Evaluating the efficacy of environmental DNA (eDNA) as an early detection tool for the Mohawk Watershed's newest aquatic invader, the bloody-red shrimp, *Hemimysis anomala*.

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Invasion history

The bloody-red shrimp *Hemimysis anomala* (hereafter BRS) is a recent Ponto-Caspian aquatic invasive species that was first reported in 2006 in Lakes Ontario and Michigan (Pothoven et al, 2007; Walsh et al, 2012) and has now become firmly established in the Great Lakes, St. Lawrence River and other inland lakes of New York, including Oneida, Cayuga and Seneca Lakes (Brown et al., 2014). Our research team most recently discovered multiple reproducing populations of BRS in the Erie Canal and Mohawk River as far east as Waterford, NY (Brown et al., 2014; Boscarino, unpubl.), These results strongly indicate that the Erie Canal and Mohawk River are serving as major vectors of spread for this species towards the Hudson River.

Importance of early detection in the case of BRS

This project seeks to develop an effective early detection method for BRS as they continue their expansion throughout the Hudson-Mohawk River watershed. Early detection is critical for management success and to limit the cost of control measures (Anderson, 2005; Vander Zanden, 2010). Efforts to detect non-native species in the early stages of an invasion are often hindered by inadequate sampling methods that are often cost-ineffective or simply ineffective at low densities. These constraints lead to most species being detected well after they have become established and control options at that point are limited or nonviable (Crooks and Soulé, 1999). At present, we lack a reliable method for detecting BRS at low densities or sampling for organisms like BRS that inhabit tight, rocky interstitial spaces on the benthic floor during the day. BRS are difficult to detect by routine sampling, as the species typically does not enter the water column until twilight, initial densities can be low and most lake-users lack the net systems used by researchers to sample zooplankton populations. Importantly, BRS migrate from rocky crevices and man-made structures after dark, with ontogenetic differences in the timing and extent of their movement, which make detection, accurate density estimates and demographic analyses a further challenge (Boscarino et al, 2012).

Evaluating the effectiveness of environmental DNA (eDNA) to detect the presence of BRS in invaded systems

Background on eDNA as a research tool

We hypothesize that the application of molecular tools (environmental DNA) can detect the presence of BRS more reliably and with less effort than using traditional surveying techniques. Environmental DNA is a new technique with high potential for invasive-species detection work. Species-specific DNA fragments are cast into the environment through feces, urine, and the shedding of cells, and can be detectable for days to weeks following an organism's presence (Thomsen et al, 2012). This technique has been successfully used to delimit non-native species range expansion (Ficetola et al, 2008; Jerde et al, 2011; Thomsen et al, 2012; Goldberg et al, 2013). Although initial work concentrated on fish and amphibians in aquatic systems, application to invertebrates has also been fruitful (Thomsen et al, 2012; Goldberg et al, 2013).

BRS-specific eDNA methodology and protocol

In this study, we performed a series of controlled laboratory studies to determine the sensitivity of eDNA detection to variations in BRS density and length of time in water samples. We aimed to develop a relationship between density and eDNA concentration, using literature-established sequencing detection methods to help guide our initial explorations (Goldberg et al., 2013). Individual BRS for use in our experiments were sampled from Seneca Lake and Cayuga Lake, New York and live BRS were then stored in three 15 gallon aquaria. We compared several

different mitochondrial-DNA (mt-DNA), ribosomal-RNA (rRNA), and nuclear DNA sequences of different haplotypes of BRS and a variety of other mysids, including the native *Mysis diluviana,* through further literature searches. This allowed us to identify several potential sequences unique to BRS that could be used as eDNA primers in PCR amplification. Several organisms from our stock tanks were used in a DNA extraction protocol to establish a store of pure BRS DNA. We created a number of quantitative PCR plates that tested the effectiveness of various primers and ran them each for 40 thermal cycles. Quantitative PCR was chosen over other methods because it can be considered, observed and interpreted in real-time, allowing the researcher to see when signals reach a threshold that to be considered a positive (i.e, positive detection) result. This allows us to determine primer sensitivity, and determine overall efficacy of each primer as suitable for detecting BRS and at which densities it requires. Ultimately, while the rRNA primers were the most sensitive (i.e., samples tested with the rRNA primers were amplified to a detectable level most quickly in the thermal cycles), we found that the mt-DNA primers were the most consistently reliable options. We used mt-DNA primers in all future qPCR tests, specifically primers located on subunit 1 of a BRS cytochrome oxidase (COI) gene. Initially, we ran the various primers at a temperature gradient in order to determine the most effective annealing temperature for the DNA. It was determined that the range of temperatures between 54ºC and 55ºC were the ideal annealing temperature for the COI primer we selected. Once the proper primers had been determined, we tested several different dilutions (1-8 parts per hundred) of the collected BRS DNA to determine at what concentration eDNA results became unreliable as a detection method. Additionally, we performed DNA extraction on vacuum filtered water samples of various volumes from the aquaria holding the sample BRS populations (quantities of 100mL, 250mL, 500mL, and 750mL were drawn through filters that we then performed DNA extraction protocol on). The vacuum experiments were performed to determine whether and at what quantities water samples could be used to detect presence or absence of BRS.

Discussion

In performing the dilution experiments, our goal was to determine the smallest concentration of DNA that could effectively be used in PCR amplification to detect the presence of BRS. We found that a concentration as little as one "micromolar DNA" could be detected reliably in a PCR well. This established a baseline for future experimentation. In our filtered water experiments, we found that volumes of water as low as 250 mL could be vacuumed through a filter and reliably be used to detect presence of BRS. However, it should be noted that the samples were taken from relatively small tanks, all with moderate to high densities of BRS, and that samples taken from the field would likely only yield reliable results with a higher volume of water being filtered. Future experimentation would require several field validation experiments and further exploration for ideal primer pairs.

Our results indicate that eDNA has strong potential as a method of detection for BRS in invaded systems. While we have not as of yet obtained a substantial body of field validation results, we believe that the use of these primers on the COI gene are most conducive to reliable results. Future field validation experiments will help tease apart whether this technique could be used as an early detection tool (i.e., detect presence of BRS at low densities) or simply as a tool to monitor established spread without the need for more costly and labor intensive plankton net sampling. Given the relative ease of collecting a water sample versus having the proper net equipment and pier access to engage in plankton sampling, we feel strongly that at the very minimum, eDNA will be a great citizen science tool for BRS after field validation studies are completed and the PCR protocol fully developed.

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EXTRAS

We will test the efficacy of traps and environmental DNA (eDNA); both traps and eDNA have high potential for use in research and citizen-based monitoring programs. The applications of eDNA to invertebrate and to lotic systems are advancing rapidly (e.g., Deiner and Altermatt, 2014).

Our primary objective in this study is to investigate the efficacy of utilizing environmental DNA (eDNA) technology to help identify and inform early detection and rapid response protocols for BRS.

A major outcome of this objective will be our contribution to better monitoring protocols and citizen-science programs throughout the Great Lakes, Finger Lakes and Mohawk where the species range is rapidly expanding (addresses GLAA Goal 7).

The development and application of a low-cost trap and eDNA detection protocol are new techniques expected from our work. These will be useful to determine rates and modes of spread, as well as in risk assessment, throughout the Great Lakes basin and beyond. Traps and molecular tools will also boost citizen-science programs.