Six decades of Lake Ontario ecological history according to benthos

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Abstract

The Laurentian Great Lakes have experienced multiple anthropogenic changes in the past century, including cultural eutrophication, phosphorus abatement initiatives, and the introduction of invasive species. Lake Ontario, the most downstream lake in the system, is considered to be among the most impaired. The benthos of Lake Ontario has been studied intensively in the last six decades and can provide insights into the impact of environmental changes over time. We used multivariate community analyses to examine temporal changes in community composition over the last 54 years and to assess the major drivers of long-term changes in benthos. The benthic community of Lake Ontario underwent significant transformations that correspond with three major periods. The first period, termed the pre/early *Dreissena* period (1964-1990), was characterized by high densities of *Diporeia*, Sphaeriidae, and Tubificidae. During the next period defined by zebra mussel dominance (the 1990s) the same groups were still prevalent, but at altered densities. In the most recent period (2000s to present), which is characterized by the dominance and proliferation of quagga mussels deeper into the lake, the community has changed dramatically: *Diporeia* almost completely disappeared, Sphaeriidae have greatly declined, and densities of quagga mussels, Oligochaeta and Chironomidae have increased. The introduction of invasive dreissenids has changed the Lake Ontario benthic community, historically dominated by *Diporeia*, Oligochaeta and Sphaeriidae, to a community dominated by quagga mussels and Oligochaeta. Dreissenids, especially the quagga mussel, were the major drivers of these changes over the last half century.

Keywords: Lake Ontario, benthic invertebrates, long-term changes, community analysis, invasive species, *Dreissena*.

Introduction

Freshwaters are among the most endangered ecosystems due to increasing human impacts over the past century (Strayer and Dudgeon, 2010; Geist, 2011). The Laurentian Great Lakes, which support large populations and major economies in North America, have experienced impacts of multiple anthropogenic stressors in the past century, including watershed deforestation, overfishing, cultural eutrophication and contamination, and introduction of invasive species (Beeton, 1965; Stoermer et al., 1993; Mills et al., 1993b; Sturtevant et al., 2019). Lake Ontario is the smallest lake in the system by surface area (18 960 km^2) with approximately 20% of Great Lakes basin population living in the watershed; but most of the lake's inflow (86%) comes from the upper Great Lakes via the Niagara River (Mills et al., 2003). Lake Ontario's position as the most downstream lake in the system is part of the reason it was considered among the most impaired of the Great Lakes (Allan et al., 2013). For instance, sediments in Lake Ontario exhibited elevated contamination compared even to Lake Erie (Marvin et al., 2002). Lake Ontario was often the first to be colonized by Atlantic Ocean invaders such as sea lamprey (*Petromyzon marinus*) and alewife (*Alosa pseudoharengus*) that harmed native fish populations (Christie, 1972; Mills et al., 2003). Similar to the other Great Lakes, with the exception of perhaps Lake Superior, Lake Ontario experienced degradation in water quality, loss of shallow water habitat, and shifts in food webs in the 1950s and 1960s (Mills et al., 2003). Cultural eutrophication, a major destabilizing force of the Lake Ontario ecosystem from the 1940s to the 1970s, led to nuisance algal blooms and water quality deterioration (Schelske, 1991). The 1972 Great Lakes Water Quality Agreement (GLWQA) between the United States and Canada resulted in controls on phosphorus loadings to each of the Great Lakes, and initiated ecosystem recovery (Mills et al., 2003). However, the pivotal events for the ecosystem in the 1990s were not planned, science-based management actions, but rather the invasions of exotic species from Eurasia (Mills et al., 2003), of which the most impactful were the dreissenids (*Dreissena polymorpha*, the zebra mussel, and *Dreissena rostriformis bugensis*, the quagga mussel).

Benthic invertebrate community assessment has proven useful in both classifying and monitoring the impacts of anthropogenic stress in aquatic systems due to the relative longevity of taxa, wide range of functional traits, and well-documented responses to different stressors (Barbour et al., 1999; Hilsenhoff, 1988; Howmiller and Scott, 1977; Nalepa and Thomas, 1976; Milbrink, 1973; Burlakova et al., 2018a, 2018b). Analysis of temporal trends in the structure of Lake Ontario benthic macroinvertebrate assemblages could provide a broad perspective into the lake's environmental history since the 1960s. Further, among the deep Great Lakes (all lakes except Lake Erie), Lake Ontario has the longest history of dreissenid invasion (since 1989 for zebra mussels, Griffiths et al., 1991, and since 1990 for quagga mussels, Mills et al., 1993a), and can therefore provide valuable insights into effects of dreissenids on benthic communities.

The benthos of Lake Ontario has been studied intensively, particularly in the last six decades (Mozley, 1990; Nalepa, 1991). The first benthic samples were collected in Lake Ontario in 1872 (Nicholson, 1873), and the first large systematic survey was conducted in 1964. Llake-wide benthic surveys have been carried out almost every decade since then. Multiple studies have analyzed temporal changes in the benthic community on a lake-wide basis (Nalepa, 1991; Barton and Anholt, 1997; Dermott and Geminiuc, 2003; Lozano et al., 2003; Mills et al., 2003; Lozano, 2011; Birkett et al., 2015), for parts of the lake (e.g., Stewart and Haynes, 1994; Haynes et al., 2005; Dermott et al., 2012; Barrett et al., 2017), or have focused on changes in specific taxa (e.g., Sly and Christie, 1992; Watkins et al., 2007). Most studies agree that the most important changes were the decline in abundance of major benthic groups during the 1960s, and the collapses in *Diporeia* and Sphaeriidae populations in the late 1990s. Although some recent works have analyzed long-term benthic community changes in specific portions of the lake (Dermott et al., 2012; Barrett et al., 2017); to date no studies have followed the dynamics of benthic changes since the 1960s, and no studies have provided a detailed account of changes in taxonomic community structure over the entire lake.

In this study we compare results of our lake-wide survey that we conducted in 2018 with historical data to examine the long-term changes in the benthic community of Lake Ontario over the last half century. We used multivariate community analyses to examine the temporal changes in benthic community composition, and to assess the major drivers of the changes.

Methods

2018 sampling protocol

In August - September 2018, a total of 165 benthic samples was collected from 55 stations in Lake Ontario (Fig. 1) aboard the U.S. Environmental Protection Agency's Great Lakes National Program Office (US EPA GLNPO) R/V *Lake Guardian*. Nine of these stations were sampled in August 14-15, 2018 during the US EPA GLNPO Great Lakes Biological Monitoring Program's Long-Term Monitoring (LTM, Great Lakes Monitoring, 2021) summer survey, and 46 stations were sampled between September 10-18, 2018 during the Lake Ontario Cooperative Science and Monitoring Initiative (CSMI) benthic survey (Cooperative Science and Monitoring Initiative, 2021). Field operations were conducted as per US EPA Standard Operating Procedures for Benthic Invertebrate Field Sampling SOP LG406 (US EPA, 2018).

Triplicate samples for benthic invertebrates were collected at each station using a Ponar grab (sampling area 0.0523 m^2) to determine benthic density (number of individuals per m^2) and total wet biomass (g/m^2). Samples were elutriated through a 500 µm mesh sieve and preserved with neutral buffered formaldehyde with Rose Bengal stain to a final formalin concentration of 5 - 10%. Organisms were picked out of samples under low magnification using a dissecting microscope. *Dreissena* spp. were identified to species, counted, shell length measured to nearest 0.1 mm with a caliper, and weighed (shell and tissue wet weight) by 5-mm size fractions to the nearest 0.0001 gram after being blotted dry on absorbent paper. All *Dreissena* collected during these 2018 surveys were quagga mussels (*D. rostriformis bugensis*). Other major taxonomic groups (Amphipoda, Chironomidae, Oligochaeta, Mollusca) were identified, counted, and weighed after being blotted dry. After counting and weighing by relative size category (extra-large, large, medium, and small), Chironomidae and Oligochaeta were mounted on slides for identification and identified using a compound microscope; other organisms were identified using a dissecting microscope. Adult oligochaetes were identified to species; immatures were taken to the lowest taxonomic level possible, usually family, and included in abundance estimates. Oligochaete fragments were excluded from density but used for biomass estimates because they could be weighed but not attributed to individuals. Density and biomass of immature oligochaetes (in cocoons) were recorded but were not considered in density nor in biomass. Chironomidae were identified to the lowest practical taxonomic level, usually genus. Other invertebrates were identified to species, when possible. Meiobenthic organisms occasionally present in samples (e.g. Nematoda, Hydracarina, Ostracoda, benthic Cladocera, Copepoda, and Harpacticoida) were not recorded in our samples and were excluded from historical data, if present. Details are described in Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis (SOP LG407) (US EPA, 2015). For convenience, we will use a combined name "*Dreissena*" or "dreissenids" unless we refer to a particular species (*D. polymorpha, D. rostriformis bugensis*), and "*Diporeia*" when referring to *Diporeia hoyi.* A phylogeographic analysis of deep-water amphipod *Diporeia* across glaciated North America found no evidence to suggest the existence of two or more phylogenetic species (Usjak, 2010), nor does genetic analyses support the presence of more than one *Diporeia* species within a Great Lake or across the Great Lakes although the Lake Superior *Diporeia* are genetically distinguishable from the *Diporeia* in lakes Huron, Michigan, and Ontario (Pilgrim et al., 2009; Bistolas et al., 2017). We report *Diporeia* densities separately from other amphipods (e.g., *Gammarus*, *Echinogammarus*, *Hyalella*, grouped in historical analysis as "Amphipoda") due to its historical role in the food web of the Great Lakes (Gamble et al., 2011a, b).

Environmental parameters were measured using a conductivity/temperature/depth (CTD) probe and sensors attached to a Rosette sampling device, and included bottom temperature (°C), dissolved oxygen (mg/L), chlorophyll *a* fluorescence (µg/L), beam attenuation (1/m, used as a measure of turbidity), and specific conductance (µS/cm). Sediment type was assessed using major descriptive categories (1 – clay and silt, 2 – silt, 3 – silt and sand, and 4 – sand) of sediments in the benthic samples before washing. Sediment nutrients (concentration of total phosphorus (STP, mg P/g), organic carbon (SOC, mg C/g), and total nitrogen (STN, mg N/g)) were collected from a separate Ponar (SOPs LG600 -

LG602, US EPA, 2004a, 2004b, 2005) at each of the Ponar stations. In addition, we used estimates of spring, summer, and growing season (spring + summer) surface chlorophyll concentration at each of the benthos stations derived from observations collected by the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite (Lesht et al., 2016).

Historical data

The first lake-wide study of Lake Ontario benthos was conducted in September 1964, when Hiltunen (1969) collected bottom samples from 24 stations ranging in depth from 11 to 225 m (Table 1). This survey was followed by lake-wide studies conducted in November 1972 (Nalepa and Thomas, 1976) and in September 1977 (Golini, 1979). Beginning in 1990, benthic surveys were conducted on a regular basis, largely to determine carrying capacity of lake forage base to inform salmon stocking programs, and to assess the impacts of the invasion of zebra and quagga mussels: in 1990 and 1995 (Dermott and Geminiuc, 2003); 1994 and 1997 (Lozano et al., 2001); 1998, 1999, and 2003 (Watkins et al., 2007); 2008 (Birkett et al., 2015); and in 2013 (Nalepa and Baldridge, 2016). Thus, including our 2018 survey, 13 lake-wide benthic surveys were conducted in Lake Ontario over the course of 54 years (1964 - 2018), and for 11 of them we were able to acquire species-level data (Table 1). More benthic studies were conducted in 1967-1970: by Kinney (1972) and 16 surveys conducted from 1964 to 1966 by the Great Lakes Institute at the University of Toronto (Rodgers, 1972, re-analyzed later by Barton and Anholt, 1997); however, due to different design, sampling methods and seasons, and unavailability of the primary data, these surveys were not used in our analysis.

Historical surveys varied in sample design (random selection of sampling stations vs. fixed selected stations) and numbers of stations sampled per year (from 24 in 1964 to 151 in 1977). Sampling design of the first two surveys (1964 and 1972) aimed to provide a broad and representative coverage of the lake (Hiltunen, 1969) as well as depth zones (Nalepa and Thomas, 1976). The 1977 survey followed a polygonal grid pattern (Thomas, 1969) with some modifications (Golini, 1979). Benthic samples in 1994 and 1997 surveys were collected based on a randomized design using a systematic grid (Lozano et al., 2001), and 1998 and 1999 surveys followed the same design (Watkins et al., 2007). The rest of the surveys (2003, 2008, 2013, and 2018, Watkins et al., 2007, Birkett et al. 2015, Nalepa and Baldridge, 2016) followed the design of 1990 and 1995 benthic surveys conducted along a series of transects (Dermott and Geminiuc. 2003). Surveys also used different sampling gear (Smith-McIntyre dredge in 1964, Shipek grab in 1977, and Ponar in all other surveys), the mesh size of the sieve used to wash sediments (from 150 µm in 1977 to 600 µm in 1964, 1972, 1990 and 1995), and the numbers of sampling events per year (Table 1). The sampling efficiencies of Ponar and Smith-McIntyre grabs are similar (reviewed in Sly and Christie, 1992), but the Shipek grab lacks top screens to reduce the effect of an advance pressure wave on sample efficiency (Nalepa et al., 1988). We converted Shipek densities (Golini, 1979) to Ponar equivalents using taxa-specific sampling efficiency conversions presented by Sly and Christie (1992) (2.5 for Oligochaeta, 1.7 for Chironomidae, 1.4 for *Diporeia*, and 1 for other groups). The level of taxonomic identification and taxa reported varied among surveys from reporting only dreissenids and *Diporeia* to reporting all species collected (Table 1). Using original unpublished species data for 1972 sampling we corrected some of the group densities reported in Nalepa and Thomas (1976). Due to some discrepancies between benthos densities provided in the text and those given in the data appendix in Birkett et al. (2015), we reviewed the original counting sheets and corrected a few errors; as a result, our 2008 density values are not identical to the values published in Birkett et al. (2015). The weight of benthic invertebrates was measured in only five of the surveys and different units were used to report biomass, including wet weight in 1990, 1995 (Dermott and Geminiuc, 2003), and 2018 (our data), dry weight in 2008 (Birkett et al., 2015), and ash-free dry weight in 2013 (Nalepa and Baldridge, 2016). Due to limited biomass data and inconsistent metrics over time, historical comparisons were performed on the basis of density only. Because different stations were sampled throughout the years, we calculated average benthos densities (± standard error) in each lake zone (0 – 30 m, >30 – 50 m, >50 – 90 m, and > 90 m), and calculated lake-wide density for each year as depth-weighted average using means of stations located at these four depth zones considering the proportion of the total lake area represented by each zone (21.6, 11.7, 18.5, and 48.2%, respectively) (Manly, 2009) (Electronic Supplementary Material (ESM) Table S1). Please note that due to non-random nature of most survey designs, the lake-wide averages may be biased.

Data analysis

Environmental parameters were analyzed using Principal Component Analysis and were normalized prior to the analysis in the statistical software R version 3.6.1 (R Core Team, 2019). We used a non-parametric Kruskal-Wallis test to test whether the environmental parameters, total combined density and biomass of all species in the zoobenthos collected in Lake Ontario in 2018 differed among lake regions (east, central, and west), or depth zones (shallow, <30 m, intermediate: >30 - 50 m and >50 - 90 m, and deep, >90 m). These analyses were performed on data combined by depth zone using STATISTICA software (STATISTICA data analysis software system, version 13, StatSoft, Inc. 1984 - 2018, www.statsoft.com).

For all analyses on benthic community structure, we used species (or higher taxa) densities collected from all stations sampled in 1964 – 2018. Because the level of taxonomic resolution was inconsistent throughout the years, species with rare occurrences (1 - 3 total in the whole dataset) were pooled into higher taxonomic units (e.g., family) to be able to compare the data. We used the Integrated Taxonomic Information System (ITIS, https://www.itis.gov/) for current taxonomy, but in a few cases we used taxonomic names listed in common identification guides (i.e. species groups). For example, in this paper we are using the old taxonomic names consistent with historical data: Sphaeriidae for family Pisidiidae, and the old division of class Oligochaeta (currently Clitellata) into Enchytraeidae, Lumbriculidae, Naididae, and Tubificidae, even though only three orders are currently recognized (Enchytraeida, Lumbriculida, and Tubificida) and the former families of Naididae and Tubificidae in the order Tubificida are now combined in Naididae (Erseus et al., 2008).

We analyzed changes in community structure through time with nonparametric multivariate statistical techniques on data matrices of all species and their abundances found in the community in R version 3.6.1 (R Core Team, 2019). We chose a fourth root transformation of densities for all analyses to

(1) down-weight the importance of highly abundant species (e.g., *Dreissena*), and to (2) allow both the midrange and the rare species to contribute to the similarity matrix. This choice of transformation was guided by constructing shade plots to visualize the effects of transformation on the data matrix (Clarke et al., 2014b). The similarity of the community composition was summarized by calculating Bray-Curtis similarity indices (BC) ranging from 0 (no species in common) to 1 (identical samples) (Bray and Curtis, 1957; Clarke, 1993). To visualize the differences among assemblages we used Non-metric Multi-Dimensional Scaling (NMDS), which calculates a set of metric coordinates for samples, most closely approximating their nonmetric distances. NMDS was found to be consistently reliable in a comparative study of ordination methods for community data (Kenkel and Orlóci, 1986). For complete description of the methods used please see Clarke and Green (1988), and Clarke (1993).

We tested for differences in community composition among different depth zones, lake regions, and years with Analysis of Similarities (ANOSIM), a resampling technique that uses permutation/randomization methods on BC similarity matrices to identify differences among groups of samples, after which pairwise comparisons are conducted (Clarke, 1993) in PRIMER 7. Large values of the test statistic (*R*) indicate high degree of separation of groups, and small values (close to 0), little or no separation, and its value is at least as important as its statistical significance, which is limited by the number of available permutations (Clarke et al., 2014a).

To find the Spearman rank correlation between the biotic resemblance matrix (BC of fourth-root transformed 2018 benthic density) and the seriation resemblance matrix of environmental parameters (Euclidian distances of log-transformed and normalized variables) or years we used the PRIMER RELATE algorithm (Clarke et al., 1993). We used BEST analysis on 2018 dataset to select environmental variables that best explain benthic community patterns by maximizing a Spearman rank correlation between biotic and environmental resemblance matrices (Clarke and Ainsworth, 1993).

We used “Similarity Percentage” (SIMPER) analysis to examine the contribution of each species (or aggregated the species to family or class) to the average BC similarity among communities within each level of factors tested. In addition, we determined the contribution of each species to the average BC dissimilarity between these groups. “Similarity profile” permutation tests (SIMPROF routine) were used to test for structure in the data (Clarke and Gorley, 2006). To distinguish communities among years, we used Cluster analysis followed by SUMPROF to find significant difference among clusters. To analyze the temporal changes in taxa densities, we used non-parametric Spearman correlation coefficients. All test effects were considered significant if $P < 0.05$.

We examined changes in overall benthic community structure by major taxonomic groups with non-metric multidimensional scaling (NMDS), perMANOVA, and permutational ANOVAs. NMDS was performed on fourth-root transformed data with Bray-Curtis distance using the R package “vegan” (Oksanen et al., 2019). We plotted NMDS axes according to major factors (e.g., year, depth zone) with ellipses that encompass 80% of the data for each specified group. The purpose of perMANOVAs was to test for significant differences in overall community composition over time with depth zone and stage of *Dreissena* invasion as covariates. PerMANOVAs were also performed with the R package “vegan” (Oksanen et al., 2019). In the case of significant effects of years, we followed perMANOVAs with pairwise comparisons using the R package “RVAideMemoire” (Hervé, 2020). We then performed permutational ANOVAs (R package "lmPerm"; Wheeler and Torchiano, 2016) with 5000 permutations on total benthic density and density of specific taxonomic groups to test for differences among years. Depth zone was included as a covariate. Permutational tests were chosen because data were non-normal and could not be successfully transformed. All analyses for taxonomic group data are presented with and without *Dreissena* included. We used $\alpha=0.05$ to determine significance for perMANOVAs and permutational ANOVAs.

Results

2018 Lake Ontario benthic community and environment

A total of 76 benthic invertebrate taxa was collected in Lake Ontario in 2018. The most diverse taxa were Oligochaeta (33 species and higher taxa), Chironomidae (28), Malacostraca (six total: five Amphipoda (here including *Diporeia*), and one Mysida), and Bivalvia (3). All *Dreissena* collected during this survey were quagga mussels (*D. r. bugensis*). Other classes were represented by fewer than 3 taxa, or were not identified to species level (e.g., Trepaxonemata, Hirudinea, Hydrozoa, Nemertea). Among Oligochaeta, the most diverse taxa were in the family Tubificidae (18 species and higher taxa), and Naididae (13).

The most widely occurring taxa throughout the lake were *D. r. bugensis* found at 98% of stations sampled (Table 2), followed by Oligochaeta (immature tubificids: 83%, immature lumbriculids: 78%, lumbriculid *Stylodrilus heringianus*: 60%, and tubificid *Limnodrilus hoffmeisteri*: 56%), *Mysis* (56%), and Chironomidae (*Micropsectra* sp.: 47%, *Heterotrissocladius subpilosus* group: 44%, and *Procladius* sp.: 36%). All other taxa were found in fewer than 50% of the samples.

Dreissena r. bugensis comprised a large part of lake-wide benthic density (67%), followed by Oligochaeta (28%), and Chironomidae (5%) (Table 2). Contribution of other groups (Amphipoda (other than *Diporeia*), Gastropoda, Hirudinea, etc.) into total benthic density was less than 1% each. Among Oligochaeta, the most numerous were Tubificidae and Lumbriculidae (79% and 19% of oligochaete density, respectively). *Dreissena r. bugensis* also dominated lake-wide benthos by biomass (99.7% of total wet biomass). Most of the remaining benthic biomass was comprised by Oligochaeta (0.16%) and Chironomidae (0.02%) (Table 2).

There was no difference in total benthic density or biomass (both including and excluding *Dreissena*, $P > 0.05$, Kruskal-Wallis tests) nor in community composition ($R = 0.03$, $P = 0.17$, one-way ANOSIM) among Lake Ontario regions (western, central, and eastern). In contrast, community composition and the distribution of lake-wide diversity, densities, and biomass of benthic invertebrates (excluding *Dreissena*) significantly varied among depth zones ($R = 0.65$, $P < 0.001$, one-way ANOSIM; diversity and density: $P < 0.001$; biomass: $P = 0.067$, Kruskal-Wallis test, Figure 2A, Table 2).

Species richness was the highest at shallow (<30 m) depths where the maximum densities and biomass of Amphipoda (other than *Diporeia*), Chironomidae, Hirudinea, and Gastropoda were found. Oligochaeta families Tubificidae, Naididae, and Enchytraeidae were prevalent at the >30 to 50 m depth interval, while Lumbriculidae and some Chironomidae species (*Micropsectra* sp. and *Heterotrissocladius* *subpilosus* group) were most abundant at 51 - 90 m. The deepest zone (>90 m) was dominated by *Dreissena* and Lumbriculidae (*S. heringianus*). Although the highest densities of *Dreissena* were found at shallow depths (<30 m, 5037±2132/m^2, mean ± standard error here and elsewhere unless noted), highest dreissenid biomass was found at 51 - 90 m depth (1931±236 g/m^2), largely due to mussels being larger on average compared to <30 m (Karatayev et al., this issue). There were significant positive relationships between *Dreissena* biomass and biomass of Amphipoda (other than *Diporeia*, Spearman $\rho = 0.41$, $P = 0.002$), and Oligochaeta ($\rho = 0.52$, $P < 0.001$), but a negative relationship with Sphaeriidae ($\rho = -0.46$, $P < 0.001$). There was also a significant relationship between *Dreissena* biomass and immature Oligochaeta density ($R = 0.53$, $P = 0.009$, mostly due to immature Lumbriculidae and Oligochaeta embryos), and the relationship was even stronger at stations deeper than 90 m ($R = 0.70$, $P < 0.01$).

Similar to community structure and density, there was no difference among lake regions in environmental parameters of sampled stations ($R = -0.011$, $P = 0.56$, one-way ANOSIM; $P > 0.09$, Kruskal-Wallis tests), but stations differed significantly among depth zones ($R = 0.568$, $P = 0.001$; $P < 0.02$) (Fig. 2A). Deeper stations had higher bottom oxygen, higher sediment nutrients and organic carbon, and finer sediments, while nearshore stations had higher water temperature and turbidity, and higher chlorophyll *a* content at the bottom (Fig. 2B). The concentration of organic carbon and total phosphorus increased significantly from nearshore to offshore, from 23.9±5.8 mg C/g, and 0.64±0.06 mg P/g respectively at <30 m to 42.5±2.0 mgC/g, and 1.04±0.08 mgP/g in the >90 m depth zone ($P < 0.03$, post-hoc tests). There was a significant correlation between the benthic community and environmental parameters (Spearman $\rho = 0.56$, $P = 0.001$, RELATE). Factors that best described the benthic community structure included depth, bottom turbidity, and remotely-sensed averaged spring and summer surface chlorophyll a (Spearman $\rho = 0.756$, BEST).

Long-term changes in Lake Ontario benthic community

Community changes

Species data were available for 1964, 1972, 1990, 1994 (*Dreissena* was present but not recorded), 1995, 1997-1999, 2008, 2013, and 2018. Community structure based on the density of all macroinvertebrate taxa differed significantly among years and especially among depth zones (year (excluding 1994): R = 0.499, P = 0.001; depth: R = 0.653, P = 0.001, two-way ANOSIM, Fig. 3 A-F). There was a significant temporal trend in community composition both lake-wide (Spearman $\rho = 0.275$, P = 0.001, RELATE) and within each depth zone (Spearman $\rho = 0.436$, P = 0.001, RELATE).

NMDS plot based on densities of all benthic taxa revealed three different groups (Fig. 3C): one with pre/early *Dreissena* years (1964, 1972, and 1990), a second group containing 1994 - 1999 data (period of high zebra mussel density and dominance), and finally the recent period of quagga mussel dominance (2008 - 2018) with the most clustered data points. These groups were also supported by cluster analysis: all three periods were significantly different (P = 0.001, SIMPROF test). NMDS by species required three axes for convergence for analyses including *Dreissena* (stress = 0.14, ESM Figs. S1 - S3) and excluding *Dreissena* (stress = 0.15, ESM Figs. S4 - S6).

We found that the *Dreissena* period revealed by NDMS and cluster analyses was a more important factor influencing species composition (R = 0.606, P = 0.001, two-way ANOSIM) than depth (R = 0.541, P = 0.001). The largest differences were found between the pre/early *Dreissena* and quagga mussel periods (R = 0.83, P = 0.001, pairwise tests after ANOSIM), and zebra mussel and quagga mussel periods (R = 0.67, P = 0.001). The difference between communities in pre-*Dreissena* and zebra mussel periods was smaller, but still significant (R = 0.475, P = 0.001).

The distinction among the three periods was driven by different assemblages of dominant species. Thus, *Diporeia* was the most contributing species (23%) to the similarity of the pre/early *Dreissena* period (1964, 1972, and 1990, 54% group similarity, SIMPER) together with Tubificidae (21%) Sphaeriidae (17%), Lumbriculidae (16%), and Chironomidae (8%). The same taxa were important during years of zebra mussel dominance (1995 – 1999): Tubificidae (22%), Lumbriculidae (18%), Sphaeriidae (18%), and *Diporeia* (12%). During the recent period of quagga mussel dominance (2008 - 2018), Tubificidae had the largest contribution to group similarity (28%), followed by the quagga mussel (27%), Chironomidae (21%), and Lumbriculidae (17%). The major contributors to the dissimilarity between pre-*Dreissena* and current quagga mussel-dominated communities (60% dissimilarity) were higher densities of *Diporeia* (14%) and Sphaeriidae (10%) in 1965 - 1990, and higher densities of quagga mussels (15%), Chironomidae (9%), and Tubificidae (8%) in 2000s. The major difference between periods of zebra mussel dominance (1995 - 1999) and quagga mussel dominance (2008 - 2018, dissimilarity 49%) was due to the higher abundance of quagga mussel (11%) and Chironomidae (11%) in recent years, and decline in *Diporeia* (13%) and Sphaeriidae (12%) since 2000s.

Dynamics of major taxonomic groups

Dominance of major taxonomic groups changed over time, and the largest changes were found in *Diporeia*, Sphaeriidae, Oligochaeta, and *Dreissena* (Fig. 4, 5). The decline in *Diporeia* was significant (Spearman $\rho = -0.50$, $P < 0.001$), and its dynamics and magnitude depended on the depth zone (Fig. 5). The earliest changes were seen in the shallow zone (<30 m) where densities of *Diporeia* were quite steady from 1964 to 1977 (1200 – 1700/m^2), then dropped to <100/m^2 in 1990s, and no *Diporeia* has been recorded at <30 m since 2003. At the intermediate depth zones (>30 - 90 m) *Diporeia* densities declined from approx. 10,000/m^2 in 1964 to approx. 2,000/m^2 in 1970s, increased to 8,000/m^2 in 1994, and then sharply declined in 1995 at >30 - 50 m depths, and a few years later, in 1997, at >50 - 90 m depths. At depths >90 m the trends were similar but less pronounced, and densities fluctuated between 400/m^2 and 2000/m^2 until 2003, then declined to <1/m^2 in 2013 and 2018.

Additional data for 1997 were available for analysis of temporal changes in major taxonomic groups (ESM Table S1). Dominant taxa varied by depth zone, and shallower depths typically had the highest benthic macroinvertebrate densities (Fig. 5, 6). Permutational ANOVAs showed significant effects of year and depth zone on total benthic density (P-values <0.0001 with and without *Dreissena;* Total benthos including *Dreissena* $R^2_{adj}=0.21$; Total benthos excluding *Dreissena* $R^2_{adj}=0.25$). Year and depth zone also had significant effects on all major taxonomic groups (all P-values <0.0001): *Diporeia*

(R^2_{adj}=0.24), Amphipoda (R^2_{adj}=0.20), Oligochaeta (R^2_{adj}=0.17), Chironomidae (R^2_{adj}=0.16), *Dreissena* (R^2_{adj}=0.21), Sphaeriidae (R^2_{adj}=0.30), and Gastropoda (R^2_{adj}=0.13).

Sphaeriidae in general followed the same trend as *Diporeia*, gradually declining (ρ = -0.41, P <0.001) from a lake-wide maximum of 2,324±492/m^2 in 1964 to <10/m^2 in 2008 - 2018, and currently are occasionally found only at depths >50 m.

Oligochaeta declined (ρ = -0.14, P <0.001) from the highest densities (app. 10,000/m^2) in the shallow zone (<30 m) in 1964 and 1990 to <1000/m^2 in 2008, but increased again in the last five years (2013 - 2018) (Fig. 4 - 6, ESM Table S1). These changes were mostly driven by large declines in pollution tolerant Tubificidae (ρ = -0.24, P <0.001) that comprise from 20 to 95% of all Oligochaeta densities (Fig. 6). The largest changes in Tubificidae occurred in the shallow zone (ρ = -0.63, P <0.001), declining over ten-fold from their peak densities in 1960s and 1990s until 2008, and then increased again in 2013 - 2018. Densities of pollution-intolerant deep-water family Lumbriculidae were elevated from 1960 to mid-1990 at <90 m depths, and then declined. Naididae, small oligochaetes associated with macrophytes and bottom algae, were highest in the shallow zone (<30 m) during the zebra mussel peak in 1994, but recently started to increase at depths <50 m. Despite the decline in total Oligochaeta density, the lake-wide proportion of immature oligochaetes (all families combined) of total density increased significantly (P <0.001, ANOVA) during three *Dreissena* periods from 23% in pre-invasion period to over 50% in zebra and quagga mussel dominated years, with the highest increase (from 7% to 54% respectively) at intermediate depth zone (>30 - 90 m).

Dreissena (ρ = 0.71, P <0.001) and Chironomidae (ρ = 0.20, P <0.001) were the only taxa that showed increasing density trends with time, especially at intermediate (>30 – 90 m) depths (Fig. 5). Zebra mussels were abundant at depths <30 m following their invasion, then reached a maximum in 1995, but were rapidly replaced by quagga mussels which are now found in all depth zones at high densities (Fig. 4; Karatayev et al., this issue). Amphipoda (excluding *Diporeia*), Gastropoda, Hirudinea, and Trichoptera all peaked in the shallow zone in mid-1990s, and then declined (ESM Table S1, Fig. 5). Isopoda were found at their peak densities in 1964 and declined thereafter (ρ = -0.23, P <0.001). Polychaeta, *Manayunkia* *speciosa*, often common at depths <30 m in fine sands along the south shore in 1964 - 1998, was not found in the last 10 years. As a result of all the negative and positive changes in individual taxa, total benthic density did not exhibit overall trends lake-wide ($\rho = 0.07$, $P = 0.04$), but other benthos (everything except *Dreissena*) declined lake-wide ($\rho = -0.29$, $P < 0.001$). The decline was most pronounced at depths <90 m ($\rho = -0.45$; $P < 0.0001$), most likely due to the strong declines in *Diporeia* and Sphaeriidae.

Clear differences among years, depth zones, and stage of *Dreissena* invasion were evident in NMDS plots (Fig. 7), perMANOVAs, and permutational ANOVAs of groups. During first and second stage, nearshore zones had relatively high abundance of Gastropoda and Amphipoda, while deeper zones were characterized by *Diporeia* (Fig. 7B; 7E). NMDS plots demonstrated a shift from a community characterized by *Diporeia* and Sphaeriidae to a community of *Dreissena*, Oligochaeta, and Chironomidae over time (Fig. 7A; 7D). High abundance of quagga mussels corresponded to a community with proportionally more Oligochaeta and Chironomidae, but less *Diporeia* (Fig. 7C; 7F). The NMDS with *Dreissena* required four axes and had lower stress value (<0.1) indicating a good ordination with no real prospect of a misleading interpretation (Clark et al., 2014b), and without *Dreissena* required three axes to facilitate convergence (stress = 0.08 with *Dreissena* and stress = 0.11 without *Dreissena*, see ESM Figs. S7 - S12 for plots of all axes).

We found a significant effect of year on taxonomic composition by density of major groups with depth and *Dreissena* period as covariates (perMANOVA, $\text{pseudoF}_{1,688}=103.9$, $P=0.001$, $R^2=0.11$). Only the years 1994 and 2003 were excluded from this analysis because of incomplete data (no dreissenids recorded in 1994 and only dreissenids and *Diporeia* recorded in 2003, Table 1). All pairwise comparisons between years showed significant differences except 1964 versus 1990, 1972 versus 1990, 1990 versus 1995, 1997 versus 1998, 1998 versus 1999, and 2013 versus 2018. The effects of both covariates were significant as well (depth zone: $\text{pseudoF}_{3,688}=34.9$, $P=0.001$, $R^2=0.11$; *Dreissena* period: $\text{pseudoF}_{2,688}=17.0$, $P=0.001$, $R^2=0.04$). In pairwise comparisons, all depth zones and *Dreissena* periods were significantly different.

PerMANOVA results were similar when *Dreissena* were excluded from analyses. Year had a significant effect on taxonomic composition (pseudo$F_{1,737}$=46.2, P=0.001, R^2=0.05) and all pairwise comparisons between years were different except 1964 versus 1990, 1964 versus 1995, 1972 versus 1990, 1990 versus 1995, 1990 versus 1997, 1997 versus 1998, 1997 versus 1999, and 2013 versus 2018. Depth zone and stage of *Dreissena* invasion also had significant effects on taxonomic group composition (depth zone: pseudo$F_{3,737}$=38.4, P=0.001, R^2=0.12; *Dreissena* period: pseudo$_{2,737}$=24.7, P=0.001, R^2=0.05) with significant contrasts between all pairwise comparisons.

Discussion

The benthic macroinvertebrate community of Lake Ontario underwent significant transformations in the last six decades that can be described by three major periods defined by the primary driver of change – dreissenid mussels. The first pre- and early *Dreissena* period (1964 - 1990) was characterized by high densities of *Diporeia*, Sphaeriidae, and Tubificidae. Owing to the longer history of dreissenid invasion in Lake Ontario compared to other deep Great Lakes and intensified survey efforts early in the invasion, we were able to detect the second period of zebra mussel dominance (the 1990s) and its effect on the benthic community. Because it was of short duration, this period was largely overlooked in other Great Lakes (Karatayev et al., accepted) making it difficult to separate the effects of zebra and quagga mussels. During the period of zebra mussel dominance, the same groups were still prevalent, but their densities changed. The third period was characterized by quagga mussel dominance (2000 - 2018). The proliferation of quagga mussels in the nearshore as well as deeper into the lake in the last two decades has resulted in perhaps the most dramatic community changes: *Diporeia* almost completely disappeared, Sphaeriidae declined, while densities of quagga mussels, Oligochaeta, and Chironomidae increased.

These conclusions are based on the analysis of multiple studies of Lake Ontario benthos. Even though the exact status of the Lake Ontario benthic community before the mid-1960s is hard to establish because of the lack of lake-wide surveys, it was likely typical of other deep Great Lakes dominated by *Diporeia*. According to Cook and Johnson (1974), the proportion of oligochaetes relative to *Diporeia* increased along a natural gradient from 60-80% *Diporeia*, 10-20% Oligochaeta, 5-15% Sphaeriidae, and 0-5% Chironomidae in lakes Superior, Huron, and Michigan, to 50% *Diporeia* and 30% Oligochaeta in Lake Ontario.

Pre- and early Dreissena *period*

The first lake-wide survey of Lake Ontario benthos was done in 1964 (Hiltunen, 1969) during the period of cultural eutrophication (Beeton, 1965). At that time the most common taxon throughout the lake was Oligochaeta (mostly tolerant to organic pollution Tubificidae and intolerant Lumbriculidae), followed by *Diporeia,* and Sphaeriidae (Table 3). *Diporeia* in 1964 was three times more abundant in the shallow zone (<48 m) than in the deep zone (>92 m), but their densities were reduced in nearshore areas close to sources of urban pollution. Hiltunen (1969) described the two most important patterns in benthic communities of Lake Ontario: reduction in taxonomic diversity and five-fold decline in densities with increasing depths from shallow to deep zone, and drastically different species composition at polluted stations (near Toronto, off Rochester and near the mouth of the Niagara River) characterized by high abundance of Tubificidae.

The next lake-wide survey was carried out in 1972 (Nalepa and Thomas, 1976, Table 3) and was comparable to the 1964 study (Hiltunen, 1969) because both surveys used grabs of similar efficiency, and both were conducted in the fall (Nalepa, 1991; Table 1). *Diporeia* distribution in 1972 shifted from shallow to deeper waters, especially along the southern shore, and comprised 22, 61 and 58% of all organisms collected at shallow, intermediate, and deep zones, respectively. Eutrophic stations sampled in 1964 (near the mouth of the Niagara River and just off Toronto) were still dominated by Tubificidae, while oligotrophic Lumbriculidae (*S. heringianus*) comprised the majority of Oligochaeta in deeper areas of the lake. Chironomidae, especially pollution tolerant *Procladius* sp. and *Chironomus* spp., were generally restricted to the shallow-water stations, while pollution-intolerant *Heterotrissocladius* spp. were widespread in deeper areas.

In general, during the mid-1960s and early 1970s, benthic populations in shallow waters were co-dominated by *Diporeia* and oligochaetes (40% of the total for each) (Kinney, 1972), while populations in the deep waters (>90 m) were dominated by *Diporeia* (50%; oligochaetes: 30%, reviewed in Cook and Johnson, 1974). Mozley (1990) suggested that there were no large changes in Lake Ontario *Diporeia* populations from 1920s to early 1970s. He compared density estimates by Adamstone (1924) with densities in 1960s and early 1970s (Hiltunen, 1969; Kinney, 1972; Nalepa and Thomas, 1976), although Adamstone's estimates may be lower than actual densities due to larger meshes of sieves.

In 1977 (Golini, 1979) densities of *Diporeia* were comparable to the previous surveys in 1964 - 1972 (Kinney, 1972; Nalepa and Thomas, 1976), but abundance of Oligochaeta was almost four times lower than in 1964 (Fig. 4). *Diporeia* and Oligochaeta comprised similar percentages of total benthos (app. 30%) in the shallow zone, but *Diporeia* was still dominant deeper (>40%). Golini (1979) partially attributed these lower densities of Oligochaeta to a shift of the status of Lake Ontario towards more oligotrophic conditions. Lake productivity had begun to decline steadily since the mid-1970s as a result of phosphorus control measures initiated by the Great Lakes Water Quality Agreement of 1972. Phosphorus loadings into Lake Ontario in the early 1980s were reduced by 39% and mid-lake spring concentrations of soluble reactive phosphorus were reduced by 33% (Stevens and Neilson, 1987). This decline in total phosphorus led to a 43 – 85% decrease in phytoplankton biomass in the 1980s compared to the early 1970s (Gray, 1987; Millard et al., 2003). Similar reductions in phosphorus and phytoplankton biomass in western Lake Erie (Carr and Hiltunen, 1965; Dermott, 1994; Wright, 1955) and Saginaw Bay (reviewed in Nalepa et al., 1998, 2002) in the 1970s as compared to the 1960s led to large declines in oligochaetes in these regions over the same time period. Nevertheless, in Lake Ontario during the 1970s, oligochaetes still comprised a large portion of the shallow and deepwater benthos (34% and 53% of total benthic density respectively), and *Diporeia* was dominant at intermediate depth zone (43% and 52%).

In the next decade, in the 1980s, the most remarkable long-term change in the benthic community was the increase in abundances of *Diporeia*. Barton and Anholt (1997) suggested that the positive changes in *Diporeia* densities in 1980s were likely attributed to predation release (due to increased predation on and associated declines in sculpins), and changes in zooplankton communities. Large-scale management activities on the lake accelerated throughout the 1970s with the implementation of the GLWQA, including the start of annual releases of hatchery-reared lake trout for population restoration in 1973. The numbers of yearling lake trout released into Lake Ontario doubled in 1980 (Christie et al., 1987). Lake trout prey heavily on sculpins, and the result was a 33% reduction in sculpin populations (Sly and Christie, 1992). Because sculpins are a major predator of *Diporeia* in Lake Ontario, *Diporeia* abundance increased by 25% in eastern Lake Ontario between 1970 and 1984 (Sly and Christie, 1992).

Overall, we found that despite the large changes in the densities of major taxonomic groups, the structure of the community was not altered between the mid-1960 and the end of 1980, and the same taxa (*Diporeia*, Tubificidae, and Sphaeriidae) dominated Lake Ontario benthos.

Period of zebra mussel dominance

Increases in *Diporeia* densities continued into the 1990s. According to Lozano et al. (2003), densities of *Diporeia* in 1997 were significantly greater at >70 m depths than in 1972. In addition to *Diporeia*, other taxa including Amphipoda, Oligochaeta, Hirudinea, Gastropoda, and Trichoptera increased in densities in the 1990s (Fig. 5, ESM Table S1; Dermott and Geminiuc, 2003; Haynes et al., 1999; Lozano and Nalepa, 2003). The observed increases in other benthic taxa in the early 1990s might be also due to the introduction of *Dreissena*, especially the zebra mussel, which was abundant in the shallow zone already in early 1990s (Fig. 5). Extensive filter feeding mussel communities increased water clarity, and reduced water-column nutrients and phytoplankton density (reviewed in Estepp and Reavie, 2015). The effect of zebra mussels on benthic communities is well known and described in studies from both Europe and North America, including the Great Lakes (reviewed in Karatayev et al., 1997, 2002, 2007; Higgins and Vander Zanden, 2010). Zebra mussels enrich sediments with organic matter through deposition of feces and pseudofeces coated in a mucus that are rich in bacteria, an additional food source for littoral benthos (Izvekova and Lvova Katchanova, 1972; Howell et al., 1996; Thayer et al., 1997; Stewart et al., 1998). Further, *Dreissena* are ecosystem engineers that provide new habitat and nutrition for littoral benthic macroinvertebrates (Karatayev et al., 1983, 1997, 2002; Stewart et al., 1998, 1999; Mayer et al., 2014), algae and microorganisms growing on the surface of dreissenid shells provide additional food for sediment surface-dwelling benthos (Makarevich et al., 2008), and interstitial spaces between individual mussels offer refuge and feeding ground for epifaunal benthos (Angelini et al., 2011, Bateman and Bishop, 2017). Through filter feeding, *Dreissena* also increase light availability promoting macrophyte and algal growth, hence increasing overall productivity within the littoral zone (reviewed in Karatayev et al., 1997; Zhu et al., 2006; Higgins and Vander Zanden, 2010). As a result, dreissenids cause radical changes in the benthic community both taxonomically and functionally (Lvova-Kachanova and Izvekova, 1978; Karatayev et al., 1997, 2002; Burlakova et al., 2005, 2012; Ward and Ricciardi, 2007). Native suspension feeders (i.e. some Chironomidae, Sphaeriidae, Unionidae etc.) can be out-competed by zebra mussels, while collector-gatherers feeding on the sediments (i.e., Oligochaeta) and bottom algae, and predators can increase in abundance due to the increase in habitat complexity and the novel food source provided by zebra mussels in the littoral zone (Stewart et al., 1998; Dermott and Geminiuc, 2003; reviewed in Ward and Ricciardi, 2007; Burlakova et al., 2012). Thus, Lozano et al. (2003) found an increase in average species richness in shallow waters of Lake Ontario at stations where *Dreissena* were present, and several species of Tubificidae*,* Naididae*,* and non-suspension feeding Chironomidae were more abundant or only found at stations where dreissenids were present. Likewise, during the first decade of the *Dreissena* invasion in Lake Erie, the densities of chironomids, oligochaetes, and native *Gammarus* increased, while the abundance of native *Pisidium* and *Sphaerium* declined compared to the previous decade (Burlakova et al., 2014). However, most importantly, these quantitative changes were not accompanied by changes in taxonomic structure of the community, and community structure was not significantly different between the pre- and early *Dreissena* period (1964 – 1990) and the period of zebra mussel dominance in the 1990s. The same dominant species (*Diporeia* and Oligochaeta) were still present throughout the community until 1999.

Period of quagga mussel dominance

The largest changes in the community, however, happened in the early 2000s, when quagga mussels began to dominate not only the nearshore dreissenid community, but also spread into deeper regions of the lake. The replacement of zebra by quagga mussels in nearshore regions was first observed in 1995 (Mills et al., 1999) and was complete at depths <30 m by 1998 - 1999. The decline of *Diporeia*, Sphaeriidae, and Oligochaeta coincided with the dramatic expansion of quagga mussels (Watkins et al., 2007). In the shallow zone, *Diporeia* started to decline in 1990s and was no longer recorded after 2003. In the intermediate depths, *Diporeia* densities sharply declined at >30 - 50m depths in 1995 and declined at >50 - 90 m depths a few years later, in 1997. In the deepest zone, trends were generally similar to trends at intermediate depths but less pronounced until 2003. After 2003 densities at depths >90 m steadily declined to <1/m^2 by 2013 - 2018 (Table 3). Food competition with *Dreissena* is one of the leading hypotheses of *Diporeia* decline across all deep Great Lakes colonized with quagga mussels (Nalepa et al., 2009; Barbiero et al., 2011, 2018; Ryan et al., 2012; Watkins et al., 2012) and an attractive one, as both species feed on the settling spring diatoms and this mechanism would explain the compelling negative correlation between quagga mussels and *Diporeia* (Vanderploeg et al., 2010). *Diporeia* started to decline after the arrival of zebra mussels, but the decline was likely exacerbated by the arrival of quagga mussels in Lake Ontario (Dermott, 2001, Watkins, 2007, 2013) and similarly in Lake Michigan (Nalepa et al., 2006, 2009). Both *Diporeia* and dreissenids, however, co-occur in the New York Finger Lakes (Dermott et al., 2005, Watkins et al., 2012, Foley et al., 2014). Further, the maintenance of high *Diporeia* condition also in the presence of quagga mussels is not consistent with food limitation (Nalepa et al., 2006, Ryan et al., 2012, Foley et al., 2014). An alternative explanation could be the impact of a pathogen (Hewson et al., 2013). McKenna et al. (2017) used a model to test two hypotheses for causes of *Diporeia* loss, competition with quagga mussels or a pathogen causing increased mortality and found that the elevated mortality scenario supported a disease-like cause for loss of this native amphipod. The correlation between *Diporeia* declines and quagga mussel increases could be due to *Diporeia* preventing mussel settlement. In the Baltic Sea, Segerstråle (1978) found that high densities of *Pontoporeia affinis* (a similar amphipod to *Diporeia* and previously in the same genus) prevented recruitment of newly-settled larvae of the Baltic clam *Macoma baltica*. Thus, an initial decline in *Diporeia* due to a pathogen would allow quagga mussels to expand, which is consistent with the decline of *Diporeia* at sites prior to the arrival of quagga mussels (Watkins et al., 2007, Nalepa et al., 2009). However, searches for viral or bacterial pathogens have not yet identified a likely candidate (Winters et al., 2014, 2015, Bistolas et al., 2017). Therefore, the exact mechanism of *Diporeia* decline is still unclear (reviewed in Watkins et al., 2012, 2013). Modeling (McKenna et al., 2017) predicted possible recovery of *Diporeia* after stabilization of dreissenid populations. While only a single individual of *Diporeia* per year was found in 2014, 2015, and 2018 at all nine permanent LTM stations, in 2019 we found over 30 individuals combined at two deep stations (Burlakova and Karatayev, unpublished data).

Sphaeriidae, previously found in abundance at all depths, followed the same trend as *Diporeia* and are currently found at low densities only at depths >90 m. Previously common in the nearshore, the filter-feeding polychaeta *M. speciosa* was not found in the last 10 years. The decline in native filter-feeders after *Dreissena* invasion is well described in literature and was expected (Karatayev et al., 1997), although in several other lakes Sphaeriidae, formerly abundant in profundal zones, declined to almost extirpation long before the introduction of *Dreissena* (e.g. Lake Mendota, Karatayev et al., 2013, and Oneida Lake, Karatayev et al., 2014).

The decline in Oligochaeta from 1964 to 2008, however, was reversed in the last ten years (Fig. 6, ESM Table S1), mostly due to increases in pollution tolerant Tubificidae and Naididae. Small Naididae, usually associated with macrophytes and benthic algae, recently started to increase at depths down to 50 m, likely due to proliferation of bottom algae and periphyton deeper into the lake (Leon Katona, Wright State University, personal communication). In spite of the decline in total Oligochaeta density compared to 1964, the proportion of immature oligochaetes increased significantly in zebra- and quagga mussel dominated years. It is likely that conditions in *Dreissena* aggregations promote Oligochaeta reproduction as evidenced from the positive relationship of *Dreissena* biomass and density of immature Oligochaeta. Facilitation of deep-water benthos, mainly Oligochaeta, by quagga mussels has recently been shown in lakes Michigan and Huron (Burlakova et al., 2018a; Mehler et al., 2020).

Two previous studies of long-term trends in benthic communities (Dermott et al., 2012; Barrett et al., 2017) in the Bay of Quinte and on an artificial reef in southwestern Lake Ontario also found large significant changes in community composition among three time periods: before *Dreissena* invasion, during the time of zebra mussel dominance (1990s), and later in the 2000s. However, these shallow (<60 m) systems were heavily dominated by round gobies in the period following zebra mussel dominance. Round goby (*Neogobius melanostomus*) were first found in Lake Ontario in 1998, nine years after the first record of dreissenids in the lake (Owens and Dittman, 2003) and have been abundant in the lake since 2005 (Weidel et al., 2019). In 2018 we expected to find a decline in dreissenids at <30 m depths where estimated goby density was >4 fish/m^2 because gobies can consume >40 mussels of 4 – 12 mm in size per day (Naddafi and Rudstam, 2014). Surprisingly, we found no changes in total *Dreissena* densities although the lack of 5 – 10 mm mussels in 2008, 2013, and 2018 suggested an effect of round goby at depths <60 m (Karatayev et al., this issue). Likewise, we did not find declines in other potential round goby food items, Chironomidae and Oligochaeta (Brush et al., 2012) that instead increased at depths <50 m during the quagga mussel-dominated period, although there was a decline in Amphipoda (Fig. 5).

In conclusion, we believe that the large shifts in community structure in the last 54 years were due to a complete change of the dominant species complex in Lake Ontario as a result of dreissenid invasion. Karatayev et al. (2015) hypothesized that by having different spatial distributions in a waterbody, and different druse-forming habits, these two species of *Dreissena* can have very different effects on benthic invertebrate communities. Because Lake Ontario has the longest history of dreissenid invasion among other deep Great Lakes, we were able to detect a period of zebra mussel dominance and its effect on Lake Ontario benthic community. This short period was largely overlooked since the first lake-wide surveys of dreissenids in other deep Great Lakes happened well after zebra mussel invasion (Karatayev et al., accepted) making it difficult to separate the effects of zebra and quagga mussels. We found significant changes in benthic community structure between periods of zebra and quagga mussel dominance: a community once characterized by *Diporeia* and Sphaeriidae shifted to a community dominated by quagga mussels and Oligochaeta. A single species, *D. r. bugensis*, now accounts for 67% of all benthic density and 99% of biomass. Establishment of *D. r. bugensis* was associated with the almost complete extirpation of the two previously most common taxa, *Diporeia* and Sphaeriidae.

What can we expect in the next decades? It would be reasonable to suggest that after thirty years of Lake Ontario colonization *Dreissena* populations have stabilized and may be beginning to decline. Yet Karatayev et al. (this issue) reported a significant increase in lake-wide density and biomass of *Dreissena* in 2018 suggesting that the *D. r. bugensis* population in Lake Ontario is still growing. Although in Lake Michigan *Dreissena* populations started declining after 2010 at depths <90 m (Nalepa et al., 2020; Mehler et al., 2020), it is likely that factors such as food limitation have caused this decline (Barbiero et al., 2018). However, similar declines in phytoplankton and spring diatom blooms have not been observed in Lake Ontario (Rudstam et al., 2017; US EPA, 2019) which may explain the continued increase in the biomass of Lake Ontario mussels. Therefore, unless quagga mussels decline as a result of increased goby predation as seen in some smaller lakes (Rudstam and Gandino, 2020, see discussion in Karatayev et al., this issue), the Lake Ontario benthic community is likely to continue to be co-dominated by quagga mussels and oligochaetes into the future.

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Table 1. Lake-wide benthic surveys conducted in Lake Ontario in 1964 - 2018.

Sampling date	Number of stations	Sampler	Sampler area, m^2	Mesh size, µm	Taxonomic resolution	Reference
1964, September	24	Smith-McIntyre	0.0625	600	Species	Hiltunen, 1969
1972, November	55	Ponar	0.050	600	Species	Nalepa and Thomas, 1976
1977, September	151	Shipek	0.0416	150	Groups*	Golini, 1979
1990, October	25	Ponar	0.053	600	Species	Dermott and Geminiuc, 2003
1994, August	51	Ponar	0.048	500	Species (excluding *Dreissena*)	Lozano et al., 2001, Watkins et al., 2007
1995, October	41	Ponar	0.053	600	Species	Dermott and Geminiuc, 2003
1997, September	68	Ponar	0.048	500	Species	Lozano et al., 2001
1998, September	114	Ponar	0.048	500	Species	Watkins et al., 2007
1999, August	67	Ponar	0.048	500	Species	Watkins et al., 2007
2003, August - October	36	Ponar	0.053	500	*Diporeia, Dreissena*	Watkins et al., 2007
2008, August-September;	51	Ponar	0.048	500	Species	Birkett et al., 2015

2009, September**						
2013, July, August	45	Ponar	0.048	500	Species	Nalepa and Baldridge, 2016
2018, August, September	55	Ponar	0.0523	500	Species	Our data

* samples were identified to lower taxonomic level (species and genus), but the primary data were unavailable.

** 44 stations were sampled in August 2008, 3 stations in September 2008, and 4 stations in September 2009. All data from this survey are attributed to 2008 in the analyses.

Table 2. Average (± standard error) density (ind./m^2) and wet biomass (g/m^2) of major taxonomic groups of benthic invertebrates collected at 55 stations in Lake Ontario in 2018 averaged by depth zones and depth-weighted lake-wide. We report *Diporeia* densities separately from other Amphipoda.

Taxa	0 - 30m	>30 - 50m	>50 - 90m	>90m	Lake-wide
Stations #	*13*	*3*	*16*	*23*	*55*
Density (ind./m^2):					
Amphipoda	33±16	0	2±1	0	7±4
Chironomidae	569±116	74±49	408±90	88±29	249±34
Diporeia	0	0	0	0.3±0.3	0.1±0.1
Dreissena	5037±2133	4587±1965	4749±532	3554±501	4215±576
Sphaeriidae	0	0	2±2	18±5	9±2
Gastropoda	57±57	0	0	0	12±12
Hirudinea	2±2	0	0	0	0.4±0.4
Mysidae	1±1	0	16±4	40±10	22±5
All Oligochaeta	3681±940	5494±4300	1516±263	426±79	1921±545
Turbellaria	5±3	21±13	6±2	1±1	5±2
Others	16±8	36±19	14±4	5±2	13±3
All benthos	9401±2919	10212±6256	6711±665	4131±580	6455±1011
All benthos w/o *Dreissena*	4364±978	5626±4295	1964±259	577±88	2239±547
Biomass (g/m^2):					
Amphipoda	0.08±0.04	0	0.01±0.01	0	0.02±0.01
Chironomidae	0.44±0.07	0.05±0.02	0.25±0.06	0.08±0.02	0.19±0.02
Diporeia	0	0	0	0.002±0.002	0.001±0.001
Dreissena	1432±455	1007±228	1931±236	539±112	1044±123
Sphaeriidae	0	0	<0.01	0.02±0.01	0.01±0.01

Gastropoda	0.15±0.15	0	0	0	0.03±0.03
Hirudinea	<0.001	0	0	0	<0.001
Mysidae	0.004±0.004	0	0.19±0.05	0.62±0.24	0.33±0.12
All Oligochaeta	1.87±0.63	2.68±1.73	2.56±0.42	1.07±0.28	1.71±0.29
Turbellaria	0.001±0.001	0.003±0.002	0.001±0.001	<0.001	0.001±0.0001
Others	0.04±0.03	0.03±0.02	0.04±0.01	0.02±0.01	0.03±0.01
All benthos	1435±456	1009±229	1934±237	541±112	1047±124
All benthos w/o *Dreissena*	2.58±0.72	2.76±1.73	3.05±0.42	1.81±0.49	2.32±0.36

Figure legends

Figure 1. Location of benthic stations in Lake Ontario sampled during August – September 2018, including the Lake Ontario Cooperative Science and Monitoring Initiative (CSMI) stations (black circles) and GLNPO long-term monitoring (LTM) stations (open circles). Vertical lines overlaying offshore depositional basins delineate the tentative partitioning of stations by region (western, central, and eastern) during the survey.

Figure 2. A) Non-parametric multi-dimensional scaling (NMDS) ordination plots of Lake Ontario benthic community structure in 2018 based on density (ind./m^2) of benthic taxa (Stress = 0.14). Stations are indicated by depth zones and ellipses encompass 80% of the data in each group. *Dreissena* was not included in this analysis. B) Principal components analysis plot of environmental parameters of stations sampled in Lake Ontario in 2018. The first 2 PC axes explained 62% of the variation. Parameters included depth (m), bottom temperature (°C), bottom dissolved oxygen (mg/L), bottom beam attenuation (Turbidity, 1/m), bottom specific conductance (µS/cm), and the surface remote-sensed summer chlorophyll *a* (µg/L). Sediment characteristics included sedimentary total phosphorus (STP; mg P/g), organic carbon (SOC; mg C/g), total nitrogen (STN; mg N/g), and descriptive sediment type (Substrate; 1 – clay and silt, 2 – silt, 3 – silt and sand, and 4 – sand). The stations are color-coded by depth zones. Vector length is proportional to loading of each environmental parameter.

Figure 3. Non-parametric multi-dimensional (NMDS) ordination plots of Lake Ontario benthic community structure by species (or in some cases by higher taxa) in 1964-2018 based on density (ind./m^2). First two NMDS axes are presented including *Dreissena* (A-C) and excluding *Dreissena* (D-F). Data are grouped and color coded by year (A and D), depth zone (B and E), and *Dreissena* period (C and F). See ESM for all axes (ESM Figs. S1 - S6). Ellipses encompass 80% of data within each group.

Figure 4. Average depth-weighted densities of major taxonomic groups that were consistently counted over time for the entire lake with major events highlighted. The following years in our data set were excluded due to incomplete data: 1994 and 2003. Missing data were simulated using splines then all data were smoothed with a general additive model (GAM) function. Displayed data are additive (stacked). See Fig. 5 for raw data.

Figure 5. Average depth-weighted (±SE) densities of major taxonomic groups that were consistently counted over time for the entire lake (first column) and averages by depth zone (remaining columns). Note: *Diporeia* and other Amphipoda are presented separately.

Figure 6. Average depth-weighted (±SE) densities of all Oligochaeta and Oligochaeta families that were counted over time for the entire lake (left column) and averages by depth zone (remaining columns).

Figure 7. First two axes of NMDS by major taxonomic group including *Dreissena* (A-C) and excluding *Dreissena* (D-F). Data are grouped by year (A and D), depth zone (B and E), and *Dreissena* period (C and F). See ESM for all axes (ESM Figs. S7 - S12). Ellipses encompass 80% of data within each group.

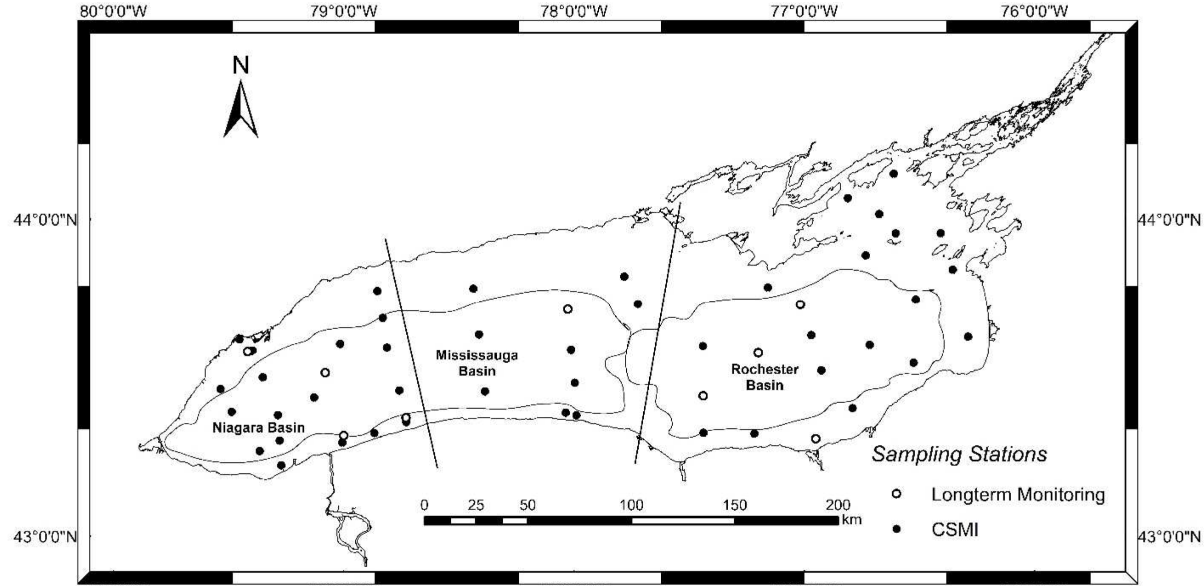
80°0'0"W
79°0'0"W
78°0'0"W
77°0'0"W
76°0'0"W
44°0'0"N
43°0'0"N
N
Mississauga Basin
Rochester Basin
Niagara Basin
Sampling Stations
○ Longterm Monitoring
● CSMI
0
25
50
100
150
200
km

Figure 1.

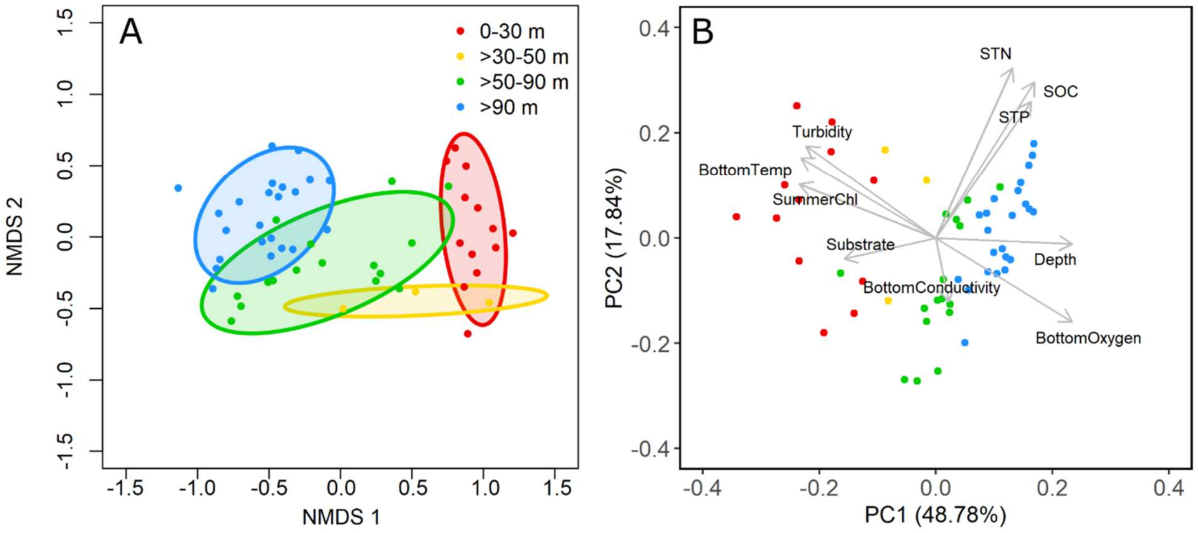
A
0-30 m
>30-50 m
>50-90 m
>90 m
NMDS 1
NMDS 2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
B
STN
SOC
STP
Turbidity
BottomTemp
SummerChl
Substrate
Depth
BottomConductivity
BottomOxygen
PC1 (48.78%)
PC2 (17.84%)
-0.4
-0.2
0.0
0.2
0.4

Figure 2

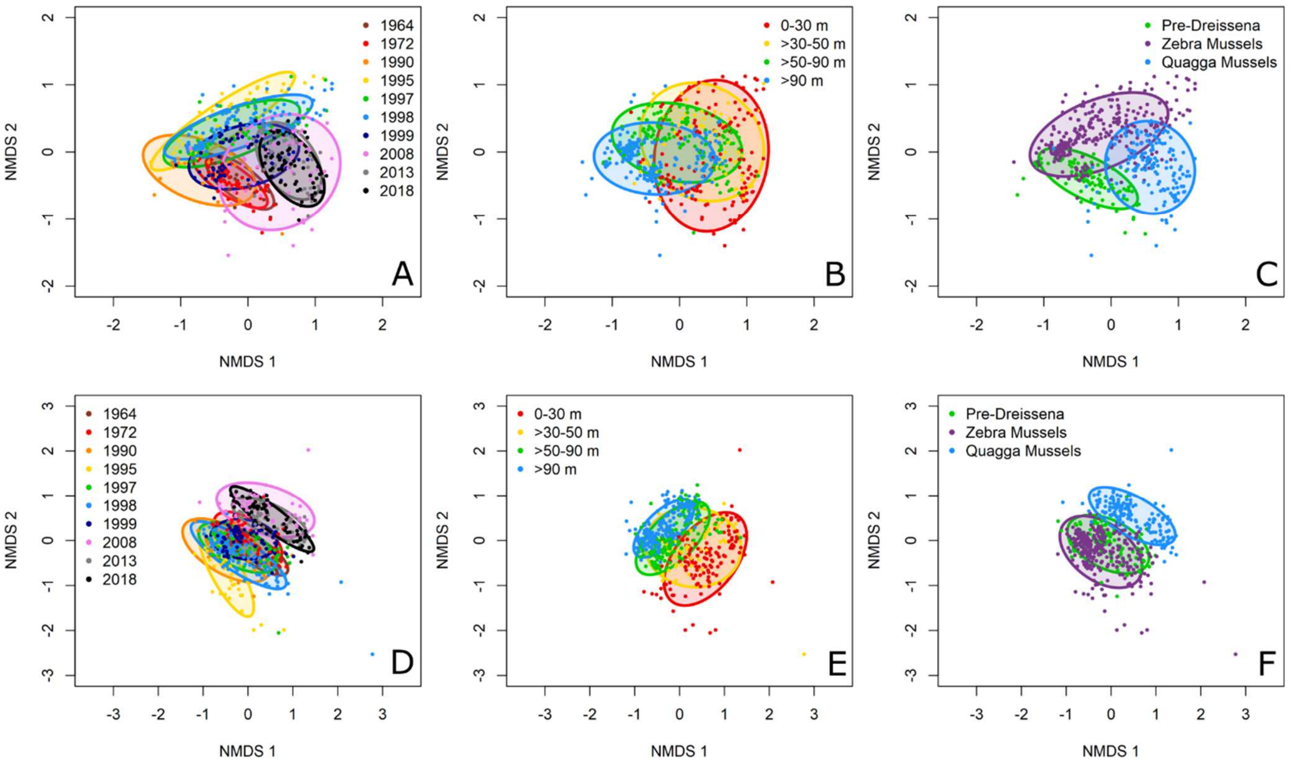
A
B
C
D
E
F
NMDS 1
NMDS 2
1964
1972
1990
1995
1997
1998
1999
2008
2013
2018
0-30 m
>30-50 m
>50-90 m
>90 m
Pre-Dreissena
Zebra Mussels
Quagga Mussels
-2
-1
0
1
2
-3
3

Figure 3

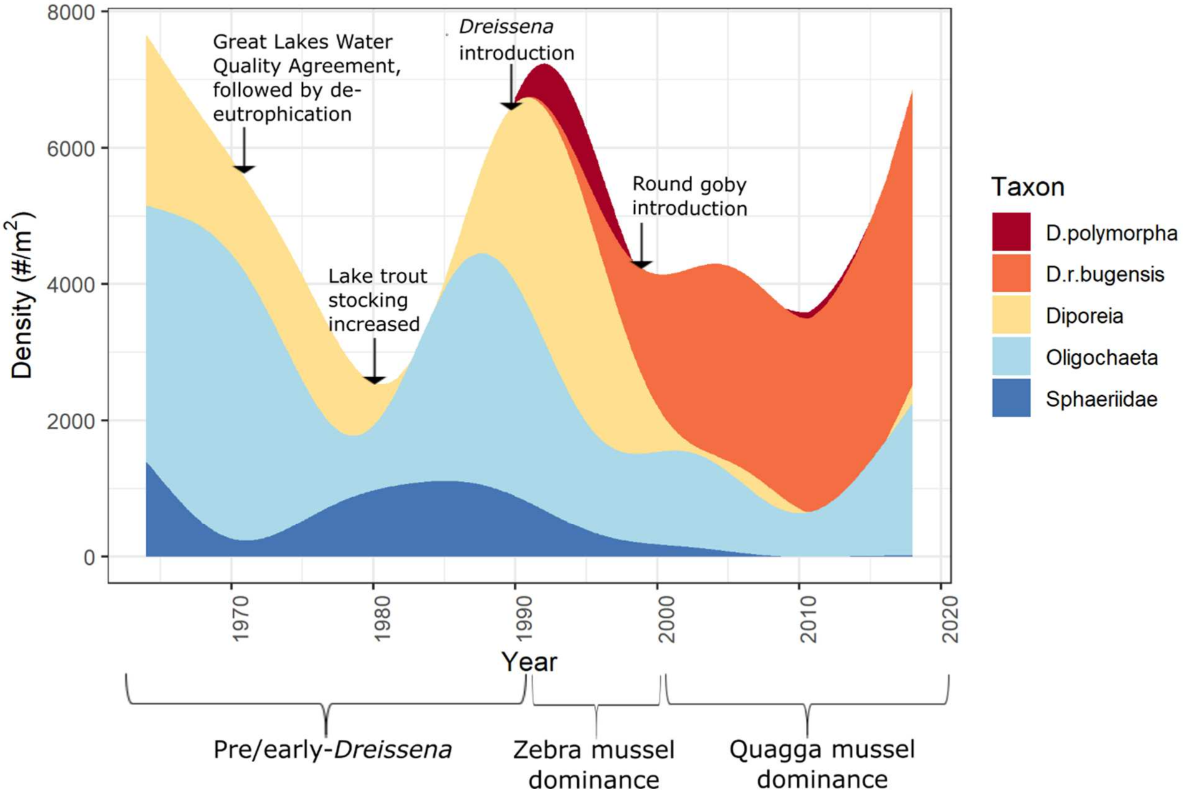
8000
6000
4000
2000
0
Density (#/m^2)
Great Lakes Water Quality Agreement, followed by de-eutrophication
Lake trout stocking increased
Dreissena introduction
Round goby introduction
1970
1980
1990
2000
2010
2020
Year
Taxon
D.polymorpha
D.r.bugensis
Diporeia
Oligochaeta
Sphaeriidae
Pre/early-*Dreissena*
Zebra mussel dominance
Quagga mussel dominance

Figure 4

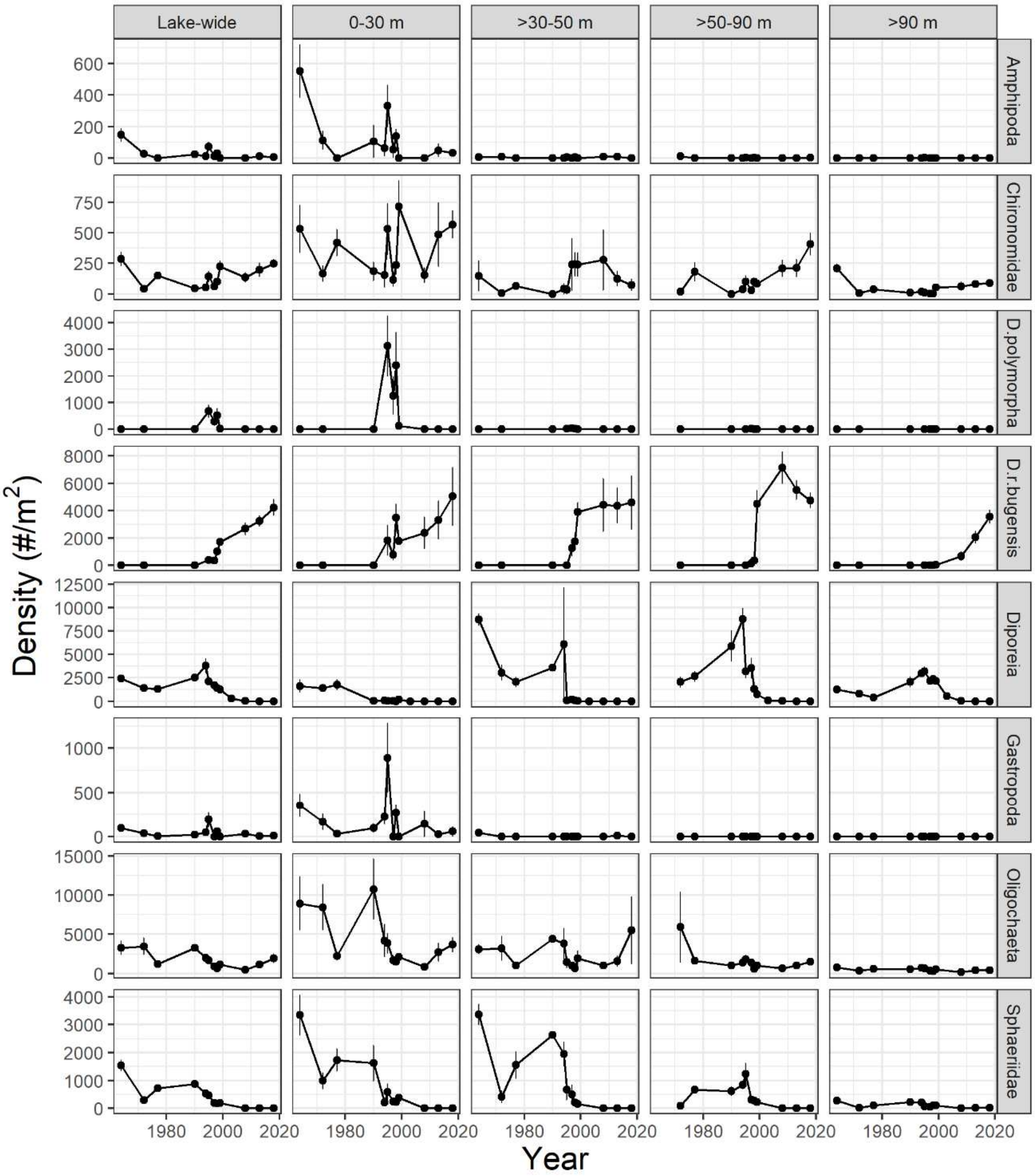
Lake-wide
0-30 m
>30-50 m
>50-90 m
>90 m
Amphipoda
Chironomidae
D.polymorpha
D.r.bugensis
Diporeia
Gastropoda
Oligochaeta
Sphaeriidae
Density (#/m2)
Year
1980
2000
2020
0
200
400
600
250
500
750
1000
2000
3000
4000
6000
8000
2500
5000
7500
10000
12500
15000

Figure 5

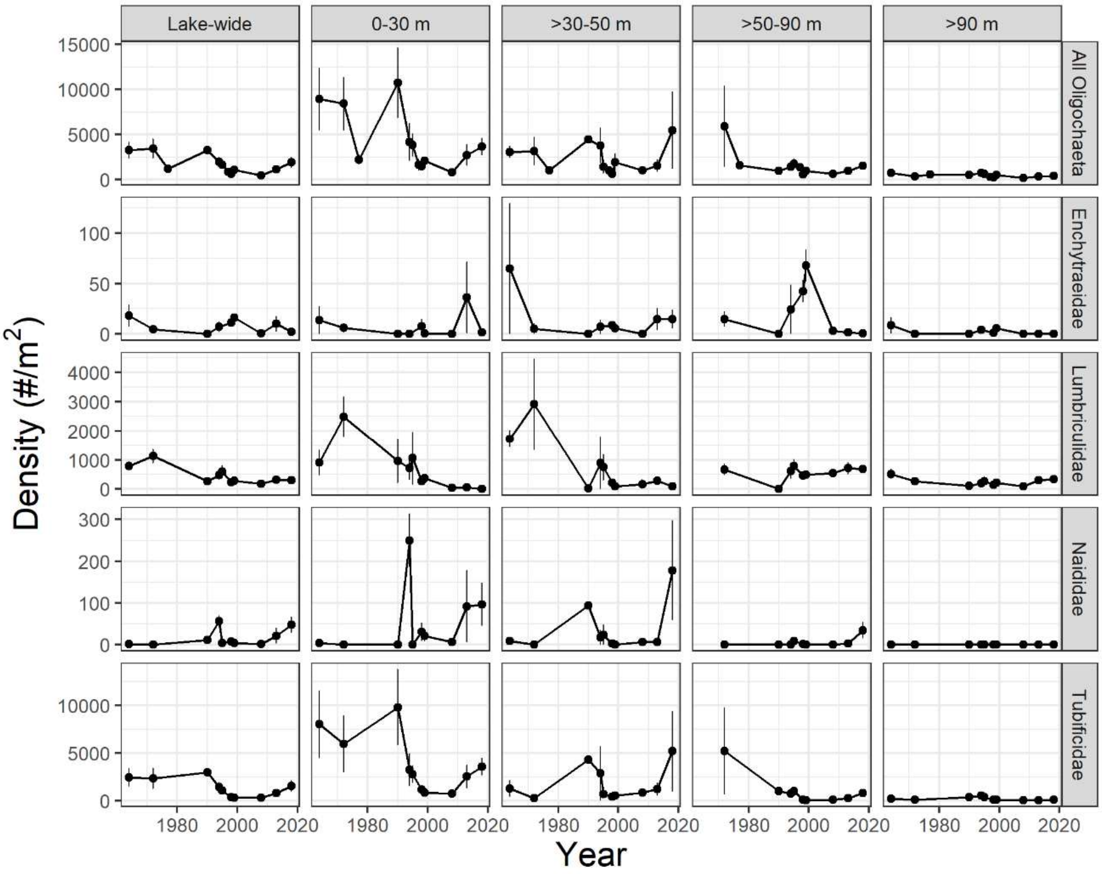
Lake-wide
0-30 m
>30-50 m
>50-90 m
>90 m
All Oligochaeta
Enchytraeidae
Lumbriculidae
Naididae
Tubificidae
Density (#/m²)
Year
15000
10000
5000
0
100
50
0
4000
3000
2000
1000
0
300
200
100
0
10000
5000
0
1980
2000
2020

Figure 6

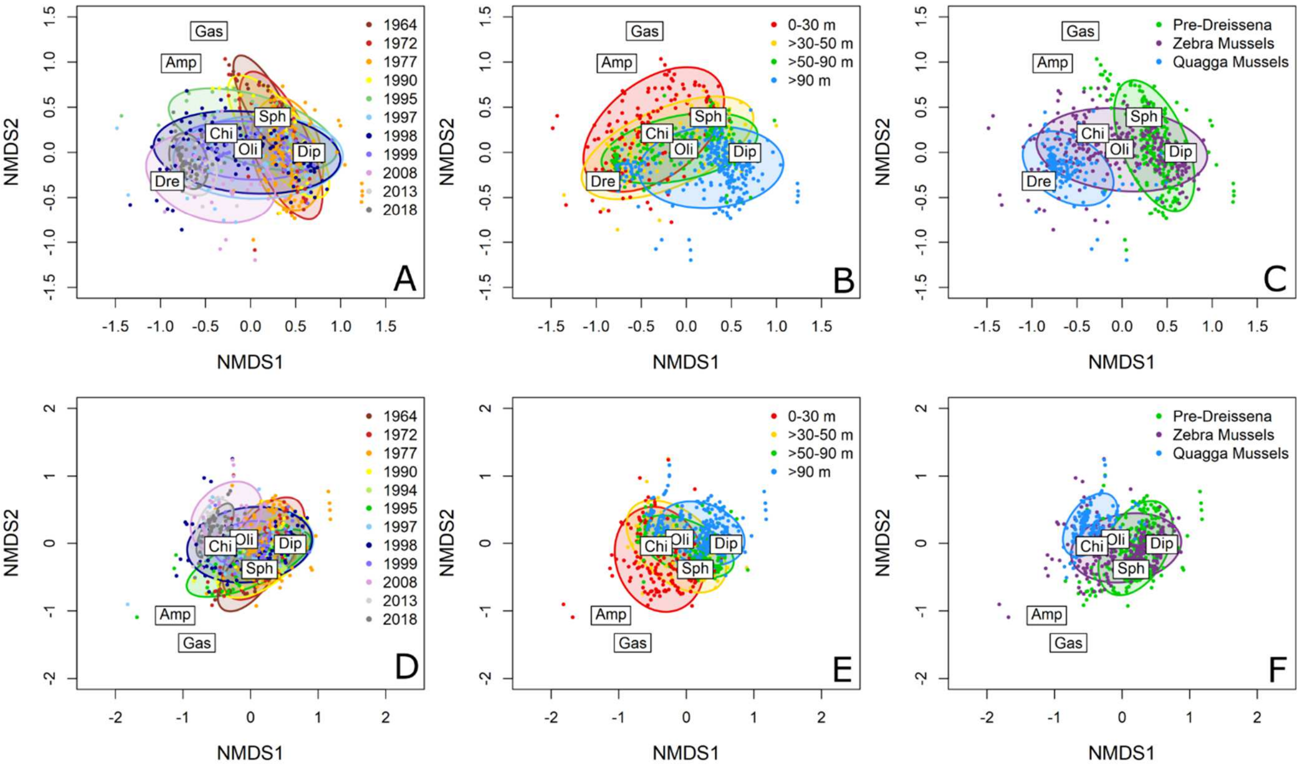
A
B
C
D
E
F
NMDS1
NMDS2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
-2
-1
0
1
2
Gas
Amp
Chi
Oli
Sph
Dip
Dre
1964
1972
1977
1990
1994
1995
1997
1998
1999
2008
2013
2018
0-30 m
>30-50 m
>50-90 m
>90 m
Pre-Dreissena
Zebra Mussels
Quagga Mussels

Figure 7