OHIO BIOLOGICAL SURVEY NOTES

The Impacts of Long-Term, Episodic Applications of the Lampricide TFM on the Common Mudpuppy (*Necturus maculosus maculosus*) in a Northeast Ohio Stream

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Abstract: The Common Mudpuppy (*Necturus maculosus*) is a totally aquatic salamander species found in numerous streams and lakes within the Laurentian Great Lakes watershed. It is a non-targeted species that is sensitive to the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) and frequently sustains high mortality during applications. The Schnabel capture-mark-recapture method was used to examine population stability over a period of 30 years of episodic exposure to TFM in the Grand River of northeastern Ohio. Population estimates varied substantially over time, but the negatively sloped trend line indicated that the population was in decline. Three additional sets of morphometric data all indicated that the size (total length) of adult males and females within the river decreased over time with the periodic application of TFM. Fecundity of female mudpuppies is understood to increase with body size; therefore, a decrease in body size results in reduced fecundity and fewer eggs laid per year. Young-of-the-year and larvae of one or two years of age appear to be more sensitive to TFM than older juveniles and adults; consequently, with lampricide applications at three- to five-year intervals, fewer juveniles attain the age of six to eight years (the age of sexual maturity and first reproduction). Status of this riverine population of mudpuppies is in decline, and the decline is not only attributable to the lethal concentration of TFM during applications but to the chronic, sublethal effects upon reproductive recruitment. Immigration upstream from Lake Erie or downstream from above the initial TFM application point need to be studied for potential contribution to recruitment through immigration. The three largest tributaries to the reach of the Grand River treated with lampricide do not support mudpuppies upstream of their mouths and do not subsize the riverine population.

Keywords: Common Mudpuppy, TFM, population decline, amphibian decline, capture-mark-recapture

INTRODUCTION

The Common Mudpuppy, *Necturus maculosus maculosus* (Rafinesque 1818), is a large, totally aquatic, paedomorphic species of salamander found in the Great Lakes, some inland lakes, and many mid-sized to large streams. Its geographical distribution extends form southeastern Manitoba to southern Quebec, south to northern Georgia, and west to eastern Kansas (Petranka 1998). The species is considered to have declined in the Great Lakes states, including Indiana (Hoffman et al. 2014, Minton 2001), Michigan (Harding 1997, Mifsud 2014), Minnesota (Minnesota DNR 2022), New York (Gibbs et al. 2007, Hunsinger 2001), and Ohio (Pfingsten and White 1989, Sipes 1964). Currently, the status of the mudpuppy is critically imperiled in Illinois (S1/S2), imperiled in Indiana (S2), vulnerable in Pennsylvania and Minnesota (S3), vulnerable to apparently secure in Michigan, New York, and Wisconsin (S3/S4), and secure in Ohio and in Ontario (S4; NatureServe Explorer 2021, Ohio DNR 2020). Habitat degradation, pollution, and sedimentation and siltation are often cited as causes contributing to noted declines (Hoffman et al. 2014, McDaniel et al. 2009, Pfingsten and White 1989). Accumulated evidence indicates that mudpuppies are sensitive to environmental contaminants, such as organochlorine pesticides, polychlorinated biphenyls, and mercury (Bonin et al. 1995, Gendron et al. 1997). Development of the "dead zone" in the hypoxic/anoxic waters of the eastern basin of Lake Erie contributes to the growth of *Clostridium botulinum* type E. Neurotoxins produced by this anaerobic bacterium kill numerous organisms, many of which are fed upon by mudpuppies, which in turn perish by the thousands (Hannett et al. 2011, Lipps and Matson 2013). Commercial harvesting of mudpuppies by biological supply companies has been noted as a probable contributor to local declines in Wisconsin (Casper 1998) and in the Sandusky Bay of Ohio (Pearse 1921, Pfingsten and White 1989). Bishop (1941) was the first to draw attention to declines of the mudpuppy within several streams of the Lake Ontario drainage in western New York in *Salamanders of New York* (Hunsinger 2001, Schmidt et al. 2004).

Chemical lampricides have been used to kill and control the invasive sea lamprey (*Petromyzon marinus*) in tributaries of the Great Lakes and Lake Champlain and are known to cause mortality in riverine populations of mudpuppies (Chellman et al. 2017, Gilderhus and Johnson 1980, Matson 1990). Sea lamprey, native to the North Atlantic, invaded Lake Erie and the upper Great Lakes following the opening of the Welland Canal (Pearse et al. 1980) or its improvements in 1919 (Great Lakes Fisheries Commission 2000). The first record of sea lamprey in the Ohio waters of Lake Erie was in 1927 (Trautman 1981). Since 1958, the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been applied at three- to five-year intervals to streams of the upper Great Lakes that support sea lamprey ammocoetes (Applegate et al. 1958, 1961; Gilderhus and Johnson 1980; Howell 1966). Frequency of TFM application in Lake Erie tributaries was formerly dependent upon the numbers of sea lamprey ammocoetes as determined through Quantitative Assessment Surveys (QAS) conducted by the United States Fish and Wildlife Service Sea Lamprey Control Program (hereafter Program) personnel and upon total lengths (TL) of ammocoetes relative to the size of transformers (the size at which transformation to the parasitic phase occurs). In 2005, the Program began switching QAS to a new survey methodology termed Rapid Assessment (RA), a less intensive method employing catch-per-unit-effort (Jubar et al. 2021). Also in 2005, the concept of "Expert Judgement" (EJ) was incorporated into the Lake Erie stream selection strategy. Expert Judgement category 1 utilizes accumulated information concerning consistent annual recruitment, which resulted in the treatment history of every two to six years into stream treatment prioritization (Grunder et al. 2021). Six tributaries to Lake Erie meet EJ criteria; two of these, Grand River and Conneaut Creek, are Ohio streams. Subsequently, these two streams are now on a fixed accelerated treatment cycle of three years (Jubar et al. 2021). Formerly, stream treatments in the Great Lakes were timed to subject all ammocoete stages detected through surveys to the lampricide before they attained transformer size (National Research Council of Canada [NRCC] 1985). Since 2009, scheduling Ohio streams for treatment is based upon the confirmed detection of one sea lamprey ammocoete > 100 mm total length, an individual likely to transform the following year (Grunder et al. 2021).Application of TFM to tributaries of Lake Erie began in 1986 (Wills and Keerns 2015; pers. obs.), whereas it was first applied to streams of the Lake Champlain watershed in 1990 (Vermont Department of Environmental Conservation 1994). The first streams treated with TFM in Ohio were Conneaut Creek on 18 October 1986 and the Grand River on 26 April 1987. A third stream, the Huron River, was added to the Ohio list of treated streams on 7 May 2018 (USFWS LBS Treatment data).Non-targeted species mortality caused by TFM has been recorded for numerous taxa including invertebrates (Dermott and Spence 1984, Gilderhus and Johnson 1980, Jeffrey et al. 1986, Smith 1967), fishes (Bills and Johnson 1992, Boogaard et al. 2003, Kane et al. 1985), and amphibians (Boogaard et al. 2003, Chellman 2011, Gilderhus and Johnson 1980). Following the first 20 years of use of TFM as a management tool for sea lamprey control in streams, Gilderhus and Johnson (1980) reported that field survey reports often cited mortality of mudpuppies. They noted mortality of mudpuppies in 32% of tributaries to Lake Superior and 36% of those to Lake Michigan; they also noted that 18% of those surveys cited high numbers killed. Similarly, field surveys conducted during applications of TFM to Conneaut Creek and the Grand River in Ohio cited high mortality of mudpuppies and sensitive non-targeted fishes. In 2008, the Ohio Environmental Protection Agency (OEPA) designated a section of the Grand River as a mudpuppy sanctuary. The TFM concentration flowing through the sanctuary during stream treatments was not to exceed the minimum lethal concentration (MLC) for effective control of sea lamprey. Adult mudpuppies are sensitive to TFM concentrations slightly higher than MLCs of sea lamprey ammocoetes at variable pH concentrations. In laboratory tests conducted simultaneously with lake sturgeon and ammocoetes, the no-observable-effect concentrations (NOECs) of TFM on adult mudpuppies (mean TL > 30 cm) ranged from 1.1–1.6 times the MLCs for ammocoetes (Boogaard et al. 2003). Therefore, an adequate margin of safety was found to exist for adult mudpuppies to survive exposure during treatments at or slightly above the MLC for sea lamprey (Boogaard et al. 2003). The sensitivity and NOEC for adults stressed during mating and spawning periods may be elevated and those of larval and juvenile mudpuppies may be different from those of adults (Boogaard et al. 2003). Several studies of the effects of TFM on young-of-the-year (YOY) (Boogaard 2008, Durfrey and Neuderfer 2009) and on juvenile mudpuppies (Neuderfer 2002, Neuderfer et al. 2004) determined that the sensitivity of mudpuppies to TFM was age specific and that adults were more resistant, whereas YOY and one-year-old age classes were at substantially greater risk of TFM-induced mortality. Boogaard et. al (2008) suggested that numerous YOY were unlikely to survive routine TFM applications greater than the 1.0 MLC needed to kill ammocoetes, and that YOY mortality in stretches downstream of boost stations could be nearly complete. Less is known concerning the sensitivity of two- to five-year-old juveniles. Kaye et al. (2012) field tested sensitivity of YOY or mudpuppies having a TL approaching 4 cm in the Sturgeon River of Michigan. At the pH and alkalinity during the study, 4.8% of the mudpuppies died in the traps. Clearly, the sensitivity of YOY requires further testing in the field.

Overall, the purpose of this report is to examine the impacts of long-term, repeated, episodic applications of TFM on the stability of the riverine population of the mudpuppy in the Grand River of Lake and Ashtabula counties, Ohio (Fig. 1). The primary method used was to compare population estimates made by using capture-mark-recapture data accumulated at

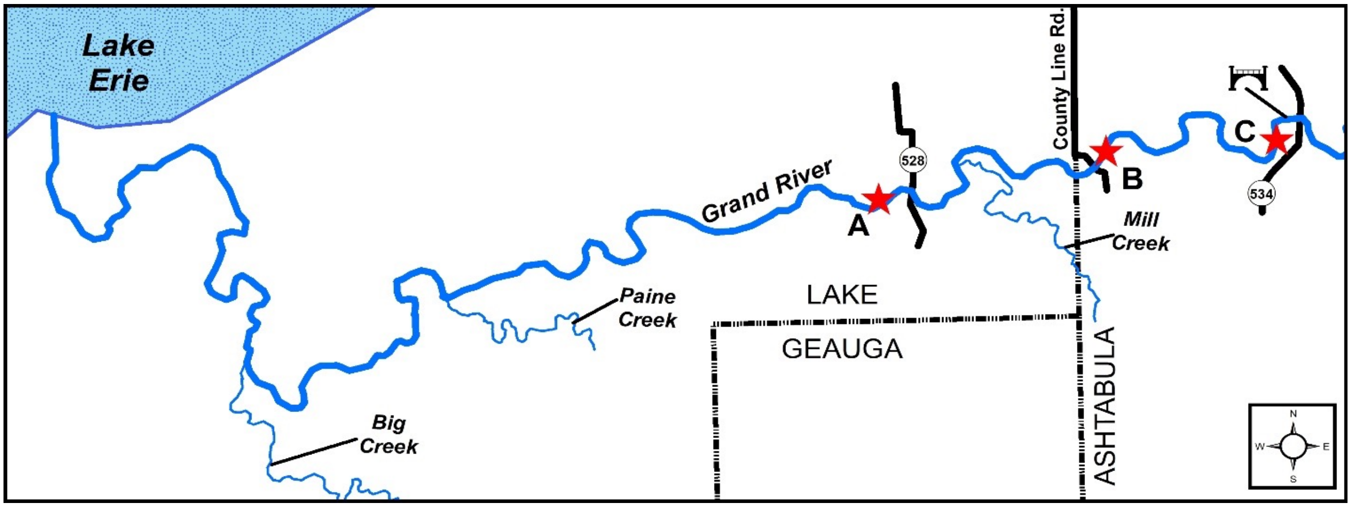

Fig. 1. Map of the Grand River flowing through Lake and Ashtabula counties in northeast Ohio: A) location of the research site at Hidden Valley downstream of Ohio Route 528 (RM 22); B) location of the first TFM application on the Grand River in 1987 upstream of County Line Road (RM 25.9); C) location of the Harpersfield Dam (RM 30.9). The initial source of all TFM applications after 1987 was positioned downstream of the dam and covered bridge.

a research site (included within the mudpuppy sanctuary) subjected to repeated applications over time, and to examine the trends. However, three additional datasets were available for analysis that approach the question of population stability from different perspectives. Each of the following three datasets include the TL of individuals; therefore, size trends over time with multiple exposures to lampricide could be a productive measure of population stability.

Dataset 1: During the population study in the sanctuary, the TL of all captured mudpuppies was measured; consequently, the TLs of animals in the population examined were compared over time to evaluate size change of individuals in the population.

Dataset 2: The U.S. Fish and Wildlife Service (USFWS) began a program of trapping sea lamprey at the Harpersfield Dam in 1984. The Grand River is one of five index streams on Lake Erie where capture-mark-and-recapture methods are employed to estimate the adult sea lamprey population (currently using the Peterson Method). The dam is the structure preventing sea lamprey from gaining access to extensive spawning grounds farther upstream (Fig. 1). The primary application point source for pumping TFM into the river was positioned near the Ashtabula-Lake County line in 1987 (Fig. 1); thereafter, it was located just downstream of the Harpersfield Dam during following applications. Mudpuppies were also captured in USFWS live traps, and in many years, the number of mudpuppies captured and their TLs were recorded.

Dataset 3: During the day of lampricide application and often one or two following days, salvage operations (field surveys) to recover dead mudpuppies and other non-targeted and targeted animals from the river were conducted by personnel and staff of USFWS, Ohio Department of Natural Resources (ODNR), the OEPA, and multiple regional stakeholder organizations. Nearly all salvaged mudpuppies from the Grand River treatments (as well as those from Conneaut Creek) were preserved in the herpetology collections of the Cleveland Museum of Natural History (CMNH). Total lengths and standard lengths of many of the salvaged mudpuppies have been measured and incorporated into this report.

METHODS

The Grand River is a tributary to the west-central sub-basin of Lake Erie in Northeastern Ohio. It drains an area of 1844 km^2 (712 mi^2; ODNR 1954). The study site was located in Hidden Valley Metropark (HV), Madison Township, Lake County (Thompson, Ohio 7.5’ topographic map, 1960, photorevised 1970) at 41°44’ N latitude and 81°03’ W longitude (RM 22). Originally (1987–1988), the study site was 600 m in length (Matson 1990), but it was extended to 700 m in 1989 (Matson 1998). Prominent features of the site included two meanders, one riffle, one to three gravel bars (dependent upon water level), and several incised channel-margin pools. One prominent bar was seldom totally submersed and existed either as an island bar or as a peninsula. Water depth ranged up to 2 m during the summer months but was subject to dramatic fluctuations during the remainder of the year and following heavy precipitation events. Substrate within the river channel included silt, silt-detritus, sand, gravel, cobble, glacial erratics, and both siltstone slabs and bedrock of the Chagrin Member of the Ohio Shale (Swinford 1985). Substrate composition was not evenly distributed within the site; consequently, suitable mudpuppy habitats within the site were patchy.

Fieldwork was conducted from mid-March through mid-July during 1987 and 1988; thereafter, it extended from late

May or early June through mid- to late August in the years between 1989 and 2017. Four methods were used over the years to capture mudpuppies (see Matson 1990). The method found most effective and the only one used after 1988 was manual elevation of slabs to one side and sweeping the footprint of the slab with the side of the foot downstream toward a 1.8 m seine. This method of capturing mudpuppies in riverine environments has also been found to be more effective than trapping during the summer months (Haines 2020). Geographic coordinates of capture locations for each animal were not determined in the initial 1987–1988 study. During the following years, locational coordinates of the slab under which a mudpuppy was captured were determined using one of two methods. The first, described in Matson (1998), was used from 1989 to 2007 and involved trigonometric calculation from established set points on shore. The second, beginning in 2008, used a hand-held Garmin GPS unit to obtain longitudinal and latitudinal coordinates. Coordinates were obtained for additional demographic, movement, and activity range studies to be presented elsewhere.All mudpuppies were marked with a year of first capture toe-clip during 1987–1988. Total length was measured to the nearest millimeter in graduated plastic cylindrical tubes; the mudpuppy was then returned to and released at the point of capture. From 1989–2007, mudpuppies < 60 mm TL were measured to the nearest mm and given year of capture toe-clips; those 60–140 mm TL were measured to the nearest 5 mm in graduated plastic tubes, given a unique toe-clip number employing the method of Martoff (1953), and then released at the point of capture. Mudpuppies > 140 mm TL were measured to the nearest 5 mm in graduated plastic tubes, sexed when feasible as described by Bishop (1926, 1941), and marked using a numbered plastic t-tag inserted through the proximal one-third of the caudal musculature beneath the vertebral column. Standard length (SDL) was measured to the nearest 5 mm in graduated plastic tubes on all mudpuppies having a TL > 60 mm beginning in 1989. Each mudpuppy was then returned to the point of capture and released.

Passive integrated transponders (PIT tags) were used beginning in 2008 (Microchip ID Systems). PIT tags were injected into the musculature just beneath the skin of the ventral-lateral portion of the tail approximately 25 mm caudad the vent. Mudpuppies ≥ 200 mm TL received a 12.5 mm PIT tag, whereas beginning in 2015, mudpuppies ≥ 110 mm TL received a 6.3 mm mini PIT tag. Mudpuppies with TL ≥ 60 mm but < 110 mm were toe-clipped and given a unique number (Martoff 1953). Mudpuppies < 60 mm TL were given year of capture toe-clips. After 1988, hatching-year larvae were noted and released at the point of capture without marking. Unmarked individuals < 60 mm TL were not included in population estimates after 1988—they were not detected after the first year of capture, probably attributable to inaccurate toe clipping and/or regeneration of digits. Fieldwork was conducted at the research site in 2018, but no new animals were marked. Mudpuppies recaptured from other years and those not previously marked were measured and sexed, and their site-of-capture coordinates were taken for activity range and movement study. The measurement data were used, but no population estimate was feasible.

Capture-mark-recapture (CMR) methods were used to analyze the population data accumulated over time. The Schnabel method of population censusing and estimation (Schnabel 1938, Seber 1973) was used to estimate population size before and after treatments of the river with TFM. Years having high precipitation and unpredictable high-water events prevented realization of this plan during several applications of the lampricide. Chapman's Poisson table was used to calculate 95% confidence interval estimates (CIE) for ≤ 50 recaptures. The standard normal approximation to the Poisson was used to estimate 95% CIE for > 50 recaptures (Seber 1973). Population estimates were compared using the normal approximation z-statistic (Seber 1973). Mean TL for mudpuppies captured each year within the study site having TL > 99 mm was graphed and the trend line was plotted. Mean TL for mudpuppies with TL > 99 mm was calculated for each year the project was conducted for mudpuppies salvaged during TFM treatments and for mudpuppies trapped at the Harpersfield Dam; trend lines were plotted for years 1987–2018 (1984–2018 for animals trapped at the dam). Total length > 99 mm was chosen to standardize comparisons and reduce bias between the three meristic datasets because the methods of capturing or salvaging animals do not detect one to three-year-old larvae and small juveniles equally well. Trapping produces the fewest small mudpuppies, whereas salvage operations conducted concurrently or following TFM applications produce far greater numbers. Despite that, many are overlooked, remain undetected in deep water, are concealed in cracks in the bedrock or other substrate, or are readily consumed by scavengers and predators.

The U.S. Fish and Wildlife Service maintains a sea lamprey trapping station during the vernal upstream movement and spawning period at the Harpersfield Dam, Harpersfield Township, Ashtabula County, OH (Fig. 1). The initial point source of TFM applications in 1987 was approximately 100 m upstream of County Line Road, which separates Lake and Ashtabula counties; thereafter, it was located approximately 0.1 mile downstream of the dam. Mudpuppies were frequently captured in the lamprey traps. Mean TL of mudpuppies with TL > 99 mm trapped each year for which data were available beginning in 1984 and extending through 2018 was calculated, graphed, and the trend line was plotted.TFM was applied to the Grand River eight times from 1987 through 2017 at three- to five-year intervals, except for the 12-year period from 1987 to 1999 (Table 1). Field salvage operations by kayak, canoe, or wading were conducted by personnel of the USFWS, ODNR, OEPA, interested stakeholders, and private volunteers to salvage dead target and non-targeted taxa, mostly fishes and amphibians. Salvage operations began about 0.1 mile (about 0.26 km) downstream of the Harpersfield Dam (the primary application point after 1987) and extended up to approximately 22 river

1986	1999	2003	2006	2008*	2009	2013	2017**

*New 18-month protocol initiated; treated spring 2008 and fall 2009.
**Treatment scheduled for 2016 delayed until 2017 due to high water.

Table 1. Years in which the Grand River was treated with the lampricide TFM.

Year	Marked	Recaptures	$\hat{N}$	Lower CIE	Upper CIE
1987	212	35	471	333	671
1988	290	130	193	180	215
1989	63	10	132	68	274
1990	139	37	198	142	280
1991	416	174	333	288	389
2008	15	0	111	10	
2009	169	45	227	167	309
2010	164	47	210	153	277
2011	15	0	82	7	
2012	69	9	167	83	362
2013	35	1	177	61	
2014	101	15	240	140	427
2015	67	8	185	88	427
2016	213	48	354	264	478
2017	199	47	314	233	425

Table 2. Mark-and-recapture data, population estimates, and 95% confidence intervals for the Common Mudpuppy at the Hidden Valley Grand River study site, Lake County, Ohio.

Year	Days	Workers	Hours	Captures	Captures/ hour
1987	16	5	400	212	0.43
	7	2	91	(total)	
1988	10	5	250	290	0.65
	15	2	195	(total)	
1989	11	2	143	63	0.44
1990	14	3	273	139	0.51
1991	40	4	1040	416	0.4
2008	5	3	87	16	0.18
2009	15	4	390	169	0.43
2010	13	4	338	164	0.49
2011	5	3	98	15	0.15
2012	5	3	98	69	0.7
2013	4	3	78	35	0.45
2014	7	3	137	101	0.74
2015	5	3	98	67	0.68
2016	13	3	254	213	0.84
2017	10	3	201	199	0.98

Table 3. Number of Common Mudpuppies captured per hour at the Hidden Valley Grand River study site, Lake County, Ohio.

miles (35.4 km) downstream (Cheryl Kaye, USFWS, pers. comm.). Most field surveys conducted during and following each treatment salvaged numerous mudpuppies. Several applications were conducted during high water, when turbidity was high and visibility was low, the flow rate was high, and the depth made walking in the river hazardous (e.g., 1999). Few targeted or non-targeted individuals of any species were salvaged during those high-water operations. Dead mudpuppies were examined for marks and PIT tags, and most were preserved in the herpetology collection of the Cleveland Museum of Natural History. Salvaged adult mudpuppies and juveniles > 99 mm TL were dissected in the laboratory, sexed using both external morphology and internal gonadal observation, and the TL and SDL were measured to the nearest 1 mm. Ova of sexually mature and juvenile females were measured to the nearest 0.1 mm using a dial micrometer (Matson 2013). Mean TL for salvaged mudpuppies with TL > 99 mm was calculated for individuals from each TFM application, graphed, and the trend line was plotted.

RESULTS

Capture-mark-recapture data for mudpuppies at the Grand River site for the years 1987–2017 are presented in Table 2. The number of mudpuppies captured per year ranged from 15–416 in years 2011 and 1991, respectively. Recaptures were 0 and 174 during those same years. Population estimates over all years when mudpuppy recaptures exceeded one ranged from 132–471. Three of the estimates, years 2008 ($\hat{N} = 111$), 2011 ($\hat{N} = 82$), and 2013 ($\hat{N} = 177$), are included in Table 2, but they had ≤ 1 recaptures and were conducted in wet years with high water, which resulted in few field days, few mudpuppies captured or recaptured, and low catch per unit effort in 2011(Table 3). Upper CIE are not included in the table (Table 2) nor in the graph (Figure 2) for those years because they would have exceeded the y-axis (2013 with one degree of freedom) or could not be determined with zero degrees of freedom (0 recaptures; Chapman's Poisson distribution table and Seber 1973). Wide-ranging estimates between years may in part be attributable to differences in water levels, the number of workers and the level of effort directed to the study, variable stream dynamics, efficiency of the methodology and

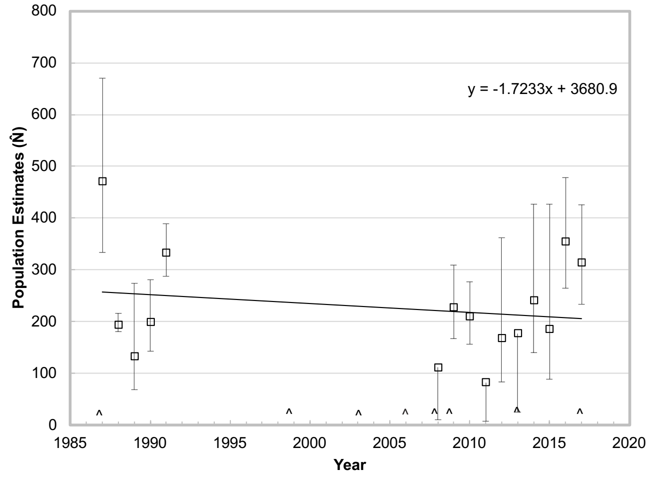

Fig. 2. Plot of the annual population estimates with 95% confidence interval estimates for the Common Mudpuppy in the Grand River in Lake County, Ohio, for the years 1987–2017. Carets above the x-axis indicate years of TFM application.

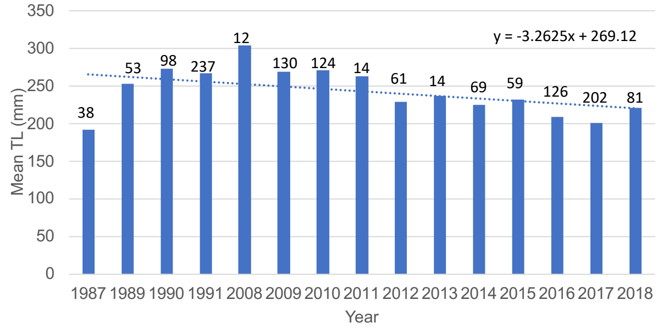

Fig. 3. Mean TL of Common Mudpuppies > 99 mm TL captured at the research site in the Grand River, Lake County, Ohio, for the years 1987–2018. Numbers above the bars indicate the sample size for each year.

techniques of the field crew, annual variation in mudpuppy movements, and/or unknown factors. Population estimates with CIE and the negatively sloped trend line ($y = -1.7233x + 3280.6$) are plotted in Fig. 3 and show a decline in population size over time.

Pairwise comparison of population estimates between consecutive year estimates indicated several significant changes in population size. Year estimates between 1987 and 1988, $\hat{N} = 471$ and 193, respectively, were significantly different ($p < 0.001$); those of 1990 and 1991, $\hat{N}=198$ and 333, were different ($p<0.01$); and those of 1991 and 2009, $\hat{N} = 333$ and 227, were different ($p < 0.05$). The differences between 1987 and 1988 (a drought year) follow the first application of TFM in April 1987; those between 1990 and 1991 may be due to the large degree of effort in 1991 and drought conditions, which resulted in a high number of mudpuppy captures and recaptures. During the significant drought years of 1988 and 1991, the water level was extremely low, and a sizeable percentage of the river channel was exposed and without water. These conditions would have displaced mudpuppies using exposed refugia to areas maintaining water flow, thereby increasing their density during the summer months. Drought conditions such as those of 1988 and 1991 have not occurred since 1991. Significant differences between estimates for 1991 and 2009 are probably attributable to applications of TFM in 1999, 2003, and 2008. Differences between other consecutive pairwise population estimates were not statistically significant. The long gap between estimates for 1991 and 2008 was due to the 12-year period from 1987 to 1999 without scheduled TFM applications and too-wet years within the period 1999–2008, with high water levels. Of those population comparisons, only the 1991/2009 comparison was separated by multiple TFM applications. The trend of estimated population size plotted against time in years has a negative slope, indicating a decline in estimated population size over time (Fig. 3).The number of mudpuppies captured per hour at the study site ranged from 0.15–0.98 (Table 3). Lowest catch per hour occurred during 2011, when water levels in the river were greatly elevated due to precipitation; consequently, fewer field days were devoted to the project and fewer animals were captured because capture efficiency of the methodology employed varies inversely with water level. Highest catch per hour occurred during each of the last two years of the project (2016–2017), when July and August were dry and water levels were very low.Analysis of the size data on mudpuppies having a $TL > 99$ mm captured at the population research site indicates that TL decreased approximately 50 mm from 1987 through 2018, as shown by the negative slope of the trend line $y = -3.2625x + 269.12$ (Fig. 3).

Salvage operations downstream of the Harpersfield Dam (a distance of over 20 river miles that includes the research site) during TFM applications and conducted one to several days afterward detected many dead non-targeted organisms. Mudpuppies of all size and age cohorts were salvaged during those operations. Mean TL of mudpuppies having a $TL > 99$ mm also decreased temporally; data and the trend line $y = -2.1607x + 222.91$ are plotted in Fig. 4.

Mean TL of mudpuppies trapped at the Harpersfield Dam having a $TL > 99$ mm exhibited a trend line having a negative slope ($y = -6.8033x + 208.92$, Fig. 5), indicating a temporal decrease in size of approximately 50 mm from 1984 to 2018. Data for years not represented in the plot were not available; in some years, measurements were not recorded or animals trapped were ≤ 99 mm TL. The number of mudpuppies trapped annually varied considerably, perhaps reflecting differences in annual movement patterns or trap nights.During the early 1990s, a channel margin pool that had been highly productive for capturing one- to three-year-old mudpuppies during March and April through intensive seining was rapidly filled through stream dynamics and deposition, and within three years was no longer available (see Matson 1998, Fig. 28-2). This pool, which was over 1 m deep in early spring and positioned laterally to a meander riffle, appeared to serve as

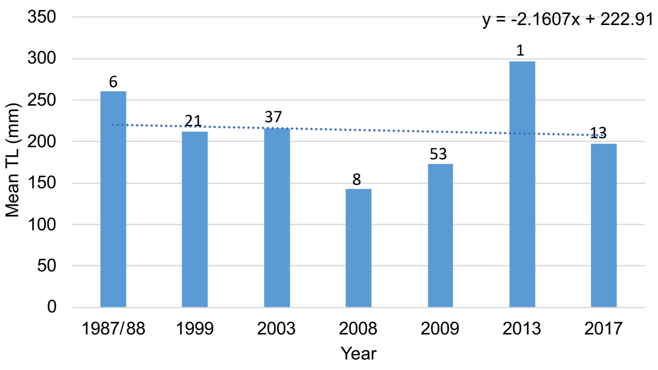

Fig. 4. Mean TL of Common Mudpuppies > 99 mm TL salvaged from the Harpersfield Dam in the Grand River downstream approximately 22 river miles to the mouth of Big Creek, Lake County, Ohio, for the years 1987–2017. Numbers above the bars indicate the sample size for each year. These data include mudpuppies salvaged from the Hidden Valley research site.

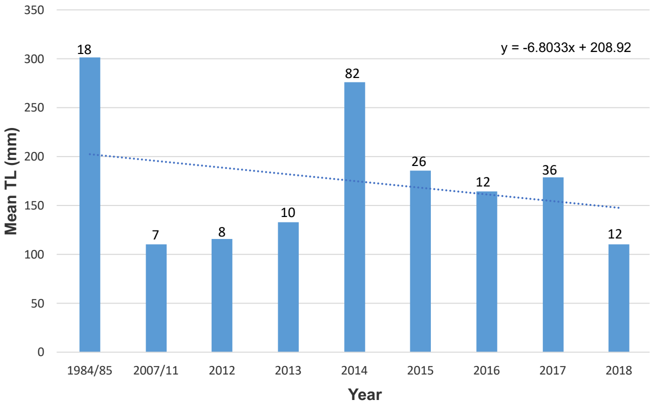

Fig. 5. Mean TL of Common Mudpuppies > 99 mm TL trapped at the Harpersfield Dam in the Grand River, Lake County, Ohio, for the years 1984–2018. Numbers above the bars indicate the sample size for each year. Data courtesy of the USFWS Sea Lamprey Control Program.

an overwintering nursery for young mudpuppies where few adults were captured. By late April, mudpuppies had dispersed and were distributed elsewhere in the stream (see Matson 1990, Fig. 2). Thereafter, fewer one- to two-year-old larvae were encountered, and the successful method of capturing mudpuppies became limited to slab tipping (described in Methods). Consequently, the proportion of one to two-year-old larval mudpuppies captured changed over time and consisted more of individuals ≥ three years old and hatchlings. It is feasible that the loss of the nursery pool may have contributed to the observed population decline. However, numerous nests with both eggs and later hatchlings continued to be found, and one- to three-year old larvae remained common within the site. Young-of-the-year larvae remain common in salvage collections made during TFM field collections.

DISCUSSION

Non-target mortality observed during and for several days immediately following TFM applications is readily apparent during field surveys. Populations of several species in the family Ictaluridae—namely Stonecat Madtoms (*Noturus flavus*) and Brindled Madtoms (*Noturus miurus*)—generally incur the greatest loss of individuals among fishes observed in Ohio streams, the Grand River, and Conneaut Creek. Among amphibian taxa found dead during lampricide treatments, only the mudpuppy generally suffers high mortality. However, in tributaries used as stations to boost the attenuated lampricide concentration in the chemical bank during the stream treatment, extensive losses can occur to plethodontid stream salamanders such as the Northern Two-lined Salamander (*Eurycea bislineata*), which develops over two to three years in gilled aquatic larval stages (Pfingsten 2013; pers. obs., Mill Creek, 2017: 197 salvaged in 0.8 miles of stream, most recovered in lower 0.5 mile). TFM concentrations exceeding minimum lethal concentrations (typically applied at 1.5 the MLC) required to kill sea lamprey are applied in tributary boost streams (Boogaard et al. 2003, 2008). Salamanders of several genera (*Desmognathus* and *Eurycea*) distributed in streams of the eastern Great Lakes states, Ontario, and Quebec within the Great Lakes drainage system have gilled larval stages and may easily be overlooked during stream surveys. The impact of TFM on these species would be of interest, as would the NOECs as a first measure to maintain local species richness and biodiversity.

CMR data (Fig. 2 and Table 2) indicate a temporal downward trend in the size of the mudpuppy population. These data are supported by observations of mortality made during field surveys. During field surveys, reaches of the river providing little or no preferred or suitable habitat yield few dead or dying mudpuppies, unless the animals are transported downstream by the current. However, numerous dead or dying mudpuppies are often found in stream stretches providing suitable or preferred habitat. The research site has subsections that provide optimum habitat and support numerous mudpuppies of all size and age cohorts, and observed mortality in these subsections can be high. For example, in October 2009, 25 mudpuppies of many size and age cohorts, including four toe-clipped individuals, were salvaged from a section of stream less than 40 m in length on one side of the river. (Hatching-year larvae were not included in this number.)

The slope of the trend line (Fig. 2) indicates that the size of the population is in slow to moderate decline. Movement patterns of mudpuppies within the river may partially explain the rate of decline. Relatively little is understood about the general or seasonal movements of mudpuppies. In a previous study using the same site, Matson (1998) found 86% of marked mudpuppies between 1989 and 1991 (382 mudpuppies with 609 captures) were captured only one or two times and were considered transients. Similarly, Shoop and Gunning (1967) marked 69 mudpuppies in Big Creek, Louisiana, and found that 52 of 69 animals (about 75%) were captured only once or twice over a two-year period; those captured only once could represent transients. Sajdak (1982) marked 141 mudpuppies with 47 recaptures in the Mukwonago River of Wisconsin. He suggested that mudpuppies were sedentary and widely dispersed during the summer months, but they made seasonal movements in autumn to breeding areas and in spring to summer activity ranges. If animals did not return to their former summer activity ranges, they could be transients. The low rate of recapture in these studies may in part reflect biases inherent in the methodologies being used for capture, or to the time of year in which the projects were conducted, resulting in failure to recapture mudpuppies actually present. Although the population estimates are themselves of interest, the overall negative slope of the trend line over 30 years is of more consequence regarding the status of the population. Recruitment is known to be occurring at the site because we continue to locate nests and hatchlings, and because we continue to find all size and age cohorts during salvage surveys (pers. obs.; salvage collections preserved at CMNH) throughout the treated portion of the river. It is feasible that the riverine population derives a subsidy through immigration upstream from Lake Erie or from the downstream movement over the Harpersfield Dam (RM 30.9) from the upper Grand River. Pope (1964) noted that the large Lake Michigan mudpuppy population near Chicago seemed to make annual migrations up tributary streams and rivers. Major tributaries downstream of the Harpersfield Dam, Big Creek (RM 9.3), Paine Creek (RM 14.3), and Mill Creek (RM 24.7) do not support local subpopulations of mudpuppies except at their mouths in the Grand River (Matson 1998); they therefore could not serve as reservoirs to repopulate or subsidize the riverine population. Additional research concerning the movements of mudpuppies is needed to gauge the importance of these potential subsidies.

Each of three sets of measurement data on TL of male and female mudpuppies indicates a decrease in body size over time. The sex of mudpuppies in the Grand River was determined through gonadal observation of animals salvaged throughout the river during TFM applications. Sex ratio of males to females in the stream was 1:1 (Matson 2013), indicating that males and females probably have equal susceptibility to TFM. Fecundity in an aquatic egg-laying amphibian species is understood to increase with the size of the female and assumes that mature ova size remains constant, but that the body volume available for the storage of ova in the coelomic cavity is increased (Duellman and Trueb 1986). This relationship exists in mudpuppies. Shoop (1965), studying *Necturus m. louisianensis* in Big Creek, Louisiana, found that the number of ovarian eggs (those >1.0 mm in diameter) varied directly with female size (TL). Gendron (2000), sampling in the Great Lakes system and the St. Lawrence and Ottawa rivers, found that fecundity varied from 11–217 eggs and was dependent upon locality and TL of the female. Mean clutch size in the Grand River, based upon 13 nests at the study site, was 83 (range 60–120; Matson 1998). Mudpuppy reproductive recruitment in the Grand River is predicted to further decrease over time with continued application of TFM as a result of the noted decline in population size (both males and females) attributed to TFM, coupled with the decrease in female size resulting in a diminished number of eggs laid. Chellman (2011) noted a nearly significant reduction in the size (TL) of females trapped in the Lamoille River, Vermont, during 2008–2010 before and after TFM application. He attributed the reduction in body size to differences in water temperature between years and to female trapping and movement responses to those environmental changes. A portion of that noted reduction may have been the result of the TFM application.

The reduction in mudpuppy size (TL) also indicates that fewer juveniles are attaining older adult size due to delayed maturity of both males and females. Bishop (1926) thought that mudpuppies in New York state reached sexual maturity and the age of first reproduction at six years when about 200 mm TL. The growth rate was 30–35 mm/year for the first five years and then slowed. Pope (1947) considered mudpuppies in the Chicago area to reach sexual maturity at seven to eight years of age, whereas Gendron (2000), using growth line skeletochronology, found the growth rate to be high until eight or nine years of age, at which time sexual maturity was reached and growth slowed to 10 mm or less/year. Streams in Ohio were treated with TFM on a three- to five-year schedule; however, the Grand River is now an EJ category 1 stream and is scheduled to be treated on an accelerated cycle of three years. Therefore, larval and juvenile mudpuppies must survive a minimum of two applications before achieving the age of first reproduction. Mudpuppies are a long-lived species with delayed maturity, reaching 23 years (Senning 1940) or older; the record longevity is 34 years (Gendron 2000). The reduction of body size in the riverine population indicates that fewer females have the longevity to attain large size, when they would have the greatest fecundity and potentially make the greatest positive contribution to the stability of the population. Species with delayed maturity and reduced recruitment respond relatively slowly to perturbations (Wheeler 2003). As described by Wheeler (2003), what is required is more information on the survivorship of various life stages. If YOY and one-year-old mudpuppies are more sensitive to TFM than adults, as found by Durfrey and Neuderfer (2009) and Boogaard (2008), then recruitment through reproduction will be further reduced with each TFM application. More information concerning the age structure and composition and TFM sensitivity is needed to more completely understand the lethal and cumulative sublethal impacts of TFM on mudpuppy populations. A population known to be declining in size and to have a decreasing percentage of young is in serious condition (Alexander 1958).

Reproduction in mudpuppies has not been thoroughly investigated. Basic information, such as the frequency of ovulation and whether spawning of adult females is annual, biennial, or otherwise, has not been adequately determined. Matson (2013, Fig. 23-6) presented data indicating that a small portion of females examined did not develop mature ova every year and probably breed biennially. These data are measurements of ova over several months of the year. More data are needed during summer and early fall to test this contention. It is feasible that the lower rate of ova development may be an indicator that those females were less efficient predators and could not channel the necessary nutrients and energy into development of ova. It may also indicate that those July females spawned very early and that their ova had enlarged toward mating during the next fall breeding cycle. If some females reproduce biennially rather than annually, then recovery from perturbations such as TFM would further slow any recovery and contribute to the rate of population decline.

Observed mortality of both target and non-target species during a TFM application can be very low during high-water events, when nearly all field survey work must be conducted from watercraft, and when turbidity is high, preventing location of dead animals in the water column, on the substrate, or in cracks in the bedrock. Detection of changes in the population status of target and non-target species then requires pre- and post-treatment methodologies to assess changes and to evaluate the impact of the application. Long-term study at a selected site in the Grand River known to provide suitable habitat to support numerous mudpuppies indicates that the population has declined over 30 years of episodic exposure to TFM. Population decline has resulted directly through mortality caused by TFM, but more importantly, by chronically affecting the life and reproductive cycles of survivors.Other factors, including pollutants such as organochlorine pesticides, polychlorinated biphenyls, mercury (Bonin et al.1995, Gendron 1997), or others that have not been investigated or yet identified at HV, could be affecting mudpuppies in the Grand River. Increased siltation and sedimentation in the Grand River over the past 30 years could be an important factor effecting the suitability of the site for mudpuppies. Over the years, channel margin pools have been lost through filling, and rock slabs known to function as refugia and to serve as nest rocks have been covered over. During the one-in-500-year flood of July 2006, some of the large siltstone slabs were either covered by silt debris or moved downstream by the current. Habitat modification may therefore be contributing to the population decline observed at HV; however, it does not adequately explain the decrease in body size throughout the section of the Grand River subjected to repeated applications of TFM.

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