SWIMMER'S ITCH SOLUTIONS

MISIP Research Project:

Understanding How Snail Infection Levels Affect *q*PCR results

Final Report

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by

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SPECIALIZING IN EDUCATION AND CONTROL

Introduction

Snail infection level has traditionally been the most used and most reliable metric for assessing swimmer's itch control measures. Snail surveys effectively assess how much avian schistosome transmission to snails has occurred over the previous year, and are good predictors of, and correlate well with, the number of cercarial dermatitis cases. Another method, quantitative PCR (*q*PCR) has emerged as an additional tool to assess swimmer's itch levels by estimating the number of cercariae in a given volume of water or a given length of beach sampled by plankton tow. This offers advantages but also presents challenges: cercariae are shed from infected snails that are distributed in a non-uniform manner, and cercariae are planktonic, making them subject to currents and winds. Therefore, *q*PCR holds tremendous promise for spot sampling (i.e., in a specific location at a specific time of day), but its reliability as a lake-wide swimmer's itch control metric has yet to be tested. Progress was certainly made in 2017, but there appear to be several questions that still need to be addressed to continue to refine best practices.

Research questions to be addressed

- What is the temporal and spatial variation in cercarial counts in lake water samples as quantified by *q*PCR? If the same location with the same number of snails infected is measured over the course of several days, are the estimates consistent?
- How do *q*PCR estimates of cercariae compare to snail infection rates? How many infected snails have to be present in a given area to reliably detect cercariae? Can *q*PCR substitute for snail surveys?
- How does the relocation of common merganser broods affect the correlation between snail infection levels and qPCR estimates of cercarial densities?

Experimental design and methodology

- We have conducted our own *q*PCR studies, and we have also been in contact with Patrick Hannington about their *q*PCR efforts, and both groups have agreed to continue to share best practices and methodologies determined by this year's experimental *q*PCR work. We anticipate that the research of both groups will be improved by future communications and sharing of results.
- Snails were collected and water samples were taken simultaneously from four different Michigan lakes (Crystal Lake, Glen Lake, Higgins Lake, and Larks Lake)
    - 10 sites on Crystal Lake (July 12-13, 2018)
        - Total of 2112 snails and 40 water samples
    - 2 sites on Glen Lake (July 23, 2018)
        - Total of 457 snails and 8 water samples
    - 4 sites on Higgins Lake (July 19, 2018 and August 3, 2018)
        - Total of 460 snails and 12 water samples
    - 5 sites on Larks Lake (August 4, 2018)
        - Total of 842 snails and 16 water samples
- Avian schistosome levels were measured by microscopic examination of the snails and by *q*PCR analyses of the water samples. DNA extraction and *q*PCR analysis were conducted in the laboratory of Randy DeJong at Calvin College. Resulting avian schistosome levels are compared and statistically analyzed.

Results

For the purpose of making direct comparisons, a categorial swimmer's itch assessment index was generated based on snail infection level and *q*PCR analyses of water samples (Table 1). While these various levels and categories (ideal, tolerable, moderate, severe, epidemic) might seem arbitrary, they are based on several years of professional experience working on swimmer's itch on numerous lakes in the USA.

Site-specific comparisons of snail infection levels and *q*PCR analyses of water samples can be made at 10 locations on Crystal Lake (Figure 1), 2 locations on Glen Lake (Figure 2), 4 locations on Higgins Lake (Figure 3), and 5 locations on Larks Lake (Figure 4).

Conclusions

Visual inspection of the above maps reveals many sites where there is agreement between the two methods. Of eight sites with positive snails, seven were also found to be positive by qPCR. Three sites without infected snails were negative by qPCR, whereas only one site with infected snails was negative by qPCR. In addition, it is encouraging that some of the highest levels detected by qPCR were from sites with higher snail infection rates. There are ten sites where no infected snails were found, but qPCR detected cercariae in the water, usually at low levels, indicating that qPCR is overall more sensitive to detect the presence of swimmer's itch. These are not surprising results since cercariae are moved by wind and currents, as well as the fact that 25L of water is a relatively small sample.

The question then becomes whether the two measures are statistically correlated. A plot of the data shows the substantial variation in the data (Figure 5). In consultation with an excellent biological statistician (Stacy DeRuiter, Calvin College), the data were entered into a generalized linear model and the factors of lake, time of collection, and downwind status were examined for confounding effects. Of these, downwind was a significant factor, as predicted. Overall, the analysis found a weak, but significant, correlation between snail infection rates and qPCR. We stress here that though we believe the analysis valid, it is preliminary and the degree of correlation and statistical values may change as the most optimal analysis is reached and/or more data are added.

Table 1. A categorial swimmer's itch assessment index based on snail infection level or *q*PCR analyses of water samples.

Snail Infection Level	Category	*q*PCR (cercariae/25 L)
< 0.24%	**Ideal**	< 5
0.25-0.49%	Tolerable	5.0-9.9
0.50-0.99%	Moderate	10.0-29.9
1.00-1.99%	Severe	30.0-99.9
> 2.00%	**Epidemic**	> 100

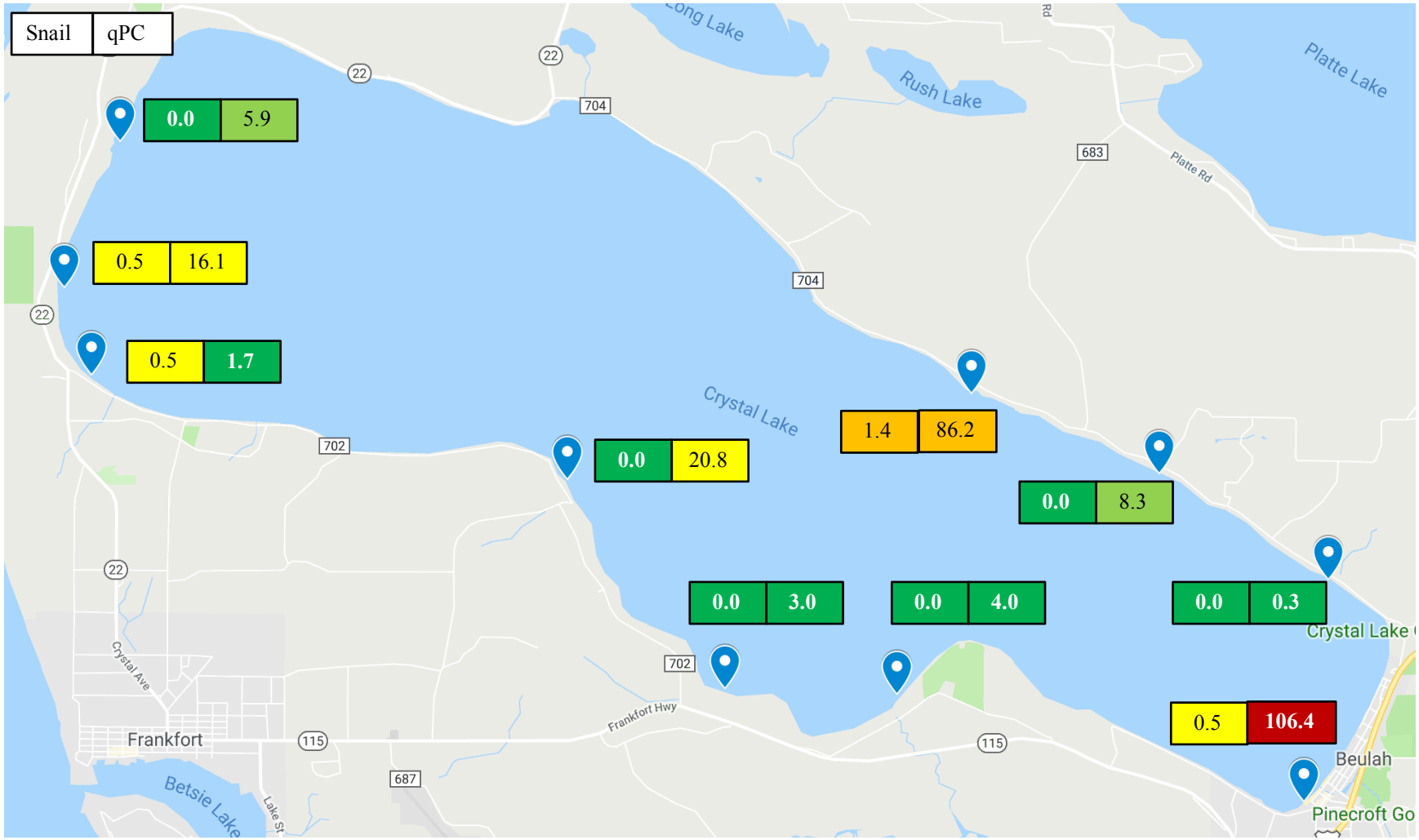

Figure 1. A comparison of avian schistosome snail infection levels (% infected) (boxes on left) and *q*PCR tests of water samples (# cercariae/25L) (boxes on right) at 10 locations on Crystal Lake (Benzie County, MI). All samples (2112 snails, 40 water) were collected on July 12-13, 2018.

Figure 2. A comparison of avian schistosome snail infection levels (% infected) (boxes on left) and *q*PCR tests of water samples (# cercariae/25L) (boxes on right) at 2 locations on Glen Lake (Leelanau County, MI). All samples (457 snails, 8 water) were collected on July 23, 2018.

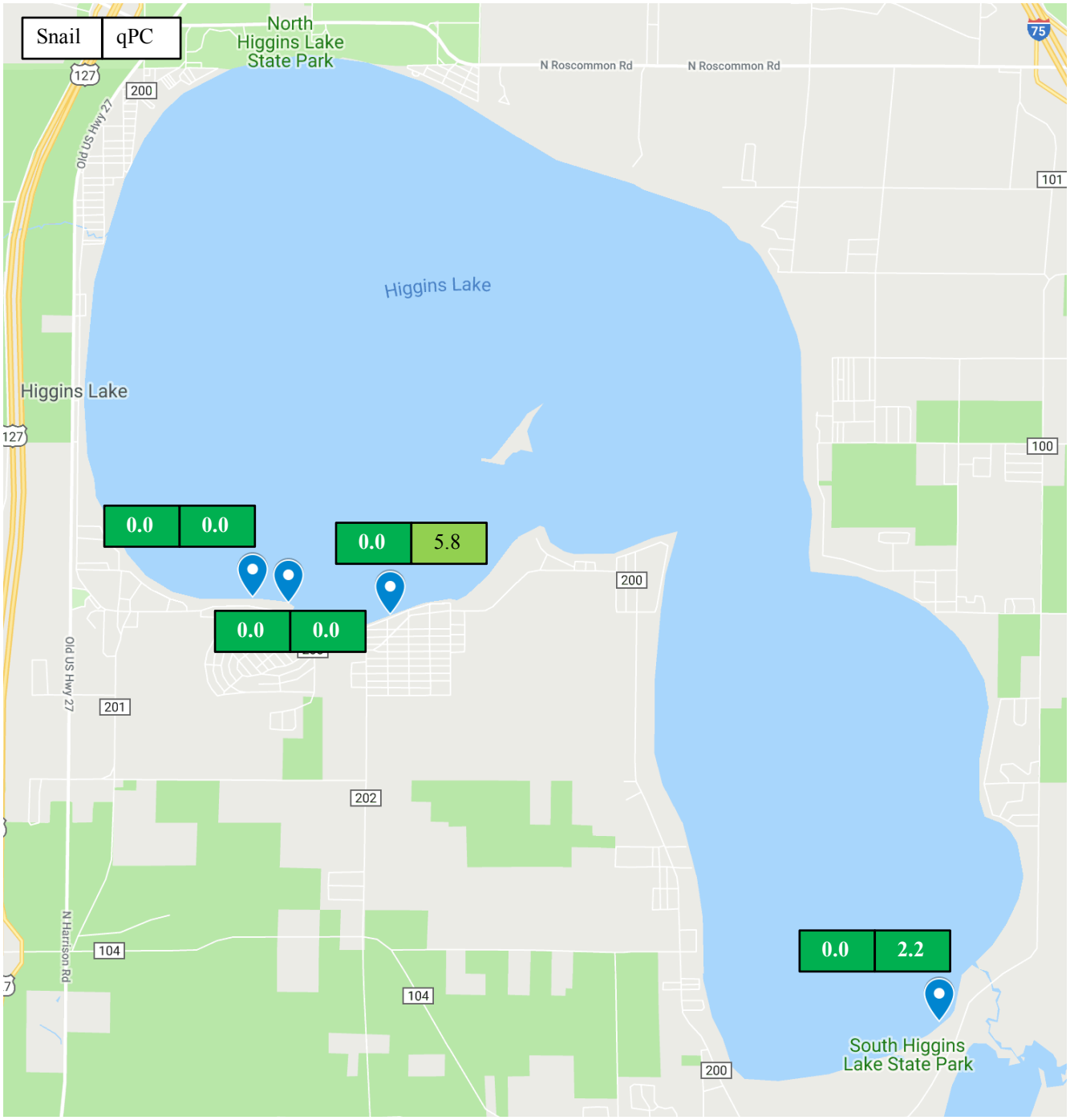

Figure 3. A comparison of avian schistosome snail infection levels (% infected) (boxes on left) and *q*PCR tests of water samples (# cercariae/25L) (boxes on right) at 4 locations on Higgins Lake (Roscommon County, MI). The two west-most samples (304 snails, 8 water) were collected on July 19, and the two east-most samples (156 snails, 4 water) were collected on August 3, 2018.

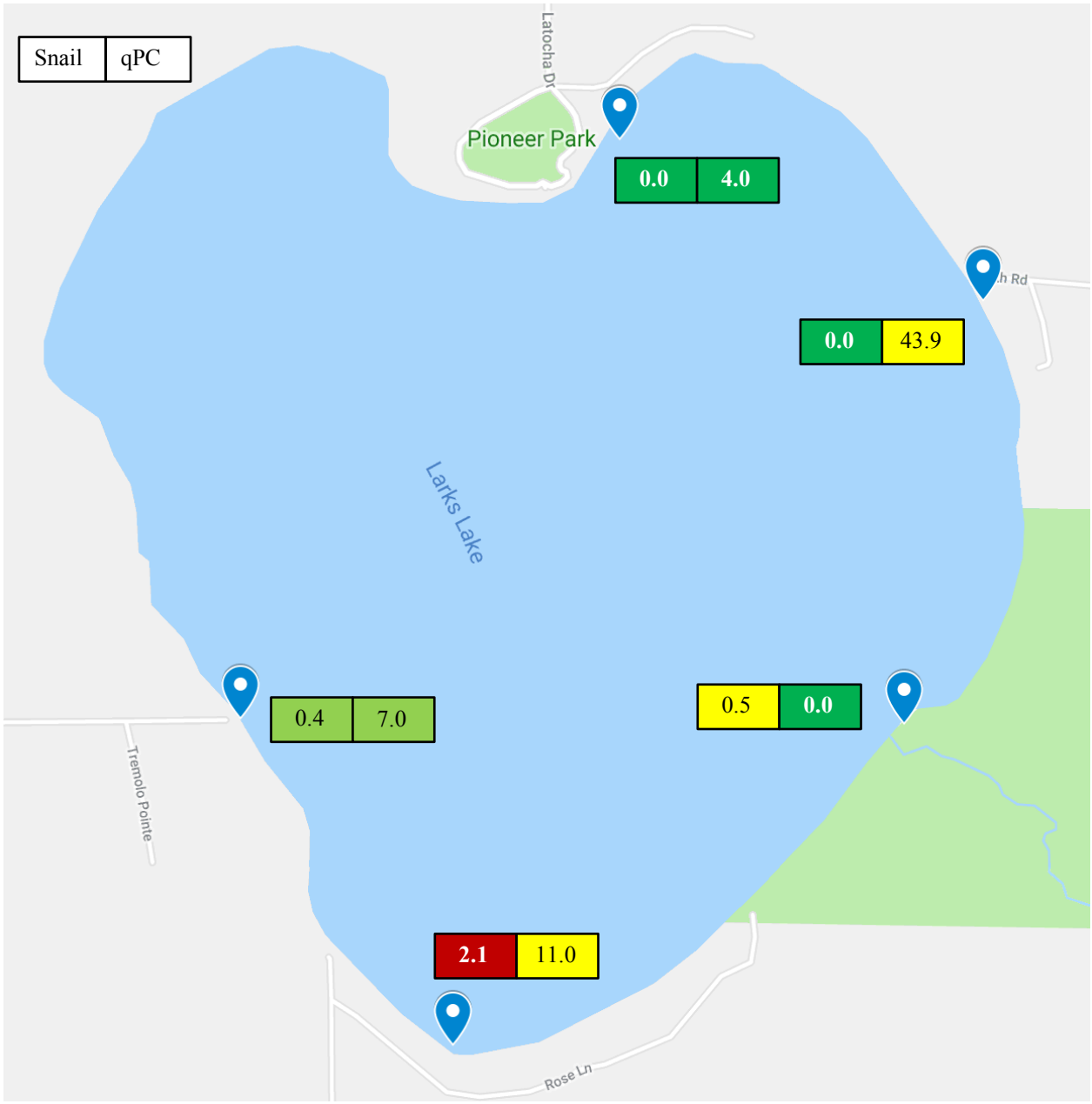

Figure 4. A comparison of avian schistosome snail infection levels (% infected) (boxes on left) and *q*PCR tests of water samples (# cercariae/25L) (boxes on right) at 5 locations on Larks Lake (Emmet County, MI). All samples (842 snails, 16 water) were collected on August 4, 2018.

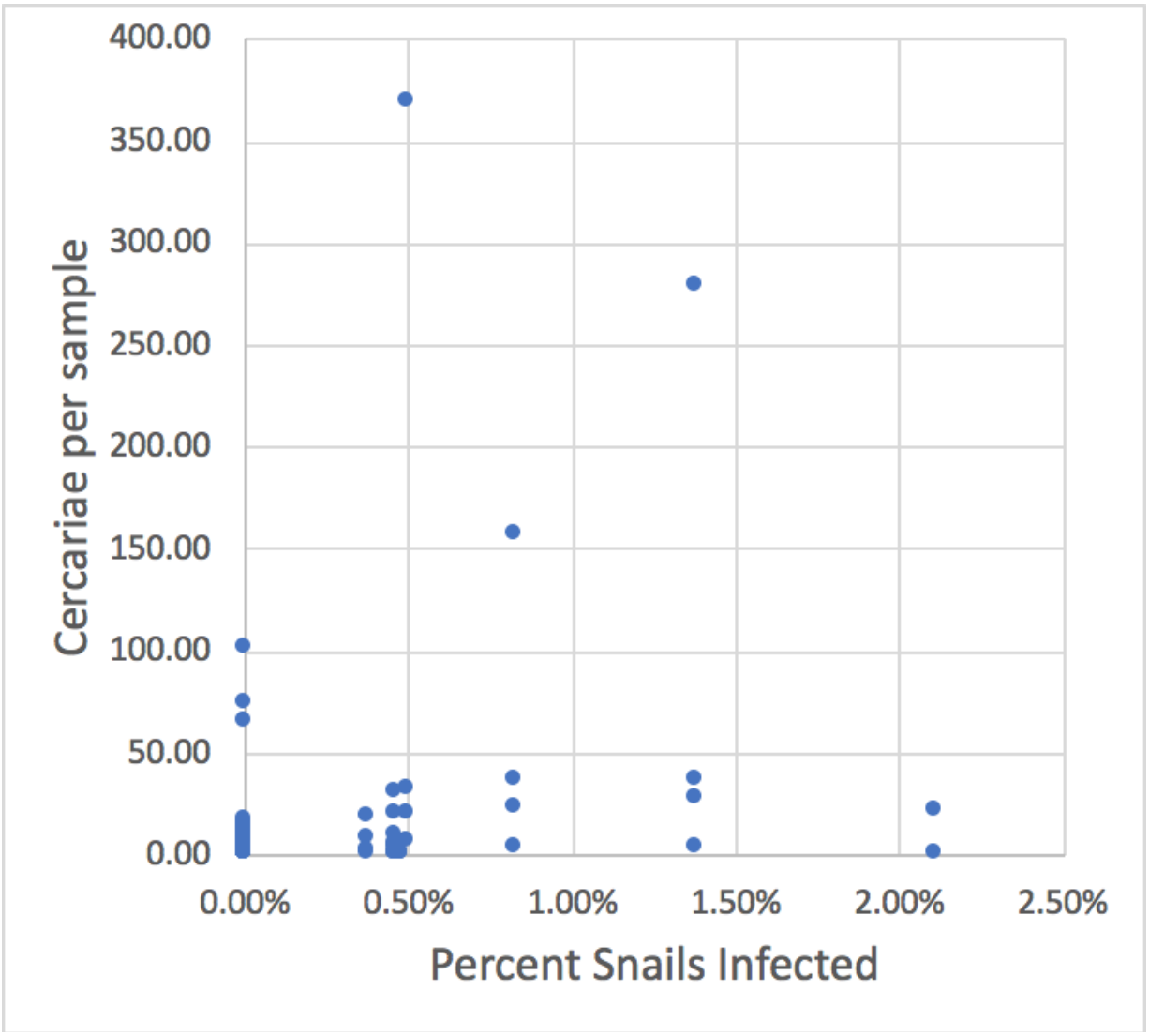

Figure 5. Relationship between two swimmer's itch metrics: Snail infection level (% infected) and *q*PCR analysis of water samples (number of avian schistosome cercariae/25L). Data collected July 12-August 4, 2018 at 21 unique locations on four Michigan lakes (Crystal, Glen, Higgins, and Larks).