PROGRESS REPORT: Michigan Swimmer's Itch Survey 2016
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Summary:

- Our primary goal was to measure avian schistosome parasites and potential snail intermediate hosts in northwest Michigan lakes, and to test for relationships with various hypothesized environmental drivers. We originally sought to sample ≥20 sites and ultimately obtained data from 38 sites on 16 lakes, with financial and volunteer support from local lake associations.
- With the help of local volunteers, we collected daily filtered-water samples from each site for a 28-day period in July–August for qPCR analysis of the abundance of avian schistosome cercariae in the water. **Analysis is complete for approximately half of the (over 1000) samples, and we are on track to finish this task by the end of March.**
- We conducted 3-5 surveys at each site to observe site characteristics, snail abundance, zooplankton abundance, mussel (zebra & quagga) abundance, water turbidity, and water alkalinity. We trapped crayfish on two occasions at each site, and we measured mussel settlement using stacked-PVC samplers. We obtained hourly data for temperature and light intensity using data loggers. We measured growth rates of attached algae (periphyton) by measuring algae accumulation on plexiglass samplers, using a fluorometric assay. **These measurements are fully compiled; see Figures 15-22.**
- We collected water samples on two dates from each site for measuring nutrient levels (nitrates+nitrites & orthophosphorus) and pesticides. So far we have conducted measurements of 2,4-D and glyphosate using ELISA test kits, but we may conduct additional pesticide measurements depending on budget and time limitations. We also collected sediment grab samples from each site for analysis of phosphorus and total organic carbon, and we plan to compile landscape-level data on land use (e.g., % urbanization). **These measurements are still in progress but will likely be complete by the end of March.**
- Several interesting patterns are evident in the dataset so far. The best and most consistent predictor of total snail abundance was water turbidity, with snails being much more abundant at sites with clearer water. However, there were no correlations between attached algae (periphyton) growth and either turbidity or snail abundance, suggesting that the water clarity effect on snails might not be mediated by attached algae growth in these lakes (as hypothesized). Further analysis is needed to follow up on this result, including separate analyses of each snail genus. The best predictors of turbidity were water temperature and mussel abundance on samplers, suggesting that water clarity was driven in part by lake-level abundance of invasive zebra and quagga mussels. Zebra mussel abundance and biomass, in turn, appears to be driven largely by water alkalinity (correlated with calcium availability), the local availability of hard substrates to settle on, and local crayfish abundance.

INTRODUCTION

Swimmer's itch is a nasty itchy rash (Fig. 1) caused by avian schistosomes, snail-borne parasites closely related to the causative agents of human schistosomiasis (*Schistosoma* spp.) (Colley *et al.*, 2014; Horak *et al.*, 2015). Avian schistosomes include a diverse group of trematode (flatworm) parasites, most in the genus *Trichobilharzia*, that normally use water birds as their definitive hosts (Brant & Loker, 2009). Infected snails release infectious stages called "cercariae", which swim through the water in search of a bird to infect (Fig. 2). However, cercariae sometimes mistake humans for birds and penetrate our skin instead (Horak *et al.*, 2015). They cannot complete their life cycles in humans but often cause a severe itchy rash that can take several days to clear (Fig. 1) (Horak *et al.*, 2015; Verbrugge *et al.*, 2004). People with no skin reaction might experience asymptomatic larval migrans (worms migrating through the body), with unknown long-term effects (Horak *et al.*, 2015). Anecdotal evidence suggests that swimmer's itch is increasing in Michigan inland lakes, where it discourages swimming and other recreational activities (Muzzall *et al.*, 2003).

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 (Kolarova *et al.*, 2010). 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 (Kolarova *et al.*, 2010). **We sought to fill this knowledge gap by conducting a large-scale survey, using a recently developed qPCR (DNA-detection) method (Jothikumar *et al.*, 2015).**

Hypothesized drivers of avian schistosomes and snail intermediate hosts

Studies on other trematode parasites have shown that pollution from fertilizer, herbicides, and insecticides can all increase the densities of snail intermediate hosts, leading to more parasites (Halstead *et al.*, 2014; Rohr *et al.*, 2008). These chemicals typically act by either increasing growth of the snails' food (algae growing on surfaces) or killing off insect predators of snails (Halstead *et al.*, 2014; Rohr *et al.*, 2008). Fertilizers add nutrients (mostly nitrates and/or phosphates) that can otherwise limit algal growth, leading to eutrophication and increased abundance of attached algae (Johnson & Chase, 2004; Rohr *et al.*, 2008). Insecticides primarily act by decreasing the abundance of snail predators, such as crayfish (Halstead *et al.*, 2014). Herbicides typically affect snail populations by clearing the water of floating algae (phytoplankton), leading to clearer water, more light penetration, and more growth of attached algae on surfaces (Halstead *et al.*, 2014; Rohr *et al.*, 2008). This effect was the most important factor driving trematode parasites of amphibians, where the common agricultural herbicide atrazine was the most important predictor of snail abundance and trematode infection (Rohr *et al.*, 2008). **We therefore predicted positive relationships between water clarity (1/turbidity), attached algae growth, and snail abundance (Fig. 3a).**

Invasive zebra and quagga mussels are also important components of lake ecosystems in northern Michigan, where these mussels are often dominant members of the benthic community. Zebra mussels are known to impact water clarity by filtering algae out of the water column, and this effect might also lead to increased snail abundance via increased growth of attached algae. Mussel growth rates are sometimes limited by calcium availability, which tends to correlate with alkalinity. Crayfish are important predators of zebra mussels, and their presence has been shown

to induce reduced feeding rates by mussels. **We therefore predicted positive relationships between alkalinity, mussel abundance, and water clarity (1/turbidity) (Fig. 3a).**

Temperature also influences parasite development and production of cercariae, with potential implications for climate effects (McCreesh & Booth, 2013; Paull *et al.*, 2015). Other potentially important factors for cercaria distribution and abundance include wind and wave action, substrate composition, and bird visitation (Muzzall *et al.*, 2003). **We therefore predicted positive relationships between snail abundance, cercaria abundance, bird visitation, and temperature (Fig. 3a).**

METHODS (summary)

Sampling sites & times

We sampled at 38 sites on 16 inland lakes in northwestern Michigan (Table 1; Fig. 4). These sites represented a wide range of lake sizes, shoreline types (e.g., beach vs. marsh), and levels of human activity. Each sampling site was restricted to a 15 m stretch of shoreline, and extended at least 15 m into the littoral zone (out to 0.8 m depth) and 15 m into the riparian zone (Fig. 5). We conducted all aquatic sampling within the limits of this riparian zone. We placed a buoy at each site at 60 cm depth marking the location of temperature/light loggers and periphyton samplers. One exception was Deer Lake, where slope was so shallow we decided to place the buoy at only 45 cm depth. Sampling occurred during a 28-day period in July-August 2016, starting on July 5. Daily measurements started 2-3 weeks earlier at selected sites on Crystal Lake and Intermediate Lake. Snail surveys started the week prior to this sampling period and occurred every 2 weeks at most sites (3 total surveys) and weekly at a few selected sites (5 total surveys).

Volunteers were encouraged to collect daily data describing weather conditions, air temperature, on-shore wind speed and direction. They were also asked to tally any sightings of birds within visual range of the study site. Before daily sampling began, two HOBO® pendant data loggers and a marker buoy were installed at each site at 60 cm depth. Both loggers recorded hourly temperature readings, but one logger at each site also monitored hourly light intensity and was therefore anchored to ensure the light detector remained horizontal and facing upwards.

Habitat assessment

The habitat at each site was assessed in the littoral zone (15 m by 15 m in water; Fig. 4) and the riparian zone (15 m by 15 m on land; Fig. 4) using a standardized checklist modeled after a shoreline assessment protocol developed by the Environmental Protection Agency. For all components of the site assessment, 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). The riparian ground cover was observed, checking for the abundance of vegetation (woody shrubs, saplings, herbs, & grasses) and barren areas (dirt/sand/rock). Riparian canopy and human influence were also classified and recorded. Habitat in the littoral zone was assessed by noting the presence/absence and abundance of various substrates (boulder, cobble, gravel, sand, muck) and types of aquatic vegetation (submergent, emergent, floating, and total plant cover).

Avian schistosome cercaria sampling:

Daily cercaria samples were collected by trained volunteers, who were provided with labelled 15-mL centrifuge tubes, a 1-liter pitcher, a nitex mesh filter (custom-made using 35 um mesh, Fig. 6), a squirt bottle filled with tap water, another squirt bottle filled with 70% ethanol, a small funnel, an anemometer, and a stand to hold the filter during filtration (if necessary). Cercariae are likely

to have aggregated distributions in the water, so to reduce the chances of missing small clumps of cercariae we sought to collect a distributed sample from each sampling site. We therefore asked volunteers to filter 24 separate 1-L scoops of water for each daily sample at each sampling site. To collect the sample, volunteers entered the water near the left boundary of the site while holding the 1-liter pitcher and the nitex mesh filter, collecting water from the surface using the pitcher and pouring it through the filter as they moved in a zig-zag pattern throughout the littoral zone of the sampling site. Volunteers were allowed to collect their water samples in a bucket prior to filtration, allowing sediment to settle to the bottom of the bucket prior to decanting the water through the mesh filter. Avian schistosome cercariae are known to move toward light and stay near the water surface (Brachs & Haas, 2008; Cort & Talbot, 1936), so they are unlikely to have been lost through this procedure. Once the lake water had been filtered, the squirt bottle containing tap water was used to rinse any residual material into one corner of the filter, followed by a final rinse with 70% ethanol to wash the sample into a 15 mL centrifuge tube using a small funnel. The use of 70% ethanol killed and preserved the DNA of any cercariae captured in the sample. Volunteers rinsed and back-rinsed their filters daily with tap water to keep the mesh clean. Samples were stored at room temperature until they were transported back to Oakland University at the end of the survey for further processing.

Avian schistosome DNA detection – ***measurements in progress***

Samples were centrifuged at 1500 rpm for 2 minutes to concentrate the sample for removal of ethanol preservative. Remaining ethanol evaporated off in a 55C drying oven, and samples were frozen until DNA extraction. DNA was extracted using a proteinase K extraction procedure with bead beating following a modified version of Qiagen's DNA isolation from their mouse tail tissue protocol (Qiagen 2010). To cut costs, we mixed our own lysis buffer (0.1 M Tris pH 8.5, 0.2 M NaCl, 5 mM EDTA, 0.4% SDS; Qiagen 2010) and used 0.5 grams of acid-washed glass sandblasting abrasive for bead beating. Extractions were carried out using 1 mL of lysis buffer and 10 µL of Proteinase K unless samples had more than 1 gram of initial sediment/debris, in which case we added additional extraction reagents to maintain a ratio of 1 mL lysis buffer + 10 µL Proteinase K per 1 g sample. Samples were securely capped and placed in a 55°C water bath and vortexed for 5 seconds every 15 minutes for 2 hours. After the 2-hour extraction process, samples were centrifuged at 1500 rpm for 2 minutes, and supernatant containing potential schistosome DNA was pipetted into a pre-labeled 1.5 mL microcentrifuge tube and stored at -20°C.

We used a real-time PCR TaqMan assay based on the assay described by Jothikumar et al. (2015), who designed primers to target 18S ribosomal RNA (rDNA) gene sequences based on a diverse panel of schistosome isolates representing 13 genera and 20 species. We utilized the custom primers and probe described by Jothikumar et al. (2015), which were designed to target a highly conserved region to maximize detection of itch-causing cercariae. The TaqMan probe was labeled with FAM (6-Carboxyfluorescein) at the 5' end and black hole quencher (BHQ-1) at the 3' end. The TaqMan real-time PCR assay consisted of a 25 µL final reaction volume containing TaqMan Universal PCR Master Mix with 0.25 uM of each forward and reverse primer, 0.1 uM TaqMan probe, and 5 µL DNA sample (1:100 dilution). We added ~1.4 µg/µL of bovine serum albumin (BSA) to our PCR reactions to help reduce inhibition due to humic substances (Garland *et al.*, 2010). All reactions also contained a TaqMan Exogenous Internal Positive Control (IPC) primer-probe and template set to distinguish true target negatives from PCR inhibition (Garland *et al.*, 2010; Hyatt *et al.*, 2007). All amplification reactions for the TaqMan assay were performed on Bio-Rad CFX Connect Real-time PCR Detection System using the CFX Manager Software 3.1.

Quantitation cycles (Cq values) were recorded for each unknown sample and were compared to a standard curve in order to calculate the number of cercariae per liter of lake water. Standards were created by counting 5 *Trichobilharzia* sp. cercariae using microscopy and seeded into each of six 1.5 mL microcentrifuge tubes containing 500 µl of 70% ethanol, and then subjecting them to the DNA extraction procedure outlined above.

For our 2015 survey, all filtered water samples were assayed in singlicate to control costs. For the 2016 survey, we decided to divide each sample in half and extract them in duplicate, to help us to analyze potential sources of error in the assay. Individual extractions have so far been assayed via qPCR in singlicate, but we might eventually re-run some of our positive samples to facilitate this analysis of sources of measurement error. **Note that we experienced delays last semester due to supply problems (e.g., back-ordered qPCR plates and reagents). Nevertheless, all >2000 sample DNA extractions are complete and approximately half of the DNA samples have been assayed with qPCR. We anticipate completing qPCR by the end of March.**

Quadrat surveys – snail & local mussel abundance

Each site was visited 3-5 times to measure the abundance and diversity of aquatic snails within the littoral zone, which we divided into 3 depth areas (0-40 cm, 40-80 cm, and >80 cm). Square-foot PVC quadrat sampling frames (0.09 m^2; Fig. 7) were haphazardly tossed within each depth zone until a total of 4 frames were counted in each. A clear-bottom viewing bucket was used to observe and count the number of snails on the substrate within the boundaries of each frame (Fig. 7). Densities were recorded for snails and mussels, identified to the genus level. Notes on other organisms (e.g., crayfish) were made as they were encountered. Snails were also collected from each depth zone and preserved in 70% ethanol to verify the proportion of snails from each species.

Mussel samplers:

Zebra mussel settling rates were measured by placing two samplers (Fig. 8) at each site in July and leaving them undisturbed through September, when new mussels settle and attach onto available substrates (Mackie *et al.*, 1990). We 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 (Herman & Wickman, 2014) and comprised of a stacked array of three roughened PVC plastic sheets (Fig. 8). Samplers were collected in October. They 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, we massed 10 randomly selected mussels from each sampler. We then divided the total mussel biomass per sampler by the mean mass per mussel to estimate the total number of mussels settled.

Crayfish trapping:

At each site, we conducted two crayfish trapping sessions spaced two weeks apart. For each trapping session, we set three traps overnight, 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. We 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 (Fig. 9). All crayfish caught were documented with photographs.

Periphyton growth (attached algae)

Three plexiglass tiles (10×10 cm) were sanded, rinsed, and anchored above the benthos at a depth of ~30 cm below the surface (Fig. 10). After three weeks, tiles were removed, placed in pans, and carefully brushed clean of periphyton. Vacuum filtration was used to concentrate the sample

onto a GF/F glass microfiber filter (0.7 µm; Whatman Inc, Kent, U.K.). Filters were stored in foil envelopes at -20 °C until fluorometric analysis. Filters were incubated for 24 hours in the dark in a 90% methanol solution to promote algal cell lysis following a modified version of the EPA method 445.0 (Arar & Collins, 1997). 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 two tiles to obtain an index of periphyton growth potential at each site. Algae from the third tile were preserved in Lugol's solution for possible future analysis of taxonomic composition.

Zooplankton samples:

We sampled for zooplankton on three occasions at each site. So far we have analyzed two zooplankton samples from each site. Each sample consisted of three 5 m horizontal drags with a standard 8 inch zooplankton net. Samples were preserved in 50 mL Lugul's solution for later analysis. We sub-sampled 2 mL from each sample, counted the three most abundant taxa (Copepoda, Cladocera, and Ostracoda), and took notes of other rare taxa.

*Water chemistry & Sediment analysis – **measurements in progress***

Analyses of water chemistry and sediment composition from each site are still in progress, in collaboration with Dr. David Szlag (OU Chemistry Department), Dr. Scott Tiegs (OU Biology), and their students. We collected paired water grab samples at two time points from each site, using acid-washed 250 mL brown HDPE sample bottles. These were rinsed three times with lake water and then filled at a water depth of 50 cm during each sampling event. Samples were placed on ice and frozen within 24 hours, and stored at -20° C until nutrient analysis could be completed. Samples are currently being assayed for phosphate (ortho; EPA 365.1), nitrate/nitrite (EPA 353.2), and ammonia salicylate (EPA 350.1) using an AQ1 Discrete Analyzer (Seal Analytical Inc., Mequon, WI, USA). We conducted two sediment grabs at each site using a Petite Ponar sampler, and collected two sediment mini-cores from each grab.

The Szlag lab is currently re-running water samples to verify their initial measurements and analyzing a second set of phosphorus measurements for the sediment samples. We used standard Abraxis ELISA test kits to measure concentrations of two common herbicides (2,4-D and Glyphosate) in each water sample, and we are interested in also measuring concentrations of triazine herbicides, carbamate herbicides, and organophosphate insecticides. My students are also working to complete an analysis of total organic carbon in the sediment samples, in collaboration with Dr. Tiegs.

*Land use variables – **measurements in progress***

For the 2015 survey, my student Madelyn Messner assessed the major land use types at the lake-watershed level and within a one-mile perimeter around each lake using a GIS-based watershed-mapping tool. The Long-Term Hydrologic Impact Assessment (L-THIA) model (Lim *et al.*, 2001) is an accessible online tool that assesses the water quality impacts of land use change using data from the 2006 National Land Cover Database (Fry *et al.*, 2011). Maddie utilized the L-THIA for the Great Lakes Watershed Management System to generate percent land use data for the watershed of each lake in the 2015 survey (12 digit HUC regions), and for lake polygons that encompassed all of the land within a one-mile perimeter of the shoreline. Percent land use was summarized into the following categories: urban, cropland, pastureland, forest, and water; later we combined cropland and pastureland to create an 'agriculture' land use category. Urban land use is defined by open space/city park, low-density residential (1/3-2 acre lots), high-density residential

(townhomes to 1/4 acre lots), commercial/industrial/transportation, and barren land. Agricultural land use includes grassland, pasture/hay, and generalized cropland agriculture. Forested areas are defined as those with deciduous, mixed, or shrub/scrub forest types (Lim *et al.*, 2001). We plan to calculate these same variables for all lakes involved in the 2016 survey.

PRELIMINARY RESULTS & DISCUSSION

A note on statistics for non-scientists. For statistical tests, $P < 0.05$ is generally referred to "statistically significant" by most biologists and represents a common rule-of-thumb cutoff for deciding that the observed pattern probably isn't due to random chance alone (i.e., something is probably going on). Technically, the "P-value" represents the probability of observing a pattern as or more extreme than your dataset, assuming the "null hypothesis" of no effect is actually true.

Predictors of snail abundance

Although several variables still need to be compiled, a few patterns stand out in the dataset so far. The best predictors of total snail abundance were a negative effect of water turbidity ($F_{1,35} = 12.1$, $P = 0.001$; Fig. 11a) and a positive effect of cobble substrate in the littoral zone ($F_{1,35} = 5.8$, $P = 0.021$; Fig. 11b). Snail counts and turbidity readings were log-transformed prior to analysis to improve normality. **This strong correlation between turbidity and snail abundance is consistent with our original hypothesis that snail abundance is driven by water clarity.**

The best predictors of water turbidity so far are mussel biomass on samplers ($F_{1,26} = 14.0$, $P < 0.001$; Fig. 12a) and water temperature ($F_{1,26} = 19.5$, $P < 0.001$; Fig. 12b). This is consistent with findings of prior studies that zebra mussels increase water clarity by filtering phytoplankton out of the water. However, there was no detectable relationship between turbidity and periphyton growth, or between periphyton growth and snail abundance (both $P > 0.1$; Fig. 11c & 12d), contrary to our hypothesized mechanism linking water clarity and snail abundance. The best predictor of periphyton growth (so far) is water alkalinity ($F_{1,36} = 5.6$, $P = 0.023$; Fig. 12c).

Further analysis of individual snail taxa and additional predictors might help explain the lack of any apparent relationship between snails and periphyton, or between periphyton and turbidity, despite the very strong correlation between turbidity and snails. One possibility is that snail abundance is more tightly linked to periphyton growth out in deeper water, where the effects of water turbidity on light penetration might be more of a limiting factor for attached algae. To test this hypothesis, we would need to conduct additional sampling in deeper water (see Future Directions).

Predictors of mussel abundance

We measured mussel abundance using two very different sampling methods, first via quadrat sampling and second by suspending standardized PVC samplers in the water column. We expected these techniques to measure different things about mussel abundance, with quadrat sampling measuring the local density of already-established adult mussels, and samplers providing an index of potential settlement rates by new juveniles. We expected the samplers to provide a better indicator of lake-wide mussel abundance and their potential to impact water quality. Unfortunately, several of the zebra mussel samplers were lost, removed, or damaged during the course of the survey. However, we still detected some very interesting patterns.

Our results bore these predictions out very well. Mussel densities in quadrats was best predicted by our index of "gravel" substrate, suggesting that local densities are limited by substrate availability ($F_{1,36} = 16.7$, $P < 0.001$; Fig. 13a). There was no correlation between the two measures

of zebra mussel abundance (P > 0.1; Fig. 13b). The best predictor of mussel biomass on samplers was the alkalinity of the water ($F_{1,28} = 7.6$; P = 0.010; Fig. 13c).

There was also an intriguing relationship between crayfish density and the average mass of mussels on samplers ($F_{1,17} = 4.7$, P = 0.045; Fig. 13e), after controlling for an apparent effect of mussel density-dependence on this variable ($F_{1,17} = 7.0$, P = 0.017; Fig. 13f). This is consistent with prior studies that showed negative effects of crayfish on mussel filtration rates, probably due to anti-predator behavior (i.e., holding the shell closed). To our knowledge, this is the first time this pattern has been found in a field study, and it suggests that this anti-predator response to crayfish might have ecologically relevant effects on mussel feeding behavior in natural lakes.

The best predictor of crayfish (so far) was a negative effect of our index of sand substrate ($F_{1,36} = 4.9$, P = 0.034; Fig. 11d), suggesting that crayfish prefer locations with less open sand habitat.

Predictors of zooplankton abundance

Both copepods and cladocerans tended to increase with total zooplankton (Fig. 14a, b). However, in our analyses of potential predictors, these groups seemed to respond to different environmental predictors. Copepods were more abundant at sites with steeper slopes (Fig. 14c), and cladocerans increased with nighttime temperature and mussel abundance on samplers (Fig. 14d, f). Ostracods seemed to respond negatively to water alkalinity (Fig. 14e). Further analysis is needed of additional environmental predictors – in particular, water chemistry might play an important role in limiting these populations.

Site-level data

Summaries of site-level data collected so far are provided in Figs 15-22.

PRELIMINARY CONCLUSIONS

We plan to wait till our datasets are complete to draw any firm conclusions. However, there are a few interesting patterns in the dataset so far, summarized in Fig. 3b.

First, our results seem to confirm that water clarity is an important predictor of snail abundance. Further analysis is needed to determine whether and how different snail taxa respond to different environmental predictors. This pattern is consistent with our core a priori hypothesis that snail populations are responding to variation in food availability, which is limited by light penetration to the benthos. It is also consistent with findings from our 2015 survey showing apparent relationships between snail abundance, light intensity, and periphyton growth rates.

However, there were no clear relationships between periphyton growth (attached algae) on our samplers and either snails or turbidity, contrary to the hypothesis that snail populations are linked to water clarity via its effects on attached algae. **We are interested in hearing any ideas people might have to help explain this pattern.** Our current working hypothesis is that our periphyton measurements, which we conducted in shallow water, might not be representative of periphyton growth in deeper waters where snails spend much of their time in clear-water lakes. Turbidity might be more of a limiting factor for periphyton growth in deeper water, because light must travel a greater distance through the water and there might be fewer effects of other variables such as shade from aquatic vegetation or trees on the shoreline.

One of our most interesting results came from measurements of mussel settling rates, which turned out to be one of the best predictors of water turbidity. Zebra and/or quagga mussels were found in the quadrat samples of all sites in this study. However, their settling rates on samplers might be a better proxy for lake-wide mussel abundance, which in turn might be a more important driver of water clarity (1/turbidity) than mussel densities at local sites. Mussels were more

abundant on samplers in sites with high alkalinity, likely because their population growth rates are limited by the availability of calcium for making shells.

FUTURE DIRECTIONS

Dr. Raffel recently received word that his NSF-CAREER grant is being recommended for funding. This is a five-year grant supporting work on a proposed metabolic theory approach to describing the temperature-dependence of infectious diseases. This work will focus primarily on a fungal disease of amphibians, which will draw some of my attention away from swimmer's itch for the next few years. Our lab has also received funding to conduct a MDEQ-sponsored study of harmful algal blooms (HAB) starting in Summer 2017. This project is in collaboration with Dr. David Szlag (OU Chemistry) and researchers at Wayne State, University of Kentucky, and Lake Superior State University.

Due to the interesting mussel patterns from the 2016 survey, we are considering including zebra mussel and periphyton sampling, including measurements from deeper water, as part of our 2017 HAB survey. The MDEQ study will necessarily focus on lakes with known histories of HABs and is meant to cover a larger spatial area extending from southeast through northwestern Michigan. However, we hope to include some of the same lakes from our 2016 survey in this new effort, some of which will serve as "control" sites. **Our tentative list of lakes for this new survey – which was developed with input from MDEQ staff – includes Platte Lake, Little Glen Lake, Lake Margrethe, Deer Lake, Lime Lake, and Intermediate Lake.** Note that since this study is funded by MDEQ, we do not need to solicit donations to support our 2017 survey efforts. However, we hope local lake associations and riparian owners will be willing to support this project by suggesting sampling sites, keeping an eye on our buoys following deployment, and perhaps occasionally offering places for students to stay while sampling. **We will also be seeking to recruit citizen-scientist volunteers to record observations of potential cyanobacteria (blue-green algae) blooms using a custom iphone app developed by our collaborator at the University of Kentucky.**

In terms of swimmer's itch specific projects, my student Jason Sckrabulis plans to continue laboratory experiments and model development for the temperature-dependence of cercaria production by avian and human schistosomes. He is also interested in further developing and testing his cercaria trap device. We currently lack specific funds to support these continued lab experiments. However, Al Flory from Crystal Lake has offered to provide some summer support for Jason's studies, which will include analysis of data collected by Crystal Lake over the last few years. Madelyn Messner is planning to defend her Master's thesis soon, and we hope to submit the results from her 2015 study for publication in a high-profile journal.

Finally, Ron Reimink of Freshwater Solutions has offered to continue providing us with a source of *Trichobilharzia* infected snails, which will help to facilitate Jason's work. We hope to increase our level of collaboration and data sharing with both Mr. Reimink and Dr. Patrick Hanington to help them refine their cercaria sampling protocols. I have also agreed to act as a scientific consultant, as needed, to assist Mr. Reimink in the design and analysis of studies to test his interesting ideas about merganser behavioral ecology.

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Walloon Lake Association, Lime Lake Association, Higgins Lake Property Owners Association, Twin Lakes Property Owners Association, Elk-Skegemog Lake Association, Crystal Lake & Watershed Organization, Lake Margrethe Foundation Fund, Hamlin Lake Preservation Society, Portage Lake Watershed Forever, and Intermediate Lake Association. Jennadene McBride was supported by the OU Summer Student Campus Corps program. Aleena Hajek and Alex Bageris received funds from the Honors College to help support their components of the project. Finally, this work was partially funded by Startup funds and a Faculty Fellowship award to Dr. Raffel from Oakland University.

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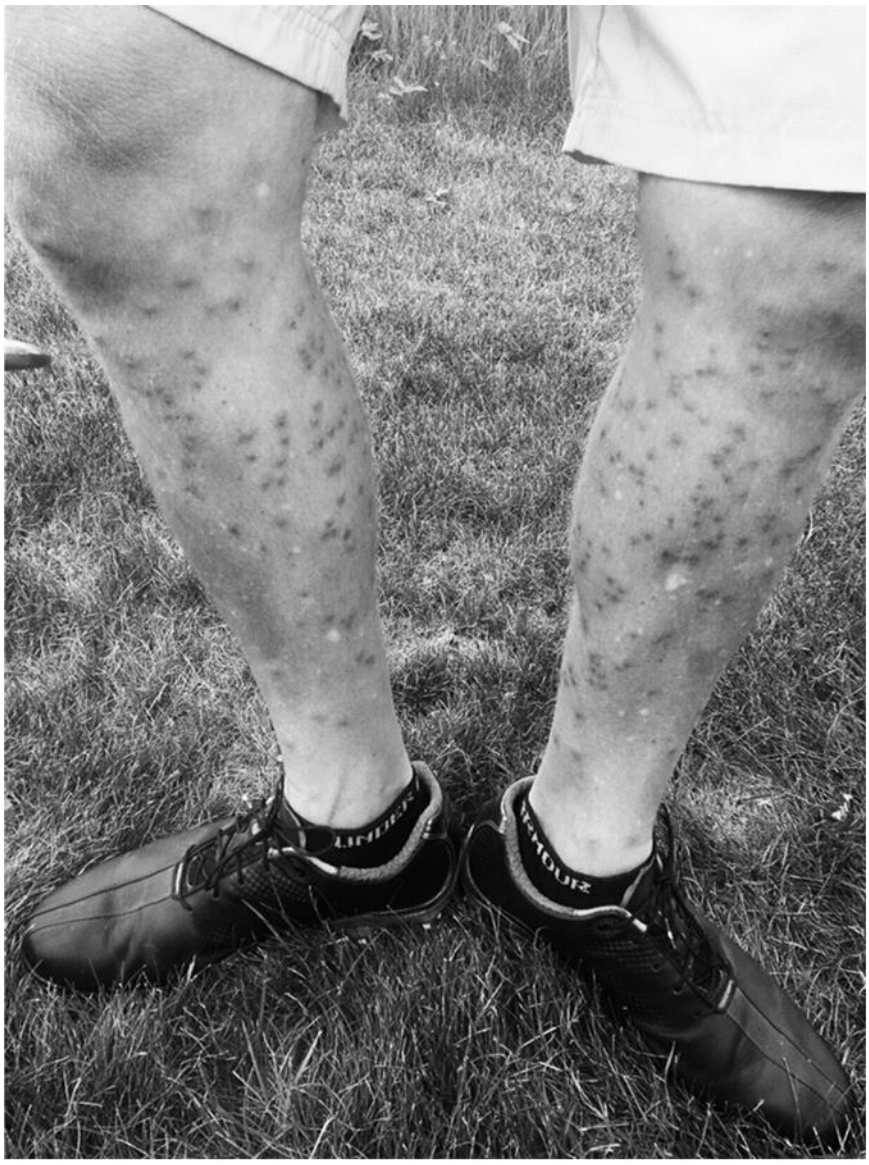

Fig. 1. Severe case of cercarial dermatitis ("swimmer's itch"). This picture was taken ~2 days following exposure to avian schistosomes in Crystal Lake (Benzie Co, MI). Raised papules indicate attempted skin penetration by cercariae.

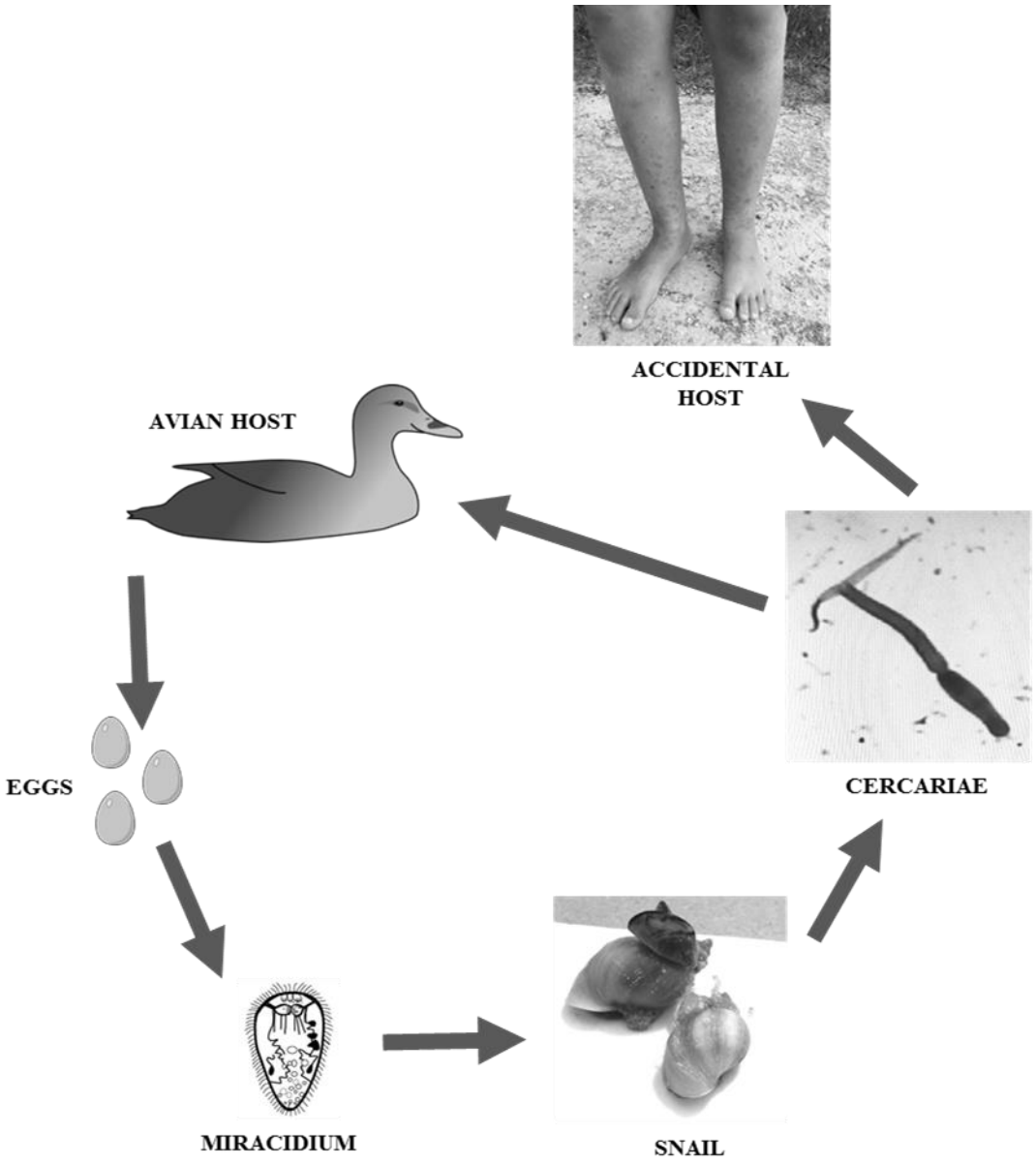

Fig. 2. Typical life cycle for an avian schistosome parasite in the genus Trichobilharzia. Adult blood flukes like in avian hosts where they reproduce sexually and make eggs, which are expelled in (usually) host feces. Aquatic snails are second intermediate hosts where the parasites reproduce asexually, releasing thousands of swimming cercariae into the water. Cercariae often mistake humans for suitable hosts and try to penetrate the skin, resulting in the itchy rash known as “swimmer’s itch”.

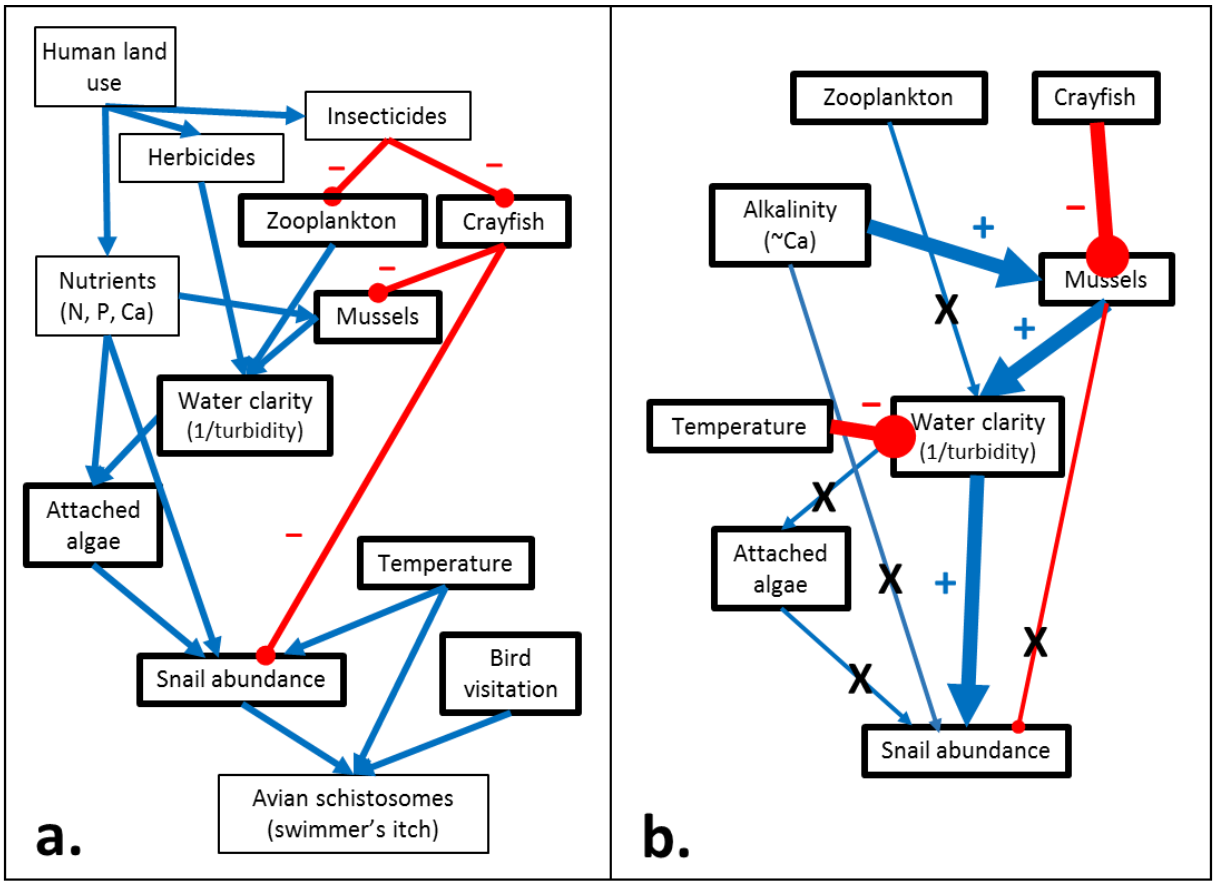

Figure 3. (a) A priori hypothesized drivers of snail and avian schistosome abundance. Boxes with thick borders represent variables with complete datasets so far. (b) Correlations detected so far. These results suggest potentially important relationships between invasive mussel abundance (as measured by settling rates), water clarity (1/turbidity), and snail abundance. The best predictor of mussel settling rates (so far) was water alkalinity. The best predictors of water clarity were temperature and mussel abundance. Water clarity was the best predictor of snail abundance, though patterns of attached algae growth did not support our proposed mechanism linking water clarity to snails. X's indicate hypothesized relationships that were not supported by our analysis.

Table 1. Site identifiers and 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

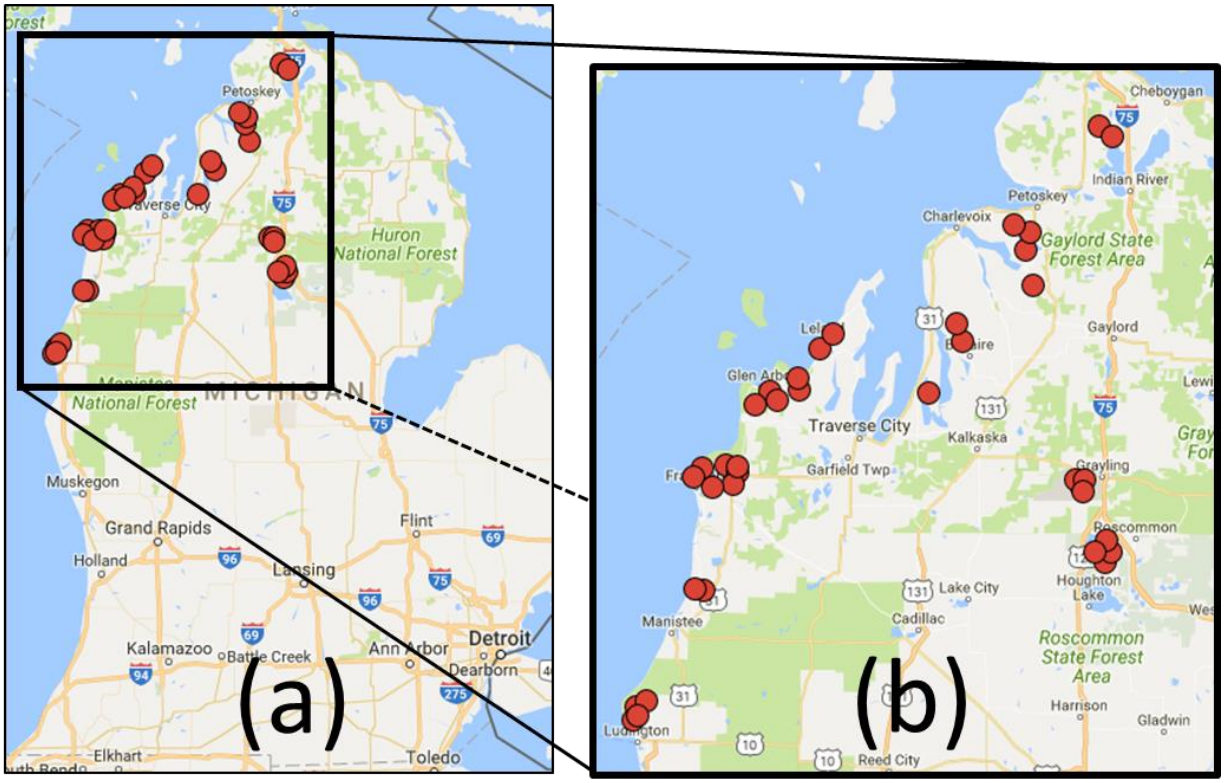

Figure 4. Map of study sites in northwest Michigan. (a) Full state; (b) Inset.

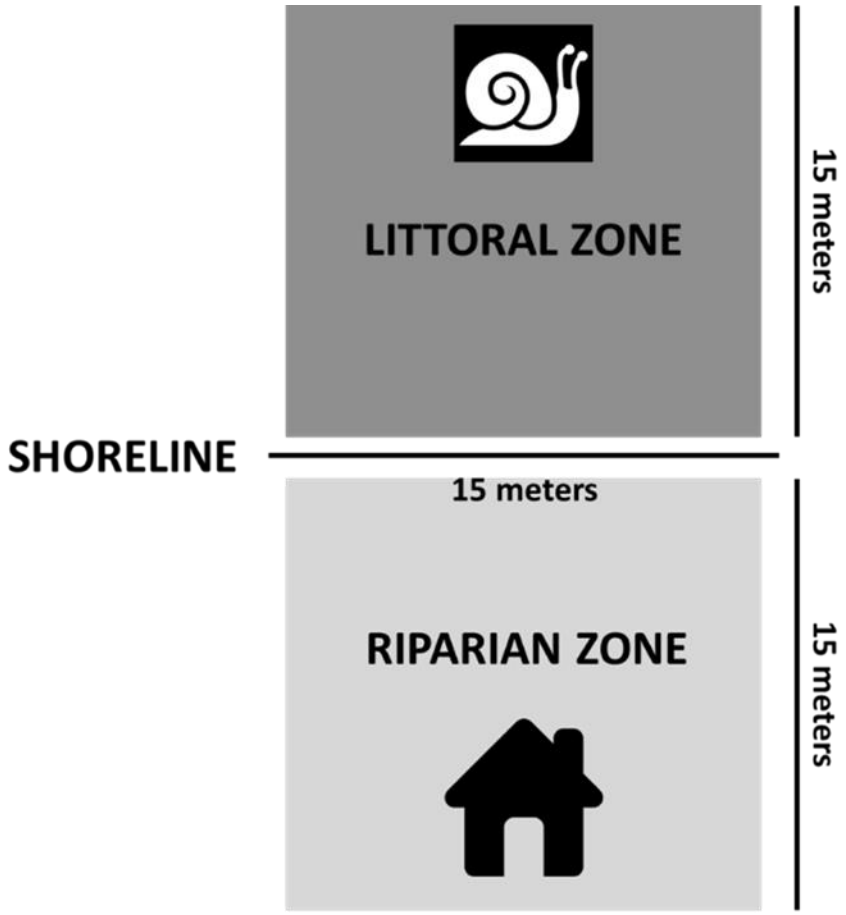

Figure 5. Sampling plan for each study site. Aquatic sampling was conducted within the littoral zone, whereas local land use was assessed within the riparian zone.

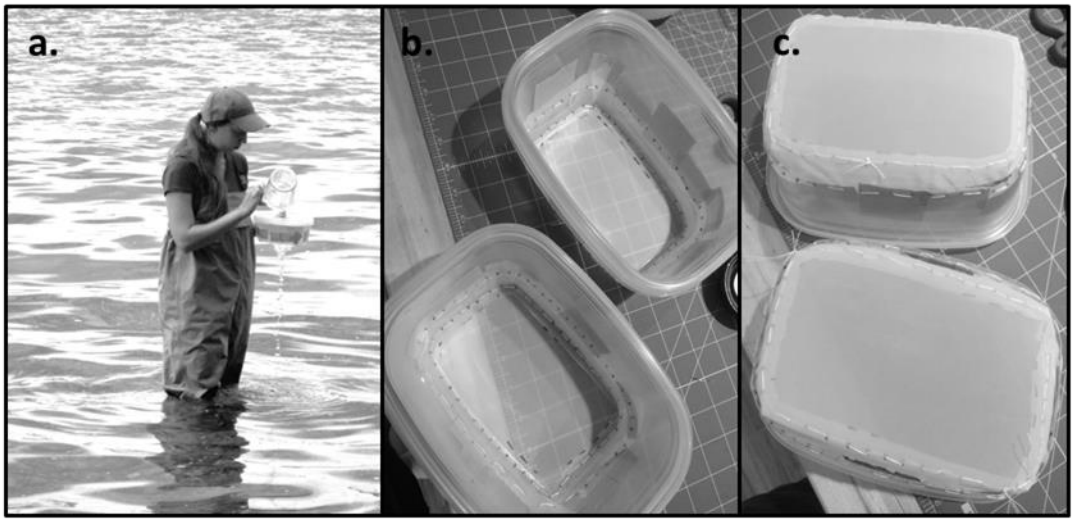

Figure 6. Method for filtering cercariae from surface waters (a), using custom-built nitex filters (b, c). In 2016, volunteers filtered 24+ liters of water each day and rinsed the filtrate in a vial with 70% ethanol to preserve the cercaria DNA.

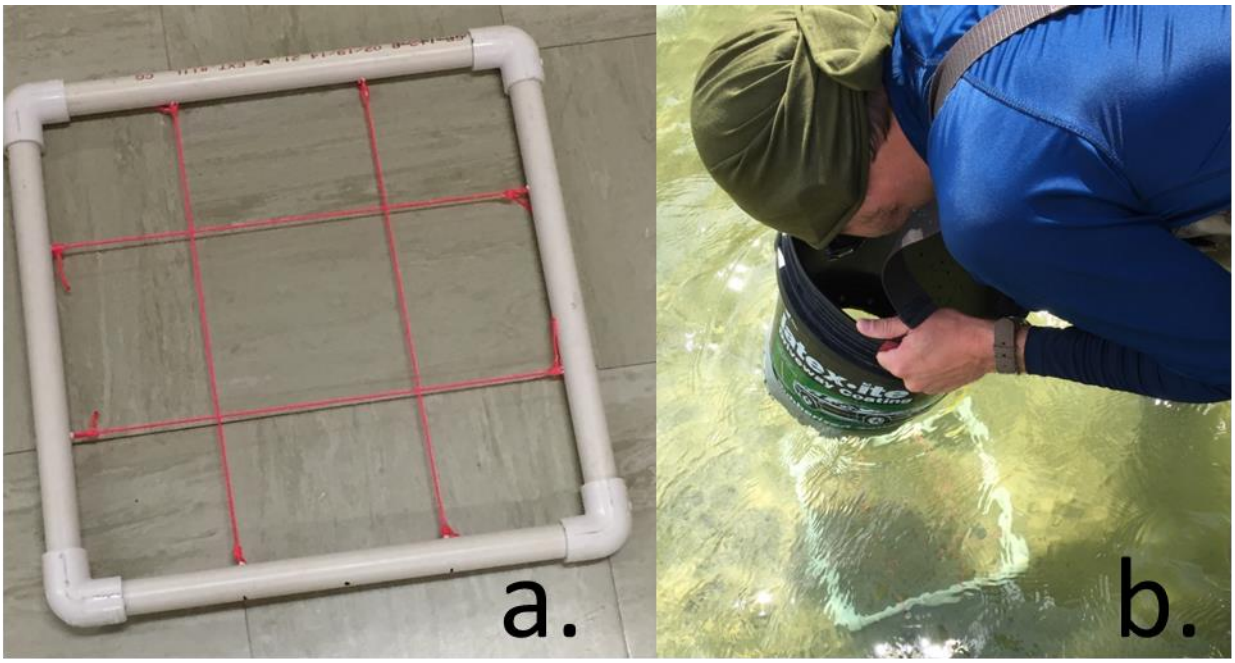

Figure 7. Quadrat sampling methods. (a) Quadrat sampler made of PVC pipe and string. (b) Counting snails and mussels using a view bucket. Each site was surveyed 3-5 times in July 2017. Each survey consisted of 15 haphazardly tossed quadrat samples, distributed to include 5 samples at each of three depth ranges.

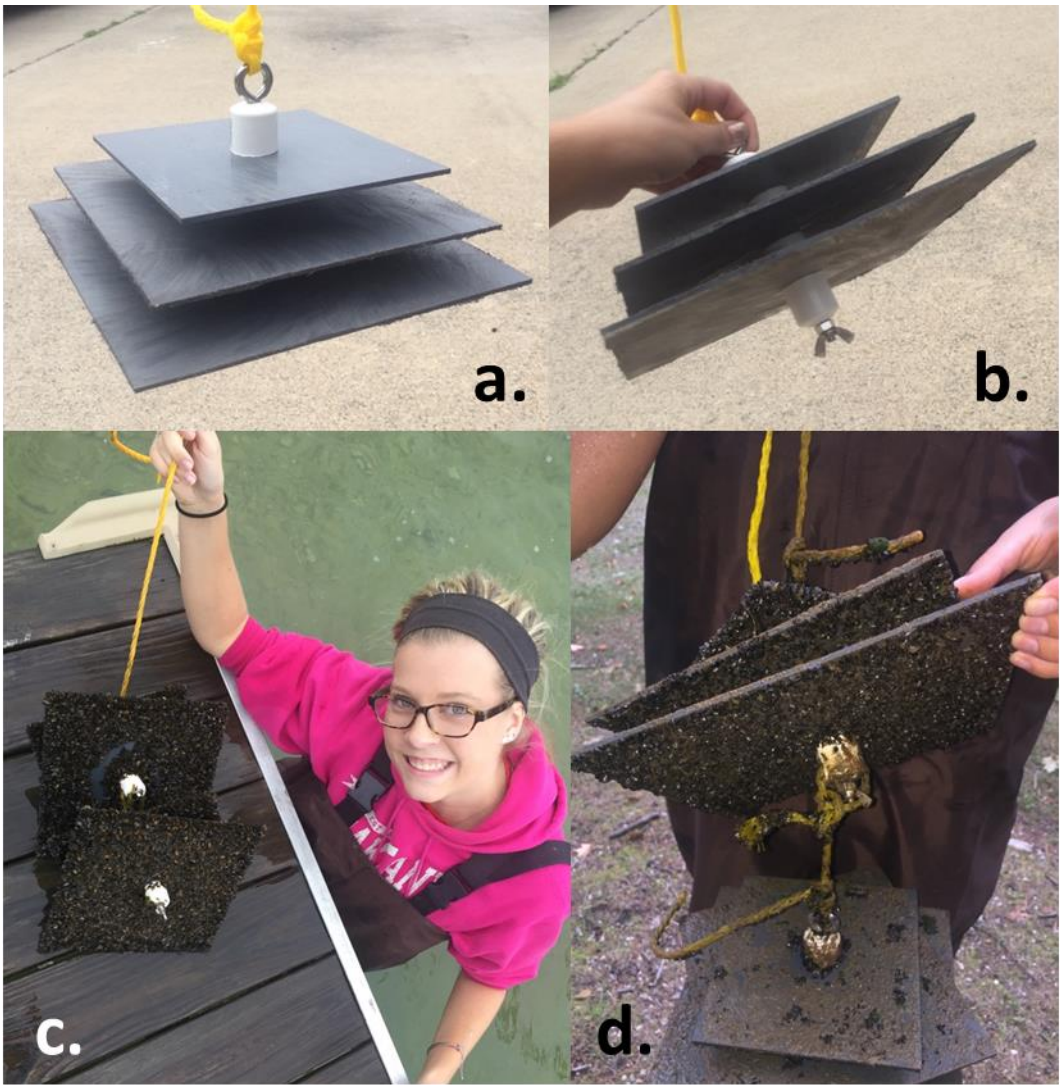

Figure 8. Method for measuring mussel settling rates at each site. Samplers were built from stacks of gray PVC plastic plates. Two samplers were suspended in the water column at each site, either from a fixed dock or a floating buoy.

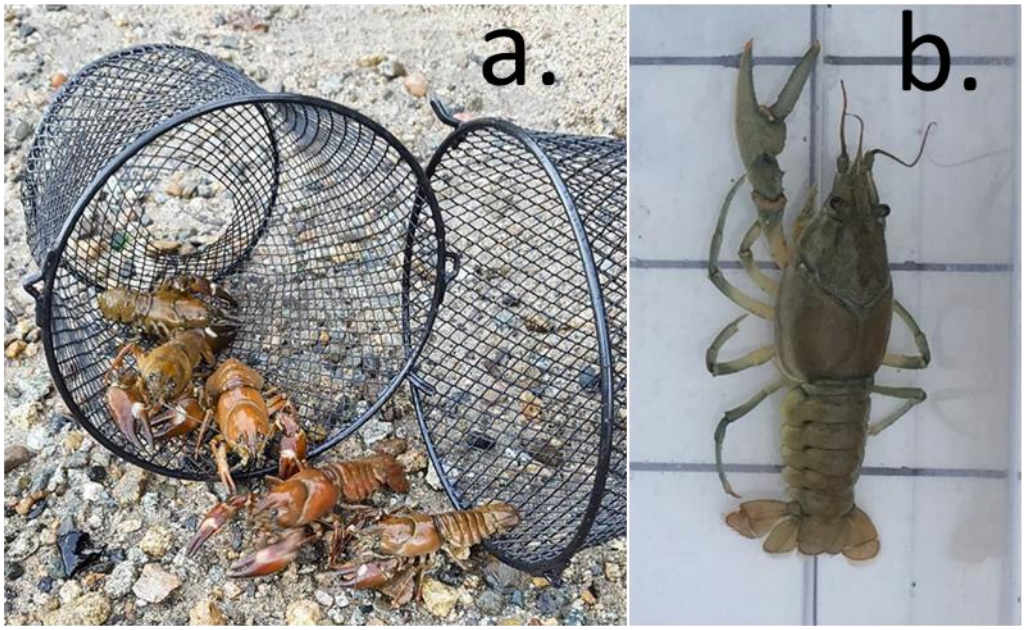

Figure 9. Crayfish sampling with a baited minnow trap. We set three traps overnight at each site on two separate occasions. Each was baited with tuna. Total of 6 trapping nights per site. Photo “a” from: www.vancouverisawesome.com. Photo “b” taken at Little Traverse Lake by Aleena Hajek.

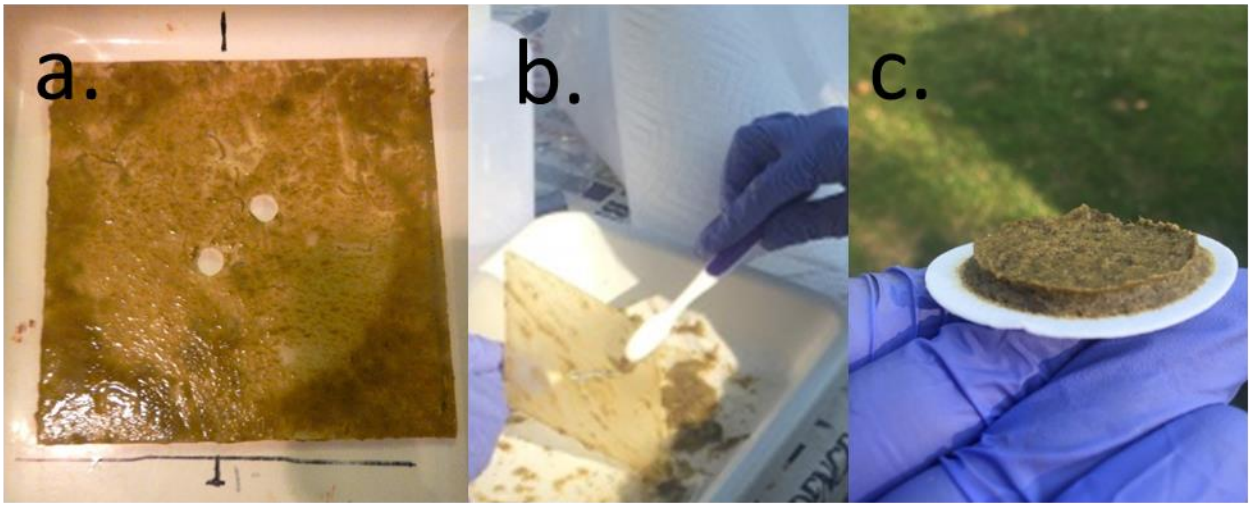

Figure 10. Periphyton sampling methods. Three roughened plexiglass plates were floated horizontally in the water column at each site, at a standard depth, and left for a set period of time. They were then scrubbed clean and any organic material was vacuum filtered onto filter paper. We then extracted chlorophyll from the sample (methanol extraction) and measured relative fluorescence units (RFU) with a 96-well plate fluorometer.

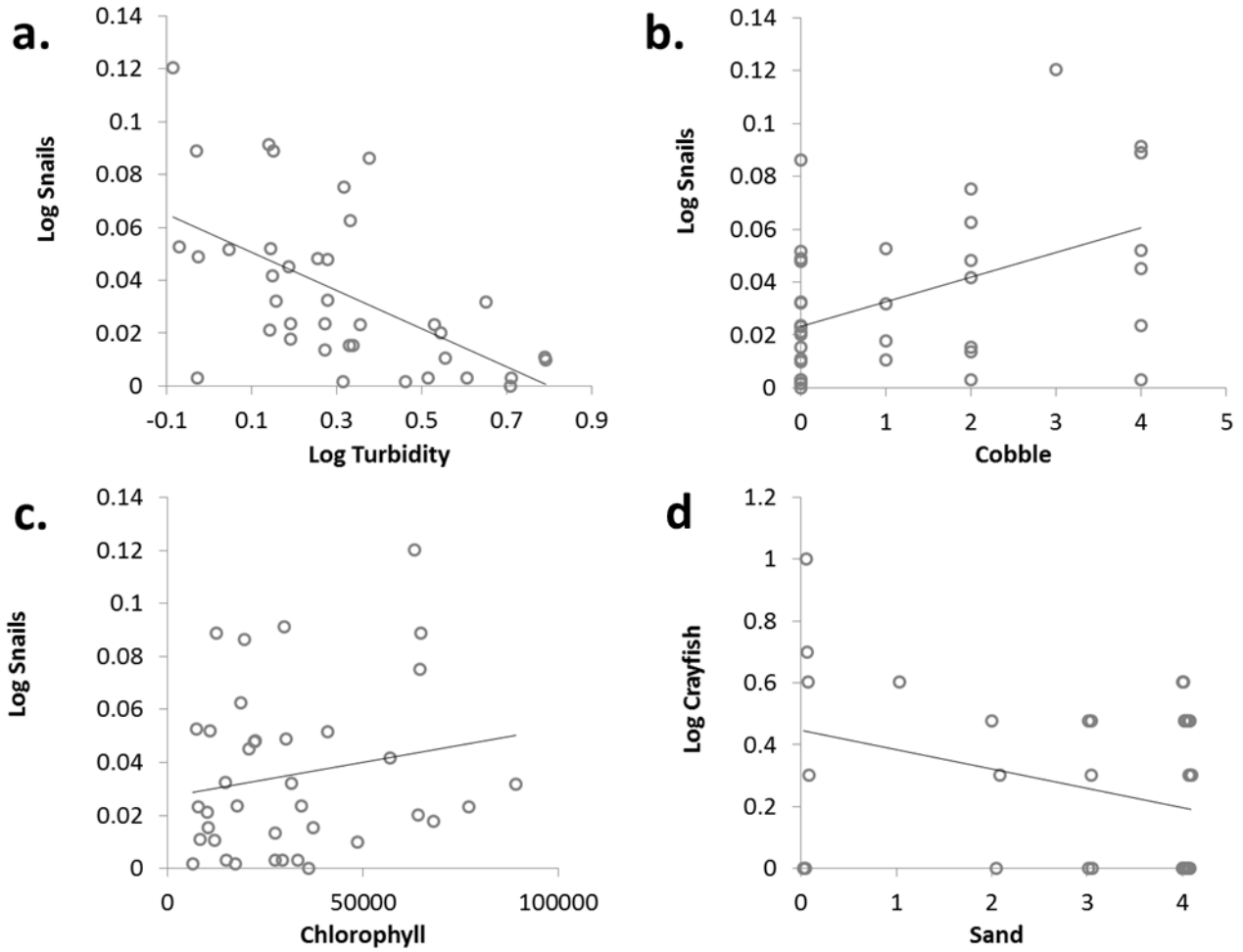

Figure 11. Preliminary results: predictors of snail and crayfish abundance. Showing relationships between: (a) snail abundance and water turbidity; (b) snail abundance and an index of cobble substrate; (c) snail abundance and periphyton growth (relative fluorescence units: no significant correlation); (d) crayfish abundance and an index of sand substrate.

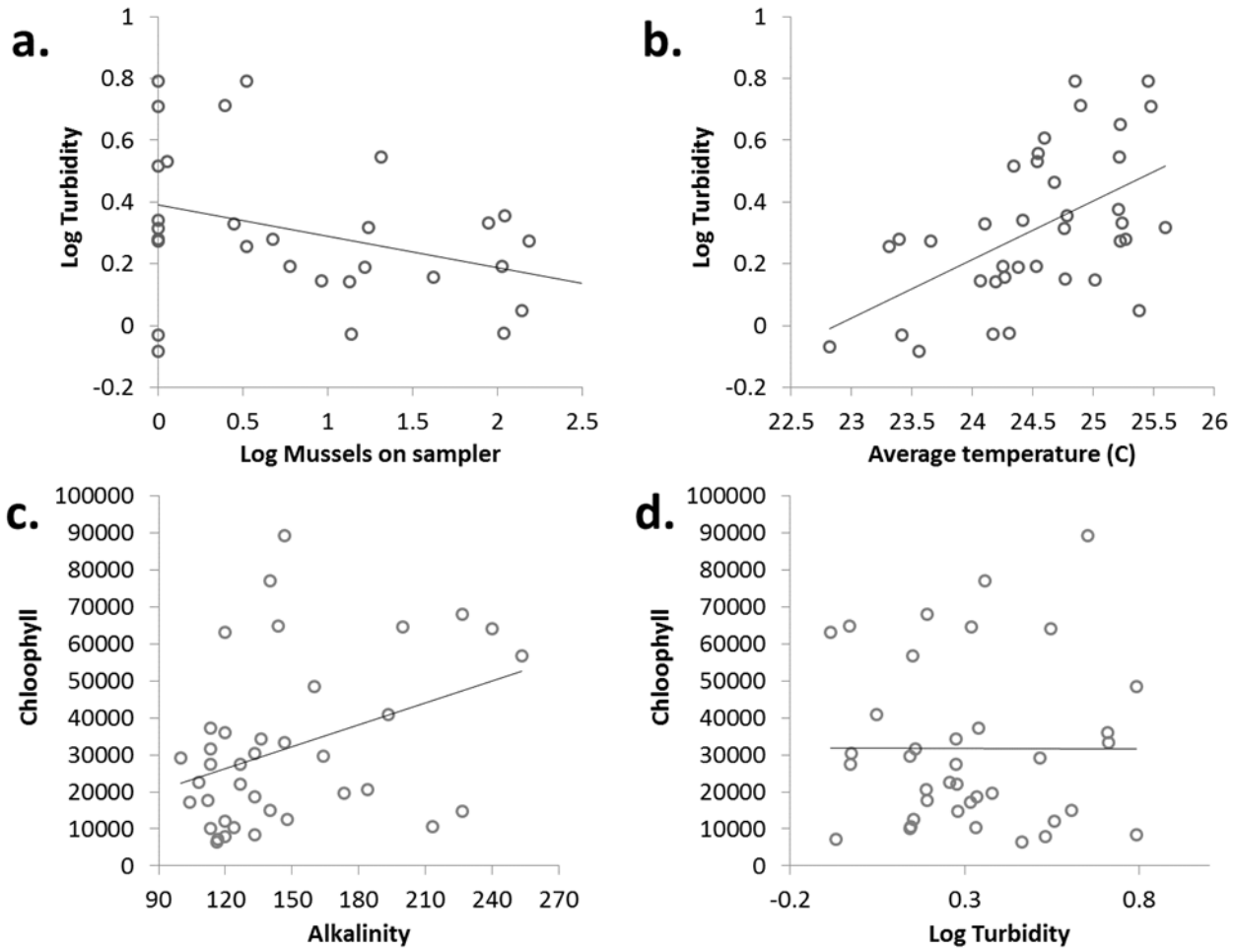

Figure 12. Preliminary results: predictors of water turbidity and periphyton (attached algae) growth on tiles. The best predictors of turbidity were (a) mussel abundance on samplers and (b) average temperature. Chlorophyll was (c) weakly correlated with alkalinity and (d) NOT correlated with water turbidity.

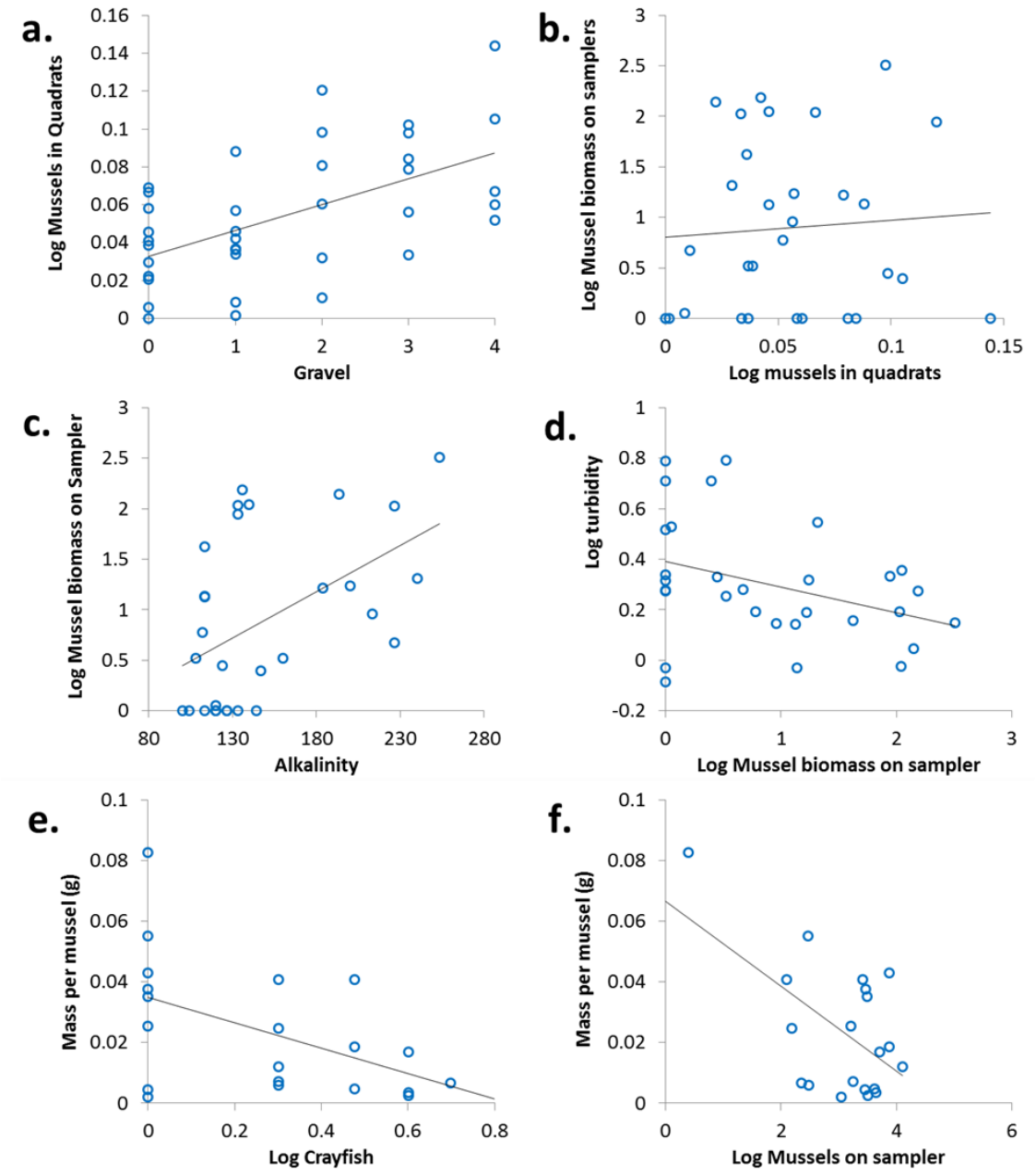

Figure 13. Preliminary results: predictors of quagga/zebra mussel abundance in quadrat surveys (established adult mussels) and on mussel samplers (mussel settling rates). (a) Relationships between: (a) adult densities at local sites and an index of gravel substrate in littoral zone; (b) mussel biomass on samplers and mussel densities in quadrats; (c) mussel biomass on samplers and alkalinity; (d) mussel biomass on samplers and water turbidity; (e) mass per mussel on samplers and crayfish abundance; (f) mass per mussel on sampler and mussel density on sampler.

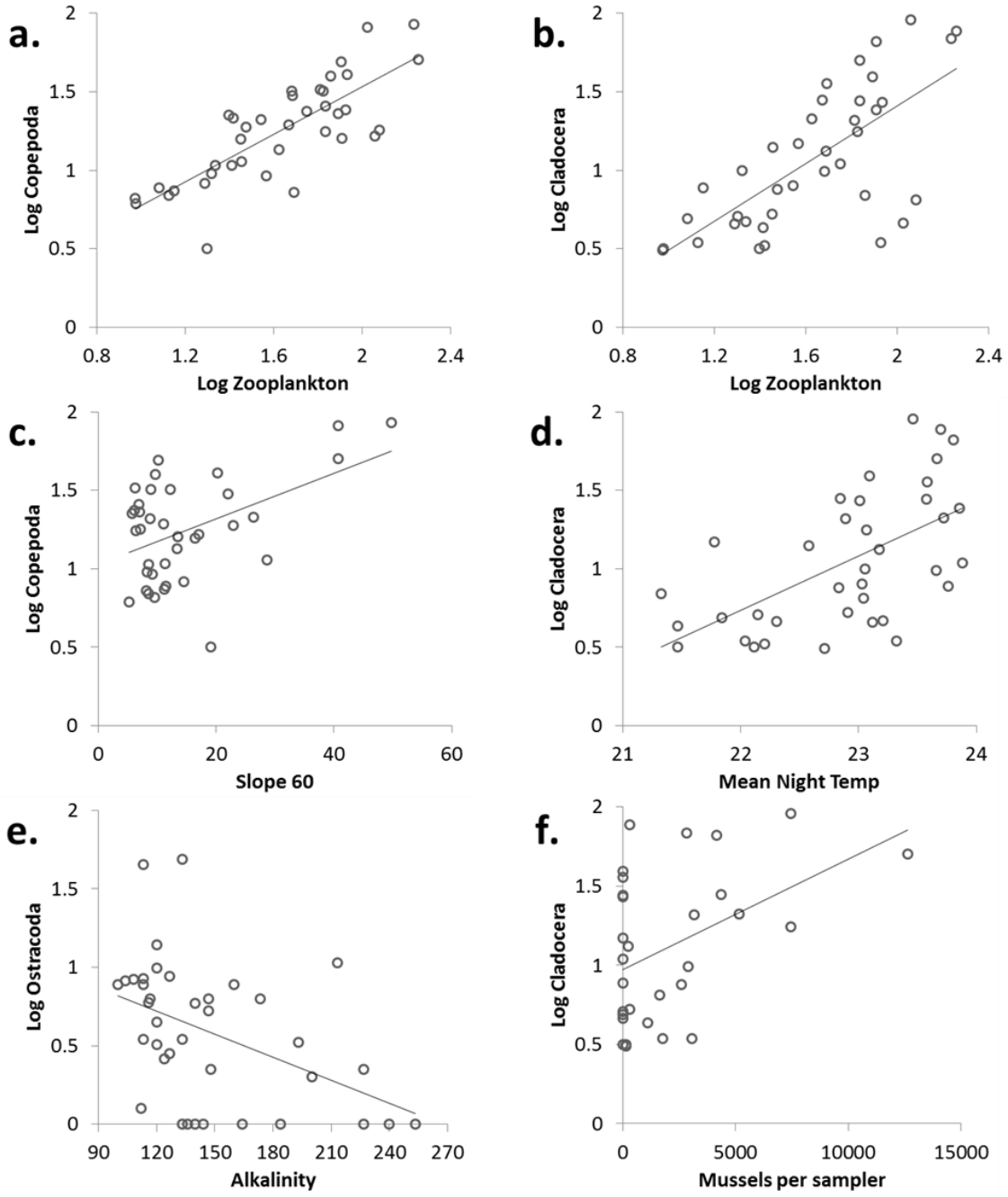

Figure 14. Preliminary results: patterns for specific zooplankton taxa, including: (a) relationship between copepods and total zooplankton; (b) relationship between cladocerans and total zooplankton; (c) relationship between copepods and shore slope; (d) relationship between cladocerans and nighttime temperature; (e) relationship between ostracods and water alkalinity; and (f) relationship between cladocerans and mussel settling rates.

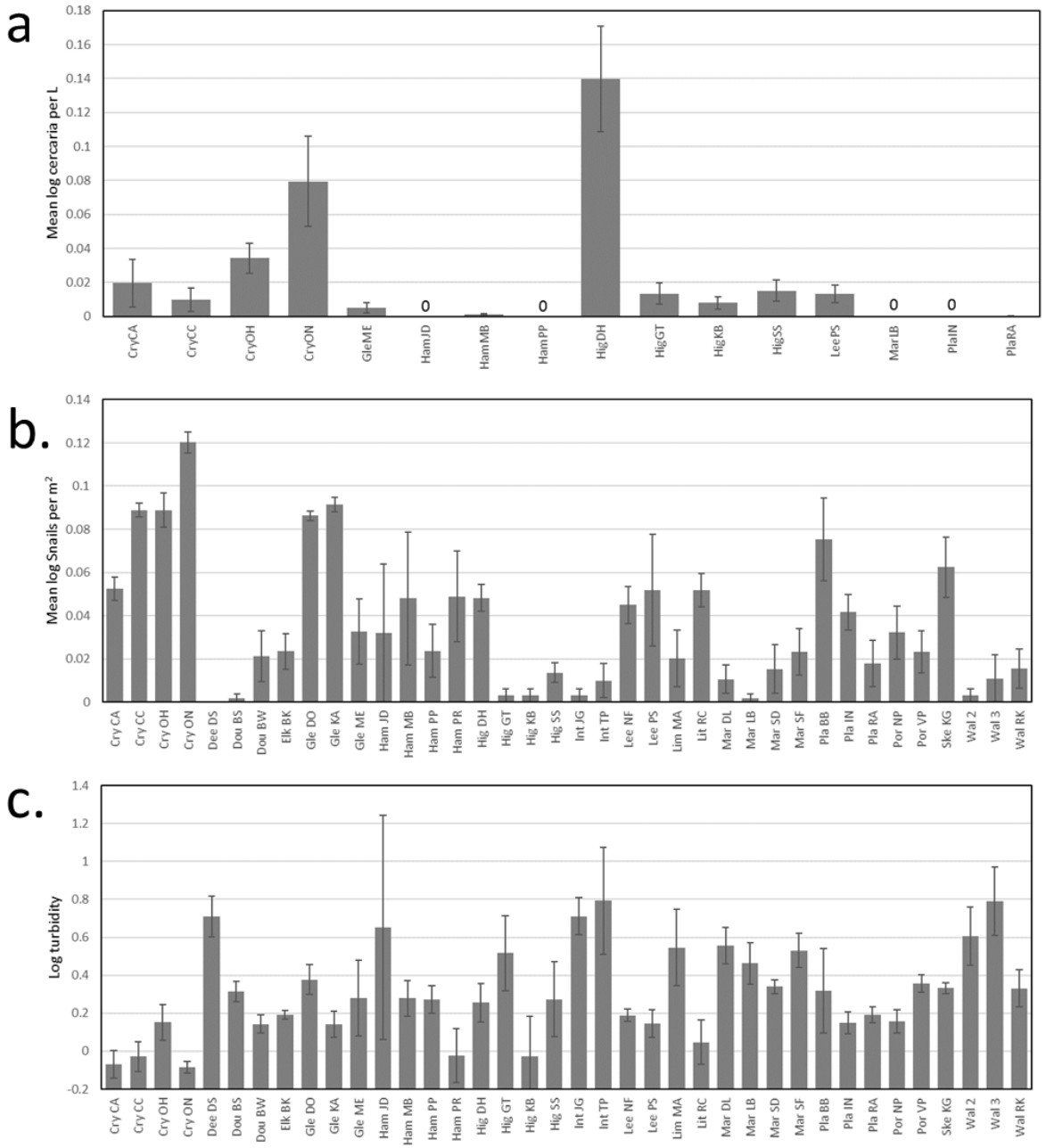

Figure 15. Site-level measurements: (a) cercaria abundance in the water (**INCOMPLETE: approximately half of samples have been fully analyzed using qPCR**); (b) total snail abundance (average over 3-5 sampling occasions); (c) turbidity of the water (average of samples over 3-5 sampling occasions). Error bars = SE.

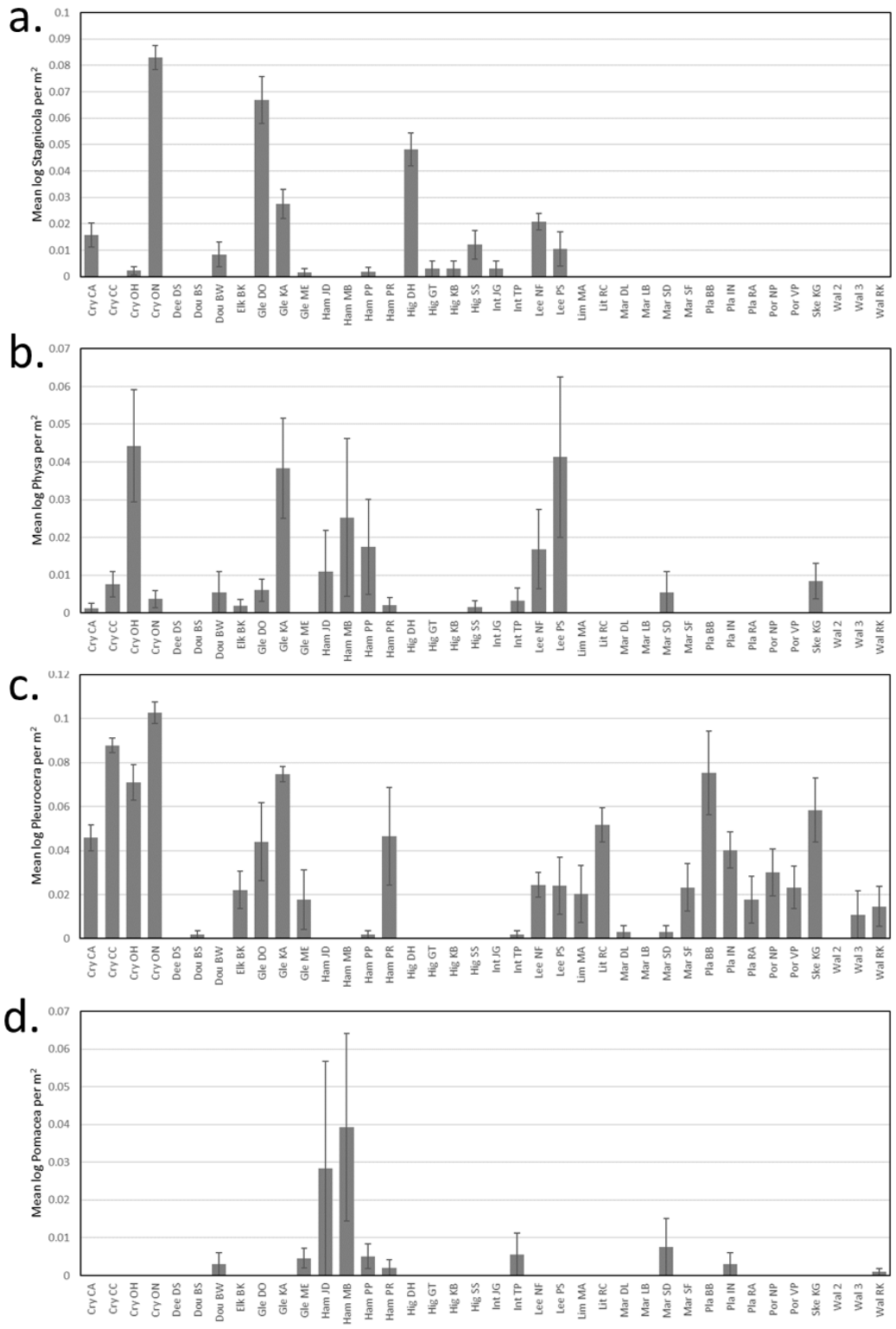

Figure 16. Site-level measurements of four specific snail taxa: (a) *Stagnicola* spp; (b) *Physa* spp.; (c) *Pleurocera* spp.; (d) *Pomacea* spp. Error bars = SE.

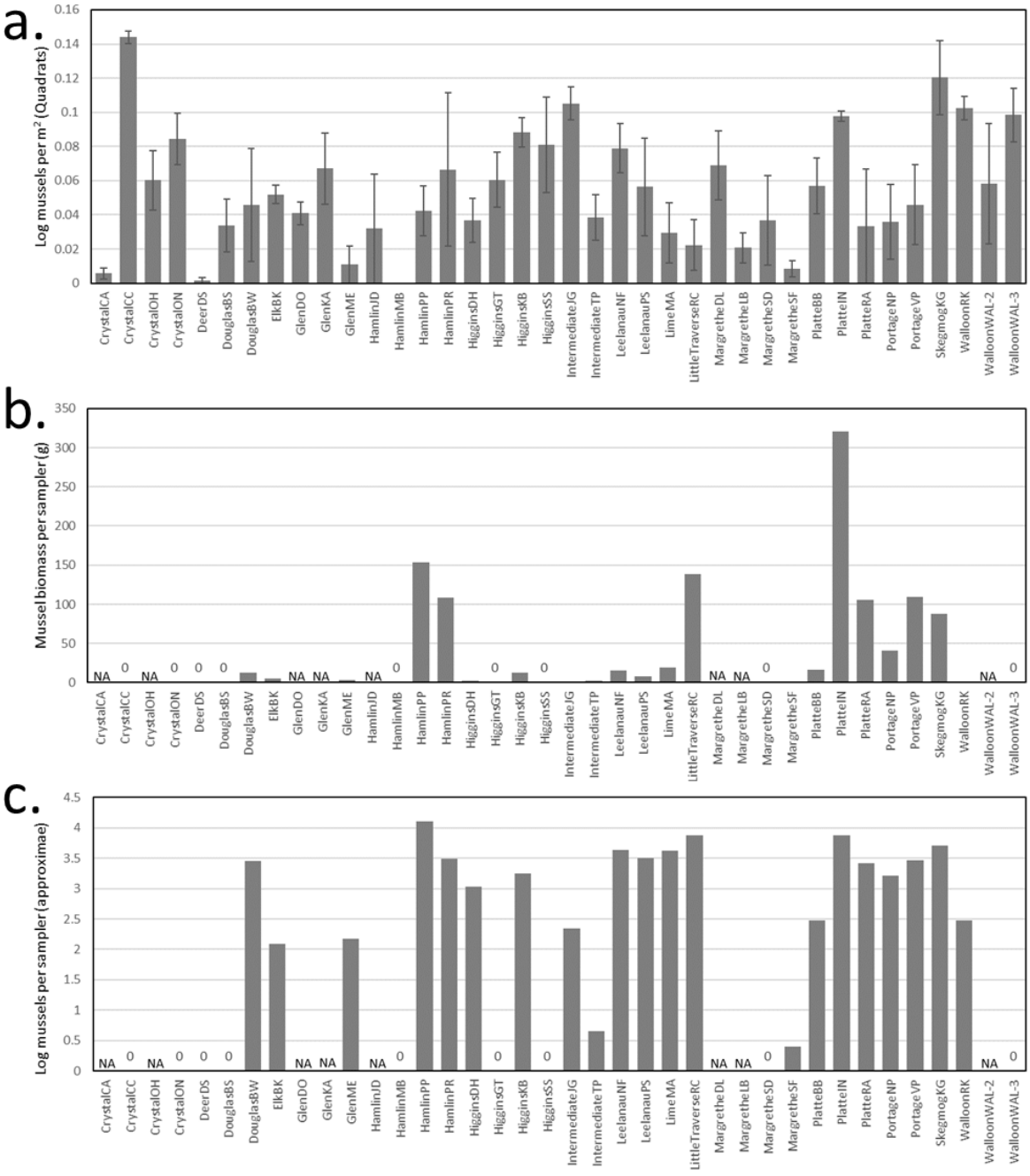

Figure 17. Site-level measurements of mussel abundance: (a) adult mussels in quadrat samples (average of 3-5 sampling occasions); (b) average biomass of mussels settled on each sampler; (c) approximate number of mussels settled on each sampler. Error bars = SE. "NA" indicates a missing datapoint (usually due to a missing sampling device).

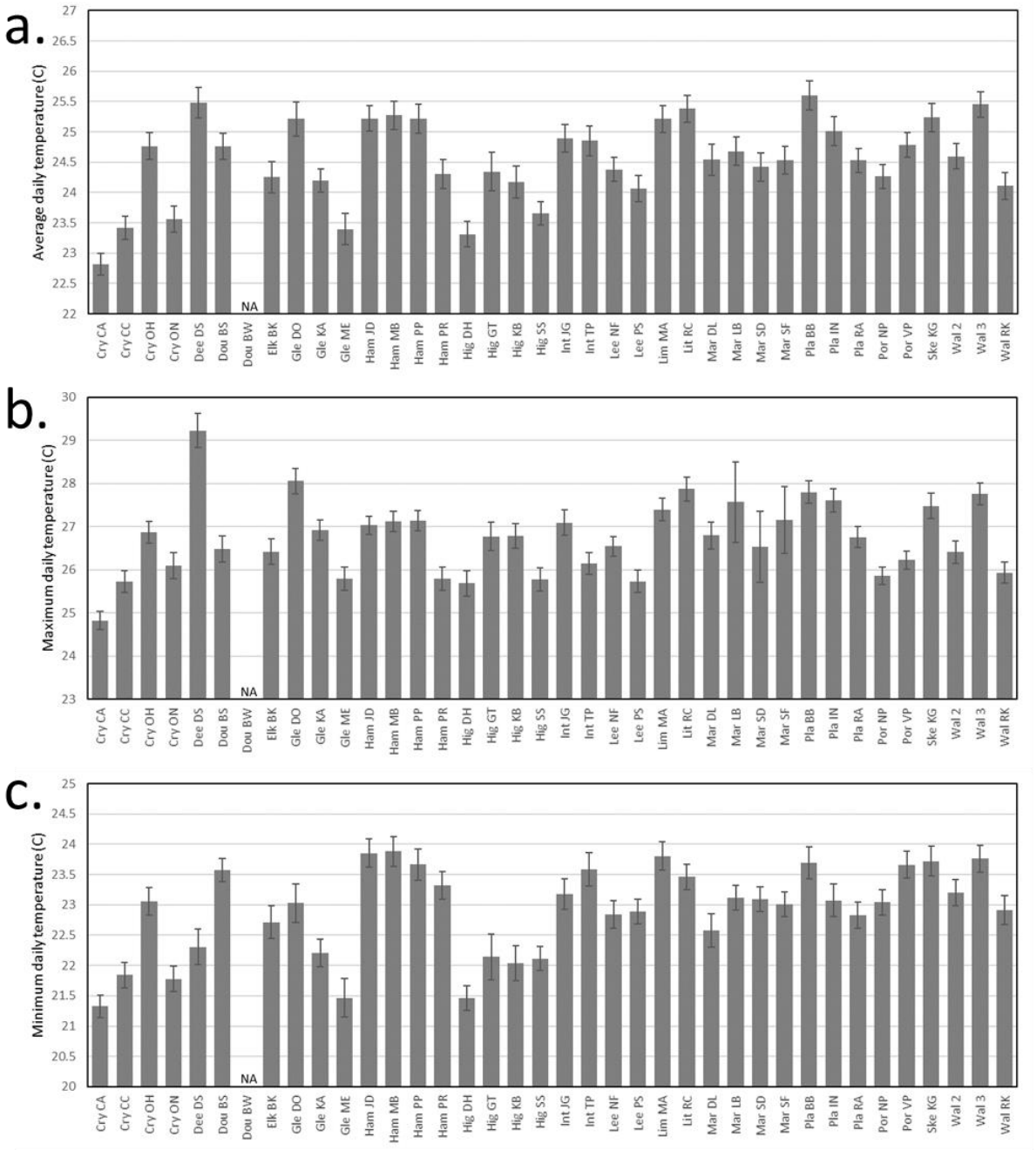

Figure 18. Site-level water temperature measurements: (a) average temperature; (b) mean of maximum daily temperature; (c) mean of minimum daily temperature. Error bars = SE for sites with multiple samples. "NA" indicates a missing datapoint (both data loggers were lost).

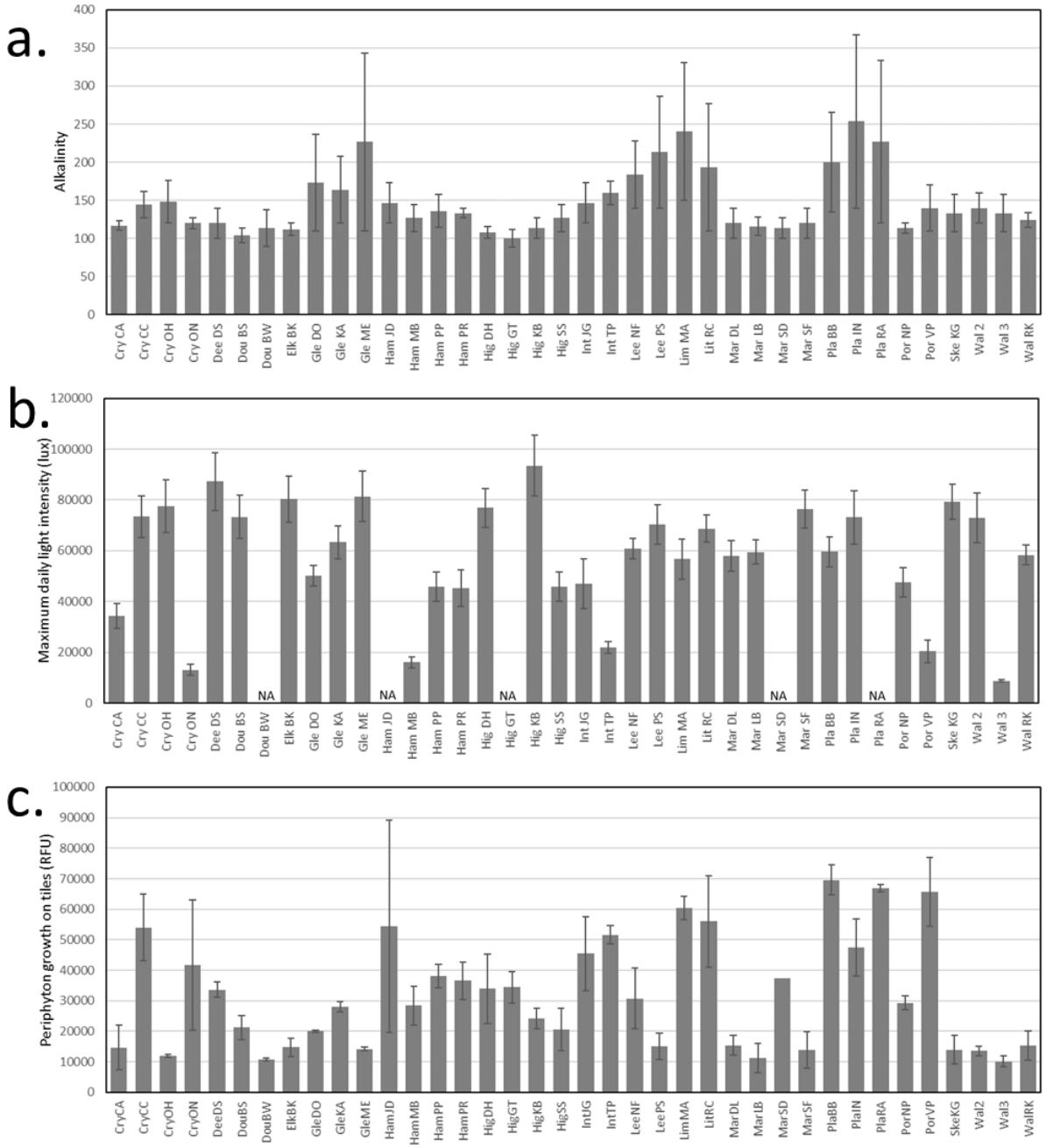

Figure 19. Site-level measurements: (a) water alkalinity (average of 3-5 sampling occasions); (b) light intensity; (c) periphyton growth (average relative fluorescence units of algae from 1-2 samplers). Error bars = SE for sites with multiple samples. "NA" indicates a missing datapoint; five light loggers were lost during the course of the survey.

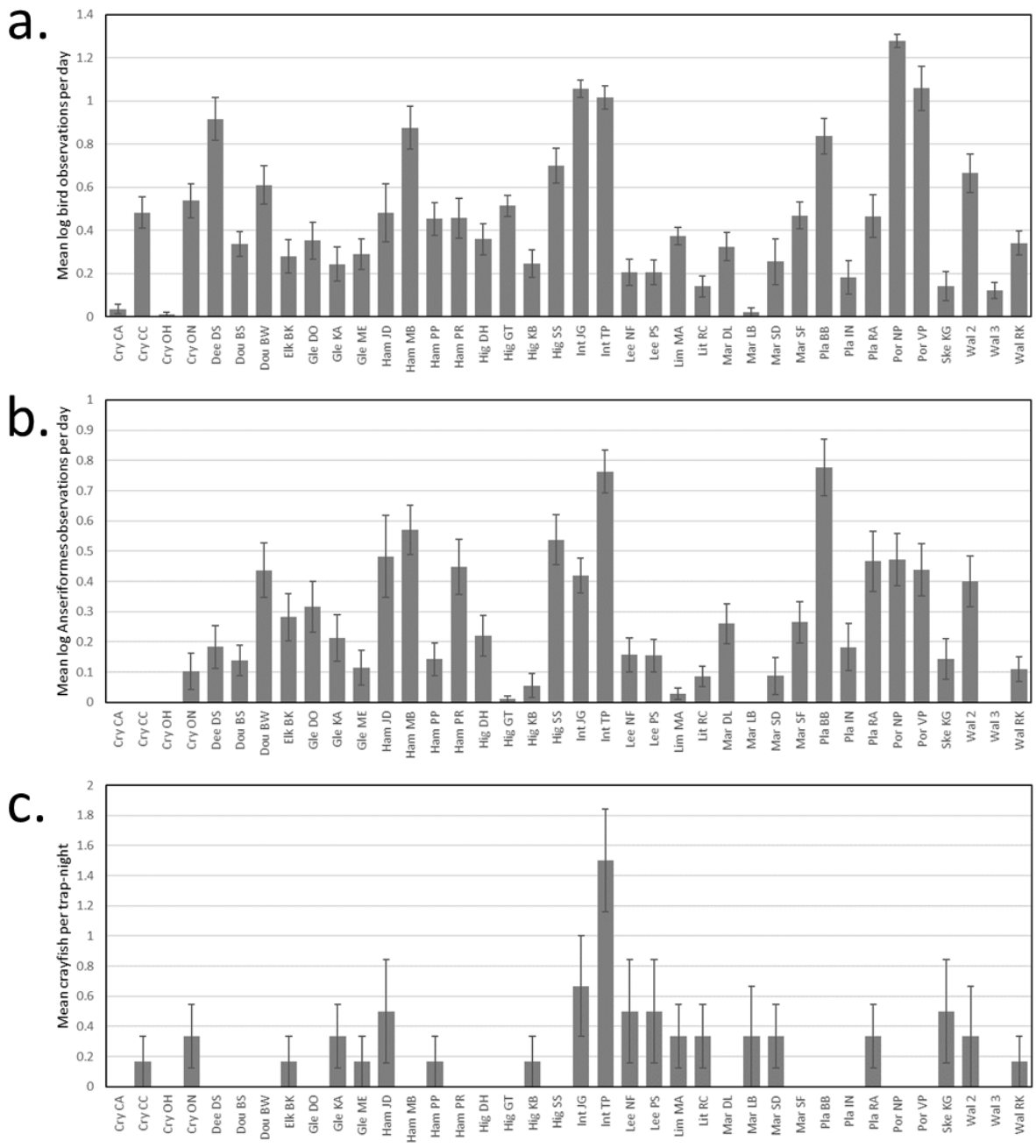

Figure 20. Site-level abundance of birds and crayfish: (a) total birds daily abundance (volunteer observations); (b) duck & goose daily abundance (volunteer observations); (c) crayfish abundance (mean across 6 trap-nights). Error bars = SE.

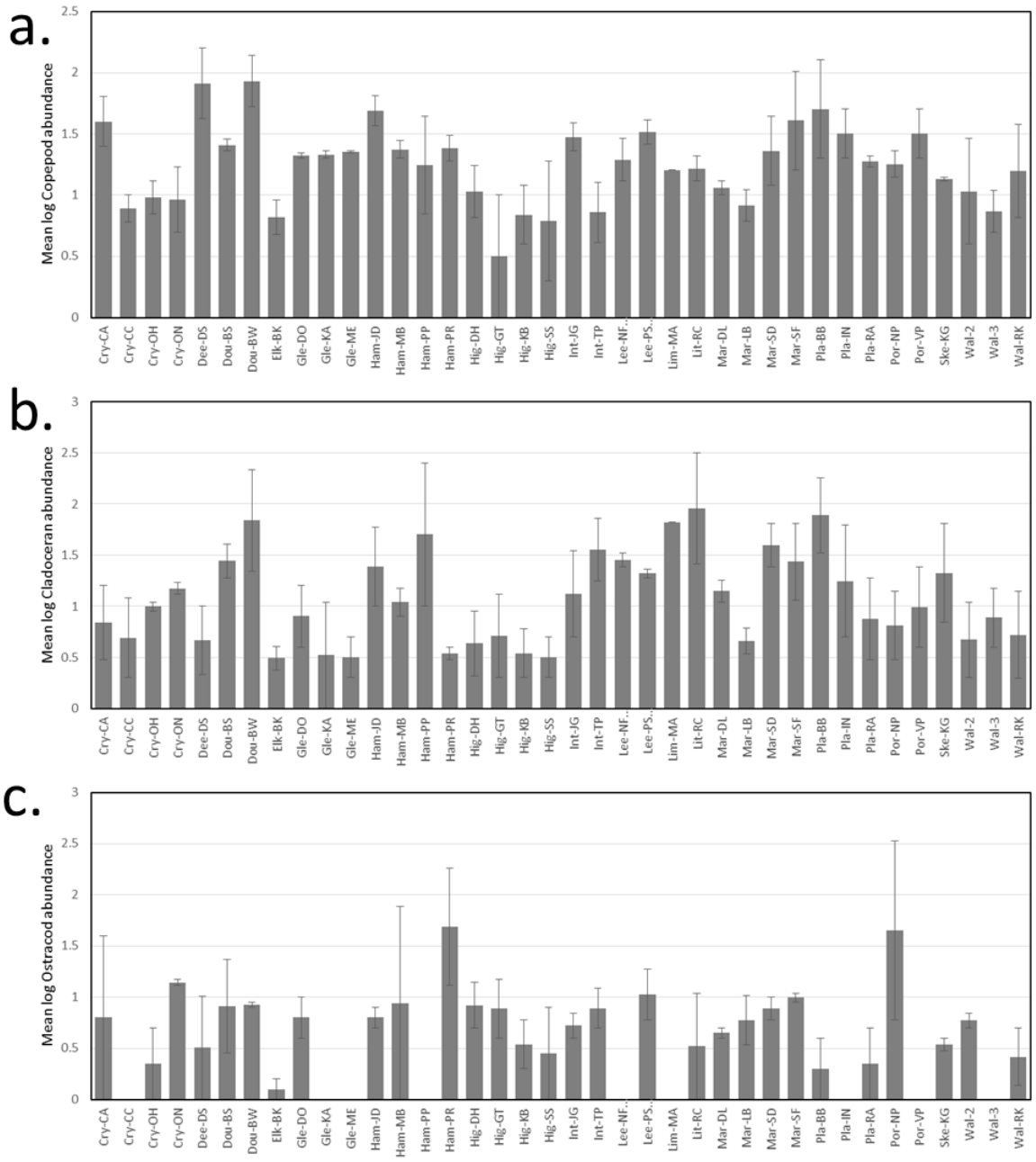

Figure 21. Site-level measurements of zooplankton taxa from tow-net sampling. (a) Copepoda; (b) Cladocera; (c) Ostracoda.

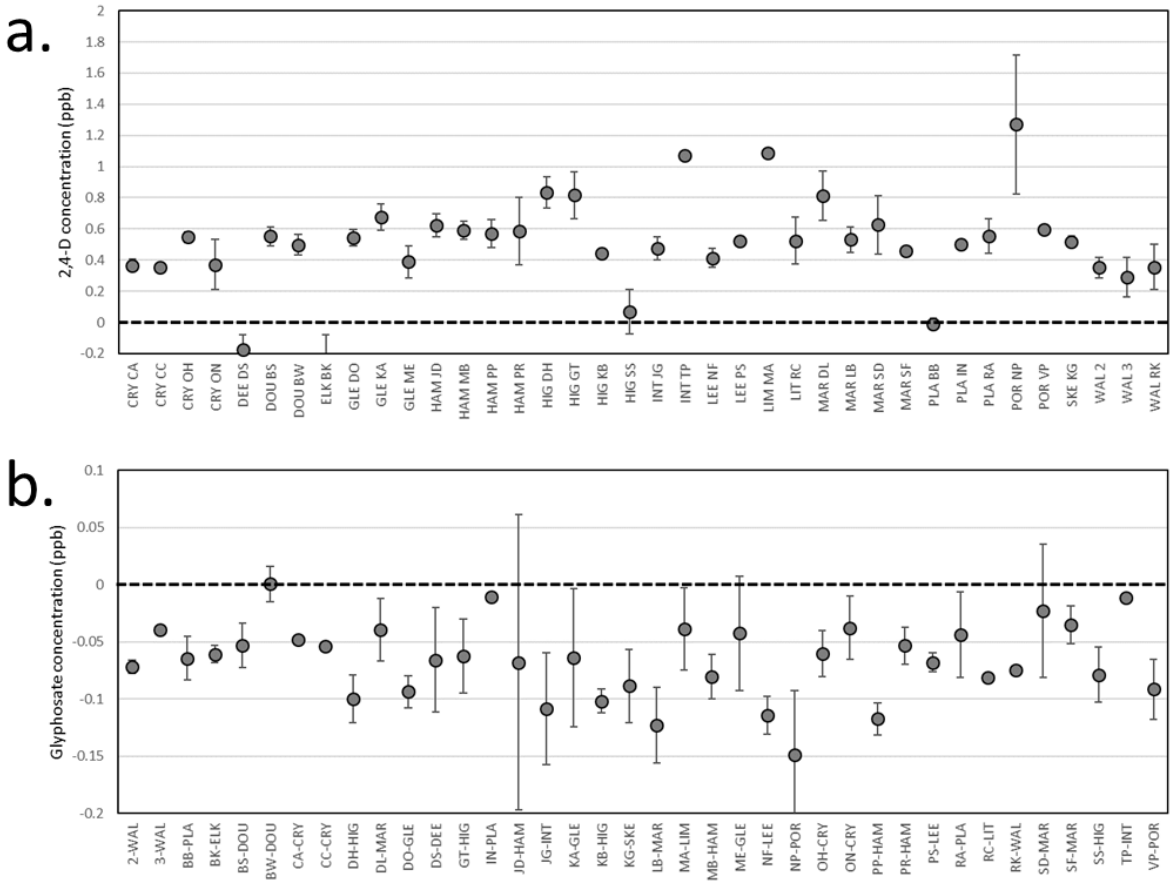

Figure 22. Site-level ELISA measurements of two commonly applied herbicides. (a) 2,4-D (active ingredient in most "Weed & Feed" lawncare products and many anti-weed products used in lakes); (b) Glyphosate (active ingredient in Roundup). Although many sites had detectable (but low) levels of 2,4-D, NO sites had detectable levels of glyphosate in the water. This might be due to its very short half-life. Also note the dashed horizontal line, which represents the expected ELISA result for negative controls. Values less than the dashed line are effectively zeroes (no detectable reaction). Error bars = SE.