

Factors affecting rotifer assemblages along a nearshore to offshore transect in southeastern Lake Michigan

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Abstract

Rotifer assemblages have been widely used as indicators of trophic conditions. Our goal was to evaluate whether differences in rotifer assemblages along a nearshore to offshore transect in southeastern Lake Michigan were consistent with productivity differences among sites. Sampling took place in March-December during 2013-2019 at a nearshore (17-m deep), mid-depth (45-m deep), and offshore site (110-m deep). *Conochilus* and *Keratella* were present in most samples (84-100%) and were the most abundant taxa overall at each site, combining for around 64-69% by density of the rotifers. Although the productivity gradient was not large between sites, there was a progression with the highest contributions of *Keratella*, *Conochilus*, and *Kellicottia* occurring at the nearshore, mid-depth, and offshore sites, respectively, which is consistent with a gradient of decreasing productivity from nearshore to offshore. The rotifer community also demonstrated strong seasonal patterns. In particular, there was a progression from dominance by illoricate forms, particularly *Synchaeta*, early in the season, to loricate and colonial forms in the summer and fall. Redundancy analysis indicated that in addition to productivity (i.e., total phosphorus), predatory factors (e.g., cyclopoid copepods, *Cercopagis*, *Bythotrephes*) had a strong influence on the rotifer community and seasonal patterns. Although productivity was important in structuring rotifer assemblages along our nearshore to offshore gradient, other factors, especially those related to predation, are difficult to separate from productivity effects. This complexity somewhat limits the usefulness of rotifers as an indicator group for productivity among sites that do not have extreme differences in trophy within a lake.

Introduction

Rotifer dynamics can be important for understanding energy transfer and nutrient cycling in freshwater lakes because of their high grazing rates and their role as an important food source for invertebrate predators (Makarewicz and Likens, 1979; Mazumder et al., 1992; Williamson, 1983). Rotifers can also account for much of the zooplankton biomass or production in some systems (Makarewicz and Likens, 1979; Mazumder et al., 1992). Rotifers can be an important indicator group because they have a high rate of increase and rapid population turnover, making them sensitive to environmental changes (Gannon and Stemberger, 1978; Makarewicz and Likens, 1979).

Rotifer assemblages have been widely used as indicators of water quality or trophic conditions throughout the world (Duggan et al., 2001; Gannon and Stemberger, 1978; Sládeček, 1983), including the Great Lakes (Barbiero and Warren, 2011). Furthermore, rotifers can be classified into feeding guilds (Obertegger et al., 2011) to provide additional information on ecosystem processes (Obertegger and Manca, 2011). However, trophic conditions seldom act in isolation, and other factors likely influence rotifer community composition and abundance as well, including predation, competition, temperature, and food resources (Devetter, 1998). These multiple factors likely complicate assessing the roles of top down and bottom up factors on rotifer communities (Gannon and Stemberger, 1978; Makarewicz and Lewis, 2015; Stich et al., 2018).

The pelagic zone in mid-depth and offshore regions of Lake Michigan underwent rapid changes in trophic conditions in the 2000s, including declining spring total phosphorus levels (Mida et al., 2010) and the loss of the spring phytoplankton bloom (Fahnenstiel et al., 2010; Pothoven et al., 2016). This change was largely attributed to direct and indirect filtering activities of mussels (Fahnenstiel et al., 2010; Vanderploeg et al., 2010). In turn, there have been changes in zooplankton community composition and abundance, including declining cyclopoids and herbivorous cladocerans (Barbiero et al., 2019; Vanderploeg et al., 2012). Although there have been declines in spring chlorophyll concentrations and total phosphorus (TP) in some nearshore areas, it appears that inputs from tributaries can help dampen the potential effects of mussel filtering compared to the offshore, especially during the spring (Pothoven and Vanderploeg, 2020).

There have also been changes in the predatory cladoceran community over time, including the arrival of the invasive *Bythotrephes longimanus* in 1986 (Evans, 1988) and *Cercopagis pengoi* in 1999 (Charlebois et al., 2001), which in turn has affected the native predatory cladoceran *Leptodora kindtii* (Cavaletto et al., 2010). Predatory zooplankton populations also vary seasonally and spatially between nearshore and offshore, with *Cercopagis* most abundant early in the summer and in the nearshore and

Bythotrephes more abundant later in the year and in the offshore (Cavaletto et al. 2010; Pothoven and Fahnenstiel, 2015).

Rotifer populations could be responding to the strong seasonal patterns in the environmental factors as well as to spatial differences between the nearshore and offshore in Lake Michigan. Our goal was to evaluate whether differences in rotifer assemblages along a nearshore to offshore transect in southeastern Lake Michigan were consistent with trophic differences along the transect. Our expectation was that the nearshore site would have a rotifer assemblage reflective of the more productive conditions in the region compared to sites further offshore. The rotifer community structure was also evaluated within the context of seasonal patterns and other factors besides trophic status that could affect rotifers through predation and competition and alter the expected trophic influences on rotifer assemblages.

Methods

Sampling took place in southeastern Lake Michigan at a nearshore site (17-m deep), a mid-depth site (45-m deep), and an offshore site (110-m deep) near Muskegon Michigan during 2013-2019 (see Fig. 1 in Pothoven and Fahnenstiel, 2015). The stations are part of a National Oceanic and Atmospheric Administration (NOAA) long term research project in the area. Sampling took place once each month during March-December each year, although some sampling efforts did not take place as planned due to weather constraints.

During each sampling event, plankton were collected using duplicate tows of a 50-cm diameter, 3-m long, 64- μ m mesh conical net. The net was hauled vertically through the water column at a speed of 0.5 m/s from 1-2 m above bottom to the surface. Plankton were anesthetized with Alka-Seltzer and preserved in 2-4% sugar buffered formalin solution. We assumed net clogging was not an issue due to the length of the net relative to its diameter as well as to the low chlorophyll concentrations in the region, particularly at the deep offshore site.

In the laboratory, an aliquot was taken with a Henson-Stempel pipette so that at least 600 target individuals (i.e., rotifers, nauplii, veligers) were counted from each sample. Rotifers were identified to genus and a subsample of 20 individuals for the most abundant groups in each sample was measured for biomass calculations. Abundance was determined based on net area and tow length, and the average abundance from duplicate tows at each site on a given day was used for analysis. Rotifer measurements were used to calculate biomass using equations from A. Ruttner Kolisko (in Bottrell et al. 1976) and converted to dry weight by multiplying by 0.1, except for *Asplanchna* (0.039). Subsamples of

up to 20 individual nauplii and veligers were also measured for each sample date and lengths were used to calculate dry biomass (Bowen et al., 2018; Culver et al., 1985).

During each sampling event, discrete samples of water were taken from one depth in the upper water column (generally 5-m from surface) with a modified Niskin bottle and poured into an acid-cleaned polycarbonate carboy, which was held in a cooler in the dark until returning to the laboratory and from which all water samples were taken to determine chlorophyll *a* and total phosphorus (TP) concentrations. See Pothoven and Vanderploeg (2020) for processing details for chlorophyll *a* and TP. Secchi depth transparency was measured with a black/white 25-cm diameter disk. Water temperature and specific conductance were recorded using a Seabird SBE19 or SBE19plus CTD (Conductivity-Temperature-Depth) profiler. Whole water tows with a 153- μ m mesh zooplankton net were also used at each site to collect copepods, herbivorous cladocerans, and predatory cladocerans. See Pothoven and Fahnenstiel (2015) for details on zooplankton sampling and analysis methods.

As part of a companion study during 2018-2019, discrete water samples were collected with a Niskin bottle from within the epilimnion (5-m depth) at the 110-m station on nine dates. For each sample, 15-L of water was filtered through a 35- μ m sieve and preserved in sugar buffered formalin. In the laboratory, all rotifers from each sample were counted. We assumed that the concentration of rotifers in the discrete sample at 5-m was representative of rotifer abundance above the hypolimnion, where we assumed most rotifers would be found (Duffy and Liston, 1978; Stemberger and Evans, 1984). Based on temperature profiles, abundance of rotifers was then converted to a whole water column abundance (assuming all rotifers were found above the hypolimnion) for comparison to abundance from the whole water column 64- μ m net tows to evaluate the possibility that the 64- μ m net tows underestimated rotifer abundance (Thomas et al., 2017). Rotifer abundance in the 64- μ m net was plotted as a function of abundance from the 35- μ m estimates, and regression line was determined. We evaluated whether the slope of the regression line differed from 1 and the intercept differed from 0 based on 95% confidence intervals.

To determine the trophic dynamics of the rotifer community, we used the feeding-guild ratio (GR), calculated as (raptorial abundance – microphagous abundance) / total abundance (Smith et al. 2009). GR ranges from -1 to + 1, with GR < 0 indicating dominance by microphagous groups, and GR > 0 indicating dominance by raptorial groups. Rotifers classified as raptorial included *Ascomorpha*, *Asplanchna*, *Collotheca*, *Ploesoma*, *Polyarthra*, *Synchaeta*, and *Trichocerca*, with all other groups collected classified as microphagous, i.e., all malleate and malleoramate groups (Grothe and Grothe, 1977; Stemberger, 1979). Raptorial rotifers feed through active grasping, piercing, or pumping actions

of single food items, whereas microphagous rotifers collect multiple small (15-20 μm) food items (Arfé et al., 2019; Obertegger et al., 2011). Feeding strategy indices such as GR focus on the functional status of a rotifer community and have been used to help understand rotifer community relationships with ecological processes and changes (Arfé et al., 2019; Obertegger et al., 2011; Wen et al., 2017). A general linear model was used to evaluate GR, with site, month, year, and site x month and site x year as factors.

To evaluate rotifer community composition among sites, we used a permutational multivariate analysis of variance (PERMANOVA). For this analysis, only rotifer genera that were found in at least 3% of the samples were used. Rotifer abundance was standardized to the total for each sample site and date, which were used to create a Bray-Curtis similarity matrix. We used fixed effects of site, month, and year as factors, with the highest-level interaction removed due to missing cells. Tests were run with Type III sum of squares, unrestricted permutation of raw data, and 9999 permutations. The PERMDISP routine was used to follow up PERMANOVA to evaluate whether significant differences were due to spatial differences in assemblages or were a function of heterogeneous multivariate dispersions. PERMANOVA and PERMDISP were run using PERMANOVA + for PRIMER (Anderson et al., 2008).

An exploratory analysis of the relationship between rotifer community composition and environmental factors was carried out using CANOCO 5.1 (ter Braak and Šmilauer, 2018). Initial analysis indicated that the maximum gradient length was 2.4 units long with a linear model recommended, so a redundancy analysis (RDA) was used to help explain the variation in rotifer community composition by select environmental variables. The rarest species (< 3% of samples) were excluded from analysis and rotifer composition (percent abundance) was used as the response variable. Response data were arcsin transformed, centered to zero and standardized to unit variance. Selected factors included day of year (DOY), trophic state factors (i.e., chlorophyll concentration, TP concentration, and Secchi depth), water temperature (at 5 m depth), the most abundant potential predators (i.e., *Cercopagis*, *Bythotrephes*, *Leptodora*, cyclopoid copepods, diaptomid copepods, and *Limnocalanus*), and the most abundant potential competitors (i.e., *Daphnia*, *Bosmina*, nauplii, and dreissenid veligers). Predator and competitor data are reported as number/ m^2 to help facilitate comparisons of total abundance across sites with differing depths. Based on program recommendations, chlorophyll and water temperature were log (10x) transformed, and all other factors were log (x+1) transformed, except day of year, Secchi depth and TP, which were not transformed.

Results

Conochilus and *Keratella* were present in most sampling efforts (84-100%) and were the most abundant taxa overall at each site, combining for around 64-69% of the rotifers (Table 1). Overall, the

percent contribution of *Conochilus* to overall abundance was higher at the mid-depth and offshore sites than at the nearshore site, where *Keratella* contributed more to the overall abundance than at the other two sites. *Synchaeta* were primarily important in the spring (Fig. 1), with the highest percent contribution at the nearshore site, whereas the highest overall percent contributions of *Kellicottia* and *Collotheca* were at the offshore site (Table 1). The overall percent contribution of *Polyarthra* was similar among sites.

On average, rotifer population development began in May (nearshore) or June (mid depth and offshore), with *Synchaeta* and *Keratella* generally the most abundant groups (Fig. 1). Peak abundance occurred in July on average, as *Conochilus* and *Keratella* abundance increased. Abundance generally decreased into the fall, especially at the nearshore and mid depth sites. By fall, *Conochilus* was the most abundant group. The highest contributions of *Kellicottia* and *Polyarthra* generally took place during fall. Illoricate forms accounted for >54% of the rotifers at all sites during May and June, but during summer and fall, loricate and colonial forms dominated (Fig. 1).

The assemblage of rotifer taxa differed among sites, (Pseudo $F_{2,93}=2.5$, $P=0.017$), with pairwise comparisons indicating that the nearshore site differed from the other two sites, which did not differ from each other. The site \times month interaction was significant (Pseudo $F_{18,93}=1.5$, $P=0.015$), but the site \times year interaction was not (Pseudo $F_{12,93}=0.4$, $P=0.44$). Overall, PERMDISP analysis indicated no overall difference in dispersion among sites ($F_{2,188}=2.6$, $P=0.18$).

On average, $GR < 1$ indicating dominance by microphagous feeders for most sampling events, except at the offshore site in March, at all sites in May, and at the mid-depth and nearshore sites in June (Fig. 2). Overall, GR was -0.35 , -0.37 , and -0.41 at the nearshore, mid-depth, and offshore sites, respectively, with no overall difference among sites ($F_{2,138} = 0.32$, $P = 0.73$).

There were strong seasonal patterns in environmental variables with differences in levels among sites (Fig. 3). TP and chlorophyll concentrations were highest in spring and at the nearshore site. The difference in trophic state among sites was most evident in spring (i.e., May) when average TP, chlorophyll a , and Secchi depth were $8.3 \mu\text{g/l}$, $4.8 \mu\text{g/l}$, and 4.8 m , respectively, at the nearshore site, compared to $4.1 \mu\text{g/l}$, $1.6 \mu\text{g/l}$, and 14.8 m at the mid depth site and $2.4 \mu\text{g/l}$, $0.6 \mu\text{g/l}$, and 18 m at the offshore site. Surface temperatures varied little among sites except in spring when warming occurred earlier at the nearshore site. The predator *Cercopagis* was most abundant in summer and at the nearshore site (based on areal density), whereas *Bythotrephes* was most abundant in the fall and at the deeper sites. Cyclopoid copepods were most abundant in summer, whereas *Daphnia* were most abundant in the fall, with areal densities of both groups highest at the two deeper sites.

For the RDA analysis, Secchi depth, *Limnocalanus*, chlorophyll, and copepod nauplii were not significant predictors and were not included in the ordination. With the remaining environmental factors, the RDA explained 32% of species-environment variability. Of the explained fitted variation, 85% was explained by the first two axes. Most environmental factors were associated with axis 1, especially DOY, *Bythotrephes* and veligers, which all increased with axis 1 and TP which decreased with axis 1 (Fig. 4). *Cercopagis* and *Leptodora* both increased with axis 2 and were influential factors. *Keratella* was the group most strongly associated with axis 2, increasing with both *Cercopagis* and *Leptodora*. *Conochilus* was positively associated with DOY and *Bythotrephes* and negatively associated with TP. *Kellicottia* was also negatively associated with TP and positively associated with cyclopoid and *Daphnia*. *Synchaeta* was most strongly positively associated with TP and was negatively associated with most other environmental factors

Based on our limited data from the offshore site, there was no evidence that the 64- μm net tows underestimated rotifer abundance compared to grab samples filtered through a 35- μm sieve (Fig. 5a). However, data were highly variable and the regression line was not significant ($R^2=0.28$, $P=0.16$), with a slope of 0.6 (95% CI= - 0.25 to 1.44) and intercept of 2080 (95% CI= - 2105 to 6265). There was some evidence that the rotifer community composition might have differed somewhat between methods, with a smaller proportion of the small bodied *Polyarthra* in the 64- μm net compared to the 35- μm sieve samples (Fig. 5b).

When combined with crustacean zooplankton biomass from the 153- μm tows and nauplii and veliger biomass from the 64- μm net tows, rotifers accounted for a small percentage of the community biomass, i.e., 1.3% (offshore) to 2.4% (nearshore).

Discussion

We found differences in rotifer assemblages across our three sampling sites, especially between the nearshore site and the two deeper sites further offshore. Rotifers are considered an indicator of trophic conditions within water bodies, but the comparison of rotifer communities is often made across a wide range of trophic conditions within or across bodies of water (Gannon and Stemberger, 1978; Oh et al., 2017). However, even within intermediate conditions, rotifers community composition can provide insight into trophic state (Barbiero and Warren, 2011). Although the productivity gradient was not large among our sites, with nearshore generally falling into an oligo-mesotrophic classification and the other two sites into an ultra-oligotrophic state (Vollenweider and Kerekes, 1982; Wetzel, 2001), they did represent a range of productivity. The nearshore region of southeast Lake Michigan is in close proximity to river nutrient inputs, whereas the mid depth and offshore regions have become

increasingly oligotrophic as a result of direct and indirect mussel filtering activities (Fahnenstiel et al., 2010; Pothoven et al., 2016; Pothoven and Vanderploeg, 2020).

Our results are generally consistent with other studies within the Great Lakes, where it is thought that the sequence of relative abundance for the common groups *Polyarthra*, *Keratella*, *Conochilus*, and *Kellicottia* represents a progression from more productive to less productive conditions (Barbiero and Warren, 2011; Gannon and Stemberger, 1978). Even though *Keratella* and *Conochilus* were the most abundant taxa at all our sites, we did see a progression where the highest contribution to the overall community by *Keratella* was at the nearshore site, by *Conochilus* at the mid depth site, and by *Kellicottia* at the offshore site, consistent with a gradient of decreasing productivity from nearshore to offshore. Overall, *Polyarthra* were not abundant and accounted for a similar percentage of the community at all sites, suggesting factors other than productivity were influencing their distribution in our sampling area. The importance of small bodied *Polyarthra* may have also been underrepresented at all sites due to sampling with 64- μm mesh nets. Rotifer groups that are reflective of eutrophic conditions (i.e., *Filinia*, *Trichocerca*, *Brachionus*; see Gannon and Stemberger, 1978) were rare or absent at all sites, although for those that were present, their relative contributions were highest at the nearshore site.

Although the rotifer community differences among our sites were consistent with a gradient in productivity, there was also evidence that other factors were important. First, the PERMANOVA analysis indicated a significant site \times month interaction, which somewhat complicated interpretations. This could be related in part to the strong seasonal patterns noted for some rotifer groups, as well as environmental factors. For example, differences in measures of productivity (TP, chlorophyll, Secchi depth) among sites were strongest in spring, with less difference during the later months when rotifer abundance was highest. Second ordination analysis indicated that although TP was an important environmental factor, chlorophyll and Secchi depth were not, and other environmental factors related to temporal dynamics, predation, and competition had an influence on rotifer community structure across sites. Finally, GR did not differ among sites, indicating that feeding strategy did not differ among sites, with microphagous rotifers that feed on small particles dominating at all sites for most months except early in the season.

Rotifer community structure is known to show a seasonal progression in water bodies throughout the world (Devetter, 1998; Makarewicz and Lewis, 2015; Stemberger and Evans, 1984; Yin et al., 2018). In our study, there was a general progression from a community dominated by *Synchaeta* in the spring to one dominated by *Conochilus* and *Keratella* the summer and by *Conochilus* in the fall.

Contributions by *Kellicottia* were highest in the late summer or fall and by *Polyarthra* in the fall. Seasonal patterns are affected strongly by water temperature (Yin et al., 2018). This pattern is evident in our study with the earlier development of the rotifer population at the nearshore site which warmed up sooner than the deeper sites and is consistent with observations made in 1979 in southeast Lake Michigan (Stemberger and Evans, 1984). However, factors other than temperature are known to influence the seasonal dynamics of rotifers, including the development of predator populations (Stemberger and Evans, 1984) and changes in preferred food availability (Devetter, 1998).

Predation pressure by cyclopoid copepods is a well-known driver of rotifer community structure and in particular the seasonal dynamics of *Synchaeta* (Devetter, 1998; Mazumder et al., 1992; Plaßmann et al., 1997; Stemberger and Evans, 1984). A combination of enclosure and field studies found that soft-bodied *Synchaeta* are a preferred prey of cyclopoid copepods, and predation pressure by cyclopoids was adequate to explain seasonal declines of *Synchaeta* in Lake Constance, Germany (Plaßmann et al., 1997). In Lake Michigan, the shift from a rotifer population dominated by *Synchaeta* in the spring to one dominated by spiny or colonial forms in the summer was attributed to the development of the cyclopoid copepod population (Stemberger and Evans, 1984). In Lake Ontario, increases in cyclopoid abundance between 1970 and 1982 led to the near elimination of *Synchaeta* (Mazumder et al., 1992). Our ordination analysis shows there was a negative association between cyclopoid abundance and *Synchaeta* contribution to the community. However, *Synchaeta* was also negatively associated with other predatory or competitive factors and strongly positively associated with TP concentrations, which were also highest in the spring, especially at the nearshore site. Furthermore, GR levels were only positive in the spring, coinciding with the dominance by the raptorial feeding *Synchaeta*. More productive conditions in the spring could have contributed to the early development of *Synchaeta* prior to the increases in cyclopoids and other predatory and competitive factors.

Although it is possible that cyclopoid copepods are in decline in Lake Michigan, especially in the offshore (Vanderploeg et al., 2012), other predatory invertebrates, including the invasive predatory cladoceran, *Cercopagis pengoi*, also undergo population growth in early summer. *Cercopagis* was found in Lake Michigan in 1999 and is known to impact rotifer communities (Witt et al., 2005). *Cercopagis* abundance generally peaks in early summer, coinciding with the observed decrease in *Synchaeta* and shift in community structure to loricate and colonial groups in our study. A negative association between *Cercopagis* and *Synchaeta* is consistent with lab and field studies in invaded systems in Europe (Lehtiniemi and Linden, 2006, Põllumäe and Kotta, 2007). In Lake Ontario, the arrival of *Cercopagis* was associated with long term declines in the soft bodied *Polyarthra* (Barbiero and Warren, 2011;

Makarewicz and Lewis, 2015). This predation impact led a decoupling of the *Polyarthra* and TP relationship (Barbiero and Warren, 2011). Ordination analysis in our study indicated a negative association between *Polyarthra* and *Cercopagis* as well, and *Polyarthra* were most important in the community structure in the fall, after *Cercopagis* had become rare or absent. On the other hand, the loricate *Keratella* appeared to be positively associated with *Cercopagis*, consistent with other studies (Barbiero and Warren, 2011; Pöllumäe and Kotta, 2007). In fact, ordination analysis indicated that the higher importance of *Keratella* at the nearshore site might be more closely related to the higher abundance of *Cercopagis* at that site than to higher productivity, considering *Keratella* appeared to have limited association with TP. Rotifer community structure can also be influenced by other invertebrate predators, including *Leptodora kindtii* (Pöllumäe and Kotta, 2007) and diaptomid copepods (Williamson, 1987; Williamson and Vanderploeg, 1988), which are the dominant zooplankton group in the lake and can be also be competitors with rotifers.

Another invasive predatory cladoceran, *Bythotrephes longimanus*, appears to have a strong influence on rotifer community dynamics. Although *Bythotrephes* may prey on larger rotifers such as *Asplanchna* (Vanderploeg et al., 1993), their impact on rotifers is largely considered indirect (Barbiero and Warren, 2011; Hovius et al., 2006). We noted that the colonial *Conochilus* was positively associated with *Bythotrephes*. *Conochilus* have become a dominant component of the zooplankton community in Lake Michigan since the arrival of *Bythotrephes* in the late 1980s (Barbiero and Warren, 2011; Branstrator and Lehman, 1991), and the positive association between *Conochilus* and *Bythotrephes* has been observed in other lakes, including Canadian Shield lakes (Hovius et al., 2006) and Lake Maggiore in Italy (Arfè et al., 2019). One possible mechanism is that *Bythotrephes* reduce *Leptodora* numbers, providing a release from predation for *Conochilus* (Barbiero and Warren, 2011; Branstrator and Lehman, 1991). Another possible mechanism is that *Bythotrephes* reduce *Daphnia* abundance, freeing *Conochilus* from competition for resources and allowing rapid population increases (Arfè et al., 2019; Barbiero and Warren, 2011; Hovius et al., 2006). Other zooplankton, especially large cladocerans such as *Daphnia*, are known to reduce the abundance of some rotifers through exploitative competition (Fussmann, 1996) and mechanical interference (Gilbert, 1988; Gilbert and Stemberger, 1985). However, our ordination did not show a negative association between *Conochilus* and *Daphnia*, and this colonial species is likely not affected by mechanical interference from *Daphnia* (Fussmann, 1996).

We did not directly address dreissenid mussel abundance as a factor. Although dreissenids can directly filter rotifers (MacIsaac et al., 1991), this was unlikely to be important at the mid-depth and offshore sites where stratification provided a refuge for rotifers from direct filtration. Dreissenid

mussels in the nearshore of our sampling region can be abundant, but they tend to be very patchy even within a year and are difficult to quantify. For example, on average, dreissenid mussel densities at a nearby 25-m site were high in the nearshore during 2013-2014 ($9,444/\text{m}^2$), but densities ranged between 266 and $39,879/\text{m}^2$ (S. Pothoven, unpublished data). Dreissenid mussel densities averaged $6,104/\text{m}^2$ (range 3,738-9926) and $11,671/\text{m}^2$ (range 8,106-15,792) at the nearshore and nearby offshore site (100-m), respectively during 2013-2019. Dreissenids could indirectly affect rotifers through filtering activities that reduce productivity, with mussels in nearshore or mid depth areas likely also influencing trophic conditions in the offshore (Hecky et al., 2004; Vanderploeg et al., 2010). Unlike adult dreissenids, veligers are found in the water column and could be potential competitors with rotifers. However, if food is not limiting, even high densities of veligers might not lead to reductions in rotifer populations (Winkler et al., 2005). We did find that high abundance of veligers was positively associated with higher contributions of some rotifer groups such as *Polyarthra*, and low contributions from other groups such as *Keratella*, indicating that veliger interactions with rotifers might vary across rotifer groups. These associations could also simply indicate an alignment of seasonal patterns of some rotifer groups with veliger population dynamics.

Trophic gradients could also be expected to produce differences in rotifer abundance (Makarewicz and Lewis, 2015). It is somewhat difficult to compare volumetric abundance across sites of varying depths, but overall average areal abundance was not higher at the nearshore site ($182,312/\text{m}^2$) compared to the mid depth ($259,009/\text{m}^2$) and offshore ($311,445/\text{m}^2$) sites. Furthermore, if we assume that all rotifers were found above the metalimnion at the offshore and mid depth sites during July, when on average abundance was highest, there is little difference in the concentration of rotifers among sites, with average July abundances of $26,832/\text{m}^3$ at the nearshore site, $30,005/\text{m}^3$ at the mid depth site, and $25,475/\text{m}^3$ at the offshore site. Thus, as noted for community composition, it is likely that no single factor controls total abundance of rotifers across depths within our sampling region.

We found that rotifers accounted for a small fraction of crustacean zooplankton biomass. Despite a low biomass, rotifers can still account for much of the secondary production in a lake due to their high turnover rates compared to other zooplankton (Makarewicz and Likens, 1979). The low biomass contribution by rotifers contrasts other studies within the Great Lakes that found rotifers accounted for over half the zooplankton biomass (Mazumder et al., 1992; Thomas et al., 2017). One reason for the difference is that biomass of rotifers in these studies was based at least in part on data collected with smaller mesh (20-30 μm) than used in our study (64- μm). Although we did not find strong evidence for underestimation of rotifer abundance in our 64- μm net tows compared to 35- μm sieve,

data were limited and highly variable, and the potential underestimation of rotifers in 64- μm net tows is well documented (Chick et al., 2010; Thomas et al., 2017). However, despite this potential bias, other studies done with 64- μm nets have effectively documented patterns and changes in rotifer abundance and community composition, including for small bodied *Polyarthra* (Barbiero et al., 2011; Duffy and Liston, 1978; Makarewicz and Lewis, 2015). Another difference between our study and some other studies is that our net tows were done throughout the entire water column rather than just the top 20-m, resulting in lower volumetric abundances, especially at our offshore site, compared to those reported in other studies (Barbiero et al., 2011).

Overall, although trophic status did appear to be a factor that was important in structuring rotifer assemblages along our nearshore to offshore gradient, other factors, especially those related to predation, appear as important and are difficult to separate from productivity effects. This complexity somewhat limits the usefulness of rotifers as an indicator group for productivity among sites that do not have extreme differences in trophy within the lake. Considering the seasonal variation in both rotifer groups and environmental factors, future analyses that look at variation in rotifer assemblages and environmental factors within a site and/or within a specific season would help provide a better understanding of the impact of specific factors, including trophic status, on potential annual variation in rotifer assemblages or among sites within a given season.

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Table 1. Overall average abundance, % of total abundance, and frequency occurrence (%) of rotifer groups at a nearshore, mid-depth, and offshore site in southeastern Lake Michigan during 2013-2019.

Group	Near			Mid			Off		
	#/m ³	%	Freq %	#/m ³	%	Freq %	#/m ³	%	Freq %
<i>Ascomorpha</i>	152	1.2	43.9	80	1.3	39.1	36	1.2	37.7
<i>Asplanchna</i>	582	4.5	89.4	187	3.0	79.7	92	3.2	77.0
<i>Collotheca</i>	424	3.2	37.9	312	5.1	39.1	318	10.9	45.9
<i>Conochilus</i>	4169	32.0	97.0	3368	54.6	84.4	1417	48.7	83.6
<i>Filinia</i>	34	0.3	19.7	2	<0.1	9.4	<1	<0.1	4.9
<i>Kellicottia</i>	364	2.8	90.9	333	5.4	84.4	212	7.3	85.2
<i>Keratella</i>	4277	32.8	100	874	14.2	100	441	15.2	95.1
<i>Monostyla</i>	13	0.1	18.2	2	<0.1	9.4	2	0.1	13.1
<i>Notholca</i>	41	0.3	47.0	12	0.2	31.2	4	0.1	26.2
<i>Ploesoma</i>	385	3.0	74.2	171	2.8	64.1	76	2.6	68.8
<i>Polyarthra</i>	847	6.5	93.9	355	5.8	87.5	177	6.1	82.0
<i>Synchaeta</i>	1710	13.1	89.4	460	7.4	79.7	132	4.5	75.4
<i>Trichocerca</i>	20	0.2	21.2	3	<0.1	10.9	2	0.1	13.1
<i>Trichotria</i>	3	<0.1	4.5	1	<0.1	1.6	1	<0.1	3.3
Other	<1	<0.1	1.5	6	0.1	1.6	<1	<0.1	1.6
Total	13022			6167			2911		

Figure Legends

Fig. 1. Average monthly rotifer abundance (top), percent contribution (middle,) and percent contribution of illoricate, loricate, and colonial forms (bottom) at a nearshore, mid-depth and offshore site in southeast Lake Michigan during 2013-2019.

Fig. 2. Average monthly guild ratio (GR) for rotifers from a nearshore, mid-depth, and offshore site during 2013-2019. GR ranges from -1 to + 1, with GR < 0 indicating dominance by microphagous groups and GR > 0 indicating dominance by raptorial groups.

Fig. 3. Average monthly values for environmental factors used in redundancy analysis (RDA) at a nearshore, mid depth, and offshore site during 2013-2019. Secchi depth and chlorophyll were not significant and were not included in the RDA, but are shown here to demonstrate productivity differences among sites.

Fig. 4. RDA species + environmental variables biplot, showing relation of rotifer groups to selected environmental variables. Only environmental factors with a significant effect are shown.

Fig. 5. a) Whole column rotifer abundance from the offshore in 2018-2019 determined using 64- μm nets as a function of whole water column rotifer abundance estimates derived from discrete water samples taken in the epilimnion and filtered through a 35 μm mesh (see methods for details). The dashed line represents a 1:1 relationship, and the solid line is the regression through the points. b) Overall rotifer community composition for whole water column samples collected in 64- μm net tows and for discrete samples from the epilimnion and filtered through 35- μm mesh.









