DAPHNIA VERTICAL POSITION AND IMPLICATIONS FOR THE
IMPACT OF THE INVASIVE ZOOPLANKTON PREDATOR,
B.YTHOTR.PHES LONGIMANUS, ON PLANKTON COMMUNITIES IN
SOUTH-CENTRAL ONTARIO

by

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Abstract

Species diversity and identity play an important role in determining community structure and regulating ecosystem processes. However, species interactions are often characterized by the mean effect or response, often ignoring intraspecific variation in trait expression. In this thesis, I examined the ecological consequences of intraspecific trait variation in *Daphnia* vertical position in plankton communities invaded by the presence of the invasive zooplankton predator, Spiny water flea (*Bythotrephes longimanus*). In the first chapter, I assessed the factors influencing *Daphnia* vertical position response to *Bythotrephes* presence using a field survey of invaded and uninvaded lakes across a gradient of abiotic variables in south-central Ontario. In the second chapter, I examined the influence of *Daphnia* vertical position on *Bythotrephes* impacts on plankton communities using mesocosm experiments where I manipulated *Bythotrephes* presence and *Daphnia* vertical position. In the final chapter, I used mesocosm experiments to determine if differences in the vertical position of dispersing *Daphnia* influenced recovery in *Daphnia* populations invaded by *Bythotrephes*. Results from my field survey show that *Daphnia* vertical position response to *Bythotrephes* is species specific, with only *D. mendotae* and *D. longiremis* vertical position influenced by *Bythotrephes* presence. In invaded lakes, *D. mendotae* vertical position in dependent on water clarity and *Bythotrephes* density. Results from my mesocosms experiment suggest that *Daphnia* vertical position influences the type and magnitude of *Bythotrephes* impacts on *Daphnia* populations, resulting in indirect impacts on small cladocerans, large cladocerans and copepodids. Furthermore, differences in *Daphnia* vertical position in invaded mesocosms influenced algal densities, resulting in trophic cascades in mesocosms where more *Daphnia* were hypolimnetic. There was no effect of differences in vertical position of dispersing *Daphnia* on Daphnia populations invaded by *Bythotrephes*. Taken
together, these results suggest that *Daphnia* vertical position response to *Bythotrephes* is species specific and that differences in *Daphnia* vertical position result in differences in *Bythotrephes* impacts on plankton communities in invaded lakes.
Co-Authorship

This thesis conforms to the publication format as outlined in the Department of Biology Guide to Graduate Studies, with consolidated Acknowledgements sections. The contributions made by co-authors are outlined below:

Chapter 2: Dr. Shelley Arnott provided assistance with the survey design, interpretation of data, analysis and manuscript preparation.
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List of Abbreviations, Acronyms, and Symbols

AICc – Akaike Information Criterion corrected for small sample sizes
Ca – Dissolved Calcium
CAISN – Canadian Aquatic Invasive Species Network
DOC – Dissolved Organic Carbon
FDR – False Discovery Rate
GLHT – Generalized Linear Hypothesis Testing
GLM – Generalized Linear Model
GLMM – Generalized Linear Mixed Model
NA – Not applicable
NASA – National Aeronautics and Space Administration
PAR – Photosynthetically Active Radiation
TP – Total Phosphorus
UV A – Ultraviolet Radiation A
UV B – Ultraviolet Radiation B
Zmax – Maximum depth of a lake
Chapter 1

General Introduction

Species diversity and identity play an important role in shaping community structure and regulating ecosystem function, with dominant species, keystone species, ecosystem engineers and foundation species having a disproportionate impact on the abundance and diversity of other species (Brooks and Dodson 1965, Paine 1966, Carpenter et al. 1987, Jones et al. 1994, Schmitz and Suttle 2001, Whitham et al. 2006). In ecological contexts, species interactions are often characterized by a mean effect or response, known as “the mean field theory”, which can obscure the effects of intraspecific differences in trait expression in these interactions (Violle et al. 2012). Despite a large degree of intraspecific variation in trait expression, both within and between populations, observed in many systems (Cam et al. 2002, Bolnick et al. 2003, Hallgrimsson and Hall 2003), and the impact of these differences on the type and strength of ecological interactions (MacArthur and Levins 1967, Abrams and Matsuda 1997), mean field theory is a widely applied in community ecology at the species level, with a focus on assessing differences in traits between species. However, an increasing number of studies are examining the role of intraspecific trait variation in shaping and maintaining community structure and ecosystem function (Treseder and Vitousek 2001, Johnson and Agrawal 2005, Post el a. 2008, Duffy 2010, Schreiber et al. 2011, Jung et al. 2014, Lajoie and Vellend 2015, Glassmire et al. 2016).
Sources of within species variation in trait expression

Individuals within a species or population can vary in their expression of a number of ecologically important traits including anti-predator defenses (Arnold and Bennett 1984, Duffy 2010), parasite resistance or exposure (Pearl et al. 2008, Johnson et al. 2009, Ganz and Ebert 2010), tolerance to abiotic disturbances (Fangue et al. 2008, Meyer et al. 2009), resource use and preference (reviewed in Bolnick et al. 2002), and competitive ability (Metcalf 1986, Aarssen 1992, Lankau and Strauss 2007). These differences are a consequence of heritable genetic differences between individuals or phenotypic plasticity in trait values across varying environmental conditions. The impact of this variation on community structure and ecosystem function depends on whether this variation is a consequence of heritable differences or phenotypic plasticity. If within population differences in trait expression are a result of inherited genetic differences, population level means are expected to change as the abiotic and biotic environment filter out traits that confer low fitness (Moran et al. 2016). In contrast, phenotypic plasticity allows individuals to adjust trait expression in response to abiotic and biotic cues, buffering populations against natural and anthropogenic disturbances (Moran et al. 2016), although the degree of plasticity is itself inherited and subject to selection (Crispo 2008). Both heritable genetic differences and phenotypic plasticity can be influenced by epigenetic changes (also known as maternal effects or transgenerational plasticity), affecting gene expression but not the genetic code, and can be inherited over short and intermediate time scales. For example, rats exposed to pesticides show negative fitness across three generations due to the inheritance of DNA methylation induced by exposure in the first generation (Manikkam et al. 2012).
Ecological consequences of intraspecific trait variation

Differences in trait expression, both within and between populations impact species interactions, community structure, and ecosystem function by increasing trait variation in a number of ways. For species interactions that depend non-linearly on trait values, variation around a trait mean can alter interaction strength, a phenomenon known as Jensen’s inequality (Ruel and Ayres 1999). When species’ trait variation influences an ecological interaction in a non-linear manner, this variation can alter the value of the average interaction strength (Bolnick et al. 2011). For a variable trait $x$, with trait mean $\bar{x}$, and non-linear function $f(x)$, Jensen’s inequality predicts that the average value of $f(x)$ will be larger or smaller than the function evaluated at the trait mean $f(\bar{x})$. This suggests that populations with different degrees of variance in trait expression but identical mean traits differ in mean interaction strengths (Ruel and Ayres 1999, Bolnick et al. 2011), resulting in different demographic rates and community dynamics (Okuyama et al. 2008). Population level differences in trait expression have also been shown to protect populations from extreme temporal fluctuations in population density via a mechanism known as portfolio effect (Bolnick et al. 2011), where population level differences in trait expression increase temporal stability in average population densities due to complementary or independent dynamics of individuals with varying traits (Lhomme and Winkel 2002). This has been observed for Alaska salmon in Bristol Bay, where asynchrony in annual run timing between local populations led to a 2.2 fold reduction in variability in annual returns as compared to homogeneous populations (Schindler et al. 2010).
Intraspecific variation within a population can alter the number and strength of species interactions, influencing food web topology. Increased population niche width as a result of population level differences in trait expression can also influence the strength of intraspecific competition, by reducing niche overlap between individuals with divergent trait expression, a phenomenon known as niche complementarity (Resuch et al. 2005, Hughes et al. 2009).

Population level differences in trait expression can also weaken pair-wise associations as only a subset of individuals in each species participate in each interaction (Allesina et al. 2008, Bolnick et al. 2011). Increased niche width as a result of intraspecific trait variation can increase the number of species interactions, thereby increasing the connectivity in a food web. Increased connectivity as a result of intraspecific variation enhances the robustness of food webs, reducing the probability of local extinctions and increasing food web stability (Dunne et al. 2002, Gross et al. 2009). Furthermore, reducing the strength of species interactions dampens oscillations between consumer and resource populations, increasing food web stability (McCann et al. 1998).

Population level differences in trait expression have been shown to play an important role in shaping community structure and regulating ecosystems function across a wide range of systems. At the ecosystem level, differences in alewife *Alosa pseudoharengus* migratory behaviour (anadromous versus landlocked), caused differences in body size that resulted in trophic cascades in the presence of anadromous individuals as compared to landlocked individuals (Post et al. 2008). Differences in tolerance to toxic cyanobacteria between two genotypes (tolerant and sensitive) of water flea (*Daphnia pulicaria*), resulted in differences in phytoplankton primary production (Chislock et al. 2013). Differences in black cottonwood
(Populus trichocarpa) genotype and three-spine stickleback (Gasterosteus aculeatus) ecotype has been shown to influence macroinvertebrate species richness and phosphorus availability in aquatic ecosystems (Rudman et al. 2015). Furthermore, differences in trait expression can provide biological insurance against disturbances. In eel grass (Zostera marina) beds, increasing genotypic diversity, linked to increasing trait diversity has been shown to confer greater resistance to and rapid recovery from Brant goose (Branta bernicla subsp. nigricans) grazing (Hughes and Stachowicz 2004). For the common grass Festuca lenensis, population level differences in water uptake influenced their ability to withstand warmer, drier climate regimes expected under climate change (Liancourt et al. 2013). However, most researchers investigating the role of intraspecific variation in trait expression in ecological communities typically measure direct changes in abundance (reviewed by Des Roches et al. 2018). Indirect impacts such as changes in community composition or productivity (reviewed by Des Roches et al. 2018) and the magnitude of intraspecific effects remains understudied.

Anthropogenic activities not only influence habitat quality and availability and the nature of species interactions in ecological communities, but also impose strong selective pressures that result in rapid changes in trait expression in affected populations or lead to local extinctions (reviewed in Olden et al. 2004, Palkovacs et al. 2012). Despite well documented impacts of human induced trait changes in wild populations (reviewed by Palkovacs et al. 2012) and the importance of population level differences in trait expression in altering species interactions and shaping community structure, few studies have examined if these differences influence how anthropogenic disturbances impact ecological communities. In this thesis, I examined if
population level differences in *Daphnia* daytime vertical position influenced the impacts of the invasive Spiny water flea (*Bythotrephes longimanus*) on the structure and function of freshwater plankton communities.

*Variation in Daphnia daytime vertical position*

*Daphnia* are pelagic, filter feeding zooplankton that occur in lakes and ponds globally. They are a primary consumer of phytoplankton and key food source for secondary consumers, serving as an important link in the transfer of energy to higher trophic levels (Miner et al. 2012). The global pervasiveness of *Daphnia* as a genus is due in part to their ability to modulate the expression of morphological, behavioural and life history traits in response to a wide range of biotic and abiotic conditions (reviewed by Miner et al. 2012). For example, numerous *Daphnia* species have been shown to shift their vertical position in response to predator presence and density (Wright and O’Brien 1982, Leibold 1990, 1991, De Meester et al. 1996, Boeing et al. 2004, Rautio et al. 2004, Boeing et al. 2006), food availability (Johnsen and Jakobsen 1987, Leibold 1990), thermal gradients (Kessler and Lampert 2004, Pangle and Peacor 2010), exposure to ultra-violet (UV) radiation (Rhode et al. 2001, Raitou et al. 2004, Boeing et al. 2004, Hanson and Hylander 2009, Rose et al. 2012), and inter- and intraspecific competition (Lampert 2005). *Daphnia* have been shown to move upwards in the water column to feed on algae (Johnsen and Jakobsen 1987), avoid benthic predators (Wright and O’Brien 1982, Leibold 1990, 1991) and reduce the metabolic costs of residing in low temperatures (Kessler and Lampert 2004), and move downwards to avoid visual predators (De Meester et al. 1996), exposure to UV radiation (Hanson and Hylander 2009), and reduce negative effects of competition with other conspecifics,
daphniids, and non-daphniid zooplankton species for algal resources in the epilimnion (Lampert 2005). Furthermore, there exists large variation in the expression and magnitude of shifts in daytime vertical position in response to these stimuli. For example, *Daphnia pulex* show a large degree of inter-clonal variation in the depth of their vertical position response when exposed to kairomones of the phantom midge larva (*Chaoborus* sp.) and fish (Boeing et al. 2006). Large variation in vertical position response to predators was also detected for *Daphnia magna* clones exposed to fish kairomones (De Meester 1996).

*Daphnia vertical position response to the invasive zooplankton predator, Bythotrophes longimanus*

The spiny water flea, *Bythotrophes longimanus*, is an invertebrate predator that invaded the Laurentian Great Lakes in the 1980s from Europe, and has spread to more that 180 lakes (EDDMaps 2018) on the Canadian Shield as well as many watersheds in the Northern United States (Branstrator et al. 2006; Kerfoot et al. 2011). *Bythotrophes* is a voracious zooplankton predator, consuming three quarters of its body weight per day (Lehman et al. 1997). Its invasion in North American lakes has been linked to reduced richness, abundance, and production of freshwater zooplankton, primarily associated with loss of cladocerans (Yan et al. 2001, Boudreau and Yan 2003, Strecker et al. 2006, 2011, Strecker and Arnott 2008, 2010, reviewed by Azan et al. 2015), with cascading impacts on phytoplankton production (Strecker et al. 2008, Walsh et al. 2016). For Harp Lake in Ontario, Canada, where *Bythotrophes* was first detected in 1993 (Yan et al. 2001), cladoceran richness was reduced by 36% post-invasion (Boudreau and Yan 2003) and the average cladoceran species per count declined from 5.8 to 2.44 species (Yan
et al. 2001). This loss of cladoceran taxa is linked to *Bythotrephes* preference for cladocerans over other zooplankton taxa (Vanderploeg et al. 1993; Wahlstroöm and Westman 1999; Boudreau and Yan 2003; Strecker et al. 2006).

*Bythotrephes* is found primarily in the light penetrating regions of the epilimnion and upper metalimnion (Pangle and Peacor 2006, Young and Yan 2008) as it is a visual predator, requiring light to feed (Pangle and Peacor 2009, Jokela et al. 2013). The spatial restriction of *Bythotrephes* to these light penetrating upper layers creates a hypolimnetic refuge, where *Daphnia* can avoid predation. The use of this refuge has been observed for *D. mendotae* populations in Lake Michigan (Pangle and Peacor 2006, Pangle et al. 2007, Bourdeau et al. 2013 and *D. retrocurva* populations in Lake Erie (Pangle et al. 2007). Furthermore, lab experiments have shown that this response is linked with exposure to *Bythotrephes* kairomones, with *D. mendotae* shifting downwards in experimental columns (Bourdeau et al. 2013). By allowing *D. mendotae* individuals to avoid *Bythotrephes* predation and persist in invaded lakes, this downwards shift in vertical position can indirectly impact community composition and primary production in invaded lakes, potentially leading to different outcomes between invaded lakes where this behavioural response occurs as compared to those where this response is absent. However, the prevalence of this vertical position response to *Bythotrephes* in *Daphnia* populations across the invaded range is unknown. Additionally, despite the large degree of variation observed for *Daphnia* daytime vertical position, the influence of differences in *Daphnia* vertical position on the impacts of *Bythotrephes* on plankton communities in invaded lakes is not well understood.
**Objectives**

The goal of this thesis was to examine if differences in *Daphnia* vertical position mediates *Bythotrephes* impacts on plankton communities in invaded lakes in south-central Ontario. In the first chapter, I assessed if *Bythotrephes* presence is linked with a deeper vertical position in multiple *Daphnia* species and populations across the Canadian Shield using a field survey of 45 invaded and uninvaded lakes. For the second chapter, I experimentally manipulated *Daphnia* vertical position in *Bythotrephes* present and absent mesocosms to examine the influence of differences in *Daphnia* vertical position on *Bythotrephes* impacts on zooplankton and phytoplankton communities. In my final chapter, I examined the effect of differences in vertical position of dispersing *Daphnia* on daphniid populations in *Bythotrephes* invaded systems using mesocosm experiments.
References


Chapter 2

Anti-predator behaviour of *Daphnia* to the invasive predator *Bythotrephes longimanus* is influenced by predator density

Sarah S. Hasnain and Shelley E. Arnott
Abstract

*Bythotrephes longimanus*, an invasive zooplankton predator, has been shown to negatively impact zooplankton abundance and diversity in North American lakes. Previous studies have shown that *Daphnia* populations in lakes move to deeper waters during the day, in the presence of *Bythotrephes*, a visual predator occupying well-lit regions. However, *Daphnia* vertical position can be influenced by a variety of abiotic factors including resource availability and UV radiation. Here I examined the effect of abiotic variables on *Daphnia* daytime vertical position, a potential behavioural anti-predator response to *Bythotrephes*. I conducted a survey to determine 1) if *Daphnia* daytime vertical position differed between invaded and uninvaded lakes and 2) if *Daphnia* vertical position in invaded lakes was affected by water chemistry variables linked to water clarity, UV exposure, and phytoplankton production. Invaded lakes had a lower proportion of epilimnetic *Daphnia* as compared to uninvaded lakes. At the species level, differences in vertical position between invaded and uninvaded lakes were only observed for *Daphnia mendotae* and *Daphnia longiremis*. For *D. mendotae*, vertical position was also influenced by *Bythotrephes* density and dissolved organic carbon levels. My results show that *Daphnia* vertical position response to *Bythotrephes* is variable, depending on species and local abiotic conditions, with important implications for community structure and ecosystem function in invaded lakes.
Introduction

The introduction of predators to aquatic ecosystems has resulted in large negative impacts on some native communities (Elton, 1958; Mack et al., 2000; Salo et al., 2007; Sih et al., 2010). Best known examples include the decline of haplochrome cichlid populations following the introduction of the Nile Perch in Lake Victoria (Goldschmidt et al., 1993) and loss of invertebrate and amphibian populations with trout stocking into alpine lakes (Knapp and Matthew, 2000; Knapp, 2005). However, these large negative impacts are not consistently observed across ecological communities. In some cases, the establishment of an invasive predator has led to negligible (Bruno et al., 2005) or even positive impacts on native communities (Rodriguez, 2006). The ability of some native prey species to deploy effective morphological, behavioural, or life-history responses in the presence of introduced predators influences the direction and magnitude of their ecological impacts (Cox, 2004; Strauss et al., 2006; Carthey and Banks, 2014).

The deployment of an effective anti-predator response is dependent on the ecological context in which the affected prey species exist. There is ample evidence that prey response to predators is influenced by both the biotic (e.g. presence of multiple predators, predator density) and abiotic environment (e.g., resource availability, light availability, soil and water chemistry, temperature) in which the predator-prey interaction occurs. For example, Daphnia anti-predator response to the invertebrate predator, Chaoborus, is influenced by both resource availability (Leibold, 1990) and the presence of UV radiation (Boeing et al., 2004). When novel predators are introduced, native prey response must be placed within the existing abiotic and predator context. The deployment of any anti-predator response would be dependent on not only the recognition of
the introduced predator as a threat, but also the ability of native species to assess predation risk relative to other predators (Carthey and Banks, 2014) and trade-offs associated with being exposed to potential suboptimal abiotic conditions as a result of this response (Boeing et al., 2004; Larsson and Lampert, 2012).

For the invasive Spiny Water Flea, *Bythotrephes longimanus* (hereafter *Bythotrephes*), there is evidence that some native prey species can successfully deploy anti-predator response in its presence. *Bythotrephes* is an invertebrate zooplankton predator that was introduced into the North American Great Lakes in 1980s (MacIsaac et al., 2000). It has since spread to more than 179 inland lakes in Ontario (EDDMaps, 2017) as well as some lakes in northeastern and midwestern United States (Branstrator et al., 2006), causing declines in richness and abundance of native crustacean zooplankton, especially cladocerans (Yan et al., 2001; Yan and Boudreau, 2002; Boudreau and Yan, 2003; Strecker and Arnott, 2005; Strecker et al., 2006; Strecker and Arnott, 2008; Strecker and Arnott, 2010). *Bythotrephes* is a visual predator, relying on light to detect and capture prey species (Pangle and Peacor, 2009; Jokela et al., 2013) and is therefore restricted to depths in stratified lakes where light penetration occurs, i.e., the epilimnion and upper metalimnion (Lehman and Cáceres, 1993; Straile and Hälßich, 2000; Pangle et al., 2007; Young and Yan, 2008). This spatial restriction creates a potential refuge at darker depths that zooplankton may inhabit to avoid *Bythotrephes* predation. Field observations indicate that zooplankton, particularly *Daphnia* and copepods, migrate deeper in the water column during the daytime allowing them to avoid *Bythotrephes* predation (Lehman and Cáceres, 1993; Pangle et al., 2007; Bourdeau et al., 2013; Jokela et al., 2011). Recent laboratory experiments have
demonstrated that this downward movement by *Daphnia mendotae* is in response to a chemical cue (kairomone) produced by *Bythotrephes* (Pangle and Peacor, 2006; Bourdeau et al., 2013).

Daytime vertical position in *Daphnia* is influenced by many biological and water chemistry factors. *Daphnia* shift their vertical position to deeper in the water column to avoid exposure to ultra-violet radiation in lakes with greater water clarity (determined by the concentration of dissolved organic carbon; Morris et al., 1995; Wissel and Ramacharan, 2003; Boeing et al., 2004; Kessler et al., 2008; Hansson and Hylander, 2009; Williamson et al., 2011) and to avoid predation from pelagic visual predators (Leibold, 1990; Kessler et al., 2008; Larsson and Lampert, 2012). *Daphnia* adopt a shallower vertical position in the presence of benthic predators (Leibold, 1990; Leibold, 1991; Boeing et al., 2004), to feed on algal resources (Johnsen and Jakobsen, 1987; Leibold, 1990), which are most abundant in the epilimnion, and to avoid metabolic costs associated with cold, hypolimnetic waters (Kessler and Lampert, 2004). In invaded lakes, all these factors could influence *Daphnia* anti-predator response to *Bythotrephes*.

The deeper vertical position of two *Daphnia* species occurring in Lake Michigan and Lake Erie; *D. mendotae* and *D. retrocurva*, in response to *Bythotrephes* in both laboratory experiments (*D. mendotae*) and field observations (*D. mendotae* and *D. retrocurva*) suggest that *Daphnia* can behaviourally avoid *Bythotrephes* predation (Pangle et al., 2007; Bourdeau et al., 2013), although with fitness costs (Pangle et al., 2007). A study of the diurnal vertical position of zooplankton in 14 invaded south-central Ontario lakes found that *Daphnia* migrate away from the well-lit surface waters during the day and return to the epilimnion at night (Jokela et al., 2011), suggesting that native *Daphnia* across the invaded range may be able to behaviourally respond to
Bythotrephes predation. Unfortunately, *Daphnia* in this study were not identified to species so it is unknown whether this behaviour is prevalent across all species and populations in the region. Additionally, it is unknown how conflicting selective pressures from different abiotic and biotic variables across a range of lake ecosystems may modify this response.

The objective of this paper was to examine: 1) if *Daphnia* daytime vertical position differed between invaded and uninvaded lakes and 2) if *Daphnia* vertical position in invaded lakes was influenced by *Bythotrephes* density and abiotic variables governing light availability, UV exposure (dissolved organic carbon; DOC, secchi disk depth (Secchi), and total phosphorus (TP)), and phytoplankton production; total phosphorus (TP). Given that *Bythotrephes* is a visual predator, I expected fewer *Daphnia* in the epilimnion in invaded lakes as light penetration is the greatest in this region of the water column. Furthermore, I expected *Daphnia* to remain mostly epilimnetic in invaded lakes with greater concentration of DOC and shallower Secchi depths, as less light penetration in the water column would reduce *Bythotrephes* predation on epilimnetic individuals. Considering the strong positive association between TP and phytoplankton production (Dillon and Rigler, 1974), TP provides an indirect measure of resource availability for *Daphnia*. I expected that in lakes with larger TP concentrations, the maximum phytoplankton biomass would be shallower, especially for green algae (Longhi and Beisner, 2009), resulting in a greater proportion of epilimnetic *Daphnia* even in the presence of *Bythotrephes*. I did not expect larger TP concentrations to influence the availability of phytoplankton in the meta- and hypolimnion as the presence of deep chlorophyll maxima is strongly linked with nitrogen, rather than phosphorus availability (Saros et al., 2005).
Methods

Lake Survey

To examine if abiotic variables influence the impact of *Bythotrephes* on vertical position of *Daphnia* species in south-central Ontario, I conducted a survey of 45 lakes from July to August 2013 (Table S1). Lakes were chosen along a gradient of dissolved organic carbon (DOC), total phosphorus (TP), and Secchi disk depth (Secchi), based on samples collected by the Canadian Aquatic Invasive Species Network (CAISN) 2011 survey (N. Yan unpublished data, also see Cairns et al. 2011 for details about sampling methods, values provided in Table S2). All lakes were thermally stratified (Kalff, 2002). Because I was sampling across broad water chemistry gradients, I sampled *Daphnia* from each thermal layer of as many lakes as logistically possible during the months of July and August when lakes are or nearly completely thermally stratified in south-central Ontario. I was able to sample up to three times as many lakes as sampled in previous surveys that detected an impact of *Bythotrephes* on *Daphnia* density (Boudreau and Yan, 2003; Strecker et al., 2006) and vertical position (Jokela et al., 2011).

Zooplankton sampling and enumeration

Zooplankton were collected during the day (between 10am and 4pm) at the deepest location of each lake using a 50 µm mesh closing net with a 35-cm inner diameter. Temperature (°C) was measured using an YSI Model 550 Dissolved Oxygen and Temperature probe at 1m depth intervals to determine the depth of the epi-, meta-, and hypolimnion. A single zooplankton sample was collected from each thermal layer and preserved in 90% ethanol. Daytime vertical
position of several *Daphnia* species has been shown to remain constant during the ice free period (Threlkeld 1979, Kawabata 1989, Matthes 2004), suggesting that a single sample from each thermal layer provides an accurate representation of *Daphnia* vertical position. Since the number of individuals sampled from each thermal layer would be affected by the volume of that layer, with more individuals sampled from larger layers as compared to smaller ones, all *Daphnia* in a sample volume representing 100L of water in each thermal layer were enumerated to standardize effort. *Daphnia* were identified to species using Ward and Whipple, 1959; Witty, 2004; and Haney et al., 2013. Since *Daphnia pulex* and *Daphnia pulicaria* can only be distinguished using genomic methods, they were grouped as *D. pulex/pulicaria*.

**Bythotrephes density**

*Bythotrephes* densities in lake ecosystems vary throughout the year (Yan and Pawson, 1997), as well as between years (Pothoven et al., 2008). In south-central Ontario, Strecker and Arnott (2008) have shown that *Bythotrephes* densities vary significantly in invaded lakes throughout the year. Furthermore, a large degree of spatial heterogeneity in *Bythotrephes* density has been observed in invaded lakes in this region (Yan et al., 2002). Logistical constraints allowed us to sample *Bythotrephes* once in each lake during the day, which tends to under-represent densities and ignores seasonal variation in abundance. Therefore, I determined the invasion status of each lake based on *Bythotrephes* presence detected in my study lakes by Jokela et al. (2011), who conducted an intensive nighttime macro-invertebrate survey of 80 lakes using five vertical tows per lake. All *Bythotrephes* individuals in my daytime samples were
enumerated. Densities in my samples were up to an order of magnitude less than those in Jokela et al. (2011). This suggests that densities in my samples under-represented *Bythotrephes* densities in the lakes that I assessed. Additionally, *Bythotrephes* were not detected in my samples for many lakes where *Bythotrephes* presence had been observed by Jokela et al. (2011). I used densities in my samples as a qualitative measure of *Bythotrephes* abundance in my survey lakes rather than precise density values. Lakes with *Bythotrephes* presence detected by Jokela et al. (2011), but no *Bythotrephes* individuals detected in my samples were classified as low density lakes, while those with both *Bythotrephes* observed by Jokela et al. (2011) and *Bythotrephes* individuals present in my samples were classified as high density lakes.

*Statistical Analysis*

Of the 45 lakes surveyed, 5 lakes; Three Mile Lake, Otter Lake, Portage Lake and Silver Lake 1 and Silver Lake 2, contained 10 or fewer *Daphnia* individuals in the entire water column sample (epi-, meta- and hypo- samples combined). These lakes were excluded from analyses examining *Daphnia* vertical position, as differences of a few individuals between the epi-, meta- and hypolimnion could have a large effect on my estimates of vertical position. Additionally, due to lack of Secchi depth data available for Kapikog Lake, I removed it from all analyses of *Daphnia* vertical position. All explanatory variables; *Bythotrephes* density, DOC, TP, and Secchi were tested for collinearity, with DOC and Secchi found to be highly collinear with each other. All analyses were conducted in R version 3.4.2 (R Core Team, 2017).
I used conditional inference trees to assess: 1) if the proportion of epilimnetic *Daphnia* across my survey lakes was influenced by *Bythotrephes* presence and, 2) if DOC, Secchi, TP and *Bythotrephes* density influenced the proportion of epilimnetic *Daphnia* in invaded lakes. Tree-based models such as conditional inference trees, are useful for examining complex ecological datasets (reviewed in De'ath and Fabricius, 2000; Olden et al., 2008), especially where potential explanatory variables may be collinear, with non-linear relationships and higher order interactions. Conditional inference trees use the following algorithm: 1) test independence of explanatory variables to each other and the response variable, 2) if this is rejected, the explanatory variable with the greatest association to the response variable is chosen, measured by a p-value, 3) if the p-value is less than the alpha (\( \alpha = 0.05 \)), the data is split into sections based on the explanatory variable and the algorithm searches for the next variable with the greatest association. This process continues until the value of \( x \), where \( x = 1 - p \) and where \( p \) is the p-value obtained from significance testing in step 2, does not exceed the minimum criterion. For this analysis, the stop criterion was based on p-values obtained from permutation tests, maximizing the absolute values of the standardized linear statistic (Hothorn et al., 2006), with a minimum of 8 lakes per split and a minimum of 5 lakes in each terminal node.

All conditional inference trees were constructed in a step-wise procedure. First, a tree was constructed to assess if the proportion of epilimnetic individuals for total *Daphnia* and the most common *Daphnia* species differed between invaded and uninvaded lakes. If a significant effect of *Bythotrephes* presence was detected on the proportion of epilimnetic *Daphnia* individuals, an additional tree was constructed to assess the impacts of DOC, TP, Secchi and *Bythotrephes*
density on the proportion of epilimnetic *Daphnia* in invaded lakes. Conditional inference trees were constructed using the party (version 1.0.18) package in R, as it is not susceptible to “variable selection bias” (Hothorn et al., 2006; Strobl et al., 2009). Variable selection bias refers to tree algorithms which favour variables that have many potential splitting points. Additionally, conditional inference trees constructed using the party package employ a conditional permutation scheme where the variable to be permuted is conditional on other correlated variables which are held fixed, allowing for highly correlated variables to be used when constructing these trees (Strobl et al. 2009).

Conditional inference trees were constructed for total *Daphnia* (all *Daphnia* species pooled together), as well as the most common *Daphnia* species (found in >20% of all the lakes assessed): *D. mendotae, D. catawba, D. longiremis, D. ambigua* and *D.pulex/pulicaria*. Due to the reduced sample sizes for species specific conditional inference trees (i.e. not every species was present in every lake), the minimum criteria for the number of lakes for each terminal node was set to 4. If no effect of *Bythotrephes* presence on the proportion of epilimnetic individuals was detected, I did not assess the influence of DOC, Secchi, TP and *Bythotrephes* density on the proportion of epilimnetic individuals in invaded lakes. The mean (± SD) proportion of epilimnetic individuals for each significant split is reported in the results.

**Results**

Each of my 45 study lakes used to assess vertical position contained at least one species of *Daphnia*. Twenty-five lakes were invaded while fourteen lakes were not invaded by
Bythotrephes. The most widely distributed Daphnia species were D. mendotae (65% occurrence), D. longiremis (48% occurrence), D. catawba (37.5% occurrence), D. ambiguа (35% occurrence), and D. pulex/pulicaria (30% occurrence). Three other species; D. parvula, D. dubia, and D. retrocurva were also detected, albeit in less than 10% of the lakes sampled.

In invaded lakes, fewer individuals were present in the epilimnion on average as compared to uninvaded lakes (Invaded: 43 ± 24% epilimnetic individuals, Uninvaded: 61 ± 28% epilimnetic individuals, conditional inference tree, p<0.001) (Figure 2.1a). Conditional inference trees identified Bythotrephes density (p<0.001) as statistically significant predictors of the proportion of total Daphnia in the epilimnion in invaded lakes. For invaded lakes with low Bythotrephes density, on average more individuals were epilimnetic as compared to those with high Bythotrephes density (Figure 2.1b, high density: 21 ± 28% epilimnetic individuals, low density: 56 ± 26% epilimnetic individuals).

For the most common species in my dataset, on average, 77 ± 26% of D. catawba, 57 ± 32% of D. ambiguа, 25 ± 31% of D. longiremis, 43 ± 31% of D. mendotae, and 20 ± 23% of D. pulex/pulicaria were epilimnetic across all lakes (Figure 2.2). Conditional inference trees constructed for individual species that are the most common in my dataset, D. mendotae, D. catawba, D. ambiguа, D. longiremis, and D. pulex/pulicaria, only detected an effect of Bythotrephes presence (p<0.001) on D. mendotae and D. longiremis vertical position.

For D. mendotae, fewer individuals were epilimnetic in invaded lakes as compared to uninvaded ones (Figure 2.3a, Invaded: 39 ± 32% epilimnetic individuals, Uninvaded: 56 ± 26% epilimnetic individuals). In invaded lakes, conditional inference trees identified both
Bythotrephes density (p<0.001) and DOC levels (p<0.001) as significant predictors of the proportion of epilimnetic D. mendotae. For lakes with low Bythotrephes density, 53 ± 32% of D. mendotae were epilimnetic. For lakes with high Bythotrephes density, the proportion of epilimnetic D. mendotae individuals was 4% greater in lakes with DOC levels greater than 4.2 mg/L as compared to those where DOC levels were less than or equal to 4.2 mg/L (Figure 2.3b, DOC ≤ 4.2 mg/L: 19 ± 20% epilimnetic individuals, DOC > 4.2 mg/L: 23±26% epilimnetic individuals). For D. longiremis, fewer individuals were epilimnetic in uninvaded lakes as compared to invaded ones (Figure 2.4, Invaded: 31.4 ± 33% epilimnetic individuals, Uninvaded: 15.1 ± 19% epilimnetic individuals). For D. ambiguа, D. catawba, and D. pulex/pulicaria, there was no difference in vertical position between invaded and uninvaded lakes.

Discussion

My results show that overall, fewer Daphnia occupy epilimnetic habitat in lakes invaded by Bythotrephes. This result is consistent with the patterns observed for D. mendotae and D. retrocurva populations in Lake Michigan and Lake Erie where individuals were found to adopt a deeper vertical position in response to Bythotrephes (Pangle and Peacor, 2007), a response induced specifically by Bythotrephes kairomones for Lake Michigan D. mendotae (Pangle and Peacor, 2006; Bourdeau et al., 2013), and also observed in Daphnia from 15 lakes in south-central Ontario (Jokela et al., 2011). At the species level, a smaller proportion of D. mendotae individuals were also present in the epilimnion in invaded lakes. Together, these results suggest that the smaller proportion of epilimnetic individuals observed for total Daphnia in invaded lakes reflects the deeper vertical position of D. mendotae, in the presence of Bythotrephes.

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D. mendotae vertical position in invaded lakes

I observed a smaller proportion of epilimnetic *D. mendotae* individuals in invaded lakes with high *Bythotrephes* density, suggesting a deeper *D. mendotae* vertical position in these lakes. This deeper vertical position could be a result of phenotypic plasticity (i.e. induced response) in response to *Bythotrephes* or a behavioural adaptation resulting from selection against individuals spatially overlapping with *Bythotrephes*. *Bythotrephes* is a voracious predator, killing approximately three-quarters of its body weight per day (Lehman et al., 1997). In invaded lakes, *Bythotrephes* predation on spatially overlapping epilimnetic *D. mendotae* would impose a strong selective pressure for individuals with a deeper vertical position, shifting population vertical position to deeper in the water column and reducing intra-population variation in vertical position. However, shifts in *Daphnia* vertical position can also be induced by a variety of predators (Dodson, 1988; Watt and Young, 1994; Nesbitt et al., 1996; von Elert and Pohnert, 2000; Stirling and Roff, 2007; Bourdeau et al., 2013), including *Bythotrephes* (Bourdeau et al., 2013). Exposure of Lake Michigan *D. mendotae* to *Bythotrephes* kairomone has been shown to induce a deeper vertical position in lab experiments, suggesting that the deeper *D. mendotae* vertical position in the presence of *Bythotrephes* observed in my system could also be an inducible response.

*Daphnia* daytime vertical position also has a strong genetic component, with high intra-population variation in vertical position observed in response to a variety of predators and their kairomones (De Meester, 1996; Cousyn et al., 2001; Boeing et al., 2006), which adds an
additional layer of complexity when determining which mechanism is driving *D. mendotae* vertical response to *Bythotrephes*. The ability to induce changes in vertical position in response to predators in *Daphnia* is genetically constrained (Rozenberg et al., 2015), potentially resulting in some individuals being able to induce a deeper vertical position in the presence of *Bythotrephes* or its kairomones as compared to others. Predation on spatially overlapping *Daphnia* individuals could also lead to selection towards individuals that can induce a deeper vertical position, thereby shifting populations towards deploying an induced response in the presence of *Bythotrephes* or its kairomones. Given the scope of my study, I do not know which of these mechanisms is underlying the vertical position of *D. mendotae* in the presence of *Bythotrephes* in my system.

**Dissolved organic carbon and *D. mendotae* vertical position**

My results show that water clarity has a small influence on *D. mendotae* vertical position in invaded lakes. The proportion of epilimnetic *D. mendotae* in high *Bythotrephes* density lakes where DOC levels were greater than 4.2 mg/L was 4% greater as compared to those with DOC levels less than or equal to 4.2 mg/L. DOC absorbs both ultra-violet (UV) and photo-synthetically active radiation (PAR), limiting light penetration in the water column (Morris et al., 1995) and reducing *Bythotrephes* predation. At DOC concentrations of 4.2mg/L or greater, light penetration in the water column is substantially reduced as compared to clear water, with only 1% of PAR (intensity of 5.2 μmol/m²-second reaching 4m, determines from average solar radiation for south-central Ontario from July 1st to August 31st 2013 (NASA Atmospheric Science Data Center) and relationships between DOC and light attenuation from Williamson (2009), chlorophyll *a* and TP
(Dillon and Rigler, 1974) and diffuse attenuation coefficient (Morris et al. 1995)). At 1% PAR penetration (intensity of 5.2 μmol/m²-second), Bythotrephes can only ingest up to 1.06 D. mendotae individuals per hour, compared to 2.3 or more individuals per hour at ambient light levels (calculated from Pangle and Peacor, 2009). For most lakes in my study region, the bottom of epilimnion ranged from 3-5m, suggesting that D. mendotae could find an optical refuge from Bythotrephes in the lower depths of the epilimnion when DOC levels are greater than 4.2mg/L. It is also possible that small difference in the proportion of epilimnetic D. mendotae observed between lakes with DOC levels less than or equal to 4.2 mg/L and those with greater DOC levels is a result of low sample size for high Bythotrephes density lakes present in my survey (n =8).

DOC can also affect the penetration of UV radiation in lakes, which negatively impacts Daphnia survival and reproductive success (Zagarese et al., 1994; Williamson et al., 1994; Grad et al., 2001). In lakes with high overall UV irradiance, Daphnia have been shown to adopt a deeper vertical position to escape these impacts (Zagarese et al., 1994; Boeing et al. 2004). However, only 1% of surface UVA and UVB penetrated the water column between 0-2m across all my survey lakes (based on published relationships between dissolved organic carbon and UV attenuation depth (Morris et al., 1995; Williamson et al., 1996), and likely did not influence D. mendotae vertical position.

D. longiremis vertical position

Sixteen percent more D. longiremis individuals were present in the epilimnion in invaded lakes as compared to uninvaded lakes. Despite a widespread occurrence across the lakes sampled,
more than 10 *D. longiremis* individuals were detected in only 6 lakes (Figure S2.1). It is possible that the increase in epilimnetic individuals observed in invaded lakes is due to the influence of lakes with few *D. longiremis* individuals, where a difference of a few individuals between the epilimnion and other thermal layers resulted in a large increase in the proportion of epilimnetic individuals. The increase in the proportion of epilimnetic *D. longiremis* in invaded lakes could also be driven by factors independent of *Bythotrephes* presence. *D. longiremis* is a species that resides primarily in the hypolimnion (Vanderploeg et al., 2009). On average 55.3% of *D. longiremis* individuals were hypolimnetic across my survey lakes. Field and Prepas (1997) have shown that *D. longiremis* vertical distribution is influenced by dissolved oxygen levels, with individuals shifting upwards in the water column when dissolved oxygen is less than 1mg/L in the hypolimnetic region. Dissolved oxygen levels below 1mg/L have been observed in the hypolimnion for 98 lakes during the 2010 and 2011 CAISN surveys in south-central Ontario, including five lakes in my survey where *D. longiremis* were present. It is possible that the greater proportion of epilimnetic *D. longiremis* individuals observed in invaded lakes is a result of an upwards shift in vertical position as due to extreme hypoxia in the hypolimnion of these lakes. However, I could not assess if dissolved oxygen levels influenced the vertical position of *D. longiremis* in my study lakes.

*Bythotrephes* density in invaded lakes

When analyzing the impacts of *Bythotrephes* density on *Daphnia* vertical position, I only sampled each lake once, likely underestimating daytime *Bythotrephes* densities in my study
lakes. Furthermore, multiple year field surveys of *Bythotrephes* populations suggest that there is both intra and inter-annual variation in density (Yan and Pawson, 1998; Yan et al. 2001; Pothoven et al., 2008; Strecker and Arnott, 2008), which I was unable to assess in this study. Comparing the density data from my field survey to the intensive night sampling survey conducted by Jokela et al. (2011) six years prior to this study confirmed this, with my samples underestimating densities in some lakes by one order of magnitude. Therefore, the densities that I used in my analysis are not a precise measure of *Bythotrephes* abundance across my survey lakes. Nevertheless, I observed a strong correlation (Pearson correlation = 0.822, p <0.0001) between *Bythotrephes* densities in my samples and those of Jokela et al. (2011), suggesting that lakes with large densities of *Bythotrephes* in 2007 also had large densities in my 2013 survey. My results suggest that water clarity has a small influence on *D. mendotae* vertical position at high *Bythotrephes* densities when predation pressure is likely strong, with high DOC levels restricting water clarity and likely providing an optical refuge for *D. mendotae* in the epilimnion. For lakes with low *Bythotrephes* density, lower probability of encountering *Bythotrephes* in the epilimnion substantially reduces predation risk for *D. mendotae* regardless of light conditions, leading to the shallower vertical position observed.

**Implications**

The ability of some *Daphnia* species to thrive in invaded communities has important implications for aquatic ecosystems. *Daphnia* are primary consumers of algae and a key food source for secondary consumers. Reduced *Daphnia* density due to *Bythotrephes* predation can
lead to trophic cascades, resulting in increased phytoplankton biomass in invaded lakes, with the strength of the cascade likely dependent on lake trophic status (Strecker and Arnott, 2008; Walsh et al., 2016). However, by adopting a deeper vertical position to reduce habitat overlap with *Bythotrephes*, *D. mendotae* may be able to maintain high abundance in invaded lakes and continue grazing on phytoplankton, potentially dampening these trophic cascades. Considering these potentially contrasting impacts on primary production and algal biomass in invaded lakes, the trophic consequences of species specific *Daphnia* vertical position response to *Bythotrephes* should be examined in future studies.
References


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Figures

**Figure 2.1:** a) Proportion of total *Daphnia* in the epilimnion in *Bythotrephes* invaded (present, n = 25) and uninvaded (absent, n = 14) lakes. Asterisk (*) denotes statistically significant differences (p<0.05). b) Boxplots identifying *Bythotrephes* density (individuals/L) as a significant predictor influencing the proportion of total *Daphnia* in the epilimnion in invaded lakes.
Figure 2.2: Proportion of epilimnetic *D. ambigua*, *D. catawba*, *D. longiremis*, *D. mendota* and *D. pulex/pulicaria* in 39 lakes in south central Ontario.
Figure 2.3: Boxplots identifying a) the proportion to epilimnetic *D. mendotae* in *Bythotrephes* invaded and uninvaded lakes and b) identifying values of *Bythotrephes* density (individuals/L) and DOC (mg/L) as significant splits influencing the proportion of *D. mendotae* in the epilimnion in invaded lakes.
Figure 2.4: Boxplots identifying the proportion to epilimnetic *D. longiremis* in *Bythotrephes* invaded (Y) and uninvaded (N) lakes.
Chapter 3

*Daphnia* vertical position influences *Bythotrephes* impacts on zooplankton and algal communities

Sarah S Hasnain and Shelley E Arnott
Abstract

Predation plays an important role in structuring and maintaining ecological communities. Population level differences in predator trait expression have been shown to influence predator impacts on prey species, altering ecological interactions and trophic dynamics. However, the effect of inter-population differences in prey traits on the impacts of predation on ecological communities remains poorly understood, especially for introduced predators where differences in prey traits could influence the outcome of biological invasions. In this chapter, I examined if population level differences in *Daphnia* vertical position influenced the impacts of the invasive predator *Bythotrephes* on plankton communities by examining impact on *Daphnia*, non-daphniid cladoceran zooplankton, copepod zooplankton, and algal abundance. My results show that differences in *Daphnia* vertical position resulted in differences in *Bythotrephes* impacts on small and large cladocerans. *Bythotrephes* predation had a greater impact on per capita small and large cladoceran densities in mesocosms where a greater proportion of hypolimnetic *Daphnia* were present. Furthermore, per capita density in algal biomass increased in invaded mesocosms with a greater proportion of hypolimnetic *Daphnia*. Taken together, these results suggest that population level differences in *Daphnia* vertical position influence the magnitude and type of *Bythotrephes* impacts on zooplankton communities.
**Introduction**

Predation is an important mechanism structuring many aquatic food webs (Brooks and Dodson 1965, Paine 1966, 1974, Polis and Holt 1992). Predators impact prey population dynamics by directly reducing prey densities and impose a strong selective pressure on prey species trait expression, often inducing behavioural, morphological and life history changes (reviewed in Lima 1998, Tollrian and Harvell 1999, Lass and Spaak 2003, Benard 2004). These changes in prey density and phenotype result in indirect effects on food web structure and ecosystem function (Colwell and Fuentes 1975, Paine 1980, Carpenter et al. 1985, Schmitz et al. 2004, Trussell et al. 2003, Grabrowski and Kimbro 2005, Schmitz 2008). For example, reductions in prey density due to predation or change in prey trait expression can increase resource availability, leading to trophic cascades (Paine 1980, Carpenter et al. 1985, Trussell et al. 2003, Schmitz et al. 2004). However, this research assumes that mean trait values or species identity sufficiently characterize predator-prey interactions, ignoring potential effects of intraspecific variation in both predator and prey traits on ecological communities.

Within a species, individuals can vary across a wide variety of behavioral, morphological, physiological and life history traits. For species occupying habitat along wide environmental gradients or in landscapes with large degrees of spatial and temporal heterogeneity, variation in selection pressures can often result in population level differences in trait expression, a phenomenon known as phenotypic divergence (reviewed by Crispo 2008, Pfennig et al. 2010). From an evolutionary perspective, these population level differences in trait expression are a first step in the process of ecological speciation (reviewed by Dieckmann et al. 2004, Pfennig et al.
2010). However, considerably less attention has been paid to the ecological consequences of differences in trait expression between populations. Some studies have shown that these differences can influence species abundance and diversity at the community level (Post et al. 2008, Palkovacs and Post 2009) and affect the type and magnitude of food web interactions (Post et al. 2008, Ingram et al. 2011, Weis and Post 2013, Benesh and Kalbe 2016, Fryxell and Palkovacs 2017). For example, differences in the duration of residence and foraging morphology between two populations of alewives, *Alosa pseudoharengus*, influenced prey biomass and altered the strength of trophic cascades (Post et al. 2008). Most studies examine ecological impacts of inter-population trait variation through predator-prey interactions (but see Benesh and Kalbe 2016), focusing on the consequences of differences in predator traits. The ecological impacts of inter-population differences in prey traits on predator-prey dynamics remain poorly understood (but see Lenhart et al. 2018).

The introduction of predators outside their native range has been shown to result in greater negative impacts on prey communities as compared to native predators (Elton, 1958; Mack et al., 2000; Salo et al., 2007; Sih et al., 2010) as prey are unable to detect or respond appropriately to the predator due to the lack of shared evolutionary history with native prey species (Cox and Lima, 2006, Banks and Dickman 2007, Sih et al., 2010, Carthey and Banks 2014, Carthey et al. 2017). However, these studies assume that introduced predator impacts on native prey are uniform across the invaded range, despite the ubiquity of intraspecific and inter-population variation in traits across ecological systems (Bolnick et al. 2011). Prey naïveté to introduced predators is dependent on a number of factors, including the ecological novelty of the predator to
the prey species, the suite of anti-predator defenses available, and the degree of specialization of predator recognition templates and anti-predator defenses possible (Carthey and Banks 2014). Furthermore, inter-populational differences in behavioural, morphological, and life history traits may confer protection from predation for some prey, but not others. For example, in lakes invaded by the spiny water flea, *Bythotrephes longimanus* (hereafter Bythotrephes), a Eurasian visual zooplankton predator (Pangle and Peacor 2009, Jokela et al. 2013) spatially restricted to the upper light penetrating regions of the water column (Pangle and Peacor 2006, Pangle et al. 2007), predation is only expected to impact prey populations that spatially overlap with this predator.

*Bythotrephes* is a voracious zooplanktivore (Lehman et al. 1997) and its introduction into North American freshwater systems has resulted in devastating impacts on native zooplankton communities, resulting in reduced biomass and species diversity in invaded lakes, especially for cladocerans (Yan et al. 2001; Boudreau & Yan 2003; Barbiero and Tuchman 2004, Strecker and Arnott 2005; Strecker et al. 2006; Kelly et al. 2013, reviewed by Azan et al. 2015, Kerfoot et al 2016). Strong predation on cladocerans, particularly *Daphnia* which are important grazers (Vanderploeg et al. 1993, Schulz and Yuritsa 1999), has been linked to trophic cascades leading to increased phytoplankton production in invaded lakes (Strecker and Arnott 2008, Walsh et al. 2016). In some lakes, *Daphnia* populations (Pangle and Peacor 2006, Pangle et al. 2007, Jokela et al. 2011, Bourdeau et al. 2013, Chapter 2) reside in darker portions of the water column in invaded lakes, where *Bythotrephes* predation is reduced (Pangle and Peacor 2009, Jokela et al. 2013). This deep vertical position allows these *Daphnia* to avoid *Bythotrephes* predation and can
potentially have large food web consequences. Despite evidence suggesting a large degree of
variation in daytime vertical position across Daphnia populations in natural systems (De Meester
2006), the influence of population-level differences in Daphnia vertical position on Bythotrephes
impacts in freshwater ecosystems remains unknown.

The goal of this chapter was to determine if differences in Daphnia vertical position
influence the effect of Bythotrephes on plankton communities in invaded lakes. To accomplish
this, I manipulated Bythotrephes presence in mesocosms with three-tier food webs which were
stocked from lakes where Daphnia populations exhibited different mean vertical positions
(Figure 3.1) and examined if the impacts of Bythotrephes on the density of Daphnia, non-
daphniid cladoceran zooplankton, copepod zooplankton and algae differed across mesocosms
with different daytime vertical distributions. I expected predation on Daphnia to be greater in
mesocosms with greater spatial overlap with Bythotrephes, i.e. shallow vertical position as
compared to those where spatial overlap was reduced, i.e. deep vertical position. Detailed
predictions of outcomes for all zooplankton taxa assessed and algal densities across all
experimental treatments are provided in Table 3.1.

Methods

Study Site and Experimental Design

From July 7th to August 1st 2014, I conducted a field mesocosm experiment to assess the
influence of differences in Daphnia vertical position on effects of Bythotrephes predation on
Daphnia competitor and algal density. Impacts of Bythotrephes predation on zooplankton community structure and algal production can occur over short periods of time that are comparable to the three-week length of our experiment (Strecker and Arnott 2005, Jokela et al. 2017, Arnott and Azan 2017). Mesocosms were set up in Fletcher Lake (45.20.452’N, 78.47.798’W) located in Haliburton County, ON (Table 3.2), where Bythotrephes was first detected in 2006 (Cairns 2007). Five mil food grade polythene enclosures, 1m in diameter and 13 m deep (Filmtech Plastics, Brampton, Ontario), were closed at the bottom and suspended from floating wooden frames anchored in the lake. Each mesocosm was filled with water that was pumped from 1.5m and filtered through an 80-µm mesh to exclude crustacean zooplankton, but allow most phytoplankton to pass through. Each mesocosm was covered with screen mesh to prevent colonization by aerial insects.

To assess if differences in Daphnia vertical position can influence the impacts of Bythotrephes predation on plankton communities, I manipulated Bythotrephes presence and absence in mesocosms across a gradient of Daphnia vertical position. I quantified Daphnia vertical position based on their numeric proportion in regions of the water column (based on temperature gradients) likely to have reduced predation by Bythotrephes; hypolimnetic representing the dark and cold bottom waters (generally 9-13m), and non-epilimnetic representing the cooler, darker waters below the epilimnion (generally from 5 to 13 m). Bythotrephes predation was expected to be significantly reduced in non-epilimnetic region due to reduced light availability and completely absent in the hypolimnion, where light penetration does not occur, as it is a visual predator requiring light to feed (Jokela et al. 2009). Examining the
effect of both the proportion of hypo- and non-epilimnetic individuals allowed me to separately assess the effects of reducing and eliminating *Bythotrephes* predation in invaded mesocosms. Mesocosms were stocked with *Daphnia* from populations in two uninvaded lakes; Bonnie Lake (45.17.36'N 79.06.45'W; Bracebridge Municipality, Table 3.2) and Echo Lake (45.17.36'N, 79.06.45'W; Lake of Bays Municipality, Table 3.2), in south-central Ontario that exhibited contrasting vertical position. Echo lake contained mostly epilimnetic individuals (>60%), while Bonnie Lake *Daphnia* were mostly hypolimnetic (>60% individuals). Each mesocosm was randomly inoculated with an ambient density of zooplankton sampled from the same volume of water from either Echo or Bonnie lake, or half volumes for mesocosms stocked from both lakes, resulting in 16 mesocosms stocked with Echo Lake zooplankton communities, 16 mesocosms stocked with Bonnie Lake zooplankton communities and eight mesocosms stocked from both lakes. When stocked into mesocosms filled with water from Fletcher Lake, the vertical position of *Daphnia* did not reflect the position in their source lakes. Instead, stocking mesocosms from these uninvaded lakes with different *Daphnia* vertical positions created a gradient of daphniid vertical position from 20 to 100% epilimnetic (Figure S3.1). There were significant differences in the abundance of zooplankton between Echo and Bonnie Lake, which influenced starting zooplankton densities in my mesocosms. Overall zooplankton density was greater in enclosures stocked with zooplankton from Echo Lake as compared to Bonnie Lake (Figure S3.2); log normally distributed linear model with lake origin (either Echo Bonnie, or Both) as a predictor variable and zooplankton density as a response variable (p < 0.0001).
Half of the mesocosms were randomly assigned the invasion treatment. *Bythotrephes* individuals were collected from Fletcher Lake and Lake of Bays, Muskoka, Ontario (45°15.00’N, 79°04.00’W) using an 80 µm mesh net. *Bythotrephes* were stocked at a density of 10 individuals per m$^3$ of epilimnion volume (23 individuals per mesocosm) only once at the beginning of the experiment. There were no *a priori* differences in the proportion of hypo- and non-epilimnetic *Daphnia* between invaded and uninvaded mesocosms in Week 0 (Figure S3.2, Gamma GLMs, hypo: $p = 0.911$; non-epi: $p = 0.792$).

**Sampling protocol and identification**

Each mesocosm was sampled prior to the addition of *Bythotrephes* (Week 0) to determine abundance and depth distribution of zooplankton and phytoplankton. Samples were also taken at the end of the study (Week 3). Epi-, meta- and hypolimnetic boundaries were determined weekly using the thermal profile of Fletcher Lake using a YSI model 600 OMS V2. Zooplankton samples from each mesocosm were collected by towing a closing net with an 80 µm mesh (15 cm diameter) through each thermal layer (starting 20cm above the enclosure bottom for the hypolimnion). Samples were preserved in 90% ethanol.

Zooplankton were enumerated using subsamples of a known volume and identifying all individuals within each subsample until no new species were found three subsamples in a row. A minimum of seven sub-samples was counted for each thermal layer in each mesocosm. All specimens were identified to the species level using Ward and Whipple 1959, Smith and Fernando 1978, De Melo and Hebert 1994, Witty 2004, and Haney et al. 2013. I grouped
Bosmina freyi and Bosmina liederi as “Bosmina freyi/liederi” and Daphnia pulex and Daphnia pulicaria as “Daphnia pulex/pulicaria” due to morphological similarities between these species. Juvenile copepods were identified as either nauplii or copepodids, without distinguishing between cyclopoid and calanoid juveniles as both have similar diets and occupy a similar trophic position in aquatic food webs (Brandl 1998, Finlay and Roff 2004).

Water samples were collected from the middle of the meta- and hypolimnion using a 2L Van Dorn sampler prior to the application of the Bythotrephes treatment (Week 0) and at the end of the study. For the epilimnion, water samples were collected from 10 cm below the surface of the mesocosm by submerging the sample container. Each sample was stored in an opaque 500ml or 1L container. I determined total algal biomass as well as biomass for green algae, cyanobacteria, diatoms and cryptophytes by analyzing a well-mixed 25ml subsample within 24 hours of sample collection using BBE moldaenke Algae Lab Analyser (BBE moldaenke; Schwentinental, Germany), which was zeroed with distilled water prior to the assessment of each sample.

Statistical analysis

All analyses were conducted in R v 3.2.4 (R Core Development Team 2016) using packages vegan v 2.3.2 (Oksanen et al. 2013), bbmle v 1.0.17, glmmADMB v 0.7.7 and fitdistrplus 1.0.7, with α = 0.05.

I calculated the density of each species in each thermal layer for every mesocosm by estimating the total number of individuals present in subsamples. Species specific density in each
layer was calculated by dividing the total number of individuals present in a single sample by the volume sampled with a single vertical tow. To calculate the total density of a species in a mesocosm, I summed the total number of individuals in the epi- meta- and hypolimnion samples for each mesocosm and divided this by the total volume of the mesocosm that would be sampled in a single vertical tow. Since I could not conclusively state whether the absence of a species in our sample represented a true absence in our mesocosm, I added a number representing the minimum density that could be detected (1 individual / total volume of mesocosm sampled in a single vertical tow) to densities of all taxa assessed in samples from Week 0 and Week 3. I added one third of the minimum detection density to each layer to represent the presence of a single undetected individual in that layer, for each species. For algae, I also added a minimum detection biomass (0.01 µg/L; representing the measurement resolution of the algae analyzer that I used) to the biomass for all algal groups as it was unclear if a biomass of 0 represented a true absence, a lack of detection due to sampling, or measurement error. Because of differences in starting densities between mesocosms stocked from Bonnie Lake and those stocked from Echo or both lakes (Figure S3.2), I standardized the change in density between Week 0 (prior to the application of the invasion treatment) and Week 3 by calculating per capita change in density for each species and functional group for each mesocosm; calculated as density in Week 3 divided by density in Week 0. I also calculated change in biomass per unit in biomass for algae (Week 3 biomass divided by Week 0 biomass) to standardize change across different starting algal biomass in my mesocosm.
Considering that *Bythotrephes* has been shown to negatively impact cladoceran density (Yan et al. 2001, Yan et al. 2002, Boudreau and Yan 2003, Strecker et al. 2006) and preferentially feed on larger bodied cladocerans, especially *Daphnia* (Schulz and Yurista 1999), I separately assessed *Bythotrephes* impacts on *Daphnia*, small (<0.85mm) and large cladocerans (>1.0mm). Non-daphniid cladoceran species were categorized as small or large based on average body length (Barnett et al. 2007) of the species present in our mesocosms. Small cladocerans included *Bosmina freyi/liederi, Eubosmina tubicen, Eubosmina longispina, Chydorus sphaericus, Eubosmina coregoni, Ceriodaphnia lacustris, Simocephalus vetulus*, and *Scapholeberis mucronata*. Large cladocerans included *Holopedium glacialis* and *Sida crystallina*. All non-daphniid cladoceran species included have been shown to be negatively impacted by *Bythotrephes* presence (reviewed in Azan et al. 2015). Copepod zooplankton were split into calanoid copepods, cyclopoid copepods, nauplii and copepodids, representing different trophic positions and feeding strategies (calanoid and cyclopoid copepods) and juvenile stages (nauplii and copepodids). Calanoid copepods are stationary suspension feeding herbivore-omnivores that could compete with *Daphnia* and other cladocerans for access to algal resources while cyclopoid copepods are raptorial feeding omnivore-carnivores (Barnett et al. 2007), that could be indirectly impacted by *Bythotrephes* predation on small cladocerans.

To determine if *Daphnia* vertical position influenced the impacts of *Bythotrephes* on zooplankton communities, I assessed the effect of *Daphnia* vertical position and *Bythotrephes* presence on per capita change in density of small and large cladocerans, copepods, and change in biomass per unit biomass for algae using Gamma or log-normally distributed generalized linear
models (GLM). The proportion of hypo- and non-epilimnetic Daphnia in week 0 (prior to Bythotrephes addition) were used as measures of Daphnia vertical position. I also used Gamma or log-normally distributed GLMs to assess the impacts of Daphnia vertical position and Bythotrephes presence on the most common small and large cladoceran species. To confirm if any observed per capita changes density for total Daphnia, Daphnia species, total small cladoceran, and small cladoceran species were a result of Bythotrephes predation in the epilimnion, I also assessed differences in per capita change in density between the epi-, meta- and hypolimnion using Gamma or log-normally distributed generalized linear mixed models (GLMM) with mesocosm included as a random intercept. Mean per capita change in density ± standard deviations are provided for statistically significant differences in per capita density between thermal layers.

Due to differences in starting densities between mesocosms, it is possible that any significant influence (p<0.05) of Daphnia vertical position on Bythotrephes impacts for any zooplankton group could be confounded by density dependent Bythotrephes predation. To ensure that density dependent predation was not confounding Bythotrephes impacts on per capita change in density, I examined the correlation between total zooplankton density in week 0 and per capita change in density for taxa where Daphnia vertical position was found to influence per capita density. As per capita density is calculated by dividing densities in week 3 by densities in week 0, I expected per capita density for each taxa assessed to be spuriously correlated with total zooplankton density in week 0. Therefore, I assessed if the correlation coefficient obtained from this analysis was significantly different (p<0.05) from coefficients obtained by bootstrapping the

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correlation between taxon per capita change in density and total zooplankton density in week 0 (n = 1000). There was no significant differences between correlation coefficients obtained from my data and the mean of the bootstrapped coefficients for any taxon assessed, suggesting that per capita change in density observed in my mesocosms was not influenced by differences in starting densities across my mesocosms (Figure S3.3).

Through the course of my experiment, it was possible that *Daphnia* could change their vertical position in response to individual mesocosm environments. For invaded mesocosms with greater spatial overlap between *Daphnia* and *Bythotrephes* (fewer hypo- and non-epilimnetic *Daphnia*), *Daphnia* may adopt a deeper depth distribution in response to *Bythotrephes* kairomone (Bourdeau et al. 2013). Additionally, in uninvaded mesocosms with more hypo- and non-epilimnetic *Daphnia*, metabolic costs associated with residing in deeper, colder waters with potentially lower algal biomass and quality could result in *Daphnia* migrating to warmer, algae abundant epilimnetic waters (Johnsen and Jakobsen 1987, Leibold 1990, Raitou et al. 2003, Kessler and Lampert 2004). To determine if total *Daphnia* and individual *Daphnia* species shifted their depth distributions during the experiments, either as a result of direct predation or an induced response to *Bythotrephes* predation, I assessed differences in the proportion of hypo and non-epilimnetic individuals with experiment week (categorical variables with two levels; Week 0 or Week 3) and *Bythotrephes* (categorical variables with two levels; Present or Absent) as interacting explanatory variables using either gamma or log-normal distributed generalized linear mixed models (GLMMs) with a log link and mesocosm as a random effect. Any changes in the
proportion of hypo- and non-epilimnetic *Daphnia* between Week 0 and Week 3 were interpreted as shifts in vertical position during the experiment.

For all GLMs and GLMMs, I used AICc values to assess model fit between Gamma and log-normal distributions, choosing the distribution with the lowest AICc value for the full model assessed. Model fit was also assessed by visually examining plots of residual versus fitted values and square root of the standard deviance of residuals versus fitted values. Cook’s distance was used to identify influential points, those with leverage greater than 1.0. Minimum adequate models were chosen using log-likelihood ratio tests based on Crawley’s (2005) procedure. If influential points were detected, robust GLMs were constructed using the robustbase package (version 0.93-3), fitting a Gamma or log-normal generalized linear model using Mallows or Huber type robust estimators (Cantoni and Ronchetti (2001), Cantoni and Ronchetti (2006)). which downweights the effect of influential points on model fit. For robust glms, minimum adequate models were also chosen based on Crawley’s (2005) procedure with Wald-type tests. For GLMMs, if statistically significant interactions were detected, I used generalized linear hypothesis tests for assessing multiple comparisons using the multcomp package (version 1.3-8).

Performing a large number of hypothesis tests simultaneously can increase the probability of wrongly rejecting a null hypothesis due to random chance (Pike 2011). To correct for this, I used Benjamini-Hochberg (1995) false discovery rates (FDR), which control the number of false positives (when the null hypothesis is rejected despite being true). Details about this procedure are provided in Appendix 3. This method was used to assess p-values obtained from GLMs examining per capita change and layer density, for each zooplankton group (i.e. *Daphnia*, small
The proportion of hypo and non-epilimnetic total Daphnia in both invaded and uninvaded mesocosms did not change through the duration of our experiment (Gamma GLMMs, significant random effects, Table 3.3). Overall, total Daphnia densities were greater in Week 3 as compared to Week 0. The effect of Bythotrephes on per capita change in total Daphnia density was influenced by the proportion of total hypolimnetic Daphnia (Fig 3.2a, Gamma GLMs, Table 3.4). In uninvaded mesocosms with a greater proportion of hypolimnetic Daphnia in week 0, per capita total Daphnia density increase was larger as compared to mesocosms with a lower proportion of hypolimnetic Daphnia. Invaded mesocosms had a smaller per capita increase compared to uninvaded mesocosms (Table 3.4). There was no effect of the proportion of non-
epilimnetic total *Daphnia* in week 0 or its interaction with *Bythotrephes* on per capita change in *Daphnia* density. Despite an overall negative effect of *Bythotrephes* on per capita change in *Daphnia* density across the entire water column, there was no significant effect of *Bythotrephes* on per capita change in total *Daphnia* density across the individual thermal layers (Gamma GLMMs, significant random effects, Table 3.5). However, I detected a greater per capita increase in *Daphnia* density in the epilimnion as compared to the meta- and hypolimnion (Figure 3.2b, Table 3.5, Epi = 3.88 ± 6.74, Meta = 2.59 ± 3.37, Hypo = 1.74 ± 1.46).

**Daphnia Species**

At the species level, there was no significant difference in the proportion of hypo- and non-epilimnetic *D. catawba* during the experiment across invaded and uninvaded mesocosms (Gamma GLMMs, significant random effects, Figure S3.4, Table 3.3). There was no effect of *Bythotrephes*, the proportion hypo- and non-epilimnetic total *Daphnia* or *D. catawba* in week 0 on the per capita change in *D. catawba* density (Gamma GLMs, Table 3.5). Per capita increase in *D. catawba* metalimnetic densities was smaller in invaded mesocosms as compared to uninvaded mesocosms (Uninvaded = 6.48±10.3, Invaded = 0.97±2.49), with no effect on epi- or hypolimnetic per capita change in density (Figure 3.3, Gamma GLMMs, significant random effects, Table 3.5, generalized linear hypothesis testing: meta p = 0.02).

There was no significant difference in the proportion of hypo- and non-epilimnetic *D. ambiguа* during the experiment across invaded and uninvaded mesocosms (Gamma GLMMs, significant random effects, Table 3.3, Figure S3.4). Fewer hypo- and non-epilimnetic *D. ambiguа*
were present in all mesocosms in week 3 as compared to week 0 (Gamma GLMMs, significant random effects, Figure S3.4). *Bythotrephes* presence, the proportion of total hypolimnetic *Daphnia* and the interaction between these variables had no impact on per capita change in *D. ambigua* density (Gamma GLMs, Table 3.4). Per capita increase in *D. ambigua* density was larger in invaded mesocosms with a smaller proportion of total non-epilimnetic *Daphnia* in week 0 as compared to uninvaded mesocosms (Figure 3.4a, Gamma GLMs, Table 3.4). Per capita increase in *D. ambigua* density was similar between invaded and uninvaded mesocosms with a greater proportion of non-epilimnetic *Daphnia* (Figure 3.4a, Table 3.4). A larger per capita increase in *D. ambigua* density was also observed in mesocosms with a greater proportion of non-epilimnetic *D. ambigua* individuals (Figure 3.4b, Gamma GLMs, Table 3.4). Mean per capita increase in *D. ambigua* density in the metalimnion and hypolimnion density was smaller than the epilimnion (Figure 3.4c, Table 3.5, significant random effects, layer: \( p < 0.001 \), FDR is significant, Epi = 14.25 ± 28.4, Meta = 1.23 ± 1.61, Hypo = 1.48 ± 2.25), with no difference between invaded and uninvaded mesocosms.

For *D. mendotae*, there were fewer hypolimnetic individuals in uninvaded mesocosms in Week 3 as compared to Week 0 (Gamma GLMMs, Table 3.3, GLHT: \( p = 0.018 \), Figure S3.4). There was no change in the proportion of non-epilimnetic *D. mendotae* between Week 0 and Week 3 in either invaded or uninvaded mesocosms. Fewer non-epilimnetic individuals were present in uninvaded mesocosms in Week 3 as compared to invaded mesocosms (Table 3.3, GLHT: \( p = 0.018 \)). Total *Daphnia* depth distribution did not influence the impact of *Bythotrephes* on per capita change in *D. mendotae*. Per capita change in density for *D. mendotae*
was lower in invaded mesocosms as compared to uninvaded mesocosms (Figure 3.5a). A larger per capita increase was observed for *D. mendotae* in uninvaded mesocosms with more hypolimnetic *D. mendotae* individuals as compared to invaded mesocosms (Figure 3.5b). The impact of *Bythotrephes* on *D. mendotae* varied between thermal layers. Per capita increase in epilimnetic *D. mendotae* density was smaller in invaded mesocosms as compared to uninvaded mesocosms (Figure 3.5c, Table 3.5, Gamma GLMMs, significant random effects, generalized linear hypothesis testing; *epi* *p* = 0.002, Uninvaded =40.0 ± 67.7, Invaded =9.5 ± 5.1) with no differences observed in the meta- and hypolimnion.

Fewer hypolimnetic *D. pulex/pulicaria* were present in invaded mesocosms in Week 3 as compared to uninvaded mesocosms (Gamma GLMMs, significant random effects, generalized linear hypothesis testing, *p* = 0.043, Table 3.3, Figure S3.4). There were no differences in the proportion of non-epilimnetic *D. pulex/pulicaria* individuals in both invaded and uninvaded mesocosms. Only the proportion of total non-epilimnetic *Daphnia* impacted per capita increase in *D. pulex/pulicaria* density, with greater per capita increase in density observed in mesocosms with greater proportion of total non-epilimnetic individuals (Fig 3.6a). A greater per capita increase in *D. pulex/pulicaria* density was also observed in mesocosms with a greater proportion of hypo- and non-epilimnetic *D. pulex/pulicaria* (Figure 3.6b). For *D. pulex/pulicaria*, there was a decrease in per capita density in the epi- and metalimnion in invaded mesocosms (Epi = 0.77 ± 0.41, Meta = 1±0) as compared to uninvaded mesocosms (Epi = 3.4 ± 7.3, Meta = 5.0 ± 10.1), with no impact observed in the hypolimnion (Figure 3.6c, Table 3.5, significant random effects; generalized linear hypothesis testing; *epi*: *p* = 0.02, *meta*: *p* = 0.009).
**Small cladoceran density**

Small cladocerans were mostly epilimnetic across all mesocosms during the experiment (Figure S3.5). There was a general increase in small cladoceran density in all mesocosms during the experiment. In uninvaded mesocosms, larger per capita increases in total small cladoceran density were observed in mesocosms with a greater proportion of hypolimnetic total *Daphnia* (Figure 3.7a, Gamma GLMs, Table 3.4). Total *Daphnia* depth distribution influenced the magnitude of *Bythotrephes* impact on total small cladocerans, with a smaller per capita increase in density observed in invaded mesocosms with a greater proportion of hypolimnetic *Daphnia* as compared to uninvaded mesocosms (Table 3.4). The proportion of non-epilimnetic *Daphnia* in week 0 did not influence the effect of *Bythotrephes* on per capita change in total small cladoceran density (Table 3.4), although greater increase in per capita density was observed in all mesocosms with more non-epilimnetic *Daphnia* (Table 3.4). In invaded mesocosms there was a smaller increase in per capita total small cladoceran density in the metalimnion as compared to uninvaded mesocosms (Uninvaded = 118.1 ± 357.9, Invaded = 25.2 ± 19.7, GLHT: p = 0.02) but no difference in the epilimnion or hypolimnion (Figure 3.7b, Gamma GLMMs, Table 3.5, significant random effects).

*B. freyi/liederi, E. tubicen,* and *E. longispina* densities increased through the course of the experiment. Invaded mesocosms had a smaller per capita increase in *B. freyi/liederi, E. tubicen* and *E. longispina* density as compared to uninvaded mesocosms (Table 3.4, Figure 3.8a, c and e). Per capita change in *B. freyi/liederi* density was greater in mesocosms with greater proportion of
total hypo- and non-epilimnetic total *Daphnia* (Figure 3.8a, Table 3.4). Per capita change in *B. freyi/liederi* density in invaded mesocosms as compared to uninvaded mesocosms was smaller in the metalimnion (Uninvaded = 92.6 ± 319.2, Invaded = 10.9 ± 15.6) and larger in the hypolimnion (Uninvaded = 1.5 ± 2.3, Invaded = 18.2 ± 50.7) (Figure 3.8b, Table 3.5, Gamma GLMMs, significant random effects, generalized linear hypothesis testing: meta p = 0.0358, hypo: p = 0.006).

There was no effect of proportion of total hypo- and non-epilimnetic *Daphnia* on the per capita change in *E. tubicen* density (Figure 3.8c, Table 3.4). The increase in per capita *E. tubicen* density in the epilimnion was larger as compared to meta- and hypolimnion (Figure 3.8d, Table 3.5, Epi = 70.1 ± 114.4, Meta = 5.7 ± 14.7, Hypo = 1.7 ± 2.7). There was a smaller per capita increase in *E. longispina* density (Figure 3.8e, Table 3.4), in mesocosms where fewer epilimnetic *Daphnia* were present, i.e. greater proportion of total hypo- and non-epilimnetic *Daphnia*. For *E. longispina*, epilimnetic per capita increase in density was smaller in the presence of *Bythotrephes* (Uninvaded = 105.7 ± 86.7, Invaded = 54.2 ± 62.0), with no differences in per capita density between invaded and uninvaded mesocosms in the meta- and hypolimnion (Figure 3.8f, Table 3.5, generalized linear hypothesis testing, epi: p <0.001).

**Large cladoceran density**

Large cladocerans were mostly epilimnetic across all mesocosms during the experiment (Figure S3.5). Total large cladoceran densities decreased in most mesocosms during the experiment. There was no interaction between the proportion of hypolimnetic *Daphnia* and
Bythotrephes on per capita change in density (Table 3.4). Per capita large cladoceran density increased in uninvaded mesocosms with greater proportion of non-epilimnetic total Daphnia in week 0, while there was a small decrease in per capita change in density in invaded mesocosms (Figure 3.9a, Table 3.4). There was no effect of proportion of hypolimnetic Daphnia in week 0, Bythotrephes or their interaction on per capita change in H. glacialis density (Table 3.4). In invaded mesocosms with greater proportion of total non-epilimnetic Daphnia, I observed a small per capita decrease in H. glacialis density as compared to mesocosms with fewer total non-epilimnetic Daphnia (Figure 3.9b, Table 3.4). In uninvaded mesocosms, an increase in per capita H. glacialis density was observed in mesocosms with fewer epilimnetic Daphnia. There was no effect of Bythotrephes in epi-, meta- or hypolimnetic per capita change in density for large cladocerans or for H. glacialis (Table 3.5).

Copepod density

Both calanoid and cyclopoid copepods were mostly epilimnetic across all mesocosms during the experiment (Figure S3.5). Overall, calanoid density decreased through the course of my experiment. Daphnia vertical position had no influence on the impact of Bythotrephes on per capita change in calanoid density (Gamma GLMs, Table 3.5). Additionally, there was no effect of Bythotrephes or proportion of total hypo- or non-epilimnetic Daphnia in week 0 on per capita change in calanoid density (Table 3.5). Cyclopoid density increased throughout the experiment in most mesocosms. There was no effect of Bythotrephes, proportion total hypo- and non-epilimnetic Daphnia in week 0 or the interaction between them on per capita change in density.
(Gamma GLMs, Table 3.4). In almost all mesocosms, copepodid density decreased with time. Per capita decrease in density was smaller in uninvaded mesocosms with a greater proportion of total hypolimnetic *Daphnia* as compared to invaded mesocosms (Figure 3.9c, Gamma GLMs, Table 3.4). There was no interactive effect of the proportion of non-epilimnetic *Daphnia* in week 0 and *Bythotrephes* on per capita change in copepodid density, although a smaller decrease in per capita copepodid density was observed in mesocosms with more total non-epilimnetic *Daphnia* (Table 3.4). There was no effect of *Bythotrephes* or proportion of total hypo- and non-epilimnetic *Daphnia* on the per capita change in nauplii density.

**Algal biomass**

In all mesocosms, total algal biomass increased through the course of the experiment, with most of the production occurring in the epilimnion. Algal biomass per unit biomass was greater in invaded mesocosms with more hypolimnetic *Daphnia* (Figure 3.10a, Gamma GLMs, Table 3.4, FDR significant). There was no effect of total non-epilimnetic *Daphnia* on *Bythotrephes* impacts on total algal biomass per unit biomass (Table 3.4).

Cyanobacteria were the most abundant algal functional group, followed by green algae, diatoms, and cryptophytes across our mesocosms. For all algal groups, biomass was greater in week 3 as compared to week 0 in the majority of mesocosms. For green algae, there was no effect of *Bythotrephes* nor an interaction between *Bythotrephes* and total *Daphnia* vertical position on change in biomass per unit biomass (Gamma GLMs, Table 3.4). A larger increase in green algal biomass per unit biomass was observed in mesocosms with greater proportion of hypolimnetic
Daphnia in week 0, while there was no effect of the proportion of non-epilimnetic Daphnia in week 0 (Figure 3.10b, Table 3.4). For cyanobacteria, I observed a larger increase in biomass in uninved mesocosms with fewer hypolimnetic Daphnia (Figure 3.10c, Table 3.4). There was no effect of Daphnia vertical position on change in cyanobacteria biomass per unit biomass observed in invaded mesocosms. For diatoms and cryptophytes, there was no effect of Daphnia depth distribution or Bythotrephes presence on change in biomass per unit biomass (Table 3.4).

**Discussion**

Differences in the vertical position of Daphnia in our mesocosms resulted in differences in the effect of Bythotrephes predation on the change in abundance of several zooplankton groups (e.g. small cladocerans, copepods) as well as individual species (e.g. H. glacialis). These impacts consequently influenced the change in biomass per unit biomass for total algae as well as individual algal groups (e.g cyanobacteria). Most notably, I found evidence of trophic cascades in invaded mesocosms where spatial overlap between Daphnia and Bythotrephes was low, i.e. more hypo and non-epilimnetic Daphnia. The strength of this trophic cascade was linked to the degree of spatial overlap between total Daphnia and Bythotrephes, with stronger cascades in invaded mesocosms with the least spatial overlap between Bythotrephes and Daphnia. Previous studies have attributed trophic cascades associated with Bythotrephes invasion as the consequence of reduced Daphnia and cladoceran density due to predation (Strecker and Arnott 2008, Walsh et al. 2016). My results suggest that differences in Daphnia vertical position can also contribute to trophic cascades in Bythotrephes invaded systems.
Impacts on Daphnia

Total *Daphnia* vertical position did not change during the course of the experiment. The vertical position of *Daphnia* in the water column did not influence the impacts of *Bythotrephes* on total *Daphnia* abundance. However, *Bythotrephes* predation on *Daphnia* in invaded mesocosms, due to preferential predation (Schulz and Yurista 1999), resulted in smaller increases in abundances as compared to uninvaded mesocosms, matching observations from numerous field surveys and mesocosm experiments (Yan et al. 2002, Barbiero and Tuchman 2004, Strecker and Arnott 2005, Pangle and Peacor 2006, Pangle et al. 2007, Strecker and Arnott 2008, Pangle and Peacor 2009, Strecker and Arnott 2010, Jokela et al. 2013, reviewed by Azan et al 2015, Azan and Arnott 2017). The lack of impact of *Daphnia* vertical position on total *Daphnia* abundance in the presence of *Bythotrephes* could be due to similarity between the impacts of *Bythotrephes* predation and the metabolic costs of occupying deeper colder hypolimnetic waters (Kerfoot 1985, Dawidowicz and Loose 1992, Loose and Dawidowicz 1994, Cole et al. 2002, Kessler and Lampert 2004, Pangle et al. 2007, Pangle and Peacor 2010) across my mesocosms.

*D. ambiguа* was the only species for which *Bythotrephes* impacts on abundance was influenced by total *Daphnia* vertical position, with a smaller increase in abundance in invaded mesocosms with a greater proportion of non-epilimnetic total *Daphnia* as compared to uninvaded mesocosms. In contrast, I detected a larger increase in *D. ambiguа* abundance in mesocosms with more non-epilimnetic *D. ambiguа* individuals. This increase in density is driven by the upwards movement of *D. ambiguа* individuals from the meta- and hypolimnion towards
the epilimnion, as observed by the decrease in the proportion of hypo- and non-epilimnetic individuals in week 3. These results suggest that *Bythotrephes* predation on and competition for algae with larger bodied *D. mendotae* and *D. pulex/pulicaria* (Schulz and Yuritsa 1999) influenced *D. ambigua* abundance in my mesocosms. In uninvaded mesocosms, smaller per capita increases in magnitude were observed in uninvaded mesocosms where a greater proportion of total *Daphnia* were epilimnetic, likely a result of competition with larger *Daphnia* species for algal resources. The similarity in the magnitude of increase in *D. ambigua* abundance in both invaded and uninvaded mesocosms with more non-epilimnetic *D. ambigua* individuals in week 0 is a result of movement into the epilimnion by hypo- and non-epilimnetic individuals in week 3. This movement into the epilimnion across all mesocosms regardless of *Bythotrephes* presence suggests that not only are the costs of competition for algal resources with larger *Daphnia* species and *Bythotrephes* predation on *D. ambigua* abundance similar to each other in magnitude and direction, but that the metabolic costs of occupying deeper waters are larger in magnitude as compared to benefits of avoiding competition and predation in the epilimnion.

Total *Daphnia* vertical position did not influence the impacts of *Bythotrephes* presence on per capita change in the abundance of *D. pulex/pulicaria, D. mendotae* and *D. catawba*. The vertical position of *D. catawba, D. mendotae* and *D. pulex/pulicaria* did not change during the experiment. Invaded mesocosms had smaller increase in *D. pulex/pulicaria* abundance as compared to uninvaded mesocosms. A smaller increase in *D. mendotae* abundance was observed in invaded mesocosms as compared to uninvaded mesocosms. Coupled with the lack of per capita increase in epilimnetic density between invaded and uninvaded mesocosms, these results suggest
Bythotrephes predation on epilimnetic individuals resulted in smaller per capita increase in abundance in invaded mesocosms. There was no effect of Bythotrephes on D. catawba density. It is possible that preferential Bythotrephes predation on larger bodied Daphnia (Schulz and Yuritsa 1999), such as D. pulex/pulicaria and D. mendotae over the smaller species D. catawba could be underlying this trend. A smaller increase in D. catawba metalimnetic abundance in invaded mesocosms as compared to uninvaded ones suggests that there is some Bythotrephes predation on this species, but it is not sufficient to impact D. catawba abundance at the mesocosm level.

Indirect impacts on other zooplankton groups

Differences in Daphnia vertical position resulted in differences in the impacts of Bythotrephes on small cladocerans, especially B. freyi/leideri and E. longipsina. These results may be linked to Bythotrephes preference for large bodied cladocerans such as Daphnia (Schulz and Yurista 1999) in mesocosms where more Daphnia are epilimnetic, leading to a smaller decline in total small cladoceran abundance as predicted. In mesocosms with fewer epilimnetic Daphnia, Bythotrephes predation likely extended to smaller cladocerans resulting in the larger decline in density observed. In the literature, Bythotrephes invasion has shown to reduce small cladoceran abundance (Vanderplog et al. 1993, Yan and Pawson 1997, Walstrom and Westman 1999, Yan et al. 2001, Barbiero and Tuchman 2004, Strecker et al. 2006, Strecker and Arnott 2008, Kelley et al. 2013, Kerfoot et al. 2016), although this effect has not been consistently observed across all studies (Lehman and Caeceres 1993, Barbiero and Tuchman 2004, Strecker and Arnott 2005, Hessen et al. 2011). Additionally, there is a large variation in the magnitude of
Bythotrephes impacts observed, with declines in small cladoceran abundance ranging from 40-126% in invaded lakes (Vanderplog et al. 1993, Yan et al. 2001, Kelley et al. 2013, Kerfoot et al. 2016). My results suggest that Daphnia vertical position could be an important factor explaining the variation in the effect and magnitude of Bythotrephes impact observed across these studies.

Differences in Daphnia vertical position in invaded mesocosms also resulted in different Bythotrephes impacts on large cladocerans abundance. Large cladoceran abundance increased in invaded mesocosms where Daphnia were shallower in the water column. At the species level, H. glacialis abundance increased in invaded mesocosms with shallower Daphnia vertical position. These results suggest that despite its large body size, Bythotrephes preferentially feed on Daphnia as compared to H. glacialis. This may be due to the protective gel surrounding the body of H. glacialis, which can impede capture and ingestion by predators (Azan et al. 2015).

However, the decline in H. glacialis abundance in invaded mesocosms where a greater proportion of Daphnia have a deeper vertical position, suggests that Bythotrephes do feed on H. glacialis when fewer Daphnia are present in the epilimnion. These results also suggest that variability in the impact of Bythotrephes on H. glacialis abundance depends on the abundance of epilimnetic Daphnia. The lack of Bythotrephes impact on H. glacialis or increase observed in some studies (Yan et al 2001, Yan and Pawson 1997, Dumitru et al. 2001, Strecker and Arnott 2005, Fernandez et al. 2009, Kelly et al. 2012, Jeziorski et al. 2015) and the decline in abundance in others (Lehman 1991, Walstrom and Westman 1999, Barbiero and Tuchman 2004, Strecker et al. 2006) may be a consequence of differences in Daphnia vertical position across the lakes assessed and has yet to be examined in any experimental or observational studies.
There was no effect of *Bythotrephes* or *Daphnia* vertical position on calanoid or cyclopoid abundance. The lack of *Bythotrephes* impact on adult calanoids and cyclopoids is surprising as negative impacts of *Bythotrephes* on cyclopoids such as *Mesocyclops edax* (Makarewicz et al. 1995, Yan and Pawson 1997, Yan et al. 2001, Yan et al. 2002, Boudreau and Yan 2003, Strecker and Arnott 2005, Strecker et al. 2006, Kelly et al. 2012) and calanoids such as *Leptodiaptomus minutus* (Strecker and Arnott 2005, Strecker et al. 2006, Hessen et al. 2011, Bourdeau et al. 2011, Kelly et al. 2012); both species present in my mesocosms, have been observed across the majority of studies in the literature. One possible explanation for this lack of *Bythotrephes* effect may be that prey preference for larger bodied cladocerans (Schulz and Yurista 1999) combined with faster escape responses for copepods as compared to cladocerans (Pichlová-Ptácˇníková and Vanderploeg 2011), resulted in greater overall predation on cladocerans. Additionally, copepod species, *Diacyclops thomasi* and *Leptodiaptomus minutus*, in Lake Michigan have been shown to migrate to the hypolimnion to avoid *Bythotrephes* predation in Lake Michigan. However, I did not observe any change in copepod vertical position across my invaded and uninvaded mesocosms; the majority were epilimnetic (Figure S3.5), suggesting that copepods were able to avoid *Bythotrephes* predation without changing their vertical position. For calanoid copepods, I did not observe any effect of *Daphnia* vertical position on per capita change in calanoid density in uninvaded mesocosms. This is surprising as both *Daphnia* and calanoids primarily feed on algae, therefore less competition for algae between *Daphnia* and calanoids is expected in mesocosms with fewer epilimnetic *Daphnia* leading to an increase per capita calanoid density. It
is possible that I was unable to detect the impacts these competitive interactions due to the short time frame of my experiment.

Copepodid abundance was lower in invaded mesocosms with more Daphnia that were deeper in the water column, suggesting that Bythotrephes do feed on copepodids when fewer Daphnia are present in the epilimnion, a result also observed by Strecker et al. (2007). I did not detect any effect of Bythotrephes on nauplii abundance in my mesocosm, an observation that contradicts other studies where fewer nauplii were observed in invaded lakes as compared to uninvaded ones (Kerfoot et al. 2016). One possible explanation could be that Bythotrephes preference for larger bodied prey (Schulz and Yurista 1999) resulted in predation on Daphnia, small cladocerans and large cladocerans rather than nauplii which occupy the smaller end of the zooplankton size spectrum in my mesocosms. Considering that nauplii represented 11- 43% of abundance across all mesocosms in week 3, similar to total Daphnia abundance (1 – 54%), density dependent predation did not influence nauplii per capita change in density in my mesocosms.

Algal Density and Trophic Cascades

Differences in Bythotrephes impacts on small cladocerans, large cladocerans and copepodids due to differences in Daphnia vertical position impacted primary production in my mesocosms, resulting in trophic cascades. Increase in total algal biomass per unit biomass was greater in invaded mesocosms as compared to uninvaded mesocosms, with the greatest increase in magnitude observed in mesocosms with greatest proportion of hypolimnetic Daphnia. This is
likely a result of reduced small and large cladoceran abundance due to *Bythotrephes* predation and reduced epilimnetic grazing by *Daphnia*. The invasion of *Bythotrephes* in some northern temperate lakes in North America has been associated with trophic cascades due to reduction in grazing zooplankton biomass (Walsh et al. 2016) which has been observed in some lakes but not others (Strecker and Arnott 2008). My results suggest that in addition to the nutrient status of these lakes (Walsh et al. 2016), *Daphnia* vertical position could be an important factor explaining the variable impacts of *Bythotrephes* on primary production observed across these studies.

There was no influence of *Daphnia* vertical position on *Bythotrephes* impacts on any algal taxonomic group. Neither *Daphnia* vertical position nor *Bythotrephes* presence impacted change in diatom or cryptophyte biomass per unit biomass in my mesocosms. *Daphnia* grazing on green algae was reduced in mesocosms with a greater proportion of hypolimnetic *Daphnia*, likely resulting in the increase in green algal biomass observed in these mesocosms. Cyanobacterial biomass per unit biomass declined in uninvaded mesocosms with a greater proportion of hypolimnetic *Daphnia*, likely driven by indirect changes in competitive dynamics with green algae as a result of *Daphnia* vertical position.

*Daphnia* vertical position

The depth distribution of total *Daphnia* and individual species remained consistent over the duration of the experiment, suggesting that *Bythotrephes* presence did not induce a deeper vertical position response. This contrasts with laboratory studies where *D. mendotae* individuals from Lake Michigan adopt a deeper vertical position when exposed to *Bythotrephes* kairomone.
(Bourdeau et al. 2013) as well as my results in Chapter 2 where *D. mendotae* have a deeper vertical position in invaded lakes across south-central Ontario as compared to uninvaded lakes. One possible reason for this mismatch could be that vertical response to *Bythotrephes* is developed either through selection of individuals that have a deeper vertical position or can be induced to do so as a result of strong predation pressure over many generations (Lehman et al. 1999), and therefore might not be observable in my experiment due to its short duration. *Daphnia* vertical position response to predators is also genetically constrained, with high intra-population variation in the magnitude of vertical position responses induced (De Meester 1996, Cousyn et al. 2001, Boeing et al. 2006). It is possible that some individuals were shifting their vertical positions in response to *Bythotrephes*, but I was unable to detect this as there was no effect of *Bythotrephes* presence on the vast majority of daphniid individuals in my mesocosms.

There was large variation in *Daphnia* vertical position, in both invaded and uninvaded mesocosms only receiving individuals from populations with shallow (Echo Lake) or deep (Bonnie Lake) vertical positions, despite both lakes of origin having similar water chemistry (Table 2) and predator community compositions (*Chaoborus* and fish were present in both lakes). Given that *Daphnia* daytime vertical position is influenced by a variety of biotic and abiotic factors ranging from water clarity (Wissel and Ramacharan 2003, Boeing et al., 2004, Kessler et al., 2008, Hansson and Hylander 2009, Williamson et al. 2011) to resource availability and the vertical position of predators (Johnsen and Jakobsen 1987, Leibold 1990, Watt and Young 1994, Kessler et al. 2008, Larson and Lampert 2012, Bourdeau et al. 2013), one possibility could be that stocked individuals were responding to the abiotic conditions of Fletcher Lake. However,
Fletcher Lake has similar water chemistry as Bonnie and Echo lake (Table 3.2), suggesting that this is likely not the case. As *Daphnia* vertical position is also genetically constrained (De Meester 1996, Cousyn et al. 2001, Boeing et al. 2006, Rozeberg et al. 2015), it is also possible that removing these individuals from the biotic and abiotic contexts that inform their vertical position and introducing them into a new context without the presence of any predators allowed for the varying vertical positions present in these populations to be observed.

**Conclusions**

Food web and ecosystem consequences of differences in *Daphnia* vertical position remain largely ignored, despite literature suggesting that inter-population trait variation, especially in predator-prey interactions, may be the primary mechanism maintaining food web complexity in ecological systems and driving trophic cascades. This study highlights the strong influence of *Daphnia* vertical position on interactions between zooplankton groups, ultimately affecting primary production in lake ecosystems, regardless of *Bythotrephes* presence. My results also provide the first experimental evidence suggesting that differences in *Daphnia* depth distribution influence the impacts of *Bythotrephes* predation on small and large cladocerans abundances, resulting in increased algal biomass. Understanding the influence of *Daphnia* vertical position on the structure and functioning of lake ecosystems will improve our ability to predict food web and ecosystem impacts of future invasions.
References


Lemoine, N. P. 2017. Predation risk reverses the potential effects of warming on plant-herbivore
interactions by altering the relative strengths of trait- and density-mediated interactions. The American Naturalist, 190: 337-349.


### Table 3.1: Expected outcomes for Daphnia, non-daphniid small (<0.85mm) and large cladocerans (>1.0mm), calanoid and cyclopoid copepods, and algal abundance in Bythotrephes invaded and uninvaded mesocosms with shallower and deeper Daphnia vertical position. Expected outcomes were based on expected densities in final experiment week (Week 3) as compared to initial conditions (Week 0).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Bythotrephes</th>
<th>Daphniid vertical position</th>
<th>Expected outcome</th>
<th>Potential mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daphnia</td>
<td>Absent</td>
<td>Shallow</td>
<td>Large increase</td>
<td>Feeding on abundant epilimnetic algae</td>
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<tr>
<td>Daphnia</td>
<td>Absent</td>
<td>Deep</td>
<td>Small decrease/no change</td>
<td>Metabolic costs of occupying colder hypolimnion</td>
</tr>
<tr>
<td>Daphnia</td>
<td>Present</td>
<td>Shallow</td>
<td>Large decrease</td>
<td>Bythotrephes predation</td>
</tr>
<tr>
<td>Daphnia</td>
<td>Present</td>
<td>Deep</td>
<td>Small decrease/no change</td>
<td>Metabolic costs of occupying colder hypolimnion, and no impact of Bythotrephes predation due to lack of spatial overlap</td>
</tr>
<tr>
<td>Small cladocerans</td>
<td>Absent</td>
<td>Shallow</td>
<td>Small decrease</td>
<td>Competition with Daphnia for abundant epilimnetic algae</td>
</tr>
<tr>
<td>Small cladocerans</td>
<td>Absent</td>
<td>Deep</td>
<td>Large increase</td>
<td>No competition with Daphnia for abundant algal resources</td>
</tr>
<tr>
<td>Small cladocerans</td>
<td>Present</td>
<td>Shallow</td>
<td>Small decrease/no change</td>
<td>Bythotrephes predation, and less competition with Daphnia for algal resources as a result of Bythotrephes predation on Daphnia.</td>
</tr>
<tr>
<td>Small cladocerans</td>
<td>Present</td>
<td>Deep</td>
<td>Large decrease</td>
<td>Bythotrephes predation</td>
</tr>
<tr>
<td>Large cladocerans</td>
<td>Absent</td>
<td>Shallow</td>
<td>Small decrease/no change</td>
<td>Competition with Daphnia for abundant epilimnetic algae</td>
</tr>
<tr>
<td>Large cladocerans</td>
<td>Absent</td>
<td>Deep</td>
<td>Large increase</td>
<td>No competition with Daphnia for abundant epilimnetic algae</td>
</tr>
<tr>
<td>Large cladocerans</td>
<td>Present</td>
<td>Shallow</td>
<td>Large increase</td>
<td>Less competition with Daphnia for abundant epilimnetic algae due to Bythotrephes predation</td>
</tr>
<tr>
<td>Large</td>
<td>Present</td>
<td>Deep</td>
<td>Large increase</td>
<td>No competition with Daphnia</td>
</tr>
<tr>
<td>Cladocerans</td>
<td>Calanoids</td>
<td>Cyclopoids</td>
<td>Total Algae</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-----------</td>
<td>------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td><strong>Absent</strong></td>
<td>Shallow</td>
<td><strong>Present</strong></td>
<td>Shallow</td>
<td></td>
</tr>
<tr>
<td><strong>Small decrease/no change</strong></td>
<td>Competition with <em>Daphnia</em> for abundant epilimnetic algal resources</td>
<td>Predation by <em>Bythotrephes</em> on epilimnetic cyclopoids</td>
<td>Feeding on epilimnetic algae by <em>Daphnia</em>, small cladocerans, large cladocerans and calanoids</td>
<td></td>
</tr>
<tr>
<td><strong>Deep</strong></td>
<td><strong>Small increase</strong></td>
<td><strong>No change</strong></td>
<td><strong>Large decrease</strong></td>
<td>Less removal of epilimnetic algae by <em>Daphnia</em> due to lack of spatial overlap. Feeding on epilimnetic algae by small cladocerans, large cladocerans and calanoids.</td>
</tr>
<tr>
<td><strong>Shallow</strong></td>
<td><strong>Small increase</strong></td>
<td><strong>No change</strong></td>
<td><strong>Small increase</strong></td>
<td>Less removal of epilimnetic algae by <em>Daphnia</em>, small cladocerans and large cladocerans due to <em>Bythotrephes</em> predation</td>
</tr>
</tbody>
</table>
Total Algae | Present | Deep | Large increase | Less removal of epilimnetic algal by *Daphnia* due to lack of spatial overlap and *Bythotrephes* predation on small cladocerans.
---|---|---|---|---
Table 3.2: Area, maximum depth (Zmax), pH, average total phosphorus (TP), dissolved organic carbon (DOC) and calcium (Ca) for Fletcher, Echo and Bonnie lakes based on data provided from the Canadian Aquatic Invasive Species Network (CAISN) surveys (2008, 2011). Mesocosms were suspended in Fletcher Lake. Zooplankton were stocked from either Echo, Bonnie or combination of both lakes.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Area (ha)</th>
<th>Maximum Depth (m)</th>
<th>pH</th>
<th>TP (ug/L)</th>
<th>DOC (mg/L)</th>
<th>Ca (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fletcher</td>
<td>266.28</td>
<td>23.2</td>
<td>6.12</td>
<td>5.0</td>
<td>4.6</td>
<td>2.34</td>
</tr>
<tr>
<td>Echo</td>
<td>215.6</td>
<td>11.9</td>
<td>6.46</td>
<td>9.0</td>
<td>6.6</td>
<td>2.74</td>
</tr>
<tr>
<td>Bonnie</td>
<td>39.3</td>
<td>21.9</td>
<td>6.78</td>
<td>5.6</td>
<td>2.3</td>
<td>2.85</td>
</tr>
</tbody>
</table>
**Table 3.3:** Summary of results from Gamma distributed GLMMs assessing the differences in the proportion hypo- (Hypo) and non-epilimnetic *Daphnia* between invaded and uninvaded mesocosms (Byth; N or Y) across experimental weeks 0 and 3 (Week; 0 or 3). FDR refers to the false discovery rate adjusted p-values with statistical significance denoted in brackets. FDR adjustments were not performed for statistically significant (p<0.05) interactions between *Bythotrephes* and Week. Generalized linear hypothesis tests performed (GLHT) for relevant comparisons when significant interactions between predictor variables were detected. P-values for GLHT comparisons were not FDR adjusted as these values are Bonferroni corrected (statistically significant values in bold). None refers to no significant predictors detected, with no relevant p or FDR values reported (NA). Degrees of freedom (df) are provided in brackets, next to p-values.

<table>
<thead>
<tr>
<th>Taxa/species</th>
<th>Response variable</th>
<th>Significant Predictor(s)</th>
<th>p-value (df)</th>
<th>FDR</th>
<th>Non-significant predictors</th>
<th>GLHT comparisons</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total <em>Daphnia</em></td>
<td>Hypo</td>
<td>Byth x Week</td>
<td>0.03 (76)</td>
<td>NA</td>
<td>None</td>
<td>N0-N3, Y0-Y3, N3-Y3</td>
<td>0.07, 0.09, 0.07</td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Byth x Week, Byth, Week</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td><em>D. ambigua</em></td>
<td>Hypo</td>
<td>Week</td>
<td>0.02 (79)</td>
<td>0.06 (significant)</td>
<td>Byth x Week, Byth</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>Week</td>
<td>0.02 (79)</td>
<td>0.06 (significant)</td>
<td>Byth x Week, Byth</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td><em>D. catawba</em></td>
<td>Hypo</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Byth x Week, Byth, Week</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>Byth x Week</td>
<td>0.0008 (76)</td>
<td>NA</td>
<td>None</td>
<td>N0-N3, Y0-Y3, N3-Y3</td>
<td>0.973, 0.593, 0.99</td>
</tr>
<tr>
<td><em>D. mendotae</em></td>
<td>Hypo</td>
<td>Byth x Week</td>
<td>0.03 (76)</td>
<td>NA</td>
<td>None</td>
<td>N0-N3, Y0-Y3, N3-Y3</td>
<td>0.018, 0.698, 0.09</td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>Byth x Week</td>
<td>&lt;0.0001 (76)</td>
<td>NA</td>
<td>None</td>
<td>N0-N3, Y0-Y3, N3-Y3</td>
<td>0.091, 0.950, 0.018</td>
</tr>
<tr>
<td>Taxa/species</td>
<td>Response variable</td>
<td>Significant Predictor(s)</td>
<td>p-value (df)</td>
<td>FDR</td>
<td>Non-significant predictors</td>
<td>GLHT comparisons</td>
<td>p-value</td>
</tr>
<tr>
<td>-------------------</td>
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<td>------------------------</td>
<td>---------</td>
</tr>
<tr>
<td><em>D. pulicaria/pulex</em></td>
<td>Hypo</td>
<td>Byth x Week</td>
<td>0.03 (76)</td>
<td>NA</td>
<td>None</td>
<td>N0-N3, Y0-Y3, N3-Y3</td>
<td>0.878</td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Byth x Week, Byth, Week</td>
<td>NA</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.043</td>
<td>0.043</td>
</tr>
</tbody>
</table>
Table 3.4: Summary of results from gamma and log-normal distributed GLMs assessing the impact of *Bythotrephes* (Byth), proportion of hypolimnetic total *Daphnia* in week 0 (Hypo) and proportion of non-epilimnetic total *Daphnia* in week 0 (Non-epi) on per capita change in *Daphnia*, other cladoceran groups, copepod densities and algal biomass. The impact of *Bythotrephes* and the vertical position of each *Daphnia* species on its per capita change in density were also assessed; represented by the abbreviated species name (Amb = *D. ambigua*, Cat = *D. catawba*, Men = *D. mendotae*, and Pul = *D. pulex/pulicaria*) and the proportion of hypolimnetic (Hypo) and non-epilimnetic (Non-epi) individuals. The influence of the proportion of hypo- and non-epilimnetic total *Daphnia* or each *Daphnia* species on *Bythotrephes* impacts were assessed in separate models. FDR refers to the false discovery rate adjusted p-values with statistical significance provided inside brackets. FDR adjustments were not performed for statistically significant (p<0.05) interactions between *Bythotrephes* and *Daphnia* vertical position. Degrees of freedom (df) are provided in brackets, next to p-values.

<table>
<thead>
<tr>
<th>Taxa/Species</th>
<th>Significant predictor(s)</th>
<th>p-value (df)</th>
<th>FDR</th>
<th>Outcome(s)</th>
<th>Non-significant predictor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total <em>Daphnia</em></td>
<td>Byth x Hypo</td>
<td>0.019 (36)</td>
<td>NA</td>
<td>Greater per capita increase in uninvaded mesocosms with more hypolimnetic <em>Daphnia</em> as compared to those with fewer <em>Daphnia</em>.</td>
<td>Byth x Non-epi Non-epi</td>
</tr>
<tr>
<td>D. ambigua</td>
<td>Non-epi</td>
<td>0.0003 (38)</td>
<td>0.0009 (Significant)</td>
<td>Greater increase in per capita density in mesocosms with greater proportion of total non-epilimnetic <em>Daphnia</em></td>
<td>Byth x Hypo, Byth x Amb Hypo, Hypo, AmbHypo, Byth,</td>
</tr>
<tr>
<td></td>
<td>Byth x Amb</td>
<td>0.04 (38)</td>
<td>NA</td>
<td>Per capita decrease in density in invaded mesocosms with more non-epilimnetic <em>Daphnia</em> as compared to uninvaded.</td>
<td></td>
</tr>
<tr>
<td>D. catawba</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No significant effect of any predictor variable.</td>
<td>Byth x Hypo, Byth x Non-epi, Byth x Cat Hypo, Byth x Cat Non-epi, Byth, Hypo, Non-epi, Cat Hypo, Cat</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Taxa/Species</th>
<th>Significant predictor(s)</th>
<th>p-value (df)</th>
<th>FDR</th>
<th>Outcome(s)</th>
<th>Non-significant predictor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Bythotrephes</strong></td>
<td></td>
<td></td>
<td><strong>Bythotrephes</strong>, with a smaller per capita density in invaded mesocosms as compared to uninvaded.</td>
<td>Non-epi</td>
</tr>
<tr>
<td><em>D. mendotae</em></td>
<td>Byth</td>
<td>0.0007 (38)</td>
<td>0.021 (significant)</td>
<td>Smaller per capita increase in density in invaded mesocosms as compared to uninvaded.</td>
<td>Byth x Hypo, Byth x Non-epi, Hypo, Non-epi, Men Non-epi</td>
</tr>
<tr>
<td></td>
<td>Byth x Men Hypo</td>
<td>0.0003 (37)</td>
<td>NA</td>
<td>Larger per capita increase in density in invaded mesocosms with more hypolimnetic individuals as compared to those with fewer hypolimnetic individuals.</td>
<td></td>
</tr>
<tr>
<td><em>D. pulex/pulicaria</em></td>
<td>Byth</td>
<td>0.006 (38)</td>
<td>0.02 (significant)</td>
<td>Smaller per capita increase in density in invaded mesocosms as compared to uninvaded</td>
<td>Byth x Hypo, Byth x Non-epi, Hypo, Byth x Pul Hypo, Byth x Pul Non-epi</td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>0.03 (38)</td>
<td>0.04 (significant)</td>
<td>Larger per capita increase in density in mesocosms with greater proportion of non-epilimnetic <em>Daphnia</em> in week 0.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pul Hypo</td>
<td>&lt;0.0001 (38)</td>
<td>0.00000585 (significant)</td>
<td>Larger per capita increase in density in mesocosms with a greater proportion of hypolimnetic individuals in week 0.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pul Non-epi</td>
<td>&lt;0.001 (38)</td>
<td>0.0007482 (significant)</td>
<td>Larger per capita increase in density in mesocosms with greater proportion of non-epilimnetic individuals in week 0.</td>
<td></td>
</tr>
</tbody>
</table>

**Other cladocerans**

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<table>
<thead>
<tr>
<th>Taxa/Species</th>
<th>Significant predictor(s)</th>
<th>p-value (df)</th>
<th>FDR</th>
<th>Outcome(s)</th>
<th>Non-significant predictor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Small cladocerans</td>
<td>Byth x Hypo</td>
<td>0.05 (37)</td>
<td>NA</td>
<td>Greater per capita increase in density in uninvaded mesocosms with more hypolimnetic <em>Daphnia</em>. Smaller per capita increase in density in invaded mesocosms with more hypolimnetic <em>Daphnia</em>.</td>
<td>Non-epi x Byth</td>
</tr>
<tr>
<td></td>
<td>Byth</td>
<td>0.03</td>
<td>0.045 (significant)</td>
<td>Smaller per capita increase in density in invaded mesocosms as compared to uninvaded mesocosms.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>0.02 (39)</td>
<td>0.045 (significant)</td>
<td>Greater increase in per capita density when more <em>Daphnia</em> are non-epilimnetic.</td>
<td></td>
</tr>
<tr>
<td><em>B. freyi/liederi</em></td>
<td>Byth</td>
<td>0.014 (37)</td>
<td>0.02 (significant)</td>
<td>Smaller per capita increase in density in invaded mesocosms</td>
<td>Byth x Hypo, Byth x non-epi</td>
</tr>
<tr>
<td></td>
<td>Hypo</td>
<td>0.012 (37)</td>
<td>0.036 (significant)</td>
<td>Greater per capita increase in mesocosms with more hypolimnetic total <em>Daphnia</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>0.0002 (37)</td>
<td>0.0006 (significant)</td>
<td>Greater per capita increase in mesocosms with more non-epilimnetic total <em>Daphnia</em></td>
<td></td>
</tr>
<tr>
<td><em>E. tubicen</em></td>
<td>Byth</td>
<td>0.03 (38)</td>
<td>0.12 (significant)</td>
<td>Smaller per capita increase in invaded mesocosms</td>
<td>Byth x Hypo, Byth x Non-epi, Hypo, Non-epi</td>
</tr>
<tr>
<td><em>E. longispina</em></td>
<td>Byth</td>
<td>0.03 (38)</td>
<td>0.045 (significant)</td>
<td>Smaller per capita increase in invaded mesocosms.</td>
<td>Byth x Hypo, Byth x Non-epi</td>
</tr>
<tr>
<td></td>
<td>Hypo</td>
<td>0.0002 (38)</td>
<td>0.0007 (significant)</td>
<td>Greater per capita increase in mesocosms with more hypolimnetic total <em>Daphnia</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>&lt;0.0001(38)</td>
<td>0.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taxa/Species</td>
<td>Significant predictor(s)</td>
<td>p-value (df)</td>
<td>FDR</td>
<td>Outcome(s)</td>
<td>Non-significant predictor(s)</td>
</tr>
<tr>
<td>----------------------</td>
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<td>--------------</td>
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<td>------------------------------------------------------------------------------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>(significant)</td>
<td></td>
<td></td>
<td>Greater per capita increase in mesocosms with more non-epilimnetic total <em>Daphnia</em></td>
<td></td>
</tr>
<tr>
<td>Large cladocerans</td>
<td>Byth x Non-epi</td>
<td>0.03 (36)</td>
<td>NA</td>
<td>Increase in per capita density in uninvaded mesocosms with greater proportion of non-epilimnetic total <em>Daphnia</em>. Decrease in per capita change in invaded mesocosms.</td>
<td>Byth x Hypo, Hypo</td>
</tr>
<tr>
<td><em>H. glacialis</em></td>
<td>Byth x Non-epi</td>
<td>0.03 (36)</td>
<td>NA</td>
<td>In uninvaded mesocosms, smaller per capita decrease in mesocosms with more epilimnetic total <em>Daphnia</em>. Greater decrease in invaded mesocosms with more non-epilimnetic total <em>Daphnia</em>.</td>
<td>Byth x Hypo, Hypo</td>
</tr>
<tr>
<td><strong>Copepods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calanoids</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes</em> or total <em>Daphnia</em> vertical position on per capita change in calanoid density.</td>
<td>Byth x Hypo, Byth, Hypo, Byth x Non-epi, Byth, Non-epi</td>
</tr>
<tr>
<td>Cyclopoids</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes</em> or total <em>Daphnia</em> vertical position on per capita change in cyclopoid density.</td>
<td>Byth x Hypo, Byth, Hypo, Byth x Non-epi, Byth, Non-epi</td>
</tr>
<tr>
<td>Copepodids</td>
<td>Byth x Hypo</td>
<td>0.0002 (36)</td>
<td>NA</td>
<td>Smaller per capita decrease in density in uninvaded mesocosms with more total hypolimnetic <em>Daphnia</em>.</td>
<td>Byth x Non-epi, Hypo</td>
</tr>
<tr>
<td></td>
<td>Non-epi</td>
<td>&lt;0.0001 (38)</td>
<td>0.0003 (significant)</td>
<td>Smaller decrease in per capita copepodid density in mesocosm with more total non-epilimnetic <em>Daphnia</em>.</td>
<td></td>
</tr>
<tr>
<td>Nauplii</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes</em> or total <em>Daphnia</em> vertical position on per capita change in nauplii density.</td>
<td>Byth x Hypo, Byth, Hypo, Byth x Non-epi, Byth, Non-epi</td>
</tr>
<tr>
<td>Taxa/Species</td>
<td>Significant predictor(s)</td>
<td>p-value (df)</td>
<td>FDR</td>
<td>Outcome(s)</td>
<td>Non-significant predictor(s)</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------</td>
<td>--------------</td>
<td>-----</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Algae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Algae</td>
<td>Byth x Hypo</td>
<td>0.001 (36)</td>
<td>NA</td>
<td>Per capita increase in algal biomass was greater in invaded mesocosm with more hypolimnetic <em>Daphnia</em></td>
<td>Byth x Non-epi, Non-epi</td>
</tr>
<tr>
<td>Green Algae</td>
<td>Hypo</td>
<td>&lt;0.0001 (38)</td>
<td>0.0005 (significant)</td>
<td>Greater per capita increase in green algae in mesocosms with more hypolimnetic <em>Daphnia</em></td>
<td>Byth*Non-epi, Hypo, Non-epi</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Byth x Hypo</td>
<td>0.05 (36)</td>
<td>NA</td>
<td>In uninvaded mesocosms, per capita increase in biomass was smaller in mesocosms with a greater proportion of hypolimnetic total <em>Daphnia</em>.</td>
<td></td>
</tr>
<tr>
<td>Diatoms</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes or Daphnia</em> vertical position on per capita biomass</td>
<td>Byth x Hypo, Byth x Non-epi, Byth, Hypo, Non-epi</td>
</tr>
<tr>
<td>Cryptophytes</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes or Daphnia</em> vertical position on per capita biomass</td>
<td>Byth x Hypo, Byth x Non-epi, Byth, Hypo, Non-epi</td>
</tr>
</tbody>
</table>
Table 3.5: Summary of results from Gamma distributed GLMMs assessing the impacts of *Bythotrephes* presence (Byth) on the per capita change in density of total *Daphnia*, total small cladocerans, total large cladocerans as well as *Daphnia*, small and large cladoceran species in the epi-, meta- and hypolimnion (Layer). FDR refers to the false discovery rate adjusted p-values with statistical significance denoted in brackets. FDR adjustments were not performed for statistically significant (p<0.05) interactions between *Bythotrephes* and Layer. Degrees of freedom (df) for residual deviance are provided in brackets, next to p-values.

<table>
<thead>
<tr>
<th>Taxa/Species</th>
<th>Significant predictor(s)</th>
<th>p-value (df)</th>
<th>FDR</th>
<th>Outcome(s)</th>
<th>Non-significant predictor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total <em>Daphnia</em></td>
<td>Layer</td>
<td>0.01 (118)</td>
<td>0.03  (Significant)</td>
<td>Greater per capita increase in density in the epilimnion as compared to the meta- and hypolimnion</td>
<td>Byth x Layer, Byth</td>
</tr>
<tr>
<td><em>D. ambigua</em></td>
<td>Layer</td>
<td>&lt;0.0001 (118)</td>
<td>&lt;0.0001 (Significant)</td>
<td>Greater per capita increase in density in the epilimnion as compared to the meta- and hypolimnion</td>
<td>Byth x Layer, Byth</td>
</tr>
<tr>
<td><em>D. catawba</em></td>
<td>Byth x Layer</td>
<td>&lt;0.0001(116)</td>
<td>NA</td>
<td>Smaller per capita increase in metalimnetic density in metalimnion in invaded mesocosms as compared to uninvaded mesocosms</td>
<td>None</td>
</tr>
<tr>
<td><em>D. mendotae</em></td>
<td>Byth x Layer</td>
<td>0.0002 (116)</td>
<td>NA</td>
<td>Smaller increase in density in the epilimnion in invaded mesocosms</td>
<td>None</td>
</tr>
<tr>
<td><em>D. pulex/pulicaria</em></td>
<td>Byth x Layer</td>
<td>0.03 (116)</td>
<td>NA</td>
<td>Decrease in per capita density in the epi- and metalimnion in invaded mesocosms</td>
<td>None</td>
</tr>
<tr>
<td>Total Small Cladocerans</td>
<td>Byth x Layer</td>
<td>0.0005 (116)</td>
<td>NA</td>
<td>Smaller increase in per capita metalimnetic density in invaded mesocosms</td>
<td>None</td>
</tr>
<tr>
<td><em>B. freyi/liederi</em></td>
<td>Byth x Layer</td>
<td>0.027 (116)</td>
<td>NA</td>
<td>Smaller increase in per capita meta- and hypolimnetic density in invaded mesocosms</td>
<td>None</td>
</tr>
<tr>
<td><em>E. tubicen</em></td>
<td>Layer</td>
<td>&lt;0.0001 (118)</td>
<td>0.0003</td>
<td>Greater per capita increase in density the epi- as compared to meta- and hypolimnion</td>
<td>Byth x Layer, Byth</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Taxa/Species</th>
<th>Significant predictor(s)</th>
<th>p-value (df)</th>
<th>FDR</th>
<th>Outcome(s)</th>
<th>Non-significant predictor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. longispina</em></td>
<td>Byth x Layer</td>
<td>0.02 (116)</td>
<td>NA</td>
<td>No change in per capita metalimnetic density in invaded mesocosms as compared to increase in density in uninvaded mesocosms</td>
<td>None</td>
</tr>
<tr>
<td>Total Large cladocerans</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes</em> or thermal layer on per capita change in density.</td>
<td>Byth x Layer, Byth, Layer</td>
</tr>
<tr>
<td><em>H. glacialis</em></td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>No effect of <em>Bythotrephes</em> or thermal layer on per capita change in density.</td>
<td>Byth x Layer, Byth, Layer</td>
</tr>
</tbody>
</table>
Figures

**Figure 3.1:** Food web diagram depicting interactions in the tri-trophic food web consisting of *Bythotrephes longimanus*, major zooplankton taxonomic groups and algae in my mesocosm experiment. Solid arrows indicate predator-prey interactions with widespread evidence in the literature, while dashed lines indicate predator-prey interactions with less or contradicting evidence in the literature. Arrow direction indicates the direction of energy movement in the food web.
Figure 3.2: a) Effect of proportion of total hypolimnetic *Daphnia* in week 0 on the per capita change in total *Daphnia* density in uninvaded (open circles, dotted line) and invaded (filled circles, solid line) mesocosms, and b) per capita change in total *Daphnia* density in the epi-, meta- and hypolimnion of my mesocosms. Different letters denote statistically significant differences (p <0.05). Shaded regions represent the 95% confidence interval estimated from the best fitting model.
Figure 3.3 Per capita change in *D. catawba* density in the epi-, meta-, and hypolimnion in uninvaded (N) and invaded mesocosms. Different letters denote statistically significant differences (p < 0.05). Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3.
Figure 3.4: Effect of a) proportion of total non-epilimnetic *Daphnia* and b) proportion of non-epilimnetic *D. ambigua* in week 0 on the per capita change in *D. ambigua* density in uninvaded (open circles, dotted line) and invaded (filled circles, solid line) mesocosms and, c) per capita change in *D. ambigua* density in the epi-, meta- and hypolimnion of uninvaded (N) and invaded (Y) mesocosms. Different letters denote statistically significant differences (p<0.05). Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3. Shaded regions represent the 95% confidence interval estimated from the best fitting model.
Figure 3.5: Effect of proportion of total hypolimnetic *Daphnia* and b) proportion of hypolimnetic *D. mendotae* in week 0 on the per capita change in *D. mendotae* density in uninvaded (open circles, solid line) and invaded (filled circles, dotted line) mesocosms and, c) per capita change in *D. ambigua* density in the epi-, meta- and hypolimnion of uninvaded (N) and invaded (Y) mesocosms. Different letters denote significant differences (p <0.05). Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3. Shaded region represents the 95% confidence interval estimated from the best fitting model.
Figure 3.6: Effect of a) proportion of total hypolimnetic *Daphnia* and b) proportion of hypolimnetic *D. pulex/pulicaria* in week 0 on the per capita change in *D. pulex/pulicaria* density in uninvaded (open circles, dotted line) and invaded (filled circles, solid line) mesocosms and, f) per capita change in *D. pulex/pulicaria* density in the epi-, meta- and hypolimnion of uninvaded (N) and invaded (Y) mesocosms. Different letters denote statistically significant differences (p<0.05). Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3. Shaded regions represent the 95% confidence interval estimated from the best fitting model.
Figure 3.7: a) Effect of proportion of total hypolimnnetic *Daphnia* on per capita change in density for a) total small cladocerans and b) total small cladoceran density in the epi-, meta-, and hypolimnion of uninvaded (N, open circles, dotted line) and invaded (Y, filled circles, solid line) mesocosms. Different letters denote statistically significant differences. Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3.
Figure 3.8: First column: The effect of proportion of hypolimnetic *Daphnia* on per capita change in density for a) *B. freyi/liederi*, c) *E. tubicen* and e) *E. longispina* in uninvaded (open circles, dotted line) and invaded (filled circles, solid line) mesocosms. Second column: Per capita change in b) *B. freyi/liederi*, d) *E. tubicen* and f) *E. longispina* density. Different letters denote statistically significant differences. Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3. Shaded regions represent the 95% confidence interval estimated from the best fitting model.
Figure 3.9: Effect of proportion of total non-epilimnetic *Daphnia* on per capita change in density for a) total large cladocerans, b) *H. glacialis*, and c) copepodid density in uninvaded (open circles, solid line) and invaded (filled circles, dotted line) mesocosms. Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3. Shaded regions represent the 95% confidence interval estimated from the best fitting model.
Figure 3.10: Effect of proportion of total hypolimnetic *Daphnia* on change in biomass per unit biomass for a) total algae, b) green algae and c) cyanobacteria density in uninvaded (open circles, dotted line) and invaded (filled circles, solid line) mesocosms. Values above the dashed line at 1 indicate increasing density and below indicate decreasing density between experiment week 0 and 3. Shaded regions represent the 95% confidence interval estimated from the best fitting model.
Chapter 4

Are the impacts of dispersal on Daphnia populations in systems invaded by Bythotrephes influenced by Daphnia vertical position?

Sarah S Hasnain and Shelley E Arnott
Abstract

Dispersal is an important process that determines the structure and function of ecological communities. Although the role of dispersal amount, frequency, condition and pathway quality on the establishment success of arriving individuals is well understood, the ecological consequences of differences in trait expression in these individuals remains ignored. In this chapter, I examine if differences in the vertical position of the source population of dispersing Daphnia influence the impacts of the Spiny Water Flea, Bythotrephes longimanus, on resident Daphnia populations in invaded system. To determine this, I conducted a mesocosm experiment stocked with and receiving dispersing Daphnia from populations with different vertical positions; shallow, deep or both in the presence and absence of Bythotrephes. My results show that the population from which dispersers originated and traits associated with these populations do not influence total Daphnia density or the density of any species in Bythotrephes invaded mesocosms. Only D. mendotae abundance was influenced by the vertical position of resident Daphnia, with no impact of Bythotrephes observed in mesocosms stocked from populations with deep vertical position while density declined in mesocosms stocked from populations with shallow vertical position. These results suggest that differences in trait expression of dispersing individuals do not influence the impact of Bythotrephes predation in invaded populations and that trait expression of resident individuals can play an important role influence in mediating the impacts of this invasive species.
Introduction

In ecological communities, community assembly and dynamics are simultaneously structured by processes at both the local and regional level (Leibold and Norberg 2004, Leibold et al. 2004, reviewed by Guichard 2017). These processes include local biotic and abiotic conditions (Cadotte et al. 2006, Orrock and Watling 2010, Cadotte and Tucker 2017), the amount, frequency and condition of the dispersing individuals, (Howeth and Leibold 2010a, Fronhofer et al. 2015, Sinclair and Arnott 2017) and quality of dispersal pathway (reviewed by Wilson et al. 2009). Among these factors, the influence of the traits of the arriving individuals on recipient communities remains understudied (but see De Meester et al. 2002, Pantel et al. 2011, 2015, De Bie et al. 2012). In most theoretical models and empirical experiments, the ecological consequences of differences in trait expression between dispersing individuals of the same species is ignored. However, intraspecific trait variation has been shown to influence population dynamics, allowing some individuals of the same species to persist in certain abiotic and abiotic conditions, while others perish (Duffy 2010, Jung et al. 2010). Furthermore, differences in trait expression between individuals of the same species has been shown to result in differences in community structure and ecosystem function (Post et al. 2008, Ingram et al. 2011, McPeek 2014, Lajoie and Vellend 2015, Chapter 3), although this effect has not been consistently observed (Kichenin et al. 2013).

The arrival of individuals from the regional species pool can increase the abundance of local populations (reviewed in Furrer and Pasinelli 2015) and introduce new genotypes for selection (Bohonak and Jenkins 2003, Bell and Gonzalez 2011, Bourne et al. 2014, Low-décarie et al. 2015), providing spatial insurance (Loreau et al. 2003) against losses in community
diversity and ecosystem function as a result of perturbations. However, dispersal can also introduce genotypes maladapted to the local abiotic and biotic environment, preventing populations from adapting to local conditions (De Meester et al. 2002, Urban 2006, Nosil, Vines and Funk 2015). These contrasting impacts of dispersal on population dynamics in recipient environments have been well studied from an evolutionary perspective (reviewed by Gonzalez et al. 2013). Greater levels of intraspecific genotypic and phenotypic diversity in dispersers increase establishment success, resulting in recovery for resident populations undergoing decline (reviewed by Rius and Darling 2014). In addition, the genetic and trait composition, and identity of the resident population influences the establishment success of arriving individuals (De Meester et al. 2007, Vellend et al. 2009, Pantel et al. 2011). Dispersers with different traits from residents are more likely to establish as they can occupy different niches (i.e. limiting similarity; MacArthur and Levins 1967) as compared to those with similar traits as residents due to local adaptation as a result of priority effects, a phenomenon known as community monopolization (De Meester et al. 2002). However, the ecological consequences of intraspecific trait differences in dispersing individuals on the structure and function of recipient populations and communities remain understudied (but see Low-décarie et al. 2015).

epilimnion and upper metalimnion, resulting in a spatial refuge for prey species against predation in the colder hypolimnetic waters (Young and Yan 2008). Some Daphnia, especially Daphnia mendotae have been shown to be able to avoid Bythotrephes predation by adopting a deeper daytime vertical position (Chapter 3, Pangle and Peacor 2006, Pangle et al. 2007, Bourdeau et al. 2013), despite large metabolic costs associated with occupying the colder regions (Loose and Dawidowicz 1994, Lampert et al. 2003, Pangle and Peacor 2006, 2010, Pangle et al. 2007). The degree of spatial overlap between Daphnia and Bythotrephes can influence the impacts of this predator on zooplankton communities in invaded systems, resulting in differences in direction and magnitude of Bythotrephes impacts on non-daphniid cladocerans and juvenile copepods (Chapter 3).

Although Daphnia daytime vertical position is influenced by a variety of factors (Wright and Brien 1982, Johnsen and Jakobsen 1987, Reede and Ringelberg 1995, Leech and Williamson 2001, Kessler and Lampert 2004), it is a genetically influenced phenotype, with population and clone specific differences observed (De Meester and Dumont 1988, Meester 1994, King and Miracle 1995, De Meester 1996, Boeing et al. 2006) in response to different biotic and abiotic factors present. Variation in local biotic and abiotic conditions across the landscape can select for different phenotypes occupying different vertical positions (De Meester 1996) or plasticity in vertical position response, resulting in differences in Daphnia vertical position across a region (Chapter 2). Dispersal from the regional species pool has been experimentally shown to affect zooplankton abundance and diversity in invaded systems (Strecker and Arnott 2010), although the impacts of differences in vertical position of arriving Daphnia individuals on recipient populations remains unknown. Considering that adopting a hypolimnetic vertical position allows
Daphnia to avoid Bythotrephes predation and persist in invaded systems (Chapter 3), differences in vertical positions of dispersers are expected to influence establishment success and recovery in daphniid populations in these systems.

The objective of this chapter was to determine if dispersing Daphnia from populations with different vertical positions influenced recovery of Daphnia in systems invaded by Bythotrephes longimanus. I specifically chose to assess impacts on Daphnia as reductions in daphniid abundance and diversity due to Bythotrephes invasion are widely observed in the literature (Chapter 3, reviewed by Azan et al. 2015). To determine this, I conducted a mesocosm experiment stocked with Daphnia from populations with different vertical positions; shallow, deep or both in the presence and absence of Bythotrephes and then introduced dispersing Daphnia from these populations (Figure 1). In uninvaded mesocosms, total Daphnia abundance was expected to increase in mesocosms stocked with Daphnia from populations with deep vertical position after receiving dispersers from populations with shallow vertical position and vice versa as dispersing individuals could establish in niches (shallow or deep depth) not occupied by resident Daphnia. For invaded mesocosms, those stocked from populations with deep vertical position would be less vulnerable to Bythotrephes predation as compared to those stocked from shallow populations due to reduced spatial habitat overlap. For this reason, total Daphnia density was expected to increase in invaded mesocosms stocked with Daphnia from shallow populations and receiving dispersers from deep populations. For mesocosms stocked from deep populations and receiving dispersers from shallow populations, I expected no change in total Daphnia density after dispersers were added as large spatial overlap between Bythotrephes and Daphnia was expected to prevent dispersing individuals from establishing in
these mesocosms. For mesocosms stocked with and receiving *Daphnia* from populations with both shallow and deep vertical position, I expected no effect of dispersal in invaded mesocosms due to *Bythotrephes* predation on shallow individuals and niche saturation preventing dispersers from deep populations from establishing. Detailed expectations for combinations of stocking origin, *Bythotrephes* presence and dispersal origin treatments examined in this study are provided in Table 4.1.

**Methods**

*Study Site and Experimental Design*

From July 7th to August 31st 2014, I conducted a field mesocosm experiment to assess if the introduction of *Daphnia* from populations with different *Daphnia* vertical positions influenced the *Daphnia* abundance in populations invaded by *Bythotrephes*. Mesocosms were stocked with zooplankton communities from two uninvaded lakes in south-central Ontario with daphniid populations with shallow (Echo Lake: 45.17.36'N, 79.06.45'W, more than 60% of individuals are epilimnetic) or deep (Bonnie Lake: 45.17.36'N 79.06.45'W, more than 60% of individuals are hypolimnetic) vertical position. Eight mesocosms were stocked from either Echo or Bonnie lakes. Four mesocosms were stocked from both lakes (Both treatment), establishing a control to observe *Bythotrephes* impacts on *Daphnia* abundance when *Daphnia* from both populations with shallow (mostly epilimnetic) and deep (mostly hypolimnetic) vertical position are present. Half of the mesocosms were stocked with *Bythotrephes* following the collection of zooplankton samples in Week 0, to establish the invaded treatment. Mesocosms were attached to wooden frames suspended 10cm above the water level in Fletcher Lake (Chapter 3), an invaded
lake in south-central Ontario. Each mesocosm was haphazardly assigned a location on experimental rafts (four mesocosms per raft) to prevent any factors associated with mesocosm position to influence the results observed. One mesocosm (invaded, stocked from Bonnie and receiving Echo dispersers) was removed from all analyses due to the presence of a smallmouth bass detected in Week 6 of the experiment. Additional details about zooplankton and *Bythotrephes* stocking, and mesocosms set-up are provided in Chapter 3.

To test the impacts of dispersing *Daphnia* from populations with different vertical position (Bonnie or Echo) on *Daphnia* abundance in the presence of *Bythotrephes*, dispersers from either Bonnie or Echo lakes were added to mesocosms stocked with zooplankton from Bonnie or Echo lakes. For mesocosms stocked with zooplankton from both lakes, dispersers from both lakes were added. Zooplankton sampling prior to the start of the experiment (Week 0) showed a large variation in *Daphnia* vertical position across all mesocosms, regardless of the stocking treatment (Bonnie, Echo or Both; Figure S3.2). This suggests that daphniid vertical position in our mesocosms did not reflect the vertical position of *Daphnia* in their lake of origin. Dispersers were introduced four weeks after the start of the experiment (Week 4). To ensure that only individuals with shallow and deep vertical positions from these lakes were introduced as dispersers in my mesocosms, dispersers were added from only the epilimnion of Echo Lake (0-6m) or the hypolimnion of Bonnie Lake (11-21m). I also corrected for the large differences in daphniid densities between Echo and Bonnie (Chapter 3), by averaging the total number of individuals found in a single haul from each lake and only adding densities representing 10% of this average density (or 5% from both lakes for the Both treatment). Thus, the same densities of *Daphnia* were added as dispersers for each dispersal treatment. A total of 27 zooplankton species
were present in Bonnie and Echo lakes, belonging to the following taxonomic families: Daphniidae, Bosminiidae, Chydoridae, Sididae, Holopediidae, Cyclopidae and Calanoïdæ. A minimum of one species from each taxonomic family was found in each lake.

**Sampling protocol and identification**

To determine abundance and depth distribution of *Daphnia* in the water column, the epi-, meta-, and hypolimnion of each mesocosm was sampled both prior to the application of the dispersal treatment (Week 3) and at the end of the study (Week 7). Zooplankton samples for each thermal layer (starting 20cm above the enclosure bottom for the hypolimnion) were collected by towing a closing net with an 80 μm mesh (15 cm diameter) and preserved in 90% ethanol. All zooplankton were enumerated using sub-samples of a known volume and identifying all individuals within each sub-sample until no new species were found three sub-samples in a row, with a minimum of seven sub-samples counted for each thermal layer. All specimens were identified to the species level using Ward and Whipple 1918, Smith and Fernando 1978, De Melo and Hebert 1994, Witty 2004, and Haney et al. 2013, and *Daphnia pulex* and *Daphnia pulicaria* were grouped as “*Daphnia pulex/pulicaria*” due to morphological similarities between these species.

**Statistical analysis**

All analyses were conducted in R v 3.2.4 (R Core Development Team 2016) using packages bbmle v 1.0.17, lme4 v 0.7.7 and fitdistrplus v 1.0.7, with α = 0.05. The density for *Daphnia* in each thermal layer for every mesocosm was calculated using the same sub-sampling
procedure as outlined in Chapter 3. Due to differences in zooplankton densities stocked from Echo and Bonnie lakes, I standardized the change in density between Week 3 (prior to the application of the dispersal treatment) and Week 7 by calculating a per capita change in density for total *Daphnia* and the most common *Daphnia* species for each mesocosm (present in >20% of all mesocosm samples); calculated as density in Week 7 divided by density in Week 3.

To assess if per capita change in density of total *Daphnia* and the most common *Daphnia* species in mesocosms was influenced by the dispersal of *Daphnia* from populations with different vertical positions, I used log-normal or gamma distributed generalized linear models (GLM) with a log link function with *Bythotrephes* presence (categorical variable, two levels: absent or present), stocking origin (categorical variable, three levels: Bonnie, Echo or Both) and dispersal origin (categorical variable, three levels: Bonnie, Echo or Both) as interacting explanatory variables. A statistically significant three-way interaction between *Bythotrephes*, stocking origin and dispersal origin was interpreted as evidence that the effect of *Bythotrephes* presence or absence on per capita change in *Daphnia* density depended on the lake of origin stocked and from which dispersers were introduced. Statistically significant interactions between *Bythotrephes* and stocking origin or *Bythotrephes* and dispersal origin suggested that the impact of *Bythotrephes* on per capita *Daphnia* density was dependent on the lake from which zooplankton were stocked or the lake from which dispersers were introduced. Significant interactions were assessed using the multcomp (version 1.3-8) to conduct generalized linear hypothesis tests (GLHT) and determine significant comparisons. Effect sizes were calculated as differences in median per capita change in density between treatments found to be significantly different.
It is possible that the dispersing individuals may have shifted their vertical position in response to differences in biotic and abiotic factors in my mesocosms as compared to sources lakes or due to unknown factors. In invaded mesocosms, dispersing *Daphnia* with greater spatial overlap with *Bythotrephes* may adopt a deeper vertical position due to exposure to *Bythotrephes* kairomone (Bourdeau et al. 2013). In uninvaded mesocosms, metabolic costs associated with occupying deeper waters could result in *Daphnia* with a deeper vertical position migrating to warmer epilimnetic waters (Kessler and Lampert 2004). The addition of *Daphnia* as part of the dispersal treatment could also influence the vertical position of resident *Daphnia* as increased density could alter competition for algal resources (Lampert 2005). To determine if the vertical position of total *Daphnia* or individuals species shifted during the experiment, I assessed differences in the proportion of epi- and hypolimnetic individuals with experiment week (Week 3 or Week 7), *Bythotrephes* (present or absent), stocking origin (Bonnie, Echo, or Both) and dispersal origin (Bonnie, Echo, or Both) as interacting explanatory variables using either gamma or log-normal distributed linear mixed models (GLMMs) with a log link and mesocosm as a random effects intercept (lme4, version 3.4.2). I chose to assess changes in the proportion epi- and hypolimnetic *Daphnia* as these regions are likely to be strongly impacted by *Bythotrephes* presence due to predation in the epilimnion, and the introduction of dispersing individuals via the establishment of shallow individuals in the epi- and deep individuals in the hypolimnion. Effect sizes were calculated as difference in median proportion of hypo- and epilimnetic individuals (±SD) between treatments that were found to be significantly different.

For all GLMs and GLMMs, AICc values (R package bbmle) and visual examination of plots of residual versus fitted values and square root of the standard deviance of residuals versus
fitted values were used to assess model fit for gamma and log-normal distributions. Cook’s distance was used to identify influential points, identified as those with leverage greater than 0.5. Minimum adequate models were chosen using log-likelihood ratio tests based on Crawley’s (2005) procedure. If influential points were detected, models were run with and without these points to determine their effect on model fit. P-values from GLMs and GLMMs for each zooplankton group and species assessed were corrected for false positives using the Benjamini-Hochberg (Benjamini and Hochberg 1995) false discovery rates. P-values for statistically significant three way interaction between *Bythotrephes*, stocking origin, and dispersal origin was not FDR corrected as no further significance tests were performed. For statistically significant interactions (after FDR corrections), the multcomp package (version 1.3-8) was used to conduct general linear hypothesis tests (GLHT) to determine statistically significant comparisons. P-values obtained from GLHTs were not FDR corrected as Bonferroni corrections were already performed. Details about the Benjamini-Hochberg false discovery rate corrections are provided in Appendix 3.

**Results**

*Total Daphnia*

Before the addition of dispersing individuals, the proportion of epi- and hypolimnetic *Daphnia* individuals between mesocosms stocked from Bonnie, Echo or both lakes was significantly different from each other. In Week 3, there were more hypo- and fewer epilimnetic *Daphnia* in both invaded (Hypo Week 3: 28.9 ± 4.6%, Hypo Week 7: 31.8 ± 3.4%, Epi Week 3: 41.8 ± 9.4, Epi Week 7: 36.6 ± 6.5) and uninvaded (Hypo Week 3: 28.3 ± 1.0%, Hypo Week 7:
32.1± 4.3% , Epi Week 3 : 42.7 ± 1.1%, Epi Week 7: 35.7 ± 2.0%) mesocosms as compared to Week 7 (Figure 4.1a, Table 4.1, Gamma GLMMs, random effects are significant). In Week 3, there were more hypo- and fewer epilimnetic *Daphnia* in mesocosms stocked from Bonnie Lake (Hypo: 32.3 ± 1.2%, Epi: 34.4±2.5%) as compared to those stocked from Echo (Hypo: 23.3±4.4%, Epi: 53.2 ± 8.7%) or both (Hypo: 26.6± 4.7%, Epi: 44.7±9.7%) lakes (Figure 4.1b, Table 4.1, Gamma GLMMs, random effects are significant).

Invasion status influenced how per capita change in total *Daphnia* density responded to dispersal treatments. While there was no effect of dispersal origin on per capita change in total *Daphnia* density in uninvas oste mesocosms, per capita increase in density was greater in invaded mesocosms receiving dispersers from Bonnie Lake as compared to those receiving dispersers from both lakes (Figure 4.2, Table 4.2, Bonnie Dispersal = 8.7 ± 15.8 , Both Dispersal = 3.3 ± 5.4). Additionally, stocking origin influenced per capita change in total *Daphnia* density. Greater per capita increase in density observed for mesocosms stocked from Bonnie Lake as compared to those stocked from Echo or from both lakes (Figure 4.2, Table 4.2, Bonnie = 5.9 ± 16.7 , Echo = 1.5± 1.9, Both = 1.6 ± 5.4).

For mesocosms stocked from Echo or both lakes, the proportion of epi- and hypolimnetic *Daphnia* significantly differed between Weeks 3 and 7. There was an increase in the proportion of hypolimnetic *Daphnia* in mesocosms stocked from Echo and both lakes (Figure 4.1b, Echo Week 3 = 23.2 ± 4.4%, Echo Week 7 =31.6 ± 3.5 %, Both Week 3 = 26.5 ± 4.7%, Both Week 7 = 31.5 ± 0.9%). The proportion of epilimnetic individuals decreased in mesocosms stocked from Echo and Both (Echo Week 3 = 53.2 ± 8.7%, Echo Week 7 = 36.7± 6.8%, Both Week 3 =44.6 ± 9.4% , Both Week 7 = 36.4 ± 1.5%). The proportion of hypolimnetic *Daphnia* in uninvas oste
mesocosms was also significantly different between Weeks 3 and 7. There were more hypolimnetic total *Daphnia* in uninvaded mesocosms in Week 7 as compared to Week 3 (Table 4.2, Week 3 = 28.3 ± 1.0%, Week 7 = 32.1 ± 4.3%).

*Daphnia* species

The four most common *Daphnia* species present in our mesocosms were *D. catawba*, *D. ambiguа*, *D. mendotae* and *D. pulex/pulicaria*. Dispersal origin did not influence per capita change in density for any of these species in invaded mesocosms. Stocking origin only influenced per capita change in density for *D. catawba* and *D. mendotae*, depending on invasion status. For *D. catawba*, there were significant differences in the proportion of hypo- and epilimnetic individuals in Week 3 between mesocosms stocked from Bonnie, Echo or both lakes. Mesocosms stocked from Bonnie Lake had more hypolimnetic *D. catawba* individuals in uninvaded and invaded mesocosms in Week 3 as compared to those stocked from Echo Lake (Figure 4.3, Table 4.2, Gamma GLMMs, non-significant random effects, Bonnie Uninvaded = 31.9 ± 3.3%, Echo Uninvaded = 11.1 ± 4.9%, Bonnie Invaded = 32.7 ± 1.7%, Echo Invaded = 16.8 ± 6.7%). There were fewer epilimnetic *D. catawba* individuals in uninvaded and invaded mesocosm stocked from Bonnie Lake as compared to those stocked from Echo Lake in Week 3 (Table 4.2, Bonnie Uninvaded = 32.3 ± 3.4%, Bonnie Invaded = 34.1 ± 2.1%, Echo Uninvaded = 76.6 ± 10.9%, Echo Invaded = 66.1 ± 13.1%). Per capita change in *D. catawba* density decreased across all mesocosms except for uninvaded mesocosms stocked from Bonnie Lake (Figure 4.4a, Table 4.3). Per capita increase in density in uninvaded mesocosms stocked from Bonnie Lake was greater than uninvaded mesocosms stocked from Echo Lake (Figure 4.4a, Table 3, Bonnie = 1.6 ± 1.1,
In invaded mesocosms stocked from Echo Lake, reduction in per capita density was smaller as compared to uninvaded mesocosms (Figure 4.4a, Table 3, Uninvaded = 0.1 ± 0.1, Invaded = 0.9 ± 1.5). There was no impact of *Bythotrephes* presence on *D. catawba* in mesocosms stocked from Bonnie Lake.

For *D. mendotae*, there were no significant differences in the proportion of epi- or hypolimnetic individuals between mesocosms stocked from Bonnie, Echo or both lakes, regardless of *Bythotrephes* presence (Figure 4.5c, Table 4.2, Gamma GLMMs, non-significant random effects). There was no significant difference in per capita change in density between invaded and uninvaded mesocosms stocked from Bonnie, Echo or both lakes. Overall, increase in per capita density in mesocosms stocked from Bonnie Lake was larger as compared to mesocosms stocked from Echo Lake (Figure 4.4b, Table 4.3, Bonnie = 1.6 ± 1.5, Echo = 0.4 ± 0.7). The reduction in per capita density in invaded mesocosms stocked from Bonnie Lake was smaller as compared to invaded mesocosms stocked from Echo Lake or both lakes (Table 4.3, Bonnie = 2.1 ± 2.1, Echo = 0.6 ± 1.0, Both = 0.3 ± 0.3). Change in per capita density in uninvaded mesocosms stocked from Bonnie Lake was greater as compared to mesocosms stocked from Echo Lake (Table 4.3, Echo = 0.3 ± 0.2, Bonnie = 1.2 ± 0.7). There was no difference in per capita density between uninvaded mesocosms stocked from Bonnie Lake and those stocked from both lakes.

*D. ambigua* vertical position differed between mesocosms based only on stocking origin. In mesocosms stocked from Bonnie, fewer individuals were epilimnetic and more individuals were hypolimnetic as compared to those stocked from Echo, regardless of experiment week (Figure 4.6, Table 4.3, Gamma GLMMs, non-significant random effects, Bonnie: Epi = 33.3 ±
4.3% , Hypo = 33.3 ± 0.8%, Echo: Epi = 31.2 ± 1.6%, Hypo = 32.3 ± 2.2%). There was no difference in per capita change in density between invaded and uninvaded mesocosms. However, the interaction between stocking and dispersal origin influenced per capita change in *D. ambigu*ua density (Figure 4.4c, Table 4.3). Per capita decline in density was less in mesocosms stocked from Echo receiving Echo dispersers as compared to those stocked from Bonnie and receiving Echo dispersers (Table 4.3, Echo Stocking & Disperal = 1.0 ± 0.7, Bonnie Stocking & Dispersal = 4.1 ± 3.9). In mesocosms stocked and receiving dispersers from Bonnie Lake, there was no change in per capita density as compared to those stocked from Bonnie and receiving Echo dispersers, where per capita density decreased. Per capita change in density in mesocosms stocked and receiving dispersers from Bonnie Lake was smaller as compared to mesocosms stocked from Echo receiving Bonnie dispersers (Bonnie Stocking & Dispersal = 0.9 ± 0.2, Bonnie Stocking & Echo Dispersal =4.1 ± 3.9 , Echo Stocking & Dispersal = 3.2 ± 4.2).

For *D. pulex/pulicaria*, there was no effect of *Bythotrephes* presence, stocking or dispersal origin on vertical position between Week 3 and Week 7, with 33% of individuals occupying the hypo- and 33% of individuals occupying the epilimnion. There was no also effect of *Bythotrephes* presence, stocking or disperser origin on per capita change in *D. pulex/pulicaria* density.

*Change in vertical position*

At the species level, only *D. catawba* vertical position was influenced by *Bythotrephes* presence, stocking origin, and dispersal origin through the duration of the experiment (Figure 4.4, Table 4.2, Gamma GLMMs, non-significant random effects). For invaded mesocosms, vertical
position of *D. catawba* changed between Week 3 and 7 depending on stocking origin and dispersal origin, with an overall increase in the proportion of hypolimnetic individuals and decrease in the proportion of epilimnetic individuals. There was an increase in the proportion of hypo- and a decrease in the proportion of epilimnetic individuals between Week 3 and 7 in mesocosms stocked from and receiving dispersers from both lakes (Figure 4.4 a & b, Table 4.2, Both Origin & Dispersal, Week 3: Epi = 64.7 ± 15.9%, Hypo = 15.9 ± 8.0%; Week 7: Epi = 43.3 ± 4.3%, Hypo = 28.4 ± 23.3%). There was an increase in the proportion of hypo- and decrease in the proportion of epilimnetic individuals between Week 3 and 7 in mesocosms stocked from and receiving dispersers from Echo Lake (Figure 4.4, Table 4.2, Echo Stocking & Dispersal, Week 3: Epi = 69.1 ± 15.6%, Hypo = 14.5 ± 7.7%; Week 7: Epi = 42.8 ± 2.6%, Hypo = 28.0 ± 1.1%). In Week 7, the proportion of hypolimnetic *D. catawba* was greater in invaded mesocosms stocked with and receiving dispersers from Bonnie as compared to those stocked from Echo and receiving Bonnie dispersers (Figure 4.4a & b, Table 4.2, Bonnie Stocking & Dispersal: Hypo = 33.3 ± 1.1%; Echo Stocking & Bonnie Dispersal: Hypo = 25.2 ± 4.4%), while the proportion of epilimnetic individuals was greater in mesocosms stocked from Echo and receiving Bonnie dispersers (Epi = 44.7 ± 2.4%) as compared to those stocked from and receiving Bonnie dispersers (Epi = 33.3 ± 0.3%).

For *D. mendotae*, the proportion of hypolimnetic individuals increased between Week 3 and 7 across all treatments. There were more hypolimnetic individuals in Week 7 as compared to Week 3 (Figure 4.5a & b, Table 4.3, Gamma GLMMs, non-significant random effects, Week 3 = 27.5 ± 4.7%, Week 7 = 31.4 ± 2.9%). The proportion of epilimnetic *D. mendotae* changed between Week 3 and 7 decreased depending on invasion status and stocking treatment. There
were fewer epilimnetic *D. mendotae* individuals in invaded mesocosms in Week 3 as compared to uninvaded mesocosms (Uninvaded = 35.3± 9.8%, Invaded = 38.4 ± 8.7%) and in uninvaded mesocosms in Week 7 as compared to Week 3 (Figure 4.5b, Gamma GLMMs, non-significant random effects, Table 4.2, Uninvaded: Week 3 = 46.3 ± 9.8%, Week 7 = 37.1 ± 4.3%). In mesocosms stocked from Echo Lake, there was a decrease in the proportion of epilimnetic individuals in Week 7 (Figure 4.5d, Table 4.2, Week 3 = 47.0 ± 9.7%, Week 7 = 37.1 ± 7.0%), with no differences observed for Bonnie Lake and Both mesocosms.

**Discussion**

Studies have shown that dispersal from the regional species pool can rescue populations from demographic extinction, restore community structure and ecosystem function (Loreau et al. 2003, reviewed in Furrer and Pasinelli 2015). In particular, the addition of dispersers from the regional species pool to *Bythotrephes* invaded zooplankton communities has been shown to mitigate the negative impacts on the abundance of *Daphnia* and other large cladocerans (Strecker and Arnott 2010). In my mesocosms, I did not observe any effect of population from which dispersing *Daphnia* originated, and the vertical position associated with these populations on *Daphnia* density in *Bythotrephes* invaded mesocosms. There were no differences in per capita density in invaded mesocosms between those receiving hypolimnetic dispersers from Bonnie Lake and epilimnetic dispersers from Echo Lake. This contrasts with my expectations that lack of spatial overlap between *Bythotrephes* and hypolimnetic dispersers from Bonnie Lake would allow these dispersers to establish and result in increased *Daphnia* density in mesocosms stocked from Echo Lake as compared to those stocked from and receiving Echo Lake dispersers. Taken
together, these results suggest that the traits associated with the population from which 
dispersers originate are not an important factor influencing recovery in invaded systems.

At the species level, there was no effect of the population from which dispersers originate or the vertical position associated with these populations on the density of *D. catawba*, *D. mendotae*, *D. ambigua* and *D. pulex/pulicaria* in invaded mesocosms. This is a surprising result as I expected as dispersing individuals from the hypolimnion of Bonnie Lake would establish in the hypolimnion of invaded mesocosms stocked from Echo Lake, where predation by *Bythotrephes* on epilimnetic individuals due to spatial overlap reduced density resulting in no change or a small increase in density in these mesocosms. There are a number of possible mechanisms underlying the lack of effect observed. One possible explanation is that although only epilimnetic individuals from Echo Lake and hypolimnetic individuals from Bonnie Lake were dispersed, these individuals may not have maintained their vertical position after being introduced in our mesocosms, resulting in a lack of effect. Considering that in Week 0, the proportion of hypolimnetic *Daphnia* in mesocosms stocked from Echo, Bonnie or both lakes did not reflect the traits associated with these populations (Chapter 3), it is possible that the hyp- and epilimnetic individuals dispersed did not immediately adopt the vertical position they occupied in their home lakes. Additionally, it is possible that *Bythotrephes* predation on dispersing hypolimnetic individuals in invaded mesocosms reduced their densities before these individuals could shift to the hypolimnion resulting in the lack of impact observed.

The effect of traits of dispersing individuals on the populations in which they arrive could be dependent on other factors influencing colonization success such as the quantity of dispersing individuals (Sinclair and Arnott 2017), the frequency of dispersal events (Howeth and Leibold
2010a, 2010b) and the condition of dispersing individuals (Fronhofer et al. 2015, Sinclair and Arnott 2017). Although my results show that population from which dispersers originate and the trait associated with these dispersers are not a factor influencing colonization success, it is possible that this lack of effect is a result of insufficient number and frequency of individuals dispersed. Although in my mesocosms dispersers were introduced at a volume representing 10% of the total volume of a single mesocosm, a level greater than used in similar experimental manipulations (Forrest and Arnott 2006, Strecker et al. 2010), this amount may be insufficient to observe any effect of dispersal origin. Furthermore, dispersers were introduced only once at the beginning of the experiment. It is possible that increasing the quantity of dispersing individuals and the frequency of dispersal events could lead to an observable effect of dispersal origin and traits of dispersing individuals on recipient communities.

Stocking origin also influenced per capita change in Daphnia density, with greater per capita increase in density observed in mesocosms stocked from Bonnie as compared to Echo or both lakes. Prior to the addition of dispersers, more hypolimnetic and fewer epilimnetic Daphnia were present in mesocosm stocked from Bonnie as compared to those stocked from Echo or both lakes. A greater proportion of hypolimnetic daphniids in mesocosms stocked from Bonnie Lake could have resulted in lower niche occupancy in the resource rich epilimnion as compared to mesocosms stocked from Echo or both lakes, where most individuals were epilimnetic and may have allowed more dispersing individuals to establish in the epilimnion as compared to mesocosms stocked from Echo or both lakes. However, mesocosms stocked from Bonnie lake also had on average 0.40 individuals/L less Daphnia than mesocosms stocked from Echo Lake and 0.31 individuals/L less Daphnia than mesocosms stocked from both lakes at the beginning of
the experiment. We addressed this disparity by examining the difference in per capita densities rather than absolute densities in our analyses, but it is possible that low *Daphnia* abundance in mesocosms stocked from Bonnie lake resulted in low niche occupancy, thereby allowing more dispersers to establish and persist as compared to mesocosms stocked from Echo Lake or both lakes where niche occupancy was higher due to higher starting densities. Given the scope of our experiment, we cannot distinguish whether the influence of stocking origin on *Daphnia* per capita density is driven by differences in vertical position, starting densities or both factors.

*Change in vertical position*

* *Daphnia* vertical position changed throughout the course of this experiment, with the magnitude of change depending on stocking origin and *Bythotrephes* presence. The proportion of hypo- and epilimnetic total *Daphnia* only changed in uninvaded mesocosms, with the proportion of hypolimnetic individuals increasing between Week 3 and Week 7. This concurrent decrease in epilimnetic individuals and an increase in hypolimnetic individuals suggests a potential downwards movement of epilimnetic individuals into the hypolimnion. For uninvaded mesocosms, this downwards movement into the hypolimnion could be a result of increased competition for algal resources between residents and dispersers due to increased density (Lampert 2005). A decrease in epilimnetic individuals and an increase in hypolimnetic individuals was also only observed in mesocosms stocked from Echo Lake or both lakes, where most individuals were epilimnetic in Week 3, further suggesting that competitive interactions in the epilimnion could be driving this shift in vertical position. There was no change in proportion of epi- or hypolimnetic individuals in mesocosms stocked from Bonnie Lake, where more than
30% of individuals were hypolimnetic prior to the addition of dispersers, likely a result of niche saturation preventing non-hypolimnetic individuals from occupying the hypolimnion or genotypic differences resulting in preference for hypo- or epilimnetic habitat.

**Impacts on Daphnia species**

For *D. mendotae*, the vertical position of resident *Daphnia* played an important role in mediating the impacts of *Bythotrephes* in invaded mesocosms. There was no difference in *D. mendotae* per capita density between invaded and uninvaded mesocosms stocked from Bonnie Lake, where a larger proportion of resident *Daphnia* and *D. mendotae* were hypolimnetic, thereby reducing spatial overlap between *Bythotrephes* and *D. mendotae*. Both invaded and uninvaded mesocosms stocked from Echo lake declined in *D. mendotae* in density by the end of the experiment. This decline in density is likely linked to the movement of epilimnetic individuals into the hypolimnion in uninvaded Echo Lake mesocosms, as indicated by the concurrent decrease in the proportion of epilimnetic individuals in uninvaded mesocosms and an overall increase in the proportion of hypolimnetic individuals. This downwards movement of epilimnetic individuals in uninvaded mesocosms stocked from Echo Lake, suggests that the lack of difference between invaded and uninvaded Echo mesocosms is likely a result of the metabolic costs associated with occupying deeper waters in uninvaded mesocosms and in those stocked from Bonnie Lake (Pangle and Peacor 2006, Pangle et al. 2007). One possible reason for this downwards movement in uninvaded mesocosms could be escape from the negative indirect effects of strong competition for algal resources in the epilimnion. In Chapter 3, I observed an increase in per capita algal density in uninvaded mesocosms where a greater proportion of
*Daphnia* were hypo- and non-epilimnetic suggesting that competition for epilimnetic resources was suppressing resource availability in mesocosms with more epilimnetic *Daphnia* and cladoceran species. For invaded mesocosms, it is likely that the large degree of spatial habitat overlap between *Bythotrephes* and *D. mendotae*, which were mostly epilimnetic in invaded Echo Lake mesocosms in Week 3, resulted in predation on epilimnetic *D. mendotae* leading to the decline in per capita density observed.

Across all mesocosms, the proportion of hypolimnetic *D. mendotae* increased between Weeks 3 and 7. This increase in hypolimnetic individuals occurred concurrently with a decrease in epilimnetic individuals in uninvaded mesocosms stocked from Echo, suggesting that a downwards movement from the epi- to the hypolimnion in these mesocosms is likely underlying this trend. There was no change in the proportion of epilimnetic *D. mendotae* and no difference in invaded mesocosms, a surprising result as *D. mendotae* have been shown to adopt a deeper vertical position in the presence of *Bythotrephes* kairomones (Bourdeau et al. 2013), and numerous *D. mendotae* populations have been shown to reside in the hypolimnion in invaded lakes (Pangle and Peacor 2006, Pangle et al. 2007, Chapter 2). However, I also did not observe a downwards vertical movement for epilimnetic *D. mendotae* between Weeks 0 and 3 (Chapter 3) in invaded mesocosms. These results suggest that the deeper vertical position observed for many *D. mendotae* populations in invaded lakes (Pangle and Peacor 2006, 2009, Chapter 2) may be caused by selection for individuals and clonal lines that reside in the hypolimnion rather than a plastic response to *Bythotrephes* presence, although predator recognition may evolve with extended exposure (as seen in Lake Michigan *D. mendotae* (Bourdeau et al. 2013)).
D. catawba density declined across most mesocosms during my experiment, regardless of Bythotrephes presence. This decline in per capita density reinforces my observations in Chapter 3, where decline or no change in density was observed across all mesocosms, regardless of invasion status or vertical position of resident total Daphnia or D. catawba and is likely linked to competition with other for algal resources, especially in mesocosms stocked from Echo or both lakes where a greater proportion of D. catawba were epilimnetic in Week 3. The concurrent decrease in the proportion of epi- and increase in the proportion of hypolimnetic individuals during my experiment in both uninvaded and invaded mesocosms stocked from Echo Lake or both lakes, also suggests that competition for epilimnetic resources may be driving the movement of epilimnetic individuals into the hypolimnion. Competition for epilimnetic resources with other Daphnia and cladocerans species could have negatively impacted per capita change in density (Boersma 1995, Burns 2000, Johnson and Havel 2001), either through exploitation of algal resources (Kruetzer and Lampert 1999) or chemical interference (Burns 2000). The increase in algal density observed in mesocosms with a greater proportion of hypolimnetic Daphnia in Chapter 3 demonstrates that grazing by Daphnia in the epilimnion substantially reduces total algal density and suggests that strong competition for algal resources does exist in mesocosms with a large number of epilimnetic Daphnia and other cladocerans.

There was also no effect of Bythotrephes presence on D. ambiguа or D. pulex/pulicaria per capita density in my mesocosms. For D. ambiguа, this result differs from the my observations in Chapter 3 where density was smaller in invaded mesocosms as compared to uninvaded mesocosms, depending on total Daphnia vertical position. This lack of impact may be a result of low densities of D. ambiguа present in Week 3, representing 3% of all Daphnia in my
mesocosms, likely leading to low encounter rates with *Bythotrephes*. This is also true for *D. pulex/pulicaria*, which consisted less than 1% of all *Daphnia* in my mesocosms in Week 0, 0.7% in Week 3 and 6% in Week 7, leading to low encounter rates with *Bythotrephes* in invaded mesocosms. There was also no effect of *Bythotrephes* or dispersal origin on *D. ambigua* and *D. pulex/pulicaria* vertical position, a pattern consistent with the lack of *Bythotrephes* impact on *D. ambigua* and *D. pulex/pulicaria* abundance.

**Conclusions**

The population from which dispersers originated and traits associated with these populations did not influence *Daphnia* density, regardless of *Bythotrephes* presence in my mesocosms. However, the vertical position of resident *Daphnia* influenced the impacts of *Bythotrephes* on *D. mendotae* density, with reduced impact of *Bythotrephes* predation observed in mesocosms stocked from populations deeper resident *Daphnia* vertical position. These results suggest that although a deeper *Daphnia* vertical position can mitigate the impacts of *Bythotrephes* on *Daphnia* populations in invaded lakes, the population of origin of dispersers and the associated *Daphnia* vertical position do not influence recovery of *Daphnia* populations in *Bythotrephes* invaded systems. Future studies should examine if local conditions and regional processes such as dispersal quantity and rate affect the impact of within species differences in trait expression in dispersers on the structure of recipient populations and communities.
References


### Tables

**Table 4.1:** Expected outcomes for total *Daphnia* density in *Bythotrephes* invaded and uninvaded mesocosms stocked from populations with shallow, deep or both vertical position (Stocking Origin) and receiving dispersing *Daphnia* from these populations (Dispersal Origin).

<table>
<thead>
<tr>
<th>Stocking Origin</th>
<th>Dispersal Origin</th>
<th><em>Bythotrephes</em></th>
<th>Expected outcome</th>
<th>Potential mechanism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echo</td>
<td>Echo</td>
<td>Absent</td>
<td>No change</td>
<td>Niches are saturated preventing dispersing <em>Daphnia</em> from establishing.</td>
</tr>
<tr>
<td>Bonnie</td>
<td>Bonnie</td>
<td>Absent</td>
<td>No change</td>
<td>Niches are saturated preventing dispersing <em>Daphnia</em> from establishing.</td>
</tr>
<tr>
<td>Echo</td>
<td>Bonnie</td>
<td>Absent</td>
<td>Small Increase</td>
<td>Establishment of deep dispersing <em>Daphnia</em> due to lack of <em>Daphnia</em> with deep vertical position</td>
</tr>
<tr>
<td>Bonnie</td>
<td>Echo</td>
<td>Absent</td>
<td>Small Increase</td>
<td>Establishment of shallow dispersing <em>Daphnia</em> due to lack of <em>Daphnia</em> with shallow vertical position</td>
</tr>
<tr>
<td>Both</td>
<td>Both</td>
<td>Absent</td>
<td>No change</td>
<td>Niches are saturated preventing dispersing <em>Daphnia</em> from establishing.</td>
</tr>
<tr>
<td>Echo</td>
<td>Echo</td>
<td>Present</td>
<td>Small decrease/no change</td>
<td><em>Bythotrephes</em> predation on epilimnetic individuals prevents shallow dispersing <em>Daphnia</em> from establishing.</td>
</tr>
<tr>
<td>Bonnie</td>
<td>Bonnie</td>
<td>Present</td>
<td>No change</td>
<td>Niches are saturated preventing dispersing <em>Daphnia</em> from establishing.</td>
</tr>
<tr>
<td>Echo</td>
<td>Bonnie</td>
<td>Present</td>
<td>Large increase</td>
<td>Lack of <em>Bythotrephes</em> predation on <em>Daphnia</em> with deep vertical position allows dispersing <em>Daphnia</em> to establish.</td>
</tr>
<tr>
<td>Bonnie</td>
<td>Echo</td>
<td>Present</td>
<td>No change</td>
<td><em>Bythotrephes</em> predation prevents shallow dispersers from establishing.</td>
</tr>
<tr>
<td>Both</td>
<td>Both</td>
<td>Present</td>
<td>No change</td>
<td><em>Bythotrephes</em> predation on shallow dispersing <em>Daphnia</em>. Niche saturation prevents establishment of deep dispersing <em>Daphnia</em>.</td>
</tr>
</tbody>
</table>
Table 4.2: Summary of results from gamma distributed GLMMs assessing the impacts of *Bythotrephes* presence (Byth; Y or N), stocking origin (Origin (O); Bonnie, Echo or Both) and dispersal origin (Dispersal (D); Bonnie, Echo or Both) on the proportion of epi- (Epi) and hypolimnetic (Hypo) total *Daphnia* as well as the four most common *Daphnia* species; *D. ambigua*, *D. catawba*, *D. mendotae*, and *D. pulex/pulicaria* between weeks 3 and 7 (Week; 3 or 7). FDR refers to the false discovery rate adjusted p-values with statistical significance denoted in brackets. FDR adjustments were not performed for statistically significant (p<0.05) interaction between *Bythotrephes*, Origin, Dispersal and Week. Generalized linear hypothesis tests were performed (GLHT) for relevant comparisons when significant interactions between predictor variables were detected. P-values for GLHT comparisons were not FDR adjusted as these values are Bonferroni corrected (statistically significant values provided in brackets in bold).

<table>
<thead>
<tr>
<th>Taxa/Species</th>
<th>Vertical position</th>
<th>Significant predictor(s)</th>
<th>P-value</th>
<th>FDR</th>
<th>GLHT comparisons</th>
<th>Non-significant predictor(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Daphnia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>Origin x Week</td>
<td>&lt;0.0001</td>
<td>0.0005 (significant)</td>
<td>Bonnie 3 – Echo 3 (&lt;0.001) Bonnie 3 – Both 3 (&lt;0.001) Echo 3 – Echo 7 (&lt;0.001) Both 3 – Both 7 (0.002)</td>
<td>Byth x Dispersal x Origin x Week, Byth x Dispersal, Dispersal</td>
</tr>
<tr>
<td></td>
<td>Hypo</td>
<td>Origin x Week</td>
<td>0.0009</td>
<td>0.06 (significant)</td>
<td>Both 3 – Bonnie 3 (&lt;0.001) Bonnie 3 – Echo 3 (&lt;0.001) Echo 3 – Echo 7 (&lt;0.001) Both 3 – Both 7 (0.005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Byth x Week</td>
<td>0.042</td>
<td>0.15 (significant)</td>
<td>N3 – N7 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>D. ambigua</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>Origin</td>
<td>0.0001</td>
<td>0.001 (significant)</td>
<td>NA</td>
<td>Byth x Dispersal x Week x Origin, Byth x Dispersal x Week, Byth x Origin x Week, Dispersal x Week, Byth x Week, Dispersal, Byth</td>
</tr>
<tr>
<td></td>
<td>Hypo</td>
<td>Origin</td>
<td>0.0009</td>
<td>0.06 (significant)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>D. catawba</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>Origin x Byth x Week x Dispersal</td>
<td>0.042</td>
<td>NA</td>
<td>Both Y 3 Both – Both Y 7 Both (0.02) Echo N 7 Echo – Echo N 3 Echo (&lt;0.01)</td>
<td>None</td>
</tr>
<tr>
<td>Taxa/Species</td>
<td>Vertical position</td>
<td>Significant predictor(s)</td>
<td>P-value</td>
<td>FDR</td>
<td>GLHT comparisons</td>
<td></td>
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<td>---------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Hypo</td>
<td>Origin x Byth x Week x Dispersal</td>
<td>0.049</td>
<td>NA</td>
<td></td>
<td>Echo N 3 Echo – Bonnie N 3 Echo (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bonnie N 3 Bonnie – Echo N 3 Bonnie (&lt;0.01)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Bonnie Y 3 Bonnie – Echo Y 3 Bonnie (&lt;0.01)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Both Y 3 Both – Both Y 7 Both (0.02)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Echo N 3 Echo – Echo N 7 Echo (&lt;0.01)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Echo N 3 Bonnie – Echo N 7 Bonnie (&lt;0.01)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Echo N 3 Echo – Bonnie N 3 Echo (&lt;0.01)</td>
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<td></td>
<td></td>
<td>Bonnie N 3 Bonnie – Echo N 3 Bonnie (&lt;0.01)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Bonnie Y 3 Bonnie – Echo Y 3 Bonnie (&lt;0.01)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Bonnie N 3 Echo – Echo N 3 Echo (&lt;0.01)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Bonnie Y 7 Bonnie – Echo Y 7 Bonnie (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Taxa/Species</td>
<td>Vertical position</td>
<td>Significant predictor(s)</td>
<td>P-value</td>
<td>FDR</td>
<td>GLHT comparisons</td>
<td>Non-significant predictor(s)</td>
</tr>
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<td>---------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>D. mendota</td>
<td>Epi</td>
<td>Byth x Week</td>
<td>0.051</td>
<td>0.24 (not significant)</td>
<td>N 3 – N 7 (0.001)</td>
<td>Byth x Dispersal x Week x Origin, Byth x Dispersal x Week, Byth x Origin x Week, Dispersal x Week, Dispersal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y3 – N3 (0.03)</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Origin x Week</td>
<td>0.053</td>
<td>0.16 (significant)</td>
<td>Echo 3 – Echo 7 (0.01)</td>
<td></td>
</tr>
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<tr>
<td>Hypo</td>
<td>Byth</td>
<td></td>
<td>0.04</td>
<td>0.22 (significant)</td>
<td>NA</td>
<td>Byth x Dispersal x Week x Origin, Byth x Dispersal x Week, Byth x Origin x Week, Dispersal x Week, Dispersal</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Week</td>
<td>0.0001</td>
<td>0.001 (significant)</td>
<td>NA</td>
<td>Byth x Dispersal x Week x Origin, Byth x Dispersal x Week, Byth x Origin x Week, Dispersal x Week, Dispersal</td>
</tr>
<tr>
<td>D. pulex/pulicaria</td>
<td>Epi</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>Byth x Dispersal x Week</td>
<td></td>
<td>0.023</td>
<td>NA</td>
<td>None</td>
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</tbody>
</table>

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Table 4.3: Summary of results from gamma distributed GLMMs assessing the differences in per capita change in density between experimental weeks 3 and 7 for total *Daphnia* as well as the most common *Daphnia* species: *D. ambigua*, *D. catawba*, *D. mendotae*, and *D. pulex/pulicaria* in invaded and uninvaded mesocosms (*Bythotrephes*; N or Y), with different stocking ((O); Bonnie, Echo, or Both) and different dispersal origins (Dispersal origin (D); Bonnie, Echo, or Both). FDR refers to the false discovery rate adjusted p-values with statistical significance denoted in brackets. FDR adjustments were not performed for statistically significant (p<0.05) interaction between *Bythotrephes*, Origin and Dispersal. Generalized linear hypothesis tests were performed (GLHT) for relevant comparisons when significant interactions between predictor variables were detected. P-values for GLHT comparisons were not FDR adjusted as these values are Bonferroni corrected (statistically significant values in brackets and bold).

<table>
<thead>
<tr>
<th>Taxa/species</th>
<th>Significant Predictor(s)</th>
<th>p</th>
<th>FDR</th>
<th>Non-significant predictors</th>
<th>GLHT comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total <em>Daphnia</em></strong></td>
<td><em>Bythotrephes</em> x Dispersal origin</td>
<td>0.027</td>
<td>0.07</td>
<td><em>Bythotrephes</em> x Stocking origin</td>
<td>BothY – BonnieY (0.014)</td>
</tr>
<tr>
<td>Stocking origin</td>
<td></td>
<td>0.0006</td>
<td>0.003</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td><strong>D. ambigua</strong></td>
<td>Stocking origin x Dispersal origin</td>
<td>0.002</td>
<td>0.009</td>
<td><em>Bythotrephes</em></td>
<td>Echo(O &amp; D)-Bonnie (O)Echo (D) (0.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bonnie(O &amp; D)-Bonnie (O) Echo (D) (0.015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bonnie (O &amp; D) -Echo (O) Bonnie (D) (0.0483)</td>
</tr>
<tr>
<td><strong>D. catawba</strong></td>
<td><em>Bythotrephes</em> x Stocking origin</td>
<td>0.0002</td>
<td>0.04</td>
<td><em>Bythotrephes</em> x Dispersal origin, Dispersal origin</td>
<td>EchoY – EchoN (0.003)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>EchoN – BonnieN (&lt;0.001)</td>
</tr>
<tr>
<td><strong>D. mendotae</strong></td>
<td><em>Bythotrephes</em> x Stocking origin</td>
<td>0.013</td>
<td>0.07</td>
<td><em>Bythotrephes</em> x Dispersal origin, Dispersal origin</td>
<td>MixY-BonnieY (0.04)</td>
</tr>
<tr>
<td><strong>D. pulicaria/pulex</strong></td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td><em>Bythotrephes</em> x Dispersal origin</td>
<td>None</td>
</tr>
<tr>
<td>Taxa/species</td>
<td>Significant Predictor(s)</td>
<td>p</td>
<td>FDR</td>
<td>Non-significant predictors</td>
<td>GLHT comparisons</td>
</tr>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>origin, <em>Bythotrephes</em>, Dispersal origin</td>
<td></td>
</tr>
</tbody>
</table>

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Figure 4.1: Proportion of total hypolimnetic *Daphnia* in experiment weeks 3 and 7 in mesocosms with a) *Bythotrephes* present or absent and b) stocked from Bonnie, Echo or both (Both) lakes. Different letters represent statistically significant differences (p<0.05).
Figure 4.2: a) Per capita change in total *Daphnia* density between experiment weeks 3 and 7 in uninvaded (Absent) and invaded mesocosms receiving dispersing *Daphnia* from Bonnie, Echo or Both lakes b) Per capita change in total *Daphnia* density between experiment weeks 3 and 7 in mesocosms stocked from Bonnie, Echo or Both lakes. Values above the dashed line at 1 represent increase in per capita density between week 3 and 7, while values below this line represent decrease in density. Different letters represent statistically significant differences (p<0.05).
Figure 4.3: Upper row: Proportion of hypolimnetic *D. catawba* in experiment weeks 3 and 7 in *B. bythotrephes* invaded and uninvaded mesocosms stocked from Bonnie, Echo or both lakes (in bold) and receiving dispersers from Echo, Bonnie or both lakes. Lower row: Proportion of hypolimnetic *D. catawba* in experiment weeks 3 and 7 in *B. bythotrephes* invaded mesocosms stocked from Bonnie (Bonnie), Echo (Echo) or both lakes (Both) and receiving dispersers from Echo, Bonnie or both lakes. Different letters represent statistically significant differences (P <0.05).
Figure 4.4: Per capita change in a) *D. catawba*, b) *D. mendotae* and c) *D. ambiguа* density between experiment weeks 3 and 7 in mesocosms stocked from Bonnie, Echo or both lakes with *Bythotrephes* absent or present and receiving dispersers from Bonnie, Echo or both lakes. Values above the dashed line at 1 represent increase in per capita density between week 3 and 7, while values below this line represent decrease in density. Different letters represent statistically significant differences (p <0.05).
Figure 4.5: a) Proportion of hypolimnetic *D. mendotae* in all mesocosms in experiment Weeks 3 and 7. b) Proportion of epilimnetic *D. mendotae* in a) *Bythotrephes* uninvaded (Absent) and invaded (Present) mesocosms and b) stocked from Echo, Bonnie or both lakes (Both) in experiment Weeks 3 and 7. Different letters represent statistically significant differences (p<0.05).
Figure 4.6: Proportion of hypolimnetic *D. ambigua* in mesocosms stocked from Bonnie, Echo or both lakes (Both) throughout the experiment. Different letters represent statistically significant differences (p<0.05).
Chapter 5

General Conclusions

Summary

Each chapter of my thesis examines one aspect of the interaction between *Daphnia* vertical position and *Bythotrephes* presence, providing a comprehensive understanding of the impacts of *Bythotrephes* on *Daphnia* vertical position and the influence of *Daphnia* vertical position on *Bythotrephes* impacts in invaded lakes. Taken together, these chapters show that not only can invasive species change trait expression within a species in impacted systems, but that within-species differences in trait expression affect the magnitude and direction of invasive species impacts on populations and communities in these systems.

*Bythotrephes* presence has been linked with shifts in the vertical position of resident *D. mendotae* from the epi- to the hypolimnion in Lake Michigan and for *D. retrocurva* in Lake Erie (Pangle and Peacor 2006, Pangle et al. 2007). For Lake Michigan *D. mendotae*, this movement into the hypolimnion has been experimentally demonstrated to be a plastic response to *Bythotrephes* kairomone (Bourdeau et al. 2013). In Chapter 2, I showed that a hypolimnetic *D. mendotae* vertical position is not a population specific response in Lake Michigan but is prevalent across multiple populations in south-central Ontario. My results also show that this response is species-specific, with a deeper vertical position observed only for *D. mendotae*, despite other Daphnia species present in these invaded lakes. Furthermore, I showed that *D. mendotae* vertical position in invaded lakes is finely tuned to *Bythotrephes* density and water clarity, with more epilimnetic individuals present in low density lakes with high DOC levels as compared to those with low DOC levels, and lakes with high *Bythotrephes* density.
In Chapter 3, I showed that population level differences in *Daphnia* vertical position prior to Bythotrephes invasion influenced the magnitude and direction of *Bythotrephes* impacts on small cladocerans, large cladocerans and copepodids. Furthermore, my results showed that differences in *Daphnia* vertical position in invaded lakes can result in differences in algal production leading to differences in the magnitude of trophic cascades. This study provides the first experimental evidence that differences in *Daphnia* vertical position can not only affect the impacts of Bythotrephes on *Daphnia*, but also other zooplankton species and influence the strength of trophic cascades in invaded systems.

In Chapter 4, I showed that differences in the population of origin of dispersing *Daphnia* and the daytime vertical position associated with these populations does not influence the impacts of *Bythotrephes* on *Daphnia* populations in invaded mesocosms. The dispersal of *Daphnia* into invaded mesocosms resulted in recovery in *Daphnia* density, which was reduced due to Bythotrephes predation in these mesocosms. My results also demonstrated that the vertical position of resident *Daphnia* is an important factor influencing the impacts of *Bythotrephes* on invaded *Daphnia* populations, further confirming that *Daphnia* vertical position influences the impacts of *Bythotrephes* in invaded systems.

**Implications and future directions**

The results of my thesis chapters provide a comprehensive example of the importance of differences in trait expression play in regulating the structure of populations and communities. Existing research on the impacts of within-species differences in trait expression often focus on direct impacts on abundance, with few studies examining indirect impacts on community composition and productivity (reviewed by Des Roches et al. 2018). Additionally, the
consequences of within-species differences in trait expression on communities impacted by natural and anthropogenic disturbances remains understudied. My work provides a unique contribution by showing that the within-species differences in traits can indirectly impact community structure by affecting the abundance of other species across multiple trophic levels. Furthermore, my work shows that within-species differences in trait expression can influence the type and magnitude of population and community level consequences of biological invasions. These contributions have practical implications. My work shows that within-species differences in expression of traits that provide competitive advantages against or confer protection from invasive species may be able to mitigate the negative impacts of invaders on ecological systems.

While the importance of within-species differences in trait expression on species interactions, community structure and ecosystem function is beginning to be understood, we still lack a framework for identifying and characterizing which traits define the ecological niche of a species and play an importance role in the organization of ecological communities. The development of this framework is important in order to predict the impacts of anthropogenic change on ecological systems. While results from the chapters of my thesis and other studies have shown that within-species difference in trait expression plays an important role in structuring ecological communities and determining the impacts of natural and anthropogenic disturbance (Post et al. 2008, Schindler et al. 2010, Chislock et al. 2013), the next step is to characterize trade-offs between multiple traits in order to determine how multiple traits interact with each other to limit trait expression and reparameterize interspecific interactions such as competition, predator-prey interactions, host-disease dynamics and mutualisms in terms of a small number of traits in order to predict change in community composition.
Chapter 2 Appendix

Table S2.1: Geographical coordinates, presence/absence of *Bythotrephes* and *Daphnia* species for each of the 45 lakes sampled in South-Central Ontario.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Latitude (N), Longitude (W)</th>
<th><em>Daphnia</em> species present</th>
<th><em>Bythotrephes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Axe</td>
<td>45°23', 79°30'</td>
<td><em>D. catawba, D. ambigua</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Bear</td>
<td>45°20', 48°72'</td>
<td><em>D. dubia, D. mendoza, D. longiremis, D. pulex/pulicaria, D. ambigua</em></td>
<td>Present</td>
</tr>
<tr>
<td>Bigwind</td>
<td>45°03', 79°03'</td>
<td><em>D. catawba, D. mendoza, D. pulex/pulicaria, D. longiremis, D. ambigua, D. dubia</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Blackstone</td>
<td>45°13', 79°32'</td>
<td><em>D. mendoza, D. retrocurva</em></td>
<td>Present</td>
</tr>
<tr>
<td>Bonnie</td>
<td>45°08', 79°15'</td>
<td><em>D. pulicaria, D. catawba, D. longiremis</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Buck # 1</td>
<td>45°24', 79°23'</td>
<td><em>D. catawba, D. ambigua, D. mendoza</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Buck # 2</td>
<td>45°23', 78°59'</td>
<td><em>D. catawba, D. ambigua, D. longiremis, D. pulex/pulicaria, D. longiremis, D. mendoza, D. dubia</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Camel</td>
<td>45°09', 79°25'</td>
<td><em>D. catawba, D. dubia</em></td>
<td>Present</td>
</tr>
<tr>
<td>Camp</td>
<td>45°26', 78°54'</td>
<td><em>D. catawba, D. ambigua, D. mendoza, D. dubia</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Clear</td>
<td>45°59', 79°33'</td>
<td><em>D. longiremis</em></td>
<td>Present</td>
</tr>
<tr>
<td>Clinto</td>
<td>45°19', 78°52'</td>
<td><em>D. pulex/pulicaria</em></td>
<td>Present</td>
</tr>
<tr>
<td>Crosson</td>
<td>45°08', 79°03</td>
<td><em>D. catawba, D. ambigua</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Location</td>
<td>Latitude, Longitude</td>
<td>Species</td>
<td>Status</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>Crown</td>
<td>45°23', 78°40'</td>
<td><em>D. catawba, D. ambiguа, D. pulex/pulicaria, D. longiremis</em></td>
<td>Present</td>
</tr>
<tr>
<td>Dickie</td>
<td>45°08', 79°05'</td>
<td><em>D. catawba, D. ambiguа</em></td>
<td>Present</td>
</tr>
<tr>
<td>Dyson</td>
<td>45°12', 79°39'</td>
<td><em>D. longiremis, D. mendotae</em></td>
<td>Present</td>
</tr>
<tr>
<td>Echo</td>
<td>45°10', 79°03'</td>
<td><em>D. catawba, D. mendotae, D. ambiguа</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Fifteen Mile</td>
<td>45°20', 78°58'</td>
<td>D. catawba, D. ambiguа, D. pulicaria, D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Fletcher</td>
<td>45°21', 78°47'</td>
<td><em>D. ambiguа, D. pulex/pulicaria, D. longiremis, D. mendotae</em></td>
<td>Present</td>
</tr>
<tr>
<td>Grandview</td>
<td>45°12', 79°03'</td>
<td>D. pulex/pulicaria, D. longiremis</td>
<td>Absent (presence detected in 2015)</td>
</tr>
<tr>
<td>Harp</td>
<td>45°23', 79°08'</td>
<td>D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Henshaw</td>
<td>45°06', 79°35'</td>
<td><em>D. catawba, D. ambiguа, D. pulicaria</em></td>
<td>Present</td>
</tr>
<tr>
<td>Horseshoe</td>
<td>45°18', 79°51'</td>
<td>D. longiremis</td>
<td>Present</td>
</tr>
<tr>
<td>Kapikog</td>
<td>45°09', 79°54'</td>
<td><em>D. dubia, D. mendotae, D. longiremis</em></td>
<td>Present</td>
</tr>
<tr>
<td>Leonard</td>
<td>45°04', 79°27'</td>
<td>D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Livingston</td>
<td>45°22', 78°43'</td>
<td>D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Longline</td>
<td>45°25', 78°97</td>
<td><em>D. catawba, D. ambiguа, D. pulex/pulicaria</em></td>
<td>Absent</td>
</tr>
<tr>
<td>Louie</td>
<td>45°23', 78°43'</td>
<td><em>D. catawba, D. ambiguа, D. pulex/pulicaria, D. mendotae</em></td>
<td>Absent</td>
</tr>
<tr>
<td>McKay</td>
<td>45°03', 79°10'</td>
<td>D. catawba, D. ambiguа, D. longiremis</td>
<td>Present</td>
</tr>
<tr>
<td>Nine Mile</td>
<td>44°57', 79°35'</td>
<td>D. catawba, D. ambiguа, D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Otter</td>
<td>45°17', 78°53'</td>
<td>D. pulex/pulicaria</td>
<td>Present</td>
</tr>
<tr>
<td>Location</td>
<td>Latitude, Longitude</td>
<td>Species</td>
<td>Status</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------</td>
<td>------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Oudaze</td>
<td>45°27', 79°11'</td>
<td>D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Oxtongue</td>
<td>45°22', 79°11'</td>
<td>D. dubia, D. mendotae, D. longiremis</td>
<td>Present</td>
</tr>
<tr>
<td>Paint</td>
<td>45°13', 78°55'</td>
<td>D. longiremis</td>
<td>Present</td>
</tr>
<tr>
<td>Portage</td>
<td>45°13', 79°48'</td>
<td>D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Round</td>
<td>45°28', 79°24'</td>
<td>D. catawba, D. ambigua, D. mendotae</td>
<td>Present</td>
</tr>
<tr>
<td>Silver # 1</td>
<td>45°07', 79°34'</td>
<td>D. longiremis</td>
<td>Present</td>
</tr>
<tr>
<td>Silver # 2</td>
<td>44°54', 79°19</td>
<td>D. mendotae, D. dubia</td>
<td>Present</td>
</tr>
<tr>
<td>Smoke</td>
<td>45°31', 78°41'</td>
<td>D. longiremis, D. mendotae, D. dubia</td>
<td>Present</td>
</tr>
<tr>
<td>Solitaire</td>
<td>45°23', 78°08'</td>
<td>D. dubia, D. mendotae, D. pulex/pulicaria</td>
<td>Absent</td>
</tr>
<tr>
<td>Sucker</td>
<td>45°15', 79°41'</td>
<td>D. mendotae, D. catawba, D. longiremis</td>
<td>Present</td>
</tr>
<tr>
<td>Three Mile Lake</td>
<td>45°11', 79°27'</td>
<td>No Daphnia</td>
<td>Present</td>
</tr>
<tr>
<td>Walker</td>
<td>45°23', 79°25'</td>
<td>D. mendotae, D. pulex/pulicaria, D. ambigua</td>
<td>Present</td>
</tr>
<tr>
<td>Waseosa</td>
<td>45°25', 79°16'</td>
<td>D. mendotae, D. catawba</td>
<td>Present</td>
</tr>
<tr>
<td>Wolf</td>
<td>45°42', 78°70'</td>
<td>D. catawba, D. ambigua, D. mendotae</td>
<td>Absent</td>
</tr>
<tr>
<td>Young</td>
<td>45°12', 79°33'</td>
<td>D. mendotae</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table S2.2: Minimum and maximum values for physical and water chemistry parameters for the 45 lakes sampled for this study (Field survey) which were chosen from the CAISN survey 2006 and 2010 survey of 500 lakes in South-Central Ontario.

<table>
<thead>
<tr>
<th>Physical/Water Chemistry parameter</th>
<th>Field survey minimum value</th>
<th>Field survey maximum value</th>
<th>CAISN survey minimum value</th>
<th>CAISN survey maximum value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved Organic Carbon (mg/L)</td>
<td>1.8</td>
<td>11.65</td>
<td>0.5</td>
<td>33.4</td>
</tr>
<tr>
<td>Total Phosphorus (μg/L)</td>
<td>3.06</td>
<td>21.1</td>
<td>0.6</td>
<td>66</td>
</tr>
<tr>
<td>Maximum Depth (m)</td>
<td>1.6</td>
<td>58</td>
<td>1.6</td>
<td>93</td>
</tr>
<tr>
<td>Secchi Disk Depth (m)</td>
<td>1.1</td>
<td>7.95</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Figure S2.1: The total number of *D. longiremis* individuals present in the entire water column for each lake included in the vertical position analysis (n = 39). Points above the solid, dotted and dashed lines represent lakes with more than 0, 5 or 10 *D. longiremis* individuals present in the entire water column.
Chapter 3 Appendix

Details of False Discovery Testing

I used Benjamini-Hochberg (1995) false discovery rates (FDR) to correct for false positives (when the null hypothesis is rejected despite being true) in my results which become more likely when a large number of statistical tests are performed simultaneously. In this procedure, a proportion of the number of tests declared significant are assessed by using an algorithm that sets a significance threshold at the highest P-value at which the inequality $P_i \leq iq/m$ holds true. Q is the false discovery rate set by the user and i represents the comparison number (from $i = 1$ to $m$, the maximum number of comparisons performed). P-values smaller than $iq/m$ correspond to false discovery rate adjusted statistically significant comparisons. This method was used to assess p-values obtained from GLMs examining per capita change and layer density where applicable, for each zooplankton and algae group (i.e. *Daphnia*, small cladocerans, large cladocerans, etc) as well as individual species (i.e. *Daphnia*, small cladoceran, and large cladoceran species). Although the false discovery rate controls the maximum number of false positives accepted as true, it also increases the possibility of a true statistically significant effect considered as non-significant, i.e. false negative. Considering the logistical constraints in replication for this experiment, the FDR was set to 0.20, implying that up to 20% of false positives would be accepted as statistically significant in order to minimize the possibility of a true statistically significant effect to be rejected. Effects of independent variables in all models were considered statistically significant only if p-values from the relevant statistical tests were significant after being FDR adjusted, with “FDR significant” indicating effects which were significant after FDR adjusted and “FDR non-significant” indicating effects which were not significant. P-values equivalent to alpha values ($a = 0.05$) were considered significant, if the FDR adjusted value was significant. FDR adjusted significance tests were performed for each set of comparisons in Libre Office Calc version 4.2.8.2 using the spreadsheet provided as supplement by Pike (2011).
Figure S3.1: The proportion of hypo- and non-epilimnetic *Daphnia* across all forty mesocosms prior to the start of the experiment (Week 0). Red dots indicate mesocosms in which were invaded by *Bythotrephes* in Week 1, while black dots indicate mesocosms which remained uninvaded.
Figure S3.2: Differences in zooplankton density (individuals/L) in mesocosms stocked from Echo, Bonnie or Both Lakes prior to the start of the experiment.
Figure S3.3: Sampling distributions of bootstrapped correlation coefficients between per capita change in density for a) *Daphnia*, b) small cladocerans, c) large cladocerans and d) copepodids. Dashed line represents the value of the coefficient obtained from correlation analysis between taxa per capita change in density and total zooplankton density (individuals/L) in week 0.
Figure S3.4: The proportion of hypolimnetic a) Total *Daphnia*, b) *D. catawba*, c) *D. ambigua*, and non-epilimnetic d) *D. mendotae*, and e) *D. pulex/pulicaria* across invaded (Y) and uninvaded (N) mesocosms in Week 0 (0) and Week 3 (3).
Figure S3.5: The proportion of hypolimnetic total *Daphnia*, small cladocerans, large cladocerans, Copepods, Nauplii, and Copepodids in invaded (Y) and uninvaded (N) mesocosms in Week 0 (0) and Week 3 (0).