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Filamentous green algae (Cladophora glomerata) in near shore Lake Ontario: an investigation of tissue and water nutrient dynamics through a period of growth and decomposition.

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Filamentous green algae (*Cladophora glomerata*) in near shore Lake Ontario: an investigation of tissue and water nutrient dynamics through a period of growth and decomposition.

A Thesis in Biology

By

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Dedication

First, I would like to thank Dr. Pennuto. The wealth of knowledge you have taken the time to share with me -through academic, professional and personal conversation and time- has shaped me into an actual scientist, and not just some guy with an interest in science. Your tutelage and patience have helped me, not only in my study of biology, but in how I now choose to interact with and raise my young son. I could not thank you enough and you have become a true mentor to me.

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Dr. Karatayev – While we didn't ultimately have a great deal of personal interaction, the wealth of literature you have contributed throughout your career has undoubtedly helped to shape our current and future knowledge of the Ecology within my home region of the Great Lakes. You are truly a giant in your field and I was honored to have any help you could offer with my work.

To my parents, James and Candace Wagner, I don't know where I would be in life without you. Thank you for never giving up on me and supporting me on the hundreds of various endeavors I have taken throughout my life. I love you both and as I am writing this, I can't help but think that I have finally (almost) finished this degree I have worked on for years! It would have never happened without you.

Most importantly, to my wife, Natalie. I would have never been able to go back to school, and pursue this dream, without your help. You have been my support as I've needed it, my escape when I was having a tough time and my love when it was tough to see the good and the reason. I only hope that all this time and sacrifice spent working on school pays off for us and our family. I love you more than I can describe.

Finally, my son, Jameson. I hope that someday, if you are feeling like life is too big and too much, you can look at me and understand that it is always possible to figure out a new direction in life and never too late to start work toward what you want. I am 40-years-old and finally finishing my degree. Be your own greatest competition and don't compare yourself or your time to others. Everyone's life is different and, as long as you don't give up, you can get to where you want to be. Love you Bear-bear!

Table of Contents

Chapter 1

List of Tables

List of Figures

Chapter 2

List of Tables

List of Figures

ABSTRACT OF THESIS

Cladophora glomerata, a filamentous green alga abundant in the Laurentian Great Lakes, has long been considered a nuisance throughout the region. Previous phosphorus (P) abatement practices and legislation successfully reduced the abundance of the algae, but with the introduction of dreissenid mussels, a resurgence has been observed. Though there is substantial literature and modeling of the growth cycle of *Cladophora*, relatively little research has been dedicated to the decomposition stage of the algae, a period which may contribute to a substantial influx of nutrients to near-shore environmental regions. By first examining a period of *in-situ* growth within Lake Ontario, I established a baseline of expected biomass accrual and nutrient content of both healthy algal tissue and water in the sample area. While nuisance biomass levels were not observed during this period, tissue nutrients suggested P limitation in the region even though the water column was dynamic and well mixed. *In-situ* growth dynamics were coupled with a full factorial *in-vitro* experiment examining the loss of nutrients, from tissue to water through decomposition. An abiotic variable (wave action), and a biotic variable (herbivorous crayfish, *Faxonius propinquus*), were introduced as decomposition contributors. While all treatments resulted in greater than half of the tissue mass lost, there did not appear to be any treatment effect with all replicates experiencing similar loss throughout the 20 days. Although the algae was detached when collected, it was still alive and productive for up to 10-days after the start of the experiment, evidenced by tissue and water nutrient changes. While final nutrient concentrations in tissue and water did mirror each other for all treatments, the capacity for crayfish to limit the epiphytic community appeared to have the greatest effect in contributing to nutrient loss to the water column.

Chapter 1

In-situ Growth and Nutrition Dynamics of *Cladophora glomerata* in Nearshore Lake Ontario

Abstract

Cladophora glomerata remains a nuisance alga along the nearshore of Lake Ontario in most years. I assessed biomass, spatial coverage, water column profiles, and tissue nutrient changes from late May through late August of 2019 to characterize the state of *Cladophora* in this region. Profile data suggest that this area is highly dynamic, with multiple upwelling events contributing to drastic changes in temperature throughout the growth season. Areas occupied by the algae remained chemically homogenous out to 9 m depth suggesting strong mixing throughout the water column. *Cladophora* biomass never appeared to reach nuisance levels (>50 g/m^2) across the sampling region, however the 3 m contours typically had the greatest total biomass, reaching nuisance conditions on occasion. Tissue carbon and nitrogen declined throughout the growing season, as did chlorophyll *a* content. Phosphorus levels remained unchanged throughout this period of growth.

Introduction

A resurgence of *Cladophora glomerata* is occurring throughout the Great Lakes with nuisance levels detected in all of the lakes, except Lake Superior (Auer et al. 2010). High *Cladophora* biomass in the Great Lakes was observed as early as the 1930's, washing up and fouling beaches throughout the region. With increased legislation and technologies in the 1970/1980's, in the form of the Great Lakes Water Quality Agreement and phosphorus abatement practices, algal biomass was reduced to below nuisance levels (Auer 2014). Soluble reactive phosphorus (SRP) concentrations in Lake Ontario open water dropped, from a 1972 average of ~13 μg P/L to a current, fairly stable mean of ~2 μg P/L (Dove and Chapra 2015). Although *Cladophora* levels were initially reduced, due to declining SRP, the late 1990's through recent times have again experienced increased growth (Pennuto et al. 2012, Dayton et al. 2014, Bootsma et al. 2015, Howell and Dove 2017, Watkins et al. 2017). It has been suggested that *Cladophora* is likely to reach nuisance levels (> 50 g/m^2) at sites with increased urban influence (Higgins et al. 2012) and that the ecosystem engineering attributed to dreissenid mussel colonization plays a large role in resurgence of *Cladophora* (Ozersky et al. 2009, Kuczynski et al. 2016).

The dreissenid mussel colonization resulted in three distinct habitat changes favoring *Cladophora* growth. First, mussels increase light penetration and photosynthetic depth as small particulate organic matter is filtered from the water column. Second, the mussel shells and byssal threads increase the amount of hard surface area available for attachment by the algae. Third, as mussels process food, there is a subsequent release of bioavailable P due to the excretion of feces and pseudofeces (Dayton et al. 2014, Kuczynski et al. 2016). Research on these three engineering processes by the mussels worked toward one of goals of the 2018 cooperative

science and management initiative (CSMI) activities for Lake Ontario. These activities attempt to provide data important in development and validation of lake wide water quality models and the Great Lakes *Cladophora* growth model (CGM) by collecting monthly field data from nearshore locations. The environmental data collection efforts help to enhance temporal resolution of *Cladophora* growth.

The prediction of spatiotemporal patterns in *Cladophora* biomass and estimation of sloughing events is important to successful management of the Great Lakes basin. Several growth models, ranging from relatively simple empirical regression models (Parker and Maberly 2000) to complex multi-compartment models (Auer and Canale 1982, Higgins et al. 2005) have been developed. Ultimately, these models require measurement of multiple physiological and environmental parameters at various scales to enhance predictive capacity. One such parameter, diffusive flux, requires the presence of a concentration boundary layer at a relatively fine spatiotemporal scale near *Cladophora* attachment sites. It may be possible for such layers to develop in calm lake conditions with areas of high mussel density (Ackerman et al. 2001, Boegman et al. 2009). Indeed, Dayton (2011) observed such nutrient concentration boundary layers near mussel and *Cladophora* beds in Lake Michigan.

This study involved high resolution spatiotemporal sampling of near-bottom water column nutrients, algal tissue nutrients, and *Cladophora* mass and coverage to better understand *in-situ* algal growth dynamics. My primary objectives involved documenting *Cladophora* % coverage, biomass and tissue nutrient concentrations in Lake Ontario at 3 depths (3, 6 and 9 m) on 10 dates over the growing season within a sampling area near Olcott, NY where high algal abundance is expected (Fig. 1). Additionally, I compared nearbed vs. surface nutrient

concentrations on the same 10 dates to determine if *Cladophora* influenced microscale water column nutrients.

Methods

Benthic *Cladophora* and bottom water samples were retrieved by divers approximately every ten days from each of three depth contours (3, 6 and 9 m) in nearshore Lake Ontario over the growing season of 2018 (Fig. 1). Additionally, a boat crew collected surface water samples and an array of profile data from each dive site on each date.

Profiles

From each dive site on each collection date, a surface crew drew a single water sample using a field-rinsed van Dorn bottle from 1 m depth. The water was field-filtered (GF/C) into a 125 mL poly bottle and stored on ice in a cooler until returning to the lab. Once in the lab, water samples were frozen at -20º C until shipment to the National Water Quality Lab at Heidelberg University (NCWQR) for processing. Secchi disc depth was determined. Temperature, conductivity, and oxygen profiles were collected using a YSI Quanta meter, with dissolved oxygen (D.O.) calibrated according to manufacturer's instructions prior to each field day. Bottom temperature was also obtained from 3 anchored temperature loggers (Optic Tidbit®), one each at 3, 6, and 9 m programmed to collect data at 6-hr intervals over the growing season. Light penetration was obtained using a Li-Cor Model LI-193 with spherical quantum sensor.

Nearbed water chemistry

Once on the bottom, the objective for divers was to collect water within 2-5 cm of the substrate surface, or from within a *Cladophora* patch, directly over the selected cobble. Triplicate water samples were pulled into 200-mL syringes and brought to the surface (9 total samples). On the surface, the boat crew immediately filtered 100 mL of sample through lab-rinsed GF/C filters into single-use poly bottles which were stored in the dark on ice until returning to the lab. Samples were frozen at -20º C until shipment was made to NCWQR. NCWQR analyzed samples for seven constituents: ammonia (NH_3), chloride (Cl^-), sulfate (SO_4^-), nitrite (NO_2), nitrate (NO_3), silicon dioxide (SiO_2), and soluble reactive phosphorous (SRP). Nitrogen constituents (i.e, NH_3, NO_2, and NO_3) were summed to represent total inorganic nitrogen (TIN).

Benthic Cladophora collections and processing

After reaching the substrate, divers first made observations of *Cladophora* percent coverage and mat depth. Coverage and mat depth were estimated from a series of underwater images (n = 2-to-5) (GoPro 4) taken within the sample area (approximately 25 m^2). Coverage in each image was estimated visually and mat depth was determined from images of the bottom mat with a scale object in view. Divers then collected replicate (n = 5), 0.25 m^2 quadrat air-lift samples on each sample date and depth to quantify benthic algal biomass. Divers placed quadrats haphazardly on the lake bottom and airlifted all material within the quadrat into large mesh bags (0.5 mm mesh) attached to the air-lift tube. Sample bags were taken to the surface and stored on ice in the dark until returning to the lab. In the lab, samples were gently washed over a sieve series to remove mud and silt. Samples were then floated in a shallow tray and hand-picked to remove all algae. The entire algal collection was wet-weighed by placing between layers of

paper towel and applying pressure to remove as much water as possible prior to obtaining the mass. From this mass, several subsamples were removed and processed for either chlorophyll *a*, dry mass, or carbon, nitrogen, and phosphorous content. All mussels and invertebrates were preserved for other assessments and not reported here.

Chlorophyll *a* analysis followed procedures of Steinman et al. (2006). In short, a squeeze-dried fresh sample of algae (5-400 mg wet weight) was placed in a foil-covered scintillation vial with 10 mL of methanol and place in a refrigerator to steep for 2 hr. After 2-hr, a 2-mL aliquot was transferred to a 3.5 mL cuvette and read on a spectrophotometer at 750 (for turbidity correction) and 664 nm. Samples were then acidified with 0.1 mL of 0.1 N HCl and re-read at 750 and 665 nm following a 90-sec wait. Chlorophyll a content (µg/g) was determined as:

Chlorophyll *a* = 26.7 (E664b – E665a) * (V_{ext} / mass (mg)) * L (cm)

Where: (E664b – E665a) = turbidity-corrected absorbance value at 664 nm before acidification minus turbidity-corrected absorbance after acidification, V_{ext} = volume of the extract in mL, mass is the weight of *Cladophora* placed in the methanol (mg), and L = length of the light path thru cuvette (cm).

To obtain dry biomass, the remaining algal sample was first weighed (wet weight, g), placed in a pre-weighed weigh boat, and dried at 60º C to a constant weight (dry weight). For samples with enough material, two subsamples were removed to determine carbon/nitrogen and phosphorous content. Carbon/nitrogen ratios were determined on a CE Elantech CHN analyzer (formerly Carlo-Erba). Dried samples (approx. 5 mg) were packed into tin capsules and combusted at 900º C (sample mean mass (mg + standard error) = 4.7 $\pm$ 0.19, n = 157)). Aspartic acid (N = 10.52%, C = 36.09%) and BBOT (N = 6.51, C = 72.53) standards were used to establish calibration curves and standards were run every 10 samples. Relative percent difference

values for both standards were within acceptable limits (Aspartic acid: N = 2.67%, C = 0.06%; BBOT: N = 0.08%, C = 0.19%). Tissue P levels were determined using the ascorbic acid reduction method (APHA 4500-P E; APHA 1998) after digestion in 12 N sulfuric acid and ammonium persulfate.

Statistical analyses

Chlorophyll *a* and dry mass estimates were examined for season and depth changes using a 2-way ANOVA. Seasons were categorized as spring (30 May-26 Jun), mid-summer (8 Jul-29 Jul), and late summer (7 Aug-31 Aug). Chlorophyll and dry mass data met variance and normality assumptions.

Water column chemistry was assessed for depth differences by comparing surface measurements of each constituent with bottom values using paired t-tests. Since these investigations indicated no differences (all $P > 0.05$), I assessed season and depth effects on nutrient conditions with a 2-way ANOVA on individual constituents (TIN, SRP, SiO_2, Cl, and SO_4) to assess fine-scale *Cladophora*/mussel impacts on bottom water chemistry. *Cladophora* tissue nutrients (i.e., C, N, P, and CN ratio) as well as chlorophyll *a* were examined with 2-way ANOVAs with season and depth as main effects. All nutrient chemistry data met normality and variance assumptions.

Results

Temperature and dissolved oxygen

Profiles of various parameters indicated that the nearshore zone under study in this 90-day project was well-mixed. On each sampling date, surface and bottom temperatures were

generally within 3ºC of each other (Fig. 2a). At least two upwelling events (Day 149/150 and Day 189), when water temperature declined by at least 10º C relative to the previous sampling date, were encountered during the 90-day sampling period. However, the bottom-moored temperature loggers revealed more frequent temperature declines and a dynamic temperature regime in the nearshore, with at least 5 upwelling events recorded (Fig 2b) where temperatures declined by at least 12º C within 24-48 hr (min = 12º, max = 17º C). On all sample dates and depths, dissolved oxygen was near or above saturation levels, depending on temperature (90-day mean = 10.7 ±0.59 mg/L (se), Table 1).

Light penetration (%PAR), Secchi disc depth, and conductivity

Light penetration profiles suggested ample light for *Cladophora* growth reached the bottom on most sampling dates (Table 1). In general, light at the bottom over the 6 and 3-meter contours exceeded 20% of surface PAR, although % PAR at 9 meters was less than 10% on roughly half of the sample dates (mean = 9.4% over the season). Similarly, absolute PAR levels at the 9-m stations had a season average of 69 µmol photons * m-2 * sec-1. Secchi disc depths were in good agreement with light transmission observations from the PAR sensor at 9 meters (r = 0.83, P = 0.006). Over the sampling period, Secchi disc depth averaged 6 meters, with a maximum reading of 8.2 m in mid-June (Table 1). Conductivity profiles showed no obvious differences between surface to bottom samples and the mean across the study period was 297 µS/cm over the season. (Table 1).

Water column nutrient chemistry

Water column nutrients, like the profile observations, suggested a well-mixed nearshore zone as no surface and bottom comparisons were significantly different (all paired t-test $P > 0.05$). Two-way ANOVAs indicated four of five constituents (Cl, SO_4, SRP, and TIN) were significantly different across season (all $P < 0.01$, Table 2) and either declined with season (TIN and SRP) or had a mid-summer dip (SO_4 and Cl). Only SRP showed a significant difference with depth ($P = 0.034$), and the depth*season interaction was not significant.

Cladophora growth, coverage, tissue nutrients, and chlorophyll a

During the summer 2019 growing season, *Cladophora* coverage was roughly 35% within the whole sample area, but with considerable variability. The 9-m sample sites consistently had the least algae, whereas the 6- and 3-m depths had higher coverage estimates (Fig. 3). Mean coverage by depth was 39, 59, and 5% for the 3, 6, and 9 meter depths. Coverage was strongly correlated with percent cobble substrate ($r = 0.55$, $P = 0.007$; Fig. 4). In addition to *Cladophora*, mid and late summer growths of *Spirogyra* were observed by divers throughout the 6 and 3-meter locations, but not quantified.

Cladophora biomass exhibited complex patterns over depth and season, mostly increasing in mass thru mid-summer, but then declining rapidly at the 6-m stations (Fig. 5a). There was still substantial biomass collected at the 3-m depth stations into the middle of August. ANOVA outputs suggested this 'depth*season' interaction was significant ($F_{4,61} = 2.74$, $P = 0.037$) and that both depth and season were highly significant ($F_{2,61} = 12.07$ and 7.78, respectively; both $P < 0.001$). Although biomass occasionally exceeded the nuisance threshold, on most dates and depths it was below these levels. Similarly, although the growth pattern suggested maximum biomass up until the end of July, followed by a decrease, I did not observe a

robust sloughing event. Nor was there evidence of shoreline windrows of algal mats through the sampling window. The overall appearance and nutrient condition of *Cladophora* stands showed marked changes through the sampling season and with depth. Visually, *Cladophora* stands got 'duller' through the season as they began to both senescence and become covered with layers of silt.

Chlorophyll *a* levels, on average, declined with season (Fig. 5b), even though ANOVA results suggested a significant interaction between season and depth (Table 3). The significant interaction reflects the continued decline in chl *a* at the 6-m stations after mid-summer while both the 3- and 9-m stations each remained relatively unchanged (Fig. 5b). Chlorophyll *a* content correlated well with tissue carbon content ($r = 0.51$, $P = 0.003$), but was not correlated with either N or P content, nor with dry mass (all $P > 0.05$).

ANOVAs for carbon, nitrogen, and phosphorous content, and tissue C:N showed no 'season*depth' interaction effects for any tissue nutrient. Three of four measures (C, N, and C:N ratio) showed strong season and depth differences (Table 4). Phosphorous did not show any season or depth response. All tissue nutrients were lowest at 3-m stations and highest at 9-m stations over the sampling season (Fig. 6). The C:N ratio pattern across depth followed from the individual C and N levels, resulting in the highest C:N at the 3 m site and the lowest C:N at the 9 m sites. Similarly, tissue nutrients exhibited consistent patterns through the season. Nitrogen and carbon declined with season, whereas C:N ratio increase and P levels in tissue remained constant (Fig. 6).

Discussion

The summer 2019 growing season did not produce nuisance *Cladophora* levels in this region of Lake Ontario, even though there was ample substrate for attachment and abundant sunlight for photosynthesis. For example, estimates of critical irradiance (ICR) needed to assure net photosynthesis range from ~10 to 45 µmol photons * m^{-2} * sec^{-1} (Graham et al. 1982, Necchi 2004). The season average for this project at the 9-m stations surpassed this minimum threshold with a mean of 69 µmol photons * m^{-2} * sec^{-1}, even though on one sample date the level was a low of 9.3 (Table 1). Conversely, SRP levels were very low and tissue P data suggested the *Cladophora* in this region was P-limited the entire growing season even though tissue P levels were high enough for growth. Nutrient content of *Cladophora* tissue may provide some evidence as to when a sloughing event might occur, since senescing tissues invest less in pigment production and starch storage. It is generally believed that *Cladophora* requires a minimum tissue P level of 0.035 to 0.06% for growth (Auer and Canale 1982, Tomlinson et al. 2010) and levels below 0.1% indicate P-limitation (Auer and Canale 1982). Tissue P levels were fairly consistent throughout the 2019 growing season in this region of Lake Ontario, and indicated a season-long, P-limitation (season average = 0.048 $\pm$ 0.004 (s.e.). Although tissue %P remained constant over the sampling season, %C, %N, and chl *a* content declined through time. This observation makes some sense since the chlorophyll molecule is carbon, and to a lesser extent, nitrogen rich, but contains no P. In the end, the lack of a discrete sloughing event precluded making a link between the onset or timing of sloughing and either internal tissue nutrient levels or external environmental conditions.

All of the profile data and fine-scale nutrient assessments indicated that this nearshore region of the lake was a well-mixed, homogenous zone from surface to bottom and from the 3-m depth contour out to the 9-m contour. However, a comparison of the bottom-moored temperature

loggers to our profile temperatures reveals how temporally dynamic this region actually is. Divers encountered an upwelling event on Day 189 (17 June) when water temperatures were approximately 12^{o} C colder than the previous sample date 10 days earlier. Loggers indicated there were at least five upwelling events over the 90-day sample window, but our actual sample dates occurred in between each of these upwelling events (Fig. 2). Without more data it is difficult to determine the influence of these events on the growth dynamics of *Cladophora* during the summer 2019 growing season, although some data are suggestive of a rapid algal response to rapidly changing temperatures. For example, on Day 189, chl *a* content exhibited a large decline at all depths relative to the preceding sample date, followed by an increase at all depths on the subsequent sample date (Fig. 5b). In fact, it was the only date when mean chl *a* differed significantly from the previous and subsequent sample date ($t = 4.26$ and 2.22; $P < 0.001$ and $P = 0.039$, respectively). Low temperature exposure should limit carbon fixation in algae, which, if light levels remain unchanged, should reduce its ability to photosynthesize (Davison 1991). This might also explain the poor correlation between chl *a* content and dry mass across the sampling season, since dry mass might not respond to temperature as rapidly as the metabolic machinery producing chlorophyll.

Previous work showed a dynamic relationship between *Cladophora* and dreissenid mussels where nutrient release by mussels could fuel local algal production (e.g., Ozersky et al. 2009). Similarly, Ackerman et al. (2001) and Boegman et al. (2009) had suggested that concentration boundary layers (either seston or nutrients) could occur near the sediment-water interface under conditions of low mixing (i.e., little-to-no wind) and high mussel density. In a detailed near-bed nutrient flux study, Dayton (2011) used ‘peepers’ (modified Hesslein samplers) to understand if and when phosphorous concentration boundary layers were present over mussel

beds in Lake Michigan. A temporal signature of mussel nutrient release was estimated from samples of water collected within a dome over the mussels. He estimated under the best conditions (high mussel density, low mixing) a nutrient boundary layer 5-15 cm deep might form at the sediment-water interface (Dayton 2011, Dayton et al. 2014). The present work sought to capture that nearbed dynamic flux in an open system by collecting nearbed water samples from locations 2-5 cm above the sediment surface and from within a *Cladophora* mat with attached mussels. Coupled with our observations of multiple upwelling events, plus a noticeable along-shore current, this collection protocol might be viewed as a field test of whether a concentration gradient might be present at non-quiescent conditions. We found no detectable, fine-scale nutrient gradient (SRP or TIN), suggesting rapid and thorough fine-scale mixing of mussel-excreted nutrients in the southern Lake Ontario shore. Thus, at the microscale level sampled in this study, it appears that nutrient release/uptake from mussel/*Cladophora* assemblages, when present at the densities or biomass observed during summer 2019, has little absolute effect on water column nutrient concentrations.

Cladophora coverage across the 3-to-9 meter nearshore averaged about 35%, which is in good agreement with Brooks et al. (2015) estimate of 40% coverage for the Lake Ontario nearshore observed with Landsat imagery. Shallow depth stations, however, had considerably more percent coverage than the 9-m station with estimates of 39 and 59% coverage (3 and 6 meters) compared to 5% coverage for the 9-m stations. Similarly, percent coverage at different depths varied across the sampling season. Whereas the 9-m depth stations always had limited *Cladophora* coverage and the 3-m stations showed a late summer spike and collapse, the 6-m stations had broad coverage from early summer through the end of sampling (Fig. 3). Although there were obvious depth differences in percent coverage, across depths *Cladophora* was

restricted to substrates with hard surfaces (Fig. 4), as per natural history observations of its need for firm attachment sites (Higgins et al. 2008, Auer et al. 2014). Patterns in biomass followed patterns in percent coverage within some, but not all depth; 3-m stations exhibited a spike in early summer and late summer and 9-m stations always were low, but at the 6-m stations biomass peaked in mid-summer before declining by the end of the sampling. Although biomass occasionally exceeded the general 'rule-of-thumb' for nuisance levels (i.e., 50 mg/m2), on most sample dates and depths it was below nuisance levels. This is in agreement with an earlier study in this region of Lake Ontario from 2008 when Pennuto et al. (2102) documented a season average of ~48 g/m^2 (dry mass). Although growth patterns suggested maximum biomass up until the end of July, followed by a decrease, I did not observe a robust sloughing event. Nor was there evidence of shoreline windrows of algal mats through the sampling window. Late season growths of *Spirogyra* at the 6- and 3-m stations were noted but not quantified.

However, there are examples of local agal dry mass levels considerably higher in Lake Ontario. In 2008, Pennuto (unpublished report) measured levels as high as ~250 g/m^2 (dry mass) in a small embayment near the FitzPatrick nuclear power plant outside of Oswego, NY. Anecdotally, on 16 June 2020, I returned to the 2019 sample location to collect *Cladophora* for a decomposition and nutrient release experiment and encountered a massive sloughing event with abundant windrows of *Cladophora* adrift over the 6-to-9-m contours. These observations suggest there are very localized phenomenon at play in dictating when, where, and how much *Cladophora* occurs in a specific location of nearshore Lake Ontario and more long-term observations are warranted to tease out these local drivers.

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Table 1. Profile data summary for nearshore Lake Ontario in summer 2019. 'Surface' and 'bottom' refer to readings at the 9-m station on each sample date. PAR/%PAR = photosynthetically active radiation (µmol m^2 sec^{-1}) and the percent PAR at the 9-m station relative to the surface reading on that date.

			Conductivity (µS/cm)		**Dissolved oxygen (mg/L)**	
Date	**Secchi disc (m)**	**PAR/% PAR**	surface	bottom	surface	bottom
30 May	6.8	9.31/2.2	267	268	14.0	12.1
17 Jun	8.2	139.7/13.7	292	293	11.0	12.6
26 Jun	5.6	50.7/9.8	263	265	10.7	10.6
8 Jul	5.0	75.9/10.2	289	290	13.3	13.4
18 Jul	4.3	33.01/7.5	271	273	8.8	9.9
29 Jul	7.5	89.13/7.5	267	265	12.3	8.9
7 Aug	7.2	37.8/9.6	267	276	9.4	8.7
20 Aug	7.4	74.7/10.3	271	274	9.8	9.6
30 Aug	7.5	111.0/13.3	270	251	9.9	9.7

Table 2. ANOVA output investigating season and depth effects on water column nutrients in nearshore Lake Ontario. All df = 2, 90 so this column was not included. No 'season*depth' interaction term was significant so only the main effects F and P values are shown here. Significant effects appear in bold.

	Depth			Season	
	F	**P**		**F**	**P**
Cl	0.11	0.897	Cl	6.18	**0.003**
SO_4	0.09	0.918	SO_4	7.58	**0.001**
TIN	0.79	0.453	TIN	32.78	**<0.001**
SRP	3.49	**0.034**	SRP	8.89	**<0.001**
SiO_2	0.05	0.951	SiO_2	2.30	0.106

Table 3. ANOVA output investigating season and depth effects on chlorophyll *a* content in *Cladophora* tissue.

Source	df	F	P
Season	2	36.58	<0.001
Depth	2	4.39	0.015
Season * depth	4	3.27	0.016
Error	70		

Table 4. ANOVA output investigating season and depth effects on *Cladophora* tissue nutrient content from nearshore Lake Ontario. All df = 2, 40 so this column was not included. No 'season*depth' interaction term was significant so only the main effects F and P values are shown here. Significant effects appear in bold.

	Depth			**Season**	
	F	**P**		**F**	**P**
Carbon	5.07	**0.011**	Carbon	42.71	**<0.001**
Nitrogen	20.43	**<0.001**	Nitrogen	23.99	**<0.001**
Phosphorous	2.91	0.066	Phosphorous	0.99	0.379
C:N ratio	54.88	**<0.001**	C:N ratio	5.17	**0.010**

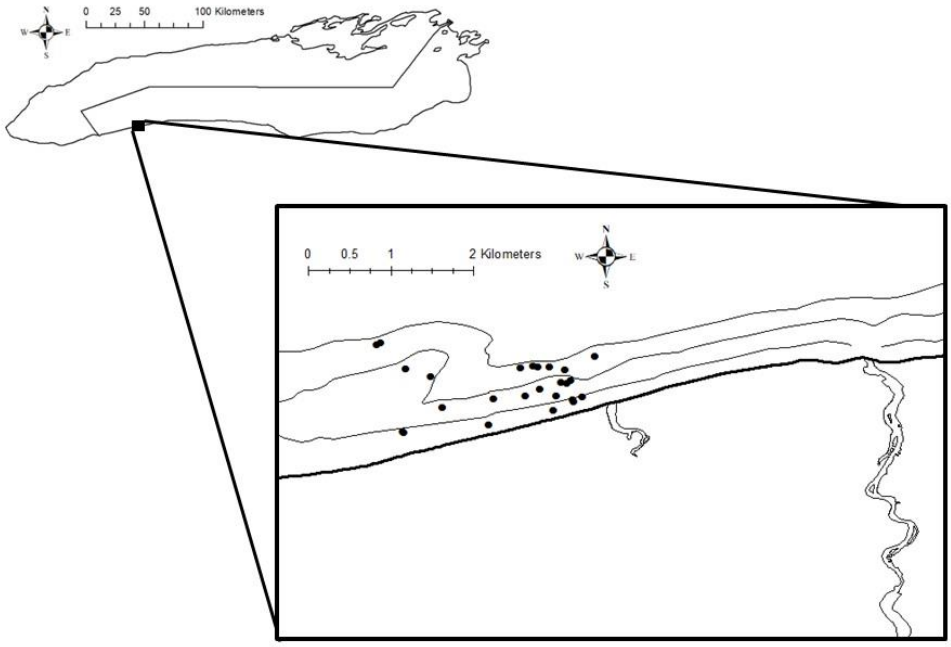

Figure 1. Locations for sample collections (indicated by dots) at 3, 6, and 9 m depth (indicated by profile lines). Every 10 days, divers collected benthic water and benthos air-lift samples and a boat crew collected surface water and profile data.

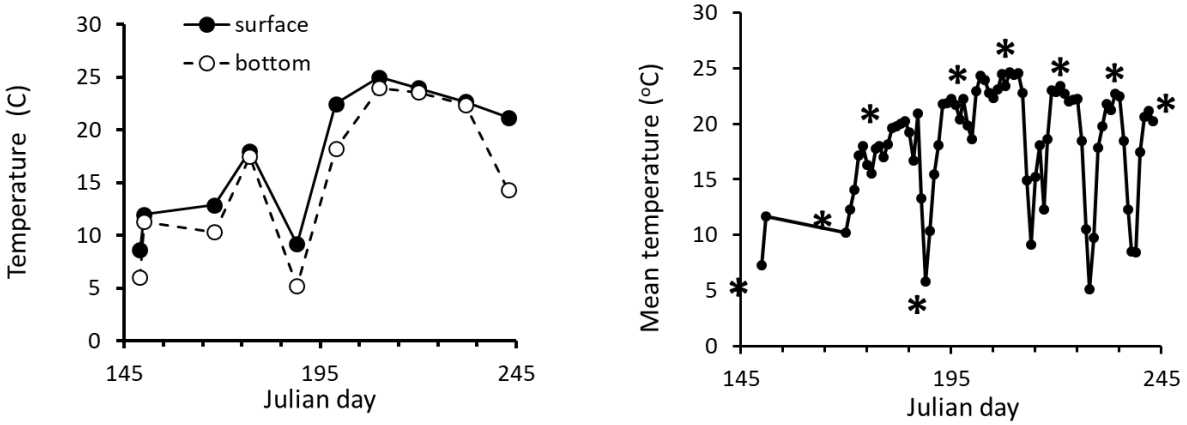

Figure 2. Surface and bottom temperature (left panel) from profile recordings the day of sampling and average daily bottom temperature from three temperature loggers over 90-day sampling period (right panel). Asterisks in right panel represent dates divers collected samples and dates for surface profiles. Note that on only one sample day did divers experience an upwelling event (Day 189).

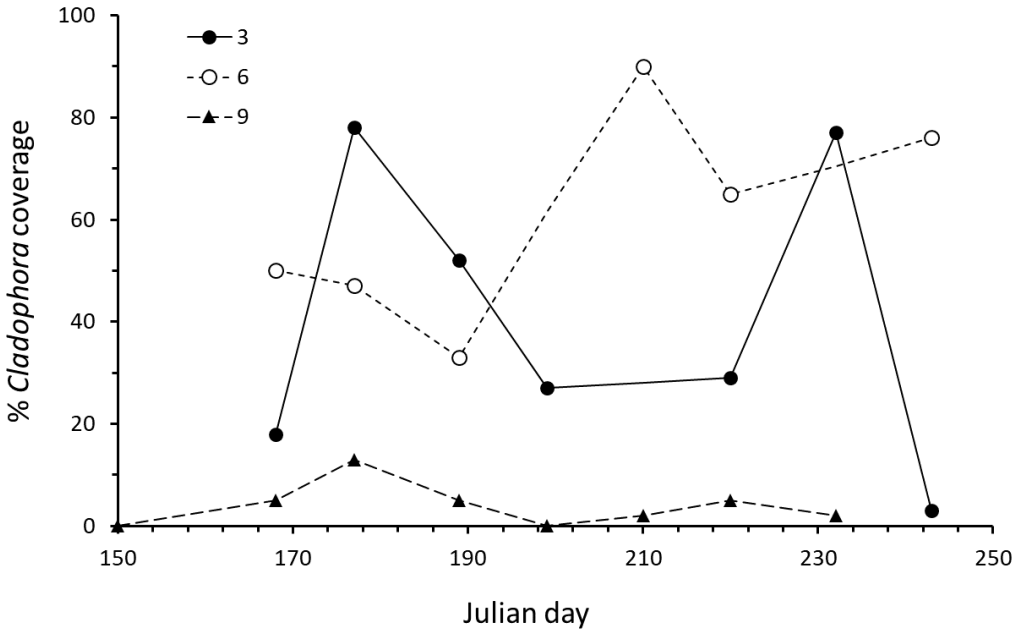

Figure 3. *Cladophora* coverage thru time at various depths in nearshore Lake Ontario during the 2019 sampling season.

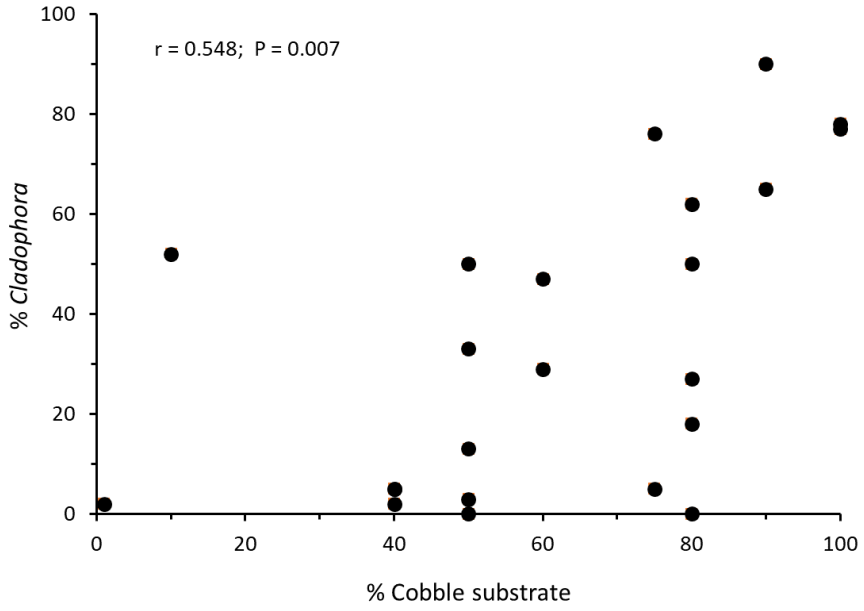

Figure 4. Relationship between *Cladophora* percent coverage and percent cobble substrate.

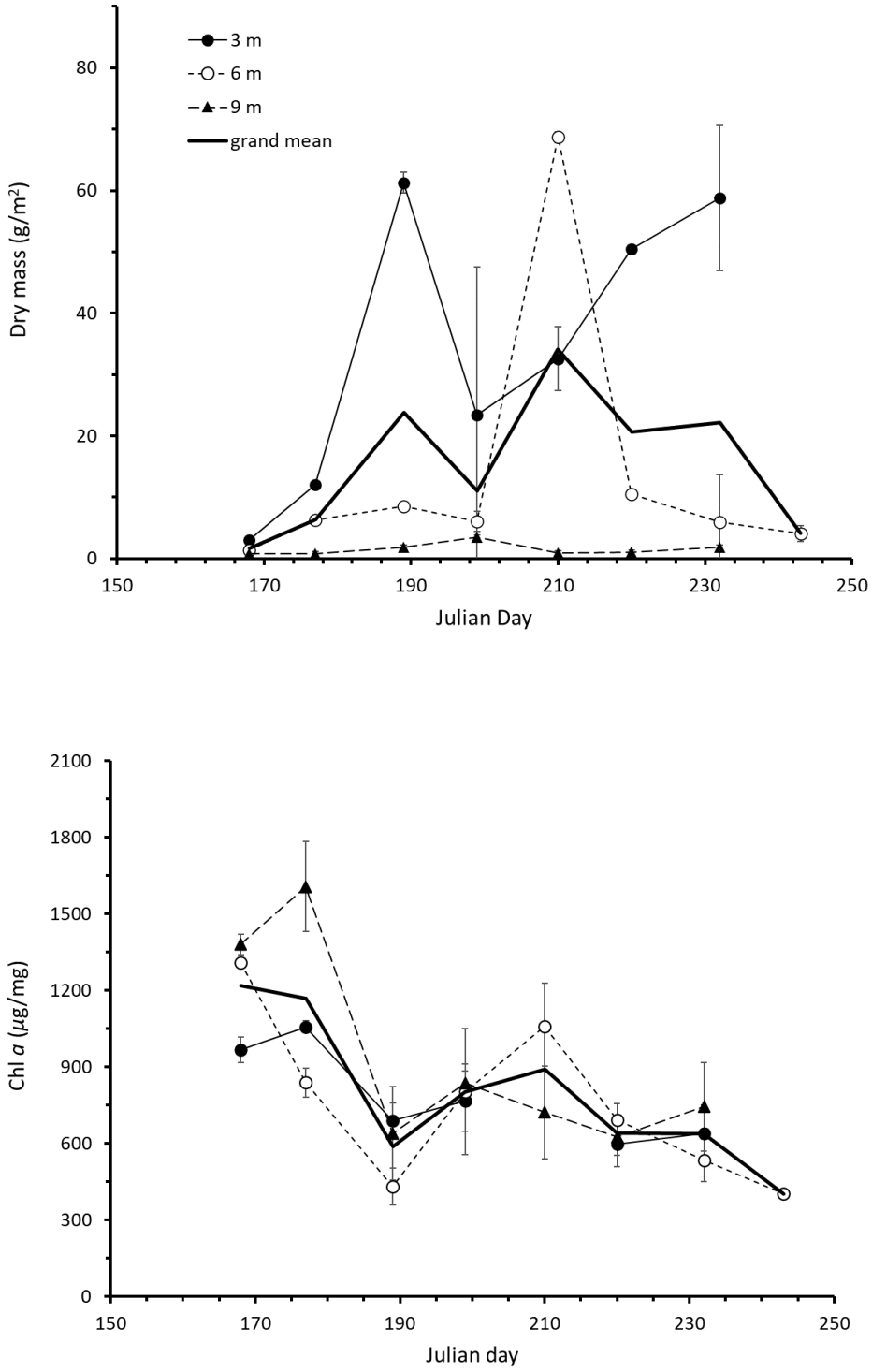

Figure 5. Mean *Cladophora* dry mass (g/m2) (top) and chlorophyll *a* (bottom) at various depths over the 2019 sample season in nearshore Lake Ontario. Error bars = 1 s.e. Bold line is the mean value across depths on each date.

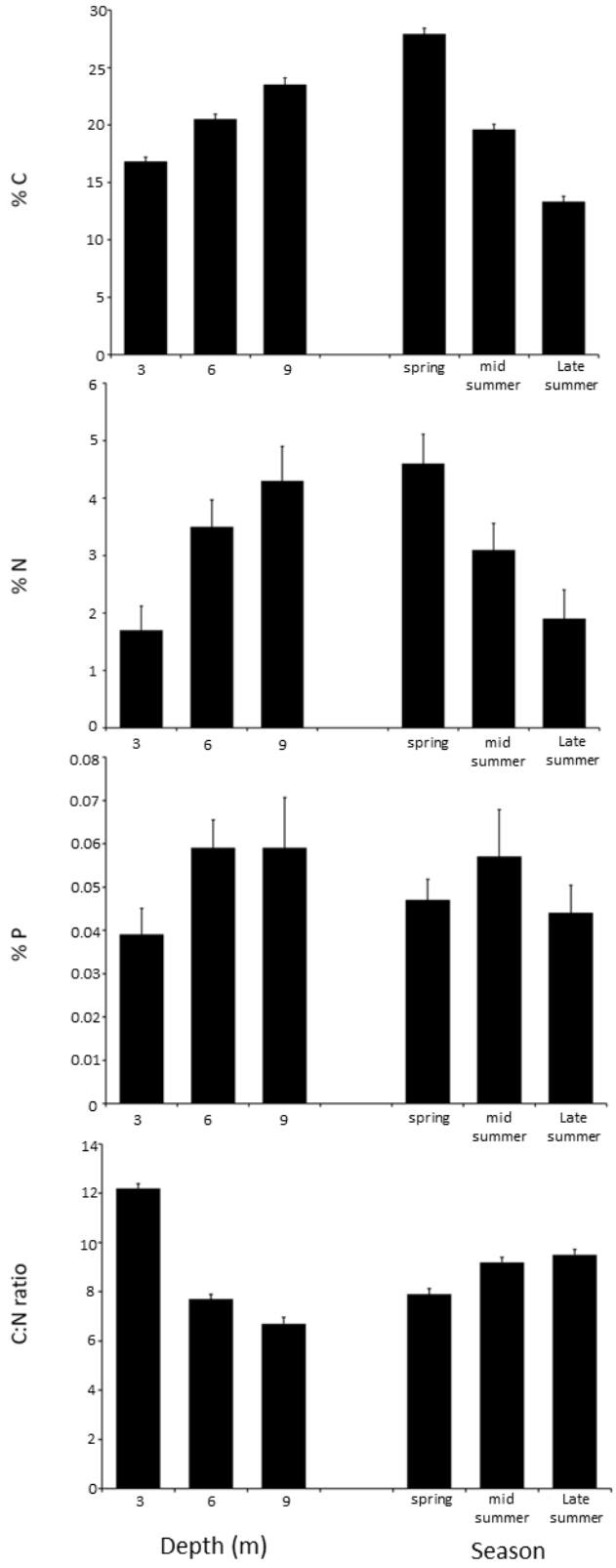

Figure 6. *Cladophora* tissue nutrient content at various depths over the 2019 sample season in nearshore Lake Ontario. All error bars = 1 s.e.

Chapter 2

In-vitro Decomposition and Nutrient Efflux of *Cladophora glomerata* in the Presence of Abiotic and Biotic Stressors

Abstract

Decomposition dynamics of *Cladophora glomerata* have been overlooked, even though extensive investigations into best management practices for growth are available. A rapid loss of significant quantities of tissue-bound nutrients has the potential for large impacts throughout the nearshore environment. In this study, I quantified the transfer of tissue-bound nutrients to the water column in the presence of an abiotic force (wave action) and a biotic force (Northern Clearwater Crayfish, *Faxonius propinquus*). I hypothesized that algae in all treatments would lose biomass throughout the 20-day trial, with nutrient efflux from tissue observed as increases in water column concentrations and that that the sheer stress from wave action would lead to more physical breakdown during decomposition than herbivory by the crayfish. I found that, after sloughing, the algae remained quite alive and productive over the initial 10-days of the experiment, evidenced by substantial loss in water column phosphorus. Ultimately, all treatments lost greater than 50% of their biomass over 20-days. A large amount of phosphorus and nitrogen was released to the water column. The crayfish treatments may have limited epiphytic diatom communities, which were suspected of rapid absorption of soluble phosphorus as it was lost by the decomposing algae. Although *Cladophora* continued to photosynthesize after sloughing, by the end of this 20-day trial, the majority of tissue was broken down. The actual nutrient efflux from the decomposing tissue to bioavailable forms in the water column was variable and likely depended more on microbial communities present over activities by larger invertebrates or physical stresses.

Introduction

Decomposition is a fundamental process in every ecosystem, whereby tissue-bound nutrients are released back to the environment. For aquatic systems, this process has been examined intensively in streams, but less so in lakes. This lack of lacustrine research is likely driven by the relative importance of leaf litter as an energy source to streams (Stoler and Relyea 2011). Studies of litter decomposition in lentic systems often focus on ephemeral pools since the temporary nature of these systems requires a reliance on carbon input from terrestrial sources. These studies reveal that litter species identity determines nutrient accessibility and quality following decomposition, and ultimately this affects the structure of recipient communities (Rubbo et al. 2008). In larger lake systems, there may be abundant submerged and emergent nearshore plants, whose decomposition may lead to substantial fluxes of soluble nutrients.

Cladophora glomerata is a large, filamentous macroalga that attaches to hard substrates via holdfasts. It has long been viewed as a nuisance algal species in shallow, nearshore regions of the Laurentian Great Lakes (Higgins et al. 2008a). Decades of ecosystem engineering by Ponto-Caspian dreissennids have led to increased water clarity, increased surface area for attachment, and altered benthic phosphorus availability, resulting in *Cladophora* resurgence (Hecky et al. 2004, Malkin et al. 2008). Following summer growth and maturation, *Cladophora* eventually breaks free from its holdfast, usually en-masse, in an event called sloughing. In nuisance years, immense amounts of sloughed biomass can then be transported in the water column by waves and currents, eventually settling in the benthos, washing up as shore windrows, or possibly being pulled into water-intakes, clogging infrastructure (Higgins et al. 2008b).

Cladophora is a filamentous alga composed of long aggregate filaments like a single organism, but it can also be thought of as a community of unicellular individuals. Each filament

is attached to the substrate by a root-like structure, the holdfast, but the individual cells composing the filament have little-to-no intercellular transport and interact directly with the environment for metabolic purposes. The mechanism of nutrient uptake is not well understood though research in this direction is occurring (Jia et al. 2021). Filamentous algae can have substantial variability in life stage and metabolic activity throughout a single filament. Even though filamentous algal cells do not rely on each other for transference of nutrition or other chemical processes, the community of cells within a filament shares a fate when succumbing to or overcoming external stress. A filament community might escape herbivory from gape-limited consumers through its collective size, but remain susceptible to sloughing once mechanical breakdown occurs anywhere along the filament.

Water currents abound in the habitats where *Cladophora* is found and are important in mixing of the water column. Similarly, the constant ebb and flow of water in the environment assures the removal of wastes and the influx of potential nutrient resources (Higgins et al. 2008a). As a filament grows and extends upward through the water column, there is the potential for variable light availability along the filament length. Younger cells, found nearer the water surface, may shade the more benthic portions of the filament, much like the canopy structure in a forest community. Older cells, typically nearer the holdfast in a filament, also accumulate epiphyton, reducing the surface area available for the filament to intercept sunlight. This self-shading with filament growth appears to strongly affect photosynthetic processes along a given filament (Higgins et al. 2008b). Thus, for each individual filament, it is possible for death and decomposition to begin in basal cells while growth and metabolic activity occurs in apical cells.

There is a substantial literature base supporting the modelling of the growth cycle of *Cladophora* in many different locations and habitats (Canale and Auer 1982, Higgins 2005,

Tomlinson et al. 2010). Although nutrient uptake and biomass accrual occur during the growth stage, many of the problems associated with this filamentous alga occur after sloughing, while decomposition is occurring. As with any organism, during decomposition, the foundational nutrients stored in tissue are returned to the ecosystem. It is generally accepted that the decomposition process is influenced by several mechanisms: leaching, mechanical degradation, and biological degradation (Best et al. 1990).

Leaching is a passive process by which solutes are lost from the tissues to the surrounding environment and it occurs during all life stages, as well as after senescence. As a passive process, it is relatively slow but is continually occurring. Mechanical degradation involves environmental interaction. Abiotic forces, such as wind or water current, physically break up materials. This effectively reduces the gross dimensions of the organism to smaller particles and increases the surface area available for increased interactions. Biological degradation requires interaction with other biota, be it microbial communities or invertebrate shredder functional groups. The material is effectively reduced in size as grazing occurs (Bohman and Tranvik 2001). This eventually allows for microbial digestion to absorb or release tissue-bound solutes to the ecosystem. Although nuisance conditions of *Cladophora* are characterized by immense mats of floating or washed-up plant material (>50 g/m^2), the rate of the decomposition process of *Cladophora* remains unknown. There may be potential for ecosystem-wide effects resulting from a large pulse of formerly tissue-bound nutrients to the environment.

Once *Cladophora* has sloughed from the substrate, both physical and biological processes may influence its decomposition and eventual nutrient release. A study of estuarine *Cladophora* in the Baltic Sea found biomass and nitrogen reduction occurred at a similar rate over a 14-day

period, while phosphorus loss occurred independently (Paalme et al. 2002). In this study, I used a full-factorial, randomized block design, *in-vitro* experiment to examine the decomposition of *Cladophora* tissue over 20-days. I observed and measured changes in biomass and nutrient content of *Cladophora* tissue in the presence of two variables that might influence decomposition rate: crayfish consumption and wave action.

Along with the changes in tissue chemistry, I also assessed changes in water chemistry to understand the release of nutrients to the ecosystem as decomposition progressed. Fundamentally, decomposition should lead to mineralization of *Cladophora* tissue and reduction of mass with time. Thus, nutrients stored within the tissue eventually efflux to the water column or are incorporated into consumer tissue, including microbes. Additionally, if the mass of algal tissue varies in life stage, it is possible for an uptake of nutrients to initially occur before all cells senesce and begin to decompose, making interpretation of soluble nutrient concentrations difficult. Still, *Cladophora* in the presence of crayfish attack or wave action is likely to decompose more quickly and return nutrients to the system more completely than when these variables are absent or in the presence of an individual agent. Thus, I hypothesized that 1) *Cladophora* would lose mass over time in all treatments, 2) physical forces would dominate over biological forces in advancing tissue breakdown, and that 3) nutrient concentrations in the water would track losses from the algal tissue as decomposition progressed.

Materials and Methods

A full-factorial, randomized block design with four treatments and five replicates per treatment was used to investigate the role of biotic and abiotic variables on *Cladophora* breakdown. The four treatments were: 1) *Cladophora* alone (control), 2) *Cladophora* with

crayfish (biotic variable), 3) *Cladophora* with wave action (abiotic variable), and 4) *Cladophora* with both crayfish and waves (interaction). The trial began on 6 July, 2020 and ran for 20 days.

Experimental set-up

Twenty aquaria (23L, 41.3 x 21.6 x 26 cm) were arranged among four shelves with every treatment on each shelf, in a lab space with 12:12 light:dark lighting and controlled temperature (21ºC). Each aquaria received 7 L of carbon filtered city water to begin the experiment. Aquaria were brought back to volume every five days with distilled water to maintain the starting volume of 7 L. In addition to the experimental aquaria, five 'extra' tanks with 7 L of filtered city water held crayfish-only (i.e., no *Cladophora*) to provide some insight into any potential dissolved nutrients released by these decomposers. Each of the 20 test aquaria received ~15g (wet weight) of freshly-collected, recently sloughed, *Cladophora* added in a single mass, plus its treatment.

A large mass of sloughed, floating *Cladophora* was harvested from a near-shore area of Lake Ontario on 3 July 2020 (Fig. 1). Massive windrows of this alga were composed predominantly of newly detached *Cladophora*, as evidenced by the bright green color, with smaller amounts of *Spirogyra* and *Lemna*. Algae was collected from surface water windrows using a hand-held net (1.0 mm mesh) attached to a long pole and scooping from a small boat. Material was placed into zipper top plastic bags with lake water and placed on ice in a cooler until return to the lab. In the lab, collected material was floated in a large white dissecting tray. Algal strands were removed, lightly swirled to remove macroinvertebrates, and set aside. This process led to the removal of large surface debris and other plant fragments (e.g., bird feathers, *Lemna*, sticks, leaves, floating trash, etc.). Cleaned algae was refrigerated for up to three days prior to use in the experiment.

After filling with 7 L of filtered city water, each tank received ~15 g of the rinsed algae. Clumps of algae were picked from the collected mass and pressed between 2-ply brown paper towels to remove excess water before wet mass was determined. Five random tanks, one per shelf block, each received a single crayfish consumer (northern clearwater crayfish, *Fraxonius propinquus*), hand-collected from Ellicott Creek two days prior to use. This species previously had been identified as a *Cladophora* consumer functioning as a keystone species in streams of northern Michigan (Creed 1994). Each crayfish used in the experiment was weighed and sexed prior to their addition to aquaria receiving the biotic treatment. Any crayfish mortality during the experiment was replaced with a crayfish of similar size and sex. Mass was also recorded at the end of the 20-day trial.

To create the turbulence treatment, I designed a wave machine that induced a constant 'sloshing' of the aquaria contents for those tanks with the 'wave' treatment (Fig. 2a, 2b). An electric motor with a rheostatic control was attached to a drive shaft and mounted to a wood tower. Small belts connected long PVC 'arms' to pulleys on the drive shaft to induce consistent motion across each shelf. Short PVC arms with plastic fins were then attached to each long PVC arm. The plastic fins remained just under the water surface while imparting waves at a frequency of approximately 15 'sloshes' per minute (motor rheostat set at 15 rpm). This wave frequency created continual water movement and maintained the algae in suspension, but was slow enough to prevent any splashing that might result in water loss from each system. The motor ran continuously for the 20 days, except it was turned off once, between Day 9 and 10 (~10 hours) to allow suspended tissue to settle and collection of water samples for nutrient analysis (see below).

Data Collection

On Day 0 (July 6th, 2020) of the experiment, five individual portions of algal tissue were removed from the initial mass collected, before additional portions were added to test aquaria, to create a wet mass-to-dry mass conversion equation. These algal masses (~15 g ww), were pressed between 2-ply paper towels and weighed. The samples were placed in pre-tared aluminum weigh boats and dried for at least 48 hrs at 60° C in a drying oven. After drying, samples were placed in a dessicator until room temperature and re-weighed to obtain dry weight. This resulted in a wet mass-to-dry mass linear regression equation of $\text{DM (g)} = 0.5989(\text{WW}) - 6.8264$ ($R^2 = 0.8423$). All initial wet weights for the experiment were converted to dry weights using this equation.

I also determined algal chlorophyll *a* (*chl a*) content and carbon, nitrogen, and phosphorous content (C:N:P) on starting algal samples. For *chl a*, five, small samples of algae (~0.2 g ww) were placed in foil-wrapped scintillation vials with 10 mL of methanol and steeped in the refrigerator (~4ºC) for 24 hr. Pigment content was determined with a HACH DR/4000U spectrophotometer following procedures in Steinman et al. (2006). In short, working in subdued light, pigment-extracted aliquots (3 mL) were placed in cuvettes and read at 750 and 664 nm before, and 750 and 665 nm after acidification with 0.1 mL of 0.1N HCl and absorbance values recorded. These values were inserted into the equation:

$$\text{Chlorophyll } a \left(\frac{\mu g}{g}\right) = 26.7 \left(E_{664b} - E_{665a}\right) * \left(\frac{V_{met}}{M_{ww}}\right)$$

where:
26.7 = absorbance correction;

E_{664b} = (ABS of sample 664 nm – ABS of blank at 664 nm) – (ABS of sample at 750 nm – ABS of blank at 750 nm);

E_{665a} = (ABS of sample at 665 nm – ABS of blank at 665 nm) – (ABS of sample at 750 nm – ABS of blank at 750 nm);

V_{met} = Volume (mL) methanol;

M_{ww} = wet weight (g) of tissue sample.

For CN analysis, dried initial samples were ground in a mini-Wiley mill to homogenize. Five replicate subsamples for each treatment (~5 mg each) were packaged in tared tin capsules and analyzed in a CE Elantech, Flash 2000 Organic Elemental Analyzer. The analyzer ran at a combustion temperature of 900ºC and was calibrated using BBOT, aspartic acid and acetanilide (Lot #: 300113, 080118, and 011018; respectively). Standards were run as unknowns every 10 samples to allow relative percent difference calculation for assessing quality assurance.

A last sample was dried and digested for tissue P analysis by the ascorbic acid method, procedure 4500-P-E (APHA 2005). Three replicate samples per treatment (~0.1g) were gently boiled in (50 mL) of distilled water with 1 µl of 0.1 N sulfuric acid and 0.4 mg of potassium persulfate until about half its volume remained. After returning the reduced sample to their initial volume (50 mL) by addition of distilled water, a pre-weighed, commercially available molybdenum blue reagent (Hach 2106069 PhosVer 3 Phosphate Reagent Powder Pillow, 10mL) was added. After a 90-second wait period for color development, samples were read at 890 nm on a HACH D/R 4000 spectrophotometer for total phosphorus (TP) content.

On Day 20, crayfish were removed and weighed. The entire aquarium contents were passed through a 74-µm sieve to collect any remaining *Cladophora*. Small samples were removed, recorded, and used for *chl a*. All remaining tissue was placed in a pre-weighed aluminum weigh boat and processed for dry-weight as outlined above. These dried samples were then processed for CN analyses and TP content by the same methods used for initial (Day 0) samples.

Along with final tissue analyses, 100-mL water samples were drawn from each aquarium on Days 0, 10 and 20 using a syringe and filtered through a GF-C glass fiber filter (~0.7 µm

nominal pore size). Filtered samples were frozen at -20º C and sent to the National Center for Water Quality Research at Heidelberg University for nutrient and chemical analysis (NH_3, Cl^-, SO_4, SiO_2, NO_2, NO_3 and SRP). Ammonia, nitrite, and nitrate values were combined and reported as total nitrogen (TN). A total of 60 frozen samples were analyzed (i.e., 5 Day-0 samples for starting nutrient concentrations, 5 distilled water samples, 25 Day-10 samples, and 25 Day-20 samples).

Statistical Analyses

Final *Cladophora* percent mass remaining on Day 20 was analyzed for treatment differences using a one-way ANOVA on arcsin-transformed data. Variance and normality assumptions were met.

Tissue nutrient and chl *a* concentrations were compared for any changes over time or by treatment using repeated-measures ANOVAs (RMANOVA) across two dates (start and finish) and the four treatments. Since 'time' was the only significant factor, no post-hoc testing was required as there were only two dates. Box and whisker plots were created to visualize the direction and magnitude of change with time for each nutrient (% content of C, N and P) as well as chl *a* (µg/g). Assumptions of sphericity, variance and normality were all met for each set of data.

Water chemistry data received from Heidelberg University were also fit using RMANOVA. These data were collected across three dates (Days 0, 10, and 20) and four treatments and were assessed for effects of time, treatment, and any interaction. Any significant main effects were followed up with post-hoc testing (Tukey HSD) to tease out where pairwise

differences occurred. Data were transformed using *ln*(x+1) to meet assumptions of sphericity, variance and normality.

Results

Mass change

Over the 20-day trial, *Cladophora* in all treatments lost mass and ANOVA outcomes for the arcsine-transformed percent remaining dry-mass did not indicate any differences among treatments ($F_{3,16} = 0.7876$, $P = 0.52$). The mean percentage of remaining *Cladophora* by dry weight for each treatment was: crayfish only = 30.2, wave action only = 33.4, crayfish with wave action = 30.6, and no treatment = 33.2 (Fig. 3). There was a grand average loss of 68.2% of tissue mass amongst all aquaria over the 20-days.

Tissue Nutrients

RMANOVAs of tissue C, N, and P revealed that there was a significant change in each of the measured tissue nutrients over time (all P-values <0.001; Table 1). However, there was no treatment nor time*treatment interaction. Tissue chl *a* content also declined significantly over the experiment duration ($F_{1,16} = 1904.8$, $P < 0.001$), but was unaffected by the treatments and exhibited no significant time*treatment interaction. Both tissue C and chl *a* measurements exhibited a strong decrease in concentration through time, while tissue N and P showed a slight increase in percent content over time (Fig 4).

Visually, the *Cladophora* tissue progressed from a rich and deep green color on Day 0 with noticeable, branching filamentous structure to a greenish-brown color with no filaments intact on Day 20 (Fig 5). Treatments with turbulence resulted in the algae remaining in

suspension for the duration of the experiment (Fig 6A), whereas replicates of the control treatment all remained in a single mass at the bottom of each aquarium (Fig 6B). In the crayfish-only treatment (no turbulence), the alga was spread evenly across the bottom of the aquaria (Fig 5C).

Water Chemistry

RMANOVAs for all water chemistry components (SiO_2, Cl^-, SO_4, SRP and TN) showed significant differences between times and among treatments, but also exhibited significant time*treatment interactions (Table 2), making interpretation difficult. Three nutrients (TN, SO_4, and SiO_2) increased in the water column over the experiment duration, with crayfish treatments diverging from non-crayfish treatments by Day 20 (Figs. 7B, 7C, and 7D). The chloride treatment showed no obvious pattern of change over the experiment, increasing in some and decreasing in others (Fig. 7E). Notably, SRP declined dramatically by Day 10, but then increased in all treatments, though much more sharply in non-crayfish treatments (Fig. 7A).

Discussion

Sloughed *Cladophora* lost over half of its initial biomass (mean = 68.2%) to decomposition in the 20-day experiment. Results suggest neither herbivory nor wave action accelerated mass losses since percent remaining in controls was not different than any treatment. The scope of this experiment certainly did not include all possible influencers to decomposition, as the design was limited to determining if mechanical breakdown due to sheer stress by turbulence or herbivory had any effect on the decomposition rate of *Cladophora*. It may be possible for other effectors, such as temperature or the presence of various other organisms, to

have greater impact on the decomposition process of this algae. Regardless, it is clear this alga lost mass rapidly under these experimental conditions.

Water column nutrient analyses over the test duration suggested sloughed algae was not dead algae, and continued some nutrient uptake over the first 10 days. Soluble phosphorus concentrations declined through Day 10 across all treatments, indicating metabolic demand by either *Cladophora*, epiphytic diatoms, bacteria, or some combination of these consumers (Fig. 6A). The return of water-column SRP to Day 0 levels in the non-crayfish treatments indicates *Cladophora* was senescing and releasing SRP into the tanks. The other dissolved nutrients did not show the same responses, with all the reactive nutrients (TN, SiO_2, and SO_4) increasing over the duration of the trial (Figs. 7B, 7C, 7D).

Changes in tissue nutrients support observed changes in water column nutrients. The phosphorus and nitrogen content in remaining *Cladophora* tissue was higher at the end of the experiment than at the beginning (Fig 4B, 4C), whereas tissue C and chl *a* content were lower on Day 20 than Day 0. Senesced *Cladophora* should have a lower metabolic demand for carbon, which, in turn, should lead to a reduction in the presence of the carbon-rich chl *a* molecule (Fig 4D). Higher tissue N and P concentrations at the end of the experiment are plausibly explained by an increase in bacterial abundance on decomposing *Cladophora* tissue. In general, as leaf litter decays over time in aquatic environments microbial biomass increases as the community absorbs both tissue-bound and leached nutrients (Benfield 1996).

In a study of decomposition of estuarine *Cladophora* in the Baltic Sea (Paalme et al. 2002) used both *in situ* and *in vitro* experiments to document a 65% mass reduction in tissue through the first 20 days post-sloughing, with little additional increase thereafter (through 35 total days), as well as a net increase of tissue P for the first 14 days under aerobic conditions.

They also showed a slight increase in tissue nitrogen. My observations mirror each of these observations of Paalme et al. (2002).

Although there appeared to be no influence on *Cladophora* tissue degradation by including crayfish in the experiment, water chemistry data seem to indicate their presence affects the individual systems as a whole. SRP, TN and SiO_2 all showed significant differences in the aquaria which included crayfish compared to those that did not. The initial decline in water-column SRP, followed by increases in both TN and SRP suggest uptake by *Cladophora* until production declines. After Day 10, TN and SRP increase in the water, whereas chl *a* and tissue C content decline. But crayfish treatments diverge from non-crayfish treatments in this pattern. Possibly, the decline in SRP in crayfish treatments is explained by crayfish excreta that are in a more bio-available form and are quickly taken up by microbes and stimulatory to microbial growth, or perhaps crayfish exoskeletons harbor large microbial communities which rapidly take up available nutrients. Similarly, the increases in dissolved silica are not easily explained by *Cladophora* decomposition. Perhaps epiphytic diatoms attached to *Cladophora* filaments are also dying as the algae senesces, releasing silica. These observations require further investigation.

Although water column nutrient concentrations in treatments containing crayfish diverge from non-crayfish treatments by Day 20, crayfish mass was unchanged over the trial duration (Day 0 = 5.9 g; Day 20 = 5.8 g; $t = 0.0015$, $df = 9$, $P > 0.5$) and the algal mass lost in their tanks did not differ from non-crayfish treatments. These observations suggest no herbivory on *Cladophora* by crayfish occurred. But if these animals did not eat, I would expect them to exhibit some mass loss over the trial duration, especially in the treatments with turbulence since it would be necessary to expend energy to overcome the currents. It is possible that the crayfish switched

to filter-feeding ingestion, which could substantially affect any diatom communities within the enclosures. Food availability can cause a dietary shift and change in feeding mode in various species of crayfish, allowing direct consumption of diatoms (Tran and Manning 2019). The frustules of diatoms are composed predominantly of silica (Desikachary and Dweltz 1961) and when broken down, result in abundant SiO_2 in the water. It is plausible that diatom predation by the crayfish resulted in the higher-than-expected levels of SiO_2 in the crayfish tanks (Fig 7C). In addition, when phosphorus is limited, similar to my Day 10 findings (Fig 7A), some diatoms will down-regulate nitrogen uptake (Alipanah et al. 2018). Thus, it is possible the diatom communities are influencing the observed increase in water column N in the presence of reduced SRP (Fig 7B).

In conclusion, these data suggest sloughed *Cladophora* harbors potentially large epiphytic communities (probably diatoms and microbes) that contribute to post-senescence nutrient dynamics. Initially, *Cladophora* tissue and/or epiphytic communities continue to take up SRP for the first 10 days, followed by nutrient release to the water column as both *Cladophora* tissue breaks down and biofilm communities die back. Over half of the algal tissue mass is lost within 20 days via these actions, and neither the presence of wave action nor herbivorous crayfish appear to alter this rate. Thus, depending on the total mass of algae present following a sloughing event, a large pool of bioavailable phosphorous and nitrogen will enter the water column within 20 days. For SRP, water column concentrations will return to pre-sloughing levels. Water column TN and SiO_2, on the other hand, will increase by roughly three-fold relative to pre-sloughing levels.

It has been established that sessile *Cladophora* filaments may harbor >60% additional mass due to epiphytes during late season conditions (Higgins et al. 2008b). This research may

suggest that, upon sloughing, these epiphytic communities may have a rapid means of transportation through the movement of sloughed algal mats. Despite our many manipulations of *Cladophora* filaments before introduction to the experiment (i.e., post-sloughing collection, rinsing, introduction to filtered water, addition of wave action and a predator), it appears that epiphytic communities were able to thrive. While *Cladophora* itself may hold nutrients vital to primary production in the nearshore region of the Great Lakes, it may also be a mobile habitat, increasing the surface area required for movement by small, otherwise sessile ecosystem contributors while also providing their metabolic needs. Along with contributions to nearshore food web dynamics by the algae, contributions by the community it brings with it should also be considered. While the recent resurgence of *Cladophora* is attributed to *Dreissena* in the Great Lakes, it is likely that *Cladophora* isn't the only organism benefitting from increased algal biomass.

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Table 1. RMANOVA output examining tissue nutrient and *chl a* concentrations among four treatments across two dates (Day 0, Day 20). Significant outcomes in bold.

	Source	df	F	P
Carbon	treatment	3	0.248	0.862
	error	16		
	time	1	286.277	**<0.001**
	time*treatment	3	0.420	0.741
	error	16		
Nitrogen	treatment	3	1.618	0.225
	error	16		
	time	1	20.618	**<0.001**
	time*treatment	3	0.925	0.451
	error	16		
Phosphorus	treatment	3	0.574	0.640
	error	16		
	time	1	16.257	**<0.001**
	time*treatment	3	0.592	0.629
	error	16		
chl a	treatment	3	2.134	0.136
	error	16		
	time	1	1904.803	**<0.001**
	time*treatment	3	1.437	0.269
	error	16		

Table 2. RMANOVA output examining water column nutrient concentrations among four treatments across three dates (Day 0, Day 10, Day 20). Significant outcomes in bold.

	Source	df	F	P
Soluble Reactive Phosphorus	treatment	3	19.15	**<0.001**
	error	16		
	time	2	369.30	**<0.001**
	time*treatment	6	15.04	**<0.001**
	error	32		
Chloride	treatment	3	11.31	**<0.001**
	error	16		
	time	2	6.43	**0.001**
	time*treatment	6	4.75	**0.001**
	error	32		
Silicon Dioxide	treatment	3	566.48	**<0.001**
	error	16		
	time	2	273.27	**<0.001**
	time*treatment	6	11.57	**<0.001**
	error	32		
Total Nitrogen	treatment	3	18.36	**<0.001**
	error	16		
	time	2	695.27	**<0.001**
	time*treatment	6	5.01	**0.001**
	error	32		
Sulfate	treatment	3	16.02	**<0.001**
	error	16		
	time	2	74.35	**<0.001**
	time*treatment	6	5.71	**<0.001**

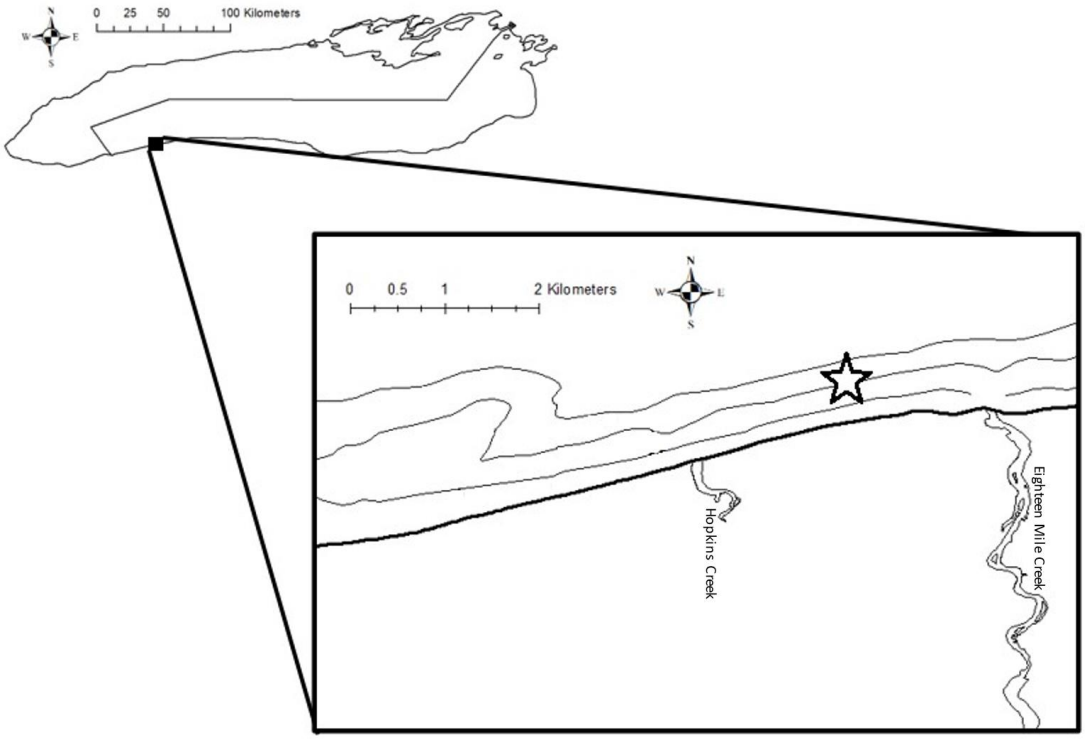
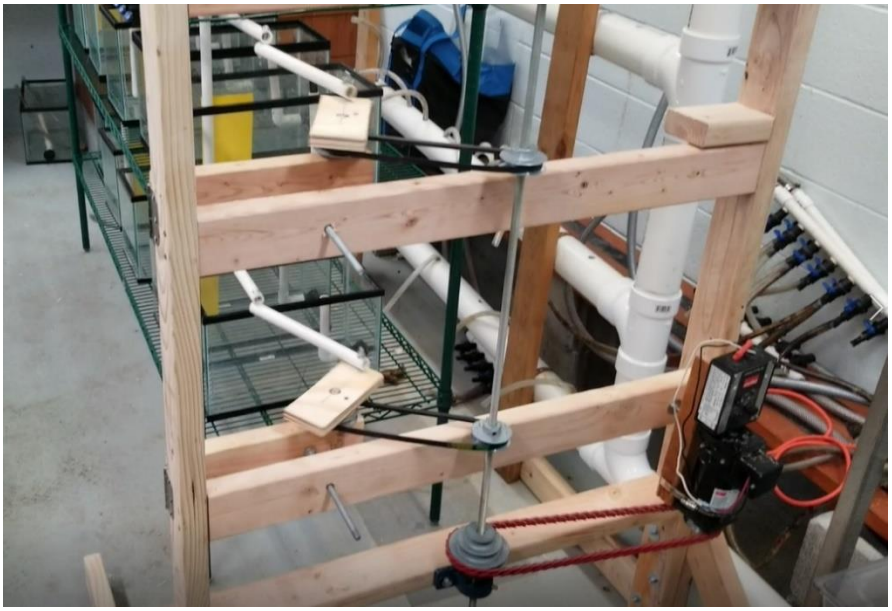


Figure 1. Map of collection area near south-western shore of Lake Ontario. The contour lines in the inset image signify 3, 6 and 9 m depth while the star indicates the collection area, approximately 1km west of 18-Mile Creek.

A. 

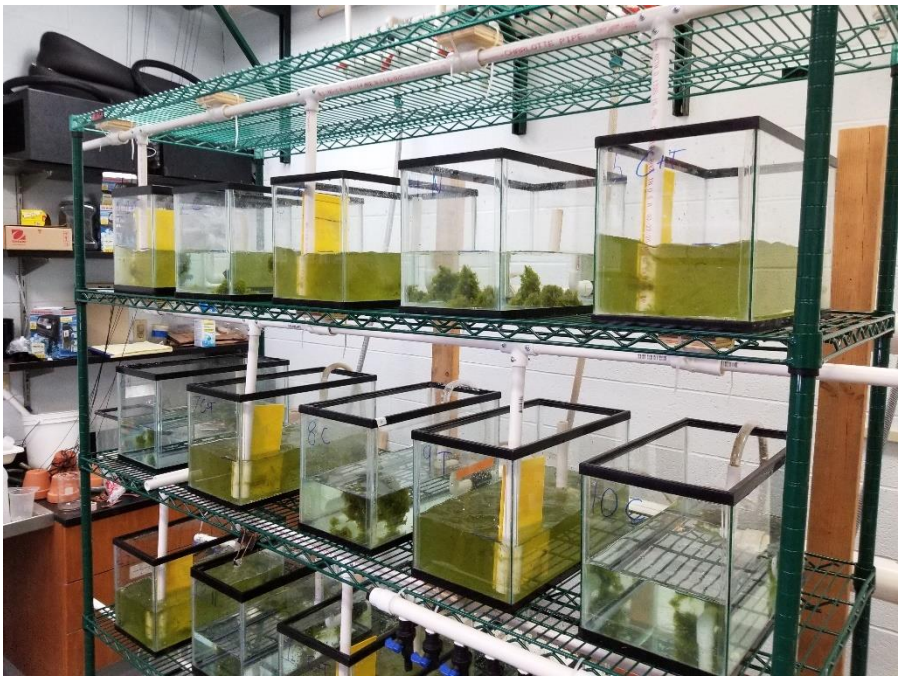
B. 

Figure 2. Experimental aquarium design testing wave and crayfish effects on *Cladophora* decomposition. A: Rheostat-controlled motor attached to drive shaft pulleys with aquaria in background. B: Day 0 aquaria with 7 L filtered city water and 'fins' inside aquaria receiving turbulence treatment.

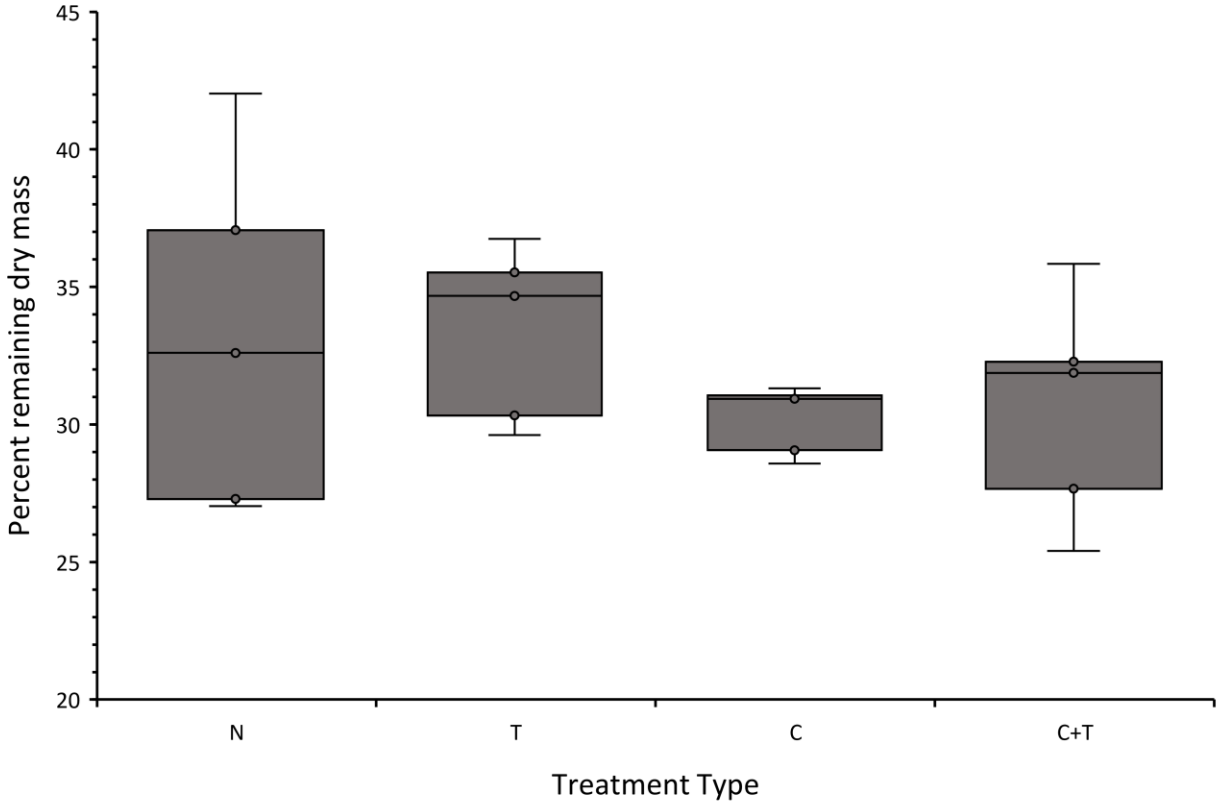


Figure 3. Plot of remaining dry mass after 20-day trial by treatment type. Treatments are identified as follows: No treatment = N, Turbulence only = T, Crayfish only = C, Crayfish and turbulence = C+T. High and low values of boxplots show quartiles with the line within each box indicating the median. Whiskers identify the highest and lowest values respectively and open circles indicate individual data points.

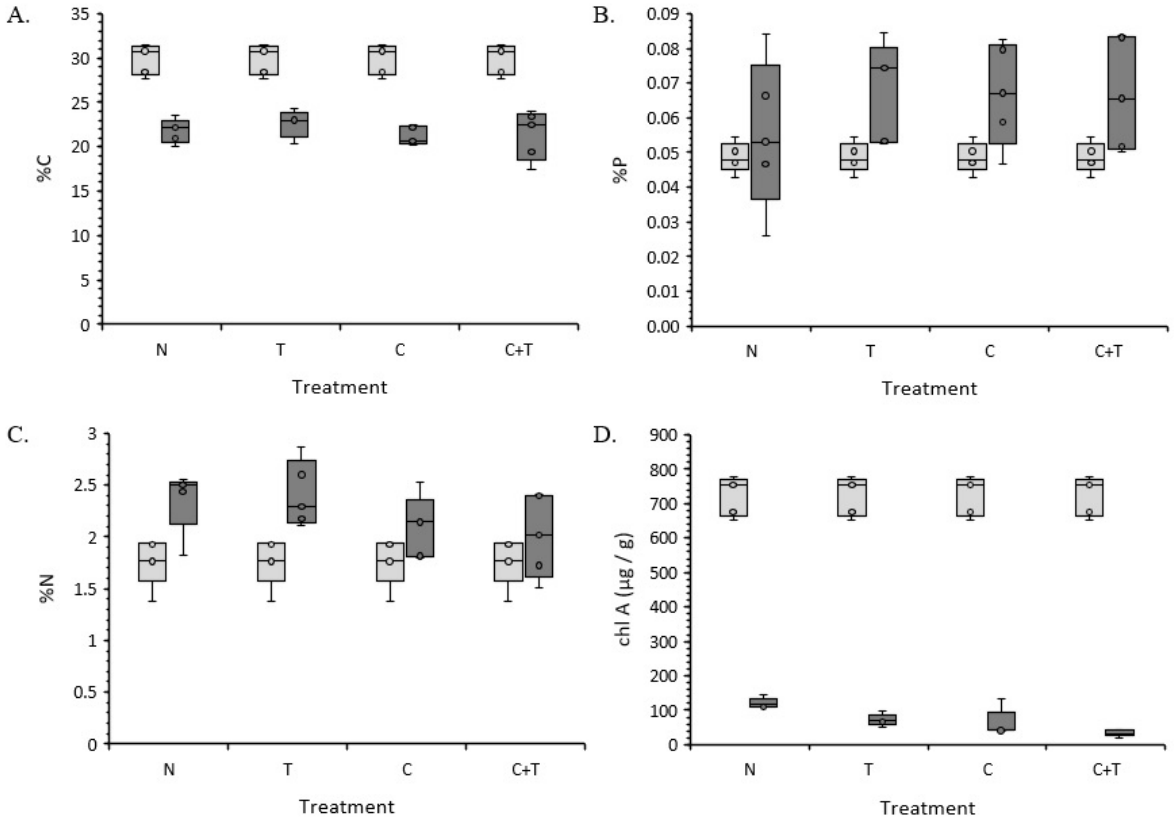
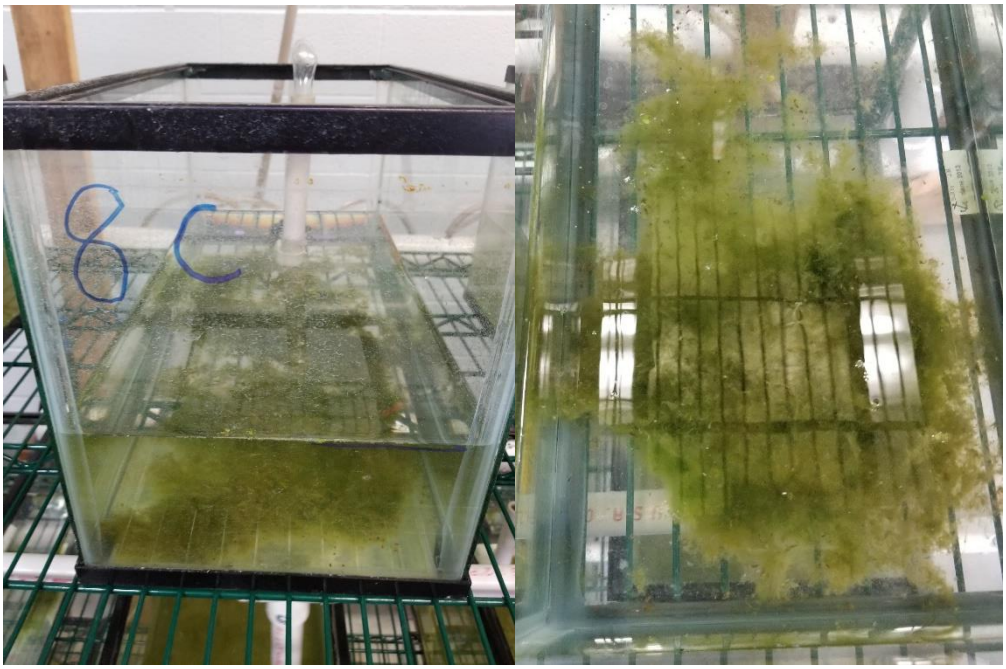


Figure 4. Changes in C, N, P and *chl a* tissue content of *Cladophora* between Day 0 and Day 20 of experiment. High and low values of boxplots show quartiles with the line within each box indicating the median. Whiskers identify the highest and lowest values respectively and open circles indicate individual data points. Light gray boxes represent Day 0 and dark gray boxes represent Day 20. For treatments, No treatment (control) = N, Turbulence only = T, Crayfish only = C, and Crayfish and turbulence = C+T.

A.



B.

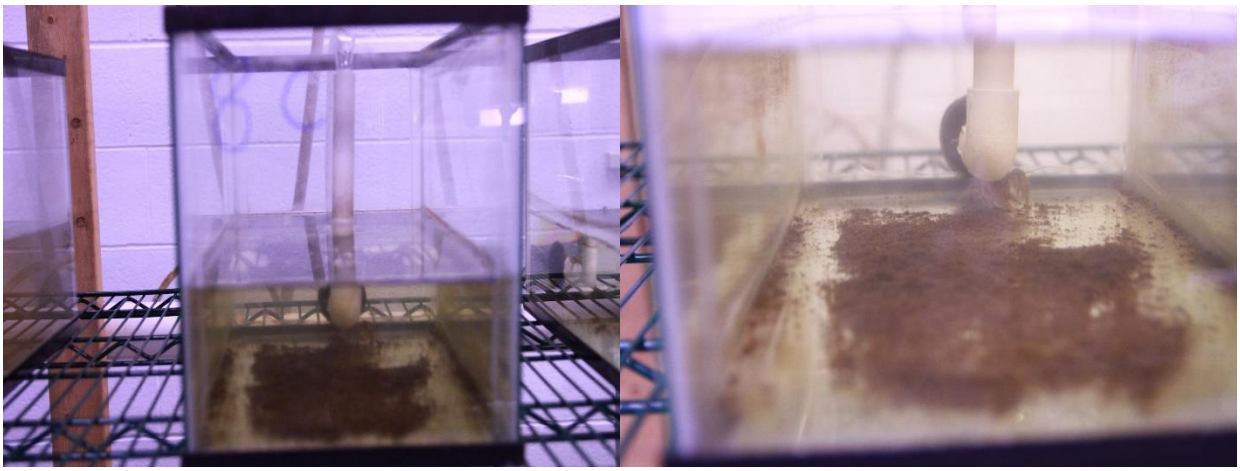


Figure 5. *Cladophora* on day 0 (A.) and day 20 (B.). The initial fresh algae had visibly reduced in dimension as well as changed from green, with visible branching and algal filamentous structure to brown sediments, no longer resembling filamentous algae.

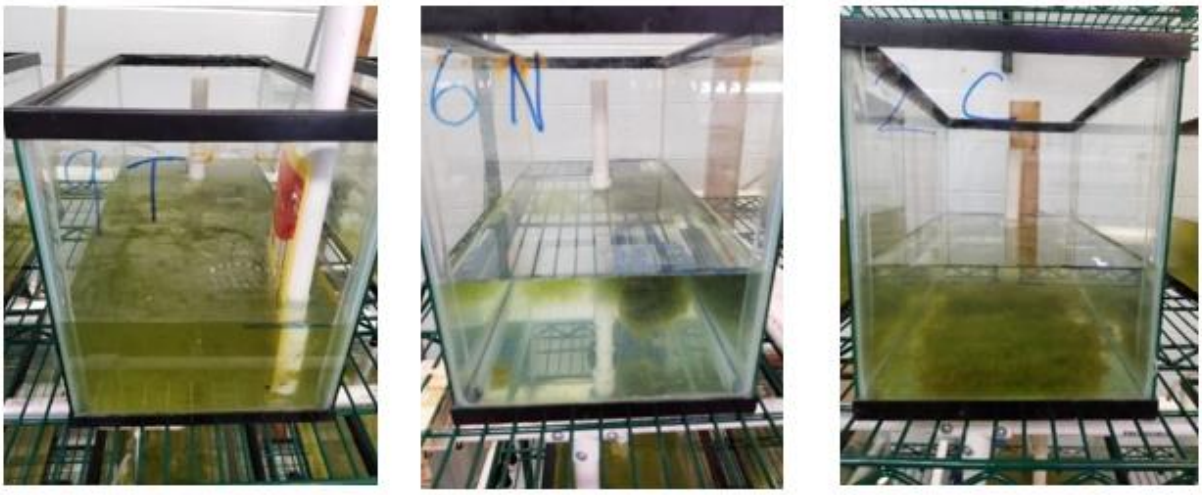


Figure 6. Images from Day 5 of the experiment. Panel A shows a turbulence treatment with algae remaining in suspension. Panel B shows a control treatment with neither turbulence nor crayfish included, and algae remained in a single mass. Panel C shows a crayfish treatment with no induced turbulence, and algae were spread across the aquaria due to crayfish bioturbation.

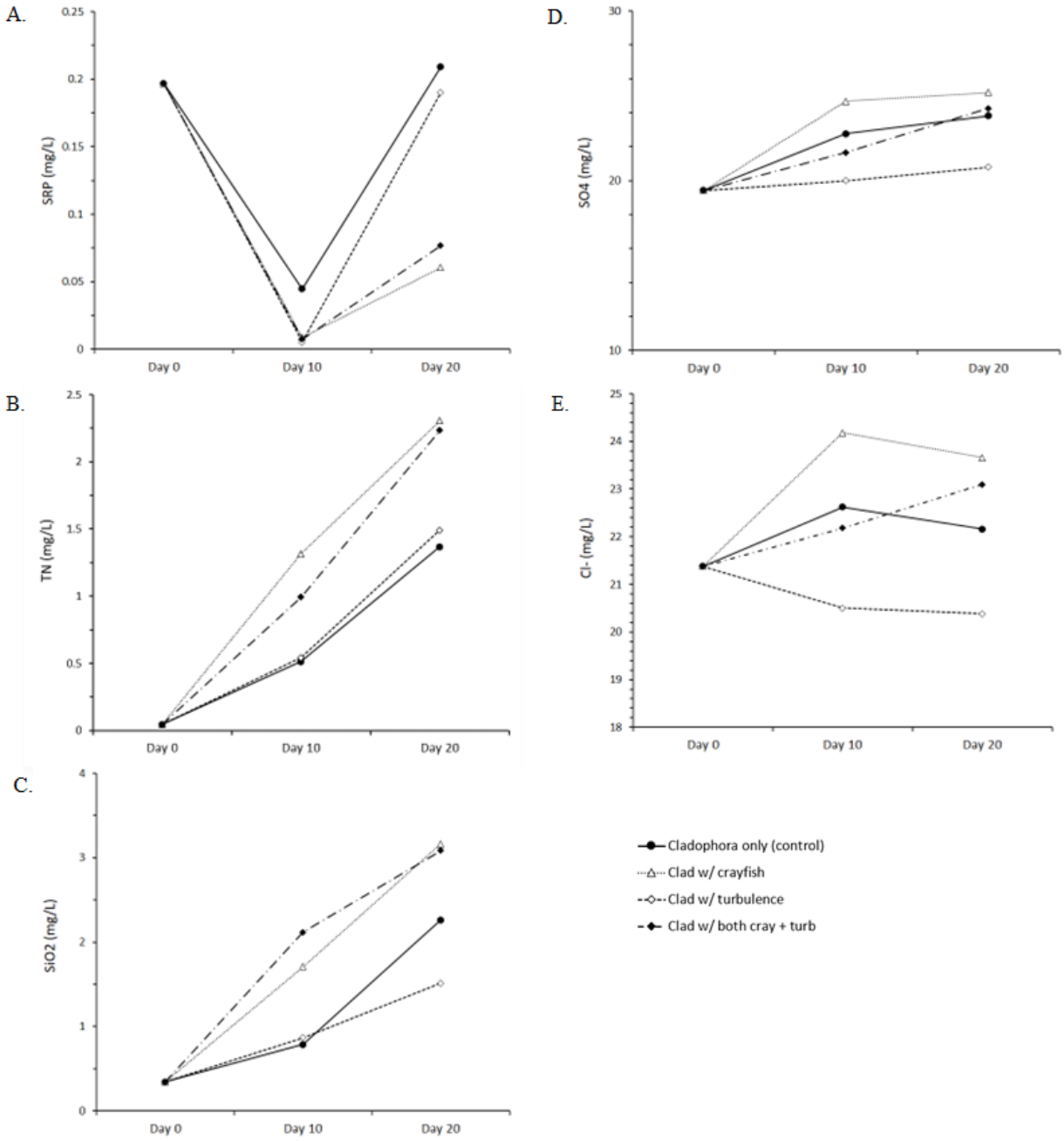


Figure 7. Water column mean concentrations of nutrients on three sample dates over the experiment duration. Panels A, B, C, D, and E are soluble reactive phosphorous (SRP), total nitrogen (TN), silicon dioxide (SiO_2), sulfate (SO_4) and chloride (CL^-).