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Effects of propagule pressure, environmental factors, and climate change on success and impacts of benthic aquatic invasions

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EFFECTS OF PROPAGULE PRESSURE, ENVIRONMENTAL FACTORS, AND CLIMATE

CHANGE ON SUCCESS AND IMPACTS OF BENTHIC AQUATIC INVASIONS

By

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Abstract

A. S. Brainard. Effects of Propagule Pressure, Environmental Factors, and Climate Change on Success and Impacts of Benthic Aquatic Invasions, 206 pages, 10 tables, 28 figures, 2018. APA style guide used.

Nonnative species introductions are linked to anthropogenic drivers, including transport of species into novel habitat(s), changes in local environmental factors that may facilitate invasions, or large-scale shifts in abiotic conditions with climate change. In freshwater ecosystems, transport of nonnative species often occurs via boats (e.g., trailers, boat props, bilge water). Environmental factors include differences in watershed land use and water quality (e.g., transparency, nutrient and chlorophyll-a concentrations, pH, and conductivity). Climate change may increase water temperatures, affecting lake stratification, and causing low or pulsed dissolved oxygen concentrations. Both understanding factors causing spatial variability of invasive species and assessing potential negative impacts to ecosystems are important. For example, some invasive species are more tolerant or adapted to increased water temperature or lower dissolved oxygen, and may persist in such environments, while natives decline. This research assessed propagule pressure, environmental factors, and climate change, focusing on benthic macrophytes and macroinvertebrates. First, propagule risk (proxy for propagule pressure, capturing extent to which invasives might be introduced from different populations) and environmental conditions were correlated with richness and abundance of invasive macrophytes in 20 lakes; results suggest that propagule risk was the driver of invasive macrophytes in the lake communities studied. Negative impacts on macrophyte communities from the introduction of a dominant macroalgae, *Nitellopsis obtusa*, were also evaluated. Increased abundance of *N. obtusa* was correlated with reductions in total and native macrophyte richness, a pattern consistent across depths, suggesting that *N. obtusa* can displace species in lake communities. Finally, bioenergetics and nutrient/trace metal sediment release from the nonnative oligochaete, *Branchiura sowerbyi*, were compared to those of native benthic invertebrates (*Hexagenia* and *Chironomus riparius*) under various water temperature and DO concentrations, to understand ecosystem implications of *B. sowerbyi*'s introduction. Results suggest that *B. sowerbyi* may be more tolerant of conditions under predicted future climate, and thus may spread and become more dominant in benthic communities, with implications for sediment nutrient and contaminant dynamics. Results of this research offer insight into ecological processes involved in benthic aquatic invasions, including factors that may lead to their success, and the consequences for ecosystems and native species once established.

Key Words: invasive species, climate change, propagule pressure, environmental factors, macrophytes, benthic invertebrates, bioenergetics, bioturbation, bioirrigation

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CHAPTER 1: INTRODUCTION

Invasive species are recognized as a major threat to global biodiversity (Clavero and

García-Berthou 2005; Molnar et al. 2008), with direct impacts on economies and public health

(Schaffner et al. 2013). For instance, reductions in species richness (Hejda et al. 2009; Brainard

and Schulz 2017 [Chapter 3]) and evenness (Hejda et al. 2009) due to invasive species have been

documented. Economically, invasive species have been estimated to cost the United States up to

$120 billion annually (Pimentel et al. 2005). Certain invasive species (e.g., invasive mosquitoes)

can cause direct human health concerns (Schaffner et al. 2013), while other invasives can

indirectly influence public health. For instance, the invasion of dreissenid mussels (*Dreissena*

polymorpha and *Dreissena bugensis*) directly resulted in phytoplankton assemblages in some

North American lakes to be dominated by cyanobacteria (Vanderploeg et al. 2001), which can

produce toxins harmful to humans, domestic pets, and livestock (Sivonen and Jones 1999). Other

invasive species can either directly or indirectly have negative effects on public health by

altering ecosystems to reduce availability of goods (e.g., agricultural and forest products) and/or

services (e.g., clean drinking water, recreation opportunities) (Pejchar and Mooney 2009).

Climate change is another stressor on ecosystems worldwide (Rosenzweig et al. 2007;

Adrian et al. 2009). For example, increased air temperatures are expected to increase surface

water temperatures. Evidence currently suggests a strong link between increased air temperatures

and those in the epilimnion of many freshwater lakes globally (O'Reilly et al. 2015; Livingstone

2003; Arhonditsis et al. 2004). In addition, a rise in water temperatures will likely result in

greater strength of thermal stratification for many lakes, as greater differences in temperatures

between surface (influenced by air temperatures) and deeper layers cause greater density

differences and more resistance to mixing (Adrian et al. 2009). As a direct result of longer

periods of stratification, decreased dissolved oxygen (DO) is likely to occur at depth (Hanson et al. 2006). Reduced DO at the sediment-water interface has implications for nutrient cycling, availability of dissolved oxygen for biota and habitat suitability (De Stasio et al. 1996; Jansen and Hesslein 2004; Wilhelm and Adrian 2008) - although the extent of impacts will be regional and likely dependent upon other variables (e.g., lake morphology). Nevertheless, climate change threatens to alter lake ecosystem processes (both abiotic and biotic). Lakes are sensitive to climate variability, and thus lakes are good model study systems to evaluate the effects of climate change (Adrian et al. 2009).

Invasive species and climate change potentially could act synergistically to degrade ecosystems (Hellmann et al. 2008). Increased temperatures in a region will likely facilitate a novel suite of potential invader that could establish (Holzapfel and Vinebrooke 2005), become abundant, and exert negative effects (conversely, however, some coldwater-adapted invasive species may be reduced) (Rahel and Olden 2008). Climate change will likely shift abiotic conditions to exacerbate impacts of certain invasive species (Hellmann et al. 2008; Rahel and Olden 2008). For example, Taniguchi et al. (1998) examined competition for food among invasive brown trout (*Salmo trutta*) and native brook trout (*Salvelinus fontinalis*) at various temperatures, and found invasive brown trout were superior competitors relative to brook trout at increased water temperature (yet competition for food was similar at colder temperatures). Further, abundances of certain native species may decline as environmental conditions shift away from those to which they are best adapted (Byers 2002); this may facilitate the expansion or establishment of invasives (Hellmann et al. 2008). Beyond increased water temperature, climate change may alter other, related abiotic factors in aquatic system such as dissolved oxygen. Aquatic invasive species that can tolerate increased periods of hypoxia, or larger

fluctuations of dynamic dissolved oxygen conditions, may be at an advantage relative to other species.

Benthic organisms are vital to healthy freshwater ecosystem functions (Covich et al. 1999). Macrophytes and macroinvertebrates, specifically, play critical roles in ecosystem primary production, habitat availability, and secondary production within food webs (Likens 1975; Waters 1977; Hall et al. 2006; Christie et al. 2009). However, the importance of the benthos in limnology has largely been understudied (Vadeboncoeur et al. 2002). In a literature meta-analysis, Vadeboncoeur et al. (2002) evaluated the number of studies that quantified primary producer productivity and invertebrates (both productivity and biomass) between pelagic and benthic organisms, and found that 91% of studies only quantified pelagic primary production. Additionally, 73% of studies measured only pelagic invertebrate (zooplankton) productivity or biomass (Vadeboncoeur et al 2002). However, evidence suggests that benthic primary producers may contribute nearly 50% of whole-lake productivity, and benthic invertebrates contributing up to approximately 40% of total invertebrate secondary production or biomass (Vadeboncoeur et al. 2002). Thus, there appears to be a discrepancy in the research focus of the benthos relative to its importance in freshwater ecosystems.

Benthic invasive species can have direct negative effects on ecosystems that have been invaded (such as those described above), and indeed, some of the most notorious invaders in North America are benthic species. For example, Eurasian watermilfoil (*Myriophyllum spicatum*), an invasive macrophyte, has been shown to displace native macrophytes in Lakes Mendota (Lind and Cottam 1969) and Wingra (Nichols and Mori 1971) in Wisconsin. Infestations of *Hydrilla verticulata*, another invasive macrophyte in North America, have been shown to cause reduced recreational fishing opportunities and revenue generated from sport

fishing (Colle et al. 1987). These are just a few examples, among many, of negative ecological and economic effects resulting from benthic aquatic invasive species.

The impacts of some benthic invasive species may not always be as apparent as those described above, and may vary over time. Even invasive species that are documented to have negative effects in one location, may not be as detrimental in others. For example, Eurasian watermilfoil displacement of native macrophytes was not observed in some locations where the plant simply established in previously unvegetated areas of littoral zones (Keast 1984). Negative impacts from invasions also may have temporal implications; Strayer et al. (2006) evaluated the impacts of invasive species over time, and suggested that these effects may be realized only due to changes in community composition or characteristics of species within a community. However, those authors did not include changes in abiotic environmental conditions (e.g., such as those with climate change) in contributing to the subsequent increase in abundance and effects of invasive species over time. While the effects of invasive species on native communities, populations, or ecosystem processes may not be large or apparent under current conditions, changes in temperature or dissolved oxygen concentrations in freshwater ecosystems may cause reductions in native species (not directly related to the invader), and create conditions that favor an invasive species over natives. Further, some invasive species (e.g., *Nitellopsis obtusa*) are cryptic in nature, making their negative effects on systems invaded ambiguous due to limited documentations of their presence. Indeed, the effects of many invasive species likely change over time (Strayer et al. 2006), and negative effects are not realized until invasive populations reach an abundance that is noticeable and/or quantifiable.

The overall objective of this research was to investigate benthic invasion ecology patterns and processes related to anthropogenic actions such as overland transport, alteration of

environmental factors, and climate change. The research herein focuses on macrophytes and benthic macroinvertebrates.

Chapter 2 addresses two factors that have been hypothesized to lead to invasions, propagule pressure and anthropogenic alteration of environmental factors, in relation to presence (richness) and abundance (both biomass and dominance) of invasive macrophytes in lakes. By selecting study lakes with an *a priori* range in propagule pressure (e.g., extent of boat movements to other waterbodies), and secondarily assessing abundance of invasive macrophytes in relation to environmental conditions, the objective of this study was to determine the relative importance of propagule pressure versus conditions that may favor invasive species. It was hypothesized that increased propagule pressure would lead to greater abundance of invasive macrophytes. Additionally, increased extent of degraded natural environmental conditions (e.g., watershed land use types, increased water phosphorus concentrations) were predicted to result in increased abundance of invasive macrophytes.

Chapter 3 addresses the impacts from the invasion of a cryptic macroalgae invader, *Nitellopsis obtusa*, on macrophytes communities. *Nitellopsis obtusa*, first discovered in North America in the 1970s (Geis et al. 1981; Karol and Sleith 2017), can form dense mats that previously have been implicated in the displacement of native macrophytes (although evidence for this is largely anecdotal). Additionally, it has been suggested that *Nitellopsis obtusa* invades deeper water locations, limiting the ability of early detection monitoring to accurately document its presence. I predicted that, when high in abundance, *Nitellopsis obtusa* would result in decreased macrophyte richness and abundance of other macrophytes, and would be observed in deeper water sampling locations within study lakes.

In Chapter 4, the bioenergetics performance of a nonnative oligochaete, *Branchiura sowerbyi*, is compared to those of important and abundant native invertebrates (*Hexagenia* spp. and *Chironomus riparius*) that are found in many freshwater systems in the northeastern U.S., including the Laurentian Great Lakes. Survivability and bioenergetics endpoints (respiration, nutrient excretion and egestion, and growth) were compared between the three macroinvertebrates across temperature and dissolved oxygen (DO) conditions that are projected with climate change. The effects of climate change are often viewed only through the lens of temperature on species responses (Rahel and Olden 2008). In this chapter, examining responses of the three organisms to dissolved oxygen offers insight into other abiotic changes that are predicted to occur with climate change. It was hypothesized that increased water temperature and decreased DO would result in favorable conditions for *Branchiura sowerbyi*, due in part to its suggested ability to tolerate such conditions. As a result, survivability of *Branchiura sowerbyi* was predicted to be greater in warmer water temperatures with reduced DO compared to the native macroinvertebrates. Additionally, decreased respiration, excretion/egestion of metabolic wastes, and growth of *Branchiura sowerbyi* was hypothesized to be lower than *Hexagenia* spp. and *Chironomus riparius* due to its ability to tolerate environmental conditions suggested with climate change.

Chapter 5 evaluates sediment phosphorus and trace metal release due to the bioturbation (sediment mixing) and bioirrigation (sediment ventilation) of *Branchiura sowerbyi* compared to those of native *Hexagenia* spp. and *Chironomus riparius* macroinvertebrates. Because *Branchiura sowerbyi* is larger than the two native invertebrates, and its burrowing behavior is different (does not construct structures, and feeds with anterior end into the sediment with wastes deposited at the sediment-water interface), it was predicted that *Branchiura sowerbyi* would

result in greater phosphorus and trace metal release compared to those of the native macroinvertebrates.

Altogether, this research highlights the importance of benthic aquatic invasive species in freshwater ecosystems, and the influences of anthropogenic activities, such as propagule pressure and climate change, in driving both invasion success (presence and abundance) and impacts.

CHAPTER 2: PRESENCE AND ABUNDANCE OF INVASIVE MACROPHYTES INCREASE ACROSS A RANGE OF PROPAGULE RISK

INTRODUCTION

Negative impacts of invasive species are recognized as a leading cause of anthropogenic change (Vitousek et al. 1997), including in aquatic ecosystems (Gallardo et al. 2015). Invasives, like most species, are not evenly distributed across landscapes (Ruiz et al. 2013). Understanding factors that can lead to their spatial variability in presence and abundance is a main goal in invasion ecology to prioritize prevention efforts, and understand ecological factors that might contribute to vulnerability or resistance of particular habitats.

Inland lakes are model systems to test patterns of spatial variability in invasive species because they act as islands in the terrestrial landscape (Browne 1981). Major transport vectors, such as recreational boats, are quantifiable. Additionally, lakes and watersheds have clearly defined boundaries that allow for environmental factors (e.g., water chemistry, land use) to be quantified accurately.

Propagule pressure, which incorporates the number of individuals of a species introduced into an area in a given event (propagule size) plus the number of introduction events (propagule number) (Lockwood et al. 2005), has been documented to be a critical component of invasion success (Colautti et al. 2006, Simberloff 2009). Repeated introductions (e.g., large propagule number, or many separate introduction events) can promote invasive species establishment and/or dominance by increasing genetic diversity of invader populations (Roman and Darling 2007), increasing the likelihood of introducing genotypes pre-adapted to conditions in the novel system (Le Roux et al. 2008). Propagule pressure is difficult to measure accurately, and researchers often construct models or conduct controlled experiments to overcome the challenge of quantifying propagule pressure. For example, Chadwell and Engelhardt (2008) utilized

greenhouse experiments to test the role of propagule pressure on colonization success of *Hydrilla verticillata* by introducing plant fragments to mesocosms at various densities. Marchetti et al. (2004) estimated propagule pressure of invasive fish in California based on historic records of introductions, and Leung et al. (2006) used gravity models to estimate movement of recreational boaters between lakes.

A leading vector of invasive species to freshwater ecosystems, such as inland lakes, is overland transport from recreational boating (Johnson et al. 2001). One approach to estimate propagule pressure from recreational boating is to quantify the potential risk of introduction of invasive individuals, or propagule risk. Lo et al. (2012) utilized a similar approach to estimate propagule pressure from commercial shipping activity in Canada by utilizing shipping data (region of vessel origin, and vessel size to estimate ballast volume). Thus, each waterbody that boaters had previously visited can represent increased risk by moving individuals from one lake to another, and is an indirect estimate of potential introduction events.

Invasive macrophytes are a well-suited functional group to address spatial variability in aquatic systems where they have invaded. Macrophytes have predictable growing seasons and are relatively straightforward to sample compared to mobile or inconspicuous taxa such as fish or zooplankton. Macrophytes can be transported by recreational boats (Rothlisberger et al. 2010), and prevention programs for aquatic invasive species aim to educate boaters about invasive macrophytes at boat launches (Sharp et al. 2016). When abundant, macrophytes can reduce lake property values (Horsh and Lewis 2009) and limit recreational opportunities. Therefore, preventing invasive macrophyte introductions is a common priority for resource managers.

Beyond propagule risk, environmental factors that increase resource availability or alter natural habitat may directly promote invasive establishment and abundance (Jauni et al. 2015), or

indirectly increase the success of invasive species by reducing that of native species (Shea and Chesson 2002). Resource availability in lakes can be increased through land use changes in the watershed (Arbuckle and Downing 2001; Jennings et al. 2003). For example, watersheds with increased impervious surfaces can result in greater nutrient loads to lakes compared to those that are forested (Beaulac and Reckhow 1982, Lee et al. 2009). Degraded water quality, often influenced by watershed land use, may influence abundance of invasive species. Phosphorus, often the limiting nutrient for primary production in freshwater systems (Schindler et al. 2008), can be analyzed as total phosphorus concentration, providing a direct indictor of eutrophication. Increased phosphorus generally increases water column phytoplankton abundance, which decreases water transparency, thus reducing light penetration necessary for macrophyte production (Chambers and Kalff 1985). Invasive plants have been shown to display high trait plasticity (Funk 2008), and thus may be more successful than native plant species in a eutrophying system as conditions change to low light, high nutrient states.

Total habitat size also may influence species colonization and success (Debinski and Holt 2000). With increased habitat area (e.g., island size in biogeography), more species are likely to be present (Arrhenius 1921), which may be attributed to greater habitat complexity (Heck and Wetstone 1977; Gratwicke and Speight 2005). In freshwater systems, available habitat size is a function of lake size, or for benthic primary producers specifically (i.e., macrophytes), relates to littoral zone area conducive to photosynthesis.

Propagule introductions and altered environmental factors (e.g., disturbance) often can be correlated; thus their influences on invasions have been difficult to evaluate independently. For example, urbanized areas with greater anthropogenic influences (e.g., runoff) are often areas that receive increased propagules due to increased access. A meta-analysis conducted by Anderson et

al. (2015) suggested that tourist locations concentrate people in small areas, increasing habitat disturbance while simultaneously providing a near-constant supply of propagules. Therefore, evaluating the risk of propagule introductions on invasive success from those of environmental factors requires a study system where they are not correlated, posing a challenge for field-based observations where controlling variables is difficult. One approach is to select systems with a known range in the independent variable of interest (e.g., propagule risk), then evaluate others (e.g., environmental factors) with perhaps less range of variability, to determine any additional effects.

Here, I assessed invasive macrophyte richness and abundance (biomass (g/m^2) and dominance (% biomass of total assemblage)) in 20 lakes across New York chosen based on a likely range in propagule risk. Previous research has identified which aquatic taxa are more probable to be transported by recreational boats (Johnson et al. 2001, Rothlisberger et al. 2010), but documented influences on invaded communities are rare, and when quantified, usually involve models or experiments rather than observations of distribution and abundance in natural systems. I hypothesized that increased propagule risk would lead to greater richness, biomass (g/m^2) and dominance (%) of invasive macrophytes in lakes (Figure 2-1). As more potential propagules are introduced, the likelihood that both presence (e.g., establishment) and abundance (e.g., biomass and dominance) within the invaded community will increase by overcoming population and environmental stochasticity. Over time, perhaps as more genotypes are introduced through propagules from different sources, the greater the likelihood that an invasive species can increase in abundance by becoming a better competitor for resources or occupy microhabitat(s) in the novel environment.

Secondarily, I assessed the effects of environmental factors on richness and abundance of invasive macrophytes across the study lakes. I hypothesized that increased measures of eutrophication and habitat degradation, would lead to greater presence and abundance of invasive macrophytes (Figure 2-1). I additionally evaluated available habitat (lake area, depth, littoral zone area, and littoral:total area) on invasive macrophyte richness and abundance, and hypothesized that increased available habitat would result in greater richness and abundance of invasive macrophytes. As available habitat area increases, the number of species (including invasives) generally increases. Similarly, variability of habitat likely also increases as total habitat area increases. Invasive plants are often capable of thriving in a wide range of habitat conditions (Rejmánek and Richardson 1996), thus I predict their per-area abundance to increase in lakes with greater available habitat.

My ultimate goal was to increase the fundamental understanding of the role of propagule pressure and environmental factors in macrophyte invasions by evaluating which were better predictors of within-lake richness and abundance of invasive macrophytes.
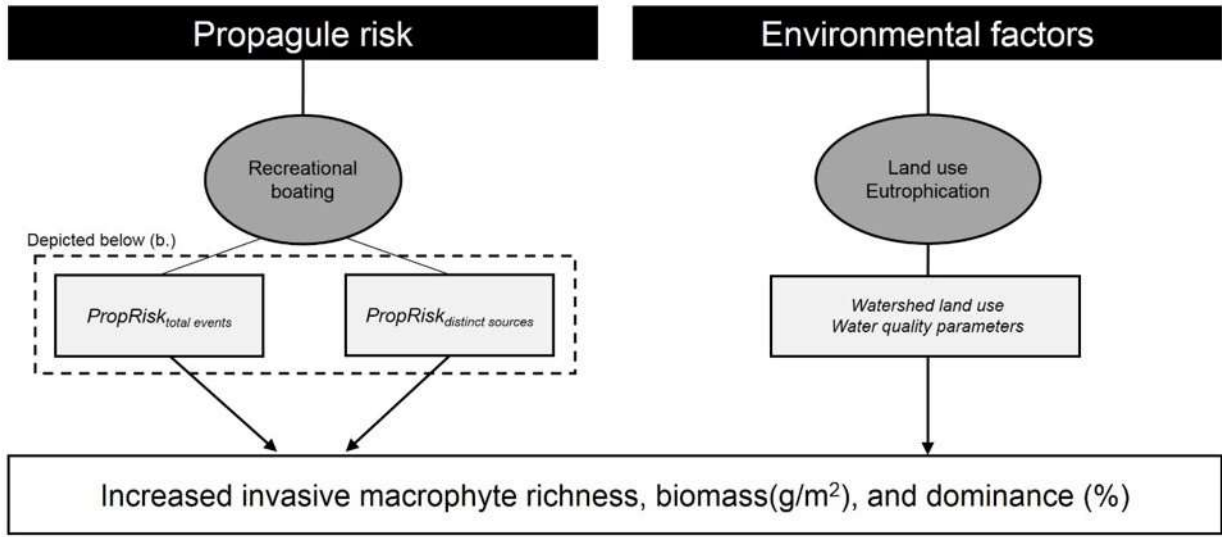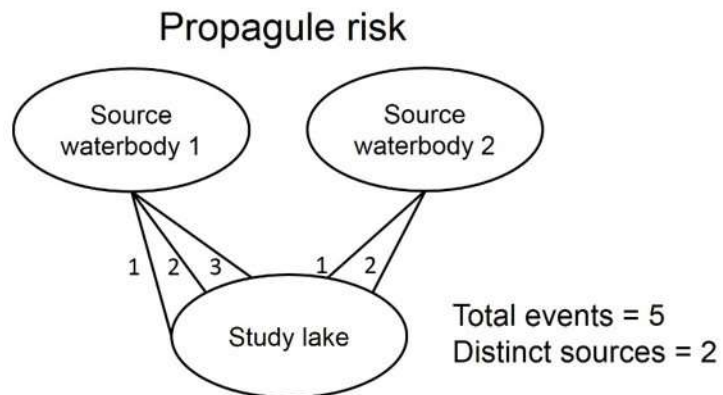
Figure 2-1. (a) Conceptual diagram of predicted effects of anthropogenic propagule risk and disturbance on invasive macrophyte biomass (g/m^2) and dominance (%). (b) Depiction of total propagule risk ($PropRisk_{total\ events}$) and distinct propagule risk ($PropRisk_{distinct\ sources}$), as determined by waterbodies boaters had visited previously, based on questionnaire responses.

METHODS

Study area

      I selected 20 lakes from two geographic regions of New York State – six within Central New York (CNY) and 14 Adirondack (ADK) lakes (Figure 2-2). Lakes were selected to represent an *a priori* range in propagule risk based on boater visitation numbers and thus previous boat trips to other waterbodies (Figure 2-3). Five of the lakes are not publicly-accessible, four in CNY (Song, Crooked, and Echo Lakes and Gatehouse Pond) and one ADK lake (Arbutus Pond). The remaining 15 lakes have public access boat launches, varying in popularity with recreational boaters (Holmlund et al. 2016). Watersheds and surrounding shorelines are relatively undisturbed compared to more urbanized areas.
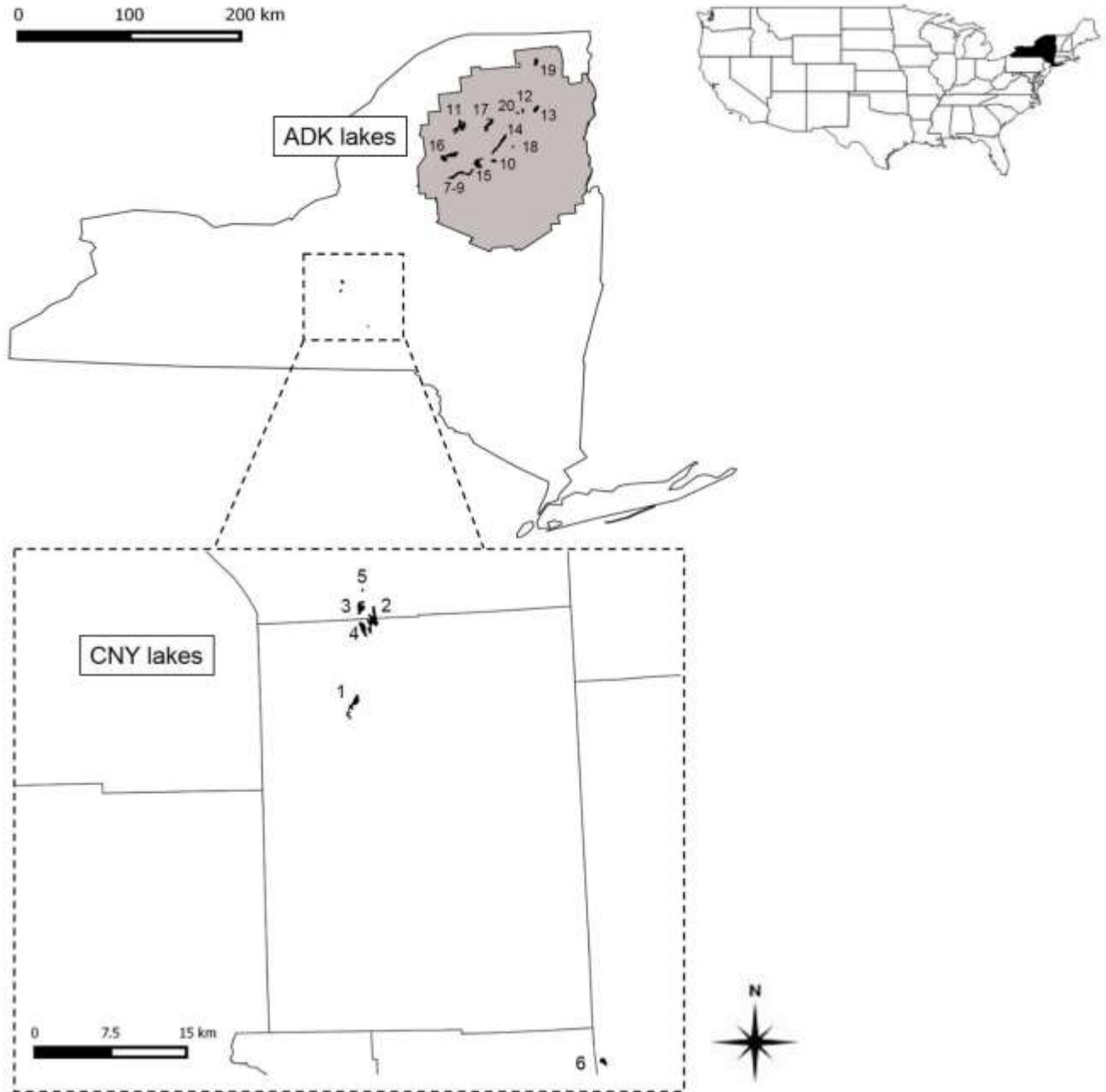
Figure 2-2. Location of study lakes in New York, USA. 1 = Little York Lake, 2 = Tully Lake, 3 = Crooked Lake, 4 = Song Lake, 5 = Gatehouse Pond, 6 = Echo Lake, 7 = Fourth Lake, 8 = Seventh Lake, 9 = Eighth Lake, 10 = Blue Mountain Lake, 11 = Cranberry Lake, 12 = Lake Flower, 13 = Lake Placid, 14 = Long Lake, 15 = Raquette Lake, 16 = Stillwater Reservoir, 17 = Tupper Lake, 18 = Arbutus Pond, 19 = Chateaugay Lake, 20 = Second Pond.
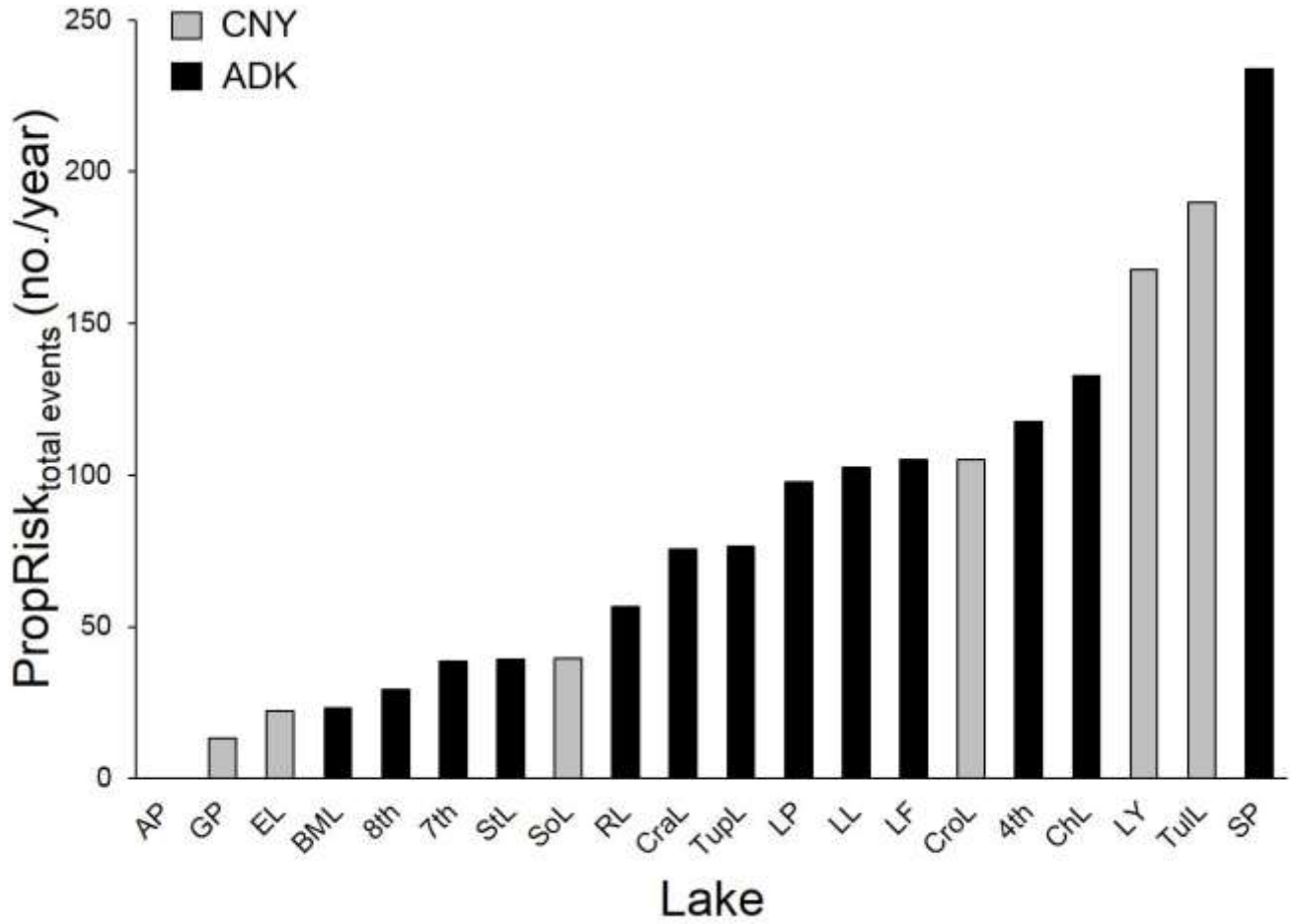
Figure 2-3. Gradient of PropRisk$_{total\ events}$, defined as the average annual sum of waterbodies previously visited by boaters for a boating season (152 days, May 1 through September 30), for the 20 study lakes. CNY = Central New York; ADK = Adirondacks.

Macrophyte sampling

Five of the CNY lakes were sampled in 2011 and 2012, and the remaining lake (Echo Lake) sampled in 2014 (due to access limitations). Eleven of the ADK lakes were sampled in 2013, and three were sampled in 2014. In each lake (and each year, where multi-year data were collected), I sampled macrophytes from 10 sites in each lake's littoral zone. Macrophyte samples were collected from quadrats (0.25 m^2) by SCUBA. At each sampling site, I used a transect line run parallel to shore that was 1% of the total lake shoreline length as a guide to bound the lateral extent of sites.

For the five CNY lakes sampled in 2011 and 2012, three quadrats were collected from three depth intervals (shallow, < 1 m; intermediate, 1-2 m; deep, > 2 m) per site (n = 90 quadrats per lake per year). For the remaining lakes, I determined that 70 quadrats per lake was an adequate minimum sample size to characterize abundance differences based on prospective power analysis (> 0.8 degree of confidence), conducted using data from the 2011 and 2012 samples using the software package, G*Power (Version 3.1, Faul et al. 2007). Thus, macrophytes were collected from seven quadrats per site in 2013 and 2014, four from shallow (< 2 m) and three from deep (≥ 2 m) depth intervals (n = 70 quadrats).

In each quadrat, I separated and identified species (Crow and Hellquist 2000 a,b) and determined each species' biomass by dry weight (60°C for a minimum of 24 h) (Vis et al. 2003). I calculated two measures of abundance as response variables: invasive macrophyte biomass (g/m^2) and dominance (% biomass of total assemblage). Additionally, a subset of CNY lakes (Little York Lake, Tully Lake, and Gatehouse Pond) are dominated by macroalgae, including *Chara* spp. and the invasive *Nitellopsis obtusa* (Brainard and Schulz 2017). Thus, in addition to abundance values of all macrophytes (including macroalgae), I determined biomass and

dominance of invasive macrophytes without including macroalgae, to analyze patterns of the higher plants.

Propagule risk

I utilized questionnaires to determine numbers of waterbodies boaters had previously visited (Syracuse University Institutional Review Board 11-182). Specifically, I quantified total propagule risk, defined as the sum total number of waterbodies previously visited by boaters (counting multiple trips to the same waterbody as multiple events) annually, representing total possible introduction events (PropRisk$_{\text{total events}}$), and distinct propagule risk defined as the discrete number of source waterbodies previously visited per year (PropRisk$_{\text{distinct sources}}$) (Figure 2-1b). Both estimates of propagule risk represent potential 'exposure' to macrophytes introduced from other waterbodies to a study lake. For example, if three respondents indicated they had previously visited Source Waterbody 1 in questionnaires gathered for a given study lake, and two respondents previously visited Source Waterbody 2, the PropRisk$_{\text{total events}}$ for the study lake would equaled five (Source Waterbody 1 times 3 + Source Waterbody 2 times 2), and the PropRisk$_{\text{distinct sources}}$ equaled two (Source Waterbody 1 and Source Waterbody 2) (Figure 2-1b).

For CNY lakes, propagule risk was quantified through boat launch questionnaires at the two-public access lakes (Little York and Tully Lakes) and homeowner questionnaires at all six lakes. Boat launch users were asked to provide the names of waterbodies they had previously visited. Over 90% of boat launch respondents indicated they had previously visited another waterbody within a year time period, thus I assumed the other waterbodies listed in 2011 and 2012 were annual estimates. Boat launch questionnaire response rate was 94%. Homeowner questionnaires were distributed to all properties on each lake, and participants were asked to list waterbodies they themselves had visited in the past, plus previous waterbodies visited for other

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boats granted access to the study lake by homeowners (if known). Homeowner questionnaire response rate was 31% (145 returned out of 468 distributed) – a potential source of bias in response rate may be homeowners that did not want to self-report their poor boating practices, however, because questionnaires were distributed to every property and the reasonable return rate, the questionnaires likely captured homeowner boating behavior. To quantify propagule risk from homeowner questionnaires, I divided total and distinct waterbodies by the number of respondents for each lake to generate average risk per homeowner, and then multiplied values by total number of homeowners. Propagule risk values from boat launch and homeowner questionnaires were summed for the two CNY publicly-accessible lakes where both were quantified (Little York and Tully Lakes).

For ADK lakes, propagule risk variables were quantified from boat launch surveys conducted by Paul Smith's College Adirondack Watershed Institute (AWI) Watershed Stewardship Program. All available data from questionnaires for my study lakes from 2002 to 2012 were used to quantify propagule risk. Boaters at ADK launches were asked to list waterbodies they had previously visited within the past two weeks, and $\text{PropRisk}_{\text{total events}}$ and $\text{PropRisk}_{\text{distinct sources}}$ were determined by summing responses in a given year. When multiple years of boat launch questionnaire data were available, annual estimates of propagule risk were averaged.

Boat launch visitations vary by day of week (Holmlund et al. 2016); I determined a day-specific (Monday-Sunday) boat launch use factor (D_{uf}) by calculating the proportion of boats visiting on a given day of week to the total annual boat visitations for lakes where such data were available in the two geographic regions (Table 2-S1). I then estimated propagule risk for the

boating season (from May 1 through September 30, 152 days), and summed across each of the seven days of the week, as follows:

$$PropRisk_{total\ events} = \sum_{Sunday}^{Monday} \frac{T_w}{Total\ Annual\ Days\ Questionnaires\ Collected} \ * \ (D * D_{uf})$$

$$PropRisk_{distinct\ sources} = \sum_{Sunday}^{Monday} \frac{D_w}{Total\ Annual\ Days\ Questionnaires\ Collected} \ * \ (D * D_{uf})$$

Where T_w and D_w represent total and distinct waterbodies visited, respectively, and D, a constant, (21.7) is the number of days available on average for each given day of week during the 152 day boating season. For example, to calculate $PropRisk_{total\ events}$, I determined the sum total number of waterbodies visited per year for a given lake, standardized by the number of days that questionnaire information was collected, and weighted each lakes' value by the probability a visitation occurred for a given day of the week over the boating season (D_{uf}). I averaged values for lakes that had multiple years of boat launch data available.

Environmental factors

Watershed land use

I delineated lake watersheds (ESRI ArcMap, Version 10.1, ESRI 2011) and utilized the National Land Cover Database 2011 (NLCD 2011, Homer et al. 2015) to determine percent residential (developed – open space, low intensity, and medium intensity), urban (developed - high intensity), agricultural (pasture/hay and cultivated crops), and barren (including strip mines and gravel pits) land uses, based on total watershed area.

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Water quality parameters

I obtained water quality parameters including transparency (m), chlorophyll-a (µg/L), total phosphorus (µg/L), conductivity (µS/cm), and pH for 12 of the study lakes that had data available through the Citizen Statewide Lake Assessment Program (CSLAP) or Adirondack Lake Assessment Program (ALAP) (Table 2-S2). Transparency for each lake was measured as Secchi depth. Chlorophyll-a and total phosphorus from surface water samples were collected by a Kemmerer sampler (CSLAP, 1.5 m) or integrated tube sampler (ALAP, 0-2 m). Conductivity and pH data were measured in the laboratory from frozen water samples. Water quality parameter data collection methods are available for CSLAP (Mueller and Kishbaugh 2015) and ALAP (Laxson et al. 2016) programs. I averaged parameters for study lakes when multiple years of data were available (Table 2-S2).

Lake morphology

Average and maximum lake depths (m) and lake areas (km^2) were obtained from lake-specific CSLAP and ALAP annual reports (Table 2-S2) or from New York State Department of Environmental Conservation (NYSDEC) contour maps. To determine littoral zone area (km), I used the image processing software ImageJ (Version 1.50, Schneider et al. 2012) to calculate the surface area of a lake that was ≤ 3 m, and assumed this depth represented the maximum depth limit of littoral zones across the study lakes. Depth contour maps were not available for three of the study lakes (Crooked and Echo Lakes, Gatehouse Pond), thus littoral zone areas were not determined for these waterbodies (Table 2-S2).

Statistical analysis

I estimated the influence of propagule risk on invasive macrophyte richness, biomass (g/m^2), and dominance (%) by linear regression. Biomass data were log (x+1) transformed to

retain macrophyte data for lakes that did not have invasive macrophytes (resulting in zero biomass values) and to meet normality assumptions of regression. Dominance data (%) were arcsine transformed to stabilize variance (Gotelli and Ellison 2013). I additionally used linear regression to test the potential influence of watershed land uses, water quality parameters, and lake morphology on richness, biomass, and dominance of invasive macrophytes. Percent watershed land use variables were arcsine transformed to meet assumption of normality.

I also used a forward multiple linear regression model with propagule risk ($PropRisk_{total\ events}$ and $PropRisk_{distinct\ sources}$), watershed land use metrics, and water quality (transparency, chlorophyll-a, total phosphorus, conductivity, and pH) as explanatory variables to test if inclusion of propagule risk with environmental factors increased explanatory variability in invasive richness and abundance compared to that of separate linear regressions. Forward selection multiple regression models are used to identify a subset of significant predictors of a dependent variable, while maintaining parsimony (Blanchet et al. 2008). Criteria for the forward selection regression models included an F statistic ≤ 0.05, while variables that had $F > 0.1$ were removed. In addition to evaluating change in the explained variability, the multiple regression model provided insight into which explanatory variables best described richness and abundance of invasive macrophytes in the study lakes.

I tested correlations between propagule risk and watershed land uses, water quality, and lake morphology variables using Spearman's rank correlation. To provide insight into regional variation among the study lakes, I used Mann-Whitney tests to evaluate if response (invasive macrophyte richness and abundance) and explanatory variables (propagule risk, land use, water quality, and lake morphology) differed by region (CNY and ADK). All statistical analyses were conducted in SPSS (Version 24, IBM Corp. 2016).

RESULTS

   Richness and abundance of invasive macrophytes varied across study lakes (Tables 2-S3 and 2-S4); however, mean invasive richness (CNY = 2.0 ± 1.7; ADK = 1.1 ± 0.9, $p = 0.274$), biomass (CNY = 60.0 ± 57.4 g/m^2; ADK = 2.9 ± 6.1 g/m^2; $p = 0.153$) and dominance (CNY = 26.9 ± 31.6 %; ADK = 13.5 ± 20.7 %; $p = 0.547$) were not significantly different between CNY and ADK regions (Mann-Whitney test, Table 2-1). Propagule risk also did not significantly differ between regions (PropRisk$_{total\ events}$ − $p = 0.904$; PropRisk$_{distinct\ sources}$ − $p = 0.076$) (Table 2-1).

   Watershed agricultural ($p < 0.001$) land uses were significantly different between regions, residential ($p = 0.076$), urban ($p = 0.091$), and barren ($p = 0.109$) land uses were not. Urban and barren watershed land use categories were uncommon in these watersheds (Table 2-1). Mean chlorophyll-a ($p = 0.048$), total phosphorus ($p = 0.003$), conductivity ($p = 0.010$), and pH ($p = 0.003$) were significantly different in lakes between regions (Table 2-1), and on average, were higher in CNY compared to ADK lakes (Table 2-1). Water transparency was not significantly different between regions ($p = 0.149$, Table 2-1). Mean lake surface area ($p = 0.002$) was significantly different between the regions, with greater surface areas in ADK lakes (Table 2-1). Average ($p = 0.547$) and maximum depths ($p = 0.312$), as well as littoral:total surface area ratios ($p = 0.197$) did not differ by region (Table 2-1).

   Estimates of propagule risk were significantly correlated with certain watershed land use, water quality, and lake morphology variables (Table 2-2). Specifically, PropRisk$_{total\ events}$ was positively correlated with urban watershed land use ($p = 0.009$) (Table 2-2). PropRisk$_{distinct\ sources}$ was positively correlated with agricultural land use ($p = 0.011$), total phosphorus ($p = 0.037$), conductivity ($p = 0.003$), and pH ($p = 0.019$) (Table 2-2). Agricultural land use, total phosphorus,

conductivity, and pH were significantly different between regions (Table 2-1), and thus regional

differences may bias the importance of these factors in propagule risk correlations.

Table 2-1. Mean biomass (g/m^2) and dominance (%) of invasive macrophytes, propagule risk, watershed land use (%), site-scale land use, water quality, and lake morphology variables between Central New York (CNY) and Adirondack (ADK) lakes. Significant differences (Mann-Whitney) are shown in bold.

Variable	CNY Mean	ADK Mean	Mann Whitney p-value
Macrophyte presence and abundance			
Invasive richness	2.0	1.1	0.274
Invasive biomass (g/m^2)	60.0	2.9	0.153
Invasive dominance (%)	26.9	13.5	0.547
Propagule risk			
PropRisktotal	89.6	80.6	0.904
PropRiskdistinct	71.9	23.2	0.076
Watershed land use			
Residential LU (%)	12.9	0.5	0.076
Urban LU (%)	24.2	0.3	0.091
Agriculture LU (%)	1.3	0.2	**< 0.001**
Barren LU (%)	0.1	0.2	0.109
Water quality			
Transparency (m)	3.5	4.6	0.149
Chlorophyl-a (μg/L)	4.5	2.3	**0.048**
Total phosphorus (μg/L)	15.2	7.2	**0.003**
Conductivity (μS/cm)	256.9	34.4	**0.010**
pH	7.6	6.9	**0.003**
Morphology			
Avg depth (m)	4.4	7.6	0.547
Max depth (m)	16.4	20.2	0.312
Lake area (km^2)	0.4	10.6	**0.002**
Littoral:Total	0.6	0.4	0.197

Table 2-2. Significance (p-values) of Spearman's rank correlation coefficients between propagule risk and watershed land use, water quality parameters, and lake morphology for all 20 study lakes. Significant correlations (and associated correlation values in parentheses) shown in bold.

	PropRisk$_{total\ events}$	PropRisk$_{distinct\ sources}$
Watershed land use		
Residential LU (%)	0.333	0.156
Urban LU (%)	**0.009 (0.565)**	0.333
Agriculture LU (%)	0.109	**0.011 (0.553)**
Barren LU (%)	0.566	0.997
Water quality		
Transparency (m)	0.253	0.837
Chlorophyll-a (μg/L)	0.812	0.319
Total phosphorus (μg/L)	0.491	**0.037 (0.606)**
Conductivity (μS/cm)	0.067	**0.003 (0.769)**
pH	0.195	**0.019 (0.663)**
Morphology		
Average depth (m)	0.794	0.855
Maximum depth (m)	0.700	0.772
Lake area (km^2)	0.782	0.640
Littoral:total ratio	0.082	0.115

Propagule risk

      I found that greater invasive macrophyte richness was significantly correlated with increased $PropRisk_{total\ events}$ across all lakes ($p = 0.032$, $R^2 = 0.231$), but not within regions (CNY – $p = 0.061$; ADK – $p = 0.431$). Biomass (g/m^2) of invasive macrophytes significantly increased with greater $PropRisk_{total\ events}$ across all lakes ($p = 0.021$, Figure 2-4a) and within ADK ($p = 0.013$), but not within the CNY region ($p = 0.233$). Similarly, invasive macrophyte dominance (%) significantly increased with increasing $PropRisk_{total\ events}$ ($p = 0.024$, Figure 2-4b) and was significant within ADK region ($p = 0.004$), but not in CNY ($p = 0.703$).

      Increased $PropRisk_{distinct\ sources}$ also was associated with greater richness of invasive macrophytes ($p = 0.002$, $R^2 = 0.409$), but was not significant in CNY ($p = 0.074$) or ADK ($p = 0.414$) regions. Increased $PropRisk_{distinct\ sources}$ resulted in greater invasive macrophyte biomass across all lakes ($p = 0.001$, Figure 2-4c), but this relation was not significant in either the CNY ($p = 0.280$) or ADK regions ($p = 0.084$). Greater dominance of invasive macrophytes was not significantly correlated with increased $PropRisk_{distinct\ sources}$ across all lakes ($p = 0.185$, Figure 2-4d), however I did observe significantly greater dominance of invasive macrophytes with higher $PropRisk_{distinct\ sources}$ in the ADK region ($p = 0.022$).

      To test the influence of propagule risk on the higher plant community, I excluded macroalgae biomass from total macrophyte biomass and reanalyzed responses of invasives to propagule risk. I found invasive biomass increased with greater $PropRisk_{total\ events}$ ($p = 0.001$, Figure 4e) and these regressions were significant in both CNY ($p = 0.05$) and ADK ($p = 0.013$) regions. Similarly, greater $PropRisk_{total\ events}$ resulted in significantly increased dominance of invasive higher plants ($p < 0.001$, Figure 2-4f) in both regions (CNY – $p = 0.017$; ADK – $p = 0.005$). Invasive biomass of higher plants increased significantly with greater $PropRisk_{distinct\ sources}$

($p < 0.001$, Figure 2-4g) in the CNY ($p = 0.032$) region, but not in the Adirondacks ($p = 0.083$). Finally, greater PropRisk$_{distinct\ sources}$ was associated with increased invasive dominance of higher plants across all lakes ($p = 0.023$, Figure 2-4h) and within both regions (CNY $- p = 0.031$; ADK $- p = 0.024$).

Patterns of biomass and dominance of invasive macrophytes with versus without macroalgae (higher plants only) differed only with propagule risk, and not with watershed land use, water quality, or lake morphology variables. Thus, I examined the influence of environmental factors (watershed, water quality parameters) and lake morphology on the complete macrophyte assemblages (including macroalgae) in the study lakes.
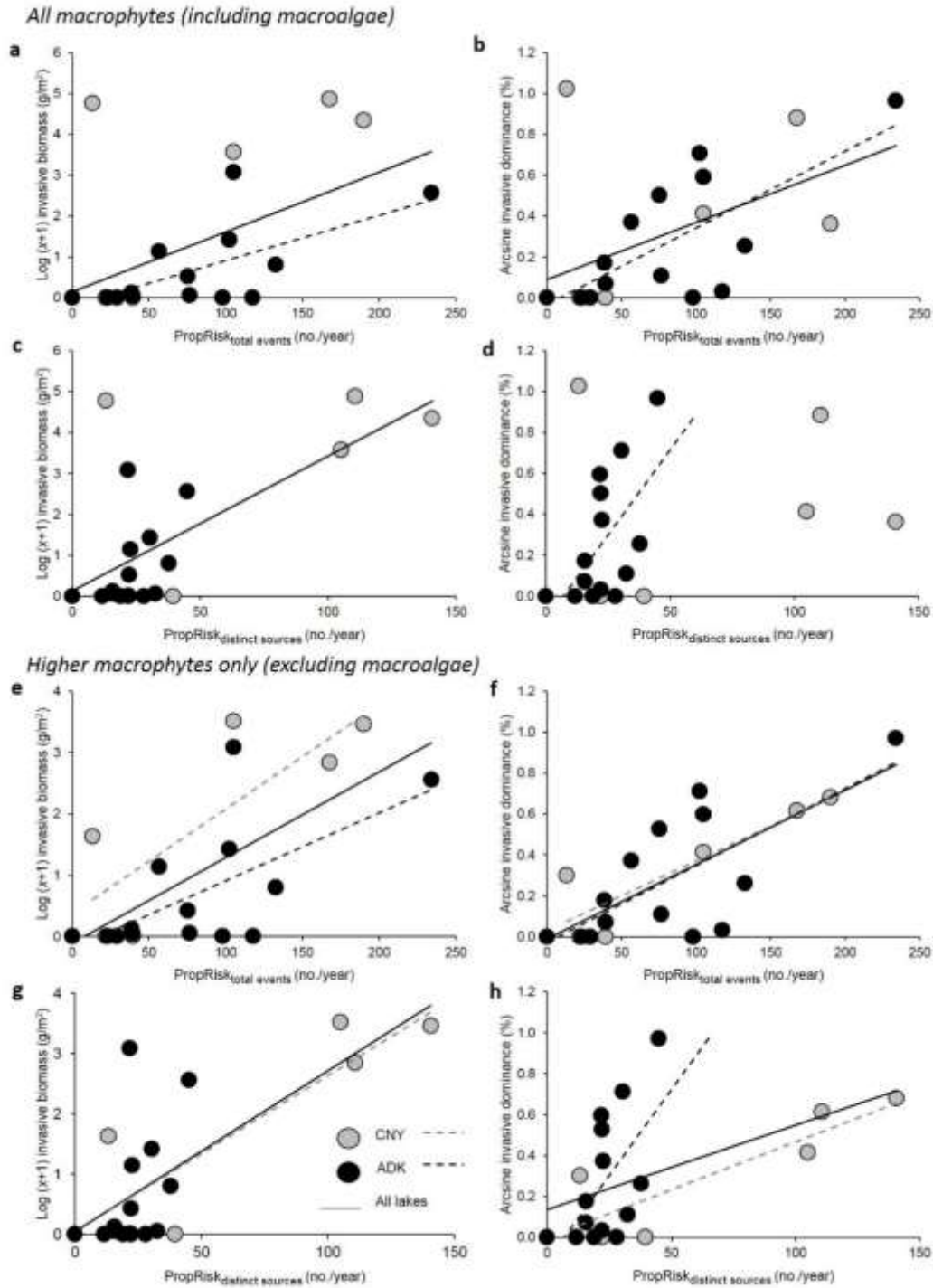
Figure 2-4a-h. Biomass (g/m^2) and dominance (%) of invasive macrophytes as a function of PropRisk$_{\text{total events}}$ (a, c, e, g) and PropRisk$_{\text{distinct sources}}$ (b, d, f, h) with all macrophytes (a-d) and higher plants only (excluding macroalgae) (e-h) in Central New York (CNY) and Adirondack (ADK) lakes. Regression equations are provided in Table 2-S5.

Environmental factors

Watershed land use

Richness of invasive macrophytes was greater with increased residential ($p = 0.025$) and agriculture ($p = 0.043$) watershed land uses (Table 2-3). Additionally, greater percentage of residential land use was associated with significantly greater biomass ($p = 0.013$, Figure 2-5a) but not dominance ($p = 0.412$, Table 2-3) of invasive macrophytes. In addition, greater agricultural land use in the watershed was associated with significantly greater invasive biomass of macrophytes ($p = 0.001$, Figure 2-5b), but was not a strong predictor of dominance of invasive macrophytes ($p = 0.183$, Table 2-3). Urban and barren watershed land uses were not significantly correlated with biomass or dominance of invasive macrophytes (Table 2-3).

Water quality parameters

I found that greater conductivity was associated with increased invasive macrophyte richness ($p = 0.011$, Table 2-3) and biomass ($p = 0.001$, Figure 2-6a, Table 2-3), but was not correlated with invasive dominance ($p = 0.070$, Table 2-3). Similarly, greater pH resulted in significantly greater biomass of invasive macrophytes ($p = 0.014$, Figure 2-6b), but not greater richness ($p = 0.061$) or dominance of invasives ($p = 0.258$) (Table 2-3). Transparency, chlorophyll-a, and total phosphorus were not correlated with increased richness, biomass, or dominance of invasive macrophytes (Table 2-3).

Table 2-3. Linear regressions of invasive biomass (g/m^2) and dominance (%) with watershed land use categories, water quality parameters, and lake morphology. Significant regressions (p-values < 0.05) are shown in bold. NS indicates regression was not significant ($\alpha = 0.05$). Invasive biomass and dominance data were (x+1) or arcsine transformed, respectively, and land use data were arcsine transformed to meet assumption of normality.

Watershed land use	Invasive richness			Invasive biomass (g/m^2)			Invasive dominance (%)		
	β	p	R^2	β	p	R^2	β	p	R^2
Residential LU (%)	0.499	**0.025**	0.249	0.543	**0.013**	0.295	0.194	NS	0.038
Urban LU (%)	0.237	NS	0.056	0.293	NS	0.086	0.067	NS	0.004
Agriculture LU (%)	0.457	**0.043**	0.209	0.703	**0.001**	0.495	0.310	NS	0.096
Barren LU (%)	-0.195	NS	0.038	-0.272	NS	0.074	-0.272	NS	0.074
Water quality	β	p	R^2	β	p	R^2	β	p	R^2
Transparency (m)	-0.192	NS	0.037	-0.162	NS	0.026	-0.267	NS	0.071
Chlorophyll-a (μg/L)	0.288	NS	0.083	0.347	NS	0.120	0.111	NS	0.012
Total phosphorus (μg/L)	0.498	NS	0.248	0.571	NS	0.326	0.276	NS	0.076
Conductivity (μS/cm)	0.702	**0.011**	0.493	0.837	**0.001**	0.700	0.541	NS	0.292
pH	0.556	NS	0.309	0.685	**0.014**	0.469	0.355	NS	0.126
Lake morphology	β	p	R^2	β	p	R^2	β	p	R^2
Mean depth (m)	-0.099	NS	0.010	-0.401	NS	0.160	-0.448	**0.047**	0.201
Maximum depth (m)	0.023	NS	0.001	-0.182	NS	0.033	-0.270	NS	0.073
Lake area (km^2)	-0.089	NS	0.008	-0.383	NS	0.147	-0.099	NS	0.010
Littoral:total	0.475	NS	0.226	0.742	**0.001**	0.551	0.876	**< 0.001**	0.767
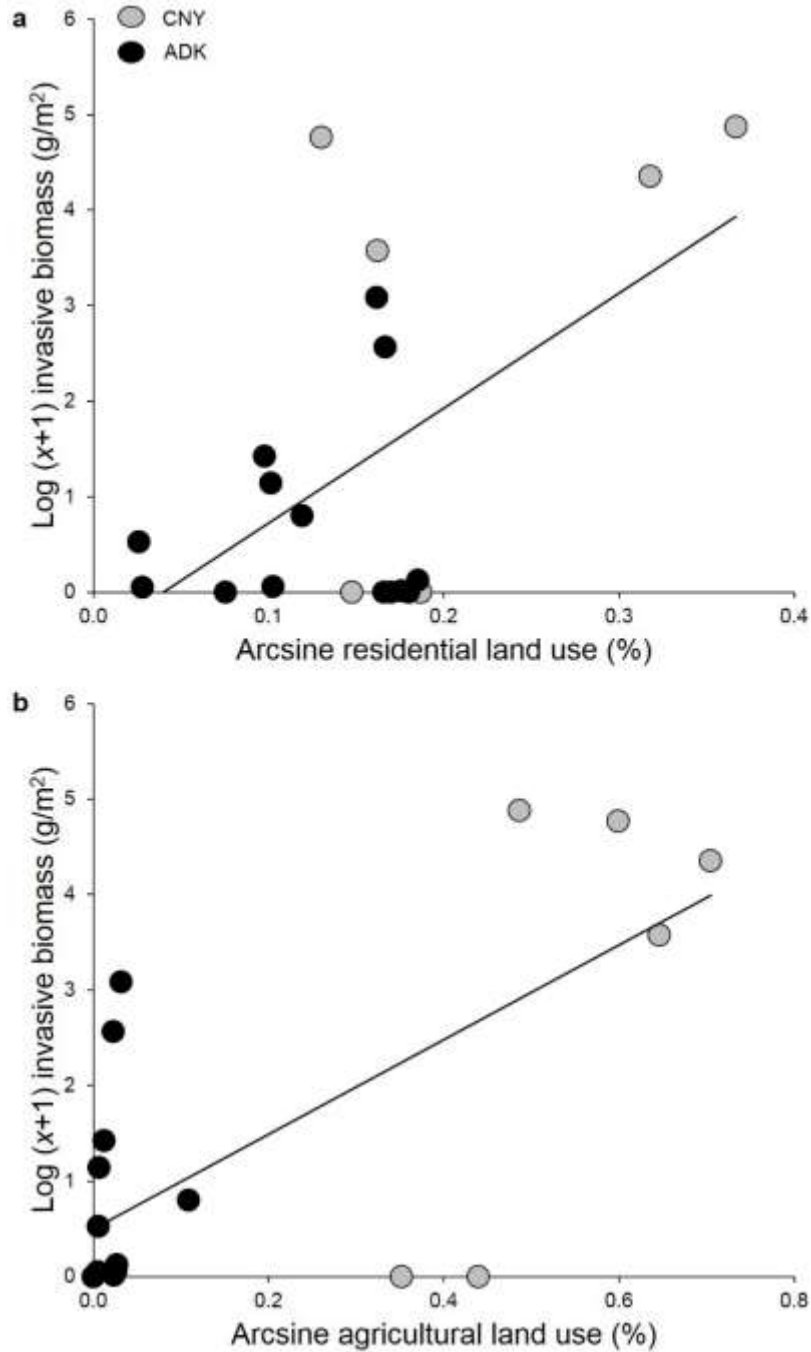
Figure 2-5. Biomass of invasive macrophytes (g/m^2) as a function of increased watershed (a) residential land use (%) and (b) agricultural land use (%). Biomass data were (x+1) transformed; land use data were arcsine transformed to meet assumption of normality. Regressions:
(a) Log (invasive biomass + 1) = 12.03(residential land use %) - 0.478, p = 0.013, R^2 = 0.295;
(b) Log (invasive biomass + 1) = 4.96(agricultural land use %) + 0.499, p = 0.001, R^2 = 0.495.
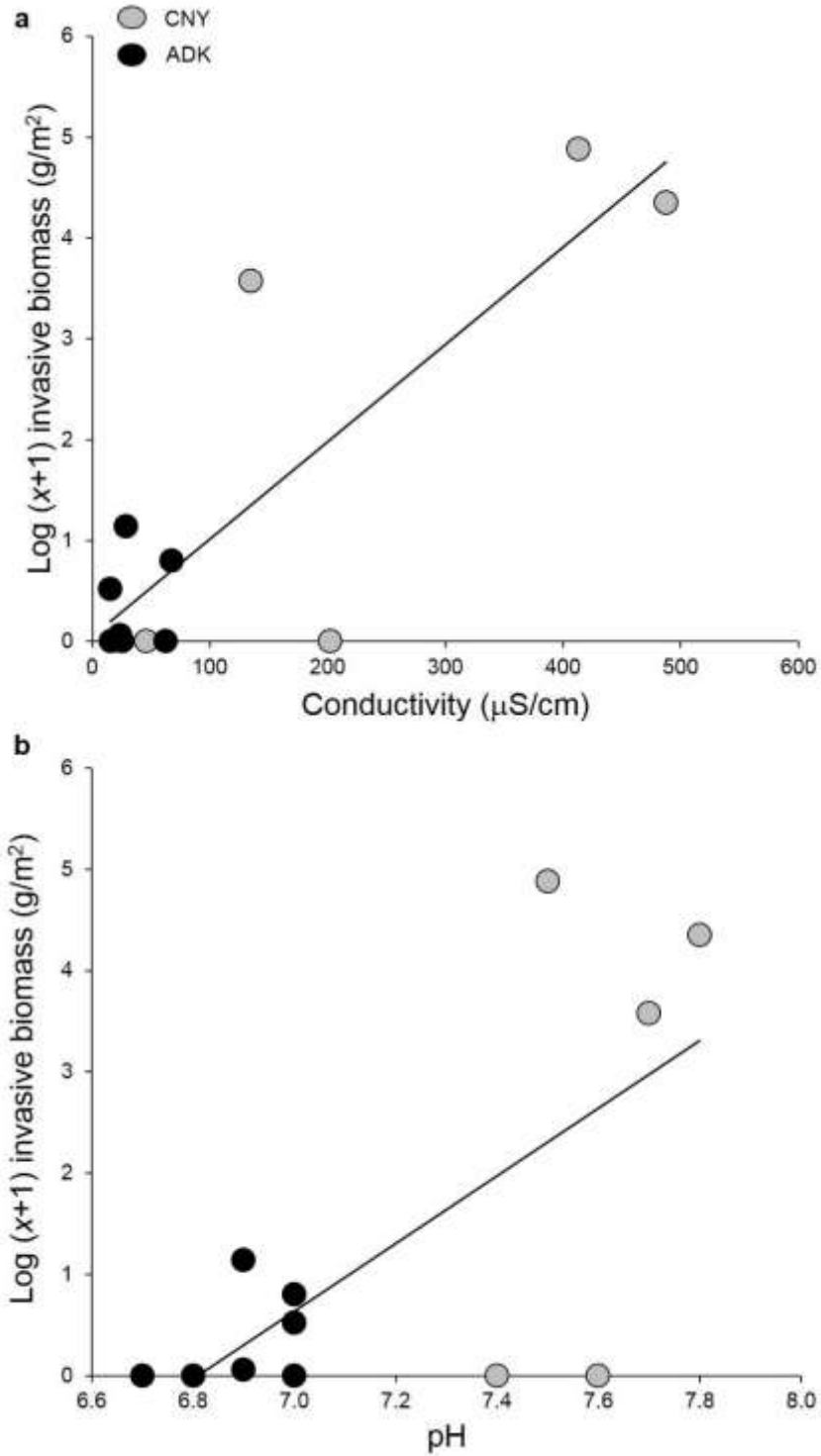
Figure 2-6. Total phosphorus (μg/L) in relation to agricultural land use of Central New York (CNY) and Adirondack (ADK) study lakes. Land use variables were arcsine transformed to meet assumption of normality. Regression: Total P = 14.69(agricultural) + 7.124, p = 0.001, $R^2 = 0.842$.

Lake morphology

Average lake depth did not significantly influence richness, biomass of invasive macrophytes ($p = 0.080$), but lakes with increased mean depth had decreased invasive macrophyte dominance ($p = 0.047$, Table 2-3). I did not observe an effect of maximum lake depth on richness ($p = 0.923$), biomass ($p = 0.443$) or dominance ($p = 0.249$) of invasive macrophytes (Table 2-3). Similarly, richness and abundance of invasive macrophytes were not significantly correlated with lake surface area (Table 2-3). However, greater littoral zone to lake surface area (littoral:total) was associated with both increased biomass ($p = 0.001$) and dominance ($p < 0.001$) of invasive macrophytes (Table 2-3).

Propagule risk and environmental factors

For richness of invasive macrophytes, the PropRisk$_{distinct sources}$ was the only explanatory variable that best fit the forward selection multiple regression model ($p < 0.001$, Table 2-4). The explained variability in richness of invasive species richness increased by 35% by including environmental factors (watershed land use and water quality parameters) compared to distinct propagule risk alone.

Biomass (g/m^2) of invasive macrophytes was also best described by PropRisk$_{distinct sources}$ only ($p < 0.001$, Table 2-4). Compared to the explained variability by the simple linear regression model of PropRisk$_{distinct sources}$ ($R^2 = 0.462$), the multiple regression model that included an evaluation of environmental factors increased the explained variability by 42%.

The forward selection multiple regression model indicated that PropRisk$_{total events}$ and urban watershed land use were the best predictors of dominance (%) of invasive macrophytes ($p = 0.002$, Table 2-4), increasing the explained variability in dominance of invasive macrophytes in the study lakes by 50% compared to propagule risk alone.

Table 2-4. Multiple regression (forward selection) models for invasive richness, biomass (g/m^2) and dominance (%). Propagule risk (total events and distinct sources, no./yr), watershed land uses (residential, urban, agriculture, and barren, %), and water quality parameters (transparency (m), chlorophyll-a (chl-a, $\mu g/L$), total phosphorus (TP, $\mu g/L$), conductivity ($\mu S/cm$), and pH) were explanatory variables put into the regression model.

Explanatory variables	Best fit variables - forward selection		
	Invasive richness	Invasive biomass (g/m^2)	Invasive dominance (%)
PropRisk$_{total\ events}$ PropRisk$_{distinct\ sources}$ Residential LU Urban LU Agricultural LU Barren LU Transparency Chl-a TP Conductivity pH	PropRisk$_{distinct\ sources}$ $p < 0.001$ $R^2 = 0.757$ Invasive richness = 0.028(PropRisk$_{distinct\ sources}$) - 0.065	PropRisk$_{distinct\ sources}$ $p < 0.001$ $R^2 = 0.885$ Invasive biomass = 0.039(PropRisk$_{distinct\ sources}$) - 0.585	PropRisk$_{total\ events}$, Urban LU $p = 0.002$ $R^2 = 0.697$ Invasive dominance = 0.005(PropRisk$_{total\ events}$) - 9.7(Urban LU) - 0.072

DISCUSSION

Propagule risk

Previous studies of propagule pressure have evaluated likelihood of dispersal by using

models to predict locations of high introduction potential (Buchan and Padilla 1999, 2000; Leung

et al. 2006), reported which invasive macrophytes species can be transported by boats

(Rothlisberger et al. 2010), or used an experimental approach to quantify importance of

propagule pressure to invasion success (Chadwell and Engelhardt 2008). I utilized propagule

risk, a measure of the degree of exposure to invasive propagules, and demonstrated that it can be

predictive of invasive macrophyte richness, biomass, and dominance in lakes (Figure 2-4).

While I did not directly assess genetic diversity of invasive populations in this study,

propagule risk may be linked indirectly to increased potential genetic diversity of invading

populations (Roman and Darling 2007). Repeated introductions of nonnative individuals from

multiple outside source locations likely results in greater genetic diversity of the colonizing

population (Dlugosch and Parker 2008). For example, Kirk et al. (2011) found that an admixture

of genotypes from long- and short-distance source populations increased genetic diversity of

Phragmites australis in the St. Lawrence/Great Lakes region. Similarly, Brown and Stepien

(2009) compared population genetics of round goby (*Neogobius melanostomus*) in North

America to those in native populations in Eurasia, and concluded that propagules from multiple

sources likely supplemented genetic diversity within the gobies' invasive range. Increased

genetic diversity of colonizing populations is more likely to introduce genotypes that are pre-

adapted to local conditions in the introduced habitat (Ruis and Darling 2014). For example, a

lake with low propagule risk (i.e., Stillwater Reservoir) would be hypothesized to have less

genetic diversity in an invasive macrophyte population compared to a lake with increased

propagule risk. However, the patterns depicted here with propagule risk and abundance of invasive macrophytes may simply be a temporal increase in populations through natural reproduction and spread. Further research that evaluates the extent of genetic diversity, across ranges of propagule risk, in contrast to time since establishment may further refine this concept.

In addition to possible link between propagule risk and genetic diversity driving the patterns observed with invasive macrophytes in these study lakes, other processes related to propagule pressure may be in play. For example, high levels of propagule pressure influence source-sink dynamics of invading populations, providing a steady supply of individuals into new areas that may not be well-suited habitat for long-term establishment and success of the population (Pulliam 1988; Sepulveda 2018). In these instances, however, invasive species are often documented in sampling programs due to a certain threshold of individuals being introduced at a high rate (Sepulveda 2018). In addition, certain invasive species may be successful in their invasive range due to absence of natural constraints found in native ranges, or have certain attributes that promote their invasive ability (Pearson et al. 2018), and thus their apparent success in novel environments may not be strongly linked to propagule pressure.

Three of the CNY lakes included in my study were dominated by macroalgae (*Chara* spp. and *Nitellopsis obtusa*), which comprised approximately 70% to 88% of total standing biomass in these systems. Abundant macroalgae populations are often found in hard-water lakes (Kufel and Kufel 2002), such as those in the CNY region (Table 2-S2). Macroalgae were rare in ADK lakes (comprised exclusively of native *Nitella* sp.) and relative abundance was low (maximum % standing biomass of macroalgae was 1.2% in Tupper Lake). To account for differences in regional abundance of higher plants and macroalgae, I both analyzed the whole dataset and reexamined after excluding macroalgae. In the CNY region, where macroalgae were

dominant, I found that greater propagule risk was significantly associated with biomass and dominance of higher plants (Figures 2-4e-h), but this relationship was not significant when all invasive macrophytes, including macroalgae, were included (Figures 2-4a-d). These results suggest that an extremely abundant invasive population (e.g., *Nitellopsis obtusa*) that becomes dominant in communities may limit the establishment and/or abundance of other invasive macrophytes (e.g., *Myriophyllum spicatum*), even though propagules of other invasive macrophytes are likely being introduced based on high levels of propagule risk.

The similar relationships between dominance of invasive macrophytes and total propagule risk in CNY and ADK lakes suggests that the influence of total propagule risk was similar across regions (regression slopes - CNY = 0.003, ADK = 0.004, Figure 2-4f, Table 2-S5). However, lower distinct source propagule risk values were associated with a higher percent dominance of invasives in ADK compared to CNY lakes (Figure 4h, Table 2-S5). Perhaps ADK source waterbodies pose a greater risk as sources of invasive propagules, based on invaded status and potentially higher dominance of invasive macrophytes. Assessing invasive populations from source waterbodies is a logical step to further refine estimates of propagule introduction risk to a receiving lake (e.g., weighting factor of source populations).

Propagule risk estimates from homeowners' boat usage were lower than those from boaters using the launches for the publicly-accessible CNY lakes. For example, in Little York Lake I determined $PropRisk_{total\ events}$ from homeowners to be 16 versus 152 from boat launch visitations, which suggests homeowners are a small fraction of propagule risk compared to transient recreational boats using launches. Indeed, Johnson and Carlton (1996) characterized the overland transport of resident boats as 'rare' in the Great Lakes region, and I suspect movement of homeowner's boats between lakes in the Adirondacks is minimal. However, my estimates of

propagule risk in ADK lakes are likely conservative relative to those in CNY, as I did not survey homeowners and propagule risk was quantified solely from boat launch questionnaires in ADK lakes. Nevertheless, including estimates of overland transport of boats from lake homeowners could be incorporated into vector analyses, in addition to information from boat launches, to better predict the risk of propagule introductions. Finally, using questionnaires to assess propagule pressure of a waterbody has been shown to be an effective method (Chivers and Leung 2012), providing insight into recreational boating behavior. Measuring propagule pressure directly from boats is both time intensive and cost prohibitive, thus utilizing questionnaires to indirectly measure of propagule pressure is a practicable approach to estimate invasion risk for a lake.

Environmental factors

Increased invasive richness and biomass of invasives were observed with increased residential and agricultural land uses (Figure 2-5, Table 2-3). Residential and agricultural land uses are often associated with increased nutrient inputs in lakes. For example, Soranno et al. (1996) estimated that phosphorus loading to waterbodies prior to human disturbance of watersheds (i.e., pre-settlement conditions) was one-sixth that of current loadings. Indeed, increased agricultural watershed land use was correlated with greater total phosphorus in the study lakes ($p = 0.001$, Figure 2-7). However, unlike propagule risk, which was independent of region (Figure 2-3), mean estimates of agricultural land use significantly varied between regions (Table 2-1), thus confounding my ability to isolate the influence of watershed land use on invasive macrophyte richness and abundance in these study lakes.
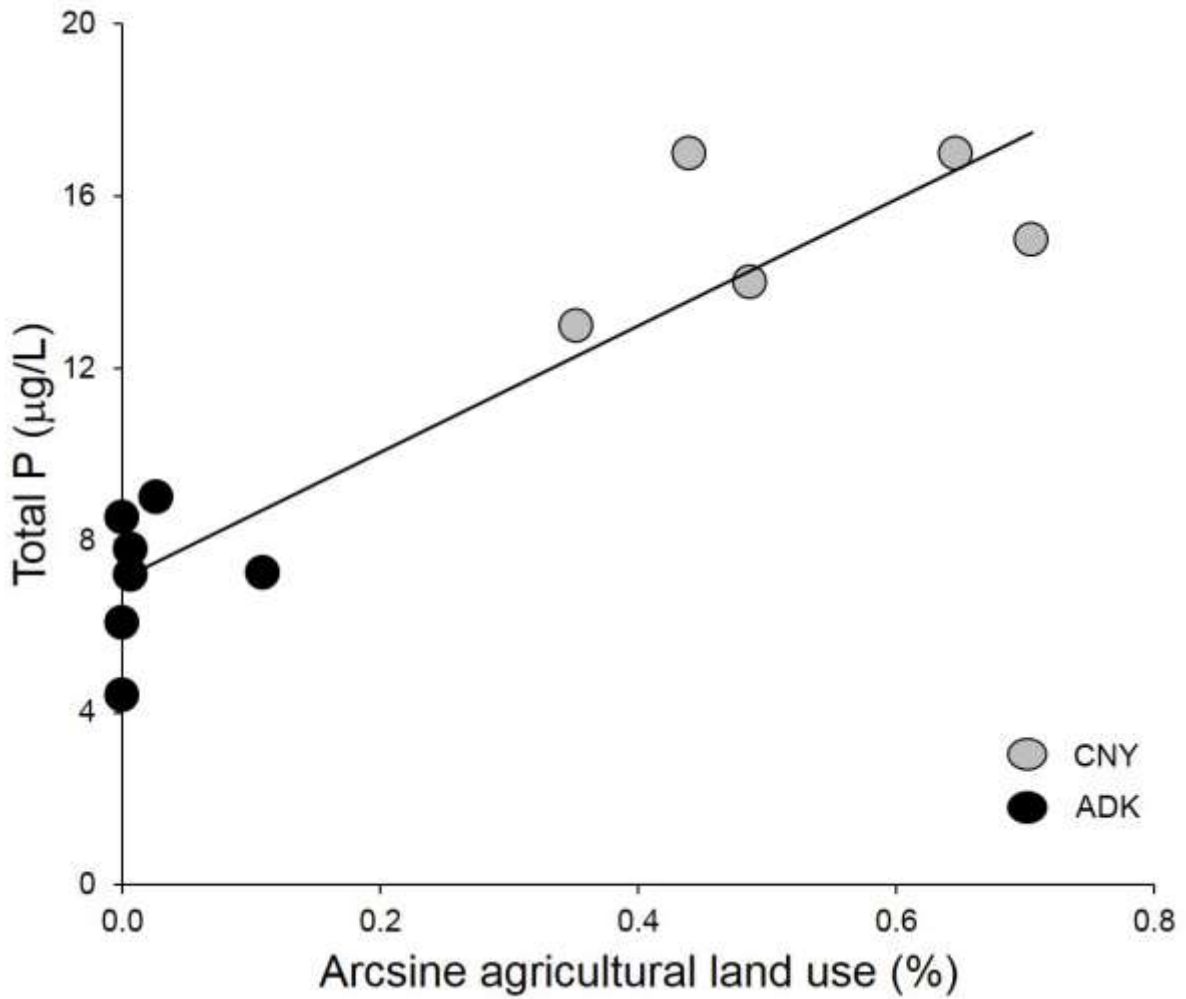
Figure 2-7. Total phosphorus (μg/L) in relation to agricultural land use of Central New York (CNY) and Adirondack (ADK) study lakes. Land use variables were arcsine transformed to meet assumption of normality. Regression: Total P = 14.69(agricultural) + 7.124, p = 0.001, $R^2 = 0.842$.

Water quality

Conductivity and pH (similar to watershed land use) were highly variable and significantly different between regions (Table 2-1). The observed increase in biomass of invasive macrophytes with greater lake conductivity and pH (Figure 2-6) likely reflects differences in macrophyte biomass among regions (CNY and ADK). Interestingly, I did not observe a significant effect of total phosphorus or chlorophyll-a concentrations on biomass or dominance of invasive macrophytes, but like conductivity and pH, total phosphorus and chlorophyll-a both were significantly different between regions. However, increased total phosphorus was correlated with greater total (native and nonnative) macrophyte biomass ($p = 0.012$), suggesting that increased phosphorus concentrations can influence overall community abundance of macrophytes in the study lakes, but not that of invasive macrophytes specifically.

Lake morphology

Unexpectedly, abundance of invasive macrophytes was not significantly correlated with increasing lake surface area. However, for macrophytes, habitat is defined less by total surface area then by area of the littoral zone, where light penetration is sufficient to support primary production of rooted plants. Thus, littoral zone surface area standardized by total lake surface area (littoral:total) may be a more realistic predictor of macrophyte habitat size, and predictably resulted in greater biomass ($p = 0.001$) and dominance ($p < 0.001$) of invasive macrophytes at larger proportions of littoral:total area (Table 2-3). Mean lake depth is also related to littoral:total surface area, and invasive macrophyte dominance increased with decreasing average depth, thus, as available habitat area for invasive macrophytes increased in the study lakes, abundance similarly increased, as might be predicted based on spatial patterns of community structure (Tokeshi 1993).

Propagule risk and environmental factors

　　While my objective was to assess the importance of propagule risk and environmental factors independently, combining these predictors in a multivariate regression increased predictive explanatory power for richness and abundance of invasive macrophytes. Britton-Simmons and Abbott (2008) similarly concluded that propagule pressure and disturbance combined to regulate success of invasive *Sargassum muticum* in short- and long-term experiments. However, in this study, $PropRisk_{total\ events}$ was positively correlated with urban watershed land use, and $PropRisk_{distinct\ sources}$ was correlated with increased agricultural land use (Table 2-2), potentially overestimating the combined influence of propagule risk and watershed land use on invasive macrophyte abundance across the study lakes.

　　These results beg the question – is the influence of propagule risk or environmental factors more important in macrophyte invasions to lakes? The strength of this study is that the lakes comprised a known and large range in propagule risk (Figure 2-3); I secondarily assessed the influence of environmental factors on invasive abundance. Many of the environmental estimates showed significant regional differences, and a limited range (compared to propagule risk), confounding my ability to isolate their effects on abundance of invasive macrophytes. However, propagule risk is a clear driver influencing the success of invasive macrophytes in these study lakes, as indicated by results of the forward selection multiple regression that included propagule risk as best predictor of richness and abundance. To directly assess the influence of environmental factors considered here (e.g., watershed land use, water quality parameters) on within-lake abundance of invasive macrophytes would require selecting lakes across an *a priori* range of environmental factors independent of propagule pressure, which was not possible in these open access lakes, but might be possible in experimental lake areas.

CONCLUSIONS

I have demonstrated a direct link between propagule risk, richness and abundance of invasive macrophytes in lake communities, perhaps due to increasing genetic diversity of invading populations leading to greater likelihood of establishment and subsequent increases in abundance. Propagule risk may be a useful approach to investigate how genetic diversity contributes to success of colonizing species by directly assessing variability in introduced genotypes across a recognized range of exposure from source populations. More generally, my results suggest propagule risk can be used to highlight locations at risk of nonnative individuals being introduced, helping prioritize efforts to prevent the spread of invasive species.

CHAPTER 3: IMPACTS OF THE CRYPTIC MACROALGAL INVADER, *NITELLOPSIS OBTUSA*, ON MACROPHYTE COMMUNITIES

INTRODUCTION

Nitellopsis obtusa is an invasive macroalgae with potential to dominate nearshore areas once established in North American aquatic ecosystems. First verified in North America in the St Lawrence River in 1978 (Geis et al. 1981), *Nitellopsis* has since spread to water bodies in Indiana, Michigan, Minnesota, New York, Pennsylvania, Vermont, and Wisconsin (Pullman and Crawford 2010, Kipp et al. 2014, Sleith et al. 2015). *Nitellopsis* is native to Europe and Asia, where it is considered rare in certain parts of its range (Simons and Nat 1996, Kato et al. 2014).

Nitellopsis is unique among macroalgae in the family Characeae because of the presence of star-shaped bulbils, which are specialized for vegetative reproduction (Sleith et al. 2015). These bulbils are the source of the common name of this taxon—starry stonewort. Observations of star-shaped bulbils can easily distinguish *Nitellopsis* from other genera in the family Characeae (Figure 3-1a,b), but general similarities in morphology among taxa in this family can make identification difficult. Distinguishing between native macroaglae and *Nitellopsis* also can be difficult because bulbils can be less abundant in mid-summer months (Hackett et al. 2014) or go unnoted because of lack of awareness of the possible presence of this invader. *Nitellopsis* closely resembles native taxa such as *Nitella* spp. and *Chara* spp. because whorled branches arise from stem nodes in all 3 genera (Figure 3-1c,d). When bulbils are not present or easily observed (Figure 3-1e), *Nitellopsis* can be distinguished from native macroalgae based on irregular branching of the whorls (Figure 3-1c,d) and absence of a garlic-like odor produced by many *Chara* spp. (Pullman and Crawford 2010, Hackett et al. 2014). Ultimately, similarities between *Nitellopsis* and native macroalgae, especially for observers unfamiliar with *Nitellopsis*, may lead

to underreporting of this invasive.

The discovery of *Nitellopsis* in the St. Lawrence River indicates that its introduction to North America probably was via transoceanic transport in the ballast of ships. Mechanisms of subsequent secondary spread to inland water bodies have been debated. Potential vectors include hydrochory, whereby secondary spread occurs through water currents (Sytsma and Pennington 2015). Dispersal by animals, referred to as zoochory (Honnay et al. 2010, Sytsma and Pennington 2015), may also be an important mechanism for spread of *Nitellopsis* (Pulllman and Crawford 2010), mainly through waterfowl dispersal. Sleith et al. (2015) conducted a survey of lakes in New York and found that *Nitellopsis* often was present in public lakes near boat launches, but did not occur in many lakes characterized as undeveloped with minimal public access. This result suggests that *Nitellopsis* may be transported secondarily to new water bodies through overland 'hitchhiking' on recreational boats or trailers.

No matter the mechanism of spread, *Nitellopsis* has established itself rapidly in littoral zones of inland waterbodies in the northeastern and midwestern USA. For example, Sleith et al. (2015) surveyed 390 water bodies in New York in an effort to confirm historical accounts and identify new locations where *Nitellopsis* was present. They documented *Nitellopsis* at 18 locations where it was previously undocumented. *Nitellopsis* typically occurs in water 3 to 4 m deep (Kipp et al. 2014), where it can grow as tall as 2 m and create dense mats (Pullman and Crawford 2010). The method used by Sleith et al. (2015) to survey for *Nitellopsis* (wading in shallow water) may have led to false negatives in water bodies where *Nitellopsis* was present because it tends to inhabit water deeper than can be sampled by wading.

Despite its relatively rapid range expansion and ability to attain high densities in freshwater systems, published reports of negative effects associated with *Nitellopsis* invasions

are largely anecdotal and generally lack empirical data. A Web of Science® (Thomson Reuters, Philadelphia, Pennsylvania) literature search (key words = *Nitellopsis obtusa* AND biomass) returned 17 published references, of which only 7 explicitly reported densities (Schloesser et al. 1986, Nichols et al. 1988, Griffiths et al. 1991, Blindow 1992, Ruiters et al. 1994, Lewandowski and Ozimek 1997, Hilt et al. 2010). I found no published accounts of *Nitellopsis* effects on native macrophyte communities in its nonnative range, but Pullman and Crawford (2010) noted anecdotally that extirpation of some native species seems to occur. Thus, effects of invasion of *Nitellopsis* are not well known (Pullman and Crawford 2010) even though 25 y have passed since the first call to action to document effects of this invasion (Crowder and Painter 1991).

Here, I report probable effects of the establishment of *Nitellopsis* on macrophyte communities in four lakes in central New York. I also show that the distribution of *Nitellopsis* can be influenced by water depths in parts of littoral zones. My goal is to determine if *Nitellopsis* introductions pose ecological or recreational threats and, given its range expansion, to stress the importance of early-detection monitoring programs designed specifically to identify this often-overlooked cryptic invader.
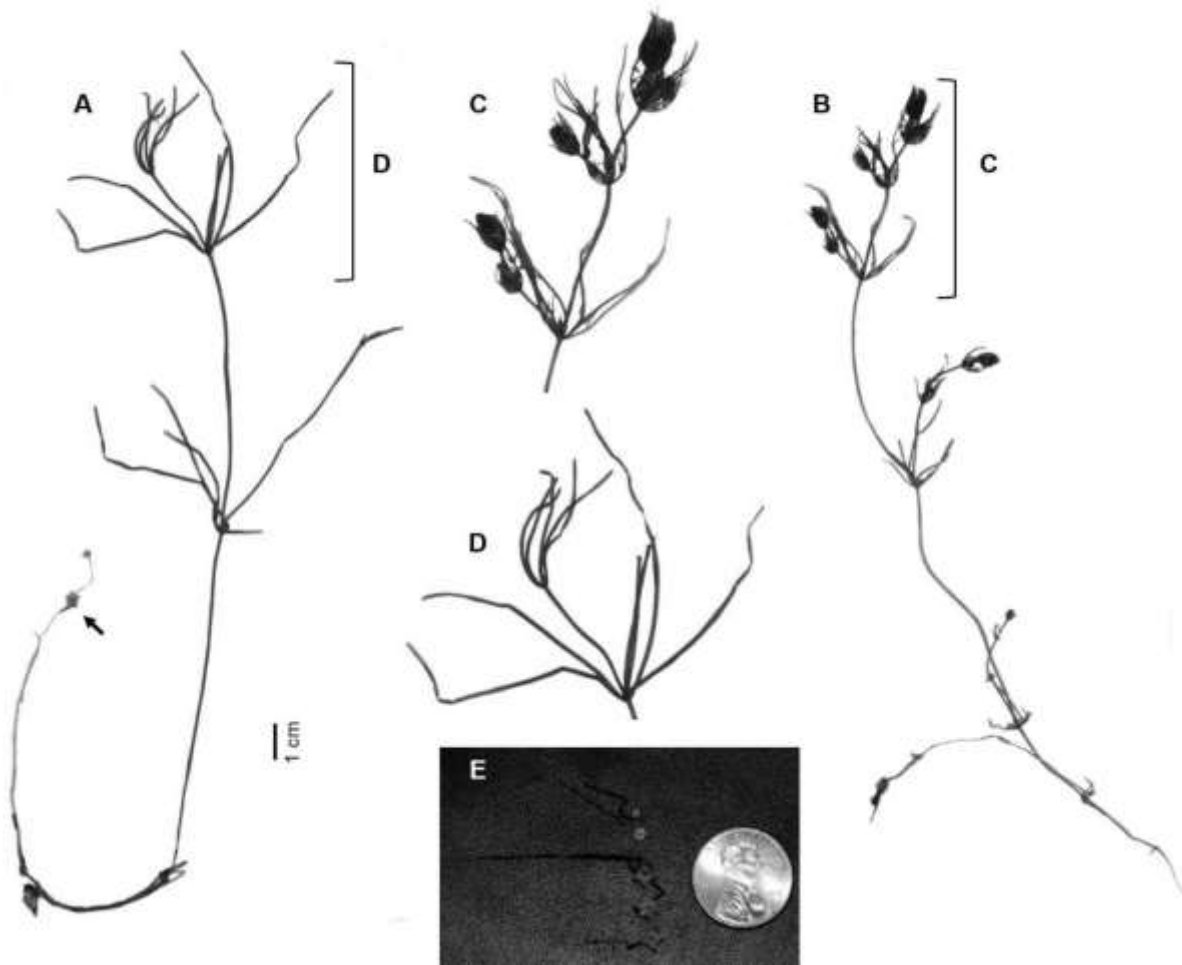
Figure 3-1. Photographs of *Nitellopsis obtusa* (a) and *Chara* sp. (b), both obtained from Tully Lake (1 cm scale is applicable to a and b). Whorled branches arise from stem nodes in both taxa. Detailed branching pattern of whorls show the regular pattern and more even size of leaflets in *Chara* sp. (c) and more irregular branching of whorls in *N. obtusa* (d). *Nitellopsis obtusa* often has characteristic star-shaped bulbils at the base of the plan (arrow in a) which can vary greatly in size (e).

METHODS

Study area

  I selected four lakes (Gatehouse Pond, Crooked, Tully, and Little York) in a lake district near Tully, New York, where *Nitellopsis* is present (Figure 3-2). Geology of the region was influenced by Pleistocene glaciation that formed the Finger Lakes region, directly to the west, and the landscape is characterized by many small kettle depressions (Kappel and Miller 2003). Watersheds of the study lakes are characterized by mixed forest and agricultural land use with minimal residential development (Anderson et al. 1976). Little York and Tully Lakes have public-access boat launches. Crooked Lake and Gatehouse Pond are private access waterbodies, but Gatehouse Pond was accessible to recreational boats before the late 1980s.
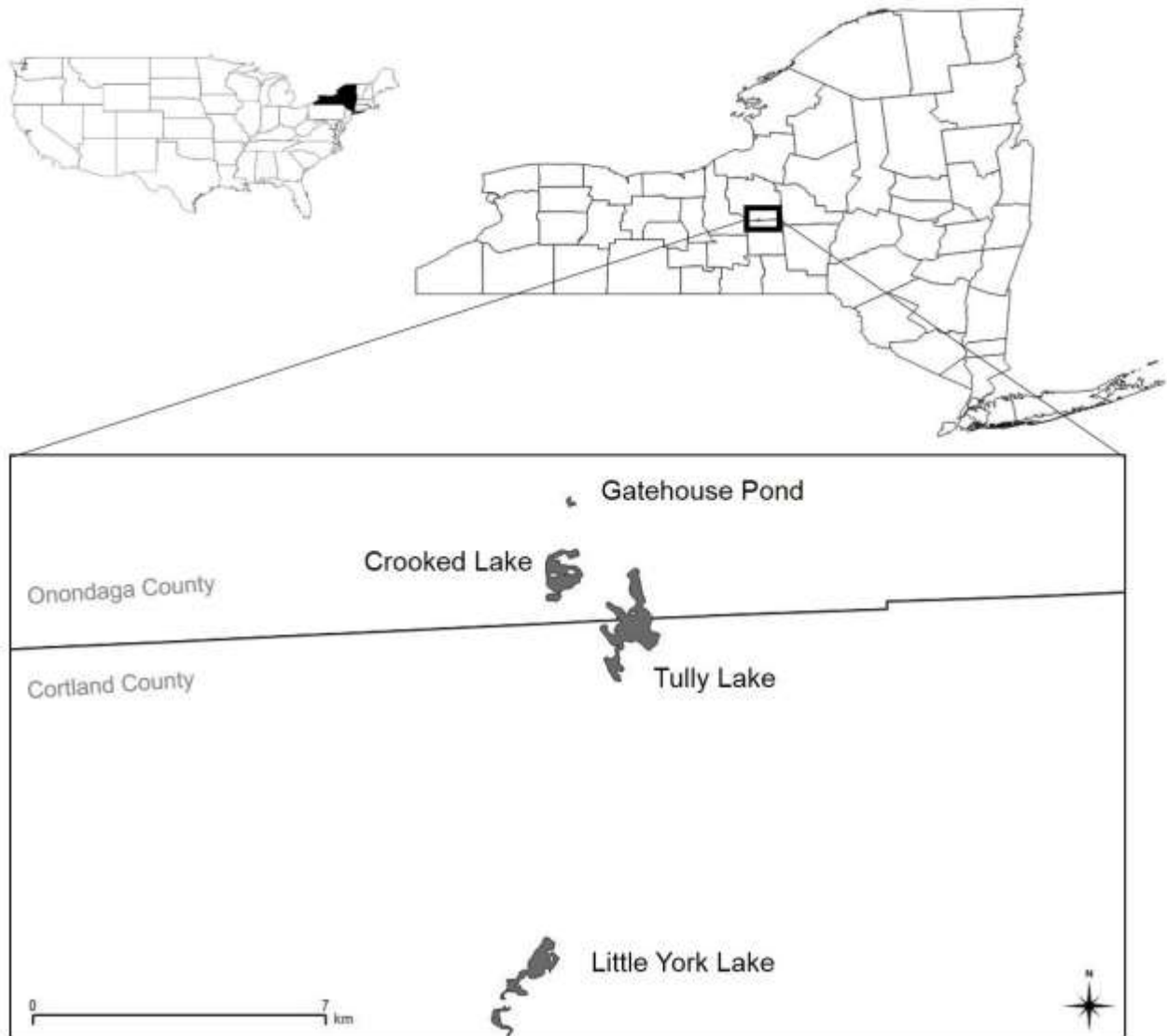
Figure 3-2. Location of study lakes in New York, USA.

Data collection

I collected samples at 10 sites in each lake between August and September in 2011 and 2012. I spaced sampling sites equidistantly around littoral zones, and collected samples from quadrats (0.25 m^2) by SCUBA diving at 3 depth intervals: 1) shallow, <1 m; 2) intermediate, 1 to 2 m; and 3) deep, >2 m (Figure 3-3). I used a transect line that was 1% of the total shoreline distance and parallel to shore as a guide to space quadrats equally at sampling sites (Figure 3-3). At each depth, I collected all macrophytes present in quadrats at 3 replicate locations (Figure 3-3). In total, I collected samples from 90 quadrats per lake per year. I identified macrophytes in each quadrat (Crow and Hellquist 2000a, b) and dried them at 60°C for 24 h to measure dry biomass (g).

Data analysis

I measured biomass of each species for each replicate sample, and calculated the total biomass of native and nonnative species other than *Nitellopsis* (total non-*Nitellopsis* biomass). I estimated total species richness, including *Nitellopsis,* other nonnative species, and native species, by totaling the number of macrophyte taxa identified at each depth (Table 3-S1). I tested the potential influence of *Nitellopsis* biomass on species richness by regressing total species richness (including *Nitellopsis*) on average *Nitellopsis* biomass for each depth (e.g., *Nitellopsis* biomass was averaged across all quadrats in deep locations that had 3 species present). I then used linear regression to estimate the influence of *Nitellopsis* biomass on native species richness (excluding *Nitellopsis* and other nonnative macrophytes) at each of the 3 depths. I also used linear regression to examine whether increased *Nitellopsis* biomass in a quadrat was associated with decreased total non-*Nitellopsis* or native macrophyte biomass, and tested for homogeneity of slopes among regressions for the 3 depths with analysis of covariance (ANCOVA). Biomass

data for each quadrat were $\log(x + 1)$-transformed to adhere to assumptions of regression and to enable us to retain macrophyte data for quadrats that had only *Nitellopsis*, which resulted in 0 biomass values for all other macrophytes. I used analysis of variance (ANOVA) to test whether *Nitellopsis* and macrophyte biomass differed within lakes and whether *Nitellopsis* frequency differed among depths within lakes. A post hoc multiple comparison test (Tamhane's T2) was used to test significance between the three depths. I conducted all statistical analyses in SPSS (version 23; IBM, Armonk, New York).
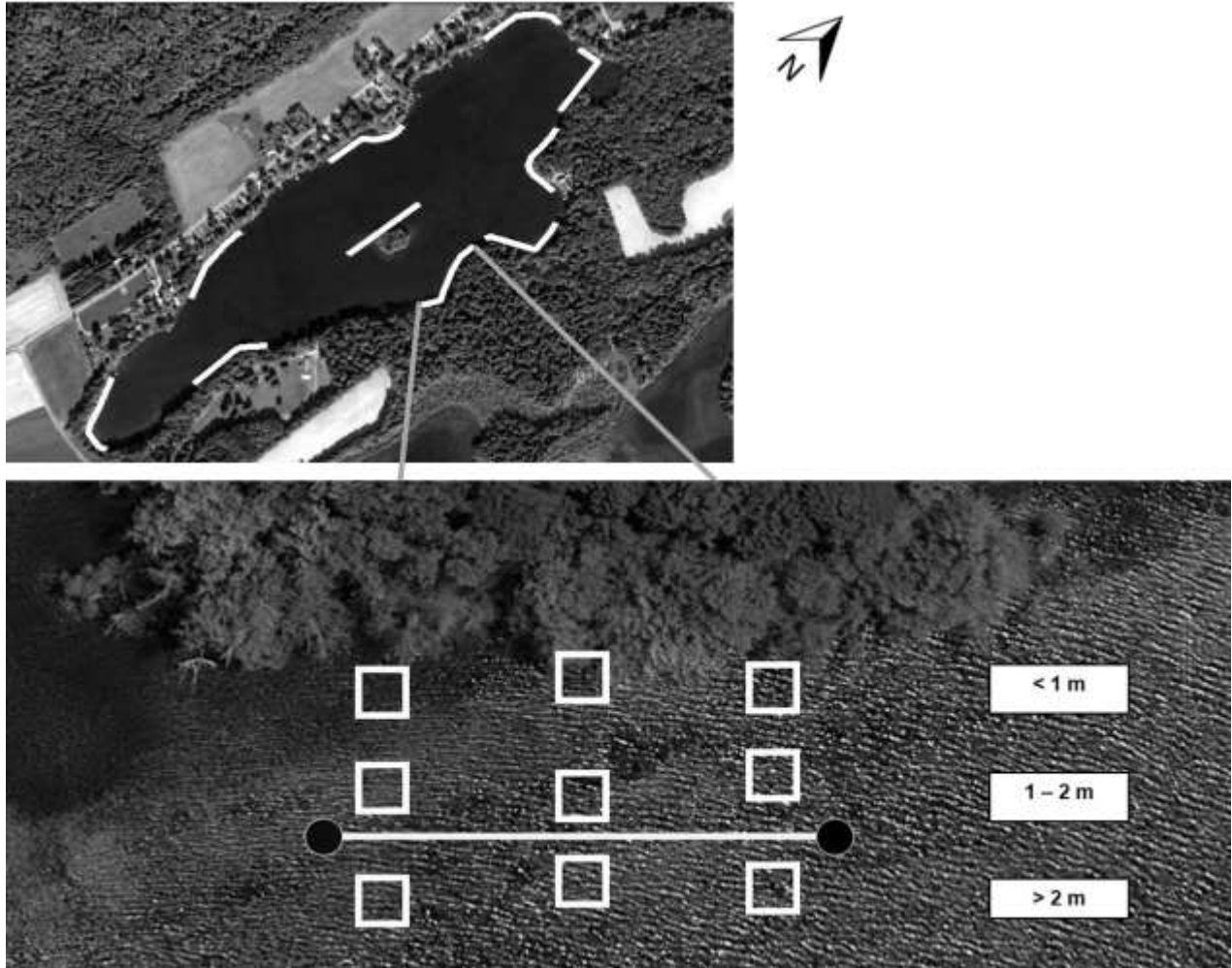
Figure 3-3. Example of a typical macrophyte sampling site with three depth intervals (shallow [<1 m], intermediate [1-2 m], and deep [>2 m]), and a transect line parallel to shore. Three replicate quadrats were collected at each depth. Aerial imagery courtesy of Good Earth®.

RESULTS

Nitellopsis biomass in individual lakes ranged from 3.3 to 140.7 g/m^2, and total biomass ranged from 47 to 567.8 g/m^2. Non-*Nitellopsis* species richness was greatest in intermediate quadrats, with a maximum of 10 species/quadrat (mean $\pm$ SD, 4.0 $\pm$ 1.8 species/quadrat). In deep quadrats, the maximum was 9 species/quadrat (3.5 $\pm$ 2.1 species/quadrat). Non-*Nitellopsis* species richness was lowest in shallow quadrats, with a maximum of 7 species/quadrat (3.1 $\pm$ 1.5 species/quadrat).

Total species richness decreased as *Nitellopsis* biomass increased (Figure 3-4a, Table 3-S2) at all 3 depths (shallow: $p < 0.001$, intermediate: $p = 0.029$, deep: $p = 0.002$), and regression slopes did not differ among depths (shallow: $b = -0.0154$, intermediate: $b = -0.0164$, deep: $b = -0.0176$; ANCOVA, $p = 0.939$). Note that at high *Nitellopsis* biomass, species richness approaches one indicating *Nitellopsis* may be the only species present. Native species richness also decreased as *Nitellopsis* biomass increased at all depths (shallow: $p = 0.02$, intermediate: $p = 0.05$, deep: $p < 0.001$; Figure 3-4b, Table 3-S2), and regression slopes did not differ among depths (ANCOVA, $p = 0.435$). Non-native macrophyte species richness was not related to *Nitellopsis* biomass at any depth (shallow: $p = 0.065$, intermediate: $p = 0.980$, deep: $p = 0.770$). Total non-*Nitellopsis* biomass and native species biomass both decreased as *Nitellopsis* biomass increased across all quadrats (total: $p < 0.001$, native: $p < 0.001$; Figure 3-5a,b). *Nitellopsis* biomass explained slightly >10% of the variation in total and native macrophyte biomasses ($R^2 = 0.115$ and 0.124, respectively).
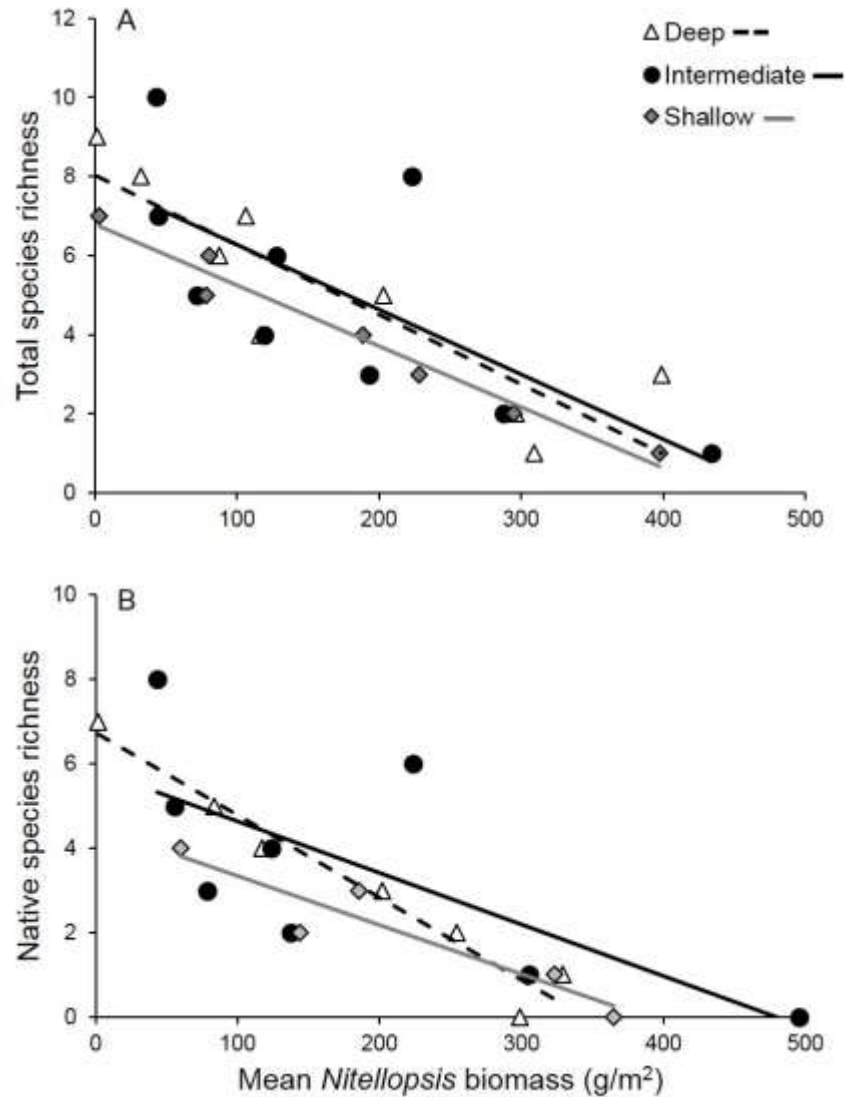
Figure 3-4. Total (a) and native (b) species richness as a function of Nitellopsis biomass at shallow, intermediate, and deep sampling locations. Regressions are:

total richness$_{shallow}$ = –0.0154(Nitellopsis biomass) + 6.7968, p < 0.001, R^2 = 0.97;

total richness$_{intermediate}$ = –0.0164(Nitellopsis biomass) + 7.9233, p = 0.029, R^2 = 0.52;

total richness$_{deep}$ = –0.0176(Nitellopsis biomass) + 8.0321, p = 0.002, R^2 = 0.77;

native richness$_{shallow}$ = –0.01(Nitellopsis biomass) + 4.51, p = 0.02, R^2 = 0.87;

native richness$_{iintermediate}$ = –0.01(Nitellopsis biomass) + 5.85, p = 0.05, R^2 = 0.50;

native richness$_{deep}$ = –0.020(Nitellopsis biomass) + 6.73, p < 0.001, R^2 = 0.95.
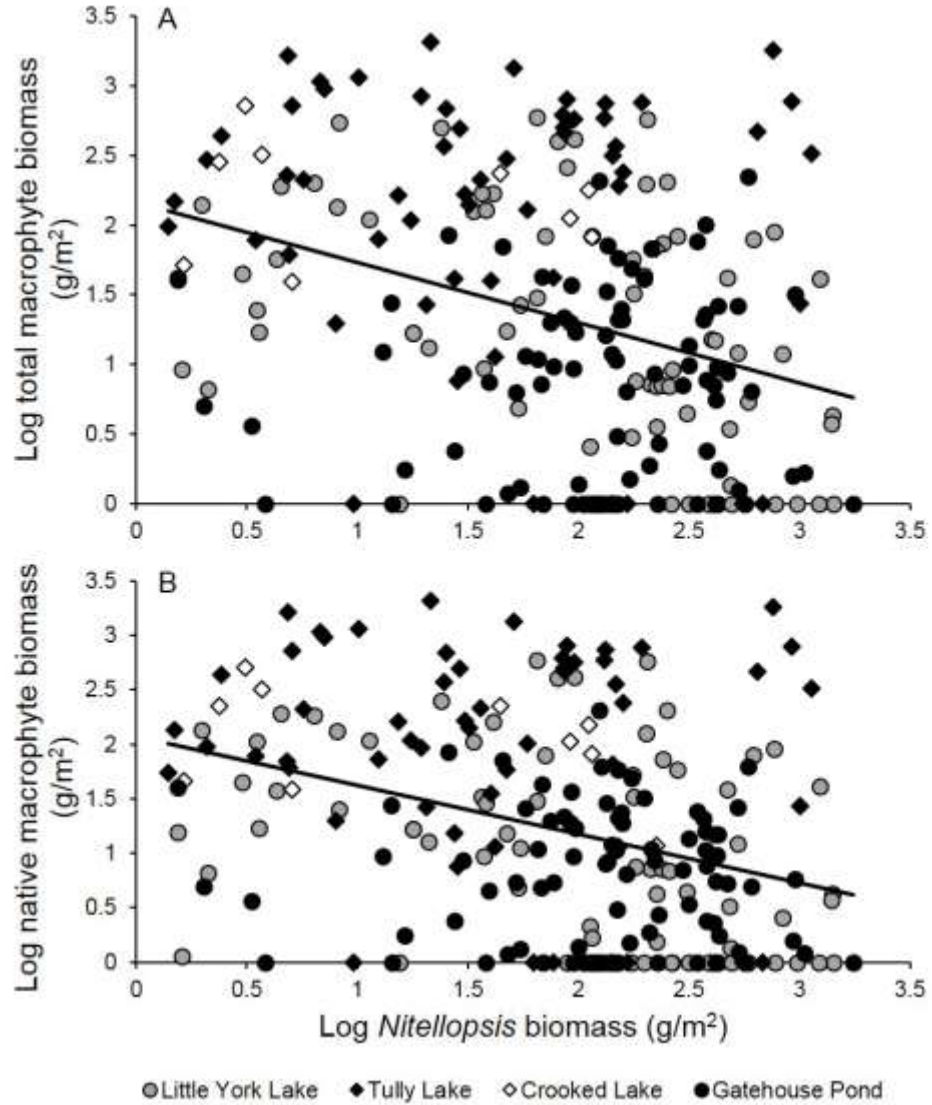
Figure 3-5. Total non-*Nitellopsis* (a) and native (b) macrophyte biomass as a function of *Nitellopsis* biomass in quadrats. Data were log(x + 1)-transformed. Regressions: total non-*Nitellopsis* biomass = −0.43(*Nitellopsis* biomass) + 2.17, p < 0.001, R^2 = 0.115. Native biomass = −0.45(*Nitellopsis* biomass) + 2.07, p < 0.001, R^2 = 0.124.

In some lakes, *Nitellopsis* biomass exceeded total non-*Nitellopsis* biomass (Figure 3-6, Table 3-S3). For instance, mean *Nitellopsis* biomass was significantly greater than total non-*Nitellopsis* biomass ($p < 0.001$) in Gatehouse Pond, and tended to be greater in Little York Lake (120 g/m^2 vs 99 g/m^2). Mean total non-*Nitellopsis* biomass was significantly greater than mean *Nitellopsis* biomass in Tully and Crooked Lakes ($p < 0.001$ for each lake).

I found a significant effect of depth on *Nitellopsis* frequency at Tully Lake ($p = 0.007$) and Gatehouse Pond ($p < 0.001$). *Nitellopsis* was more frequent in deep than in shallow quadrats at Tully Lake ($p = 0.005$) and Gatehouse Pond ($p < 0.001$) (Figure 3-7, Table 3-S4). *Nitellopsis* was additionally more frequent in intermediate than shallow quadrats in Gatehouse Pond ($p < 0.001$). In Little York and Crooked Lakes, *Nitellopsis* frequency did not differ significantly among depths (Little York Lake: $p = 0.534$, Crooked Lake: $p = 0.135$).
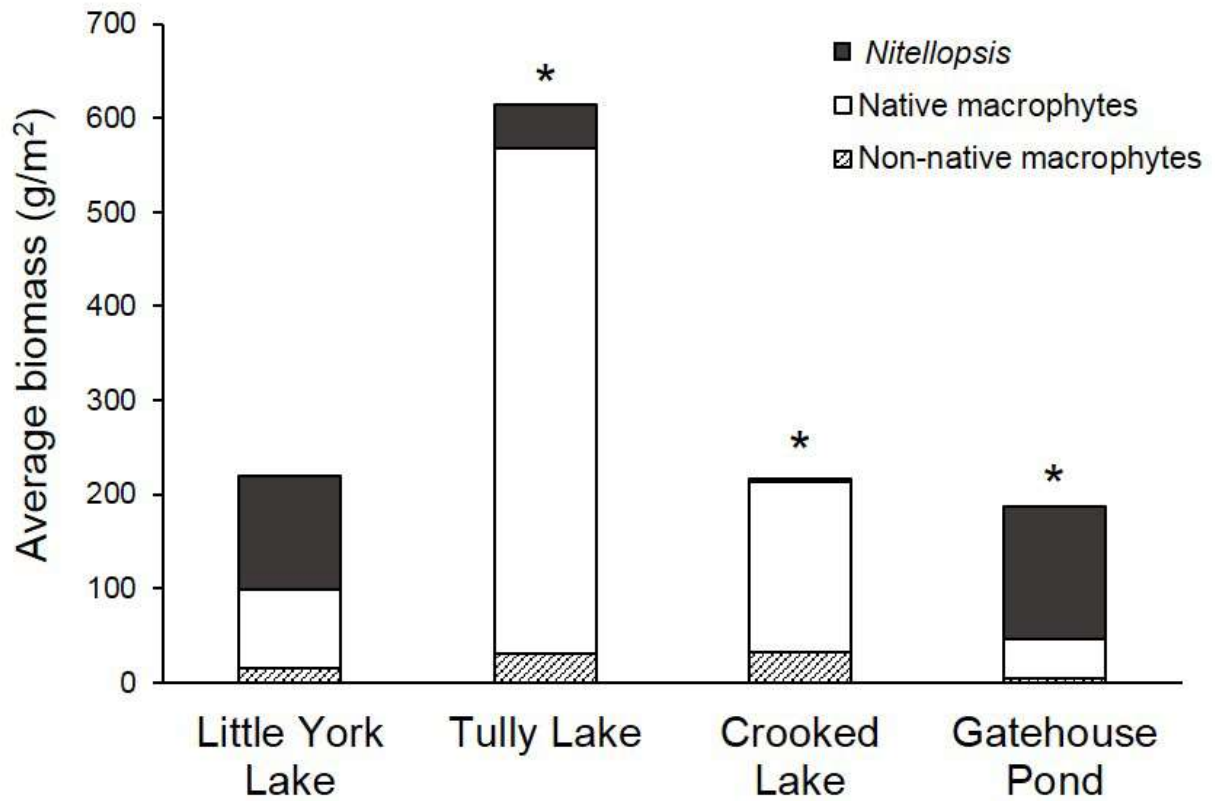
Figure 3-6. Mean (n = 180) biomass of *Nitellopsis*, and native and nonnative macrophytes averaged across depths from samples collected in 2011 and 2012 at each lake. * indicates a significant difference between total non-*Nitellopsis* and *Nitellopsis* biomass (2-sample t-tests, Little York Lake: p = 0.388, Tully Lake: p < 0.001, Crooked Lake: p < 0.001, Gatehouse Pond: p < 0.001).
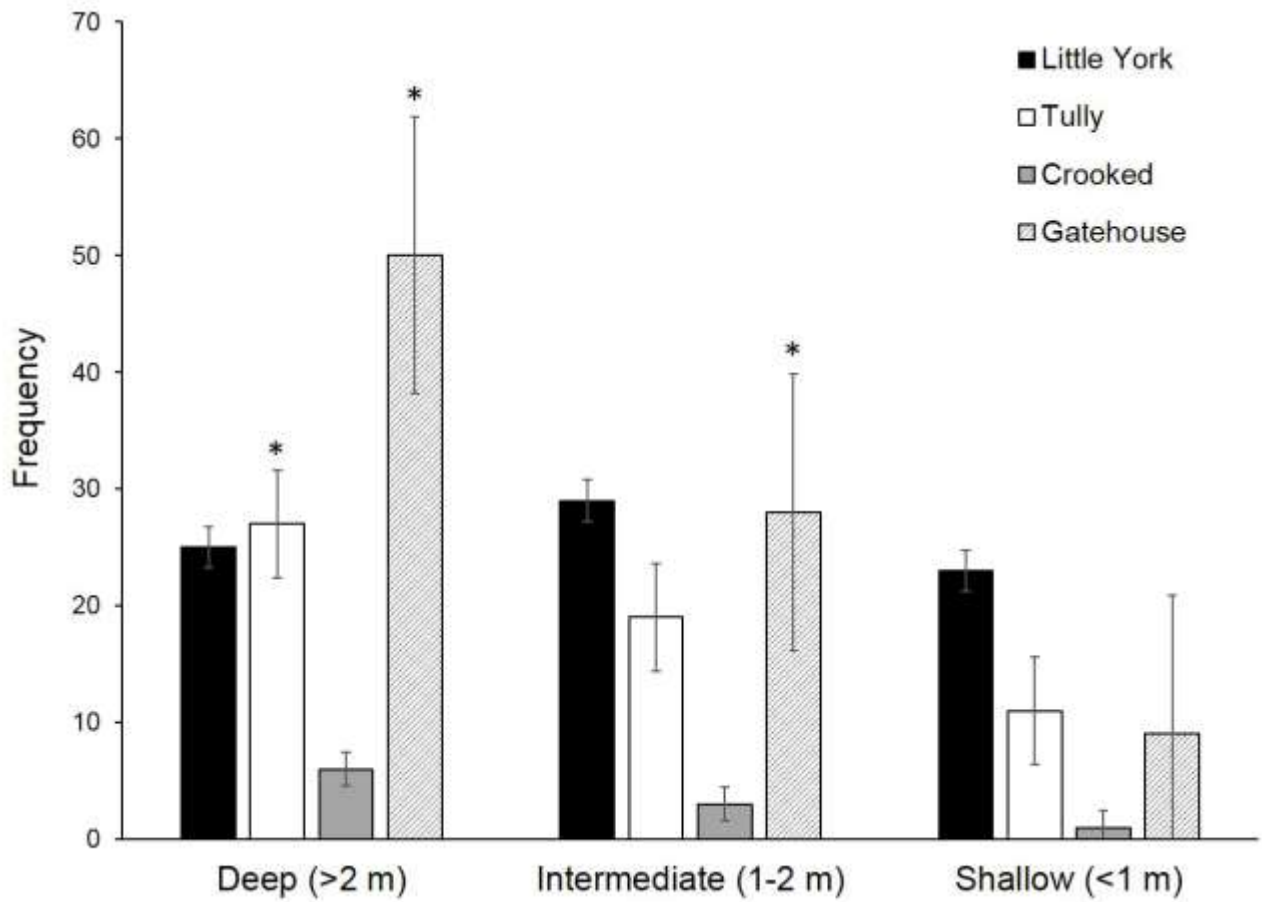
Figure 3-7. Mean (± SE; n = 60) frequency of *Nitellopsis* in quadrats collected from shallow (<1 m), intermediate (1–2 m), and deep (>2 m) sampling locations in each lake. Frequencies are means for samples collected in 2011 and 2012. * indicates *Nitellopsis* was significantly more frequent in deep or intermediate than in shallower quadrats for this lake.

Table 3-1. Number of quadrats in which *Nitellopsis* was sampled across deep, intermediate, and shallow locations by lake.

Lake	*Nitellopsis* frequency (number of quadrats)		
	Deep (>2 m)	Intermediate (1–2 m)	Shallow (<1 m)
Little York Lake	25	29	23
Tully Lake	27	19	11
Crooked Lake	6	3	1
Gatehouse Pond	50	28	9

DISCUSSION

My results substantially augment the few reported abundances of this invasive macroalgal species in North America. Schloesser et al. (1986) found a peak *Nitellopsis* biomass of 289 g/m^2 in the St Clair–Detroit River system, and Geis et al. (1981) reported *Nitellopsis* biomass as high as 72% of the total standing crop of littoral macrophytes in the St Lawrence River. In 2 of the lakes I sampled (Little York Lake and Gatehouse Pond), *Nitellopsis* biomass exceeded that of all other macrophyte species combined, with magnitude similar to the high overall biomass per area reported by Geis et al. (1981) (Figure 3-6). Benthic primary producer biomass in temperate regions often peaks in July (Wetzel 2001). However, *Nitellopsis* biomass generally reaches its maximum in September (Schloesser et al. 1986, Nichols et al. 1988), although seasonal growth maxima may vary based on local site conditions (e.g., nutrient availability). My samples were collected in August through September, likely capturing peak *Nitellopsis* biomass in the study lakes.

The regressions of *Nitellopsis* biomass with macrophyte richness had similar negative slopes among depths (Figure 3-4a,b). Pullman and Crawford (2010) characterized *Nitellopsis* as most abundant in deeper water where fewer species are present, suggesting *Nitellopsis* may simply colonize empty habitat rather than displace native species. If invasion of empty habitats had occurred, one would expect to find fewer macrophyte species and higher *Nitellopsis* biomass in deep than in shallower water. Instead, I found strong negative relationships between *Nitellopsis* biomass and macrophyte richness at all depths. My results suggest *Nitellopsis* has displaced macrophytes in the littoral zone across depths, rather than simply filling empty benthic habitats. Moreover, total biomass of non-*Nitellopsis* macrophytes was negatively related to *Nitellopsis* biomass (Figure 3-5a,b), but *Nitellopsis* biomass explained less of the variance in

non-*Nitellopsis* biomass than in species richness (Figure 3-4a,b), which suggests that other factors in addition to the presence of *Nitellopsis* (e.g., substrate characteristics, light availability, etc.) determine macrophyte biomass.

Recreational access differed among the study lakes, and this difference may be related to abundance and distribution of *Nitellopsis*. Little York and Tully Lakes have public-access boat launches, whereas Crooked Lake does not. Average *Nitellopsis* abundance was higher in Little York and Tully Lakes (120 and 46 g/m^2, respectively) than in Crooked Lake (3.3 g/m^2). Gatehouse Pond, which currently is private but had a public boat launch before the late 1980s, had dense beds of *Nitellopsis* with biomass similar to that in the 2 public lakes (141 g/m^2). Given the close proximity of the lakes, migratory and resident waterfowl probably move among lakes. I have sampled 2 other private access lakes in the area (Song Lake within the lake district and Echo Lake, ~ 30 miles to the south) and found no *Nitellopsis*. Thus, recreational boats are a more likely vector than waterfowl for secondary spread of *Nitellopsis* into new water bodies.

Documented effects of *Nitellopsis* on native flora and fauna are sparse. However, increased abundance of *Nitellopsis* can to limit fish-spawning habitat. Pullman and Crawford (2010) reported that bass and sunfish species did not spawn in dense beds of *Nitellopsis*. Only the Redear Sunfish (*Lepomis microlophus*) is known to use *Nitellopsis* beds for reproduction. An unsuccessful attempt was made to clear thick *Nitellopsis* beds to restore fish spawning habitat in Big Lake, Michigan, but *Nitellopsis* recolonized cleared areas too quickly to allow enough time for fish spawning (Pullman and Crawford 2010).

Nitellopsis biomass typically decreases in November (Nichols et al. 1988), which probably reduces dissolved O_2 available for other biota in late autumn and winter because of decomposition (Kipp et al. 2014). Documented negative effects of *Nitellopsis* on benthic

macroinvertebrates are lacking (Kipp et al. 2014), but a seasonal decline in dissolved O_2 associated with *Nitellopsis* senescence could be detrimental to long-term viability of benthic flora and fauna. In addition, late-season decay of large beds of *Nitellopsis* probably affects nutrient release from sediments by decreasing redox potential and increasing internal nutrient loading. However, these potential negative effects of this invader have not been quantified.

Budgetary and time constraints often limit the scope of aquatic invasive species monitoring. Typically, stakeholders are asked to look for possible invaders and report suspicious sightings to regulatory management agencies. However, *Nitellopsis* often is found in deep water (>2 m) (Figure 3-7) and could easily be missed by observers. Furthermore, *Nitellopsis* can be confused easily with native macroalgae (e.g., *Nitella* spp. and *Chara* spp.). When at low abundance, *Nitellopsis* is often found scattered among native macrophyte species, such as *Utricularia vulgaris* (Pullman and Crawford 2010), *Vallisneria americana*, *Najas flexilis*, *Elodea canadensis*, and the invasive macrophyte *Myriophyllum spicatum* (Schloesser et al. 1986), and can be overlooked easily. I have observed dense beds of *Nitellopsis* intermixed within larger beds of *Chara*, further confounding its detection by observers.

The potential invasive range of *Nitellopsis* throughout the northeastern and Midwestern USA (Escobar et al. 2016) coupled with reduction in species richness of macrophytes when *Nitellopsis* is abundant (this study), implementation of early monitoring methods that target *Nitellopsis* (e.g., sample deep water sites or underwater observations by SCUBA or snorkelling) is warranted. Evidence suggests that *Nitellopsis* is moved through the landscape by recreational boating. Thus, prevention programs that target public boat launches may be effective in detecting and preventing *Nitellopsis* spread before introduction and establishment.

CHAPTER 4: SURVIVAL AND BIOENERGETICS OF A NONNATIVE BENTHIC INVERTEBRATE COMPARED TO NATIVES IN ELEVATED TEMPERATURES AND REDUCED DISSOLVED OXYGEN: IMPLICATIONS FOR CLIMATE CHANGE IN THE GREAT LAKES

INTRODUCTION

Climate change is expected to fundamentally alter freshwater ecosystems, through increased water temperatures leading to increased strength of summer thermal stratification, and decreased dissolved oxygen (DO) concentrations at depth. Reductions in DO below thermoclines are predicted specifically in the Great Lakes (Magnuson et al. 1997). Climate change in the Great Lakes region is also predicted to result in greater precipitation, as records suggest annual precipitation has increased by 2.1% each decade since the early 1900s (Meyer et al. 1999). For areas that do not thermally stratify for extended periods (e.g., nearshore western basin of Lake Erie), increased precipitation and associated runoff with climate change may increase nutrient inputs (Jeppesen et al. 2009), leading to greater system productivity. In contrast to seasonally stratified areas, as productivity increases in shallower water nearshore, oxygen concentrations may exhibit a diurnal pulse characterized by higher concentrations during the day coupled with hypoxic conditions at night as greater biomass (from the increased productivity) is present for respiration.

Aquatic invasive species are an additional stressor that can profoundly alter natural processes of ecosystems and pose threats to native biota (Molnar et al. 2008). For example, increased light penetration into the water column and associated clear water phase(s) has been attributed to the dreissenid mussel invasion in the Great Lakes due to high filtering rates of phytoplankton (MacIsaac 1996). Further, following the invasion of dreissenid mussels, increased concentrations of organic pesticides were documented in waterfowl that were consuming them (MacIsaac 1996). Dreissenid mussels filter large amounts of water (up to 132 m^3 m^{-2} day^{-1},

MacIsaac et al. 1992), which can include pesticides from runoff, which are then concentrated in the mussel's tissue and subsequently biomagnified in waterfowl after consumption. This negative impact on waterfowl is one example of an altered biotic interaction resulting from an aquatic invasive species. More generally, Dextrase and Mandrak (2006) suggest that native fish and mollusk species, in particular, are threatened by aquatic invasive species in Canada and the Great Lakes basin.

Climate change and aquatic invasive species may act synergistically to negatively impact aquatic systems. Warmer water temperatures are likely to increase the establishment of invasive species with ranges historically limited by temperature, facilitating range expansion spatially, and/or resulting in increased population abundance locally (Hellman et al. 2008; Bartolai et al. 2015). In Lake Superior, for example, increased water temperatures have been predicted to facilitate expansion of dreissenid mussels based on thermal habitat requirements (Hall and Stuntz 2008). More broadly, climate change may fundamentally alter the species composition of aquatic communities (Rahel and Olden 2008) through shifts in thermal optima that may favor invasive species over natives. Additionally, secondary effects of increased water temperatures such as reduced (hypoxic) or diurnal fluctuations in (pulsed) DO concentrations at the sediment-water interface (such as those expected in nearshore Lake Erie, which is shallow yet highly productive, Makarewicz 1993) may favor species that are able to tolerate DO reductions.

Branchiura sowerbyi, a nonnative burrowing oligochaete in the Great Lakes, is a potential species whose success may be increased under projected conditions for climate change scenarios – specifically increased water temperatures and reduced or more variable DO concentrations. *B. sowerbyi* is native to subtropical and tropical regions of Asia (Mills et al. 1993), and was first reported in North America in 1930 in Ohio (Spencer 1932). Currently, *B.*

sowerbyi has been documented in Lakes Erie (Hiltunen 1969), Huron (Spencer and Hudson 2003), St. Clair and Michigan (Leibig et al. 2012). *B. sowerbyi* is the largest tubificid in the Great Lakes (Hiltunen 1969), reaching up to 15 cm in length (Wang and Matisoff 1997).

Because the life cycle of *Branchiura sowerbyi* is intimately tied to temperature (Aston 1968), coupled with its ability to survive in depleted DO for extended durations (Aston 1973), the potential exists for *B. sowerbyi* to become a dominant component of the benthos in nearshore habitats of the Great Lakes in future climatic conditions. The thermal optimum for *B. sowerbyi* was reported as approximately 25°C (Aston 1968); however, Carroll and Dorris (1972) found that *B. sowerbyi* went through two life cycles in an Oklahoma stream with warmer water temperatures, and a single life cycle in a neighboring stream with colder water temperatures, so the growth response to temperature by *B. sowerbyi* may be more complex than originally thought. *B. sowerbyi* is hypothesized to tolerate reduced DO concentrations because of external gills (Aston 1973), unlike any native aquatic oligochaete in North America (Peckarsky et al. 1990). In the western basin of Lake Erie, *B. sowerbyi* has increased in average abundance from 75 individuals m^{-2} in the early 1980s to 122 individuals m^{-2} in 2003-2004 (Soster et al. 2011).

In contrast to *Branchiura sowerbyi*, native burrowing mayflies (*Hexagenia* spp.) may be intolerant of future water conditions (e.g., increased temperatures and/or reduced DO concentrations) projected with climate change and thus may be at risk of population decline. *Hexagenia* have been a major component of the Lake Erie benthos and an important component of food webs (Bridgeman et al. 2006) contributing to secondary production of benthivorous fish. Lake Erie populations of *Hexagenia* crashed in the mid-20th century, largely attributed to cultural eutrophication and pollution (Carr and Hiltunen 1965). In recent years, populations have rebounded (Krieger et al. 1996), and are hypothesized to have recovered because of improved

water quality from nutrient reductions programs (e.g., Great Lakes Water Quality Agreement, GLWQA). The invasion of dreissenid mussels may have also coincidentally benefited the recovery of *Hexagenia* populations, whereby dense clusters of dreissenid mussels may provide some refuge from fish predation (DeVanna et al. 2011).

In the western basin of Lake Erie, *Hexagenia* spp. densities have increased from a maximum of 76 individuals m^{-2} in the early 1990s up to approximately 700 individuals m^{-2} in 2003-2004 (Soster et al. 2011). As further evidence of the recovery of *Hexagenia* in Lake Erie, Soster et al. (2011) reported no *Hexagenia* were present in samples collected in the western basin in the early 1980s, but approximately 20 years later in the early 2000s, Krieger et al. (2007) reported mean abundance in the western basin ranging from 60 to 427 individuals m^{-2}.

Hexagenia, in particular, are intolerant of low DO concentrations (Rasmussen 1998), and the primary strategy to deal with low DO is a behavioral adaptation to aerate U-shaped burrows in an attempt to flush in available oxygen (Charbonneau and Hare 1998; Gallon et al. 2008). Further, Gallon et al. (2008) reported that *Hexagenia* were inactive (no movement) < 1% of time over a 24 hour period of observation. More specifically, constant beating of gills was recorded for up to 9.5 hours in duration without break (Gallon et al. 2008). Constant motion exhibited by *Hexagenia* may contribute to its inability to tolerate low DO – movement requires oxygen, and as concentrations of DO decline in the benthos, a sufficient amount of available oxygen may not be present to maintain metabolism.

Chironomus riparius is another native burrowing invertebrate that is found at high densities in productive nearshore areas (Bairlein 1989; Penttinen and Holopainen 1995), such as the western basin of Lake Erie. In contrast to *Hexagenia*, *C. riparius* can tolerate conditions in which DO is limited through a physiological adaptation – presence of hemoglobin within its

hemolymph (fluid equivalent to blood in invertebrates). Hemoglobin facilitates efficient capture and utilization of available O_2 (Choi et al. 2000). Additionally, *C. riparius* behaviorally respond to hypoxia by reducing feeding activity and allocating more time to ventilating burrows and expending energy (Stief et al. 2005) to acquire available oxygen. Further, during prolonged periods of reduced DO, *C. riparius* have been shown to abandon burrows and construct chimneys that both increase access to available oxygen (overcome the oxygen depletion layer at the sediment-water interface) and increase microbial food availability, as chimneys can be highly colonized by bacteria (Stief et al. 2005).

While reported densities of *Chironomus riparius* in the western basin of Lake Erie are limited, the abundance of Chironomidae (the taxonomic family that contains the genus *Chironomus*) has declined from approximately 3,600 individuals m^{-2} in the early 1980s down to around 1,000 individuals m^{-2} in 2003-2004 (Soster et al. 2011). Soster and McCall (1990) reported *C. riparius* densities in the western basin of Lake Erie up to approximately 3,000 individual m^{-2}.

One approach to determine how environmental conditions can influence the viability of individuals and populations is through bioenergetics modeling. A bioenergetics model is a mass-balance equation that parcels energy consumed into distinct physiological endpoints, such as respiration, waste excretion and/or egestion, and growth (both somatic and reproductive) (Ney 1993). Because of their relative simplicity, bioenergetics models can be useful to evaluate implications of climate change (Railsback and Rose 1999). For example, Petersen and Kitchell (2001) used bioenergetics to assess how climate regimes influenced predation on juvenile salmon, and Jenkins and Keeley (2010) applied bioenergetics to habitat quality for cutthroat trout under climate change predictions. Additionally, Wu and Or (2005) quantified growth and

reproduction of amphipods in the low DO conditions that may be exacerbated with climate change.

Bioenergetics modeling also can be applied to invasive species in an effort to assess their invasibility and likelihood to become locally abundant. Gutiérrez-Yurrita and Montes (1999) evaluated the bioenergetics of the red swamp crayfish (*Procambarus clarkii*) in Spain to refine species management alternatives. Bioenergetics models also have been used to evaluate growth of zebra mussels (Schneider 1992), and to predict the likelihood Asian carp will invade the Great Lakes (Cooke and Hill 2010). Finally, bioenergetics models have been used to evaluate the synergy between climate change and invasions. Penk et al. (2015) evaluated how warmer water temperatures influenced physiological performance of an invasive mysid (*Hemimysis anomala*) compared to a functionally similar native species (*Mysis salemaai*), and found that the invasive *Hemimysis* increased its feeding rate with elevated temperatures to conserve assimilation. In contrast, the native *Mysis* did not increase its rate of feeding when temperatures were increased, resulting in decreased ingestion and assimilation, leading the authors to conclude that the native mysids would have reduced growth compared to the invasive *Hemimysis* under climate change.

Here, I examine the influence of temperature and DO on the survival and bioenergetics of the nonnative *Branchiura sowerbyi* in comparison to native *Hexagenia* spp. and *Chironomus riparius*. My objective was to understand if elevated water temperatures and/or reduced DO conditions would result in asymmetries in survival and bioenergetics performance of the nonnative oligochaete compared to the two native benthic invertebrates.

I first determined the abundance and biomass of each organism in nearshore Lake Erie at three locations (Old Woman Creek, Sandusky, and Maumee Bays) to observe if patterns of abundance and biomass were related to current water temperature and DO concentrations. Field

samples were also used to derive growth rates, considered to be rates at high DO concentrations, based on means and variability of DO measured in 2013. I hypothesized that *Branchiura sowerbyi* would be found at a higher abundance in locations with increased water temperatures, and in particular given its apparent ability to tolerate low oxygen, decreased DO concentrations compared to native *Hexagenia* and *C. riparius*.

I then quantified survival and bioenergetics parameters (respiration, excretion plus egestion, and growth rates) of each organism across gradients of temperature and DO concentrations in laboratory experiments. I hypothesized that survival of *Branchiura sowerbyi* would be greater at high temperatures and low DO compared to that of *Hexagenia* and *Chironomus riparius* as a direct result of *B. sowerbyi's* superior tolerance of projected climate change conditions. Additionally, because the life cycle of *Branchiura sowerbyi* is closely tied to thermal conditions, I hypothesized that *B. sowerbyi* would have increased respiration, excretion/egestion, and growth rates in high temperatures compared to native *Hexagenia* spp. and *Chironomus riparius*. Further, I hypothesized that *B. sowerbyi* would exhibit lower respiration, excretion/egestion, and growth rates compared to the native species in low DO treatments, as metabolic activity would be reduced to combat adverse low oxygen surroundings.

I then measured bioenergetics parameters to model consumption rates of each organism and simulated growth rates under hypoxia. I predicted that *Branchiura sowerbyi* consumption rates would be greater at higher temperatures, and lower than the native organisms' consumption rates at low DO concentrations due to ability to tolerate such conditions. Additionally, I hypothesized that growth in hypoxia would be lower for *B. sowerbyi* in comparison to that of the native species because overall metabolic activity would be reduced as a mechanism to tolerate adverse oxygen settings.

Ultimately, my goal was to address how climate change may alter the physiological processes and success of the nonnative *Branchiura sowerbyi* compared to functionally similar native invertebrates in nearshore Lake Erie. Conditions that may favor *B. sowerbyi* under climate change, leading to increased abundance or relative composition of the benthic community, could have fundamental effects on ecosystem function, including increased sediment bioturbation or decreased forage base for important recreational fisheries.

METHODS

Benthic Invertebrate Sampling

To determine abundances and biomasses of *Branchiura sowerbyi, Hexagenia* spp., and *Chironomus riparius* in nearshore western Lake Erie, benthic samples were collected monthly from May through October, 2013 at three sites per location (Old Woman Creek [(OWC], Sandusky, and Maumee Bays). Five replicate samples were collected per location each month with a petite Ponar benthic sampler. Samples were washed through a 500 μm sieve to remove sediment and large debris, then preserved in 70% ethanol to be transported back to the laboratory.

In the lab, sampled were stained with Rose-Bengal solution to facilitate sample processing. The number of *Branchiura sowerbyi*, *Hexagenia* spp., and *Chironomus riparius* in each sample was counted, and converted to abundance (no. m^{-2}) based on the surface area sampled by the petite Ponar (0.023 m^2). Biomass (g DW m^{-2}) values for each organism were determined from two replicate samples per month, determined by drying individuals at 60°C for 24 h (Salonen et al. 1976) and weighing on a microbalance (Mettler Toledo UTM20). Masses of organisms by location were averaged to determined average monthly biomass.

Water Parameter Sampling

I sampled temperature (°C), dissolved oxygen (DO, %), total phosphorus (TP, mg L^{-1}), and total nitrogen (TN, mg L^{-1}) monthly at each location from May through October, 2013. At each location each month, I collected temperature and DO using a mulitprobe (YSI 6600) from 15 locations, and TP and TN water samples were collected from the three benthic invertebrate sampling locations. Water samples were analyzed on an autoanalyzer (Bran Luebbe AA3), USEPA method 365.3 (USEPA 1983).

Survival

Survival was quantified at four temperatures (4°C, 10°C, 20°C, and 30°C) at high DO (at saturation), hypoxic (< 2 mg L^{-1}), and pulsed (12 hr high O$_2$, 12 hr hypoxic) DO treatments. Pulsed DO was achieved by bubbling in oxygen (high O$_2$) or N$_2$ gas (hypoxic) to achieve target DO concentrations, over a 24 hr diel cycle. Survival was quantified based on experiment design of the laboratory growth experiment (described below), and was quantified as the number of individuals that were alive at the end of 14 (*Branchiura sowerbyi* and *Chironomus riparius*) or 12 days (*Hexagenia*), from the initial five individuals per organism and temperature-DO treatment. Additionally, a separate experiment was conducted to determine sediment nutrient release (SNR) from the study organisms (Brainard and Schulz, unpublished data) at 20°C and 30°C. The SNR experiment consisted of six *Branchiura*, seven *Hexagenia*, and 10 *Chironomus* in mesocosms (0.002 m^3), conducted over nine days. Percent survival from growth and SNR experiments was combined to determine average survival (%) for each temperature-DO combination.

Bioenergetics

I collected *Branchiura sowerbyi* to use in laboratory experiments from Tully Lake, located approximately 30 miles south of Syracuse, NY. *B. sowerbyi* were not transported from Lake Erie sampling sites due to concerns of moving of a nonnative species across a large geographic extent, and mortality during long transport. *Hexagenia* eggs were obtained from Dr. Jan Ciborowski (University of Windsor) and stored at 4°C until hatched at 10°C in aerated tanks. *Chironomus riparius* eggs were obtained from Aquatic Research Organisms (Hampton, NH) and were similarly stored at 4°C until hatched at 10°C. Organisms were kept in tanks (10 gallon) and fed daily; food consisted of a slurry of baker's yeast, alfalfa powder, and Tetramin® fish food flakes in deionized water, following Winter et al. (1996). Prior to bioenergetics experiments (described below), organisms were acclimated to experimental temperatures at a rate of 3°C per day.

I report mass-specific values for bioenergetics parameters. Masses were determined by drying individuals at 60°C for 24 h (Salonen et al. 1976) and weighing on a microbalance (Mettler Toledo UTM20).

Respiration

Respiration rates (μg O_2 mgDW^{-1} hr^{-1}) were quantified at 4°C, 10°C, 20°C, and 30°C at high O_2 (mg L^{-1} at saturation) and hypoxic (< 2 mg L^{-1}) dissolved oxygen concentrations in a fully factorial design, using a microrespirometer (Hansatech Oxygraph). Twenty individuals of *Branchiura sowerbyi*, and ten individuals of *Hexagenia* and *Chironomus riparius* were used for each temperature-dissolved oxygen treatment across a range of individual masses (Table 4-S1). A greater number of *B. sowerbyi* were used for respiration compared to *Hexagenia* and *C. riparius* due to lack of published respiration rates for this species.

For each experimental trial, the oxygen chamber of the microrespirometer was inverted to permit sediment to be placed in the chamber, providing a burrowing medium to make conditions inside the experimental chamber more natural. Sediment used in the respiration experiment was collected from Tully Lake, NY, and processed through a 500 μm sieve to homogenize sediment and remove large debris and other invertebrates. Sediment was stored at 4°C and brought to appropriate experimental temperatures prior to conducting replicate respiration trials. All water used was collected from Skaneateles Lake (an oligotrophic lake and used in all bioenergetics experiments for consistency), and filtered (Whatman GF/F). For high DO treatments, O_2 was bubbled into the water prior to conducting trials. For hypoxic treatments, N_2 gas was bubbled in to lower DO concentrations below 2 mg L^{-1}.

For each replicate respiration trial, the rate of O_2 change of sediment and overlying water alone was quantified first, for approximately 45 minutes, to determine baseline respiration of any microorganisms present in the sediment and/or water. A single individual was then placed within the chamber and allowed to respire until the rate of O_2 change was constant (generally 45 minutes). Respiration rates (μg mgDW^{-1} hr^{-1}) were then calculated after correcting for initial sediment and water only rates.

Respiration rates were converted to g C gDW^{-1} day^{-1} for use in bioenergetics models – mass of O_2 was converted to mass of carbon by a conversion factor of 0.949 (Lampert 1987, in Yurista and Schulz 1995).

Excretion/egestion

Mass-specific excretion (soluble waste products) plus egestion (undigested solids) rates (μmol L^{-1} mgDW^{-1} hr^{-1}) of total phosphorus (TP) were quantified at 4°C, 10°C, 20°C, and 30°C at high O_2 (mg L^{-1} at saturation) and hypoxic (< 2 mg L^{-1}) dissolved oxygen concentrations in a

fully factorial design. Excretion/egestion was calculated as the difference in TP concentrations from control replicates to those containing organisms. Each excretion/egestion replicate included six individuals of each organism (Figure 4-S1). Control treatments consisted of water only, and concentrations were used to correct for background phosphorus concentrations.

Filtered Skaneateles Lake water, with low nutrients concentrations, was used to allow for a change in phosphorus to be measured. For each temperature-DO treatment combination, a single individual was placed in a 50 mL Falcon tube containing 40 mL of water. After 5 hours, individuals were removed and dried to determine dry mass. TP was measured with unfiltered water within the Falcon tubes using the persulfate digestion method on a spectrophotometer. Excretion/egestion rates were then determined after correcting for the average phosphorus concentrations in control treatments.

Total phosphorus concentrations were used for consumption and growth under hypoxia models. Excretion/egestion rates of TP were converted to g C gDW^{-1} day^{-1} to be used in consumption and hypoxic growth model calculations – μmol of P was converted to mass of carbon by first converting mol to mass (molecular weight of P = 30.97), then by calculating TP to soluble reactive phosphorus (SRP) as a 4:1 ratio (Madeira et al. 1982). SRP was then converted to units of carbon based on a 122:1 carbon to SRP conversion factor for mayfly excretion rates (James et al. 2007).

Growth

Experiment

Growth (mgDW day^{-1}) was measured in the laboratory at 4°C, 10°C, 20°C, and 30°C fully crossed with high DO, hypoxic, and pulsed dissolved oxygen concentrations (Figure 4-S2). Growth for *Chironomus riparius* was not determined at 10°C due to mortality in the cultured

stock (Figure 4-S2). *Branchiura* and *Chironomus* growth were measured over 14 days. High mortality was observed in *Hexagenia* treatments initially, and new treatments were subsequently established, thus *Hexagenia* growth experiments consisted of 12 days. Organisms were fed daily with a mixture of baker's yeast (15 g), Tetramin® fish food flakes (20 g), alfalfa powder (15 g), in 500 mL of deionized water, following Winter et al. (1996).

For each temperature-DO combination, five organisms of a given species were placed in containers (1600 cm^2) alone (e.g., no treatments included more than one taxon) with 5 cm of sediment (Tully Lake) and 1 L of overlying water (Skaneateles Lake). For high DO treatments, O_2 was continually bubbled into containers; hypoxic DO treatments were set up by bubbling in N_2 gas to lower DO concentrations below 2 mg L^{-1}, and containers were then sealed. For pulsed DO treatments, DO concentrations were raised to saturation (O_2 bubbled) and then reduced to below 2 mg L^{-1} (N_2 bubbled) on a 12 hr cycle.

Photographs of each individual used in the growth experiment were taken on a dissecting microscope (Leica MZ125) and used to determine initial masses (mg) from length-weight regressions (Figure 4-S3). Lengths were determined using ImageJ. Final masses were then determined by dry weight. Growth (mgDW day^{-1}) was calculated only for individuals that were alive at the end of the experiment, and was determined as the difference in final mass from initial, divided by the duration (days).

Field-derived growth estimates

Growth rates were estimated from invertebrates collected from field samples in OWC, Sandusky and Maumee Bays from May through October, 2013. Lengths of 507 individuals (214 *Branchiura sowerbyi*, 110 *Hexagenia*, and 183 *Chironomus riparius*) were determined by

photographs using a dissecting microscope (Leica MZ125) and masses were determined from length-weight relationships (Figure 4-S3).

Invertebrates of each species were ordered by weight (e.g., lowest weight to highest weight), and I determined the rate of change of masses as a power function. I assumed seasonal growth for each organism began on March 1 (day 1) and ended on October 31 (day 245) (Armitage et al. 1995; Péry and Garric 2006). The average monthly mass for each organism (May through October) approximately aligned with ordered masses; thus I assumed that an individual's ordered mass corresponded to the number of days between March 1 and October 31.

I calculated growth rates for each organism (mg mgDW^{-1} day^{-1}) as the difference in mass (an individual's mass minus smallest organism measured) divided by the product of the rate of change and number of days to reach the organisms mass. For organisms in a given month, I plotted growth rates as a function of mass (mg) to derived monthly growth rates, which were then used to determine functional relationships with temperature based on monthly water temperatures collected 1 m above the sediment in the field.

Field-derived growth rates were determined for three different organism sizes (25% quartile, median, and 75% quartile, based on variability in measured field masses) at 4°C, 10°C, 20°C, and 30°C, and were converted to g C gDW^{-1} day^{-1} for use in modeling consumption rates. Masses of organisms were converted to units of carbon by a 47% conversion factor (Rothlisberger et al 2008). Note that dissolved oxygen concentrations measured in the field approximately 1 m above the sediment-water interface (day measurements) were only below 30% DO at a single location in OWC during the August sampling (5.6%), thus field-derived growth rates were assumed to be high DO rates in the consumption model.

Modeled Consumption and Growth in Hypoxia

Consumption rates (g C gDW day^{-1}) were estimated for high DO conditions with three

sizes (25% quartile, median, and 75% quartile organism mass based on field samples, Table 4-1)

for each organism using respiration, excretion/egestion, and field-derived growth rates, as

follows (Equation 1):

Consumption = Respiration$_{high\ DO}$ + Excretion/Egestion$_{high\ DO}$ + Growth$_{high\ DO}$ (1)

Respiration and excretion/egestion functional relationships used in the consumption

model were those from the high DO laboratory treatments, to correspond to conditions for field-

derived growth rates.

Table 4-1. Masses (25% quartile, median, and 75% quartile) of each organism, based on organism sizes measured from field samples, used to determine mass-specific consumption and hypoxic growth rates.

Organism	25% Quartile (mg)	Median (mg)	75% Quartile (mg)
Branchiura	2.8	3.9	5.3
Hexagenia	0.1	0.4	0.9
Chironomus	0.2	0.4	0.8

I assumed the modeled consumption rates were maximum values, as they were derived with high DO relationships. I used the simulated consumption rates, and functional relationships of respiration and excretion/egestion under hypoxia (measured) to model hypoxic growth for three sizes (25%, median, and 75% masses) of each organism, as follows (Equation 2):

$$Growth_{hypoxia} = Consumption_{high\ DO} - Respiration_{hypoxia} - Excretion/Egestion_{hypoxia} \qquad (2)$$

Because consumption rates were assumed to be maximum values, hypoxic growth rate estimates are likely conservative, as consumption under hypoxic conditions may be less than under high DO conditions.

Statistical Analysis

I used one-way ANOVA to test if field abundances and biomasses of organisms were different between locations (OWC, Sandusky, and Maumee Bays). Additionally, I used multiple regression with temperature, DO, TP, and TN as explanatory variables and organism abundance to evaluate if abiotic variables correlate with benthic invertebrate abundance.

Two-way ANOVA was used to determine effects of temperature and dissolved oxygen (explanatory variables) on survival and bioenergetics parameters (response variables). To meet assumptions of normality, respiration rates were log-transformed, TP excretion/egestion rates were log $(x+1)$ transformed, laboratory growth rates were log $(x+0.5)$ transformed. I used one-way ANOVA to test if field-derived growth rates differed by size.

To analyze within and among organism differences in consumption and hypoxic growth, I used one-way ANOVA with temperature and organism size as explanatory variables. Consumption rates were log-transformed, and hypoxic growth values were log $(x+0.01)$ transformed.

Bonferroni post-hoc tests were used to determine significance within explanatory

variables. All statistics were conducted in SPSS (version 24; IBM, Armonk, New York).

RESULTS

Field abundances (number of individuals m^{-2}) of organisms were significantly different

by location (OWC, Sandusky, and Maumee Bays). *Branchiura sowerbyi* abundance was

significantly greater in OWC (143 ind. m^{-2} ± 116) compared to Sandusky (22.3 ind. m^{-2} ± 14.4)

(one-way ANOVA, $p < 0.001$) and Maumee Bays (7.3 ind. m^{-2} ± 5.5) (one-way ANOVA,

$p < 0.001$), and *B. sowerbyi* abundance was significantly greater in Sandusky compared to

Maumee Bay (one-way ANOVA, $p < 0.001$). Thus, abundance of *B. sowerbyi* was greatest in

OWC.

Hexagenia spp. abundance was significantly lower in OWC (17.8 ind. m^{-2} ± 55.6)

compared to Maumee Bay (140 ind. m^{-2} ± 233) (one-way ANOVA, $p < 0.001$), but abundance in

OWC was not significantly different than that in Sandusky Bay (18.9 ind. m^{-2} ± 31.3) (one-way

ANOVA, $p = 1.0$). Further, abundance of *Hexagenia* was significantly lower in Sandusky Bay

compared to Maumee Bay (one-way ANOVA, $p < 0.001$). Thus, *Hexagenia* was most abundant

in Maumee Bay, and least abundant in OWC and Sandusky Bay.

Abundances of *Chironomus riparius* were significantly greater in OWC

(376 ind. m^{-2} ± 454) compared to Maumee Bay (153 ind. m^{-2} ± 144) (one-way ANOVA,

$p < 0.001$), but *C. riparius* abundance in OWC was not significantly different than that in

Sandusky Bay (322 ind. m^{-2} ± 249) (one-way ANOVA, $p = 1.0$). *C. riparius* abundance in

Sandusky Bay was significantly greater compared to that in Maumee Bay (one-way ANOVA, p

< 0.001), thus *C. riparius* was most abundant in OWC and Sandusky Bay and least abundant in

Maumee Bay.

In OWC, *Branchiura sowerbyi* abundance was significantly greater than that of *Hexagenia* (one-way ANOVA, $p = 0.009$), but lower than that of *Chironomus* (one-way ANOVA, $p < 0.001$), and *Hexagenia* abundance in OWC was significantly lower than that of *Chironomus* (one-way ANOVA, $p < 0.001$) (Figure 4-1a); thus, *Chironomus* were most abundant in OWC.

In Sandusky Bay, *B. sowerbyi* abundance was significantly lower than that of *Chironomus* (one-way ANOVA, $p < 0.001$), but *B. sowerbyi* abundance was not significantly different than that of *Hexagenia* (one-way ANOVA, $p = 1.0$) (Figure 4-1a). *Hexagenia* abundance in Sandusky Bay was significantly lower than that of *Chironomus* (one-way ANOVA, $p < 0.001$) (Figure 4-1a). Thus, *C. riparius* was the most abundant study organism in Sandusky Bay.

Biomass (g DW m-2) of the three study organism differed by location. *Branchiura sowerbyi* biomass was significantly greater than both *Hexagenia* ($p < 0.001$) and *Chironomus riparius* ($p < 0.001$) at OWC (Figure 4-1b). Similarly, the biomass of *B. sowerbyi* was greater than *Hexagenia* and *C. riparius* in both Sandusky and Maumee Bays (p-values < 0.001, Figure 4-1b). However, the biomass of *Hexagenia* and *C. riparius* were not significantly different at the three locations (p-values > 0.05). Thus, *B. sowerbyi* abundance was less than the two native invertebrates, particularly in *C. riparius* (Figure 4-1a), the average biomass of *B. sowerbyi* in the western basin of Lake Erie was significantly greater (Figure 4-1b).
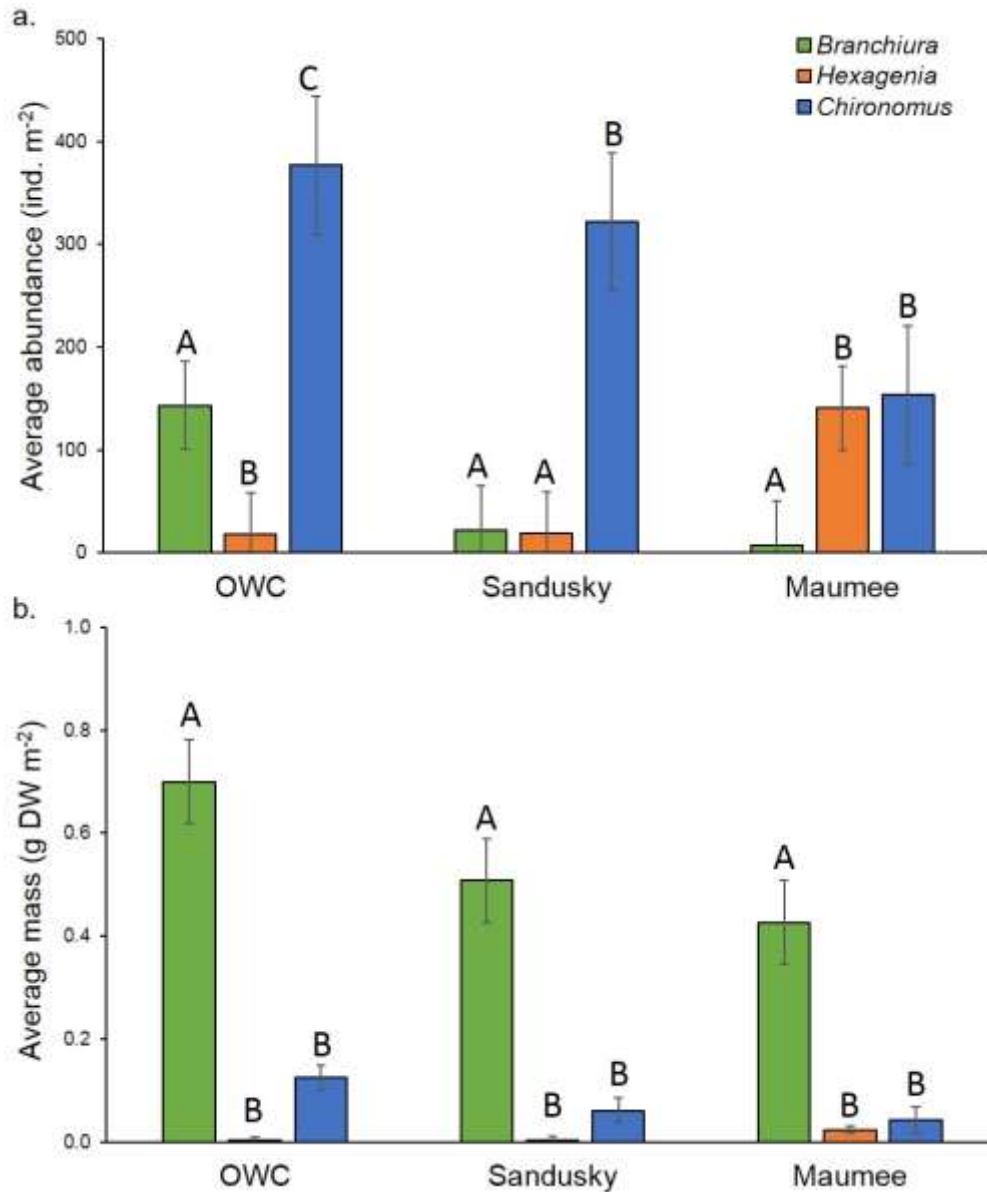
Figure 4-1. (a) Field abundances (no. m^{-2}) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) in Old Woman Creek (OWC), Sandusky and Maumee Bays. Letters indicate significance between organisms at a location, based on one-way ANOVAs. Bars represent standard error. (b) Biomass (g DW m-2) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) in Old Woman Creek (OWC), Sandusky and Maumee Bays. Letters indicate significance between organisms at a location, based on one-way ANOVAs. Bars represent standard error.

B. sowerbyi abundance in Maumee Bay was significantly lower than those of *Hexagenia* (one-way ANOVA, $p < 0.001$) and *C. riparius* (one-way ANOVA, $p < 0.001$) (Figure 4-1), but *Hexagenia* abundance was not significantly different than that of *C. riparius* (one-way ANOVA, $p = 1.0$). Thus, *B. sowerbyi* was less abundant in Maumee Bay compared to the native invertebrates.

I found that field abundances of organisms were not significantly correlated with temperature, based on linear regression (*Branchiura sowerbyi* – $p = 0.956$, $R^2 = 0.00$; *Hexagenia* – $p = 0.369$, $R^2 = 0.003$; *Chironomus riparius* – $p = 0.998$, $R^2 = 0.00$). However, *B. sowerbyi* abundance was significantly influenced by DO concentrations, as measured in the field ($p < 0.001$, $R^2 = 0.240$), and abundance was inversely related to DO (i.e., greater abundance of *B. sowerbyi* at lower DO concentrations). Similarly, linear regression indicated field abundance of *C. riparius* was significantly correlated with DO ($p < 0.001$, $R^2 = 0.059$), with more individuals present at lower DO. *Hexagenia* abundance was not significantly correlated with DO concentrations ($p = 0.110$, $R^2 = 0.010$).

Using multiple regression, I found that overall abundance of organisms was significantly correlated with abiotic variables (temperature, DO, TP, and TN) for *Branchiura sowerbyi* ($p < 0.001$, $R^2 = 0.283$), *Hexagenia* ($p = 0.001$, $R^2 = 0.070$), and *Chironomus riparius* ($p < 0.001$, $R^2 = 0.134$).

Further, *Branchiura sowerbyi* abundance was significantly influenced by the abiotic variables (temperature, DO, TP, and TN) in OWC ($p < 0.001$, $R^2 = 0.237$) and Maumee Bay ($p = 0.017$, $R^2 = 0.134$), but *B. sowerbyi* abundance was not correlated with abiotic variables measured in Sandusky Bay ($p = 0.146$, $R^2 = 0.082$).

Hexagenia spp. abundance was significantly correlated with the abiotic variables (temperature, DO, TP and TN) in Sandusky Bay ($p < 0.001$, $R^2 = 0.284$) and Maumee Bay ($p < 0.001$, $R^2 = 0.475$), but *Hexagenia* abundance was not significantly correlated with temperature, DO, TP, and TN in OWC ($p = 0.072$, $R^2 = 0.102$).

Chironomus riparius abundance was significantly correlated with abiotic variables (temperature, DO, TP and TN) in OWC ($p < 0.001$, $R^2 = 0.262$), Sandusky ($p < 0.001$, $R^2 = 0.297$), and Maumee Bays ($p = 0.010$, $R^2 = 0.147$).

Biomasses of the study organisms were not significantly influenced by temperature (*Branchiura sowerbyi* – p = 0.629; *Hexagenia* – $p = 0.682$; *Chironomus riparius* – $p = 0.723$), based on linear regression. Similar to estimates of organism abundance, *B. sowerbyi* biomass was significantly influenced by bottom dissolved oxygen concentrations ($p = 0.030$, $R^2 = 0.262$), with greater biomass at lower DO concentrations. Similarly, linear regression indicated field biomass of *C. riparius* was significantly correlated with DO ($p = 0.006$, $R^2 = 0.406$), with more individuals present at lower DO concentrations. *Hexagenia* biomass was not significantly correlated with DO concentrations ($p = 0.476$, $R^2 = 0.040$).

Using multiple regression, I found that biomass values of the study organisms were not significantly correlated with abiotic variables (temperature, DO, TP, and TN) for *Branchiura sowerbyi* ($p = 0.262$, $R^2 = 0.314$), *Hexagenia* ($p = 0.539$, $R^2 = 0.248$), and *Chironomus riparius* ($p = 0.131$, $R^2 = 0.423$).

WITHIN ORGANISM

Survival

Survival (%) of *Branchiura sowerbyi* was not significantly influenced by temperature (two-way ANOVA, $p = 0.797$); there were no significant differences in survival between temperature treatments (all p-values = 1.0; 4°C – 100% ± 0; 10°C – 100% ± 0; 20°C – 92% ± 13; 30°C – 92% ± 29).

Additionally, survival of *Branchiura sowerbyi* was not significantly influenced by dissolved oxygen (two-way ANOVA, $p = 0.457$). More specifically, survival of *B. sowerbyi* at high DO (98% ± 6.3) was not significantly different than that in hypoxic treatments (86% ± 33) ($p = 0.705$). Survival in high DO was also not significantly different than that in pulsed DO treatments (96% ± 7.8) ($p = 1.0$), and hypoxic treatment survival did not differ from that in pulsed DO ($p = 1.0$). I did not observe a significant two-way interaction between temperature and dissolved oxygen on *B. sowerbyi* survival (Figure 4-2).

Hexagenia spp. survival was not significantly influenced by temperature (two-way ANOVA, $p = 0.365$). Specifically, survival of *Hexagenia* was not significantly different at 4°C (27% ± 30) compared to 10°C (53% ± 31) ($p = 1.0$), 20°C (66% ± 34) ($p = 0.877$), or 30°C (69% ± 37) ($p = 0.526$). Further, *Hexagenia* survival was not significantly different at 10°C compared to 20°C ($p = 1.0$) or 30°C ($p = 1.0$), and survival at 20°C was not significantly different than that at 30°C ($p = 1.0$).

Figure 4-2. Survival (%) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) in high DO (solid), hypoxic (hashed), and pulsed DO (light colored) treatments at 4°C, 10°C, 20°C, and 30°C. Lower case letters indicate significance between dissolved oxygen treatments for a given organism at a particular experimental temperature, based on two-way ANOVAs. Upper case letters signify significance in survival among organisms by temperature (one-way ANOVAs). Bars represent standard error. NS = no survival.

Hexagenia survival was significantly different in dissolved oxygen treatments (two-way

ANOVA, $p = 0.008$). Survival was significantly greater in high DO (83% ± 26) compared to

hypoxic (38% ± 30) treatments ($p = 0.007$). However, *Hexagenia* survival was not significantly

different between high and pulsed DO (65% ± 37) ($p = 0.388$), and survival was not different

between hypoxic and pulsed treatments ($p = 0.240$). I also did not observe a significant two-way

interaction between temperature and DO on *Hexagenia* survival (Figure 4-2).

Chironomus riparius survival was not significantly influenced by temperature (two-way

ANOVA, $p = 0.133$). Specifically, *C. riparius* survival at 4°C (13% ± 23) was not significantly

different than survival at 20°C (48% ± 29) ($p = 0.231$) and at 30°C (51% ± 30) ($p = 0.179$).

Survival did not differ at 20°C compared to 30°C ($p = 1.0$).

There was not a significant effect of dissolved oxygen on survival of *Chironomus*

riparius (two-way ANOVA, $p = 0.236$). Survival was not significantly different in high DO

treatments (59% ± 29) in comparison to hypoxia (32% ± 30) ($p = 0.738$) or pulsed DO (46% ±

30) treatments ($p = 1.0$), and *C. riparius* survival in hypoxia was not different than in pulsed DO

($p = 0.783$). I did not find a significant two-way interaction between temperature and DO on *C.*

riparius survival (Figure 4-2).

Respiration

Branchiura sowerbyi respiration rates ($\mu g\ O_2\ mgDW^{-1}\ hr^{-1}$) were significantly influenced

by temperature (two-way ANOVA, $p < 0.001$). Specifically, respiration rates at 4°C (0.65 $\mu g\ O_2$

$mgDW^{-1}\ hr^{-1}$ ± 0.56) were significantly lower than rates at 10°C (2.2 $\mu g\ O_2\ mgDW^{-1}\ hr^{-1}$ ± 2.6) (p

< 0.001), and rates at 4°C were significantly higher compared to those at 20°C

(0.33 $\mu g\ O_2\ mgDW^{-1}\ hr^{-1}$ ± 0.33) ($p = 0.018$). Respiration rates of *B. sowerbyi* were not

significantly different at 4°C compared to 30°C (0.77 $\mu g\ O_2\ mgDW^{-1}\ hr^{-1}$ ± 0.65) ($p = 0.249$).

Further, *B. sowerbyi* respiration rates at 10°C were significantly higher than those at 20°C ($p < 0.001$), and 30°C ($p < 0.001$), and rates were significantly lower at 20°C compared to 30°C ($p < 0.001$). Respiration rates of *B. sowerbyi* were greatest at 10°C (Figure 4-3).

Respiration rates of *Branchiura sowerbyi* also were significantly influenced by dissolved oxygen (two-way ANOVA, $p < 0.001$), and were significantly greater in high DO compared to hypoxic treatments ($p < 0.001$). I observed a significant two-way interaction between temperature and dissolved oxygen ($p < 0.001$). Respiration rates were significantly greater in high DO compared to hypoxia at 4°C ($p < 0.001$), 10°C ($p = 0.001$), and 20°C ($p < 0.001$), but respiration rates were not significantly different at 30°C between dissolved oxygen treatments ($p = 0.204$) (Figure 4-3).

Respiration rates of *Hexagenia* spp. also were significantly influenced by temperature (two-way ANOVA, $p = 0.040$) (Figure 4-3). However, I did not observe significant differences among temperatures (4°C-10°C – $p = 1.0$; 4°C-20°C – $p = 0.067$; 4°C-30°C – $p = 1.0$; 10°C-20°C – $p = 0.101$; 10°C-30°C – $p = 1.0$; 20°C-30°C – $p = 0.265$).

Respiration rates of *Hexagenia* were influenced additionally by dissolved oxygen (two-way ANOVA, $p < 0.001$). Rates were significantly greater in high DO compared to hypoxic treatments ($p < 0.001$). I did not observe an overall significant two-way interaction between temperature and dissolved oxygen ($p = 0.549$), however, respiration rates were significantly greater in high DO treatments compared to hypoxia at 10°C ($p = 0.003$) and 20°C ($p = 0.045$) (Figure 4-3). Respiration rates of *Hexagenia* were not significantly different in dissolved oxygen treatments at 4°C ($p = 0.068$) or 30°C ($p = 0.353$) (Figure 4-3).
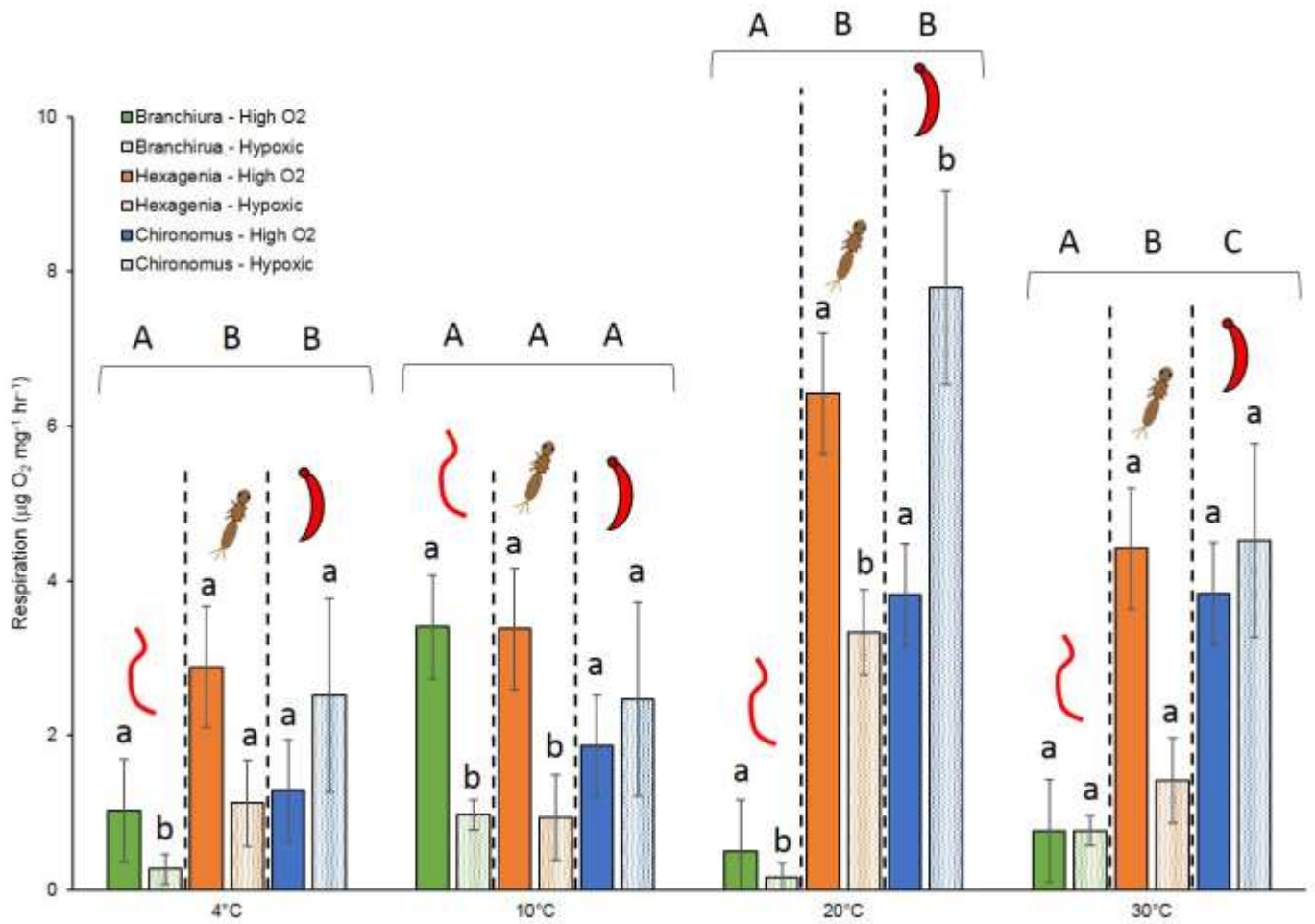
Figure 4-3. Respiration rates (μg O_2 mgDW^{-1} hr^{-1}) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) in high DO (solid) and hypoxic (hashed) treatments at 4°C, 10°C, 20°C, and 30°C. Note that figure depicts non-transformed values; respiration rates were log transformed to meet assumption of normality for statistical analyses. Lower case letters indicate significance between dissolved oxygen treatments for a given organism at an experimental temperature, based on two-way ANOVAs. Upper case letters signify significance in respiration rates among organisms by temperature (one-way ANOVAs). Bars represent standard error.

Respiration rates of *Chironomus riparius* were significantly influenced by temperature (two-way ANOVA, $p < 0.001$). Rates at 4°C (1.9 µg O_2 mgDW^{-1} hr^{-1} ± 1.6) were significantly lower compared to rates at 20°C (5.8 µg O_2 mgDW^{-1} hr^{-1} ± 4.1) ($p < 0.001$) and 30°C (4.2 µg O_2 mgDW^{-1} hr^{-1} ± 2.4) ($p < 0.001$). Additionally, respiration rates of *C. riparius* were significantly lower at 10°C (2.2 µg O_2 mgDW^{-1} hr^{-1} ± 1.5) compared to rates at 20°C ($p = 0.003$) and 30°C ($p = 0.013$). At 4°C, respiration rates were not significantly different than at 10°C ($p = 1.0$), and rates at 20°C were not significantly different than those at 30°C ($p = 1.0$).

Respiration rates of *Chironomus riparius* were significantly influenced by dissolved oxygen (two-way ANOVA, $p < 0.001$). Unlike for *B. sowerbyi* and *Hexagenia*, respiration of *C. riparius* was significantly lower in high DO compared to hypoxic conditions ($p = 0.005$). I did not observe a significant two-way interaction between temperature and dissolved oxygen on respiration rates of *C. riparius* ($p = 0.316$); however, respiration rates were significantly lower in high DO compared to hypoxia at 20°C ($p = 0.007$) (Figure 4-3). *C. riparius'* respiration rates were not significantly different under different dissolved oxygen treatments at 4°C ($p = 0.087$), 10°C ($p = 0.320$), or 30°C ($p = 0.828$) (Figure 4-3).

Total Phosphorus (TP) Excretion/Egestion

TP excretion/egestion release rates of *Branchiura sowerbyi* were significantly influenced by temperature (two-way ANOVA, $p = 0.002$). More specifically, excretion/egestion release rates of TP were significantly lower at 10°C (0.006 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.01) compared to at 20°C (0.07 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.08) ($p = 0.002$). TP excretion/egestion release rates of *B. sowerbyi* were not significantly different at 4°C (0.03 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.06) compared to at 10°C ($p = 1.0$), or at 4°C compared to at 20°C ($p = 0.068$), or at 4°C compared to at 30°C

(0.03 μmol L^{-1} mgDW^{-1} hr^{-1} ± 0.02) (p = 1.0). Further, I did not observe a significant difference in TP excretion/egestion for *B. sowerbyi* at 10°C compared to at 30°C (p = 0.083) or at 20°C compared to at 30°C (p = 1.0).

I did not observe a significant effect of dissolved oxygen on *Branchiura sowerbyi* excretion/egestion release rates of TP (two-way ANOVA, p = 0.849), and nor was there a significant two-way interaction between temperature and dissolved oxygen (p = 0.358). Thus, TP excretion/egestion rates of *B. sowerbyi* were not significantly different in high DO compared to hypoxic treatments at the experimental temperatures in this study (Figure 4-4).
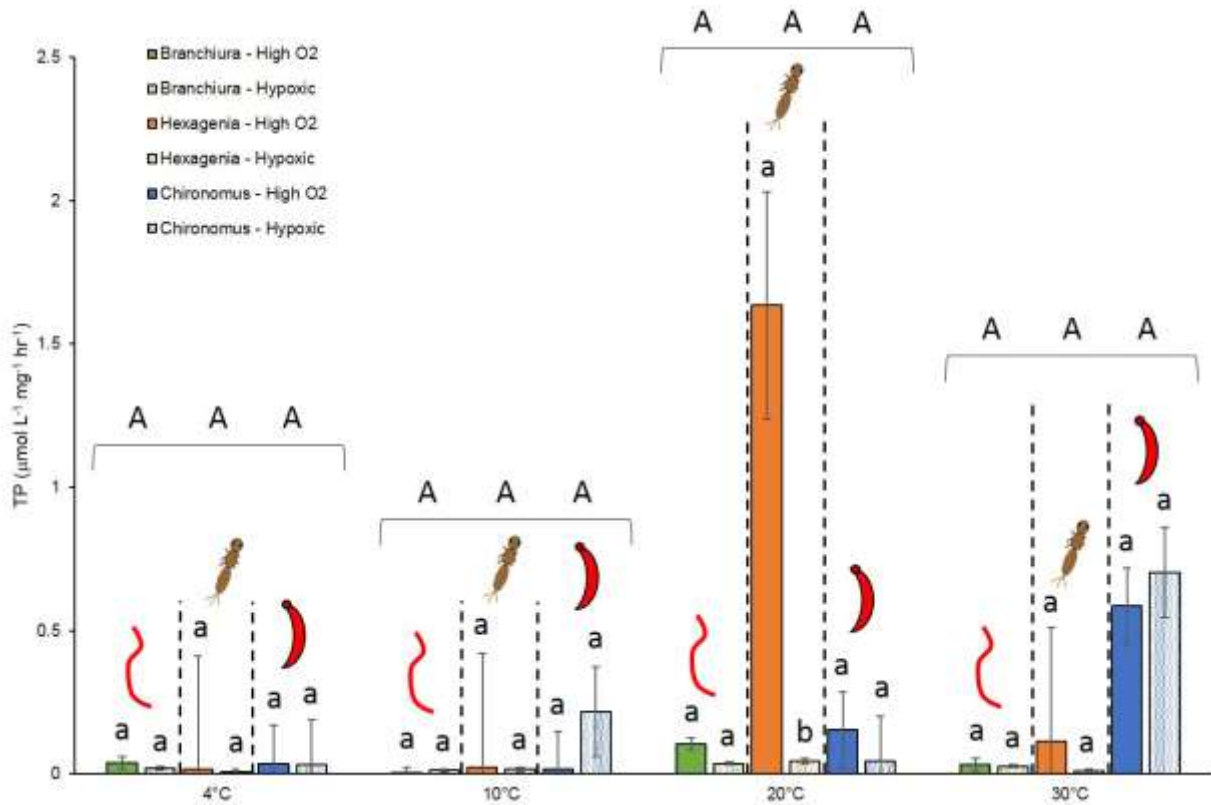
Figure 4-4. Total phosphorus (TP) excretion/egestion release rates (μmol L^{-1} mgDW^{-1} hr^{-1}) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) in high DO (solid) and hypoxic (hashed) treatments at 4°C, 10°C, 20°C, and 30°C. Note figure depicts non-transformed values; TP excretion/egestion rates were log (x+1) transformed to meet assumption of normality for statistical analyses. Lower case letters indicate significance between dissolved oxygen treatments for a given organism at an experimental temperature, based on two-way ANOVAs. Upper case letters signify significance in respiration rates among organisms by temperature (one-way ANOVAs). Bars represent standard error.

Hexagenia spp. excretion/egestion release rates of TP were influenced by temperature (two-way ANOVA, $p = 0.003$). I found that less TP was released at 4°C (0.01 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.02) compared to 20°C (0.84 µmol L^{-1} mgDW^{-1} hr^{-1} ± 2.7) ($p = 0.004$). Additionally, TP excretion/egestion release rates of *Hexagenia* were significantly lower at 10°C (0.02 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.03) compared to at 20°C ($p = 0.008$). I did not observe differences in TP excretion/egestion release rates at 4°C compared to 10°C ($p = 1.0$), at 4°C compared to 30°C (0.06 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.13) ($p = 1.0$), 10°C compared to 30°C ($p = 1.0$), or 20°C compared to 30°C ($p = 0.124$).

Dissolved oxygen significantly influenced TP excretion/egestion release rates of *Hexagenia*, and release was significantly greater in high DO compared to hypoxia ($p = 0.014$). Overall, the two-way interaction of temperature and dissolved oxygen on TP release rates of *Hexagenia* was not significant ($p = 0.448$), however, rates were significantly greater in high DO treatments compared to hypoxia at 20°C ($p = 0.024$) (Figure 4-4). TP excretion/egestion release rates of *Hexagenia* were not significantly different by dissolved oxygen treatment at 4°C ($p = 0.796$), 10°C ($p = 0.457$), or 30°C ($p = 0.080$).

TP release rates of *Chironomus riparius* were significantly influenced by temperature (two-way ANOVA, $p = 0.014$). Specifically, rates were lower at 4°C (0.03 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.07) compared to at the highest experimental temperature, 30°C (0.64 µmol L^{-1} mgDW^{-1} hr^{-1} ± 1.2) ($p = 0.036$). Further, TP release rates from *C. riparius* were significantly lower at 10°C (0.12 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.35) compared to 30°C ($p = 0.049$). I did not observe significant differences in TP release rates for *C. riparius* at 4°C compared to 10°C ($p = 1.0$), at 4°C compared to 20°C (0.10 µmol L^{-1} mgDW^{-1} hr^{-1} ± 0.15) ($p = 0.417$), at

10°C compared to 20°C ($p = 0.538$), or at 20°C in comparison to 30°C ($p = 1.0$). Thus, the highest TP release rates for *C. riparius* were observed at 30°C (Figure 4-4).

Similar to TP release patterns of *Branchiura sowerbyi*, I did not observe a significant influence of dissolved oxygen on TP release rates of *C. riparius* (two-way ANOVA, $p = 0.568$), and the two-way interaction between temperature and dissolved oxygen did not result in different release rates ($p = 0.263$). Thus, TP excretion/egestion release rates of *C. riparius* were not significantly different between dissolved oxygen treatments at 4°C ($p = 0.653$), 10°C ($p = 0.354$), 20°C ($p = 0.326$), or 30°C ($p = 0.130$).

Growth – experiment and field estimates

Branchiura sowerbyi growth rates measured in the laboratory experiment were significantly influenced by temperature (two-way ANOVA, $p = 0.028$), but growth rates were not different among the dissolved oxygen treatments (two-way ANOVA, $p = 0.295$). *B. sowerbyi* growth at 4°C (-0.11 ± 0.16 mgDW day^{-1}) was significantly lower compared to growth at 30°C (0.14 ± 0.17 mgDW day^{-1}, $p = 0.022$), but was not significantly different between other temperatures (4°C-10°C – $p = 0.201$; 4°C-20°C – $p = 1.0$; 10°C-20°C – $p = 1.0$; 10°C-30°C – $p = 1.0$; 20°C-30°C – $p = 0.376$). Growth rates of *B. sowerbyi* were only significantly greater than 0 at 30°C (one sample t-test, $p = 0.029$; Figure 4-5).

Growth rates of *Branchiura sowerbyi* were not different between high DO (0.06 ± 0.19 mgDW day^{-1}) and hypoxia (-0.06 ± 0.14 mgDW day^{-1}, $p = 0.549$), high DO and pulsed DO (-0.02 ± 0.21 mgDW day^{-1}, $p = 0.364$), or hypoxia and pulsed DO treatments ($p = 1.0$). Additionally, there was not a significant two-way interaction between temperature and dissolved oxygen ($p = 0.687$).

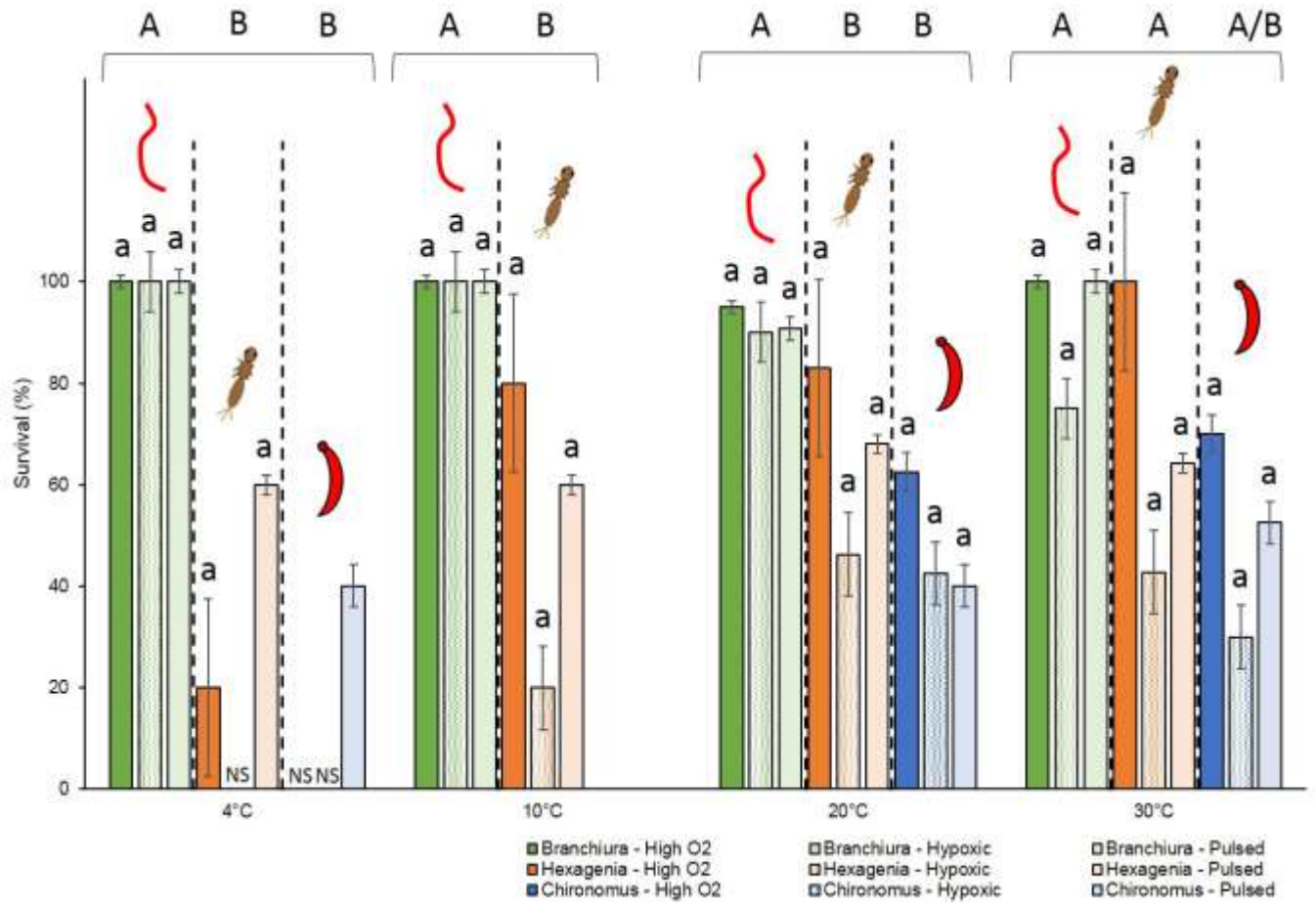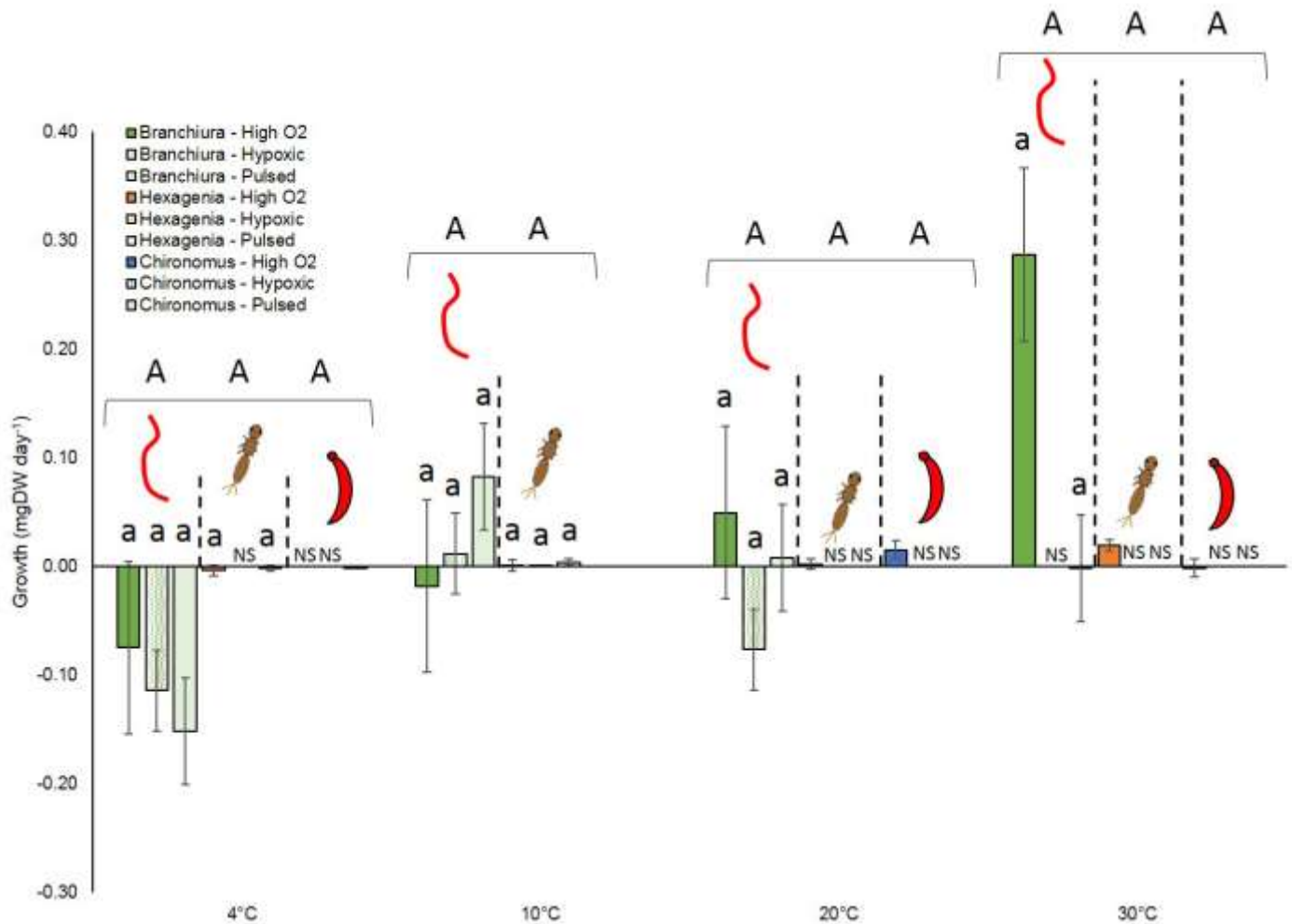Figure 4-5. Laboratory growth rates (mgDW day⁻¹) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) in high DO (solid), hypoxic (hashed), and pulsed DO (light colored) treatments at 4°C, 10°C, 20°C, and 30°C. Note that figure depicts non-transformed values; growth rates were log (*x*+0.5) transformed to meet assumption of normality for statistical analyses. Lower case letters indicate significance between dissolved oxygen treatments for a given organism at an experiment temperature, based on two-way ANOVAs. Upper case letters signify significance in growth rates among organisms by temperature (one-way ANOVAs). Bars represent standard error. NS = no survivors in growth experiment at end of 14 (*Branchiura sowerbyi* and *Chironomus riparius*) or 12 days (*Hexagenia* spp.).

Growth rates of *Branchiura sowerbyi* estimated from field samples were not significantly influenced by temperature (one-way ANOVA, $p = 0.666$) and *B. sowerbyi* growth rates were not significantly different by organism size (one-way ANOVA, $p = 0.938$.
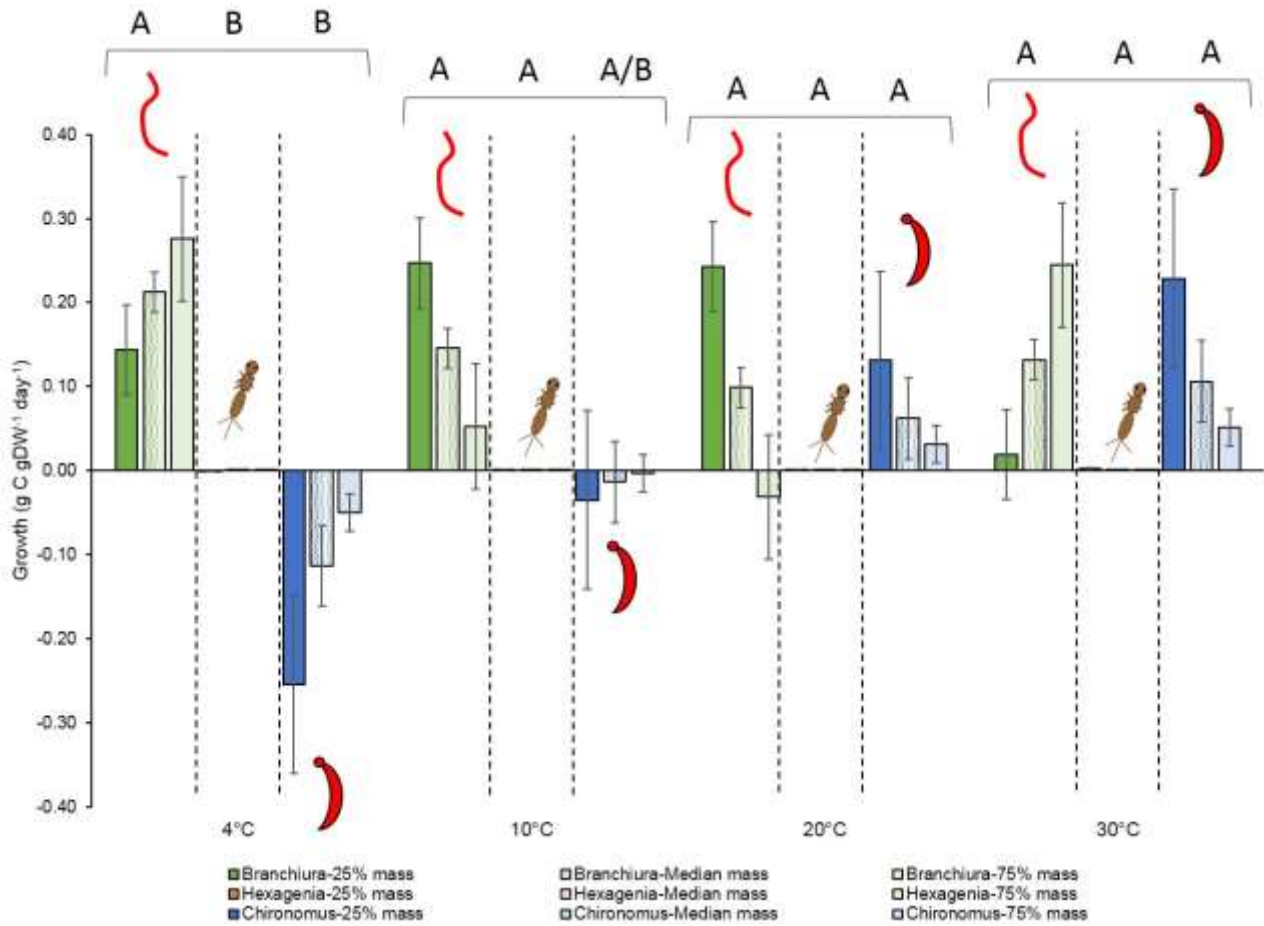
Figure 4-6. Growth rates (g C gDW^{-1} day^{-1}) derived from field samples for *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) for small (25% quartile mass, solid bars), intermediate (median mass, hashed bars), and large (75% quartile mass, light colored) individuals. Note that figure depicts non-transformed values; growth rates were log (x+0.01) transformed to meet assumption of normality for statistical analyses. Letters indicate significance between organisms at 4°C, 10°C, 20°C, and 30°C, based on one-way ANOVAs. Bars represent standard error.

Growth rates of *Hexagenia* measured in the laboratory were not significantly influenced by temperature (two-way ANOVA, $p = 0.665$; all temperature combination p-values = 1.0), or dissolved oxygen (two-way ANOVA, $p = 0.984$; all DO combination p-values = 1.0). I did not observe a significant two-way interaction between temperature and dissolved oxygen on *Hexagenia* growth rates ($p = 0.971$).

Growth rates of *Hexagenia* estimated from field samples also were not significantly influenced by temperature (one-way ANOVA, $p = 0.346$), and additionally were not different among *Hexagenia* of different sizes (one-way ANOVA, $p = 0.211$).

Growth rates of *Chironomus riparius* measured in the laboratory experiment were not significantly different in different temperatures (two-way ANOVA, $p = 0.065$), although growth rates tended to be greater at 20°C (Figure 4-5). Due to low survival of *C. riparius* in the different dissolved oxygen treatments (particularly in hypoxic and pulsed treatments at higher temperatures), the influence of DO on growth of *C. riparius* was not able to be analyzed. Of note, I observed no growth (no individuals surviving) in hypoxic treatments for *C. riparius* at any of the experimental temperatures (Figure 4-5).

Conversely to the laboratory experiment, field-derived growth rates of *Chironomus riparius* were significantly different by temperature (one-way ANOVA, $p = 0.010$). *C. riparius* growth rates were significantly lower at 4°C compared to 20°C ($p = 0.047$) and 30°C ($p = 0.013$). Growth rates were not significantly different between other temperature treatments (p-values > 0.05). Thus, growth rates of *C. riparius* were lowest at 4°C and significantly greater at higher temperatures. *C. riparius* growth rates did not vary by organism size (one-way ANOVA, $p = 0.994$).

Modeled Consumption and Growth in Hypoxia

Branchiura sowerbyi consumption rates were not influenced by temperature (one-way ANOVA, $p = 0.682$), and were not significantly different based on organism size (one-way ANOVA, $p = 0.943$) (Figure 4-7).

Modeled growth rates of *Branchiura sowerbyi* under hypoxia were not influenced by temperature (one-way ANOVA, $p = 0.076$), and were also not significantly different by size (one-way ANOVA, $p = 0.997$) (Figure 4-8).

Hexagenia consumption rates similarly were not different among temperatures ($p = 0.729$), but were significantly different between sizes of organisms ($p = 0.008$). Consumption rates for the small individual (0.44 ± 0.25 g C gDW^{-1} day^{-1}) was higher than for the intermediate (0.10 ± 0.03 g C gDW^{-1} day^{-1}, $p = 0.027$) and large individual (0.05 ± 0.005 g C gDW^{-1} day^{-1}, $p = 0.012$). In addition, the consumption rate of the intermediate-sized *Hexagenia* was not significantly higher than that of the large individual ($p = 1.0$). Therefore, smaller *Hexagenia* had the highest consumption rates (Figure 4-7).
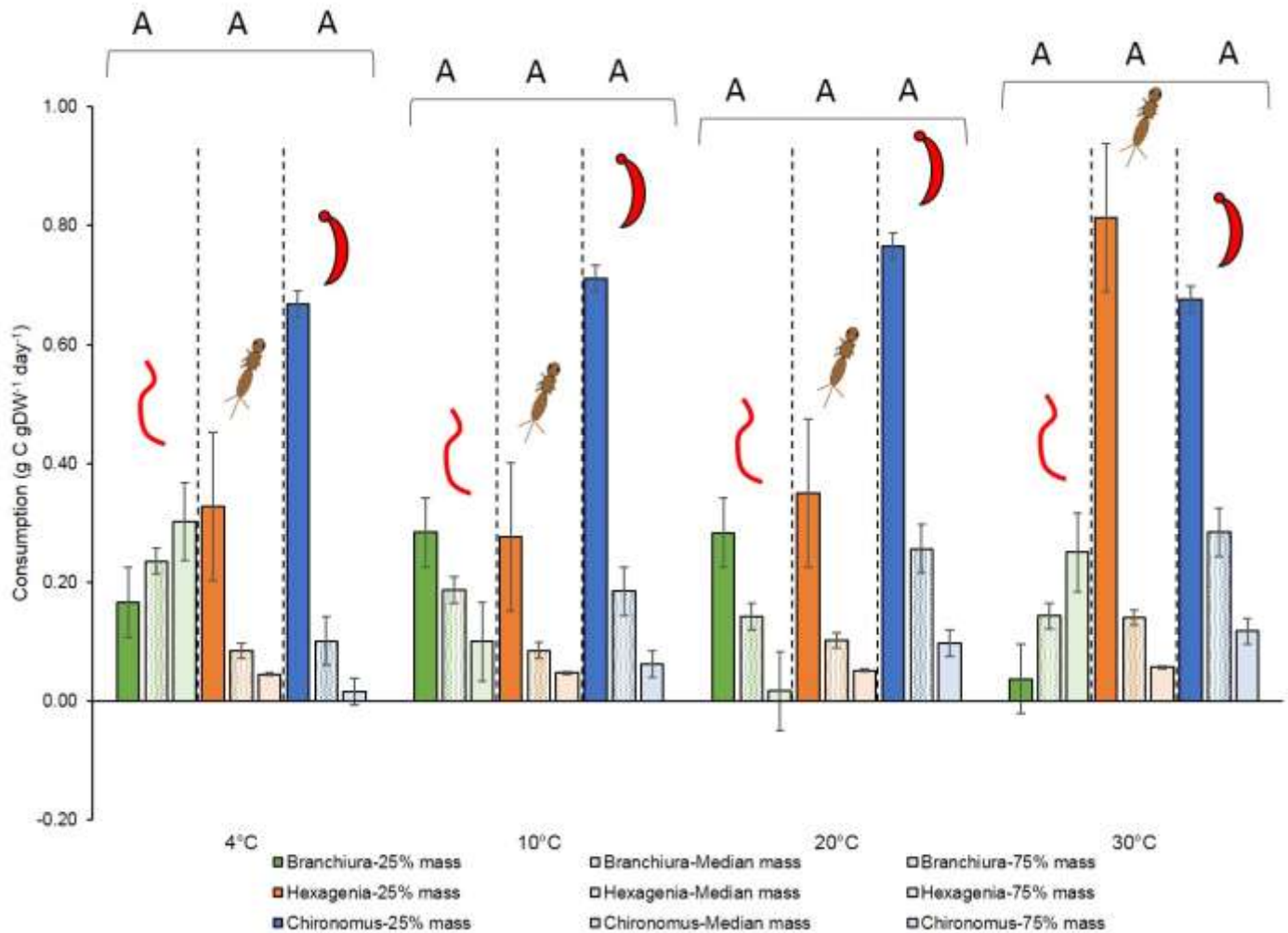
Figure 4-7. Modeled consumption rates (g C gDW^{-1} day^{-1}) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) for small (25% quartile mass, solid bars), intermediate (median mass, hashed bars), and large (75% quartile mass, light colored) individuals. Note that figure depicts non-transformed values; consumption rates were log-transformed to meet assumption of normality for statistical analyses. Letters indicate significance between organisms at 4°C, 10°C, 20°C, and 30°C, based on one-way ANOVAs. Bars represent standard error.

Growth rates under hypoxia for *Hexagenia* were not different among temperature treatments (one-way ANOVA, $p = 0.623$), but were significantly different by size (one-way ANOVA, $p = 0.043$). Rates were not significantly higher for the small individuals (0.21 ± 0.19 g C gDW^{-1} day^{-1}) compared to the intermediate (0.02 ± 0.01 g C gDW^{-1} day^{-1}, $p = 0.106$) or the large sized individual (-0.001 ± 0.002 g C gDW^{-1} day^{-1}, $p = 0.068$). Growth rates of *Hexagenia* were also not significantly different between the intermediate and large individuals ($p = 1.0$) (Figure 4-8).

Consumption rates of *Chironomus riparius* were not influenced by temperature (one-way ANOVA, $p = 0.976$), but were influenced by size (one-way ANOVA, $p < 0.001$). Like those of *Hexagenia*, consumption rates of *C. riparius* were significantly higher for the small (0.71 ± 0.04 g C gDW^{-1} day^{-1}) compared to intermediate (0.21 ± 0.08 g C gDW^{-1} day^{-1}; $p < 0.001$) and large (0.07 ± 0.04 g C gDW^{-1} day^{-1}, $p < 0.001$) individuals. Consumption rates of the intermediate *C. riparius* were significantly greater than the large sized *C. riparius* ($p = 0.034$). Consumption rates of *C. riparius* were, therefore, were highest for smaller individuals (Figure 4-7).

Modeled growth rates for *C. riparius* under hypoxic conditions were not influenced by temperature (one-way ANOVA, $p = 0.985$), but were significantly different by size class (one-way ANOVA, $p < 0.001$). Growth rates of small *C. riparius* (0.62 ± 0.07 g C gDW^{-1} day^{-1}) were significantly higher than for intermediate (0.14 ± 0.10 g C gDW^{-1} day^{-1}, $p < 0.001$) and larger (0.02 ± 0.05 g C gDW^{-1} day^{-1}, $p < 0.001$) individuals. However, growth rates between intermediate and larger sized *C. riparius* were not different ($p = 0.142$). Thus, smaller *C. riparius* had higher growth rates under hypoxia (Figure 4-8), in contrast to no size-dependency in growth rates when DO was high (field derived growth rates).
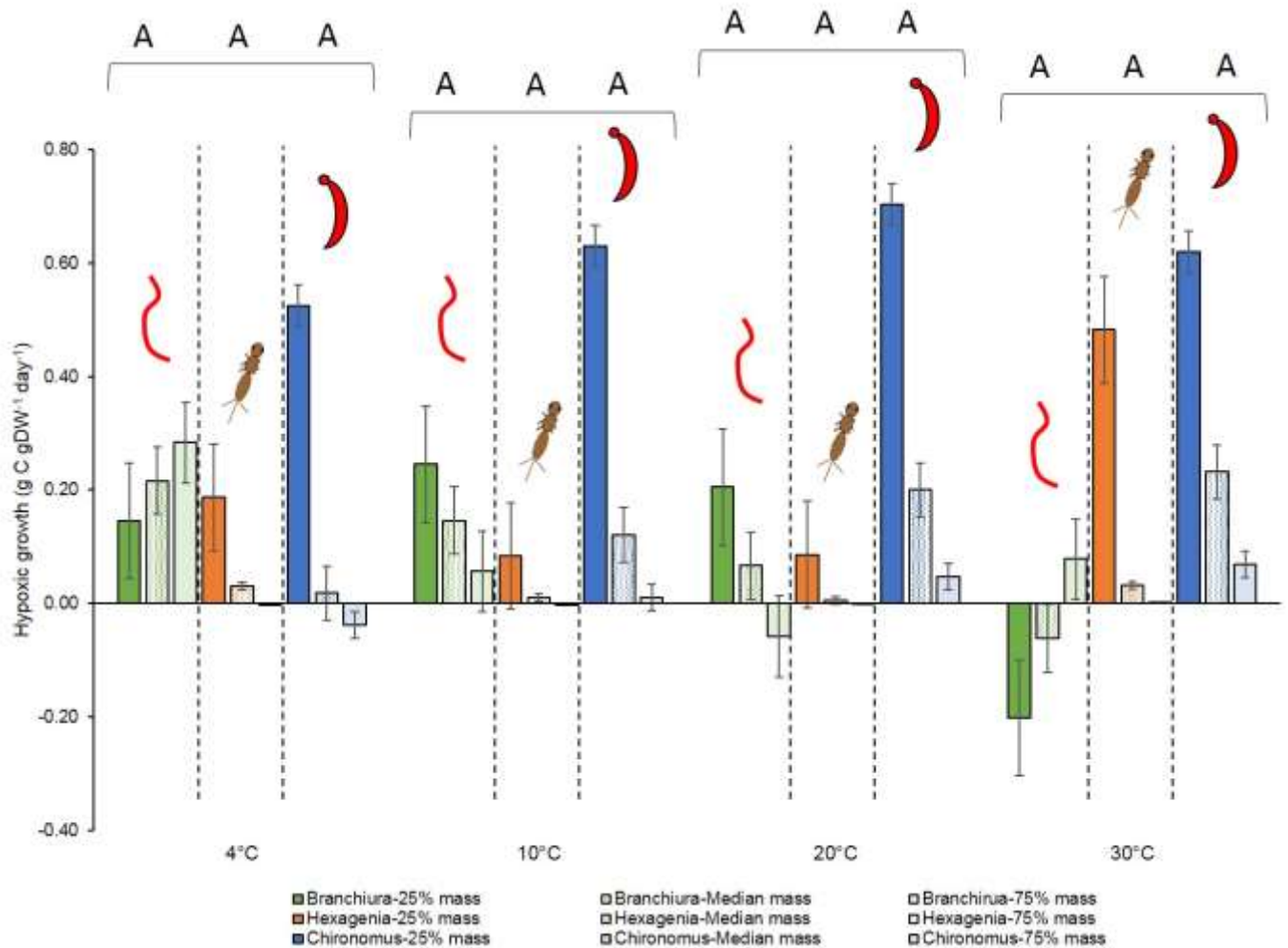
Figure 4-8. Modeled growth rates under hypoxic conditions (g C gDW^{-1} day^{-1}) of *Branchiura sowerbyi* (green bars), *Hexagenia* spp. (orange bars), and *Chironomus riparius* (blue bars) small (25% quartile mass, solid bars), intermediate (median mass, hashed bars), and large (75% quartile mass, light colored) individuals. Note that figure depicts non-transformed values; statistics were analyzed with growth rates log (x+0.1) transformed to meet assumption of normality. Letters indicate significance between organisms at 4°C, 10°C, 20°C, and 30°C, based on one-way ANOVAs. Bars represent standard error.

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Survival

Survival of *Branchiura sowerbyi* was significantly greater compared to that of *Hexagenia* (one-way ANOVA, $p < 0.001$) and Chironomus (one-way ANOVA, $p < 0.001$), but survival was not significantly different between *Hexagenia* and *Chironomus* (one-way ANOVA, $p = 0.100$).

I found that survival significantly differed by organism (two-way ANOVA) at 4°C ($p < 0.001$), 10°C ($p = 0.035$), 20°C ($p = 0.001$), and 30°C ($p = 0.001$). At 4°C, *Branchiura sowerbyi* survival was significantly greater than those of *Hexagenia* ($p = 0.006$) and *C. riparius* ($p = 0.001$), but *Hexagenia* survival was not significantly different than that of *Chironomus* ($p = 1.0$) (Figure 4-2). *B. sowerbyi* survival was significantly greater than that of *Hexagenia* at 10°C ($p = 0.035$) (Figure 4-2). At 20°C, *B. sowerbyi* survival was greater than those of *Hexagenia* ($p = 0.040$) and *C. riparius* ($p = 0.001$), but survival of *Hexagenia* was not significantly different compared to that of *C. riparius* ($p = 0.564$) (Figure 4-2). At 30°C, *B. sowerbyi* survival was not different than that of *Hexagenia* ($p = 0.088$), but *B. sowerbyi* survival was significantly greater than that of *Chironomus* ($p = 0.001$); *Hexagenia* survival was not significantly greater than that of *C. riparius* at 30°C ($p = 0.277$) (Figure 4-2).

Survival of the study organisms was different in high DO ($p = 0.002$), hypoxic ($p < 0.001$), and pulsed ($p < 0.001$) dissolved oxygen treatments, based on results from a two-way ANOVA. *Branchiura sowerbyi* survival was not significantly different than that of *Hexagenia* ($p = 0.477$), but *B. sowerbyi* survival was significantly greater than *Chironomus riparius* ($p = 0.001$) in high DO treatments; survival was not significantly different between *Hexagenia* and *C. riparius* in high DO ($p = 0.078$). In hypoxia, *B. sowerbyi* survival was greater than those of *Hexagenia* ($p < 0.001$) and *C. riparius* ($p < 0.001$), but survival of *Hexagenia* and *C. riparius*

did not differ ($p = 1.0$). Similarly, survival of *B. sowerbyi* was significantly greater than those of *Hexagenia* ($p = 0.012$) and *C. riparius* ($p < 0.001$) in pulsed DO, but survival of *Hexagenia* and *C. riparius* in pulsed treatments were not significantly different ($p = 0.478$).

Respiration

Respiration rates of *Branchiura sowerbyi* were lower than those of *Hexagenia* ($p < 0.001$) and *Chironomus riparius* ($p < 0.001$) (one-way ANOVA, Figure 4-3). Specifically, respiration rates were significantly different among organisms at 4°C ($p < 0.001$), 20°C ($p < 0.001$), and 30°C ($p < 0.001$), but were not significantly different at 10°C ($p = 0.530$) (two-way ANOVA, Figure 4-3). At 4°C, *B. sowerbyi* respiration rates were significantly lower than those of *Hexagenia* ($p = 0.001$) and *C. riparius* ($p = 0.001$), but *Hexagenia* and *C. riparius* respiration rates did not differ ($p = 1.0$) (Figure 4-3). At 20°C, *B. sowerbyi* respiration rates were also significantly lower than those of *Hexagenia* ($p < 0.001$) and *C. riparius* ($p < 0.001$), but respiration rates were not different between *Hexagenia* and *C. riparius* ($p = 0.700$) (Figure 4-3). Further, respiration rates of *B. sowerbyi* at 30°C were significantly lower compared to those of *Hexagenia* ($p < 0.001$) and *C. riparius* ($p < 0.001$), and respiration rates of *Hexagenia* were significantly lower than that of *C. riparius* ($p = 0.002$) (Figure 4-3).

In high DO conditions, *Branchiura sowerbyi* respiration rates were significantly lower than those of *Hexagenia* ($p < 0.001$) and *Chironomus riparius* ($p < 0.001$); respiration rates did not differ between *Hexagenia* and *C. riparius* ($p = 0.743$) when DO was at saturation. Similarly, respiration rates of *B. sowerbyi* were significantly lower than those of *Hexagenia* ($p < 0.001$) and *C. riparius* ($p < 0.001$) in hypoxia. However, unlike under high DO, respiration rates of *Hexagenia* in hypoxia were significantly lower compared to those of *C. riparius* ($p < 0.001$).

Total Phosphorus Excretion/Egestion

I did not observe differences in mass-specific excretion/egestion release rates among organisms (one-way ANOVA, $p = 0.204$) (Figure 4-4). *Branchiura sowerbyi* TP release rates were not significantly different than those of *Hexagenia* ($p = 1.0$) or *Chironomus riparius* ($p = 0.244$), and *Hexagenia* release rates did not differ from those of *C. riparius* ($p = 0.703$).

Additionally, I found that overall TP release rates differed by organisms at 30°C (two-way ANOVA, $p = 0.042$), but were not significantly different at 4°C ($p = 0.896$), 10°C ($p = 0.571$), or 20°C ($p = 0.606$). However, at 30°C, *Branchiura sowerbyi* release rates were not significantly different than those of *Hexagenia* ($p = 1.0$) or *Chironomus riparius* ($p = 0.098$), and *Hexagenia* release rates did not differ from that of *C. riparius* ($p = 0.074$). While not significantly different, *C. riparius* TP release rates at 30°C were, on average, higher than *B. sowerbyi* and *Hexagenia* (Figure 4-4).

Growth – experiment and field estimates

Laboratory growth rates were not significantly different among species (one-way ANOVA, $p = 0.621$, all p-values between organisms = 1.0). Additionally, I did not observe a significant difference in growth rates among the organisms at 4°C ($p = 0.175$), 10°C ($p = 0.948$), 20°C ($p = 0.810$), or 30°C ($p = 0.542$) (Figure 4-5). Growth rates were also not significantly different among organisms in high DO ($p = 0.959$), hypoxic ($p = 0.695$), or pulsed DO ($p = 0.587$).

Growth rates measured from field samples were significantly different among taxa (one-way ANOVA, $p < 0.001$). Specifically, *Branchiura sowerbyi* growth rates were significantly greater than those of *Hexagenia* ($p = 0.001$) and *Chironomus riparius* ($p = 0.003$). Growth rates of *Hexagenia* were not different than those of *C. riparius* ($p = 1.0$), although *C. riparius* growth

rates were generally greater than those of *Hexagenia*. Thus, growth rates measured from field samples (assumed to be under high DO conditions) were highest for *B. sowerbyi* and lowest for the two native invertebrates, *Hexagenia* and *C. riparius* (Figure 4-6).

Further, growth rates of *Branchiura sowerbyi* were significantly greater than those of *Hexagenia* ($p = 0.034$) and *Chironomus riparius* at 4°C ($p = 0.003$), but growth rates of *Hexagenia* and *C. riparius* did not differ ($p = 0.164$). *B. sowerbyi* growth was greater than *C. riparius* at 10°C ($p = 0.035$), but not *Hexagenia* ($p = 0.056$) (Figure 4-6). At 20°C, growth rates among the organisms were not statistically different (p-values > 0.05) (Figure 4-6). Similarly, growth rates among the organisms were not different at 30°C (p-values > 0.05) (Figure 4-6).

Growth rates among organisms varied between intermediate sized organisms. Specifically, growth rates of the intermediate sized *Branchiura sowerbyi* were significantly greater than *Hexagenia* ($p = 0.025$) and *Chironomus riparius* ($p = 0.037$). Growth rates of the intermediate sized *Hexagenia* and *C. riparius* were not statistically different ($p = 1.0$). Growth rates of the small and large individuals among organisms were not different (p-values > 0.05).

Modeled Consumption and Growth in Hypoxia

Modeled consumption rates were not significantly different among species (one-way ANOVA, $p = 0.205$). However, in general, *Branchiura sowerbyi* rates were lower than those of *Hexagenia* and *Chironomus riparius*, and *Hexagenia* consumption rates were lower than that of *C. riparius*. Thus, consumption rates of *B. sowerbyi* were greater than those of the two native invertebrates, although these differences were not statistically significant. Based on two-way ANOVA results, at 4°C, consumption rates did not differ among the three study organisms ($p = 0.832$) (Figure 4-7). Additionally, consumption rates did not vary among organisms at 10°C ($p = 0.600$), 20°C ($p = 0.475$), and 30°C ($p = 0.642$) (Figure 4-7).

I observed that consumption rates differed among organisms at small ($p = 0.005$), but not intermediate ($p = 0.069$), and large ($p = 0.148$) organism sizes (one-way ANOVA). The consumption rate of the small *Branchiura sowerbyi* did not differ from that of small *Hexagenia* ($p = 0.170$), but were significantly lower than that of the small *Chironomus riparius* ($p = 0.004$).

Growth rates under hypoxia were not different among species (one-way ANOVA, $p = 0.053$). However, growth rates of *Branchiura sowerbyi* (0.09 ± 0.15 g C gDW^{-1} day^{-1}) were generally greater than those of *Hexagenia* (0.08 ± 0.14 g C gDW^{-1} day^{-1}), but *B. sowerbyi* growth rates under hypoxia were lower than *Chironomus riparius* (0.26 ± 0.28 g C gDW^{-1} day^{-1}). Additionally, growth rates in hypoxia were not significantly different by organism at 4°C ($p = 0.704$), 10°C ($p = 0.450$), 20°C ($p = 0.223$), or 30°C ($p = 0.121$) (Figure 4-8).

Modeled growth rates under hypoxia differed among organisms at small ($p = 0.004$), but not intermediate ($p = 0.197$), or large ($p = 0.343$) sizes (one-way ANOVA). The growth rate of the small *Branchiura sowerbyi* did not differ from those of *Hexagenia* ($p = 1.0$) but was significantly less than growth rates of the small *Chironomus riparius* ($p = 0.005$). The growth rate of the small *Hexagenia* was significantly lower than that of *C. riparius* ($p = 0.020$). Thus, small *C. riparius* had significantly greater growth rates under hypoxia compared to the other two study organisms. At the intermediate and large organism sizes, growth rates under hypoxia among the three study organisms were not significantly different (all p-values > 0.05).

DISCUSSION

Measured field abundances, biomass, survival, and bioenergetics parameters differed among the nonnative *Branchiura sowerbyi* compared to native *Hexagenia* spp. and *Chironomus riparius* along temperature and DO concentration gradients, with implications for climate change in nearshore habitats in the western basin of Lake Erie.

Field Abundances and Biomass

Field abundances of *Branchiura sowerbyi* and *Chironomus riparius* were greatest in OWC, while *Hexagenia* was most abundant in Maumee Bay (Figure 4-1a). These abundance patterns in nearshore western Lake Erie may be related, in part, to the dominant sediment type in each location. For example, sediments in nearshore coastal wetlands (e.g., OWC) are generally higher in organic matter (OM) and detritus, with finer sediment (silt) compared to open water areas (e.g., Sandusky and Maumee Bays) in Lake Erie (Williams et al. 1976). Carroll and Dorris (1972) showed that *B. sowerbyi* had greater production in areas that had higher OM in sediment. I observed that *B. sowerbyi* were larger in OWC (5.3 mg) compared to Sandusky (4.0 mg) and Maumee Bays (3.3 mg), highlighting that the organic-rich sediment or other conditions in OWC may explain the spatial pattern in abundance of *B. sowerbyi* compared to the two native invertebrates across the study locations, and in nearshore habitats of the Great Lakes generally. However, the biomass of *B. sowerbyi* was significantly greater than the two native invertebrates at all sample locations.

Because OWC likely has increased OM, decreased DO at the boundary of the sediment-water interface and within upper layers of sediment due to microbial respiration and greater biological oxygen demand (BOD) may be expected, compared to Sandusky and Maumee Bays. *Hexagenia* is intolerant of low DO concentrations (Britt 1955; Carr and Hiltunen 1965; Winter et al. 1996), and decreased DO due to increased OM content of the sediment may contribute to their low abundance in OWC. For example, my field data from 2013 (May-October) indicated that the average DO (% saturation) in OWC (62%) was lower than those in Sandusky (96%) and Maumee Bays (89%), again suggesting that *Hexagenia* could be limited in OWC by DO concentrations. I measured DO concentrations during daylight (approximately mid-morning to

late afternoon) when photosynthesis of primary producers was occurring; thus my field data likely represent the high end of DO concentrations, compared to values that would be experienced at night.

I did not observe a significant correlation between water temperature and abundance of the three study organisms. However, field abundances and biomass values of *Branchiura sowerbyi* and *Chironomus riparius* were correlated with DO concentrations. Interestingly, increased abundance and biomass of *B. sowerbyi* and *C. riparius* were observed at lower DO, suggesting a tolerance to reduced DO concentrations and/or these species occupy habitats with DO conditions that prevent spatial overlap with other species that are intolerant to low DO, such as *Hexagenia* spp.

Survival

Survival was not significantly influenced by temperature for the three study organisms. However, *Hexagenia* in particular, showed a non-significant increasing trend in survival from low (4°C) to high (30°C) temperatures in high DO treatments. Winter et al. (1996) reported a similar low survival of *Hexagenia* (approximately 20%) near 4°C, and increasing survival (approximately 60-80%) above 8°C, similar to my results (Figure 4-2). Interestingly, *Hexagenia* was the only organism whose survival was influenced by DO across temperatures, with survival in pulsed DO greater than hypoxic treatments. Under increased periods of hypoxia predicted with climate change, populations of *Hexagenia* may be negatively impacted. Britt (1955) reported that populations of *Hexagenia* in western Lake Erie experienced mortality (up to 465 dead nymphs in a single sediment sample) during a stratification event that lasted only a few days in length. My results indicate that an increase in pulsed DO in the benthos could also hinder survival of *Hexagenia*.

The effect of DO on survival of *Branchiura sowerbyi* and *Chironomus riparius* were not significant, perhaps due to behavioral and/or physiological adaptations. Additionally, *B. sowerbyi* and *C. riparius* abundance in OWC (Figure 4-1), with the lowest minimum DO (5.6%) and lowest average DO (62.5%) in 2013 sampling, may further suggest adaptations are sufficient to overcome reduced DO availability. Ultimately, survival of the study organisms in different temperature and DO treatments provides insight into the potential population dynamics that may occur in nearshore habitats of the Great Lakes with climate change

Bioenergetics

Decreased respiration rates of *B. sowerbyi* suggest a comparatively lower demand for oxygen. Results of the respiration experiment suggest that *B. sowerbyi* can tolerate limited DO, perhaps due to a lower O_2 metabolic demand leading to an ability to inhabit reduced DO conditions. The respiration rates of the three study organisms align with my measures of field abundances, which showed *B. sowerbyi* at greater abundance in OWC with the lowest DO concentrations.

Respiration rates of *Chironomus riparius* were highest of the three study organisms, and interestingly, were greater under hypoxic compared to high DO treatments (Figure 4-3). *C. riparius* has been previously characterized as euryoxic, tolerant of a wide range of DO concentrations (Penttinen and Holopainen 1995). Koln (1989) reported that respiration of *C. riparius* was greater in reduced DO treatments compared to high oxygen concentrations at two temperatures (10°C and 20°C). This pattern was attributed to increased undulation activity as a behavioral adaptation to offset declining DO. I found a similar pattern of increased respiration rates of *C. riparius* under hypoxic conditions at each experimental temperature (significantly different respiration rates by DO at 20°C, Figure 4-3). Additionally, Stief et al. (2005) suggested

that *C. riparius* behaviorally decreased its feeding rate and allocated more energy to burrow ventilation under hypoxic conditions. *C. riparius* can also shift from burrowing to creating chimney structures when DO is limited, which was shown to produce similar concentrations of available DO to that of the overlying water column (Stief et al. 2005). Burrows, in contrast, had 50% lower DO compared to overlying water concentrations. Chimney structures were thought to increase available DO by breaking through the 400 to 600 μm thick diffusive boundary layer above the sediment surface (Stief et al. 2005). Thus, although *C. riparius* had the greatest measured respiration rates, suggesting a high demand for O_2 to carry out metabolic processes, its complex behavioral and physiological (e.g., presence of hemoglobin in the hemolymph) adaptations likely allow *C. riparius* to occupy areas of reduced DO. These adaptations may explain the field abundances of *C. riparius*, which showed an increase in the number of individuals present at lower DO concentrations.

Given the results of this survival experiment, which indicated that *Branchiura sowerbyi* survival was greater than those of *Hexagenia* spp. and *Chironomus riparius* at increased water temperatures and low DO concentrations, I suggest that *B. sowerbyi* populations may persist, but *Hexagenia* and *C. riparius* populations could decline with predicted climate change. This potential relative increase in *B. sowerbyi* benthic assemblage composition (due to declines in *Hexagenia* and *C. riparius*), coupled with lower overall respiration rates compared to those of the native invertebrates, may ultimately result in less oxygen demand from the benthos and more O_2 available for other benthic processes.

Mass-specific excretion/egestion rates of TP generally increased with temperature for all organisms (Figure 4-4). A similar pattern of increased rates of phosphorus release at higher temperatures was reported by Devine and Vanni (2002) for tubificid oligochaetes, chironomids,

and *Chaoborus* (a midge larva in the Order Diptera). However, similar to Devine and Vanni (2002), I did not observe a difference in TP excretion/egestion rates among the study organisms. Devine and Vanni (2002) concluded that the benthic invertebrates studied are functionally redundant in terms of nutrient release. Therefore, the influence of excretion/egestion rates on nutrient dynamics likely is linked directly to the relative abundance of the benthic invertebrate community, rather than by which species are present (or dominant). Locations that facilitate greater overall abundance of burrowing invertebrates, such as those studied here and by Devine and Vanni (2002), may be more prone to a greater influence of nutrient excretion/egestion as a source of system productivity.

Based on results of the survival experiment, if *B. sowerbyi* populations remain constant while native invertebrate numbers decline under climate change, the net result may be a decrease in overall nutrient excretion/egestion of TP from the benthic invertebrate community in nearshore Great Lake environment. Future research focusing on population modeling of these three study organisms would provide additional insight into the effects of climate change on population dynamics and could be linked directly to specific TP release rates from excretion/egestion to evaluate the influence on available nutrients and feedback to system productivity.

Growth rates of *Branchiura sowerbyi* in the laboratory experiment increased with temperature. Interestingly, growth rates of *Hexagenia* and *Chironomus riparius* were not correlated with temperature. However, growth rates of *Hexagenia* and *C. riparius* were greater at higher temperatures (20°C and 30°C, Figure 4-5), suggesting a positive effect of temperature on growth rates (although not significant in my data set). Indeed, Péry and Garric (2006) showed that growth of *C. riparius* increased linearly with temperature. I additionally did not observe a

difference in laboratory growth rates among organisms by DO, but overall growth rates were generally lower in reduced DO treatments (hypoxic and pulsed) compared to high DO, especially for *B. sowerbyi* (Figure 4-5). Winter et al. (1996) demonstrated that *Hexagenia* growth increased linearly with increasing DO with growth negligible around 1 mg L^{-1} (hypoxia). The effect of DO on growth rates of *Hexagenia* and *Chironomus riparius* was influenced by limited survival, which ultimately reflects long-term population viability of *Hexagenia* and *C. riparius* in nearshore habitats with climate change.

I found that field-derived growth rates of *Chironomus riparius* (assumed to be in high DO based on distribution of DO data from each study location), were significantly influenced by temperature, and that field-derived growth rates did not vary by size for the three study organisms. However, growth rates differed among organisms, particularly at lower temperatures (4°C and 10°C, Figure 4-6). These results suggest that low temperatures may favor *B. sowerbyi* growth, relative to the two native invertebrates. Additionally, growth of *B. sowerbyi* was relatively consistent between temperatures, compared to the two native invertebrates (Figure 4-6), thus increasing water temperature regimes may result in greater growth of the two native invertebrates (particularly *C. riparius*).

I did not observe a temperature effect on simulated consumption or growth under hypoxia rates, but rates were significantly different by organism size (mg) for *Hexagenia* and *Chironomus riparius*. Increased consumption and growth in hypoxia rates were greater for smaller *Hexagenia* and *C. riparius* individuals. The size differences in rates (both consumption and growth) among organisms may be related to indeterminate (*B. sowerbyi*) versus determinate (*Hexagenia, C. riparius*) growth patterns, which can influence key traits of an organism's life history (Mumby et al. 2015).

CONCLUSIONS

I have demonstrated that benthic invertebrate species in nearshore habitats of the Great Lakes may experience the effects of climate change differently, based on different bioenergetic responses to increased water temperatures and hypoxic and/or more variable (pulsed) DO concentrations. Although *Branchiura sowerbyi* is not currently widespread or regionally dominant in the Great Lakes, given its low respiration and high growth rates plus its ability to survive adverse abiotic conditions compared to the native invertebrates, climate change may result in greater dominance of *B. sowerbyi* in the benthos. Certain nearshore habitats in the Great Lakes, such as coastal wetlands (e.g., OWC) or backwater areas with suitable characteristics, could be sentinel sites to monitor *B. sowerbyi* population dynamics.

Hexagenia spp. populations that were once dominant in the benthos, having since recovered from previous declines after eutrophication in the mid to late 20th century, may be particularly threatened by climate change effects. In recent times, the western basin of Lake Erie is experiencing dynamic patterns of agricultural runoff, leading to increased system productivity (Michalak et al. 2013). *Hexagenia* spp. previously have demonstrated an ability to be resilient to water quality declines in Lake Erie; however, with climate change as an additional stressor, population declines of *Hexagenia* may be greater, or recovery could be hindered, compared to the past. Indeed, results of the survival experiment demonstrate that *Hexagenia* are not well-suited to overcome increased water temperatures and reduced DO concentrations. Continued and thorough monitoring programs that target *Hexagenia* spp. in the western basin of Lake Erie should be implemented to capture population trends and validate the results of my experiments.

An increase in pulsed DO conditions is likely to occur with climate change for nearshore, highly productive littoral zones of freshwater ecosystems that do not seasonally stratify due to increased productivity during daylight (when primary production is occurring) and increased

respiration at night. However, the effects of pulsed DO conditions are rarely quantified on the performance of aquatic organisms, especially in the context of a changing climate. My results showed a decline in native invertebrate (*Hexagenia* and *Chironomus riparius*) survivorship in pulsed DO conditions compared to treatments with high DO, indicating these populations could be at risk. Alternatively, the effects of pulsed DO conditions in nearshore areas may ameliorate those of prolonged hypoxia, as survivorship was greater in pulsed versus hypoxic DO. Thus, nearshore areas may serve as refugia for native invertebrates that are stressed by low DO concentrations in otherwise seasonally stratified habitats, aiding populations to persist under projected climate change conditions in the Great Lakes. Research that investigates the effects of pulsed or diurnal DO concentrations on aquatic organisms are warranted to fully characterize the impacts of climate change on freshwater ecosystems.

CHAPTER 5: INTERNAL FLUX OF PHOSPHORUS AND METALS FROM SEDIMENT BY A NONNATIVE OLIGOCHAETE AND NATIVE INVERTEBRATES VARIES AMONG ORGANISMS, TEMPERATURES, AND DISSOLVED OXYGEN CONCENTRATIONS

INTRODUCTION

External inputs of nutrients and contaminants (e.g., trace metals) have plagued the Laurentian Great Lakes since the 1850s (Kemp et al. 1976), resulting in degraded sediment and water quality (Schelske and Hodell 1995; Smirnov et al. 1998; Painter et al. 2001; Marvin et al. 2004a; Marvin et al. 2004b). As a result of excessive nutrient and contamination inputs, many aspects of Great Lakes ecosystems have been negatively impacted. For example, eutrophication of Lake Erie resulted in greater phytoplankton production (both maximum abundance and seasonal duration) (Davis 1964), with associated reduced dissolved oxygen concentrations at depth (i.e., hypoxia and anoxia) from microbial respiration (Manny 1991). High inputs of contaminants resulted in the persistence of bioaccumulating and toxic pollutants in food webs (Environment Canada and USEPA 2003), leading to contamination of species at higher trophic levels, such as fish (De Vault et al. 1996) and piscivorous water birds (Grasman et al. 1998).

Following the bi-national commitment between the United States and Canada to address degradation of the Great Lakes (known as the Great Lakes Water Quality Agreement), signs of recovery from impairment were observed. For instance, recovery of burrowing mayflies (*Hexagenia* spp.), which were thought to be locally extinct in Lake Erie by the mid-20th century (Manny 1991), is directly attributed to increased sediment and water quality from external nutrient abatement programs (Krieger et al. 1996). Fish populations, such as walleye (*Sander vitreus*), have also showed signs of recovery beginning in the 1970s, due (in part) to improvements in water quality (Hatch et al. 1987).

Recently, water quality concerns have once again been raised, particularly in Lake Erie (Sekaluvu et al. 2018). Decreased water quality has been attributed to external nutrient loading in the western basin from agriculture runoff (Michalak et al. 2013; Conroy et al. 2014). As a result of increased nutrients in Lake Erie, an increase in cyanobacterial blooms has been documented (Michalak et al. 2013), negatively impacting ecosystem function (e.g., decreased water clarity) (Paerl and Huisman 2008) and raising public health concerns (Brooks et al. 2016).

Beyond external loading of nutrients and contaminants, biotic processes may contribute to decreased water quality by acting as internal sources that remobilize historic inputs, thus increasing system productivity (nutrients) or making contaminants bioavailable. For example, invasive dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) have been shown to alter nutrient dynamics by shifting concentrations of nutrients to nearshore zones as a result of filtering large volumes of phytoplankton and other suspended particles. The mussels subsequently release high concentrations of nutrients nearshore through excretion/egestion (termed the 'nearshore phosphorus shunt', Hecky et al. 2004). In addition, high abundances of dreissenid mussels and round gobies (*Neogobius melanastomus*), another infamous invasive species in the Great Lakes, have shifted food webs from being dominated by pelagic processes to an increased influence of benthic processes (Mills et al. 2003; Hecky et al. 2004). This shift in the predominant interconnections of ecological communities in the Great Lakes has resulted in novel pathways to mobilize historic contamination of metals in sediments to higher trophic levels and the pelagic (Southwood Hogan et al. 2007).

Further, bioturbation (mixing of sediment particles) and bioirrigation (sediment ventilation) by burrowing invertebrates are biotic processes that can lead directly to increased nutrient and contaminant internal loading (Matisoff and Wang 2000; Meysman et al. 2006a;

Norkko et al. 2012). Bioturbation/bioirrigation are recognized forms of 'ecosystem engineering'

processes (Meysman et al. 2006a), whereby the physical environmental is highly modified by an

organism such that it exerts a strong influence on other organisms (Jones et al. 1994; Hastings et

al. 2007). Specifically, bioturbation results in the direct mixing or redistribution of sediment

particles (Meysman et al. 2006a), which can lead flux of nutrients and contaminants from

sediment to the water column by suspending particles or exposing deeper sediment layers

(Schaller 2014). Bioirrigation can release nutrients and/or contaminants from sediment through

transport in pore-water caused by benthic invertebrates flushing burrows with water to aerate

with dissolved oxygen, filter feed, or remove metabolic wastes (Meysman et al. 2006b). For

example, Fukuhara and Sakomoto (1987) found that tubificids and chironomids caused sediment

mobilization of phosphorus (P) and ammonia (NH_3), and demonstrated sediment nutrient release

at higher densities of organisms (> 0.2 g m^{-2} nitrogen; > 8 mg m^{-2} phosphorus at the highest

densities for *Chironomus plumosus*). Additionally, Chaffin and Kane (2010) showed that total

reactive phosphorus (TRP) and soluble reactive phosphorus (SRP) releases from sediment were

greater in *Hexagenia* microcosm treatments (TRP maximum approximately 200 μg L^{-1}; SRP

maximum approximately 100 μg L^{-1}) compared to control treatments (maximum concentrations

of TRP and SRP below 10 μg L^{-1}). He et al. (2015) found increased thallium release from

sediment in chironomid treatments (approximately 38 mg L^{-1}) compared to sediment-only

controls (approximately 33 mg L^{-1}) early in a release experiment, and the difference in measured

thallium concentrations were attributed to bioturbation/bioirrigation by the chironomid larvae.

Thus, biological mixing and ventilation of sediment by macrofauna has been shown to have a

direct impact on the release of both nutrients and contaminants.

The burrowing activity of *Branchiura sowerbyi*, a large nonnative benthic oligochaete present in the Great Lakes, may cause increased nutrient and trace metal release from sediment compared to activities of native burrowing invertebrates in nearshore habitats, where it is dominant. First documented in North America in 1930 (Spencer 1932), *B. sowerbyi* is now present in many inland waterbodies and in Lakes Erie (Hiltunen 1969), Huron (Spencer and Hudson 2003), St. Clair and Michigan (Leibig et al. 2012). Mature *B. sowerbyi* can reach lengths of up to 10 cm in length (Wang and Matisoff 1997). Aster (1973) suggested that *B. sowerbyi* is tolerant to a wide range of environmental conditions (Aster 1973). In Lake Erie, maximum densities of *B. sowerbyi* up to 584 individuals per m^{-2} have been reported (Soster 1984).

B. sowerbyi feeds, like most tubificids, with its head down into the sediment ingesting particles to consume associated detritus, and then eliminates waste to the surface at the sediment-water interface (Wang and Matisoff 1997; Ståhl-Delbanco and Hansson 2002). Thus, *B. sowerbyi* is primarily involved with bioturbation by mixing sediment particles as it burrows to feed, and contributes less to bioirrigation because it does not actively aerate a burrow structure (although some bioirrigation effects are likely as water is permitted into sediment layers during and post-burrowing).

In addition to the nonnative *Branchiura sowerbyi*, the native invertebrates *Hexagenia* spp. and *Chironomus riparius* can be dominant in nearshore benthic communities of the Great Lakes. *Hexagenia* spp. were once abundant, but populations crashed in the mid-20th century due to decreased water quality, principally low dissolved oxygen concentrations (Krieger et al. 1996). However, *Hexagenia* populations have since rebounded (Schloesser and Nalepa 2001) and Krieger et al. (2007) reported average densities of *Hexagenia* in Lake Erie of 215 individuals per m^2 from 1997 to 2003. *Hexagenia* spp. construct U-shaped burrows that are aerated with

overlying water (Matisoff and Wang 2000). Smaller *Hexagenia* nymphs filter feed by flushing of constructed burrows, while larger nymphs ingest sediment particles (Keltner and McCafferty 1986; Matisoff and Wang 1998). Late-instar *Hexagenia* typically range in size from 2 to 3.5 cm (Matisoff and Wang 2000). *Chironomus riparius* are smaller than both *B. sowerbyi* and *Hexagenia*, ranging in size from 1 to 2 cm in length as fourth instars (Mackey 1977), but also construct burrow structures that are actively aerated (Matisoff and Wang 2000). In Lake Erie, *C. riparius* maximum reported densities are approximately 3,000 individuals per m^{-2} (Soster and McCall 1990). *C. riparius* feeds with its head up in the sediment column, and releases waste downward (Ståhl-Delbanco and Hansson 2002), as opposed to the burrowing and feeding behavior of tubificids such as *Branchiura sowerbyi*. Thus, due to burrow construction and active aeriation, *Hexagenia* spp. and *C. riparius* are involved with both bioturbation and bioirrigation.

I utilized laboratory microcosm experiments to compare sediment burrowing depths, nutrients (total phosphorus (TP) and total dissolved phosphorus (TDP)), and trace metal sediment release among the nonnative *Branchiura sowerbyi*, and the native *Hexagenia* spp. and *Chironomus riparius*. Due to their larger size and ability to tolerate decreased dissolved oxygen conditions in the sediment column, I hypothesized that *B. sowerbyi* would mix sediment to greater depths compared to the native invertebrates. Similarly, corresponding to increased burrow depths and differences in feeding behavior (principally wastes being eliminated at the sediment-water interface), I hypothesized that *B. sowerbyi* would result in greater TP, TDP, and trace metal release to overlying water compared to the native burrowing invertebrates.

Ultimately, my goal was to determine if the introduction of *Branchiura sowerbyi* into the Great Lakes has ecosystem implications through a greater influence on internal loading of

phosphorus and trace metals compared to two important and abundant native benthic invertebrates (*Hexagenia* spp. and *Chironomus riparius*).

METHODS

Bioturbation/bioirrigation depth

Depth of bioturbation/bioirrigation for study organisms was quantified in glass microcosms by placing glass microbeads on the sediment surface at the start of the trace metal experiment (see specific trace metal experiment details, below), and was conducted at 20°C. Microcosms (10.2 cm in length and width, 12.7 cm in height) contained 5 cm of sieved sediment (500 μm sieve to remove gross organic material and other macrofaunal invertebrates) (Matisoff and Wang 2000), and 1000 mL of overlying filtered lake water. Sediment was collected from Port Bay, Lake Ontario and water was collected from Skaneateles Lake. Bioturbation/bioirrigation depth experiment included two replicate microcosms for each organism (*Branchiura sowerbyi*, *Hexagenia* sp., *Chironomus riparius* separately), with five individuals in each replicate microcosm, plus control treatments that had no benthic macroinvertebrates added (sediment and overlying water only).

Glass microbeads (Recollections[TM] Signature) were added to microcosms to cover the sediment surface. Microbead depth per microcosm was generally 0.1 cm on top of the sediment layer. After 168 hours (7 days), I collected sediment cores manually to quantify the number of microbeads present at three sediment depth intervals – (1) 0-1 cm, (2) 1-2 cm, and (3) 2+ cm. Three replicate sediment cores were collected per replicate treatment using tygon tubing (0.6 cm inner diameter) by slowly pushing tubes through the sediment column, applying a rubber stopper to the exposed opening, and carefully lifting tubes with sediment out of microcosms. Sediment was then immediately frozen until processed.

On frozen sediment cores, a dremel hand tool was used to cut sections by depth interval. Core sub-sections were placed in separate scintillation vials to thaw, and the number of microbeads present in each sediment core was counted on a dissecting microscope (Leica MZ125) using zooplankton counting trays. Microbeads present were converted to number per volume of sediment (no. cm^{-3}) based on the depth interval and inner diameter of the tubing used to collect cores.

Sediment phosphorus release

Phosphorus (P) sediment nutrient release of the three study organisms was quantified using a microcosm experiment, conducted within experimental chambers at the Center for Integrated Research and Teaching in Aquatic Sciences (CIRTAS) at SUNY College of Environmental Science and Forestry (SUNY ESF). Experimental temperatures chosen were 20°C and 30°C, selected based on current water temperatures in nearshore western basin of Lake Erie (19.6°C, as measured during the 2013 field sampling season nearshore western basin of Lake Erie from May through October) and results from a bioenergetics experiment with the three study organisms, which suggested differences in organism performance at 30°C compared to 20°C (Brainard and Schulz, unpublished data). In addition, Coyer and Magnum (1973) suggested that biological mixing of sediment increased by a factor of two with a 10°C increase in water temperature, suggesting the effect of temperature on sediment nutrient release would likely be observable between 20°C and 30°C.

In addition to the temperature variability, I utilized three different dissolved oxygen concentrations to quantify sediment nutrient release, fully crossed with the two temperature treatments. The dissolved oxygen treatments consisted of: (1) high O_2 (mg L^{-1} at saturation, O_2 bubbled into treatments throughout the experiment). (2) hypoxic O_2 (N_2 bubbled to lower DO to

< 2 mg L^{-1} and microcosms then sealed to inhibit atmospheric O_2 intrusion). (3) pulsed O_2 (O_2 or N_2 bubbled into microcosms to raise or lower oxygen levels, respectively, on a 12/12 hour cycle).

Microcosms consisted of 5 cm of sediment and 1000 mL of overlying lake water (sediment – Port Bay, Lake Ontario; lake water – Skaneateles Lake). Sediment was sieved (500 µm) to remove gross organic material and other macrofaunal invertebrates. Port Bay sediment was selected due to representativeness of nearshore Great Lake sediment conditions (fine silts with relatively high organic content), and was stored at 4°C until used in sediment nutrient release experiments. Skaneateles Lake water was used in the sediment nutrient release experiment due to its low nutrient concentration in order to facilitate measuring changes in P concentrations in experimental treatments.

Three replicates for each organism (*Branchiura sowerbyi*, *Hexagenia* spp., and *Chironomus riparius*) were conducted at each temperature (20°C and 30°C), dissolved oxygen (high, hypoxic, and pulsed) combination, in addition to three control replicates for each temperature/DO combination.

Densities used for each organism's treatments corresponded approximately to field densities reported in the Great Lakes (Table 5-1). I collected *Branchiura sowerbyi* from Tully Lake (30 miles south of Syracuse, NY) to avoid overland transport of a nonnative invertebrate from Lake Erie. *Hexagenia* eggs were obtained from Jan Ciborowski (University of Windsor), and *Chironomus riparius* eggs were obtained from Aquatic Research Organisms, Inc. Eggs were hatched at 10°C in aerated tanks. Stock organisms were fed daily by providing 2 mL of food (baker's yeast, alfalfa powder, and Tetramin® fish food in deionized water, following Winter et al. 1996) to tanks in which organisms were held (10 gallon tanks, with 20 cm of sediment from

Port Bay, Lake Ontario). Organisms were acclimated to experimental temperatures for 72 hours prior to beginning sediment nutrient release experiments, at a rate of 3°C change per day; food was not applied to experiment microcosms during the experimental duration to avoid adding phosphorus to water columns. I assumed that the organic sediment used in microcosms provided an adequate food source to sustain normal invertebrate activity.

Total phosphorus (TP) and total dissolved phosphorus (TDP) were measured in each replicate microcosm by collecting 250 mL of overlying water with a 60 mL syringe every 8, 24, 48, 72, 166, and 216 hrs (total experimental duration = 9 days). In addition, TP and TDP concentrations from the filtered Skaneateles Lake water were initially determined, and represented initial P concentrations (time = 0). The times chosen to obtain water samples was selected based on organisms burrowing and/or constructing burrows immediately after being placed in microcosms (also observed by Matisoff and Wang 2000). In addition, results from a pilot experimental showed higher rates of P increase in overlying water at the beginning of experiments, thus, an increased number of sampling events were included earlier in the experimental design to better capture potential sediment nutrient flux dynamics over time. After each sampling interval, an equivalent 250 mL of filtered Skaneateles Lake water was added back to each microcosm to maintain a constant water volume and to represent typical turnover rates in nearshore Great Lake coastal habitats (~ 25% volume overturn daily, Looi, unpublished data). Phosphorus concentrations at each sampling time were adjusted by known concentrations of TP and TDP in the filtered Skaneateles Lake water to account for the possible dilution effect.

Table 5-1. Densities of macroinvertebrates used in SNR experiments, and corresponding field densities (number per m^2) as reported in the literature for the western basin of Lake Erie. (a) Soster 1984, (b) Soster et al. 2011, (c) Soster and McCall (1990).

Density	*B. sowerbyi*	*Hexagenia*	*Chironomus* spp.
Number in microcosm	6	7	10
Corresponding invertebrate density (no. m^{-2})	582	679	970
Reported field density (no. m^{-2})	584[a]	698[b]	625[b] – 3,000[c]

Trace metal release

I quantified trace metal release from sediment in microcosms for the three study organisms at 20°C. Each replicate microcosm (n = five per organism plus five controls) contained 5 cm of sieved sediment (Port Bay, Lake Ontario) and 500 mL of filtered overlying lake water (Skaneateles Lake).

Water samples (120 mL) were collected every 24 hours over a 7 day period. Following each sampling time, 120 mL of Skaneateles Lake water were added to each replicate to represent turnover rates in nearshore Great Lakes coastal habitats, and trace metal concentrations were adjusted to account for potential dilution effect.

Each water sample was analyzed for nine trace metals using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Fe, Mg, Mn, Si, Sr, and Zn), or inductively coupled plasma mass spectrometry (ICP-MS; Al, As, and Cr) at the Analytical & Technical Services laboratory at SUNY ESF, following U.S. EPA Method 6010C (USEPA 2007) and U.S. EPA Method 6020A (USEPA 1998).

Statistical Analysis

I used one-way ANOVAs to test if the number of microbeads differed by sediment depth (0-1 cm, 1-2 cm, and 2+ cm) and for differences between organism treatments. Number of microbeads (no. cm^{-3}) was log (x+1) transformed to meet the assumption of normality (Gotelli and Ellison 2013).

The effects of temperature and dissolved oxygen on total and total dissolved phosphorus nutrient release were analyzed with one-way ANOVAs. Phosphorus concentrations were log transformed to meet the ANOVA assumption of normality. Finally, I tested if trace metal concentrations differed by organisms using one-way ANOVA (trace metal concentrations were

log transformed to meet assumptions of normality). Bonferroni post-hoc tests were used to determine significant differences within explanatory variables. All statistics were conducted in SPSS (version 24; IBM, Armonk, New York).

RESULTS

I observed that water clarity (directly linked to bioturbation) varied within accumulation tanks among organisms. For instance, *Branchiura sowerbyi* tanks were visibly less turbid (Figure 5-1a) compared to those of *Hexagenia* spp. (Figure 5-1b) and *Chironomus riparius* (not depicted). In all cases, tanks were aerated (with air stones), thus observed turbidity in tanks can be attributed to burrowing invertebrate activity rather than suspension of sediment from infused oxygen.
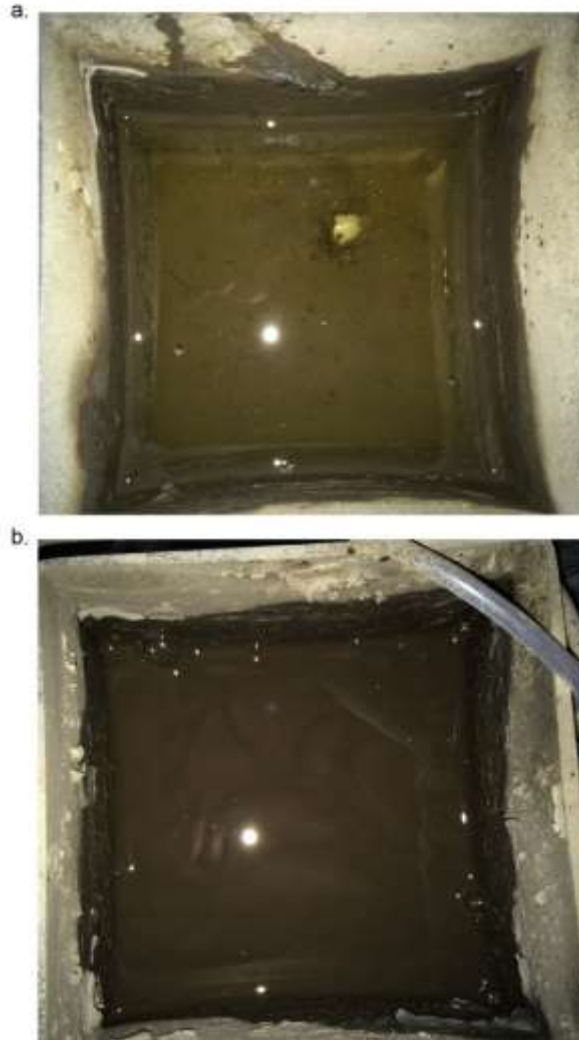
Figure 5-1. Photographs showing water turbidity (correlated to bioturbation/bioirrigation) of (a) *Branchiura sowerbyi* and (b) *Hexagenia* spp. Water clarity was consistently observed to be greater in *B. sowerbyi* containers compared to those containing *Hexagenia* and *Chironomus riparius* (not pictured).

Bioturbation/bioirrigation depth

Microbeads were not distributed uniformly with depth at the end of the bioturbation/bioirrigation depth experiment ($p < 0.001$). There were significantly more microbeads toward the surface (0-1 cm sediment interval, 662.4 no. cm^{-3} ± 509.4) compared to the deepest sediment interval (2+ cm, 16.8 no. cm^{-3} ± 15.7, $p < 0.001$). Additionally, there were significantly more microbeads in the intermediate sediment interval (1-2 cm, 283.3 no. cm^{-3} ± 268.1) compared to the deepest interval ($p < 0.001$). However the number of microbeads did not differ between the two top sediment layers (0-1 cm and 1-2 cm, $p = 0.461$) (Figure 5-2). Thus microbeads, which were akin to sediment particles in this experiment, were mixed beyond 2 cm in the mescosms, but the majority of the microbeads remained in the upper 2 cm of sediment.

The number of microbeads present at each depth interval varied among organism treatments. Specifically at the 0-1 cm interval, there were a greater number of microbeads in the *Chironomus riparius* (1008 no. cm^{-3} ± 376.6) and *Hexagenia* treatments (988.7 no. cm^{-3} ± 501.3) compared to the *Branchiura sowerbyi* (338.1 no. cm^{-3} ± 419.9) and control treatments (314.3 no. cm^{-3} ± 285.1), although the differences among organism were not statistically significant (p-values between organism treatments > 0.05) (Figure 5-2).
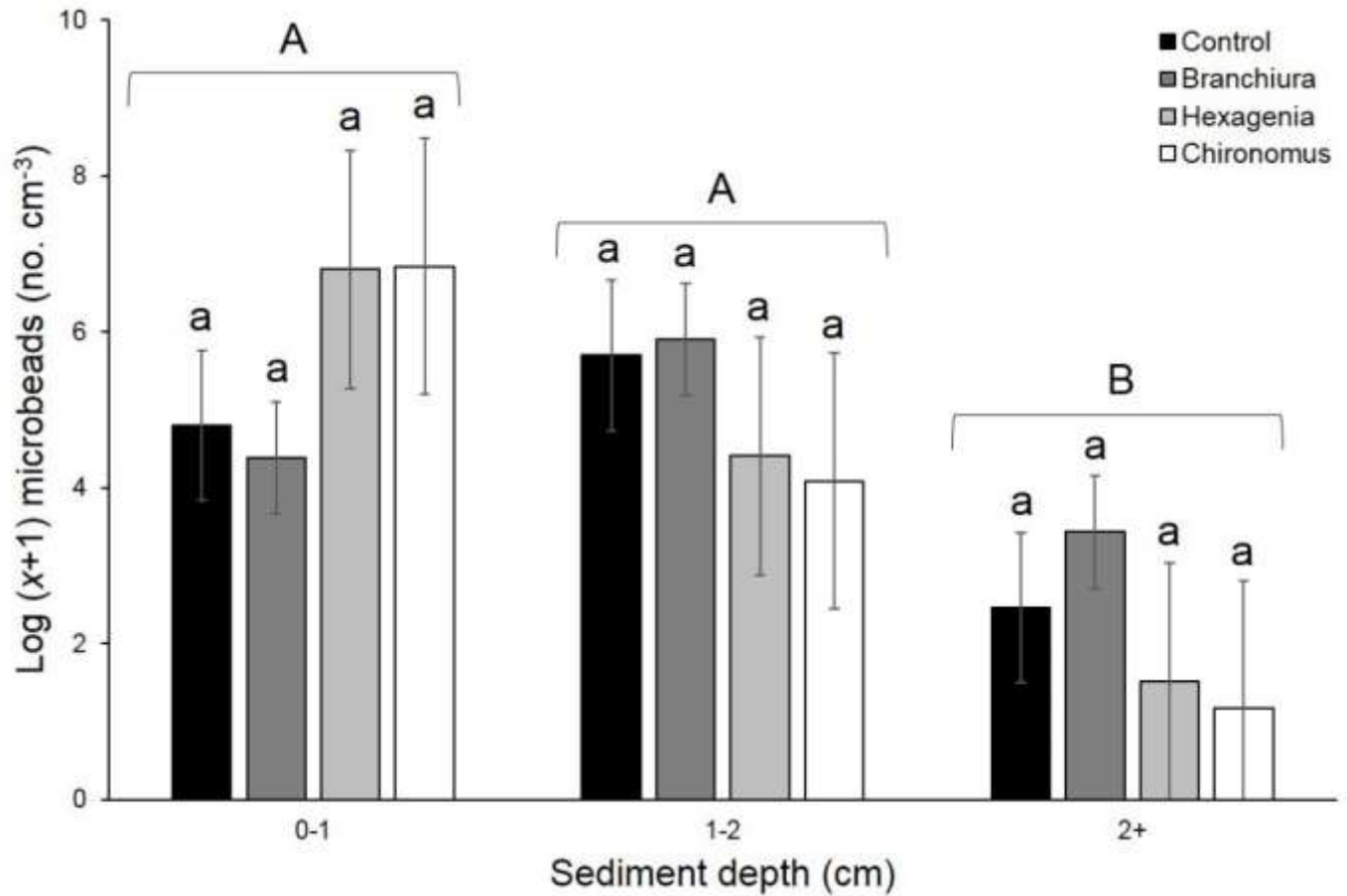
Figure 5-2. Number of microbeads (no. cm^{-3}, log $x+1$ transformed) found at sediment depth (cm) in control (black bars), *Branchiura* (dark grey bars), *Hexagenia* (light grey bars), and *Chironomus* (white bars) treatments. Significance between number of microbeads found at different sediment depth intervals indicated by upper case letters, while there were no significant differences in the number of microbeads between organism treatments within a given depth, as indicated by lower case letters. Bars represent standard error (n = 6 measurements per bar).

At the 1-2 cm sediment interval, there were generally more microbeads in the *Branchiura sowerbyi* (435.7 no. cm^{-3} ± 208.1) and control treatments (454.8 no. cm^{-3} ± 367.4) compared to *Hexagenia* (95.8 no. cm^{-3} ± 44.6) and *Chironomus riparius* treatments (147.0 no. cm^{-3} ± 150.9). Differences in the number of microbeads at the 1-2 cm sediment interval were not statistically significant between organism treatments (all p-values > 0.05) (Figure 5-2). Within the 2+ sediment depth interval, the number of microbeads in the *Branchiura sowerbyi* treatment (31.5 no. cm^{-3} ± 12.0) was generally greater than in control (18.5 no. cm^{-3} ± 15.9), *Hexagenia* (11.9 no. cm^{-3} ± 16.1), and *Chironomus riparius* treatments (5.4 no. cm^{-3} ± 4.9), although there was not a significant different among organism treatments (all p-values > 0.05) (Figure 5-2). Contrary to the surface (0-1 cm) depth interval, which showed a general increase in the number of microbeads in *Hexagenia* and *C. riparius* treatments, there was a trend of more microbeads at depth in *B. sowerbyi* treatments at depth (2+ cm interval) (Figure 5-2).

Sediment phosphorus release

Temperature

I found that sediment nutrient release rates for TP differed by temperature, with increased concentrations of TP at the higher temperature (30°C, 0.55 mg m^{-2} hr^{-1} ± 0.81) compared to 20°C (0.36 mg m^{-2} hr^{-1} ± 0.49) ($p < 0.001$, Figure 5-3). More specifically, TP release rates were significantly different among organisms at 20°C ($p = 0.008$) (Figure 5-3a), but I did not observe an effect of organism at 30°C ($p = 0.159$) (Figure 5-3b).

At 20°C, sediment release rates of TP from *Hexagenia* (0.043 mg m^{-2} hr^{-1} ± 0.049, $p = 0.009$) and *Chironomus riparius* (0.045 mg m^{-2} hr^{-1} ± 0.064, $p = 0.037$) were greater than in control treatments (0.025 mg m^{-2} hr^{-1} ± 0.039), but TP release rates from *Branchiura sowerbyi* (0.030 mg m^{-2} hr^{-1} ± 0.036) were not different from controls ($p = 0.460$) (Figure 5-3a). TP release

rates at 20°C in *B. sowerbyi* treatments were not significantly different than in *Hexagenia* ($p =$ 0.912) or *C. riparius* ($p = 1.0$) treatments, and TP release in *Hexagenia* treatments did not differ from those in *C. riparius* treatments ($p = 1.0$) (Figure 5-3a). Thus, at 20°C, sediment TP release was greater in native benthic invertebrate treatments compared to controls, but did not differ significantly from release by the nonnative, *B. sowerbyi*. I estimated annual TP sediment nutrient release from *B. sowerbyi*, *Hexagenia* spp., and *C. riparius* at 20°C to be 254.9, 376.2, and 390.7 mg m^{-2} yr^{-1}, respectively.

At 30°C, rates of TP release in *Branchiura sowerbyi* (0.41 mg m^{-2} hr^{-1} ± 0.47, $p = 1.0$), *Hexagenia* (0.63 mg m^{-2} hr^{-1} ± 1.02, $p = 1.0$), and *Chironomus riparius* (0.71 mg m^{-2} hr^{-1} ± 1.01, $p = 0.528$) treatments did not differ from those in controls (0.47 mg m^{-2} hr^{-1} ± 0.57) (Figure 5-3b). TP release rates from sediments at 30°C in *B. sowerbyi* treatments also did not differ from those of *Hexagenia* ($p = 1.0$) or *C. riparius* ($p = 0.193$), and TP release rates between *Hexagenia* and *C. riparius* were not different ($p = 0.864$) (Figure 5-3b). Therefore, at elevated water temperatures, there was not a significant difference in TP release among the study organisms and controls. When hourly estimates of TP sediment release at 30°C were scaled up to annual values, I found that *B. sowerbyi* was estimated to release 3,611 mg m^{-2} yr^{-1}, *Hexagenia* spp. 5,486 mg m^{-2} yr^{-1}, and *C. riparius* annual TP release was 1719 mg m^{-2} yr^{-1}, indicating that annual release rates of TP from sediment are likely to be much greater in elevated water temperatures.
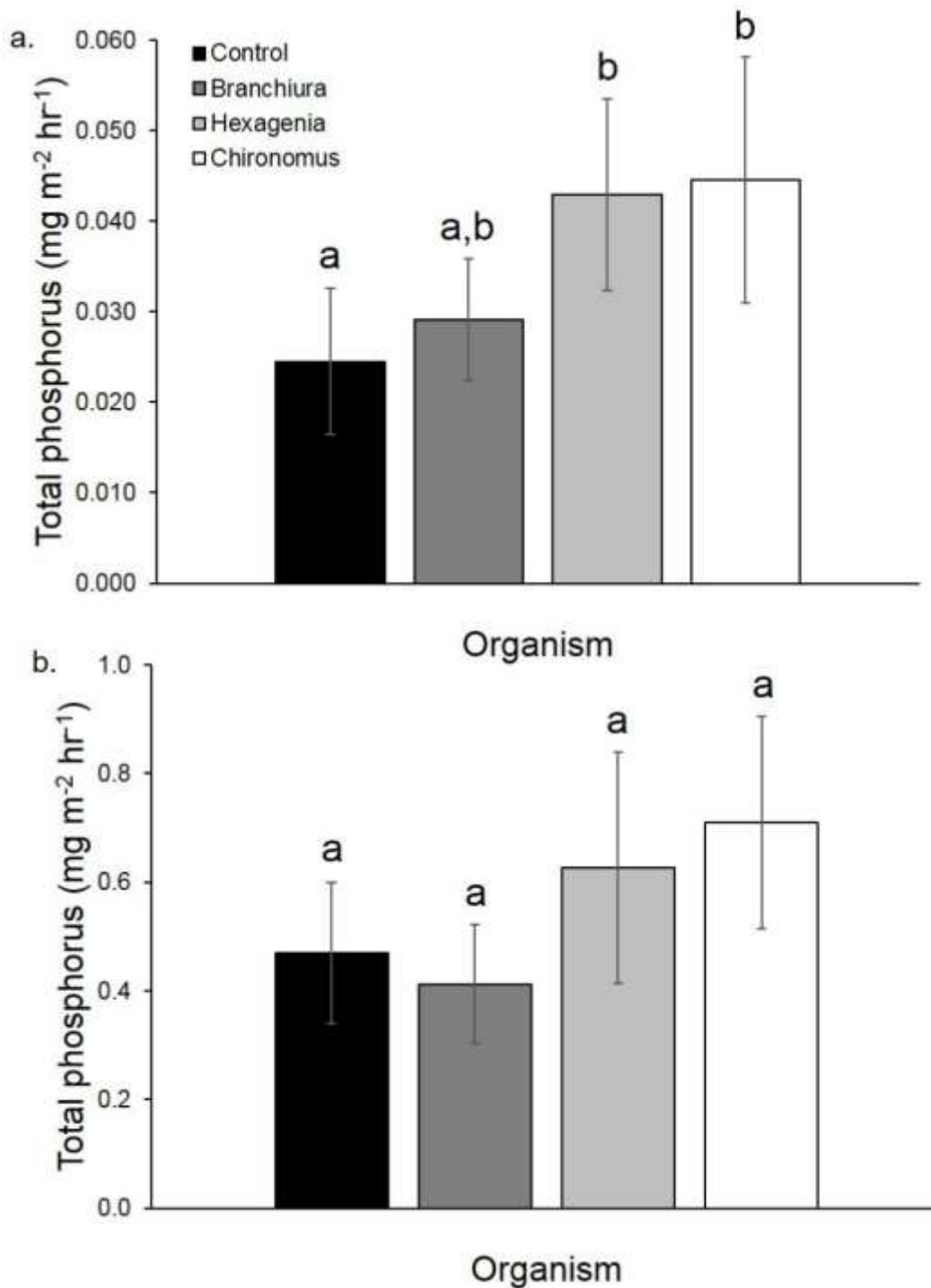
Figure 5-3. Release rates of total phosphorus (TP) from sediments (mg m^{-2} h^{-1}) in control (black), *Branchiura* (dark grey), *Hexagenia* (light grey), and *Chironomus* (white) treatments at (a) 20°C and (b) 30°C water temperatures. Note that figure represents non-transformed data, but statistical analysis was conducted with log transformed TP release rates to meet normality assumption of ANOVA. Significance between TP concentrations between organisms indicated by lower case letters. Bars represent standard error (n = 54 measurements per bar).

For TDP release rates from sediments, I found a significant effect of temperature, where increased TDP was observed at 30°C (0.25 mg m^{-2} hr^{-1} ± 0.37) compared to 20°C (0.01 mg m^{-2} hr^{-1} ± 0.01, $p < 0.001$) (Figure 5-4). Similar to TP concentrations, increased TDP differed among organisms at 20°C ($p = 0.044$) (Figure 5-4a), but not at 30°C ($p = 0.442$) (Figure 5-4b).

At 20°C, however, TDP release rates of the study organisms did not differ. More specifically, TDP release rates of *Branchiura sowerbyi* (0.009 mg m^{-2} hr^{-1} ± 0.105, $p = 1.0$), *Hexagenia* (0.013 mg m^{-2} hr^{-1} ± 0.013, $p = 0.084$), and *Chironomus riparius* (0.013 mg m^{-2} hr^{-1} ± 0.019, $p = 0.158$) did not significantly differ from those in control treatments (0.007 mg m^{-2} hr^{-1} ± 0.009) (Figure 5-4a). TDP release rates from the *B. sowerbyi* treatment did not differ from those in the *Hexagenia* ($p = 0.639$) or *C. riparius* ($p = 1.0$) treatments, and TDP release rates of *Hexagenia* did not differ from that of *C. riparius* ($p = 1.0$) (Figure 5-4a). Annual estimates of TDP release rates from sediment by *B. sowerbyi*, *Hexagenia* spp., and *C. riparius* were estimated to be 75.6, 110.4, and 111.0 mg m^{-2} yr^{-1}, respectively, at 20°C.

TDP sediment release rates from *Branchiura sowerbyi* (0.19 mg m^{-2} hr^{-1} ± 0.24, $p = 1.0$), *Hexagenia* (0.24 mg m^{-2} hr^{-1} ± 0.38, $p = 1.0$), and *Chironomus riparius* (0.35 mg m^{-2} hr^{-1} ± 0.55, $p = 1.0$) treatments were not different from those in control treatments (0.21 mg m^{-2} hr^{-1} ± 0.22) at 30°C (Figure 5-4b). Additionally, TDP release rates from the *B. sowerbyi* treatment did not differ from those in *Hexagenia* ($p = 1.0$) or *C. riparius* ($p = 1.0$) treatments, and TDP release rates from sediments in the *Hexagenia* treatment were not different from those of *C. riparius* ($p = 0.689$) (Figure 5-4b). Per year TDP release rate estimates from sediments for *B. sowerbyi* (1697.8 mg m^{-2} yr^{-1}), *Hexagenia* spp. (2119.3 mg m^{-2} yr^{-1}), and *C. riparius* (3029.5 mg m^{-2} yr^{-1}) at 30°C all were greater than TDP release rate estimates at 20°C.
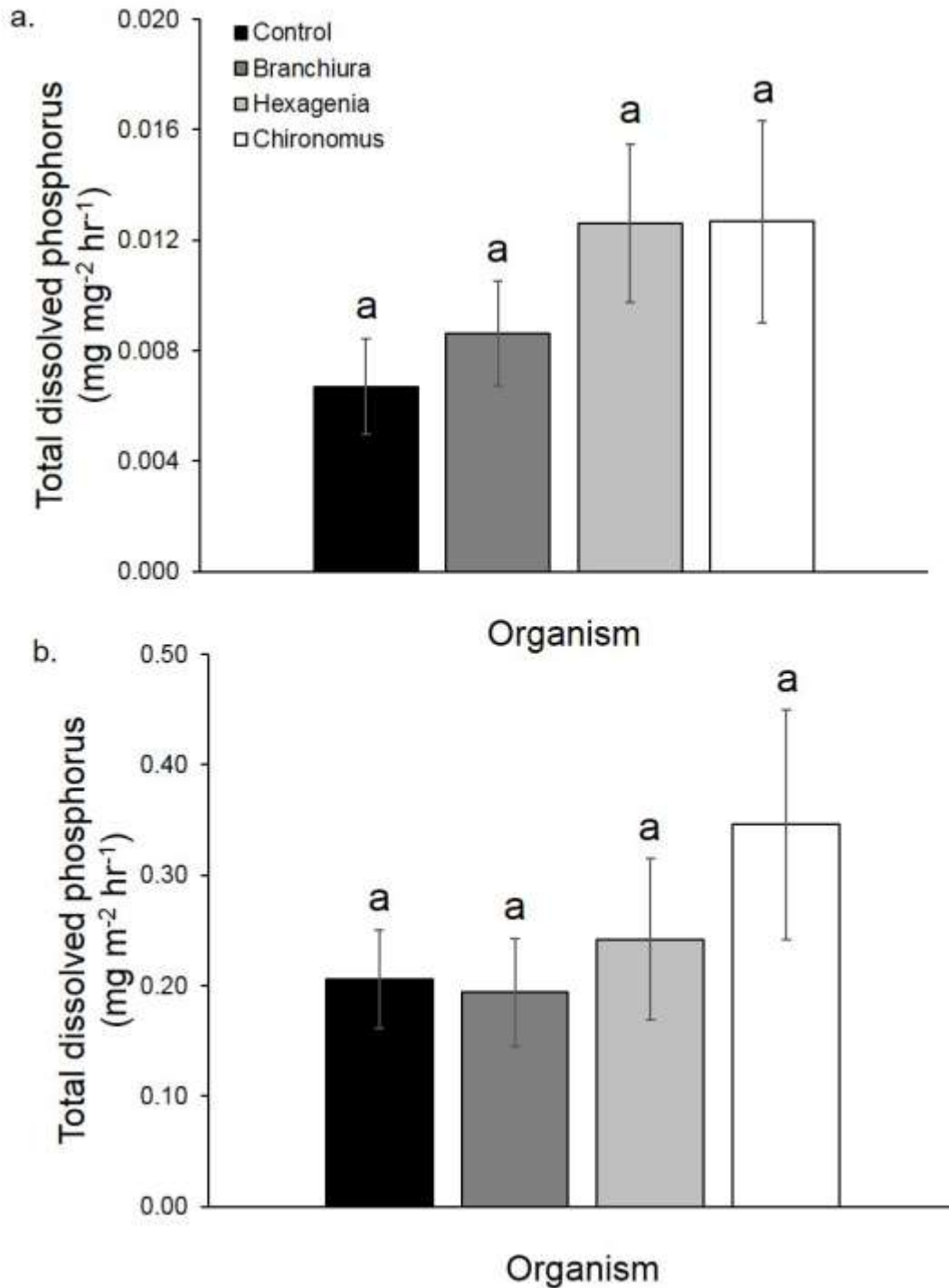
Figure 5-4. Total dissolved phosphorus (TDP) sediment release rates (mg m^{-2} hr^{-1}) in control (black), *Branchiura* (dark grey), *Hexagenia* (light grey), and *Chironomus* (white) treatments at (a) 20°C and (b) 30°C water temperatures. Note that figure represents non-transformed data, but statistical analysis was conducted with log transformed TDP release rates to meet normality assumption of ANOVA. Significance between TDP concentrations between organisms indicated by lower case letters. Bars represent standard error (n = 54 measurements per bar).

Dissolved oxygen

I found release rates of TP from sediments varied between dissolved oxygen treatments ($p < 0.001$) (Figure 5-5a), with release rates significantly greater in hypoxic treatments (0.46 mg m^{-2} hr^{-1} ± 0.90) compared to high (0.31 mg m^{-2} hr^{-1} ± 0.53, $p < 0.001$) and pulsed DO (0.12 mg m^{-2} hr^{-1} ± 0.18, $p < 0.001$) (Figure 5-5a). Release rates of TP from sediment did not differ in high compared to pulsed DO treatments ($p = 0.141$), however, there was a general trend of lower TP release from sediment in pulsed compared to high DO (Figure 5-5a).

The release of TP from sediments was not significantly different between the study organisms in the three DO conditions (high – $p = 0.625$; hypoxic – $p = 0.510$; pulsed – $p = 0.482$) (Figure 5-5a). Specifically, in high DO, TP release rates of the three study organisms did not differ from that in controls (0.23 mg m^{-2} hr^{-1} ± 0.38) (*Branchiura sowerbyi* – 0.27 mg m^{-2} hr^{-1} ± 0.47, $p = 1.0$; *Hexagenia* – 0.26 mg m^{-2} hr^{-1} ± 0.44, $p = 1.0$; *Chironomus riparius* – 0.47 mg m^{-2} hr^{-1} ± 0.75, $p = 1.0$) (Figure 5-5a). I also did not observe differences in TP release rates among organisms (all p-values = 1.0, Figure 5-5a). Similarly, TP release rates from organisms were not different in hypoxic DO compared to controls (0.43 mg m^{-2} hr^{-1} ± 0.66) (*B. sowerbyi* – 0.30 mg m^{-2} hr^{-1} ± 0.43; *Hexagenia* – 0.62 mg m^{-2} hr^{-1} ± 1.2; *C. riparius* – 0.50 mg m^{-2} hr^{-1} ± 1.1; all p-values = 1.0), and there was not a difference in TP release rates among organisms (all p-values = 1.0) (Figure 5-5a). Likewise, *B. sowerbyi* ($p = 1.0$), *Hexagenia* ($p = 1.0$), and *C. riparius* ($p = 0.842$) release rates of TP were not different from controls (0.08 mg m^{-2} hr^{-1} ± 0.09) in pulsed DO treatments. There were no differences in release rates of TP among organisms (*B. sowerbyi* – 0.09 mg m^{-2} hr^{-1} ± 0.12; *Hexagenia* – 0.13 mg m^{-2} hr^{-1} ± 0.18; *C. riparius* – 0.16 mg m^{-2} hr^{-1} ± 0.27; all p-values = 1.0) (Figure 5-5a).
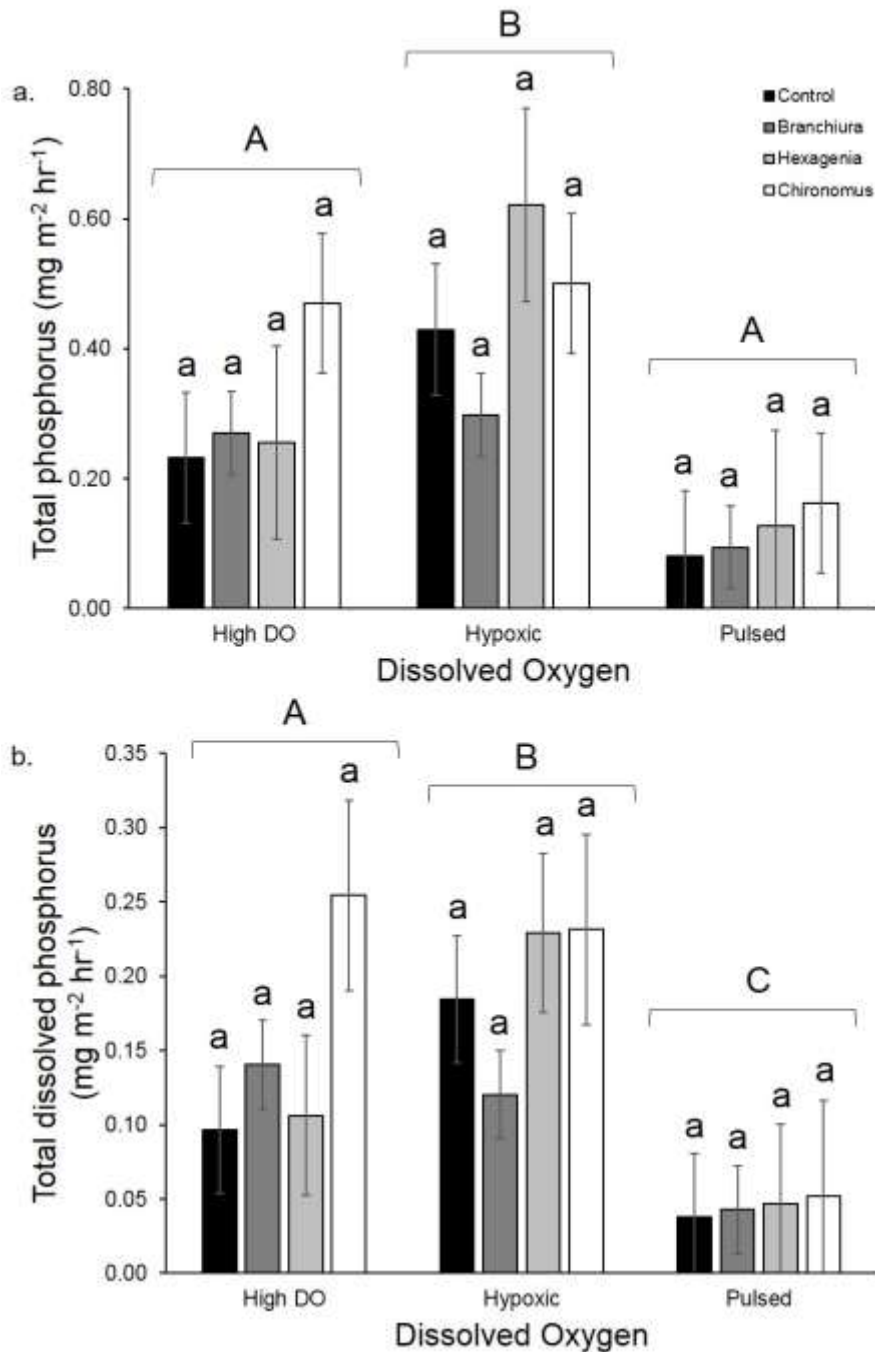
Figure 5-5. (a) Total phosphorus (TP) and (b) total dissolved phosphorus (TDP) sediment release rates (mg m^{-2} hr^{-1}) in control (black), *Branchiura* (dark grey), *Hexagenia* (light grey), and *Chironomus* (white) treatments in high DO, hypoxic, and pulsed DO conditions. Note that figure represents non-transformed data, but statistical analysis was conducted with log transformed TP and TDP release rates to meet normality assumption of ANOVA. Significance between oxygen treatments indicated by upper case letters, and differences in phosphorus concentrations between organisms for a given oxygen treatment indicated by lower case letters. Bars represent standard error (n = 36 measurements per bar).

Release rates of TDP differed among DO concentrations ($p < 0.001$), where release rates were significantly greater in hypoxic (0.19 mg m^{-2} hr^{-1} ± 0.40) compared to both high (0.15 mg m^{-2} hr^{-1} ± 0.28, $p = 0.001$) and pulsed DO treatments (0.04 mg m^{-2} hr^{-1} ± 0.07, $p < 0.001$) (Figure 5-5b). Also, TDP release rates in high DO were significantly greater than in pulsed DO ($p = 0.031$) treatments (Figure 5-5b). Thus, release rates of TDP were greatest in hypoxic conditions, and lowest in pulsed DO conditions (Figure 5-5b).

Release rates of TDP, however, did not differ significantly within DO treatments by organism (high – $p = 0.705$; hypoxic – $p = 0.696$; pulsed – $p = 0.870$) (Figure 5-5b). In each DO condition (high, hypoxic, and pulsed), TDP release rates from the study organisms did not differ from controls (all p-values = 1.0), and release rates of TDP did not differ between organisms in any DO treatment (Figure 5-5b).

Trace metal release

Trace metal release rates from sediment were different among treatments containing different study organisms for three of the nine metals analyzed (Table 5-2). Sediment release rates of Al from *Chironomus riparius* treatments (5.3 mg m^{-2} hr^{-1} ± 6.9) were not different from those in control treatments (4.7 mg m^{-2} hr^{-1} ± 7.4, $p = 1.0$); however, release rates of Al from *Branchiura sowerbyi* (13.7 mg m^{-2} hr^{-1} ± 21.8, $p = 0.005$) and *Hexagenia* (12.2 mg m^{-2} hr^{-1} ± 18.1, $p = 0.012$) treatments were significantly greater than release from controls (Figure 5-6a). The release rates of Al from sediment were not different among the three study organisms (Figure 5-6a), however, there was a general trend of increased Al sediment release rates in *B. sowerbyi* and *Hexagenia* treatments compared to those in the *C. riparius* treatment. Annual estimates of Al release rates from sediment ranged from 0.047 kg m^{-2} yr^{-1} (*C. riparius*) up to 0.12 kg m^{-2} yr^{-1} (*B. sowerbyi*).

Table 5-2. ANOVA results of trace metal concentrations released by organisms (mg m^{-2} hr^{-1}), log transformed). Significantly different ($\alpha = 0.05$) trace metal concentrations between organisms shown in bold; NS = not significant.

Metal	Organism	
	p	R^2
Aluminum (Al)	**0.002**	**0.107**
Arsenic (As)	NS	0.014
Chromium (Cr)	NS	0.030
Iron (Fe)	**0.004**	**0.094**
Magnesium (Mg)	NS	0.001
Manganese (Mn)	NS	0.036
Silicon (Si)	NS	0.017
Strontium (Sr)	NS	0.004
Zinc (Zn)	**< 0.001**	**0.576**

Sediment release rates of Fe from *Hexagenia* (120.4 mg m^{-2} hr^{-1} ± 126.5, $p = 0.112$) and

Chironomus riparius (86.3 mg m^{-2} hr^{-1} ± 84.1, $p = 1.0$) treatments did not differ from those in the

controls (66.5 mg m^{-2} hr^{-1} ± 84.5); however, release rates of Fe from *Branchiura sowerbyi*

treatments (179.5 mg m^{-2} hr^{-1} ± 177.3) were significantly greater than those from control

treatments ($p = 0.002$, Figure 5-6b). Release rates of Fe from *B. sowerbyi*, however, were not

different from those of *Hexagenia* ($p = 1.0$) or *C. riparius* ($p = 0.158$), and Fe release rates from

sediment were not significantly different between *Hexagenia* and *C. riparius* treatments ($p = 1.0$)

(Figure 5-6b). Annual sediment release rates of Fe ranged from 0.76 kg m^{-2} yr^{-1} (*C. riparius*) to

1.57 kg m^{-2} yr^{-1} for *B. sowerbyi*.
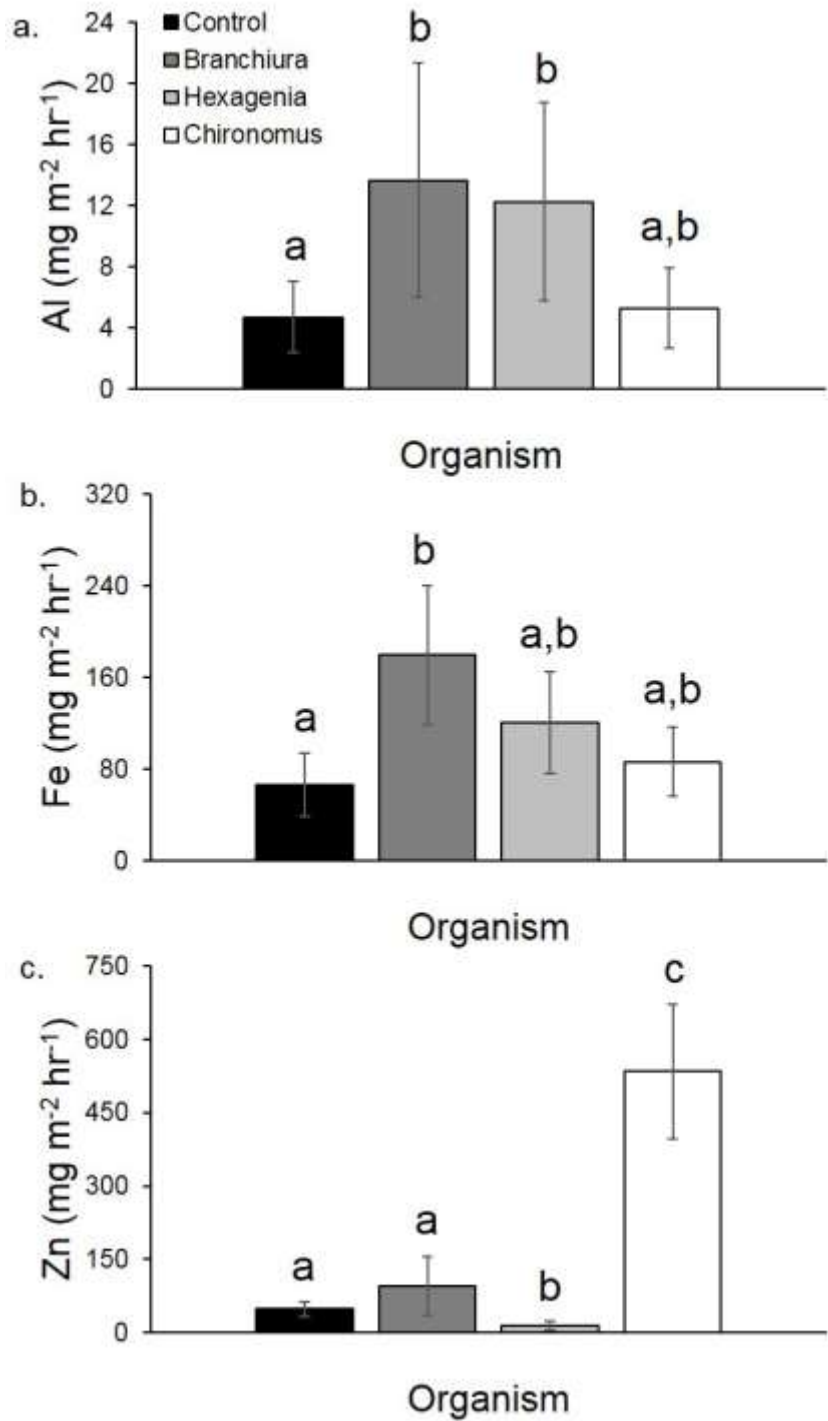
Figure 5-6. (a) Aluminum, (b) iron, and (c) zinc trace metal release rates from sediments (mg m^{-2} hr^{-1}) in control (black), *Branchiura* (dark grey), *Hexagenia* (light grey), and *Chironomus* (white) treatments at 20°C. Note that figure represents non-transformed data, but statistical analysis was conducted with log transformed trace metal release rates to meet normality assumption of ANOVA. Significance between trance metal concentrations indicated by lower case letters. Bars represent standard error (n = 35 measurements per bar).

I observed that sediment release rates of Zn from *Branchiura sowerbyi* treatments (95.1 mg m^{-2} hr^{-1} ± 180.9) were not different from control treatment release (47.8 mg m^{-2} hr^{-1} ± 60.9, *p* = 1.0). However, Zn release rates in *Hexagenia* treatments (14.3 mg m^{-2} hr^{-1} ± 32.8) were significantly lower (*p* < 0.001), and release rates in *Chironomus riparius* (834.6 mg m^{-2} hr^{-1} ± 1069, *p* < 0.001) were significantly greater than those in controls (Figure 5-6c). Further, Zn release rates from sediment in *B. sowerbyi* treatments were significantly greater than those in *Hexagenia* treatments (*p* < 0.001), but those in *B. sowerbyi* treatments were lower than in *C. riparius* treatments (*p* < 0.001) (Figure 5-6c). Finally, Zn release rates in *Hexagenia* treatments were significantly lower than in *C. riparius* treatments (*p* < 0.001); hence, *C. riparius* resulted in significantly greater Zn release rates than did the other two study organisms. On an annual basis, I found that Zn release rates from sediment by *C. riparius* were highest among the study organisms, and estimated to be 4.7 kg m^{-2} yr^{-1}, while *Hexagenia* spp. Zn release rates were lowest and calculated to be 0.13 kg m^{-2} yr^{-1}.

DISCUSSION

I have demonstrated that bioturbation/bioirrigation from burrowing benthic invertebrates can cause phosphorus and trace metal release from sediments, and be a source of internal loading to the Great Lakes.

Additionally, release rates of phosphorus and trace metals can differ between organisms, and I qualitatively observed that native *Hexagenia* spp. and *Chironomus riparius* decreased water clarity compared to the nonnative *Branchiura sowerbyi* (Figure 5-1). Decreased water clarity (or conversely, increased turbidity) by organisms is likely correlated with the differences observed in phosphorus and trace metals release from sediment, driven by differences in the extent of bioturbation/bioirrigation. For example, Matisoff and Wang (2000) reported that

Hexagenia expels large quantities of sediment from burrows that increases water turbidity in a microcosm, which aligns with my observations (Figure 5-1). The increased turbidity from bioturbation and/or bioirrigation can result in mobilization of phosphorus and trace metals to overlying water.

The vertical influence of bioturbation/bioirrigation in sediment layers ultimately influences the degree to which constituents are released to the overlying water. I observed a general trend of greater (although not significant) number of microbeads at the surface of sediment layers by *Hexagenia* spp. and *Chironomus riparius* compared to *Branchiura sowerbyi* and control treatments (Figure 5-2). *Hexagenia* and *C. riparius* actively aerate burrow structures, pulling in water with greater concentrations of DO and expelling pore water from burrows to the water column (Charbonneau and Hare 1998). In doing so, microbeads (acting as sediment particles) were likely transported to the surface to a greater extent compared to the *B. sowerbyi* treatments. Conversely, *B. sowerbyi* burrows into sediment head down (Ståhl-Delbanco and Hansson 2002) to feed, and does not behaviorally aerate sediment layers. Thus, when burrowing, sediment particles are expected to be pushed downward into the sediment and not mixed back to the surface, as suggested by the greater number of microbeads in *B. sowerbyi* treatments at the deepest sediment interval (Figure 5-2).

I observed that release rates of TP and TDP were greater at 30°C compared 20°C (Figures 5-3 and 5-4, respectively). Climate change is expected to increase water temperatures in the Great Lakes (Magnuson et al. 1997). Higher rates of phosphorus released from sediment in warmer water temperatures has implications for internal loading from climate change. Based on these experimental results I predict that bioturbation/bioirrigation under higher temperatures will

likely result in increased rates of phosphorus release from sediment, and thus a greater influence of internal nutrient loading, acting as a positive feedback loop on eutrophication.

Sediment release rates of TP and TDP were not significantly different among organisms at 20°C (Figure 5-3) or 30°C (Figure 5-4). However, TP release rates caused by *Hexagenia* spp. and *Chironomus riparius* were approximately 20% and 69% greater than controls at 20°C, respectively. Sediment release rates of TDP from the study organisms were not greater than controls, suggesting that bioturbation may be the dominant mechanism by which these particular burrowing invertebrates cause sediment phosphorus flux, and that these organisms do not directly increase availability of dissolved phosophorus. For example, bioturbation from burrow construction, which was immediately observed when organisms were placed into microcosms (and also noted by Matisoff and Wang (2000)), likely suspended sediment and associated TP. Subsequent sediment suspension and flux of TP likely is facilitated by burrow aeration. On the other hand, the influence of bioirrigation (ventilation of sediment, increasing flux of soluble constituents) appears to be secondary, as no differences in release rates of TDP were observed among *Hexagenia, C. riparius* and control treatments (Figure 5-4).

Respiration rates among the study organisms differed at 20°C and 30°C (Brainard and Schulz, unpublished data). At both temperatures, *Branchiura sowerbyi* had decreased rates of respiration compared to both *Hexagenia* spp. and *Chironomus riparius*. Increased respiration rates of *Hexagenia* spp. and *C. riparius* compared to *B. sowerbyi* suggest increased overall activity of the native invertebrates, and thus likely contributed to the pattern of greater TP flux rates to overlying water, particularly at 20°C (Figure 5-3a). Increased activity, related to greater rates of respiration, may be driven by burrowing aeration behavior of *Hexagenia* spp. and *C. riparius*.

Increased rates of TP and TDP sediment release occurred in hypoxia compared to high and pulsed DO (Figure 5-5), suggesting an abiotic influence of sediment phosphorus release in the low DO conditions in my experiment (i.e., redox conditions causing P release). However, there was an increase in flux of TP and TDP within organism treatments relative to controls ranging from 0.045 to 0.047 mg m^{-2} yr^{-1} (particularly in *Hexagenia* spp. and *Chironomus riparius* treatments, Figure 5-5) representing a percent difference from controls up to 25%. This increase in TP and TDP flux from native invertebrate treatments suggests invertebrates can increase phosphorus release (in addition to abiotic influences) through bioturbation/bioirrigation during prolonged hypoxia. Periods of increased hypoxia are predicted with climate change in the Great Lakes (Magnuson et al. 1997) due to increased water temperatures causing greater productivity of primary producers (e.g., phytoplankton). As phytoplankton are decomposed by microbes, a decline in DO can occur, depending on mixing patterns. Thus, abiotic and biotic driven sediment release of phosphorus from historic external loading when hypoxic conditions persist may increase internal loading and lead to eutrophication.

High DO conditions (mg L^{-1} at saturation) were assumed to permit normal bioturbation/bioirrigation activity of invertebrates due to a lack of low DO-related physiological and/or behavioral stress. Phosphorus sediment release in pulsed DO treatments, with oxygen concentrations lowered and raised daily, simulating diurnal changes in anoxia nearshore, were lower than release rates in high DO (Figure 5-5). Pulsed DO may be predicted to cause half the normal bioturbation/bioirrigation activity compared to high DO, as oxygen concentrations were reduced to hypoxic levels for half of the total experiment duration, with invertebrates likely stressed by low DO. The release rates of phosphorus in high (0.17 mg m^{-2} hr^{-1}) and pulsed DO (0.047 mg m^{-2} hr^{-1}) suggest that normal bioturbation/bioirrigation activity in pulsed DO resulted

in release rates greater than half those in high DO. In highly productive nearshore zones of the

Great Lakes, where DO can be high during day through photosynthesis and decline at night from

respiration, the effects of bioturbation/bioirrigation by benthic invertebrates on internal nutrient

loading may be ameliorated compared to loading during persistent high or hypoxic DO states.

Respiration rates among the study organisms did not differ among DO treatments (high,

hypoxic, and pulsed), however, a pattern of decreased respiration rates by *Branchiura sowerbyi*

compared to *Hexagenia* spp. and *Chironomus riparius* was observed under hypoxic conditions

(Brainard and Schulz, unpublished data). Further, survival of *B. sowerbyi* individuals compared

to the two native invertebrates was 46% to 56% greater in hypoxia (survival average of 20°C and

30°C treatments). These patterns of reduced respiration but greater survival, suggest that *B.

sowerbyi* can tolerate low DO environments better than native *Hexagenia* spp. and *C. riparius*,

perhaps through reducing overall activity. Reductions in activity (e.g., bioturbation/bioirrigation)

would be predicted to result in less mobilization of phosphorus from sediment to overlying

water, as indicated by generally lower TP and TDP release rates by *B. sowerbyi* in hypoxia

(Figure 5-5).

I found that release rates from sediment of Al, Fe, and Zn differed among organisms and

controls, with increased flux rates of Al by *Branchiura sowerbyi* and *Hexagenia*, increased rates

of Fe release by *B. sowerbyi*, and increased rates of Zn release by *Chironomus riparius* (Figure

5-6). Average released concentrations of Al, Fe, and Zn (as µg L^{-1}) exceeded freshwater acute

and/or chronic U.S. EPA aquatic life criteria (Table 5-3). Observed trace metal concentrations

released are dependent on the baseline values within sediment used in experiments; nevertheless,

my results indicate that when metal concentrations are high in sediment,

bioturbation/bioirrigation from benthic invertebrates can cause elevated concentrations of metals in overlying water.

Toxicity of Al to aquatic biota is largely driven by pH, and can be a limiting factor to production and abundance of aquatic organisms (e.g., plants and invertebrates) at low pH (Sparling and Lowe 1996). For instance, elevated Al (> 500 µg L-1) in low pH environments can directly influence ion regulation and respiration efficiency of aquatic invertebrates (Sparling and Lowe 1996). Aquatic invertebrates also can accumulate high levels of Al in tissues, and the pathway of accumulation is primarily through adsorption (process by which dissolved solids adhere to a surface) rather than through assimilation (absorption and assimilation) (Sparling and Lowe 1996). Nearshore zones of the Great Lakes generally exhibit circumneutral or slightly basic pH. For example, the average pH in 2013 from May through October, in the three nearshore areas of Lake Erie (Old Woman Creek, Sandusky and Maumee Bays) I sampled was 8.5. Thus, elevated internal loading of Al from bioturbation/bioirrigation of burrowing invertebrates is not likely to result in high levels of toxicity to biota, relative to other freshwater ecosystems that may be more at risk due to lower pH levels (e.g., the Adirondacks in the northeast United States, Driscoll et al. 1980).

Iron is one of the most common metals found in aquatic environments (Khalaf et al. 1985), and is considered low toxicity to aquatic biota (Vuori 1995). Certain forms of Fe in aquatic systems, however, (e.g., ferric hydroxide and Fe-hummus precipitates) can cause direct effects through altered metabolic function and osmoregulation of aquatic biota (Vuori 1995). Xing et al. (2010) studied the effect of Fe accumulation on the small, floating macrophyte *Spirodela polyrrhiza*, and found that at tissue concentrations of 1000 µg L^{-1} (chronic effect threshold value by U.S. EPA, Table 5-3), *S. polyrrhiza* displayed localized necrosis of tissue.

Further, Fe co-precipitation of other metals can act to reduce water column concentrations, but increase the amount of metals available to benthic species through dietary intake, leading to toxic effects with food webs (Vuori 1995). At the cellular level of an individual, increased concentrations of Fe can cause the formation of hydroxyl free radicals (Vuori 1995), resulting in cellular deterioration. The amount of internal loading of Fe relative to external sources (Table 5-3) for Lake Erie, in particular, suggests Fe toxicity may be occurring in nearshore zones of the Great Lakes, even though Fe is generally considered non-toxic. The extent to which biota (e.g., invertebrates, fishes) show accumulation of Fe within tissues should be further quantified, and mechanisms of Fe-derived food web toxicity within the Great Lakes further explored.

Elevated Zn concentrations have been documented to be directly toxic to aquatic organisms. For example, increased concentrations of Zn can cause gill epithelial damage in rainbow trout, leading indirect mortality by tissue hypoxia (Skidmore and Tovell 1972; Burton et al. 1972). Cusimano et al. (1986) tested the toxicity of Zn to steelhead trout (*Salmo gairdneri*), and found that at increased pH (up to 7.0) resulted in faster mortality of fish, measured by LC50, compared to low pH values (4.7). Given the relatively neutral-to-alkaline pH concentrations in the Great Lakes, negative effects from elevated internal loading of Zn from bioturbation/bioirrigation may pose greater threats to fish populations than Al and Fe, particularly where *Chironomus riparius* are dominant in nearshore zones (Table 5-3).

Table 5-3. Concentrations of Al, Fe, and Zn ($\mu g \ L^{-1}$) measured in sediment release experiment, compared to U.S. EPA acute and chronic aquatic life water quality criteria concentrations ($\mu g \ L^{-1}$). Bold values indicate exceedances of acute threshold values, and italicized values indicate exceedances of chronic values.

Treatment	Al ($\mu g \ L^{-1}$)	U.S. EPA criteria ($\mu g \ L^{-1}$)		Fe ($\mu g \ L^{-1}$)	U.S. EPA criteria ($\mu g \ L^{-1}$)		Zn ($\mu g \ L^{-1}$)	U.S. EPA criteria ($\mu g \ L^{-1}$)	
		Acute	Chronic		Acute	Chronic		Acute	Chronic
Branchiura sowerbyi	***853***			*5392*			***2443***		
Hexagenia spp.	***764***	750	87	*3616*	N/A	1000	***367***	120	120
Chironomus riparius	*331*			*2593*			***13737***		
Control	*293*			*1998*			***1229***		

Kemp et al. (1976) estimated external inputs of phosphorus and Zn from natural and anthropogenic sources to Lake Erie, and Kelly et al. (1991) derived external input rates of Cr to Lake Erie (Table 5-4). In comparison to my experimentally-derived internal loading estimates, the rates of internal to external loading can be substantial (Table 5-4). For phosphorus, specifically, internal loading at 20°C is far less than external loads, however, internal phosphorus loading from sediment due to bioturbation/bioirrigation exceeds those of external inputs at 30°C (Table 5-4). Therefore, warmer water temperatures associated with climate change in the Great Lakes may result in greater concentrations of phosphorus within waterbodies than what is coming from outside sources (e.g., tributaries, atmospheric inputs). Internally mobilized trace metals from sediment to overlying water may far exceed those of external sources (Table 5-4). Although external loading of nutrients and contaminants into the Great Lakes has largely been reduced, my results suggest that bioturbation/bioirrigation from benthic invertebrates can remobilize historic inputs resulting in a greater contribution of internal loading to currently measured concentrations.

Table 5-4. Experimentally-derived internal loading rates (kg m^{-2} yr^{-1}) of phosphorus at 20°C and 30°C, Zn, and Cr compared to external load estimates.

Organism	Internal Loading (kg m^{-2} yr^{-1})	External Loading[a,b] (kg m^{-2} y^{-1})	Internal:External (kg m^{-2} yr^{-1})
Total Phosphorus - 20°C			
Branchiura sowerbyi	0.0003	0.0015	0.17
Hexagenia spp.	0.0004	0.0015	0.25
Chironomus riparius	0.0004	0.0015	0.26
Total Phosphorus - 30°C			
Branchiura sowerbyi	0.004	0.0015	2.37
Hexagenia spp.	0.005	0.0015	3.60
Chironomus riparius	0.006	0.0015	4.08
Zn			
Branchiura sowerbyi	0.83	0.00035	2401
Hexagenia spp.	0.13	0.00035	360
Chironomus riparius	4.68	0.00035	13500
Cr			
Branchiura sowerbyi	0.015	0.0000001	129337
Hexagenia spp.	0.004	0.0000001	36746
Chironomus riparius	0.004	0.0000001	38277

[a] Phosphorus and Zn external load rates derived from Kemp et al. (1976)
[b] Cr external load rates derived from Kelly et al. (1991)

CONCLUSIONS

My results suggest burrowing invertebrate taxa act to mix (bioturbate) and ventilate (bioirrigate) sediment layers differently. *Branchiura sowerbyi* likely mixes particles deeper, while *Hexagenia* spp. and *Chironomus riparius* mix and ventilate sediment such that particles are moved to the surface. However, these differences in behavioral bioturbation/bioirrigation do not appear to result in differences in TP and TDP flux rates to overlying water from the different taxa, and with the exception of Zn flux from *C. riparius*, do not result in differences in trace metal release. Thus, the introduction of *B. sowerbyi* into the Great Lakes likely has not resulted in greater flux of nutrients and contaminants compared to native burrowing invertebrates. However, internal loading of phosphorus due to bioturbation/bioirrigation, relative to external loads, is likely to be greater in warmer water temperatures associated with climate change in the Great Lakes. Thus, as water temperatures rise, increased eutrophication from legacy nutrients is predicted through the release of phosphorus (Table 5-4). Further, internally-derived metal concentrations from sediment to water far exceed those of external inputs, suggesting that invertebrate macrofauna likely play a critical role in the impairment of Great Lakes water quality by mobilizing legacy nutrient and contaminant elements.

CHAPTER 6: CONCLUSIONS

Anthropogenic influences are inherently linked to species invasions, and include the transport of species to novel environments and alterations in environmental conditions that may promote their success or enhance their negative effects.

Chapter 2

The results from Chapter 2 provide evidence of the direct link between propagule pressure and the presence and abundance (both biomass and dominance) of invasive macrophytes in lake communities. More specifically, results highlight the importance of propagule risk, defined as the extent to which invasives might be introduced from different source populations, as a useful approach to evaluate both invasive macrophyte presence and abundance. Propagule risk also may be linked directly to genetic diversity of invading species, such that increased genetic diversity in an invasive population may be predicted when more individuals from other source waterbodies are continually introduced; this may contribute to the strong effect of propagule pressure on success of invasions and should be studied further.

Further, results suggest a large magnitude of boat movement among lakes in the northeastern U.S. In the context of climate change, one might predict future increased boat movement among lakes, given decreases in ice cover duration as air temperatures increase. The ability for increased 'exposure' to a lake of propagules from invaded waterbodies, given that boating activity can persist for a greater time per year, may lead to increased secondary spread of invasive macrophytes among lakes in the future. The extent of boat movement among lakes with greater boating opportunities in a warmer climate should be evaluated to expand our understanding of invasion dynamics and management efforts to prevent negative impacts from aquatic invasions.

This chapter also secondarily evaluated the effects of environmental factors (watershed land use, water quality parameters) and lake morphology in relation to the presence and abundance of invasive macrophytes. The results of environmental factor influence on invasive macrophytes, in particular, highlighted regional differences between central New York (CNY) and Adirondack (ADK) lakes, confounding the ability to isolate environmental effects on the presence and abundance of invasive macrophytes in the complete dataset. To assess directly the influence of environmental factors on macrophyte invasions, the selection of study lakes with an *a priori* range in environmental factors would be necessary. Such a selection of certain environmental factors as explanatory variables may be possible in experimental lake areas where environmental conditions are more controlled or managed, or in a set of mesocosm studies *in situ*.

Chapter 3

Chapter 3 significantly augments the few reported abundance values for *Nitellopsis obtusa* in North America, and demonstrates that this invasive macroalgae can reduce richness and biomass of other macrophytes when highly abundant. With the potential invasive range of *Nitellopsis obtusa* (Escobar et al. 2016) and the now documented effects on other macrophytes, the importance for early monitoring methods that target *Nitellopsis obtusa* specifically are stressed. Further, other ecosystem effects of *Nitellopsis obtusa* are not fully understood, such as the impacts on macroinvertebrate and fish communities, and influences on abiotic environmental conditions. For example, the seasonal senescence of large standing stocks of *Nitellopsis obtusa* likely results in reduced DO concentrations at depth, and this reduction in available O_2 may be exacerbated with climate change as thermal stratification intensifies.

A possible further extension to the research in Chapters 2 and 3 may be to evaluate the population genetics of invading *Nitellopsis obtusa*, whose range is expanding in the Northeast (Sleith et al. 2015). One might predict greater genetic diversity in *N. obtusa* in lakes with high propagule risk compared to lakes with lower propagule risk.

Chapter 4

Climate change likely will alter the bioenergetic performance of both invasive and native benthic macroinvertebrates. Chapter 4 demonstrated that increased water temperatures and reduced DO concentrations may lead to different bioenergetic performance of the nonnative *Branchiura sowerbyi* in comparison to native *Hexagenia* spp. and *Chironomus riparius*. Although *Branchiura sowerbyi* is not dominant in many locations in the Great Lakes, given its low respiration and high growth rates, plus its ability to survive adverse abiotic conditions (e.g., hypoxia) compared to the two native invertebrates studied, climate change may result in greater dominance of *Branchiura sowerbyi* in the benthos of the Great Lakes. Additionally, the results of Chapter 4 have implications for the ongoing recovery of *Hexagenia* spp. in the Great Lakes. Once highly abundant, *Hexagenia* spp. populations crashed in the mid-20th century due to mainly to eutrophic conditions resulting in intolerable DO conditions. Populations of *Hexagenia* have since rebounded, but current eutrophic conditions (e.g., western Lake Erie) may result in decreased population densities of *Hexagenia*. Coupled with increased water temperatures and reduced DO at depth, as predicted with climate change, these abiotic conditions may limit subsequent recovery of *Hexagenia*. Finally, the results from the bioenergetics measurements of the macroinvertebrates studied indicate the importance of pulsed DO conditions on physiological performance. In nearshore areas of the Great Lakes, that may be predicted to have greater influence of pulsed DO conditions, the negative effects of climate change on macroinvertebrates

may be ameliorated compared to prolonged hypoxic conditions (such as those in more open water habitats). The impacts of pulsed DO in the context of climate change should be further evaluated by freshwater ecologists to fully understand the impacts of climate change on freshwater populations, as the response to hypoxia alone did not predict the response to pulsed oxygen in this study.

Chapter 5

There was generally not a significant difference in phosphorus sediment release between *Branchiura sowerbyi* and native *Hexagenia* spp. and *Chironomus riparius*, as evaluated in Chapter 5. However, the bioturbation and bioirrigation activities of burrowing invertebrates as a source of internal release from legacy inputs may be a significant source, potentially leading to continued decline in freshwater ecosystem health even as external inputs are reduced. Pulsed DO conditions appear to ameliorate the effects of sediment phosphorus release, in particular, suggesting potentially lower release of sediment phosphorus in nearshore areas with pulsed compared to high and hypoxic DO conditions. These nearshore areas with diurnal fluctuations of DO may be refuge areas to sustain populations (see Chapter 4), while contributing less phosphorus to overall internal inputs of nutrients promoting eutrophication.

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APPENDICES

Chapter 2

Table 2-S1. Day-specific boat launch usage factors, calculated as proportion of the number of boat launch visits for each day of the week relative to total boat launch visits across all days of the week. The year surveyed is listed in parentheses after the lake name.

Lake	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Source
Lake Placid 2006	-	0.10	0.09	0.13	0.18	0.27	0.23	AWI 2006
Lake Placid 2007	0.18	0.13	0.13	0.13	0.11	0.17	0.15	AWI 2007
Cazenovia 2011	0.12	0.10	0.10	0.11	0.15	0.24	0.19	Village of Cazenovia 2011
Cazenovia 2012	0.09	0.07	0.13	0.12	0.15	0.22	0.21	Village of Cazenovia 2012
Raquette Lake 2008	0.13	0.04	0.02	0.02	0.29	0.26	0.25	AWI 2008
Upper St. Regis 2008	0.07	0.15	0.15	0.14	0.19	0.15	0.20	AWI 2008
Lake Placid 2009	0.15	-	-	0.12	0.21	0.24	0.24	AWI 2009
Upper St. Regis 2009	0.06	0.01	0.15	0.14	0.14	0.22	0.21	AWI 2009
Lake Placid 2010	0.12	0.09	0.09	0.08	0.10	0.20	0.16	AWI 2010
Fourth Lake 2011	0.01	0.00	0.00	0.01	0.01	0.02	0.02	AWI 2011
Saratoga 2011	0.09	0.09	0.12	0.12	0.13	0.24	0.23	AWI 2011
Saratoga 2012	0.08	0.07	0.06	0.09	0.13	0.29	0.28	AWI 2012
AVERAGE (SD)	0.1 (± 0.05)	0.08 (± 0.05)	0.1 (± 0.05)	0.1 (± 0.04)	0.15 (± 0.07)	0.21 (± 0.07)	0.2 (± 0.07)	

Table 2-S2. Morphological characteristics and water quality parameters for the 20 study lakes. Littoral zone areas were not calculated for Crooked Lake, Gatehouse Pond, and Echo Lake, because bathymetric maps are not available for these waterbodies. Water quality parameters are included for lakes with available data through the New York State Citizens Statewide Lake Assessment Program (CSLAP) and Adirondack Lake Assessment Program (ALAP). CNY = Central New York; ADK = Adirondacks.

Lake	Region	Latitude	Longitude	MORPHOLOGY				WATER QUALITY						
				Avg Depth (m)	Max Depth (m)	Lake Area (km²)	Littoral:Total Area	Transparency (m)	Chl-a (µg/L)	Total P (µg/L)	Conductivity (µS/cm)	pH	Years Included	Source
Little York Lake	CNY	42°42′14.97″	76°09′17.82″	4.6	22.9	0.4	0.94	4.1	1.7	14	413.5	7.5	2010 - 2013	CSLAP
Tully Lake	CNY	42°46′36.55″	76°08′04.96″	4.5	10.4	0.9	0.57	4.2	4.9	15	487.5	7.8	2012 - 2015	CSLAP
Crooked Lake	CNY	42°47′12.34″	76°09′00.11″	4.9	24.4	0.4	--	3.3	7.3	17	134.8	7.7	2010 - 2013	CSLAP
Song Lake	CNY	42°46′03.52″	76°08′47.43″	4.0	8.6	0.4	0.33	3.2	4.1	17	202.5	7.6	2010 - 2014	CSLAP
Gatehouse Pond	CNY	42°48′04.46″	76°08′55.73″	3.7	22.9	0.0	--	--	--	--	--	--	--	--
Echo Lake	CNY	42°23′08.62″	75°51′02.30″	4.8	9.2	0.2	--	2.9	4.4	13	46.3	7.4	2010 - 2014	CSLAP
Fourth Lake	ADK	43°45′24.21″	74°50′45.12″	10.2	24.4	8.3	0.12	--	--	--	--	--	--	--
Seventh Lake	ADK	43°44′38.43″	74°44′27.84″	12.2	26.5	3.4	0.28	--	--	--	--	--	--	--
Eighth Lake	ADK	43°46′37.15″	74°42′10.30″	11.9	24.7	1.2	0.14	--	--	--	--	--	--	--
Blue Mountain Lake	ADK	43°51′42.34″	74°27′34.64″	7.6	30.5	5.0	0.02	8.0	0.8	6.1	62.7	7.0	2014, 2015	ALAP
Cranberry Lake	ADK	44°10′26.58″	74°50′01.68″	1.8	11.6	27.5	0.42	3.4	4.0	7.8	15.5	7.0	2014, 2015	ALAP
Lake Flower	ADK	44°18′54.05″	74°07′29.88″	1.5	3.0	0.7	0.92	--	--	--	--	--	--	--
Lake Placid	ADK	44°19′10.56″	73°58′28.60″	15.8	46.0	7.9	0.07	6.4	1.4	4.4	25.7	6.7	2014	ALAP
Long Lake	ADK	44°00′05.56″	74°23′49.72″	4.0	13.1	16.6	0.99	--	--	--	--	--	--	--
Raquette Lake	ADK	43°50′04.88″	74°38′29.09″	13.4	29.0	21.0	0.20	3.5	2.0	7.2	29.0	6.9	2014, 2015	ALAP
Stillwater Reservoir	ADK	43°53′37.47″	75°01′02.18″	1.9	9.8	25.2	0.33	--	--	--	--	--	--	--
Tupper Lake	ADK	44°10′01.97″	74°32′15.60″	11.9	30.5	19.7	0.16	4.4	2.3	9.0	24.0	6.9	2014, 2015	ALAP
Arbutus Pond	ADK	43°59′13.24″	74°14′27.76″	1.9	7.6	0.5	0.46	3.3	3.3	8.6	16.3	6.8	2014, 2015	ALAP
Chateaugay Lake	ADK	44°44′18.00″	73°58′06.09″	10.1	23.8	10.4	0.20	3.4	2.8	7.3	67.4	7.0	2014, 2015	ALAP
Second Pond	ADK	44°17′01.13″	74°10′46.65″	1.5	2.4	0.3	1.00	--	--	--	--	--	--	--

Table 2-S3. Macrophyte species present within each study lake (X). Invasive macrophyte species highlighted in grey.

Macrophyte	CNY Region						ADK Region													
	Little York Lake	Tully Lake	Crooked Lake	Song Lake	Gatehouse Pond	Echo Lake	Fourth Lake	Seventh Lake	Eighth Lake	Blue Mtn Lake	Cranberry Lake	Lake Flower	Lake Placid	Long Lake	Raquette Lake	Stillwater Reservoir	Tupper Lake	Arbutus Pond	Upper Chateaugay Lake	Second Pond
Alisima sp.													X							
Bidens beckii				X																
Brasenia schreberi			X			X					X						X			
Callitriche sp.						X														
Ceratophyllum demersum	X	X	X		X														X	X
Ceratophyllum echinatum			X		X															
Chara	X	X	X	X	X	X	X						X						X	
Cyperaceae sp											X									
Elatine minima																	X			
Eleocharis acicularis				X								X	X		X				X	X
Eleocharis acicularis inundata																		X		
Eleocharis palustris						X														
Eleocharis robbinsii						X														
Eleocharis sp.		X																		
Elodea canadensis	X	X	X	X	X		X	X	X		X	X		X			X		X	
Elodea nuttallii								X			X			X						
Eriocaulon aquaticum						X	X	X	X	X	X	X	X	X	X	X	X	X		X
Gramineae											X									
Isoetes echinospora			X	X			X	X	X		X	X	X	X			X			
Isoetes lacustris													X							
Isoetes sp.						X	X						X					X	X	X
Isoetes tuckermanii							X	X	X	X			X	X	X	X	X	X	X	
Juncus filiformis																X				
Juncus militaris																X				
Juncus pelocarpus							X	X	X	X	X					X				
Juncus sp.													X						X	

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Species																					
Lobelia dortmanna							x	x	x	x			x	x	x	x	x	x	x		
Myriophyllum alterniflorum	x	x	x	x	x																
Myriophyllum heterophyllum	x						x	x			x	x		x	x			x			x
Myriophyllum sibiricum		x		x	x															x	
Myriophyllum spicatum	x	x	x		x			x				x								x	
Myriophyllum tenellum						x	x		x	x			x		x		x	x	x		
Najas fle1ilis	x	x	x		x		x													x	
Najas guadalupensis													x							x	
Neobeckia aquatica				x																	
Nitella sp.							x	x			x	x		x	x			x		x	
Nitellopsis obtusa	x	x	x		x																
Nuphar advena	x		x	x	x															x	
Nuphar sp.								x	x												
Nuphar variegata			x			x				x	x	x		x	x			x			
Nymphaea odorata		x				x														x	x
Pontederia cordata						x														x	
Potamogeton amplifolius						x															
Potamogeton crispus	x	x	x																		
Potamogeton diversifolius							x														
Potamogeton epihydrus							x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Potamogeton gramineus																				x	x
Potamogeton illinoensis	x	x	x	x	x	x	x		x			x		x		x		x		x	x
Potamogeton nodosus																					x
Potamogeton pectintus	x	x	x		x															x	
Potamogeton perfoliatus							x													x	
Potamogeton praelongus	x	x	x	x			x					x								x	
Potamogeton pusillus																			x		
Potamogeton richnardsonii							x					x			x						
Potamogeton robbinsii							x	x	x			x			x				x	x	x
Potamogeton zosteriformis	x	x	x	x	x															x	
Ranunculus flammula							x														

Species																					
Sagittaria aquaticum													x								
Sagittaria cuneata							x	x	x			x	x		x						
Sagittaria graminea																			x	x	x
Sagittaria sp.						x									x						
Schoenoplectus subterminalis								x	x	x	x	x	x	x	x	x					
Scirpus sp.		x												x							
Scirpus validas																					
Sparganium sp.						x	x	x		x	x	x	x	x			x	x	x	x	
Utricularia inflata							x	x					x		x	x					
Utricularia minor											x	x									
Utricularia purpurea								x	x		x	x	x	x			In	x			
Utricularia sp.							x						x							x	
Utricularia vulgaris	x	x	x		x							x		x							
Vallisneria americana	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x		
Zosterella dubia	x	x	x		x																

Table 2-S4. Macrophyte abundance (total and invasive), invasive macrophyte richness, propagule risk, and watershed land use for the 20 study lakes. For determination of PropRisk$_{total\ events}$ and PropRisk$_{distinct\ sources}$ see Figure 1b. CNY = Central New York; ADK = Adirondacks.

Lake	Region	Total Biomass (g/m²)	Invasive Biomass (g/m²)	Invasive Biomass (%)	Invasive richness	Prop Risk$_{total\ events}$ (no./yr)	Prop Risk$_{distinct\ sources}$ (no./yr)	Watershed Land Use			
								Residential (%)	Urban (%)	Agriculture (%)	Barren (%)
Little York Lake	CNY	219.5	131.0	59.7	4	168	111	12.9	0.0	21.9	0.0
Tully Lake	CNY	613.0	76.7	12.5	3	190	141	9.8	0.4	42.0	0.0
Crooked Lake	CNY	216.5	34.7	16.0	3	105	105	2.6	0.0	36.3	0.0
Song Lake	CNY	56.2	0.0	0.0	0	40	40	3.5	0.0	18.1	0.0
Gatehouse Pond	CNY	160.4	117.5	73.2	2	13	13	1.7	0.0	31.8	0.0
Echo Lake	CNY	124.9	0.0	0.0	0	22	22	2.2	0.0	11.9	0.0
Fourth Lake	ADK	17.3	0.01	0.1	2	118	22	3.1	0.0	0.1	0.0
Seventh Lake	ADK	4.5	0.1	2.9	3	39	16	3.4	0.0	0.1	0.0
Eighth Lake	ADK	33.9	0.0	0.0	0	29	19	2.9	0.0	0.0	0.0
Blue Mountain Lake	ADK	30.5	0.0	0.0	0	23	12	3.2	0.0	0.0	0.3
Cranberry Lake	ADK	2.9	0.7	23.1	1	75	22	0.1	0.0	0.0	0.0
Lake Flower	ADK	66.4	20.8	31.4	2	105	22	2.6	0.1	0.1	0.0
Lake Placid	ADK	27.6	0.0	0.0	0	98	28	2.7	0.1	0.0	0.0
Long Lake	ADK	7.4	3.1	42.5	2	102	30	1.0	0.0	0.0	0.0
Raquette Lake	ADK	16.1	2.1	13.2	1	56	23	1.0	0.0	0.0	0.1
Stillwater Reservoir	ADK	10.9	0.05	0.5	1	39	16	0.1	0.0	0.0	1.1
Tupper Lake	ADK	4.9	0.06	1.2	2	77	33	1.0	0.0	0.1	0.0
Arbutus Pond	ADK	23.5	0.0	0.0	0	0	0	0.6	0.0	0.0	0.0
Chateaugay Lake	ADK	19.3	1.2	6.4	1	133	38	1.4	0.0	1.2	0.2
Second Pond	ADK	17.7	12.0	67.7	1	234	45	2.8	0.1	0.1	0.0

Table 2-S5. Linear regression equations, p-values, and R^2 values for significant correlations between variables explaining propagule risk ($PropRisk_{total\ events}$ and $PropRisk_{distinct\ sources}$), and invasive biomass (g/m^2) and dominance (%) as response variables. NS = not significant. CNY = Central New York, ADK = Adirondacks.

Response		All macrophytes (including macroalgae)		Higher plants only (excluding macroalgae)	
		Invasive biomass (g/m²)	Invasive dominance (%)	Invasive biomass (g/m²)	Invasive dominance (%)
$PropRisk_{total\ events}$	All	0.015(x) + 0.151, p = 0.021, R² = 0.261	0.003(x) + 0.090, p = 0.024, R² = 0.252	0.014(x) - 0.107, p = 0.001, R² = 0.444	0.004(x) − 0.011, p < 0.001, R² = 0.573
	ADK	0.011(x) - 0.194, p = 0.013, R² = 0.413	0.004(x) - 0.034, p = 0.004, R² = 0.507	0.011(x) − 0.202, p = 0.013, R² = 0.412	0.004(x) − 0.030, p = 0.005, R² = 0.499
	CNY	NS	NS	0.017(x) + 0.381, p = 0.05, R² = 0.643	0.003(x) + 0.029, p = 0.017, R² = 0.792
Response		**Invasive biomass (g/m²)**	**Invasive dominance (%)**	**Invasive biomass (g/m²)**	**Invasive dominance (%)**
$PropRisk_{distinct\ sources}$	All	0.033(x) + 0.120, p = 0.001, R² = 0.462	NS	0.027(x) + 0.050, p < 0.001, R² = 0.557	0.004(x) + 0.136, p = 0.023, R2 = 0.255
	ADK	NS	0.017(x) - 0.118, p = 0.022, R² = 0.365	NS	0.017(x) - 0.114, p = 0.024, R² = 0.358
	CNY	NS	NS	0.026(x) + 0.050, p = 0.032, R² = 0.722	0.005(x) - 0.001, p = 0.031, R² = 0.727

Table 3-S1. Macrophyte species sampled by lake.

Species	Little York Lake	Tully Lake	Crooked Lake	Gatehouse Pond
Brasenia schreberi			X	
Ceratophyllum demersum	X	X	X	X
Ceratophyllum echinatum			X	X
Chara	X	X	X	X
Eleocharis sp.		X		
Elodea canadensis	X	X	X	X
Isoetes echinospora			X	
Myriophyllum alterniflorum	X	X	X	X
Myriophyllum heterophyllum	X			
Myriophyllum sibiricum		X		X
Myriophyllum spicatum	X	X	X	X
Najas flexilis	X	X	X	X
Nitellopsis obtusa	X	X	X	X
Nuphar advena	X		X	X
Nuphar variegata			X	
Nymphaea odorata		X		
Potamogeton crispus	X	X	X	
Potamogeton illinoensis	X	X	X	X
Potamogeton pectintus	X	X	X	X
Potamogeton praelongus	X	X	X	
Potamogeton zosteriformis	X	X	X	X
Scirpus sp.		X		
Utricularia vulgaris	X	X	X	X
Vallisneria americana	X	X	X	X
Zosterella dubia	X	X	X	X

Table 3-S2. Total and native species richness and average *Nitellopsis* biomass (g/m^2) at deep (>2 m), intermediate (1–2 m), shallow (<1 m) sample locations across the 4 study lakes.

Depth	Total macrophytes		Native macrophytes	
	Species richness	*Nitellopsis* biomass (g/m^2)	Species richness	*Nitellopsis* biomass (g/m^2)
Deep	1	309.0	0	298.6
	2	296.1	1	329.0
	3	399.1	2	254.1
	4	116.1	3	202.0
	5	203.2	4	117.6
	6	87.7	5	83.9
	7	106.0	7	1.5
	8	32.6		
	9	1.5		
Intermediate	1	434.1	0	495.6
	2	287.5	1	305.1
	3	193.2	2	137.6
	4	119.0	3	78.7
	5	72.2	4	123.6
	6	127.8	5	55.8
	7	44.5	6	223.2
	8	223.2	8	43.5
	10	43.5		
Shallow	1	397.4	0	365.0
	2	294.7	1	323.2
	3	228.1	2	144.0
	4	188.9	3	185.2
	5	78.2	4	59.9
	6	80.2		
	7	3.3		

Table 3-S3. Mean biomass of *Nitellopsis*, native macrophytes, and nonnative macrophytes by lake.

Lake	*Nitellopsis* (g/m^2)	Native macrophytes (g/m^2)	Nonnative macrophytes (g/m^2)
Little York Lake	120.1	83.5	15.8
Tully Lake	45.9	536.9	30.9
Crooked Lake	3.3	180.6	32.6
Gatehouse Pond	140.7	42.9	4.1
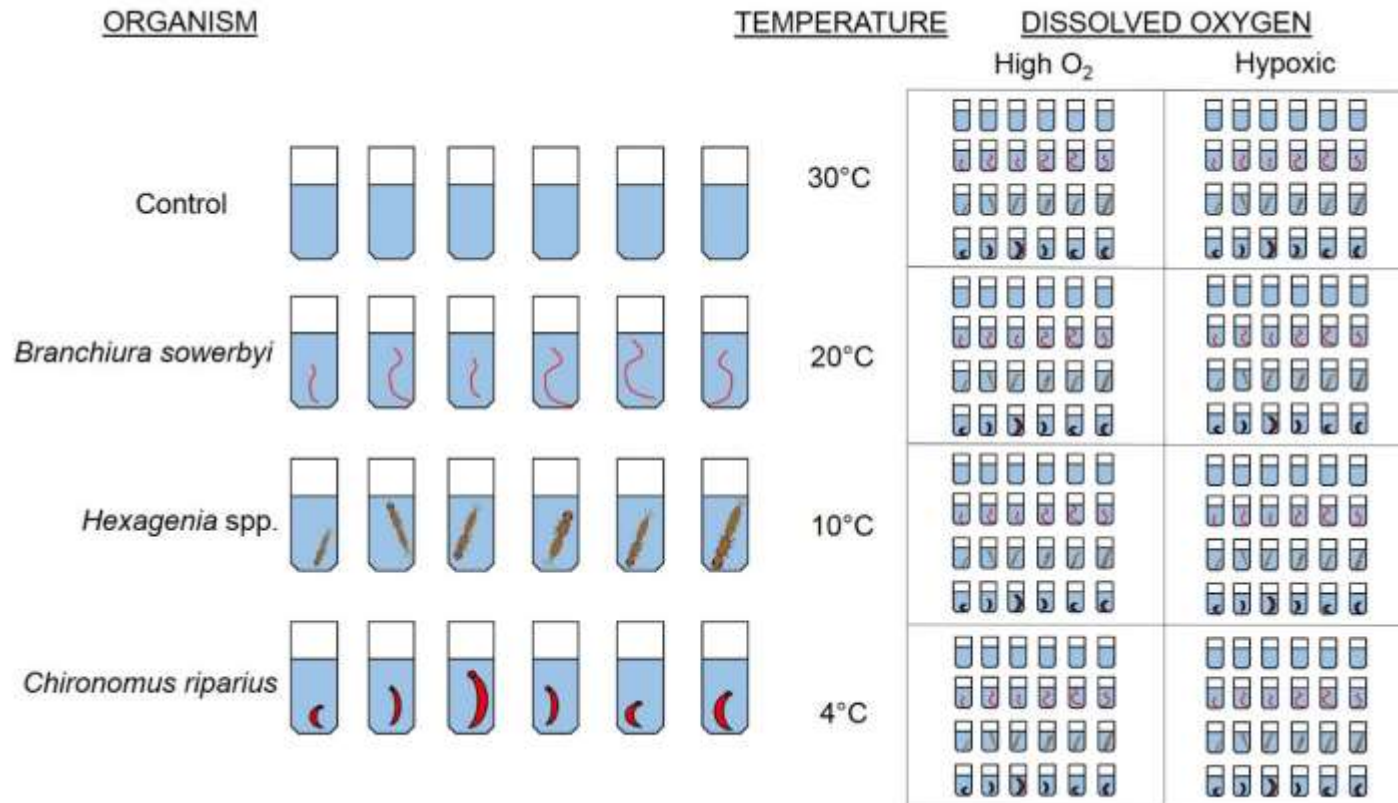
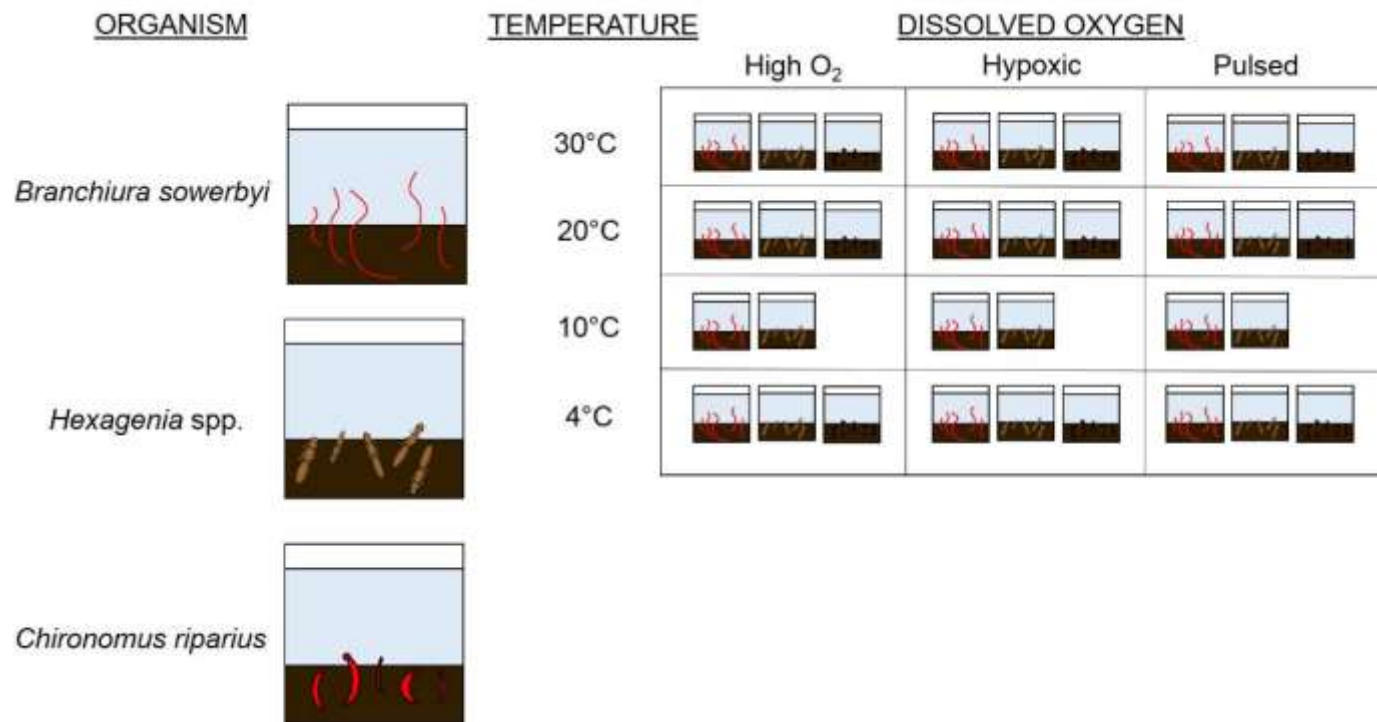Figure 4-S1. Depiction of experimental design used for excretion/egestion experiments.

Figure 4-S2. Depiction of experimental design used for laboratory growth experiments.
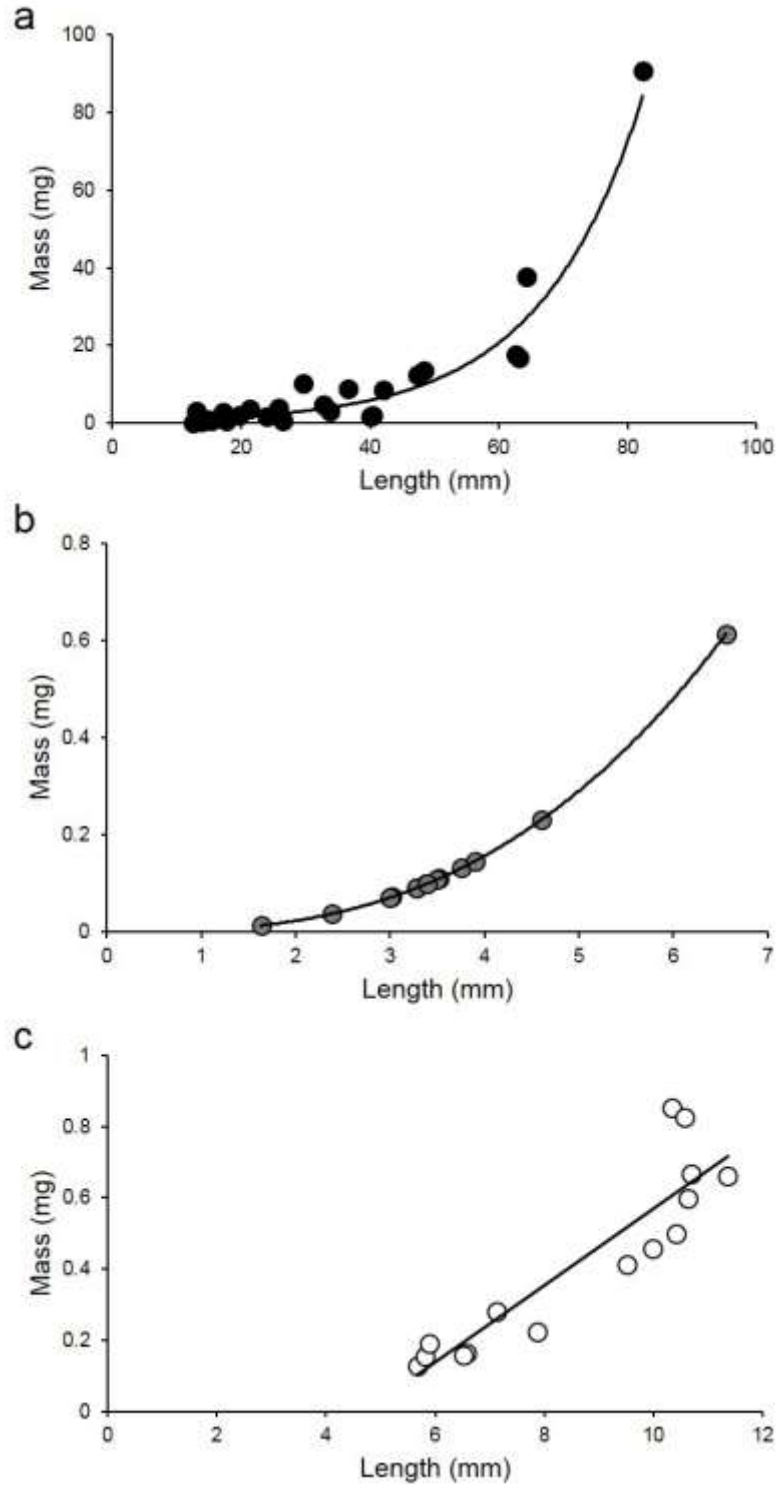
Figure 4-S3. Length-weight regressions for (a) *Branchiura sowerbyi*, (b) *Hexagenia* spp., and (c) *Chironomus riparius*. Regression equations: (a) Mass $= 0.4799$ e$^{0.0628(\text{length})}$, $p < 0.001$, $R^2 = 0.750$; (b) Mass $= 0.0034$ (length)$^{2.764}$, from Benke et al. (1999); (c) Mass $= 0.108$ (length) $- 0.513$, $p < 0.001$, $R^2 = 0.820$.

RESUME

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Education

	Name and Location	Dates	Degree
High School:	Owego Free Academy (OFA) Owego, NY	1998-2002	Regents Diploma
College:	West Chester University West Chester, PA	2006-2008	Bachelor of Science
Graduate School:	West Chester University West Chester, PA	2008-2010	Master of Science
	SUNY ESF Syracuse, NY	2011-2018	Doctor of Philosophy

Employment

Employer	Dates	Job Title
O'Brien & Gere	9/2014 - current	Scientist