Elsevier Editorial System(tm) for Journal of Great Lakes Research
Manuscript Draft

Manuscript Number: GLR-D-16-00188R2

Title: Biotic and abiotic factors influencing zooplankton vertical distribution in Lake Huron

Article Type: Full length article

Keywords: Invasive species; DVM; Mysis; Bythotrephes; rotifer; vertical migration

Corresponding Author: Dr. Carly J. Nowicki,

Corresponding Author's Institution: University of Toledo

First Author: Carly J. Nowicki

Order of Authors: Carly J. Nowicki; David B Bunnell, PhD; Patricia M Armenio; David M Warner, PhD; Henry A Vanderploeg, PhD; Joann F Cavaletto; Christine M Mayer; Jean V Adams

Abstract: The vertical distribution of zooplankton can have substantial influence on trophic structure in freshwater systems, particularly by determining spatial overlap for predator/prey dynamics and influencing energy transfer. The zooplankton community in some of the Laurentian Great Lakes has undergone changes in composition and declines in total biomass, especially after 2003. Mechanisms underlying these zooplankton changes remain poorly understood, in part, because few studies have described their vertical distributions during daytime and nighttime conditions or evaluated the extent to which predation, resources, or environmental conditions could explain their distribution patterns. Within multiple 24-hour periods during July through October 2012 in Lake Huron, we conducted daytime and nighttime sampling of zooplankton, and measured food (chlorophyll-a), temperature, light (Secchi disk depth), and planktivory (biomass of Bythotrephes longimanus and Mysis diluviana). We used linear mixed models to determine whether the densities for 22 zooplankton taxa varied between day and night in the epi-, meta-, and hypolimnion. For eight taxa, higher epilimnetic densities were observed at night than during the day; for four of these taxa a general linear model revealed these patterns were best explained by biomass of Bythotrephes longimanus (Leptodiaptomus ashlandi) or Mysis diluviana (Leptodiaptomus minutus) or Secchi disk depth (Diacyclops thomasi, cyclopoid copepodites). By investigating the potential effects of both biotic and abiotic variables on the vertical distribution of crustacean zooplankton and rotifers, we provide descriptions of the Great Lakes zooplankton community and discuss how future changes in food web dynamics or climate change may alter zooplankton distribution in freshwater environments.

Ms. Ref. No.: GLR-D-16-00188R2
Title: Biotic and abiotic factors influencing zooplankton vertical distribution in Lake Huron
Journal of Great Lakes Research

Robert Hecky
Editor
Journal of Great Lakes Research

June 1, 2017

RE: Revision submission for Journal of Great Lakes Research, Ms. Ref. No.: GLR-D-16-00188R2

Dear Dr. Hecky,

Thank you for the opportunity to revise our manuscript entitled ***Biotic and abiotic factors influencing zooplankton vertical distribution in Lake Huron*** by Carly J. Nowicki, David B. Bunnell, Patricia M. Armenio, David M. Warner, Henry A. Vanderploeg, Joann F. Cavaletto, Christine M. Mayer, and Jean V. Adams for publication in the *Journal of Great Lakes Research*.

We have carefully addressed the comments and suggestions provided by the Associate Editor. After consideration, we agreed with the editor's assessment to average zooplankton densities in the hypolimnion. We reran our analysis with the average and updated all figures to reflect these new values. There were no changes with our statistical results. Also, we address all minor comments. We are confident that you will find the manuscript satisfactory for publication.

The submitted manuscript has been approved by all authors and none of the authors have declared a conflict of interest in completing this study. This manuscript has not been published elsewhere and we believe that the *Journal of Great Lakes Research* is the ideal publication for this manuscript.

Sincerely,

Carly J Nowicki

Carly J. Nowicki

Primary Address:
College of Natural and Social Sciences
Madonna University
36600 Schoolcraft Road
Livonia, MI 48150
734-432-5517
Email: cnowicki@madonna.edu

Response to Associate Editor's comments:

Thank you for addressing the concerns of the reviewers. I believe you now have a solid analysis of zooplankton data that is important for our understanding of ecosystem changes in Lake Huron. I still have a few suggestions that I would like you to address before we can accept this paper.
I do have one concern about the methods. On line 228 you state that you summed the densities in the two hypolimnion samples at the deep station. These are volumetric densities and you should therefore average them, not sum them. If you did sum those densities, you may need to rerun the analyses with the average (or perhaps skip the deep hypolimnion sample if you want to be more consistent with the shallow stations). I do not think this will make a large change in the results, but do not know.

Response: We agree with the editor's assessment. In our revision, we reran the analyses after averaging densities in the upper and lower hypolimnion. This changed our hypolimnion densities and we updated all figures to reflect the new values. However, there were no changes in our statistical results. The Methods section was revised to include the average.

I also have the following minor comments with line number:
Line 26 Abstract. Zooplankton communities have not changed in all Laurentian Great Lakes (in particular Superior, but also others depending on the time frame you refer to). Please reword.

Response: The sentence was reworded to "The zooplankton community in some of the Laurentian Great Lakes has undergone changes in composition and declines in total biomass, especially after 2003."

Paragraphs about factors affecting DVM: Overall a nice section. I still had a little problem with the difference between proximate and ultimate factors. For example, I was a little surprised at inclusion of light and temperature effects separately to the effect of light and temperature on your main hypothesis of DVM being a tradeoff between predation rate and growth rates. Light is discussed as a synchronized migration with moon light using a paper from a Brazilian lake. Is that migration different from avoidance of visual predators? There is a section on UV light both here and in the discussion, and UV could be important perhaps, but can you back that up with information on UV penetration in Lake Huron? I think those data must be available. Most UV light papers are from smaller, soft-water lakes if I remember this correctly.

Response: We acknowledge the close link between light and water clarity and visual predator avoidance. In the Introduction, we modified this section (lines 83-89) to better describe this relationship and acknowledge the proximate effect of light. Rejas et al. 2007 was also reviewed and the migration of species with moon light was associated with the predator avoidance hypothesis; therefore, this sentence was modified to reflect this association. Also, we performed a more thorough literature search for data on UV penetration in Lake Huron during the time of our study (2012) but the literature was very limited. We found one study that measured UV radiation penetration in Lakes Huron, Erie and Ontario (Smith et al. 2004) and one UV attenuation study in Lake Erie (Smith et al. 2011). The Smith et al. (2004) study found that photic depths of UV radiation varied widely from 6-12m in Lake Huron. This information was added to the discussion section (lines 481-484).

Line 104 what is meant with "endured these disturbances", probably do not need that part of the sentence.

Response: This part of the sentence was removed.

Line 125-127. I am surprised by this sentence. Vertical distribution have been studied for many years so are there really no studies on the effect of biotic and abiotic factors on distributions? Some of these studies may be from the 1960s or earlier. In Lake Ontario, there have been studies by Ora Johannsson, Sprules, and Watkins. Megard studied distributions with acoustics in Lake Superior. I would also check in the lake books edited by Munawar. If you consider mysids a zooplankton there are plenty of studies in these lakes. Did you review these studies and found them inadequate?

Response: This sentence was not clearly written. Our intent was to highlight that there are limited studies that have examined the <u>*concurrent*</u> *effects of several abiotic and biotic factors on zooplankton distribution patterns in Lake Huron. Many studies have examined one or two factors at a time, but very few have analyzed the effects of temperature, water clarity, food availability,* Mysis *predation, and* Bythotrephes *predation within the same study and during both night and daytime sampling. We reworded this sentence to hopefully make this clearer.*

Line 139 - you also consider temperature as an abiotic factor, but do not list an expectation - would you get larger or smaller differences between day and night in the epilimnion if the epilimnetic temperature is high? Would be nice to know your expectations.

Response: We added a sentence at this location (line 142) describing our temperature expectations.

Line 159. The reviewers wanted to see some explanation for how temperature profiles were used to determine the strata, not only that they were used.

Response: Using the temperature profiles, the thermocline was identified and used to define the metalimnion. This information was added to the sentence.

Line 180 - what instar are you talking about? Instars of Bythotrephes? Copepods?

Response: This sentence is referring to instars of Bythotrephes*. However, the 600 individual zooplankton that were counted excluded* Bythotrephes*. Therefore, "instar" was removed in this location.*

Line 271. You present more complex models with higher AIC than the minimum, suggesting you continued looking at the potential models beyond reaching the minimum AIC value. I think that is appropriate, so change the method description. I would like to see all models within delta AIC<2 (some suggest using delta AIC<5 or7) of the minimum value.

Response: We changed the method description to explain that we reported models that were considered but disregarded in the final step based on higher AIC_C *values. We have received the editor's suggestion to include all models within delta AIC < 2 of the minimum value; however, given that all of the models within* $AIC_C < 2$ *would be too numerous and complicated, we decided to omit these results from the manuscript.*

Line 300 spelling of epilimnetic

Response: Fixed

Line 303 - did you specify dry weight earlier? Remind readers that the biomass is in dry weight here.

Response: Yes, dry weight was specified in the Methods section. We added "dry weight" to this sentence.

Line 360 - are you limiting the comparison to Lake Huron and Michigan? If so state that.

Response: The sentence was reworded to reflect only Lake Huron and Lake Michigan.

Line 362 - Predator avoidance is the ultimate reason, light is the proximate factor to avoid visual predators. These are not mutually exclusive. Water clarity is a light effect.

Response: This sentence was reworded to tone down our statement that water clarity may be an independent factor in zooplankton DVM.

Line 284 missing parenthesis around 2015

Response: Fixed.

Line 396. Statement sounds like Barbiero studied rotifers for some years after 2006. This is not the case. Adjust text accordingly.

Response: The timeframe for the Barbiero and Warren study was added to the sentence.

Line 413 light

Response: Fixed

Line 430. Not only did Byth biomass not help predicting zooplankton distributions, the one instance it was significant appear to be in the opposite of predictions. That is not strong support for a Byth effect. What do you mean by "excluded Daphnia mendotae"?

Response: This sentence was a typo from the previous revision (prior to the new analysis). The sentence was reworded and reference to Daphnia mendotae *was removed.*

Line 434 - Also fish abundance and distribution. Can add some information on your investigations of fish here.

Response: We removed the acoustic information from the paper, given the fact that fish were only sampled in 50% of the months.

Line 435 - Repeated sentence?

Response: Yes, this repeated sentence was removed.

Line 463. Bythotrephes is a visual predator. So why would not their presence in the epilimnion lead to a larger proportion of the copepod in the epi at night?

Response: We agree with the editor's assessment, and, therefore, removed the last part of this sentence.

Line 495 What would you expect to be the effect of temperature? Some zooplankton prefer cold temperature, some do not. High epilimnetic temperature would lead to low movement into the epi of some copepods and more movement by some cladocerans (my expectations). This makes is hard to predict community effect.

Response: Our predictions of the effects of temperature were added to both the Introduction (line 142) and the Discussion (lines 497- 498).

References - check for consistent capitalization

Response: Done.

Table 3 table headings - You have a statement about epilimnetic differences for meta and hypolimntion samples. Remove the word epilimnetic.

Response: Done. Good catch!

Table 4 - State if all or only some two way interactions were included in the analyses

Response: At each step, only predictor variables could be added, no interaction terms. This information was added to the caption for Table 4.

Line 685 untransformation of the least square mean. Should it be untransformed least square mean? Have not heard the term untransformation before.

Response: Changed to untransformed.

References:

Smith, R. E., Allen, C. D., & Charlton, M. N. (2004). Dissolved organic matter and ultraviolet radiation penetration in the Laurentian Great Lakes and tributary waters. *Journal of Great Lakes Research*, *30*(3), 367-380.

Smith, R. E. H., Furgal, J. A., Charlton, M. N., Greenberg, B. M., Hiriart, V., & Marwood, C. (1999). Attenuation of ultraviolet radiation in a large lake with low dissolved organic matter concentrations. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*(8), 1351-1361.

Title: Biotic and abiotic factors influencing zooplankton vertical distribution in Lake Huron

Carly J. Nowicki[a] [*], David B. Bunnell[b], Patricia M. Armenio[b] David M. Warner[b], Henry A. Vanderploeg[c], Joann F. Cavaletto[c], Christine M. Mayer[a], Jean V. Adams[b]

[a]University of Toledo, Department of Environmental Science, 2801 West Bancroft Street, Toledo, Ohio, USA

[b]U.S. Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, Michigan, USA

[c]National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, 4840 S. State Road, Ann Arbor, Michigan, USA

[*] Corresponding author:
Office address:
Carly J. Nowicki
College of Natural and Social Sciences
Madonna University
36600 Schoolcraft Road
Livonia, MI 48150
734-432-5517
Email: cnowicki@madonna.edu

Abstract

The vertical distribution of zooplankton can have substantial influence on trophic structure in freshwater systems, particularly by determining spatial overlap for predator/prey dynamics and influencing energy transfer. The zooplankton community in some of the Laurentian Great Lakes has undergone changes in composition and declines in total biomass, especially after 2003. Mechanisms underlying these zooplankton changes remain poorly understood, in part, because few studies have described their vertical distributions during daytime and nighttime conditions or evaluated the extent to which predation, resources, or environmental conditions could explain their distribution patterns. Within multiple 24-hour periods during July through October 2012 in Lake Huron, we conducted daytime and nighttime sampling of zooplankton, and measured food (chlorophyll-*a*), temperature, light (Secchi disk depth), and planktivory (biomass of *Bythotrephes longimanus* and *Mysis diluviana*). We used linear mixed models to determine whether the densities for 22 zooplankton taxa varied between day and night in the epi-, meta-, and hypolimnion. For eight taxa, higher epilimnetic densities were observed at night than during the day; for four of these taxa a general linear model revealed these patterns were best explained by biomass of *Bythotrephes longimanus* (*Leptodiaptomus ashlandi*) or *Mysis diluviana* (*Leptodiaptomus minutus*) or Secchi disk depth (*Diacyclops thomasi*, cyclopoid copepodites). By investigating the potential effects of both biotic and abiotic variables on the vertical distribution of crustacean zooplankton and rotifers, we provide descriptions of the Great Lakes zooplankton community and discuss how future changes in food web dynamics or climate change may alter zooplankton distribution in freshwater environments.

Keywords: Invasive species; DVM; *Mysis; Bythotrephes;* rotifer; vertical migration

Introduction

Within a 24-hour period, zooplankton actively migrate within the water column to alter spatial overlap with their food sources, predators, and other environmental variables (Balcer et al., 1984; Loose and Dawidowicz, 1994; Peacor et al., 2005; Wells, 1960). For example, some cladocerans (e.g., daphnids, bosminids, *Holopedium gibberum*) and cyclopoid copepods (e.g., *Diacyclops thomasi*) have been documented to undergo diel vertical migration (DVM), descending during the day and ascending to surface waters at night (Hutchinson, 1967; Wells, 1960). These migrational patterns can influence trophic dynamics in freshwater systems because zooplankton are vital conduits in the pelagic food web, linking primary producers and higher trophic levels (Carpenter and Kitchell, 1996). The primary mechanism believed to regulate vertical migration is light-mediated predator avoidance (Dodson, 1990; Lampert, 1993), with descent associated with escaping predation under higher light levels, and ascent associated with exploitation of food and warmer water temperatures when the risk of predation is lower. In other words, zooplankton vertical migration is likely driven by a tradeoff between higher predation risk in the surface waters and decreased capacity for growth and reproduction in colder, deeper waters (Loose and Dawidowicz, 1994; Pangle et al., 2007). Other environmental variables, however, also have been hypothesized to influence zooplankton vertical migration, such as temperature (Cooke et al., 2008) , resource availability (Johnsen and Jakobsen, 1987; Makinol et al., 1996), light (Rejas et al., 2007), and UV levels (Fischer et al., 2006; Rhode et al., 2001).

The Laurentian Great Lakes offer relatively unique habitat among freshwater lakes, given their deep depths (exceeding 100 m) and mix of native and nonindigenous planktivores that could influence vertical migration patterns of herbivorous and omnivorous zooplankton species. The vertical migration patterns of predatory invertebrates are relatively well described. The native invertebrate planktivore, *Mysis diluviana* (opossum shrimp, hereafter called *Mysis*) has demonstrated its own light-mediated pattern of DVM (Beeton and Bowers, 1982; Beeton, 1960; Bowers, 1988), remaining near the bottom of the lake during the day and then moving upward in the water column to feed at night (Beeton, 1960). These movements can influence night-time movements of their prey, driving some zooplankton species further upward towards the surface (Peacor et al., 2005). Alternatively, the nonindigenous predatory cladoceran, *Bythotrephes longimanus* Leydig (spiny water flea, hereafter called *Bythotrephes*), is an epilimnetic predator that has also been documented to influence zooplankton vertical position in both the laboratory (Pangle and Peacor, 2006) and in the field (Bourdeau et al., 2011; Bourdeau et al., 2015). In the Great Lakes, *Bythotrephes* occupies the epilimnion during both day and night in the summer months (Lehman and Caceres, 1993; Ptáčníková et al., 2015), but has been documented to occur ~15-20 m deeper (into the metalimnion) during the day and then ascending to the epilimnion at night during autumn (Lehman and Caceres, 1993). In smaller Canadian lakes, *Bythotrephes* migration was relatively weak, especially in the presence of hypolimnetic planktivores (Young and Yan, 2008).

Other environmental factors beyond planktivore distribution can also vary with depth and influence the vertical distribution of zooplankton in the Great Lakes. For example, several *Daphnia* species will escape to deeper vertical layers to avoid damaging ultraviolet (UV) radiation at the surface during the day (Rhode et al., 2001). Light and water clarity may also be proximate factors for influencing migration associated with the avoidance of visual predators

(Bourdeau et al., 2011; Bourdeau et al., 2015). Even some species will synchronize movements and emergence with lunar phases to avoid predation (Rejas et al., 2007). Food and temperature are also significant influential factors driving many zooplankton to ascend to warmer, chlorophyll-rich surface strata at night (Bourdeau et al., 2015; Johnsen and Jakobsen, 1987). Williamston et al. (2011) hypothesized that water transparency plays a key role in regulating these major drivers of zooplankton DVM. Moreover, temperature gradients may be more influential than food on zooplankton vertical distribution (Loose and Dawidowicz, 1994) as colder temperatures in the hypolimnion are less metabolically favorable for many zooplankton species (Bourdeau et al., 2015; Dawidowicz and Loose, 1992) and may cause zooplankton to rise in the water column. Even those species that tend to occupy the relatively warm waters near the interface of the epilimnion and metalimnion, such as *Leptodiaptomus* as*hlandi* and *Leptodiaptomus minutus*, exhibit a nighttime migration toward the even warmer surface waters (Balcer et al., 1984). As a result, only by simultaneously measuring both biotic and abiotic characteristics of the food web during both day and night can scientists deduce which factors influence the vertical distribution of zooplankton species in the Great Lakes during both daytime and nighttime conditions.

Over the last century, most of the Laurentian Great Lakes have undergone substantial anthropogenic disturbances, including proliferation of nonindigenous species, nutrient fluctuations, and changing climate (Bunnell et al., 2014; Gronewold et al., 2013). Lake Huron, the second largest of the Great Lakes in terms of surface area (Beeton, 1984), is unique in that near coincident changes in phytoplankton, zooplankton, and forage fishes occurred around 2003 that have persisted to the present. The establishment of dreissenid mussels in Lake Huron in

1989 (Griffiths et al., 1991), along with mandated reductions in phosphorus loading, caused significant reductions in nutrients (Bunnell et al. 2014), declining pelagic primary production (Fahnenstiel et al., 1995a; Fahnenstiel et al., 1995b; Reavie et al., 2014), and increases in water clarity (Bunnell et al. 2014). Total zooplankton biomass declined 70% after 2003, relative to 1998 - 2002 (Barbiero et al., 2012), and cladoceran and cyclopoid copepods reached record-low biomass (Barbiero et al., 2009). At higher trophic levels, the collapse of alewife and the Chinook salmon fishery around 2003 was due to excessive predation by salmonines (He et al., 2014), and reductions in lower trophic level biomass (Kao et al., 2016). With biological changes occurring at all levels of the ecosystem, Lake Huron provides an appropriate case-study to examine how multiple trophic factors (top-down and bottom-up) and other environmental variables likely influence food web structure, including vertical distributions of the zooplankton community. One application of this study could be increased knowledge of the extent to which light-dependent planktivores, such as fish larvae or *Bythotrephes*, overlap with rotifers or crustacean zooplankton in the epilimnion and metalimnion during daytime hours.

Although many recent studies in the Great Lakes have described the vertical distribution patterns of crustacean zooplankton during the daytime hours (Bourdeau et al., 2011; Bourdeau et al., 2015), nighttime distribution patterns have been rarely documented. We know of few studies in the Great Lakes that were designed to determine the influence of multiple abiotic factors in conjunction with planktivores on both the diurnal and nocturnal vertical distribution of zooplankton. Furthermore, the description of vertical distribution patterns of rotifer in the Great Lakes is limited. Herein, our objective was to (1) determine the densities of crustacean zooplankton and rotifers in three vertical strata (epilimnion, metalimnion, hypolimnion) during the day and night; (2) within a given stratum, determine which zooplankton species exhibited differences in densities between the day and night; (3) when density differences within a stratum occurred, evaluating how the biomass of planktivores (i.e., *Bythotrephes*, *Mysis*), chlorophyll a, water temperature, and Secchi depth could explain the patterns.

Given the importance of non-consumptive (e.g., Pangle et al., 2007) and consumptive (e.g., Bunnell et al., 2011) effects of *Bythotrephes*, we hypothesized that higher *Bythotrephes* densities would cause Lake Huron zooplankton to be more abundant in the epilimnion at night than during the day. Similarly, we hypothesized that higher densities of *Mysis* would induce zooplankton to move up into the epilimnion at night (Peacor et al., 2005). In terms of abiotic factors, we believed clearer water (i.e., deeper Secchi depths) could cause zooplankton to migrate well below the epilimnion during the day, while warmer epilimnetic temperatures may cause some species to move towards the surface at night, and contribute to higher differences in epilimnetic densities between day and night.

Methods

Field survey design

We conducted monthly sampling events from July through October 2012 of zooplankton, *Bythotrephes*, *Mysis*, and water quality parameters. Planktivorous fish were estimated during July and September only, and therefore were not included in our analyses but can be considered present at all sampling sites. Sampling occurred offshore at two ports, Thunder Bay and

Hammond Bay, Lake Huron, and at two site depths (46 m and 82 m) at each port (Supplemental Figure 1). These sites were selected as part of ongoing surveys for the Cooperative Science and Monitoring Initiative. Daytime and nighttime sampling occurred between approximately 0845-2115 hours and 2130-0413 hours, respectively. At the start of each sampling event, vertical whole water column profiles of temperature and fluorescence were determined using an instrument equipped with a CTD (conductivity-temperature-depth) sensor (Seabird), and a fluorometer (TurnerScufa Cyclops 7 or WET Labs ECO-AFL/FL). The instrument was acclimated for one minute just below the surface of the water before decent to the substrate surface. Downcasts of temperature and fluorescence profiles (averaged each 1 m) were used to determine the location of vertical strata where zooplankton could be sampled with a closing net; temperature was the primary determination of the vertical strata, as the thermocline was identified and used to define the metalimnion. Three strata (epilimnion, metalimnion, and hypolimnion) were established for the 46 m sites, and four strata (epilimnion, metalimnion, upper hypolimnion, and lower hypolimnion) were established for the 82 m sites, with the exception of Thunder Bay in September where the 82 m site was only sampled at three strata (epilimnion, metalimnion, and hypolimnion). Water clarity was estimated by recording the Secchi disk depth on the shaded side of the research vessel during daytime only. Light was also measured as photosynthetically active radiation at most sampling locations; however, instrumentation varied among sampling events and some measurements were confounded by instrument malfunction. As a result, we used Secchi depth as an indicator of daytime light conditions.

Zooplankton (and planktivore) vertical position

Density (number per m^3) of crustacean zooplankton and rotifers was estimated in each vertical stratum during day and night with a closing 64-µm mesh net (0.5 m diameter) equipped with a flowmeter. Duplicate samples were taken from pre-determined start and end depths of each stratum (see Table 1 and Table 2). After the animals were washed down to the cod end, the sample was bathed for 2 - 5 min in antacid to narcotize organisms before being preserved in 5% formalin. In the laboratory, samples were stained with Phloxine B for ease of species identification. In the laboratory, each sample was thoroughly mixed and then subdivided into 1 ml aliquots, and at least 600 individual zooplankton (crustacean zooplankton and rotifers, excluding *Bythotrephes*) per sample were counted and identified to the lowest taxonomic level possible and to life stage (i.e., adult, copepodite, nauplii) under a dissecting microscope. Given the considerable abundance of invasive dreissenid mussel veligers in Lake Huron, they were not included in our count to 600 so that we could better estimate densities of rotifers and nauplii. If fewer than 100 total macrocrustaceans (i.e., cladocerans and adult and copepodite copepods) were counted, additional 1 ml aliquots of just those macrocrustaceans were counted so that at least 100 macrocrustaceans were counted in the sample. This method ensured that densities of major macrocrustacean taxa in Lake Huron were accurately represented. The abundance of *Bythotrephes* (and other rare predator cladocerans) was determined by counting all individuals in the entire preserved sample.

Planktivore biomass

In addition to estimating their stratum-specific densities with a 64-µm mesh zooplankton net, *Bythotrephes* were also collected during the day using whole water column vertical zooplankton tows (153-µm mesh, 0.5 m diameter), also bathed in antacid for 2 - 5 minutes and preserved in 5% formalin. We assumed this larger-mesh sample better represented *Bythotrephes* biomass in the entire water column. In the laboratory, formalin was strained from the sample and all contents were put into a glass dish to count all individuals from the sample. Spine lengths of up to 20 individuals per instar were measured. Mysids were collected at night in triplicate with whole water column vertical tows using 1000-µm mesh nets (1 m diameter). The cod ends were bathed in antacid for 2 - 5 minutes to narcotize the organisms and the animals were then preserved in 90% ethanol. In the laboratory, all the individuals in the sample were counted and measured (using ImagePro Plus 6.2 software) from the tip of the rostrum to the cleft of the telson. *Bythotrephes* and *Mysis* biomass were estimated by applying the appropriate length:dry weight regression (Garton et al., 1990; Rudstam et al., 2008) and multiplying mean weight by areal density to calculate areal biomass (dry mg/m^2).

Abiotic variables

Food availability was characterized by chlorophyll-*a* (µg/L) concentrations within each stratum. To measure chlorophyll-*a,* a Niskin bottle collected water samples within each stratum, either coincident or just following deployment of the CTD instrument. Chlorophyll-*a* was extracted from each water sample by first filtering 100 - 200 mL of water through a glass filter (47 mm GF/F) immediately after collection, and then freezing the filter in aluminum foil for no longer than 3 weeks. In the laboratory, samples were extracted with N, N-dimethylformamide following Speziale et al. (1984), and analyzed fluorometrically under low light levels on a 10AU fluorometer (Turner Designs). Temperature profiles were used to examine how temperature may affect zooplankton vertical distribution by calculating the mean temperature of each stratum and location of the thermocline. The thermocline depth was defined as the midpoint of two points of the temperature profile where the slope was at a maximum, indicating a rapid change in temperature (Fiedler, 2010).

Statistical analyses

The abundance (#/m^3) of each crustacean zooplankton taxon was calculated for each replicate strata sample in each time of the day. For copepods, species counts represent adult life stages; copepodites were summed as either calanoid or cyclopoid, and nauplii were grouped as a different taxon for all copepods. For *Bythotrephes*, we summed all instars in our counts. Using the volume sampled for each replicate, we calculated the mean density of the two replicate samples in each stratum and time of day. Because we were interested in comparing the vertical distributions across both bottom depths (that had different numbers of hypolimnetic strata), we then averaged the mean densities for upper and lower hypolimnion strata at the 82 m sites.

To determine whether the density of zooplankton in each vertical stratum varied between day and night in our samples (N = 96 across all ports, bottom depths, months, time of day, and strata), we used a linear mixed model (SAS Institute Version 9.4) estimated with the restricted maximum likelihood.   For a given taxon, we first normalized the data by adding the smallest non-zero density to all densities and then transformed (natural log) the density estimate. We included several predictor variables in the model that we hypothesized could influence density, including fixed (time of day, vertical stratum, bottom depth, time of day×stratum interaction) and random (port×month interaction) effects. We were most interested in whether or not the time of day×stratum interaction was significant for each taxon, and reported the Type III F-statistic and associated P-value. A significant interaction would indicate that densities in a given stratum varied between day and night. To further investigate, we reported the predicted density (as a least squares mean, LSMean) for a given time of day and stratum (i.e., across all months, ports, and depths). We made pairwise comparisons among the LSMean densities for each of the three strata to determine if densities differ between day and night (experiment-wise error rate of $\alpha = 0.05$).

For taxa with a significant time of day×stratum interaction, the density was always higher in the epilimnion stratum at night than during the day; there was no consistent pattern for any of the other vertical strata. As a result, we fit general linear models (GLM) to determine which predictor variables most parsimoniously explained variation in differences in epilimnetic densities (Epi_Diff) between the day and night for these select taxa. The predictor variables were: (1) *Bythotrephes* biomass (Bytho), (2) *Mysis* biomass (Mysis), (3) mean epilimnetic temperature (Temp), (4) mean epilimnetic chlorophyll (Chl), and (5) Secchi disk depth (Secchi). Because we were interested in attributing observed differences to these abiotic and abiotic effects, we did not include identifiers of space (ports and bottom depths) nor time (month) as predictors in our model.

We considered including site depth (46 m or 82 m) as predictor variable given the potential for epilimnion in the 82 m site to be deeper than the epilimnion in the 46 m site. But, because the depth of the epilimnion was not predictably deeper at 82 m than at 46 m (see Tables 1, 2), we did not include bottom depth as a predictor variable. We also considered metalimnetic temperature, the difference between epilimnetic and metalimnetic temperature, and thermocline depth as potential predictor variables, but they were each correlated with epilimnetic temperature. We also considered metalimnetic chlorophyll and the difference between epilimnetic and metalimnetic chlorophyll as potential predictor variables but they were both correlated with epilimnetic chlorophyll. We evaluated potential multicollinearity among the final predictor variables by calculating variance inflation factors (threshold of $VIF > 4$) and condition indices (threshold of $CI > 30$) for all combinations of variables.

We used a stepwise model selection process whereby Akaike's information criterion (corrected for small sample size, AIC_C) was used to determine whether additional variables should be added to the model (SAS Software, Version 9.4). The stepwise selection procedure started with only the estimated intercept, and the first predictor variable was added based on the lowest AIC_C. If the intercept-only model had the lowest AIC_C then the selection process was stopped; otherwise, predictor variables were added and removed until the model with the lowest AIC_C was determined. We also reported the model that was considered, but disregarded, in the final step for inclusion based on its higher AIC_C value.

For taxa without significant time of day×stratum interaction terms, we sought to determine how zooplankton densities varied between the three strata independent of time of day.

Hence, we removed the time of day×stratum interaction from the linear mixed model, and reported the Type III F-statistic and associated P-value for the effect of stratum, as well as the predicted density (as LSMean) for each stratum. We made pairwise comparisons of the LSMean densities among the three strata (experiment-wise error rate, α = 0.05).

Results

Day versus night differences in stratum densities

Twenty two of the 46 zooplankton taxa we identified during the study were sufficiently numerous in the samples to include in the linear mixed models (see Table 3). Eight of these 22 taxa had a significant time of day×stratum interaction term (Figure 1, Table 3), which indicated that the densities in the vertical strata differed between day and night. These eight taxa included three cladocerans (*Bosmina* spp., *Daphnia galeata mendotae, and Bythotrephes*), three calanoid copepod species (*L. ashlandi, L. minutus, and Epischura lacustris*) and two cyclopoid copepods (*Diacyclops thomasi*, cyclopoid copepodites). Pairwise comparisons revealed higher densities in the epilimnion during the night than during the day for all eight taxa. These results were complemented by higher densities during the day in the metalimnion for three taxa (*L. minutus*, *E. lacustris*, *D. thomasi*) and in the hypolimnion for four taxa (*D. galeata mendotae, Bosmina* spp., *D. thomasi*, cyclopoid copepodites).

Can predictor variables explain day vs. night differences in the epilimnion?

Estimates for predictor variables varied across sampling sites and within vertical strata (Tables 1, 2). Maximum chlorophyll-*a* concentrations primarily occurred within the epilimnion or metalimnion. At the 46 m sites, the maximum chlorophyll-*a* occurred at a vertical depth range of 5.5 – 37.5 m, whereas the range was 7.5 – 45 m at the 82 m sites. For both depths, the maximum chlorophyll-*a* value was shallowest in October. Temperature profiles showed distinct temperature ranges for the epilimnion (13.7 – 22.2 °C) and hypolimnion (4.49 – 6.96 °C), and epilimnetic temperatures decreased from July to October. The depth of the thermocline ranged 15 – 38 m, with the greatest depths occurring in October at both the 46 m and the 82 m sites. Secchi disk depth ranged 9.5 – 17.5 m at the 46 m site and 10.5 – 18 m at the 82 m site.

Bythotrephes biomass (dry weight) ranged from 15.7 – 66.3 mg/m^2 (122.9 – 435.7 individuals/m^2; Table 2). In Hammond Bay, *Bythotrephes* had the highest biomass in September (54.6 mg/m^2, 82 m), and in Thunder Bay *Bythotrephes* biomass peaked in July (66.3 mg/m^2, 82 m; Table 2). *Mysis* biomass had a wide range from 0.44-113.35 mg/m^2, with a peak (113.35 mg/m^2) occurring in August (82 m) at Hammond Bay, and July (95.61 mg/m^2; 82 m) at Thunder Bay.

For the eight taxa with a significant interaction term for time of day×stratum, we used a GLM to seek to explain variation in the difference between nighttime and daytime epilimnetic densities. The model selection process for *L. ashlandi* revealed the most parsimonious model to include only *Bythotrephes* biomass among the possible predictor variables (Table 4, Figure 2a), and a scatterplot between *Bythotrephes* biomass and the difference in night and day epilimnetic densities illustrated a weak inverse relationship. This inverse pattern was opposite from our expectation of higher *Bythotrephes* densities causing higher differences in day versus night epilimnetic densities if high *Bythotrephes* densities were icnducing a large proportion of *L. ashlandi* to migrate below the epilimnion during the day. For *L. minutus*, the most parsimonious model included only *Mysis* (Table 4, Figure 2b), and the scatterplot revealed greater differences in epilimnetic densities when *Mysis* densities increased. Higher density differences could result from *Mysis* causing more *L. minutus* to migrate up to the epilimnion at night to avoid planktivory as *Mysis* ascends the water column. For both *D. thomasi* and cyclopoid copepodites (most of which were likely immature *D. thomasi*), the most parsimonious model included Secchi disk depth (Table 4, Figure 2c,d). The greatest differences in epilimnetic densities were associated with higher Secchi depths, which could be interpreted as more *D. thomasi* migrating to sub-epilimnetic waters during the day when light penetration was higher. For the other four zooplankton taxa, however, model selection revealed that the intercept-only model was most parsimonious and none of the predictor variables helped explain variation in differences in epilimnetic densities between day and night (Table 4).

Vertical position of other zooplankton

For those zooplankton taxa without a significant interaction term for time of day×stratum, our study afforded an opportunity to describe the predominate vertical layer that was occupied based on day and night sampling. For 9 out of the 10 rotifer genera, densities varied among the three vertical layers (Figure 3, Table 3). For most genera, densities did not differ between the epilimnion and metalimnion (e.g., *Ascomorpha, Colletheca, Conochilus, Gastropus, Keratella,* *Polyarthra*, *Synchaeta*), but densities in those two layers were higher than in the hypolimnion. For only two genera (i.e., *Asplanchna*, *Ploesoma*) were densities in the epilimnion significantly higher than those in the metalimnion (and hypolimnion). *Kellicottia* individuals were unique given that the highest densities were in the metalimnion (Fig. 3f), and densities in the hypolimnion were higher than in the epilimnion.

We also evaluated whether densities varied among vertical strata for four other crustacean zooplankton taxa that, similar to the rotifers, did not differ in their densities between day and night for a given stratum (Table 3). The densities of *Limnocalanus macrurus* were highest in the hypolimnion, intermediate in the metalimnion, and lowest in the epilimnion (Fig. 4a). *Leptodiaptomus sicilis* was somewhat similar, except that its highest densities occurred in the hypolimnion and metalimnion, which were not different from one another, but both higher than those observed in the epilimnion (Figure 4b). Calanoid copepodites, conversely, attained their highest densities in the epilimnion and metalimnion (Figure 4c). Finally, copepod nauplii were estimated to have similarly high densities across the three strata (Figure 4d).

Discussion

Day and nighttime vertical distribution patterns of zooplankton can strongly influence trophic interactions (i.e., predator-prey) and energy dynamics in freshwater ecosystems. Therefore, describing zooplankton vertical distribution patterns in the Laurentian Great Lakes, particularly following dramatic system perturbations (i.e., invasive species, nutrient fluctuation, climate change), is critical for improved understanding of trophic interactions within the food web. Our results revealed eight zooplankton taxa exhibited differences in daytime and nighttime densities within at least one vertical stratum: *Daphnia galeata mendotae*, *Bosmina* spp. *Bythotrephes*, *Leptodiaptomus ashlandi*, *Leptodiaptomus minutus*, *Epischura lacustris*, *Diacyclops thomasi*, and cyclopoid copepodites. These results complement previous research on daytime vertical distributions patterns of zooplankton in Lake Huron and Lake Michigan (Beeton, 1960; Bourdeau et al., 2011; Bourdeau et al., 2015; Peacor et al., 2005). Furthermore, while predator avoidance has been touted as the primary driver of zooplankton DVM (Dodson, 1990; Lampert, 1993) and changes in zooplankton daytime vertical migration in the Great Lakes (Bourdeau et al., 2011; Bourdeau et al., 2015; Peacor et al., 2005), our results suggest that other factors (i.e., water clarity) may play a role in influencing zooplankton vertical distribution in the Great Lakes.

Reexamining zooplankton densities in Lake Huron

A comparison of our results to historical findings in Great Lakes reveals significant changes in zooplankton vertical distribution over time. In Lake Michigan in the 1950s, *Daphnia* were rarely found below the metalimnion (~ 10 – 20 m) during the day and migrated to the surface (< 5 m) a few hours after sunset (Wells, 1960). Haney and Hall (1975) also observed similar distribution patterns and magnitudes in *Daphnia* in Michigan inland lakes. However, our results showed that *Daphnia* in Lake Huron in 2012 had relatively high densities in the hypolimnion (~ 30 – 80 m) during the day. These results coincide with other recent studies that found deeper daytime distributions (~ 30 m) of *Daphnia galeata mendotae* in Lakes Michigan and Erie (Bourdeau et al., 2015; Pangle et al., 2007). A similar shift was also observed in *Bosmina*, with our results revealing greater densities of *Bosmina* in the hypolimnion than the epi- or metalimnion during the day; however, historical values determined this species to be found at ~ 10 – 20 m during the day (Wells, 1960). Wells (1960) also found that diaptomids (*L. ashlandi* and *L. minutus*) strongly favored the upper strata, with high abundance in the epilimnion during the day and an increase in abundance at the surface (< 5 m) at night, and *L. ashlandi* was found at slightly deeper depths than *L. minutus.* In contrast, our study found that both species had higher densities in the metalimnion during the day. Bourdeau et al. (2015) also observed deeper daytime distributions for these species in Lake Michigan, with *L. ashlandi* being found as deep as ~37 m and *L. minutus* as deep as ~27 m. Of the species examined in our study, there appears to be an overall downward shift in zooplankton day- and nighttime vertical distributions, which likely causes an increase in vertical movements in Lake Huron.

In addition to examining species with changes in diurnal distribution, our results provide a necessary update of mean densities for important crustacean zooplankton and rotifers in Lake Huron. Barbiero et al. (2012) reported an overall decline in the zooplankton community in Lake Huron from 2003 to 2006, accompanied by a large increase in *Limnocalanus macrurus* dominating the hypolimnion and an increase in calanoid copepods. Similarly, in 2012 *Limnocalanus macrurus* had its highest densities in the hypolimnion, while calanoid copepodites had higher mean densities in the epilimnion and metalimnion. Furthermore, dramatic shifts have been reported in the rotifer community, with *Conochilus* becoming the dominant species, in data spanning 1983-2006 (Barbiero and Warren, 2011). Our results showed that *Conochilus* continued to dominate the rotifer community in 2012 with densities more than 20 times the density of any other rotifer species examined in our study. Historically, *Keratella* was the dominant rotifer, but declined after the *Bythotrephes* invasion (Barbiero and Warren, 2011), and this species remained uncommon in Lake Huron in 2012 (< 15 individuals in the water column for each port, depth, and month).

Unfortunately, it is difficult to evaluate changes in rotifer vertical distribution compared to past observations because of a lack of layered rotifer studies in the Great Lakes. Our results filled a knowledge gap in rotifer vertical distribution. In general, rotifers were most concentrated in the epilimnion and metalimnion in Lake Huron; only *Synchaeta* was just as abundant in the hypolimnion as in the other two layers. In the EPA monitoring program in August, rotifer densities are only estimated in the top 20 meters of water, which corresponded to our epilimnion samples in August 2012. Our results illustrate that rotifer densities remain relatively abundant in waters deeper than 20 m, even for the most abundant *Conochilus*. One implication of these results for higher trophic levels is the high densities of rotifers in the epi- and metalimnion could conceivably reduce the probability of starvation for first-feeding fish larvae that otherwise could have limited small crustacean zooplankton to consume given the declines of small cladocerans and cyclopoid copepods in Lake Huron (Barbiero et al. 2009).

Biotic and abiotic factors influencing zooplankton distribution

More recently, several studies in the Great Lakes have aimed to explain changes in zooplankton abundance and distribution, and our work complements these studies by concurrently examining previously studied factors (i.e., *Bythotrephes* and light) in conjunction with new factors (i.e., *Mysis*). One primary focus of recent studies examining changes in zooplankton abundance and position in the water column is the presence of *Bythotrephes*. Since its invasion in the Great Lakes in the 1980s, *Bythotrephes* has had both predatory and non-consumptive effects on the zooplankton community. In Lake Michigan, *Bythotrephes* was implicated in altering the composition of the daphnid community to favor dominance of *Daphnia galeata mendotae*, in part because of its superior ability to migrate to deeper waters and avoid predation by *Bythotrephes* (Lehman and Caceres, 1993). These patterns were further verified by laboratory studies that found *Bythotrephes* kairmones to induce vertical migration of *Daphnia galeata mendotae* to deeper, colder depths and large reductions in population growth (Pangle and Peacor, 2006). Similarly, these nonlethal effects of *Bythotrephes* have been observed in copepods and other cladoceran species in Lakes Michigan and Erie (Bourdeau et al., 2011; Pangle et al., 2007). We predicted that *Bythotrephes* biomass would be a significant factor in zooplankton day versus nighttime epilimnetic densities in Lake Huron in 2012, given evidence that *Bythotrephes* abundance can explain up to 50% of the variation in the vertical distribution of zooplankton in Lake Michigan when compared with other factors (i.e., stratum temperatures, light attenuation, and sampling date; Bourdeau et al. 2015). However, our results were inconsistent with previous ones because *Bythotrephes* biomass only accounted for variation in day versus night epilimnetic densities for one species, *L. ashlandi*, in Lake Huron in 2012.

Several factors may explain our inability to detect a *Bythotrephes* effect, including insufficiently low biomass of *Bythotrephes* to elicit zooplankton responses, or influence of other predators (i.e., *Mysis*) that were not considered in previous studies. The maximum *Bythotrephes* biomass estimated from Lake Huron in 2012 (54.0 mg/m^2) was lower than those observed in previous studies. Bunnell et al. (2011) reported maximum *Bythotrephe*s biomass to be 72 mg/m^2 in Lake Huron in 2007. In Lake Michigan in 2004 - 2007, *Bythotrephes* abundance peaked in late July at approximately 85 mg/m^2 at a 45 m site and at about 280 mg/m^2 at a 110 m site (Bourdeau et al., 2011). Furthermore, on average, *Mysis* biomass (53.52 mg/m^2) was more than 1.5 times greater than *Bythotrephes* (35.32 mg/m^2), although *Bythotrephes* has a higher per capita consumption rate (Bunnell et al. 2011). The low *Bythotrephes* abundance observed during our study may have facilitated the native planktivore, *Mysis*, having a greater effect on zooplankton (i.e., *L. minutus*) vertical distribution in Lake Huron.

Despite mysids co-evolving with native zooplankton in the Great Lakes, the possibility that *Mysis* influences zooplankton migration patterns has only rarely been evaluated, and our study provides further evidence of the role of *Mysis* in inducing zooplankton migration. In laboratory experiments, *Daphnia* responded to kairomones of *Mysis* by changing their vertical position in the water column (Peacor et al., 2005); however, these patterns have not been well documented in the field. *Mysis* undergoes extensive DVM driven by its sensitivity to light, and in Lakes Huron and Michigan, *Mysis* remains at the bottom of the lake during the day, and ascends through the metalimnion at night (Beeton, 1960). The extent of *Mysis* vertical movement in the water column, along with its consumption of copepods (Johannsson et al., 2001; O'Malley and Bunnell, 2014), could induce zooplankton species that occupy the hypolimnion or metalimnion during the day to move up to shallower waters at night. Previous studies indicate *L. minutus* primarily occupies the epilimnion and metalimnion during the day, but then migrates up to shallower epilimnetic waters at night (Balcer et al., 1984; Barbiero and Tuchman, 2004a). Our study suggests that the upward nighttime migration of *Mysis* (~27 m) through the metalimnion, may be driving the vertical nighttime ascent of *L. minutus*. In fact, *L. minutus* density had almost a 4-fold increase in the epilimnion from day to night.

In addition to planktivory, water clarity (i.e., Secchi depth) also influenced zooplankton epilimnetic densities in Lake Huron. Increased water clarity can increase the amount of light penetrating the water column. Ambient levels of UV radiation can be lethal to some zooplankton (Hunstman, 1924), and several *Daphnia* species will escape to deeper strata to avoid UV radiation at the surface (Rhode et al., 2001). In fact, UV radiation in different strata could be more intense and drive some species further down into the water column, resulting in a deeper average daytime depth (see Fischer et al., 2006; Leech and Williamson, 2001; Rhode et al., 2001). Our models could not explain the differences in day versus night densities of *Daphnia galeata mendotae* in Lake Huron, but perhaps inclusion of UV radiation would have been more helpful. Ultraviolet radiation in Lake Huron can vary and photic depths of UV light have been reported from 6 – 12m (Smith et al. 2004). However, significant changes in water clarity due to increased filtration by dreissenid mussels may have increase UV penetration by the time our study was conducted in 2012. For instance, secchi depth did explain differences in epilimnetic densities of *D. thomasi* and cyclopoid copepodites during the day (low) and night (high). Given the increasing water transparency associated with the proliferation of invasive mussels (Barbiero and Tuchman, 2004b; MacIsaac, 1996), our results are important for predicting future changes in zooplankton vertical distribution as water clarity continues to fluctuate in the Great Lakes. In fact, Williamson et al. (2011) emphasizes a central role of water transparency in controlling the influence of predation, resource availability, temperature, and light penetration on zooplankton vertical migration patterns. This “transparency-regulatory hypothesis” provides a more flexible explanation for variation in zooplankton vertical distribution that includes multiple driving factors. Our results fit within this type of framework and demonstrate that many different variables are influencing zooplankton vertical distribution in Lake Huron.

Surprisingly, epilimnion temperature and mean chlorophyll in the epilimnion were not selected for any best fit models. We predicted that warmer epilimnetic temperatures would increase migration of most species examined in this study, based on previous research. Prior evidence suggests that warmer surface waters are a primary driver of zooplankton upward migration (Dawidowicz and Loose, 1992; Loose and Dawidowicz, 1994; Williamson et al., 1996; Winder et al., 2003). Also, recent studies in Lake Michigan found that *Daphnia* migrational patterns depended heavily on the location of the thermocline and *Daphnia* consistently migrated to a temperature range of 5 - 8 °C during the day and then ascended to warmer epilimnetic waters at night (Vanderploeg et al., 2015). Likewise, hypolimnion temperature was shown to influence the daytime depth of species that prefer warmer surface waters such as nauplii, diaptomid copepodites, and *L. ashlandi* (Bourdeau et al., 2015). However, we did not observe any of these patterns in our study. Epilimnetic temperature had a relatively narrow temperature range (14.6 – 22.2 °C), which may have limited the model’s ability to discern its effect on mean epilimnetic density. Also, studies outside of the Great Lakes have showed that zooplankton vertical migration patterns are based on clear feeding patterns with seasonal variation (Makinol et al., 1996), however our results did not show evidence of this pattern for any of the species examined.

Conclusion

The characterization of zooplankton vertical distribution in large, deep freshwater ecosystems and the factors that influence their patterns remains understudied. Our study was the first to concurrently document daytime and nighttime densities of some zooplankton species in Lake Huron and provide an important update on current zooplankton vertical densities and the environmental factors influencing their distribution. Although measuring changes in zooplankton community composition is important for interpreting and predicting ecological changes in the food web, understanding how these animals move within the water column provides improved understanding of vertical energy transfer. For example, the day and nighttime position of different zooplankton species are needed to inform trophic models (e.g., bioenergetics, Ecopath) that require knowledge of predator/prey overlap. Our study suggests that predation pressure and water clarity are important drivers that regulate zooplankton vertical distribution. With the ongoing effects of invasive species, exacerbated by ongoing and predicted changes in water transparency, it is important to understand both biotic and abiotic factors that may influence the movement and distribution of secondary producers in freshwater systems, and the impact these movements may have on the entire food web.

Acknowledgements

We would like to thank Scott Peacor at Michigan State University and Ed Rutherford and Jim Liebig at the NOAA-GLERL laboratory for their input on data manipulation. Funding for this project was provided by the Environmental Protection Agency and the Great Lakes

Restoration Initiative. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Balcer, M.D., Korda, N.L., Dodson, S.I., 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. Univ of Wisconsin Press.

Barbiero, R.P., Balcer, M., Rockwell, D.C., Tuchman, M.L., 2009. Recent shifts in the crustacean zooplankton community of Lake Huron. Canadian Journal of Fisheries and Aquatic Sciences 66, 816-828.

Barbiero, R.P., Lesht, B.M., Warren, G.J., 2012. Convergence of trophic state and the lower food web in Lakes Huron, Michigan and Superior. J Great Lakes Res 38, 368-380.

Barbiero, R.P., Tuchman, M.L., 2004a. Changes in the crustacean communities of Lakes Michigan, Huron, and Erie following the invasion of the predatory cladoceran *Bythotrephes longimanus*. Canadian Journal of Fisheries and Aquatic Sciences 61, 2111-2125.

Barbiero, R.P., Tuchman, M.L., 2004b. Long-term dreissenid impacts on water clarity in Lake Erie. J Great Lakes Res 30, 557-565.

Barbiero, R.P., Warren, G.J., 2011. Rotifer communities in the Laurentian Great Lakes, 1983–2006 and factors affecting their composition. J Great Lakes Res 37, 528-540.

Beeton, A., Bowers, J., 1982. Vertical migration of *Mysis relicta* Loven, Ecology of Mysidacea. Springer, pp. 53-61.

Beeton, A.M., 1960. The vertical migration of *Mysis relicta* in Lakes Huron and Michigan. Journal of the Fisheries Research Board of Canada 17, 517-539.

Beeton, A.M., 1984. The world's Great Lakes. J Great Lakes Res 10, 106-113.

Bourdeau, P.E., Pangle, K.L., Peacor, S.D., 2011. The invasive predator *Bythotrephes* induces changes in the vertical distribution of native copepods in Lake Michigan. Biol. Invasions 13, 2533-2545.

Bourdeau, P.E., Pangle, K.L., Peacor, S.D., 2015. Factors affecting the vertical distribution of the zooplankton assemblage in Lake Michigan: The role of the invasive predator *Bythotrephes longimanus*. J Great Lakes Res 41, Supplement 3, 115-124.

Bowers, J.A., 1988. Diel vertical migration of the opossum shrimp *Mysis relicta* in Lake Superior: Observations and sampling from the Johnson-Sea-Link II submersible. Bulletin of marine science 43, 730-738.

Bunnell, D.B., Barbiero, R.P., Ludsin, S.A., Madenjian, C.P., Warren, G.J., Dolan, D.M., Brenden, T.O., Briland, R., Gorman, O.T., He, J.X., 2014. Changing ecosystem dynamics in the Laurentian Great Lakes: bottom-up and top-down regulation. BioScience 64, 26-39.

Carpenter, S.R., Kitchell, J.F., 1996. The trophic cascade in lakes. Cambridge University Press.

Cooke, S.L., Williamson, C.E., Leech, D.M., Boeing, W.J., Torres, L., 2008. Effects of temperature and ultraviolet radiation on diel vertical migration of freshwater crustacean zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 65, 1144-1152.

Dawidowicz, P., Loose, C.J., 1992. Metabolic costs during predator-induced diel vertical migration of *Daphnia*. Limnol. Oceanogr. 37, 1589-1595.

Dodson, S., 1990. Predicting diel vertical migration of zooplankton. Limnol. Oceanogr.

Fahnenstiel, G.L., Bridgeman, T.B., Lang, G.A., McCormick, M.J., Nalepa, T.F., 1995a. Phytoplankton Productivity in Saginaw Bay, Lake Huron: Effects of Zebra Mussel (*Dreissena polymorpha*) Colonization. J Great Lakes Res 21, 464-475.

Fahnenstiel, G.L., Lang, G.A., Nalepa, T.F., Johengen, T.H., 1995b. Effects of zebra mussel (*Dreissena polymorpha*) colonization on water quality parameters in Saginaw Bay, Lake Huron. J Great Lakes Res 21, 435-448.

Fiedler, P.C., 2010. Comparison of objective descriptions of the thermocline. Limnology and Oceanography: Methods 8, 313-325.

Fischer, J.M., Nicolai, J.L., Williamson, C.E., Persaud, A.D., Lockwood, R.S., 2006. Effects of ultraviolet radiation on diel vertical migration of crustacean zooplankton: an in situ mesocosm experiment. Hydrobiologia 563, 217-224.

Garton, D.W., Berg, D.J., Fletcher, R.J., 1990. Thermal tolerances of the predatory cladocerans *Bythotrephes cederstroemi* and *Leptodora kindti*: relationship to seasonal abundance in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 47, 731-738.

Griffiths, R.W., Schloesser, D.W., Leach, J.H., Kovalak, W.P., 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. Canadian Journal of Fisheries and Aquatic Sciences 48, 1381-1388.

Gronewold, A.D., Fortin, V., Lofgren, B., Clites, A., Stow, C.A., Quinn, F., 2013. Coasts, water levels, and climate change: A Great Lakes perspective. Climatic Change 120, 697-711.

Haney, J., Hall, D.J., 1975. Diel vertical migration and filter-feeding activities of Daphnia. Arch. Hydrobiol 75, 413-441.

He, J.X., Bence, J.R., Madenjian, C.P., Pothoven, S.A., Dobiesz, N.E., Fielder, D.G., Johnson, J.E., Ebener, M.P., Cottrill, R.A., Mohr, L.C., Koproski, S.R., 2014. Coupling age-structured stock assessment and fish bioenergetics models: a system of time-varying models for quantifying piscivory patterns during the rapid trophic shift in the main basin of Lake Huron. Canadian Journal of Fisheries and Aquatic Sciences 72, 7-23.

Hunstman, A., 1924. Limiting factors for marine animals. I: The lethal effect of sunlight. Contributions to Canadian Biology and Fisheries 2, 81-88.

Hutchinson, G., 1967. A Treatise on Limnology. II. Introduction to lake biology and their limnoplankton. A Treatise on Limnology: II. Introduction to lake biology and their limnoplankton.

Johannsson, O.E., Leggett, M.F., Rudstam, L.G., Servos, M.R., Mohammadian, M.A., Gal, G., Dermott, R.M., Hesslein, R.H., 2001. Diet of *Mysis relicta* in Lake Ontario as revealed by stable isotope and gut content analysis. Canadian Journal of Fisheries and Aquatic Sciences 58, 1975-1986.

Johnsen, G.H., Jakobsen, P.J., 1987. The effect of food limitation on vertical migration in *Daphnia longispina*. Limnol. Oceanogr. 32, 873-880.

Kao, Y.-C., Adlerstein, S.A., Rutherford, E.S., 2016. Assessment of top-down and bottom-up controls on the collapse of alewives (*Alosa pseudoharengus*) in Lake Huron. Ecosystems, 1-29.

Lampert, W., 1993. Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis, Diel vertical migration of zooplankton, pp. 79-88.

Leech, D.M., Williamson, C.E., 2001. In situ exposure to ultraviolet radiation alters the depth distribution of Daphnia. Limnol. Oceanogr. 46, 416-420.

Lehman, J.T., Caceres, C.E., 1993. Food-web responses to species invasion by a predatory invertebrate - *Bythotrephes* in Lake Michigan. Limnol. Oceanogr. 38, 879-891.

Loose, C.J., Dawidowicz, P., 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology, 2255-2263.

MacIsaac, H.J., 1996. Potential abiotic and biotic impacts of zebra mussels on the inland waters of North America. American Zoologist 36, 287-299.

Makinol, W., Haruna, H., Ban, S., 1996. Diel vertical migration and feeding rhythm of *Daphnia longispina* and *Bosmina corexoni* in Lake Toya, Hokkaido, Japan. Hydrobiologia 337, 133-143.

O'Malley, B.P., Bunnell, D.B., 2014. Diet of *Mysis diluviana* reveals seasonal patterns of omnivory and consumption of invasive species in offshore Lake Michigan. Journal of Plankton Research 36, 989-1002.

Pangle, K.L., Peacor, S.D., 2006. Non-lethal effect of the invasive predator *Bythotrephes longimanus* on *Daphnia mendotae*. Freshwater Biology 51, 1070-1078.

Pangle, K.L., Peacor, S.D., Johannsson, O.E., 2007. Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. Ecology 88, 402-412.

Peacor, S.D., Pangle, K.L., Vanderploeg, H.A., 2005. Behavioral response of Lake Michigan *Daphnia mendotae* to *Mysis relicta*. J Great Lakes Res 31, 144-154.

Ptáčníková, R., Vanderploeg, H.A., Cavaletto, J.F., 2015. Big versus small: Does *Bythotrephes longimanus* predation regulate spatial distribution of another invasive predatory cladoceran, *Cercopagis pengoi*? J Great Lakes Res 41, Supplement 3, 143-149.

Reavie, E.D., Heathcote, A.J., Shaw Chraïbi, V.L., 2014. Laurentian Great Lakes phytoplankton and their water quality characteristics, including a diatom-based model for paleoreconstruction of phosphorus. PLoS ONE 9, e104705.

Rejas, D., De Meester, L., Ferrufino, L., Maldonado, M., Ollevier, F., 2007. Diel vertical migration of zooplankton in an Amazonian Várzea Lake (Laguna Bufeos, Bolivia). Studies on neotropical fauna and environment 42, 71-81.

Rhode, S.C., Pawlowski, M., Tollrian, R., 2001. The impact of ultraviolet radiation on the vertical distribution of zooplankton of the genus *Daphnia*. Nature 412, 69-72.

Rudstam, L.G., Schaner, T., Gal, G., Boscarino, B.T., O'Gorman, R., Warner, D.M., Johannsson, O.E., Bowen, K.L., 2008. Hydroacoustic measures of *Mysis relicta* abundance and distribution in Lake Ontario. Aquatic Ecosystem Health & Management 11, 355-367.

Smith, R. E., Allen, C. D., Charlton, M. N. 2004. Dissolved organic matter and ultraviolet radiation penetration in the Laurentian Great Lakes and tributary waters. Journal of Great Lakes Research, 30(3), 367-380.

Speziale, B.J., Schreiner, S.P., Giammatteo, P.A., Schindler, J.E., 1984. Comparison of N,N-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. Canadian Journal of Fisheries and Aquatic Sciences 41, 1519-1522.

Vanderploeg, H.A., Pothoven, S.A., Krueger, D., Mason, D.M., Liebig, J.R., Cavaletto, J.F., Ruberg, S.A., Lang, G.A., Ptáčníková, R., 2015. Spatial and predatory interactions of visually preying nonindigenous zooplankton and fish in Lake Michigan during midsummer. J Great Lakes Res 41, 125-142.

Wells, L., 1960. Seasonal abundance and vertical movements of planktonic crustacea in Lake Michigan, in: Interior, D.o. (Ed.). U.S. Fish and Wildlife Service, Washington, pp. 343-367.

Williamson, C.E., Fischer, J.M., Bollens, S.M., Overholt, E.P., Breckenridge, J.K., 2011. Toward a more comprehensive theory of zooplankton diel vertical migration: Integrating ultraviolet radiation and water transparency into the biotic paradigm. Limnol. Oceanogr. 56, 1603-1623.

Williamson, C.E., Sanders, R.W., Moeller, R.E., Stutzman, P.L., 1996. Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migration theory. Limnol. Oceanogr. 41, 224-233.

Winder, M., Buergi, H.R., Spaak, P., 2003. Seasonal vertical distribution of phytoplankton and copepod species in a high-mountain lake. Archiv für Hydrobiologie 158, 197-213.

Young, J.D., Yan, N.D., 2008. Modification of the diel vertical migration of *Bythotrephes longimanus* by the cold-water planktivore, *Coregonus artedi*. Freshwater Biology 53, 981-995.

Table 1. Summary of abiotic and biotic variables associated with sampling zooplankton in vertical strata near Hammond Bay, Lake Huron in July-October 2012.

Month	**Depth (m)**	**Secchi depth (m)**	***Bytho-trephes* (mg/m^2)**	***Mysis* (mg/m^2)**	**Thermo-cline depth (m)**	**Layer name**	**Layer Depth**	**Mean Temp.**	**Chl-a**
July	46	16.5	22.85	5.65	18	Epi	0-12	22.0	0.57
						Meta	12-24	14.0	0.56
						Hypo	24-44	4.7	0.61
	82	18	15.68	54.20	15	Epi	0-12	20.5	0.31
						Meta	12-22	12.2	0.55
						U. Hypo	22-60	5.3	3.24
						L. Hypo	60-82	4.0	0.52
Aug.	46	9.5	24.92	5.17	28	Epi	0-25	19.3	0.76
						Meta	26-33	8.3	0.56
						Hypo	34-42	5.0	0.20
	82	14	26.42	113.35	24	Epi	0-22	20.3	0.65
						Meta	23-40	9.9	1.06
						U. Hypo	41-60	5.3	0.50
						L. Hypo	61-81	4.1	0.36
Sept.	46	13	34.00	99.78	21	Epi	0-15	17.6	2.77
						Meta	16-30	11.8	4.22
						Hypo	31-44	5.7	4.51
	82	10.5	54.60	52.44	23	Epi	0-15	19.8	2.93
						Meta	15-32	8.9	7.18
						U. Hypo	32-55	4.8	3.92
						L. Hypo	55-82	4.3	3.11
Oct.	46	11	30.44	93.73	21	Epi	0-10	14.2	1.19
						Meta	11-28	9.8	0.66
						Hypo	29-41	4.5	0.34
	82	11.5	46.95	63.34	27	Epi	0-20	13.7	1.31
						Meta	21-30	9.0	1.15
						U. Hypo	31-55	4.5	0.38
						L. Hypo	56-79	4.1	0.19

Table 2. Summary of abiotic and biotic variables associated with sampling zooplankton in vertical strata near Thunder Bay, Lake Huron in July-October 2012.

Month	**Depth (m)**	**Secchi depth (m)**	***Bytho-trephes* (mg/m^2)**	***Mysis* (mg/m^2)**	**Thermo-cline depth (m)**	**Layer name**	**Layer Depth**	**Mean Temp.**	**Chl-a**
July	46	9.5	25.09	7.76	23	Epi	0-9	22.2	0.47
						Meta	9-35	12.9	0.71
						Hypo	35-44	5.4	0.61
	82	15.5	66.31	95.61	31	Epi	0-15	21.1	0.32
						Meta	15-30	14.1	0.77
						U. Hypo	30-60	6.4	1.53
						L. Hypo	60-80	4.7	0.58
Aug.	46	17.5	34.58	37.76	27	Epi	0-25	19.9	0.52
						Meta	26-35	12.5	0.70
						Hypo	36-44	7.0	0.57
	82	14.5	29.21	95.50	30	Epi	0-20	20.5	0.68
						Meta	21-42	10.6	0.83
						U. Hypo	43-62	5.3	0.73
						L. Hypo	63-82	4.7	0.36
Sept.	46	10.5	18.28	16.41	24	Epi	0-16	19.9	0.90
						Meta	16-30	10.7	0.91
						Hypo	30-42	5.7	0.66
	82	12.5	53.02	11.27	38	Epi	0-30	14.7	1.11
						Meta	30-50	7.9	0.60
						Hypo	50-80	5.1	0.35
Oct.	46	12.5	34.35	0.44	34.5	Epi	0-26	14.7	1.31
						Meta	27-37	10.9	0.61
						Hypo	38-41	6.0	0.34
	82	13	37.77	55.08	32.5	Epi	0-14	14.6	1.20
						Meta	15-40	11.0	0.69
						U. Hypo	41-60	5.5	0.34
						L. Hypo	61-78	5.3	0.34

Table 3. Summary of linear mixed model results to determine whether the density of zooplankton in each vertical stratum varied between day and night (time of day×stratum interaction); when significant, pairwise comparison results are shown for each layer (Epi. = epilimnion, Meta. = metalimnion; Hypo. = Hypolimnion). For each taxa, we also report whether the density of zooplankton varied between strata, independent of time of day. Zooplankton taxa are ordered alphabetically within a coarse taxonomic category. Sampling occurred during July-October, 2012, in Lake Huron.

Coarse zooplankton category	**Zooplankton taxa**	**Time of day × stratum interaction effect**		**Do densities differ between day and night for these layers?**			**Vertical stratum effect**	
		F statistic	**P-value**	**Epi.**	**Meta.**	**Hypo.**	**F statistic**	**P-value**
Cladoceran	*Bosmina longirostiris*	27.61	<0.0001	Yes	No	Yes	20.46	<0.0001
	Bythotrephes longimanus	5.89	0.0041	Yes	No	No	70.20	<0.0001
	Daphnia galeata mendotae	74.96	<0.0001	Yes	No	Yes	10.37	0.0004
Copepod	calanoid copepodite	2.25	0.11	No	No	No	7.56	0.0010
	cyclopoid copepodite	13.58	<0.0001	Yes	No	Yes	37.06	<0.0001
	Diacyclops thomasi	44.52	<0.0001	Yes	Yes	Yes	10.35	<0.0001
	Epischura lacustris	17.39	<0.0001	Yes	Yes	No	35.12	<0.0001
	Leptodiaptomus ashlandi	7.31	0.0012	Yes	No	No	53.47	<0.0001
	Leptodiaptomus minutus	19.95	<0.0001	Yes	Yes	No	165.50	<0.0001
	Leptodiaptomus sicilis	1.27	0.29	No	No	No	94.00	<0.0001
	Limnocalanus macrurus	0.94	0.40	No	No	No	51.42	<0.0001
	nauplii	0.34	0.72	No	No	No	0.37	0.69
Rotifer	*Ascomorpha* spp.	1.33	0.27	No	No	No	14.37	<0.0001
	Asplanchna spp.	0.06	0.94	No	No	No	40.40	<0.0001
	Collotheca spp.	0.15	0.86	No	No	No	57.76	<0.0001
	Conochilus spp.	0.58	0.57	No	No	No	52.99	<0.0001
	Gastropus spp.	0.43	0.65	No	No	No	17.88	<0.0001
	Kellicottia spp.	0.30	0.74	No	No	No	27.00	<0.0001
	Keratella spp.	0.94	0.40	No	No	No	12.67	<0.0001
	Ploesoma spp.	0.78	0.46	No	No	No	64.78	<0.0001
	Polyarthra spp.	0.25	0.78	No	No	No	20.07	<0.0001
	Synchaeta spp.	1.64	0.20	Yes	No	No	0.42	0.66

Table 4. Outcomes of the stepwise model selection process to determine the best fit model explaining variation in differences in epilimnetic densities of zooplankton between day and night in Lake Huron in 2012. At each step, only predictor variables could be added (i.e., no interaction terms). The best fit model was determined by selecting the model with the lowest corrected Akaike's information criterion (AIC_C) among the following predictor variables: *Bythotrephes* biomass (Bytho), *Mysis* biomass (Mysis), Chlorophyll *a* (Chl), mean epilimnetic temperature (Temp), and Secchi disk depth (Secchi). The best fit model (identified by the lowest AICc) for each zooplankton taxon is in bold. The last row for each taxon shows the model with the additional variable (that led to the lowest AICc) that was considered before being rejected owing to comparisons with the simpler model.

Species	**Model**	**AIC_C**
Bosmina spp.	**intercept**	**33.30**
	Chl	33.96
Bythotrephes	**intercept**	**13.07**
	Secchi	15.25
Daphnia galeata mendotae	**intercept**	**42.04**
	Mysis	43.31
Leptodiaptomus ashlandi	intercept	34.27
	Bytho	**33.96**
	Bytho+Secchi	35.29
Leptodiaptomus minutus	intercept	17.19
	Mysis	**14.23**
	Mysis+Bytho	16.78
Epischura lacustris	**intercept**	**29.86**
	Temp	31.95
Diacyclops thomasi	intercept	39.62
	Secchi	**35.21**
	Secchi+Mysis	36.21
cyclopoid copepodites	intercept	29.60
	Secchi	**27.95**
	Secchi+Temp	29.05

Figure Captions

Figure 1. Predicted mean density (untransformed least square mean) of daytime (open bars) and nighttime (filled bars) zooplankton abundance as a function of vertical stratum (Epi = Epilimnion, Meta = Metalimnion, Hypo = Hypolimnion) from samples collected at 46 and 82 m sites near Thunder Bay and Hammond Bay, Lake Huron from July-October 2012. The plotted zooplankton taxa (panels a-h) were the eight zooplankton taxa (of 22 tested) where a linear mixed model identified a significant interaction between time of day×stratum. Asterisks indicated where pairwise comparison of mean densities between daytime and nighttime (within a stratum) revealed significant differences (experiment-wise error rate, $\alpha = 0.05$).

Figure 2. Scatter plots of predictor variables versus the difference in nighttime and daytime epilimnetic zooplankton densities for four zooplankton taxa (panels a-d) from samples collected at 46 and 82 m sites near Thunder Bay and Hammond Bay, Lake Huron from July-October 2012. Only predictor variables that were selected during a stepwise model selection in a general linear model are plotted (see Table 4). Vertical lines represent the predicted difference from the best fit model.

Figure 3. Predicted mean density (untransformed least square mean) of rotifer abundance as a function of vertical stratum (Epi = Epilimnion, Meta = Metalimnion, Hypo = Hypolimnion) from samples collected at 46 and 82 m sites near Thunder Bay and Hammond Bay, Lake Huron from July-October 2012. A linear mixed model for each of the plotted rotifer genera (panels a-j) failed to identify a significant interaction between time of day×stratum (Table 4). For all taxa but *Synchaeta* spp., mean densities varied among the three vertical strata (Table 4). Within each panel, different letters above the bars indicate significant differences in mean density using pairwise comparisons (experiment-wise error rate, α = 0.05).

Figure 4. Predicted mean density (untransformed least square mean) of crustacean zooplankton abundance as a function of vertical stratum (Epi = Epilimnion, Meta = Metalimnion, Hypo = Hypolimnion) from samples collected at 46 and 82 m sites near Thunder Bay and Hammond Bay, Lake Huron from July-October 2012. A linear mixed model for each of the plotted taxa (panels a-d) failed to identify a significant interaction between time of day×stratum (Table 4). For all taxa, mean densities varied among the three vertical strata. Within each panel, different letters above the bars indicate significant differences in mean density using pairwise comparisons (experiment-wise error rate, α = 0.05).

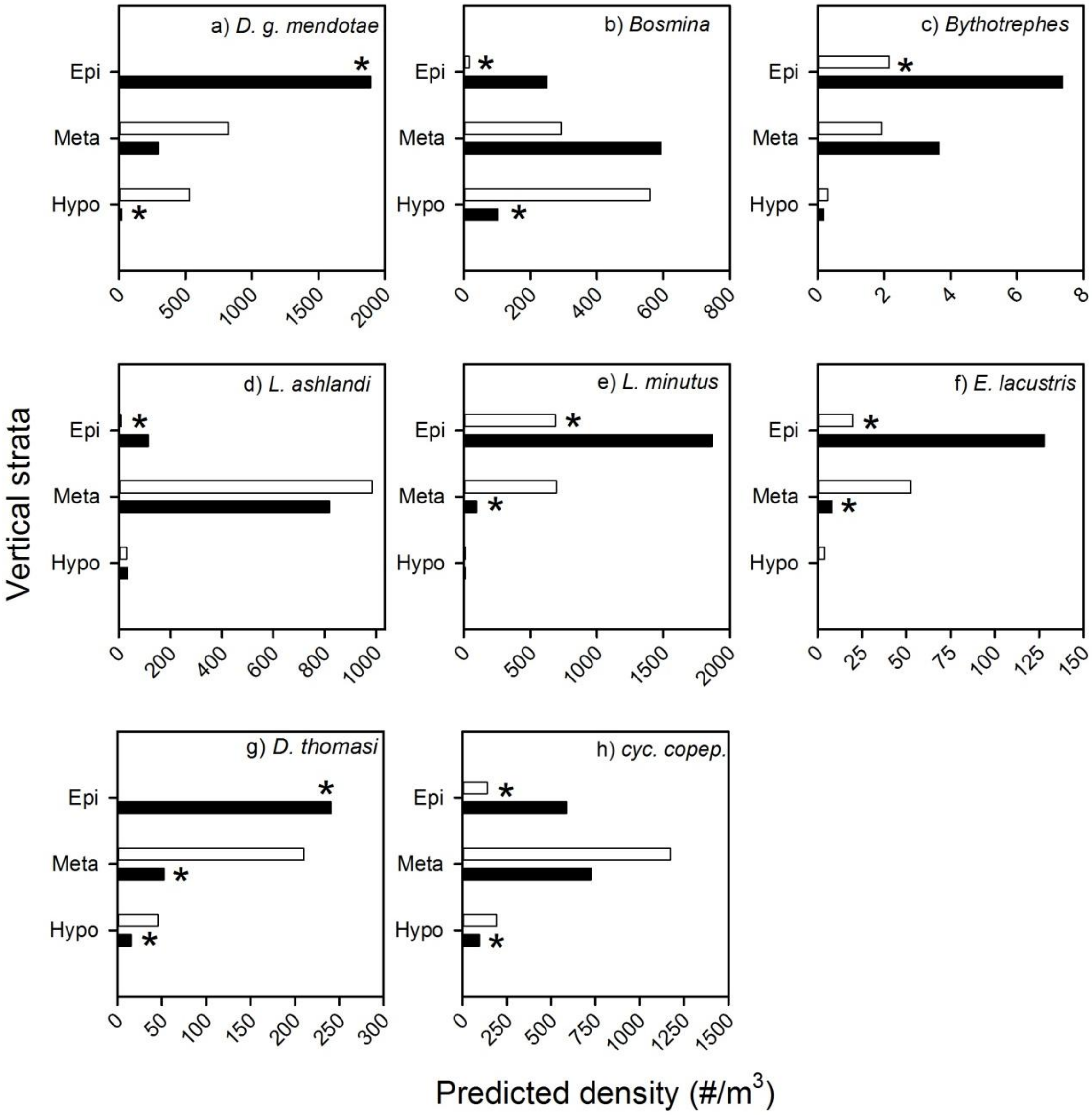
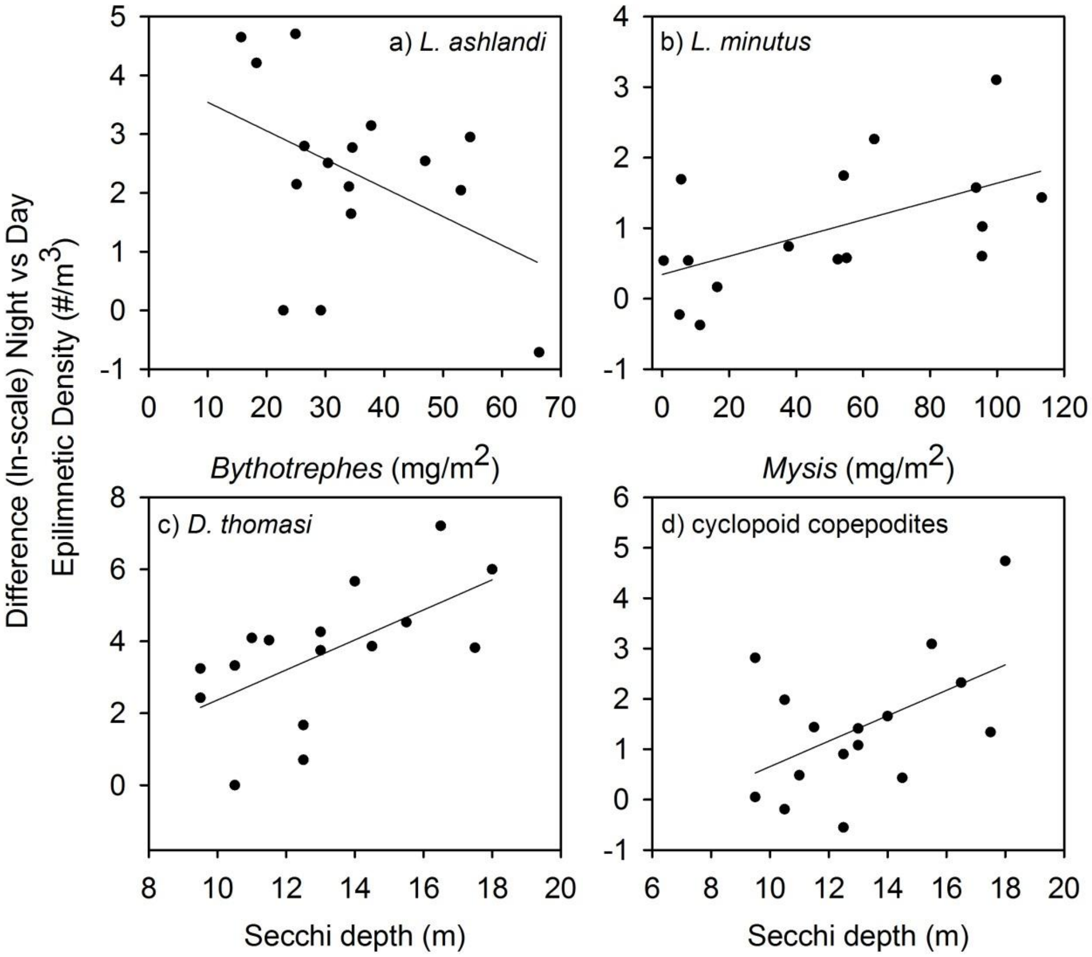
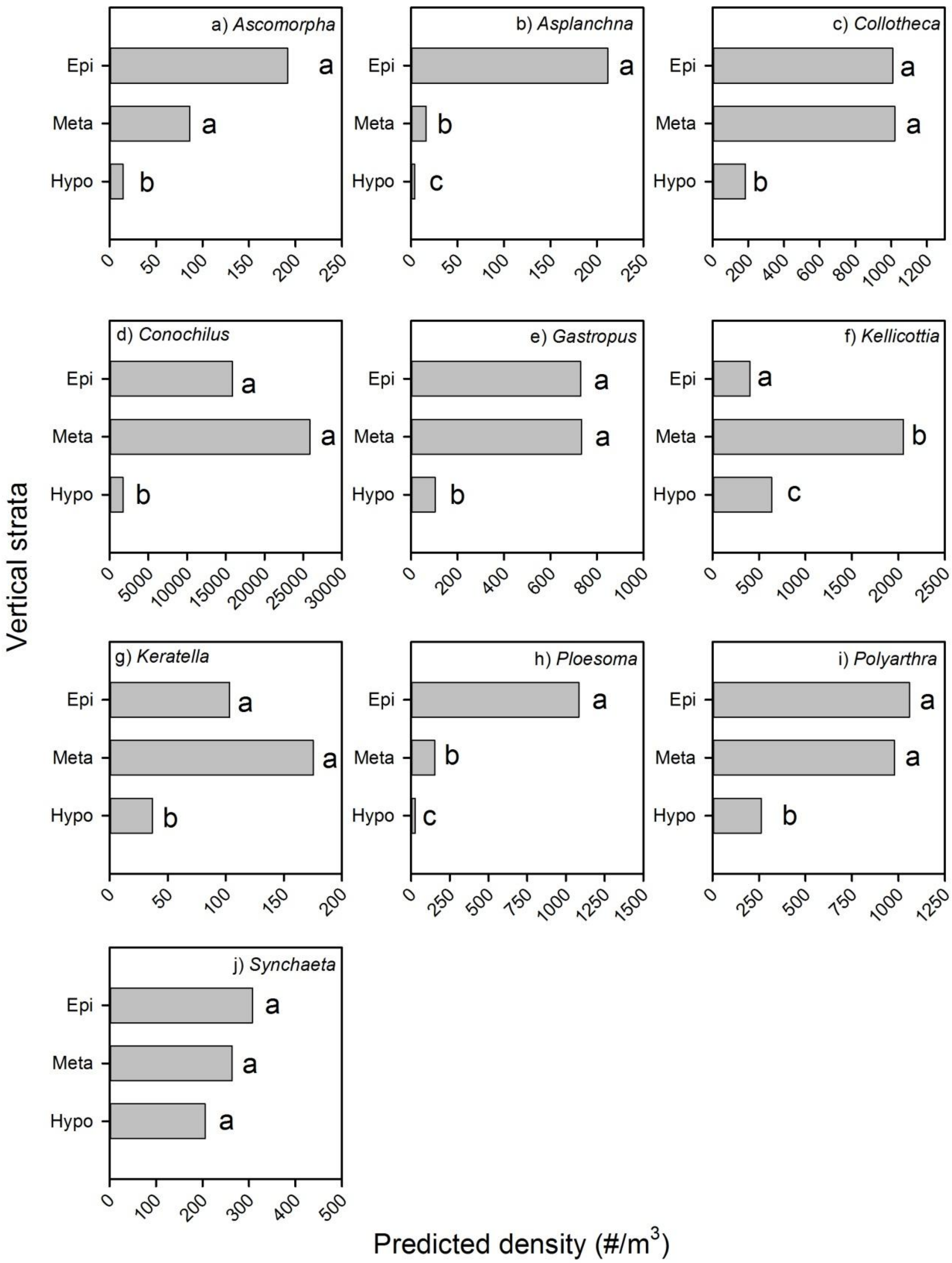

a) *D. g. mendotae*
b) *Bosmina*
c) *Bythotrephes*
d) *L. ashlandi*
e) *L. minutus*
f) *E. lacustris*
g) *D. thomasi*
h) *cyc. copep.*
Epi
Meta
Hypo
Vertical strata
Predicted density (#/m3)
0 500 1000 1500 2000
0 200 400 600 800
0 2 4 6 8
0 200 400 600 800 1000
0 25 50 75 100 125 150
0 50 100 150 200 250 300
0 250 500 750 1000 1250 1500
*

Figure 1



Difference (ln-scale) Night vs Day
Epilimnetic Density (#/m^3)
a) *L. ashlandi*
5
4
3
2
1
0
-1
0
10
20
30
40
50
60
70
Bythotrephes (mg/m^2)
b) *L. minutus*
4
3
2
1
0
-1
0
20
40
60
80
100
120
Mysis (mg/m^2)
c) *D. thomasi*
8
6
4
2
0
8
10
12
14
16
18
20
Secchi depth (m)
d) cyclopoid copepodites
6
5
4
3
2
1
0
-1
6
8
10
12
14
16
18
20
Secchi depth (m)

Figure 2



748
749 Figure 3

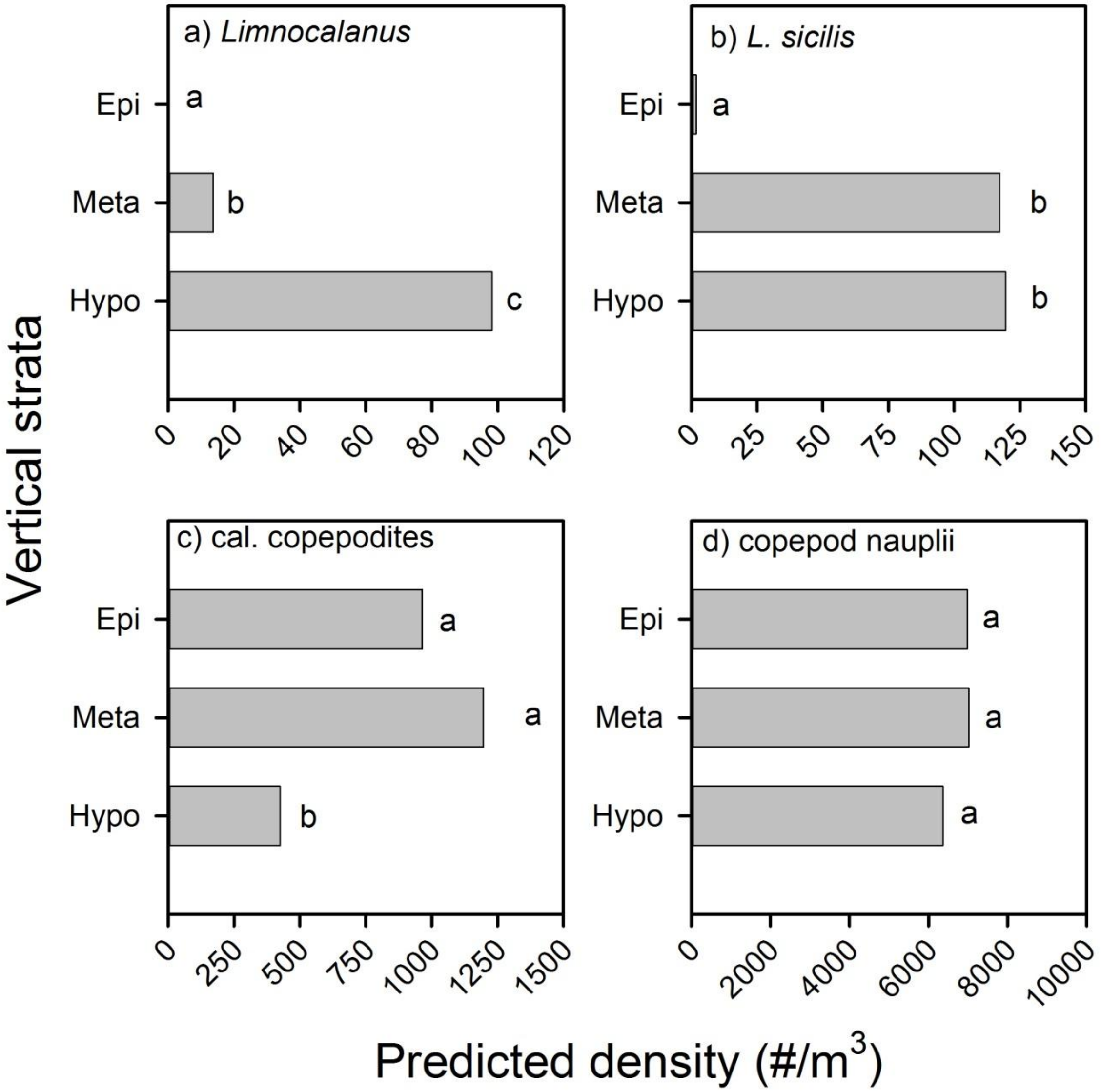

a) *Limnocalanus*
b) *L. sicilis*
c) cal. copepodites
d) copepod nauplii
Epi
Meta
Hypo
a
b
c
a
b
b
a
a
b
a
a
a
0
20
40
60
80
100
120
0
25
50
75
100
125
150
0
250
500
750
1000
1250
1500
0
2000
4000
6000
8000
10000
Vertical strata
Predicted density (#/m3)

Figure 4

Supplementary Figure 1