the SCDRS. Given the low detection probability of some survey techniques and bias towards older age classes, this technique may be a useful tool for better determining Mudpuppy presence in aquatic habitats. This may include sites where traditional sampling methods are difficult or cost prohibitive. In addition to its function for Mudpuppies, there is potential for this method to detect other cryptic aquatic species. Future assessments should include additional surveys targeting Mudpuppies along the Huron River to verify the positive eDNA result where the species has not been detected through trapping.

Appendix 5.1 Tissue Sample Tables

Range Wide Mudpuppy Tissue Samples				
Region	Location/Water Body	Year	Submitted By	Number of Samples
New York	St. Lawrence River	1993	C. Bishop	1
		1995		3
Canada	Batiscan River	1993		1
		2015		2
	St. Lawrence River	2015	NA	15
	Des Prairies River	1993	C. Bishop	1
		2015		2
	Long Point, Lake Erie	1995	C. Bishop	16
	Kemptville Creek	2014	Kiley Briggs	12
	Lake St. Louis	2015	NA	6
	Lake St. Pierre	2015	NA	4
	Ottawa River	2015	NA	2
	Outaouais River	NA	Jean-Francois Desroches	2
	Lake Saint Francis	2015	NA	6
	Lake St. Clair	1995	C. Bishop	5
	Richelieu River	2015	NA	5
	St. Laurent River	NA	Jean-Francois Desroches	19
Minnesota	Big Cobb River	2011	Kristen Larson	1
	Big Cormorant Lake	2014		8
	Cannon River	2015		1
	Chippewa River	2011		2
	Clearwater River	2013		1
	Grindstone River	2015		23
	Hay Creek	2015		8
	Kettle River	2015		26
	Lake Latoka	2014		17
	Lower Tamarack River	2015		11
	Minnesota River	2013		4
		2014		26
		2015		72
	Mississippi River	2012		11
		2013		19
		2014		10
	Otter Tail River	2013		8
	Red Lake River	2013		8
	Sand Creek	2015		4
	Snake River	2015		23
	St. Croix River	2010		6
		2011		8
		2012		1
		2015		40
	Sunrise River coll	2015		3
	Wolfe Island	2015		8
	Yellow Medicine River	2011		2

Table 1. Mudpuppy tissue samples collected from across the species range.

Range Wide Mudpuppy Tissue Samples				
Region	Location/Water Body	Year	Submitted By	Number of Samples
Ohio	Conneaut Creek	NA	Tim Matson	30
	Gibraltar Island	2015	Katy Greenwald	1
	Lake Erie Islands	2001	Rich King	1
		2003		2
		2004		1
		2005		6
		NA		12
Kentucky	Licking River	2015	Mason Murphy	5
Missouri	Gasconade River	2009	MODNR	1
		2010		3
		2011		1
		2012		3
Michigan	Lincoln Lake	2014	MDNR	3
Iowa	NA	NA	Jeff LeClere	29

Table 1 (cont.). Mudpuppy tissue samples collected from across the species range.

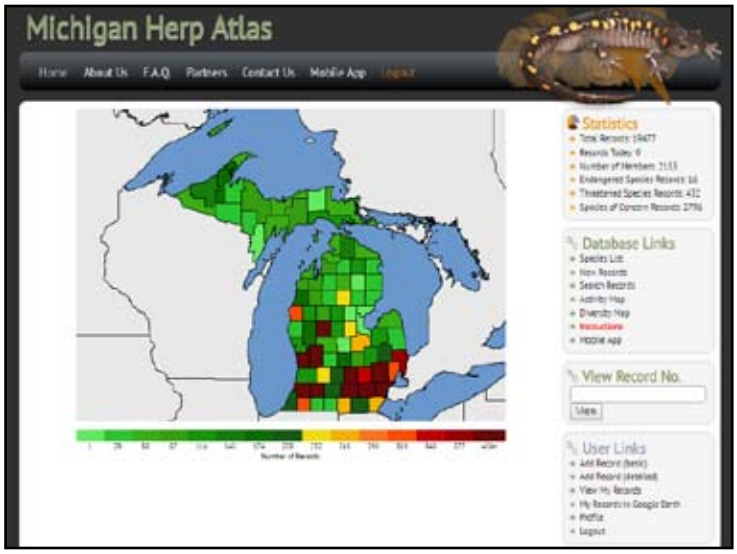
SCDRS Project Area Mudpuppy Tissue Samples						
Agency	*Year*	*St. Clair River*	*Lake St. Clair*	*Detroit River*	*Lake Erie (Mouth of Detroit River)*	*Huron River Watershed*
C. Bishop	1995	X	X	38	X	X
MDNR	2015	0	2	X	X	X
	2016	4	14	X	X	X
USFWS	2014	12	0	1	9	X
	2015	1	0	47	0	X
	2016	0	0	0	0	X
USGS	2014	4	1	13	0	X
	2015	18	0	6	0	X
	2016	15	3	0	0	X
HRM	2014	8	2	0	X	X
	2015	8	6	1	0	2
	2016	6	1	8	0	0
	2017	0	X	6	0	X

Table 2. Mudpuppy tissue samples collected from project partners within the SCDRS project area. X represents areas not sampled

6. Outreach and Education

Objective

Complementary to field assessments, habitat restoration, and genetic analyses, considerable collaborative effort was placed on incorporating a campaign to educate and engage the public about Mudpuppies. The overall goal was to promote the project objectives and to help foster support for conservation of this species and their habitat as well as help gather additional opportunistic data within the project area. Objectives included increasing awareness of Mudpuppies and the critical role they play in Great Lakes ecosystems; dispelling myths and misinformation about this species; and encouraging community members to help provide observations to supplement distributional data through submissions of Mudpuppy observations to the Michigan Herp Atlas.



The Michigan Herp Atlas online database where both environmental professionals and the general public can submit observations of amphibians and reptiles to help monitor species populations in the state.

The Michigan Herp Atlas is a publicly accessible (though spatially restricted) database of herpetofauna observations within the state of Michigan. Initiated by the MDNR in 2004 and expanded through a partnership with HRM in 2010, this valuable citizen science resource was created to provide evidence of trends in species distributions and population health over time. The Michigan Herp Atlas presents an opportunity for everyone to contribute to the conservation of amphibians and reptiles. Importantly, it allows for future evaluation of Michigan's herpetofauna status and conservation measures to be made based on up-to-date science.

Methods

Outreach efforts were conducted primarily by HRM and our project partner, Michigan Sea Grant. Part of the National Sea Grant network, Michigan Sea Grant supports a variety of research and education projects in coastal communities. During HRM's sampling events in the SCDRS project area, staff interacted with interested local community members including many anglers, hunters, and outdoor enthusiasts. Conversations were

focused on providing information about the Mudpuppy, their importance as a bioindicator species, how they help to control invasive species, threats facing Mudpuppies, efforts underway to protect them, and how citizens can get involved through the Michigan Herp Atlas. Anecdotal accounts (including historic frequency of capture when compared to present) and potential sample location information were gleaned from the public when possible. Public engagement with community members and others who rely on the Great Lakes for recreation and income was also conducted at several Michigan Sea Grant workshops where HRM gave brief presentations on the project and results to date. At the workshops, HRM talked directly to anglers about the importance of Mudpuppies for Great Lakes ecosystem health and how to properly remove hooks and release them if accidently caught while fishing. HRM also participated in the grand opening of the Blue Water River Walk restoration site where community members were informed about the construction of Mudpuppy habitat.

HRM engaging the public while conducting Mudpuppy surveys within the SCDRS by providing information about the species and their ecological importance.

Materials produced as part of the outreach objectives included interpretive signage, informational flyers, online articles, news articles, and educational videos relating to Mudpuppies. Interpretive signage describing Mudpuppies and their ecological value was designed using Microsoft Publisher for installation at the new restoration sites within the Metroparks. Educational flyers designed using Microsoft Publisher and Adobe InDesign CS3 were distributed to the general public by HRM and project partners at events, through social media, and via email listserves. Several measures were taken to reach community members through multiple forms of media including publications and online videos.

Results

Throughout the fieldwork conducted between 2014 and 2017, HRM staff engaged in several conversations with local community members about Mudpuppies. Anecdotal data collected suggests that observations of Mudpuppies have become less frequent in the last 20 years within the SCDRS. This statement was comparatively the most frequently heard from locals who regularly fished and recreated in this area. Other commonly heard statements were related to the myths and perceived nuisance that Mudpuppies pose to game fish. After explaining to engaged anglers about the role Mudpuppies play in the ecosystem including, as predators of invasive round gobies, their positions largely changed and anglers committed to stop throwing them onto the ice when ice fishing. Several individuals even became interested in learning more about how to help increase Mudpuppy numbers in the region and help conserve their populations. Many were also very willing to provide localities for their observations and when possible, these reports were used to modify sample locations within the region to include sites where Mudpuppies have been reported. Other anecdotal data from

HRM presented at several fisheries workshops including one focused on Lake St. Clair.

the public included interesting behavior patterns of Mudpuppies in heavily urbanized segments of the Detroit River. While surveying the St. Clair River in 2015, HRM spoke with a retired Detroit Police underwater recovery specialist. He shared that Mudpuppies often used artificial structures such as sunken cars or boats as refugia in the Detroit River. It was apparently not uncommon for 30 or more Mudpuppies to be recovered from a car once returned to the surface. Based on additional research of social media including online blogs, this behavior has been observed numerous times and there are reports of Mudpuppies using sunken cars as structures for adhering their eggs to (Derringer 2009).

HRM participated in four fisheries workshops hosted by Michigan Sea Grant extension in partnership with fisheries agencies and stakeholder organizations. These annual workshops offered to the public focus on current research and information related to the regional status of Great Lakes fisheries. HRM gave brief presentations introducing the local citizens to the Mudpuppy project and its objectives. Through these events, attendees were informed about the importance of Mudpuppies, and encouraged to return them to the water when caught. Attendees were also asked to submit their observations to the Michigan Herp Atlas. Stakeholders at these meeting were diverse and included anglers, charter boat captains, resource professionals, and other interested groups.

Interpretive signage was provided to the Huron-Clinton Metropolitan Authority for display around the new restoration sites at the Lake St. Clair and Lake Erie Metroparks. These resources were also provided to the John Ball Zoo in Grand Rapids, Michigan, for exhibit signage that describes the importance of Mudpuppies. Informative flyers and handouts were distributed at various events over the span of the project period. In addition to providing handouts, presentations and posters were provided by HRM and our project collaborators at the International Association for Great Lakes Research Conference, Joint Meeting of Ichthyologists and Herpetologists, Michigan Wetland Association Conference, Midwest Fish and Wildlife Conference, Michigan Partners in Amphibian and Reptile Conservation Conference, Midwest Partners in Amphibian and Reptile Conservation Conference, the St. Clair-Detroit River System Initiative Meeting, and the Stewardship Network Conference. HRM, EMU, and other project partners shared results and future objectives of the project at several privately held team workshops as well as through group emails and conference calls. In December 2015, an online article, The Misunderstood Mudpuppy was published by Michigan Sea Grant on their webpage that provided basic information on the Mudpuppy including a list of common myths and the associated facts (Bohling 2015). Michigan Sea Grant also assisted in the development of a series of educational videos that describe the species, their ecological importance, and steps to take when a Mudpuppy is accidentally captured on a fishing hook. The first video also titled The Misunderstood Mudpuppy is now available on YouTube with additional videos scheduled for release in early 2018, which will also be viewable on YouTube as well as Michigan Sea Grant

Michigan Sea Grant

Michigan Sea Grant filming captive Mudpuppies at the Potter Park Zoo in Lansing, Michigan as part of the development of educational videos.

Interpretive signage like this display at the Blue Water River Walk is an effective tool for providing information to local citizens.

social media pages (http://msue.anr.msu.edu/news/video_features_michigans_largest_fully_aquatic_salamander_msg18_bohling18?platform=hootsuite).

Discussion

Education and community engagement can be an effective tool and strategy for conservation projects by helping to ensure the short and long-term protection of habitats and species. Public understanding and education regarding riverine systems in the United States and the threats they face has been considered limited for several decades (Doppelt 1993). The National Research Council has stressed the importance of outreach and endorses citizen environmental education in order to develop widespread stewardship of aquatic resources and to address the training of the next generation of scientists and policy makers (National Research Council 1996). Outreach programs are also an effective tool for improving the recovery success of rare and imperiled species. Through these programs, residents from local communities can gain a greater understanding of their local ecosystems and scientists can gather data previously unavailable through traditional research (Brewer 2002).

Throughout this project, HRM and project partners were successful in raising awareness for the Mudpuppy including its needs as a sensitive indicator species and its population health within SCDRS. Teaching local community members about its importance as a bioindicator species and the current status of Mudpuppies in the region also provided opportunities to discuss water quality and habitat degradation in the SCDRS and other aquatic systems within the Great Lakes. Many of those who corresponded with field crew were enthusiastic and eager to learn more about Mudpuppies and learn of the efforts to improve water quality and fish and wildlife habitat in this region.

Studies focused on environmental education indicate that conservation strategies are often considered more valuable and effective when incorporating citizen participation (Bjorkland and Pringle 2001). A central focus of public engagement in this project was encouraging community members to participate in citizen science by submitting their observations the Michigan Herp Atlas. A detectable increase in reported Mudpuppy observations was observed in the online atlas database including observations from recent restoration sites. Prior to initiating this work, the database included 25 records of Mudpuppy observations from across the state that were entered between 2010 and summer 2014. Following outreach activities between fall 2014 and winter 2017, 34 Mudpuppy observation records were submitted including several historic sightings (MIHerpAtlas 2018). In this case, providing Michigan

Mudpuppy observations submitted to the Michigan Herp Atlas increased following outreach efforts as part of this work.

citizens with the opportunity to directly contribute to the conservation of a native species appears to have been successful. New observations have included restoration locations where anglers have not seen Mudpuppies in over ten years. To date, the Michigan Herp Atlas represents the largest collection of Mudpuppy locality data for the state and has provided a valuable resource for recent conservation projects in the Great Lakes region including, efforts to restore populations of the imperiled Salamander Mussel (Lambert, Hinz Jr. et al. 2016).

An effort to spread awareness regarding the myths surrounding Mudpuppies and discouraging persecution of the species has been successful. Reports from project partners at Lake St. Clair Metropark and the MNDR Lake St. Clair Fisheries Research Center indicate that far fewer Mudpuppies have been found frozen on the ice by anglers in recent years. Anglers have also encouraged other anglers to put them back. In addition to resources published through the Michigan Sea Grant, other institutions have begun to participate in informing Michigan citizens about Mudpuppies and the threats facing the species (Eckert 2016). Educational videos planned for future release will likely build on this new foundation of knowledge and provide anglers and outdoor enthusiasts additional resources such as how to safely remove fishing hooks and release Mudpuppies back to the water. Continuing to incorporate interpretive signage that provides important facts about the Mudpuppy may be another effective tool for further spreading awareness about the imperiled species in the SCDRS. Public involvement and engagement will be critical in helping foster support for future restoration along the project corridor and helping shift the mindset to protect these important species in the SCDRS.

Appendix 6.1 Outreach and Education Flyers

Flyer created by HRM to educate the public about Mudpuppies, the role they play in the Great Lakes, and what can be done to help conserve Michigan populations. Flyers were distributed at various public events including workshops and conferences.

The Mudpuppy - (*Necturus maculosus*)

What is A Mudpuppy?

- Michigan's largest, fully aquatic salamander.
- This cold water adapted species can be found in lakes and rivers throughout the Great Lakes Region. They prefer aquatic habitat with abundant cover such as rocks, logs, leaf litter, or other debris.

Jason Foit

Mudpuppies have bushy red external gills they use to breathe underwater. They have slimy skin and bodies that are brownish-gray with scattered dark spots and a yellowish belly.

Why are They Important?

- "Bioindicators" – They are sensitive to pollutants and poor water quality and therefore act as an early warning system for environmental problems.
- They play a critical role in local ecosystems as the only intermediate host to the State Endangered Salamander Mussel. They are also known predators of the round goby, a problematic invasive species.
- Great Lakes populations are declining and their true abundance is unknown.

Mudpuppies are a declining species in the Great Lakes region. Help conserve these unique animals by submitting your observations of Mudpuppies to the Michigan Herp Atlas.

What You Can Do!

- Mudpuppies are a misunderstood species. Due to their unusual appearance and incorrect assumption as game fish predators, when are caught on fishing lines these harmless salamanders are frequently thrown on land or the ice and left to die. If you catch a Mudpuppy while fishing, please carefully remove any hooks and return them to their natural habitat.
- Report any observations of Mudpuppies to the Michigan Herp Atlas at www.miherpatlas.org

Mudpuppies are often caught on fishing lines. If you accidently catch one of these aquatic salamanders, carefully remove the hook and release it .

www.Flikr.com

Interpretive signage developed by HRM for potential use at sites with restored Mudpuppy habitat.

7. Conclusion and Future Objectives

The Mudpuppy is recognized as an important indicator species due to its sensitivity to water quality and habitat disturbance. It is a critical component of healthy, functioning Great Lakes ecosystems, notably as the only host organism for the state endangered Salamander Mussel. Populations of this fully aquatic salamander are known to be declining in Michigan and it has been identified as a focal species for the St. Clair-Detroit River System in the Michigan Wildlife Action Plan. Prior to this work, Mudpuppy research had been limited in the Great Lakes region, and information on population status and genetic diversity was lacking. This project was conducted to fill in the large gaps in data as well as help conserve the species through restoration and public outreach campaigns. Restoration and conservation of Mudpuppy populations will help create and sustain healthy Great Lakes habitats and aid in the recovery of other rare and imperiled species. Efforts led by HRM and EMU, with a wealth of support from project partners and collaborators, were successful in accomplishing the objectives developed to reach these goals.

Field Research

Several years of collaborative intensive sampling and field assessments resulted in new, current spatial distribution and relative abundance data for the project area. The true status of a cryptic and elusive species such as the Mudpuppy can be difficult to fully determine within a short study period (Graeter, Buhlmann et al. 2013). Information gathered through this work will serve as a critical baseline for developing a more complete understanding of Mudpuppy health in the Great Lakes. These data can be used to determine where conservation management should be prioritized within the SCDRS to restore Mudpuppy habitat and where measures should be taken to protect robust populations already present. This work also helped identify areas that should be further evaluated to investigate potential factors that may be inhibiting Mudpuppy presence and dispersal within the system. As a long-lived and fully aquatic species, Mudpuppies rely on healthy, permanent water body habitats. Population viability and genetic flow are contingent on contiguity of habitats. Based on information gathered by both HRM and project partners, relatively healthy Mudpuppy populations appear to be present in the St. Clair River, Lake St. Clair, and Detroit River, while no Mudpuppies were detected from western Lake Erie or most of the Huron River.

No Mudpuppies were detected in southwestern Lake Erie despite significant survey effort placed on that region from HRM's nearshore and USFWS fish sampling. The success in capturing Mudpuppies utilizing the same

techniques within other regions of the study area and observations as close as the mouth of the Detroit River to Lake Erie, compared to the total lack of captured Mudpuppies from the sample sites along the western shore of Lake Erie reveal a critical area in the SCDRS that should be the focus of future assessments. Given that the SCDRS has undergone several significant environmental disturbances in recent years, the lack of Mudpuppies may be a result of these disturbances. In addition to known threats to the health and biodiversity of the Lake Erie basin, climate change remains another factor that may affect this ecosystem more drastically than others in the Great Lakes particularly for cold water adapted species like the Mudpuppy (Pearsall, Carton de Grammont et al. 2012). Given their affinity for inhabiting cooler water habitats, determining the population health of Mudpuppies in Lake Erie may provide an effective tool for tracking the long-term effects of climate change and increasing water temperatures on the ecosystem.

The numerous dams on the Huron River were identified early on as focal points for assessing potential barriers to Mudpuppy movement. The lack of Mudpuppy captures and limited records from years prior to this study suggest the heavily fragmented nature of the river may be among the most significant factors leading to small or possibly locally extirpated Mudpuppy populations in the Huron River. Other factors may include nutrient loading, historic contaminants, seasonally shallow warm water, and persecution. Additional field assessments and site evaluations including analyses of water quality and adjacent land uses should be conducted for both Lake Erie and the Huron River.

Ultimately, the population density and spatial distribution data gathered for Mudpuppies can be used as a tool to help conserve the imperiled Salamander Mussel, which relies on the Mudpuppy as an obligate host and critical habitat during the larval stage. (Environment and Climate Change Canada, Canadian Wildlife Service et al. 2016; Bogan, Woolnough et al. 2017). Genetic variability can be an effective tool to compare the dispersal capabilities of Mudpuppies and the issues barriers may place to them, fish, and other wildlife species. Known distribution of Mudpuppies can also be used to more effectively utilize resources and determine priority sampling areas where Salamander Mussels may potentially be present.

Restoration

Mudpuppy habitat restoration monitoring data supports the value of creating and restoring aquatic ecosystems with this species as a conservation target. Significant efforts from this work were focused on both conducting restoration monitoring and implementing new habitat restoration within the SCDRS.

The presence of dams including the ones located at Hydro Park (A) and Argo Park (B) on the Huron River likely affect Mudpuppy dispersal and this region warrants further assessments.

Creation of habitat and the positive results from the monitoring efforts at several restoration sites were among contributing factors that led to the removal of the Beneficial Use Impairment (BUI) for loss of fish and wildlife habitat on the St. Clair River, moving it one step closer to delisting the AOC. A majority of individuals captured by HRM during this project were collected from restoration locations where Mudpuppy habitat structures have been placed. Noteworthy observations of unique habitat use by Mudpuppies included the presence of eggs on telemetry anchors and sunken cars, young age classes using fish egg mats, and adults using sunken vehicles for refugia. These findings demonstrate the potential of the Mudpuppies adaptive nature and opportunities for creativity in designing future Mudpuppy restoration projects. Such methods may be particularly valuable in areas where natural habitat is very limited, such as shorelines dominated by seawall. Successful restoration sites may be able to serve as an important example to which future restoration projects can be matched. Prioritizing the creation of aquatic habitat in the SCDRS and the Great Lakes region where it has been reduced from historic levels will help bolster populations of Mudpuppies leading to more feasible recovery of the endangered salamander mussel.

Restoration sites including the Blue Water River Walk (A) and Cottrellville (B) have demonstrated the value of creating Mudpuppy habitat along the SCDRS.

Genetic Research

Genetic analyses have helped describe the diversity of Mudpuppy populations within the project area, within the Great Lakes region, and across the species' range. Genetic variability can be an effective tool to compare the dispersal capabilities of this species to the issues that barriers pose to them, fish and other wildlife species. This work was also instrumental in assessing and developing new sampling techniques for detecting Mudpuppies. A subsample of the over 700 tissue samples submitted from across the Mudpuppy range show genetic diversity trends that were previously unassessed and unknown.

Phylogeographic analysis showed that there is an evolutionary split on a range-wide scale between eastern and western populations of the Mudpuppy. Michigan populations fall within the eastern clade, with a level of genetic diversity that is similar to other locations (but relatively low overall for the locus we sequenced, with some populations consisting of only a single haplotype). These results imply that varying conservation strategies for the species are warranted, depending on the location and targeted populations. From a management perspective, if translocation is ever considered, animals should not be moved across this east/west boundary. Effort to improve adult survivorship, increase breeding opportunities and success, and juvenile survivorship are all important goals to sustain a long-term genetically viable population.

Despite significant effort to assess genetic variability locally,

the population genetic analysis ran into numerous technical difficulties and work is still underway. Completed results are expected in spring 2018. In spite of these issues, preliminary results showed a surprisingly high level of connectivity among sites within the SCDRS. This result should be interpreted with caution, as it is based on a very small number of molecular markers. It is possible that re-analysis will show that there is in fact substructure among these sites. Currently, results show that Mudpuppies collected from eastern and western Lake Erie are genetically distinct, but there are no such genetic substructures within the SCDRS, suggesting Mudpuppies are likely capable of dispersing within this region. Further monitoring for the isolated eastern Lake Erie population is warranted to help determine potential factors inhibiting gene flow. Assessing the physical movement of Mudpuppies through telemetry and mark recapture is a critical next step in not only helping understand the genetics, but the overall natural history of this species.

HRM collecting Mudpuppy tissue samples for genetic analyses. This project has resulted in important information regarding genetic diversity of Mudpuppy populations within the SCDRS.

A strong positive correlation was found between trapping data and eDNA analysis. Every site with positive trapping records also resulted in a positive eDNA screen. The only discrepancy resulted from two sites on the Huron River, which were positive for eDNA, but where mudpuppies have never been captured. This indicates that Mudpuppies may in fact be present at these locations, and perhaps future trapping could be conducted to corroborate this result. This initial success suggests that eDNA could be a promising, cost-effective method for assessing intensive Mudpuppy sampling locations. Developing survey methodologies with higher success rates of Mudpuppy detection will also benefit the management and conservation of the rare Salamander Mussel.

Continued success of identifying Mudpuppy presence through eDNA may lead to less time instensive sampling techniques such as trapping.

Further investigating genetic differentiation in the Huron River as well as the Lake Erie populations will be valuable to more accurately assess health of the SCDRS. Additional trapping and collection of genetic samples is warranted in these regions to better understand the factors limiting population size and degree of genetic flow. The lack of Mudpuppy detection calls for additional review and analyses of this region. Additional targeted survey work may help reveal factors inhibiting the species presence and if detected, can provide additional tissue samples for genetic study to better depict their spatial distribution and population health.

U.S. Fish and Wildlife Service

Strong project partnerships and collaboration between various organziations was critical in the success of this project.

Outreach and Education

Public engagement efforts were effective at spreading awareness for the Mudpuppy as a bioindicator of environmental health as well as increasing reporting of observations of the species in the project area. Encouraging the public to submit Mudpuppy observations to the Michigan Herp Atlas resulted in valuable data that provides further insight into the status of populations in the state and efficacy of restoration efforts. Anecdotal data gathered while engaging local anglers support the understanding that Mudpuppy populations in the Great Lakes have declined, and helped identify additional sampling locations within the project area. Information like this has historically been overlooked by researchers and managers; however, it can serve as a valuable component when assessing populations for long-term management and conservation especially when accompanied by photo vouchers. Direct conversations and distribution of educational materials were effective at informing local citizens and natural resource professionals about the importance of Mudpuppies on the landscape. The increase in reported Mudpuppy observations and decrease in reports of persecution of the species indicate these efforts have been effective.

This novel study was successful in evaluating a critical bioindicator species utilizing a multi-faceted, collaborative approach. As a result, previously unknown data is now available for Mudpuppies along significant stretches of the Michigan shoreline of the SCDRS. A better understanding of species status, spatial distribution, population health, and genetic structure is essential for best management of this declining salamander as well as the dependent Salamander Mussel. Understanding Mudpuppy populations in the Great Lakes will provide insight into areas within this watershed that likely support diverse aquatic organisms and healthy aquatic habitats. Monitoring of Mudpuppy health in correlation to surrounding land uses may be a useful tool for identifying major threats in the region. Furthermore, continued decline in Mudpuppy populations will reduce the potential for recovery of Salamander Mussels as the Mudpuppy is functionally critical habitat for this unique mussel species. The support obtained through this project is a testament to the value and power of scientific collaboration and the need for a greater understanding and conservation of Mudpuppies in the Great Lakes.

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