Manuscript Title: Inter population variation in behaviour of native prey (*Daphnia*) mediates the impact of the invasive spiny water flea (*Bythotrephes cederströmii)* on plankton communities

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SSH and SEA developed the original idea and experimental system; SSH and SEA contributed to experimental design; SSH carried out the experiment; SSH ran all analyses; SSH wrote the first draft and all authors revised it.

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. We examined if population level differences in *Daphnia* vertical position influenced the impacts of the invasive predator *Bythotrephes cederströmii* on major zooplankton and algal groups. Our results show that differences in *Daphnia* vertical position resulted in differences in *Bythotrephes* impacts on small cladocerans, with a larger reduction small cladoceran density in mesocosms where *Daphnia* adopted a deeper vertical position. Furthermore, we observed a greater increase in algal biomass 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 (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; 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 naiveté 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. 2012, 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, Hasnain and Arnott 2019) 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 1993, De Meester 1996, Tessier and Leibold 1997, Michels and De Meester 2004, Boeing et al. 2006), the influence of population-level differences in *Daphnia* vertical position on *Bythotrephes* impacts in freshwater ecosystems remains unknown.

Our goal was to determine if differences in *Daphnia* vertical position influence the effect of *Bythotrephes* on plankton communities in invaded lakes. To accomplish this, we manipulated *Bythotrephes* presence in mesocosms with three-tier food webs which were stocked from lakes where *Daphnia* populations exhibited different mean vertical positions 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. We 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.

Methods

Study Site and Experimental Design

From July 7th to August 1st 2014, we 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 1), 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, we manipulated *Bythotrephes* presence and absence in mesocosms across a gradient of *Daphnia* vertical position. We quantified *Daphnia* vertical position based on their numeric proportion in the region of the water column (based on temperature gradients) likely to have reduced predation by *Bythotrephes*; the hypolimnion representing the dark and cold bottom waters (generally 9-13m). *Bythotrephes* predation was expected to be 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). Mesocosms were stocked with *Daphnia* from populations in two uninvaded lakes; Bonnie Lake (45.17.36'N 79.06.45'W; Bracebridge Municipality, Table 1) and Echo Lake (45.17.36'N, 79.06.45'W; Lake of Bays Municipality, Table 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 0 to 40% hypolimnetic (Figure S1). There were significant differences in the abundance of zooplankton between Echo and Bonnie lakes, which influenced starting zooplankton densities in our 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 S2, 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. We grouped *Bosmina freyi an*d *Bosmina liederi a*s "*Bosmina freyi/liederi"* and *Daphnia pulex a*nd *Daphnia pulicaria a*s "*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. We 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; SchwentinentalSchwentinental, 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 bbmle v 1.0.17, glmmADMB v 0.7.7, fitdistrplus 1.0.7, piecewiseSEM v 2.1.2, robustbase v 0.93-3 with $\alpha = 0.05$.

We calculated the density of each species in each thermal layer for every mesocosm by estimating the total number of individuals present in sub samples. 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, we 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 we could not conclusively state whether the absence of a species in our sample represented a true absence in our mesocosm, we 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. We 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, we also added a minimum detection biomass (0.01 µg/L; representing the measurement resolution of the algae analyzer that we 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 S2), we 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. We 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 our mesocosms. It is possible that the proportion of total hypolimnetic *Daphnia* may have changed during the mesocosm experiment due to differences in abiotic and biotic variables between the lakes of origin and Fletcher lake. Details about the procedures used to assess changes in proportion of total hypolimnetic *Daphnia* are provided in supplementary materials.

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), we 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 are 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 (copepodids and nauplii). 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.

We used piecewise structural equation modelling (piecewise SEM, Shipley 2000, Grace 2006) to explore causal relationships between per capita change in density of major zooplankton groups (see above), change in biomass per unit in biomass for total algae, *Bythotrephes* presence, and the proportion of total hypolimnetic *Daphnia* in week 0. For the full model, we only fit paths between *Bythotrephes* presence, proportion of total hypolimnetic *Daphnia* in week 0, and the per capita change in density for each zooplankton group (Figure 1). Paths for calanoid and cyclopoid copepods also included per capita change in copepodid density as an explanatory variable since copepodids mature into adult calanoid and cyclopoid copepods, directly impacting the per capita change in density for these taxonomic groups. Paths for total algal density included per capita change in density for all

herbivorous zooplankton groups, i.e. *Daphnia,* all cladocerans, copepodids, and calanoid copepods as the density of these groups directly reduce total algal density across time. Collinearity between per capita change in density of all taxonomic groups was accounted for by explicitly treating these as correlated errors (not as a directed path). Proportion of total hypolimnetic *Daphnia* in week 0 and *Bythotrephes* presence were included as exogenous variables and potential paths were fit with GLMs with a normal distribution and a log link function. For paths for per capita changes in calanoid and cyclopoid densities, per capita change in copepodid density was included as an exogenous variable. For the path for per capita change in total algal density, per capita change in densities for all herbivorous zooplankton groups were also fit as exogenous variables. After fitting this full model, we fit a nested version containing only the significant links identified in the full model to confirm, using AIC, that the full model is not more likely than the reduced model, given the data. Separate piecewise SEMs were also fit for the four major algal groups; green algae, cyanobacteria, diatoms, and cryptophyta using the protocol outlined for the full model, with the nested version also confirmed using AIC.

Significant relationships were defined as those with p-values <0.05 for pathways with per capita change in density with either *Bythotrephes* presence, *Daphnia* vertical position, or both. Paths with significant causal relationships between per capita change in density for total algae or a zooplankton group, *Bythotrephes* presence, and *Daphnia* vertical position were re-fit with either normal or gamma distributed generalized linear models to assess the possibility of a significant interaction between the explanatory/exogenous variables. Piecewise SEMs were also fitted for the most common species in each zooplankton group with statistically significant causal relationships detected in the full model, with *Bythotrephes* presence and proportion of total hypolimnetic *Daphnia* in week 0 as exogenous variables, excepting paths for the most common calanoid and cyclopoid species where per capita change in copepodid density was also included. Significant causal relationships in piecewise SEMs for both common species were also re-fit with GLMs to assess if there was a significant interaction between *Daphnia* vertical position and *Bythotrephes* presence.

For all GLMs, we 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 fitting a Gamma or log-normal GLM using Mallows or Huber type robust estimators (Cantoni and Ronchetti (2001), Cantoni and Ronchetti (2006)), which down weights the effect of influential points on model fit. For robust GLMs, minimum adequate models were chosen based on Crawley's (2005) procedure with Wald-type tests.

Results

We found that per capita change in density for *Daphnia*, small cladocerans, and cyclopoids was predicted by the presence of *Bythotrephes* and the proportion of total hypolimnetic *Daphnia* in week 0 (Figure 2a). Per capita change in juvenile copepod density was only predicted by proportion of total hypolimnetic *Daphnia* in week 0 (Figure 2a). Change in biomass per unit of biomass for total algae was predicted by per capita change in density of large cladocerans and juvenile copepods, and the proportion of hypolimnetic *Daphnia* in week 0 (Figure 2a).

The proportion of hypolimnetic total *Daphnia* in both invaded and uninvaded mesocosms did not change through the duration of our experiment. 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* (Figure 3a, Gamma GLMs, Table S3). 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.

Small cladocerans were mostly epilimnetic across all mesocosms during the experiment. There was a general increase in small cladoceran density in all mesocosms during the experiment. We observed a significant interaction between *Bythotrephes* presence and the proportion of total hypolimnetic *Daphnia* in week 0 on per capita change in small cladoceran density. In uninvaded mesocosms, larger per capita increases in total small cladoceran density were observed in mesocosms with a greater proportion of total hypolimnetic *Daphnia* in week 0 (Figure 3b, Gamma GLMs, Table S3). 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* in week 0 as compared to uninvaded mesocosms (Figure 3b, Table S3). Both calanoid and cyclopoid copepods were mostly epilimnetic across all mesocosms during the experiment. Cyclopoid density increased throughout the experiment in most mesocosms.

In all mesocosms, total algal biomass increased through the course of the experiment, with most of the production occurring in the epilimnion. We observed a significant interaction between *Bythotrephes* presence and proportion of hypolimnetic *Daphnia* in week 0. 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* in week 0 (Figure 3c, Gamma GLMs, Table S3). Cyanobacteria were the most abundant algal functional group, followed by green algae, diatoms, and cryptophytes across our mesocosms. Density for all algal groups increased through the course of the experiment. Per capita change in biomass per unit biomass for green algae was predicted by the proportion of total *Daphnia* in the hypolimnion, and per capita change in density of large cladocerans, calanoids, and juvenile copepods. Green algal biomass increased in mesocosm with a greater proportion of hypolimnetic *Daphnia* in week 0. There was no effect of *Bythotrephes* presence, the proportion of total hypolimnetic

Daphnia in week 0, or per capita change in density in any zoooplankton group on the change in biomass per unit biomass for diatoms, cyanobacteria, and cryptophyta.

Most common species

For *Daphnia,* the most common species were *Daphnia catawba* and *Daphnia mendotae*, with mean±SD density of 86.5±107.4 and 20.2±16.7 individuals per litre across all mesocosms at the end of the experiment. *Bythotrephes* presence predicted per capita change in density for *D.* m*endotae* (Figure 3b, p = 0.05). Per capita change in density for *D. mendotae* was lower in invaded mesocosms as compared to uninvaded mesocosms (Figure 4a). There was no effect of *Bythotrephes* presence or proportion of total hypolimnetic *Daphnia* on per capita change in *D. catawba* density.

For small cladocerans, the most common species were *Bosmina freyi/leideri, Eubosmina tubicen,* and *E. longispina,* with mean±SD density of 67.1±99.4, 21.0±24.3, and 17.7±16.8 individuals per litre across all mesocosms at the end of the experiment. *B. freyi/liederi, E. tubicen,* and *E. longispina* densities increased through the course of the experiment. Per capita change in *B. freyi/leideri* was only predicted by the proportion of total hypolimnetic *Daphnia* in week 0 (Figure 3b, p = 0.04), with no effect of *Bythotrephes* presence. Per capita change in *B. freyi/*liederi density was greater in mesocosms with greater proportion of total hypolimnetic total *Daphnia* (Figure 4b). Both *Bythotrephes* presence and proportion of total hypolimnetic *Daphnia* in week 0 predicted per capita change in *E. longispina* density (Figure 3b, *Bythotrephes*: p = 0.05, proportion of total hypolimnetic *Daphnia:* p = 0.03). There was a smaller per capita increase in *E. longispina* density (Figure 4c), in mesocosms with a greater proportion of total hypolimnetic *Daphnia* in week 0. There was no effect of *Bythotrephes* presence or the proportion of total hypolimnetic *Daphnia* in week 0 on per capita change in *E. tubicen* density.

The most common species for copepods across our mesocosms were *Skistodiaptomus oregonensis* and *Cyclops scutifer* with mean±SD density of 14.2±20.3 and 13.4±12.0 individuals per

litre across all mesocosms at the end of the experiment. Per capita change in *C. scutifer* density was predicted by the proportion of total hypolimnetic *Daphnia* in week 0 ($p = 0.04$) and *Bythotrephes* presence ($p = 0.01$). Per capita increase in *C. scutifer* density was smaller in invaded mesocosms as compared to uninvaded mesocosms (Figure 4d). Per capita increase in *C. scutifer* density was larger in mesocosms with a greater proportion of hypolimnetic *Daphnia* in week 0 (Figure 4d). There was no effect of *Bythotrephes* presence or proportion of total hypolimnetic *Daphnia* in week 0 on per capita change in *S. oregonensis* density.

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 small cladocerans as well as individual species (e.g. *C. scutifer*). Most notably, we found evidence of trophic cascades in invaded mesocosms where spatial overlap between *Daphnia* and *Bythotrephes* was low, i.e. more hypolimnetic *Daphnia.* The strength of this trophic cascade was linked to the degree of spatial overlap between *Daphnia* and *Bythotrephe*s, with stronger cascades observed in invaded mesocosms with the least spatial overlap. 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). Our results suggest that differences in *Daphnia* vertical position can also contribute to trophic cascades in *Bythotrephes*-invaded systems.

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).

The vertical position of the most common *Daphnia* species, *D. mendotae* and *D. catawba* did not change during the experiment. A smaller increase in *D. mendotae* abundance was observed in invaded mesocosms as compared to 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. mendotae* over the smaller species *D. catawba* could be underlying this trend.

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*. Fewer total small cladocerans were present in invaded mesocosms where a greater proportion of *Daphnia* were deeper in the water column. 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 (i.e. fewer hypolimnetic *Daphnia*). In mesocosms with greater proportion of hypolimnetic *Daphnia*, *Bythotrephes* predation likely extended to smaller cladocerans resulting in the smaller increase in total small cladoceran density observed. In the literature, *Bythotrephes* invasion has been shown to reduce small cladoceran abundance (Vanderplog et al. 1993, Yan and Pawson 1997, Wahlström 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). Our 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.

For *B. freyi/leideri,* an increase in density was observed across all mesocosms as the proportion of hypolimnetic *Daphnia* increased, with a greater increase in density observed in the absence of *Bythotrephes*. For *E. longispina,* smaller increase in density was observed in mesocosms with greater proportion of hypolimnetic *Daphnia*, with invaded mesocosms exhibiting a smaller magnitude of increase as compared to uninvaded mesocosms. The contrasting effects of *Daphnia* vertical position on both of these species suggest that *B. freyi/leideri* in our mesocosms was constrained by competition for grazing with *Daphnia* (Demott 1982, Demott and Kerfoot 1982). For *E. longispina*, *Bythotrephes* predation is likely underlying the smaller increase in density observed in invaded as compared to uninvaded mesocosms. This result contradicts observations from Kelly et al. (2012), where *E. longispina* abundance increased in *Bythotrephes* invaded lakes, suggesting that *Bythotrephes* impacts on *E. longispina* may be mediated by other abiotic or biotic factors. The mechanisms mediating the lack of increase in *E. longispina* density in mesocosms with deeper *Daphnia* vertical position are unclear. It is possible that a deeper *Daphnia* vertical position increases competition between *E. longispina* and other bosminid species. However, competitive dynamics between different bosminid species, especially in the presence and absence of *Daphnia* is unknown.

There was no effect of *Bythotrephes* presence or proportion of *Daphnia* in the hypolimnion on change in total calanoid abundance or on the density of *S. oregonensis*. The lack of *Bythotrephes*

impact on adult calanoids is surprising as negative impacts of *Bythotrephes* on calanoids (Strecker and Arnott 2005, Strecker et al. 2006*,* Hessen et al. 2011, Bourdeau et al. 2011, Kelly et al. 2012) has been observed across the majority of studies in the literature. Furthermore, the lack of effect of proportion of hypolimnetic *Daphnia* on calanoid density also contradicts expected increases in calanoids due to reduced competition between *Daphnia* and calanoids for algae in lake ecosystems (Sommer et al. 2003). Both *Daphnia* and calanoids primarily feed on algae, therefore less competition for algae between *Daphnia* and calanoids is expected in mesocosms with greater proportion of hypolimnetic *Daphnia,* leading to an increase per capita calanoid density. It is possible that we were unable to detect the impacts of these competitive interactions due to the short experiment time frame.

We observed separate negative effects of *Bythotrephes* and the proportion of hypolimnetic *Daphnia* on change in total cyclopoid density. A larger increase in *C. scutifer* density was observed in uninvaded mesocosms with a greater proportion of hypolimnetic *Daphnia* as compared to invaded mesocosms, suggesting that *Bythotrephes* presence negatively impacts *C. scutifer* density. Kelly et al. (2012) also observed a similar negative impact of *Bythotrephes* on *C. scutifer* abundance across 212 Canadian and 342 Norwegian lakes. In contrast, no effect of *Bythotrephes* presence on *C. scutifer* abundance was observed by Hessen et al. (2011), while a positive effect was observed by Walseng et al. (2015) for 1541 and 1735 Norwegian lakes. Although the effect of *Bythotrephes* on *C. scutifer* remains unresolved in observational studies, our results provide the first evidence of negative *Bythotrephes* impacts on *C. scutifer* abundance in an experiment setting.

Algal Density and Trophic Cascades

Differences in *Bythotrephes* impacts on *Daphnia* and small cladocerans due to differences in *Daphnia* vertical position impacted primary production in our mesocosms, resulting in trophic cascades. Increase in total algal 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 cladoceran abundance due to *Bythotrephes* predation and reduced epilimnetic grazing by *Daphnia*. The invasion of *Bythotrephes* in some northern temperate lakes in North American 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). Our 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 effect of *Bythotrephes* presence on any algal taxonomic group. *Daphnia* grazing was reduced in mesocosms with a greater proportion of hypolimnetic *Daphnia,* likely resulting in the increase in green algal biomass observed in these mesocosms.

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. Our 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.

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Tables

Table 1: 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.

Lake	Area (ha)	Maximum Depth (m)	pH	TP (µg/L)	DOC (mg/L)	Ca (mg/L)
Fletcher	266.28	23.2	6.12	5.0	4.6	2.34
Echo	215.6	11.9	6.46	9.0	6.6	2.74
Bonnie	39.3	21.9	6.78	5.6	2.3	2.85

Figures

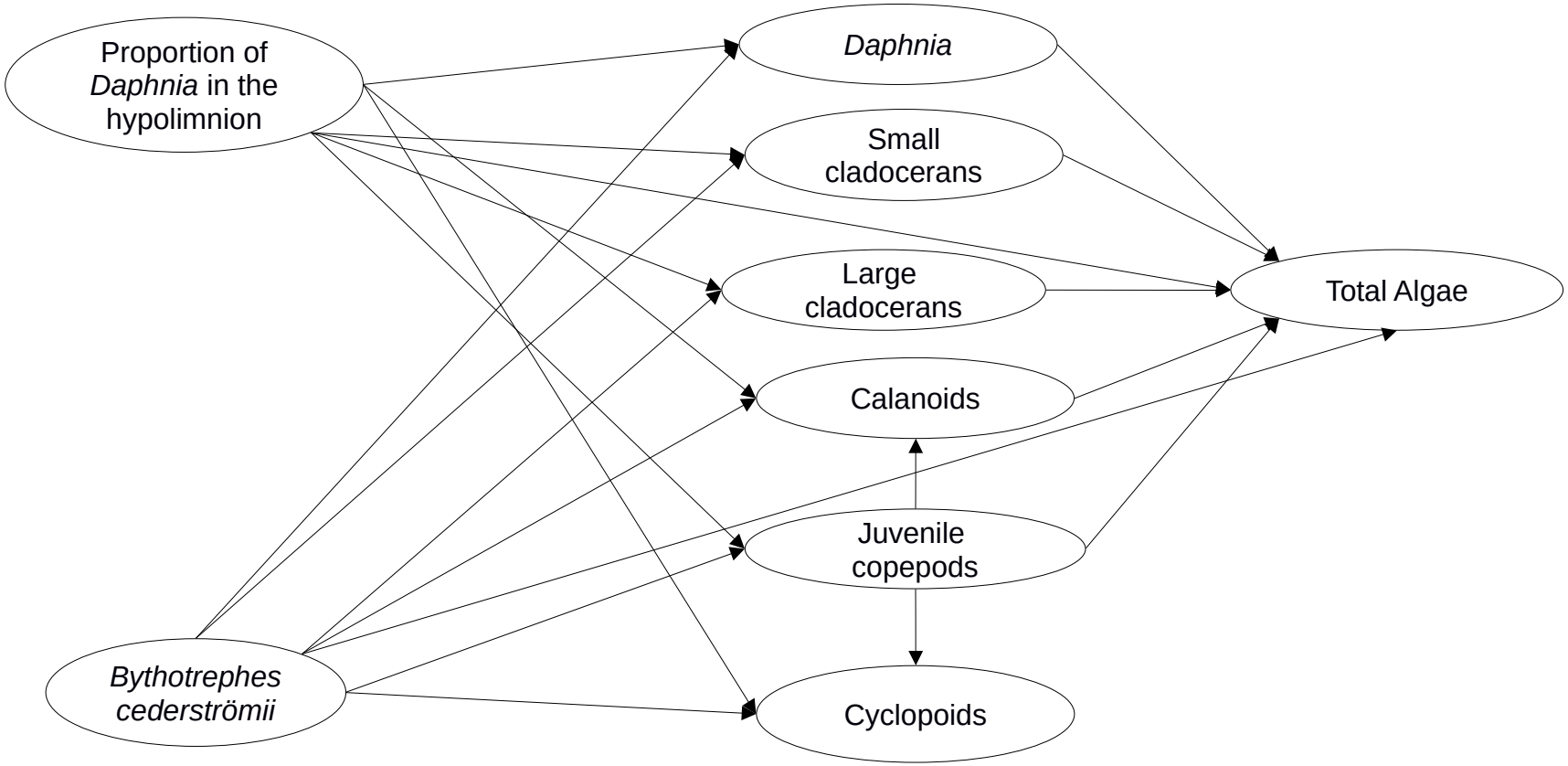

Figure 1: Visualization of the full structural equation model (full model) used to assess the impacts of proportion of hypolimnetic *Daphnia* and the presence of *Bythotrephes cederströmii* on per capita change in *Daphnia*, Small Cladocerans, Large Cladocerans, Calanoids, Cyclopoids, and Juvenile copepods. Model assumes that all paths (represented by arrows) between per capita change density of each zooplankton group, the proportion of hypolimnetic *Daphnia* and the presence of *Bythotrephes* are possible. For per unit change in total algal biomass, the path includes all zooplankton groups except cyclopoids, the presence of *Bythotrephes*, and proportion of hypolimnetic *Daphnia.*

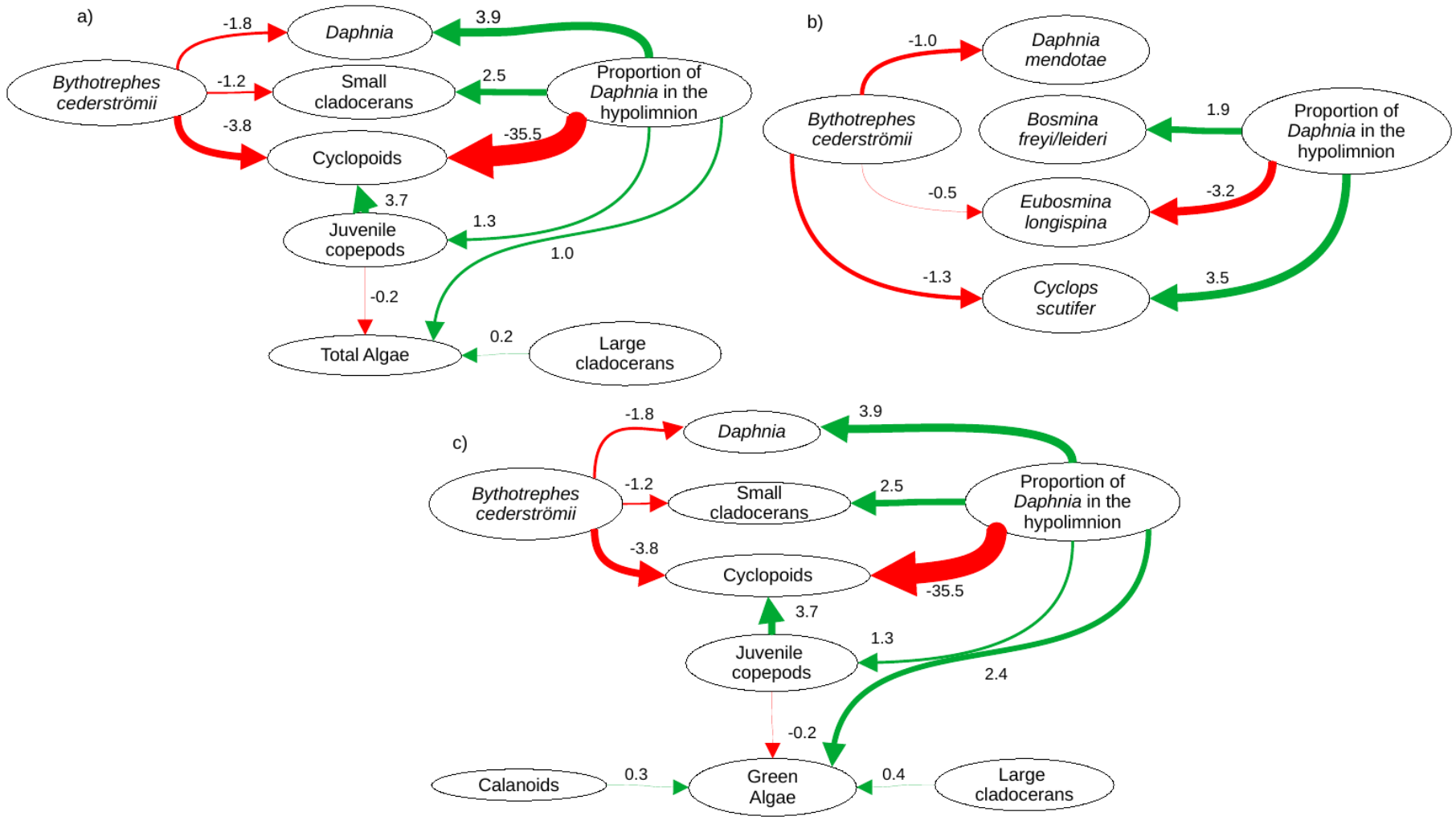

Figure 2: Visualization of the best structural equation model predicting per capita change in density a) for zooplankton groups and change in biomass per unit biomass for total algae, b) most common *Daphnia,* small cladoceran and cyclopoid species, and c) zooplankton groups and change in biomass per unit biomass for green algae. Arrows represent standardized path coefficients that are statistically significant ($p < 0.05$); associated information includes standardized coefficient value. Arrow width is scaled with the size of the coefficient, wider arrows represent larger coefficients. Green arrows signify positive path coefficients, and red arrows signify negative path coefficients.

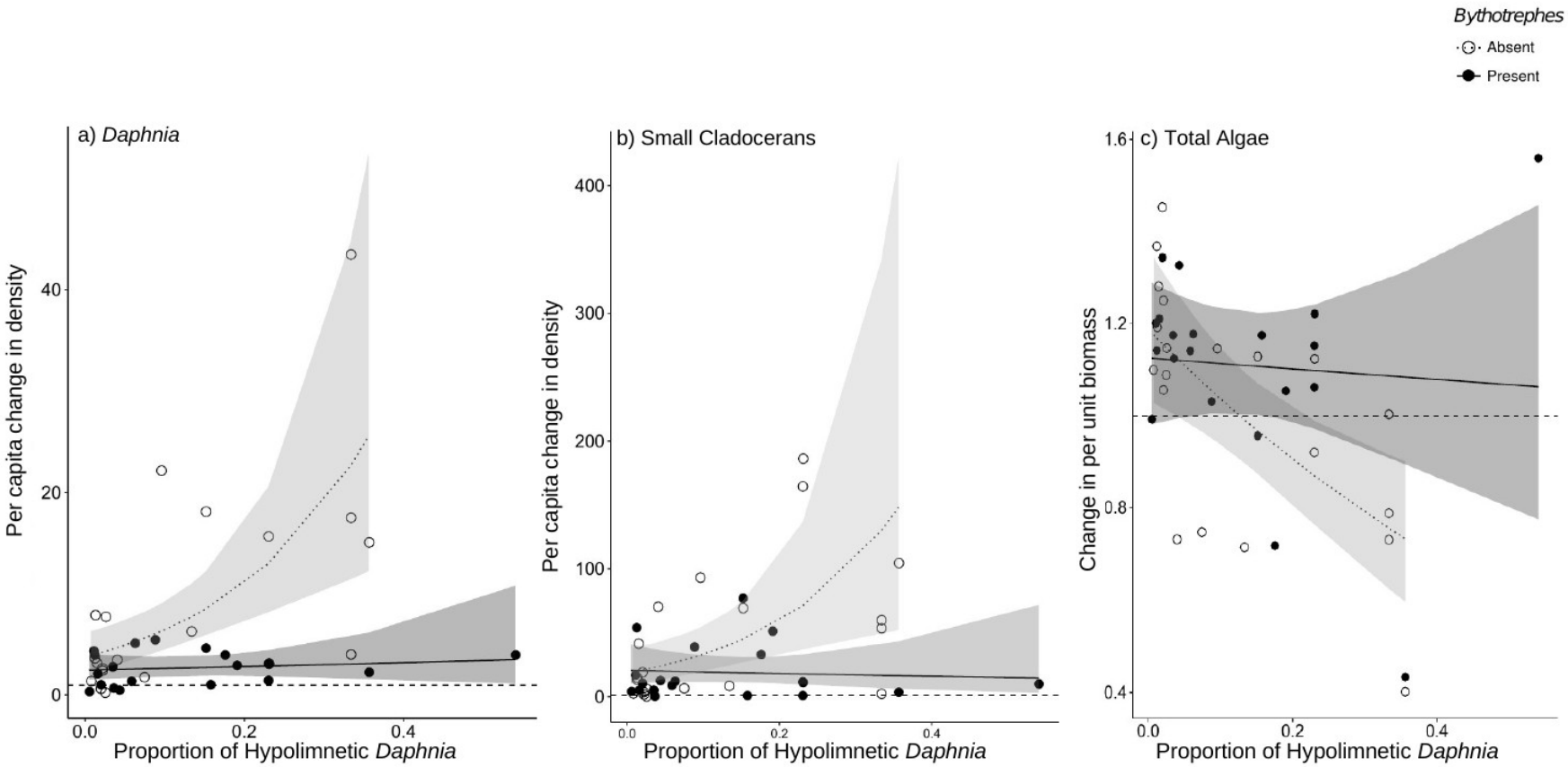

Figure 3: Effect off proportion of total hypolimnetic *Daphnia* in week 0 on the per capita change in a) Total *Daphnia* and b) Small Cladoceran density, and change in biomass per unit biomass for c) Total Algae in *Bythotrephes cederströmii (Bythotrephes)* uninvaded (N) and invaded (Y) mesocosms. Values 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 generalized linear or robust regression model.

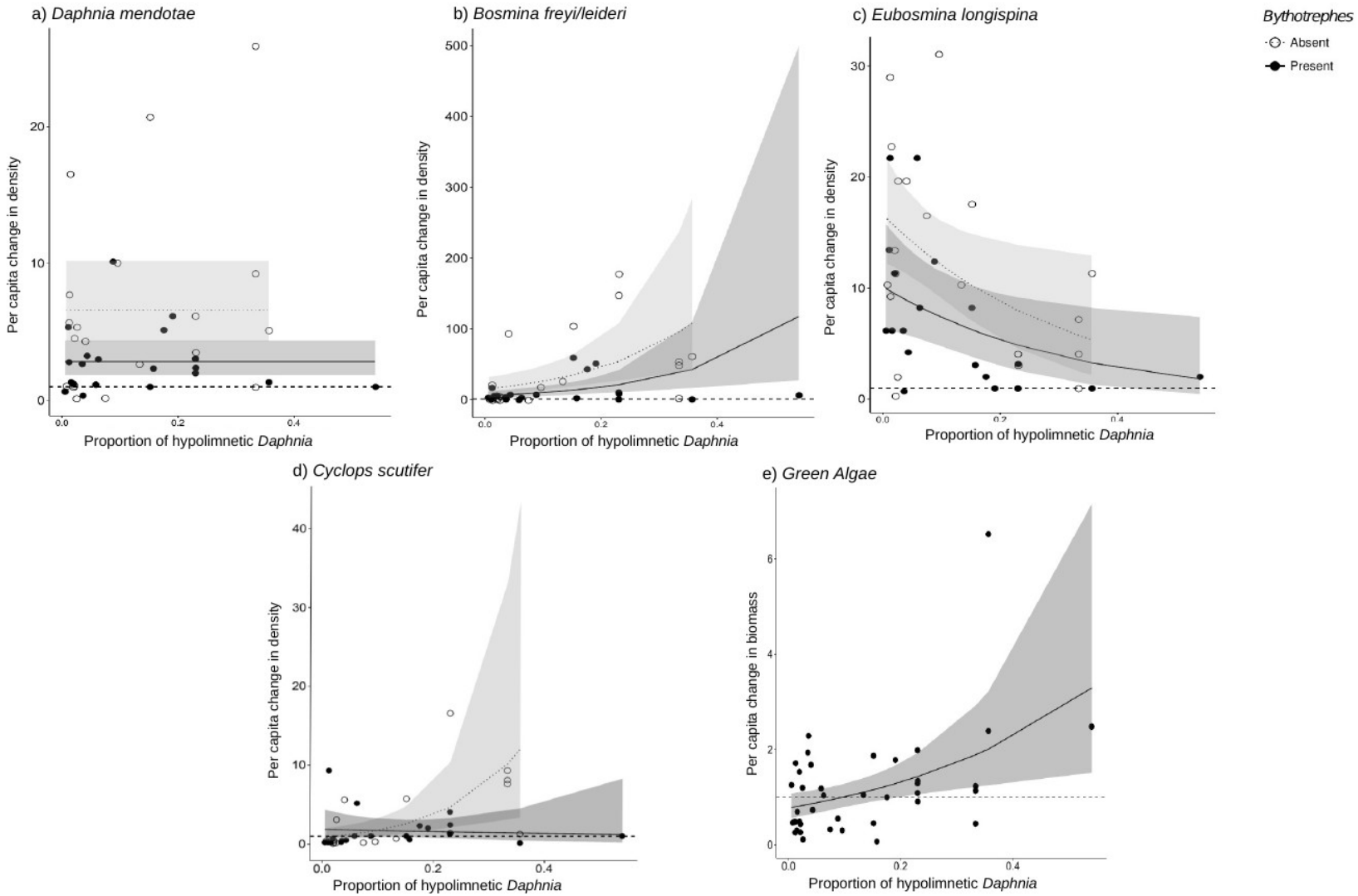

Figure 4: Effect off proportion of total hypolimnetic *Daphnia* in week 0 on the per capita change in a) *D. mendoate,* b) *B. freyi/leideri*, c) *E. longispina*, d) C. scutifer density, and change in biomass per unit biomass for e) Green Algae in *Bythotrephes cederströmii (Bythotrephes)* uninvaded (N) and invaded (Y) mesocosms. For green algae, no effect of *Bythotrephes* presence was detected, with all points represented in black. 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 generalized linear model or robust regression.