Evaluation of Degradation of Benthos in the Kalamazoo and Clinton Rivers: Pilot Health Survey of Native Mussels

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Cover Photos: Inset left, young giant floater and Wabash pigtoe from site 5 in the Kalamazoo River; Inset right, fluted-shell with a zebra mussel attached from site 15a in the East Fork West Branch St. Joseph River; Background, Kalamazoo River at site 11a looking downstream from US-131, Plainwell, MI (Photos by Peter J. Badra).

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Introduction

The Clinton and Kalamazoo Rivers are Areas of Concern (AOCs), as defined in the Great Lakes Water Quality Agreement, where beneficial uses have been impaired by impacts. The importance of the Great Lakes ecosystem to the health and economy of the region compelled the U.S. and Canada to create the Great Lakes Water Quality Agreement. The agreement is a commitment between the U.S. and Canada to restore and protect the waters of the Great Lakes. Addressing beneficial use impairments is a necessary step in restoring AOCs and fulfilling the Great Lakes Water Quality Agreement. Degradation of benthos and degradation of fish and wildlife populations are two of the beneficial use impairments identified in the Clinton River and Kalamazoo River AOCs in Michigan.

Native mussels (Unionidae) are an integral benthic component of aquatic ecosystems, playing an important role in cycling nutrients and structuring food webs (Vaughn and Hakenkamp 2001). Being long lived benthic organisms, they are prone to bioaccumulate contaminants within their tissues, e.g. clubshell (Pleurobema clava) can have a maximum life span of 50 years or more (Badra and Goforth 2001). Mussels are particularly sensitive to heavy metals, ammonia, pesticides, and other contaminants (Keller and Zam 1991; Van Hassel and Farris 2007). The rivers and lakes of Michigan support 43 species of native unionid mussels. Eighteen of these are state listed as threatened or endangered, and five are federally listed as endangered. The Clinton watershed has historically supported 31 mussel species, and the Kalamazoo watershed has historically supported 28 mussel species (University of Michigan Museum of Zoology Mollusk Collection; Badra 2010; Mulcrone and Mehne 2001; Strayer 1980). The status of these benthic species is indicative of the quality and function of the river ecosystems they inhabit.

This project is part of a larger effort to address the beneficial use impairments of degradation of benthos, and degradation of fish and wildlife populations by evaluating the health of freshwater mussels in two AOCs through mussel population surveys, contaminants analyses, and mussel health/disease parameters. The role of Michigan Natural Features Inventory (MNFI) in this effort is to perform mussel surveys, help determine which mussel species would be the best candidates for histopathological and toxicological assessment, and to determine locations of mussel populations that would be appropriate for tissue collection to carry out the future assessment. MNFI's survey and sampling efforts were coordinated with USFWS Michigan Ecological Services Field Office staff in East Lansing. Mussel tissue samples were not collected during this project, as originally planned, in order to allow tissue collection to be timed with a future histopathological/toxicological assessment. Survey effort was shifted in part from the Kalamazoo and Clinton Rivers to the St. Joseph River to better coordinate efforts with a separate large-scale mussel survey in the Kalamazoo River watershed.

The goal of this project is to facilitate future investigations into the potential of contaminants within Great Lakes AOCs to be limiting factors to the growth, health, and sustainability of mussel species, and to assess the current status of these important benthic species including listed species. There is a need to develop methods of assessing mussel health such as these to identify and address impacts from contaminants (Waller and Cope 2019). Mussel surveys were performed in the Kalamazoo and Clinton AOC watersheds, as well as the St. Joseph watershed (Maumee drainage). The St. Joseph is a non-AOC watershed that differs in its history of chemical impacts from the Kalamazoo and Clinton watersheds, and could be used as a control. Updated information on the location and status of Federally and State listed mussel species was generated by this project and is another benefit of this effort.

Methods

Locations of survey sites in the Kalamazoo River were determined based on evidence for extant mussel populations from past surveys or museum records within an area with known PCB contamination. USFWS Michigan Ecological Services Field Office staff provided input on potential suitable site locations. Kalamazoo River survey sites were located in the main stem near, and in Kalamazoo, MI, where there is a history of PCB contamination. Survey site locations in the Clinton and St. Joseph River watersheds were based primarily on evidence of mussel populations, especially federal and state listed species, from past surveys.

Mussel surveys took place in wadable habitats less than approximately 70cm deep. The search area at each site was measured to standardize sampling effort among sites and allow unionid mussel density estimates to be made. When feasible, the search area extended from bank to bank in order to include the widest range of microhabitats. Live unionids and shells were located with a combination of visual and tactile means. Glass bottom buckets were used to facilitate visual detection. Frequent hand sweeps through the substrate (5-10cm deep) were made to help ensure that buried individuals were being detected, including smaller sized unionid mussels. In order to maximize detection of clubshell (a species known to often bury itself particularly deep within stream substrates) and other mussel species that might be buried in the substrate at sites within the St. Joseph River, special attention was paid to tactilely locating mussels 5-10cm down into the substrate.

Live individuals were identified to species and placed back into the substrate anterior end down (siphon end up) in the immediate vicinity of where they were found. All listed mussel species were returned to the substrate where they were found. Shells were also identified to species and returned to the site they were found. The number of individuals of each unionid mussel species was recorded at each site. External annular rings were counted, and length, width, and thickness measurements made on a sub-set of live individuals found. Asian clams (*Corbicula fluminea*) and zebra mussels (*Dreissena polymorpha*) were noted when found. Latitude and longitude of survey sites was recorded with handheld Garmin GPS units. The field crew usually consisted of two MNFI staff but ranged from 1-4 people.

Access to survey sites in the Kalamazoo and Clinton watersheds was made through publicly owned land. A canoe with electric trolling motor was used to access survey sites 1-5 in the Kalamazoo River. Access to survey sites in the St. Joseph watershed required walking through privately owned land. Landowners were contacted by phone and/or mail to obtain permission to access survey sites. Chemical resistant PVC waders and shoulder length PVC gloves were used when surveying sites in the Kalamazoo River to minimize contact with sediments potentially contaminated with PCBs.

Habitat data were recorded to describe and document stream conditions at the time of the surveys. Substrate within each search area was characterized by estimating percent composition of each of the following six particle size classes (diameter): boulder (>256 mm); cobble (256-64 mm); pebble (64-16 mm); gravel (16-2 mm); sand (2-0.0625 mm); and silt/clay (<0.0625 mm) (Hynes 1970). Woody debris, aquatic vegetation, exposed solid clay substrate, and eroded banks were noted when observed. The percentage of the search area with pool, riffle, and run habitat, and a rough characterization of current speed were estimated visually. Conductivity, pH, and water temperature were recorded with an Oakton handheld meter. Alkalinity and hardness were measured with LaMotte kits at selected sites.

Results

Kalamazoo River

Fourteen sites were surveyed in the Kalamazoo River mainstem (Table 1). A total of 16 unionid mussel species were found including the state threated slippershell (*Alasmidonta viridis*) and purple wartyback (*Cyclonaias tuberculata*). Five species of special concern were also detected (Table 2). The two most abundant species were mucket (*Actinonaias ligamentina*) and Wabash pigtoe (*Fusconaia flava*)(Figure 1). Mucket was found at densities up to 0.39 indvs./m^2 at site 11a and Wabash pigtoe was found at 0.16 indvs./m^2 at site 1. Four of the 25 muckets found at site 11a were marked with an X etched in their shells (Figure 2). It is not known who marked them. The non-native Asian clam was found in very high densities at several sites, but it was most prevalent at sites 3, 4, and 9., where there were hundreds of live individuals per square meter. Zebra mussels were found only at site 8 and were represented by shells only.

Substrate was generally suitable for unionid mussels except for site 6, which had at least two separate glaciers of loose sand moving downstream through it, site 12, which was dominated by boulders that may have been placed in the river and on the banks as part of a remediation effort, and site 13, which was dominated by coarse organic debris from phragmites (*Phragmites australis*)(Table 3). Physical and chemical habitat measures at each site are given in Table 4.

Table 1. Kalamazoo River watershed: Locations of native mussel survey sites, Summer 2017.

Site #	Waterbody	Access	Latitude (N)	Longitude (W)
1	Kalamazoo River Mainstem	Canoe carry in by baseball park	42.29012	-85.55834
2	"	Canoe downstream of baseball park	42.29553	-85.57288
3	"	Canoe from Commerce Lane	42.32370	-85.57569
4	"	Canoe ~100m downstream from site 3	42.32407	-85.57655
5	"	Canoe from Commerce Lane	42.32561	-85.57598
6	"	Upstream of DNR ramp	42.48196	-85.78908
7	"	Upstream of site 6	42.48129	-85.78482
8	"	Boat ramp at Allegan Dam	42.56788	-85.95236
9	"	River trail G Avenue	42.33252	-85.58218
10	"	D Avenue	42.37589	-85.57878
11a	"	US131 by Meijers	42.45442	-85.65542
11b	"	US131 by Meijers	42.45432	-85.65485
12	"	Meijers parking lot	42.45305	-85.66249
13*	"	Schulz boat ramp	42.64416	-86.18992
13*	"	Schulz boat ramp	42.64570	-86.18979

* A point to point meander search was performed at site 13

Table 2. Kalamazoo River watershed: Numbers of live unionid mussels (#), relative abundance (RA), and density (D, indvs./m^2) recorded at each survey site. An S indicates only shells of a species were found, the number shells found are given in parentheses for listed mussel species if no live were found (S(#)). Presence of non-native bivalves is noted along with a rough density estimate. (Fed E= Federally endangered, E= State endangered, T= State threatened, SC= species of special concern)

Kalamazoo		1			2	3			4			5		
Common name	Species	#	RA	D	#	#	RA	D	#	RA	D	#	RA	D
Mucket	*Actinonaias ligamentina*	13	0.31	0.10		S			2	0.22	0.02			
Elktoe	*Alasmidonta marginata* (SC)	5	0.12	0.04		S			S			S		
Slippershell	*Alasmidonta viridis* (T)	S(2)												
Purple wartyback	*Cyclonaias tuberculata* (T)													
Spike	*Eurynia dilatata*	S				S			S					
Wabash pigtoe	*Fusconaia flava*	21	0.50	0.16		9	1.00	0.07	5	0.56	0.05	6	0.75	0.06
Plain pocketbook	*Lampsilis cardium*	2	0.05	0.02					1	0.11	0.01			
White heelsplitter	*Lasmigona complanata*	S							1	0.11	0.01	1	0.13	0.01
Fluted-shell	*Lasmigona costata* (SC)	1	0.02	0.01		S			S					
Fragile papershell	*Leptodea fragilis*													
Round pigtoe	*Pleurobema sintoxia* (SC)													
Giant floater	*Pyganodon grandis*	S							S			1	0.13	0.01
Mapleleaf	*Quadrula quadrula*													
Strange floater	*Strophitus undulatus*													
Paper pondshell	*Utterbackia imbecillis* (SC)													
Ellipse	*Venustaconcha ellipsiformis* (SC)													
	Total # individuals and density	42		0.33	0	9		0.07	9		0.09	8		0.08
	# species live	5			0	1			4			3		
	# species live or shell	9			0	5			8			4		
	Area searched (m^2)	128			255	128			96			96		
Asian clam	*Corbicula fluminea*	~100/m^2			~20/m^2	>100/m^2			>100/m^2			~10/m^2		
Zebra mussel	*Dreissena polymorpha*													

Table 2. Continued...

Kalamazoo		6	7	8			9			10			11a		
Common name	Species	#	#	#	RA	D	#	RA	D	#	RA	D	#	RA	D
Mucket	*Actinonaias ligamentina*						1	0.50	0.01	16	0.52	0.13	25	0.83	0.39
Elktoe	*Alasmidonta marginata* (SC)									2	0.06	0.02			
Slippershell	*Alasmidonta viridis* (T)														
Purple wartyback	*Cyclonaias tuberculata* (T)						S(1)[A]								
Spike	*Eurynia dilatata*			S(2)			S			S					
Wabash pigtoe	*Fusconaia flava*			2	1.00	1.00	S			S					
Plain pocketbook	*Lampsilis cardium*						S[A]			2	0.06	0.02			
White heelsplitter	*Lasmigona complanata*						S(1)			8	0.26	0.06	5	0.17	0.08
Fluted-shell	*Lasmigona costata* (SC)									2	0.06	0.02			
Fragile papershell	*Leptodea fragilis*			S(2)											
Round pigtoe	*Pleurobema sintoxia* (SC)			S(11)											
Giant floater	*Pyganodon grandis*						1	0.50	0.01	1	0.03	0.01			
Mapleleaf	*Quadrula quadrula*			S(2)											
Strange floater	*Strophitus undulatus*						S[A]			S					
Paper pondshell	*Utterbackia imbecillis* (SC)						S								
Ellipse	*Venustaconcha ellipsiformis* (SC)						S(2)[A]								
	Total # individuals and density	0	0	2		0.01	2		0.02	31		0.24	30		0.47
	# species live	0	0	1			2			6			2		
	# species live or shell	0	0	5			10			9			2		
	Area searched (m^2)	120	128	250			128			128			64		
Asian clam	*Corbicula fluminea*			<1/m^2			100's/m^2			S100/m^2			S50/m^2		
Zebra mussel	*Dreissena polymorpha*			S~5/m^2											

[A] Found outside of measured survey area during a meander search

Table 2. Continued...

Kalamazoo		11b			12			13
Common name	Species	#	RA	D	#	RA	D	#
Mucket	*Actinonaias ligamentina*	10	0.71	0.16	1	1.00	0.01	
Elktoe	*Alasmidonta marginata* (SC)	S(2)						
Slippershell	*Alasmidonta viridis* (T)							
Purple wartyback	*Cyclonaias tuberculata* (T)	S(1)						
Spike	*Eurynia dilatata*							
Wabash pigtoe	*Fusconaia flava*							
Plain pocketbook	*Lampsilis cardium*							
White heelsplitter	*Lasmigona complanata*	3	0.21	0.05				
Fluted-shell	*Lasmigona costata* (SC)	1	0.07	0.02				
Fragile papershell	*Leptodea fragilis*							
Round pigtoe	*Pleurobema sintoxia* (SC)							
Giant floater	*Pyganodon grandis*							
Mapleleaf	*Quadrula quadrula*							
Strange floater	*Strophitus undulatus*							
Paper pondshell	*Utterbackia imbecillis* (SC)							
Ellipse	*Venustaconcha ellipsiformis* (SC)							
	Total # individuals and density	14		0.22	1		0.01	0
	# species live	3			1			0
	# species live or shell	5			1			0
	Area searched (m^2)	64			156			[B]
Asian clam	*Corbicula fluminea*	S50/m^2			S10/m^2			
Zebra mussel	*Dreissena polymorpha*							

[B] Approximately 160m^2 meander search

Figure 1. Six Wabash pigtoe (*Fusconaia flava*), one giant floater (*Pyganodon grandis*, center), and one white heelsplitter (*Lasmigona complanata*, top) found live at site 5 in the Kalamazoo River. Photo by Peter J. Badra.

Figure 2. Four of the eleven mucket (*Actinonaias ligamentina*) found live at site 11a in the Kalamazoo River were marked with an X etched into their shells. It is not known who marked them. Photo on left shows one side of the mussels and photo on the right shows the other. Photo by Peter J. Badra.

Table 3. Kalamazoo River watershed: Percentage of each substrate particle size class estimated visually at each survey site. Diameter of each size class: boulder (>256mm), cobble (256-64mm), pebble (64-16mm), gravel (16-2mm), sand (2-0.0625mm), silt/clay (<0.0625mm).

Site #	Boulder	Cobble	Pebble	Gravel	Sand	Silt	Note
1			35	25	20	20	Rip-rap on banks
2		2	15	15	23	45	
3				40	50	10	
4			20	40	30	10	
5					40	60	
6					30[A]	70	River ~400m wide
7					50	50	
8			10	20	50	20	
9				10	10	10	70% Asian clam shell
10		10	35	25	20	10	
11a-b		10	25	25	30	10	
12	90[B]	1	1	2	2	2	~2% of bottom was clay
13							100% Coarse organic debris[C]

[A] At least two separate glaciers of loose sand are moving through this site.

[B] Boulders appear to have been placed on the river bottom and along banks.

[C] A 5-10m zone of phragmites (*Phragmites australis*) along the shore was the source of the coarse organic debris.

Table 4. Kalamazoo River watershed: Physical habitat characteristics and water chemistry measures recorded at each survey site.

Site #	Current speed (m/sec.)	Aquatic vegetation?	Woody debris?	Eroded banks?	%Pool	%Riffle	%Run	Water clarity	pH	Conductivity (µS)	Water temp. (C)
1	near zero	Y	Y	Y[A]			100[B]	very clear	8.75	634	22.2
2	0.3	Y	N	N			100	very clear	8.77	638	22.7
3	0.3	Y	Y	N		50	50	very clear	8.51	752	20.5
4	0.3	Y	Y	N			100	medium	8.55	759	20.8
5	0.1	N	Y	N			100	medium	8.49	760	21.3
6	..	Y	Y	..			100	very clear	8.68	693	22.5
7	..	N	N	..			100	very clear	8.74	702	22.6
8	near zero	Y	N	Y			100	medium	8.77	639	22.2
9	0.25	Y	Y	N			100	very clear	8.42	729	21.7
10	1.0	Y	Y	N	20		100	very clear	8.60	732	23.3
11a-b	1.0	Y	N	N		20	80	very clear	8.68	760	23.5
12	1.0	Y[C]	N	N[D]			100	very clear	8.73	759	24.7
13	near zero	Y[E]	Y	N	100			medium	8.02	639	19.4

[A] Rip-rap present.

[B] Search area was in a large eddy.

[C] Algae was very abundant.

[D] Boulders appear to have been placed on the river bottom and along banks.

[E] River bottom was 100% covered with aquatic vegetation.

Clinton River Watershed

Eight sites were surveyed in the Clinton River watershed (Table 5). A total of 18 mussel species were found including shells of the state endangered round hickorynut (*Obovaria subrotunda*) at site 5 and 6 in the Clinton River mainstem (Figure 3). Five species of special concern were also found (Table 6). Low densities of mussels were found at all sites surveyed in the Clinton. The top two most abundant species were fatmucket (*Lampsilis siliquoidea*) and white heelsplitter (*Lasmigona complanata*), though these were found at only 0.03 and 0.06 indvs./m^2 respectively (Figure 4). Asian clams and zebra mussels were both present at sites 3, 5, and 6, but only Asian clam was found live in high densities (site 6). Several grove snails (*Cepaea nemoralis*), a non-native land snail, were found incidentally along the Clinton River at site 5.

Substrate was generally suitable for unionid mussels except for sites 1 and 4, which were dominated by silt. Site 3 had an unusually high amount of cobble that may have been placed there to attempt to control erosion of the stream bank (Table 7). Sites 1 and 2 in the North Branch Clinton River had heavily eroded streambanks (Figure 5). Stream water was very turbid at sites 1, 2, 4, and 8. Relatively high pH was recorded at site 5 in the Clinton River mainstem and high conductivity was recorded at sites 5-8 in the North Branch and mainstem of the Clinton River (Table 8).

Table 5. Clinton River watershed: Locations of native mussel survey sites, Summer 2017.

Site #	Waterbody	Access	Latitude (N)	Longitude (W)
1	North Branch Clinton River	Nicholson Nature Center	42.62272	-82.89703
2	North Branch Clinton River	Nicholson Nature Center	42.62631	-82.88921
3	Clinton River	MacArthur Park	42.5974	-82.8711
4	Clinton River	Boat ramp w/canoe E. of Gratiot Ave.	42.58159	-82.87688
5	Clinton River	Hike downstream from Frank Bud Park	42.58722	-82.92233
6	Clinton River	Frank Bud Park	42.58741	-82.92786
7	North Branch Clinton River	Hike downstream from Dunham Rd.	42.61661	-82.90590
8	North Branch Clinton River	Hike downstream from Dunham Rd.	42.61462	-82.90562

Figure 3. Three of the ten round hickorynut (*Obovaria subrotunda*, state threatened) shells found at site 6 in the Clinton River. Photo by Peter J. Badra.

Table 6. Clinton River watershed: Numbers of live unionid mussels (#), relative abundance (RA), and density (D, indvs./m^2) recorded at each survey site. An S indicates only shells of a species were found, the number shells found are given in parentheses for listed mussel species if no live were found (S(#)). Presence of non-native bivalves is noted along with a rough density estimate. (Fed E= Federally endangered, E= State endangered, T= State threatened, SC= species of special concern)

Clinton		1			2			3	4			5
Common name	Species	#	RA	D	#	RA	D	#	#	RA	D	#
Mucket	*Actinonaias ligamentina*											S(1)
Three-ridge	*Amblema plicata*	S(4)										S(1)
Pimpleback	*Cyclonaias pustulosa*											
Wabash pigtoe	*Fusconaia flava*				S(3)							
Plain pocketbook	*Lampsilis cardium*				S(1)							S(1)
Fatmucket	*Lampsilis siliquoidea*	2	0.67	0.01	3	0.60	0.02					S(1)
White heelsplitter	*Lasmigona complanata*				1	0.20	0.01					
Fragile papershell	*Leptodea fragilis*	S(3)			S(3)							
Round hickorynut	*Obovaria subrotunda* (E)											S(1)
Pink heelsplitter	*Potamilus alatus* (SC)											
Kidney-shell	*Ptychobranchus fasciolaris* (SC)											
Giant floater	*Pyganodon grandis*	1	0.33	0.01	S(2)							
Mapleleaf	*Quadrula quadrula*											
Strange floater	*Strophitus undulatus*											
Paper pondshell	*Utterbackia imbecillis* (SC)											
Deertoe	*Truncilla truncata* (SC)				1	0.20	0.01					
Ellipse	*Venustaconcha ellipsiformis* (SC)								2	1.00	0.01	
Rainbow	*Villosa iris* [A] (SC)	S(1)										
	Total # individuals and density	3		0.02	5		0.03	0	2		0.01	0
	# species live	2			3			0	1			0
	# species live or shell	5			7			0	1			5
	Area searched (m^2)	160			200			23	136			128
Asian clam	*Corbicula fluminea*							<1/m^2				~50/m^2
Zebra mussel	*Dreissena polymorpha*							~10/m^2	<1/m^2			<1/m^2

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

Table 6. Continued...

Clinton		6			7			8		
Common name	Species	#	RA	D	#	RA	D	#	RA	D
Mucket	*Actinonaias ligamentina*							3	0.38	0.04
Three-ridge	*Amblema plicata*				S			S		
Pimpleback	*Cyclonaias pustulosa*							1	0.13	0.01
Wabash pigtoe	*Fusconaia flava*	S(1)			1	0.08	0.01			
Plain pocketbook	*Lampsilis cardium*	S(3)								
Fatmucket	*Lampsilis siliquoidea*				4	0.31	0.03	2	0.25	0.03
White heelsplitter	*Lasmigona complanata*				1	0.08	0.01	4	0.50	0.06
Fragile papershell	*Leptodea fragilis*	S(1)			1	0.08	0.01	S		
Round hickorynut	*Obovaria subrotunda* (E)	S(10)								
Pink heelsplitter	*Potamilus alatus* (SC)							S		
Kidney-shell	*Ptychobranchus fasciolaris* (SC)							S		
Giant floater	*Pyganodon grandis*				1	0.08	0.01	S		
Mapleleaf	*Quadrula quadrula*				S					
Strange floater	*Strophitus undulatus*				4	0.31	0.03			
Paper pondshell	*Utterbackia imbecillis* (SC)				S					
Deertoe	*Truncilla truncata* (SC)				S(1)					
Ellipse	*Venustaconcha ellipsiformis* (SC)									
Rainbow	*Villosa iris* [A] (SC)				1	0.08	0.01	1	0.13	0.01
	Total # individuals and density	0		0.00	13		0.10	11		0.16
	# species live	0			7			5		
	# species live or shell	4			11			10		
	Area searched (m^2)	116			128			68		
Asian clam	*Corbicula fluminea*	>50/m^2								
Zebra mussel	*Dreissena polymorpha*	S(2)								

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

Figure 4. *From right to left...* Four white heelsplitter (*Lasmigona complanata*), three mucket (*Actinonaias ligamentina*), two fatmucket (*Lampsilis siliquoidea*) and a shell of a mucket below, one rainbow (*Villosa iris* (=*Cambarunio iris*), species of special concern), and one pimpleback (*Cyclonaias pustulosa*) from site 8 in the North Branch of the Clinton River. Photo by Peter J. Badra.

Table 7. Clinton River watershed: Percentage of each substrate particle size class estimated visually at each survey site. Diameter of each size class: boulder (>256mm), cobble (256-64mm), pebble (64-16mm), gravel (16-2mm), sand (2-0.0625mm), silt/clay (<0.0625mm).

Site #	Boulder	Cobble	Pebble	Gravel	Sand	Silt	Note
1						100[A]	
2				15	15	70	
3		60[B]	5	5	5	25	
4						100	
5		20	30	20	10	10	10% clay hardpan
6			10	50	30	10	
7		30	20	10	10	10	20% clay hardpan
8	10	40	20	10	10	10	

[A] Thick layer of silt on top of sand

[B] Appeared to have been placed in order to control river bank erosion

Figure 5. Heavily eroded stream banks at sites 1 and 2 in the North Branch of the Clinton River. A sign warning of flooding and fast currents posted at trails near the two sites. Photos by Peter J. Badra.

Table 8. Clinton River watershed: Physical habitat characteristics and water chemistry measures recorded at each survey site.

Site #	Current speed (m/sec.)	Aquatic vegetation?	Woody debris?	Eroded banks?	%Pool	%Riffle	%Run	Water clarity	pH	Conductivity (µS)	Water temp. (C)
1	0.1	N	Y	Highly	50		50	low	..	..	..
2	<0.1	Y	Y	Highly	50		50	low	..	..	..
3	0.2	Y	Y	N			100	medium	8.01	247	21.9
4	zero	Y	Y	N	100			low	8.20	294	23.6
5	0.5-1.0	N	Y	Y			100	medium	8.85	949	22.0
6	1.0	Y	Y	Y			100	very clear	8.56	948	22.4
7	0.5	N	Y	Y			100	medium/low	8.16	994	22.4
8	0.25	N	Y	Y			100	low	8.33	990	23.1

St. Joseph River Watershed (Maumee Drainage)

Twenty-nine sites were surveyed in the St. Joseph River watershed (Table 9)(Figure 6). A total of 17 mussel species were documented including the federally endangered clubshell (*Pleurobema clava*), the state threatened slippershell, and state threatened wavy-rayed lampmussel (*Lampsilis fasciola*)(Figure 7). Live clubshells were found at three sites (14a-c) located adjacent to each other near the Dimmers Rd. crossing of the East Fork West Branch. Shells of clubshell were found at eight sites (Table 10). All of these were in the East Fork of the West Branch except for one shell found at site 13a downstream of the confluence of the East and West Forks. The East Fork West Branch St. Joseph River at the Montomery Rd. crossing was surveyed in 2017 (Zanatta pers. com. 2019). This site was not surveyed in 2018 or 2019 to avoid any potential impacts to the habitat or mussels from surveying too frequently.

The two most abundant species were three-ridge (*Amblema plicata*) and kidney-shell (*Ptychobranchus fasciolaris*). Maximum density for these species was 3.63 indvs./m^2 and 1.63 indvs./m^2 respectively and found at site 1. Site 1 also supported the highest overall density of mussels found of all survey sites in this study, with a total density of unionid mussels at 7.75 indvs./m^2.

Live zebra mussels were found at sites 15a and 15b in the East Fork of the West Branch St. Joseph River just downstream of the Cambria Millpond. These are 4km upstream of sites 14a-c where live clubshell are present. One live zebra mussel was attached to a live fluted-shell (Lasmigona costata) (Figure 8) at site 15a. Several others were seen attached to woody debris at this site. A second site (15b) was surveyed downstream and adjacent to site 15a to better assess the status of zebra mussels. Thirty-five live zebra mussels were found in a 18m^2 search area at site 15b. Live Asian clams were seen at sites 5a, 6a, 6b, and 14a, and Asian clam shells were noted at an additional two sites. Two live Blanchard's cricket frogs (*Acris blanchardi*), a state threatened species, were found incidentally on the banks of the St. Joseph River at site 11.

Substrate composition was generally favorable for unionid mussels, including clubshell, except for sites 10 and 14a where a high proportion of silt was present (Table 11). Despite this clubshell and a relatively high density of mussels overall was found at 14a. Nearby sites 1 and 14b-c, however, had substrate typically thought of as good for supporting mussels and these sites had a higher mussel density than 14a. Alkalinity and hardness were within a range thought to be supportive of mussels and other aquatic life. Other physical habitat characteristics and water chemistry measures are given in Table 12. Site 7 was too deep to survey due to recent precipitation. Shells at this site were found on the stream bank. Dimensions (length, width, and thickness) and number of

external rings were recorded for all live clubshells found (Table 13). These data were also recorded for empty shells of clubshell and a sub-set of individuals for species other than clubshell and are available from the author.

Landowners were contacted before surveying sites on their property, and in most cases were called the morning before surveys took place. Nearly all landowners agreed to allow access, and a few were very interested in the mussel surveys. One landowner participated in surveying the section of St. Joseph River that flowed through their property.

Table 9. St. Joseph River watershed (Maumee Drainage): Locations of native mussel survey sites, Summer 2018 (sites 1-10) and 2019 (sites 11-15). Sites where live individuals (L) or shells (S) of clubshell (*Pleurobema clava*) were found in 2018/2019 are indicated.

Site #	St. Joseph River (Maumee drainage)	Access	Latitude (N)	Longitude (W)	Clubshell
1	East Fork of West Branch	Dimmers Rd.	41.79616	-84.63778	S
2a	East Fork of West Branch	Cambria Rd.	41.74653	-84.67673	
2b	East Fork of West Branch	Cambria Rd.	41.74660	-84.67627	
2c	East Fork of West Branch	Cambria Rd.	41.74691	-84.67616	
2d	East Fork of West Branch	Cambria Rd.	41.74711	-84.67478	S
3a	East Fork of West Branch	Woodbridge Rd.	41.77412	-84.65496	S
3b	East Fork of West Branch	Woodbridge Rd.	41.77380	-84.65456	S
3c	East Fork of West Branch	Woodbridge Rd.	41.77241	-84.65373	S
4	West Fork of West Branch	Bluff camp ground, Montam Rd.	41.77247	-84.76127	
5a	East Fork of West Branch	Clark Property	41.8135	-84.6532	
5b	East Fork of West Branch	Clark Property	41.81767	-84.65606	
5c	East Fork of West Branch	Clark Property	41.81791	-84.65608	
5d	East Fork of West Branch	Clark Property	41.81823	-84.65623	
6a	West Fork of West Branch	Samson Rd.	41.72249	-84.70697	
6b	West Fork of West Branch	Samson Rd.	41.72296	-84.70895	
7	West Fork of West Branch	Camden Rd.	41.75384	-84.74022	
8	East Fork of West Branch	Cambria Rd.	41.74726	-84.67392	
9	West Fork of West Branch	Bluff camp ground, Montam Rd.	41.7738	-84.76308	
10	East Fork of West Branch	Woodbridge Rd.	41.80081	-84.64430	
11	East Fork of West Branch	Cambria Rd.	41.74988	-84.67075	S
12	East Fork of West Branch	Burt Rd.	41.76624	-84.65267	S
13a	West Branch	Territorial Rd.	41.70477	-84.68879	S
13b	West Branch	Territorial Rd.	41.70521	-84.68952	
13c	East Fork of West Branch	Territorial Rd. under bridge	41.7084	-84.6896	
14a	East Fork of West Branch	Dimmers Rd.	41.79550	-84.63766	L
14b	East Fork of West Branch	Dimmers Rd.	41.79353	-84.63862	L
14c	East Fork of West Branch	Dimmers Rd.	41.79589	-84.63736	L
15a	East Fork of West Branch	Lilac Rd.	41.82024	-84.66191	
15b	East Fork of West Branch	Lilac Rd.	41.8190	-84.6598	

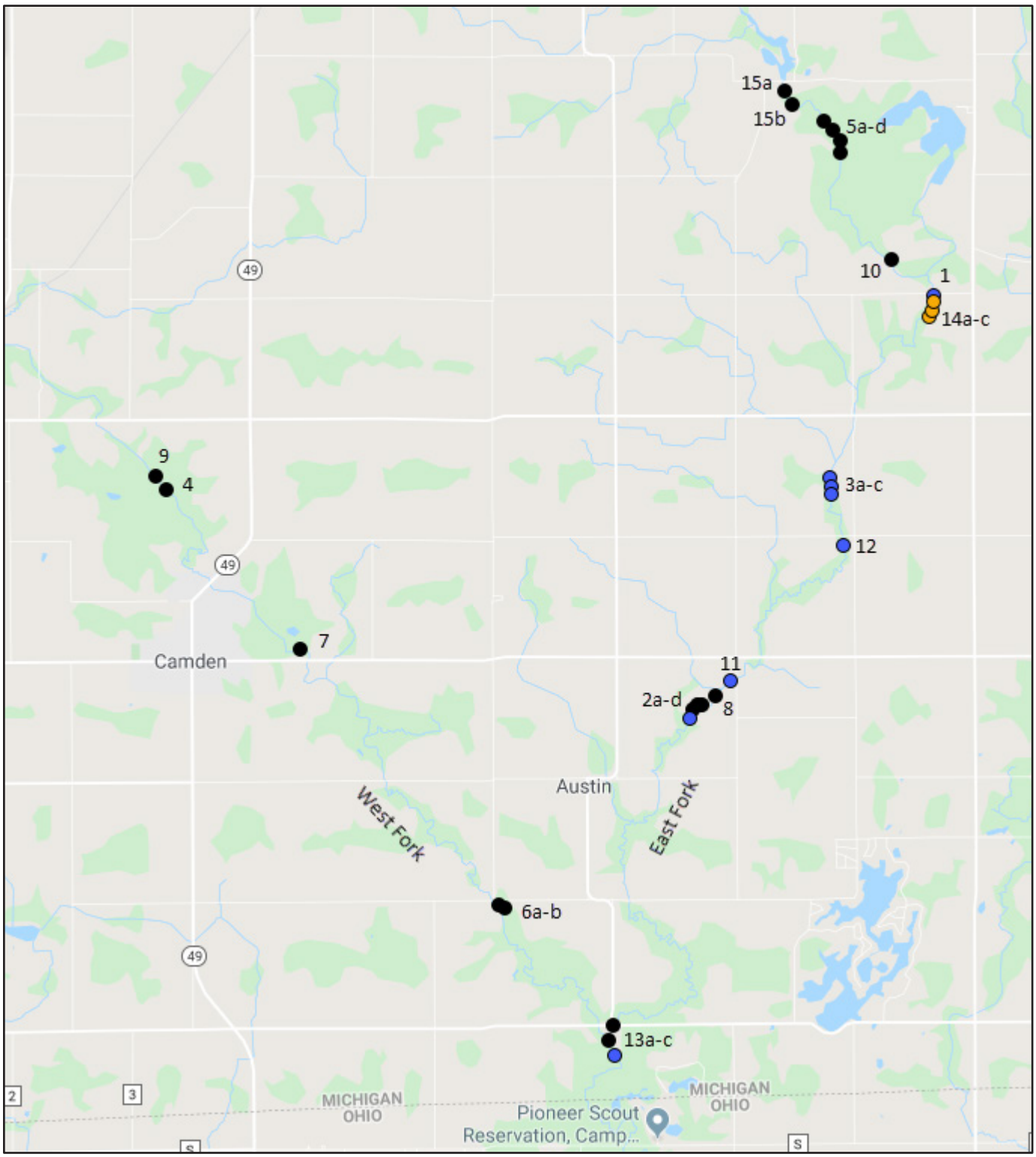

Figure 6. Map of survey sites for the St. Joseph River watershed (Maumee Drainage). Orange dots represent sites where live clubshell (*Pleurobema clava*) were found, blue dots where shells of clubshell were found, and black dots where neither live or shell were found.

Figure 7. A live wavy-rayed lampmussel (*Lampsilis fasciola*, state threatened) found at site 14c in the East Fork of the West Branch St. Joseph River. Photo by Peter J. Badra.

Table 10. St. Joseph River watershed (Maumee Drainage): Numbers of live unionid mussels (#), relative abundance (RA), and density (D, indvs./m^2) recorded at each survey site. An S indicates only shells of a species were found, the number shells found are given in parentheses for listed mussel species if no live were found (S(#)). Presence of non-native bivalves is noted along with a rough density estimate. (Fed E= Federally endangered, E= State endangered, T= State threatened, SC= species of special concern)

St. Joseph		1			2a			2b	2c			2d		
Common name	Species	#	RA	D	#	RA	D	#	#	RA	D	#	RA	D
Elktoe	*Alasmidonta marginata* (SC)											S(1)		
Slippershell	*Alasmidonta viridis* (T)											1	0.33	0.03
Three-ridge	*Amblema plicata*	29	0.47	3.63								1	0.33	0.03
Cylindrical papershell	*Anodontoides ferussacianus*													
Spike	*Eurynia dilatata*	6	0.10	0.75	S(1)									
Wabash pigtoe	*Fusconaia flava*	9	0.15	1.13								S		
Plain pocketbook	*Lampsilis cardium*	1	0.02	0.13										
Wavy-rayed lampmussel	*Lampsilis fasciola* (T)	1	0.02	0.13								S(1)		
Fatmucket	*Lampsilis siliquoidea*	S							1	0.50	0.07			
Creek heelsplitter	*Lasmigona compressa* (SC)													
Fluted-shell	*Lasmigona costata* (SC)													
Clubshell	*Pleurobema clava* (Fed. E)	S(2)										S(2)		
Round pigtoe	*Pleurobema sintoxia* (SC)	1	0.02	0.13										
Kidney-shell	*Ptychobranchus fasciolaris* (SC)	13	0.21	1.63	2	1.00	0.13					1	0.33	0.03
Giant floater	*Pyganodon grandis*	2	0.03	0.25										
Strange floater	*Strophitus undulatus*													
Rainbow	*Villosa iris* [A] (SC)								1	0.50	0.07	S(2)		
	Total # individuals and density	62		7.75	2		0.13	0	2		0.14	3		0.08
	# species live	8			1			0	2			3		
	# species live or shell	10			2			0	2			8		
	Area searched (m^2)	8			15			12	14			38		
Asian clam	*Corbicula fluminea*													
Zebra mussel	*Dreissena polymorpha*													

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

Table 10. Continued...

St. Joseph		3a			3b	3c		4			5a		5b		5c
Common name	Species	#	RA	D	#	#	RA	#	RA	D	#	RA	#	RA	#
Elktoe	*Alasmidonta marginata* (SC)										S(1)		1	0.50	
Slippershell	*Alasmidonta viridis* (T)	S(3)			S(5)	S(5)		1	1.00	0.01	S(3)				
Three-ridge	*Amblema plicata*	4	0.31	0.04	L	7	0.78				4	0.67			
Cylindrical papershell	*Anodontoides ferussacianus*							S(3)			S(1)				
Spike	*Eurynia dilatata*					S(11)					S		1	0.50	
Wabash pigtoe	*Fusconaia flava*	S(2)				S(7)									
Plain pocketbook	*Lampsilis cardium*	S(1)									S(2)				
Wavy-rayed lampmussel	*Lampsilis fasciola* (T)				S(1)	S(2)					S(1)		S(1)		
Fatmucket	*Lampsilis siliquoidea*	S(5)			L	S(5)					1	0.17	S		
Creek heelsplitter	*Lasmigona compressa* (SC)	S(1)													S(1)
Fluted-shell	*Lasmigona costata* (SC)										S(2)				
Clubshell	*Pleurobema clava* (Fed. E)	S(17)			S(1)	S(19)									
Round pigtoe	*Pleurobema sintoxia* (SC)	S(1)									1	0.17			S(1)
Kidney-shell	*Ptychobranchus fasciolaris* (SC)	9	0.69	0.09		2	0.22								
Giant floater	*Pyganodon grandis*				S(1)			S(1)							
Strange floater	*Strophitus undulatus*										S(1)		S		
Rainbow	*Villosa iris* [A] (SC)	S(3)				S(9)									
	Total # individuals and density	13		0.14		9		1		0.01	6		2		0
	# species live	2			2	2		1			3		2		0
	# species live or shell	10			5	9		3			11		5		2
	Area searched (m^2)	96			[B]	[B]		106			[C]		[D]		[D]
Asian clam	*Corbicula fluminea*							S			L				
Zebra mussel	*Dreissena polymorpha*														

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

[B] Meander search (approximately 0.5 person*hours)

[C] Meander search (approximately 2 person*hours)

[D] Meander search (approximately 1 person*hour)

Table 10. Continued...

St. Joseph		5d	6a			6b			7	8			9			10
Common name	Species	#	#	RA	D	#	RA	D	#	#	RA	D	#	RA	D	#
Elktoe	*Alasmidonta marginata* (SC)															
Slippershell	*Alasmidonta viridis* (T)					S(2)			S(3)				S(4)			
Three-ridge	*Amblema plicata*		2	0.10	0.04	3	0.12	0.07					1	1.00	0.02	
Cylindrical papershell	*Anodontoides ferussacianus*					S(1)							S(4)			
Spike	*Eurynia dilatata*		14	0.70	0.26	19	0.73	0.44								
Wabash pigtoe	*Fusconaia flava*		1	0.05	0.02	3	0.12	0.07								
Plain pocketbook	*Lampsilis cardium*															
Wavy-rayed lampmussel	*Lampsilis fasciola* (T)									S(1)						
Fatmucket	*Lampsilis siliquoidea*		3	0.15	0.06	1	0.04	0.02		1	1.00	0.02				
Creek heelsplitter	*Lasmigona compressa* (SC)															
Fluted-shell	*Lasmigona costata* (SC)															
Clubshell	*Pleurobema clava* (Fed. E)															
Round pigtoe	*Pleurobema sintoxia* (SC)															
Kidney-shell	*Ptychobranchus fasciolaris* (SC)									S(1)						
Giant floater	*Pyganodon grandis*		S(1)			S(1)										S(2)
Strange floater	*Strophitus undulatus*															
Rainbow	*Villosa iris* [A] (SC)															
	Total # individuals and density	0	20		0.37	26		0.60	0	1		0.02	1		0.02	0
	# species live	0	4			4			0	1			1			0
	# species live or shell	0	5			7			1	3			3			1
	Area searched (m^2)	[D]	54			43			[E]	63			42			[C]
Asian clam	*Corbicula fluminea*		L			L							S			
Zebra mussel	*Dreissena polymorpha*															

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

[C] Meander search (approximately 2 person*hours)

[D] Meander search (approximately 1 person*hour)

[E] Water too high to survey, shells were found on stream bank

Table 10. Continued...

St. Joseph		11			12			13a			13b			13c		
Common name	Species	#	RA	D	#	RA	D	#	RA	D	#	RA	D	#	RA	D
Elktoe	*Alasmidonta marginata* (SC)															
Slippershell	*Alasmidonta viridis* (T)				S(6)						S(2)			S(1)		
Three-ridge	*Amblema plicata*	1	0.50	0.01	22	0.76	0.06									
Cylindrical papershell	*Anodontoides ferussacianus*													S(2)		
Spike	*Eurynia dilatata*	S(7)			2	0.07	0.01	S(2)			1	0.50	0.005			
Wabash pigtoe	*Fusconaia flava*	S(5)			S(16)											
Plain pocketbook	*Lampsilis cardium*													S(1)		
Wavy-rayed lampmussel	*Lampsilis fasciola* (T)	S(6)			S(3)											
Fatmucket	*Lampsilis siliquoidea*	S(1)			S(3)									2	1.00	0.01
Creek heelsplitter	*Lasmigona compressa* (SC)				S(2)											
Fluted-shell	*Lasmigona costata* (SC)															
Clubshell	*Pleurobema clava* (Fed. E)	S(24)			S(20)			S(1)								
Round pigtoe	*Pleurobema sintoxia* (SC)				1	0.03	0.003				S(1)					
Kidney-shell	*Ptychobranchus fasciolaris* (SC)	1	0.50	0.01	4	0.14	0.01	3	1.00	0.01	1	0.50	0.005	S(3)		
Giant floater	*Pyganodon grandis*															
Strange floater	*Strophitus undulatus*										S(1)					
Rainbow	*Villosa iris* [A] (SC)	S(19)			S(9)			S(1)								
	Total # individuals and density	2		0.01	29		0.08	3		0.01	2		0.01	2		0.01
	# species live	2			4			1			2			1		
	# species live or shell	8			11			4			5			5		
	Area searched (m^2)	153			360			220			210			225		
Asian clam	*Corbicula fluminea*															
Zebra mussel	*Dreissena polymorpha*															

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

Table 10. Continued...

St. Joseph		14a			14b			14c			15a			15b		
Common name	Species	#	RA	D	#	RA	D	#	RA	D	#	RA	D	#	RA	D
Elktoe	*Alasmidonta marginata* (SC)				1	0.01	0.02							1	1.00	0.06
Slippershell	*Alasmidonta viridis* (T)				1	0.01	0.02									
Three-ridge	*Amblema plicata*	104	0.36	0.41	47	0.34	1.00	17	0.22	0.68						
Cylindrical papershell	*Anodontoides ferussacianus*	S														
Spike	*Eurynia dilatata*	70	0.24	0.28	11	0.08	0.23	13	0.16	0.52						
Wabash pigtoe	*Fusconaia flava*	39	0.13	0.16	9	0.07	0.19	7	0.09	0.28						
Plain pocketbook	*Lampsilis cardium*	6	0.02	0.02	2	0.01	0.04				S(2)					
Wavy-rayed lampmussel	*Lampsilis fasciola* (T)	1	0.003	0.004	1	0.01	0.02				S(1)					
Fatmucket	*Lampsilis siliquoidea*	6	0.02	0.02	3	0.02	0.06	5	0.06	0.20	1	0.11	0.004			
Creek heelsplitter	*Lasmigona compressa* (SC)	1	0.003	0.004												
Fluted-shell	*Lasmigona costata* (SC)	1	0.003	0.004	1	0.01	0.02	2	0.03	0.08	5	0.56	0.02			
Clubshell	*Pleurobema clava* (Fed. E)	10	0.03	0.04	8	0.06	0.17	1	0.01	0.04						
Round pigtoe	*Pleurobema sintoxia* (SC)	16	0.06	0.06	1	0.01	0.02									
Kidney-shell	*Ptychobranchus fasciolaris* (SC)	36	0.12	0.14	51	0.37	1.09	34	0.43	1.36	S(2)					
Giant floater	*Pyganodon grandis*															
Strange floater	*Strophitus undulatus*				2	0.01	0.04				3	0.33	0.01			
Rainbow	*Villosa iris* [A] (SC)	S(2)						S(1)								
	Total # individuals and density	290		1.16	138		2.94	79		3.16	9			1		0.06
	# species live	11			13			7			3			1		
	# species live or shell	13			13			8			6			1		
	Area searched (m^2)	251			47			25			259			18		
Asian clam	*Corbicula fluminea*	L(1)														
Zebra mussel	*Dreissena polymorpha*										L*			L**		

[A] *Villosa iris* = *Cambarunio iris* (Watters 2018)

L* One live zebra mussel was attached to a live fluted-shell, several others were attached to woody debris.

L** 35 live zebra mussels, none attatched to native mussels.

Figure 8. A live zebra mussel (*Dreissena polymorpha*) attached to a live fluted-shell (*Lasmigona costata*, species of special concern) at site 15a in the East Fork of the West Branch St. Joseph River. Photo by Peter J. Badra.

Table 11. St. Joseph River watershed (Maumee Drainage): Percentage of each substrate particle size class estimated visually at each survey site. Diameter of each size class: boulder (>256mm), cobble (256-64mm), pebble (64-16mm), gravel (16-2mm), sand (2-0.0625mm), silt/clay (<0.0625mm). Alkalinity and hardness were recorded for selected sites.

Site #	Boulder	Cobble	Pebble	Gravel	Sand	Silt	Alkalinity	Hardness
1				30	40	30		
2a		10	30	30	20	10	248	228
2b			10	30	40	20		
2c				25	50	25		
2d	2	13	40	20	20	5		
3a			10	35	45	10	256	288
3b				35	50	15		
3c				35	55	10	232	224
4				10	80	10		
5a-d		15	35	20	20	10		
6a		10	20	40	30			
6b			10	45	35	10		
7*								
8	10	20	30	20	10	10		
9				50	30	20		
10						100		
11	2	18	30	20	20	10	190	238
12			10	20	40	30	180	220
13a		10	30	30	20	10		280
13b		30	30	20	10	10		
13c	10	20	20	20	20	10		
14a					20	80		218
14b			25	10	40	25		
14c		5	15	15	55	10		
15a		20	25	20	30	5		
15b		25	30	15	25	5		

* Water was too high to assess substrate composition at site 7.

Table 12. St. Joseph River watershed (Maumee Drainage): Physical habitat characteristics and water chemistry measures recorded at each survey site.

Site #	Current speed (m/sec.)	Aquatic vegetation ?	Woody debris?	Eroded banks?	%Pool	%Riffle	%Run	Water clarity	pH	Conductivity (µS)	Water temp. (C)
1	0.25	N	Y	N			100	low	8.42	524	24.2
2a-d	0.25-1.0	N	Y	Y			100	medium	8.51	541	21.4
3a-b	1.0	N	Y	Y	10	5	85	very clear	8.52	587	19.5
3c	0.3	N	Y	Y	30		70	medium	8.41	537	25.8
4	0.2	N	Y	Y	10		90	medium	8.40	478	23.0
5a-d	0.1-0.5	Y	Y	N	25	10	65	very clear	..	..	..
6a	0.3	N	Y	Y			100	low	8.42	504	24.1
6b	0.3	N	Y	Y	20		80	low	8.29	501	23.6
7	..	..	Y	Y			100	low	..	..	..
8	0.5	N	Y	Y			100	medium	..	..	..
9	0.3	N	Y	N	10		90	medium	8.39	481	23.0
10	<0.1	N	Y	Y	50		50	low	8.43	531	23.9
11	0.3-0.6	Y[A]	Y	Y[B]	20	30	50	medium	8.45	541	21.2
12	0.25	N	Y	Y			100	very clear	8.51	542	22.2
13a	0.3	N	Y	Y	10	50	40	very clear	8.57	576	20.7
13b[C]	0.3-1.0	N	Y	Y		90	10	very clear	"	"	"
13c[C]	0.25	N	Y	Y	33	33	34	medium	"	"	"
14a	0.5	N	Y[D]	Y[B]			100	medium	8.37	531	21.8
14b	0.25	N	Y[D]	Y[B]			100	very clear	8.28	531	20.6
14c	0.25	Y	Y[D]	Y[B]		20	100	very clear	8.35	533	20.6
15a	0.1-0.5	N	Y	N	20	5	75	very clear	8.34	509	21.7
15b[C]	0.1-0.3	N	Y	N	10	10	80	very clear	"	"	"

[A] Algae

[B] Stream channel appeared down-cut.

[C] Water temperature, conductivity, and pH were not taken at sites 12b-c and 14b. Due to their close proximity to sites 12a and 14a respectively, they were presumed to be the same.

[D] A large amount of woody debris over 6 inches in diameter was present due to the die-off of Ash within the past 10-15 years.

Table 13. St. Joseph River watershed (Maumee Drainage): Length, width, and thickness measurements in millimeters, and the number of external annular rings for live clubshell (*Pleurobema clava*) found in the East Fork, West Branch of the St. Joseph River, Summer 2019.

Site	Length	Width	Thickness	Rings
"	47	31	17	6
"	44	29	19	6
"	66	43	27	12
"	65	38	27	17
"	77	47	34	18
"	69	43	27	18
"	68	41	27	19
"	72	47	35	20
"	81	50	31	23
14b	29	20	11	3
"	35	24	9	4
"	28	20	14	4
"	37	28	15	5
"	74	47	30	27
"	84	54	37	37
14c	80	49	32	33

Discussion

Target Species for Histopathological and Toxicological Assessment

Ideally, the two mussel species targeted for histopathological and toxicological assessment would co-occur at multiple sites in all three watersheds, they would be common non-listed species, and they would occur at high enough density that ensures collecting and sacrificing live individuals would not result in a significant negative impact to the population at each site. Based on historical museum records from University of Michigan Museum of Zoology Mollusk Collection and recent survey results compiled in Badra 2010, six non-listed mussel species have been recorded in all three watersheds. Abundance data reported in several studies of these three watersheds suggest that spike (*Eurynia dilatata*), Wabash pigtoe (Fusconaia flava), and fatmucket (*Lampsilis siliquoidea*) would be potentially suitable targets for study (Badra and Goforth 2001; Badra 2004; Paskus et al. 2012; Morowski et al. 2009; Strayer 1980; Mulcrone and Mehne 2001; D. Woolnough pers. com. 2013 and 2015).

Survey results from this survey show four common non-listed species occurring in all three watersheds (Wabash pigtoe, giant floater (*Pyganodon grandis*), plain pocketbook (*Lampsilis cardium*), and strange floater (*Strophitus undulatus*), however only Wabash pigtoe and giant floater were represented by live individuals. Wabash pigtoe and giant floater co-occur (live or shell) at Kalamazoo River sites 1, 4, 5, 9, and 10. Kalamazoo River sites

1 and 5 are the most promising in terms of abundance of live individuals of these species, though abundance may not be high enough to collect individuals from these sites. Wabash pigtoe and giant floater co-occur (live or shell) at Clinton River sites 2 and 7. They co-occur live only at Clinton River site 7. The abundance there is likely not high enough to support collection. Wabash pigtoe and giant floater co-occur (live or shell) at St. Joseph River sites 1, 6a, and 6b. Both species were found live at St. Joseph River site 1, and in high enough densities that collecting individuals there without significant negative impact would likely be possible.

In summary, survey results suggest targeting Wabash pigtoe and giant floater at Kalamazoo River site 1 or 5, Clinton River site 7, and St. Joseph River site 1 for the best chance of finding an appropriate abundance of target species for collection. Density of these two species in the St. Joseph River is high enough to ensure collection would be feasible, but additional surveys in the Kalamazoo and Clinton Rivers may be needed to locate denser occurrences. If mussels are collected from an area larger than the search area of 2019 survey sites (e.g. a 400m stream reach), there would be a greater chance of finding the number of live individuals needed for a histopathological and toxicological assessment. As more mussel survey data becomes available for these watersheds additional suitable sites may be discovered.

Listed Species Occurrences and Threats

Though one worn purple wartyback shell was found at Kalamazoo River site 9 and another at site 11b, no live individuals were detected at any of the 14 Kalamazoo River sites. Similarly, slippershell was represented by only two shells, both at site 11b. No viable populations of these species were evident within the study area.

Along with well documented impacts to the Kalamazoo River, non-native Asian clams and unstable substrate may also be negatively impacting native unionid mussels. Multiple sites in the Kalamazoo River had very high densities of Asian clams. There is some evidence that, though this species has generally been thought of to not cause harm, impacts to unionid mussels are possible when it occurs in high density through competition during filter feeding and altering physical habitat (McMahon 2000). The two slugs of loose sand moving downstream like a glacier at site 6 in the Kalamazoo River is likely excluding mussels due to its unstable nature. No native or non-native mussels were found at this site.

Round hickorynut was represented by ten worn shells at site 6 and one worn shell at site 5 in the Clinton River. No live individuals were found. Very few other records for the species in the Clinton River watershed exist. There are two pre-1935 records for round hickorynut in the Clinton River in Mt. Clemons and one mile above the mouth of the Middle Branch of the Clinton River. Neither Strayer (1980) nor Hunter (2004) recorded the species in their surveys of 76 sites in the Clinton River watershed, though their sites were upstream of the pre-1935 records. One occurrence was documented in 1988 in the North Branch Clinton River at M-59 (Hoeh 1988) and in the Middle Branch Clinton River, Shelby Township in 1993 (Sherman and Reich UMMZ# 255028). Sites 1 and 2 in the North Branch Clinton River had heavily eroded streambanks. This is likely caused by or at least exacerbated by increased imperviousness in the watershed storm and water runoff (Figure 5).

The clubshell population in Michigan is reproducing but appears to be declining over time. Clubshell density was highest at site 14b (0.17 indvs./m^2). The other location where it was previously recorded in relatively high density is the Montgomery Rd. crossing, but recent surveys indicate these numbers may have declined (D. Zanatta pers. com. 2019)(Table 14). Density of clubshell, estimated with quadrat surveys, was lower in 2003 (Badra 2004) than in 1999 (Badra and Goforth 2001). Along with the sub-population at Montgomery Rd., site 14b appears to be the last foothold this species has in Michigan.

In 1999 and in 2003, density estimates were made at site CS1 (=14b) using thirty quadrats (0.25m^2 and 0.5m^2). In 2018/2019 semi-quantitative surveys of larger areas (25 to 251m^2) were performed to maximize detection

of clubshell at each site considering its low abundance. Density estimates for semi-quantitative surveys were made though the survey techniques are somewhat different. Semi-quantitative surveys included hand sweeps 5-10cm deep into the substrate vs. excavation of substrate to 10cm for quadrat surveys. Hand sweeps were made to minimize the chances fully buried clubshell would go undetected. Semi-quantitative and quadrat surveys both allowed for visual and tactile detection of mussels.

Live clubshell as young as three years old (three external annular rings) were found at site 14b in 2019, indicating reproduction has occurred as recently as 2016 (Figure 9). Average number of external annular rings on live clubshell found in this survey was 15.75 and ranged from three to 37 (Table 13)(Figure 10). A survey targeting small young clubshell at the same site in 1999 detected two live clubshell with one external annular ring (Badra and Goforth 2001). Empty shells with three to six annular rings were also found over multiple sites the 1999 survey. Despite the apparent low abundance of clubshell present in the St. Joseph River, reproduction is still occurring. Whether or not recruitment is high enough to sustain the population into the future remains a question.

Zebra mussels are an imminent threat to the persistence of clubshell in Michigan. Live zebra mussels were found at sites 15a and 15b in the East Fork of the West Branch St. Joseph River These are 4km upstream of sites 14a-c, the most abundant occurrence of live clubshell in the state. Not only is site 14 one of the last sites for clubshell in Michigan, it is also a high-quality mussel community overall and supports multiple state listed mussel species. In 1999, a live clubshell was found only 800m downstream (CS17) of where zebra mussels were found in 2019. Sites 15a and 15b are just downstream of the Cambria Millpond, which could be source for zebra mussel veligers being carried downstream.

A response to the threat of zebra mussels in the St. Joseph River (Maumee drainage) could include…

- Investigating likely past and potential introduction points

- Surveys to determine the extent of zebra mussels in the watershed

- Physical removal of zebra mussels

- Outreach and education (e.g. signage at stream and millpond access points to increase awareness and minimize the spread of zebra mussels)

A change that was clear between 2003 and 2019 surveys at site 14a-c (CS1) is the massive die-off of ash trees. The result is more sunlight hitting the stream and large amounts of woody debris over 6 inches (15cm) in diameter down in the water (Figure 11). There might also be changes to the energy input to the river system, at least locally, with a reduction in tree leaves falling into the stream. There may be a desire to clear out large woody debris and snags from the stream. This could have a negative impact on clubshell, other mussel species, and the stream ecosystem as a whole due to trampling from machinery, boots, and removal of heavy logs. Communication with local land managers on this issue might help avoid impacts.

Both conductivity and pH measured at survey sites in the St. Joseph River were higher in 2018/2019 than in 2000. In 2000, mean values were determined from measurements taken monthly from May-October, while values for 2018 and 2019 were single measurements taken at the time of the mussel survey. The difference could be due in part to variations in precipitation. If the ratio of ground water to surface water in the St. Joseph River happened to be higher when measurements were taken in 2018/2019 than in 2000, it may be possible that slightly acid precipitation and surface water (relative to ground water), could have resulted in more acidic stream water. Changes in natural and non-natural inputs could be investigated as a potential cause for changes in pH and conductivity occurring over the past 20 years.

Table 14. St. Joseph River watershed (Maumee Drainage): A comparison of the status of clubshell (*Pleurobema clava*) found in 1998/1999 (Badra and Goforth 2001), 2003 (Badra 2003), 2014 and 2017 (Zanatta pers. com. 2019), and in this 2018/2019 study. Results of quadrat surveys in 1998/1999 are given for sites CS1-4, and in 2003 for sites CS1-2 (number live, density in individuals per m^2). All other sites in 1998/1999 and 2003 were sampled qualitatively. The number of clubshell found live in qualitative surveys in 1998, 2003, and 2014/2017 are in parenthesis. Blank cells indicate a survey was not done that year. (L= Live clubshell found, S= shells of clubshell found)

1998/1999 Site number	2018/2019 Site number	1998/1999	2003	2014/2017	2018/2019	Nearest Road
CS1	14b	L(14, 1.1)	L(3, 0.4)		L(8, 0.17)	Dimmers Rd.
CS2		L(22, 2.9)	L(3, 0.4)	L(5/2)[B]		Montgomery Rd.
CS3	12	L(3, 0.2)	S		S	Burt Rd.
CS4	11[A]	L(2, 0.2)	S		S	Cambria Rd.
CS5	3a	L(5)	L(2)		S	Woodbridge Rd.
CS6		S	S			Austin Rd.
CS7		L(1)	S			Sampson Rd.-East Fork
CS9		S				M-49
CS11	13a	S			S	Territorial Rd.
CS12	6b	S			no S or L	Sampson Rd.-West Fork
CS17	5c	S	L(1)		no S or L	1/2 mile S. of Lilac Rd.
CS18			S			N. of Austin Rd.

[A] Site 11 is about 200m downstream of site CS4.

[B] Five live clubshell were found in 5.5 person-hours in 2014 and two live were found in 9.75 person-hours in 2017 (Zanatta pers. com. 2019).

Figure 9. This live clubshell with three annular rings was the youngest individual of the species found. It indicates reproduction has occurred as recently as 2016 at site 14b in the East Fork of the West Branch St. Joseph River. Photo by Peter J. Badra.

Figure 10. This live clubshell, found at site 14b in the East Fork of the West Branch St. Joseph River was one of the oldest individuals of the species found during this survey. It is over 25 years old. Photo by Peter J. Badra.

Figure 11. The die-off of ash over the past decade has resulted in a massive input of large woody debris over 6 inches (15cm) in diameter into the St. Joseph River at sites 14a-c. Photo by Peter J. Badra.

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