PROJECT TITLE:

Survey of swimmer's itch parasites in Michigan lakes - 2016

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PROJECT SUMMARY

- Our primary goal is to measure the distribution and abundance of swimmer's itch parasites in Michigan. Swimmer's itch is caused by snail-borne flatworms that normally infect birds but sometimes try to infect humans, leading to an itchy rash. This parasite is viewed as a significant pest by riparian owners. However, few data are available to inform management or to determine which factors best predict parasite abundance.
- Our goal for 2016 is to obtain one month's worth of daily parasite data from at least 20 lakes from across Michigan. We will also collect data on environmental factors thought to increase the risk of swimmer's itch.
- This will be an expansion of survey work we conducted in 2015, which was partially funded by donations from local lake associations. The 2015 survey included 14 sites at 8 different lakes. Preliminary results show that there is variation in parasite abundance among sites and day-to-day variation within sites, and suggest that multiple environmental factors are involved in driving this variation. This apparent complexity means that we will need data from additional sites to allow robust statistical analyses of environmental predictors.
- Survey methods include collection of daily filtered-water samples by local volunteers for a 28-day period in July and August. We will analyze these samples using a new DNA-detection method for swimmer's itch parasites. We will also visit each site at the beginning and end of the survey period, to train volunteers and to measure environmental risk factors including snail densities, bird visitation, and water chemistry.
- There are limited sources of state or federal funding for swimmer's itch research, which is not (yet) viewed as a human-health problem. **We are therefore seeking financial and volunteer support from local lake organizations. We request a donation of $1000 from each participating lake (plus $500 per additional site) to cover survey costs (travel, materials & supplies, personnel). We also seek to recruit at least one local volunteer per lake to collect daily filtered-water samples in July/August 2016.**

COLLABORATORS

- Lakes participating in the 2015 survey included 8 members of the Michigan Swimmer's Itch Partnership (MSIP): Higgins Lake, Crystal Lake, Lake Leelanau, Glen Lake, Little Traverse Lake, Lime Lake, Walloon Lake, and Platte Lake. New participants for 2016 include Lake Margrethe and Lake Gogebic (pending funding). Other members of MSIP who might participate include Lake Sapphire, Lake Missaukee, Torch Lake, Burt Lake, Douglas Lake, and Long Lake (in Grand Traverse County).
- We also collaborate with Curt Blankespoor and Ron Reimink of SICON LLC, a Michigan company whose services include control of swimmer's itch by relocating bird species that harbor swimmer's itch parasites.

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PROJECT DESCRIPTION

Background

Swimmer's itch is a nasty itchy rash caused by avian schistosomes, snail-borne parasites closely related to the causative agents of human schistosomiasis (*Schistosoma* spp.) [1, 2]. Avian schistosomes include a diverse group of trematode (flatworm) parasites, most in the genus *Trichobilharzia*, that normally use water birds as their definitive hosts [3]. Infected snails release infectious stages called "cercariae", which swim through the water in search of a bird to infect. However, cercariae sometimes mistake humans for birds and penetrate our skin instead [1]. They cannot complete their life cycles in humans but often cause a severe itchy rash that can take several days to clear [1, 4]. People with no skin reaction might experience asymptomatic larval migrans (worms migrating through the body), with unknown long-term effects [1].

Anecdotal evidence suggests that swimmer's itch is increasing in Michigan inland lakes, where it discourages swimming and other recreational activities [5]. Studies on other trematode parasites have shown that pollution from fertilizer, herbicides, and insecticides can all increase the densities of snail intermediate hosts [6, 7]. These chemicals act by either increasing growth of the snails' food (algae growing on surfaces) or killing off insect predators of snails [6, 7]. Temperature also influences parasite development and production of cercariae, with potential implications for climate effects [8, 9]. Other potentially important factors for cercaria distribution and abundance include wind and wave action, substrate composition, and bird visitation [5].

A key goal of our survey is to test which environmental factors best predict among-site and day-to-day variation in cercaria abundance. This has been impossible in the past due to a lack of data. Traditional methods for detecting schistosomes involve labor-intensive screening of infected snails for cercaria production, which requires extensive training in parasite identification [10]. As a result, there are few data available for investigating large-scale spatial patterns, and no prior datasets measuring small-scale temporal (day-to-day) variation [10].

We seek to fill this knowledge gap by conducting a large-scale survey, using a newly developed qPCR (DNA-detection) method [11]. Preliminary results show that this new assay can provide accurate estimates of cercariae from filtered-water samples, particularly if the sample is diluted to avoid reaction inhibition from soil and sediment contamination (Fig. 1).

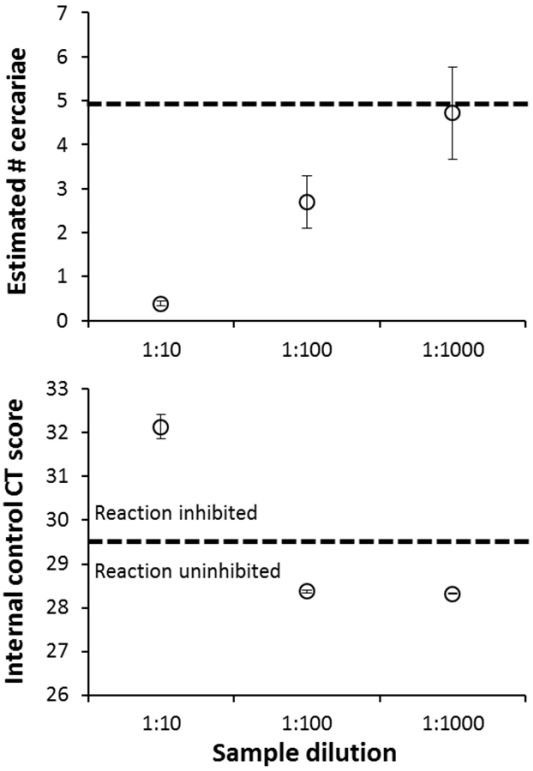

Fig. 1. Assay validation using 6 samples with known numbers of cercariae (5 cercariae per sample). The assay provided more accurate results when samples were diluted 1:1000, apparently due to reaction inhibition at higher sample concentrations. Reaction inhibition is measured using an internal positive control. Future work on this assay will focus on validating assay results for samples with different cercaria numbers, and determining how much variation there is between samples taken at the same time and place.

Proposed Methods

We will use a recently published quantitative PCR (qPCR, DNA-detection) assay to measure daily cercaria abundance in the water at each site [11]. This assay is specific to *Schistosoma* and *Trichobilharzia* spp. trematodes. Volunteers will collect daily filtered water samples over a 28 day period in July/August. Sampling involves collecting fifty one-liter samples of surface water using a standardized sampling protocol, allowing any sediment to settle, and pouring the water through a 35 um nitex filter. The sample is then rinsed into a sample tube with 70%

ethanol and stored for later analysis. Volunteers will also record daily wind speed and direction, the amount of wave action, the time spent sampling, and any birds observed during the sampling period. We will provide datasheets, a wind gauge and compass, buckets, and a filter to each volunteer. **Volunteers will need to find a pair of waders (let us know if you need help with this) and sign a liability release form.**

Before sampling begins, members of Dr. Raffel's team will (1) train and equip volunteers, (2) collect water samples for chemical analyses, (3) set up HOBO light/temperature loggers (2 depths per site) and six samplers for algal periphyton (i.e., snail food), and (4) survey the density and abundance of snails at each site. We will return at the end of the 28-day sampling period to obtain samples and loggers, collect and filter algal periphyton from samplers, and conduct another round of invertebrate surveys and water sample collection. Invertebrate surveys will use a standard visual encounter method with quadrat samplers and view buckets [5], which Maddie found to be highly effective. We will also conduct transect surveys perpendicular to the shoreline, and collect 50-100 snails to be preserved for measurement and identification in the lab.

We will quantify algal periphyton growth on samplers using a standard chlorophyll assay. At the beginning and end of the sampling period, we will measure pH and dissolved oxygen at each site, and collect water samples for water chemistry measurements. We will use standard methods to measure nutrients (Nitrogen and Phosphorus) and commercial ELISA assays (Abraxis Co.) to measure levels of triazine herbicides (e.g., Atrazine) and organophosphate insecticides (e.g., malathion).

Geographic region to be surveyed

For our 2016 survey, we are focusing on lakes in northern Michigan ("knuckles-up") including the Upper Peninsula. This is within the range of important host snail (*Stagnicola marginata*) and bird (e.g., *Mergus merganser*s) hosts of *Trichobilharzia stagnicola*, a common species that is especially problematic in beach habitats. We would like to sample a variety of shoreline types including beaches, developed property, and forest. We will consult local biologists and association leaders to select sites on each lake.

PRELIMINARY RESULTS

In summer 2015 M.S. student Maddie Messner surveyed avian schistosome abundance at 14 sites in 8 lakes in Michigan's Lower Peninsula. She pioneered the methods detailed above, overcame several challenges, and generated an extensive dataset that is already yielding interesting results.

Maddie's first challenge was to adapt the new qPCR method for use with field-collected samples, which were full of sand and debris. First, she needed to use much larger volumes of DNA-extraction buffer than in the past, which would be expensive with commercial extraction buffers. Second, she needed to minimize PCR inhibition from soil and sediment. Maddie tested several extraction procedures and selected one that uses abrasive sand and a protein-degrading enzyme to achieve high DNA yields. She then optimized the qPCR protocol to minimize reaction inhibition, and tested the accuracy and precision of the results using samples with known numbers of cercariae (Fig. 1). Accuracy was improved by diluting samples to reduce inhibition (Fig. 1). **I was pleasantly surprised by the accuracy of the new assay, relative to qPCR assays I've worked with in the past.**

In Dec 2015 and Jan 2016, Maddie processed over 400 DNA samples from the 2015 survey. She recently completed the first round of assays and is currently re-running samples

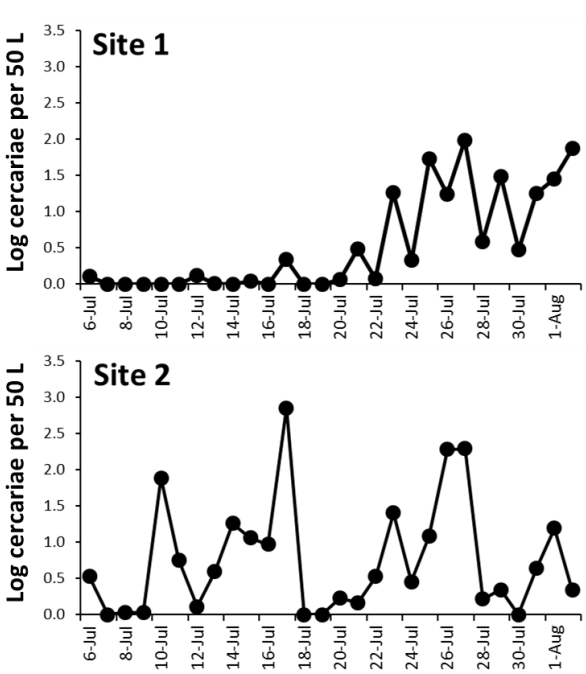

Fig. 2. Example time-series data on cercaria abundance collected by the Raffel lab and volunteers from two sites at Crystal Lake (in the Lower Peninsula), using a recently developed qPCR (DNA-detection) method.

with evidence of reaction inhibition. So far, she has found that cercaria abundance fluctuates widely from day to day, but with interesting patterns through time and highly consistent among-site differences (e.g., Fig. 2). No single environmental factor appears to account for among-site differences in cercaria abundance, but sites with high cercaria levels tended to have higher densities of *Stagnicola* sp. snails, higher bird visitation, and higher rates of algal periphyton growth. It seems clear that we need data from additional sites to allow robust statistical analyses of how environmental factors combine to influence the risk of swimmer's itch.

Another interesting pattern is that some sites had high cercaria abundance despite having very low snail densities. These sites were located hundreds of feet away from sites with higher snail densities. This result supports the idea that cercariae can drift into a beach from nearby beaches or deeper water. Based on this result, removing snails from a short stretch of beach might not be an effective control method unless drifting cercariae can also be controlled (e.g., via a floating barrier). To help account for cercaria drift at sampling sites, we plan to add a snail transect out into deeper water for the 2016 survey.

2016 SURVEY SCHEDULE

The primary daily survey will occur in July and August of 2016, with a goal of starting daily sampling on July 5 wherever possible. Participating lakes can expect an informal progress report in December 2016 and a more complete report (including data from your lake) by June 2017. We plan to present initial results of our survey work at the 2017 meeting of the Michigan Lake & Stream Association, and to publish any interesting findings in peer-reviewed journals. Estimated timing of various tasks is summarized below.

	2016								2017				
Task	M	J	J	A	S	O	N	D	J	F	M	A	M
Select sites	X	X											
Recruit & train volunteers	X	X											
Sample collection (SURVEYS)		**X**	**X**	**X**									
qPCR processing					X	X	X	X	X				
Water chemistry							X	X	X				
Statistical analyses								X	X	X	X	X	X
Report preparation								X					X

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