Examining zebra mussel and crayfish effects on swimmer's itch, a snail-borne parasitic disease

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Abstract

Swimmer's itch is caused by avian schistosomes, snail-borne parasites that normally use birds as definitive hosts but sometimes try to infect humans. Although it is clear that higher densities of waterfowl and snail hosts lead to increased swimmer's itch incidence, the effects of other ecological variables on these parasites are less well understood. Preliminary data collected by the Raffel lab in 2015 suggested links between urbanization and swimmer's itch in northern MI lakes, apparently mediated by effects of increased water clarity and growth of attached algae (i.e., snail food) on snail populations. Urbanization might lead to (1) increased introductions of invasive species like zebra mussels, which increase water clarity, and (2) insecticide runoff leading to declines in crayfish, the most important invertebrate predators of snails and mussels. My project investigated relationships between abundances of zebra mussels, crayfish, snails, and avian schistosomes in MI lakes as well as environmental and habitat data, such as water temperature, algae, and substrate type, as part of a large-scale survey effort being conducted by the Raffel lab in 2016. Our findings will help to determine the causes of swimmer's itch in northern MI lakes and inform future management efforts, so perhaps one day our kids will no longer have to worry about it.

Current Research

Avian schistosomes are caused by snail-borne trematode parasites in the genus *Trichobilharzia*, closely related to the parasites that cause human schistosomiasis (Horak et al., 2015). Avian schistosomes generally use birds as definitive hosts and cannot complete their life cycles in mammals (Blankespoor & Reimink, 1991). Several snail species act as intermediate hosts for these parasites. Infected snails release motile infective stages called cercariae into the water, and if these find a bird they will infect it by penetrating its skin (Horak et al., 2015). However, cercariae sometimes mistake humans for birds and try to penetrate our skin, leading to a massive immune response in the skin that often kills the parasite and leaves behind a nasty, itchy rash (Horak et al., 2015). Several factors are known to increase the risk of swimmer's itch, including high snail densities, high bird visitation, and warm temperatures (Horak et al., 2015).

To our knowledge, no prior studies have examined potential correlations between invasive zebra (*Dreissena polymorpha*) or quagga (*Dreissena bugensis*) mussels and cercarial dermatitis (swimmer's itch). However, zebra mussels are known to increase water clarity (decrease turbidity) by consuming phytoplankton, which are algae and bacteria that grow in the water column (Kirsch & Dzialowski, 2012). These changes in water turbidity are mostly seen in shallow ponds and lakes (MacIsaac, H. J., 1996). The pattern of mussels decreasing turbidity is supported by research on the Saginaw Bay among others. In the bay, the turbidity was 9.2 nephelometric turbidity units (NTU) in 1991 (MacIsaac, H. J., 1996). In the span of a year, it decreased to 8.3 NTU and after another, down to 3.7 NTU (MacIsaac, H. J., 1996). Turbidity has also been shown to have an inverse relationship with mussel respiration rate (Alexander Jr, J. E., Thorp, J. H. & Fell, R. D., 1994). This increased water clarity can allow more light to penetrate to the bottom of the lake, thereby increasing growth of periphyton (attached algae) that serve as

food for snails (Rohr et al., 2008). Zebra mussels can also increase nutrient (mostly phosphorus and ammonia) concentrations in water (Wojtal-Frankiewicz, 2011; Lindim, 2015) and change the species composition of phytoplankton (Baker et al., 1998), with unknown effects on growth rates of attached algae and snail populations.

If zebra mussels cause increased water clarity by consuming phytoplankton (floating algae), this should logically result in increased growth of attached algae (periphyton) and thus increased snail densities. This could lead to increased incidence of swimmer's itch where there is a greater abundance of zebra mussels. However, this potential relationship between zebra mussels and phytoplankton (floating algae) is complicated by the fact that zebra mussels rely on phytoplankton as a food source, such that phytoplankton biomass is sometimes a positive predictor of zebra mussel occurrence (MacIsaac, H. J., 1996). This leads to two possible relationships between zebra mussels and water turbidity in natural lakes. It is possible that zebra mussel abundance might have a positive correlation with phytoplankton levels and water turbidity, if zebra mussel abundance is limited by phytoplankton abundance. Conversely, we might observe a negative correlation between mussels and turbidity if zebra mussels are limited by other factors, leading to reduced phytoplankton abundance in lakes with more mussels.

Another environmental factor that can limit mussel abundance is the availability of calcium for growing shells. Zebra mussels grew best under controlled lab conditions when the water had over 8.5 mg calcium per liter, an alkalinity level of over 65 mg $CaCO_3$ per liter, and water hardness over 100 mg of $CaCO_3$ per liter (Hincks & Mackie 1997). Further, negative growth was noted with levels of calcium less than 8.5 mg per liter, alkalinity less than 17.1 mg $CaCO_3$ per liter, and water hardness less than 31 mg $CaCO_3$ per liter (Hincks, S. S. & Mackie, G. L., 1997). These variables were able to explain 60-66% of the variation that was seen in shell

length of the zebra mussels (Hincks, S. S. & Mackie, G. L., 1997). Later, alkalinity was used as a predictor variable for zebra mussels by Whittier et al. (2008). It was found that zebra mussel occurrences mostly happened in areas with a mean calcium level of at least 28 mg per liter and a 25^{th} percentile of greater than 12 mg per liter (Whittier et al., 2008). Lastly, only 2 lakes with a low alkalinity (12 mg per liter $\leq 75^{th}$ percentile < 20 mg per liter or 75^{th} percentile < 21 mg per liter and a maximum of 28 mg per liter) were reported to have zebra mussel invasions, one of which was Glen Lake, one of the 16 in our survey (Whittier et al., 2008). Therefore, alkalinity is often positively correlated with mussel occurrence and is likely that these variables will differ throughout the lakes in the survey.

Quagga mussels are a second species of invasive mussel also found in northern Michigan. Quaggas are more adapted to living in colder climates than zebra mussels (Whittier et al., 2008). Although quagga mussels do not spread as quickly as the zebra mussel, they are competitively dominant and are expected to eventually displace zebra mussels in Michigan lakes where both occur (Whittier et al., 2008). However, much less research has been done on the quagga mussel and knowledge is currently limited (Whittier et al., 2008). For example, it is not known if their environmental requirements are similar to zebra mussels, though quagga mussel shells are thinner than zebra mussel shells so they might tolerate lower calcium levels (Whittier et al., 2008). For the sake of this study, we will assume zebra and quagga mussels are similar enough ecologically to include both in among-site analyses of mussel abundance.

Crayfish are important predators on both zebra mussels and snails (Czarnoleski et al., 2011), so anything that influences crayfish populations could have indirect effects on mussel densities, snail densities, and swimmer's itch incidence (Halstead et al., 2015). Crayfish can also have non-lethal effects on zebra mussel filtration rates (Czarnoleski et al., 2011), which could

subsequently affect water clarity. Naddafi et al. found in 2007 that predatory cues from crayfish caused negative effects on the clearance rate of phytoplankton. This then has an indirect positive effect on phytoplankton due to the mussels slowing the intake (Naddafi et al, 2007). This again leads to multiple possible outcomes with different predictions. Snails and mussels provide food for crayfish, so one might expect high snail and mussel densities to support greater populations of crayfish. This would lead to positive correlations between crayfish, snails, and swimmer's itch. Alternatively, high densities of crayfish predators might reduce populations of zebra mussels and snails, resulting in negative correlations between crayfish and snails (Halstead et al., 2015). The latter possibility is more likely if crayfish densities are limited by factors other than food abundance. For example, crayfish are known to be highly sensitive to insecticides from urban and agricultural runoff, and prior studies have found that even low levels of insecticide can result in increased snail productions due to release from crayfish predation (Halstead et al., 2015). It will thus be interesting to see whether and how snail and zebra mussel abundance correlate with crayfish abundance in the lakes being studied.

The central purpose of the study was to test for potential effects of zebra mussels and crayfish on avian schistosomes and their snail intermediate hosts in northern MI lakes. In collaboration with other members of the Raffel lab, I measured these factors in 38 sites across northern MI. I examined potential predictors of mussel abundance, water clarity, growth of attached algae, and snail abundance, to test for hypothesized effects of (1) water quality on mussel abundance, (2) mussels on water clarity and periphyton growth rates, (3) crayfish on populations of zebra mussels and snails, and (4) water clarity and periphyton on populations of snail intermediate hosts for swimmer's itch parasites. I also compared and contrasted quadrat sampling versus allowing mussels to settle on an artificial substrate, as alternative ways to

measure zebra mussel abundance in northern Michigan lakes.

Methodology

My project was part of a larger effort by the Raffel lab to survey potential ecological drivers of avian schistosome parasites on 38 sites on 16 lakes in northern Michigan. A table of all the lakes, site identification codes, and latitude and longitude coordinates can be found in Table 1. Also, a map is provided in figure 1. Water temperature and light were measured continuously using data loggers using HOBO pendant data loggers. We conducted weekly surveys of snail densities at each site, in addition to growth of attached algae and water chemistry variables. I also helped conduct laboratory analysis of samples (snails, zooplankton, algae, water chemistry). The schedule of this can be seen in table 2 and 3.

At each site, I conducted two crayfish trapping sessions spaced two weeks apart. For each trapping session, I set three traps for a day each, for a total of six trapping nights at each site. Traps were baited with tuna using devices made from tea diffusers and string, positioning the bait in the middle of the trap. I used two crayfish traps (2-inch diameter opening) and one minnow trap (1-inch diameter opening) on each sampling occasion to obtain data on both large and small crayfish. This can be seen in figure 3. All crayfish caught were documented with photographs.

Zebra mussel settling rates were measured by placing two samplers at each site in July and leaving them undisturbed through the end of September, when new mussels settle and attach onto available substrates (Mackie et al., 1989). I suspended two samplers in the water column at each site, either hanging from an existing dock structure or from a buoy. Zebra mussel samplers were based on a published design (Monitoring Protocol, 2014) and comprised of a stacked array

of three roughened PVC plastic sheets. The dimensions of the sheets were 15 cm by 15 cm, 20 cm by 20 cm and 22.5 cm by 22.5 cm. This gives a total of 2,262.5 square cm of surface area for the zebra mussels to settle on per sampler. There was a 1 inch PVC pipe spacer to separate the three layers and they were all connected by a 6-inch bolt and wing nut. After being collected, the samplers were disassembled and scraped free of zebra mussels, which were preserved in 70% ethanol for analysis of wet mass and approximate counts. To determine the approximate number of mussels on each sampler, I massed 10 randomly selected mussels from each sampler. I then divided the total mussel biomass per sampler by the mean mass per mussel to estimate the total number of mussels per site. Before and after pictures of the samplers are provided in figure 2.

In addition, 3 times (week 1, week 3, and week 5 of the surveys), at every site, we conducted a quadrat survey of the substrate at each site to obtain estimates of snail and zebra mussel densities. Quadrat samplers consisted of a PVC square (one square foot) separated into 9 visual sections by string. Two strings ran vertically and two ran horizontally giving a 3 by 3 grid. This can be seen in figure 5. For each survey, we tossed random samplings of the lake bottom at three different water levels. We threw the quadrat sampler to four haphazard locations within each water depth category (0-20 cm, 20-40 cm, 40-60 cm) and used a view bucket (Fig. 4) to locate and count the snails and mussels in each quadrat. If there were too many to count, we only counted the four corners of each quadrat to obtain density estimates. Densities were recorded for both the snails and mussels and were identified to the genus level. Any other organisms, such as crayfish, were noted if encountered in quadrat sampling. Lastly, the snails were collected and preserved in 70% ethanol.

For all components of the site assessment, including cobble, a numerical score was used to indicate abundance of landscape or substrate types based on the following numeric index: 0 =

Absent, 1 = Sparse (< 10% coverage), 2 = Moderate (10−40% coverage), 3 = Heavy (40−75% coverage), and 4 = Very Heavy (> 75% coverage).

Zooplankton was also sampled at the sites on three occasions. A standard 8-inch zooplankton net with a 5-meter string attached was used. Sampling consisted of three horizontal drags. The samples were preserved in Lugul's solution. The three most abundant taxa (Copepoda, Cladocera, and Ostracoda) were recorded and notes were taken if other rare taxa were found.

Turbidity Protocol

Collect a sample in a clean container. Fill sample cell (glass vials) to the line, approximately 15 mL, then cap the cell. Handle the cell by the top to avoid getting fingerprints on the sides of the cell. Wipe the outside of the cell with a Kim wipe to remove any fingerprints or water spots. Apply a thin film of silicone oil around the outside of the cell. Wipe with a soft cloth to obtain an even film over the entire surface.Make sure the instrument is on a flat, sturdy surface then press the I/O button to turn it on. Do not hold the instrument while it's taking readings. Insert sample cell into the instrument cell so that the triangle on the sample cell is facing toward you. Close the lid. Select manual or automatic range selection by pressing the RANGE key. The display will show AUTO RNG when the instrument is in automatic range selection. Select signal averaging mode by pressing the SIGNAL AVERAGE key. The display will show SIG AVG when the instrument is using signal averaging. Use signal average mode if the sample causes a noisy signal (display changes constantly). Press READ. The display will show - - - - NTU, then the turbidity in NTU. Record the turbidity after the lamp symbol turns off.

This protocol was followed at every site and was recorded once a week as shown in table 2 and 3. A Hach portable turbidimeter was used to take samples and therefore the Hach protocol

was given and followed.

Chlorophyll A Assay Protocol

Periphyton Tiles Placement

Obtain 3 clean plexiglass tiles (10 x 10 cm) that have been secured to float and poly rope. Tie each periphyton sampler to cinder block or sufficient anchor. Each tile should be tied so that there are 30 cm of poly rope between the tile/float and the anchor. Walk out to 60 cm depth and place samplers in a triangle formation leaving at least 2 meters between each anchor. Make sure that they are all 30 cm from the water surface. Adjust the sampler so that the tile is oriented upward towards the water surface and the float is underneath the tile.

Periphyton Tile Collection (end of survey)

Use a meter stick to measure and record the final depth of each tile (write the depth on the field sheet). Remove the tile from the noodle float and place it into its corresponding Ziploc bag. Avoid touching the top of the tile. Store bagged tile in cooler on ice. Process periphyton tiles the same day as collection.

Periphyton Tile Processing and Filtration

Set up vacuum filtration apparatus. Keep tiles on ice until they are processed. Using forceps and a toothbrush - hold the tile still in the pan and brush the surface of the tile, removing periphyton and allowing it to collect in the pan. Use a squirt bottle (tap water) to rinse the tile and the toothbrush, keeping all liquid in the pan. Use vacuum pump and filter tower set up to concentrate the sample onto a Whatman GF/F glass microfiber filter (0.7 μm; Whatman Inc., Kent, U.K.). Be sure to thoroughly rinse the pan onto the top of the filter paper. If filtering in the field, keep foil squares in waterproof bag on ice and get them to a freezer within 6 hours.

Methanol Extraction for Processing Periphyton Filters

Filters will be folded and labeled in tin foil in freezer at -20 °C until fluorometric analysis. Make up 90% Methanol solution (10% water). Place each filter in its labeled tube. Add exactly 5 mL of methanol to each tube. Cap all tubes and place in fridge for a 24 hour period topromote algal cell lysis following a modified version of the EPA method 445.0.

Loading microplate for reading Chlorophyll A on Fluorometer

Centrifuge samples for 5 minutes (1500 rpm) to remove any suspended particles from the sample liquid. Pipette 200 µL of each sample. Fluorometric analysis (Synergy H1 microplate reader, Biotek, Winooski, VT, USA) was used to determine chlorophyll A in relative fluorescence units; fluorescence (emission) was recorded at the 680 nm detection wavelength using an excitation wavelength of 440 nm. We calculated the average fluorescence for the three tiles to obtain an index of periphyton growth potential at each site.

Alkalinity Protocol

Fill plastic tube full (to the top) with sample water. Pour the contents of the tube into the square mixing bottle. Add the contents of one Phenolphthalein Indicator Powder Pillow to the mixing bottle. Swirl to mix. If the water remains colorless, the phenolphthalein alkalinity is zero. In this case, proceed to Step 7. If the sample turns pink, add Sulfuric Acid Standard Solution one drop at a time. Count each drop. Swirl the mixing bottle after each drop is added. Add drops until the sample turns colorless. Multiply by 20 the number of drops of titrant used. This is the mg/L of phenolphthalein alkalinity as calcium carbonate (CaCO3). mg/L CaCO3 phenolphthalein alkalinity = number of drops x 20. (Step 7) Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the mixing bottle. Swirl to mix. Add Sulfuric Acid Standard Solution one drop at a time. Count each drop. Swirl the mixing bottle after each drop is added. Add drops until the sample turns pink. Multiply by 20 the total number of drops of titrant used in

both steps 5 and 9. This is the total mg/L of methyl orange alkalinity as calcium carbonate (CaCO3).

Alkalinity was only measured the first and final week (week 1 and 5) at each site. This gave measurements for the beginning and end of the survey. This protocol is taken from the Hach alkalinity test kit manual, seeing as this was the manufacturer that we used.

Statistical Analyses

Once all the data was compiled by late Fall 2016, correlation analysis, multiple linear regression, and structural equation modeling were used to test for relationships between variables. Prior to among-site analyses, we averaged the measurements of each predictor or response variable for each site. Count data and turbidity measurements have highly skewed distributions, so we log-transformed these variables ($\log_{10}[N+1]$ for counts) prior to calculating their averages. We next did an exploratory analysis testing for Pearson correlations between all pairwise combinations of variables measured. For each response variable of interest (snail abundance, periphyton growth, turbidity, crayfish abundance, mussels per quadrat, and mussels per sampler), we followed up on potentially explanatory ($r > 0.3$) predictor variables using multiple linear regression (function "lm" in program R). These predictors and responses are compiled into a chart in table 4. The correlations coefficients greater than +/- 0.3 are highlighted. Contributions of predictors to each model were assessed using F-tests with a Type II sums of squares procedure (function "Anova" in the "car" package). Non-significant predictors ($P > 0.05$) predictors were removed sequentially from each model via backwards selection. Program R was used for all statistical analyses (R Project Core Team, 2016). This protocol is similar to the protocol used by Alex Bageris to analyze his Honors Thesis dataset, which included several of the same variables compiled by the Raffel lab during this survey.

Results

Since there were two types of zebra mussel measurement, the quadrats and the samplers, it was interesting to see if they would provide similar data for the lakes. This ended up not being the case. For the mussel quadrats (Fig. 6), gravel ($F_{1,36}$=16.718, p value < 0.001) and cobble were significant, but no other variables such as alkalinity or crayfish abundance. However, with the mussel samplers, we do not see this correlation. It was found that the undersides of the samplers were colonized first, but when those were full, the top side would then be filled (Fig. 2). The best predictor of mussel biomass on samplers was alkalinity, which was a highly significant predictor ($F_{1,27}$ = 7.867, P = 0.009; Fig. 8). Another predictor that had a nearly significant relationship with mussel biomass, after accounting for alkalinity, was water turbidity ($F_{1,27}$ = 4.176, P = 0.051; Fig. 8).

Water turbidity was also the best predictor of snail abundance ($F_{1,35}$ = 12.101, P < 0.001). It had a negative slope, meaning the more turbid the water, the fewer snails. Snail abundance also increased with our index of cobble substrate ($F_{1,35}$=5.821, P = 0.021).

The best predictor of crayfish abundance was our index of sand substrate ($F_{1,36}$=4.850, P = 0.034), with more sandy substrate correlating with fewer crayfish (Fig. 9). Where there were more crayfish, the mass of the mussels was decreased ($F_{1,17}$ = 4.687, P = 0.045). Mussel size was also negatively affected by the density of mussels on the sampler ($F_{1,17}$=7.012, p value = 0.017) (Fig. 10).

The two most significant predictors of water turbidity were mean daily temperature ($F_{1,26}$=19.547, p value < 0.001) and mussel biomass on samplers ($F_{1,26}$=13.970, p value < 0.001; Fig. 11). Another predictor was log mussels per sampler. Lastly periphyton, measured by relative fluorescence units (RFU) of chlorophyll, was examined to see periphyton growth on surfaces.

The best predictor of periphyton growth was the alkalinity of the water ($F_{1,36} = 5.641$, $P = 0.023$; Fig. 12).

Discussion

The primary objective of this section of the research study was to determine whether zebra mussels and crayfish are important factors driving swimmer's itch incidence in northern MI lakes. Based on our hypotheses, we predicted that sites with greater zebra mussel density would increase water clarity, resulting in greater abundances of periphyton (attached algae) and snails. Some of these predictions were supported by our results. The mussels did in fact increase water clarity. This is probably because mussels filter algae out of the water, decreasing turbidity and making the water clearer. However, the predicted effect of water clarity on periphyton was not observed, and periphyton growth was not the best predictor of snail abundance. Thus, we were unable to confirm that periphyton growth rates were the primary factor linking zebra mussels and snails. One possible explanation of this lack of pattern is that our periphyton measurements were all conducted in shallow water, and that snail populations are more limited by light-limited growth of periphyton out in deeper water.

Our findings suggest a potential link between increased mussel abundance and snail abundance, which would likely lead to an increased abundance of avian schistosome cercariae in the water. Our data therefore leads us to believe that an increased swimmer's itch risk is possibly increased by zebra mussel invasion. Our results also suggest a potential role for crayfish in moderating zebra mussel impacts on water clarity. We found a negative effect of crayfish abundance on the size of mussels on samplers, consistent with prior studies that showed reduced water filtration by zebra mussels in the presence of crayfish.

The strong correlation between local mussel abundance (quadrat samples) and our index of gravel substrate suggests that substrate availability is the major regulating factor for mussel abundance at local sites. This is a density dependent factor that might set the carrying capacity of mussels in a given site. If there are more mussels, it leads to less space, and therefore mussel production will begin to cease because there is no longer an area for them to attach. In support of this idea, we also found a negative effect of mussel density on individual mussel mass on samplers (Fig 10). However, when we provided a substrate for the mussels to colonize (controlling for substrate availability), colonization rates by juvenile mussels appear to have been driven primarily by water alkalinity. As mentioned before, a minimum alkalinity is needed for shell growth of the mussels. The samplers therefore instead show the rate at which mussels colonize the substrate, rather than measuring the carrying capacity of mussels at a particular site.

Zebra and quagga mussels are already considered problematic invasive species in Michigan lakes. Our results have a few implications for organizations interested in monitoring and controlling zebra and quagga mussels. First, it seems clear that different methods of surveying mussel abundance measure different aspects of zebra mussel population dynamics. The quadrat sampling provided a fast, reliable way to measure local densities; however, these numbers are not likely to be representative of the entire lake. The samplers might be better for measuring lake-wide zebra mussel abundance and provide a measurement of the potential for rapid mussel colonization. Both samplers and quadrats provide useful information that can help provide a complete picture of zebra mussel abundance and population dynamics in each lake.

Snail location was not one of the factors studied in depth prior to conducting the study. However, after further research on the topic, snails were more likely to be found in areas where there was little cobble, due to predation factors (Kreps et al., 2012). Crayfish are known for

liking more rocky areas because it provides them shelter (Kreps et al., 2012). Therefore, this is where the crayfish density is the highest (Kreps et al., 2012). Our data did show that crayfish were less abundant in sandy areas, consistent with prior findings that crayfish prefer rocky substrates. However, we found more snails in the areas with more cobble substrate, even though those are the same areas where crayfish are likely to be found. We are uncertain what might have driven this pattern, but this result suggests that crayfish are not a strong determinant of snail densities in these lakes.

Although mussel density was never a predictor for crayfish, crayfish were instead a predictor for individual mussel mass. This is likely because when there is a risk of predation, the mussels feed less so they can keep their shells closed as an anti-predator defense. With reduced feeding rates, the mussels would not grow as rapidly and have a smaller mass to those compared to areas where the abundance of crayfish was smaller.

Lastly, since turbidity played such a significant role in predicting snails and mussels, we examined factors influencing turbidity in addition to mussels. One of the most significant predictors was mean daily temperature, with higher temperatures leading to increased turbidity (Fig. 11). One possibility is that higher temperatures led to a faster rate of chemical herbicide breakdown. Another possibility is that water temperatures simply facilitate faster growth of periphyton in the water, leading to increased turbidity.

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Table 1. Table compiling all sampling sites. Gives lake, our unique site identification code, as well as both latitude and longitude coordinates.

Lake	Site ID	GPS Coordinates
Crystal	CA	44.66502, -86.24453
Crystal	CC	44.68975, -86.20743
Crystal	OH	44.64629, -86.09312
Crystal	ON	44.63804, -86.16975
Deer	DS	45.17076, -84.97124
Douglas	BS	45.56009, -84.67489
Douglas	BW	45.58848, -84.72621
Elk	BK	44.88647, -85.36201
Glen	DO	44.85531, -86.01307
Glen	KA	44.89071, -85.95938
Glen	ME	44.86815, -85.92953
Hamlin	JD	44.06929, -86.41986
Hamlin	MB	44.02759, -86.45231
Hamlin	PP	44.05144, -86.45583
Hamlin	PR	44.01614, -86.47054
Higgins	DH	44.43672, -84.70445
Higgins	GT	44.49592, -84.69906
Higgins	KB	44.46567, -84.68034
Higgins	SS	4.464110, -84.74430
Intermediate	JG	45.02279, -85.23637
Intermediate	TP	45.06957, -85.26011
Leelanau	NF	45.04364, -85.72026
Leelanau	PS	45.00279, -85.77054
Lime	MA	44.89570, -85.84868
Little Traverse	RC	44.92495, -85.85420
Margarethe	DL	44.65653, -84.78121
Margarethe	LB	44.63559, -84.79324
Margarethe	SD	44.62734, -84.78608
Margarethe	SF	44.66086, -84.81658
Platte	BB	44.69307, -86.07571
Platte	IN	44.67515, -86.07893
Platte	RA	44.69484, -86.11979
Portage	NP	44.36551, -86.23814
Portage	VP	44.36241, -86.20688
Skegemog	KG	44.80942, -85.34577
Walloon	RK	45.32866, -85.04487
Walloon	W2	45.26464, -85.00223
Walloon	W3	45.30802, -84.98653

Table 2. Compilation of weekly schedule for the west sites.

SI SURVEY 2016- WEST SITE

	1 June 28-July1	2 July 5-8	3 July 12-15	4 July 19-22	5 July 26-29
Snail Survey	Hamlin-4 Portage-2 Crystal-4 Platte-3 Glen-3 Lime-1 LT-1 Lee-2	Hamlin-1 Crystal-2 Platte-1 Glen-1 Lee-1	Hamlin-4 Portage-2 Crystal-4 Platte-3 Glen-3 Lime-1 LT-1 Lee-2	Hamlin-1 Crystal-2 Platte-1 Glen-1 Lee-1	Hamlin-4 Portage-2 Crystal-4 Platte-3 Glen-3 Lime-1 LT-1 Lee-2
Crayfish		✓			✓
D/O	✓	✓	✓	✓	✓
Turbidity	✓	✓	✓	✓	✓
Zooplankton	✓		✓		✓
Alkalinity	✓				✓
Water Sample	✓				✓
Periphyton	SET			COLLECT	

Table 3. Compilation of weekly schedule for the east sites.

SI SURVEY 2016- EAST SITE

	1 June 28-July1	2 July 5-8	3 July 12-15	4 July 19-22	5 July 26-29
Snail Survey	Higgins-4 Margrethe-4 Elk/Ske-2 Intmdt-2 Walloon-3 Douglas-2 Deer - 1	Higgins-1 Margrethe-1 Elk-1 Intmdt-1 Walloon-1 Douglas-1	Higgins-4 Margrethe-4 Elk/Ske-2 Intmdt-2 Walloon-3 Douglas-2 Deer - 1	Higgins-1 Margrethe-1 Elk-1 Intmdt-1 Walloon-1 Douglas-1	Higgins-4 Margrethe-4 Elk/Ske-2 Intmdt-2 Walloon-3 Douglas-2 Deer - 1
Crayfish	✓			✓	
D/O	✓	✓	✓	✓	✓
Turbidity	✓	✓	✓	✓	✓
Zooplankton	✓		✓		✓
Alkalinity	✓				✓
Water Sample	✓				✓
Periphyton	SET			COLLECT	

Table 4. Chart of predictors and responses. Correlation coefficients r>0.3 are highlighted.

		Responses						
		LogCrayfish	LogTurbidity	LogSnails	Periphyton Growth (RFU)	LogMusselsQuatrats	LogMusselsSamplers	MassPerMussel
Predictors	MeanDayTemp	-0.0487291	0.44990566	-0.1020221	0.1081073	-0.1653605	-0.0158192	0.26128643
	MeanNightTemp	0.16852952	0.42372534	-0.1977547	0.20689467	-0.0803236	0.21727071	0.18663794
	MeanTemp	0.05068927	0.53367504	-0.1884645	0.21352686	-0.1434262	0.12706522	0.21604692
	Crayfish	0.92204317	0.30604207	-0.0779797	0.21846261	0.13637468	0.05559546	-0.4734733
	LogCrayfish	1	0.17204054	-0.0214194	0.23125992	0.21811181	0.17561698	-0.4918269
	AVGTurb	0.40358258	0.64821159	-0.251071	0.37172433	-0.1500889	-0.3006725	0.1269719
	LogTurbidity	0.17204054	1	-0.5711121	-0.0015602	-0.1624323	-0.3480602	0.10857145
	AVGAlk	0.28512321	-0.0551943	0.21644174	0.36806353	0.00470155	0.46278994	0.059888
	LogSnails	-0.0214194	-0.5711121	1	0.18593687	0.20729659	0.07332785	0.14791098
	Boulder	0.07265076	-0.0143205	0.06343404	0.21946169	0.1972651	0.11591793	-0.1189167
	Cobble	0.17691019	-0.3098991	0.47171164	-0.0374878	0.5456651	0.01343695	-0.1062888
	Gravel	0.30286206	-0.1990324	0.29239317	0.1419118	0.56313299	0.00304961	0.00259121
	Sand	-0.3445794	-0.2659985	0.16982931	0.26669525	-0.1573978	0.08358037	0.15444326
	Silt	0.04076859	0.22549577	-0.0645492	-0.1455183	-0.2410839	0.02425817	-0.0576486
	ChlARFU	0.23125992	-0.0015602	0.18593687	1	0.09083392	0.0951053	0.24534922
	LogMusselsQuadrats	0.21811181	-0.1624323	0.20729659	0.09083392	1	0.10904218	-0.3106244
	MusselMassPerSampler	-0.0862749	-0.271777	0.10731367	0.35746466	0.1299702	0.56678271	0.21620162
	LogMusselMass	0.08280839	-0.3555262	0.11812184	0.31951489	0.07166141	0.88795927	0.04403091
	MusselsPerSampler	0.13730509	-0.2638833	0.09715268	0.14156396	0.05405824	0.71455918	-0.2116419
	LogMusselsSamplers	0.17561698	-0.3480602	0.07332785	0.0951053	0.10904218	1	-0.5614954
	MassPerMussel	-0.4918269	0.10857145	0.14791098	0.24534922	-0.3106244	-0.5614954	1
	LogZoop	-0.071418	-0.0371994	0.09818807	0.34246568	-0.1507859	0.10996849	0.18064851

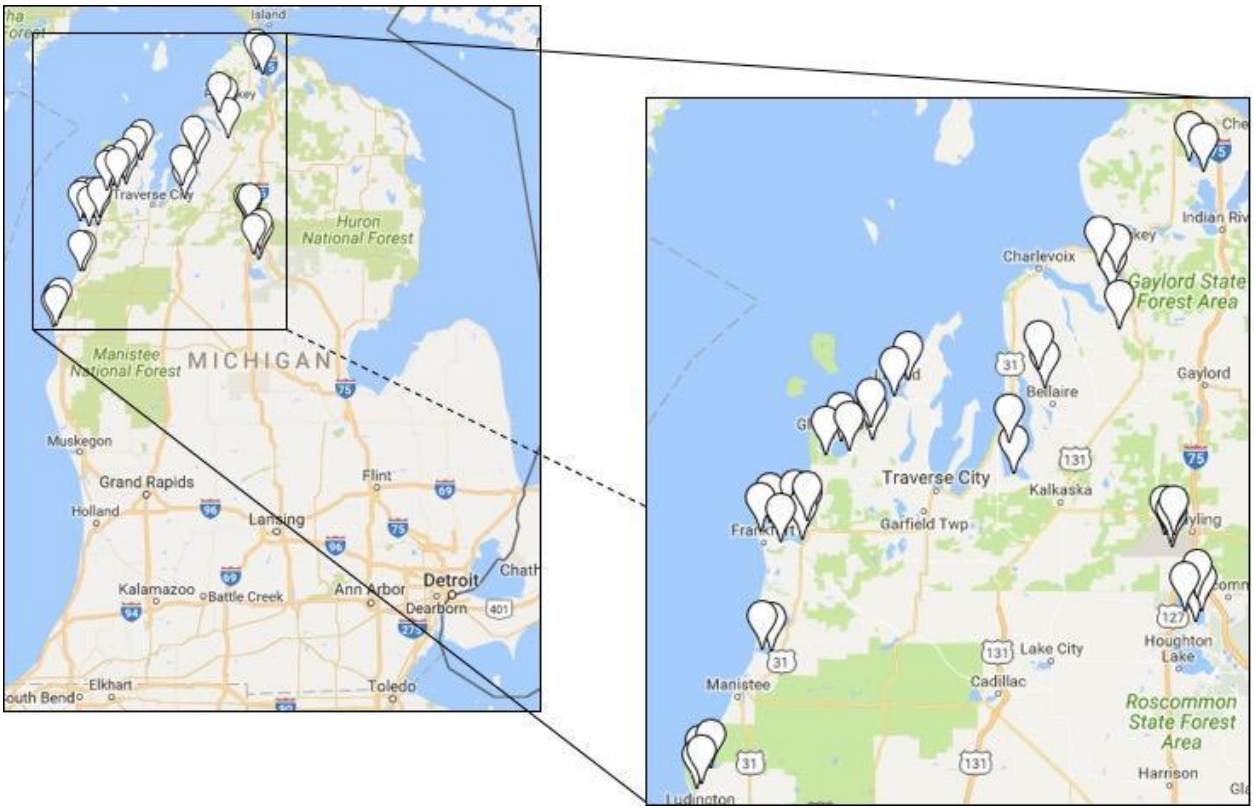

Figure 1. Map of Michigan with all 38 sites marked. Inset shows zoom of northwest Michigan. There were 16 lakes with a range of 1 to 4 sites per lake.

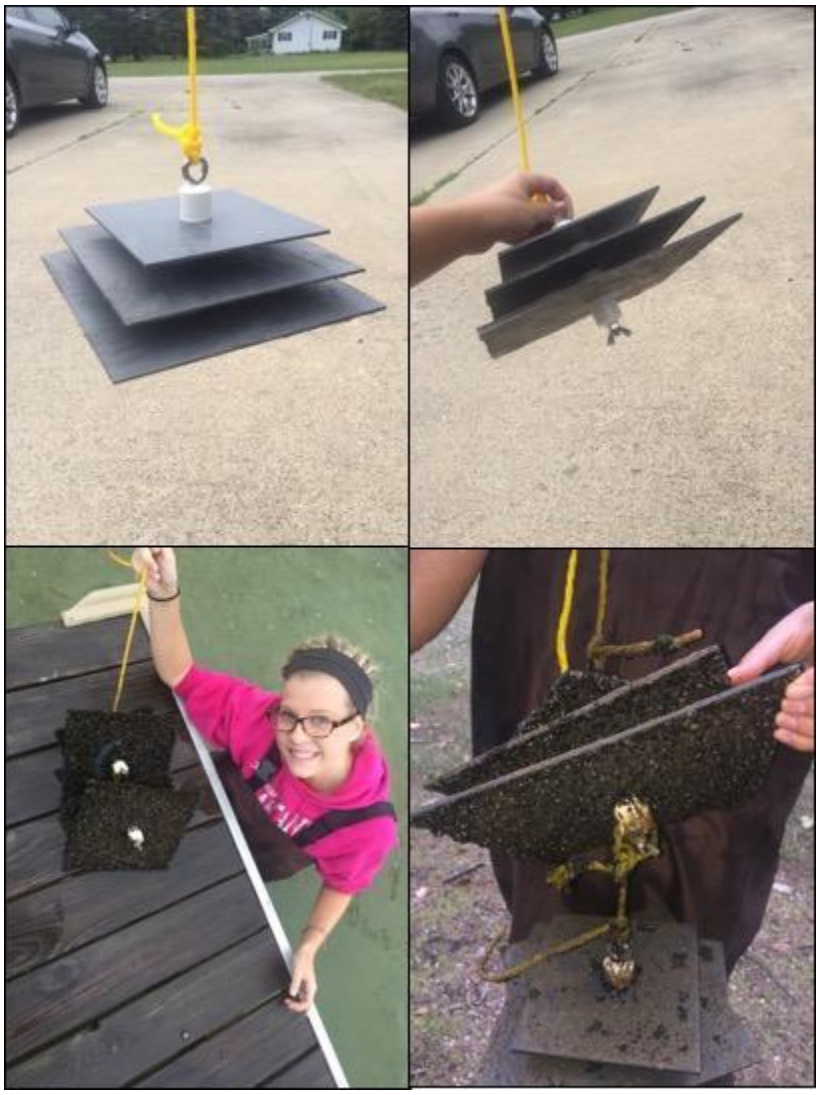

Figure 2. Top pictures show a single zebra mussel sampler after construction. When placed in the water, two samplers were tied together and hung vertically either from a dock or a buoy. This can be seen in the bottom two pictures. The bottom left is taken from Platte Lake after being in the water after being submerged for four months. Zebra mussels can be seen on the samplers. The bottom right is taken from Lake Skegmog. Here it is seen that the undersides of the plates are preferred attachment points. When the density is high enough, the tops will also be filled.

Figure 3. Set up of crayfish trap. a. and b. show minnow trap (denoted by "A - little" in our data set). c. and d. shows crayfish trap (denoted by "B - big" and "C - big" in our data set). Tuna was placed in the tea diffuser and used as bait to lure the crayfish into the traps.

Figure 4. Picture of view bucket used to more clearly view the surface of the lake. It minimized the refraction from the water, enabling a clear view.

Figure 5. Picture of quadrat used for zebra mussel and snail density sampling.

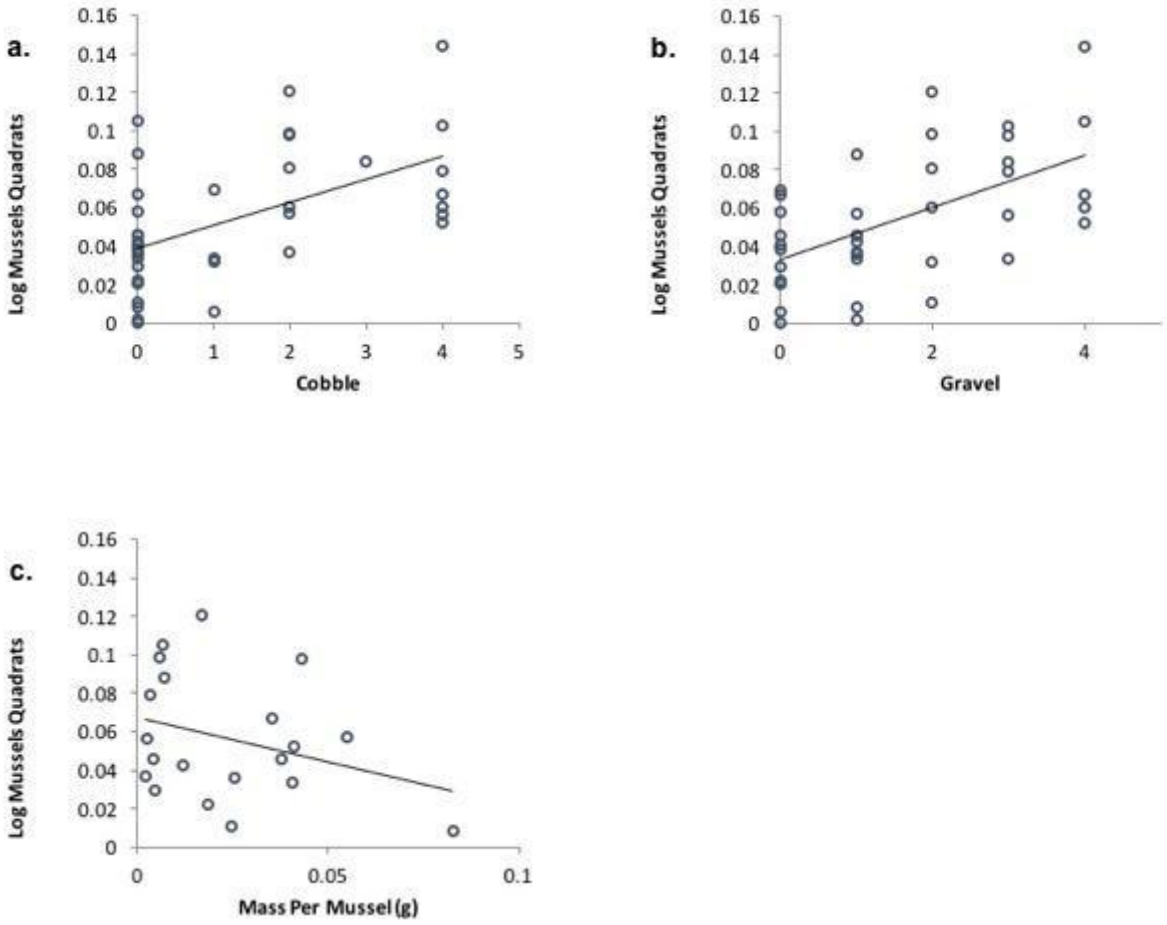

Figure 6. Log mussels quadrats has three significant predictors: cobble (a.), gravel (b.), and mass per mussel (c.). Cobble and gravel both show positive correlations whereas mass per mussel shows a negative correlation with number of mussels in the quadrat sampler.

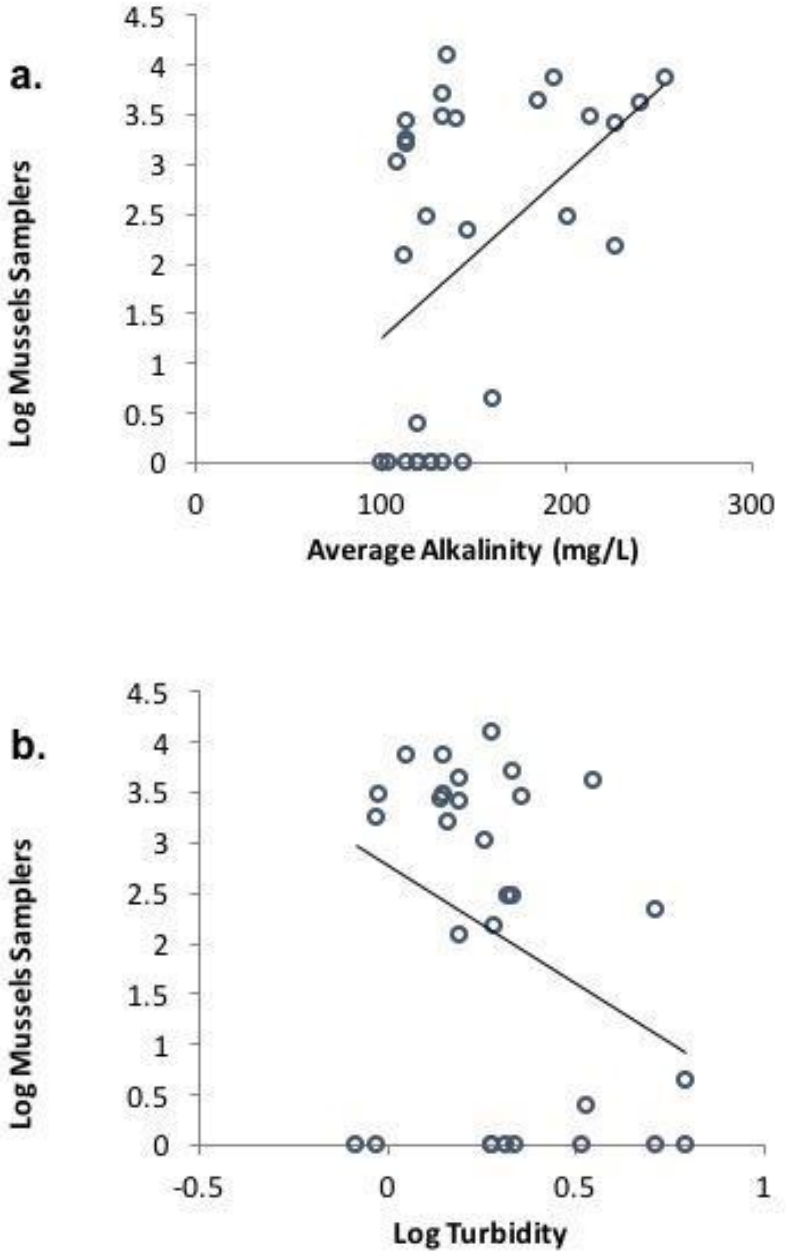

Figure 7. Average alkalinity and log turbidity are significant predictors of log mussels on the samplers. a. shows a positive correlation between alkalinity and number of mussels on the sampler. b. shows a negative correlation between turbidity and number of mussels on the sampler.

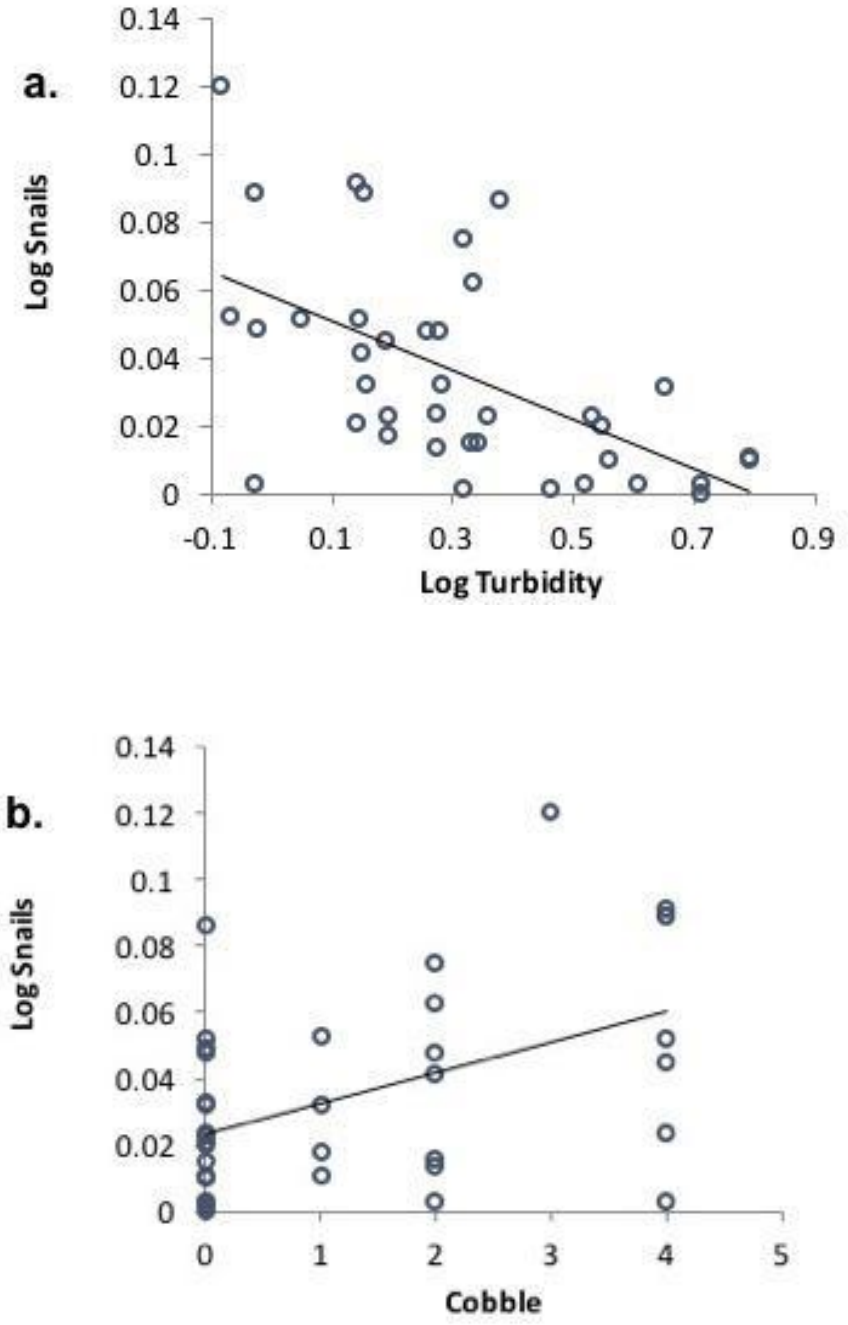

Figure 8. Log turbidity and cobble are significant predictors of log snails. a. shows a negative correlation between turbidity and snail density. b. shows a positive correlation between cobble and density of snails.

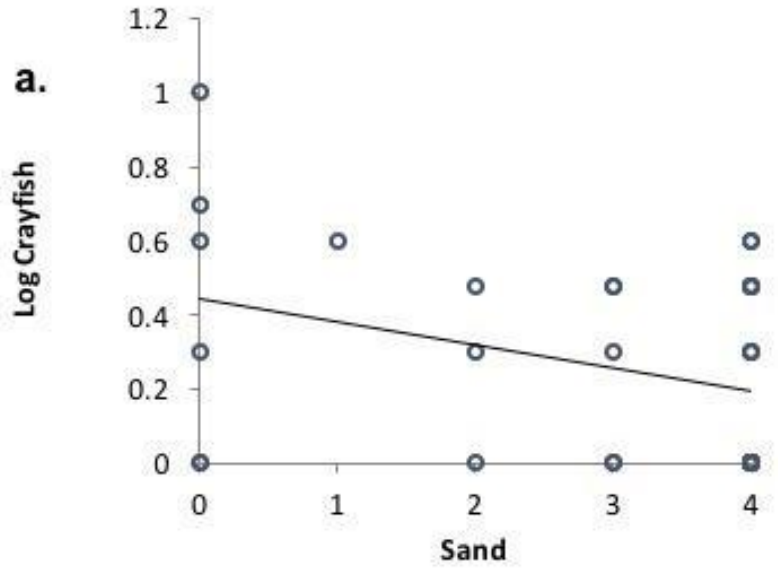

Figure 9. For log crayfish, the only significant predictor is sand. The data shows a negative correlation between sand and crayfish numbers.

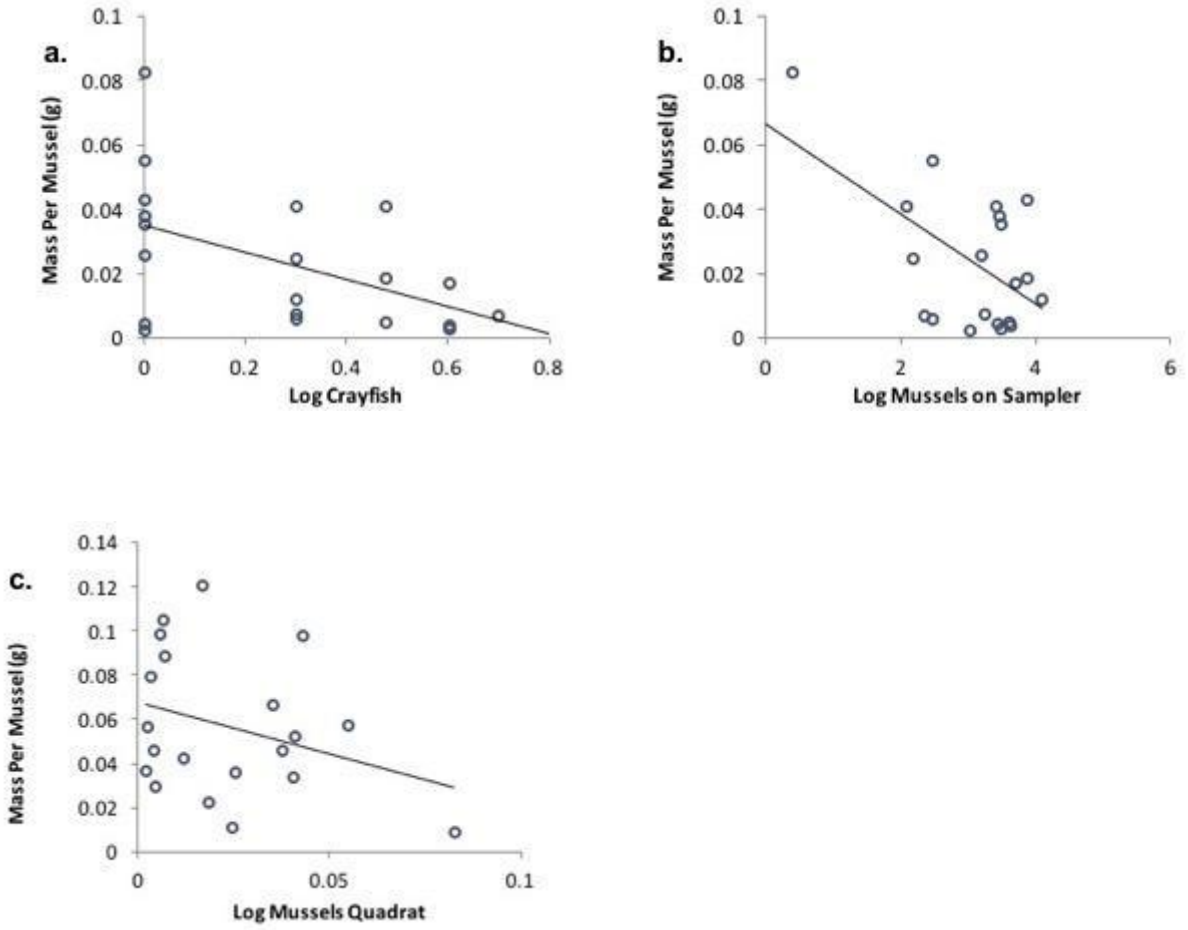

Figure 10. Predictors of mass per mussel are given. The strongest correlations are with log crayfish (a.), log mussels on sampler (b.), and log mussel quadrat (c.). All have negative correlations with mussel mass.

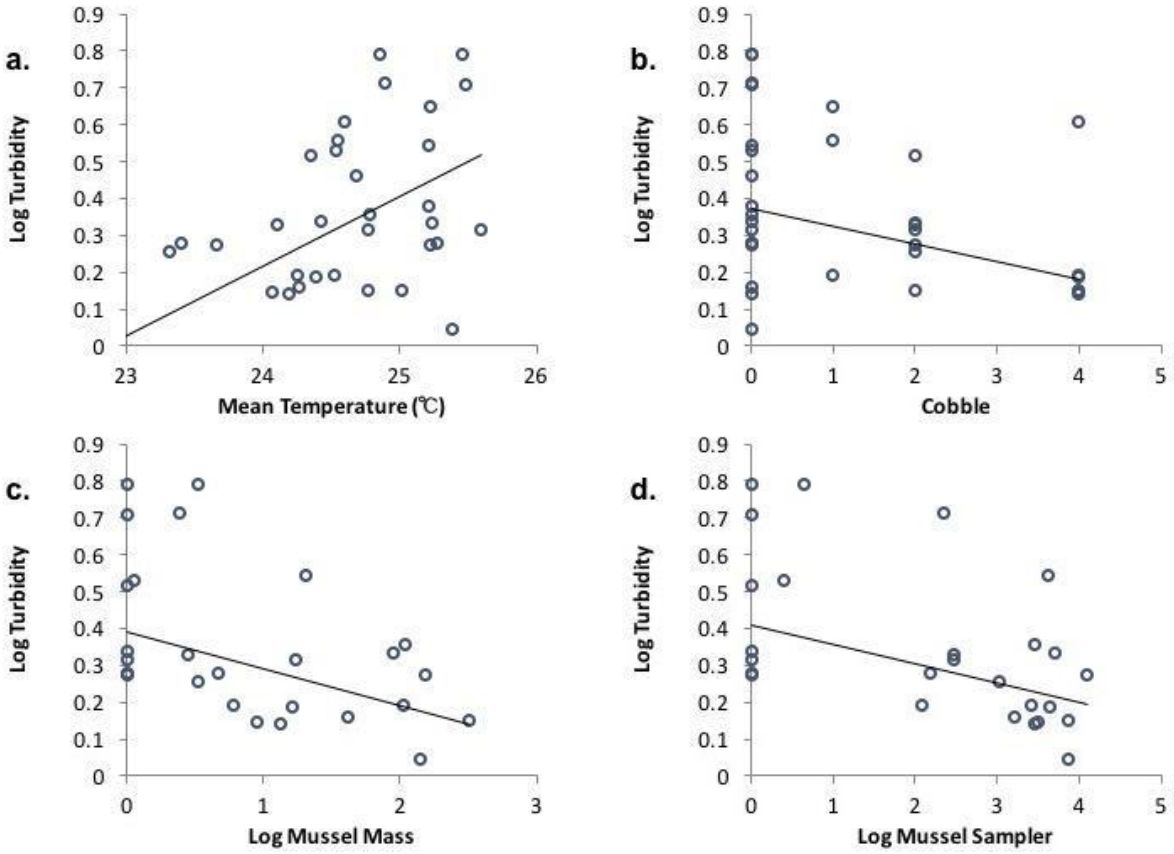

Figure 11. Log turbidity has four significant predictors: mean temperature (a.), cobble (b.), log mussel mass (c.), and log mussel sampler (d.). The graph shows that turbidity increases with increasing temperature. However, with every other predictor, turbidity has a negative correlation.

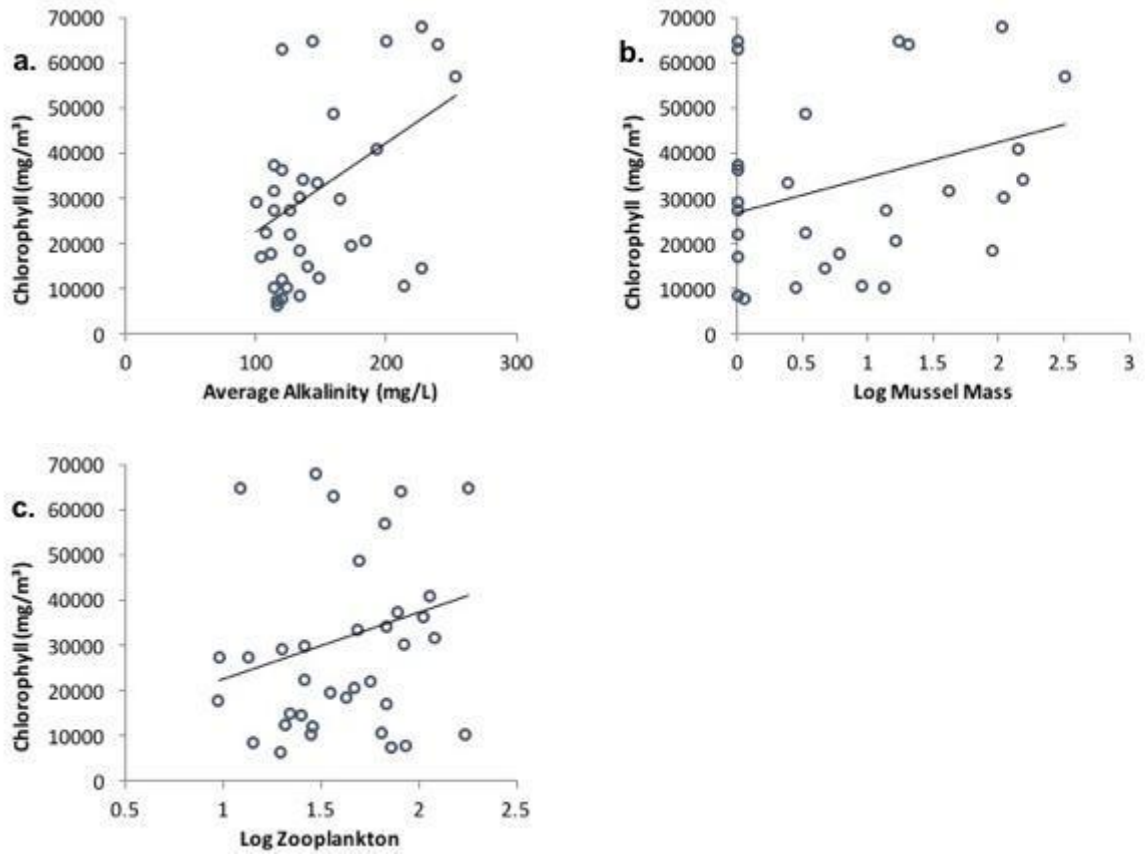

Figure 12. Chlorophyll (Periphyton Growth in predictor/response chart) has three significant predictors: average alkalinity (a.), log mussel mass (b.), and log zooplankton (c.). All three are positively correlated with chlorophyll. Note that periphyton is measured by relative fluorescence units (RFU) of chlorophyll.

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