freshwater solutions LLC
innovative swimmer's itch strategies

Comprehensive Lake Assessment Initiative

Platte Lake Improvement Association (PLIA)*
2019 Final Report

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Submitted by

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Executive Summary

Freshwater Solutions (FWS) conducted a comprehensive survey of the schistosomes that are causative agents for swimmer's itch on Big Platte Lake in 2019. Included in the survey were biodiversity assessments of the vertebrate waterfowl hosts, the invertebrate snail hosts, and the parasites through cercariae and miracidia shedding and DNA barcoding. Additionally, the magnitude of the swimmer's itch problem was assessed through qPCR analysis of swimmer's itch-causing cercariae in water samples.

Both hatch-year mallards and common mergansers were found to harbor patent adult schistosomes. Since *T. stagnicolae* was reportedly found in *S. emarginata* snails in 2018 and *T. physellae* was found in *Physa* sp. snails in 2019, we can confidently conclude that both summer resident mallards and common mergansers are the definitive hosts for at least some of the schistosomes causing swimmer's itch on Platte Lake.

Water sampling and qPCR analysis showed slightly above average numbers of cercariae in the water when compared to other lakes involved in this assessment research initiative in 2018 and 2019. There were a few hotspots with high cercariae numbers, validating a problem does exist in certain locations.

Trapping and relocating common mergansers is not likely to reduce swimmer's itch to the low levels desired, since more than one species is cycling in Platte Lake and one of those species is cycling through a very high density of mallards. Species-specific analysis of water samples showed 3 species of schistosomes in the water: *T. stagnicolae*, *T. physellae*, and the newly discovered species cycling through *Helisoma* sp. snails.

We encourage the PLIA to continue its engagement in the statewide battle against swimmer's itch and recommend the following action items for 2020:

- Educate riparians on ways to personally prevent swimmer's itch on their beach
- Join FWS in scientifically assessing personal prevention strategies
- Encourage members to report all swimmer's itch cases to "swimmersitch.ca"

FWS continues to empower lake associations to become as independent and self-sufficient as possible to help reduce yearly costs for swimmer's itch control. We lead the world in qPCR techniques for measuring avian schistosome cercariae in the water column. qPCR provides a reliable, less expensive, and easily teachable metric for assessment and we plan to offer interested lake associations mobile equipment and training for these techniques in the coming years. The PLIA will be included in those efforts and more details will be available soon. Finally, we continue our pursuit of bringing federal money to the fight with the submission of a grant proposal to the National Science Foundation. If successful, some of those monies will help pay for continued work on Platte Lake in the coming years. The following document describes, in detail, our comprehensive assessment work in 2019.

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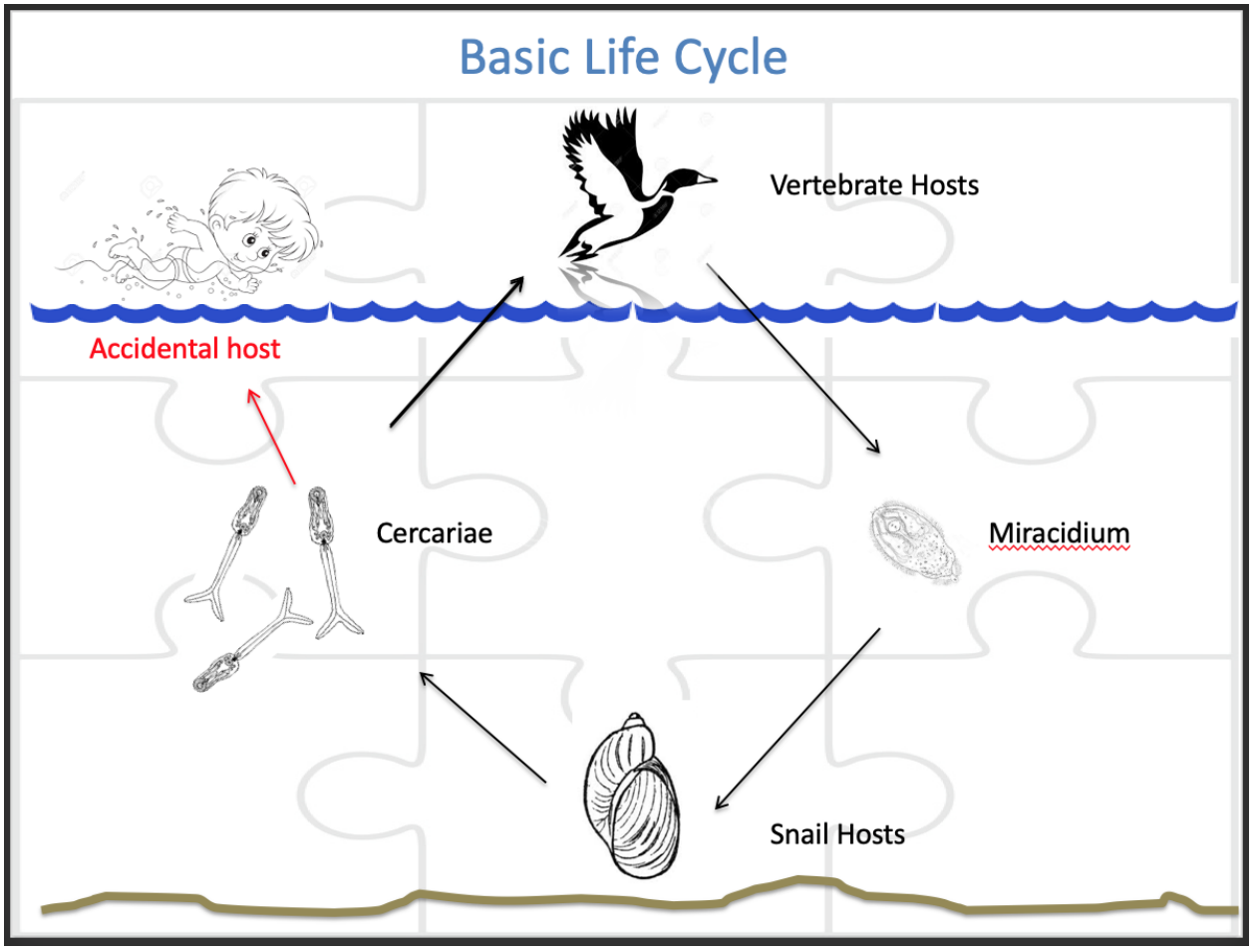
Objective 1: Determine the natural history of the parasite(s) causing swimmer's itch on Platte Lake.

A comprehensive understanding of all schistosome species causing swimmer's itch within a lake ecosystem is important when allocating funds for control, since often-limited financial resources can then be directed towards control efforts targeting the most abundant species. This increases the chance for achieving maximum results.

Freshwater Solutions' (FWS) Comprehensive Assessment Research Initiative conducted in 2018 revealed the unique biodiversity of itch-causing parasites on many NW Michigan inland lakes. *Biodiversity* is defined as the number of different species (species "richness") and the relative abundance of each species that live in a defined area. Since avian schistosomes utilize two hosts to complete their life cycle (one vertebrate and one invertebrate), there are many ways to assess schistosome parasite biodiversity on an inland lake. Fortunately, new tools are continually being developed and refined to make assessment easier, less expensive, and more comprehensive. FWS, in collaboration with Dr. Patrick Hanington (University of Alberta), is the world leader on advancing the science of swimmer's itch assessment.

The diagram below illustrates the complex life cycle of the flatworms responsible for swimmer's itch and the biological puzzle it represents.

Diagram 1: Basic Life Cycle



Our comprehensive assessment of Big Platte Lake included new molecular techniques to evaluate each piece of the complex life-cycle puzzle. By putting all the pieces together, we can provide an accurate picture of the parasites causing swimmer's itch problems for Big Platte Lake.

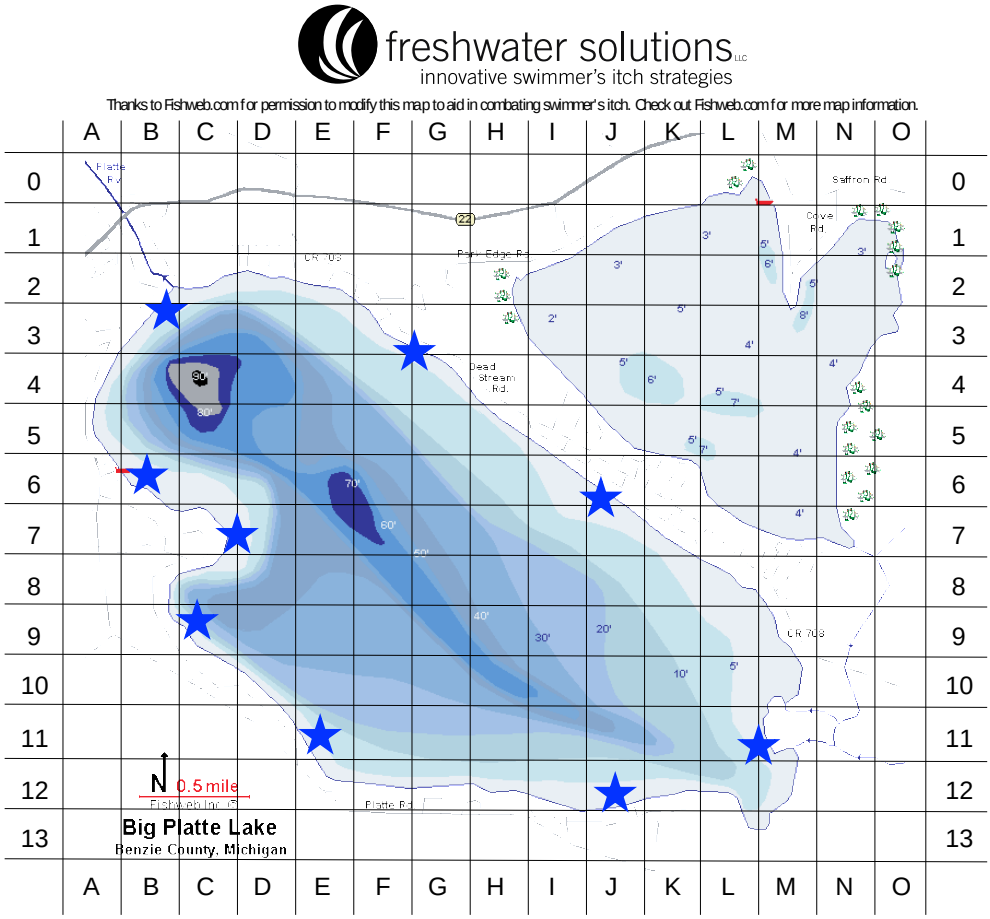
A. Determine the schistosome intermediate host(s).

•Survey locations where swimmer's itch is a perennial problem to identify the presence and density of all snails.

•Collect hundreds of snails at each site and shed them for patent infections.

PLIA representatives identified locations where swimmer's itch has been a perennial problem on Big Platte Lake. All of those locations, as well as others reportedly examined in 2018, were surveyed using full wetsuits and snorkeling and/or diving gear.

Diagram 2: Snail Collection Sites



Although our diving techniques require more time and expensive equipment, smaller snail species and those hidden in substrate easily go unnoticed using any other collecting method. Now that more schistosome parasites have been implicated in NW Michigan, some of which cycle through very small snails, our collection techniques are essential for thorough and reliable results.

To assess relative abundance, 1m2 weighted hoops were randomly tossed throughout the collection site. All snails of all species within the hoops were collected using FWS specially designed snail scoops with attached mesh bag nets.

The collected snails were placed in labeled buckets filled with fresh lake water and transported to the lab. Snails were sorted morphologically and each snail placed in its own compartment of a 12-well culture plate partially filled with conditioned water. The snails were exposed to natural lighting until dark, kept in the dark until dawn, and then exposed to natural and artificial light for several hours before each snail was examined for shedding cercariae using a dissection microscope.

A total of 2,251 snails were examined from Big Platte Lake. A patent schistosome infection was found in only one *Physa* sp. snail. None of the other snails known to harbor avian schistosomes were positive.

Table 1 – Platte Lake *Physa* sp. Cercariae

Physa sp.							
Date	Site	No. Examined	Density (m^2)	Pos. Schistosome	% infected Schistosome	Total Positive Infections	% that are infected with any parasite
6/21/19	B2	9	0.8	0	0.00%	0	0.00%
6/21/19	G3	1	0.0	0	0.00%	0	0.00%
6/21/19	J6	0	0.0	0	0.00%	0	0.00%
6/21/19	M12	0	0.0	0	0.00%	0	0.00%
6/21/19	J12	6	0.6	0	0.00%	0	0.00%
6/21/19	E11	1	0.3	0	0.00%	0	0.00%
6/21/19	C9	1	1.0	0	0.00%	0	0.00%
6/21/19	C7	2	1.0	0	0.00%	0	0.00%
6/21/19	B6	2	0.3	0	0.00%	0	0.00%
7/1/19	B6	31	0.2	0	0.00%	0	0.00%
7/1/19	C7	5	n/a	1	20.00%	1	20.00%
7/1/19	E11	1	0.1	0	0.00%	0	0.00%
7/1/19	J12	7	n/a	0	0.00%	0	0.00%

7/1/19	J6	29	1.5	0	0.00%	2	6.90%
7/1/19	B2	11	n/a	0	0.00%	1	9.09%
7/15/19	J6	70	n/a	0	0.00%	8	11.43%
Tot/Avg		**176**	**0.47**	**1**	**0.57%**	12	**6.82%**

Patent trematode infections were found in only 7.49% of all snails on Platte Lake. That's lower than most lakes FWS assessed in 2018. No conclusions can be drawn about that observation until all trematode cercariae DNA has been barcoded and a lake-wide species composition developed. This will occur as federal and/or international funding becomes available.

Table 2 – Platte Lake Snail Shedding Data

Platte Lake Snail Totals									
	Snails Examined	Snails in Hoops	Density (m_2)	% Snail Fauna	Schistosome Infections	% Schistosome	Trematode infections	% infections	% Total Infections
Stagnicola sp.	215	28	0.24	1.57%	0	0.00%	12	5.58%	21.43%
Physa sp.	176	19	0.16	1.07%	1	0.57%	12	6.82%	21.43%
Gyraulus sp.	31	11	0.09	0.62%	0	0.00%	0	0.00%	0.00%
Pleurocera sp.	1762	1675	14.44	94.15%	0	0.00%	21	2.12%	37.50%
Helisoma sp.	29	14	0.12	0.79%	0	0.00%	11	37.93%	19.64%
Campeloma sp.	8	3	0.03	0.17%	0	0.00%	0	0.00%	0.00%
Marstonia sp.	30	29	0.25	1.63%	0	0.00%	0	0.00%	0.00%
Tot/Avg	**2251**	**1779**	**15.34**	**100.00%**	**1**	**0.57%**	**56**	**7.49%**	**100.00%**

Discussion

Of all the snails found in Platte Lake known to harbor schistosomes (*Stagnicola*, *Physa*, *Gyraulus*, *Helisoma*), *Stagnicola* sp. had the highest density, followed by *Physa*, *Helisoma*, and *Gyraulus*. Compared to other NW Michigan lakes surveyed in 2018-19, densities of all 4 species were below average. *Physa* sp. were the only snails found to harbor avian schistosomes in 2019 and are known to carry several species of schistosomes that cycle through common avian hosts such as Canada geese, mallards, and common mergansers.

DNA extraction and sequencing of all non-schistosome parasites as funding becomes available should give us some baseline data to begin to assess the biodiversity and overall health of Platte Lake from a parasitological perspective.

B. Determine what parasite species are present and their relative abundance.

•Preserve pure samples of all cercariae shed from all species of snails present, extract their DNA, and sequence the DNA to compare against species housed in GenBank for species identification.

All cercariae (not just schistosomes) shed from all snail species were pipetted into individual 1.5ml conical collection tubes, centrifuged, the water decanted, and the worms preserved in 95% ethanol. The labeled tubes were placed in a conventional freezer (-20ºC) and shipped to the University of Alberta (Edmonton) for DNA extraction, sequencing, and archiving.

One snail shed schistosome cercariae from Platte Lake. In addition, 56 non-schistosome cercariae were found. Final analysis of non-schistosome cercariae is still in progress and will be reported as an addendum to this document when completed.

Table 3 – Platte Lake Cercariae Identification

Platte Lake Cercariae Barcoding			
Lake	**Location**	**Host Snail**	**Parasite**
Platte	C7	*Physa* sp.	*Trichobilharzia physellae*

Discussion

Snail shedding results revealed one species of schistosome, *T. physellae*, shed from a *Physa* sp. snail. This parasite is known to use both mallards and common mergansers as their definitive host.

C. Assess population dynamics (size, age structure, etc.) of all summer resident anatids (ducks, geese, swans).

•Conduct a boat survey of the entire lake shoreline to record summer resident anatid species, number of birds, and age categories.

Three (3) complete shoreline waterfowl surveys were conducted on Platte Lake 6 June, 9 July, and 29 July by traveling near shore at trolling speed in a motorboat with the aid of field glasses.

Table 4 – Platte Lake Waterfowl Surveys

Platte Lake Waterfowl Survey Summary 6/6/19					
	Total Birds	**AHY**	**HY**	**Broods**	**% of Population**
Mallard	118	45	73	9	60.82%
Canada Goose	21	6	15	3	10.82%
Trumpeter Swan	11	11	0	0	5.67%
Common Merganser	44	17	27	3	22.68%
Totals	**194**	**79**	**115**	**15**	**100.00%**

Platte Lake Waterfowl Survey Summary 7/9/19					
	Total Birds	AHY	HY	Broods	% of Population
Mallard	189	111	78	13	79.75%
Canada Goose	20	6	14	3	8.44%
Trumpeter Swan	1	1	0	0	0.42%
Red Breasted Merganser	1	1	0	0	0.42%
Wood Duck	8	1	7	1	3.38%
Common Merganser	18	3	15	3	7.59%
Totals	**237**	**123**	**114**	**20**	**100.00%**

Platte Lake Waterfowl Survey Summary 7/29/19					
	Total Birds	AHY	HY	Broods	% of Population
Mallard	233	177	56	10	89.96%
Canada Goose	4	2	2	1	1.54%
Trumpeter Swan	0	0	0	0	0.00%
Common Merganser	22	3	19	3	8.49%
Totals	**259**	**182**	**77**	**14**	**100.00%**

Table 5 – Waterfowl Densities Compared

Waterfowl Densities (birds/shoreline mile) - All Lakes 2018*-19								
Lake	Shoreline (mi)	Mallard	C. Goose	M. Swan	C. Merganser	H. Merganser	RB. Merganser	Total Birds
Charlevoix*	60.0	7.08	2.78	0.10	1.12	0.30	0.00	11.38
Elk*	28.0	5.25	1.54	0.14	0.54	0.00	0.21	7.68
Big Glen*	10.8	8.70	0.65	0.37	*0.28*	0.00	0.00	10.00
Little Glen*	6.4	9.22	1.25	0.00	*4.22*	0.00	0.00	14.69
NL Leelanau*	15.0	6.27	0.87	0.40	*0.07*	0.00	0.00	7.60
SL Leelanau*	26.2	14.12	1.72	0.00	*0.00*	0.08	0.00	15.84
Lime Lake*	4.2	8.33	0.95	0.00	*0.48*	0.00	0.00	9.77
Long*	16.7	6.47	0.66	0.00	0.00	0.06	0.00	7.13
Skegemog*	15.0	2.73	1.20	0.93	0.00	0.00	0.00	4.87
Walloon*	30.5	1.61	1.34	0.00	1.80	0.00	0.00	4.75
White Sand*	11.2	0.45	0.00	0.00	0.00	2.23	0.00	0.45

Pickerel	7.1	14.37	6.62	0.00	0.00	0.42	0.00	20.99
Crooked	16.3	13.62	2.33	0.86	0.00	6.56	0.00	16.81
North Torch	21.0	8.19	0.24	0.00	3.67	0.00	0.00	12.10
South Torch	20.0	3.35	2.40	0.00	0.55	0.00	0.00	6.30
Bellaire	12.0	2.83	1.17	0.00	0.00	0.17	0.00	4.00
Intermediate	14.6	12.12	0.89	1.30	1.16	0.75	0.00	15.48
Big Platte 7/29/19	9.3	25.05	0.43	0.00	2.37	0.00	0.00	27.85
Averages	**18.0**	**8.32**	**1.50**	**0.23**	**0.90**	**0.59**	**0.01**	**10.98**

Note: Italicized numbers denote common mergansers were being actively trapped and relocated, therefore numbers would fluctuate and are not reliable for comparison.

Discussion

Mallards not only dominated the waterfowl community on Platte Lake in 2019 but constituted a much larger percent of the community than other lakes in NW Michigan. Mallards are known to carry schistosome parasites that cause swimmer's itch. Due to their exceptional dominance, mallards must be considered as contributors to the swimmer's itch on Platte Lake.

The number of HY common mergansers (ducklings) decreased from 27 in early June down to 15-19 later in July. Barring predation (rarely observed), some early broods could have migrated up the river into Little Platte Lake or down the river into Loon Lake, Mud Lake, or even Lake Michigan. The same could be said for the decrease in Canada goose numbers.

D. Assess relative infection levels and species identification in definitive hosts.

•Collect avian fecal samples, where possible, and examine for avian schistosomes.

•Preserve pure samples of all miracidia obtained from examined waterfowl, extract their DNA, and sequence the DNA to compare against species housed in GenBank for species identification.

If fecal samples from HY birds (ducklings) are positive, the parasites collected (miracidia) must be cycling in the lake since the birds have never flown and could not have picked up the worms at another location. Therefore, every effort was made to collect samples from ducklings that were at least 4 weeks old (many schistosome species take ~4 weeks to become patent and produce eggs/miracidia).

Fresh waterfowl fecal samples were collected from docks, rafts, and shore where birds were observed roosting. When samples were actually seen being deposited or could be inferred (i.e. only one age group present), they were recorded as 100% certainty. If AHY and HY birds were close together, age certainties were reported as a fraction of HY to AHY birds.

Samples were placed in disposable cups with lids and stored in a cooler until returned to the lab. The samples were weighed and processed by adding conditioned water, diluted and decanted several times to clear, and placed under artificial light for >1 hour. Each sample was examined using a stereomicroscope for 3, one-minute times. Infections were recorded as the average number of miracidia observed/gram/minute.

A total of 62 fecal samples were collected, processed and analyzed from Platte Lake (28 mallards, 21 Canada geese, 12 common mergansers, and 1 red-breasted mergansers). Schistosome miracidia were found in 4 mallard samples, 11 common merganser samples, and 1 red-breasted merganser sample. Since mallards were found in such great numbers, we safely conclude they are contributors to the swimmer's itch problem on Platte Lake. Since nearly all of the common merganser samples were positive for schistosomes, we can also conclude they are contributors to swimmer's itch, even though they make up a small percent of the summer resident waterfowl community.

If enough miracidia could be collected (~4-5), they were pipetted into a 1.5ml conical collection tube, centrifuged, the water decanted, and then the miracidia preserved in 95% ethanol. The labeled tube was placed in a conventional freezer (-20ºC) and shipped to the University of Alberta for DNA extraction and sequencing. The results were then analyzed for positive species identification.

Table 6 – Platte Lake Fecal Samples

Platte Lake Waterfowl Fecal Sample Analysis					
Date	**Species**	**Location**	**Age/Certainty**	**Ave Miracidia/g/min**	**Species (if determined)**
7/9/19	Red Breasted Merganser	I6	AHY	10.95	Unknown
7/26/19	Common Merganser	A4	4/5 HY	193.59	*T. stagnicolae*
7/26/19	Common Merganser	A4	4/5 HY	54.25	*T. stagnicolae*
7/26/19	Common Merganser	A4	4/5 HY	29.91	*T. stagnicolae*
7/26/19	Common Merganser	A4	4/5 HY	40.23	*T. stagnicolae*
7/26/19	Common Merganser	A4	4/5 HY	57.14	*T. stagnicolae*
7/30/19	Mallard	B6	8/9 HY	0.23	Insufficient DNA
7/30/19	Mallard	F12	5/6 HY	1.83	*T. physellae*
7/30/19	Mallard	F12	5/6 HY	1.21	*T. physellae*
7/30/19	Mallard	F12	5/6 HY	0.36	Insufficient DNA
7/30/19	Common Merganser	K7	8/9 HY	10.61	*T. stagnicolae*
7/30/19	Common Merganser	K7	8/9 HY	9.86	*T. stagnicolae*
7/30/19	Common Merganser	K7	HY	8.87	*T. stagnicolae*
7/30/19	Common Merganser	K7	HY	13.01	*T. stagnicolae*

7/30/19	Common Merganser	K7	HY	4.76	*T. stagnicolae*
7/30/19	Common Merganser	K7	HY	3.61	*T. stagnicolae*

Discussion

DNA barcoding analysis identified the miracidia from the HY mallards as *Trichobilharzia physellae* and from HY common mergansers as *Trichobilharzia stagnicolae*. This was expected and is the most common host-parasite combination for these species in NW Michigan. One *Stagnicola emarginata* snail was reportedly shedding *T. stagnicolae* in 2018, so we can assume this species of parasite is cycling in Platte Lake. Discovering *T. physellae* in both a *Physa* sp. snail and a HY mallard duck in 2019 definitively proves this parasite as also cycling in Platte Lake.

Objective 2: Determine the level of parasite infestation on Platte Lake for data necessary to obtain a MDNR merganser trap & relocate permit in 2020 *if mergansers are implicated*.

According to current MDNR policy, to obtain a common merganser trap & relocate permit for the purpose of controlling swimmer's itch in 2020, a lake must provide a Letter of Authority documenting the swimmer's itch life cycle present on their lake and include the following information, at a minimum:

- *Presence of the swimmer's itch parasite and evidence that Common Mergansers are the host associated with the parasite's lifecycle on the lake.*
- *Documentation of Common Merganser broods on the lake.*
- *Evidence of increasing swimmer's itch cases or severity.*

In addition, one of the following criteria must be met to be eligible:

- *Snail infection rate for the lake is greater than 0.5% with a minimum sample of 1,000 snails taken from a minimum of 5 sampling locations on the lake.*
- *A qPCR assessment of the lake that is greater than 50 cercariae/25 L of water with a minimum of 5 sampling locations.*

A. Use qPCR analysis to accurately gauge schistosome cercariae levels in the water.

•Collect water samples using the FWS established collection protocol at strategic locations (approximately every mile) around the lake perimeter, extract the DNA, and run qPCR to determine the level of cercariae.

Quantitative Polymerase Chain Reaction (qPCR) amplifies the DNA of target organisms in a heterogeneous sample. When run concurrently with and compared to DNA standards, the quantity of target DNA in the sample (schistosomes in our case) can be determined. Knowing the amount of DNA in one cercaria allows for accurate estimation of the total number of cercariae in the water sample.

Water samples were collected following the FWS-developed standard protocol at each snail collection site on 21 June and again on 26 July. Samples were preserved in 95% ethanol and refrigerated. Samples were then suction filtered through a 0.4um filter disc and the DNA from all organisms extracted using a standard bead-beating method.

A single aliquot of each DNA extract was then used to run a pan-avian qPCR analysis. Species-specific qPCR analysis was also run on all positive samples using a core machine at the University of Alberta to determine relative amounts of each species.

Table 7 – Platte Lake Water Samples

Water Sample Data		
	21 June 2019	26 July 2019
Site	Avg Cer/25L	Avg Cer/25L
B2	1.25	0.00
G3	1.25	1.50
J6	>100	91.25
M11	0.00	0.00
J12	0.00	1.25
E11	>100	>100
C9	1.25	0.00
C7	46.00	1.00
B6	>100	0.00
Ratio >30/<30	**0.80**	**0.29**

Cercariae numbers provide useful information for site-specific and whole-lake assessment of swimmer's itch severity. Reporting lake-wide cercariae averages, however, is not the best metric for lake-to-lake comparisons. Just a few sites with unusually high numbers can skew results when reporting averages. Possible reasons for sporadic high numbers include accumulation due to wind or unique shoreline hydrology concentrating the cercariae. Recent scientific studies document snails under laboratory conditions releasing 10X the average number of schistosome cercariae/day on certain days and that may contribute to unusually high numbers as well.

Instead of lake-wide averages, we report the ratio of percent of sites >30 cercariae per sample to percent of sites ≤30 cercariae per sample. This ratio compares "heavy/severe" samples to "medium/light" samples on the swimmer's itch severity index found below. Obviously, a lower ratio value equates to less risk of contracting swimmer's itch.

Table 8 – Swimmer's Itch Severity Index

# Cercariae/25L	Severity Index
0-10	Light
11-30	Medium
31-100	Heavy
100+	Severe

Table 9 – Assessment Initiative Participants Comparison

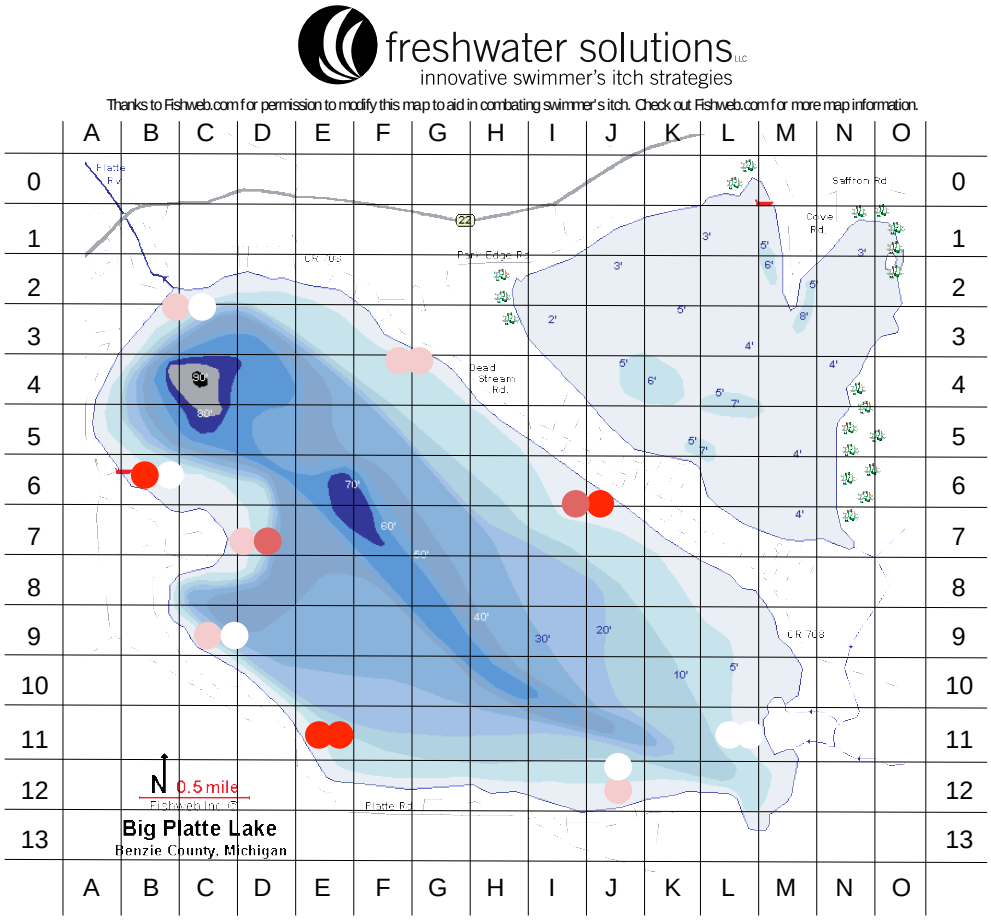
qPCR Values - All Lakes Comparison 2018			
Lake	**% Sites >30 Cer./25L**	**% Sites <30 Cer./25L**	**Ratio >30/<30**
Charlevoix	19.23	80.77	0.24
Elk	48.39	51.61	0.94
Big Glen	37.50	62.50	0.60
Little Glen	25.00	75.00	0.33
SL Leelanau	22.72	77.28	0.29
NL Leelanau	45.45	54.55	0.83
Lime	25.00	75.00	0.33
Long	13.33	86.67	0.15
Skegemog	14.29	85.71	0.17
Walloon	19.44	80.56	0.24
White Sand	12.50	87.50	0.14
qPCR Values - All Lakes Comparison 2019			
N. Torch	22.22	77.78	0.29
S. Torch	0.00	100.00	0.00
Bellaire	16.67	83.33	0.20
Intermediate	25.00	75.00	0.33
Pickerel/Crooked	10.00	90.00	0.11
Platte	33.33	66.66	0.50
Average	**22.95**	**77.05**	**0.33**

Discussion

Platte Lake cercariae numbers, with both sample dates averaged, were slightly above average from all Michigan lake participating in our comprehensive assessment research in 2018 and 2019. There were a couple hotspots on both surveys (J6, E11) with larger numbers of cercariae in the water and a couple others that showed high cercariae counts during the June survey (C7, B6), so it is reasonable some riparians reported swimmer's itch in 2019, possibly even with severe cases.

The heat map below visually shows swimmer's itch distributed around Platte Lake in 2019. Mild levels of cercariae appear to be distributed uniformly around the lake with a few locations showing very high numbers of cercariae.

Diagram 3 – Platte Lake Cercariae Heat Map



Objective 3: Train and equip designated riparians on Platte Lake to collect water samples for future qPCR analysis.

FWS, under direct leadership of Dr. Patrick Hanington from the University of Alberta, has become a world leader in qPCR analytics applied to avian schistosomes responsible for causing swimmer's itch. qPCR has many advantages over snail infection rate when assessing risk. It is much less labor intensive and therefore less expensive. It can be done in a couple of hours to provide near real-time analysis. It can assess all species of avian schistosomes cycling in the lake. And finally, volunteers with minimal training can assist in the process to help reduce costs.

FWS is committed to reducing future costs for swimmer's itch assessment and control by empowering lake associations to do more of the work themselves. Through several years of trial and error, we developed a standard water collection protocol and determined the appropriate equipment to do the job correctly.

A. Create consistent tools and methodologies to enable accurate site-to-site, lake-to-lake, and year-to-year comparisons of swimmer's itch risk via qPCR analysis.

•Construct and provide a standard water sampling kit, complete with all essential components, to the Platte Lake Association for use by volunteers for future years.

•Provide written, oral, and video instructions to volunteers on the best practice for collecting water samples for standardized qPCR analysis.

FWS, as instructed by PLIA President Wilfred Swiecki, will provide written and oral instructions with onsite training to a small group of volunteers in the spring of 2020. This increases the likelihood proper protocols will be remembered since implementation will soon follow instruction (as opposed to fall training with implementation not until the following summer). In addition, FWS created an instructional video which will be sent to PLIA representatives as an .mp4 file next spring. A complete water sampling kit was assembled and will be given to representatives on training day (unless PLIA representatives prefer to store it over the winter). The kit contains the following items:

1. 20um plankton tow
2. anemometer
3. floating thermometer
4. 1L water scoop
5. 50ml conical collection tubes
6. extension pole
7. permanent markers
8. two spray bottles
9. plastic carrying tub
10. laminated written instructions

A copy of the written instructions for water collection is included in Appendix A of this document.

Discussion

The simplicity of the collection process and the fact we have used this collection process with success the past three years provides the basis for successful volunteer water collection in future years. This provides another cost-saving opportunity for lake associations across Michigan.

FWS, in collaboration with Dr. Patrick Hanington, hopes to mobilize qPCR in Michigan beginning in 2020. Dr. Hanington has submitted a renewal grant to place 40+ qPCR machines in high schools across Alberta. The Michigan model will be patterned after Dr. Hanington's work. We will submit PLIA a formal invitation to participate when completed.

Objective 4: Collect data to strengthen and support a grant proposal to the National Science Foundation (NSF) and or the Natural Sciences and Engineering Research Council of Canada (NSERC) for multiple-year research beginning in 2020.

In recent articles published in peer-reviewed journals, swimmer's itch is being described globally as an "emerging disease". Despite that fact, obtaining government funds to combat swimmer's itch remains a challenge, both in the United States and abroad. FWS, Dr. Hanington, and a parasite ecologist from the University of Wisconsin, plan to submit a grant proposal to the NSF this fall/winter for work in 2020.

If funded, Platte Lake will be included in the project with some of the future PLIA costs covered with grant money. Regardless of our success on this application, FWS will continue to work towards garnering state and federal dollars to combat swimmer's itch on Platte Lake.

A. Use patent snail infections to obtain cercarial types for cross-continent comparisons.

- ***Preserve pure samples of all cercariae shed from host snails, extract their DNA, and sequence the DNA to compare against species found across the continent.***

- ***Preserve snail tissue from all types found on each lake, extract the DNA, and sequence the DNA to compare against species found across the continent.***

A total of 11,905 snails from 52 sites on 11 lakes in Michigan and Wisconsin were collected and analyzed for patent trematode infections in 2018. With the comprehensive assessment of Platte Lake in 2019, those totals rise to 14,156 snails from 61 sites on 12 lakes. A total of 2,080 infections were discovered and preserved for DNA sequencing.

Table 10 – Total Snail Infections (all species)

Lake	Total Snails	Total Infections	Percent Infection
Charlevoix	928	24	2.59%
Elk	1367	357	26.12%
Big and Little Glen	1610	94	5.84%
South Lake Leelanau	1312	204	15.55%
North Lake Leelanau	2616	600	22.94%
Lime	143	9	6.29%
Long	733	53	7.23%
Skegemog	1427	492	34.48%
Walloon	1173	119	10.14%
White Sand	596	72	12.08%
Platte Lake (2019)	2251	56	7.49%
Totals	**14156**	**2080**	**14.69%**

Representative snails from each lake were preserved in 95% ethanol and shipped to the University of Alberta for DNA extraction, sequencing, and archiving.

Table 11 – Snail Genera Identification and Densities (2018 in Italics)

Snail Genera	*Charlevoix*	*Elk*	Glen	SLLeelanau	NLLeelanau	*Lime*	*Long*	*Skegemog*	Walloon	*White Sand*	N. Torch	S. Torch	Bellaire	Platte	Intermediate	Crooked/ Pickerel
Stagnicola sp.	2.71	5.61	2.77	0.04	10.04	0.08	0.15	9.17	1.79	6.95	0.90	0.85	3.63	0.24	2.52	4.76
Physa sp.	1.37	0.24	2.79	1.02	0.12	0.01	0.27	0.61	0.32	0.40	0.54	4.52	0.92	0.16	2.04	0.10
Lymnaea sp.	--	--	--	0.00	--	0.03	--	--	--	0.10	--	--	0.03	--	--	0.01
Gyraulus sp.	0.24	0.01	0.62	6.18	0.50	0.08	1.17	--	0.15	0.10	0.10	--	0.32	0.09	1.87	0.63
Pleurocera sp.	0.85	5.22	6.16	4.42	2.10	1.56	0.01	5.59	2.18	--	3.20	14.78	0.90	14.44	0.29	1.50
Helisoma sp.	0.30	0.23	1.55	1.60	0.32	0.03	0.68	0.52	1.79	7.25	0.03	--	1.70	0.12	0.22	0.74
Campeloma sp.	--	--	0.01	0.02	--	--	--	1.15	0.10	--	0.30	--	--	0.03	--	--
Marstonia sp.	0.04	--	0.02	0.92	0.10	0.01	0.09	0.55	0.04	0.05	--	--	0.29	0.25	--	0.06
Viviparus sp.	--	--	--	--	--	--	0.84	--	--	11.75*	--	--	0.38	--	0.49	1.51
Cipangopaludina sp.	--	--	--	--	--	--	--	--	--	11.75*	--	--	--	--	--	--

* Band Mystery Snails (*Viviparus* sp.) and Chinese Mystery Snails (*Cipangopaludina* sp.) combined.

Discussion

With the exception of an unusually high density of *Pleurocera* sp. snails, Platte Lake has below average density of all other snails observed. In other words, Platte Lake does not have a diverse community of snails, but rather is dominated by one species (one that does not harbor avian schistosomes).

The effect of various swimmer's itch control measures on lake ecosystems is one focus of our work begun in 2018 as part of the comprehensive multi-lake assessment initiative. With successful funding, we plan to expand our assessment strategies over the next 3-5 years. The large database generated by this assessment initiative provides many other opportunities to gain insight into the lake ecosystem as well. For example, parasite profiles are now being used to assess ecosystem biodiversity and health. Due to their often-complex life cycles, which include multiple hosts, we can infer the presence of vertebrate and invertebrate species simply by knowing a parasite species exists in the ecosystem. FWS will continue to collaborate with scientists from the United States and Canada on these and other swimmer's itch related problems in the future.

B. Collect qPCR data, cercariae types, snail diversity, and other whole-lake ecological data for comparison of different swimmer's itch control measures to begin in 2020.

- ***Archive all water sample extracts at -80˚C at the University of Alberta.***
- ***Archive all cercariae and snail types as voucher specimens at the University of Alberta.***
- ***Create a whole-lake database of all other ecological parameters measured.***

All data collected on Platte Lake has been compiled, analyzed, and archived as baseline data for comparison to data collected in coming years. All water sample extracts are being stored at -80°C at the University of Alberta and can be retrieved for further analysis.

C. Establish "swimmersitch.ca" as the primary website for reporting swimmer's itch cases across the United States and Canada.

- ***Contact all lake associations (lakes >500 acres) across the North American continent to request their participation in reporting swimmer's itch cases in 2019.***
- ***Provide PLIA a season-end data file for all swimmer's itch cases reported from Platte Lake.***
- ***Aggregate all data by region, state, province, and country, as well as by date, severity, number, etc. to establish the first-ever cross-continent database documenting swimmer's itch cases.***

Dr. Hanington has been monitoring swimmer's itch cases throughout Alberta since 2012 via a questionnaire link on his website at "swimmersitch.ca". The scope of this reporting expanded to include the entire North American continent in 2018. We are working on establishing one swimmer's itch-reporting site for all of North America ("swimmersitch.ca"), the definitive "911" site where everyone can go to report cases of swimmer's itch. The data collected will be invaluable for not only examining cross-continent trends but also for documenting the thousands of cases necessary to apply for federal funding and international collaboration. In 2019 alone, this site recorded 946 cases of swimmer's itch from 155 unique lakes.

We are continually improving the reporting aspects of this site to make it valuable to the scientific world and also to each individual lake association. We recommend you encourage your riparians to report all cases of swimmer's itch, throughout the swim season, to this website: "swimmersitch.ca". Open the tab "Report Your Itch!" and fill in the survey.

Recommendations

Big Platte Lake showed above average number of sites with relatively high levels of cercariae in the collected water samples. This metric (#cercariae/25L) directly measures the risk of contracting swimmer's itch when bathing in the lake. The levels showed there were enough cercariae to indicate a definite problem exists at some locations.

Although no control effort will completely eliminate swimmer's itch from a lake ecosystem, knowing the complexities of the parasites involved allows us to make educated recommendations about how to best control swimmer's itch. Since Platte Lake has an extremely large population of mallard ducks and *T. physellae* was found cycling in mallards and *Physa* sp. snails, it is unlikely trapping common mergansers will produce a dramatic reduction in swimmer's itch.

Recommendation #1: Educate Platte Lake riparians on ways they can personally reduce their chance of contracting swimmer's itch.

Project '17 and '18, FWS research funded largely by lake associations in Leelanau County (Glen, Lime, Leelanau), provided discoveries into innovative site-specific control options as well as cercariae behavior. This knowledge can help riparians reduce their risk of contracting swimmer's itch.

FWS recently produced a document entitled *"Preventing Swimmer's Itch with 2020 Vision"* that describes the many options riparians have to greatly reduce their chance of getting swimmer's itch. This document can be used by lake associations to educate and empower their members in 2020 and beyond.

Recommendation #2: Encourage PLIA members to join the PLIA and FWS in assessing the efficiency of various prevention measures in 2020.

FWS will be presenting the PLIA with an opportunity to join a multiple lake project in 2020 that will assess the various prevention strategies presented in *"Preventing Swimmer's Itch with 2020 Vision"*. The details of this initiative will be shared in the coming months.

Recommendation #3: Promote the use of "swimmersitch.ca" to report all swimmer's itch cases in future years. This service is free and provides valuable data for us to garner additional state and federal funds for battling swimmer's itch. With modifications for 2020, it will also provide valuable information to each lake association.

Acknowledgements

It was our pleasure to work on Platte Lake this past summer. We try not to take for granted the natural beauty of the water and landscapes in NW Michigan, even though that is where we go to "work" every day in the summer. Thanks to the countless hours of work by volunteer members of PLIA on water and land preservation issues, we have good reason to believe we will leave our beautiful slice of earth in great shape for our children and grandchildren.

One of the best things about engaging a new lake association in our battle against swimmer's itch is the relationships that develop. Behind every good lake association are a myriad of individuals giving of their time and money to promote the noble causes of the association. Although we only had the opportunity to work closely with a few, we especially want to recognize Wilfred Swiecki. He has been very accommodating, helping make 2019 an exceptional field season. Thank you!

Finally, we could not function without the dedicated and talented work of our other team members: Chris Froelich, Kelsey Froelich, Sydney Rudko, Brooke McPhail, Dan Clyde, Matt Schuiling, and Annette Dobrzynski. Their pursuit of excellence and tireless commitment to quality is what has made FWS so successful. Without their behind-the-scenes diligence, we could not have produced the results we presented to you in this report.

Appendix A – FWS Standard Water Collection Protocol

Standard Water Collection Protocol

1. Label a 50ml conical collection tube with the following information using a permanent marker (Diagram 1). *NOTE: Marker will wash off if using 95% ethanol so be careful of spills.*

a. Lake name
b. Site location (name or identifying number)
c. Collection date & time
d. Collector's initials and cell number

2. Measure and record water temperature (in Celsius) using the floating thermometer and *average* wind speed (in km/h) using the handheld anemometer. Also record the direction *from which* the wind is blowing and whether it is an onshore, offshore, or alongshore wind. Record the date & time.

3. Collect a 1-liter water sample from the water surface between 8:00-12:00 a.m. using the one-liter measuring device (extension handle optional) and pour it through the 20um mesh plankton tow held vertically (Diagram 2).

4. Repeat step #2 another 24 times for a total of 25 liters sampled. Space the 1-liter samples evenly throughout the collection site if wading or uniformly from both sides of the length of a dock.

5. Holding the cod assembly upright, carefully unscrew it from the plankton net once the water level is well below the coupling union (Diagram 3).

6. While holding the cod end assembly in one hand, spray the mesh netting clean with tap water from the inside and outside (Diagram 4) until a small amount of water and trapped organisms remain (<50ml) in the bottom. If samples are not going to be processed the same day, repeat this process using 95% laboratory grade ethanol.

7. Carefully pour the remaining sample into the properly labeled, 50ml collection tube. Spray down the inside of the cod end assembly to capture any residue, bringing the volume to 50ml. Cap tightly. *NOTE: If too much liquid remains in the cod end assembly DO NOT OVERFILL the tube. Instead, pour all the liquid back into the cod end assembly and spray/filter for a longer period of time until the volume is less than 50ml.*

8. Refrigerate immediately and deliver for processing within a few hours. If 95% ethanol was used, refrigerate immediately and deliver within 24 hours.

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Diagram 1
Label a 50ml conical collection tube

Diagram 2
Collect a 1-liter water sample

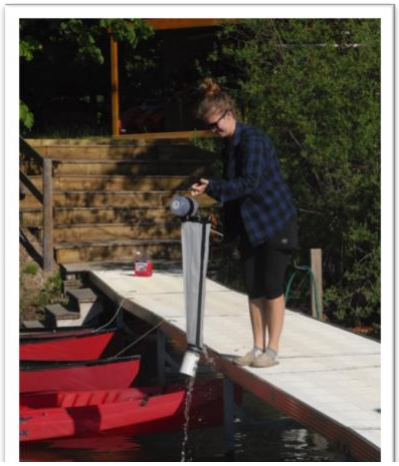

Diagram 3
Unscrew COD from the plankton net

Diagram 4
Spray the mesh netting with tap water

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Appendix B – Platte Lake Map w/Grid