Herpetologica, 76(4), 2020, 000–000

Environmental DNA Assessment Reveals Restoration Success for Mudpuppies (*Necturus maculosus*)

Jenny Sutherland[1,4,5], David Mifsud[2], Maegan Stapleton[2], Stephen F. Spear[3] and Katherine Greenwald[1]

[1] Department of Biology, Eastern Michigan University, Ypsilanti, MI 48197, USA
[2] Herpetological Resources & Management, 1101 S Main Street #110, Chelsea, MI 48118, USA
[3] The Wilds, 14000 International Road, Cumberland, OH 43732, USA

?1 Abstract: apHARDRETURNMudpuppies (*Necturus maculosus*) are secretive, fully aquatic salamanders with a range that spans much of the eastern United States and Canada including the Great Lakes region. Although this species was once abundant, there have been widespread declines due to habitat loss and modification, pollution, lampricide use, and overcollection. We compared environmental DNA (eDNA) and trapping surveys to determine Mudpuppy occupancy along the St. Clair-Detroit River System, where this indicator species could be a gauge for success of ongoing restoration. Mudpuppy eDNA was detected at all sites with positive trapping records, as well as one site where individuals have not been trapped previously. Sites with shoreline restoration had the highest occupancy estimates, whereas deep-water restoration did not affect Mudpuppy occupancy. Additionally, eDNA surveys resulted in higher detection probability than setline and minnow trap survey methods, illustrating the benefit of using eDNA to detect secretive species. This study demonstrates the success of restoration efforts in increasing the occupancy of an indicator species and can be used as a template for other restoration practices.

Key words: Amphibian; Aquatic salamander; Detection probability; Great Lakes; Indicator species; Secretive species

Species in freshwater ecosystems are understudied, but available data suggest that population declines and extinction rates greatly exceed those of terrestrial species (Ricciardi and Rasmussen 1999; Dudgeon et al. 2006; Collen et al. 2014; WWF 2016). The connectivity of lotic systems with other environments (terrestrial, groundwater, floodplain zones, and upstream–downstream habitats) allows negative land-use practices such as dredging, channelization, and damming to affect lotic systems through multiple pathways (Collen et al. 2014). This creates a cascade or accumulation effect that intensifies the effects of fragmentation, pollution, sedimentation, invasive species, and disease (Stanford and Ward 1992; Darwall et al. 2009; Feld 2013), leading to declines or extinctions of organisms in these habitats.

Amphibians can serve as indicator species of habitat degradation in fresh water ecosystems. In lotic habitats, amphibians may be secretive, which results in low detection rates and inaccurate monitoring (Stuart et al. 2004; Murphy et al. 2016). Improved survey methods with higher levels of certainty, higher rates of detection, and lower costs are needed to monitor lotic amphibian species. Environmental DNA (eDNA) analysis has made species detection easier when sampling of live organisms is harmful or logistically difficult, and it is often less costly and time-intensive than traditional survey methods (Goldberg et al. 2011; Jerde et al. 2011, 2013; Olson et al. 2012; Pilliod et al. 2013; Santas et al. 2013; Spear et al. 2015; Franklin 2016; Pitt et al. 2017; Takahashi et al. 2018). Environmental DNA has become common for detecting aquatic species and has recently been used to assess Mudpuppy (*Necturus maculosus*) populations (Spear et al. 2015; Collins et al. 2019).

Mudpuppies are fully aquatic amphibians in the Great Lakes region (Collins et al. 2019). Mudpuppies are important biological indicators because of their sensitivity to environmental stressors. They also have an important role in the ecosystem as top predators, and are obligate hosts for federally endangered Salamander Mussels (*Simpsonaias ambigua;* Watson et al. 2001; Davic and Welsh 2004). Although Mudpuppies were once abundant, recent data suggest that they are experiencing widespread population declines (Craig et al. 2015; Chellman et al. 2017). Threats to Mudpuppies in the Great Lakes region include lampricide use, habitat modification, pollution, and direct human-caused mortality (Collins et al. 2019). Mudpuppies are frequently caught by anglers, and may be killed as a result of the erroneous beliefs that Mudpuppies are poisonous, or prey on or compete with game fish (Gilderhus and Johnson 1980; Petranka 1998; Boogaard et al. 2003; Harding and Mifsud 2017). Mudpuppies are considered a Species of Special Concern in Michigan, and are a focal species along the St. Clair-Detroit River System (Harding and Mifsud 2017).

Despite declining numbers, Mudpuppy presence may be used as a gauge of restoration success (Craig et al. 2015; Collins et al. 2019), particularly in regions with a history of habitat degradation and pollution. One such location is the St. Clair-Detroit River System (SCDRS). The SCDRS is the connecting channel between the southern tip of Lake Huron and the western basin of Lake Erie, and it is part of the boundary between Canada and the United States. Since 1874 this corridor has undergone many habitat modifications, with urbanization and the need for shipping channels driving the loss of coastal wetlands, construction of sea walls, dredging, channelization, and industrialization (Bennion and Manny 2011; Haponski and Stepien 2014). This SCDRS also hosts the Round Goby (*Neogobius melanostomus*) and the Zebra Mussel (*Dreissena* spp.), both successful invasive species in the Laurentian Great Lakes, both of which are consumed by Mudpuppies (Beattie et al. 2017). In response to habitat degradation, the St. Clair and Detroit Rivers were designated as Areas of Concern (AOC) in 1987 under the

[4] Present Address: Department of Forestry and Natural Resources, Aquaculture Research Laboratory, Purdue University, 5675 W 600 N, West Lafayette, IN 47906, USA
[5] Correspondence: e-mail, sutherj@purdue.edu

TABLE 1.—Sites where minnow trap (MT) and setline (SL) data on Mudpuppy occupancy were collected from U.S. Fish and Wildlife Service (USFWS), U.S. Geological Survey (USGS), and Herpetological Resource and Management (HRM). Restoration state was either shoreline restoration (SLR) or deep-water restoration (DWR). Restoration state was left blank if there were no rock additions at this site.

Site		Sampled for eDNA	Restoration state	USFSW			USGS		HRM		
				2014	2015	2016	2015	2016	2014	2015	2016
LH	St. Clair River Headwaters			SL	MT/SL	SL					
SCR1	Blue Water Bridge		SLR						MT	MT	MT
SCR2	Keifer Park		SLR				MT			MT	MT
SCR3	Blue Water River Walk	x	SLR				MT	MT	MT	MT	MT
SCR4	Marysville	x	SLR					MT	MT	MT	MT
SCR5	East China			MT/SL	MT						
SCR6	Cottrellville	x	SLR				MT	MT		MT	MT
SCR7	Algonac State Park	x							MT	MT	MT
SCR8	Russel Island			MT	MT						
SCR9	Middle Channel		DWR	MT	MT						
LSC1	Fair Haven Boat Launch	x							MT	MT	MT
LSC2	Lake St. Clair Fisheries and Research Station								MT	MT	MT
LSC3	Lake St. Clair Metropark	x	SLR						MT	MT	MT
DR1	Belle Isle		SLR/DWR	MT					MT		MT
DR2	Milliken State Park										MT
DR3	Delray Public Access Boat Ramp			MT	MT	MT					
DR4	Fighting Island		DWR	MT/SL	MT/SL	MT/SL					
DR5	Grassy Island			MT/SL	MT/SL	MT/SL					
DR6	Hennepin Point (Grosse Ile)			MT/SL	MT/SL	MT/SL					
DR7	Turkey Island			MT/SL	MT						
DR8	Bridge to Grosse Ile			MT/SL	MT/SL	MT/SL					
HR1	Barton Hills	x								MT	MT
HR2	Gallup Park	x								MT	MT
HR3	Ypsilanti	x								MT	MT
LE1	Lake Erie Metropark										MT
LE2	Pt. Mouilee										MT
LE3	Mouth of Lake Erie			SL							MT
LE4	Sterling State Park										MT
LE5	Toledo Beach Marina										MT

Great Lakes Water Quality Agreement by the U.S. Environmental Protection Agency.

For this study, occupancy modeling was used to determine the efficacy of eDNA surveys for monitoring Mudpuppies along the SCDRS, and to determine whether restoration has resulted in an increase in Mudpuppy occupancy. We predicted that (1) Mudpuppy eDNA would be detected at sites where Mudpuppies were also trapped, (2) detection probability would be higher for eDNA sampling versus traditional trapping methods, and (3) Mudpuppy occupancy would be higher at restored versus unrestored sites. As a sensitive species, Mudpuppies are an effective biological indicator to assess the success of restoration efforts and habitat conditions along the SCDRS.

MATERIALS AND METHODS

Study Area

We studied Mudpuppy presence at 10 restored and 19 unrestored sites along the SCDRS in Michigan (Table 1; Figs. 1, 2). Shoreline restoration was defined by the addition of rocks, whereas unrestored sites did not have the addition of rocks. Deep-water restoration was defined by the addition of rocks (i.e., artificial reefs) to the bottom of the river for the benefit of fish that use broadcast spawning as their primary mode of reproduction; this restoration could also benefit Mudpuppies that use the rocks as habitat and for reproduction. A multiagency approach was used: we analyzed setline and minnow trap records from Herpetological Resource and Management (HRM; 13 d of trapping data used), U.S. Fish and Wildlife Service (USFWS; 66 d of trapping data used) and U.S. Geological Survey (USGS) Great Lakes Science Center (USGS GLSC; 64 d of trapping data used) for the years 2014–2016 (Table 1).

Of the 10 restored sites along the SCDRS, 7 sites had shoreline restoration (SLR) and 3 sites had deep-water restoration (DWR). Four of the shoreline restorations sites were located along the St. Clair River: Cottrellville (2015; 130 m of shoreline restored), Marysville (2013), Blue Water River Walk (2012; 1.31 km of shoreline restored), and Kiefer Park. At the Cottrellville site the seawall was removed, breakwaters were installed, cobble and boulders were placed throughout the shallow shelf, and trees were planted (Fig. 1A; Great Lakes Architect-Engineer Services 2014; MDEQ 2017). At the Marysville site the seawall was removed, the shoreline graded, rock riprap installed, and native emergent and submergent wetland vegetation planted (Fig. 1B; MDEQ 2017). The Blue Water River Walk restoration site was the largest restoration project on the St. Clair River (Fig. 1C). The site is a former train yard and the restoration began with the removal of 3250 t of debris. At this site, using rock and native vegetation, 0.304 ha of fish spawning habitat and 0.912 ha of nursery habitat was created, and 14 mussel and Mudpuppy structures were installed (MDEQ 2017). These structures were large flat slabs of concrete placed within the water for Mudpuppies to use as shelter, breeding, and larval care. At Kiefer Park, rocky substrates were added along the shoreline that extended down into the river.

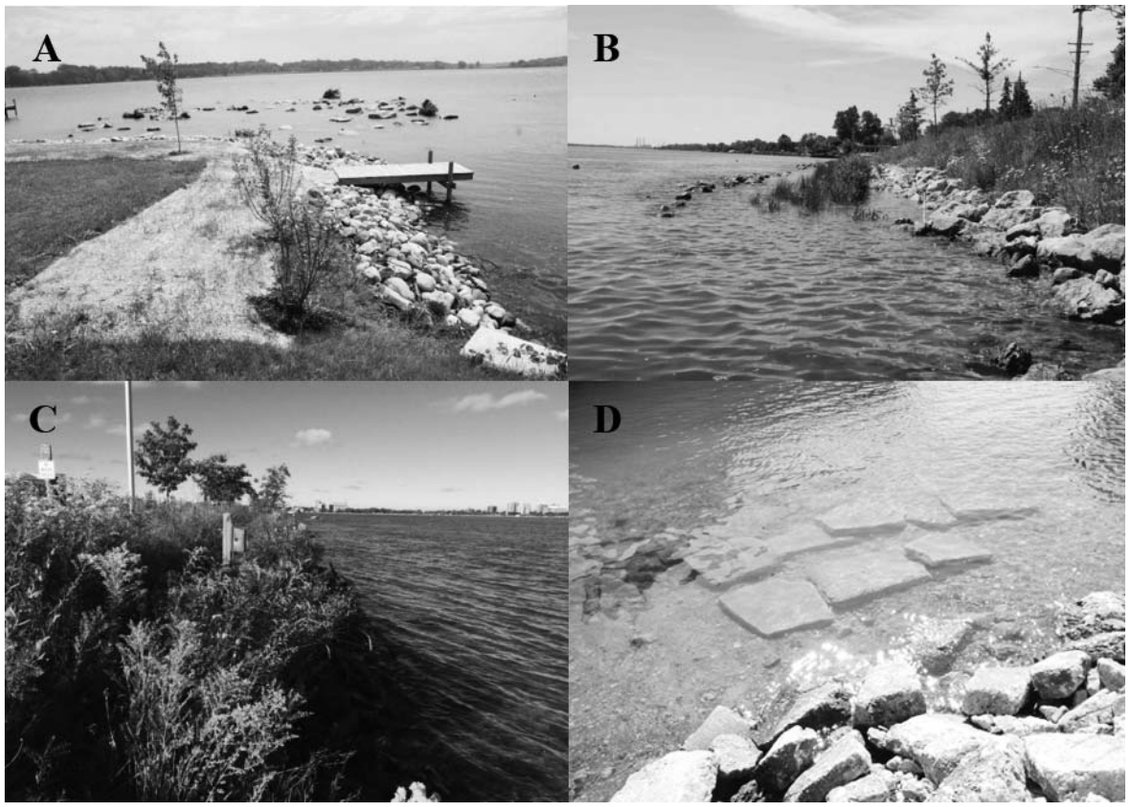

Fig. 1.—Restoration sites that were sampled for Mudpuppy eDNA: (A) Cottrellville Township Shoreline Preservation and Restorations, (B) Marysville St. Clair River Living Shoreline Restoration, (C) Blue Water River Walk, and (D) Blue Water River Walk (HRM). A color version of this figure is available online.

Restoration, albeit less extensive, also occurred at Lake St. Clair. At the Department of Natural Resources Fairhaven Boat Launch, the restoration included the addition of rocks suitable for Mudpuppies along the entire shoreline of the site and extended down into the water. At Lake St. Clair Metropark, concrete slabs were submerged along the shoreline to create habitat specifically for Mudpuppies (Fig. 1D).

Trapping

Surveying using setlines and minnow traps was conducted April through December when ice was not present on the water and gear could be deployed. The majority of sampling did not specifically target Mudpuppies, but Mudpuppies were included in the bycatch. The USFWS provided setline bycatch data, whereas USFWS and USGS both provided minnow trap bycatch data. Herpetological Resource Management conducted minnow trapping that specifically targeted Mudpuppies. Setlines were set along the bottom of the river with small and large hooks baited with dead Round Gobies along with three attached minnow traps baited with cheese cubes. Setlines were checked and reset every 24 h by USFWS (Craig et al. 2015). Shoreline minnow traps baited with cheese cubes and/or raw chicken were also set every 24 h by USGS and HRM. Shoreline and deep-water spawning reef restoration sites were targeted.

Environmental DNA

Five shoreline locations that had been restored (addition of rocks) and two unrestored locations were sampled for Mudpuppy eDNA. Within these locations there were 18 sites sampled. All locations screened for eDNA had been surveyed with minnow traps and/or setlines within the last year (Table 1). Depending on the size of the location, one to four samples were collected. Two eDNA samples were also collected from an indoor tank containing adult Mudpuppies for use as a positive eDNA control (approved by Eastern Michigan University's Animal Care and Use Committee). Overall the study included 21 1-L eDNA samples (Table 2).

We conducted water collection, filtration, and quantitative polymerase chain reaction (PCR; qPCR) using previously established protocols for Mudpuppy eDNA analysis (Spear et al. 2015; Collins et al. 2019). Water samples were collected from the shore when water temperatures were below 5°C in early spring (March–April) or autumn (November) when Mudpuppy catch-per-unit-effort was highest (Craig et al. 2015). To prevent contamination, this process was done without entering the water and while wearing gloves. To preserve DNA, 1 mL of 10% Benzalkonium chloride (BAC) at a final concentration of 0.01% was added to each 1-L container after collection of each sample before transport back to the lab for filtration (Yamanaka et al. 2016). Water samples were run through a 0.45-µm cellulose nitrate filter (Whatman International, Ltd.) in a filter cup

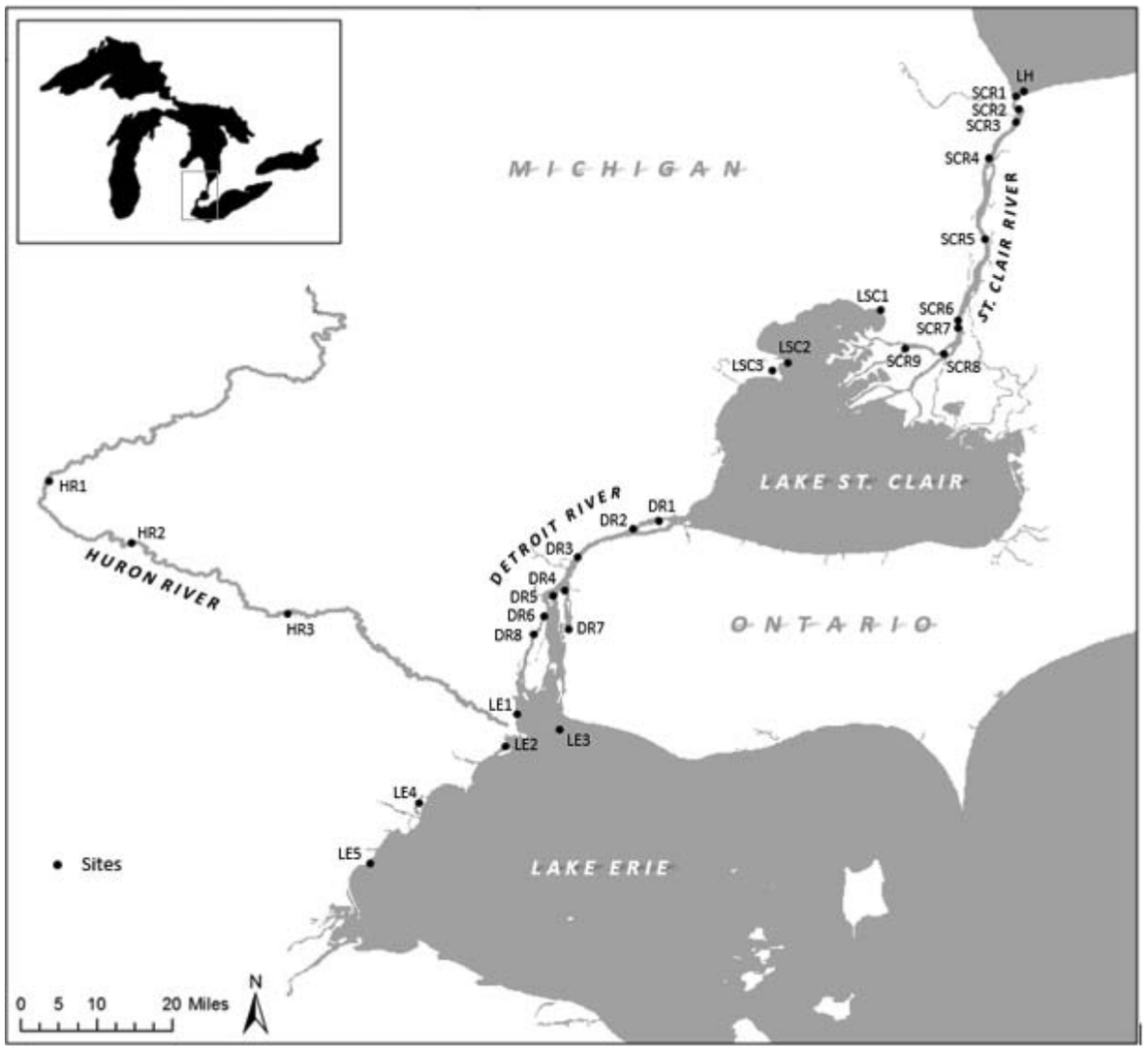

FIG. 2.—Location of 29 sampling sites along the St. Clair-Detroit River System used for Mudpuppy trapping and eDNA data collection. Sites were located in Lake Huron (LH), St. Clair River (SCR), Lake St. Clair (LSC), Detroit River (DR), Lake Erie (LE), and the Huron River (HR).

inserted into a 1-L vacuum flask. If multiple 1-L samples were taken at a site the samples were processed (filtering and qPCR) separately. After filtering, and to prevent contamination between samples, the filter paper was removed with forceps that were treated with DNA Away™ (Molecular BioProducts, Inc.). The filter paper was stored in 95% ethanol in a centrifuge tube and frozen until further processing. To test for contamination, deionized water was also filtered every time samples were filtered in the lab.

Extraction of DNA from filters was completed using methods described in Goldberg et al. (2011). We used a qPCR primer–probe set, which amplifies a 149-basepair region of mitochondrial DNA and was developed and described in Collins et al. (2019). This set amplifies DNA for the genus *Necturus; N. maculosus* is the only species within this genus located in our study area. The primer pair sequences were Nema-F: 5′-AGCAACAGCCTTTG-TAGGGTA-3′, and Nema-R: 5′-TCGCCTTATCGACGGA-GAATC-3′. The fluorescent probe was NemaProbe: Cal Flour 610-CGTACTACCATGAGGCCAAATATCCTTC-BHQ2.

We ran 15-µL qPCR reactions including 2.85 µL water, 7.5 µL QuantiTect NoRox Multiplex PCR Mix (Qiagen, Inc.), 0.4 µM primer, 0.2 µM probe, 0.6 µL TaqMan® Exogenous Internal Positive Control 10× Exo IPC Mix (Applied Biosystems), 0.3 µL of TaqMan® Exogenous Internal Positive Control 50× Exo IPC DNA (Applied Biosystems), and 3 µL of sample. Reactions were run on an ABI 7500 real-time PCR system. The qPCR cycle was as follows: initial denaturation at 95°C for 10 min, 50 cycles of 94°C for 60 s for denaturation, and annealing at 60°C for 45 s. Each sample had three replicates and was run with positive controls from tail-tip tissue extraction and negative controls of deionized water. Samples from tail-tip tissue extraction had a concentration of DNA, which was estimated using a NanoDrop™ fluorospectrometer (Thermo Fisher Scientific). These positive controls were diluted to include four different concentrations, which covered the range of

TABLE 2.—Environmental DNA results compared with detection of Mudpuppies by trapping. Numbers in parenthesis represent multiple samples being taken at a site dependent on site size, (–) indicates no detection of Mudpuppies via trapping and/or eDNA, (+) indicated a positive detection for Mudpuppies via trapping and/or eDNA. Restoration state was either shoreline restoration (SLR) or deep-water restoration (DWR), though eDNA was only collected at shoreline sites. Restoration state was left blank if there were no rock additions at this site. Two samples were collected from a tank at the Belle Isle Aquarium containing adult Mudpuppies for use as a positive eDNA control (Belle Isle Tank A and B).

Site (sample)	Restoration state	Trapping detection	eDNA detection
Huron River (1)		–	–
Huron River (2)		–	–
Huron River (3)		–	–
Lake St. Clair Metropark	SLR	–	*
Cottrellville (Up)	SLR	+	+
Cottrellville (Middle)	SLR		+
Cottrellville (Down)	SLR		+
Blue Water River Walk (Sugar)	SLR	+	+
Blue Water River Walk (Gray Fox)	SLR	+	+
Blue Water River Walk (Kramer)	SLR	+	+
Algonac State Park (Up)		+	–
Algonac State Park (Middle)			+
Algonac State Pak (Down)			+
Marysville (1)	SLR	+	+
Marysville (2)	SLR	+	+
Fairhaven Boat Launch (Up)	SLR	–	+
Fairhaven Boat Launch (Middle)	SLR		+
Fairhaven Boat Launch (Down)	SLR		–
Belle Isle Tank (A)		+	+
Belle Isle Tank (B)			+

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

DNA concentration typically used for eDNA extractions: 10^{-3} ng/μL, 10^{-4} ng/μL, 10^{-5} ng/μL, and 10^{-6} ng/μL (Spear et al. 2015). A positive eDNA control sample from an indoor tank containing adult Mudpuppies was also used.

If two out of the three replicates from a single site were positive for Mudpuppy eDNA, a positive detection of a Mudpuppy at that site was concluded (Spear et al. 2015). These three replicates were also rerun to confirm the results. If only one out of three replicates from a site was positive, the sample was also rerun. If the next three replicates were negative for Mudpuppy eDNA, the site was considered negative for eDNA. If one or more of the three replicates was positive during the second run, the site was considered positive for Mudpuppies.

Occupancy Modeling

Occupancy modeling was used to determine the detection probability and occupancy of Mudpuppies along the SCDRS using trapping and eDNA data. Calculations for this model were carried out by the program PRESENCE, which estimates detection probability and the proportion of sites occupied when the detection of the species is <1 (MacKenzie et al. 2002). The assumptions for this model are (1) the population is closed to immigration and colonization, and emigration and extinction; (2) the species is identified correctly; and (3) detecting Mudpuppies at one site is independent from detecting Mudpuppies at other sites. Despite seasonal changes in activity, Mudpuppies have small home ranges and high site fidelity (Harris 1959; Shoop and Gunning 1967; Gendron 1999; Sajdak 1982). Mudpuppies have not been shown to have a home range greater than approximately 780 m and our two closest sites were >1 km apart, so we assumed that species occupancy was constant across the three-season period as a result of Mudpuppies being long-lived and relatively sedentary (Chellman et al. 2017).

For occupancy models, sites must be surveyed (detection/no detection) ≥2 times/sampling season. Parameters include ψ_i, the probability that a Mudpuppy is present at site i, and p_{it}, the probability that a species is detected at site i at time t, assuming it is present. The method involves visiting sites multiple times within a season where the target species is either detected, with probability p, or not detected. The intent is to estimate the proportion of sites that are occupied, ψ, knowing the species is not always detected, even when present. Data from 14 setline sites and 50 minnow trap sites were used to estimate ψ and p for Mudpuppies. We varied detection probability across the 3-yr sampling period, and examined the effects of survey method on occupancy (ψ) and detectability (p).

We represented sampling events as weeklong intervals. Sites were eliminated if sampling only occurred once (i.e., during a single 1-wk period). Sampling covariates included water temperature and site restoration status (shoreline restored, shoreline unrestored, deep water restored, and deep water unrestored). Temperature covariates were z-transformed. We tested for differences between covariate occupancy and detection probability using two-sample t-tests and analysis of variance (ANOVAs) with post hoc Tukey tests. Spearman's rho test was also used to determine the correlation between temperature and detection probability using minnow traps and setlines. Sampling events for eDNA occupancy modeling were the replicates that were taken at each site. ?3

RESULTS

Trapping and Catch-per-unit-effort

Mudpuppies (n = 372) were caught using setlines (n = 172) and minnow traps (n = 200) over three field seasons (2014–2016). For setlines, there were 19 sampling events in 2014, 14 sampling events in 2015, and 16 sampling events in 2016 (49 sampling events overall at 14 sites). For minnow traps, there were 159 sampling events in 2014, 181 sampling events in 2015, and 221 sampling events in 2016 (561 sampling events overall at 50 sites). Mudpuppies were caught at 16 out of 28 sites that were surveyed. Mudpuppies were caught at 5 sites along the St. Clair River (3 restored), 1 site on Lake St. Clair (not restored), and 10 sites on the Detroit River (4 restored).

Environmental DNA

Mudpuppy eDNA was detected in water samples from nine sites and was not detected at five sites (Table 2). One of the restoration sites that only included the addition of concrete slabs (Lake St. Clair Metropark) was included in the eDNA survey, but the data could not be used because of failure of internal positive controls to amplify during PCR, possibly due to silt at the site causing inhibition. Both eDNA samples collected from the tank containing adult Mudpuppies were positive. All sites where Mudpuppies have been detected through trapping had positive eDNA results. All negative controls were negative and internal positive controls ?4

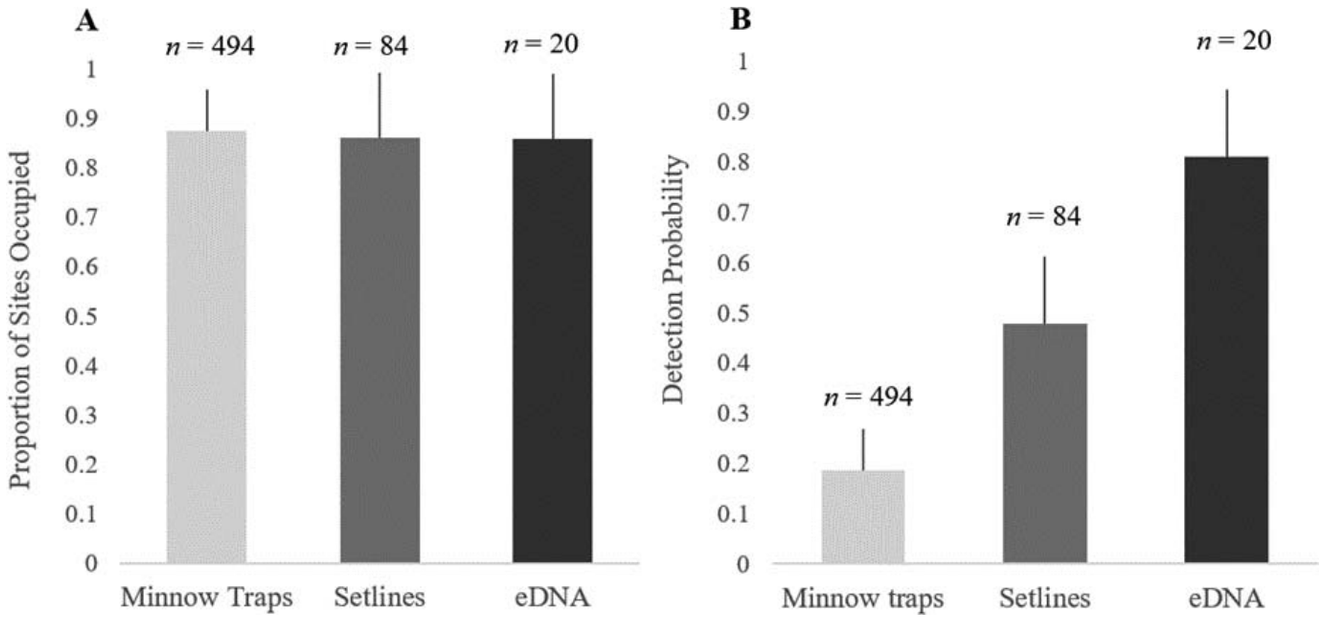

FIG. 3.—Occupancy (A) and detection probability (B) for Mudpuppies along the St. Clair-Detroit River System using the program PRESENCE and three different sampling methods (minnow traps, setlines, and eDNA). Of the 598 sampling events, 494 were minnow trap data, 84 were setline data, and 20 were eDNA data. One site was not included in the eDNA data because of inhibition of the sample.

and standards down to 10^{-6} consistently amplified. Mudpuppies have not been captured in setlines or minnow traps at the five sites where there was no Mudpuppy eDNA detection. The only mismatch was at Fairhaven Boat Launch, which had a positive detection of eDNA but Mudpuppies were not detected there by trapping.

Occupancy Modeling

The estimate of occupancy (ψ) was the same for minnow traps ($\psi = 0.87 \pm 0.08$), setlines ($\psi = 0.86 \pm 0.13$), and eDNA ($\psi = 0.86 \pm 0.13$; Fig. 3A; ANOVA $F = 0.003$, $P = 0.99$). Detection probability (p) for the 3-yr period was $p = 0.19 \pm 0.02$ for minnow traps, $p = 0.48 \pm 0.06$ for setlines, and $p = 0.81 \pm 0.10$ for eDNA (Fig. 3B). Detection probability differed among the three sampling methods (ANOVA $F = 32.34$, $P < 0.01$) and a post hoc Tukey test showed that eDNA had a higher detection probability compared with minnow traps ($P < 0.01$) and setlines ($P = 0.01$). A post hoc Tukey test also showed that setlines had a higher detection probability compared with minnow traps ($P < 0.01$).

Based on minnow trapping, shoreline restoration sites had a higher occupancy ($\psi = 0.87 \pm 0.11$) than shoreline sites without restoration ($\psi = 0.49 \pm 0.21$; Fig. 4A; two-sample t-test $t = 20.47$, df = 283, $P < 0.01$). Reef restoration sites did not have a higher occupancy than sites without a reef for either minnow trap (deep water restored $\psi = 0.77 \pm 0.11$, deep water unrestored $\psi = 0.79 \pm 0.12$; Fig. 4A; two-sample t-test = 1.19, df = 207, $P = 0.24$) or setline sampling (deep water restored $\psi = 0.80 \pm 0.17$, deep water unrestored $\psi = 0.79 \pm 0.12$; Fig. 4B; two-sample t-test = 0.78, df = 207, $P = 0.44$).

The highest detection probability for minnow traps was on the last day of sampling ($p = 0.37$) in 2014 when the surface water temperature (stated as "water temperature" going forward) was 3.03°C (Fig. 5). The highest detection probability in 2015 was $p = 0.27$ (also the last day of sampling) when the water temperature was 6.5°C. In 2016, the highest detection probability was $p = 0.39$ when the water temperature was 2.22°C, which was the coldest (water) sampling day of the year. The detection probability for minnow traps was lowest on the hottest (water temperature) sampling days of the year in 2014 and 2015; in 2016 it was the second hottest sampling day of the year by 0.3°C (Fig. 5; 2014 = 22.67°C, $p = 0.04$; 2015 = 22.25°C, $p = 0.04$; 2016 = 24.43°C, $p = 0.03$). Spearman's Rho rank correlation coefficient was $r_s = -0.97$ for minnow-trap detection probability and temperature.

In all three years the highest detection probability for setlines occurred on the coldest (water temperature) sampling day of the year (Fig. 5; 2014 = 7.15°C, $p = 0.68$; 2015 = 7.16°C, $p = 0.70$; 2016 = 4.05°C, $p = 0.93$). The detection probability was lowest for setlines during all three sampling seasons on the hottest (water temperature) sampling day of the season (2014 = 2.78°C, $p < 0.001$; 2015 = 12.76°C, $p = 0.08$; 2016 = 16.27°C, $p = 0.01$). Spearman's Rho rank correlation coefficient was $r_s = -0.98$ for setline detection probability and temperature.

Discussion

To accomplish effective wildlife management with secretive species, reliable methods must be developed to detect presence, maximize detection probability, and minimize false negatives. We used occupancy modeling and eDNA sampling to determine Mudpuppy presence at restored and unrestored locations along the St. Clair-Detroit River System. Shoreline restored sites had higher occupancy than unrestored sites, supporting our prediction that this management action is beneficial to Mudpuppies. Conversely, deep-water restoration sites with reefs did not have higher

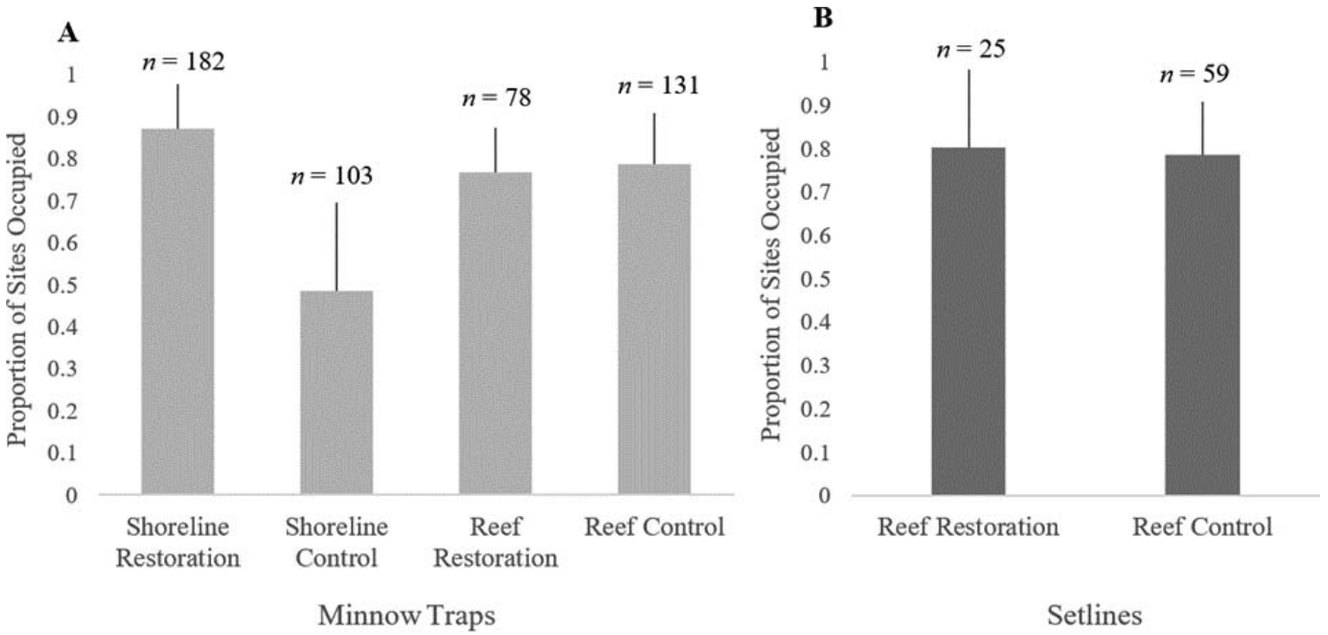

Fig. 4.—Occupancy of Mudpuppies at restoration and control sites using minnow traps (A) and setlines (B). Mudpuppies had a higher occupancy at shoreline sites when there was restoration, but not a higher occupancy at sites where there was reef restoration for minnow traps and setlines. (A) Of the 494 minnow trap samples, 182 were shoreline restoration sites, 103 were shoreline control sites (i.e., unrestored), 78 were reef restoration sites, and 131 were reef control sites (i.e., unrestored). (B) Of the 84 setline samples 25 were reef restoration sites and 59 were reef control sites (i.e., unrestored).

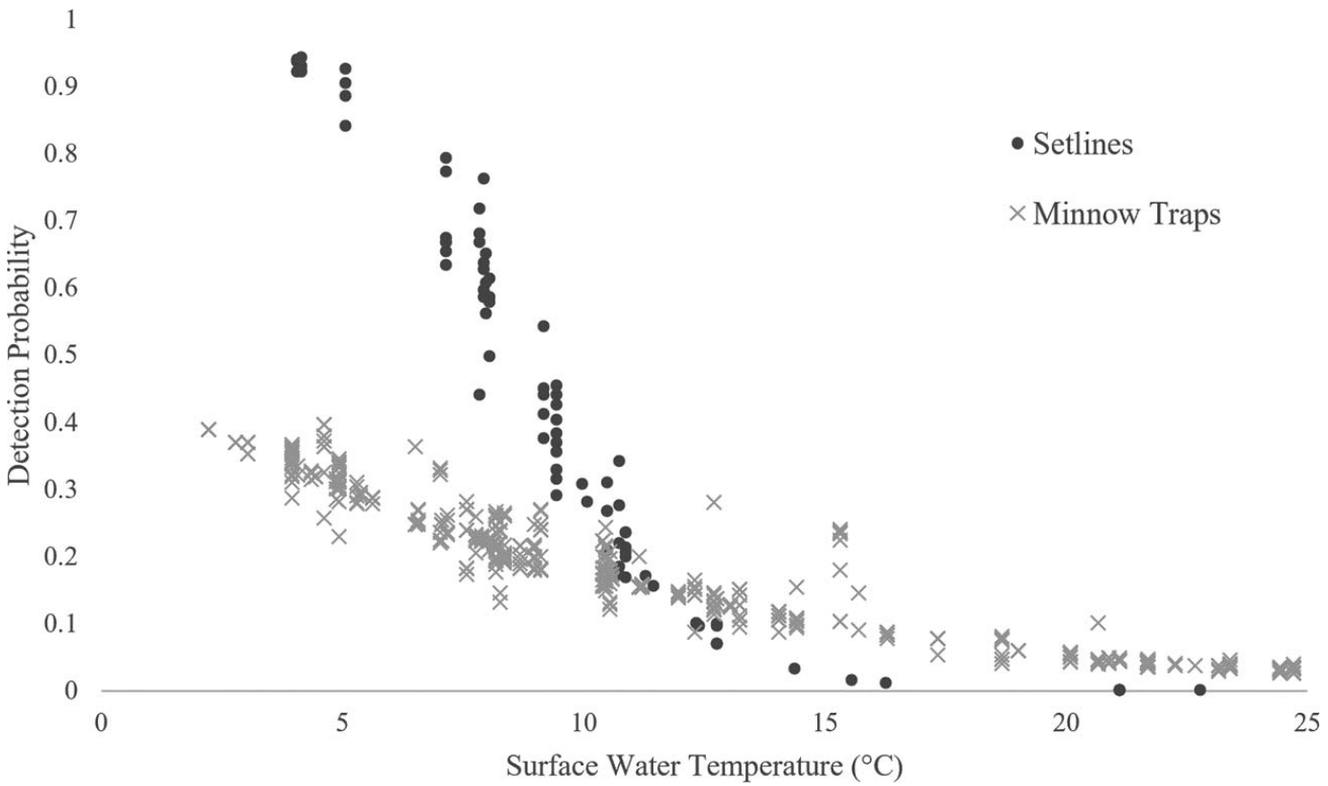

Fig. 5.—Regression for minnow traps and setlines displaying detection probability trends with temperature. Spearman's rho rank correlation $r_s = -0.97$ for minnow traps and $r_s = -0.98$ for setlines.

occupancy in minnow trap and setline surveys. All sites where Mudpuppies had been trapped had positive eDNA results (Table 2), and eDNA had the highest detection probability compared with the traditional trapping methods (minnow traps and setlines).

Our results indicate that shoreline restoration can be an important contributor to healthy habitat suitable for sensitive amphibians like Mudpuppies. Mudpuppies occur at a higher proportion of restored sites compared with unrestored sites based on our analysis of minnow trap data. The shoreline sites that were sampled for Mudpuppies had extensive restoration including the addition of large rocks known to be suitable as Mudpuppy habitat. These sites also included the addition of terrestrial and aquatic plants and the removal of seawalls. Future management along the SCDRS for Mudpuppies should continue to improve shoreline habitat.

Restoration efforts designed to increase fish spawning habitat did not increase the presence of Mudpuppies within this study. Occurrence of Mudpuppies at reef restoration sites was not higher than at sites without a reef for either minnow trap or setline sampling. It is possible that this type of restoration still benefits Mudpuppies, but that we were not able to detect it because we had bycatch data at these locations (i.e., these sites were not sampled to target Mudpuppies specifically, so some setlines and minnow traps may not have been placed directly on a reef but rather within the vicinity of the reef). Mudpuppy studies typically do not exceed a depth of 2.0 m, but Mudpuppies have been shown to inhabit deeper water and even lay eggs on artificial structures in deep water (9.8 m) along the Detroit River (Hacker 1957; Sajdak 1982; Chellman 2011; Craig et al. 2015). Future studies may revisit this question by using trapping locations that are directly on rocky substrate to determine whether reef restoration increases Mudpuppy occupancy and/or reproduction at deep-water sites.

Environmental DNA sampling successfully detected Mudpuppy presence along the SCDRS, with positive samples at all shoreline sites where Mudpuppies had been previously captured using minnow traps (Table 2). Environmental DNA had the highest detection probability of the three surveying methods (minnow traps, setline, and eDNA; Fig. 3B) although all three methods had equal occupancy (Fig. 3A). Our eDNA survey also resulted in the identification of one site (Fairhaven Boat Launch) where Mudpuppies likely occur but where detection has not occurred via trapping (Table 2). This site does contain large rocks suitable for Mudpuppy habitat and reproduction. In another recent study, Mudpuppy eDNA was detected at 6 out of 10 site locations in Ohio, but Mudpuppies were only trapped at one site (Collins et al. 2019). Environmental DNA is a powerful tool for detecting Mudpuppies and may offer a more complete picture of their distribution compared with traditional trapping methods. The use of eDNA in occupancy modeling gives an estimate of detection probability and can therefore be used to determine the number of replicates needed to confirm that Mudpuppies are absent from a site. This may be a more efficient way to survey for Mudpuppies, particularly when the goal is to assess presence across large numbers of sites. In our case, these eDNA results may help to document an additional Mudpuppy location on the SCDRS.

There was a strong relationship between water temperature and detection probability for both setlines and minnow traps (Fig. 5). Detection probability was the lowest during the warmest sampling days of the year and highest during the coldest sampling days of the year. It is thought that Mudpuppies are more active during colder months in late autumn during their breeding season and early spring when they lay their eggs (McDaniel et al. 2009; Beattie et al. 2017). This relationship was also seen on Wolf Lake, a former estuarine wetland complex to Lake Michigan, where overall Mudpuppy trapping success declined quickly at water temperatures above 14.1°C, and also in the SCDRS from 2003 to 2013 (Craig et al. 2015; Beattie et al. 2017). Other studies have suggested sampling for Mudpuppies when the water temperature is $\leq$5°C (McDaniel et al. 2009); our results corroborate this suggestion.

Summary and Conclusions

Our results demonstrate that Mudpuppy occurrence was higher at shoreline restoration sites compared with unrestored sites. Future restoration along the St. Clair-Detroit River System for Mudpuppies should continue these successful practices, including shoreline seawall removal, and the addition of large rocks and vegetation. Mudpuppy eDNA was successfully amplified at every site where Mudpuppies had been detected via trapping. Environmental DNA surveys also had the highest detection probability of the three sampling methods, whereas setlines had a higher detection probability than minnow traps. This indicates that eDNA is useful for locating Mudpuppies. There was a strong relationship between water temperature and detection probability, with detection being highest at colder water temperatures. Therefore, monitoring Mudpuppy populations will be more successful if done during colder times of the year.

Finding Mudpuppies at restoration sites along the St. Clair River has implications for removing the Beneficial Use Impairment (BUI) designation for this area. An impairment of beneficial uses is caused by changes in the chemical, physical, or biological integrity of the system, and our study indicates that the river is a step closer to being removed as an Area of Concern (MDEQ 2017:5). Mudpuppy monitoring can be used for similar conservation processes for other systems, including continued monitoring on the Detroit River to remove the same BUI designation and gauge restoration practices.

Acknowledgments.—We thank U.S. Geological Survey Great Lakes Science Center and U.S. Fish and Wildlife service for Mudpuppy bycatch data. We also thank Herpetological Resource and Management staff for collecting the data over the course of the project. Funding for the study was partially provided by the Great Lakes Fish and Wildlife Restoration Act. We thank Eastern Michigan University Biology Department for funding and support, along with the Eastern Michigan University Herpetology lab for help with field and lab work. The comments of two anonymous reviewers greatly improved the manuscript.

Literature Cited

Beattie, A.M., M.R. Whiles, and P.W. Willink. 2017. Diets, population structure, and seasonal activity patterns of Mudpuppies (*Necturus maculosus*) in an urban, Great Lakes coastal habitat. Journal of Great Lakes Research 43:132–143.

Bennion, D.H., and B.A. Manny. 2011. Construction of shipping channels in

the Detroit River: History and environmental consequences. Scientific Investigations Report 1001:48105–3331.
Boogaard, M.A., T.D. Bills, and D.A. Johnson. 2003. Acute toxicity of TFM and a TFM/Niclosamide mixture to selected species of fish, including Lake Sturgeon (*Acipenser fulvescens*) and Mudpuppies (*Necturus maculosus*), in laboratory and field exposures. Journal of Great Lakes Research 29:529–541.
Chellman, I.C. 2011. Population demographics and genetic diversity of Mudpuppies (*Necturus maculosus*). M.Sc. thesis, University of Vermont, USA.
Chellman, I.C., D.L. Parrish, and T.M. Donovan. 2017. Estimating Mudpuppy (*Necturus maculosus*) abundance in the Lamoille River, Vermont, USA. Herpetological Conservation and Biology 12:422–434.
Collen, B., F. Whitton, E.E. Dyer, J.E. Baillie, N. Cumberlidge, W.R. Darwall, C. Pollock, N.I. Richman, A.M. Soulsby, and M. Böhm. 2014. Global patterns of freshwater species diversity, threat and endemism. Global Ecology and Biogeography 23:40–51.
Collins, M.K., S.F. Spear, J.D. Groves, L.A. Williams, and S.R. Kuchta. 2019. Searching for a salamander: Distribution and habitat of the Mudpuppy (*Necturus maculosus*) in Southeast Ohio using eDNA as a rapid assessment technique. The American Midland Naturalist 182:191–202.
Craig, J.M., D.A. Mifsud, A.S. Briggs, J. Boase, and G. Kennedy. 2015. Mudpuppy (*Necturus maculosus maculosus*) spatial distribution, breeding water depth, and use of artificial spawning habitat in the Detroit River. Herpetological Conservation and Biology 10:926–934.
Darwall, W., K. Smith, D. Allen, ~~M. C. Seddon,~~ McGregor Reid, V. Clausnitzer, and V.J. Kalkman. 2009. Freshwater biodiversity: A hidden resource under threat. Pp. 43–53 in Wildlife in a Changing World: An Analysis of the 2008 IUCN Red List of Threatened Species (J.-C. Vié, C. Hilton-Taylor and S.N. Stuart, eds.). International Union for Conservation of Nature and Natural, Switzerland.
Davic, R.D., and H.H. Welsh. 2004. On the ecological roles of salamanders. Annual Review of Ecology, Evolution, and Systematics 35:405–434.
Dudgeon, D., A.H. Arthington, M.O. Gessner, … C.A. Sullivan. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews 81:163–183.
Feld, C.K. 2013. Response of three lotic assemblages to riparian and catchment-scale land use: Implications for designing catchment monitoring programmes. Freshwater Biology 58:715–729.
Franklin, T.W. 2016. Estimates of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis)* occupancy and detection using two sampling methods. Ph.D. dissertation, Appalachian State University, North Carolina, USA.
Gendron, A.D. 1999. Status Report on the Mudpuppy, *Necturus maculosus*. Committee on the Status of Endangered Wildlife in Canada, Canada.
Gilderhus, P., and B. Johnson. 1980. Effects of sea lamprey (*Petromyzon marinus*) control in the Great Lakes. Canadian Journal of Fisheries and Aquatic Science 37:1895–1905.
Goldberg, C.S., D.S. Pilliod, R.S. Arkle, and L.P. Waits. 2011. Molecular detection of vertebrates in stream water: A demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. PLoS One 6:e22746.
Great Lakes Architect-Engineer Services. 2014. Ecological Restoration Monitoring Plan Habitat Restoration Design Projects, St. Clair River Area of Concern, Michigan. Prepared for the U.S. Environmental Protection Agency. EA Science and Technology and Its Affiliate EA Engineering, Science, and Technology, USA.
Hacker, V.A. 1957. Biology and management of Lake Trout in Green Lake, Wisconsin. Transactions of the American Fisheries Society 86:71–83.
Haponski, A.E., and C.A. Stepien. 2014. Genetic connectivity and diversity of walleye (*Sander vitreus*) spawning groups in the Huron–Erie Corridor. Journal of Great Lakes Research 40:89–100.
Harding, J.H., and D.A. Mifsud. 2017. Amphibians and Reptiles of the Great Lakes Region. University of Michigan Press, USA.
Harris, J.P. 1959. The natural history of *Necturus*: I. Habits and habitats. Field Lab 27:11–20.
Jerde, C.L., A.R. Mahon, W.L. Chadderton, and D.M. Lodge. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conservation Letters 4:150–157.
Jerde, C.L., W.L. Chadderton, A.R. Mahon, M.A. Renshaw, J. Corush, M.L. Budny, S. Mysorekar, and D.M. Lodge. 2013. Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. Canadian Journal of Fisheries and Aquatic Science 70:522–526.
MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Program PRESENCE: Estimating site occupancy when detection probabilities are less than one. Ecology 83:2248–2255.
McDaniel, T.V., P.A. Martin, G.C. Barrett, K. Hughes, A.D. Gendron, L. Shirose, and C.A. Bishop. 2009. Relative abundance, age structure, and body size in Mudpuppy populations in southwestern Ontario. Journal of Great Lakes Research 35:182–189.
MDEQ (Michigan Department of Environmental Quality). 2017. Removal Recommendation, Loss of Fish and Wildlife Habitat Beneficial Use Impairment, St. Clair River Area of Concern. Report 517–284–5035. Michigan Office of the Great Lakes, Areas of Concern Program, USA.
Murphy, M.O., S.J. Price, P.M. Hime, A.N. Drayer, and D.W. Weisrock. 2016. A review of common Mudpuppy (*Necturus maculosus*) capture methods and description of a revised trap design. Herpetological Review 47:575–578.
Olson, Z.H., J.T. Briggler, and R.N. Williams. 2012. An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. Wildlife Research 39:629–636.
Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, USA.
Pilliod, D.S., C.S. Goldberg, R.S. Arkle, and L.P. Waits. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Canadian Journal of Fisheries and Aquatic Sciences 70:1123–1130.
Pitt, A.L., J.L. Shinskie, J.J. Tavano, S.M. Hartzell, T. Delahunty, and S.F. Spear. 2017. Decline of a giant salamander assessed with historical records, environmental DNA and multi-scale habitat data. Freshwater Biology 62:967–976.
Ricciardi, A., and J.B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13:1220–1222.
Sajdak, R.A. 1982. Seasonal Activity Patterns, Habitat Selection, and Population Structure of the Mudpuppy, *Necturus maculosus*, in a Wisconsin Stream. M.Sc. Thesis, University of Wisconsin–Milwaukee, USA.
Santas, A.J., T. Persaud, B.A. Wolfe, and J.M. Bauman. 2013. Noninvasive method for a statewide survey of eastern hellbenders *Cryptobranchus alleganiensis* using environmental DNA. International Journal of Zoology 2013:e174056.
Shoop, C.R., and G.E. Gunning. 1967. Seasonal activity and movements of *Necturus* in Louisiana. Copeia 1967:732–737.
Spear, S.F., J.D. Groves, L.A. Williams, and L.P. Waits. 2015. Using environmental DNA methods to improve detectability in a hellbender (*Cryptobranchus alleganiensis*) monitoring program. Biological Conservation 183:38–45.
Stanford, J.A., and J.V. Ward. 1992. Management of aquatic resources in large catchments: Recognizing interactions between ecosystem connectivity and environmental disturbance. Pp. 39–52 in Watershed Management (R.J. Naiman, ed.). Springer, USA.
Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.
Takahashi, M.K., M.J. Meyer, C. McPhee, J.R. Gaston, M.D. Venesky, and B.F. Case. 2018. Seasonal and Diel Signature of eastern hellbender environmental DNA. Journal of Wildlife Management 82:217–225.
Watson, E.T., J.L. Metcalfe-Smith, and J. Di Maio. 2001. COSEWIC Status Report on the Mudpuppy Mussel *Simpsonaias ambigua* in Canada. Committee on the Status of Endangered Wildlife in Canada, Canada.
WWF (World Wide Fund for Nature). 2016. Living Planet Report 2016: Risk and Resilience in a New Era. WWF International, Switzerland.
Yamanaka, H., T. Minamoto, J. Matsuura, … A. Kondo. 2016. A simple method for preserving environmental DNA in water samples at ambient temperature by addition of cationic surfactant. Limnology 18:233–241.

Accepted on 11 July 2020
Published on Month 2020
Associate Editor: Pilar Santidrián Tomillo

Queries for herp-76-04-02

This article has been edited and typeset from the submitted materials. Please check proofs carefully for accuracy and follow the Allen Press Guide to PDF Annotation when marking revisions. Do not edit the PDF directly.

If present, queries will be listed below with corresponding numbers in the margins or may appear as PDF comments addressed to the author or editor. If a correction is desired in response to a query, mark the necessary changes directly in the proof using the appropriate annotation tool. If no change is desired, please highlight the query number in the margins and mark "No change needed" or reply to the PDF annotation with "No change needed".

1. **Authors: Please add dates of study to Abstract. Thanks. Copy editor**
2. **Authors: Caption for figure indicates D panel shows this is Blue Water River Walk (HRM). Is that the same location? Or is the figure caption incorrect? Please double check. Thanks. Copy editor**
3. **Authors: Should there be degrees of freedom given for the *F* statistics throughout Results? If so, please style as "df = #". Thanks. Copy editor**
4. **Authors: Table 2 appears to show 8 positive detections in "Trapping detection" column and 14 positive detections in the "eDNA" column, not 9 as was listed in first sentence of "Environmental DNA section". Please cross check text against table prior to finalizing. Thanks. Copy editor**