Intra-lake trends and inter-lake comparisons of *Mysis diluviana* life history variables and their relationships to food limitation.

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Abstract

The opossum shrimp, *Mysis diluviana*, is an important member of the offshore food webs of the Laurentian Great Lakes, but its response to ecosystem changes that have occurred over the past several decades is not well understood. We combined the data of four long-term sampling programs, adding several years of data (post and prior) to previously published analyses to offer a longer-term, cross-basin analysis of *M. diluviana* populations in the Great Lakes from 1997 to 2019. Densities were high in lakes Superior and Ontario (summer values 100-300/m^2), high and variable but declining (from 200-300/m^2 in 1997-2004 to less than 100/m^2 in 2017-2019) in Lake Michigan, low (~20-50/m^2 since 2005) in Lake Huron, and very low in shallower eastern Lake Erie (<1/m^2). Biomass showed similar trends. Life history parameters (mortality, fecundity, and growth) were consistently highest in eastern Lake Erie, followed by lakes Ontario, Michigan, Huron, and Superior. Generation time was 1 year in Lake Erie and 2 years in the other lakes. Cross-basin relationships between annual *M. diluviana* areal densities and food indices (chlorophyll-a concentration and zooplankton biomass) were non-linear, increasing with food levels up to about 250 mysids/m^2 and about 650 mg dry wt/m^2. Annual growth rates were also positively correlated to both food indices in the four deep lakes, but fecundity and mortality rates were not. Our results suggest food availability is a primary factor predicting *M. diluviana* density and biomass. Density-dependent mortality and fish predation could explain some of the inter-lake differences but requires further investigations.

Keywords

Abundance; mortality, growth, fecundity, chlorophyll, zooplankton

Introduction

Mysis diluviana (formerly *Mysis relicta*) is a relatively large (up to 30-mm) shrimp-like crustacean native to the Laurentian Great Lakes (hereafter, "Great Lakes") and other deep post-glacial lakes in North America (Rudstam and Johannsson, 2009). *Mysis diluviana* is an important component of these lakes' deep offshore ecosystems, both as a predator of other zooplankton and as a prey of benthic and pelagic fishes (Gamble et al., 2011; O'Malley et al., 2017; Sierszen et al., 2014). This mysid can reach densities of over 500 /m^2 in Great Lakes offshore waters and represent up to 30% of the crustacean zooplankton biomass (Holda et al., 2019; Jude et al., 2018; Pothoven and Vanderploeg, 2017). Thus, determining if density and biomass of mysids are changing over time and which factors drive mysid population dynamics are important for understanding changing Great Lakes ecosystems. This may be particularly important after the decline in the benthic amphipod *Diporeia hoyi* in the 1990s (Nalepa et al., 2009; Burlakova et al., 2022) as *M. diluviana* is now the dominant larger deep-water crustacean in lakes Ontario, Michigan, and Huron (Pothoven and Bunnell, 2016; Bunnell et al., 2018).

Over the past 50 years, the Great Lakes have undergone substantial ecological change, both from decreasing nutrient loading and from invasive species that have restructured biological communities (Bunnell et al., 2021, 2014; Burlakova et al., 2018; Sturtevant et al., 2019). While these ecosystem changes have likely impacted *M. diluviana* ecology in the Great Lakes, it is unclear if we should expect positive or negative effects on mysid populations (Holda et al., 2019; Jude et al., 2018). For instance, total food resources of *M. diluviana* may have declined due to oligotrophication and competition with invasive species (Bunnell et al., 2011; Johannsson et al., 2011; Pothoven et al., 2010; Pothoven and Vanderploeg, 2017). However, because *M. diluviana* occurs and feeds in the deep chlorophyll layer at night, and clearer water may have increased the importance of the deep chlorophyll layer (Scofield et al., 2020), such vertical restructuring of the food web due to oligotrophication may have increased rather than decreased mysid food resources. Similarly, invasive predatory cladocerans may compete with *M. diluviana* for food (Johannsson et al., 2011) but are also known to be a food resource for *M. diluviana* (O'Malley and Bunnell, 2014; O'Malley et al., 2017). Thus, it is difficult to know *a priori* how the *M. diluviana* populations in the Great Lakes are likely to respond to recent and continuing ecosystem changes in the Great Lakes.

Several studies have been published on mysid population trends in the Great Lakes. Jude et al. (2018) compared *M. diluviana* populations across the five Great Lakes over the decade 2006 to 2016. They found offshore *M. diluviana* to be most abundant in lakes Ontario and Superior, followed by lakes Michigan, Huron, and Erie. The populations did not change significantly over time from 2006/07 to 2016 in lakes Ontario, Michigan, and Huron, but did increase slightly over time in Lake Superior (Lake Erie was not tested due to low population density and sample size). The lack of change in mysid populations reported for lakes Michigan, Huron, and Ontario could be the result of the limited time frame (2006-2016). Hypothesized causes of *M. diluviana* change (e.g., oligotrophication, loss of *Diporeia*, and invasions of predatory cladocerans and dreissenids) began prior to the mid-2000s (Barbiero et al., 2018b; Mills et al., 2003). Three studies comparing *M. diluviana* density in the late 2000s or early 2010s to those in earlier decades (1971, the late 1980s, or the late 1990s in lakes Michigan, Huron, and Ontario) documented lower densities of mysids after the mid-2000s than during earlier time periods (Bunnell et al., 2011; Johannsson et al., 2011; Pothoven and Vanderploeg, 2017).

In this paper, we used *M. diluviana* data from several annual monitoring programs to expand on the time frame and spatial extent of previous mysid studies. The additional data allowed us to expand the among-lake analysis of Jude et al. (2018) which spanned 11 years (2006-2016) with 12 years of additional data, including 9 preceding years (1997-2005) and 3 succeeding years (2017-2019). The preceding years are especially important as some of the large ecosystem changes known to have occurred in the Great Lakes (declines in nutrient concentrations, collapse of alewife, increases in dreissenid mussels) occurred during those years, especially in lakes Michigan and Huron (Barbiero et al., 2018b). In this paper we will use the term “density” for numerical density, “biomass” for biomass density and “abundance” when we are referring to both numerical density and biomass density. We also directly correlated *M. diluviana* abundance with available time series of zooplankton biomass from 1997 to 2019 (described in Barbiero et al., 2019), and an available time series of surface chlorophyll-a data from 1998 to 2019 derived from satellite data (methods in Lesht et al., 2018). The mysid data sets we analyzed included length measurements and embryo counts. We derived age distributions from the lengths for calculation of annual mortality and annual growth rates. Changes in life history variables (mortality, growth, and fecundity) both over time and across lakes could explain changes in *M. diluviana* populations and provide information on the importance of food resources and predation rates.

We used the expanded data set to ask the following questions about *M. diluviana* populations in the Great Lakes. First, are there significant differences in mysid abundance among lakes, and have such differences been maintained throughout the last two to three decades? Second, for each lake, what are the trends in abundance of mysids from the 1990s to 2019? Third, are mysid abundance related to indices of trophic state / food resources (e.g., spring phytoplankton bloom, zooplankton biomass)? Fourth, are the life history variables mortality, growth, and fecundity different among lakes? Fifth, are differences over time and among lakes in these life history variables related to mysid abundance of food resources (indicative of intra-specific competition and food limitation). ? Finally, we discuss the most likely drivers of variation in *M. diluviana* population changes across the Great Lakes given the results of these analyses.

Methods

Long-term annual monitoring datasets with Mysis *data*

Mysid density, biomass, and life history rates in the Great Lakes have been monitored by several annual programs using net sampling methods designed specifically to target *M. diluviana*. The U.S. Environmental Protection Agency's (EPA) Great Lakes National Program Office (GLNPO) biological monitoring program has included sampling of mysids at offshore stations in all five Great Lakes during spring (usually April) and summer (usually August) since 2006 (Jude et al., 2018). Although this program sampled a limited number of stations per lake (Fig. 1), it uses consistent methods in all five lakes, thereby enabling among-lake comparisons of long-term trends (Barbiero et al., 2018a). Other mysid monitoring programs focus on one or two lakes (Fig. 1). The U.S. Geological Survey's (USGS) Great Lakes Science Center (GLSC) have monitored mysids in lakes Michigan (during summer: usually August) and Huron (during late summer: usually September) since 2005 with samples taken as part of lake-wide acoustic surveys (Bunnell et al., 2011; Warner et al., 2010). The National Oceanic and Atmospheric Administration's (NOAA) Great Lakes Environmental Research Laboratory (GLERL) have monitored mysids in southeast Lake Michigan with monthly March-to-December sampling at their Muskegon transect since 1995 (with a sampling gap during 2003-2006) (Pothoven and Vanderploeg, 2017). Fisheries and Oceans Canada's (DFO) Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) have monitored mysids in Lake Ontario, collecting samples in the fall (usually October-November) and on a lake-wide basis in 1990, 1991, 1995, and from 2002 to 2017 (Johannsson et al., 2011). Earlier publications from these monitoring programs have presented data through 2013 (DFO-GLLFAS: Rudstam et al., 2017), 2015 (USGS GLSC: Bunnell et al., 2018; NOAA GLERL: Pothoven and Vanderploeg, 2017); and 2016 (EPA GLNPO: Jude et al., 2018). Here, we included additional years of data from these monitoring programs (through 2019) to estimate areal density, areal biomass, and several life history variables (fecundity, growth rate, mortality) of *M. diluviana* populations in the Great Lakes. Because the GLNPO surveys for *M. diluviana* that consistently sample all five Great Lakes, all among-lake comparisons and tests were based solely on data from GLNPO surveys. Data from the single-lake surveys conducted by USGS, NOAA, and the DFO were included in within-lake analyses of temporal trends.

Field and lab methods for targeted sampling of mysids were similar among the different monitoring programs (Johannsson et al., 2011; Jude et al., 2018; Pothoven and Vanderploeg, 2017; Warner et al., 2010). All programs used vertical plankton nets with similar specifications deployed to near the bottom of the lake (generally within 5 meters) at night to sample mysids (Table 1), which we refer to collectively hereafter as mysid nets. Samples were preserved with either ethanol or formaldehyde, and in some cases brooding females were separated into separate vials to assess brood size. All mysids were tallied in every sample, and the areal abundance calculated based on the area of the net opening. Individual mysids were assessed for standard length (tip of rostrum to end of abdomen), sex, and brooding status, which were recorded for all individuals or for a subsample when total count in a sample was high (> 100-125). Only mysids larger than 3 mm were included in the density and biomass numbers as smaller mysids may have been released from the brood pouch during sampling. Average length of late-stage mysids in the brood pouch measured 2016 to 2019 was 2.40 mm with the upper 97.5 percentile at 2.96 mm with minimal differences among lakes (N=4445, Huron, Michigan, Superior and Ontario, authors unpublished data) The mass of individual *M. diluviana* was calculated from measured standard length according to the formula ln(dry mass in g) = -12.27 + 2.72 ln(length in mm) (Johannsson et al., 2011), and areal biomass calculated as the product of mean individual mass and areal density.

Using EPA zooplankton samples to extend Mysis *time series back to 1997*

In addition to annual monitoring programs directly targeting *M. diluviana*, EPA GLNPO surveys have collected samples targeting whole-water column zooplankton at all monitoring stations since 1997 (Barbiero et al., 2019). We hereafter refer to this net as the GLNPO zooplankton net (153-µm mesh throughout net and cod end, 0.5-m diameter, 1.5 m long, towed from 100 m or 2 m above the bottom at sites shallower than 100 m). The volume sampled by the zooplankton net was measured with a calibrated flowmeter, and that volume was used to calculate volumetric density. Areal biomass of *M. diluviana* in zooplankton net samples was calculated as the product of tow depth and volumetric biomass, where volumetric biomass was estimated as the product of volumetric density and mean individual dry mass. Mean individual dry mass was estimated from up to 20 length measurements per sample and a length-mass regression [ln(dry mass in g) = -12.55 + 2.72 ln(length in mm)] based on Johannsson et al. (1995) as modified by Rudstam et al. (2008) to account for differences in how the animals were measured – end of abdomen in mysid nets versus cleft of telson in zooplankton nets). We only used zooplankton net data collected at night. Unlike mysid collections, deck lights are on during collection of zooplankton.

Although the zooplankton net frequently contained mysids when towed at night, we expected this net to have lower catch efficiency for *M. diluviana* due to the smaller mouth area, the deck lights, and a shorter tow depth to 100 m instead of the whole water column. Paired catches in the two GLNPO nets (mysid net and zooplankton net) for nighttime visits collected in 2006 to 2019 were highly correlated for both density and biomass (r^2 = 0.70 and 0.63, respectively, based on 4th-root transformed data to improve homogeneity of variance in residuals and including zero catches). The GLNPO zooplankton net underestimated mysid density by 15% and mysid biomass by 58% compared to paired samples in the GLNPO mysid net (Fig. S1-S2). Thus, the GLNPO zooplankton net data are useful but biased indicators of mysid abundance. Even so, the data provided by the GLNPO zooplankton net are important because they provide information on each lake's *M. diluviana* populations during several critical years in which we have no information from other surveys (roughly 1997 to 2005 depending on the lake). Data collected from GLNPO zooplankton net samples do not include information on sex, life stage, or reproductive status of *M. diluviana*. In addition, although the GLNPO zooplankton net data includes up to 20 length measurements per net tow, this net was selective against larger mysids, which would result in skewed size structures. Therefore, while we used these data to help inform areal density and biomass estimates in 1997-2005, we did not use them to obtain estimates of the life history variables obtained from the mysid net datasets. Note that since the GLNPO zooplankton net dataset was collected as part of the among-lake GLNPO design, we did include its abundance data in among-lake statistical tests and comparisons while including a factor for relative net efficiency to correct for bias in abundance estimates of the GLNPO zooplankton net.

Abundance analyses

Mysis diluviana areal abundance is known to increase with bottom depth. In the Great Lakes, few *M. diluviana* are encountered in water shallower than 30 m and areal abundance increase noticeably with depth at sites deeper than 30 m, plateauing at between 70 m and 100 m (Holda et al., 2021, 2019; Johannsson, 1995; Jude et al., 2018; Rudstam et al., 2008; Watkins et al., 2015). Therefore, we excluded samples collected from stations shallower than 70 m – except for eastern Lake Erie (where we excluded samples shallower than 30 m because the lake's maximum depth is around 70 m). Average areal abundance of mysids were calculated from the average values of each visit to a station (station-visit, i.e. replicates averaged and not considered independent samples). Averages were calculated separately for each agency conducting the sampling and for each gear used. For lakes Superior, Huron, and Erie, we only had samples from spring and summer (we categorized Lake Huron's late summer samples as "summer" to avoid confounding season and agency terms in later statistical models), while for lakes Michigan and Ontario we also had samples from late summer / fall. We also reported average annual abundance for each lake, season, and gear for time periods corresponding to 1990-1995 (only for Lake Ontario), 1997-2004, 2005-2012, and 2013-2019 (with minor variations among agencies due to annual programs spanning different years). We chose these time periods to include roughly equal numbers of years in each period. Using GLNPO datasets, we compared mean annual density and biomass among the lakes for each period – resulting in six ANOVA models (both response variables in each of three time periods). We $\log_e$ transformed density and biomass to satisfy assumptions of homoscedasticity and normality (adding the smallest observed value (0.15 *Mysis*/m^2 and 0.3 mg/m^2) to accommodate 0 catches). In addition to the effect of lake, we included an effect of season and, for the two most recent time periods when both the mysid net and zooplankton net were used, an effect of net type.

Within-lake temporal patterns were analyzed using generalized additive models (GAMs). We fit a separate GAM for each lake and each response variable (density and biomass) for a total of 10 GAMs. All GAM analyses were done using the function *gam* in the R package *mgcv* (Wood, 2017). In each GAM, the abundance metric was fit to a smoother function of *year* along with non-smoothed, categorical additive terms for *season* (Spring, Summer, or Fall), and for *collecting agency/net type* (GLNPO mysid net, GLNPO zooplankton net, USGS mysid net, NOAA mysid net, or DFO mysid net). Applying the smoothing function to the *year* variable allowed us to generate smoothed temporal patterns and accounted for temporal autocorrelation in our data. Including season allowed us to combine known seasonal effects such as abundance estimates in the open water being lower in spring than in summer (Johannsson, 1992; Jude et al., 2018; Pothoven and Vanderploeg, 2017). Due to non-homogenous variance and lack of normality in GAM residuals when using non-transformed abundance data, we applied fourth-root transformations to areal density and biomass data prior to fitting each GAM model. We used GAM fits to evaluate trends over time within and among lakes, while accounting for differences due to season, agency, and gear. Note that each lake-specific GAM is informed by the data from both spring and summer and from all sampling programs from that lake. We plotted the fit of all ten GAMs in terms of summer values normalized to the mysid net in the GLNPO survey, with separate panels for density and for biomass.

Comparison to lower trophic level data

We compared *M. diluviana* abundance to available data on mysid food supply to evaluate the influence of bottom-up drivers on *M. diluviana* density and biomass. The spring diatom bloom has been suggested to be important for mysids (Johannsson et al., 2001; Pothoven et al., 2010). We obtained annual spring bloom data from satellite measures of spring (lake-wide average of March-June) surface chlorophyll-a concentrations available from GLNPO for each lake for 1998-2019 (Lesht et al., 2018; U.S. EPA on-line database) (Fig. S3). We calculated a zooplankton index for each year as the average of spring and summer 0-100 m areal biomass (dry mass, excluding mysids captured in the zooplankton net) from the GLNPO stations deeper than 70 m (30 m for eastern Lake Erie) (Barbiero et al., 2019) (Fig. S4). Summer *M. diluviana* abundance (as predicted by previous GAM fits) were plotted against our annual indices of zooplankton biomass and spring phytoplankton, and relationships between food indices and *M. diluviana* density and biomass were examined with additional GAMs, where *M. diluviana* abundance was predicted as a smoothed function of a food index (phytoplankton and zooplankton). These additional GAMs included data from all lakes except eastern Lake Erie due to the limited number of mysids caught in that lake.

Life history rates

Mysis diluviana life history rates may indicate which changes in ecosystem structure (e.g., food supply, predation pressure) are impacting its populations. Mysid age distributions are most reliably determined from length distributions during summer when nearly all mysids: 1) have been released from the marsupium, and 2) belong to one of just two cohorts that do not overlap much in size (Jude et al., 2018). Because the main breeding pulse of Great Lakes *M. diluviana* is fall to spring (Holda et al., 2021, 2019; Johannsson, 1992; Pothoven and Vanderploeg, 2017), we used data from those seasons and not summer for reproductive rates. Although life history metrics for Lake Michigan were available from NOAA for the 1990s, we only used data from 2007 onwards both because comparisons of rates during the 1990s to the mid-late 2000s have already been published (Pothoven et al., 2010; Pothoven and

Vanderploeg, 2017) and although the older values represent an important baseline for comparisons, we were more interested in recent changes in life history metrics in Great Lakes *M. diluviana*. Density-dependence of life history rates was explored by comparing those rates with observed mysid density and biomass.

All individual length measurements based on summer collections (excluding the GLNPO zooplankton net samples) were pooled for each lake, year, and agency to obtain agency-specific time series of annual summer length distributions in each lake (except eastern Lake Erie). For both age classes ($a = 0, 1+$) and for all years ($t = 2006 - 2019$), summer age distributions ($P_{a,t}$) were estimated by applying Gaussian mixed models using the *Mclust()* function from the R package *mclust* (Scrucca et al., 2016) to summer length distributions. *Mclust()* output for bimodal distributions includes two means ($\bar{L}_{a,t}$), two standard deviations, and one proportion value ($P_{a=0,t}$). We report the $P_{a=0}$ values because they are important for later mortality calculations but are not individually dependent on multi-year calculations and any biases those might introduce. Mortality rates ($Mort_t$, in %) of *M. diluviana* were estimated from annual age distributions combined with annual density estimates as:

$$\mathrm{Mort_t} = 100 \cdot \left[1 - \left(\left(1 - \mathrm{P_{a=0,t+1}}\right) \cdot \mathrm{Density_{t+1}}\right) / \left(\mathrm{P_{a=0,t}} \cdot \mathrm{Density_t}\right)\right] \tag{1}$$

This gave us a mortality estimate in each lake for each year. From the mean standard lengths (mm) of each age class for each year ($\bar{L}_{a,t}$), we could estimate average growth rates of *M. diluviana* (in mm/month) for each year ($\bar{G}_t$) as:

$$\mathrm{\bar{G}_t} = \left(\mathrm{\bar{L}_{a=1+,t+1}} - \mathrm{\bar{L}_{a=0,t}}\right) / 12 \tag{2}$$

Thus, our growth and mortality rates represent the time period between the first and second summer of a *M. diluviana* cohort. We calculated these life history variables separately for each agency's dataset.

We also had information on mature, brooding (i.e., brood-carrying) females in the spring from GLNPO datasets and in the spring and fall from NOAA datasets. For each female, we measured standard length and counted the number of embryos in the marsupium. These data were used to calculate yearly mean lengths and mean brood sizes of brooding females by lake, season, and agency.

We examined life history variables for statistically significant, among-lake differences, for correlation with density and biomass, and for correlation with each food index described above. We used ANOVAs to test for among-lake differences in yearly estimates of age distribution ($P_{a=0}$), growth rate ($\overline{G}_t$), and mortality rate ($Mort_t$) based on the GLNPO dataset. For fecundity rates, we used an ANCOVA (with lake and female length as independent variables) to test for among-lake differences in length-dependent fecundity rates based on the GLNPO mysid net dataset (2006-2019). To examine correlations between mysid life history variables and mysid abundance or food indices, we fitted a linear model to each relationship for each agency dataset. From this, we reported the r^2 outputs of the model and both the sign (i.e., direction) and significance of parameter estimates. Each life history variable was compared to mysid density and biomass and food indices averaged over the portion of the year that would be relevant to the particular life history variable. Summer density of age-0 mysids was compared to mysid density and food indices from spring and summer of that year (for NOAA, this also included the previous year's fall). Summer-to-summer age-0-to-age-1+ percent annual mortality and average monthly growth rates were compared with the average densities from the current summer to the following spring. Spring brooding female length and embryos/brood were both compared with the previous year's abundance or food index (Spring – Summer, or for NOAA datasets: Spring – Fall).

Results

Abundance of Mysis diluviana

For the GLNPO data, *M. diluviana* were generally more abundant in lakes Superior, Michigan, and Ontario, less abundant in Lake Huron, and rare in eastern Lake Erie (Table 2, Fig. 2) in all three time periods that we investigated (1997-2004, 2005-2012, and 2013-2019). Lake and season showed significant effects in all six among-lake comparisons of density and biomass (three time periods x two net types = six comparisons). Net (mysid and zooplankton) effects were also significant in three of the four comparisons where it was relevant (Tables S1, S2). Abundance of *M. diluviana* in eastern Lake Erie was significantly lower than in all other lakes for all three time periods. Mysid abundance in Lake Huron was significantly lower than in lakes Superior, Michigan, and Ontario for the two more recent time periods 2005-2012 and 2013-2019, but not for 1997-2004 when only Lake Michigan had significantly higher abundance than Lake Huron. With one exception, mysid abundance in lakes Superior, Michigan, and Ontario for all three time periods were not significantly different (Fig. 2, S5). The exception was that density (but not biomass) of *M. diluviana* was significantly lower in Lake Michigan than in Lake Ontario in the most recent time period, 2013-2019 (Fig. 2).

Expanding to all available data from each lake, we generated lake-specific GAM models for density (Fig. 3, S6) and biomass (Fig. 4, S7) over a continuous time series accounting for season, agency, and net effects. These combined time series are presented in terms of summer GLNPO mysid net abundance. Density and biomass were consistently above 250 mysids/m^2 and 500 mg dw/m^2 in Lake Ontario, and above 150 mysids/m^2 and 400 mg dw/m^2 in Lake Superior, with little change in either lake since 2005. Density and biomass in Lake Huron were initially close to levels in Lake Superior, but declined from 1997 to 2010 to less than 50 mysids/m^2 and less than 100 mg dw/m^2, where they have remained through the end of the data series (2019). Eastern Lake Erie abundances were very low for the entire time series. In Lake Michigan, abundances were variable and some of the highest in the data set (200-400 mysids/m^2 and 300-1600 mg dw/m^2) in the early time periods, then declined substantially so that by 2019, Lake Michigan abundances were similar to the low levels observed in Lake Huron since 2007.

Food indices

The relationships between food indices and *M. diluviana* abundance (Fig. 5) were all significant based on GAMs (n = 84-105, edf = 2.6 – 3.7, $p < 0.001$, $r^2 = 0.35 – 0.61$). Mysid abundance increased rapidly with an increase in spring chlorophyll concentrations up to 1.5 mg/m^3 with only small changes with further increases in chlorophyll (Fig. 5). Mysid abundance also increased with zooplankton biomass up to 3 g dry wt/m^2, with less change thereafter (Fig. 5). For both indices, *M. diluviana* abundance in Lake Huron prior to 2003 were lower than predicted, while *M. diluviana* abundance in Lake Michigan during 2002-2006 were higher than predicted. Eastern Lake Erie (not included in the among-lake analyses) had very low abundance of *M. diluviana* even though spring chlorophyll concentrations were higher than observed in the other lakes. However, eastern Lake Erie zooplankton concentrations were low compared to other lakes.

Life history metrics

Cohorts of *M. diluviana* were successfully distinguished using length and the R package *Mclust()* for 83 of 86 lake- and agency-specific summer length distributions in the four deep lakes. Summer length distributions of *M. diluviana* for all agencies and lakes (except for eastern Lake Erie) were bimodal, indicating a 2-year generation time with most young released from the brood pouch in spring. For reproductive metrics in the GLNPO mysid net dataset (2006-2019), spring brooding females were present in each of the four deep lakes in all years. For eastern Lake Erie, only 5 individuals were available from three of the years in the data set. The NOAA spring and fall dataset (2007-2019) added another 236 brooding females from Lake Michigan which we included in time trend analysis for that lake. The total number of brooding females in the GLNPO and NOAA datasets combined was 1909.

In eastern Lake Erie, all *M. diluviana* individuals caught in April were adults and all but one of the mysids caught in August were juveniles. This is consistent with a 1-year generation time of *M. diluviana* in eastern Lake Erie with release of young in late spring or early summer. Because mysids were very rare in eastern Lake Erie, we pooled all the lengths measured in both seasons from all years from the GLNPO mysid net. Therefore, we have only one (multi-year) estimate of the proportion of age-0 mysids ($P_{a=0}$), monthly growth ($\bar{G}$), and annual mortality ($Mort$) for eastern Lake Erie; this precluded any analysis of time trends in that lake. The lone summer adult captured in eastern Lake Erie was a mature, non-brooding female of the same size as brooding females collected during spring. Also note that because most adults were collected during spring, we estimated growth and mortality rates of the eastern Lake Erie *M. diluviana* population based on the 8-month time period from August to April (excluding the one summer-collected adult). Similarly, we based average annual age-0 proportion of the eastern Lake Erie *M. diluviana* population on the numbers of summer juveniles and spring adults (excluding the lone summer adult). We included the five brooding females collected from eastern Lake Erie (one in spring of 2012 and two each in the springs of 2014 and 2018) in our among-lake ANCOVA analysis of fecundity, but we did not include eastern Lake Erie in analyses of time trends in fecundity.

All summer life history characteristics differed significantly across lakes (Tables S3:S6). The proportion of summer *M. diluviana* in the age-0 cohort ($P_{a=0,t}$), annual mortality rate ($Mort_t$), annual growth rate ($\bar{G}_t$) (Table 3), and embryos per brooding female (Fig. 6, Table S3) generally increased from Lake Superior to lakes Huron, Michigan, Ontario and eastern Lake Erie. For all summer life history metrics, pairwise comparisons between lakes Ontario and Superior were significantly different at either the $\alpha = 0.05$ level ($P_{a=0,t}$, Table S4), or at $\alpha = 0.10$ level ($Mort_t$, Table S5; $\bar{G}_t$, Table S6). No other pairwise comparisons of summer life history metrics between lakes were statistically significant (Tables S4-S6). However, eastern Lake Erie was excluded from ANOVA tests on summer life history metrics due to our possessing only one multi-year estimate for each such metric in that lake. Eastern Lake Erie life history variables were higher than the values from all other lakes and outside the confidence limits of the next highest value lake (Lake Ontario). Female length was significantly positively related to embryos per brooding female ($p < 0.001$, Tables S7) with no significant interactions between lake and brooding female length (df = 4 and 1175, F = 0.48, p = 0.75). The model estimated a slope of 1.5 embryos / mm brooding female length (Fig. 6). Lake was also significant, and a Tukey HSD test showed brood counts to be significantly different between most pairwise comparisons of lakes (Table S7), with the exception of two non-significant comparisons (Huron and Superior at p = 0.37, and Michigan and Ontario at p = 1.00).

Few significant relationships were observed between life history metrics and density and biomass or food indices (Table 4). However, Lake Michigan fall fecundity rates were negatively related to mysid density and biomass. Across all lakes, growth rates of *M. diluviana* showed significant positive relationships with both food indices.

Discussion

Our analyses revealed consistent patterns in mysid abundance and biomass across the lakes and trends over time. *Mysis diluviana* abundances were consistently high in lakes Superior and Ontario, initially high but variable and then declining in Lake Michigan, low in Lake Huron since a decline in the mid-2000s, and very low in eastern Lake Erie. These observations using additional datasets corroborated the findings reported by Jude et al. (2018), but our data added several new insights. The addition of pre-2006 mysid abundance data from GLNPO, NOAA, and DFO allowed us to observe 1) that the slight increase in Lake Superior in the Jude et al. (2018) data started in the early 2000s, 2) that Lake Michigan values were high and variable prior to 2007, 3) that the timing of declines in Lake Michigan between 1995 and 2007 noted by Pothoven and Vanderploeg (2017) occurred between 2003 and 2007, 4) that declines in Lake Huron from the late 1990s and early 2000s to the mid-2000s were coincident with the large decrease in zooplankton in Lake Huron occurring in 2003/2004 (Barbiero et al., 2019; Riley et al., 2008), 5) that the timing of declines in Lake Ontario between 1995 and 2002 noted by Johannsson et al. (2011) occurred prior to 1997, and 6) that low values in eastern Lake Erie have persisted at least since 1997. The inclusion of 2017-2019 mysid abundance data from all sources revealed: 1) continued stability with high values in lakes Superior and Ontario and low values in Huron and Erie, but 2) a multi-year decline in Lake Michigan (2015-2019). Our results present the status of the mysid population in all five Great Lakes up to 2019. We have a high degree of confidence in these results due to the consistency across multiple datasets contributed by the cooperating agencies.

Having observed these recent long-term patterns in Great Lakes *M. diluviana* abundance, we explored whether they were related to two food resources (spring chlorophyll-a concentration and spring-summer zooplankton biomass). Mysids of all ages utilize both phytoplankton and zooplankton in their diets (Johannsson et al., 2001, 1994; O'Malley et al., 2017; O'Malley and Bunnell, 2014). Among lakes, annual mysid abundance showed strong, significant, positive, saturating relationships with both food indices. Further, among lakes, both food resource indices were positively related to mysid annual growth rate estimates, as one would expect from the mechanistic link between food availability and *M. diluviana* density and biomass. These basin-wide relationships provided strong evidence that food availability is important for *M. diluviana* abundance and biomass in the Great Lakes. In addition, the declines in mysid abundance in lakes Michigan and Huron correspond to known declines in zooplankton biomass and spring chlorophyll-a concentrations (Barbiero et al., 2018b). Others have also reported positive correlations between mysid abundance and both chlorophyll-a concentration (Pothoven et al., 2010; Lake Michigan) and zooplankton abundance (Johannsson et al., 2011; Lake Ontario). Our results suggest these trends may explain not only within-lake trends but also among-lake patterns in mysid abundance. Apparently, phytoplankton in the deep chlorophyll layer present in the summer (Scofield et al. 2020) did not compensate for the decline in spring diatom concentrations

However, there was still much among-lake variability that food resources did not explain. First, the among-lake trend showed that mysid abundance were more strongly related to food resource indices at lower values than at higher values, suggesting a threshold beyond which food resources do not affect *M. diluviana* abundance. Second, while *M. diluviana* abundance was generally positively related to food resources, mysid abundance was lowest in eastern Lake Erie, where algae resources (but not areal zooplankton biomass) were similar to Lake Ontario. Third, there is variability in the response of mysid biomass to food resources among the four deep lakes; most notably the Lake Huron mysid abundance is lower than expected compared with the other deep lakes.

Where bottom-up effects are not driving variability in *M. diluviana* abundance, that variability is likely caused by either density-dependent effects (e.g., interference competition or cannibalism), or fish predation. Mysids interfere with feeding of conspecifics at sufficiently high concentrations in laboratory experiments (Hansson et al., 2001) and are known to cannibalize (Johannsson et al., 2001; O'Malley et al., 2017; O'Malley and Bunnell, 2014). Thus, growth rates per individual would decline and mortality rates increase at high mysid density and biomass, which may explain the apparent among-lake threshold of a 'maximum' *M. diluviana* abundance value that rarely exceeds about 200-300 mysids / m^2 even at high food resource levels. Mysid abundances over 1000 /m^2 are rare in the literature suggesting an upper limit of areal abundance for these animals (reviewed by Rudstam, 2009). However, we observed little evidence of density-dependent mortality and growth rates in our among-lake comparisons. The only variable which showed some density-dependence was fall fecundity values in Lake Michigan, which were negatively related to the preceding year's abundance levels, making Interference competition less likely. Therefore, we consider cannibalism to be a more likely mechanism as cannibalism probably affects mortality rates of young mysids prior to the first summer and our mortality rates were calculated from the first to the second summer. However, this is a hypothesis that needs to be tested.

Predation has been suggested to affect mysid abundance and distributions (McDonald et al., 1990; Boscarino et al., 2010; Johannsson, 1995). The low abundance in Lake Erie and perhaps Lake Huron may be due to higher fish predation associated with higher light levels expected on the bottom of these shallower lakes (Boscarino et al. 2010). Mysids have relatively low fecundity compared to many other aquatic animals, including fish, and therefore they are sensitive to changes in predation pressure (Rustam et al., 1998; Johannsson et al., 2003). In eastern Lake Erie, a high mortality rate estimate and a high proportion age-0 estimate (a proxy for mortality) strongly suggest that eastern Lake Erie *M. diluviana* abundance are low due to high predation by fishes in that lake. The eastern Lake Erie population has remained low since at least 1997 even though eastern Lake Erie *M. diluviana* have a shorter generation time (1 year) and significantly higher growth and fecundity rates compared with the other four lakes. Fish predation on mysids is likely high in eastern Lake Erie (Johannsson et al., 1999). Rainbow smelt (*Osmerus mordax*) – a species that feeds heavily on mysids when available (Stritzel Thomson et al., 2011) – can be abundant in eastern Lake Erie (Parker et al., 2001). In addition, eastern Lake Erie is relatively shallow, lacking a deep-water refuge from visual predation – which was hypothesized by Johannsson (1995) to be present at depths of > 100 m in Lake Ontario. The high abundance of rainbow smelt combined with scarce refuge habitat would be expected to result in high mortality rates for *M. diluviana*. Similarly, the *M. diluviana* population in Lake Champlain, a lake with high rainbow smelt populations, is low relative to the deep Great Lakes even though mysid growth rates in that lake are high (Hrycik et al., 2015). For Lake Huron, it is possible that the lower-than-expected abundance of mysids is also due to higher predation rates from abundant bloater (*Coregonus hoyi*) and rainbow smelt below the thermocline (O'Brien et al. 2022). In addition, this lake is shallower than the other three deep Great Lakes and water clarity has increased dramatically in Lake Huron over this time period (Bunnell et al., 2021), perhaps eliminating the deep-water low-light refuge in much of the lake. It is likely that rainbow smelt and bloater have a greater reliance on *M. diluviana* as food than do alewife which is still abundant in Lake Michigan (Warner et al. 2022). We do not have estimates of life history rate metrics from the years 1997-2005 when Lake Huron abundance was relatively lower than expected from the food indices, although Hinderer et al. (2012) suggested that Lake Huron mysids were in poor condition during 2007-2008.

One of the more striking results of our study is the substantial decline in Lake Michigan *M. diluviana* abundance in recent years. Our among-lake analysis indicated that declining food resources are a strong predictor of mysid abundance, so the occurrence of declining spring chlorophyll-a concentrations in Lake Michigan may explain the observed mysid decline during this time period. Additionally, an intensive study of Lake Michigan *M. diluviana* in 2015 revealed that values of age-0 mortality in 2015 were high, and values of fecundity and percentage of females brooding were relatively low when compared with rates from the previous 8 to 9 years (Holda et al., 2021). Both increasing mortality and decreasing fecundity for 2015 compared with previous years would be consistent with the initiation of the observed population decline. It is also possible that vertical migration behavior has changed over time; the proportion of *M. diluviana* migrating each night may be in decline as a response to changes in food resources in the night-time metalimnion habitat (Stockwell et al., 2020). One additional explanation is an observed shift in pelagic fish biomass from alewife-dominant to bloater-dominant. Bloater biomass increased beginning in 2017 because of strong year classes in 2015 and 2016 (Bunnell et al. 2020). This dominance of bloater persisted through 2021 (Warner et al. 2022). Although the decline in Lake Michigan mysids since 2015 is consistent with the continued decline in food resources in that lake, the decline is larger than expected perhaps because the shift in the fish community has increased fish predation on mysids.

In summary, our collaborative, Great Lakes basin-wide study of *M. diluviana* has revealed consistent patterns in density and biomass across lakes and over time, confirmed published and suspected historic trends, and highlighted the 2017-2019 decline in Lake Michigan, whereas the other lakes' populations remain relatively stable. Our study highlights the importance of bottom-up drivers to *M. diluviana* abundance, but also points at density-dependent processes that may result in threshold abundance levels in the Great Lakes, and that the higher predation risk expected in shallow eastern Lake Erie and perhaps Lake Huron is likely limiting *M. diluviana* abundance in those lakes. The among-lake life history rates we observed strongly support the predation mortality hypothesis in eastern Lake Erie, but offer little evidence of density-dependence in any of the Great Lakes. This collaboration among the agencies surveying mysids across the Great Lakes allowed for the inclusion of several independent data sets thereby providing strong support of the observed time trends.

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Tables

Table 1. Vertical plankton net specifications and typical number of replicates per site visit for each of the survey programs with annual *M. diluviana* monitoring data (EPA GLNPO = US Environmental Protection Agency Great Lakes National Program Office, NOAA = National Oceanic and Atmospheric Administration, USGS = US Geological Survey, DFO = Fisheries and Oceans, Canada). Cod-end mesh was always the same as the lower 1/3 mesh. Note that EPA GLNPO nets targeting *M. diluviana* changed form between 2011 and 2012. Since most methods are similar and density and biomass are standardized to area sampled, we refer to both collectively as 'the GLNPO *Mysis* net'. The 153-µm net is the 'GLNPO zooplankton net'.

	EPA GLNPO 2006-2011	EPA GLNPO 2012-2019	EPA-GLNPO 1997-2019	USGS	NOAA	DFO
Opening	Square	Circular	Circular	Circular	Circular	Square
Diameter or Width	1 m	1 m	0.5 m	1 m	1 m	1 m
Length	2-3 m	2 m	2 m	3 m	3 m	3 m
Upper 2/3 Mesh	1 mm/500 µm	500 µm	153 µm	1 mm	1 mm	1 mm
Lower 1/3 Mesh	250 µm	250 µm	153 µm	1 mm	1 mm	1 mm
Reps per Visit	2	2	1	1	3	2

Table 2. Among-lake comparison of annual *M. diluviana* abundance estimates by season for three different time periods (four in Lake Ontario with the 1990-1995 data from the DFO). Data separated by lake, agency, and sampling gear (mysid net or zooplankton net 'zoop'). Values shown are means of annual means for the time periods shown, ± 2 SE, where N is the number of years for which values are present. Note that bounds of time periods differ slightly between different agencies due to variation in history of annual sampling operations and inactive years. For instance, the annual DFO fall survey for *M. diluviana* was not conducted in 2018 and 2019. See Table 1 for definition of agencies. Spring refers to April-June, Summer refers to July -September, and Fall refers to October-November.

					Density (#/m^2)			Biomass (mg/m^2)		
Lake	Time Period	Agency	Net	N	Spring	Summer	Fall	Spring	Summer	Fall
Ontario	1990-1995	DFO	mysid	3	-	-	439 ± 101	-	-	2,022 ± 915
Ontario	1997-2004	GLNPO	zoop	7	82 ± 23	136 ± 46	-	120 ± 45	268 ± 147	-
Ontario	2002-2004	DFO	mysid	3	-	-	258 ± 80	-	-	895 ± 292
Ontario	2006-2012	GLNPO	mysid	7[a] – 6	102 ± 42	377 ± 282	-	287 ± 167	817 ± 337	-
Ontario	2005-2012	GLNPO	zoop	8	169 ± 85	278 ± 126	-	323 ± 324	641 ± 397	-
Ontario	2005-2012	DFO	mysid	8	-	-	208 ± 48	-	-	742 ± 157
Ontario	2013-2019	GLNPO	mysid	7	200 ± 60	278 ± 123	-	341 ± 91	591 ± 180	-
Ontario	2013-2019	GLNPO	zoop	6[b] – 7	114 ± 30	182 ± 57	-	102 ± 54	214 ± 65	-
Ontario	2013-2017	DFO	mysid	4	-	-	165 ± 39	-	-	724 ± 100
Superior	1997-2004	GLNPO	zoop	7[b] – 8	105 ± 31	125 ± 28	-	99 ± 58	154 ± 68	-
Superior	2006-2012	GLNPO	mysid	6	78 ± 15	213 ± 69	-	209 ± 42	456 ± 149	-
Superior	2005-2012	GLNPO	zoop	8	70 ± 14	146 ± 43	-	77 ± 18	175 ± 60	-
Superior	2013-2019	GLNPO	mysid	7	146 ± 29	216 ± 23	-	318 ± 65	407 ± 48	-
Superior	2013-2019	GLNPO	zoop	6[b] – 7	91 ± 33	146 ± 37	-	87 ± 25	145 ± 42	-
Michigan	1997-2004	GLNPO	zoop	7[b] – 8	159 ± 57	334 ± 127	-	307 ± 224	528 ± 311	-
Michigan	1995-2002	NOAA	mysid	4[c], 5[a], 6	154 ± 52	224 ± 82	133 ± 66	257 ± 91	619 ± 204	444 ± 194
Michigan	2006-2012	GLNPO	mysid	7	123 ± 30	158 ± 66	-	282 ± 81	408 ± 257	-
Michigan	2005-2012	GLNPO	zoop	8	97 ± 23	189 ± 64	-	127 ± 55	218 ± 120	-
Michigan	2005-2015	USGS	mysid	8	-	200 ± 73	-	-	406 ± 274	-
Michigan	2007-2015	NOAA	mysid	6	49 ± 32	99 ± 39	81 ± 29	137 ± 81	296 ± 124	298 ± 112
Michigan	2013-2019	GLNPO	mysid	7	79 ± 33	153 ± 50	-	160 ± 50	292 ± 87	-
Michigan	2013-2019	GLNPO	zoop	6[b] – 7	48 ± 28	127 ± 43	-	34 ± 18	97 ± 41	-
Michigan	2013-2019	USGS	mysid	7	-	146 ± 60	-	-	426 ± 214	-

Michigan	2013-2019	NOAA	mysid	7	36 ± 25	57 ± 29	59 ± 34	113 ± 50	167 ± 87	202 ± 120
Huron	1997-2004	GLNPO	zoop	7[b] – 8	53 ± 35	135 ± 60	-	73 ± 68	134 ± 45	-
Huron	2006-2012	GLNPO	mysid	7	17 ± 7	51 ± 11	-	42 ± 16	103 ± 27	-
Huron	2005-2012	GLNPO	zoop	8	16 ± 13	43 ± 28	-	40 ± 45	41 ± 30	-
Huron	2005-2015	USGS	mysid	8	-	64 ± 14	-	-	183 ± 67	-
Huron	2013-2019	GLNPO	mysid	7	8 ± 3	30 ± 6	-	21 ± 9	58 ± 13	-
Huron	2013-2019	GLNPO	zoop	6[b] – 7	8 ± 3	23± 12	-	7 ± 4	24 ± 20	-
Huron	2013-2019	USGS	mysid	7	-	45 ± 10	-	-	130 ± 41	-
Erie	1997-2004	GLNPO	zoop	2[b] – 6	0	3.2 ± 3.6	-	0	3.8 ± 6.8	-
Erie	2007-2012	GLNPO	mysid	3[b] – 5	0.2 ± 0.4	1.0 ± 1.1	-	1.6 ± 3.2	0.7 ± 1.1	-
Erie	2005-2012	GLNPO	zoop	4[b] – 6	0	1.4 ± 1.2	-	0	0.7 ± 1.1	-
Erie	2013-2019	GLNPO	mysid	4[a] – 5	0.6 ± 0.9	6.6 ± 6.3	-	4.4 ± 6.3	1.7 ± 1.7	-
Erie	2013-2019	GLNPO	zoop	4[b] – 5	0	3.1 ± 3.4	-	0	1.0 ± 1.4	-

a – summer sampling was conducted in fewer years than spring or fall sampling for this row.
b – spring sampling was conducted in fewer years than summer sampling for this row.
c – fall sampling was conducted in fewer years than spring and summer sampling for this row.

Table 3. Among-lake comparison of multi-year summaries of annual life history metrics based on GLNPO collections with the mysid net (2006/07-2019). Age distribution, mortality, and growth rate were calculated from annual summer length distributions and based on one estimate per summer for the number of summers indicated in the table. Growth was calculated as the difference in mean-length increment from age 0 to age 1 the following year divided by 12 to give mm/month. Mortality is the complement of age-1+ abundance in one summer over age-0 abundance the previous summer, multiplied by 100% (for eastern Lake Erie, 8-month mortality and growth estimates were converted to 12-month equivalents for this table for comparison with the other lakes). Same letters indicate lakes that are not significantly different (Tukey HSD test).

Lake	Summers	Percent Age-0	Growth (mm/mo)	Annual mortality (%)
Superior	12	71.6 ± 2.9 b	0.59 ± 0.03 b	55 ± 12 b
Michigan	13	76.2 ± 3.9 ab	0.64 ± 0.04 ab	71 ± 7 ab
Huron	13	77.0 ± 4.5 ab	0.61 ± 0.03 ab	68 ± 11 ab
Erie	Pooled	93.9	1.33	98
Ontario	12	80.8 ± 5.4 a	0.66 ±0.03 a	72 ± 12 a

Table 4. Correlation strength (r^2) and direction for the relationships between *Mysis diluviana* life-history metrics and annual values of four different indices: *M. diluviana* density (Den), *M. diluviana* biomass (Bio), satellite-derived spring surface chlorophyll-a concentration (Chl-a), and mean spring and summer zooplankton biomass (Zoo). Bold and underlined r^2 values indicate slope estimates significantly different from the null hypothesis slope of 0 (i.e., $p \leq 0.05$). Up-arrows (↑) and down-arrows (↓) indicate direction of best-fit slope estimate(s) for the trends when slope was significant or when $r^2 \geq 0.3$. Each regression is based on agency specific among-lake data from 2005-7 to 2019. Values from eastern Lake Erie were excluded due to insufficient data to produce life history metrics on an annual basis from that lake.

Index	Agency	Age-0 Cohort Proportion	Mortality rate (% / yr)	Growth Rate (mm TL/mo)	Spring embryos/brood	Fall embryos/brood	Spring TL (mm) brooding ♀	Fall TL (mm) brooding ♀
Den	GLNPO	0.07	0.04	0.00	0.02	–	0.02	–
Den	NOAA	0.00	0.00	0.01	0.06	**0.54** (↓)	0.17	0.17
Den	USGS	0.02	0.00	0.01	–	–	–	–
Bio	GLNPO	0.01	0.04	0.00	0.03	–	0.03	–
Bio	NOAA	0.00	0.00	0.00	0.02	0.30 (↓)	0.17	0.06
Bio	USGS	0.03	0.02	0.01	–	–	–	–
Chl-a	GLNPO	0.03	0.01	**0.09** (↑)	0.08	–	0.02	–
Zoo	GLNPO	0.00	0.08	**0.16** (↑)	0.09	–	0.00	–

Figures

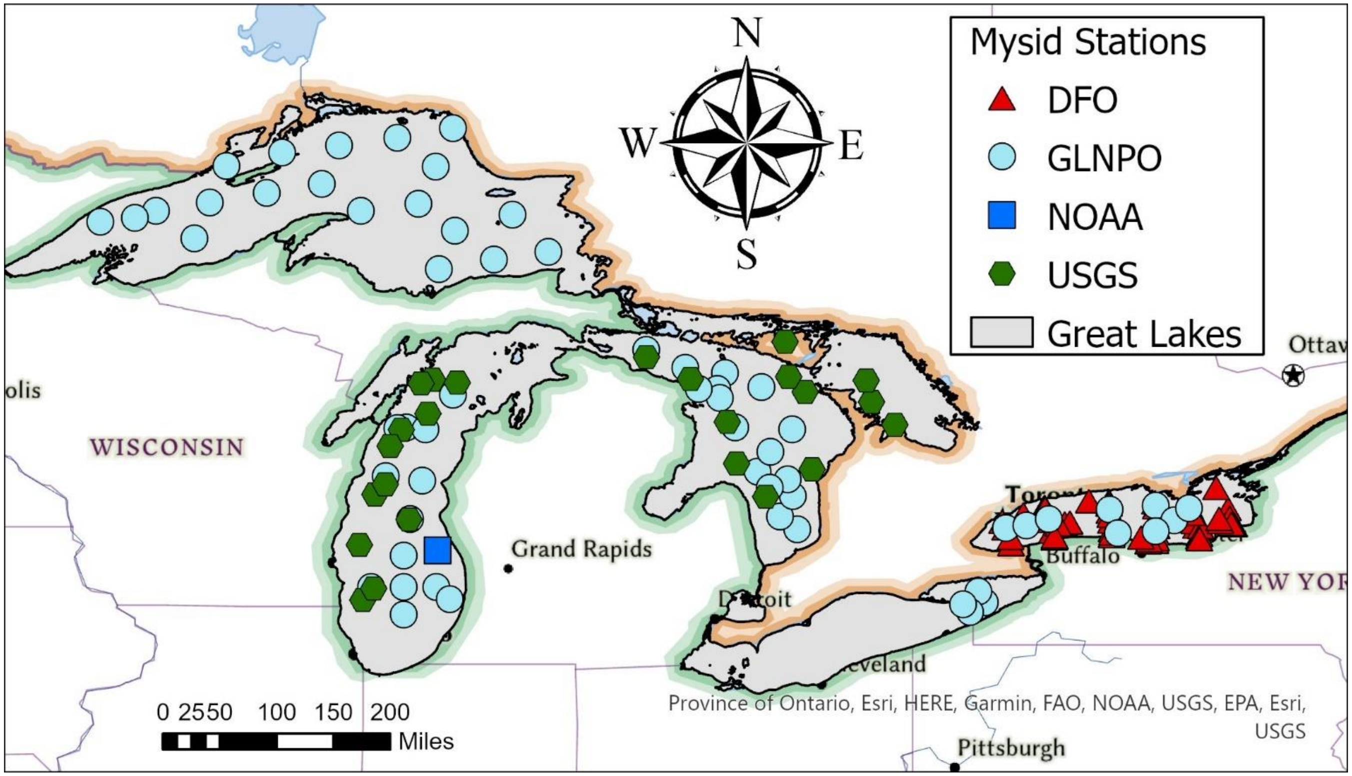

Figure 1. Map of the Laurentian Great Lakes with: 1) GLNPO monitoring stations sampled with 153-µm mesh net (1997-2019) and 250-µm mesh net (2006-2019) (light blue circles), 2) USGS station locations from representative years in lakes Michigan and Huron (green hexagons), 3) NOAA offshore Lake Michigan monitoring station in transect near Muskegon, MI (blue square), and 4) DFO monitoring stations in Lake Ontario (red triangles). Note that USGS stations were not at the same locations each year but achieved similar annual spatial coverage of lakes Michigan and Huron as depicted here in most other years included in our dataset. See legend for station identification.

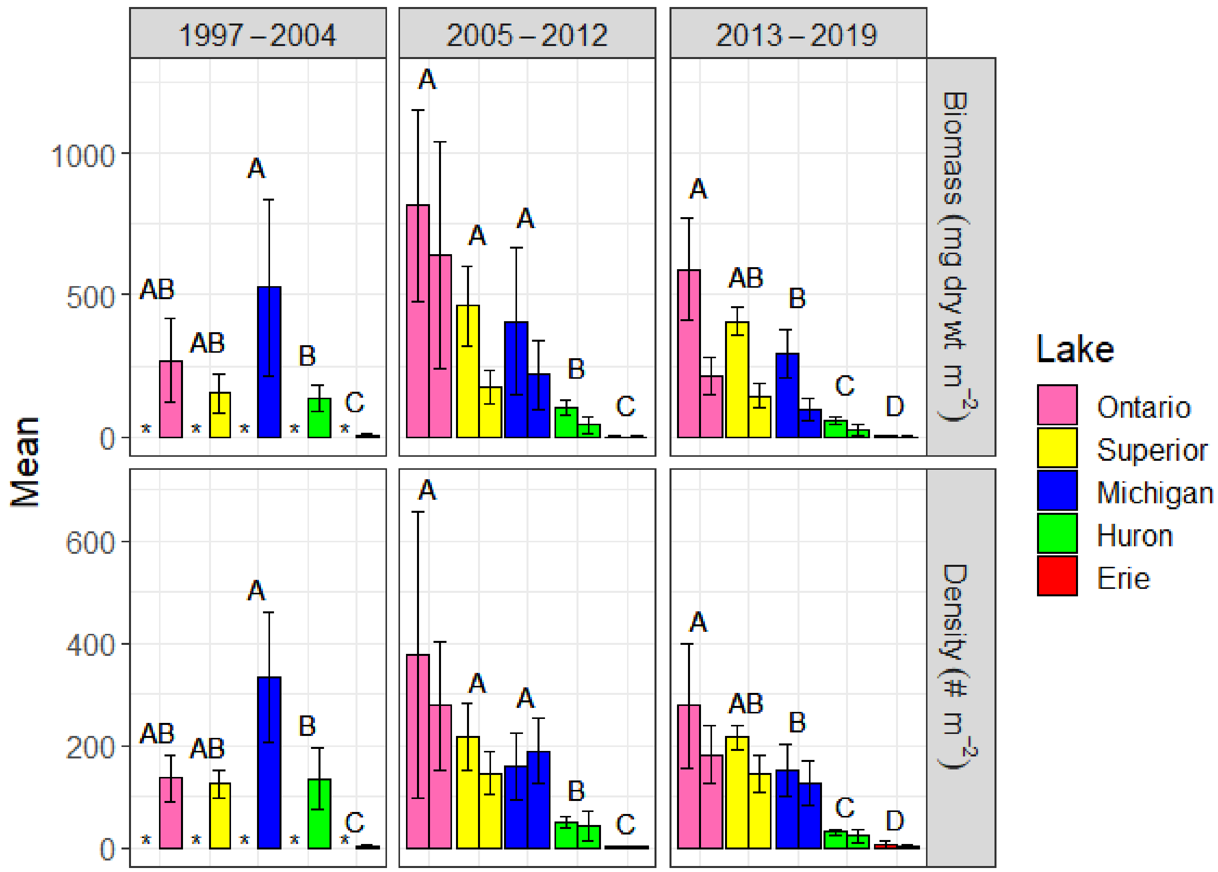

Figure 2. Summer density and biomass (mean ± 2 SE) of *M. diluviana* in each lake during three time periods for the GLNPO mysid net (left bars) and the GLNPO zooplankton net (right bars). ANOVAs of abundance as a function of lake, season, and net type (only four of the six panels) were performed (one per panel) on log$_e$-transformed data. Lake and season were significant effects in all six comparisons, and net type was significant in three of the four comparisons where it was relevant. Each of these was followed by a Tukey HSD assessment of significant pairwise differences between lakes for each time period (accounting for variation due to net types) resulting in groupings of lakes represented by the letters above the bars (see Tables S1-S6 for detailed statistics). The '*' symbols in the 1997-2004 time period highlight the absence of samples from the mysid net during those years. A plot of the same values for spring data is available as supplemental Figure S5.

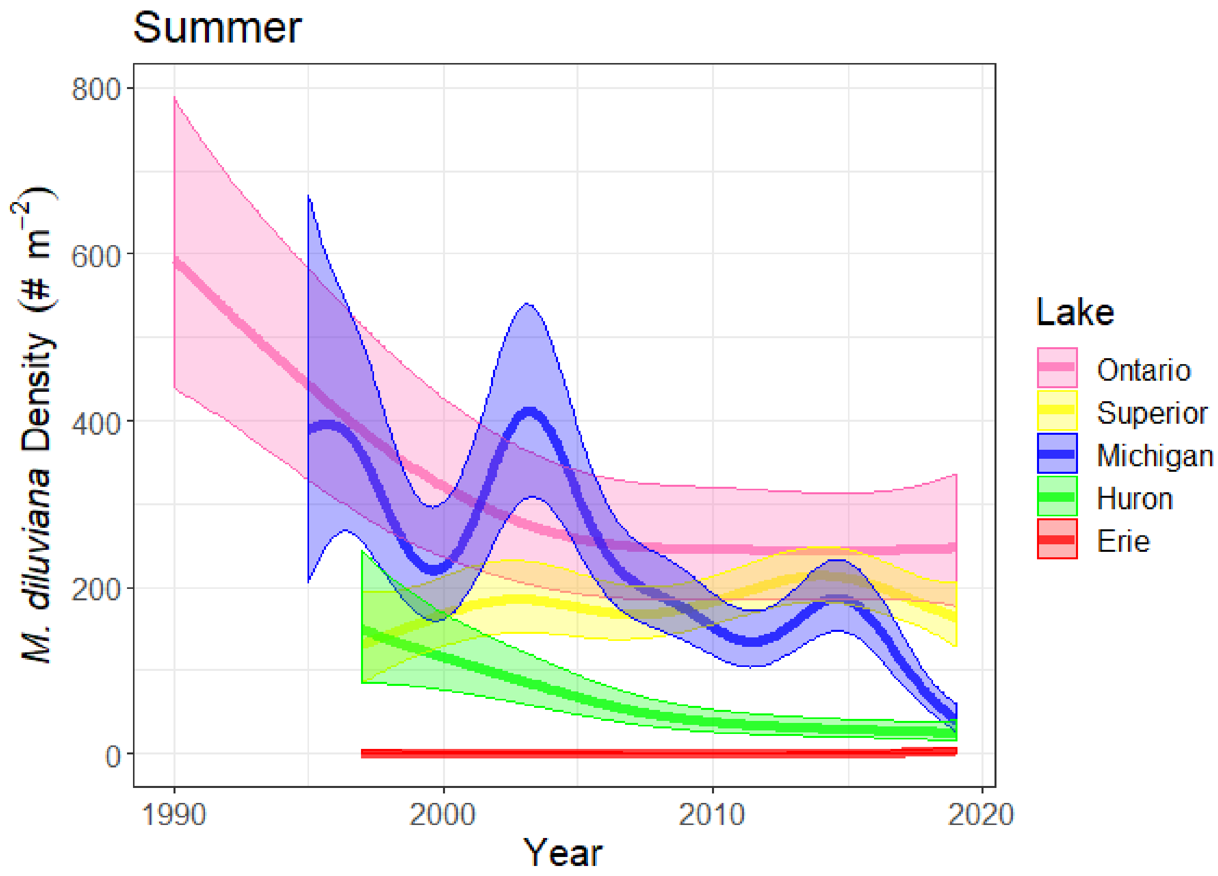

Figure 3. Summer density trends over time in each Great Lake, as predicted by lake specific GAMs fit to all available density data, with the grey area representing ± 2 SE. Values represent density references to the Summer GLNPO mysid net. Spring and Fall trends will be the same for each lake due the structure of the GAMs but have different density scale. A plot of the GAM density fits related to spring mysid net density data is available as supplementary Figure S6.

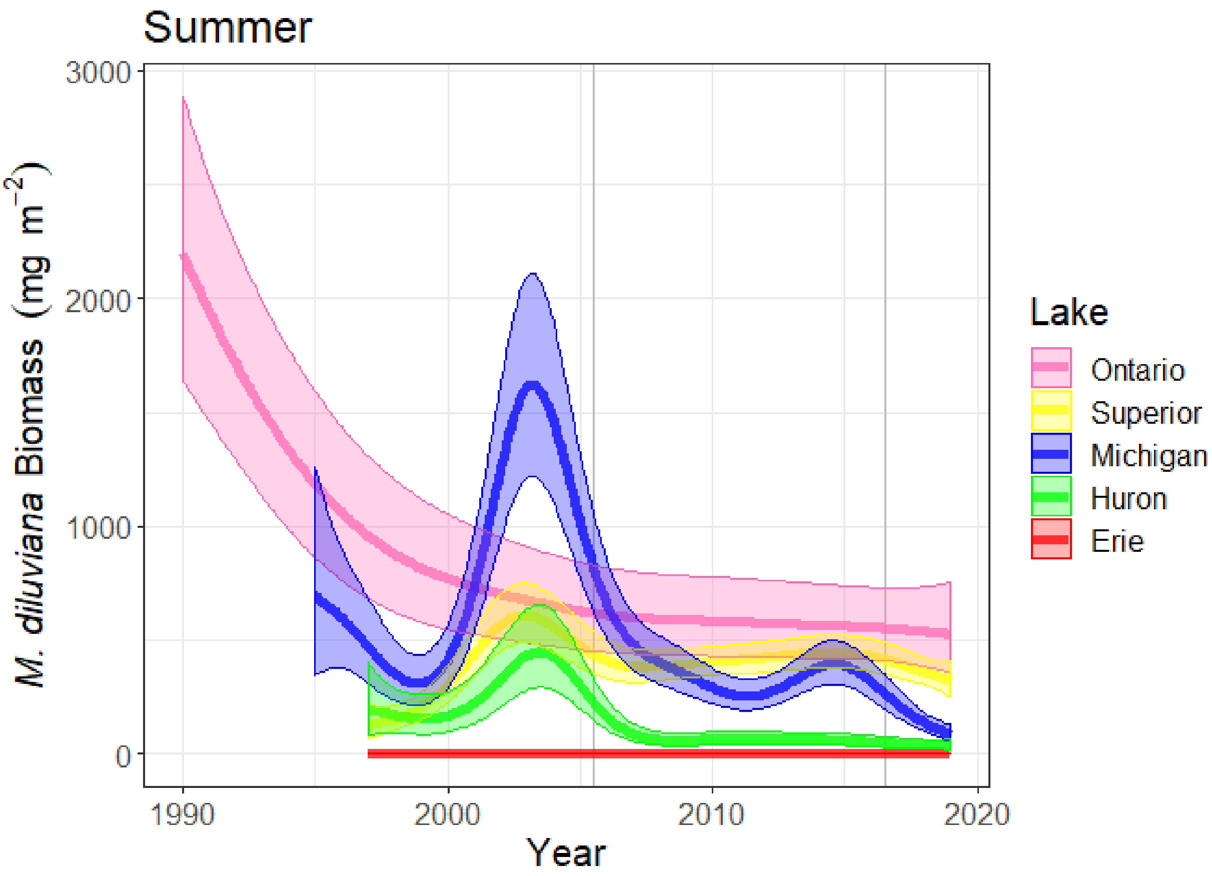

Figure 4. Summer biomass trends over time in each Great Lake, as predicted by lake specific GAMs fit to all available biomass data. Values represent biomasses as collected by the Summer GLNPO mysid net. Spring and Fall trends will show the same pattern for each lake due the GAM structures but have different biomass scales. A plot of the Spring GAM biomass fits is available as supplementary Figure S7.

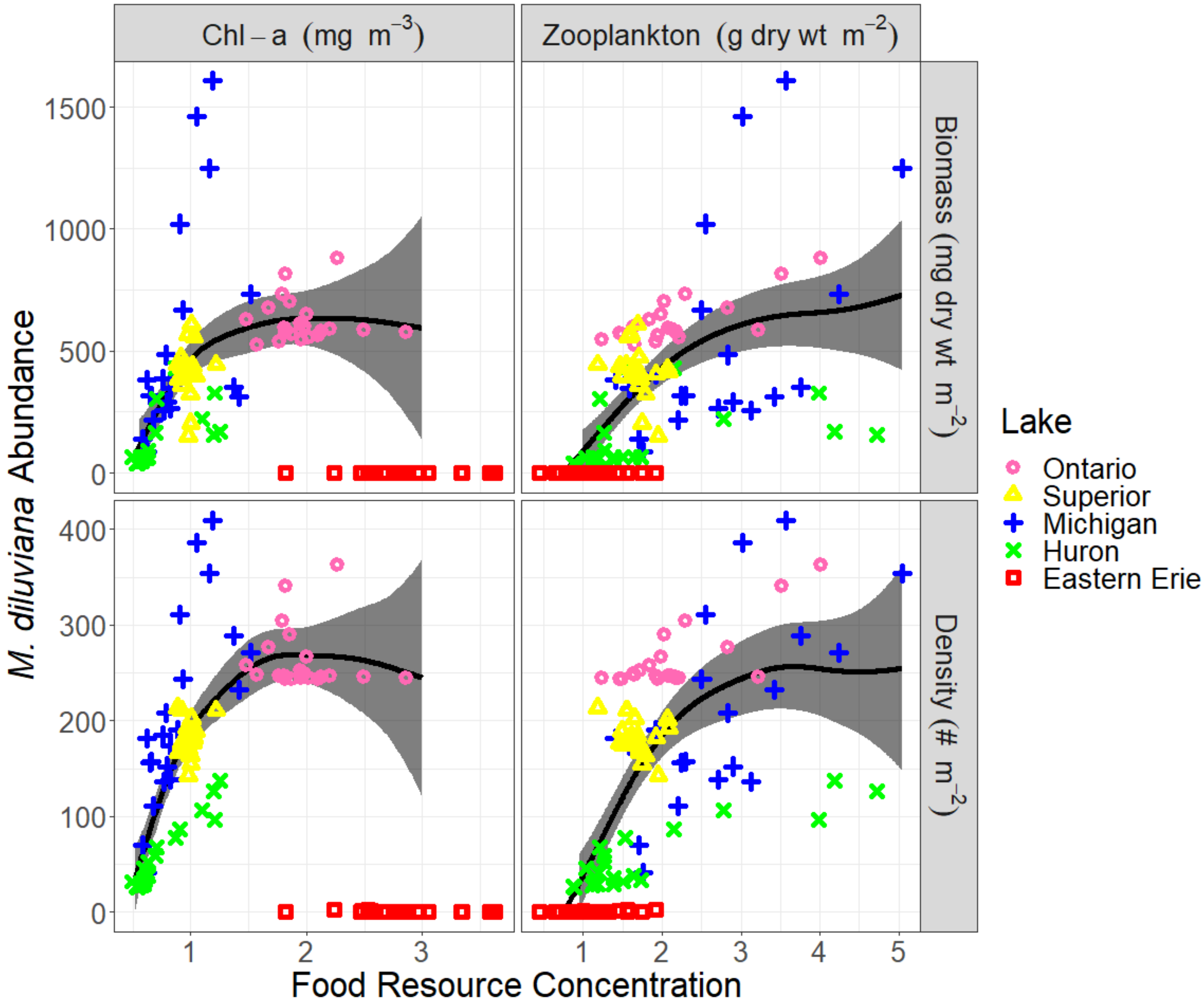

Figure 5. *Mysis diluviana* biomass and density as a function of two indices of two different food resource concentrations: spring (March-June) satellite surface chlorophyll-a and spring-summer (April and August) zooplankton dry biomass. *Mysis* biomass is in unit of mg dry wt/m^2, *Mysis* density is in #/m^2, chlorophyll is in mg/m^3, and zooplankton biomass is in g dry wt/m^2. The black line is a GAM fit to all lake data excluding Lake Erie, with a grey area representing the 95% confidence interval of the mean of the GAM fit. The GAM fits are not forced through 0.

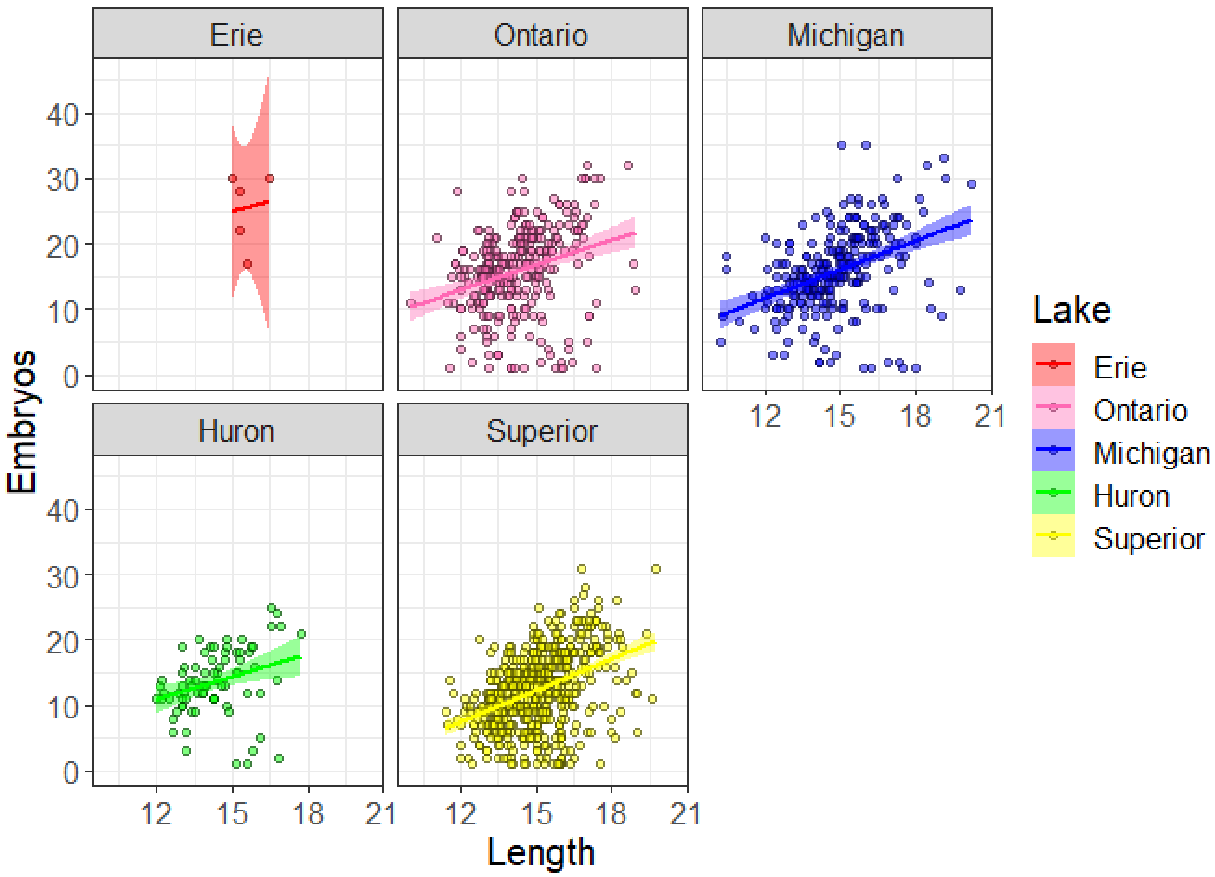

Figure 6. Brood size (count of embryos/female) as a function of brooding female length (mm). Common slope for all lakes combined) was 1.5 embryos/female/mm (ANCOVA, n = 1909, df = 1, 1179, F = 31, p < 0.001) with additive lake effect (df = 4, 1179, F = 184, p < 0.001). See table S7 for more details.

Supplementary Tables

Table S1. ANOVA tables for the among-lake comparisons of $\log_e$ *M. diluviana* density for different time periods. Analyses only includes GLNPO data for zooplankton and mysid nets.

Sources of variation	Sum of Squares	df	Mean Square	F	P
1997-2004					
Lake	202.6	4	50.7	65.3	< 0.0001
Season	8.7	1	8.7	11.2	0.001
Error	47.3	61	0.8		
Total	258.6	66			
2005-2012					
Lake	506.9	4	126.7	262.1	< 0.0001
Season	26.2	1	26.2	54.2	< 0.0001
Net	0.5	1	0.5	1	0.32
Error	61.9	128	0.5		
Total	595.5	134			
2013-2019					
Lake	429.1	4	107.3	188.8	< 0.0001
Season	25	1	25	44.1	< 0.0001
Net	6.6	1	6.6	11.6	0.001
Error	67.6	119	0.6		
Total	528.3	225			

Table S2. ANOVA tables for the among-lake comparisons of $\log_e$ *M. diluviana* biomass during 1997-2004. Analysis only includes GLNPO data for zooplankton and mysid nets.

Sources of variation	Sum of Squares	df	Mean Square	F	P
1997-2004					
Lake	307.6	4	76.9	51.2	< 0.0001
Season	8.1	1	8.1	5.4	0.023
Error	91.6	61	1.5		
Total	407.3	66			
2005-2012					
Lake	806.2	4	201.5	193.5	< 0.0001
Season	21.1	1	21.1	20.3	< 0.0001
Net	24.7	1	24.7	23.7	< 0.0001
Error	133.4	128	1		
Total	985.4	134			
2013-2019					
Lake	618.9	4	154.7	184.3	< 0.0001
Season	16.9	1	16. 9	20.1	< 0.0001
Net	61.1	1	61.1	72.8	< 0.0001
Error	99.9	119	0.8		
Total	796.8	225			

Table S3. Among-lake comparison of multi-year summaries of fecundity and related measures based on GLNPO collections with the mysid net (2006/07-2019). Mean number of embryos per brooding female and mean brooding female length (mm) were calculated based on all individual gravid females caught during this time period. Results of associated ANCOVA tests can be found in Table S7.

Lake	# females	Embryos/Brood	Length (mm)
Superior	645	12.3 ± 0.02	15.0 ± 0.005
Michigan	473	15.8 ± 0.05	14.5 ± 0.008
Huron	113	13.5 ± 0.13	14.2 ± 0.025
Erie*	5	25.4 ± 2.3	15.5 ± 0.23
Ontario	437	15.8 ± 0.05	14.3 ± 0.007

Table S4. ANOVA table for Proportion Age-0 ($P_{a=0}$). Differences significant at the α = 0.1 level are in bold, while those significant at the α = 0.05 level are in bold and underlined.

Sources of Variation	Sum of Squares	df	Mean Square	F	P
Lake	**0.0514**	**3**	**0.0171**	**3.00**	**0.040**
Error	0.2622	46	0.0057		
Total	0.3136	49			

Tukey HSD multiple comparison table for Proportion Age-0 ($P_{a=0}$). Differences significant at the α = 0.1 level are in bold, while those significant at the α = 0.05 level are both in bold and also underlined.

Lake 1	Lake 2	Difference	SE	P
Ontario	Michigan	0.05	0.04	0.46
Ontario	Huron	0.04	0.04	0.66
Ontario	**Superior**	**0.09**	**0.04**	**0.02**
Michigan	Huron	-0.01	0.04	0.99
Michigan	Superior	0.05	0.04	0.42
Huron	Superior	0.06	0.04	0.25

Table S5. ANOVA table for Mortality (Mort). Differences significant at the α = 0.1 level are in bold.

Sources of Variation	Sum of Squares	df	Mean Square	F	P
Lake	**2341**	**3**	**780**	**2.43**	**0.078**
Error	14789	46	322		
Total	17130	49			

Tukey HSD multiple comparison table for Mortality (Mort). Differences significant at the α = 0.1 level are in bold.

Lake 1	Lake 2	Difference	SE	P
Ontario	Michigan	1.1	9.8	1.00
Ontario	Huron	3.3	9.8	0.97
Ontario	**Superior**	**17.3**	**10.0**	**0.10**
Michigan	Huron	2.2	9.6	1.00
Michigan	Superior	16.2	9.8	0.12
Huron	Superior	14.0	9.8	0.22

Table S6. ANOVA table for Growth Rate ($\bar{G}$). Differences significant at the α = 0.1 level are in bold.

Sources of Variation	Sum of Squares	df	Mean Square	F	P
Lake	**0.0303**	**3**	**0.0101**	**2.53**	**0.069**
Error	0.1834	46	0.0040		
Total	0.2137	49			

Tukey HSD multiple comparison table for Growth Rate ($\bar{G}$). Differences significant at the α = 0.1 level are in bold.

Lake 1	Lake 2	Difference	SE	P
Ontario	Michigan	0.014	0.034	0.95
Ontario	Huron	0.039	0.034	0.42
Ontario	**Superior**	**0.065**	**0.035**	**0.07**
Michigan	Huron	0.026	0.034	0.73
Michigan	Superior	0.052	0.034	0.19
Huron	Superior	0.026	0.034	0.74

Table S7 ANCOVA table for Fecundity with interactive Length x Lake term. Differences significant at the α = 0.1 level are in bold, while those significant at the α = 0.05 level are in bold and underlined.

Sources of Variation	Sum of Squares	df	Mean Square	F	P
Length	**5873**	**1**	**5873**	**184**	**< 0.001**
Lake	**3999**	**4**	**1000**	**31**	**< 0.001**
Length x Lake	61	4	15	0.48	0.751
Error	37427	1175	32		
Total	47360	1184			

ANCOVA table for Fecundity (ANCOVA with additive terms only). Differences significant at the α = 0.1 level are in bold, while those significant at the α = 0.05 level are in bold and underlined.

Sources of Variation	Sum of Squares	df	Mean Square	F	P
Length	**5873**	**1**	**5873**	**31**	**< 0.001**
Lake	**3999**	**4**	**1000**	**184**	**< 0.001**
Error	37488	1179	32		
Total	47360	1184			

Tukey HSD multiple comparison table for Fecundity (based on additive ANCOVA). Differences significant at the α = 0.1 level are in bold, while those significant at the α = 0.05 level are in bold and underlined.

Lake 1	Lake 2	Difference	SE	P
Erie	**Ontario**	**9.6**	**3.5**	**0.002**
Erie	**Michigan**	**9.6**	**3.6**	**0.002**
Erie	**Huron**	**11.9**	**3.6**	**< 0.001**
Erie	**Superior**	**13.1**	**3.5**	**< 0.001**
Ontario	Michigan	0.001	0.7	1.00
Ontario	**Huron**	**2.2**	**1.0**	**0.02**
Ontario	**Superior**	**3.5**	**0.6**	**< 0.001**
Michigan	**Huron**	**2.2**	**1.0**	**0.02**
Michigan	**Superior**	**3.5**	**0.6**	**< 0.001**
Huron	Superior	1.2	1.0	0.37

Supplementary Figures

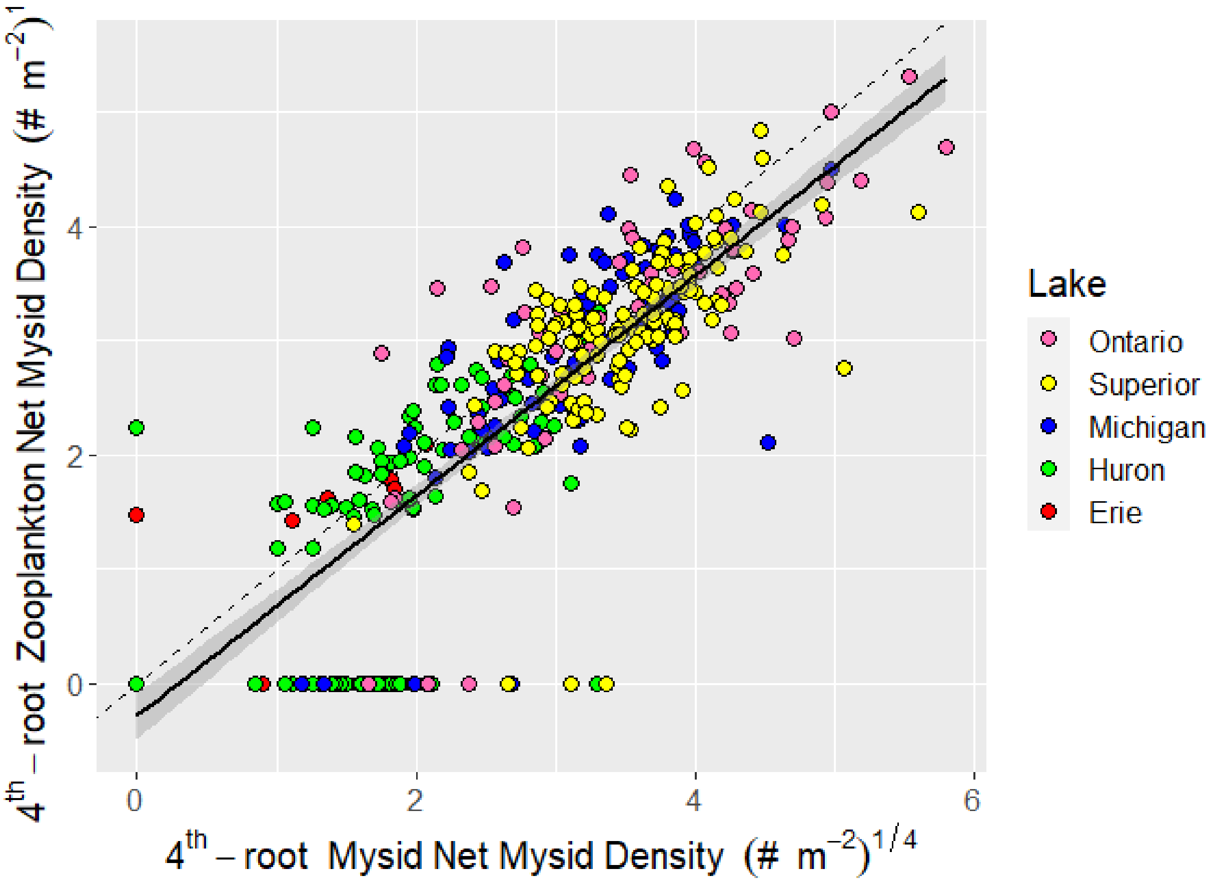

Figure S1. Areal density (#/m^2) in the 250-µm mesh net (mysid net) as a function of areal density in 153-µm mesh net (zooplankton net), with fitted predictions (solid black line), prediction confidence intervals (grey shading), and the 1:1 line (dashed line). The scale is the 4th root of abundances. Note that eight points (six from Erie and two from Huron) had zero *M. diluviana* in both nets and are therefore overlapped in this figure. The equation for the best fit line is: [Zoop Net Areal Density]$^{1/4}$ = 0.96 · [Mysid Net Areal Density]$^{1/4}$ -0.29 (df = 1, 385, F = 838, r^2 = 0.68, p < 0.001).

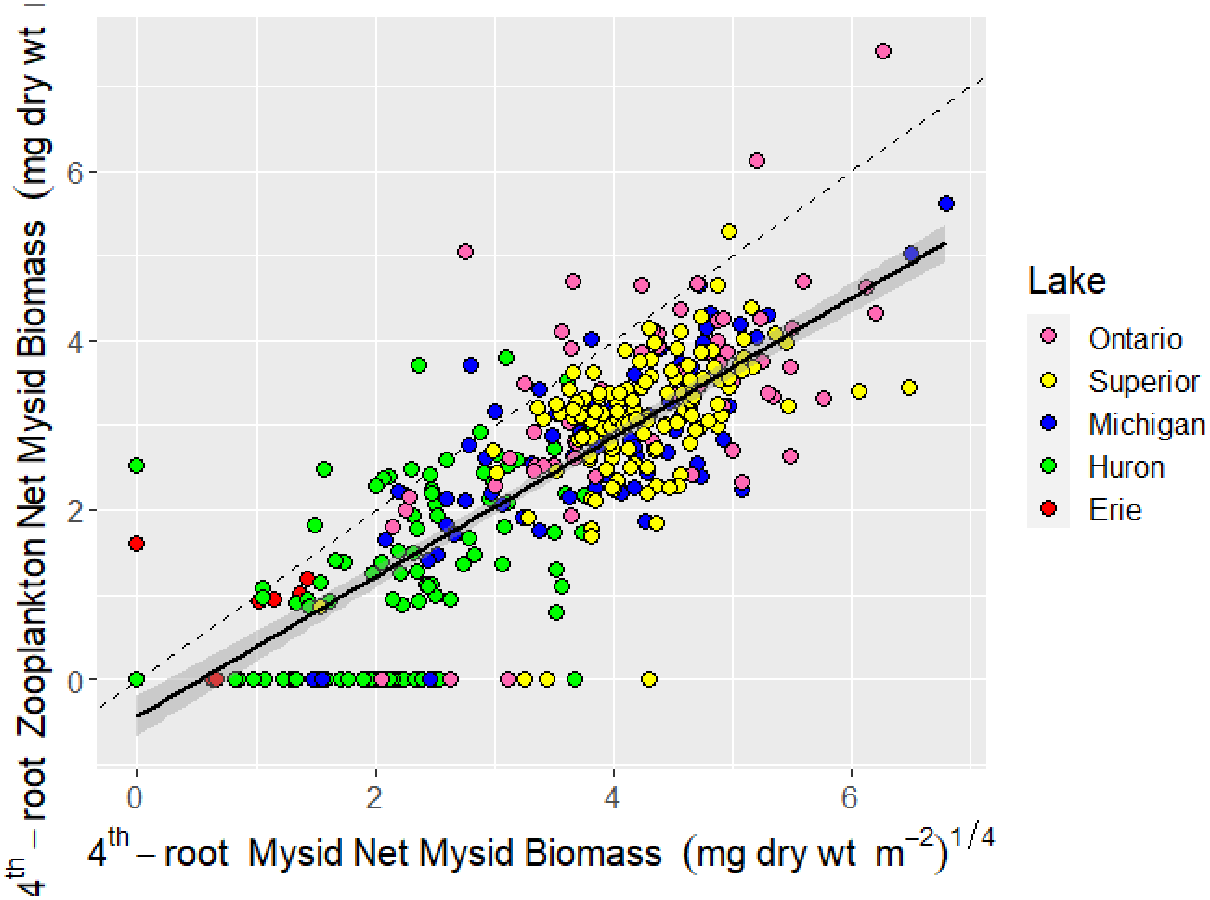

Figure S2. Areal biomass (mg/m^2) in 250-µm mesh net (mysid net) as a function of areal biomass in 153-µm mesh net (zooplankton net), with fitted predictions (solid black line), prediction confidence intervals (grey shading), and the 1:1 line (dashed line). The scale is the 4th root of biomass. Note that eight points (six from Erie and two from Huron) had zero *M. diluviana* in both nets and are therefore overlapped in this figure. The equation for the best fit line is: [Zoop Net Areal Biomass]$^{1/4}$ = 0.82 · [Mysid Net Areal Biomass]$^{1/4}$ -0.44 (df = 1, 385, F = 658, r^2 = 0.63, $p < 0.001$).

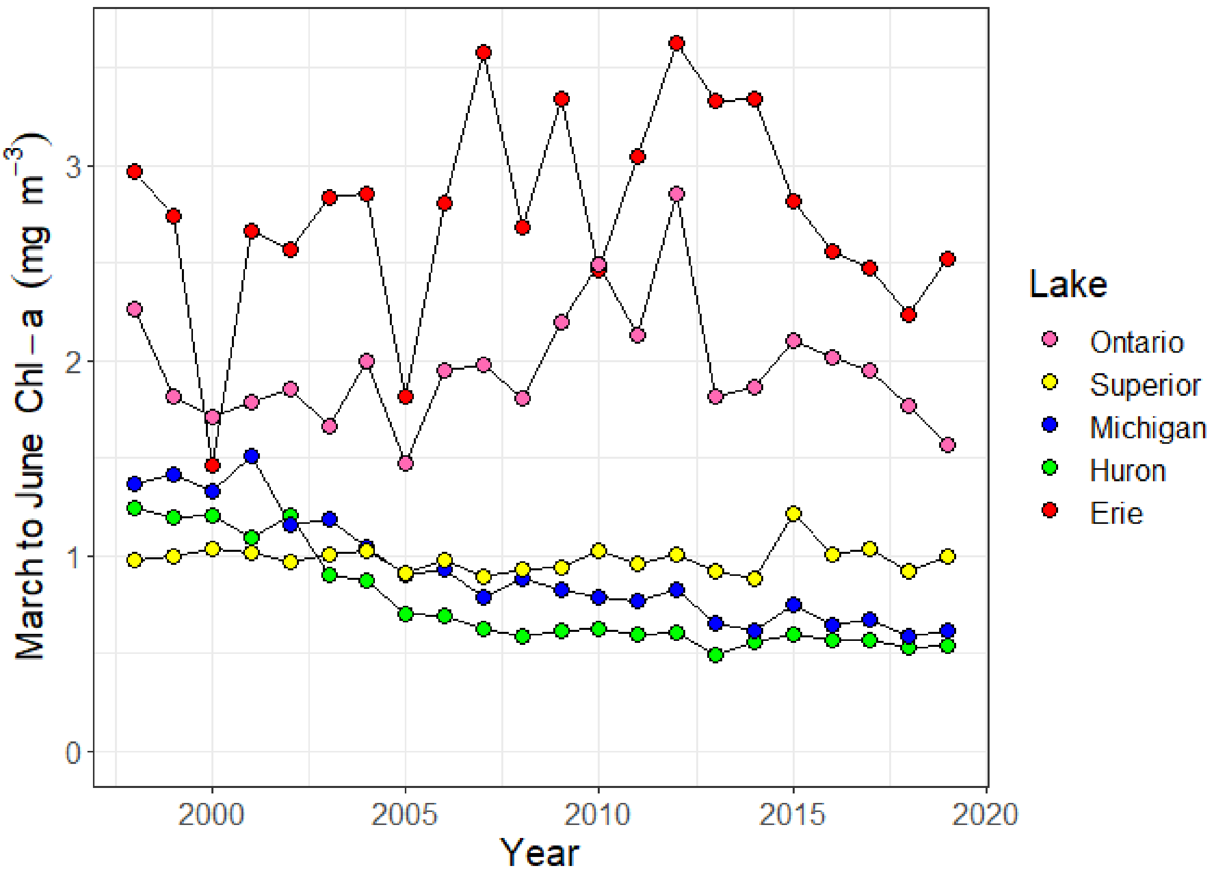

Figure S3. Annual lake-wide spring (March-June) satellite-derived surface chlorophyll-a concentrations at GLNPO monitoring stations (n stations per lake: Eastern Erie: 4, Ontario: 8, Michigan: 11, Huron: 14, Superior: 19). Lake Erie values are for the eastern basin only. Data available at the EPA GLNPO data repository and derived using methods developed by Lesht et al. (2018).

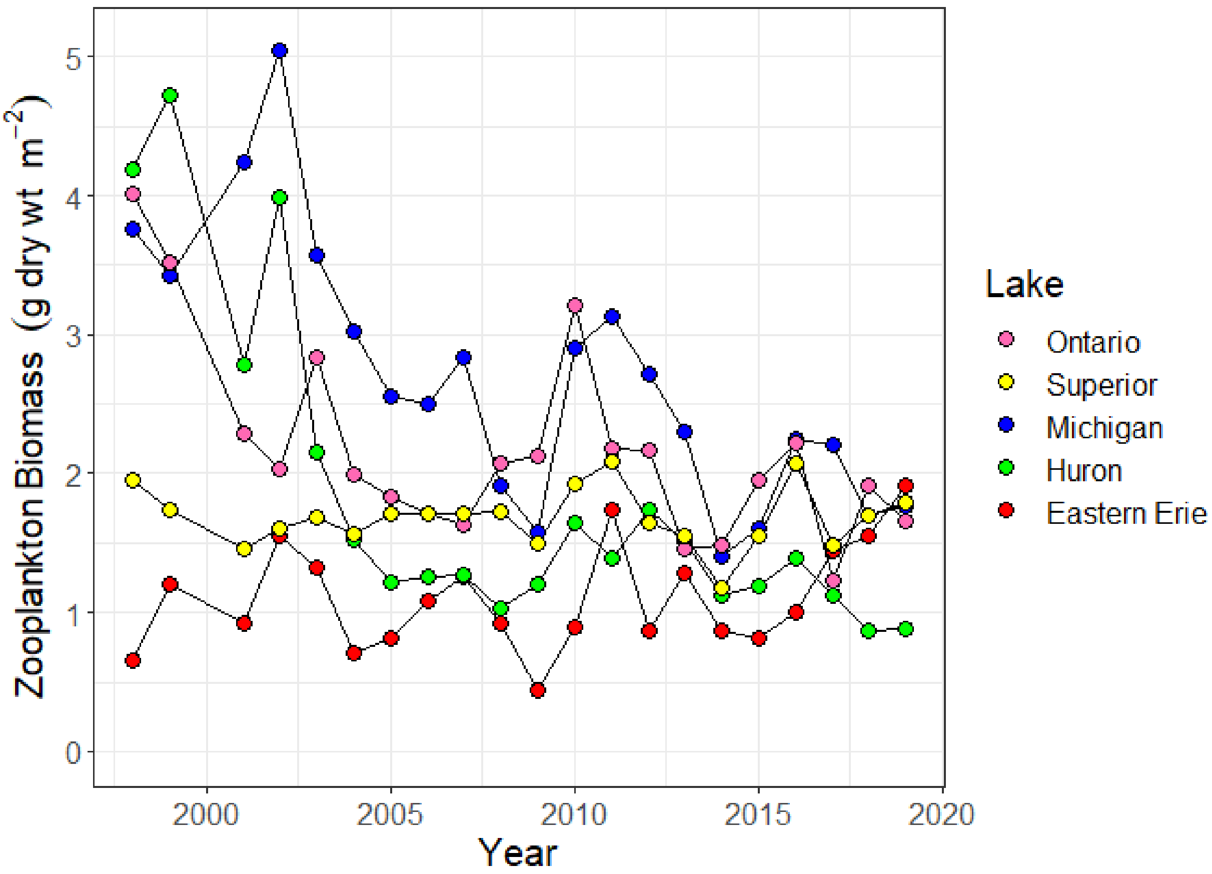

Figure S4. Annual average areal zooplankton biomass based on 153-µm, 100-m tows from GLNPO monitoring stations. Values are averages of annual spring (April) and summer (August) values. Data available in the EPA-GLNPO data repository and methods described in Barbiero et al. (2019).

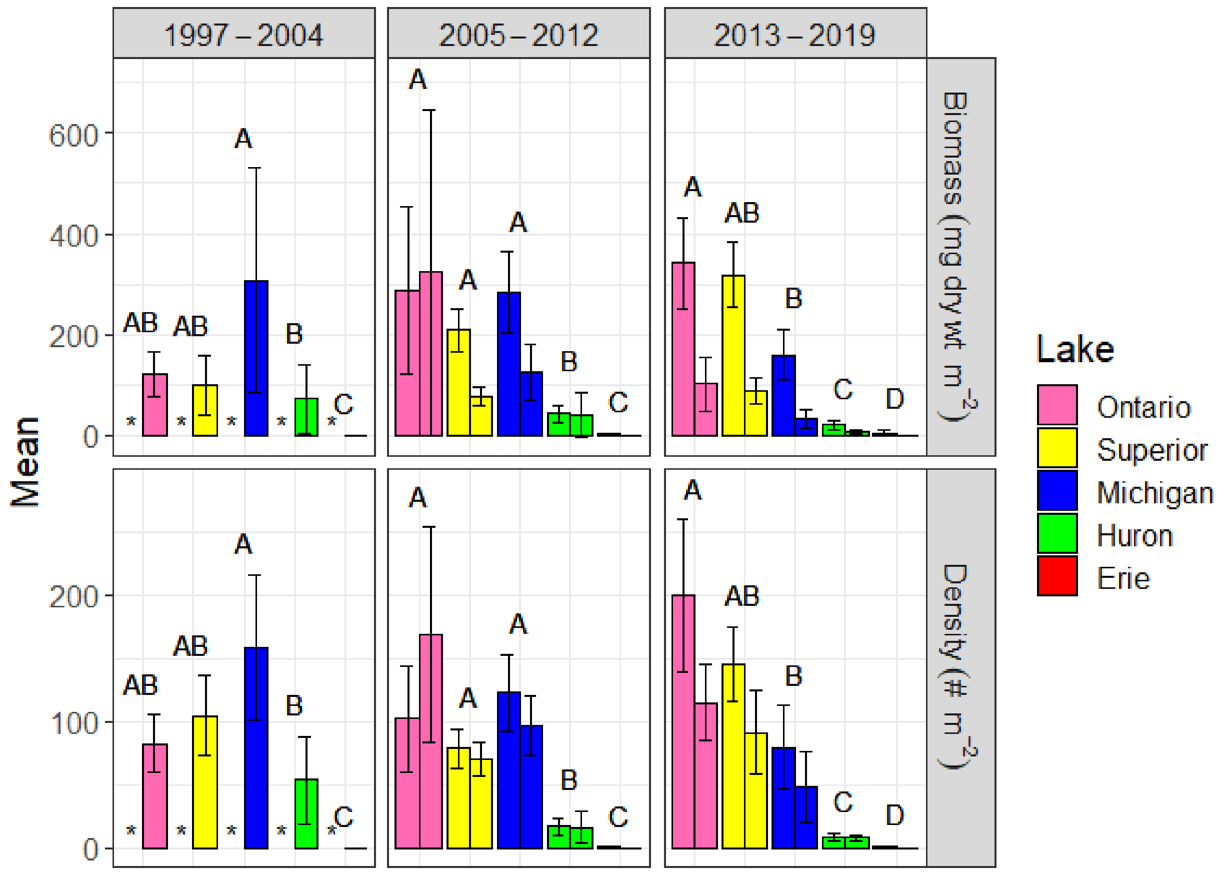

Figure S5. Spring biomass and density (mean ± 2 SE) of *M. diluviana* in each lake during three time periods for the GLNPO mysid net (left bars) and the GLNPO zooplankton net (right bars). ANOVAs of density and biomass as a function of lake, season, and net gear were performed (one per panel) on the log$_e$-transformed version of the data. Lake and season were significant effects in all six comparisons, and net type was significant in three of the four comparisons where it was relevant. Each of these was followed by a Tukey HSD assessment of significant pairwise differences between lakes for each time period (accounting for variation due to net types) resulting in groupings of lakes represented by the letters above the bars (see Tables S1-S6). The '*' symbols in the 1997-2004 time period highlight the absence of samples from the mysid net during those years. A plot of the same values for summer data is in the main text as Fig. 1.

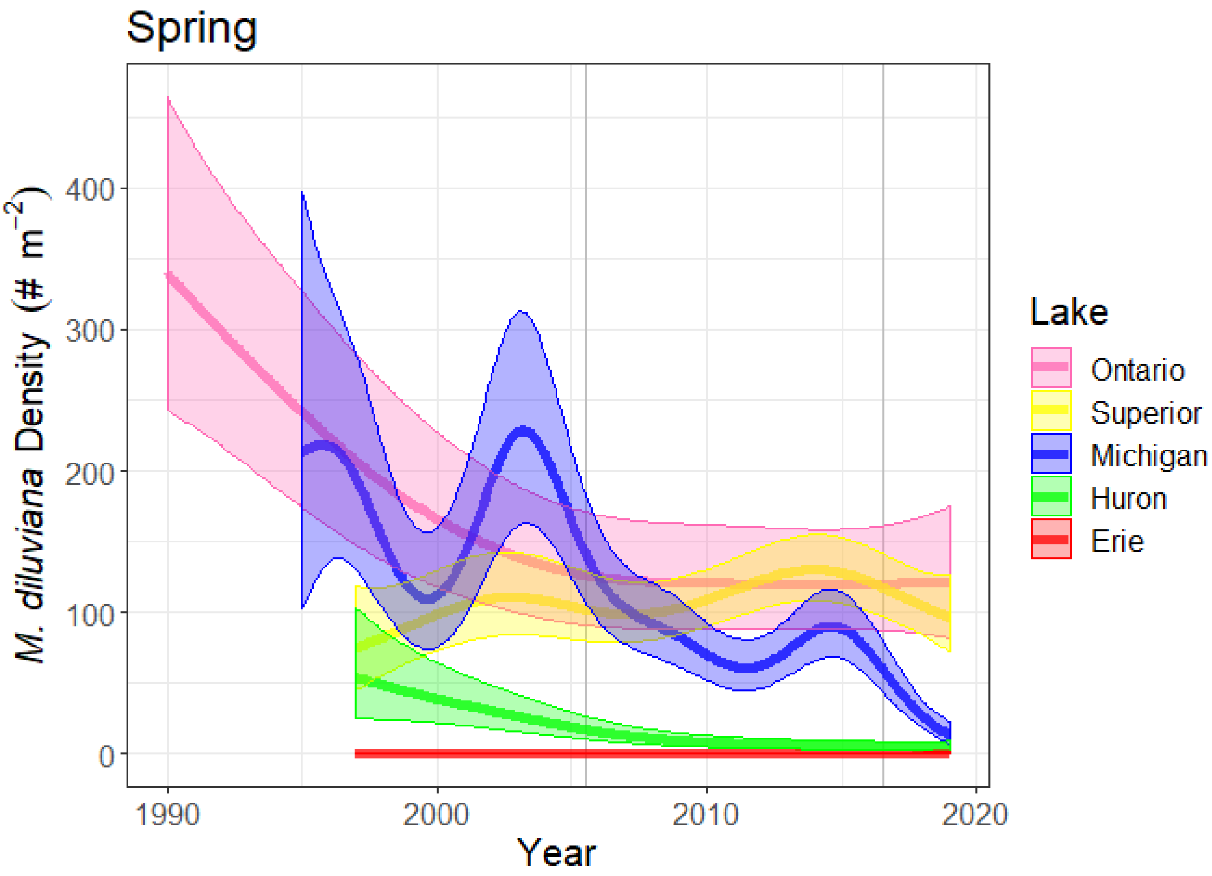

Figure S6. Spring density trends over time in each Great Lake, as predicted by lake specific GAMs fit to all available annual density data. Values represent density from the Spring GLNPO mysid net. Summer and Fall trends will have a different multiplicative intercept but will reflect the same pattern for each lake due the GAM structures. A plot of the summer GAM density fits is in the main text.

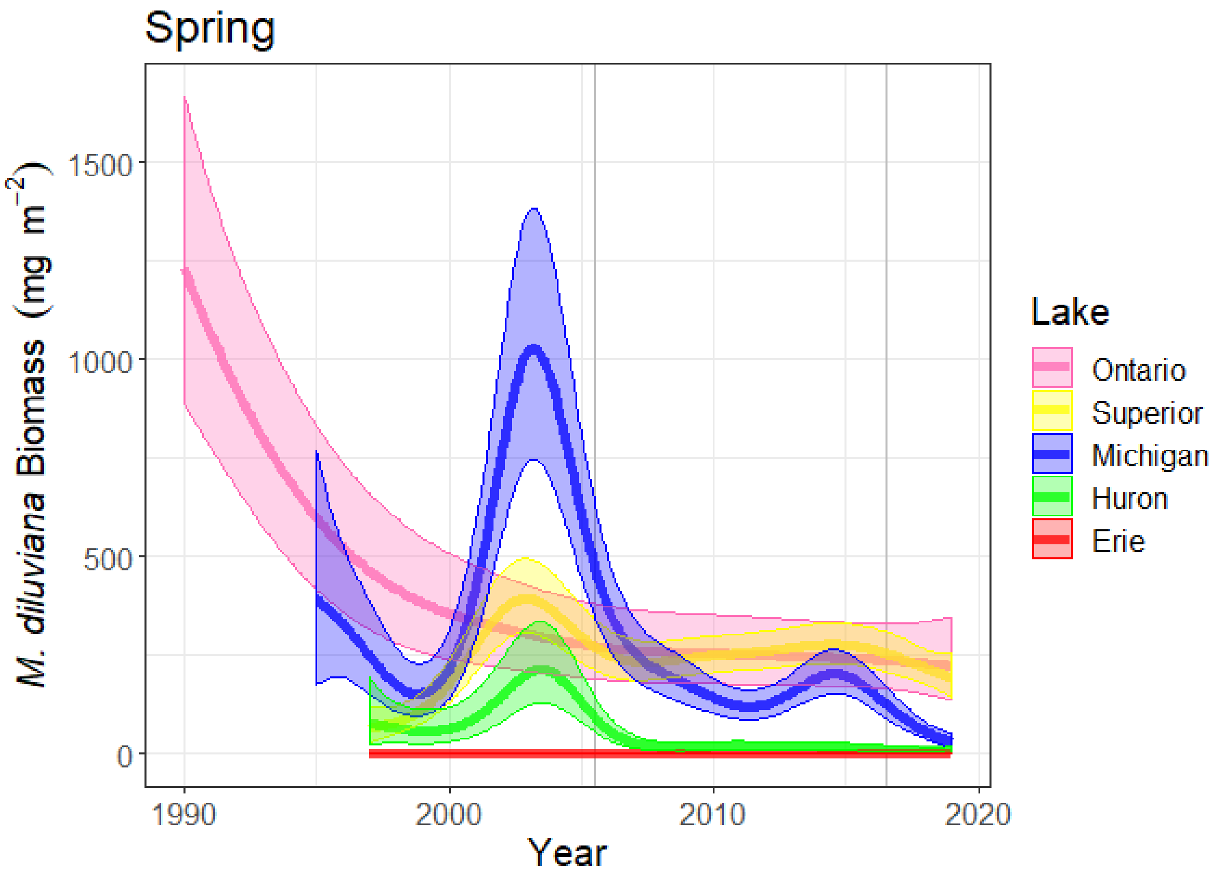

Figure S7. Spring biomass trends over time in each Great Lake, as predicted by lake specific GAMs fit to all available annual biomass data. Values represent biomasses of the Spring GLNPO mysid net. Summer and Fall trends will have a different multiplicative intercept but will reflect the same pattern for each lake due the GAM structures. A plot of the Summer GAM biomass fits is available in the main text.

Declaration of Interest Statement

The authors declare that we have no conflict of interest pertaining to the manuscript

“Evidence for bottom-up drivers of *Mysis diluviana* abundance across the Laurentian Great Lakes”

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