PRESENCE AND TROPHIC LEVEL OF FRESHWATER JELLYFISH (*CRASPEDACUSTA SOWERBII*), A CRYPTIC INVADER IN THE HUDSON RIVER BASIN, NY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The Freshwater Jellyfish (*Craspedacusta sowerbii*) is an invasive species that is relatively unstudied and underrepresented in public record due to the sporadic appearance of its observable medusa life stage. In New York State, Freshwater Jellyfish have been reported in over 110 bodies of water, which raises concern as prior research suggests several harmful interactions between *C. sowerbii* and invaded systems. This study had the dual purpose of both developing environmental DNA primers for *C. sowerbii* detection and investigating the trophic interactions of *C. sowerbii* with NY lake communities via stable isotope analysis. Sampling occurred June to September 2019, across ten lakes in the Hudson River Valley where *C. sowerbii* had been previously observed. Filtered water and sediment eDNA samples were collected, plankton tow nets pulled, and Hester-Dendy settlement plates were deployed to collect stable isotope specimens and test for *C. sowerbii* presence. eDNA samples were analyzed using real time qPCR. It was discovered that qPCR of eDNA in filtered water was the most sensitive detection method, and the least time-consuming method. Due to low catch of *C. sowerbii* medusae, stable isotope results were mostly inconclusive but may indicate that freshwater medusae do not feed on fish larvae as previously suggested. These findings could be used by scientists or managers who are seeking to track the current distribution of *C. sowerbii*, or who are interested in better understanding how the appearance of freshwater medusae may impact planktonic communities of interest.

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INTRODUCTION

Invasive species represent a growing threat to aquatic resources, displacing native species and altering ecological processes. Invasive impacts on native species radiate out to people who have social or economic investment in native systems (Pejchar and Mooney 2009). To combat these negative effects, scientists, managers, and citizens have invested significant finances and time in invasive species-focused programs; however, invasive species are typically characterized by generalist traits that make them widely successful and difficult to control. The Freshwater Jellyfish (*Craspedacusta sowerbii*) is an invasive species originating from the Yangtze River Basin, China, that has spread to every continent except Antarctica (Dumont 1994). Freshwater Jellyfish are not true jellyfish but are hydrozoans that exhibit similar life stages, including sessile polyp and free-swimming medusa stages (DeVries 1992). Freshwater jellyfish have demonstrated success in both dispersing throughout riverine systems and bypassing geographic barriers to invade new systems, and they appear to do so without any human intervention.

Most knowledge on the ecology and occurrence of *C. sowerbii* is derived from observations of the larger (5-25 mm) free-swimming medusae, rather than the tiny (0.5-2 mm) bottom-dwelling polyp stage (DeVries 1992; Jankowski 2001); however, formation of *C. sowerbii* medusae in a system is sporadic and unpredictable, and *Craspedacusta sowerbii* may exist only as polyps for years before they are reported as medusae (Dumont 1994; Fritz et al. 2009). It is commonly hypothesized that temperature is important to the appearance of medusae, although there are conflicting ideas whether rate of temperature increase (DeVries 1992) or simple value of temperature reached (Minchin et al. 2016) is more relevant. In any case, *C. sowerbii* has likely invaded significantly more freshwater

systems than presently recorded and may be influencing those aquatic ecosystems to an unknown extent.

Relatively few studies have been conducted on the ecology of Freshwater Jellyfish, but feeding experiments and gut-content analyses suggest that the species demonstrates size-selective feeding on zooplankton, and that could shift relative dominance among plankton taxa in a native community (Dodson and Cooper 1983; Spadinger and Meier 1999; Smith and Alexander 2008). Feeding experiments have also demonstrated that *C. sowerbii* is capable of killing and eating larval fishes, although it is currently unknown if medusae are significant predators of fish (Dendy 1978; Dodson and Cooper 1983; Smith and Alexander 2008). Polyp diet has been primarily associated with small crawling invertebrates, but they have been observed feeding on larval fish under experimental conditions (Bushnell and Porter 1967; Dendy 1978). Although their ecological role is currently uncertain, *C. sowerbii* have the potential to disrupt food webs and harm native aquatic organisms wherever they occur.

In New York State (NYS), there have been reports of Freshwater Jellyfish in over 100 different systems (Peard 2018). Despite the widespread occurrence of *C. sowerbii* in NYS, species information is not found on the NYS Department of Environmental Conservation website (NYSDEC 2019). Presently, *C. sowerbii* is severely understudied by scientists, and its spread is unchecked by managers; it most likely will continue to spread and impact additional lake ecosystems as increasingly warm summer temperatures open new opportunities for the species.

Study Objectives

The primary objective of this study was to test new detection methods for Freshwater Jellyfish. The hypothesis addressed in this objective is that genetic material sloughed off by *C. sowerbii* polyps and medusae will be detectable through environmental DNA (eDNA) methods, and eDNA methods will be more sensitive than other detection methods such as plankton net tows and settlement plates. Environmental DNA techniques detect free-floating genomic materials in water or sediment rather than relying on the capture or observation of physical specimens. As a result, eDNA techniques are generally more sensitive than other detection methods, and rare or cryptic species like *C. sowerbii* can be detected in bodies of water where they might otherwise go unseen (Rees et al. 2014). Recent research has also shown that cnidarians, such as *C. sowerbii,* are excellent candidates for eDNA detection (Minamoto et al. 2017). DNA primers suitable for detecting eDNA from *C. sowerbii* have been developed in collaboration with Dr. Hyatt Green, State University of New York College of Environmental Science and Forestry (SUNY-ESF) using available sequences and tools from GenBank (see Methods, below). These primers were derived from published sequences on GenBank and assessed using BLAST and OligoAnalyzer software (GenBank 2019). Species-specific sensitivity tests were conducted, both virtually and in the lab, to reduce the risk of false positives in environmental samples. Successful testing of these primers with environmental samples would provide the NYSDEC and other organizations with the ability to conveniently sample water across the state to detect new invasions of Freshwater Jellyfish.

My second study objective was to use previously unapplied methods to

investigate the ecological role of *C. sowerbii* in invaded systems, particularly in the polyp stage. The hypothesis addressed in this objective is that stable isotope analysis of $\delta^{15}N$ and $\delta^{13}C$ values will indicate that *C. sowerbii* are feeding on larval fishes as suggested in past studies (Dendy 1978; Dodson and Cooper 1983; Smith and Alexander 2008). Past research has focused on gut content analyses and feeding experiments of medusae, both of which are limited in their ability to represent natural diet over time. Stable isotope analyses of different trophic levels in a system allows assessment of relatively longer-term dietary behavior of animals in their natural habitat (Hamilton et al. 1992).

METHODS

Primer Design

Before testing of *C. sowerbii* DNA could begin with physical samples, primer sequences had to be derived *in silicio* from existing sequences available in the GenBank database (GenBank 2019). *C. sowerbii* DNA sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) under the discontinuous megablast program. The *C. sowerbii* gene sequence selected to develop primers had the accession code LN901194.1 (mitochondrial genome, partial sequence). Physical characteristics (e.g., melting temperature, dimer potential) of candidate primers was further assessed by the IDT™ OligoAnalyzer tool. Candidate primers were also tested *in vitro* with DNA extracted from *C. sowerbii* tissue and multiple species of *Hydra* using real-time quantitative polymerase chain reaction (qPCR) analysis to check for undesired amplification. After these tests were concluded, the final primer sequences derived for testing on environmental samples amplify a product length of 146bp and are as follows:

Forward Primer: 5'- GAA TCA GAA TAG GTG CTG ATA GAG AAT C -3'

Reverse Primer: 5'- CTA ATC ACG GCC TTC CTT CTG G -3'

Field Sampling

Ten lakes within the Hudson River watershed were selected for this study (Figure 1), as 42 sites in the area have already reported sightings of *Craspedacusta sowerbii*. Five sites were in northern Adirondack areas, and five were located within southern areas of the Hudson River basin.

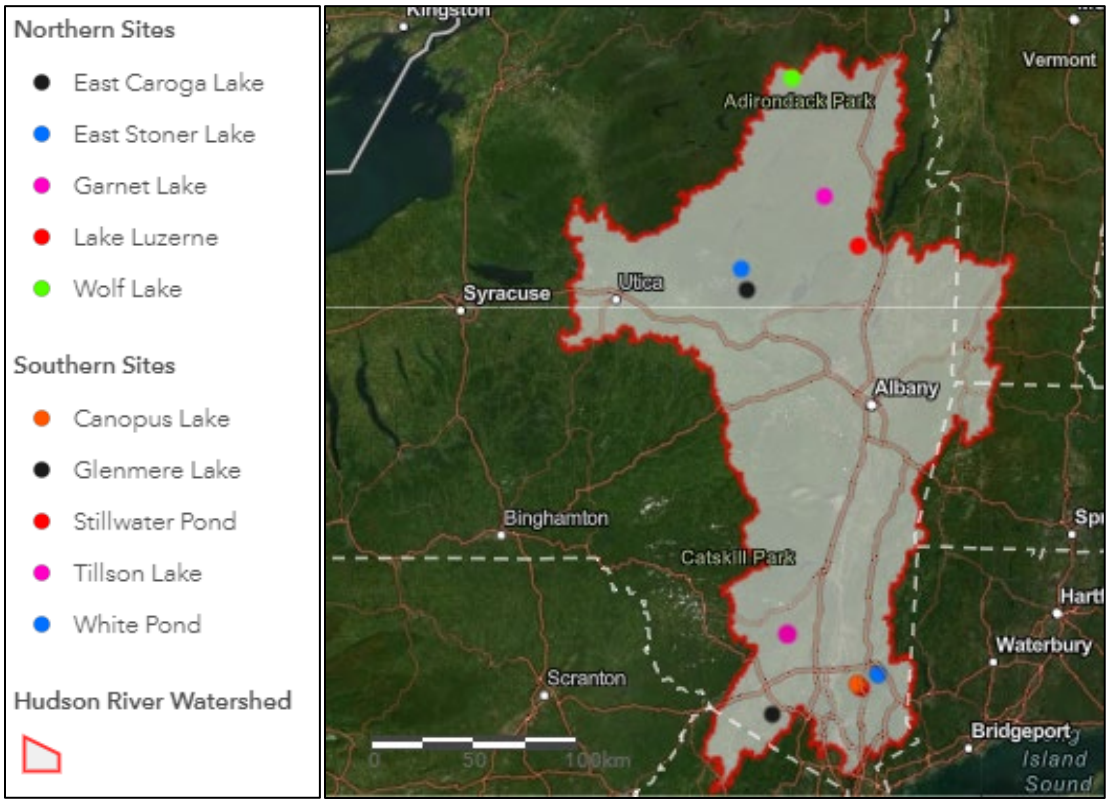

Figure 1: Field Sites for 2019 sampling season in the Hudson River Watershed.

Initial sampling began in mid-June 2019 and consisted of assessing vertical profiles of water quality with a YSI ProDSS multiparameter probe. From late June – early July 2019, each site was revisited to collect samples and deploy Hester-Dendy

settlement plate samplers (Figure 2). These 15 cm x 15 cm square settling plates were constructed based on a design tested successfully in the field and may be used as an alternate means to detect *C. sowerbii* polyps in a lake (T. Peard, personal communication). Each plate sampler consisted of six sanded acrylic plates with PVC spacers on a stainless-steel structure (Figure 2). Three plate samplers were deployed in each lake and georeferenced using a Garmin ™ Striker Plus 4 dual beam transducer (which also provided surface water temperature).

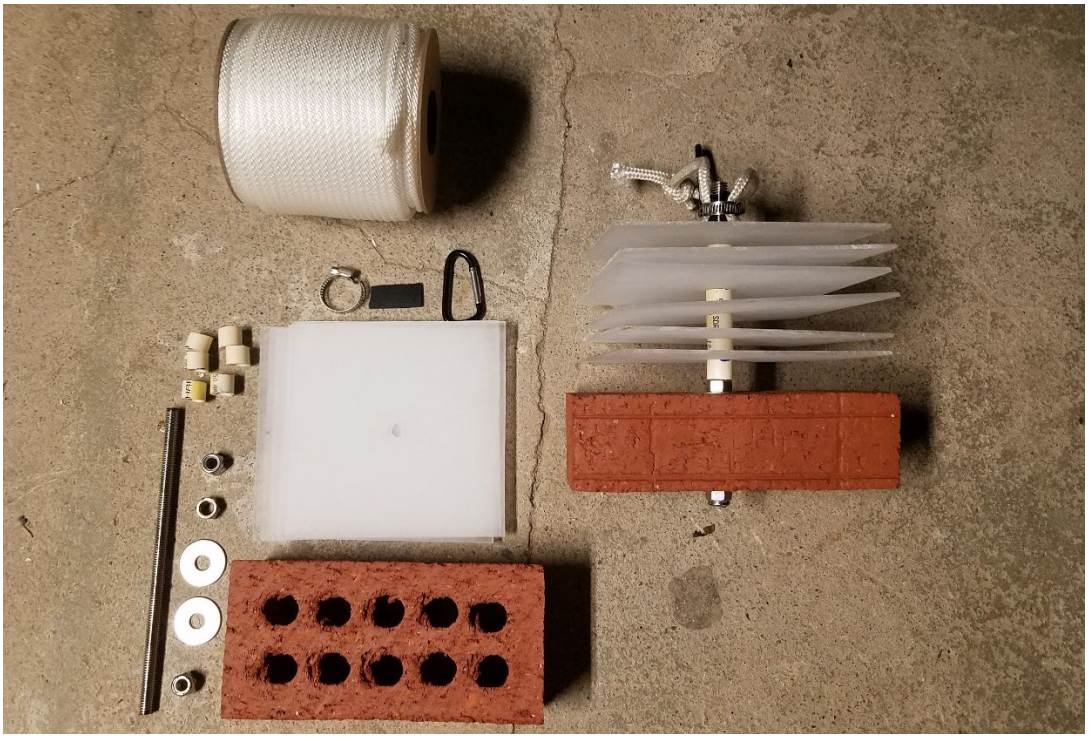

Figure 2: Hester-Dendy settlement plate design used in Summer 2019 sampling.

During deployment of settlement plates, plankton nets of 20 µm and 750 µm mesh sizes (Figure 3) were towed off the side of a canoe at 1.5 m depth for 5-10 min to collect zooplankton, fish larvae, and any medusae present. Nets were kept at depth with a 1.8 kg cannonball weight. Fish larvae and medusae densities were quantified with the 750

µm net using a General Oceanics 2030R mechanical flowmeter mounted in mouth of the net to determine volume sampled (calibrated in water flume at SUNY-ESF, June 11, 2019). Three 10-15 mL samples of surface sediment were also collected at each site with a handmade PVC-Steel gravity corer for eDNA analysis. Sterile technique was followed by soaking sediment sample gear in 10% bleach for at least 15 minutes between sites, and sample blanks were prepared at every site by measuring 15 mL of deionized water with the same gear used to measure sediment samples. Sediment samples were preserved in 3M sodium acetate and 95% ethanol on-site before transport on ice and storage at -80°C.

Figure 3: 20 µm (bottom) and 750 µm (top) mesh size nets that were towed horizontally to collect planktonic samples during Summer 2019.

Late-summer sampling occurred mid-August to mid-September 2019. Additional 750 µm plankton net tows were conducted for 5-10 min to collect large zooplankton, fish

larvae, and any medusae present. New vertical profiles of water quality were also conducted with the YSI probe. Three 2-L whole water samples were collected at each site and filtered onto 47 mm Whatman glass fiber filters in an effort to detect free-floating eDNA shed by medusae (excepting Glenmere Lake and East Caroga Lake, as they were not accessible at the time). Filter samples were put on ice immediately and stored at -80°C within 24 hours in Whirl-Pak® bags.

At this time, plate samplers were also retrieved and preserved in plastic bags with 70% ethanol for visual observations under a dissecting scope. If potential *C. sowerbii* polyps were observed on a plate, they were extracted with tweezers and placed in labeled vials of 95% ethanol at room temperature for later DNA confirmation using the Qiagen DNeasy™ Blood and Tissue Kit and SYBR qPCR analysis (Qiagen 2019).

eDNA assessment

Sediment eDNA samples were thawed and DNA was extracted using the MP Bio FastDNA™ SPIN Kit for Soil; eDNA samples on water filters had DNA extracted using the Qiagen DNeasy™ Blood and Tissue Kit. After DNA samples were extracted, total DNA concentration was measured using an Invitrogen Qubit 4 Fluorometer. Following confirmation of quality DNA, 2 µL of each sample was placed in a well with 23 µL of a mix containing the developed *C. sowerbii*-specific primer markers on a 96-well qPCR plate. Triplicates were made of every sample on a well. The plate was run with SYBR fluorescence in a QuantStudio 3 machine along with a standard and several sample blanks. Results were exported and analyzed in QuantStudio design and analysis software.

Estimation of Effort Times for Various Detection Methods

During activities associated with *C. sowerbii* detection (plankton net tows, settlement plates, and eDNA samples), time in the field and the lab was monitored and recorded a notebook then transcribed onto an Excel spreadsheet. Travel time between sites was not included to better standardize estimates of effort and is irrelevant as detection tests were conducted in the same locations. Estimates are for one trained person operating from a kayak. Effort time estimates (in units of hr elapsed * ten lakes^{-1}) were scaled to ten study lakes to demonstrate the efficiency of eDNA testing at larger sample sizes (due to equipment such as 96-well qPCR plates) in comparison to analysis of settlement plates and plankton net samples that do not have improved efficiency at larger sample sizes.

Stable Isotope Analyses

To conduct stable isotope analyses, *C. sowerbii* medusae, large zooplankton (from 750 µm tow samples), fish larvae, and small plankton samples (taken from 20 µm net tows and sieved within a 2-500 µm size range) were euthanized with MS-222 (250 mg/ml) and preserved in 95% ethanol on-site. Preserved net tow samples were then brought to the NYSDEC Forest Health Diagnostic Lab in Delmar, NY, for sorting, ID and measurement of specimens. Following sorting, specimens had ethanol rinsed off using deionized water, were dried in a 60°C oven, and pulverized in the laboratory using a mortar and pestle (Feuchtmayr and Grey 2003). After specimen preparation was complete, samples were shipped to the Cornell University Stable Isotope Laboratory (COIL, Ithaca, NY) for analysis of $\delta^{13}C$ and $\delta^{15}N$ isotope ratios. $\delta^{15}N$ levels were corrected using atmospheric values as a reference, and $\delta^{13}C$ levels were corrected using the primary reference scale of Vienna Pee Dee Belemnite. Results of these analyses were

then plotted on a $\delta^{15}N$ versus $\delta^{13}C$ scatterplot to compare values at different trophic levels within a lake. Specimens were only analyzed in lakes where medusae were successfully collected.

RESULTS

Net Tows and Settlement Plates

No specimens of *C. sowerbii* appeared in plankton net tows until mid-August, when a few individuals were collected from Tillson Lake, Lake Luzerne, and Garnet Lake (Table 1). Additional net tows in September yielded additional specimens in all three lakes previously mentioned, plus two in Stillwater Pond. Estimated medusae densities ranged from 0.018 (Lake Luzerne) to 0.24 medusae/m^3 (Stillwater Pond). Collected medusae ranged in size from 1.5 - 15 mm. When medusae began to appear in August, lake surface temperatures were above 22.5 °C (Figure 4). Lakes did not all appear to consistently increase in temperature from June to August sampling, as East Caroga Lake and East Stoner Lakes decreased in temperature. Garnet Lake was notable in having the highest measured surface temperature at 28.15 °C, the greatest number of medusae collected in the nets (11 individuals) and reported bloom conditions in late September with greatly increased medusa density (Judy Thomson, personal communication).

Table 1. **Summary of Summer 2019 *Craspedacusta sowerbii* detection results. + indicates positive detection, - indicates negative detection, N/A indicates site was unavailable on day of sampling.**

Lake	Glenmere Lake	White Pond	Stillwater Pond	Canopus Lake	Tillson Lake	Garnet Lake	Lake Luzerne	Wolf Lake	East Stoner Lake	East Caroga Lake
Sediment (eDNA)	-	-	-	+	-	-	-	-	-	-
Filtered water (eDNA)	N/A	+	+	+	+	+	-	+	+	N/A
Net Tows	-	-	+	-	+	+	+	-	-	-
Settlement Plates	+	-	-	-	-	+	-	+	+	-

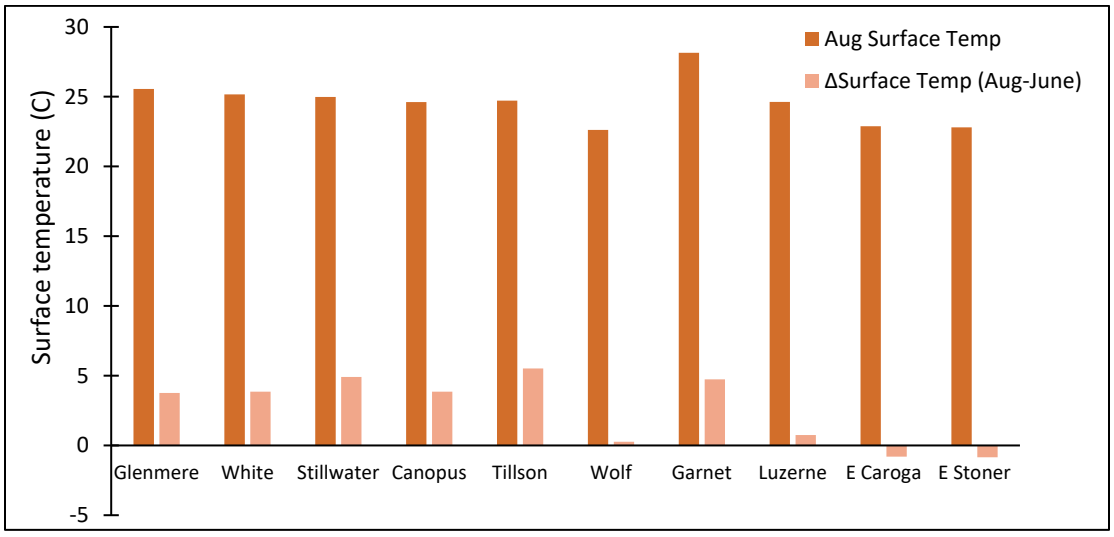

Figure 4: **August water surface temperature measurements across study sites, and difference between June and August water surface temperature measurements.**

Larval fish density varied between lakes; in White Pond, larvae were collected at a density of 4.47 larvae/m^3 whereas no larvae were collected in Wolf Lake (Figure 5). Larvae were identified as Centrarchidae, including four *Micropterus salmoides* and 24 unknown *Lepomis* spp. individuals.

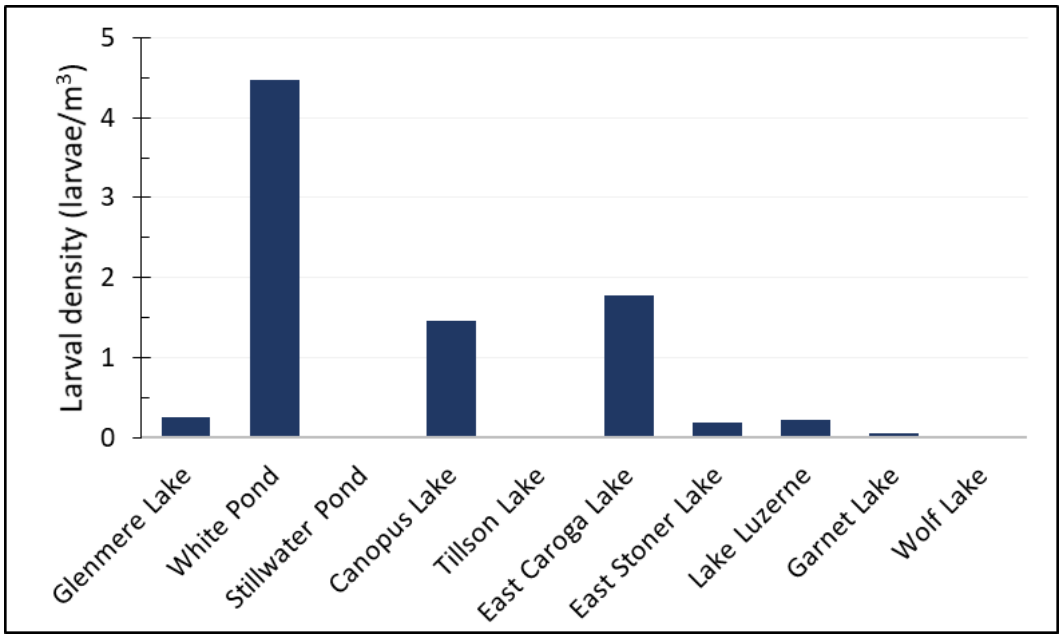

Figure 5: Estimated larval fish densities of 750 µm tow net samples across study lakes in June 2019.

During retrieval, several settlement plate samplers were lost, most likely due to theft, boat propeller damage, or movement due to storm conditions. Visual observation of settlement plates was a fairly, time-intensive process, and at this time only 12 samplers have been observed. Of the observed samplers, eight had potential *C. sowerbii* polyps (Figure 6), and two were confirmed to have polyps of that species using qPCR analysis (all triplicates returning amplification above the threshold). From the completed samples, *C. sowerbii* polyps were only detected in Wolf Lake and Glenmere Lake using settlement plates; neither of these lakes had medusae present in plankton net tows.

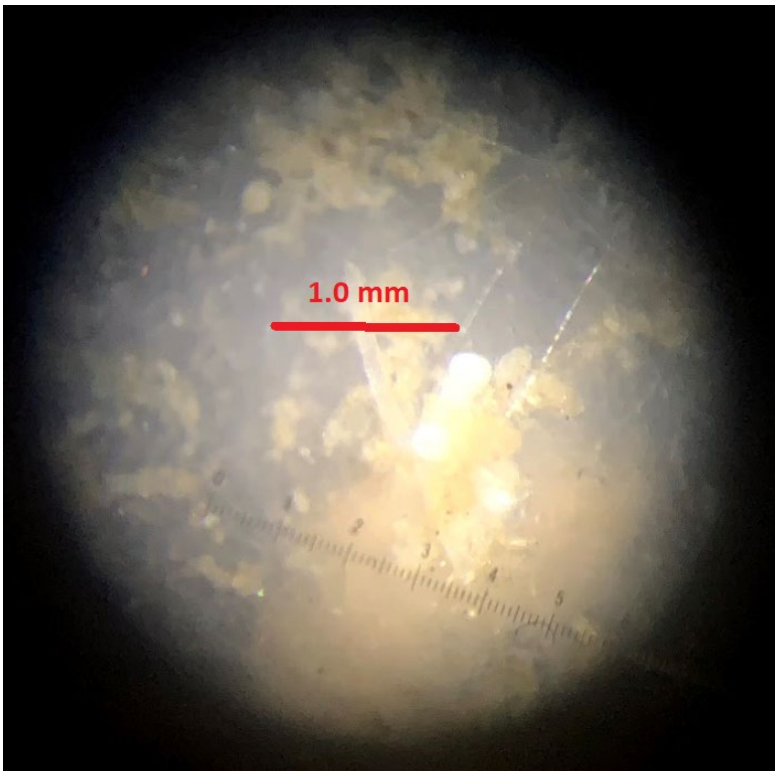

Figure 6: **Probable *Craspedacusta sowerbii* polyp specimen identified on settlement plate placed in Wolf Lake, 2 m depth, as viewed under 250x magnification.**

Environmental DNA (eDNA) samples

Sediment samples tested for eDNA had only 2 out of 30 samples return with positive detection (Table 1). These samples were both from Canopus Lake. Filtered water eDNA samples returned positive detection in 11 out of 24 samples. These samples were within East Stoner Lake, Tillson Lake, White Pond, Wolf Lake, Garnet Lake, Canopus Lake, and Stillwater Pond. Again, several eDNA samples returned with partial detection of *C. sowerbii* DNA. The reason for these partial detections may be due to the estimated concentration of *C. sowerbii* DNA, which ranged from 0.0035 to 2.7 DNA copies/µL in sediment extracts and 0.038 to 7.5 DNA copies/µL in filtered water extracts. These relatively low concentrations may have resulted in many samples with amplifications

below the threshold value ($C_T = 0.01$).

Estimated Effort Times for Various Detection Methods

Effort times for field collections plus laboratory analyses for possible *C. sowerbii* detection was estimated to be highest when using settlement plate samplers at 85.0 hr elapsed * ten lakes^{-1}, and lowest when using eDNA from filtered water at 38.9 hr* ten lakes^{-1}, although use of plankton tows was estimated to have 6.83 hr elapsed * ten lakes^{-1} fewer devoted to field collections (Figure 7). The relatively high effort time needed for use of settlement plates is partially due to the need for two sampling trips: once for deployment of samplers and again for retrieval of samplers.

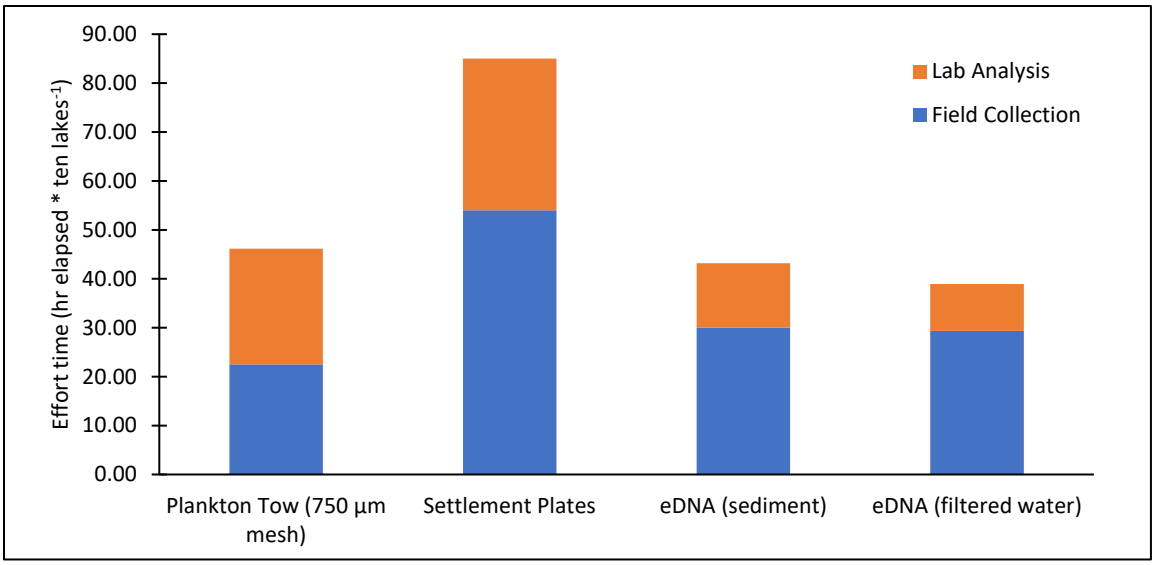

Figure 7: Comparison of effort time required to survey 10 lakes for *Craspedacusta sowerbii* among four detection methods: Plankton net tows, settlement plates, environmental DNA (eDNA) bottom sediment samples, eDNA filtered water samples.

Stable Isotope Analyses

Results of stable isotope analyses were variable among lakes, with samples from Tillson Lake having a relatively unique $\delta^{13}C$ signature, and elevated small plankton $\delta^{15}N$ levels of 5.26 and 5.56 ppt (Figure 8). Tillson Lake small plankton and separated

copepods had $\delta^{13}C$ 4-5 ppt lower than in medusae. Regarding fish larvae and medusae, in both sites where enough biomass was able to be collected to obtain a value for both, $\delta^{13}C$ was within 2 ppt, but in neither case was $\delta^{15}N$ higher in medusae than fish larvae (Figure 8; Figure 10). In Garnet Lake, medusae $\delta^{15}N$ was within 1 ppt for both larval fish and *Leptodora kindtii*, and higher (5.74 ppt) than *Bosmina* (4.11 ppt) and small plankton (2.08 – 2.18 ppt) (Figure 10).

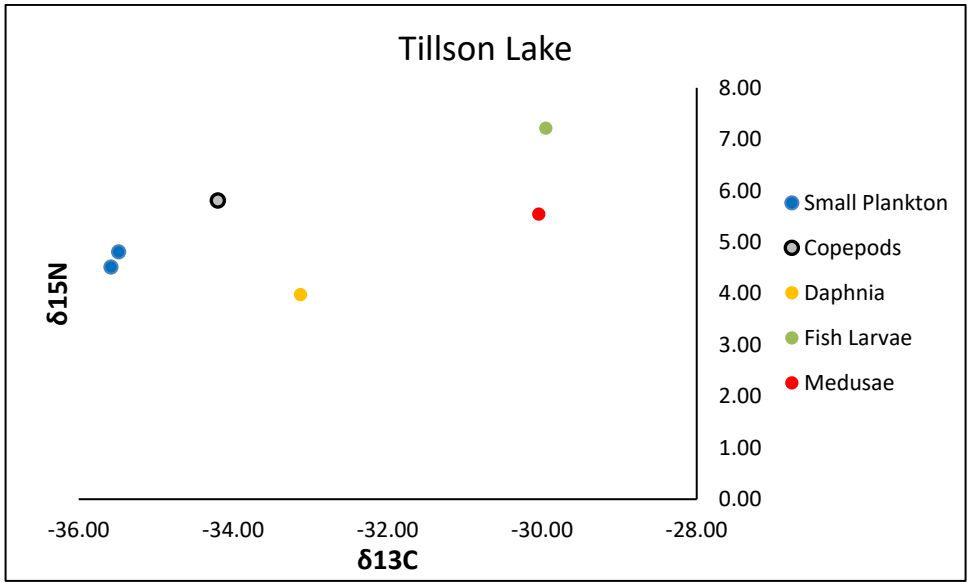

Figure 8: Scatterplot of δ15N vs. δ13C stable isotope analyses of Summer 2019 plankton tow net samples from Tillson Lake, NY. Analysis conducted by the Cornell University Stable Isotope Laboratory (COIL), Ithaca, NY.

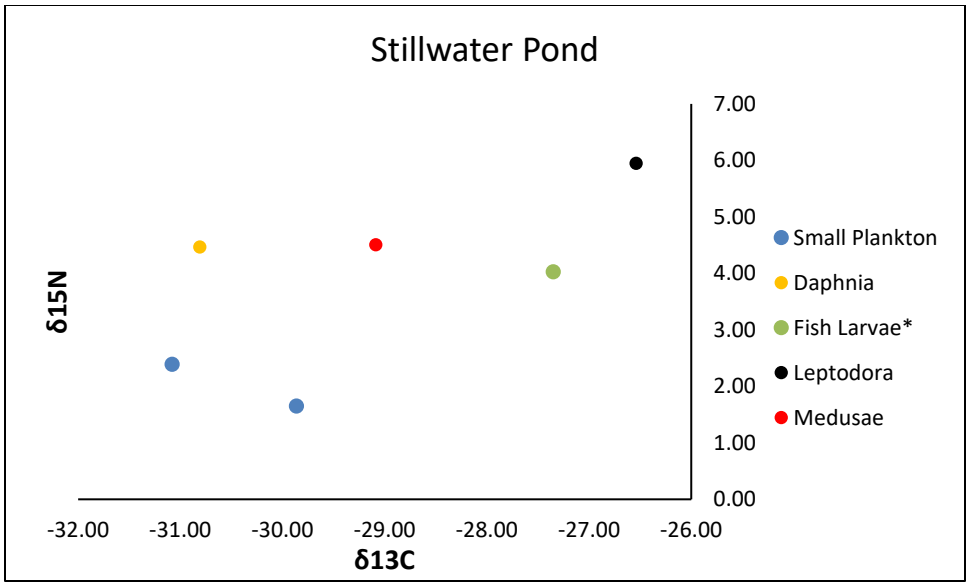

Figure 9: **Scatterplot of $\delta^{15}N$ vs. $\delta^{13}C$ stable isotope analyses of Summer 2019 plankton tow net samples from Stillwater Pond, NY. Analysis conducted by the Cornell University Stable Isotope Laboratory (COIL), Ithaca, NY. *Fish Larvae sample was below the biomass threshold and may not be accurate.**

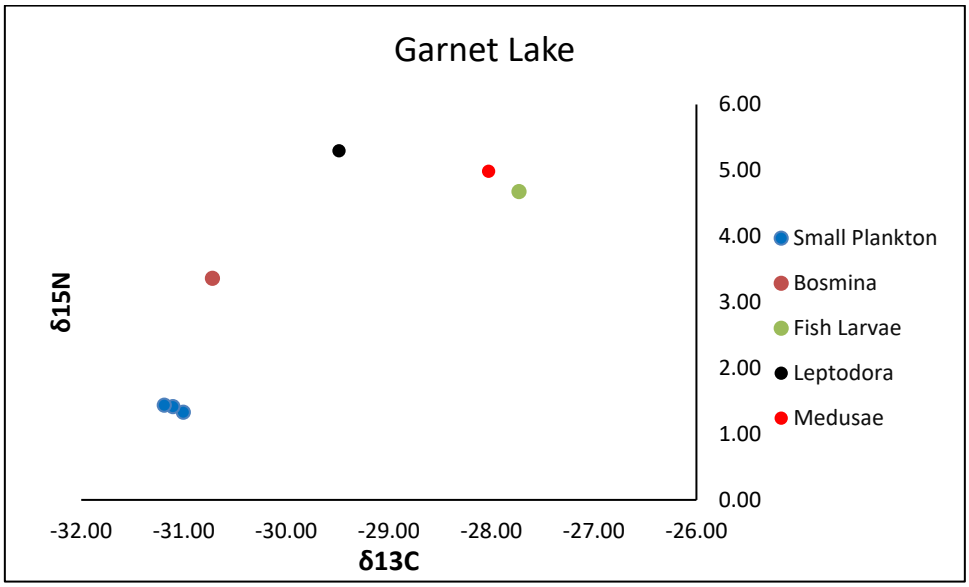

Figure 10: **Scatterplot of δ15N vs. δ13C stable isotope analyses of Summer 2019 plankton tow net samples from Garnet Lake, NY. Analysis conducted by the Cornell University Stable Isotope Laboratory (COIL), Ithaca, NY.**

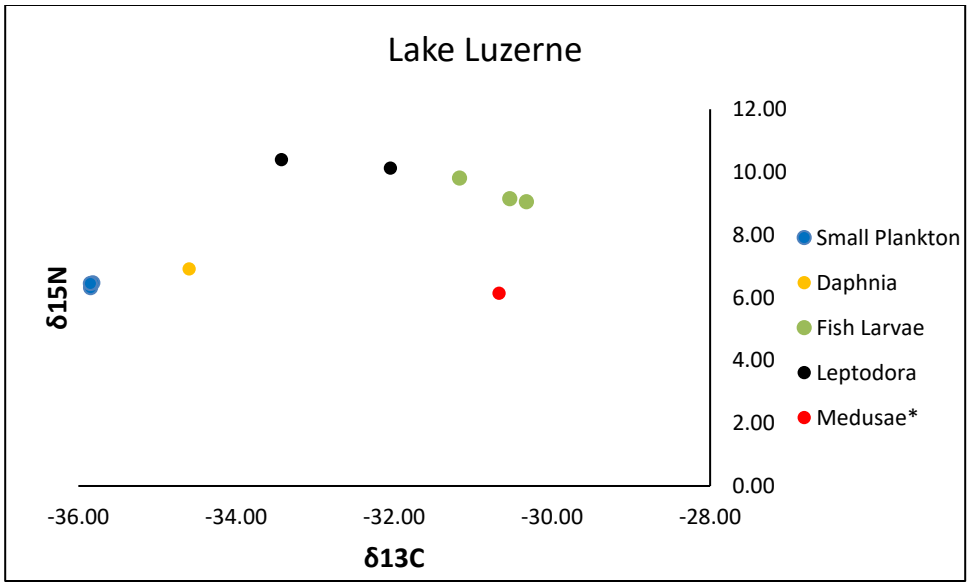

Figure 11: Scatterplot of $\delta^{15}N$ vs. $\delta^{13}C$ stable isotope analyses of Summer 2019 plankton tow net samples from Lake Luzerne, NY. Analysis conducted by the Cornell University Stable Isotope Laboratory (COIL), Ithaca, NY. *Medusae sample was below the biomass threshold and may not be accurate.

DISCUSSION

Detection of *C. sowerbii* proved to be difficult regardless of the method used, even though sampling of lakes was targeted based on location and seasonal appearance of previously reported medusae blooms. Although previous studies have hypothesized that elevated temperature is inducive to the appearance of medusae (Minchin et al. 2016)

Overall, eDNA assessment using filtered water was the most sensitive detection method for *C. sowerbii,* supporting the previously stated hypothesis. This is likely due to the season of sampling, as medusae are typically reported to be most abundant in Late Summer, when water temperatures have been in the mid-20's °C; however, the low sensitivity of sediment eDNA samples does not support the hypothesis nor align with previous eDNA studies that have demonstrated much greater DNA concentrations in

sediment samples (Minamoto et al. 2017; Turner et al. 2015). Recommendations for future use of eDNA assessment of waterbodies for *C. sowerbii* would suggest focusing on taking samples during weeks when medusae are likely present, which is with surface water temperatures having been above 20 °C for several weeks to allow polyps to produce medusae.

Altogether, these data provide interesting observations on the ecology of *C. sowerbii* in NY lakes. For one, $\delta^{15}N$ values suggest medusae do not feed at a trophic level above larval fish, but rather are feeding at a similar planktivorous level along with *Leptodora kindtii* (Leite et al. 2002). Results for $\delta^{13}C$ also may illustrate that these medusae were not feeding preferentially on copepods, even though prior feeding experimentation has demonstrated the opposite (Smith and Alexander 2008); however, the results of this study rely on very few data points due to the sparsity of medusae samples available, and should not be considered conclusive without additional sampling and isotope analyses to corroborate these observations.

As professionals and students contemplate options to locate *Craspedacusta sowerbii* for research, management, or other purposes, this study may be useful as a guide to compare effectiveness of various methods of detection. In consideration of the elusive nature of *C. sowerbii*, application of the eDNA assay developed and tested in this study would be a helpful option, as many lakes can be sampled and tested relatively quickly. Relying solely on eyewitness reports and traditional methods (such as plankton tows) will likely result in deficient evidence regarding presence of this species and its spread. The impact that *C. sowerbii* has on US lakes remains largely unknown, although observations from isotope results (e.g., the apparent lack of predation on fish larvae) should be useful to future research on the species.

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