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LAKE ONTARIO BENTHOS SURVEY COOPERATIVE SCIENCE AND MONITORING INITIATIVE 2018

Technical Report

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TECHNICAL REPORT

LAKE ONTARIO BENTHOS SURVEY

COOPERATIVE SCIENCE AND MONITORING INITIATIVE 2018

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LAKE ONTARIO BENTHOS SURVEY

COOPERATIVE SCIENCE AND MONITORING INITIATIVE 2018

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CHAPTER 1. MAJOR FINDINGS FROM THE CSMI BENTHIC MACROINVERTEBRATE SURVEY IN LAKE ONTARIO IN 2018 WITH AN EMPHASIS ON TEMPORAL TRENDS

INTRODUCTION

In this report, we present results of a benthic survey of Lake Ontario conducted as part of the United States Environmental Protection Agency (U.S. EPA) Great Lakes National Program Office (GLNPO) Great Lakes Biology Monitoring Program (GLBMP). The benthic monitoring component of GLBMP includes sample collections from a number of long-term monitoring stations (9 - 16 depending on the lake) sampled every year for each of the five Great Lakes and a much more intensive lake-wide survey conducted on each lake every 5 years as part of the Cooperative Science and Monitoring Initiative (CSMI). Consistent with the sampling scheme of previous CSMI benthic surveys, a lake-wide benthic survey was conducted in 2018 at 61 stations in Lake Ontario to assess the status of the benthic macroinvertebrate community. The primary focus of this survey was the status of benthic community, including the invasive zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. rostriformis bugensis*) in comparison with historic data.

This report contains detailed descriptions of benthic communities in Lake Ontario in 2018, including information on sampling design (station locations, sampling and laboratory procedures) and the taxonomy and abundance of benthic invertebrates. Primary information (number and biomass of each taxon in each replicate sample) can be requested from U.S. EPA GLNPO. Detailed analysis of results obtained within this study are provided in the peer-reviewed publications submitted to the special issue of the *Journal of Great Lakes Research* “Lake Ontario 2020” (Appendices 2, 3).

METHODS

Station Locations and Field Procedures

Samples for benthic macroinvertebrates were collected from August - September 2018 at 61 stations located throughout Lake Ontario (Fig. 1.1, Appendix 1), including historically sampled sites. Stations were sampled aboard the U.S. EPA R/V *Lake Guardian* using a regular Ponar grab (sampling area 0.0523 m^2, coefficient used to calculate density per m^2 = 19.12), including 9 stations sampled during the summer Long-term Monitoring (LTM) survey in August and 52 stations during the CSMI survey in September. Three replicate Ponar samples were successfully collected at 55 of the planned 61 stations, excluding 6 stations (#29, 42, 43, 62, 66, and 71B) where samples were not collected due to hard substrate. A total of 165 samples were analyzed for benthos and *Dreissena* population assessment.

Upon collection, each sample was placed separately into an elutriation device and then washed through a 500-μm mesh screen. All retained organisms and sediments were placed into a collection jar and preserved with neutral buffered formalin with Rose Bengal stain to a final concentration of 5 – 10%. Detailed methods are described in the EPA GLNPO Standard Operating Procedure for Benthic Invertebrate Field Sampling (SOP LG406, Revision 12, March 2018).

Laboratory Procedures

All organisms found in each replicate sample at the 55 Ponar stations were sorted, identified, counted, and weighted (total wet weight). Organisms were separated under low magnification using a dissecting microscope. Oligochaetes and chironomids were mounted on slides and identified using a compound microscope; other organisms were identified using a dissecting microscope. Adult oligochaetes were identified to species; immature Tubificidae, Lumbriculidae, Naididae and Enchytraeidae were identified to the lowest taxonomic level possible, usually family, and included in density and biomass estimates. Counts of oligochaete fragments were excluded from density analyses but fragment weight was considered in the determination of biomass. Immature Oligochaeta (in cocoons) were recorded but excluded both from density and biomass calculations for comparison with historic data. Chironomids were identified to the lowest practical taxonomic level, usually genus. Other invertebrates were identified to species, when possible.

Dreissena from all samples were identified to species, measured to the nearest millimeter with a caliper, counted, and the whole sample was weighed to the nearest 0.0001 g after being blotted dry on absorbent paper (total wet weight of tissue and shell, WW); details are described in the EPA GLNPO

Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis (SOP LG407, Revision 09, April 2015). All *Dreissena* collected during this survey were quagga mussels.

Figure 1.1. Location of stations in Lake Ontario sampled for *Dreissena* during August – September 2018. Please find information on station locations and depths in Appendix 1.

RESULTS AND DISCUSSION

Benthic Taxonomy, Density and Biomass

We found 76 species and higher taxa of benthic macroinvertebrates in Lake Ontario in 2018. The most diverse were Oligochaeta (33 species and higher taxa), Insecta (Chironomidae, 28), Malacostraca (6 species: 5 Amphipoda and 1 Mysida), and Bivalvia (3). Other classes were represented by less than 3 taxa, or were not identified to species level (e.g., Trepaxonemata, Hirudinea, Hydrozoa, Nemertea). Among Oligochaeta, the most diverse were Tubificidae (18 species and higher taxa), and Naididae (13).

The most widely occurred species throughout the lake was exotic bivalve *Dreissena r. bugensis*, found at 98% of all 55 benthic stations sampled, followed by Oligochaeta (immature tubificids: 83%, immature lumbriculids: 78%, lumbriculid *Stylodrilus heringianus*: 60%, and tubificid *Limnodrilus hoffmeisteri*: 56%), *Mysis* (56%), and chironomids (*Micropsectra* sp.: 47%, *Heterotrissocladius subpilosus* group: 44%, and *Procladius* sp.: 36%). All other species were found in less than 50% of the samples.

Dreissena r. bugensis comprised a large percentage of lake-wide benthos densities (67%), followed by Oligochaeta (28%), and by Chironomida (5%). Contribution of other groups (Amphipoda, Gastropoda, Hirudinea, etc.) to total benthos density was less than 1% each. Among Oligochaeta, the most numerous were Tubificidae (79%) and Lumbriculidae (19%). *Dreissena r. bugensis* dominated lake-wide benthos by biomass (99.8% of total wet biomass) (Table 1.1). The remaining benthic biomass was represented by Oligochaeta (0.15%) and Chironomidae (0.02%) (Table 1.1).

Table 1.1. Average (± standard error) density (ind. m^{-2}) and wet biomass (g m^{-2}) of major taxonomic groups of benthic invertebrates collected in Lake Ontario in 2018 averaged by depth zones and lake-wide. In 2018 benthos was collected at 55 stations. n.r. – not recorded. Number of stations given in parentheses.

Taxa	**0 - 30m (13)**	**>30 - 50m (3)**	**>50 - 90m (16)**	**>90m (23)**	**Lake-wide (55)**
Amphipoda (ind. m^{-2})	33±16	n.r.	2±1	n.r.	8±4
Amphipoda (g m^{-2})	0.08±0.04	n.r.	0.01±0.01	n.r.	0.02±0.01
Chironomidae (ind. m^{-2})	569±116	74±49	408±90	88±29	294±48
Chironomidae (g m^{-2})	0.44±0.07	0.05±0.02	0.25±0.06	0.08±0.02	0.21±0.03
Diporeia (ind. m^{-2})	n.r.	n.r.	n.r.	0.3±0.3	0.1±0.1
Diporeia (g m^{-2})	n.r.	n.r.	n.r.	0.002±0.002	0.001±0.001
Dreissena (ind. m^{-2})	5037±2133	4587±1965	4749±532	3554±501	4308±566
Dreissena (g m^{-2})	1432±455	1007±228	1931±236	539±112	1181±156
Sphaeriidae (ind. m^{-2})	n.r.	n.r.	2±2	18±5	8±2
Sphaeriidae (g m^{-2})	n.r.	n.r.	<0.01	0.02±0.01	0.010±0.004
Gastropoda (ind. m^{-2})	57±57	n.r.	n.r.	n.r.	14±14
Gastropoda (g m^{-2})	0.15±0.15	n.r.	n.r.	n.r.	0.03±0.03
Hirudinea (ind. m^{-2})	2±2	n.r.	n.r.	n.r.	0.5±0.5
Hirudinea (g m^{-2})	<0.001	n.r.	n.r.	n.r.	<0.001
Mysidae (ind. m^{-2})	1±1	n.r.	|}16±4	40±10	21±5
Mysidae (g m^{-2})	0.004±0.004	n.r.	0.19±0.05	0.62±0.24	0.31±0.11
All Oligochaeta (ind. m^{-2})	3681±940	5494±4300	1516±263	426±79	1789±367
All Oligochaeta (g m^{-2})	1.87±0.63	2.68±1.73	2.56±0.42	1.07±0.28	1.78±0.25
Others (ind. m^{-2})	16±8	36±19	14±4	5±2	12±3
Others (g m^{-2})	0.04±0.03	0.03±0.02	0.04±0.01	0.02±0.01	0.03±0.01
Turbellaria (ind. m^{-2})	5±3	21±13	6±2	1±1	4±1
Turbellaria (g m^{-2})	0.001±0.001	0.003±0.002	0.001±0.001	<0.001	0.001±0.0002
All benthos (ind. m^{-2})	9401±2919	10212±6256	6711±665	4131±580	6459±845
All benthos (g m^{-2})	1435±456	1009±229	1934±237	541±112	1183±156
All benthos w/o *Dreissena* (ind. m^{-2})	4364±978	5626±4295	1964±259	577±88	2151±384
All benthos w/o *Dreissena* (g m^{-2})	2.58±0.72	2.76±1.73	3.05±0.42	1.81±0.49	2.4±0.31

Long-Term Trends in Benthos

This section contains a brief description of trends for all major groups of benthic invertebrates in the last 50 years (except for *Dreissena*, for which trends are described in the "*Dreissena* Spatial and Temporal Trends" section of this report). This analysis is based on data from 13 lake-wide benthic surveys conducted in Lake Ontario over the course of 54 years (1964, 1972, 1977, 1990, 1994, 1995, 1997-1999, 2003, 2008, 2013 and 2018) (Hiltunen, 1969; Nalepa and Thomas, 1976; Golini, 1979; Lozano et al., 2001; Dermott and Geminiuc, 2003; Watkins et al., 2007; Birkett et al., 2015; Nalepa and Baldridge, 2016, Appendix 2). Due to different sampling locations over time, historical comparisons were performed using densities in each depth zone (<30 m, >30 – 50 m, >50 – 90 m, and >90 m) and lake-wide as a weighted average using means of stations located at 4 depth zones considering the proportion of the total lake area represented by each zone (21.6, 11.7, 18.5, and 48.2%, respectively) (Appendix 2). Detailed analysis of long-term trends in benthos is provided in the peer-reviewed publications submitted to the special issue of the Journal of Great Lakes Research "Lake Ontario 2020" (Appendices 3, 4).

Among the major long-term trends in densities of benthic macroinvertebrates in Lake Ontario, the most important were the decline in *Diporeia* (Spearman $\rho = -0.50$, $P < 0.001$) and in Sphaeriidae ($\rho = -0.40$, $P < 0.001$) at all depth zones starting in the mid-1990s, which followed a period of elevated densities at depths >30 m in late 1980s – early 1990s (Fig. 2, Appendix 2). Similar trends in *Diporeia* densities were observed in lakes Michigan (Nalepa et al., 2017) and Huron (Karatayev et al., 2020). Currently, *Diporeia* is only present at depths >90 m at very low densities (<1 m^{-2}). Total oligochaete density significantly declined in the shallow zone (<30 m) in the past several decades ($\rho = -0.14$, $P < 0.001$); the highest observed densities on record (app. 10,000 m^{-2}) occurred in 1964 and 1990, after which densities decreased to <1000 m^{-2} by 2008. However, there has been an increase in the last five years (2013 – 2018), with densities rebounding to 2,000 – 4,000 m^{-2} (Fig. 2, Appendix 2). These long-term trends were mostly driven by large changes in pollution-tolerant Tubificidae ($\rho = -0.22$, $P < 0.001$), which comprise 20 to 95% of all Oligochaeta densities. Tubificidae underwent the most dramatic changes in the shallow zone ($\rho = -0.42$, $P < 0.001$), declining over ten-fold from their peak densities in 1960s and 1990s (~9,000 m^{-2}) to 765 ± 276 m^{-2} in 2008, and then increased again to ~ 3,000 m^{-2} in 2013 – 2018. Amphipoda (excluding *Diporeia*), Gastropoda, Hirudinea, and Trichoptera densities all peaked in the shallow zone in mid-1990s, likely positively affected by aggregations of zebra mussels, and then declined (Appendix 2).

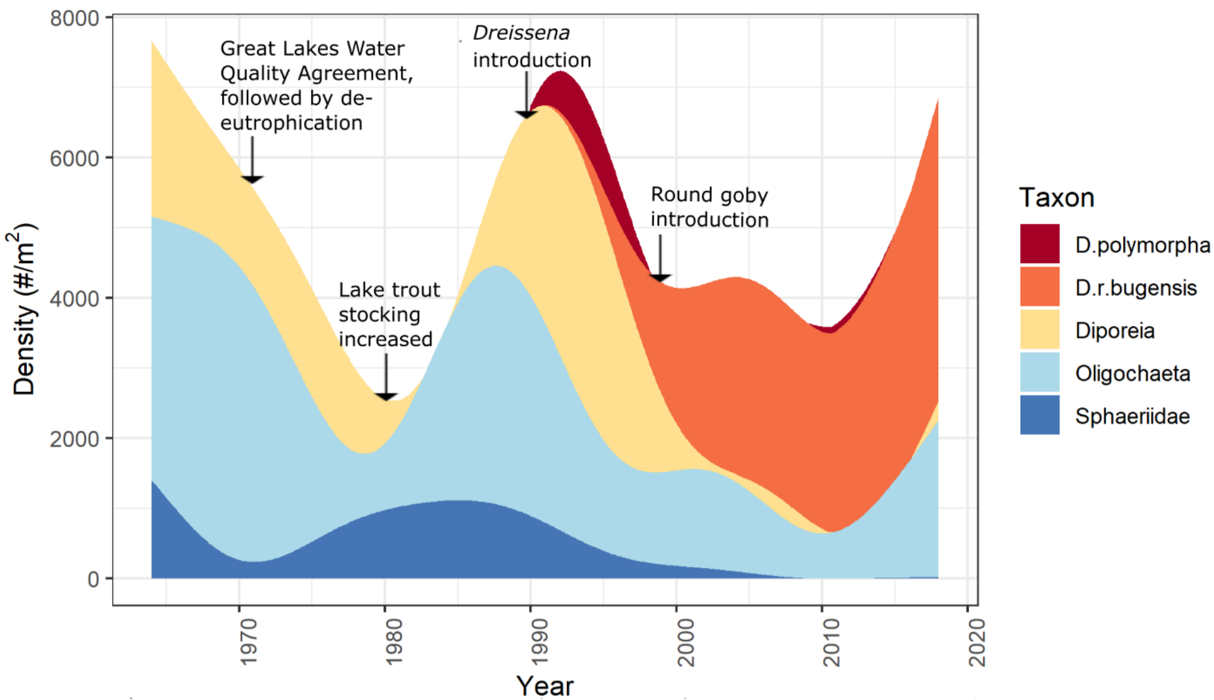

Figure 1.2. Average densities of major taxonomic groups that were consistently counted over time for the entire Lake Ontario with major events highlighted. The following years in our data set were excluded due to incomplete data: 1994 and 2003. Missing data were simulated using splines, then all data were smoothed with a general additive model (GAM) function. See Appendix 2 for data.

The only taxa that showed increasing density trends with time were *Dreissena* (lake-wide $\rho = 0.71$, $P < 0.001$) and Chironomidae ($\rho = 0.20$, $P < 0.001$), with the strongest increases occurring at intermediate depths (>30 – 90 m) (*Dreissena*: $\rho = 0.84$, $P < 0.001$; Chironomidae: $\rho > 0.30$, $P < 0.01$). As a result of mixed increasing and decreasing trends across individual taxa, total benthos density did not exhibit clear overall trends lake-wide ($\rho = 0.07$, $P = 0.04$). However, total benthos (excluding *Dreissena*) declined lake-wide ($\rho = -0.29$, $P < 0.001$). The decline in native species densities was most pronounced at depths >90 m ($\rho = -0.45$; $P < 0.0001$), primarily due to the large declines in *Diporeia* and Sphaeriidae.

Dreissena Spatial and Temporal Trends

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., 1993); therefore, trends observed in Lake Ontario may provide insight into the potential long-term dynamics of *Dreissena* populations across depth zones in other deep lakes of North America and Europe.

To document long-term trends in *Dreissena* population dynamics in Lake Ontario, we compiled a dataset of *Dreissena* spp. densities by station and depth for 1990, 1995, 1997, 1998, 1999, 2003, 2008, and 2013 (Dermott and Geminiuc, 2003; Lozano et al., 2001; Watkins et al., 2007; Birkett et al., 2015; Nalepa and Baldridge, 2016) to complement the data from 2018 presented here. To increase the spatial resolution of the 2003, 2008, and 2013 surveys, we added data from the U.S. EPA Great Lakes National Program Office (GLNPO) long-term monitoring stations (9 to 10 stations per survey, Burlakova et al., 2018). Detailed analysis of this database is provided in a paper submitted to the Special Issue of the Journal of Great Lakes Research "Lake Ontario 2020" (Appendix 3). Below is a brief analysis of *Dreissena* spp. population dynamics in Lake Ontario over the last 30 years.

Previous studies in Lake Ontario have shown that quagga mussels reached their population maximum in the shallow (0 – 30 m) to mid (>30 – 50 m) depth zone by 2003, 13 years after the first detection in Lake Ontario, and then declined (Table 1.2; Birkett et al., 2015; Nalepa and Baldridge, 2016; Karatayev et al., accepted). Such a decline may be expected if quagga mussels in shallow to mid-depth water had increased to densities greater than their carrying capacity. Similar declines in dreissenid densities in the nearshore zone, along with a shift of the maximum density to deeper areas, were also observed in lakes Michigan and Huron (Nalepa et al., 2020; Karatayev et al., 2020; Mehler et al., 2020). At depths <50 m, the decline in density occurred mainly from 2003 to 2008, and there were no significant changes from 2008 to 2018. Mussel densities in >50 – 90 m steadily declined from 2003 to 2018, but densities in deep water and lake-wide have significantly increased (Table 1.2). The increases in mussel density at depths >90 m have a strong influence on lake-wide values because by area, 48% of the lake bottom is >90 m deep.

The recent increases in lake-wide density were unexpected considering the substantial population decline recorded from 2003 to 2008 (Table 1.2). Based on observed declines in lake-wide *Dreissena* density in Michigan in 2015, 18 years after the first record of quagga mussels in the lake (Nalepa et al., 2020), we had expected the 2018 Lake Ontario surveys to indicate further declines in quagga mussel populations as well, especially given that the mussels have been present in Lake Ontario for 30 years. Contrary to our prediction, we found significant increases in *Dreissena* lake-wide density and biomass, suggesting that the mussel population in Lake Ontario is still increasing. The lake-wide average of *Dreissena* biomass was the highest observed in Lake Ontario to date at 25.2±3.3 g m^{-2} of ash-free dry tissue weight (Karatayev et al., accepted).

Table 1.2. Long-term dynamics of *Dreissena polymorpha* and *D. rostriformis bugensis* density (m^{-2}) in Lake Ontario. Average ± standard errors. Lake-wide densities were calculated as weighted averages from four depth zones. Sample size given in parenthesis.

Depth / Species	1990 (25)	1995 (41)	1997 (68)	1998 (114)	1999 (67)	2003 (46)	2008 (58)	2013 (55)	2018 (55)
0 - 30 m									
D. polymorpha	14±9	3108±1118	1259±697	2394±1259	126±59	38±36	0	0	0
D. r. bugensis	0	1798±1078	774±390	3472±1022	1786±335	7724±2936	2366±1074	2651±1177	5037±2132
Both species	14±9	4906±1716	2033±757	5867±1972	1913±333	7762±2931	2366±1074	2651±1177	5037±2133
>30 - 50 m									
D. polymorpha	0	29±29	46±39	27±11	9±9	0	0	0	0
D. r. bugensis	0	15±9	1271±608	1748±517	3899±1057	10315±4289	3536±1741	5385±1301	4587±1964
Both species	0	44±37	1317±619	1776±513	3907±1060	10315±4289	3536±1741	5385±1301	4587±1965
>50 - 90 m									
D. polymorpha	0	4±4	28±26	1±1	3±3	1±1	0	0	0
D. r. bugensis	5±5	7±5	122±55	282±114	4484±1397	7338±1835	6854±993	5355±566	4749±532
Both species	5±5	11±6	150±77	283±114	4487±1397	7339±1835	6854±993	5355±566	4749±532
>90 m									
D. polymorpha	2±2	0	0	>1±>1	0	0	0	0	0
D. r. bugensis	7±7	0	1±1	2±1	35±24	840±479	594±329	1909±398	3554±501
Both species	9±9	0	1±1	2±1	35±24	840±479	594±329	1909±398	3554±501
Lake-wide									
D. polymorpha	4±2	676±242	283±151	521±272	29±13	8±8	0	0	0
D. r. bugensis	4±4	391±233	339±111	1008±230	1688±296	4638±907	2479±393	3114±368	4216±577
Both species	8±5	1067±371	621±179	1528±431	1717±296	4646±906	2479±393	3114±368	4216±577

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CHAPTER 2. UNDERWATER VIDEO IMAGE ANALYSIS OF *DREISSENA* DISTRIBUTION IN LAKE ONTARIO IN 2018

INTRODUCTION

Incorporation of underwater image analysis into the designs of benthic surveys allows for the assessment of much larger lakebed areas, independently of substrate, in a cost- and time-effective manner. Such analysis can provide valuable information about the abundance, distribution patterns, and structure of *Dreissena* beds at various spatial scales, and it may significantly increase the precision of population size estimates (Karatayev et al., 2018).

Underwater video methods have been previously used in the Great Lakes; however, these studies were largely limited to the nearshore zone and analyzed relatively few video images per station (Custer and Custer, 1997; Ozersky et al., 2009, 2011; Lietz et al., 2015; Mehler et al., 2018). We conducted the first lake-wide *Dreissena* studies incorporating video transects in Lake Michigan in 2015, followed by Lake Huron in 2017. At each station (47 and 43 in lakes Michigan and Huron, respectively), we collected continuous video footage from 500 m-long transects along the lakebed. In 2018, we used video transects coupled with traditional grab sampling to estimate *Dreissena* coverage, density, and biomass in Lake Ontario, using procedures previously developed for Lake Michigan (Karatayev et al., 2018).

METHODS

We analyzed bottom video images taken during the 2018 CSMI study in Lake Ontario to study *Dreissena* spatial distributions and aggregation patterns along depth gradients (Fig. 2.1). Video images were obtained from a GoPro Hero 4 Black camera (hereafter GoPro) mounted on the Ponar grab and from a GoPro mounted on a benthic sled towed behind the R/V *Lake Guardian* for approximately 500 m.

Before the analysis, the quality of both Ponar and sled videos were classified as either acceptable or unacceptable for assessment of *Dreissena* density, biomass, and aggregations (Karatayev et al., 2018), and only videos of acceptable quality were used in further analysis. More than 80% of the Ponar videos and almost 60% of the sled videos had acceptable quality (Table 2.1). Half of the sled videos categorized as unacceptable had controllable issues (camera not in focus, insufficient light, sled not on bottom, etc.) while the other half had uncontrollable reasons such as algae cover or *Dreissena* buried in sediment.

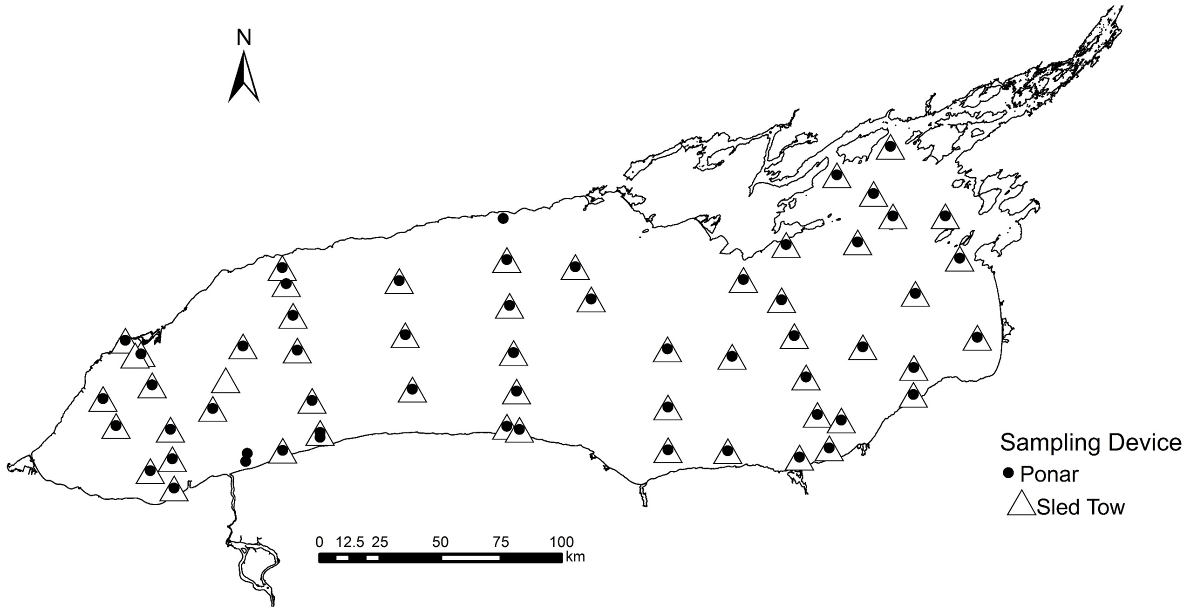


Figure 2.1. Location of stations in Lake Ontario sampled in 2018 with Ponar grabs with videos (black filled circles) and sled tows (open triangles).

Table 2.1. Number of acceptable (percent of total in parenthesis) and unacceptable bottom images collected in Lake Ontario in 2018 using GoPro cameras attached to Ponar grab and benthic sled. Unacceptable images were classified as controllable (e.g. equipment malfunction or human error) or uncontrollable (e.g. high turbidity, macrophyte coverage, etc.).

Parameters	Ponar videos (3 still images per video)	Sled videos (100 still images per video)
Number of stations (CSMI + LTM) with videos	(52 + 7)	(49 + 8)
Number of acceptable still images	123 (86%)	3300 (58%)
Number of unacceptable still images	54 (14%)	2400 (42%)
Controllable	47	900
Uncontrollable	7	1500

Videos from Ponar grabs were stopped shortly before the lake bottom was hit and a screenshot was taken. *Dreissena* mussels in each screen shot from the Ponar deployments (3 replicates at each station) were digitized in Photoshop CS6. *Dreissena* coverage was determined as percentage of each screenshot covered with mussels. To convert *Dreissena* percent coverage obtained from sled video images into density and biomass, we compared the density and biomass of *Dreissena* in three replicate Ponar samples with the mussel coverage estimated from the video images of the exact same Ponar replicate. *Dreissena* density and biomass from each Ponar replicate was paired with the coverage from still images, and the relationship between coverage and density and biomass was estimated using polynomial regression. We removed outliers using Grubbs test for outliers, which is calculated as the ratio of the largest absolute deviation from the sample mean to the sample standard deviation (Grubbs, 1969). We then used the relationship to convert *Dreissena* coverage from sled images into *Dreissena* density and biomass. We used t-tests to compare coverage among lakes using data from same depth zones, and to contrast density and biomass estimations from sled video transects with Ponar densities, and between lakes. For all tests effects were considered significant at $P < 0.05$, and marginally significant at $P < 0.10$.

RESULTS AND DISCUSSION

Benthic Sled Videos

The *Dreissena* distribution across depths estimated from sled tows had a skewed bell shape, with relatively low average coverage in the nearshore (10 – 30 m) depth zone, the highest average density in the intermediate (>30 – 100 m) depth zone, and the lowest densities in the deepest part of the lake (>100 m) (Fig. 2.2, Table 2.2). The highest absolute coverage (98.7%) was found on a rocky substrate at station ON66 (16.6 m depth).

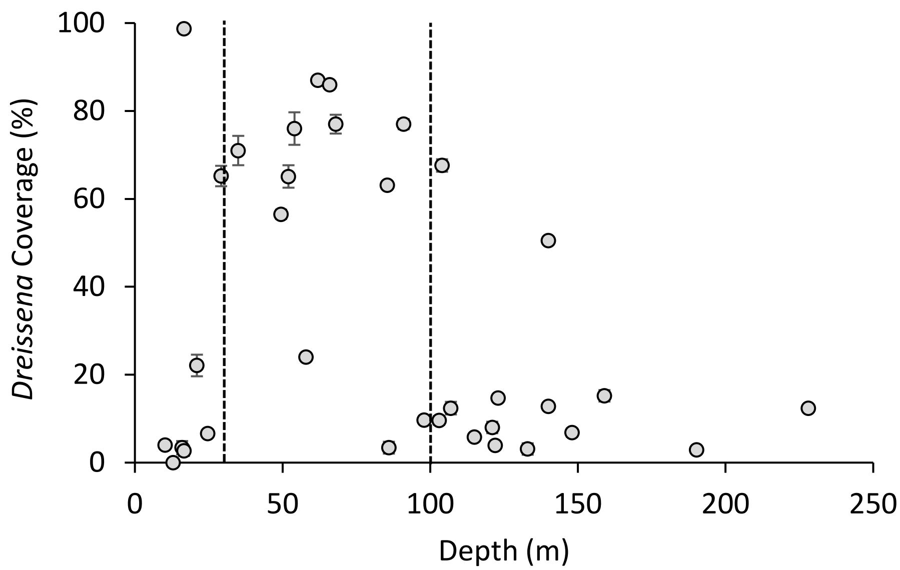


Figure 2.2. *Dreissena* percent coverage along depth gradient in of Lake Ontario in 2018. Error bars represent ±1 standard error. Dashed lines denote 30 m and 100 m depth ranges.

A similar bell-shaped distribution pattern of *Dreissena* coverage was found in our previous studies in Lake Michigan in 2015 and Lake Huron 2017 (Fig. 2.3). At the >30 – 100 m depth zone, *Dreissena* coverage was significantly higher in lakes Ontario and Michigan than in Lake Huron (P <0.001 for both t-tests), but the *Dreissena* coverage was not different between lakes Ontario and Michigan (P = 0.39). At the >100 m depth zone, *Dreissena* coverage was significantly higher in Lake Ontario compared to Lake Michigan (P <0.01), but no difference was found between lakes Ontario and Huron (P = 0.29) or lakes Michigan and Huron (P = 0.26). Lake-wide *Dreissena* coverage was significantly higher in lakes Ontario and Michigan compared to Lake Huron (P <0.001 for both tests), but there was no difference in coverage between lakes Ontario and Michigan (33% vs. 34%, P = 0.42).

Similar to other Great Lakes, in Lake Ontario there is an abundant food supply for *Dreissena* in the shallow, warm, and well mixed nearshore environment, but physical disturbance (wave and currents) limits *Dreissena* to areas with suitable substrate for attachment (e.g. gravel, rocks, bedrock). Therefore, the distribution of *Dreissena* in such areas is typically very heterogeneous, with higher densities on stable rocky substrates compared to areas with less stable substrates (Fig. 2.4A).

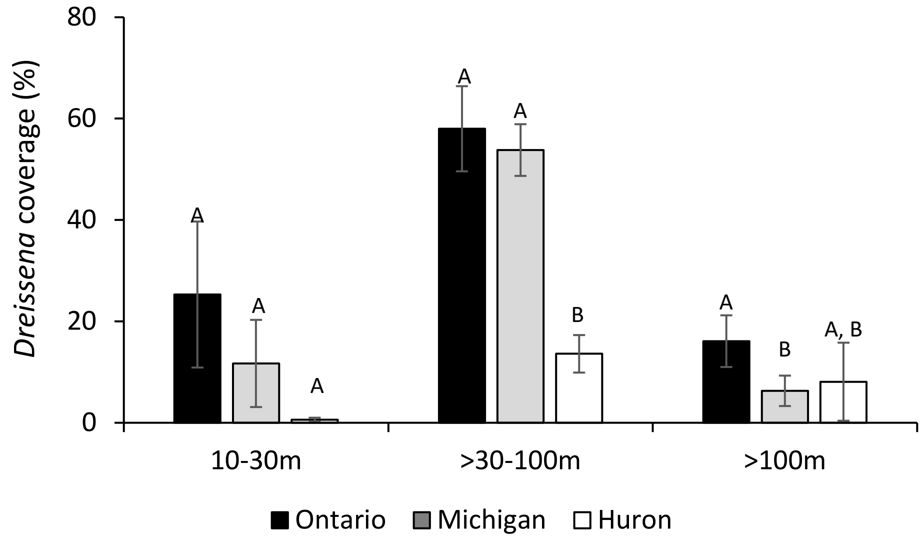


Figure 2.3. Comparison of *Dreissena* coverage in sled tows between lakes Ontario, Michigan and Huron. Different letters above each bar indicate a significant difference (P <0.05) between lakes in each depth zone.

In the mid-depth zone, where food is still available but physical disturbance is lower, *Dreissena* forms the largest aggregations (Fig. 2.4B). In the deepest zone, *Dreissena* densities are lower and individuals are almost evenly distributed on the surface of bottom sediments; this distribution pattern is likely beneficial to mussels in the profundal zone because it reduces food competition where resources are scarce (Fig 2.4C). In the deepest zones of the lake, *Dreissena* only forms sizable aggregations along ridges, trenches, or rocks emerging above the sediment surface. These irregularities in the bottom floor create turbulence that can deliver additional food to the area, thus supporting higher densities of mussels than the flat bottom areas.

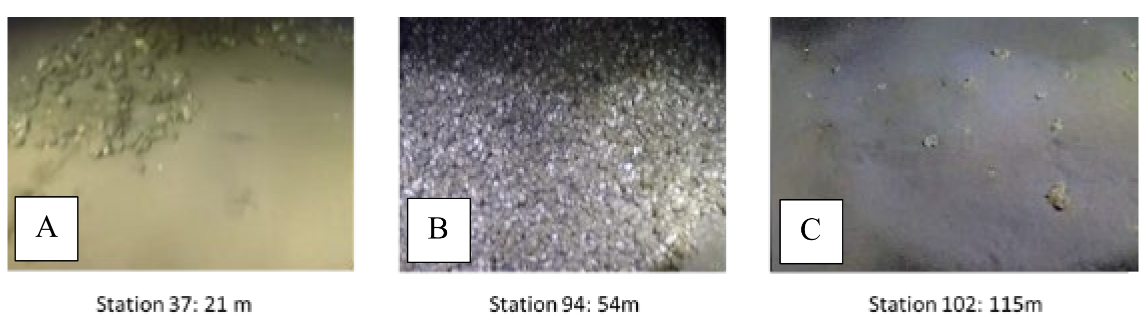
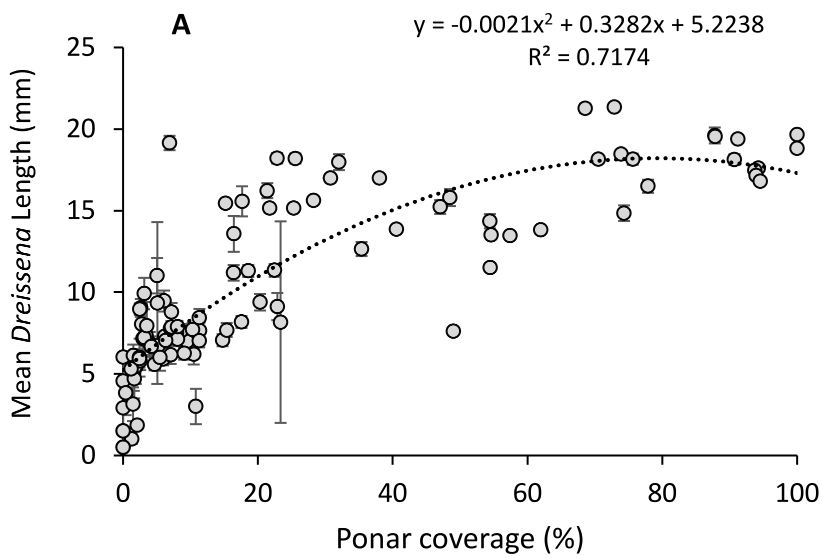


Figure 2.4. *Dreissena* representative screen shots for 10 – 30 m (A), >30 – 100 m (B), and >100 m depth zones (C). Station numbers and depth are provided for each screen shot.

Ponar Videos

Dreissena areal coverage from 123 Ponar videos (3 replicates per Ponar grab) ranged from 0% to 100% (Mean ± SE: 26.1% ± 2.8). Similar to observations based on sled tow data, the mean *Dreissena* coverage based on Ponar videos was significantly higher (52.8% ± 5.0) at intermediate depths (>30 – 100 m) compared to shallow areas (7.1% ± 2.0, t-test, $p < 0.001$) and deeper areas (>100 m) (12.4% ± 2.1, t-test, $p < 0.001$). There was a strong relationship between coverage and mean *Dreissena* length (Fig. 2.5A). The average shell length of *Dreissena* was larger (15.6 ± 1.0 mm) in areas with the highest coverage in mid-depths (>30 – 100 m) compared to mussels from shallow depths (10 – 30 m, 5.2 ± 1.5 mm) and mussels from >100 m (8.2 ± 0.9 mm), likely due to combined effects of depth-related recruitment, density-dependence, and goby consumption (Fig. 2.5B). The abundance of 5 to 12 mm dreissenids, the size range most commonly consumed by round goby, was low except at >100 m depths. Although these size distributions indicate that round goby is affecting mussel recruitment, we did not find a decline in dreissenid density in the nearshore and mid-depth ranges where goby have been abundant since 2005 (Karatayev et al., accepted, Appendix 3).



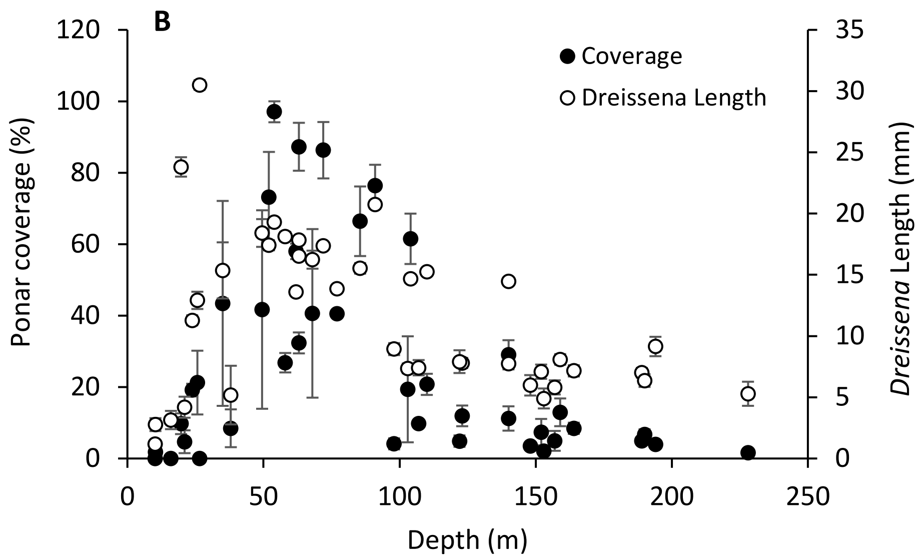


Figure 2.5. Power regression between average (+SE) *Dreissena* coverage and *Dreissena* mean length at each station collected from replicate Ponars (A) and relationship between *Dreissena* coverage, mean *Dreissena* size, and depth in Lake Ontario 2018 (B).

***Dreissena* Density: Ponar *vs*. Video Images**

The relationships between mussel density and biomass measured in Ponar grab samples and *Dreissena* percent coverage obtained from Ponar video images were best explained by second degree polynomial regression:

$\boldsymbol{Density = -1.0068(coverage)^2 + 138.22(coverage) + 1174.1}$,

multiple $R^2 = 0.53$, $p < 0.01$ (Fig. 2.6A);

$\boldsymbol{Biomass = -0.2186(coverage)^2\ x\ 44.223(coverage) + 136.53}$,

multiple $R^2 = 0.66$, $p < 0.01$ (Fig. 2.6B).

These coefficients were used to convert *Dreissena* coverage in sled tows into density and biomass. The polynomial relationships were due to the larger average *Dreissena* sizes in areas of intermediate depths and high coverage. *Dreissena* densities increased with increasing coverage up to 60%. Thereafter the curve flattened (biomass) and slightly declined (density) up to a coverage of 100%, respectively.

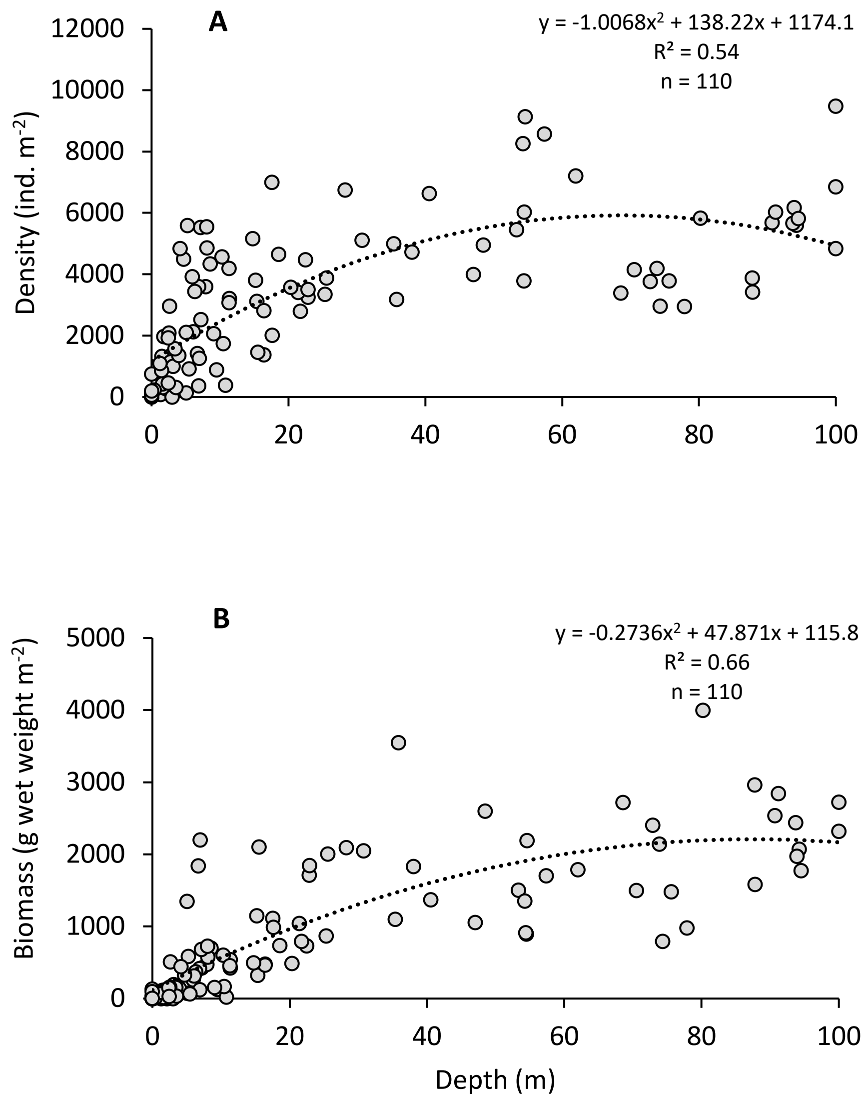


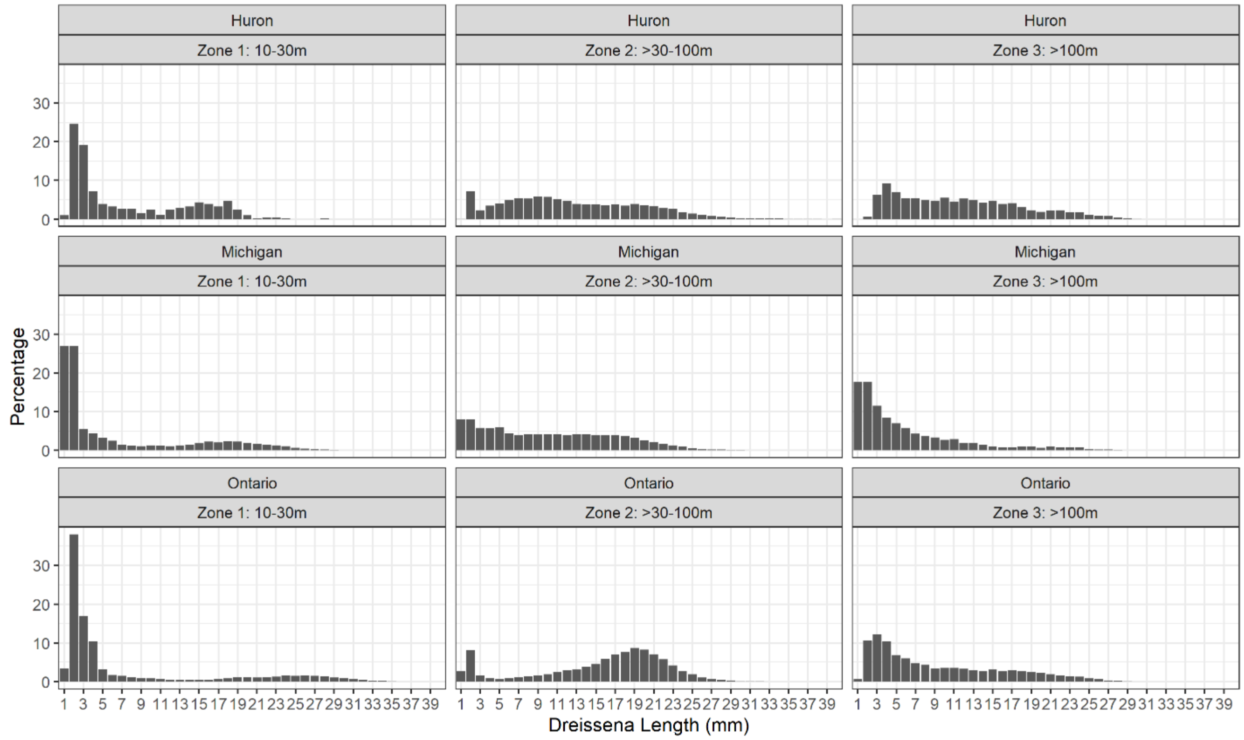
Figure 2.6. Relationship between *Dreissena* coverage in video, and density (ind. m^{-2}) and biomass (g m^{-2}) obtained from the same Ponar grabs in Lake Ontario in 2018.

We compared density and biomass estimated from benthic sled video transects with Ponar samples for 33 stations where we had useable data from both bottom grabs and sled tows (Table 2.2). There was a large but non-significant difference between mean *Dreissena* density estimated for sled tows and Ponar grabs within the 10 – 30 m depth zone ($P = 0.23$) and the >100 m depth zones ($P = 0.06$). *Dreissena* densities in the 10 – 30 m and >30 – 100 m depth zones were similar between sled tows and Ponar grabs ($P = 0.23$). Differences between mean *Dreissena* biomass based on sled tows and Ponar grabs were almost negligible for all three depth zones ($0.42 > P > 0.27$) due to high densities of small *Dreissena* (<10 mm) in those depth zones (compared to lakes Michigan and Huron), which contributed strongly to overall density but not to biomass due to their minute weight (Fig. 2.7A). At the 10 – 30 m depth zone, almost half of the total counts were very small mussels (<10 mm), while in the >100 m depth zone, only one third of the mussels were <10 mm in length (Fig. 2.7B).

Table 2.2. Average *Dreissena* percent coverage (% ± standard error), average density (m^{-2}) and average total wet biomass (g m^{-2}, shell plus tissue) across depth zones (m) from Ponar samples and estimated from video transects sampled in lakes Ontario in 2018, Huron in 2017, and Michigan in 2015. N represents the number of stations per depth zone. Data for lakes Michigan and Huron from Karatayev et al. (2018) and Karatayev et al. (2020), respectively. Asterisks indicate significate difference (t-test, $p < 0.05$) between Lake Ontario and other lakes in estimated density and biomass of *Dreissena* by depth zone.

Depth zone (m)	N	Coverage Sled (%)	Transect video density (m^{-2})	Ponar density (m^{-2})	Transect video biomass (g m^{-2})	Ponar biomass (g m^{-2})
			Lake Ontario			
10-30	7	25.3 ± 3.2	2885 ± 90	4982 ± 3382	913 ± 36	1115 ± 644
>30-100	12	58 ± 8.4	4665 ± 63	5208 ± 1495	1719 ± 32	1855 ± 1072
>100	14	16.1 ± 5.1	2724 ± 69	4368 ± 1782	703 ± 26	700 ± 405
			Lake Huron			
10-30	12	0.6 ± 0.4	82 ± 52*	65 ± 32	16 ± 10*	17 ± 12
>30-100	28	13.6 ± 3.7	1814 ± 484*	1567 ± 645	350 ± 143*	291 ± 111
>100	7	8.1 ± 7.7	1049 ± 996	1150 ± 724	202 ± 124	207 ± 124
			Lake Michigan			
10-30	9	11.7 ± 8.6	1930 ± 1418	2034 ± 931	336 ± 247	543 ± 281
>30-100	23	53.8 ± 5.1	8867 ± 849*	7201 ± 1105	1544 ± 148	1232 ± 140
>100	10	6.3 ± 3.0	1045 ± 500	1544 ± 1091	182 ± 87*	90 ± 46

A



B

Depth zone (m)	N	Percent *Dreissena* <10 mm	Percent *Dreissena* <5 mm
10 - 30	11	48 ± 28	44 ± 11
>30 - 100	16	22 ± 12	15 ± 16
>100	18	65 ± 38	43 ± 3

Figure 2.7. Size frequency distribution of *Dreissena* mussels in lakes Huron, Michigan, and Ontario (A) and percent contribution of mussels <10 mm and <5 mm to the total *Dreissena* numbers within each depth zone in Lake Ontario (B). 1 mm and 2 mm mussels were grouped together when measured for Lake Michigan samples. Therefore, the 1 mm and 2 mm size groups in Lake Michigan each represent half of the 1 - 2 mm size group.

We also compared *Dreissena* densities and biomass based on sled tows among depth zones in Lake Ontario and between Lake Ontario and lakes Huron and Michigan using t-test (Table 2.2). In Lake Ontario, the mean *Dreissena* density within the >30 – 100 m depth zone was higher but not significantly different than in both the 10 – 30 m depth zone ($p = 0.18$) and the > 100 m depth zone ($p = 0.19$). Mean *Dreissena* biomass, however, was marginally significant in >30 – 100 m depth zone compared to the 10 – 30 m depth zone ($p = 0.08$) and was significantly higher than in the > 100 m depth zone ($p < 0.001$). Lake

Ontario *Dreissena* densities within 10 – 30 m and >30 – 100 m depth zones were significantly higher compared to densities for the respective zones in Huron (Table 2.2). Compared to Lake Michigan, Lake Ontario *Dreissena* densities were higher, albeit not significantly, within the 10 – 30 m and >100 m depth zones, but were significantly lower at the 30 – 100 m depth zone. Correspondingly, depth-wise *Dreissena* biomass in Lake Ontario was higher compared to Lake Huron and Lake Michigan, but the difference was not always significant due to large dispersion in data (Table 2.2).

Use of the video transects greatly increases the number of replicates collected at each site (100 replicates/station for a video transect compared to 3 replicates/station for Ponars), which improves the quality of density and biomass estimates via increases in precision and the statistical power of testing (Karatayev et al., 2018). Due to larger sample sizes, the standard error of the station mean in video transects of Lake Ontario was on average 7.3 times lower in sled tows compared to Ponar samples, resulting in an increase in precision of the average estimation of density and biomass at the local (station) scale (Fig. 2.8A and B). At most stations, differences between *Dreissena* densities and biomass were not significant due to usually large standard errors in Ponar grab sample data because of large local patchiness in distribution and low sample size. Therefore, both methods were likely accurate in estimations of the population mean. Only at six stations we did find significant differences in *Dreissena* density and biomass values for sled tows compared to Ponar grabs. Two of those stations (O17, ON64) had a large percentage of small *Dreissena* (>80% were <10 mm), which were difficult to detected in the sled images; this detection error ultimately caused significantly lower densities and biomass in sled tows than the Ponar grabs (ON17 density: $P < 0.0001$, biomass: $P < 0.0001$; ON64 density: $P < 0.05$, biomass: $P < 0.0001$, t-tests). At four other stations, significant differences in either *Dreissena* densities, biomass, or both were the result of unusually low standard error (<10% from mean) in Ponar grab samples (ON27 biomass: $P < 0.01$; ON28 density: $p < 0.05$; ON58 density and biomass: $P < 0.05$; ON94 biomass: $P < 0.05$, t-tests) in contrast to other sites where standard error was high. These stations, all located at the 30 – 100 m depth zone, were quite homogeneously covered by large aggregations of *Dreissena* (Fig. 2.5B) likely resulting in small differences among replicates.

Similar to our previous studies (Karatayev et al., 2018; Karatayev et al., 2020), we found that at a large spatial scale (depth zone), the average *Dreissena* density and biomass were not significantly different between Ponar and video transects. The lack of significant differences between averages obtained by traditional Ponar sampling and video transects have at least two important implications: (1) Ponar grabs provide reliable estimates of *Dreissena* density; (2) the gain in precision by using video transects will be at the station scale, which is the scale used as a target in GLNPO Biology Monitoring Program to monitor changes in benthic species densities. Used in concert with traditional sampling, video sampling has the potential to greatly expand benthic monitoring capabilities. Information from underwater videos can be used to better describe small scall heterogeneity not only of biological but also physical characteristics of the benthic habitat, such as substrate and lake bottom reliefs. Additionally, information from videos are not restricted to *Dreissena* mussels, but can be used to detect other benthic-dwelling organisms such as round gobies or mysids.

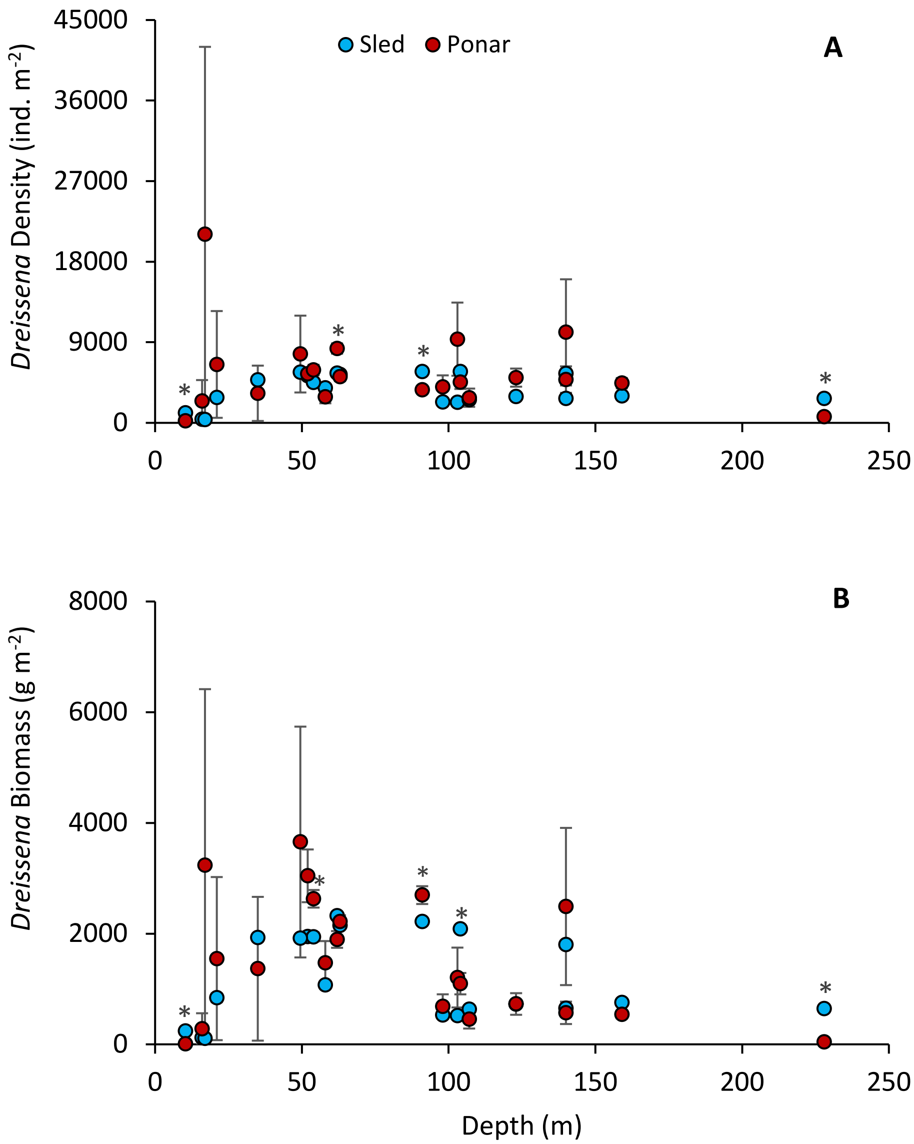


Figure 2.8. Mean *Dreissena* density (ind. m^{-2}, panel A) and mean biomass (g wet weight m^{-2}, panel B) estimated from video transects (100 screen shots analyzed per station, blue circles) and Ponar grab (3 grabs processed per station, red circles). Error bars represent ± 1 standard error. Asterisk above error bars

indicate significant differences between density and biomass based on sled tows and Ponar grabs. Only stations where *Dreissena* were found in both Ponar grabs and video transects are included.

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SUMMARY

In 2018, we conducted a lake-wide survey of benthic macroinvertebrates in Lake Ontario and compared the current status of the community with historic data. We found 87 taxa (species, genera or higher taxa) of benthic macroinvertebrates, and the most diverse were Oligochaeta (33 species and higher taxa), Insecta (Chironomidae, 28), Malacostraca (6 species), and Bivalvia (3). The most widely abundant species throughout the lake was the exotic bivalve *Dreissena r. bugensis*, which was found at 98% of all 55 benthic stations sampled, followed by Oligochaeta, *Mysis*, and chironomids. Among the major long-term changes in densities of benthic macroinvertebrates in Lake Ontario, the most important were the declines in *Diporeia* and Sphaeriidae at all depth zones, which started in the mid-1990s after a period of elevated densities in the late 1980s – early 1990s. Currently, *Diporeia* is only present at depths >90 m at extremely low densities (<1 m^{-2}). The highest densities of Oligochaeta were observed in 1964 (app. 10,000 m^{-2}), and they declined in the 1970s and 1980s, mostly due to the large decrease in pollution tolerant Tubificidae in shallow zone. Oligochaeta densities then increased during the 1990s, likely due to dreissenid invasion. Although they declined somewhat in the late 2000s, their densities have again been increasing over the past five years. The only taxa that showed long-term increases in density were *Dreissena* and Chironomidae, especially at intermediate (>30 – 90 m) depths. Contrary to our prediction, we found continued significant increases in *Dreissena* lake-wide density and biomass in 2018, suggesting that the mussel population in Lake Ontario is still increasing. The lake-wide average *Dreissena* biomass was the highest ever observed in Lake Ontario to date (at 25.2±3.3 g m^{-2} of ash-free dry tissue weight). During the 2018 CSMI survey for Lake Ontario, videos from 59 Ponar stations and 57 sled tows were used to estimate *Dreissena* distribution in the lake and were compared to results from standard Ponar sampling. *Dreissena* coverage was higher at intermediate depths (between 30 and 100 m) than at both shallow (< 30 m) and deep (> 100 m) areas. Compared to previous surveys in lakes Michigan and Huron, *Dreissena* populations in Lake Ontario had higher abundance of small *Dreissena*, especially in the shallowest depth zone (<30 m). Very small mussels (< 10 mm) were difficult to detect in underwater images, resulting in lower *Dreissena* densities in sled tows compared to Ponar grabs when high abundances of small mussels were present. However, *Dreissena* biomass estimated from Ponar and video transects were almost identical. Moreover, at the larger scale (i.e. depth zones), difference in density and biomass estimations were non-significant between sled tows and Ponar grabs. These results underscore the value that may be added to *Dreissena* monitoring efforts by incorporating underwater video imagery in monitoring, especially in areas where Ponar sampling would not be possible (e.g. rocky bottom).

ACKNOWLEDGEMENTS

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APPENDICES

Appendix 1.

Table A1. All 61 stations sampled on Lake Ontario in 2018, with information on lake basins, location (decimal coordinates), proposed (historic) and actual water depth, and main substrate.

Appendix 2.

Table A2. Long-term dynamics of density (mean ± SE, ind./m^{-2}) of major benthic taxa in Lake Ontario from 1964 to 1997 by depths zones.

Table A3. Long-term dynamics of density (mean ± SE, ind./m^{-2}) of major benthic taxa in Lake Ontario from 1998 to 2018 by depths zones.

Data sources: 1964 – Hiltunen, 1969; 1972 – Nalepa and Thomas, 1976; 1977 – Golini, 1979; 1990, 1995 – Dermott and Geminiuc, 2003; 1994, 1997 – Lozano et al., 2001; 1997, 1998, 2003 – Watkins et al., 2007; 2008 – corrected Birkett et al., 2015; 2013 – Nalepa and Baldridge, 2016; 2018 – our data.

Appendix 3. Karatayev, A. Y., L. E. Burlakova, K. Mehler, A. K. Elgin, L.G. Rudstam, J.M. Watkins, and M. Wick. Accepted. *Dreissena* in Lake Ontario 30 years post-invasion. Journal of Great Lakes Research.

Appendix 4. Burlakova, L.E., A.Y. Karatayev, A.R. Hrycik, S.E. Daniel, K. Mehler, L.G. Rudstam, J.M. Watkins, R. Dermott, J. Scharold, A.K. Elgin, and T. Nalepa. In review. Six decades of Lake Ontario ecological history according to benthos. Journal of Great Lakes Research.

Appendix 1.

Table A1. All 61 stations sampled on Lake Ontario in 2018 (including 52 Cooperative Science and Monitoring Initiative (CSMI) stations sampled September 10-18, 2018 and 9 GLNPO Long-term Monitoring stations sampled in August 2018), with information on lake basins, location (decimal coordinates), proposed (historic) and actual water depth, and main substrate. Three replicate Ponar samples were successfully collected at 55 of the planned 61 stations, excluding 6 stations (#29, 42, 43, 62, 66, and 71B, highlighted in grey) where samples were not collected due to hard substrate. All stations were sampled aboard US EPA R/V *Lake Guardian* with Ponar bottom grab (sampling area 0.0523 m^2).

Cooperative Science and Monitoring Initiative (CSMI) stations

Station	Basin	Latitude	Longitude	Proposed depth (m)	Sampling depth (m)	Substrate
6	West	43.46644	-79.5351	62	61.5	silty sand
8	West	43.62203	-79.45365	15.6	13.8	silt
9	West	43.58705	-79.39673	58	56.6	silt
12	West	43.50327	-79.35188	104.8	103.2	silt
14	West	43.39395	-79.48627	98	95.8	silt
16	West	43.27054	-79.36514	66	62.4	silty clay
17	West	43.225	-79.27148	14.4	11.1	silt
18	West	43.3034	-79.27782	85.5	83.6	silty clay
19	West	43.38353	-79.28575	107	104.1	silt, *Dreissena*
22	West	43.2968	-79.00629	11	11	silt, sand
24	West	43.43912	-79.1283	120/96	119	silt, clay
26	West	43.60797	-79.01602	120	116.5	silty clay
27	West	43.68984	-78.8309	114/100	101.3	silt
28	West	43.77517	-78.8546	65/61	60.6	sand
29	West	43.81742	-78.86992	32	29.5	no sample collected, hard substrate
32	Central	43.78277	-78.4377	78	75.3	silty clay
33	West	43.59593	-78.81265	138	135.3	silt
34	West	43.46135	-78.75918	136	134.7	silty clay
35	West	43.36185	-78.729	28	27	silt
36	Central	43.45847	-78.38702	140/160	158	silt
37	Central	43.39145	-78.03646	19	21.7	silty clay

Station	Basin	Latitude	Longitude	Proposed depth (m)	Sampling depth (m)	Substrate
38	Central	43.38287	-77.9897	20	16.5	silty sand
39	Central	43.48562	-77.99746	154	152.7	silty clay
40	Central	43.58959	-78.01297	190	181	silt
42	Central	43.83995	-78.03722	65	64.7	no sample collected, hard substrate
43	Central	43.94909	-78.04914	19	12.6	no sample collected, hard substrate
45	Central	43.82074	-77.78242	80	78.2	sand, clay
58	East	43.328	-77.43791	156/90	87.9	silty sand
61	East	43.78645	-77.15828	54	51.3	silty sand, gravel
62	East	44.88005	-76.99859	18	8.5	no sample collected, hard substrate
64	East	43.52495	-76.92603	214	211.1	silt
65	East	43.30797	-76.95077	155	144.8	silt
66	East	43.34019	-76.83732	18.5	16.5	no sample collected, hard substrate
67	East	43.4054	-76.79116	71	69.3	silty sand
69	East	43.60522	-76.71612	15.8	184.7	silt
72	East	43.54915	-76.52569	113	106.6	silt
73	East	43.63077	-76.2888	40	38.1	fine sand
74	East	43.74834	-76.51604	69	67.25	silt
75	East	43.84225	-76.35555	32	29.3	silty sand
77	East	43.95633	-76.4082	29	76	sand
80	East	44.14225	-76.61178	19	20.2	sand
81	East	44.0164	-76.67477	36.3	34.3	silty sand
82	East	44.06617	-76.81075	27	25.2	silty sand
84	East	43.8871	-76.73356	37	35	sand
94	East	43.32509	-77.21652	45	52.4	silty sand
101	Central	43.63765	-78.41327	146	145.5	silt
102	Central	43.7341	-77.72325	130	111.4	silty clay
106	East	43.95619	-76.60317	133	28.5	silt
715	East	43.63573	-76.9696	151	152.3	silt

Station	Basin	Latitude	Longitude	Proposed depth (m)	Sampling depth (m)	Substrate
716	East	43.60093	-77.4406	151	146.1	silt
71B	East	43.47727	-76.52705	11.6	10.7	no sample collected, hard substrate
93A	West	43.32743	-78.86768	19	17.4	sand

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Station	Basin	Latitude	Longitude	Proposed depth (m)	Sampling depth (m)	Substrate
ON25	West	43.51667	-79.0800	133	133	silt, hard clay on top
ON41	Central	43.7167	-78.0269	128	128	silt
ON55	East	43.4439	-77.4389	192	198	silt, few *Dreissena*
ON60	East	43.58	-77.2000	186/152	152	silt, few *Dreissena*
ON63	East	43.7317	-77.0169	87	87	silt, live *Dreissena*
ON65B	East	43.30833	-76.9500	25.5	25.5	coarse sand, lots of *Dreissena*
ON67B	West	43.37500	-78.7294	54.5	54	silt, lots of *Dreissena*
ON68B	West	43.58333	-79.4167	51.6	51.6	silt, *Dreissena*
ON69B	West	43.31833	-79.0000	15.8	13	very fine sand

Appendix 2.

Table A2. Long-term dynamics of density (mean ± SE, ind./m^{-2}) of major benthic taxa in Lake Ontario from 1964 to 1997 by depths zones. N – number of stations sampled. n.r. – data were not reported. All groups had significant P-values ($P < 0.001$) for year and depth zone in ANOVAs. Data sources: 1964 – Hiltunen, 1969; 1972 – Nalepa and Thomas, 1976; 1977 – Golini, 1979; 1990, 1995 – Dermott and Geminiuc, 2003; 1994, 1997 – Lozano et al., 2001; 1997 – Watkins et al., 2007. Lake-wide density was calculated as a weighted average using means of stations located at 4 depth zones considering the proportion of the total lake area represented by each zone (21.6, 11.7, 18.5, and 48.2%, respectively).

Taxa (by depth zone)	**1964**	**1972**	**1977**	**1990**	**1994**	**1995**	**1997**
<30 m	**N = 13**	**N = 20**	**N = 13**	**N = 7**	**N = 4**	**N = 15**	**N = 13**
Amphipoda	551±169	113±59	0±0	105±102	63±51	330±131	n.r.
Diporeia	1611±689	1412±401	1763±575	24±13	75±75	22±9	34±33
Oligochaeta	8930±3435	8426±2944	2229±531	10760±3863	4200±2079	3853±1246	1334±477
Chironomidae	533±195	166±67	417±109	184±77	155±100	532±208	n.r.
Dreissenidae	0±0	0±0	0±0	14±9	n.r.	4948±1717	2033±757
Sphaeriidae	3339±728	989±302	1736±405	1617±645	219±75	594±281	244±92
Gastropoda	353±129	168±90	35±15	97±48	224±82	892±387	n.r.
All Benthos	16047±3164	11372±3258	6486±1167	12938±3867	5475±2367	11408±2646	4169±975
Benthos w/o *Dreissena*	16047±3164	11372±3258	6486±1167	12925±3865	5475±2367	6460±1502	2136±643
31-50 m	**N = 3**	**N = 5**	**N = 16**	**N = 0**	**N = 2**	**N = 4**	**N = 11**
Amphipoda	5±5	10±10	0±0	n.r.	0±0	5±5	n.r.
Diporeia	8708±633	3045±821	2042±535	n.r.	6073±6066	58±58	163±162
Oligochaeta	3081±626	3185±1575	1016±241	n.r.	3806±1931	1447±762	629±260
Chironomidae	147±126	7±4	64±27	n.r.	42±42	34±34	n.r.
Dreissenidae	0±0	0±0	0±0	n.r.	n.r.	44±37	1316±619
Sphaeriidae	3360±372	421±220	1549±486	n.r.	1955±427	673±374	496±340
Gastropoda	45±40	3±2	3±2	n.r.	0±0	0±0	n.r.
All Benthos	15452±991	6677±2452	4702±1086	n.r.	11986±4701	2314±1100	3243±1074
Benthos w/o *Dreissena*	15452±991	6677±2452	4702±1086	n.r.	11986±4701	2270±1125	1927±987
51-90 m	**N = 0**	**N = 10**	**N = 31**	**N = 4**	**N = 10**	**N = 11**	**N = 16**
Amphipoda	n.r.	11±11	0±0	0±0	0±0	2±2	n.r.
Diporeia	n.r.	2042±572	2661±580	5883±1646	8784±1140	3154±683	3533±106

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Taxa (by depth zone)	**1964**	**1972**	**1977**	**1990**	**1994**	**1995**	**1997**
<30 m	**N = 13**	**N = 20**	**N = 13**	**N = 7**	**N = 4**	**N = 15**	**N = 13**
Oligochaeta	n.r.	5908±4499	1601±304	1006±427	1404±174	1793±544	1028±221
Chironomidae	n.r.	19±13	182±79	0±0	39±29	100±49	n.r.
Dreissenidae	n.r.	0±0	0±0	5±5	n.r.	11±6	150±77
Sphaeriidae	n.r.	101±62	675±140	624±172	839±150	1237±382	314±65
Gastropoda	n.r.	0±0	0±0	0±0	0±0	0±0	n.r.
All Benthos	n.r.	8093±4388	5127±889	7532±2178	11208±1091	6390±1171	5444±1127
Benthos w/o *Dreissena*	n.r.	8093±4388	5127±889	7527±2177	11208±1091	6379±1170	5294±1142
>90 m	**N = 8**	**N = 20**	**N = 91**	**N = 13**	**N = 35**	**N = 11**	**N = 28**
Amphipoda	0±0	0±0	0±0	0±0	0±0	4±4	n.r.
Diporeia	1253±358	780±139	391±68	2071±548	2994±322	3191±478	2168±292
Oligochaeta	773±129	371±56	599±122	521±162	742±89	671±158	224±29
Chironomidae	211±38	5±2	38±9	10±5	16±5	9±5	n.r.
Dreissenidae	0±0	0±0	0±0	7±7	n.r.	0±0	0±0
Sphaeriidae	287±80	20±12	103±15	235±114	207±88	83±28	62±15
Gastropoda	0±0	0±0	0±0	1±1	0±0	0±0	n.r.
All Benthos	2537±516	1228±171	1131±180	2932±676	4050±301	4016±566	2600±301
Benthos w/o *Dreissena*	2537±516	1228±171	1131±180	2925±673	4050±301	4016±566	2599±301
Lake-wide	**N = 24**	**N = 55**	**N = 151**	**N = 25**	**N = 51**	**N = 41**	**N = 68**
Amphipoda	146±45	27±13	0±0	23±NA	13±11	74±28	12±11
Diporeia	2416±294	1415±180	1300±179	2512±NA	3798±755	2136±263	1727±243
Oligochaeta	3262±916	3462±1064	1185±143	3275±NA	1967±504	1655±310	888±143
Chironomidae	287±59	42±15	149±28	45±NA	53±23	141±46	59±28
Dreissenidae	0±0	0±0	0±0	7±NA	n.r.	1074±370	619±179
Sphaeriidae	1535±205	290±71	730±108	884±NA	531±73	476±104	198±46
Gastropoda	100±35	37±19	8±3	22±NA	48±18	192±83	0±0
All Benthos	7961±902	5323±1115	3443±338	6841±NA	n.r.	5851±681	3538±353
Benthos w/o *Dreissena*	7961±902	5323±1115	3443±338	6834±NA	6610±789	4777±494	2920±314

Table A3. Long-term dynamics of density (mean ± SE, ind./m^{-2}) of major benthic taxa in Lake Ontario from 1998 to 2018 by depths zones. N – number of stations sampled. n.r. – data were not reported. All groups had significant P-values (P < 0.001) for year and depth zone in ANOVAs. Data sources: 1998, 2003 – Watkins et al., 2007; 2008 – corrected Birkett et al., 2015; 2013 – Nalepa and Baldridge, 2016; 2018 – our data. Lake-wide density was calculated as a weighted average using means of stations located at 4 depth zones considering the proportion of the total lake area represented by each zone (21.6, 11.7, 18.5, and 48.2%, respectively).

	1998	**1999**	**2003**	**2008**	**2013**	**2018**
<30 m	**N = 25**	**N = 9**	**N = 9**	**N = 13**	**N = 8**	**N = 13**
Amphipoda	138±47	n.r.	n.r.	1±1	48±41	33±16
Diporeia	1±1	202±138	0±0	0±0	0±0	0±0
Oligochaeta	1501±472	2100±495	n.r.	808±272	2738±1158	3681±940
Chironomidae	252±83	663±313	n.r.	154±61	486±261	569±116
Dreissenidae	5867±1972	1913±333	9193±3419	2366±1161	3302±1387	5037±2132
Sphaeriidae	235±96	375±150	n.r.	0±0	0±0	0±0
Gastropoda	271±86	n.r.	n.r.	143±143	27±26	57±57
All Benthos	8382±2285	n.r.	n.r.	3471±1251	6614±1108	9401±2919
Benthos w/o *Dreissena*	2515±592	n.r.	n.r.	1106±378	3312±1108	4364±977
31-50 m	**N = 15**	**N = 6**	**N = 5**	**N = 4**	**N = 8**	**N = 3**
Amphipoda	6±2	n.r.	n.r.	9±5	10±9	0±0
Diporeia	67±67	9±7	1±1	0±0	0±0	0±0
Oligochaeta	651±350	1911±1380	n.r.	1025±240	1552±653	5494±4300
Chironomidae	289±114	241±138	n.r.	278±248	125±60	74±49
Dreissenidae	1755±548	3907±1059	10949±5195	4419±1936	4366±1271	4587±1964
Sphaeriidae	213±70	160±76	n.r.	0±0	0±0	0±0
Gastropoda	3±2	n.r.	n.r.	0±0	14±13	0±0
All Benthos	2994±722	n.r.	n.r.	5732±1824	6067±1854	10212±6256
Benthos w/o *Dreissena*	1239±412	n.r.	n.r.	1313±351	1701±649	5626±4295
51-90 m	**N = 34**	**N = 24**	**N = 9**	**N = 15**	**N = 8**	**N = 16**
Amphipoda	2±1	n.r.	n.r.	1±1	0±0	2±1
Diporeia	1301±429	764±275	97±86	6±6	0±0	0±0

	1998	1999	2003	2008	2013	2018
Oligochaeta	564±57	995±120	n.r.	631±81	1002±218	1516±263
Chironomidae	123±33	77±16	n.r.	210±70	212±72	408±90
Dreissenidae	336±123	4487±1397	6526±2022	7149±1177	5504±700	4749±532
Sphaeriidae	280±36	231±40	n.r.	4±2	2±2	2±2
Gastropoda	0±0	n.r.	n.r.	0±0	0±0	0±0
All Benthos	2630±444	n.r.	n.r.	8003±1187	6721±750	6711±665
Benthos w/o *Dreissena*	2294±453	n.r.	n.r.	855±114	1216±184	1963±259
>90 m	**N = 40**	**N = 28**	**N = 13**	**N = 19**	**N = 21**	**N = 23**
Amphipoda	0±0	n.r.	n.r.	0±0	0±0	0±0
Diporeia	2343±336	2181±335	545±111	41±18	0±0	0±0
Oligochaeta	274±49	543±109	n.r.	169±52	381±61	426±79
Chironomidae	13±3	54±17	n.r.	63±39	80±16	88±29
Dreissenidae	2±1	35±24	1099±614	655±361	2044±456	3554±501
Sphaeriidae	108±17	104±22	n.r.	16±4	23±6	17±5
Gastropoda	0±0	n.r.	n.r.	0±0	0±0	0±0
All Benthos	2788±361	n.r.	n.r.	965±406	2529±496	4131±580
Benthos w/o *Dreissena*	2786±360	n.r.	n.r.	310±76	485±69	577±88
Lake-wide	**N = 114**	**N = 67**	**N = 36**	**N = 51**	**N = 45**	**N = 55**
Amphipoda	31±10	0±0	n.r.	1±1	12±9	7±4
Diporeia	1380±181	1238±172	281±56	21±9	0±0	0±0
Oligochaeta	647±113	1122±201	n.r.	492±71	1141±266	1921±545
Chironomidae	99±21	224±68	n.r.	135±39	197±59	249±34
Dreissenidae	1532±430	1717±296	4999±1067	2667±438	3228±420	4215±576
Sphaeriidae	180±25	192±36	n.r.	8±2	11±3	9±2
Gastropoda	59±19	0±0	n.r.	31±31	7±6	12±12
All Benthos	3999±535	4513±421	n.r.	3366±453	4599±425	6455±1011
Benthos w/o *Dreissena*	2467±237	2796±305	n.r.	700±101	1371±255	2239±547