Factors affecting the vertical distribution of the zooplankton assemblage in Lake Michigan: the role of the invasive predator *Bythotrephes longimanus.*

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ABSTRACT

We analyze six years of survey data in Lake Michigan, which spanned large ranges in the abundance of the invasive predatory cladoceran *Bythotrephes*, to quantify the effect of this predator on the daytime vertical distribution of the nine most common species and life stages of Lake Michigan zooplankton. We found that *Bythotrephes* abundance and hypolimnion depth explained almost 50% of the variation in the vertical distribution of many zooplankton. *Bythotrephes* abundance was associated with significant and large (approximately 5-11 meter) depth increases in cladocerans *Daphnia* and *Bosmina,* adult and copepodite stages of cyclopoid copepods, and adult diaptomid copepods *L. minutus*, and *L. ashlandi*; but did not significantly affect the depth of copepod nauplii, diaptomid copepodites, and adult *L. sicilis.* Whereas other environmental factors, such as light attenuation coefficient, epilimnion and hypolimnion temperature, and sampling date significantly influenced the depth of various species and life stages, the inclusion of such environmental factors into linear models did not significantly lower the predicted influence of *Bythotrephes*. These results suggest that *Bythotrephes* abundance has a significant and large influence on the vertical distribution of a large component of the zooplankton assemblage in Lake Michigan. We argue that this pattern is driven by a *Bythotrephes*-induced anti-predator response in zooplankton prey. Such effects could lead to widespread growth costs to the zooplankton assemblage due to the colder water temperatures experienced at greater depths, which could in turn affect the rapidly changing Lake Michigan food web.

Keywords Anti-predator behavior; Biological invasions; Copepods; Diel vertical migration; Phenotypic plasticity; Laurentian Great Lakes

INTRODUCTION

The vertical distribution of zooplankton in the world's lakes and oceans has critical economic and ecological importance. Zooplankton are resources for larval and young of year stages of many commercially and ecologically important fishes (Cushing, 1972; Horwood et al., 2000) and zooplankton vertical distribution may affect zooplankton growth rate (and hence abundance) and dictate their positional overlap and hence availability to fish. The vertical migrations of zooplankton also affect transport and cycling of carbon and nutrients (Steinberg et al., 2000), and so environmentally induced changes in their distribution may influence energy transfer and ecosystem functioning.

Predators are generally thought to play a central role in the vertical distribution of some zooplankton (Hayes, 2003; Lampert, 1989; Zaret and Suffern, 1976). Visually-orienting planktivores can exert strong predation pressure, which zooplankton avoid by migrating downward where it is darker (e.g., Aksnes and Giske, 1993). Based on this predation pressure, and because predation pressure may vary, many zooplankton have been shown to modify their daytime vertical position in response to changes in the abundance of fish which are visually-oriented (Bollens and Frost, 1991; Lass and Spaak, 2003; Van Gool and Ringelberg, 1998).

Bythotrephes longimanus is an invasive predatory cladoceran in the Great Lakes with the potential to strongly affect zooplankton vertical distribution. A visual predator, *Bythotrephes* can reach high densities in the epi- and metalimnion (Bourdeau et al., 2011; Pangle et al., 2007), representing a risk to zooplankton in the upper lake strata. A behavioral shift to occupy deeper, darker water below areas of high predation risk by *Bythotrephes* could

therefore be adaptive for Lake Michigan zooplankton. Such adaptive avoidance responses to invertebrate predators have been documented in zooplankton groups (cladocerans and copepods) similar to those found in Lake Michigan (Neill, 1990; Nesbitt et al., 1996).

Previous research suggests *Bythotrephes* may be affecting the vertical position of zooplankton in Lake Michigan. Laboratory studies indicate that the cladoceran *Daphnia mendotae*, and the copepods *Diacyclops thomasi* and *Leptodiaptomus minutus* respond to water-borne chemical cues from *Bythotrephes* by moving deeper in experimental water columns (Bourdeau et al., 2011; Pangle and Peacor, 2006,). Further, field surveys in the Great Lakes indicate positive associations between *Bythotrephes* abundance and the depths of *D. mendotae*, *D. retrocurva*, *Bosmina longirostris* (Lehman and Cáceres, 1993; Pangle et al., 2007) and the adult stages of cyclopoid and diaptomid copepods (Bourdeau et al., 2011).

Whereas these previous lab and field patterns suggest *Bythotrephes* is affecting zooplankton distribution in the field, potential confounding environmental influences on zooplankton depth in the field have not yet been considered. Further, previous studies have not quantified how deep zooplankton move in response to changes in *Bythotrephes* abundance - especially when considering the influence of other potentially correlated factors, such as light, temperature, and food resources. Quantifying the effect of *Bythotrephes* abundance within the context of other environmental influences in the field is important for two main reasons: (1) factors other than predator abundance can influence zooplankton vertical position, and (2) the effects of predators on zooplankton vertical distribution can be complex and indirect and cannot easily be extrapolated from laboratory studies. For example, zooplankton daytime distribution may be influenced by a number of factors other than just predator abundance;

including water temperature (Cooke et al., 2008), water transparency (e.g., Dodson, 1990), and vertical gradients of food (e.g., Leibold, 1990) and light (e.g., Van Gool and Ringelberg, 1998). Moreover, variables such as predator abundance, light, and temperature, which are often studied in isolation in the laboratory, are correlated in the field and may co-vary seasonally. Further, whereas laboratory studies can be instructive by e.g., showing that zooplankton can sense predator kairomones and that they can respond by migrating (e.g. downwards), the magnitude of the response in the field cannot be determined.

The effect *Bythotrephes* has on the vertical position of zooplankton under prevailing environmental conditions in the field is important because it could influence the effect of *Bythotrephes* on zooplankton abundance, which has been suggested to be large in the Great Lakes and other ecosystems it has invaded (Bunnell et al., 2011; Strecker et al., 2011; Vanderploeg et al., 2012). Several mechanisms may be involved. First, costs associated with *Bythotrephes*-induced vertical shifts could reduce zooplankton growth rates. One particular cost to vertical avoidance behavior is a significant reduction in growth rate due to low temperatures (Dawidowicz and Loose, 1992; Loose and Dawidowicz, 1994). Such costs could be particularly large in the summer when large lakes are thermally stratified. For example, we have proposed *Bythotrephes* negatively affects Lake Michigan *Daphnia mendotae* populations through the induction of greater downward vertical migration into cool suboptimal feeding habitats during the day (Pangle et al., 2007). In laboratory experiments, we found that *D. mendotae* incurred a 36% reduction in somatic growth rate in the presence of *Bythotrephes* kairomones by migrating to lower positions in experimental columns with a thermal gradient (Pangle and Peacor, 2006). We have predicted similar reductions in *D. mendotae's* population

growth rate in the field due to an observed correlation between deeper depths of *Daphnia* and *Bythotrephes* density (Pangle et al., 2007). Second, induced changes in zooplankton vertical distribution can affect the availability of these prey to both *Bythotrephes* and larval and young-of-the-year fish. Lastly, among-species and life stage differences in the magnitude of vertical responses and associated costs could shift the balance of competitive interactions within the zooplankton assemblage.

Here we combine six years of field survey data to quantify the relative importance and magnitude of the effect of *Bythotrephes* abundance on the daytime vertical distribution of the nine most common species and life stages of the Lake Michigan crustacean zooplankton assemblage. Previous laboratory studies have shown that several species respond to waterborne chemical cues (kairomones) from *Bythotrephes* in laboratory experiments (Bourdeau et al., 2011; Pangle and Peacor, 2006) and correlations between zooplankton depth and *Bythotrephes* abundance have been documented over shorter-term studies (Bourdeau et al., 2011; Pangle et al., 2007). Our goal here is to more thoroughly explore the effect of *Bythotrephes* on zooplankton vertical distribution in Lake Michigan with a larger data set and with a more comprehensive statistical analyses in order to both consider the influence of other (potentially confounding) environmental factors, and to estimate the magnitude of the effect (rather than simply testing whether it exists). We discuss the implications of our findings in the context of non-consumptive effects in the rapidly changing Lake Michigan food web.

METHODS

General overview

We conducted field surveys in which we sampled the daytime vertical distribution of the most abundant species and life stages of crustacean zooplankton (9 groups total), the abundance of their potential predator, the planktivorous cladoceran *Bythotrephes longimanus,* and several relevant limnological variables (e.g., light, temperature, and food) over a range of dates and locations in Lake Michigan.

We used three complimentary statistical approaches to examine if *Bythotrephes* abundance and other factors affected zooplankton vertical position. First, our principal approach was to use formal model selection and averaging of multiple linear regression (MLR) models within the context of other environmental factors that could affect zooplankton vertical distribution. Second, because zooplankton depth may be explained by several factors that may be correlated among one another, and because such correlations among explanatory variables may compromise the ability to reliably estimate their effects in MLR, we employed partial least squares regression (PLSR), which is particularly well suited to analyzing data with a sample size that is small relative to a large number of related explanatory variables. Third, we analyzed the effect of *Bythotrephes* abundance on the vertical distribution of each zooplankton group using ordinary least squares (OLS) regression. This third test was performed to better understand how environmental factors influenced the statistical significance and the magnitude of the estimate of the *Bythotrephes* effect. That is, we compared the statistical significance and the magnitude of the *Bythotrephes* abundance effect on zooplankton depth from multimodel averaging of MLR models to that obtained from the OLS regression, in which *Bythotrephes* abundance was the sole predictor variable.

Lastly, we used results from the MLR analysis to estimate the magnitude of the effect of *Bythotrephes* abundance on the vertical position of zooplankton.

Field surveys

We sampled the daytime vertical distribution of zooplankton at several offshore locations and a range of dates that spanned different *Bythotrephes* abundances. There were 25 total sampling events (i.e., a single stratified vertical distribution sample), which spanned six different years (2004-06, 2009-11) and three established offshore sampling locations (identified as M45, M60, M110) in Lake Michigan off of Muskegon, MI (see Table 1 for sampling date and location details). Each sampling event consisted of collecting lake water with a 40-meter hose connected to a diaphragm pump. The water was filtered through a 64-µm-mesh zooplankton net on the deck of a research vessel. We used two stratified pump sampling procedures: (1) collecting one cubic meter of lake water from each of five different depths ranging from 4 m to 40 m corresponding with the centers of the epilimnion, metalimnion, hypolimnion, and the transitions between them, or (2) collecting one cubic meter of lake water from ten equally spaced depth intervals by gradually moving the pump hose from 40 m depth to the surface. All sampling events were carried out at midday between 1100 and 1400 to ensure that variation in time of day that we sampled would not affect our estimates of zooplankton depth. Each depth sample was preserved in either buffered sugar-formalin solution (2004-06) or 95% ethanol (2009-11).

Vertical profiles of temperature, light, and chlorophyll fluorescence were determined with an instrument package consisting of a CTD (conductivity-temperature-depth sensor), scalar

PAR (photosynthetically active radiation) sensor, and chlorophyll fluorometer (we used concentrations observed by the fluorometer to measure chlorophyll). Unfortunately, CTD data for 5 of the sampling events (4 events at M45 in 2005, and 1 event at M45 in 2006) were lost prior to analysis. Thus we had 20 sampling events that included complete limnological data.

To ensure the integrity of our pump sampling technique, we made a comparison between *Bythotrephes* densities estimated by our pump sampling method and by net sampling. Net sampling consisted of towing a 0.5-m, 64-µm or a 1.0-m 363 µm mesh zooplankton net from the bottom of the lake to the surface. Pump sampling consisted of pumping 1 cubic meter of water from 10, 4-m interval sections of the water column. Our comparison indicated that pump derived estimates were about 2 times greater than the net derived estimates ($Y = 0.51x + 5.39$; $R^2 = 0.56$, $n = 15$, $P < 0.001$) over the range of densities observed (0 – 276 no. m^{-2})). These results are consistent with a previous study that compared pump and net sampling gears that found that pump sampling captured higher densities of animals per taxa than net sampling (Masson et al., 2004). Further, in this study we are concerned with comparing the distribution of zooplankton found at different *Bythotrephes* densities. Because we used the same pump system throughout the study, any under (or over) estimate of densities collected at each position are unlikely to affect such comparisons.

Prior to counting and identification of zooplankton, we subdivided samples using a Henson-Stemple pipette after gentle but thorough mixing. We counted at least 600 individual zooplankton in each sample during 2004-06, and at least 5% of each 1 m^3 sample during 2009-11. These methods ensured that the major crustacean zooplankton groups in Lake Michigan were likely to be included in our counts. However, because confidence in estimates of

abundance and distribution is limited by individual counts, only those samples with at least 15 individuals were included in our analyses.

We characterized daytime vertical distributions for zooplankton using a measure of zooplankton mean depth (MD), which was calculated as:

$$\mathrm{MD} = \sum_{i=1}^{j} n_i d_i z_i \Big/ \sum_{i=1}^{j} n_i zi$$

Where d_i is the depth of the center of each depth interval (in meters), n_i is the density of individuals in the depth interval (in no. m^{-3}), z_i is the number of meters represented (i.e., the thickness of the depth interval) by the ith sample, and j is the number of depth intervals sampled during each profile. *Bythotrephes* abundance (areal density [no. m^{-2}]) was calculated using volumetric densities (no. m^{-3}) from each sampled depth interval weighted by the thickness (in m) of the depth interval. The entire sample was used to determine *Bythotrephes* abundance.

Statistical analysis of field survey data

All analyses were done in R (R Development Core Team, 2013). Prior to analyses, *Bythotrephes* density was log-transformed (Log[*Bythotrephes* density + 4]; 4 representing one half the lowest detectable *Bythotrephes* density [Berry, 1987]) to normalize its distribution and to improve its linear fit with zooplankton MD (Sokal and Rohlf, 1995). Visual inspection confirmed that log-transformed data provided a more linear relationship between *Bythotrephes* abundance and zooplankton mean depth than non-transformed data.

Multi-model selection and averaging of multiple linear regression models

Multiple linear regression (MLR) models were used to test for the effect of *Bythotrephes* on zooplankton MD while taking into account other environmental factors, such as light, temperature, and food (amount of and depth of chlorophyll maximum). A subset of limnological parameters, as well as *Bythotrephes* density (BD) and sampling date (Julian date; JD) were chosen *a priori* to include only those factors known or hypothesized to influence zooplankton vertical position. These included epilimnion depth (the depth that marks the bottom of the epilimnion; ED), epilimnion temperature (the average water temperature in the epilimnion; ET), hypolimnion depth (the depth that marks the transition between the meta- and hypolimnion; HD), hypolimnion temperature (the temperature at the top of the hypolimnion; HT), Chl *a* concentration (CC) and the depth of its maximum (CMD), Secchi depth (SD) and attenuation coefficient (AC). We did not include interaction terms in the models due to the large number of potential interactions among predictor variables, the difficulty in predicting which interactions might be important and how they would affect zooplankton depth, and the low statistical power with which to detect the statistical significance of interaction terms given our sample size. We assessed potential collinearity of predictor variables using scatterplots and by calculating variance inflation factors for the selected predictor variables (Fox and Weisberg, 2011).

To determine the importance of *Bythotrephes* abundance on zooplankton depth we used formal model selection, with Akaike's information criterion corrected for small sample size (AIC_C), to compare sets of multiple linear regression models of zooplankton MD (Burnham and Anderson, 2002). We used AIC_C because it identifies models based on parsimony (i.e., more complex models must explain more variance to obtain AIC_C scores equal to simpler models). We

used Δ AIC_C values < 2 for model support because values less than 2 suggest substantial evidence for the model (Burnham and Anderson 2002). We estimated the relative importance of predictor variables from all models with Δ AIC_C values < 4. As a general rule Δ AIC_C values between 3 and 7 indicate models that have considerably less support than those with Δ AIC_C values < 2, but they do still have some explanatory power (Burnham and Anderson 2002). Given our relatively low sample size and high number of predictor variables, including more candidate models in our model selection analyses allowed us to better estimate the relative importance of *Bythotrephes* abundance among a larger subset of predictor variables.

One factor, hypolimnion depth, showed high collinearity with other predictors, however, we left it in models for MD because of its potential importance in influencing zooplankton depth. There is much disagreement over when collinearity among predictor variables begins to severely distort linear model estimation and subsequent prediction, but recent work has shown the value of using linear models in combination with threshold-based pre-selection when collinear variables are considered in the final interpretation (Dormann et al., 2013). We used the 'car' and 'MuMIn' packages of R to fit MLR models and make comparisons using AIC_C.

Partial least squares regression

When there are large numbers of predictor variables in MLR models, there can be a problem of multiple collinearity (i.e., predictor variables are not truly independent). In an effort to confirm the identification of relevant predictor variables and their relative influence on zooplankton depth from multiple regression models, we also performed partial least squares regression

analysis (PLSR) on MD. PLSR is an extension of MLR analysis in which the effects of linear combinations of several predictors on a response variable are analyzed. Associations between the response variable with latent factors extracted from predictor variables that maximize the explained variance in the response variables are established. These latent factors are defined as linear combinations constructed between predictor and response variables, such that the original multidimensionality is reduced to a lower number of orthogonal factors to detect the structure in the relationships between predictor variables and between these latent factors and the response variables. PLSR can provide reliable results when there are large numbers of predictor variables relative to the number of observations, and when predictors are highly correlated (Carrascal et al., 2009; Mevik and Wehrens, 2007). Similar results in terms of the relative importance and contribution of predictor variables, between the MLR analyses and the PLSR would add confidence to our assessment of the relative importance and influence of predictor variables in our multiple regression analyses. We used the 'pls' package to perform PLSR.

Ordinary least squares regression

To assess the significance and magnitude of the effect of *Bythotrephes* abundance on zooplankton depth for each zooplankton group, we performed OLS regression, with *Bythotrephes* density as the sole predictor of zooplankton depth in the linear model. We assess the degree to which other limnological factors could be affecting the significance and magnitude of the *Bythotrephes* abundance effect on zooplankton depth by comparing the *Bythotrephes* abundance effect on zooplankton depth from OLS regression (the unstandardized *Bythotrephes* density coefficient) to the *Bythotrephes* abundance effect on zooplankton depth

from the MLR analysis (the model-averaged unstandardized *Bythotrephes* density coefficient). Estimates of statistical significance for differences in the magnitude of the *Bythotrephes* effects (i.e., the regression coefficients) were made using the overlap rule for SE bars (Cumming et al., 2007), where for a sample size > 10, a gap between error bars between 1 and 2 SE equals *P* between 0.05 and 0.01.

Estimate of magnitude of Bythotrephes effect on zooplankton mean depth

We are unaware of a straightforward or standardized way to estimate the magnitude of a predator's (or any factor's) effect on the mean depth of zooplankton (or any species). It is a complicated problem for two main reasons. First, the magnitude of the effect of a predator on zooplankton depth is likely strongly context dependent. For example, if a second factor (e.g. another predator such as fish) is present and affecting the depth of the zooplankton, then the effect of *Bythotrephes* would likely be altered (reduced). Second, the effect of *Bythotrephes* on depth is likely highly nonlinear; at low densities no to little effect is expected, after which an increase in *Bythotrephes* density will likely cause an increasingly large effect, above which increasing density will have no further effect (e.g. a saturating response). Such a relationship has been observed in laboratory studies (Loose and Dawidowicz, 1994).

We approached this problem with a conservative approach. In our survey, *Bythotrephes* when present, ranged over two orders of magnitude, where the higher areal densities were well below that frequently observed in other surveys (see Results section). The multiple linear regression provided a coefficient for how zooplankton depth changed as a function of log base 10 of the data, i.e. the coefficient estimates how much deeper the zooplankton would be with a

10 fold increase in *Bythotrephes* density. Clearly such an application would not apply at very low *Bythotrephes* densities at which no effect is expected. However, in the range of 10-30 *Bythotrephes* to 100-300 *Bythotrephes*, which is within the range of the densities observed, it should apply. We argue that our chosen method to use the magnitude of the coefficient (e.g. a coefficient of 10 would be interpreted as high density of *Bythotrephes* leading to a 10 meter increase in the depth of the zooplankton) is conservative, given that the observed increase in *Bythotrephes* exceeds 10 fold, and our higher densities were lower than that frequently observed.

RESULTS

Bythotrephes densities in our survey ranged from 0 to 368 ind. m^{-2}. *Bythotrephes* was present in 80% (20 out of 25) of our sampling events, and reached densities > 60 ind. m^{-2} in roughly half (55%) of them. The upper range of our survey were therefore not especially high; densities above 400 ind m^{-2} were found in 5 of 6 years by Cavaletto et al. (2010) in Lake Michigan, with density reported as high as 1400 ind. m^{-2}. *Bythotrephes* have also been found to reach densities above 600 ind m^{-2} in Lake Erie (Pothoven et al., 2011). *Bythotrephes* densities were significantly correlated with sampling date ($r = 0.57$, $P < 0.05$) with the five sampling events in which *Bythotrephes* was absent collected from mid-June to early July. Copepod nauplii, cyclopoid and diaptomid copepodites, and adult *Leptodiaptomus ashlandi* were found in sufficient densities to estimate MD in 100% of our sampling events. Other zooplankton groups were found in sufficient densities to estimate MD in 96% (adult *L. minutus*), 92% (*Bosmina longirostris)*, 88% (*L. sicilis* and *Diacyclops thomasi)* and 84% *(Daphnia mendotae*) of our sampling events. The relationships between *Bythotrephes* areal density and the mean depth of

each zooplankton group can be found in Fig. 1. Fig. 2 illustrates representative vertical distributions of *Bythotrephes* and some of its main prey. A summary table of zooplankton densities and mean depths is presented in Electronic Supplementary Material (ESM) Table S1.

Multimodel selection and averaging of MLR models

Bythotrephes *abundance*

Bythotrephes abundance was important in determining the mean depth of several zooplankton groups, as inferred by its presence in the best-supported models from the multimodel selection analysis (i.e. the best supported model and those models within 2 AIC_c units of the best supported model, which are well-supported models that provide similar information content to the best supported model) (Table 2). *Bythotrephes* abundance appeared in 100% of the best supported models for *L. minutus* (4 of 4 models), 2 of 3 of the best supported models for cyclopoid copepodites and *Bosmina*; 3 of 5 for *D. thomasi* and *Daphnia*; and 1 of 3 for *L. ashlandi* and *L. sicilis*. *Bythotrephes* abundance did not appear in any models within 2 AIC_c units of the best-supported model for copepod nauplii or diaptomid copepodites.

Examining the importance of *Bythotrephes* abundance relative to other environmental predictors (RI; the sum of the Akaike weights across all models within 4 AICc units of the best model, including the best model, in which a parameter appears [Burnham and Anderson, 2002]) in determining zooplankton mean depth (Table 3) indicated that *Bythotrephes* abundance was the most important determinant of mean depth with an RI score of 1 (the maximum) for *L. minutus*, *Bosmina* (RI = 0.68), cyclopoid copepodites (0.66), and *L. sicilis* (0.47). *Bythotrephes* abundance was the second most important determinant of *Daphnia* (0.52) and *D. thomasi*

(0.64) mean depth (after hypolimnion depth [see below]). *Bythotrephes* was the fourth most important determinant of *L. ashlandi* mean depth, and was the least or second to least important determinant of diaptomid copepodite (0.10). *Bythotrephes* abundance was not an important determinant of nauplii (0.22) mean depth (Table 3).

Model averaging across the best model and those within 4 AIC_c units of the best model indicated that *Bythotrephes* abundance had statistically significant effects on the mean depth of 6 of the 9 groups; cyclopoid copepodites, *D. thomasi*, *L. minutus*, *L. ashlandi*, *Bosmina*, and *Daphnia* (Table 4), and did not significantly affect the mean depth of copepod nauplii, diaptomid copepodites, or *L. sicilis* (Table 4).

Hypolimnion depth

Hypolimnion depth was also important in determining the mean depth of several zooplankton groups (Table 2). Hypolimnion depth appeared in 4 out of 5 of the best supported models of mean depth for *D. thomasi*, 2 of 3 of the best supported models for *L. ashlandi*; 3 of 5 for *Daphnia*; 2 of 5 for diaptomid copepodites; and 1 of 3 for cyclopoid copepodites, *L. sicilis*, and *Bosmina*. Hypolimnion depth appeared 1 out of 4 of the best supported models for *L. minutus*. Hypolimnion depth did not appear in any of the best supported models describing the mean depth of copepod nauplii.

Examining the relative importance of hypolimnion depth across all models within 4 AICc units of the best model (including the best model) (Table 3) indicated that hypolimnion depth was the most important determinant of mean depth with an RI score of 0.67 for *D. thomasi, L. ashlandi* (0.69), and *Daphnia* (0.63). Hypolimnion depth was the second most important

determinant of *L. sicilis* mean depth (0.31). Hypolimnion depth was of relatively lesser importance (i.e., 3rd or 4th most important) for the mean depth of cyclopoid and diaptomid copepodites (0.23 and 0.25, respectively), *L. minutus* (0.16), and *Bosmina* (0.26).

Model averaging across the best model and those within 4 AIC_c units of the best model indicated that hypolimnion depth had statistically significant effects on the mean depth of *D. thomasi, L. ashlandi, Bosmina*, and *Daphnia* (Table 4). Hypolimnion depth did not significantly affect the mean depths of copepod nauplii, cyclopoid or diaptomid copepodites, *L. minutus*, or *L. sicilis* (Table 4).

Model averaged coefficients of MLR models indicated that zooplankton groups significantly affected by hypolimnion depth moved between 0.5 – 1.0 m deeper for every 1.0 m increase in hypolimnion depth (Table 4).

Other limnological factors

Hypolimnion temperature was the most important determinant of copepod nauplii mean depth, appearing in all 7 of the best supported models (delta $AIC_c < 4$; Table 2), giving it a RI score of 1 (the maximum) (Table 3). Copepod nauplii mean depth increased approximately 2 m for every 1 °C increase in the hypolimnion temperature (Table 4). Hypolimnion temperature was the most important determinant of diaptomid copepodite mean depth, appearing in 12 out of 14 of the best supported models (delta $AIC_c < 4$; Table 2) for an RI score of 0.86 (Table 3); and for *L. ashlandi* mean depth, appearing in 12 out of 12 of the best supported models for an RI score of 1 (Table 3). In both cases zooplankton mean depth increased approximately 2.3 m with every 1 °C increase in hypolimnion temperature (Table 4).

PLSR model check for collineartiy bias: Supporting evidence for the importance of Bythotrephes and hypolimnion depth

PLSR models also indicated that *Bythotrephes* density and hypolimnion depth had large relative contributions to latent variables that best described the mean depth of adult diaptomid and cyclopoid copepods and the cladocerans *Bosmina* and *Daphnia*. These results indicate that collinearity among predictor variables did not bias estimates of the effect of *Bythotrephes* in our MLR analysis. The detailed results of the PLSR can be found in ESM Appendix S1.

OLS Regression: analysis with Bythotrephes as only factor affecting zooplankton mean depth

Using OLS regression, *Bythotrephes* abundance had a significant positive effect on the mean depth of cyclopoid copepodites ($F_{1,13}$ = 5.31, P = 0.038, R^2 = 0.29), adult cyclopoid copepods (*Diacyclops thomasi*: $F_{1,13}$ = 14.9, P = 0.002, R^2 = 0.53), adult diaptomid copepods (*Leptodiaptomus minutus*: $F_{1,13}$ = 31.3, P < 0.001, R^2 = 0.71; *L. ashlandi*: $F_{1,13}$ = 8.59, P < 0.012, R^2 = 0.40; *L. sicilis*: $F_{1,12}$ = 6.60, P = 0.025, R^2 = 0.35), and cladocerans (*Bosmina longirostris*: $F_{1,13}$ = 5.29, P = 0.039, R^2 = 0.29; *Daphnia mendotae*: $F_{1,13}$ = 8.13, P = 0.016, R^2 = 0.42), but not for copepod nauplii ($F_{1,12}$ = 6.60, P = 0.025, R^2 = 0.35) or diaptomid copepodites ($F_{1,12}$ = 6.60, P = 0.025, R^2 = 0.35). Therefore, the only difference in statistical significance for the *Bythotrephes* effect in the OLS model compared to the MLR models was for *L. sicilis*.

Estimate of magnitude of Bythotrephes effect on zooplankton mean depth

For those species significantly affected by *Bythotrephes* abundance in the MLR analysis, mean depth increased by approximately 5 to 11 meters at the maximum observed *Bythotrephes* abundances (Table 4; Fig. 3). Downward vertical movement was particularly

dramatic for the diaptomid copepod *L. minutus*, which was 9 meters deeper; and the cladocerans *Bosmina* and *Daphnia*, which both were 11 meters deeper (Fig. 3). The magnitude of the *Bythotrephes* abundance effect on the mean depth of zooplankton predicted by the OLS model (Fig. 3) ranged from 110% higher (*Bosmina*) to 34% lower (*L. ashlandi*) than the effect indicated by multimodel averaging. However, none of the between species differences in the predicted effect on mean depth were significant (i.e. there was a large overlap in SE of *Bythotrephes* density coefficients from OLS and MLR models, suggesting $P >> 0.05$).

DISCUSSION

Our results provide compelling evidence that *Bythotrephes* has an important and large effect on the vertical distribution of several of the most common zooplankton in Lake Michigan. Even after accounting for other biotic and abiotic factors, some of which had their own significant effects on zooplankton vertical distribution, we observed that six of nine of the zooplankton groups included in the analysis were deeper to a strong and significant degree, between approximately 5 to 11 meters in response to increasing *Bythotrephes* abundance (Table 4; Fig. 3). Such findings suggest that the *Bythotrephes*-induced vertical shifts in Lake Michigan zooplankton observed in previous laboratory experiments are operating to a biologically meaningful extent in the field. Such vertical responses may have large consequences on zooplankton population growth rate, due to the temperature reduction associated with such excursions (Pangle et al., 2007).

In general, *Bythotrephes* abundance had significant effects on the vertical distribution of zooplankton groups that (a) would be most at risk of *Bythotrephes* predation, thus necessitating

the need to respond behaviorally, and that (b) also have the ability to respond efficiently (e.g. given time and energy constraints). The six groups that *Bythotrephes* affected likely all fit these criteria: *D. mendotae*, *Bosmina,* adult stages of *L. minutus*, and *L. ashlandi,* and the copepodite and adult stages of the cyclopoid copepod *D. thomasi*; all inhabit the epilminion and metalimnion in the absence of *Bythotrephes* and so would overlap considerably with *Bythotrephes* and be at risk of predation if they did not respond to *Bythotrephes*' presence. Cladocerans are thought to be the preferred prey of *Bythotrephes* (Schulz and Yurista, 1999; Vanderploeg et al., 1993) and *Daphnia*, in particular, are known to be readily consumed by *Bythotrephes* in laboratory experiments (Pangle and Peacor, 2009). Whereas copepods have been found to be lesser-preferred prey than cladocerans for *Bythotrephes*, presumably due to faster swimming speeds and greater escape response (Pichlová-Ptáčníková and Vanderploeg, 2011), *Bythotrephes* is nonetheless capable of preying on *L. ashlandi* at relatively high rates in laboratory experiments (Pichlová-Ptáčníková, unpublished). *Bythotrephes*' ability to prey on *L. ashlandi* suggests that it should also be able to prey on *L. minutus* and adult and juvenile *D. thomasi*, which because they are smaller than *L. ashlandi* are likely to be slower and less likely to escape *Bythotrephes* attacks (Muirhead and Sprules, 2003). Although prey changes in the vertical position do not completely reduce daytime vertical overlap between predator and prey, even at high predator densities (Fig. 2), we believe the response acts to reduce overlap between predator and prey.

Bythotrephes abundance did not, in contrast, significantly affect the vertical distribution of copepod nauplii and diaptomid copepodites. Whereas nauplii and copepodites overlap vertically with *Bythotrephes* and thus may be at some risk of predation, we hypothesize that

large-scale vertical responses to *Bythotrephes* may not be energetically possible (e.g., nauplii) or not worth the energetic costs (e.g., copepodites) for these juvenile stages. Instead, we hypothesize that feeding and growth maximization in warmer upper lake strata, rather than costly vertical excursions to deeper water, may be the optimal strategy for these early developmental stages. In support of this hypothesis, model averaging of MLR models indicated that warmer hypolimnion temperatures were the main drivers of deeper copepod nauplii and diaptomid copepodite distributions in Lake Michigan, respectively. Vertical overlap with *Bythotrephes* and small size predicts that both cyclopoid and diaptomid copepodites should both respond to *Bythotrephes*, however cyclopoid copepodites were found to be affected, whereas diaptomid copepodites were not. This difference may be because proportionately greater increases in energy requirements are needed to sustain larger species of copepods (Woodward et al., 2010) and the early developmental stages of diaptomid copepods, which grow to larger sizes than cyclopoids, may favor growth maximization over predator avoidance in this group of copepods. It is also possible that cyclopoid copepodites are more vulnerable to *Bythotrephes* than diaptomid copepodites.

The third group that our results indicate are not responding to *Bythotrephes* is the adult stages of the copepod *L. sicilis*. In the MLR analysis, which included the wide set of limnological factors, the effect of *Bythotrephes* abundance on *L. sicilis* mean depth was not significant (Table 4). In contrast, *Bythotrephes* abundance did have a significant effect on the adult stages of the copepod *L. sicilis* in OLS regression models where *Bythotrephes* abundance was the sole predictor (i.e., OLS analysis, Fig. 3). However, the magnitude of this latter result was one of the lowest (~4 m increase in mean depth at the maximum observed *Bythotrephes* densities) among

groups significantly affected. Taken together, we believe that the weak effect in the OLS analysis, and the non-significant effect observed in the MLR analysis, which provides more rigorous, and thus reliable results, supports the conclusion that *Bythotrephes* does not significantly affect the vertical position of *L. sicilis*. The finding that *L. sicilis* was the only adult copepod not to respond to *Bythotrephes* is also consistent with the biology of the system. *Leptodiaptomus sicilis* is restricted mainly to the meta- and hypolimnion, and so is not expected to respond to *Bythotrephes* because of its already deep distribution and thus limited vertical overlap with *Bythotrephes* in the water column.

It is noteworthy that the species that did not respond to *Bythotrephes* are the same as those that did not respond in previous laboratory findings, with one exception. *D. mendotae* and the adult stages of *L. minutus*, *D. thomasi* all show the ability to respond vertically to the presence of *Bythotrephes* in experimental water columns in the laboratory and *L. ashlandi* shows a strong (though non-signficant) trend in this direction, whereas *L. sicilis* was shown not to respond to *Bythotrephes* cues (Bourdeau et al., 2011; Pangle and Peacor, 2006). An exception is that, whereas we did find a response by *Bosmina* to *Bythotrephes* in the field in this study (see also Pangle et al., 2007 where this response was also observed in Lake Erie), we did not find a response in a laboratory study (Pangle, unpublished). Nevertheless, there is general consistency between the laboratory findings and the field results. (The responses of cyclopoid copepodites to *Bythotrephes* have not been tested in laboratory experiments owing to their small size and associated difficulty observing them.) Whereas behavior in a laboratory setting does not prove the activity occurs in the field, it is difficult to envision how species would

respond to *Bythotrephes* cues in the laboratory by vertically migrating lower in thermally stratified water columns, when the activity is not one that occurs in the field.

The zooplankton groups whose vertical distributions were significantly affected by *Bythotrephes* abundance were approximately 5 to11 meters deeper at maximum observed *Bythotrephes* densities (MLR analysis, Fig. 2). Specifically, when considering mean depth, *Daphnia* and *Bosmina* were 11 m deeper, *L. minutus* were 9 m deeper, adult and immature cyclopoid copepods were 6 m deeper and *L. ashlandi* were 5 m deeper. As presented in the methods section, there are no standardized ways to parameterize the effect of the predator on prey mean depth, which is a complicated relationship. Further, getting accurate quantitative estimates of the effects of environmental drivers on prey behavior from observational field data can be difficult due to potential correlations among predictor variables, leading to variance inflation around coefficient estimates (Graham, 1997). Whereas we cannot resolve differences in the effects of *Bythotrephes* on the different zooplankton groups due to the variation around our coefficient estimates, our approach (model averaging of coefficients, which provides better estimates of strong predictors than single model estimates [Burnham and Anderson, 2002]) provides a high degree of confidence as to which groups were significantly affected by *Bythotrephes* abundance and by how much they were affected.

We further examined how the inclusion of different environmental parameters would affect the significance and magnitude of these estimates. In particular, we were interested to know if adding additional parameters had a large influence on the significance and magnitude of the estimates. The inclusion of other limnological factors in our linear models only affected the significance of the *Bythotrephes* effect on the mean depth of 1 of the 9 groups examined (*L.*

sicilis, discussed earlier), and did not have a significant effect on the estimated magnitude of the *Bythotrephes* effect on zooplankton mean depth. Under some scenarios we expect the influence of factors other than predator abundance to be important, and hence we thought it was necessary to examine. Factors other than predator abundance have been shown to induce vertical responses in zooplankton in the field (e.g., Cooke et al., 2008; Williamson et al. 1996). If such factors are positively correlated with predation risk or directly influence predation risk and also induce responses similar in direction and magnitude to that of the predator, the effect on the prey response may be erroneously ascribed to the predator. This could be particularly problematic in large stratified water bodies like Lake Michigan, which show characteristic seasonal and vertical gradients of light and temperature - factors which may be used as proximate cues for plastic antipredator responses by zooplankton (Dodson, 1990; Miehls et al. 2013). Whereas the inclusion of other limnological factors did not significantly influence our estimates of the effect of *Bythotrephes* abundance on zooplankton mean depth, their inclusion nonetheless showed that the vertical distribution of some Lake Michigan zooplankton is being significantly influenced mainly by other limnological factors (e.g., temperature of the hypolimnion for copepod nauplii and diaptomid copepodites discussed above) or by other factors in addition to *Bythotrephes* abundance.

One factor that the analysis showed significantly influenced the distribution of several zooplankton species (*Bosmina*, *Daphnia*, *D. thomasi*, and *L. ashlandi*), was hypolimnion depth, indicating that it may serve to set a lower limit to zooplankton mean depth selection. To ensure that hypolimnion depth *per se*, and not overall water column depth, was influencing zooplankton mean depth, we examined the relationship of water column depth with

hypolimnion depth and zooplankton mean depth. We found no significant correlation between water column depth and hypolimnion depth (r = 0.42, P = 0.17), nor did we find a significant correlation between water column depth and *Daphnia* mean depth (r = 0.39, P = 0.26) nor any other zooplankton mean depth. Further, including water column depth in our multiple regression models did not affect the results of model selection. We therefore conclude that hypolimnion depth *per se* was influencing zooplankton mean depth.

Whereas hypolimnion depth significantly affected four species (*Bosmina*, *Daphnia*, *.. thomasi*, and *L. ashlandi*), we do not believe that this association was due to the reason typically suggested: that the hypoliminon is darker, and therefore is used as proxy for lower light levels that provide a refuge from visual predators (such as *Bythotrephes*) in stratified water bodies (Tessier and Welser, 1991; Lampert, 1987; Zaret and Suffern, 1976). Using the hypolimnion as a proxy for a low risk refuge from visual predators requires the assumption that the hypolimnion provides a low light environment. However, it is likely that on sunny days throughout the study period that the light extinction coefficient was low enough to support visual predation of *Bythotrephes* throughout the 40-m water column (Vanderploeg et al., this issue).

We therefore suggest two mechanisms by which hypoliminion depth may be affecting the mean depth of zooplankton prey. First, lower depths may be darker and thus reduce predation from *Bythotrephes*, but it comes at increased costs to growth associated with colder temperatures below the metalimnion-hypolimnion transtion. This tradeoff will therefore lead to zooplankton occupying deeper waters when *Bythotrephes* is at higher densities, but the hypolimnion depth would also affect the magnitude of this response.

A second, and rather intriguing mechanism for the influence of hypolimnion depth on Lake Michigan zooplankton distribution is that the hypolimnion may serve as a refuge from *Bythotrephes*, but not because it is darker, but rather because the lower temperatures of the hypolimnion limits *Bythotrephes* foraging activity, and hence risk. It is well known that lower temperatures inhibit invertebrate foraging activity (Elner, 1980; Sanford, 2002; Simonsen et al., 2009). Further, our data and others' (e.g., Vanderloeg et al. 2012) suggest that *Bythotrephes* is rarely found in the hypolimnion (*Bythotrephes* mean depth ± s.d. = 12.57 ± 6.79 m; hypolimnion mean depth ± s.d. = 26.84 ± 7.66 m). This mechanism would also involve a tradeoff with increased costs of growth to *Daphnia* associated with colder temperatures. Further, within the metalimnion, which can be several meters thick, there is a wide range of temperatures that zooplankton could select, which may reflect tradeoffs between visual predation and temperature. Our data suggest that many species' vertical distributions are in the metalimnion during the day, and some like C1-C5 diaptomids and *L. ashlandi* during both day and night (Vanderploeg et al., this issue; Bourdeau, unpublished data).Thus, the role of the hypolimnion as a thermally-, rather than (or in addition to) visually-mediated refuge from *Bythotrephes* predation in Lake Michigan deserves further study.

An alternative explanation for the association between zooplankton mean depth and *Bythotrephes* abundance that we observed is that this pattern is mostly attributable to correlated seasonal changes in both *Bythotrephes* abundance and zooplankton vertical distribution. Including Julian day as independent variable in our multiple regression model is one possible method to separate out the independent effects of sampling date, however we addressed potential collinearity between *Bythotrephes* abundance and sampling date in two

additional ways: (1) by employing residuals (a.k.a., sequential) regression on our data set to disentangle the unique and shared contributions of *Bythotrephes* density and sampling date on zooplankton mean depth; and (2) by removing early-season (i.e., spring and early summer samples in our multiple regression models and subsequent model selection analysis. (see Appendix S2 for details of each analyses). We found that even when we removed the potentially confounding effects of sampling date using these two approaches that *Bythotrephes* density continued to have large and significant effects on the mean depth of copepodite and adult cyclopoids, *L. minutus*, *L. ashlandi, Bosmina*, and *Daphnia* (Appendix S2); strengthening our argument that the observed patterns in zooplankton vertical distribution are mainly attributable to *Bythotrephes* abundance, independent of sampling date.

Because zooplanktivorous fish can induce vertical shifts in zooplankton prey (Gliwicz, 1986; Hays, 2003; Lampert, 1989), it is important to consider the potential contribution of fish to the observed patterns of zooplankton vertical distribution. Whereas fish can induce changes in zooplankton mean depth (reviewed in Lampert 1989 and Hays 2003), fish abundance would need to correlate positively with *Bythotrephes* abundance for the effect we attribute to *Bythotrephes* to be due to the effects of fish. Whereas it is difficult to reliably estimate fish abundance, we have previously found no correlation between fish abundance and *Bythotrephes* abundance (Bourdeau et al. 2011, Appendix, Fig. 4). Secondly, in contrast to *Bythotrephes* abundance, which shows a significant increase at our offshore sampling locations during our sampling season (June-September; Pearson's correlation between Log[*Bythotrephes* areal density+4] and Julian date: $r = 0.56$, $n = 25$, $P < 0.05$), fish abundance has been shown to vary very little during this same time period in offshore regions of Lake Michigan (Brandt et al.

1991). Finally, fish are less abundant than *Bythotrephes* in the offshore zone, and the primary planktivore is large alewives that reside mostly in the hypolimnion during the day (Vanderploeg et al., this issue). Consequently, if fish were responsible for inducing shifts in the vertical distribution of Lake Michigan zooplankton, we would not detect the strong associations between zooplankton vertical distribution and *Bythotrephes* abundance and Julian date that we observed during our study.

There are two potential mechanisms that could explain the observed effect of *Bythotrephes* density on zooplankton mean depth. First, a *Bythotrephes*-induced behavioral plasticity mechanism. Predator-induced behavioral plasticity in zooplankton has been observed in numerous experimental venues (including lab and field manipulations), and although it is difficult to demonstrate unequivocally in nature, it is likely widespread (Hayes, 2003; Lampert, 1989; Zaret and Suffern, 1976) and has been demonstrated in laboratory studies (Dawidowicz and Loose, 1992; Loose and Dawidowicz, 1994). Second, a differential-predation (by depth) mechanism in which predation reduces zooplankton density in upper lake strata leading to a lower mean depth of surviving individuals. Because adult copepods and cladocerans can easily traverse the observed differences in mean depths within hours (based e.g. on our own diel vertical migration studies (Pangle et al. 2007 and unpublished), the differential-predation mechanism requires that the observed depth differences represent selection on the depth preference of zooplankton prey. This is because if the effect of predation on zooplankton depth acts on a time scale slow relative to zooplankton behavioral response times (as expected in our system), then zooplankton distribution would not be affected (due to redistribution) unless there was selection on positional preference.

We believe that *Bythotrephes*--induced behavior is more plausible than differential predation based on the following factors: (1) we have demonstrated experimentally that *Bythotrephes* induces strong behavioral responses in *Daphnia* (Pangle and Peacor 2006), *Di. thomasi*, and *L. minutus* (Bourdeau et al. 2011); three of the six zooplankton groups whose vertical distribution was strongly affected by *Bythotrephes* density in the current study; (2) diel vertical migration, where prey inhabit deeper regions of the water column during the day, and upper regions at night, has been observed during our field surveys for the same zooplankton groups whose daytime distribution was strongly effected by *Bythotrephes* abundance in this study: *Daphnia* and *Bosmina* (Bourdeau, unpublished; Pangle et al., 2007), and for immature and adult cyclopoids and the adult stage of *L. minutus* (Bourdeau, unpublished); (3) total zooplankton abundance does not fall when *Bythotrephes* is present for any of the species that we find are deeper at higher *Bythotrephes* densities and (4) Lake Michigan copepods only have 1-2 generations per year. This last observation suggests that selection would need to be especially strong (within generation) to affect the habitat preference of the copepods in our study when *Bythotrephes* abundance is high, and it is difficult to envision how habitat preference could return to a shallower distribution in predator absence on such short time scales (e.g. the next year in a period when *Bythotrephes* density is low). The fact that *Bythotrephes* does not have a negative effect on abundance, let alone a strong negative effect, therefore makes the differential predation mechanism highly unlikely. In contrast, the laboratory studies documenting *Bythotrephes*-induced behavioral responses (Bourdeau et al., 2011; Pangle and Peacor, 2006), and the observations of diel vertical migration in the field

(Bourdeau, unpublished; Pangle et al., 2007), are consistent with the *Bythotrephes*-induced behavioral plasticity mechanism.

Our findings provide strong support that the non-consumptive effects (NCEs) of *Bythotrephes* mediated by predator-induced depth selection may be large for the groups (*D. thomasi*, *L. ashlandi*, *L. minutus*, *Bosmina*, and *Daphnia*) that we have shown are deeper at higher *Bythotrephes* densities. Specifically, whereas downward vertical movement by zooplankton in response to *Bythotrephes* may reduce zooplankton's risk of being consumed, the colder temperatures experienced in deeper water may have large consequences for zooplankton growth and reproduction. During summer months in Lake Michigan when *Bythotrephes* is abundant and the lake is stratified (June – September) vertical responses of the estimated magnitudes observed in our study (5-11m), could lead to temperature changes between 9-18 °C. Such changes to the thermal regime experienced by responding zooplankton could have large negative consequences for zooplankton somatic growth and reproduction (Pangle and Peacor, 2006) leading to reductions in population growth (Pangle et al., 2007).

It is interesting to note that several of the species whose depths were significantly affected by *Bythotrephes* densities in our study, including *D. thomasi*, *D, mendotae*, and the small diaptomids *L. minutus* and *L. ashlandi*, have decreased markedly in offshore abundance since recent and striking increases in *Bythotrephes* abundances in Lake Michigan (Vanderploeg et al., 2012). Whereas the direct consumptive effects of *Bythotrephes* on these prey populations is likely contributing to these declines, our results suggest that NCEs by *Bythotrephes* on these prey could be contributing strongly to net effect of *Bythotrephes* on zooplankton prey populations, thus providing an additional contributing mechanism for the

observed declines in these prey groups. Analyses explicitly linking behaviorally-mediated NCEs to zooplankton population dynamics could greatly increase our understanding of how *Bythotrephes* is impacting zooplankton dynamics in Lake Michigan.

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TABLES

Table 1. Sampling locations and dates for Lake Michigan field survey.

Latitude	Longitude	Depth (m)	Sampling months, year	No. sampling events
43°11'29" N	86°25'92" W	45	June-October 2004-06; 2009-10	14
43°11'15" N	86°27'15" W	60	August 2004	2
43°11'29" N	86°32'16" W	110	June-October 2004, 2009-11	9

Table 2. Parameters in each of the best supported multiple regression models from multimodel selection (**Δ**AIC_C, < 2); with logLik (logarithm of the likelihood function, which measures the fit of the model to the data), AIC_C score, **Δ**AIC_C, and Akaike weights. Key: AC-attenuation coefficient; BD-*Bythotrephes* density; CM-Chlorophyll maximum; CMD-Chlorophyll maximum depth; ED-Epilimnion depth; ET-Epilimnion temperature; HD-Hypolimnion depth; HT-Hypolimnion temperature; JD-Julian date; SD-Secchi depth.

Model	df	logLik	AIC_C	ΔAIC_C	Weight
Nauplii					
HT+SD	4	3.16	5.68	0.00	0.39
HT	3	0.27	7.65	1.97	0.14
Cyclopoid copepodites					
AC+ET+BD	5	-33.69	84.04	0	0.29
ET+BD	4	-36.31	84.62	0.57	0.22
HD	3	-38.73	85.64	1.59	0.13
ED	3	-38.93	86.04	2.00	0.11
Diacyclops thomasi					
ET+JD+BD	5	-30.51	77.69	0	0.21
HD	3	-35.04	78.26	0.57	0.16
ET+HD+BD	5	-30.95	78.57	0.88	0.13

HD+BD	4	-33.48	78.95	1.26	0.11
HD+HT	4	-33.71	79.42	1.72	0.09
Diaptomid copepodites					
AC+HT	4	-42.12	96.23	0.00	0.20
HT	3	-44.47	97.13	0.90	0.13
HD+HT	4	-42.61	97.23	1.00	0.12
AC+HD+HT	5	-40.48	97.63	1.40	0.10
AC	3	-44.83	97.84	1.61	0.09
Leptodiaptomus minutus					
AC+BD	4	-35.36	82.71	0.00	0.20
BD	3	-37.54	83.26	0.54	0.15
ET+BD	4	-35.64	83.28	0.57	0.15
HD+BD	4	-36.29	84.58	1.86	0.08
L. ashlandi					
HD+HT+SD	5	-34.95	86.57	0.00	0.22
HD+HT	4	-37.36	86.72	0.15	0.20
HT+JD+BD	5	-35.55	87.77	1.19	0.12
L. sicilis					
BD	3	-36.93	82.27	0.00	0.21
HD	3	-37.18	82.77	0.50	0.17
JD	3	-37.62	83.64	1.37	0.11
Bosmina longirostris					
AC+ET+BD	5	-42.80	102.26	0.00	0.36
HD	3	-47.80	103.78	1.52	0.17
BD	3	-47.84	103.86	1.60	0.16
Daphnia mendotae					

HD	3	-43.87	96.14	0.00	0.19
JD+BD	4	-42.02	96.48	0.34	0.16
BD	3	-44.06	96.52	0.39	0.15
HD+BD	4	-42.19	96.83	0.69	0.13
ET+HD	4	-42.75	97.94	1.81	0.08

Table 3. Relative importance (RI) values for multiple regression model parameters from multimodel selection based on AIC_C. RI is the sum of the Akaike weights across all models in which a parameter appears. Abbreviations are the same as in Table 2. Key: AC-attenuation coefficient; BD-*Bythotrephes* density; CMD-Chlorophyll maximum depth; ED-Epilimnion depth; ET-Epilimnion temperature; HD-Hypolimnion depth; HT-Hypolimnion temperature; JD-Julian date; SD-Secchi depth.

	AC	BD	CMD	ED	ET	HD	HT	JD	SD
Nauplii			0.16		0.30		1.00		0.55
Cyclopoid copepodites	0.29	0.66		0.21	0.51	0.23		0.05	
Diacyclops thomasi		0.64	0.08		0.50	0.67	0.18	0.31	0.03
Diaptomid copepodites	0.55	0.10	0.03	0.06		0.25	0.86	0.03	0.09
Leptodiaptomus minutus	0.41	1.00	0.03	0.07	0.26	0.16		0.09	0.10
L. ashlandi		0.27	0.06	0.04	0.11	0.69	1	0.44	0.37
L. sicilis	0.03	0.47	0.03	0.18	0.03	0.31	0.06	0.23	
Bosmina longirostris	0.50	0.68			0.52	0.26			0.05
Daphnia mendotae	0.07	0.52	0.03	0.03	0.08	0.63	0.11	0.21	0.04

Table 4. Unstandardized regression coefficients β and their standard errors (SEs) from model averaging using AIC_C weights. Abbreviations as in Table 2; *** *P*<0.001, ** *P*<0.01, **P*<0.05, *P*<0.10.

Variable	Nauplii	Cyclopoid copepodite	*Diacyclops thomasi*	Diaptomid copepodite	*Leptodiaptomus minutus*	*L. ashlandi*	*L. sicilis*	*Bosmina longirostris*	*Daphnia mendotae*
AC		-16.91. (7.88)		24.39. (12.1)	14.43. (7.7)		-8.96 (11.16)	-35.49* (15.88)	-26.04 (24.69)
BD		6.35* (2.37)	5.90* (2.53)	2.97 (2.41)	9.36*** (2.08)	4.66* (2.13)	4.69. (2.34)	11.33** (3.97)	10.96* (4.85)
CMD	-0.18 (2.25)		0.07 (0.06)	-0.08 (0.06)	0.05 (0.08)	0.1 (0.07)	-0.02 (0.1)		0.03 (0.18)
ED		0.35 (0.2)		0.23 (0.26)	0.17 (0.18)	-0.19 (0.2)	0.28 (0.24)		-0.09 (0.4)
ET		-0.84* (0.32)	-0.63* (0.25)		-0.48 (0.29)	-0.55. (0.26)	-0.2 (0.36)	-1.47* (0.61)	0.93 (0.67)
HD	0.66** (3.55)	0.43. (0.24)	0.57** (0.2)	0.44 (0.28)	0.25 (0.2)	0.82*** (0.23)	0.41. (0.22)	0.73* (0.3)	0.96* (0.38)
HT	1.77* (0.19)		0.72 (0.51)	2.30* (0.98)		2.26** (0.65)	0.43 (0.81)		1.38 (1.22)
JD		-0.06 (0.05)	0.04 (0.04)	-0.08 (0.06)	0.02 (0.03)	0.10* (0.04)	0.06 (0.03)		0.11. (0.06)
SD	-0.63 (0.3)		0.16 (0.19)	0.38 (0.33)	-0.25 (0.23)	0.43. (0.21)		0.47 (0.39)	0.38 (0.45)

FIGURES

Figure 1. Plots of mean depth as a function of Log(*Bythotrephes* areal density+4; originally measured as ind m^{-2}) for the nine most common groups of Lake Michigan zooplankton. Open circles indicate sampling events in which CTD data were lost; these sampling events were not included in our analyses.

Figure 2. Representative vertical distributions of *Bythotrephes* and three species of its zooplankton prey (*Leptodiaptomus minutus*, *Daphnia*, and *Diacyclops thomasi*) in Lake Michigan. The left panel represents a sampling profile taken at offshore station M110 on 13June 2006 (*Bythotrephes* areal density = 32 ind. m^{-2}), the right panel represents a sampling profile taken at M110 on 20 July 2010 (*Bythotrephes* areal density = 235 ind. m^{-2}). Larger symbols represent mean depths (± 1 s.d.) for each zooplankton species.

Figure 3. Plots showing unstandardized regression coefficients (± 1 SE) of the effects of *Bythotrephes* density on the vertical distribution of the nine most common zooplankton groups in Lake Michigan. Open circles with solid black error bars represent coefficients from ordinary least squares (OLS) regression; grey circles with dashed grey error bars represent multi-model averaged coefficients from multiple linear regression (MLR) models. In the case of model averaged coefficients, the error bars represent error associated with among-model estimates of coefficients. The OLS and MLR text on the plot denote whether effects of *Bythotrephes* abundance on zooplankton mean depth were significant in OLS and MLR analyses.

Figure 1

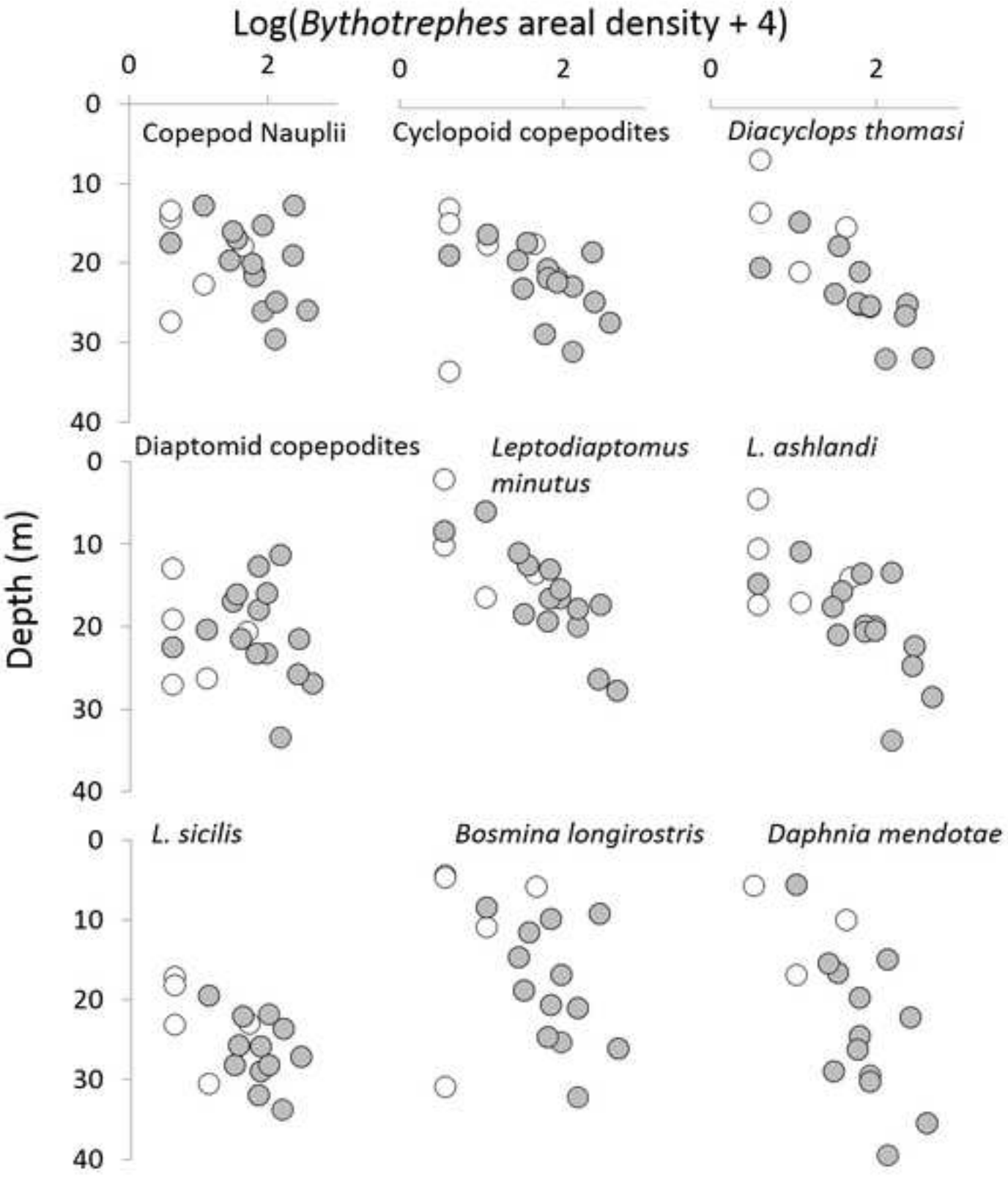

Log(*Bythotrephes* areal density + 4)
0
2
Depth (m)
0
10
20
30
40
Copepod Nauplii
Cyclopoid copepodites
Diacyclops thomasi
Diaptomid copepodites
Leptodiaptomus minutus
L. ashlandi
L. sicilis
Bosmina longirostris
Daphnia mendotae

Figure 2

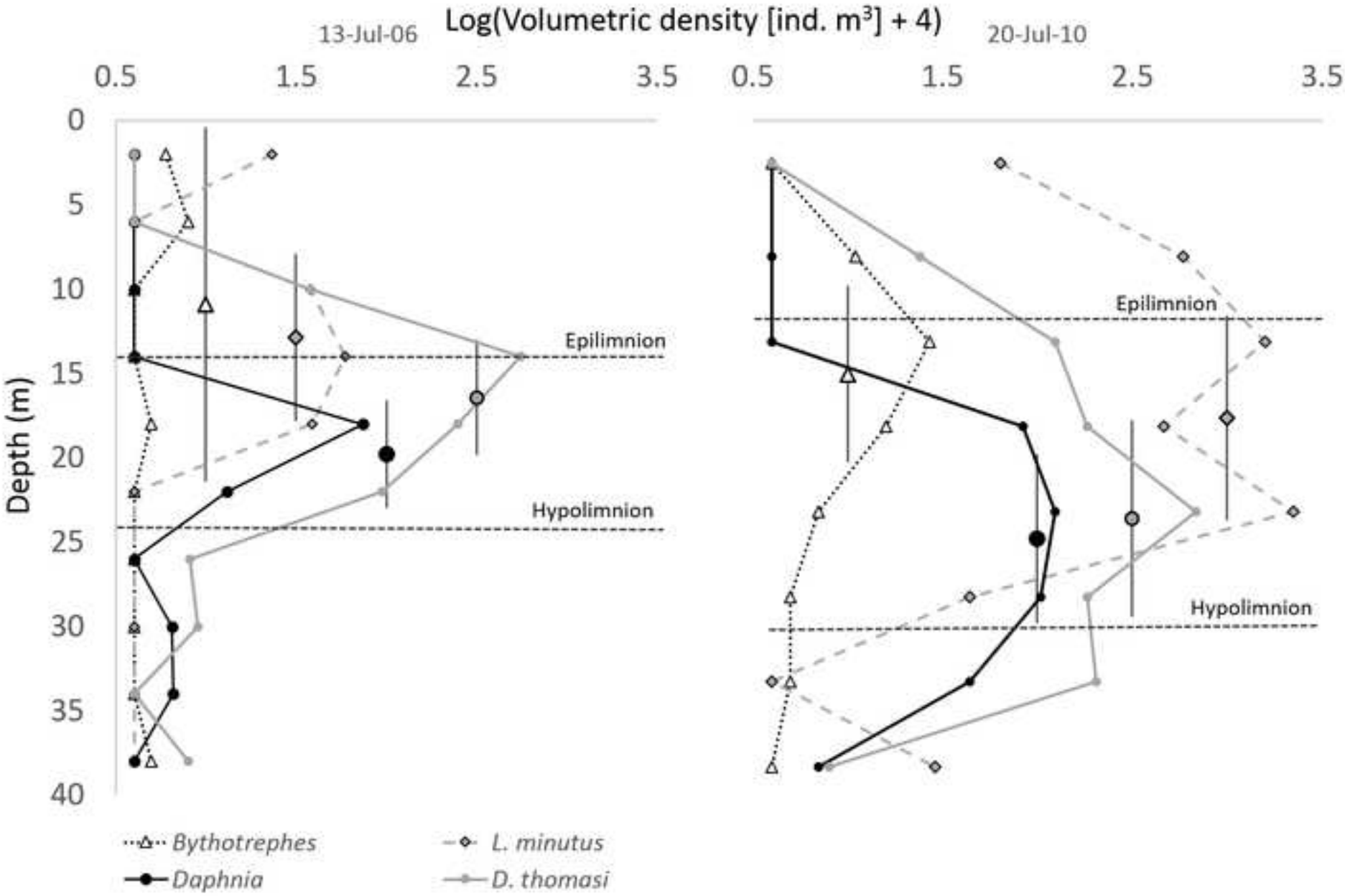
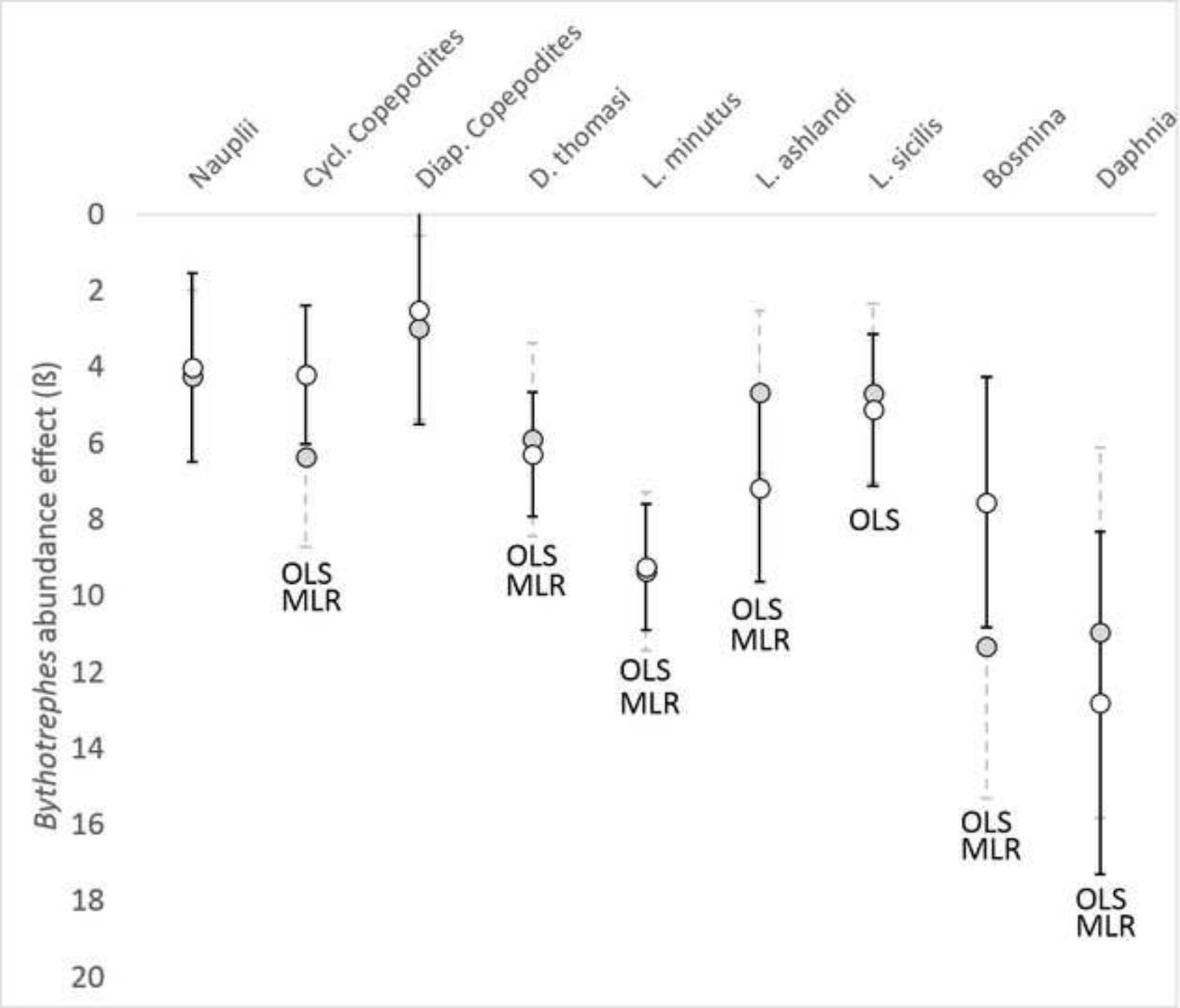

Log(Volumetric density [ind. m³] + 4)
13-Jul-06
20-Jul-10
0.5
1.5
2.5
3.5
Depth (m)
0
5
10
15
20
25
30
35
40
Epilimnion
Hypolimnion
Bythotrephes
Daphnia
L. minutus
D. thomasi

Figure 3


Nauplii
Cycl. Copepodites
Diap. Copepodites
D. thomasi
L. minutus
L. ashlandi
L. sicilis
Bosmina
Daphnia
Bythotrephes abundance effect (β)
0
2
4
6
8
10
12
14
16
18
20
OLS
MLR
OLS
MLR
OLS
MLR
OLS
MLR
OLS
OLS
MLR
OLS
MLR