Evaluation of Toronto Region Area of Concern Degradation of Phytoplankton and Zooplankton Populations and Analysis to Support Loss of Fish and Wildlife Habitat Beneficial Use Impairments

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EVALUATION OF TORONTO REGION AREA OF CONCERN DEGRADATION OF PHYTOPLANKTON AND ZOOPLANKTON POPULATIONS AND ANALYSIS TO SUPPORT LOSS OF FISH AND WILDLIFE HABITAT BENEFICIAL USE IMPAIRMENTS

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Abstract

Elmarsafy, M., Bowen, K.L., Fitzpatrick, M.A.J., Niblock, H.A., Gentile, J.N., Currie, W.J.S. Evaluation of Toronto Region Area of Concern Degradation of Phytoplankton and Zooplankton Populations and Analysis to Support Loss of Fish and Wildlife Habitat Beneficial Use Impairments. Can. Manuscr. Rep. Fish. Aquat. Sci. 3313: xii + 104 p. https://doi.org/10.60825/f2fd-pk82

This study identifies plankton communities impairments within the Toronto and Region Area of Concern (AOC) to determine food resources for forage fishes in different habitats to support an assessment of Beneficial Use Impairment (BUI) 13 (*Degradation of Phytoplankton and Zooplankton Populations*) and provide georeferenced habitat data for BUI 14 (*Loss of Fish and Wildlife Habitat*). Water quality, zooplankton abundance and biomass differed across habitat ecotypes, with Inner Harbour exhibiting very low biomass similar to the open waters of Lake Ontario and a higher proportion of small taxa like rotifers, in spite of elevated nutrients. Zooplankton prey reductions are likely due to multiple urban environmental impacts including runoff and contaminants. Ecotype impacted zooplankton productivity but not primary productivity, indicating that protected systems (Island channels, Habitat Cells and Embayments) can offer diverse physical habitats and forage for fishes. Though primary productivity rates were reduced in most habitats, productivity was shunted into bacterial growth which were highly elevated throughout the entire AOC. Seasonality in habitat characteristics differed between ecotype, notably the Cells and Islands. Seasonal plankton community assessments among a range of habitats are needed to determine how plankton are incorporated into the food web, and continued monitoring is critical to assess the effectiveness of major remediation initiatives, such as wetland creation, the ongoing Don Mouth Naturalization, and Flood Protection Projects.

Résumé

Elmarsafy, M., Bowen, K.L., Fitzpatrick, M.A.J., Niblock, H.A., Gentile, J.N., Currie, W.J.S. Evaluation of Toronto Region Area of Concern Degradation of Phytoplankton and Zooplankton Populations and Analysis to Support Loss of Fish and Wildlife Habitat Beneficial Use Impairments. Can. Manuscr. Rep. Fish. Aquat. Sci. 3313: xii + 104 p. https://doi.org/10.60825/f2fd-pk82

Cette étude identifie les dégradations des communautés planctoniques dans la secteur préoccupante (SP) de Toronto et de sa région afin de déterminer les ressources alimentaires des poissons fourrages dans différents habitats, dans le but d'étayer une évaluation de la l'altération des utilisations bénéfiques (AUB) 13 (dégradation des populations de phytoplancton et de zooplancton) et de fournir des données géoréférencées sur les habitats pour la AUB 14 (perte d'habitats où vivent les poissons et la faune). La qualité de l'eau, l'abondance du zooplancton et la biomasse variaient selon les écotypes d'habitat, le port intérieur présentant une biomasse très faible similaire à celle des eaux libres du Lac Ontario et une proportion plus élevée de petits taxons comme les rotifères, malgré des niveaux élevés de nutriments. La réduction des proies du zooplancton est probablement due à de multiples impacts environnementaux urbains, notamment le ruissellement et les contaminants. L'écotype a eu une incidence sur la productivité du zooplancton, mais pas sur la productivité primaire, ce qui indique que les systèmes protégés (channels insulaires, cellules d'habitat et baies) peuvent offrir divers habitats physiques et sources de nourriture pour les poissons. Bien que les taux de productivité primaire aient diminué dans la plupart des habitats, la productivité a été détournée vers la croissance bactérienne, qui était très élevée dans toute la zone préoccupante. La saisonnalité des caractéristiques des habitats différait selon l'écotype, notamment entre les cellules et les îles. Il est nécessaire d'évaluer les communautés planctoniques saisonnières dans divers habitats afin de déterminer comment le plancton s'intègre dans le réseau trophique, et il est essentiel de poursuivre la surveillance afin d'évaluer l'efficacité des principales initiatives d'assainissement, telles que la création de zones humides, la naturalisation en cours de l'embouchure de la Rivière Don et les projets de protection contre les inondations.

Executive Summary

The composition of zooplankton and phytoplankton communities, water properties and productivity in the Toronto Region Area of Concern was measured May to October from 2019 to summer 2022 (impacted spring 2020 and 2021 by Covid restrictions) to include relevant shallow fish habitat sites in Tommy Thompson Park and the Toronto Islands. This was completed to support an assessment of the Beneficial Use Impairment of “Phytoplankton and Zooplankton Populations” previously determined to be impaired.

Measures varied seasonally, annually, and by location across the 19 sampling sites grouped by ecotype of Inner Harbour, Islands, Embayments and Cells. Chlorophyll *a*, an indicator of phytoplankton standing crop, is in the mesotrophic range in the Inner Harbour (IH), lower than expected given that total phosphorus is within the range typically found in eutrophic systems. The IH has a rapid flushing rate of 1-2 weeks with Lake Ontario which results in phytoplankton productivity being transported out of the harbour. Phytoplankton primary productivity is highest at the Island sites and lowest in the IH and Cells. These levels are lower than expected given the high nutrient loadings but remain elevated compared to waters of Lake Ontario. Medium-sized phytoplankton (nanoplankton) are the main contributors to primary productivity.

While primary productivity is low for a shallow nearshore habitat, excessively high bacterial productivity in the IH is contributing to reduced trophic transfer efficiency because it is not a preferred food for grazing zooplankton, limiting food resources for fish. The Cells and Island sites also exhibited high bacterial productivity, in part due to being shallow wetland environments. However, the influence of the IH, including non–point source inputs from the Don River, on these habitats requires further assessment.

Multivariate analyses indicated that zooplankton community structure was shaped by both spatial and temporal factors, with temperature and chlorophyll *a* being the most important drivers. Zooplankton biomass was consistently lower in the Inner Harbour (IH) than in the Embayments, Islands, and protected Cells sites. The IH contained proportionally more small-bodied zooplankton and rotifers along with significantly lower biomass. The low biomass of crustacean zooplankton in the IH was within the same range as the less productive coastal and offshore waters of Lake Ontario which is indicative of food web impairment given the IH is a protected embayment with elevated nutrient levels. The causes of this suppression are not identified here but are likely to include multiple factors including unknown contaminants or other anthropogenic disturbances given the densely urbanized environment.

Planktivory by invertebrate predators such as the invasive spiny water flea (*Bythotrephes*) and forage fishes may be periodically high enough to suppress zooplankton in the IH, though these top-down factors are also likely to be high in the protected ecotypes which exhibit very high zooplankton biomass. A pilot study on Alewife (*Alosa pseudoharengus*) forage fish diets showed a clear preference for the largest size fraction of zooplankton, along with benthos. Reduced large zooplankton individuals within the IH (including *Daphnia* and predatory cladocerans) limits trophic transfer to forage fishes, though availability of large zooplankton prey may be higher during the fall. Studies of fish use of this ecozone during the fall period may be relevant to determine if this is an important resource or an ecological mismatch.

Resulting survey information from 2019-2022 were used to spatially delimit habitat zones within the Toronto Region Area of Concern, and seasonal (spring, summer, fall) measures relevant to fish habitat including temperature, chlorophyll *a*, zooplankton biomass and size composition

among others are provided as GIS compatible layers which can be used for future and retrospective fish habitat assessments.

Recommended BUI 13 Targets for Recovery:

- Summer zooplankton biomass in the IH should average 125 mg m^{-3}, representing ~60% of the median summer biomass observed in the Embayments (excluding Embayment 3).
- Zooplankton size structure should match that of the Embayments, with large-bodied zooplankton (> 1 mm in length, such as adult calanoids, predatory cladocerans and large *Daphnia*) comprising at least 2% of the summer community by density. Medium-sized zooplankton (0.35 to 1 mm in length, including many *Daphnia*, cyclopoids and juvenile calanoids) should comprise at least 15% of summer density.
- Reduce mean bacterial productivity in the IH by 20% to an average of 1.2 mg C m^{-3} h^{-1} (95% CI: 1.0–1.4), with values not exceeding 3 mg C m^{-3} h^{-1}.
- Lower the bacterial to primary productivity (BP:PP) ratio to 0.10 (95% CI: 0.08–0.13) through targeted reductions in bacterial productivity while maintaining primary productivity at mesotrophic levels (~20 mg C m^{-3} h^{-1}).

Overall assessment: The Inner Harbor indicates continued impairment of Phytoplankton and Zooplankton Populations (Beneficial Use Impairment #13), with consistently low zooplankton biomass, high bacterial productivity, and altered food web structure. In contrast, the Cells, Islands and Embayments support expected trophic transfer and better food resources for fish. Taken together, these findings highlight the need for ongoing research and monitoring, identification of factors impairing zooplankton populations within Inner Harbour, targeted restoration actions to improve the Inner Harbor, as well as the maintenance and protection of ecologically important wetland habitat areas in Tommy Thompson Park and Toronto Islands.

Introduction

The Toronto and Region Area of Concern (herein Toronto AOC) covers a large geographic area on the northwestern shore of Lake Ontario. It includes 42 km of waterfront, and encompasses both the Inner Harbour (IH), the Outer Harbour bounded by the Leslie Spit and nearshore waters of Lake Ontario (Fig. 1A). Beneficial Use Impairment (BUI) 3 (*Degraded Fish and Wildlife Populations*) and 14 (*Loss of Fish and Wildlife Habitat*) are currently designated as "Impaired" within the Toronto AOC (Environment Canada and Ontario Ministry of the Environment 2011; Doka et al. 2018). Similarly, an assessment of BUI 13 (Degradation of Phytoplankton and Zooplankton Populations) found that the plankton communities were impaired due to a combination of factors including: high rates of bacterial productivity, low primary productivity and excessive planktivory which would be expected to limit the amount of food resources available to fish (Currie et al. 2018). These findings also underscore the importance of planktonic communities to the assessments of BUI #3 and BUI #14. To support the assessment of these BUIs, it is imperative to assess plankton populations in the context of their importance to fish habitat and population restoration.

There are few published studies regarding plankton community composition, distribution and structure within the Toronto AOC. The on-going Water Quality Index Program surveys by the Ontario Ministry of the Environment, Conservation and Parks (OMECP) began in 1993 (Currie et al. 2015). Fisheries and Oceans Canada (DFO) completed two late-summer spatial surveys of the lower food web within Toronto Harbour in 2010 and 2013 (Currie et al. 2015), and May to October monthly sampling in 2016 (Fig 1B; Currie et al. 2018; Munawar et al. 2018; Bowen and Currie 2021). Plankton represent crucial components within aquatic food webs and ecosystems and environmental factors which affect the energy transfer from plankton to fishes are highly relevant to fish habitat assessments. In addition to planktivory, zooplankton and phytoplankton populations can be influenced by discharges of sewage and other contaminants, alterations to physical habitat and periodic mixing with Lake Ontario (Doka et al. 2018; Hlevca et al. 2018a; Howell et al. 2018; Munawar et al. 2018; Bowen and Currie 2021).

Habitat restoration efforts around Toronto have focused mostly on the creation of warmwater habitat (Veilleux 2014; Chu et al. 2014; Barnes et al. 2020; Theis et al. 2024) which aims to promote the recovery of native and predatory fishes in the Toronto AOC. However, even with habitat creation and restoration projects throughout the AOC, Indices of Biotic Integrity scores are still below RAP targets (Bowlby and Hoyle 2017; Midwood et al. 2022). The deployment of telemetry systems around Toronto is instrumental in determining habitat usage by target fish species (Brooks et al. 2017; Midwood et al. 2019b; Brownscombe et al. 2023). Further, the tagging of multiple fish species has proved useful in the assessment of restoration success, as opposed to single-species studies (Rous et al., 2017). However, given that forage fishes are generally not the focus of telemetry projects, linkages between forage species and habitat restoration actions (Rous et al. 2017) are still unclear; a knowledge gap which inhibits the restoration of predatory fishes. It was also noted that future restoration should be coordinated using a holistic approach, and with detailed spatial information of fish habitat (Choy et al. 2018). Fish habitat can be physical (i.e., substrate type, depth), limnological (i.e., temperature, water quality, dissolved oxygen) and biological (i.e., prey abundance, submerged aquatic vegetation). Restoration projects typically focus on the physical components since they can be directly managed, however, limnological factors can influence how fish use a physical habitat and the biological aspect of food resources is critical to survival.

The primary objective of this study is to expand information on spatial and temporal patterns in plankton communities in the Toronto AOC, with emphasis on their role as a food resource in prime fish habitats. In response to feedback from the Toronto Remedial Action Plan (RAP), this study aimed to expand the spatial and temporal understanding of plankton communities as a biological component of fish habitat with focus on data-deficient littoral wetland, coastal, and sheltered areas which have been the target of aquatic habitat restoration activities (Piczak et al. 2022). These areas, or ecotypes, include the Cells and Embayments within Tommy Thompson Park (TTP, also referred to as the Leslie Spit or the Outer Harbour East Headland) and channels in and around the Toronto Islands.

To effectively characterize fish habitat and food availability, this study integrates (1) A detailed quantitative and qualitative assessment of zooplankton and phytoplankton biomass across multiple ecotypes (Inner Harbor, Islands, Cells, and Embayments); (2) productivity rates for phytoplankton and bacteria; (3) high-resolution georeferenced data maps of important physical and biological parameters (e.g., temperature and zooplankton biomass); and (4) a pilot forage fish diet assessment of Alewife (*Alosa pseudoharengus)* to determine selectivity for zooplankton and benthic organisms. Taken together, this work within the Toronto AOC provides an updated status assessment of BUI 13 (*Degraded Phytoplankton and Zooplankton Populations*), to include newly-sampled wetland ecotypes of the Cells, Islands and Embayments. The findings also offer valuable information relevant to future assessments of BUI 3 (*Fish Populations*), BUI 14 (*Loss of Fish and Wildlife Habitat*) and BUI 8 (*Eutrophication and Undesirable Algae*), with a particular focus on temporally georeferenced data on water property habitat characteristics and quantity of food resources across a range of habitats within the Toronto AOC.

Methods

Sampling Areas (Ecotypes)

Although the Toronto AOC spans 42 km of waterfront along the northwestern shores of Lake Ontario (Kidd 2015), previous sampling efforts focused on the Inner Harbour (IH), where impairments had been identified (Currie et al. 2015). In addition to the IH stations sampled in 2016, sampling stations were added in three areas more suitable for warmwater fishes, including the cells and embayments of TTP and the channels within the Toronto Islands (Fig. 1; Table. 1). In the present study, sites were grouped into one of four ecotypes (Cells, Embayments, Inner Harbour, Islands), based on analytical similarities in the biota and ecotype characteristics (e.g., depth, macrophyte cover, mixing with Lake Ontario), rather than geographical position or past designation. In doing so, some areas which are commonly named as cells are now categorized as embayments and vice versa. Specifically cell 3 has become Embayment 3 and embayment C has become Cell 3. The characteristics of these ecotypes are described in more detail in the inset boxes below.

BOX 1: Cells

For this study, the ecotype classified as Cells are Cell 1 (c1), Cell 2 (c2) and Cell (originally embayment) D (cD) (Fig. 1). These are warm, shallow ponds under 2.5 m deep that are only connected to the lake via narrow, gated openings designed to exclude larger fish, although the connection at cD was mostly blocked by a beaver dam at the time of study. (Hlevca et al. 2018a) estimate flushing times of 1-11 days. Stations in c1 are characterized by rich stands of submerged aquatic plants, cD has dense floating and emergent plants, while c2 is highly turbid due to suspended sediment and currently supports little vegetation.

BOX 2: Embayments

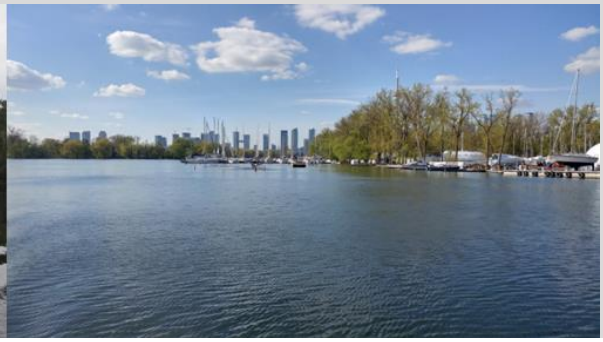
The five sites we classified as the Embayment ecotype are highly variable in depth and exposure; however, all are open to exchange with the Outer Harbour and have estimated water residence times of 3 to 8 days (Murphy et al. 2011; Hlevca et al. 2018b). They are deeper than the Cells (at 3-11 m), cooler in the summer and have sparse submerged aquatic vegetation (SAV). The Embayment locations include e6 at the north end of the Outer Harbour, Embayment (originally cell) 3 (e3) and Embayment C (eC; Fig. 1); all of which are relatively sheltered and surrounded by reclaimed parkland, light industry and marinas. Embayments A (eA) and B (eB) are at the southern end of TTP and are more connected with Lake Ontario. A large colony of cormorants and gulls resides in TTP adjacent to eB. There was also active disposal of dredged sediment occurring in e3 during the study. Periodically the Don River plume is carried into eA as it exits the IH.

BOX 3: Inner Harbour

While the IH is similar in depth to the Embayments (5-12 m), it supports less SAV (Midwood et al. 2021) and is often subject to physical disturbances from waves and boat activity. Both the Inner and Outer Harbour are subject to periodic upwellings of cold Lake Ontario water, which contribute to rapid temperature changes and hydrodynamic flushing (Hlevca et al. 2015). The IH ecotype has a water residence time of about 7-14 days, with Lake Ontario water typically entering at the Western Gap (T11) and exiting at the Eastern Gap (T13) (Haffner et al. 1982; Hlevca et al. 2018a). This area also receives nutrients, contaminants and bacteria from the Don River (near T12) which drains a large portion of the City of Toronto watershed (Howell et al. 2018; Howell and Benoit 2021), along with combined sewer overflows and direct run-off from the highly urbanized environment (Snodgrass et al. 2018; Edge et al. 2021). In this study, seven stations were classified as IH - six inside the harbour, and one just outside the eastern gap (T1) in outer harbour, which was biologically and chemically similar due to its location in the plume of water exiting the inner harbour.

BOX 4: Islands

The four Toronto Island stations in the channels between the Toronto Islands were sheltered, shallow (3-5 m depth), surrounded by urban parkland, dwellings, and marinas, and rich with SAV. Although hydrologically connected to the IH, the Island ecotype is more protected from cool lake water intrusions and mixing (Hlevca et al. 2015) and is more characteristic of a wetland.



Sonde Measurements

From May 2019 to June 2022, we conducted 14 one-day surveys on an approximate monthly basis through the growing season (mid-May to late-October). As the start of monthly sampling was delayed until August 2020 and July 2021 due to COVID-19 pandemic restrictions, sampling trips in May and June 2022 were added to provide additional spring data.

Sampling was typically conducted in the IH, Outer Harbour, Islands and Embayments on DFO's vessel, the R.V. Cisco. A multiparameter sonde (YSI EXO2) was programmed to collect spatial epilimnetic physical-chemical data every 0.5 seconds along the vessel's route within the AOC. This was carried out using a flow-through system on the vessel's deck, with water pumped from a depth of 0.3 m below the surface. The R.V. Cisco was too large to enter the cells; however, physical-chemical tow data were supplemented with vertical sonde casts taken from a smaller vessel. Sonde parameters included temperature, specific conductivity, pH, turbidity, dissolved oxygen, fluorescent dissolved organic matter (fDOM, effectively equivalent to CDOM), chlorophyll *a* (Chl *a*) and phycocyanin. In addition to these continuous measurements, vertical YSI Exo sonde profiles measuring the same parameters were taken at 19 discrete stations during each sampling trip (Fig. 1A, Table. 1). An RBR PAR-D (photosynthetically active radiation-depth) instrument determined light attenuation at most stations; however, this sensor was sometimes not available for use in the cells. Secchi depth readings were also attempted at each site using a 25-cm diameter black and white Secchi disk, although the Cells and eA and eB were sometimes too shallow and/or vegetated to provide valid measurements. At four sites in TTP (c1, c2, e3 and eC), two additional locations were sampled in each. These sites were generally located 100 to 200 m to the northeast and southwest of the central stations shown in Fig. 1A. Sampling at all sites followed methods outlined by Currie et al. 2018).

Water Collection

Integrated water samples were collected at a subset of sites listed in Table 1, from surface to 6 meters at deeper sites and surface to 1 or 2 meters at shallower sites. Water samples for nutrient analyses were prepared (i.e., filtered) on board the vessel immediately after sampling and were stored on ice in coolers. The remaining water was stored out of direct sunlight in darkened, insulated carboys for transport to DFO's Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) lab. Samples were stored overnight in a walk-in fridge (≈ 8°C) and processed the next morning. Subsamples were drawn for Chl *a*, size-fractionated primary productivity and bacterial growth assays and preserved for microscopic analyses of phytoplankton and microbial loop.

Size-fractionated primary productivity was estimated for three size categories of phytoplankton (<2 µm, 2-20 µm and >20 µm) by the standard 14-Carbon technique of (Munawar and Munawar 1996). These assays capture the assimilation of organic carbon through photosynthesis and includes carbon uptake by photosynthesizing bacteria (i.e., Cyanobacteria). Whole water samples were spiked with $Na^{14}CO_3$, incubated for 4 hours at surface temperature (± 2°C) and exposed to a constant light level of 240 µE s^{-1} m^{-2}. Because light and temperature levels were constant in these experiments, the results should be interpreted as potential rather than actual productivity. After incubation, size classes were determined by filtration of the sample through polycarbonate filters. All filters were rinsed with hydrochloric acid (0.5M) to remove excess ^{14}C-CO_2. Finally, radioactivity of each filter was determined by liquid scintillation. Heterotrophic bacterial growth rates (also referred to as bacterial productivity) were estimated by ^{3}H-Leucine incorporation into bacterial proteins following the protocol of Jorgensen (1992) and radioactivity

was determined by liquid scintillation. Detailed methods are described by Heath and Munawar 2004).

Phytoplankton samples were preserved with acidified Lugol's iodine upon collection. Identification, enumeration, and measurement used the Utermöhl (1958) inverted microscope technique following the protocols described by Findlay and Kling (1998) including taxonomic authorities. The terms 'Diatomeae' and 'diatom' are used to describe organisms of the phylum Heterokontophyta and the subphyla of Bacillariophytina (Classes: Bacillariophyceae and Mediophyceae) and Coscinodiscophytina (Class: Coscinodiscophyceae).

Microbial loop samples, including bacteria, autotrophic picoplankton (APP) and heterotrophic nanoflagellates (HNF), were fixed with 1.6% formaldehyde and enumerated using DAPI staining (Porter and Feig, 1980) under epi-fluorescence microscopy (Munawar and Weisse 1989). Cell weights used to estimate wet weight biomass were 2000 fg cell^{-1} for APP, 100 fg cell^{-1} for bacteria and 140 pg cell^{-1} for HNF (Sprules et al. 1999). Formaldehyde-preserved microbial samples degrade over time and thus maintain a shelf life of one year. Because of COVID-19 pandemic-related closures, we were unable to analyze these samples within an appropriate time frame. As such the microbial loop biomass data we obtained may not be comparable to previously published data from within the Toronto AOC or in other Great Lakes systems. However, we feel that the normalized 2019-21 data are robust enough to provide an adequate comparison of the ecotypes in the current study because all samples were in a similarly degraded state.

Water samples collected for nutrient analysis were usually submitted to ECCC's *National Laboratory for Environmental Testing* (NLET) in Burlington, ON, using Standard Operating Procedures (Environment and Climate Change Canada 2023). However, in 2021, water samples were analysed by OMECP at their Resources Road laboratory in Toronto, using protocols outlined in Table S2 of Howell and Benoit (2021) . In 2020, limited water quality parameters [total phosphorus (TP) and total nitrogen (TN)] were collected due to the closure of NLET. Extracted chlorophyll *a* was processed by GLLFAS using acetone pigment extraction (Strickland and Parsons 1968). Both labs are accredited by the *Canadian Association for Laboratory Accreditation Inc*. (CALA) and meet the ISO 17025 standard.

Zooplankton and Rotifers

Zooplankton were collected by taking a vertical total water column net haul from 1 m off bottom to the surface, usually with a metered, 64 µm mesh, 40-cm diameter Wisconsin net, and preserved in 4% sugar-buffered formalin. Zooplankton were collected in the cells using a smaller 30-cm diameter net lowered to just above the bottom, with two or three replicate tows pooled from each location to increase sample volume. When macrophytes were abundant, net hauls were taken from just above the macrophyte beds, and ideally from spots where growth was sparser. As this net was unmetered, we assumed a net efficiency of 90%. Taxonomist C. Tudorancea from Aquatic Bio-services in Kitchener ON analysed all zooplankton samples from the inner harbour and island stations, eA, eB and cD, and the first replicate (centre station) from c1, c2, e3 and eC. From the latter four locations, K. Bowen from DFO enumerated replicate samples 2 and 3, and applied mean lengths, weights and egg ratios determined from replicate 1. For each sample, each zooplankton taxon was grouped as small, medium or large based on mean lengths of <0.35 mm, 0.35-1.0 mm and >1.0 mm, respectively. Total zooplankton production was determined at each station as described in Bowen (2017) and Bowen and Currie (2021), except that means for each season were calculated separately and added together to estimate total production over the 01-May to 31-October period.

At a subset of stations (Table. 1), rotifers were collected from the integrated water samples described above. At each of these stations, a total of 8 liters of water were filtered through a 20 µm mesh sieve, and the rotifers remaining on the sieve were preserved. Zooplankton and rotifer samples were enumerated as described by (Bowen 2017; Currie et al. 2018). In 2019, rotifer samples were later halved and one half-sample from each date was combined to make a May-October composite sample for each station. In addition to the 2019 composites, the following rotifer samples were enumerated: July and September 2019, August and September 2020 and August 2021. Late-summer is believed to best represent the period of maximum rotifer abundance and diversity.

Fish Biomass Estimates

Boat electrofishing data from 2013 to 2022 at nearshore sites around Toronto Harbour and TTP were provided by the Toronto and Region Conservation Authority (TRCA), as described in Hoyle et al. (2018). Electrofishing transects were generally sampled for 1000 seconds; those with shorter intervals were corrected to provide biomass estimates per 1000 seconds. Fish species were divided into feeding guilds (Appendix 1, Table A-2) based on Scott and Crossman (1998). To compare to DFO's 2019-2022 lower food web study, electrofishing transects were divided into geographical areas as follows: Cells (c1, c2, cD), e3, Outer Harbour and Embayments, IH, Toronto Island channels, and Lake Ontario nearshore (outside of Toronto Islands and TTP; Fig. 1A; Appendix 1, Table A-3). Additional fishing locations not included were those around Essroc Quay, the turning basin and the shipping channel on the east side of the IH.

Alewife Gut Content Analysis

During the night of 27-July-2021, the boat electrofishing program of the TRCA collected nearshore adult Alewife, based on protocols outlined in Hoyle et al. 2018). Their catch included eight fish from Embayment C (eC), nine from nearshore Lake Ontario adjacent to Toronto Island's Gibraltar Point Beach (nearshore), and one from Sunfish Cut on the inner side of the islands (Fig. 1). These fish were placed on ice in the field and frozen prior to analysis at the GLLFAS lab. After thawing, fork lengths were taken to the closest mm and weights determined to 0.01 g using a Sartorius top-loading balance. Each stomach was removed intact, placed in a petri dish, cut open and the contents rinsed into the dish using a water-filled squirt bottle. Pieces of the stomach were rinsed free of food and discarded. Stomach contents were further cleaned by washing on a 64 µm sieve and preserved in 4% sugar buffered formalin. Upon later enumeration, formalin was rinsed from each sample, and the contents placed into a gridded petri dish. Macroinvertebrates, including midge larvae, adult insects and amphipods, and uncommon zooplankton, including spiny water fleas (*Bythotrephes*), fish-hook water fleas (*Cercopagis*) and large copepods were counted in the entire sample using a dissecting microscope (10 to 20X magnification). For abundant zooplankton taxa (>100 individuals), subsamples were taken using the protocol outlined for zooplankton enumeration in Bowen (2017). In the more digested samples, distinctive body parts of zooplankton and invertebrates were counted. These included the head capsules of midge larvae, the mandibles of *Bythotrephes* and *Cercopagis*, the post-abdominal claws of *Daphnia*, the heads of bosminids and the caudal rami (posteriors) of copepods (Appendix 1, Fig. A-1) These distinctive exoskeleton parts are composed of hardened chitin which although digestible to varying degrees by fishes (Gutowska et al. 2004), are sturdy and not expected to vary in digestive time between size classes. Mandible counts were divided by two to estimate the number of individuals.

The stomach contents of three fish from eC in 2021 were intact enough to allow length measurements on a total of 52 *Daphnia*. The size distribution of these *Daphnia* was compared to animals collected by DFO in the water column of eC on the closest sampling dates (13-July and 23-August). For the remaining taxa in the stomachs, animals were often too degraded to measure, so we used taxon-specific mean weight of these water column zooplankton to calculate biomass in the fish diets. For the lone fish collected near Sunfish Gap, zooplankton from i1 were used for biomass calculations and water column comparison. However, DFO did not sample zooplankton near Gibraltar Point in 2021, so five western Lake Ontario samples collected in July and August of 2021 were averaged. These included one sample from LO8 in Humber Bay collected on 24-August, and samples from each of LO2 (epilimnion) and BUR collected on 9-July and 19-August (Fig. 1B).

For estimating midge larvae weights in all samples, two nearshore fish with relatively intact stomach contents were chosen. From each stomach, four groups of 20 larvae each were blotted and weighed to 0.001 mg on a Mettler Toledo analytical balance. These values were averaged to determine the mean wet weight per animal and multiplied by 0.2 to convert to dry weight. Five amphipods from both areas were also weighed and averaged.

Comparisons to Previous Studies

To determine how the results of the present Toronto AOC survey compare to conditions elsewhere in the Lake Ontario basin, we compared our findings to other sites sampled May-October by DFO between 2014 and 2018 (Fig 1B). For summer only comparisons, data collected during the summer of 2013 was also used. Generally, the same sampling and analytical methods were used for all parameters at these stations. We carried out bi-weekly or monthly whole water column sampling in western Lake Ontario (WLO) at a 7-m deep nearshore station (BUR), and epilimnetic sampling at a 60-m deep offshore station (LO2). In eastern Lake Ontario's (ELO) Kingston Basin, epilimnetic sampling was undertaken at 35 m deep station LO81 (Bowen et al. 2022) – see Appendix 1,Table A-1 for site descriptions. The eutrophic AOC stations included the Upper Bay of Quinte at Belleville (Bowen and Johannsson 2011) and Hamilton Harbour (HH) (Bowen and Currie 2017). In 2016, the coastal area around Toronto and the IH were sampled monthly (Bowen and Currie 2017; Munawar et al. 2018). For this broad geographical comparison, the IH averages also include 2016 data. Zooplankton were collected using 64 µm mesh vertical net hauls, except in Quinte and HH where 41 L Schindler Patalas trap samples taken from multiple depths through the water column were pooled together. For rotifer comparisons, summer data were more limited and were taken from HH in 2006, 2009 and 2017 (n=24), the upper Bay of Quinte in 2009 and 2017 (N=11), eastern Lake Ontario (LO81) from 2008 to 2015 (N=37), western Lake Ontario from 2015 to 2021 (N=24) and coastal Toronto in 2016 (N=5). May-October mean rotifer biomass for other areas in the Lake Ontario basin were taken from the following sources – upper Bay of Quinte (2000-2008, N=9, Bowen and Johannsson 2011); HH (2002-2016, N=24, Bowen and Currie 2017); eastern Lake Ontario (2007-2017, N=11, Bowen et al. 2022).

Statistical Analyses

The *agricolae* package in R was used to identify differences between sites (v4.2.1, R Core Team 2021). This library provides functions for conducting analysis of variance (ANOVA) and comparing means, and *ggplot* library to create violin plots. To determine the Honestly Significant Difference (HSD) between the sites, we employed the 'HSD.test()' function from *agricolae*. The test was conducted using one-way and two-way ANOVA models, specified using the 'aov()' function.

Season was assigned to sampling dates as follows: spring (May and June), summer (July, August and early September) and fall (late-September to early November) from 2019-2022. For each season, data were examined for normality and transformed, if necessary, prior to analysis. In addition, differences among the four sampling areas were determined for the various parameters using ANOVA (JMP V15.1.0). Significant differences among areas ($p<0.05$) were determined using Tukey Honestly Significant Difference (HSD) multiple comparisons, and for non-normal zooplankton data, the non-parametric *Steel-Dwass* test was used.

Three multivariate ordination techniques were used to visualize the relationships, differences, and drivers between community structure and environmental variables across sampling locations and seasons, and to compare community structure among our targeted ecotypes. Principal Component Analysis (PCA), Nonmetric Multidimensional Scaling (NMDS), and Redundancy Analysis (RDA) were used to analyze data using the R packages *vegan* and *permute*. To spatially examine the Toronto Harbour and surrounding ecotypes, spatial R packages were used (*maptiles*, *terra*, *tidyterra*, *sf*, and *ggplot2*). Areas were identified based on the physical and chemical similarities between sites, considering factors such as water movement and zooplankton composition. Polygon boundaries were applied: (1) tracing 10 separate polygons for each ecozone within the study area and (2) tracing one polygon over the entire study area. The different zones of interest are represented by different polygons, each representing a specific boundary; such as IH, Islands, Cell 1, Cell 2, etc. The tracing process was performed in QGIS (v 3.30.2) and results imported into R as shapefiles for further processing. The polygon boundaries allowed division of the map grid to allow averages to be calculated over the four year period (2019-2022). The packages *rgeos, gstat* and *tidyverse* were used to plot sonde data for each environmental variable, applying kriging and interpolation to predict other nearby points in that area, with maximum distance from a given datapoint not greater than 0.4.

Results

Comparison of Inner Harbour to Other Lake Ontario Areas

To provide a comprehensive perspective, we compared the Toronto Harbour AOC with other areas of Lake Ontario using data collected across multiple years and seasons (May through October; see Fig.1B and Appendix 1, Table A1). This approach accounts for seasonal and interannual variability, allowing for a robust assessment of ecological conditions throughout the season. For most parameters, the Toronto IH is more similar to open water sites of Lake Ontario than it is to other AOCs in the Lake Ontario basin (Figs. 2 and 3). In general, that is positive since both the Bay of Quinte and HH are eutrophic and, as such, have significantly elevated levels of Chl a, TP and primary productivity (PP), as well as lower water clarity (higher light attenuation) (Fig. 2). However, all these parameters are elevated in the IH relative to the open waters of Lake Ontario as we would expect for an embayment. Autotrophic Picoplankton (APP) tend to be sensitive to environmental degradation (Munawar and Weisse 1989; Marshall 2002) and are usually found in higher numbers in less disturbed environments. Although in eutrophic environments, high amounts of APP can also indicate ecosystem stress by way of diminished grazing or rapid growth (Munawar et al. 2018). Generally, the highest amounts of APP biomass are found in the main lake regions, although the eutrophic Bay of Quinte has some of the highest values overall (Fig. 3A). There are fewer APP in Toronto's IH, and the lowest amount are found in HH. Bacterial growth rates are highest in the IH, Islands and Cells and are the highest observed in Lake Ontario, although followed closely by observations from the Bay of Quinte. The Embayment has similar bacterial growth rates to those seen in HH and the Lake Ontario coastal area adjacent to Toronto, while rates are much lower in the open lake (Fig. 3C). This trend is not seen in bacterial biomass, which is highest in the Bay of Quinte and HH. Bacterial biomass in the IH is similar to the offshore regions (Fig. 3D). Bacterial biomass is known to increase with increasing nutrient concentrations but overall it is much more uniform among systems of differing trophic status than autotrophic or heterotrophic plankton (Cochran and Scarborough 1995). This occurs because a large proportion of planktonic bacteria are dead or senescent and only a small proportion of the bacteria are actively growing (ibid). Furthermore, the combined biomass of cladocerans, cyclopoids, and other zooplankton species are significantly highest in HH, intermediate in the upper Bay of Quinte, and lowest in the eastern basin, western basin (LO2 and BUR) and the IH (Fig. 4A). Total zooplankton production followed this same trend, and is dominated by cladocerans in all areas (Fig. 4B). There are few differences between the IH and the open waters of Lake Ontario, except that the IH generally had lower biomass of Daphnia and higher biomass of the small cladoceran *Bosmina*.

Rotifers, a group of mostly herbivorous microzooplankton, can at times be very abundant in the Great Lakes. However, due to their small size, rotifer biomass tends to be low in all our study areas in the Lake Ontario basin. During summer, the Embayments, IH and Islands are similar to the upper Bay of Quinte, with averages in the 10 to 12 mg m^{-3} range. Biomass in the Cells was more similar to HH and the open waters of Lake Ontario, with values around 4 to 6 mg m^{-3} (Fig. 5). Overall, rotifer taxonomic composition was similar in all Lake Ontario study areas, although the large predatory rotifer *Asplanchna* tended to be more dominant in sheltered areas relative to the open lake.

The ratio of one lower food web parameter to another can provide insight on ecosystem function and transfer of energy and nutrients across trophic levels (McCauley and Kalff 1981; McQueen et al. 1986; Jeppesen et al. 2005; Heathcote et al. 2016). Unlike Toronto Harbour, algal blooms are an ongoing problem in both the Bay of Quinte and HH. In our studies, both extracted Chl *a* and Primary Productivity (PP) were higher for a given amount of TP in the Bay of Quinte and HH than any of the Toronto sites, which in spite of elevated TP, were similar to western Lake Ontario and the Toronto coastal stations (Fig. 6A and B). Conversely, the ratio of bacterial

growth rate (BG) to TP was significantly elevated at all the Toronto AOC sites and extended out to the surrounding Lake Ontario coastal sites (Fig. 6C). The PP to Chl *a* relationship showed less variability areas across the Lake Ontario basin (Fig. 6D), but had the same overall pattern as PP:TP with the lowest values in eastern Lake Ontario, Cells and Islands. The ratio of PP to Chl *a* was higher in all three AOCs and the Toronto coastal sites relative to the open waters of Lake Ontario (Fig. 6D). On average, BG:PP ratios from the Toronto Harbour AOC sites ranged from 0.11 – 0.18 (excluding the Embayments) and were about 5 – 10X higher than the other Lake Ontario sites which ranged from 0.01 – 0.04 (Fig. 6E). The average BG:PP ratio in the Embayments (0.04) was lower than the other Toronto AOC sites and similar to the Bay of Quinte. Overall, it suggests elevated bacterial activity in the Toronto Harbour area and shows that increases in bacterial growth occur at the expense of photosynthetic production. Using log-transformed total zooplankton biomass, there were more zooplankton per unit of TP at the Lake Ontario sites than in the three AOCs (Fig. 6F). Although zooplankton populations were high in both Quinte and HH, they were unable to consume the excess algae and PP in these areas, so the ratio of zooplankton to Chl *a* and PP tended to be low (Fig. 6F and with most of the other areas.

Urbanized environments contain many hard surfaces (roads, roofs, parking lots etc.) that greatly reduce the ability of a watershed to absorb stormwater. Natural surfaces in a watershed allow rain or snowmelt to slow runoff to infiltrate the soil and into the water table. In built up areas, rainfall runs over hardened surfaces and directly into the nearest creek, river or storm sewer carrying with it all it has picked up along the way. This includes dirt, debris, garbage, wildlife and pet waste, and many of these are a source of bacteria. To battle this, The City of Toronto introduced a Wet Weather Flow Master Plan in 2003 (WWFMP, City of Toronto 2003, 2023) to mitigate discharge, and the Don River mouth naturalization is a major infrastructure component. Most actions focus on preventing excess pollutant and water from entering the system at the upstream end of the system (i.e. bioswales, green roofs and downspout disconnection etc.) or on collecting and treating stormwater from CSO overflows at the downstream end of the system directly before the discharge to the harbour.

While the four wastewater treatment plants (WWTP) in Toronto are a point source of bacteria, Ashbridges Bay and Humber Bay outlets are proximate to the study area, while North Toronto Treatment Plant discharges treated effluent directly into the Don River and into the Inner Harbour. However, Toronto's sewers were historically a combined sanitary and stormwater system rather than the modern standard of separate stormwater and sanitary lines. Wet weather can overwhelm the system and cause stormwater to be combined with sanitary waste and bypass the WWTPs, leading to release directly to the watershed. A system of CSO containment tanks were installed in the 2000s to capture and divert this water back to the WWTPs, with the large infrastructure Central Waterfront Wet Weather Flow System & Connected Project . Toronto's wastewater contains multitudes of underground connections between homes and the water systems it is not uncommon for sanitary sewage lines to be directly connected to storm drains. Finding and correcting these cross corrections is another way to improve water quality in the Harbour. Microbial source tracing uses DNA found in water to identify sources of fecal contamination. Techniques can differential human, gull, cow, or dog sources of bacteria and that knowledge can lead to the source of the contamination and direct remedial actions.

Comparison of Inner Harbour to Sheltered Areas

The Cell, Embayment and Island ecotypes were added to the Toronto lower food web surveys starting in 2019 and were generally more productive than the IH, based on elevated Chl *a*, TP, PP and zooplankton biomass (Figs. 2 and 4). Some of these values, such as Chl *a* and TP were within the range observed in the Bay of Quinte. Bacterial growth rates were elevated in all Toronto ecotypes in the current study as well as the 2016 coastal sites, showing that the influence of the urban environment on bacterial production extends into the surrounding waters (Fig. 3C). Zooplankton biomass and production values at the Cell, Embayment and Island sites were also elevated relative to the open waters of Lake Ontario, and at times approached the very high levels observed in HH (Fig. 4). These parameters in the four sampling areas will be discussed further in subsequent sections.

The ratios of different parameters were generally similar in the four ecotypes, with a few exceptions. The ratios of PP to both TP and extracted Chl *a* (Fig. 6B and D) were higher in the IH relative to the Cells and Islands and the ratio of bacterial growth to TP was highest in the IH (Fig. 6C). The ratio of total zooplankton biomass to PP was higher in the Cells than the IH given the low zooplankton biomass found in the IH. There were no differences among ecotypes for the ratios of zooplankton to both Chl *a* and TP, or for Chl *a* to TP.

Comparison of Inner Harbour to Earlier Studies

While there is some year-to-year variability in the IH for most of the parameters studied, overall, the results of the current study (2019 to 2022) reinforce the findings of impairment brought forward in previous surveys (Fig. 7). When we examine summer (July to early-September) IH results over the 2013 to 2021 period, there were no annual differences in total phosphorus, light attenuation, extracted Chl *a*, bacterial growth rate, total phytoplankton biomass or total primary productivity; however, for some parameters (e.g., phytoplankton biomass) the sample size was low (Table 1). Secchi depth was lower in 2019 compared to 2016. Surface temperature in 2013 (September only) was cooler than most other years sampled, and 2021 was warmer than both 2016 and 2019.

Zooplankton + rotifer biomass was also significantly lower in early September 2013 relative to the more recent years surveyed (Fig. 8), likely driven by the upwelling of deep offshore water that occurred during that study. There were no differences in summer zooplankton + rotifer biomass between 2016 and 2021, although when rotifers were excluded, 2021 was unusually low due to the near absence of copepods. Temporal trends in Toronto IH zooplankton for the 1994 to 2016 period were examined more thoroughly in Bowen and Currie 2021).

Although variable year to year, the phytoplankton values in the IH are indicative of oligotrophic to mesotrophic conditions (Munawar et al. 2018). The phytoplankton community typically contained a mixture of Chrysophyceae, Diatomeae and Cryptophyceae (Fig. 8B). The presence of mixotrophic algae (Ochramonads, *Chrysochromulina parva*) may be a response to the elevated bacterial growth rates as these taxa are capable of ingesting bacteria for sustenance (Flynn et al. 2019) and point to our original concerns about excess bacterial growth being a driver of food web impairment in the AOC (Currie et al. 2018).

Zooplankton and Rotifers

During the 2019 to 2022 survey, total zooplankton biomass and production was consistently lower in the IH relative to the Cells, Embayments and Islands (Fig. 4). The sheltered areas added in 2019 are extremely productive compared to the open waters of Lake Ontario, with the Cells and TTP Embayments falling in the range seen in the eutrophic Upper Bay of Quinte. Zooplankton production at the Island sites approaches levels estimated for HH, which is one of the most productive embayments in the Great Lakes in terms of zooplankton (Bowen and Currie 2017).

Biomass (Fig. 9, Fig. 12; left) and densities (Fig. 10A) of all size classes of zooplankton were consistently low in the IH throughout the sampling season compared to the other ecotypes. Small zooplankton (<0.35 mm in length) consisted mostly copepod nauplii larvae, veliger larvae and small cladocerans such as *Bosmina* and *Chydorus*. There were localized high populations in the Cells (c1 and c2) in the spring, the Islands and protected Embayments in the summer, and the Cells and Embayments in the fall (Fig. 9, Fig. 12; left). Biomass of medium zooplankton (0.35 to 1.0 mm) such as cyclopoid copepods and most *Daphnia* were most abundant at e3 in the summer and cD throughout the sampling season (Fig. 12; right). Biomass of large zooplankton (>1.0 mm), including large *Daphnia*, adult diaptomid copepods and predatory cladocerans, were highest in the Cells and e3 in the spring, similar across all areas in the summer, and lowest in the Cells in the fall (Fig, 14 right). When all size classes were combined, spring zooplankton biomass was highest at some TTP sites (c1, c2 and e3) , followed by the Island sites and eC and eD (Fig. 13; left, Fig. 15). During summer when biomass reached its maximum, the highest levels were found in e3, eC, eD and the Islands. In the fall, biomass remained high at eD, followed by c2. Total biomass was consistently lowest in the IH. The most exposed embayments (eA and eB) typically supported lower zooplankton biomass than the more protected areas in TTP.

When averaged across the season, the proportion of small zooplankton was similar across all areas, but the IH had a lower percentage of medium zooplankton and a higher percentage of large zooplankton relative to the Cells and Islands (Fig 10A; Table 2). Proportions of medium and large zooplankton by density were more similar in the Embayments and the IH. By density, the proportions of large zooplankton were typically very low in all ecotypes and seasons (≤1%). Values were slightly higher in the summer in the Embayments and IH (~3.2%; Fig. 10B).

In terms of taxonomic groupings, biomass of both herbivorous cladocerans and cyclopoid copepods were generally lowest in the IH throughout the season (Fig. 15; Appendix 1, Fig. A-2). *Daphnia* in particular were low in the IH and Islands and high in the Embayments (especially e3) from late spring to early fall (Fig. 11, Appendix 1, Fig. A-3). The Cells were dominated by the small cladoceran *Bosmina*. Littoral herbivorous cladocerans (e.g., *Ceriodaphnia* and chydorids) were generally uncommon in the IH and Embayments, and highest in the shallow vegetated Cells and Island channels, especially during the summer. Predatory cladocerans were very rare in the spring in all areas, and remained so in the Cells through the summer and fall. The fish-hook water flea *Cercopagis* and *Leptodora* were the dominant predators in the summer in the Embayments, Islands and IH, followed by the larger spiny water flea (*Bythotrephes*) in the fall. Cyclopoids were mostly comprised of the predatory cool-water species *Diacyclops thomasi* in the IH throughout the season, although its biomass remained low (Fig. 15). The large predatory species *Mesocyclops* edax and littoral taxa such as *Ancanthocyclops* were more common in the other protected areas, especially during the summer. Calanoid copepod biomass values were similar in all four areas in the spring and fall, but lower in the IH relative to the Cells and Embayments in the summer. Diaptomid calanoids, especially *Skistodiaptomus oregonensis*,

were dominant in all areas in the spring and fall, and in the Cells in the summer. The warmwater calanoid *Eurytemora* was common in the summer, especially around the Islands and Embayments. Few to no dreissenid mussels were observed in the Cells, and as a result their larvae (veligers) were rarely encountered. Veligers were often abundant in the IH.

When mean lengths were weighted for the density of each taxon, zooplankton were larger in the Embayments and Islands relative to the Cells and IH (Appendix 1, Fig. A-4). This is due to the dominance of small cladocerans such as *Bosmina* in the latter two areas, along with veligers in the IH. When microzooplankton (copepod nauplii and veligers) were excluded, the remaining macrozooplankton were larger in the IH and Embayments relative to the Cells and Islands, and they were larger in the IH in the recent survey relative to 2016. *Daphnia* and cyclopoids were also generally smaller in the Cells relative to the other areas. Most of the *Daphnia* found in IH were between 0.5 and 0.9 mm in length (Fig. 11). In the summer and fall, there were very few *Daphnia* in the IH compared to the Embayments, and during summer there were proportionally fewer animals >1.0 mm in the IH (23% in Embayments vs 14% in IH; Fig 10-B). By fall, the high densities in the Embayments had fallen to the low levels found in the IH, and *Daphnia* tended to be slightly larger in the IH (10% in Embayments >1.0 mm vs 24% in IH).

Bosmina in the Cells had fewer eggs per individual than those in the Embayments and the IH, but there were no differences among areas for *Daphnia*, adult cyclopoids or adult calanoids (Appendix 1, Fig. A-5). Adult cyclopoids in the IH carried fewer eggs in 2016 than in 2019 to 2022 period. Overall the egg data suggests that zooplankton reproduction in the IH is not suppressed -relative to the other ecotypes, rather other factors such as flushing with open lake water or low survival past the embryo stage is responsible for the low IH biomass.

There was often considerable temporal variation in zooplankton populations at the Toronto study sites (Appendix 1, Fig. A-3). For example, a small nearshore species of *Daphnia* (*D. ambigua*) was abundant in c1 and c2 in late May 2019, but otherwise *Daphnia* were uncommon in these Cells. *Daphnia* were also rarely encountered in cD except for May 2022. Cladocerans often showed strong seasonal succession patterns. For example, in e3, there were very few cladocerans in May, likely because this deeper water body is slower to warm up. From June to early September, *Daphnia* were typically very abundant in e3, with the summer mean biomass reaching about 400 mg m^{-3}. This is about two orders of magnitude higher than in the IH and shallow Cells, and about five times higher than other Outer Harbour and Embayment sites. By late September, however, *Daphnia* had virtually disappeared from e3. This pond is deep enough to thermally stratify during the summer, and *Daphnia* may be taking advantage of cooler, darker water toward the bottom where they are less visible to predators. Despite similar stratification in the IH, *Daphnia* were rarely abundant there. The small tolerant cladoceran *Bosmina* is also capable of very rapid population increases when conditions suit them, and the shallow Cells and Island channels sometimes supported *Bosmina* biomass >500 mg m^{-3}. *Bosmina* populations in cD remained high into late October, after they had typically declined in other areas.

In addition to changes across the seasons, annual variations were sometimes noteworthy. For example, zooplankton biomass in c1 and c2 were consistently low in 2021 and 2022 relative to the two previous years. Copepods and large cladocerans were also unusually low in the IH during the summer of 2021. Overall, however, there were few differences in IH zooplankton biomass between the surveys conducted in 2016 and 2019-2022. Compared to the recent study, there were fewer IH veligers and *Cercopagis* and more cyclopoids in the summer of 2016, and fewer *Bythotrephes* in the fall of 2016. This interannual variation shows the

importance of sampling the lower food web over several field seasons to better understand spatial dynamics and food web interactions.

We used Non-Metric Multidimensional Scaling (NMDS) to examine the spatial and temporal distribution of zooplankton species in different sampling areas and seasons. NMDS is a multivariate technique used to reduce the dimensions of a dataset while showing the relative distances between datapoints and captures its major structure patterns (Zhu and Yu 2009). The different coloured hulls on the NMDS plot represent the four aquatic ecotypes in the Toronto study (Cells, Embayments, Islands and IH), and the zooplankton taxa are distributed throughout the hulls (Fig. 16). The points on the plot correspond to the different sampling stations during the seasons they were sampled (spring, summer, and fall) averaged across the 2019 to 2022 period. The overall stress test value performing the square transformation and Wisconsin double standardization was 0.17, indicating a good and reliable representation of the community structure. The lower the stress value closer to 0.1 the stronger the representation, i.e., values above 0.3 would indicate a poor representation (Dexter et al. 2018).

The results of the NMDS plot illustrate that the zooplankton community structure was strongly influenced by both spatial and temporal factors. The proximity and distance between the points suggests that zooplankton community composition can vary seasonally, as spring, summer and fall points have distinct clustering, with some overlap suggesting a potential transition period between seasons (Fig. 16).

Rotifers

In the eutrophic AOCs and the TTP Embayments, rotifers did not appear to be able to capitalize on higher productivity in the same manner as crustacean zooplankton, and rotifers rarely comprised more than 2% of total zooplankton biomass when averaged across the season. In less productive environments such as the IH and eastern Lake Ontario where crustacean biomass was low, rotifers comprised a higher proportion of the community (15% and 11%, respectively). Although summer rotifer biomass was not significantly different among the four Toronto ecotypes (Fig. 5), the proportion of rotifers relative to other zooplankton was highest in the IH (22%), compared to about 3% in the other areas. Summer IH biomass in 2016 was also not different relative to the recent study. *Synchaeta* was the only common genus where a difference was observed (IH >Island).

Phytoplankton Biomass and Composition

Phytoplankton communities in the IH (T4, T12, T1) were sampled during May, June, July, September and October of 2019. Total phytoplankton biomass ranged from 191 to 3169 mg m^{-3} at station T12, near the mouth of the Don River, and at the centre station (T4), phytoplankton biomass ranged from 361 to 1763 mg m^{-3}. The phytoplankton biomass range for station T1 was from 351 to 1134 mg m^{-3} (although deviations from our preservation method for the October sample meant that it was not included in this assessment). A two-way ANOVA was used to assess differences between sites, while accounting for seasonal variation, and found that only seasonal changes were significant ($F_{6,7}$=4.04, P=0.45). Overall, the phytoplankton biomass values were consistent throughout the IH, suggesting oligotrophic to mesotrophic conditions. The phytoplankton community typically contained a mixture of *Chrysophyceae, Diatomeae* and *Cryptophyceae* (Fig. 17). While composition was variable among sites, the dominant species at each of the sites were similar during each cruise (Table 3) but varied in terms of their contribution to total biomass. The presence of mixotrophic algae (*Ochramonads, Chrysochromulina parva*) may be a response to elevated bacterial growth rates, as these taxa are capable of ingesting bacteria for sustenance (Flynn et al. 2019), and echo original concerns

about excess bacterial growth being a driver of food web impairment within the Toronto AOC (Currie et al. 2018).

In other environments, like HH or the Bay of Quinte, phytoplankton biomass > 3000 mg m^{-3} would be indicative of an algal bloom. While phytoplankton levels in the Toronto AOC were typically well below this, on 17-July-2019 we identified a large accumulation of the diatom *Entomoneis palucida* at station T12 following a major storm event the previous day. Since this diatom is strongly associated with muddy stream beds (Bahls 2012), it was most likely deposited there by excess runoff from the Don River. This type of event will likely be mitigated in the future as improvements to the flow regime are realized through the implementation of the Don Mouth Naturalization and Port Lands Flood Protection Project.

Primary Productivity and Bacterial Productivity

Combining all stations and dates, total phytoplankton primary productivity (the sum of net-, nano- and pico-plankton productivity) was significantly higher in the Island ecotype than in the IH and Cells, while the Embayment was not significantly different from the others (Fig. 18). The highest rates of primary production were generated by nanoplankton (cells in the 2 – 20 µm size class) accounting for roughly 40 – 60% of the total compared to 20 – 40% for the smallest size class (APP <2 µm) and 10 – 30% for the largest size class (net plankton >20 µm) (Fig. 19).

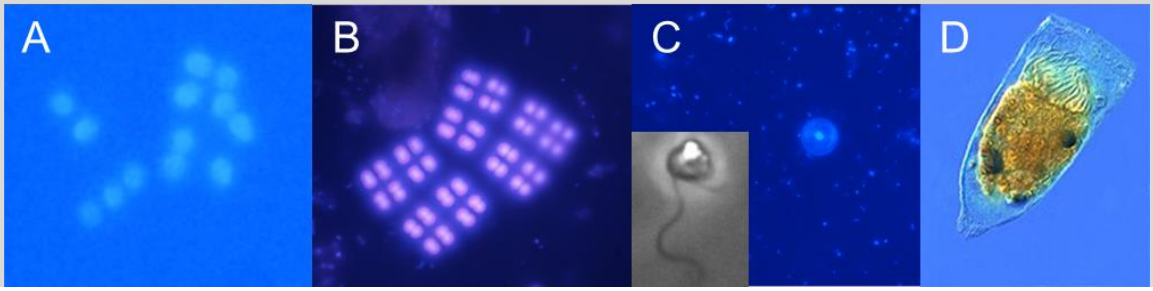
During the spring, primary productivity ranged from an average of 6.9 ± 1.6 mg C m^{-3} h^{-1} in the Cells to 22.7 ± 5.7 mg C m^{-3} h^{-1} at the Island sites with both the Embayment and the IH in the mid-range at about 13.0 mg C m^{-3} h^{-1}, although none of the areas was found to be significantly different from the others (Appendix 1, Fig. A-6). Over the summer months, average primary productivity ranged from 22.6 ± 7.4 mg C m^{-3} h^{-1} in the Cells to 51.8 ± 9.0 mg C m^{-3} h^{-1} at the island sites. This difference was statistically significant with Islands having higher productivity than the Cells and IH (Appendix 1, Fig. A-6). Over all stations and dates, summer productivity averaged 31.2 ± 3.4 mg C m^{-3} h^{-1} in the Toronto AOC. During the fall, primary productivity averaged 13.3± 1.6 mg C m^{-3} h^{-1} and ranged from a low of 8.6 ± 1.7 mg C m^{-3} h^{-1} in the IH to a high of 27.7 ± 5.9 mg C m^{-3} h^{-1} in the embayments; however these differences among areas were not significant (Appendix 1, Fig. A-6). On the whole, these results suggest that the phytoplankton community is active over the summer months and conditions range from mesotrophic to eutrophic.

Combining all stations and dates, total bacterial productivity averaged 1.8 ± 0.2 mg C m^{-3} h^{-1} and was not significantly different between areas (Fig. 18). Spring bacterial productivity averaged 1.7 ± 2.0 mg C m^{-3} h^{-1} and was lowest in the Embayments (0.5 mg C m^{-3} h^{-1}; 2 observations only), compared to the Islands (2.1 ± 0.6 mg C m^{-3} h^{-1}; Appendix 1, Fig. A-6). Summer bacterial productivity was higher than in spring and fall and averaged 2.9 mg C m^{-3} h^{-1}. In summer, bacterial productivity in the Embayments (1.5 ± 0.5 mg C m^{-3} h^{-1}) was significantly lower than in the Islands (4.6 ± 1.6 mg C m^{-3} h^{-1}; Appendix 1, Fig. A-6). Overall, bacterial productivity in the fall was lower than in summer and similar to spring (1.1± 0.2 mg C m^{-3} h^{-1}). There was no significant difference between the areas in fall (Appendix 1, Fig. A-6). The high rates of bacterial productivity identified in the previous assessment were not just limited to the inner harbour; they were observed throughout the AOC and raise concerns about the extent of bacterial contamination.

Box 5: Why Care About Bacteria?

Planktonic bacteria, while less than a micron in size, are an important component of the aquatic ecosystem as decomposers of organic matter, gaining energy through the transformation of organic carbon and nutrients, and transferring that energy to higher trophic levels. These cells may be active, slowly growing, dormant, and even dead. Most planktonic bacteria cells are inactive or dead, though the proportion of metabolically active bacteria is highly variable between systems, ranging from less than 5% in oligotrophic waters to over 50% in highly productive estuaries (Cochran and Scarborough 1995). Bacterial density and productivity rate (carbon fixation) increases with system enrichment, but this increase is generally disproportionately small relative to other plankton (Bird and Kalff 1984; Cole and Caraco 1993; Del Giorgio and Gasol 1995). Bacterial production has environmental, bottom up, and top down influences, with decomposable organic matter thought to be the main driver of growth, but also influenced by temperature. The supply of available organic matter is usually estimated by dissolved organic carbon, primary production and phytoplankton community structure of the system. Bacteria are the base member of the Microbial Loop food web, being consumed by a range of grazers from tiny heterotrophic nanoflagellates, ciliates, rotifers and even some zooplankton including *Daphnia* spp. Bacterial production is generally about 20% of phytoplanktonic production (Cole et al. 1988) but there is great variability between lakes and depths (Pace and Cole 1994). In eutrophic waters, bacteria can form additional biofilm on other plankton, benthic algae, macrophytes or detrital clumps (i.e. “lake snow”, Grossart and Simon 1998), elevating total system productivity, but the fate of this production is less known.

A) Epifluorescent micrograph of cocci bacteria; B) Epifluorescent micrograph of the colonial cyanobacteria *Merismopeda* spp.; C) Epifluorescent micrograph of heterotrophic nanoflagellates and bacteria (inset phase-contrast image of *Paraphysomonas* spp.); D) a micrograph of the tintinnid ciliate *Favella* spp. Images CC BY-SA 4.0.



Microbial Loop Biomass

For microbial loop analysis, normalized biomass values were used to compare ecotypes for bacteria, HNF and APP. This approach was adopted since the samples could not be analyzed within normal accepted time frames and sample degradation was a concern. With this consideration in mind, we felt the samples were robust enough to compare with each other but were not sufficient to compare with previous results analyzed within the sample shelf life. The Cell sites had higher bacteria concentrations than the IH but were not significantly different from the Embayment or Island sites (Fig. 20). This is consistent with expectations that wetland environments should have higher bacteria concentrations than open water environments (Bird and Kalff 1984). Neither APP nor HNF were significantly different among the ecotypes (Fig. 9). The diet of HNF consists primarily of bacteria and HNF can reproduce rapidly, which is reflected in the extreme variability of their distributions in all of the ecotypes.

Comparison of Physical and Chemical Parameters (2019-2022)

Habitat characteristics within the ecotypes for both plankton and fishes are shaped by both physical parameters such as temperature and currents, and water chemistry, including nutrients, dissolved oxygen and turbidity. Important parameters for each ecotype in the Toronto AOC are summarized in Table 4.

Temperature

The IH, Islands, Embayment and Cell sites each have different temperature characteristics that can affect fish and fish habitat suitability. These spatial differences vary by season and are influenced by water depth and amount of water exchanged with the open lake. When the seasons are pooled, the IH and Embayments were significantly cooler than the Cells and Islands (Fig. 21A). The coolest temperatures in the spring were found in the Outer Harbour, Embayments and IH where the mean surface temperatures reached 13-15°C (Fig. 22; left). The whole water column temperature at these sites averaged about 10-12 °C (Fig. 23; left, Appendix 1, Fig. A-7), making these areas more suitable for cool-water fish species such as Northern Pike and Smallmouth Bass (Scott and Crossman 1998; City of Toronto 2012). Water temperatures in the shallow Cells were significantly warmer in the spring, followed by the Island channels, reaching temperatures of 16-19°C. This provides ideal conditions for warm-water fish species such as Largemouth Bass and Bluegill (City of Toronto 2012).

During summer, shallow Cells 1 and 2 were significantly warmer than the other areas, with temperatures averaging 26°C (and sometimes as high as 28°C), and these were fairly uniform throughout the well-mixed water column. Despite the shallow depth of 2.5 m in the middle of cD, the bottom water was at times 4 to 6°C cooler than the surface, probably due to intrusion of cool lake water through the mostly blocked grate and significant floating vegetation (Fig. 22, Box 1). Summer temperatures were also elevated in the Island channels, with mean values of 25°C at the surface and 22°C through the water column. The Embayments and IH were cooler, averaging 22-23°C at the surface, and 18 - 20°C through the water column. Deeper locations in the IH, Outer Harbour and Embayment 3 typically showed thermal stratification from June to mid-September, with temperatures often in the 9-15 °C range at depths below 7 m. Examples of thermal stratification are shown in Appendix 1, Fig. A-8. These deeper waters serve as a refuge for fish and zooplankton species preferring cooler water during the summer months.

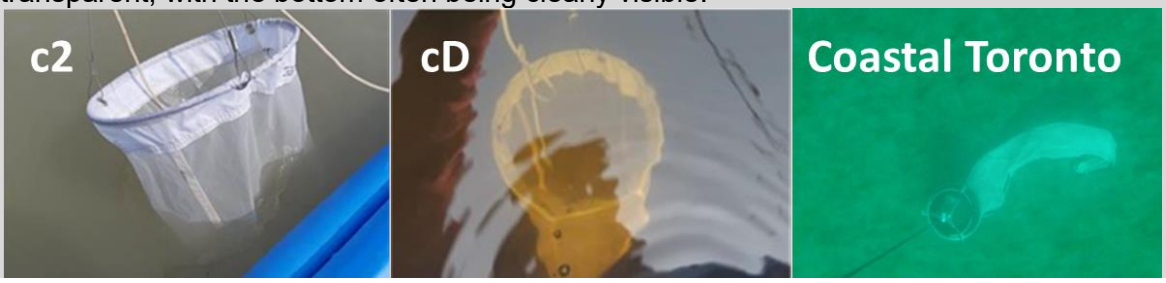
In the fall, there were no significant temperature differences among areas (Fig. 22). Thermal stratification disappeared and temperatures in the entire harbour ranged from 13-16°C by the end of October. The shallow Cells cooled the fastest as ambient air temperatures dropped. For specific sampling measurements, please refer to Appendix 1, Figs. A-9 to A-11 for individual cruise maps to observe monthly and annual variations.

Chlorophyll a

When the seasons were pooled, average water column chlorophyll (Chl *a*) measured using the EXO sonde was significantly lower within the IH (average of 2.3 µg L^{-1}) than in the cells (4.9 µg L^{-1}) and islands (6.2 µg L^{-1}), but similar to the embayments (3.6 µg L^{-1}; Fig. 21D). In the spring, islands showed the highest surface Chl *a* concentrations measured using the on-vessel flow through system (Fig. 22; right), indicating potentially higher food availability for zooplankton, which may contribute to their growth and abundance. Whole water column Chl *a concentrations* from individual stations were also significantly higher at the island sites at 8.4 µg L^{-1} relative to the other areas (Fig. 23; right, Appendix 1, Fig. A-7). In summer the highest Chl *a* values were observed at cD (11.5 µg L^{-1}, Fig. 22; right). The average water column values at the island stations (7.4 µg L^{-1}) were significantly higher than the IH (2.9 µg L^{-1}) and embayments (3.0 µg L^{-1}). In the fall, average Chl *a* values were highest in c2 and cD, and when the cells were averaged together, they had the highest Chl *a* concentration (8.5 µg L^{-1}). The other areas were not significantly different from each other, with means of 1.6 to 3.4 µg L^{-1}. In both summer and fall, the IH, OH and embayments had Chl *a* concentrations typically less than 4 µg L^{-1} (Fig. 22 and 23; right). Patterns in flow-through Chl *a* for individual dates are shown in Appendix 1, Figs. A-12 to A-14.

BOX 6: Turbidity and Clarity

Water clarity can be affected by several factors including suspended sediment and colour. Water within **c2** is usually highly turbid due to suspended clay and silt. This Cell is very shallow and sediment is easily resuspended by the wind. Water at **cD** has low turbidity from suspended particles, but is often tea-coloured due to the high levels of coloured dissolved organic carbon (DOC measured by fDOM), derived from decaying vegetation, which is common in wetlands. Coastal Toronto water is much less coloured and transparent, with the bottom often being clearly visible.



Water Clarity: Turbidity, Secchi Depth and Light Attenuation

Overall, turbidity was higher in the Cells compared to IH, Island and Embayment ecotypes (Fig. 19C) Fig. 24 right and Fig. 25; left). The Cells (and Cell 2 in particular) have the lowest recorded Secchi depth and highest light attenuation values and turbidity (e.g., suspended clay particles) relative to all other areas (Fig. 24, Fig 25; left, Fig. 26;). All these parameters indicate low water clarity in the Cells. High attenuation values mean light penetration diminishes quickly with depth and, periodically, sites in the IH such as the Don River mouth have high light attenuation, especially following rain events. While the Outer Harbour sites were not significantly different from the Islands and IH in terms of turbidity (Appendix 1, Fig. A-7), stations in the Outer Harbour occasionally had Secchi depths > 5 m. Although the water in cD may be coloured due to dissolved organic material within the water column, turbidity was typically low and the water depth was usually insufficient to take Secchi measurements. Light attenuation values ranged from 0.1 m^{-1} (clear) to 13.3 m^{-1} (like chocolate milk); both of those observations were from the IH. Attenuation rates averaged 1.2 m^{-1} over all dates and stations. Light attenuation was always

significantly higher (water murkier) in the cells compared to the IH and embayments (Fig. 18A, Appendix 1, Fig. A-6). In spring and fall, the islands also had higher light attenuation than the IH.

Specific Conductivity which can be used to estimate suspended solids and differentiate water sources, was also significantly higher in the Cells relative to the other sampling areas across all sampling seasons (Fig. 26; right, Appendix 1, Fig. A-7).

Nutrients and Chlorophyll a

Total phosphorus (TP) concentrations were elevated in all the Toronto sampling areas relative to the pelagic Lake Ontario waters (Fig. 2), ranging from 7.7 to 164 µg L^{-1} and averaging 42.5 µg L^{-1} over all dates and stations. When averaged across all seasons, the IH was similar to the Embayments but significantly lower than the Cells and Islands (Fig. 27 C). However, there was no difference in TP concentrations between the ecotypes when seasons were tested individually (Appendix 1, Fig. A-15). The highest TP concentration (164 µg L^{-1}) was observed at the Don River mouth after heavy rainfall in July 2019. Given the variation among stations, the results of chemical analysis are given as spring, summer, and fall averages by station in Appendix 1, Table A-4 and plotted in Appendix 1, Fig. A-15. The lowest recorded concentrations of Dissolved Organic Carbon (DOC) at ~ 2 mg L^{-1} were similar in all areas while the highest measures were found in the cells (maximum of 9.5 mg L^{-1}). Concentrations of DOC averaged 3.21 mg L^{-1} over all dates and stations and was found to be significantly higher in the Cells when all data were merged (Fig. 27D) and for individual seasons (Appendix 1, Fig. A-15). Dissolved inorganic Carbon (DIC) concentrations showed a similar pattern.

Total Kjeldahl Nitrogen (TKN) ranged from 0.20 to 1.10 mg L^{-1} and averaged 0.39 over all dates and stations. Inorganic forms nitrite (NO_2^-) and nitrate (NO_3^-) ranged from 0.01 to 0.73 mg L^{-1} and averaged 0.25 mg L^{-1} over all dates and stations. Concentrations of TKN was found to be significantly higher in the cells than in IH (Fig. 27B, Appendix 1, Fig. A-15), while NO_2 and NO_3 values were significantly higher in IH and lower in Cells than the other areas (Fig. 27A, Appendix 1, Fig. A-15).

Extracted Chl *a* values ranged from 0.75 µg L^{-1} in the IH in fall to 38 µg L^{-1} at the Islands sites in summer. Values averaged 9.5 µg L^{-1} over all dates and stations and were significantly lower in the IH relative to the Islands when all data were merged (Fig. 18B), as seen in the *in-situ* Chl *a* measures (Fig. 21). Individual survey data can be seen in Appendix 1, figures A-12 to A-14.

Principal Component Analysis (PCA) was used to show the relationships and patterns between key variables and sites (Cells, Embayments, IH and Islands). The biplot captured a substantial amount of total variation (48.4% PC1 and 32.5% PC2) for factors related to site and seasonality (Fig. 28). The direction and magnitude of each factor contributing to the PCA are represented by the loadings (See Appendix 1, Table A-5). The arrow vector for each factor reflects the correlation between predictor variables. In the plot, depth is the most influential factor that separates out the sites along the PC1 axis, with positive scores associating with Embayment and negative scores associating with the shallow Cells; the Island and IH sites are intermediate. Conversely, turbidity, FDOM and specific conductivity (SpCond) have strong negative scores on PC1, as the Cells have higher values for these parameters. Temperature (Temp), bacterial growth and phytoplankton productivity show negative scores on PC2 which indicates they have stronger seasonality patterns compared to the other abiotic factors (Turbidity, SpCond, FDOM and Depth).

Redundancy Analysis (RDA) was applied to determine the relationship between zooplankton taxa and environmental variables (temperature, specific conductivity, turbidity, FDOM and sonde chlorophyll) in our four ecotypes (Fig. 29). Redundancy analysis is a multivariate method used to extract and summarize variation in a set of response variables that can be explained by a set of predictor variables (McArdle and Anderson 2001). The response variable was zooplankton taxa, and the predictor variables were water quality and sampling zone. The results of the RDA showed that temperature (bit score = 0.8239) and Chl *a* (bit score = 0.5916) were the most important variables having the strongest significant relationship with zooplankton community structure.

Planktivorous Fishes in the Toronto Study Area

Electrofishing catch data during the study period provided by TRCA indicated that benthic fish, specifically Common Carp (*Cyprinus carpio*), were often the most abundant group in the Toronto area in terms of biomass (Fig. 30). They were especially abundant around the Toronto Islands and TTP embayments in the spring, which likely reflects their movement into these vegetated areas to spawn. In spring, piscivorous Northern Pike (*Esox lucius*) and Bowfin (*Amia calva*) and omnivorous Rock Bass (*Ambloplites rupestris*) were evenly distributed across the sampling areas, although biomass values of these fishes were lower in the IH, Outer Harbour and Lake Ontario nearshore adjacent to Toronto. Northern Pike and Bowfin also utilize wetland areas in the spring for spawning (Scott and Crossman 1998).

In the summer and fall of 2019 to 2021, Gizzard Shad (*Dorosoma cepedianum*), though primarily a detritovore, was the most dominant planktivore in all ecotypes except the shallow cells (Fig. 31). Biomass of this species was very high in e3 during the summers of 2019 and 2021 (only available years with fish diet data). Gizzard Shad appeared to be much less common in Embayment 3, the Outer Harbour and the Embayments in the fall. In the IH, summer Gizzard Shad biomass consistently reached moderate levels year to year. Other smaller planktivores typically comprised only a small proportion of overall fish biomass in the Toronto area, although they could be numerically abundant. Alewife (*Alosa pseudoharengus*), the predominant planktivore in the open waters of Lake Ontario, were common in the Outer Harbour area during the summer, in the IH in the fall of 2020, and the island channels in the summer of 2019 and 2020. They were otherwise uncommon, especially during the spring. Other planktivores, including Bluegill (*Lepomis macrochirus*) and Emerald Shiner (*Notropis atherinoides*), were sometimes abundant in the cells and island channels.

When we compare 2016 data to the more recent study period, Alewife were unusually abundant in the summer of 2016 in the IH and Outer Harbour areas, and in Embayment 3 in the fall (Fig. 31). Gizzard Shad were also elevated in the IH in the spring and summer of 2016 but not notably high in the other areas.

Alewife Diet Pilot Study

The diets of planktivorous fishes, both in terms of the types and amounts of algae, zooplankton and benthic invertebrates consumed, are poorly understood in the Toronto AOC. To provide insight on the efficiency of trophic transfer and further our understanding of top-down influences on zooplankton in the study area, a pilot study of Alewife diets was undertaken in the summer of 2021 (Fig. 1).

Mean Alewife lengths from eC and the Lake Ontario nearshore were not significantly different, with averages of 115 ± 11 mm and 132 ± 2 mm, respectively. Of the 18 Alewife collected, 3 nearshore and 2 eC fish had empty stomachs and were excluded. Stomach contents in the remaining fish could be quite variable even within study areas with benthic invertebrates and

midge larvae in particular comprising a high proportion of the diet biomass in some fish (Fig. 32). In eC midges comprised on average 15% of the total diet biomass but reached 61% in one fish. In the nearshore, midges made up 51% of the diet and reached over 90% in two fish. One alewife (#5) contained over 350 midge larvae. Other invertebrates, such as amphipods, accounted for 4% of diet biomass in both areas. The Alewife caught near the Toronto Islands also contained mostly benthic invertebrates (91%; Fig. 32B).

Daphnia made up an estimated 44% of the diet by weight in eC, but were rarely encountered in the nearshore Lake Ontario fish (0.3%). Comparing the sizes of *Daphnia* in both the eC stomachs and plankton samples (Fig. 33) showed that Alewife preferentially selected larger individuals. All 52 *Daphnia* measured in the stomachs were > 1mm in length, with the majority ranging from 1.3 to 1.5 mm. In the water column, only 15% of *Daphnia* were > 1mm, few were >1.2 mm, and most were 0.6 to 0.9 mm in length. *Daphnia* comprised only 5% of the zooplankton biomass in eC water, but 44% in the fish diets when benthic invertebrates were excluded (Fig. 34 A). Similarly, the large predatory cladoceran *Bythotrephes* comprised only about 1% of zooplankton biomass in the Embayment but averaged 19% of the diet. The small cladoceran *Bosmina* provided another clear example of size selectivity in Alewife feeding. *Bosmina* was the most dominant zooplankton in eC water (84%) in July 2021 but comprised only 14% in the diets. Copepods were also not a preferred diet item, comprising about 4% of the diet and about 9% in the water.

In the Lake Ontario nearshore fish, zooplankton averaged about 46% of Alewife diet biomass (Fig. 32B), although this was highly variable. *Bythotrephes* (and *Cercopagis* to a lesser extent) were the most dominant zooplankton diet items (Fig. 34B), despite their rarity in the water. One fish (#9) had an estimated 470 *Bythotrephes* in its gut, and fish #11 contained about 155 *Cercopagis* and 150 *Limnocalanus,* a large copepod. *Bosmina* and *Daphnia* together made up <1% of the diet, but in summer 2021 these two taxa averaged 16% and 18% of summer zooplankton biomass in the lake, respectively. *Dreissena* veligers were very abundant (but variable) in western Lake Ontario, averaging 54% of zooplankton biomass, but these small larvae were not observed in the stomachs. However, it should be noted that the zooplankton water column data presented here is only an estimate, given that no nearshore plankton samples were taken at the time of fish collection.

Fish Habitat Layers

Fish Habitat data layers, based on the mean seasonal (Spring, Summer, Fall) 2019-2022 values have been provided for each the ecotype microhabitats to support assessments of BUI 14 (Loss of Fish Habitat). These include data from continuous surface water sonde measures from the RV Cisco (e.g. Temperature, Conductivity, Turbidity etc.), and station measures which include sonde profiles, water properties such as Secchi depth and light attenuation, chlorophyll a, primary and bacterial productivity and zooplankton composition, size and biomass. Examples can be seen in Figs. 14,22,25. These data layers are being made available as downloadable ESRI shapefiles and the R code to produce them through the open data portal: https://open.canada.ca/data/en/dataset/fb1a47a5-de73-4fad-babc-f482f8af7c84

Discussion

Zooplankton and Fishes

The DFO lower food web study conducted in the IH in 2016, combined with other, more limited surveys in the previous decade, drew several conclusions about the status of BUI13 in the

Toronto AOC (Bowen and Currie 2021). Although the 2016 work found that zooplankton biomass in Toronto Harbour was not significantly different from adjacent nearshore areas in Lake Ontario, the IH was dominated by small taxa and contained fewer large cladocerans and copepods, and the copepods that were found carried few eggs. Compared to the current study, summer zooplankton biomass (and cyclopoids in particular) were higher in 2016 (Fig. 9A), whereas veligers and large predatory cladocerans were lower in 2016. Otherwise, the IH zooplankton populations were largely unchanged from 2016. The dominance of the zooplankton community by the small, tolerant cladoceran *Bosmina* had lessened in the current study, resulting in an increase in the overall mean size of zooplankton. Considerable year to year variation in summer IH zooplankton composition, including the proportion of larger taxa such as *Daphnia*, was noted by Bowen and Currie 2021).

The current study also demonstrated that zooplankton biomass in the IH was generally similar to the surface and nearshore waters of western Lake Ontario, but lower than levels found in more eutrophic systems such as HH and upper Bay of Quinte. The IH community also contained proportionally more small taxa, including rotifers and *Bosmina*, and fewer *Daphnia* than the open lake sites. However, given that average TP levels in the IH are about two times higher than in the west basin of Lake Ontario (26 µg L^{-1} vs 12 µg L^{-1}), one would expect zooplankton biomass to be much higher than it is. This is illustrated by the slightly lower ratios of zooplankton biomass relative to TP, Chl *a,* and primary production in the IH relative to western Lake Ontario and the coastal region adjacent to Toronto (Fig. 6).

Relative to the IH, total zooplankton biomass and production was higher in the sheltered Embayments, Cells and Island ecotypes around Toronto throughout the sampling season. Therefore, these sheltered areas are much more productive in terms of providing food for warmwater planktivorous fishes. It appears that trophic transfer operates more efficiently in these environments and zooplankton were better able to utilize production from both algae and the microbial food web. The elevated cladoceran production in the Island channels and Cells is largely driven by high populations of *Bosmina* and/or *Ceriodaphnia*, combined with warm water temperatures and greater food supply (as shown by elevated Chl *a*). The Cells and Island sites also supported higher biomass of littoral cladocerans and cyclopoids, including zooplankton taxa that are typically indicators of eutrophic conditions, such as *Chydorus sphaericus,* the cyclopoids *Mesocyclops edax* and *Acanthocyclops vernalis* (Gannon and Stemberger 1978; Haberman and Haldna 2014). These taxa are typically rare in the more exposed, unvegetated IH and Embayments. The zooplankton and rotifer communities in the IH are generally not characteristic of eutrophic environments, and most of the animals found are typical of oligotrophic open waters of the Great Lakes (Rudstam et al. 2015; Barbiero et al. 2019).

The different hulls on the NMDS plot results also provided insight on spatial patterns, as the zooplankton communities in different ecotypes were often composed of distinct taxa that may respond differently to the environmental conditions found there. In the spring for example, *Daphnia ambigua* mainly flourish in the Cells, and *Chydorus* and littoral cyclopoids associate with both the Cells and the Islands. *Bosmina* and the copepod *Diacyclops* were positioned more centrally in the plot, showing their more ubiquitous distribution. The largest populations of *Ceriodaphnia* are found in the Islands during the summer, whereas *Bythotrephes* and the calanoid *Epischura* were mostly found in the IH and Embayments in the fall. Species richness generally increased during the summertime.

The position of the hulls reinforces the similarity of ecotypes in the IH and Embayments (open water with little vegetation), compared to the Cells and Islands (shallow, more protected areas, often with abundant aquatic vegetation), however cells show to be a different ecotype from the

rest, also a pattern seen in the PCA plot (Fig. 28). The RDA showed relatively clear clustering of the different ecotypes, with the IH and Embayment sites forming one group, and the Cell and Island stations in another (Fig. 29). The RDA results also suggest that both water property variables and ecotypes contribute significantly to the variation in zooplankton community structure. Notably, *Daphnia galeata mendotae* was associated with the Embayments during the summer when water temperatures were highest. This large *Daphnia* species is especially vulnerable to fish predation, and it may have been seeking refuge in the deeper water of e3. The invasive species (e.g., *Dreissena* veligers and *Bythotrephes*) were more abundant in the lake-influenced IH and Embayments. The RDA plot also shows that the water property variables were related to the differences in zooplankton community structure between the different ecotypes. Specifically, areas with higher FDOM and Chl *a* concentrations tended to have higher abundances of small or medium-sized zooplankton (e.g., littoral cyclopoids, *Chydorus* and *Daphnia ambigua*), although it should be noted that elevated FDOM and Chl *a* were characteristic of the wetlands and are not necessarily causative in driving the higher abundances of these littoral species.

The results do not specify an environmental variable which drives veligers and *Bythotrephes* in the IH and Embayments. It is probable that the presence of hard substrates such as seawalls and pilings along the periphery of the harbour--to which adult *Dreissena* can attach--and the transportation of veligers from the lake contribute to their proliferation, as veligers can be extremely abundant in the nearshore of Lake Ontario (Bowen et al. 2018). *Bythotrephes* demonstrates a preference for cooler, pelagic waters and are likely arriving from the lake as well (Cavaletto et al. 2010; Kim 2013). At times, both *Bythotrephes* and *Cercopagis* likely exert substantial predation pressure on other zooplankton in the IH and Embayments, and may in part be responsible for diminished zooplankton biomass. Populations of both taxa are low (or absent) in the Cells, meaning that other zooplankton in the Cells are relatively free of predation from these invaders. Conversely, the predatory copepod *Mesocyclops* was more commonly found in the more protected ecotypes during the warmer months, where they may exert predation pressure on small zooplankton. These large taxa in turn tend to be more vulnerable to consumption by fishes. Overall, however, many of the zooplankton taxa cluster together toward the center of the plot, showing that they are widely distributed among ecotypes and environmental conditions.

There are a number of potential reasons for the suppression of zooplankton biomass in the IH, which are discussed in detail by Bowen and Currie (2021). Top-down (predation), bottom-up (trophic status) and environmental drivers can all play roles in structuring zooplankton communities. It appears that traditional bottom-up linkages to phytoplankton are not typically the primary drivers of zooplankton biomass within Toronto Harbour, and that factors like changes in food source (bacteria), predation, and environmental influences are more influential. It is also possible that more sensitive zooplankton taxa such as *Daphnia* are being suppressed by a chemical contaminant or other environmental disruption not measured in our study. A reduction in zooplankton size distribution and reduced trophic transfer efficiency is a known result of pesticide contamination (Hanazato 2001; Yu et al. 2020; Li et al. 2023). The range of contaminants to the Inner Harbour will include road salts, heavy metals, or pesticides found in river discharges and stormwater runoff (Winter et al. 2012; Howell and Benoit 2021), all of which may impact zooplankton growth and reproduction (Hall and Anderson 1988; Arnott et al. 2020).

Top-down influences on zooplankton populations may at times be elevated in Toronto Harbour relative to the open lake sites. Bowen and Currie (2021) suggested that the dominance of small zooplankton and the suppression of predatory cladocerans in 2016 reflected atypically high fish

predation rates in the IH that year relative to the previous decade. Our examination of electrofishing data since 2016 reinforces the idea that fish planktivory in the IH may have been unusually high that year. Although the IH zooplankton community was largely unchanged from 2016, the proportion of *Daphnia* and large predatory cladocerans has increased. This suggests a potential relaxation of planktivory in the current study, which is supported by lower biomass of Gizzard Shad in the electrofishing data. Systems with an overabundance of planktivorous fish usually contain fewer large zooplankton as planktivorous fishes selectively consume larger animals (Brooks and Dodson 1965; Mills et al. 1987).

Nearshore electrofishing data suggest that summer biomass estimates of planktivores in the IH were generally in the same range as those in the islands when Gizzard Shad were included. Biomass of other planktivore species that were likely more reliant on zooplankton were lower in the IH. Neither Alewife nor Gizzard Shad were likely effectively captured by electrofishing efforts in the IH, as TRCA surveys in this ecotype often took place in waters that were too deep to be sampled accurately (i.e., >1.5-2.0 m). Alewife is generally considered to be an offshore species, and it is likely that roaming schools moving in and out of the harbour from the open lake were missed by electrofishing surveys conducted along the shoreline. Therefore, the numbers presented here may not accurately reflect planktivory rates in the open waters. Midwood et al. (2018, 2022) conducted mid-water trawling in the IH in 2016 and 2018 and found higher catches of Alewife and Rainbow Smelt compared to more open lake areas, indicating that planktivory was high in the IH. However, it is unlikely that planktivory alone can explain the unusually low zooplankton biomass observed in the IH. It is also possible that schools of fish moving through the harbour are able to graze down the zooplankton if they are being produced at a lower rate than in the more productive, sheltered ecotypes; but these species also occur in the coastal regions around Toronto Harbour which do not show a similar reduced zooplankton biomass (Bowen and Currie 2021). More intensive hydroacoustic surveys of the open waters of the IH would be required in future studies to provide insight on forage fish populations in these areas.
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The importance of size-selective predation by Alewife was clearly evident in the pilot diet study carried out in the Toronto AOC in 2021. Alewife in eC almost exclusively consumed *Daphnia* >1 mm in length despite the relative scarcity of large *Daphnia* in the water column. They also preferentially ate both *Daphnia* and predatory cladocerans (i.e., *Bythotrephes*) and avoided

consuming *Bosmina*. At the nearshore Lake Ontario site near Gibraltar Point, zooplankton prey consumed by Alewife were mostly *Bythotrephes*. *Bosmina* and veliger larvae were virtually absent in the stomachs despite their high prevalence in the mid-summer water column, although it is possible that digestion of veliger shells made them difficult to visually identify. *Limnocalanus*, a large offshore calanoid typically residing deeper in the water column, was found in large numbers in one nearshore fish. This illustrates the nearshore movement of Alewife (Scott and Crossman 1998) and serves as a reminder that the stomach contents of fishes may not always be reflective of the area in which they were caught. Some of the Alewife from littoral regions indicated a clear importance of energy pathways from the benthos. The dominance of midge larvae in the Alewife stomachs is consistent with other observations of Alewife consuming benthic prey in the nearshore (Scott and Crossman 1998; Pothoven and Madenjian 2008), although they are typically planktivorous in deeper water. The feeding preferences of some fish species may not be easy to neatly categorize, and many species begin life feeding on zooplankton and invertebrates but switch to consuming fish as they grow larger. Though Gizzard Shad was classified as a planktivore in this study, it primarily consumes plant matter and detritus in addition to some zooplankton (Yako et al. 1996). We recommend additional diet studies of planktivorous fishes be undertaken in the Toronto area to better determine the amount and composition of zooplankton prey in their diets. The number of Alewife used in the pilot study was small (13 fish) and spatially limited. In addition to more Alewife, the inclusion of Gizzard Shad, shiners and Centrarchid panfish in the Cells and Island sites, which are important forage for piscivores, would shed light on the importance of zooplankton in diets compared to small fishes, benthos and plant material.

Temperature as Habitat

In northwestern Lake Ontario, water temperature is periodically influenced by upwellings of cold offshore water into the nearshore and even into the IH and TTP embayments (Huang et al. 2010; Murphy et al. 2011; Hlevca et al. 2015). As a result, monthly sampling frequencies are inadequate to describe temperature differences among areas in the Toronto AOC. The thermal regime in and around Toronto Harbour is better described in Hlevca et al. (2015), which utilized continuous temperature loggers. It is crucial to recognize that the temperature maps generated are only snapshots and do not reflect substantial day-to-day variability, especially during major upwelling events (see Appendix 1, Figs. A-9 to A-11 for individual day variability). These upwelling events typically take place four to 10 times during the stratified season (Hlevca et al. 2018a) and can lead to drastic changes in temperature within a matter of hours or days (Murphy et al. 2011). Water temperature is one of the most important factors influencing biochemical and physiological responses of ectothermic fishes. Peat et al. (2016) showed that Bass and Pike occupied different thermal zones in Toronto Harbour when temperatures were high, and fish moved to avoid unsuitable areas as temperature changed (Brooks et al. 2022). Rapid changes in temperature due to upwellings can result in cold shock and result in sub-lethal changes, growth impairment and, in extreme cases, mortality (Donaldson et al. 2008). Murphy et al. (2011) documented temperature changes in embayments and potential effects on young of year Bluegill, a warmwater fish species targeted by the Toronto AOC for restoration.

Water Chemistry, Chlorophyll a and Light

Physical and chemical water quality within Toronto Harbour fluctuates spatially and temporally via loading gradients (Howell and Benoit 2021), weather and lake circulation (Doka et al. 2018). Because of topography and current, the IH receives low productivity water directly from Lake Ontario through the western gap (Haffner et al. 1982). This water exits the harbour through the eastern gap and results in increased flushing of the IH compared to the Islands and some of the Cells. The hydraulic residence time of the IH is estimated to be 7 to 14 days (Hlevca et al.

2018a), compared to 95 to 140 days for eutrophic HH. Hydraulic residence time in the Cells is estimated to be between 1 and 11 days, depending on the individual cell. In the Toronto AOC, these water currents carry nutrients, bacteria and phytoplankton from the IH into the Outer Harbour and into coastal Lake Ontario, whereas algae produced in the island channels are not subjected to these physical disturbances and removal by flushing to the same degree (Hlevca et al. 2015).

Input from the highly urbanized watershed (via the Don River and stormwater infrastructure) is a major source of nutrient loading in Toronto Harbour. The area nearest the mouth of the Don River is one of the most impacted areas in the IH (Howell et al. 2018; Howell and Benoit 2021) and our highest TP concentration observed (0.164 mg L^{-1}) was at T12 in summer 2019 after a rain event. Lower values of total phosphorus are expected when flushing from the lake is higher than inflow from terrestrial sources and T4 in the central IH is consistently lower than T12 at the Don River mouth (refer to Fig. 1A for locations of sampling sites). Levels of TP found in the inner harbour are in line with levels recorded in the Inner Harbour by Howell and Benoit (2021) and along the southern shore by Makarewicz et al. (2012).

Dissolved organic carbon (DOC) compounds are excreted by living organisms as a result of metabolic processes or decomposition. As expected, higher concentrations of DOC were found in some of the enclosed Cells, such as cD which has lush macrophyte presence, and c2 which receives the direct outflow from the SAV enriched c1 (Hlevca et al. 2018a). Shallow systems are also more influenced by terrestrial inputs of organic carbon, so are expected to have more DOC across all seasons than in areas under hydrologic influence of Lake Ontario (i.e., the IH and some embayments).It is possible that the Island sites, because they are shallower than the IH and rich in macrophytes, would also show the same trend (higher DOC) as the Cells if more samples had been collected.

As inorganic forms of nitrogen are taken up by algae and macrophytes (Weisner, S.E.B. et al. 1994) and organic forms are released as waste, higher concentrations of the organic forms of nitrogen (TKN) in the Cells are expected, given that there is extensive macrophyte growth compared to the IH. Inorganic forms of nitrogen (nitrate and nitrite) are highest in the IH where there are fewer macrophytes, slightly lower in the Embayments and Islands and lowest in the Cells where macrophyte growth was greatest. Concentrations of nitrate and nitrite in the IH are in line with what was observed by Howell and Benoit (2021) in 2018 and are similar to levels in nearshore Lake Ontario.

Water clarity (i.e., sonde turbidity, Secchi depth and light attenuation) is influenced by the presence of suspended particles and dissolved organic matter (Kirk 1981) and can have significant effects on the suitability of the habitat to fish (i.e., Kovalenko et al. 2018). Our PCA showed that depth was the most influential factor separating the areas and that the shallow sites had the highest turbidity, FDOM and Specific Conductivity. The Cells are consistently murkier than the other areas due to their shallow depth and wind resuspension of bottom material and possibly the presence of Common Carp stirring up bottom sediments. Sometimes we saw elevated turbidity along the IH waterfront following rain events.

Extracted Chl *a* concentrations were higher at the Island sites than the IH (but still much lower compared to other eutrophic AOCs), and lowest in open Lake Ontario. The Cells and Embayments were not different from either the Islands or IH. Although extracted Chl *a* values are typically greater than those obtained fluorometrically with sondes, the patterns are generally similar. The sheltered Island channels warm up earlier in the spring than the IH, which promotes

higher phytoplankton growth. This is shown by higher sonde chlorophyll measurements in the Islands compared to the IH during both spring and summer, which indicates increased phytoplankton biomass and food availability for herbivorous zooplankton. In the fall, elevated Chl *a* persists in the Cells relative to the other areas, whereas Chl *a* levels begin to decline around the Islands. The surface sonde chlorophyll data has better spatial coverage, as extracted Chl *a* was measured at only a few stations that may not reflect conditions in the area as a whole. The Embayments and Outer Harbour were poorly represented by extracted Chl *a* and nutrient measurements.

Primary Productivity

Trophic status influences the availability of phytoplankton, the primary food resource of herbivorous zooplankton and, therefore, can play a large role in regulating zooplankton populations and secondary production (McQueen et al. 1986; McQueen and Post 1988; Jeppesen et al. 1997, 2005). Primary productivity is a direct measure of the photosynthetic energy (carbon) generated by phytoplankton available to higher order organisms and, thus, is an essential measure of food web function. Primary productivity is elevated in Toronto Harbour compared to open Lake Ontario waters, but this is expected in comparatively shallow, protected areas and it is well below levels observed in the eutrophic Bay of Quinte and HH. Within the Toronto AOC, primary productivity is higher in both the embayments and islands (≈25 mg C m^{-3} h^{-1}) than the cells and IH (≈15 mg C m^{-3} h^{-1}), but this difference was only significant for the cells (Fig. 18). As with Chl *a* and nutrients, the limited number of stations within this study where primary production was measured limit its statistical power.

Higher macrophyte abundance within the Cells would be expected to reduce phytoplankton primary productivity as these aquatic plants compete for nutrients and sunlight within the water column and can promote settling by reducing water movement (Ozimek et al. 1990; Van Donk and Van De Bund 2002), but few macrophytes were found in the IH. Both algal standing crop [measured either as Chl *a* (Fig. 2) or phytoplankton biomass (Fig. 8)] and primary production within the IH were lower than expected given TP concentrations that averaged >30 µg l^{-1} over the growing season, values that are typical of mesotrophic – eutrophic environments (Table 4, Fig. 2,17; Carlson 1977). As such, the role of TP as it relates to photosynthetic production requires further examination.

Of particular concern with respect to BUI 13 is determining how much of this energy generated through primary production is being transferred to zooplankton and planktivorous fishes or diverted into the microbial food web. The size fractionated primary productivity technique deployed in this study helped to provide some insight into potential energy flow through the aquatic food web. Larger net phytoplankton (> 20 µm), which include colonial and filamentous forms, are found in higher amounts in eutrophic environments and tend to be less edible to cladocerans as they can clog filter feeding structures, but may be eaten by some zooplankton (i.e., calanoid copepods). The smaller size classes, nanoplankton (2-20 um) and picoplankton (<2 um) are typically more palatable for zooplankton so a higher proportion of energy generated by these size ranges is generally desirable for efficient food web function. While phytoplankton composition data are limited to the IH in this study, it does provide insight into the potential food resources available to support zooplankton populations. The nanoplankton size class includes pennate diatoms (*Diatoma tenue* and *Navicula* spp.) as well as small and medium-sized flagellates (i.e., *Plagioselmis nanoplanktica*, *Cryptomonas* spp. and *Chrysochromulina parva*), taxa which tend to be regarded as preferred prey items for zooplankton (Balcer et al. 1984). But also found in this size class is *Fragilaria crotonensis*, a pennate diatom that aggregates into large colonies and may be less preferred by zooplankton grazers. Picoplankton in the IH are

typically small single-celled Cyanobacteria (e.g., *Chroococcus* and *Synechococcus*) and Chlorophyta (Chlorococcales) which tend to be edible and important drivers of energy exchange between phytoplankton and zooplankton, in part due to their rapid turnover rates (Munawar and Weisse 1989; Fahnenstiel and Carrick 1992; Brett et al. 2009; Carrick et al. 2015).

With respect to the role of net plankton and nanoplankton in supporting food webs, the available evidence suggests that herbivorous zooplankton are suppressed in the IH compared to the other ecotypes (Figs. 4, 6, 9-15) and that total fish biomass, including predatory fishes, is also reduced (Midwood et al. 2019a; Fig. 31). However, accurate estimates of planktivore abundances in the open waters of the IH are generally lacking, and predation on zooplankton may be high at times, as seen in 2016. These findings confirm our original assessment that organic carbon is not effectively being transferred up the food web in the IH (Currie et al. 2018) and is likely being shunted into microbial production. Neither nanoplankton nor picoplankton productivity was significantly different among ecotypes in Toronto Harbour (Fig. 20), but biomass of *Bosmina*, *Daphnia* and other herbivorous zooplankton are greater in the islands, embayments and cells, suggesting that energy transfer (food web function) may be more efficient in those areas (refer also to the discussion of trophic ratios and food webs later in this document). Importantly, our findings suggest that primary productivity in the IH is sufficient to sustain a larger zooplankton population and that other factors are regulating zooplankton biomass. However, more thorough assessments of phytoplankton taxonomy are needed to assess the quality of food resources within the different ecotypes.

Bacterial Productivity

Heterotrophic bacterial productivity (also referred to as bacterial growth) is a measure of the cellular uptake of leucine resulting from the decomposition of organic matter. In oligotrophic environments, bacteria can be an important food resource for zooplankton (Pace et al. 2004), but excess bacterial production can be shunted into heterotrophic microbes and mixotrophic plankton, and may not be as easily transferred up the food web (Sanders and Wickham 1993). In the original assessment of BUI 13 in Toronto Harbour (Currie et al. 2018), unusually high bacterial growth rates in the IH were a key factor in our recommendation to list the Toronto AOC as impaired (Currie et al. 2018). Mean bacterial growth rates from the IH in 2016 averaged 1.98 ± 0.35 mg C m^{-3} h^{-1}, and the maximum value of 5.8 mg C m^{-3} h^{-1} was the highest value we had observed in the Great Lakes up to that point. The current study found similar results. The mean bacterial growth rate over the 2019 – 2022 period was 1.9 ± 0.24 mg C m^{-3} h^{-1} in the IH with no significant differences observed between ecotypes, including the Islands (2.48 ± 0.67 mg C m^{-3} h^{-1}), Cells (1.73 ± 0.24 mg C m^{-3} h^{-1}) or Embayments (0.80 ± 0.23 mg C m^{-3} h^{-1}) (Figs. 18-19). The results show that the high bacterial growth rates are not confined to the IH and sources of bacteria including storm sewer outfalls, combined sewer overflow events and high populations of cormorants, gulls and other avifauna are likely affecting these areas. That said, recent studies conducted around the IH, Humber Bay (Sunnyside Beach) and Rouge Beach suggest that human-derived *E. coli* is a major source of bacterial contamination and has a strong positive correlation with TP concentrations (Staley et al. 2018b, 2018a; Edge et al. 2021) and this is likely the same elsewhere in the Toronto Harbour.

Bacterial production is correlated with primary production and averages about 20% of net primary production but shows a wide range of values (Cole et al. 1988). Comparative studies across a wide range of natural aquatic systems show that the proportion of planktonic bacteria that are metabolically active (i.e., growing) is generally higher in systems with higher nutrient and Chl *a* (Cochran and Scarborough 1995) and so we would expect TH to have higher BG rates than the open lake. Nevertheless, it is instructive to compare the ratio of bacterial growth

to primary productivity (BG:PP) using our extensive dataset from Lake Ontario. Toronto Harbour, not surprisingly given the high bacterial productivity, also has the highest ratio of BG:PP of the sites around Lake Ontario (Fig. 6). This ratio in IH and Cells averages 16-18% compared with 2% in offshore Lake Ontario and less than 1% in HH which despite very high PP had BG that is lower than in TH. This reiterates the fact that bacterial productivity throughout the study area is excessive. Ultimately, the fate of this excess bacterial production needs to be understood.

Heterotrophic production by the microbial food web may be an important alternate trophic pathway supporting zooplankton in the harbour. There is considerable debate as to whether zooplankton will utilize bacterial-derived carbon when photosynthetic carbon is available (Hwang and Heath 1999; Pace et al. 2004; Brett et al. 2009). Herbivorous zooplankton such as *Bosmina* and *Daphnia* can exploit bacterial food resources, although they prefer to graze on algae (Porter et al. 1983; Hart and Jarvis 1993). Microzooplankton, including rotifers and nauplii, are also able to utilize bacterial production (Christoffersen et al. 1990; Hwang and Heath 1999), and it is noteworthy that rotifers are a larger proportion of total zooplankton biomass in the IH.

Heterotrophic Nanoflagellates (HNF) are tiny (2 – 5 µm) bacterial grazers that will utilize this food resource and can grow rapidly to create large standing stocks (Hwang and Heath 1999; Attermeyer et al. 2015; Sanders et al. 2015). Our previous work from 2016 suggested that was occurring in the IH as HNF biomass was extremely high (>16 g m^{-3}), but it also followed a boom-bust pattern (Munawar et al. 2018) and similar patterns were observed in the current study (Fig. 9). Mixotrophic algae, which in the IH includes *Ochromonas* spp. and *Chrysochromulina parva*, are also capable of ingesting bacteria and utilizing this energy (Flynn *et al.*, 2019). However, comparatively low biomass of mixotrophic algae coupled with higher rates of phytoplankton primary production observed in the current and prior studies (Figs. 2C, 8B and C) suggests that bacterial ingestion is not a likely vector for energy flow through these taxa. Excess bacterial production being shunted into microbial pathways is a sign of continued impairment. Moreover, the source of this bacteria warrants consideration. Recent work (Edge et al. 2021) shows that human derived fecal matter is a major source of contamination in the IH. Given that bacterial production is elevated at all of the ecotypes we studied, not just in the IH which receives the outflows of the Don River, it is recommended that Microbial Source Tracking techniques (Edge and Hill 2007; Kinzelman et al. 2011; Staley et al. 2018b, 2018a) be deployed in the other ecotypes to identify the types and sources of bacteria as well as potential risks to human health.

Putting it Together: Food Webs

Food webs are a complex interconnection of resources and populations which results in the flow of energy through an ecosystem. These intricate linkages can be simplified into chains of direct linkages (e.g., predation or uptake) forming “trophic (or Eltonian) pyramids” (Elton 1927; Lindeman 1942). The relationships between these trophic levels can be determined by using their biomass ratios to measure energy transfer – and the efficiency of energy transfer – at each link of the food chain independently. This technique has both theoretical and empirical support and has been used previously to assess disturbances in the energy transfer between trophic levels (McCauley and Kalff 1981; Jeppesen et al. 1997, 2005). When systems deviate from expected biomass ratios (e.g., top-heavy inverted biomass pyramids; (McCauley et al. 2018), it can be an indication of alternative endogenous (e.g. changes in edibility, turnover rates, feeding guilds, etc.) or exogenous (external subsidies, allochthonous inputs, contaminant disrupters etc.) factors affecting energy source pathways. Well-functioning ecosystems have efficient

energy transfer between food web linkages, whereas inefficient energy transfer between linkages suggests a disruption in the system.

The shape of the trophic biomass pyramid is controlled by both bottom-up (resource availability) and top-down (predation) factors, and environmental drivers (McQueen et al. 1986; Carpenter et al. 2001). It is possible to determine if bottom-up or top-down disruptions are the primary cause of changes to a system, however, a clear understanding of the linkages in the ecosystem is necessary to the interpretation of the trophic ratios. Factors which influence bottom-up processes, such as how light and nutrient input control the productivity of a system, can be a dominant factor, especially in eutrophic systems but it is only part of the ecosystem equation (Lindeman 1942; McQueen et al. 1986). Top-down consumption can also impact zooplankton composition and biomass (Hrbáček et al. 1961; Brooks and Dodson 1965), which has subsequent impacts on phytoplankton (Carpenter et al. 1987). This theory of trophic cascade and active biomanipulation of lakes quickly led to efforts to change the community composition to ones favored by managers (Lammens et al. 1990).

In the Toronto AOC, as noted in the 2016 survey, we see that in spite of the elevated TP, this does not lead to increased Chl *a* (seen in the Chl:TP) or primary productivity (PP:TP) for any of the sites (Fig. 6). This in itself is indicative that the system is not operating as expected (Bowen and Currie, 2021). Algal blooms are not desired, but they are expected at these levels of TP. The primary reason algal blooms are not occurring here is that TP is being converted into bacterial biomass via elevated bacterial growth rates not seen in any other AOC site. While this BG:TP is highest in the IH, it is elevated at all Toronto sites, including the adjacent open waters of Lake Ontario . This indicates that elevated bacterial productivity is projecting into Lake Ontario itself. It is common for vegetated shallow wetlands to have slightly elevated bacterial growth (Stanley et al. 2003), but values observed in the Toronto AOC remain higher than would be expected in a healthy ecosystem.

Bacteria, while consumed by herbivorous zooplankton grazers, are not an ideal food resource (Porter et al. 1983). Algal sources are more preferred by zooplankton, but the level of phytoplankton is much reduced in favour of bacteria at all of the Toronto sites as seen by the high BG:PP ratio (Fig. 6). Furthermore, the limited algal food resources that are being produced don’t appear to be effectively used by the zooplankton in the IH, as demonstrated by the very low TZ:PP values. The TZ:TP in the IH is similar to the upper Bay of Quinte where bottom-up trophic disruption has already been identified (Currie et al. 2023). Conversely, zooplankton total biomass is typically about an order of magnitude higher in the more sheltered ecotypes and the TZ:PP and TZ:chlor ratios are elevated relative to most other sites in the Lake Ontario watershed. This shows that zooplankton are able to utilize available phytoplankton more effectively in the sheltered Toronto AOC ecotypes, and alternative endogenous pathways are also potentially being used to support their biomass, likely from submerged vegetation and periphyton (Moss 1995). Zooplankton in the wetland ecotypes may be better protected from visually feeding planktivores by seeking cover in dense macrophyte beds (Timms and Moss 1984; Burks et al. 2002) but also could experience larger densities of zooplanktivorous fishes in these zones. In the Bay of Quinte, nearshore vegetated sites had lower biomass of zooplankton compared to offshore, and adjacent nearshore zones with low macrophyte densities had even lower zooplankton biomass (Bowen et al. 2023). Poor water clarity in the cells (especially Cell 2) due to suspended sediments also likely provides an alternative protection from visual fish predation (Liljendahl-Nurminen et al. 2008; Horppila et al. 2009). Diets of forage fishes in these important fish habitat zones should be determined seasonally and compared to inner harbour sites to identify the importance of zooplankton to supporting fish populations.

The very low zooplankton biomass within the IH is likely driven by a range of factors including not having access to alternative food sources (i.e. material derived from macrophytes and associated periphyton), environmental disruption, lack of predation refugia and the extremely high level of bacteria dominating the available food within the system (Bowen and Currie 2021). This is reinforced by the planktivorous fish diet results (Figs. 32 and 32) which suggest exclusive feeding on only the very largest zooplankton (large *Daphnia* and predatory cladocerans) or on benthic food such as chironomids. Invasive predatory cladocerans in both IH and Lake Ontario also feed on zooplankton and represent an added level in the food chain. The pelagic pathway of nutrients to algae to zooplankton to fish is disrupted in Toronto by the shunting of energy into bacteria, such that alternative pathways of energy are required to support the existing biomass. There is also a high likelihood of the zooplankton being impacted by urban environmental disruptors including contaminants ranging from pesticides, tire debris, metals, and microplastics, which can cause physiological, behavioural and reproductive impacts (Hanazato 2001; Yu et al. 2020; Li et al. 2023). This lower trophic food supply disruption may limit the recovery of native fishes, including top predators, whose populations remain impaired in the Toronto AOC.

Recommendations

This study confirms that BUI 13, *Degradation of Phytoplankton and Zooplankton Populations,* remains *impaired* for the Inner Harbour within the Toronto and Region Area of Concern. Other ecotype microhabitats including the Islands, Cells and Embayments do not show this impairment. Within the IH, zooplankton biomass was significantly lower than expected considering the high total phosphorus concentrations (greater than 30 µg l^{-1} on average), likely due to a range of disruptors. This indicates a reduction in the available primary production which not being transferred up the food web, but is instead being shunted into the microbial food web. This is supported by exceptionally high bacterial growth rates (≈2 mg C m^{-3} h^{-1}) observed also across the ecotypes and the immediate coastal areas of Lake Ontario surrounding Toronto, indicating widespread, continued food web disruption and impairment within the system.

Proposed BUI 13 Targets – Inner Harbour Zooplankton

The current study demonstrates that the Embayments, Cells and Islands, generally support a well-functioning lower food web in terms of zooplankton biomass and trophic transfer efficiencies. In contrast, the IH continues to exhibit elevated microbial productivity and reduced zooplankton biomass, indicating ongoing impairment and limited food web support for fish populations. Restoring zooplankton community structure in the Inner Harbour (IH) to be more comparable to what is observed in the Embayments (Excluding Embayment 3, which is more enclosed and pond-like) is needed. Zooplankton communities in the Embayments typically reflect a more functional food web with higher biomass, balanced size structure, and greater availability of preferred prey for planktivorous fishes.

Target 1: Summer zooplankton biomass in the IH to 125 mg m^{-3}

Summer zooplankton biomass and densities in the IH were significantly lower than in the other sheltered ecotypes investigated in this study (Cells, Islands and Embayments; Table 4). As expected given differences in habitat and water depth, zooplankton community composition showed that littoral taxa typical of productive nearshore systems were important within the sheltered ecotypes, and at some sites (e.g., e3) the larger *Daphnia* preferred by forage fishes were at times dominant. To improve ecosystem function, summer zooplankton biomass in the IH should reach an average of 125 mg m^{-3} or higher. This value represents 60% of the median summer biomass observed in the Embayments (excluding Embayment 3; Table 2). The Embayment ecotype is most comparable in proximity and depth to the Inner Harbour making it a strong reference site compared to the other shallow wetland ecotypes studied. Summer (July, August and first two weeks of September) zooplankton biomass was chosen because temperature and biomass in deeper, more exposed areas such as the IH would naturally lagged and take longer to increase in the spring, and timing of fall biomass die-offs is also naturally variable among areas. The target was set using median summer values to avoid skew from high outliers and unbalanced distribution. Aiming for 60% of the Embayment median is realistic given the physical similarities between the sites. While the IH currently supports similar biomass to offshore Lake Ontario, values in the IH should be considerably higher based on system productivity and its more sheltered nature.

Target 2: Improve Composition and Size Structure of IH Zooplankton

Despite much lower biomass and densities in the IH, the proportions of medium (0.35 to 1 mm in length) and large (> 1 mm in length) zooplankton relative to the total densities in the IH during summer are currently in the same range as those observed in the Embayments, roughly 15-19% and 3% of the total density, respectively (Figure 10; Table 2). Moving forward, the percentage of medium zooplankton should be maintained at 15% or higher, and large

zooplankton 2% or higher by density. It is important that future zooplankton population gains in the IH are not achieved by simply increasing the numbers of small tolerant taxa such as veligers, *Chydorus* or *Bosmina*, which have limited value to forage fishes. Large-bodied zooplankton such as *Daphnia* are preferred prey for planktivorous fishes and their presence reflects a balanced predator-prey dynamic. When populations of large taxa are low, this can indicate that fish presence may be limited due to food availability. While not included in the above target, it should be noted that the IH currently has a higher proportion of rotifers relative to crustacean zooplankton. As a guideline, mean May to October rotifer biomass in the IH should not exceed 10% of the total zooplankton plus rotifer biomass, and it is expected that this should resolve as zooplankton biomass increases. Rotifer biomass above this threshold may indicate degraded conditions and/or bacterial driven food webs. Maintaining conditions with balanced large to small bodied zooplankton and lower proportion of rotifers reflects a more functional food web and increased food availability for fishes.

Remedial actions: Continue to improve IH water quality through watershed improvements as outlined in the Wet Weather Flow Master Plan in 2003 (WWFMP, City of Toronto 2003, 2023) and Don River Mouth Naturalization and Port Lands Flood Protection Project to reduce nutrient and watershed pollutant input. Conduct assessments of Harbour water and sediments and river water concentrations during wet and dry conditions to determine if there are sources of contaminants that could be reduced or remediated. These might include road salts, heavy metals, or pesticides found in river discharges and stormwater runoff entering the Inner Harbour (Winter et al. 2012; Howell and Benoit 2021). Targeted habitat improvement (e.g. wetland creation) within IH may allow for physical refuges for large zooplankton among plants and reduce planktivory due to increased presence of predatory fishes.

Further recommended actions: Continued and higher frequency monitoring and assessment of plankton communities are recommended for BUI 13. Consistent sampling of zooplankton through the growing season (May to November) is needed to assess community composition, population size structure and biomass, and build understanding of food web dynamics. Comprehensive seasonal analyses of open-water and nearshore planktivorous fish populations and their diets are needed to better understand their reliance on zooplankton and impacts of planktivory. Hydroacoustic surveys of the open waters of the IH are needed to better understand the movements of forage fishes in these areas, since these populations are likely to be transient users of this area, primarily feeding in coastal Lake Ontario. Using bottom-mounted hydroacoustics may be useful in gathering continuous measurements of forage fish biomass availability over longer periods of time. Maintaining TRCA fish monitoring assessments to determine fish population improvements is vital. Recommend lethal and sub-lethal (targeting growth and reproduction) toxicology studies comparing the sensitivity of small and large zooplankton taxa to heavy metals, road salts, pesticides, fecal contamination and dust to target future restoration actions. Continue to monitor the recovery of submerged aquatic vegetation in response to the WWFMP which provide can refugia for large zooplankton and improved trophic transfer to fishes.

Proposed BUI 13 Targets – Bacterial and Phytoplankton Primary Productivity

There needs to be reduced bacterial growth rates in Toronto Harbour through the reduction of bacterial and nutrient inputs. Bacterial productivity in Toronto Harbour averaged 1.9 mg C m^{-3} h^{-1} over the study period (2019-2022) with the upper quartile ranging from 2.7 – 10.9 mg C m^{-3} h^{-1}. Levels of bacterial productivity observed in Toronto Harbour are far higher than we have observed anywhere else in the Great Lakes. Related to this, the ratio of bacterial growth to primary productivity (BG:PP) at 0.13 is almost double that of Lake Ontario and triple that

observed in other habitats around the lower Great Lakes. Since primary productivity around Toronto Harbour is generally at a mesotrophic level (20 mg C m^{-3} h^{-1}, on average), a reasonable level for a nearshore environment, reductions in the BP:PP ratio have to be achieved through reductions in bacterial productivity. The expectation is that actions reducing bacterial growth will also be linked to outcomes which will reduce the overall very high total phosphorus values within all areas of the Toronto AOC.

Target 3: Reduced Bacterial Productivity. A 20% reduction in bacterial productivity to an average of 1.2 mg C m^{-3} h^{-1} (95% Confidence Interval of 1.0 – 1.4) with individual values not to exceed 3 mg C m^{-3} h^{-1}. The objective is to reduce the overall mean by eliminating the highest values but still recognize that a large urban area will likely have higher bacterial productivity than other environments.

Target 4: Reduced ratio of Bacterial Growth to Primary Productivity. A BG:PP ratio of 0.1 (95% Confidence Interval of 0.08 – 0.13) is preferred. A proportionate decline in the bacterial productivity to primary productivity ratio (BP:PP) would be expected with reduced bacterial productivity assuming primary productivity remains at similar levels even with reductions in phosphorus concentrations.

Determination of Targets 3 and 4: Both the bacterial growth (productivity) target and the bacterial growth to primary productivity (BG:PP) target were calculated from the Toronto Harbour 2019 – 2022 data set. There were two major arguments underlying these targets: 1) that bacterial productivity in TH is higher than anywhere else we have observed in the Great Lakes, and 2) that primary productivity should remain at approximately the same level as currently observed. A hard cap on bacterial productivity was set at 3 mg C m^{-3} h^{-1} because it approximates both the third quartile of the TH data set and the highest values of bacterial productivity observed in other locations around the Great Lakes. Means, standard errors and confidence intervals for bacterial productivity and BG:PP were then calculated with those upper quartile values (BG > 3 mg C m^{-3} h^{-1}) removed.

Remedial Actions: Continue to improve IH water quality through watershed improvements as outlined in the Wet Weather Flow Master Plan in 2003 (City of Toronto 2003, 2023) that aim to reduce nutrient and watershed pollutant input. Reducing organic matter inputs from the Don River and CSO overflow events would likely reduce bacterial contamination and help strengthen the food web by shifting to photosynthetic production and towards zooplankton and planktivorous fish. Work is also needed to identify sources of bacterial contamination (e.g., Microbial Source Tracking) in the inner harbour and identify which bacterial groups are being stimulated to grow in the Inner Harbour and the other habitats.

Further recommended actions: Investigate progress of WWFMP on a) water quality (i.e., TP, turbidity, Chl *a* and DOC) by monitoring nutrient levels in the harbour, especially in the area of the Don River mouth and CSO outflows, b) bacterial growth and primary productivity rates in the Inner Harbour and adjacent areas, and c) phytoplankton species composition and abundance as understanding the dynamics of the lower food web will be critical to establish the quality and availability of food sources for fish. Further research is also needed to examine the amount and proportion phosphorus being taken up by bacteria and algae and the fate of this biomass in the food web. It is recommended that 16S Amplicon Sequencing be used to identify bacterial community composition and use resultant assays to determine groups with elevated growth to guide source tracking actions.

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Tables

Table 1: Sampling station description in the Toronto AOC, 2019 to 2022. Parameters measured included zooplankton (Z), rotifers (R), water chemistry and primary production (C) and phytoplankton biomass (P).

Ecotype Designation	Station Code	2016 Station	Description	No. Reps	Depth (m)	Parameters Measured		
						2019	2020	2021-22
Inner Harbour	T11	IH1	Western Gap	1	7.6	Z	Z	Z
Inner Harbour	T12	IH12	Don R. Mouth	1	6.8	Z, R, C, P	Z, R, C	Z, R, C
Inner Harbour	T13	IH13	Eastern Gap	1	12.1	Z	Z	Z
Inner Harbour	T4	IH4	Mid Inner Harbour	1	11.5	Z, R, C, P	Z, R, C	Z, R, C, P
Inner Harbour	T5	-	island centre	1	10.1	Z	Z	Z
Inner Harbour	T6	-	island east	1	6.3	Z	Z	Z
Inner Harbour	T1	TH1	Outer Harbour Centre	1	11.5	Z, R, C, P	Z, R	Z, R
Embayment	eA	-	TTP Embayment A	1	5.2	Z	Z	Z
Embayment	eB	-	TTP Embayment B	1	3.0	Z	Z	Z
Embayment	eC	-	TTP Embayment C	3	5.5	Z, R, C	Z	Z
Embayment	e3	-	TTP Cell 3	3	9.3	Z, R, C	Z, R, C[1]	Z, R, C
Embayment	e6	-	Outer Harbour north	1	9.2	Z	Z	Z
Toronto Island	i1	-	island west	1	4.8	Z, R, C	Z, R, C	Z, R, C
Toronto Island	i2	-	island west	1	4.4	Z	Z	Z
Toronto Island	i3	-	island central	1	4.3	Z	Z	Z
Toronto Island	i4	-	island east	1	3.5	Z, R, C	Z	Z, R, C
Cell	c1	-	TTP Cell 1	3	1.3	Z	Z	Z
Cell	c2	-	TTP Cell 2	3	1.2	Z	Z, R, C	Z, R, C
Cell	cD	-	TTP Embayment D	1	2.5	Z	Z, C[2]	Z, R, C

[1] in August 2020 eC was sampled instead of e3 for rotifers, chemistry and phytoplankton

[2] in August 2020 cD was not sampled for chemistry and primary production.

Table 2: Summer (July to early September) means, standard errors and median values in Lake Ontario AOCs, Lake Ontario open water sites and Toronto AOC ecotypes for zooplankton parameters proposed as potential recovery targets in the IH.

Water Body	Area	mean	SE	median
Zooplankton Biomass (mg m^{-3})				
AOCs	Hamilton	283.4	34.1	295.7
	Quinte	96.7	16.7	83.3
Lake Ontario	East	27.1	7.3	19.3
	West	43.3	7.1	30.8
	Coastal	47.1	16.2	41.7
Toronto	Cells	199.3	40.0	106.5
	Islands	377.5	63.4	326.8
	Embayment	267.2	38.3	212.0
	Inner Harbour	52.6	5.6	41.6
Daphnia Biomass (mg m^{-3})				
	Cells	3.9	1.3	0.70
	Islands	10.8	4.7	2.44
	Embayment	81.2	21.9	32.92
	Inner Harbour	4.8	1.3	1.68
Large Zooplankton Density (%)				
	Cells	0.72	0.2	0.18
	Islands	0.54	0.3	0.26
	Embayment	3.25	0.7	2.11
	Inner Harbour	3.13	1.2	1.07
Medium Zooplankton Density (%)				
	Cells	23.9	2.5	21.70
	Islands	29.4	3.7	17.49
	Embayment	18.8	2.7	15.84
	Inner Harbour	14.7	2.1	8.98

Table 3: Phytoplankton taxa contributing over 5% to total biomass from at least one station of Inner Harbour stations T1, T4, T12 in 2019.

Date	IH 12	IH 4	TH 1
May 24	Navicula cryptocepheloides Diatoma tenue v.elongatum Plagioselmis nanoplanktica Chrysochromulina parva	Diatoma tenue v.elongatum Plagioselmis nanoplanktica Dinobryon bavaricum Chrysochromulina parva	Diatoma tenue Stephanodiscus minutulus S. hantzschii Glenodinium sp Chrysochromulina parva
June 27	Ochramonads Carteria sp Navicula cryptocephela Chlamydomonas sp	Plagioselmis nanoplanktica Ochramonads Cryptomonas marssonii Ochromonas sp Diatoma tenue v.elongatum Cryptomonas rostratiformis	Ochramonads Chrysochromulina parva Plagioselmis nanoplanktica Botryococcus braunii
July 17	Entomoneis palucida Navicula cryptocepheloides	Dinobryon divergens Ochromonads Fragilaria crotonensis Cryptomonas reflexa	Dinobryon divergens Ochramonads Synechococcus sp
Sept 5	Dinoflagellate cysts Carteria sp Fragilaria crotonensis	Ochromonads Plagioselmis nanoplanktica Cryptomonas reflexa	Fragilaria crotonensis
Oct 30	Synura sp Cryptomonas marssonii Plagioselmis nanoplanktica Botryococcus braunii Dinobryon sociale	Plagioselmis nanoplanktica Cryptomonas marssonii C. reflexa	N/A

Table 4: Spring, summer and fall averages and standard errors of select nutrient and physical parameters by ecotype (Cells, Embayment, IH and Islands) for the 2019 to 2022 period.

		Spring				Summer				Fall			
		cells	embay	inner	island	cells	embay	inner	island	cells	embay	inner	island
Station depth (m)	**Mean ± SE**	1.86 ±0.37	9.27 ±0.87	10.39 ± 0.91	4.15 ±0.28	1.55 ±0.19	8.08 ±0.82	8.79 ±0.69	4.0 ±0.32	1.5 ±0.16	9.51 ±1.04	9.33 ±0.83	4.4 ±0.31
	range	1.5-2.4	6.5-11.2	6.6-12.9	2.6-5	1.1-2.2	5.3-10.5	5.6-12.6	2.5-5.2	1-2.05	4.5-11.4	6.5-12.9	3.5-5.7
	N	4	6	10	8	6	7	12	8	8	6	11	8
Surface	**Mean**	19.43 ±2.4	14.21 ±1.3	13.45 ±1.01	16.2 ±0.91	24.83 ±1.74	20.92 ±0.93	21.07 ±0.65	23.27 ±0.83	12.70 ±1.46	12.77 ±1.64	13.54 ±0.98	13.72 ±1.35
Temperature (° C)	**range**	13.6-23.8	10.8-18.1	9.2-17.9	13.6-19.5	17-28.7	18.75-25.8	19-25.9	20.6-27.3	6.4-17.6	9.1-17.3	10.8-17.5	10-18.7
	N	4	6	10	8	6	7	12	9	8	5	10	8
Total Phosphorus	**Mean**	46.65 ± 5.97	33.27 ±3.98	32.07 ±6.95	46.45 ±7.17	53.84 ± 11.18	36.63 ±3.06	41.58 ±11.79	57.7 ±4.68	57.29 ±6.09	39.27 ±4.6	29.61 ±5.39	45.08 ±7.55
(µg L^{-1})	**range**	29-55.3	21.2-45.6	7.7-68.2	21.3-89.6	32.2-96.8	22.2-46	16.4-164	42.5-84.9	32.9-92.6	31.4-61.1	14.7-69.3	23.5-79.5
	N	4	6	10	8	5	7	12	9	8	6	11	8
Total Nitrogen	**Mean**	0.44 ±0.05	0.62 ±0.03	0.81 ±0.08	0.57 ±0.04	0.63 ±0.12	0.55 ±0.04	0.67 ±0.08	0.48 ±0.03	0.63 ±0.11	0.55 ±0.04	0.68 ±0.06	0.55 ±0.03
(mg L^{-1})	**range**	0.39-0.58	0.55-0.72	0.55-1.23	0.44-0.79	0.43-0.9	0.37-0.7	0.43-1.21	0.38-0.59	0.42-0.82	0.46-0.64	0.54-0.91	0.42-0.64
	N	4	6	10	8	4	6	10	8	4	4	7	6
Total Kjeldahl													
Nitrogen	**Mean**	0.44 ±0.07	0.34 ±0.03	0.33 ±0.04	0.38 ±0.06	0.57 ±0.11	0.34 ±0.03	0.33 ±0.04	0.34 ±0.02	0.61 ±0.13	0.36 ±0.03	0.27 ±0.02	0.35 ±0.03
(mg L^{-1})	**range**	0.29-0.61	0.25-0.45	0.2-0.55	0.26-0.76	0.38-0.85	0.27-0.47	0.21-0.61	0.3-0.44	0.39-0.86	0.27-0.4	0.22-0.36	0.25-0.44
	N	4	6	10	8	4	6	10	8	4	4	7	6
Nitrate/Nitrite (mg	**Mean**	0.038 ±0.025	0.287 ±0.009	0.497 ±0.044	0.198 ±0.032	0.062 ±0.013	0.207 ±0.029	0.346 ±0.041	0.141 ±0.023	0.041 ±0.026	0.198 ±0.039	0.409 ±0.041	0.177 ±0.025
L^{-1})	**range**	0.006-0.112	0.269-0.326	0.338-0.726	0.091-0.322	0.04-0.098	0.07-0.27	0.22-0.601	0.07-0.246	0.006-0.118	0.09-0.27	0.318-0.588	0.1-0.278
	N	4	6	10	8	4	6	10	8	4	4	7	6
Dissolved Inorganic	**Mean**	28.3 ±2.4	23.1 ±0.5	24.7 ±1.0	23.1 ±0.4	27.9 ±2.7	22.4 ±0.2	23.3 ±0.6	23.6 ±0.3	37.9 ±7.1	23.8 ±0.2	24.1 ±0.7	24.8 ±0.4
Carbon (mg L^{-1})	**range**	22.9-32.8	21.4-24.7	21-30	21.7-24.3	22.4-32.9	21.6-23.4	21-27.2	22.6-25.2	25.5-50.3	23.3-24.2	22.3-27.8	23.8-26.6
	N	4	6	10	8	4	6	10	8	4	4	7	6
Dissolved Organic	**Mean**	4.4 ± 0.4	2.6 ± 0.1	2.6 ± 0.1	3.0 ±0.2	6.6 ±1.6	2.8 ±0.1	2.8 ±0.2	3.1 ±0.1	6.0 ±1.7	2.6 ±0.2	2.4 ±0.1	2.7 ±0.2
Carbon (mg L^{-1})	**range**	3.3-5.1	2.2-3	2-3.5	2.4-4	3.7-9.4	2.5-2.9	2.3-4.9	2.7-3.5	2.8-9.5	2.3-3.1	2.1-3.1	2.3-3.5
	N	4	6	10	8	4	6	10	8	4	4	7	6
Soluble Reactive	**Mean**	2.93 ±2.04	3.78 ±1.46	3.15 ±1.82	2.13 ±1.36	11.75 ±1.7	6.75 ±1.64	6.82 ±2.66	11.68 ±3.11	5.28 ±1.69	4.58 ±1.49	9.03 ±2.46	11 ±4.48
Phosphorus (µg L^{-1})	**range**	0.4-9	0.2-8.8	0.1-17.3	0.1-11.4	9-16	1.5-11	0.3-26	1-26	1.4-9	1.8-8	1.3-21	1.3-31.6
	N	4	6	10	8	4	6	10	8	4	4	7	6
Silica (mg L^{-1})	**Mean**	0.47 ±0.22	0.55 ±0.19	1.27 ±0.27	0.74 ±0.12		0.6 ±0.07	1.12 ±0.39	0.95 ±0.07	3.81 ±3.19	1.09 ±0.02	1.35 ±0.28	1.14 ±0.27
	range	0.15-1.08	0.08-1.06	0.49-3.04	0.06-1.04		0.42-0.76	0.38-2.96	0.77-1.08	0.62-7	1.06-1.11	0.9-2.45	0.44-1.71
	N	4	6	10	8		4	6	4	2	3	5	4
Sodium (mg m^{-3})	**Mean**	33.68 ±2.27	26.6 ±3.31	41.46 ±6.71	31.78 ±3.63	27.8 ±1.41	17.17 ±0.44	24.27 ±3.01	18.83 ±0.39	24.65 ±2.99	16.43 ±0.37	23.16 ±3.3	17.15 ±0.23
	range	28.9-38.4	18.5-36.8	16.2-78.6	20.6-44.1	25.1-30.8	15.7-18.9	15.6-42.9	17-20.1	18.1-31	15.9-17.5	16.1-37.1	16.4-17.9
	N	4	6	10	8	4	6	10	8	4	4	7	6
Chl a µg L^{-1}	**Mean**	5.89 ±1.27	6.85 ±1.56	4.54 ±0.74	16.34 ±3.12	12.06 ±6.12	9.38 ±1.2	8.65 ±1.7	16.98 ±3.3	10.54 ±3.27	11.36 ±1.45	2.79 ±0.47	9.49 ±2.96
	range	2.2-7.7	2.8-13.2	1.4-9.9	5.5-29.2	2.0-35.9	5.2-15.5	3.3-23.1	7.0-38.8	2.1-29.4	7.4-17.1	0.8-6.2	3.3-25.8
	N	4	6	10	8	5	7	12	9	8	6	11	8
Particulate Organic	**Mean**	0.94 ±0.29	0.81 ±0.18	0.71 ±0.12	1.65 ±0.33		1.07 ±0.19	2.18 ± 1.54	1.16 ±0.09	0.99 ±0.25	0.84 ±0.14	0.38 ± 0.13	1.19 ±0.39
Carbon (mg L^{-1})	**range**	0.17-1.5	0.001-1.23	0.29-1.67	0.20-2.99		0.68-1.57	0.51-9.9	0.88-1.29	0.73-1.24	0.58-1.05	0.13-0.86	0.26-2.18
	N	4	6	10	8		4	6	4	2	3	5	4
Particulate Organic	**Mean**	0.18 ±0.06	0.13 ±0.03	0.12 ±0.02	0.29 ±0.06		0.17 ±0.02	0.20 ±0.1	0.20 ±0.02	0.22 ±0.08	0.15 ±0.02	0.05 ±0.01	0.19 ±0.06
Nitrogen (mg L^{-1})	**range**	0.013-0.256	0.003-0.206	0.041-0.26	0.041-0.62		0.11-0.22	0.07-0.72	0.15-0.24	0.14-0.29	0.12-0.18	0.022-0.09	0.058-0.369
	N	4	6	10	8		4	6	4	2	3	5	4

Figures

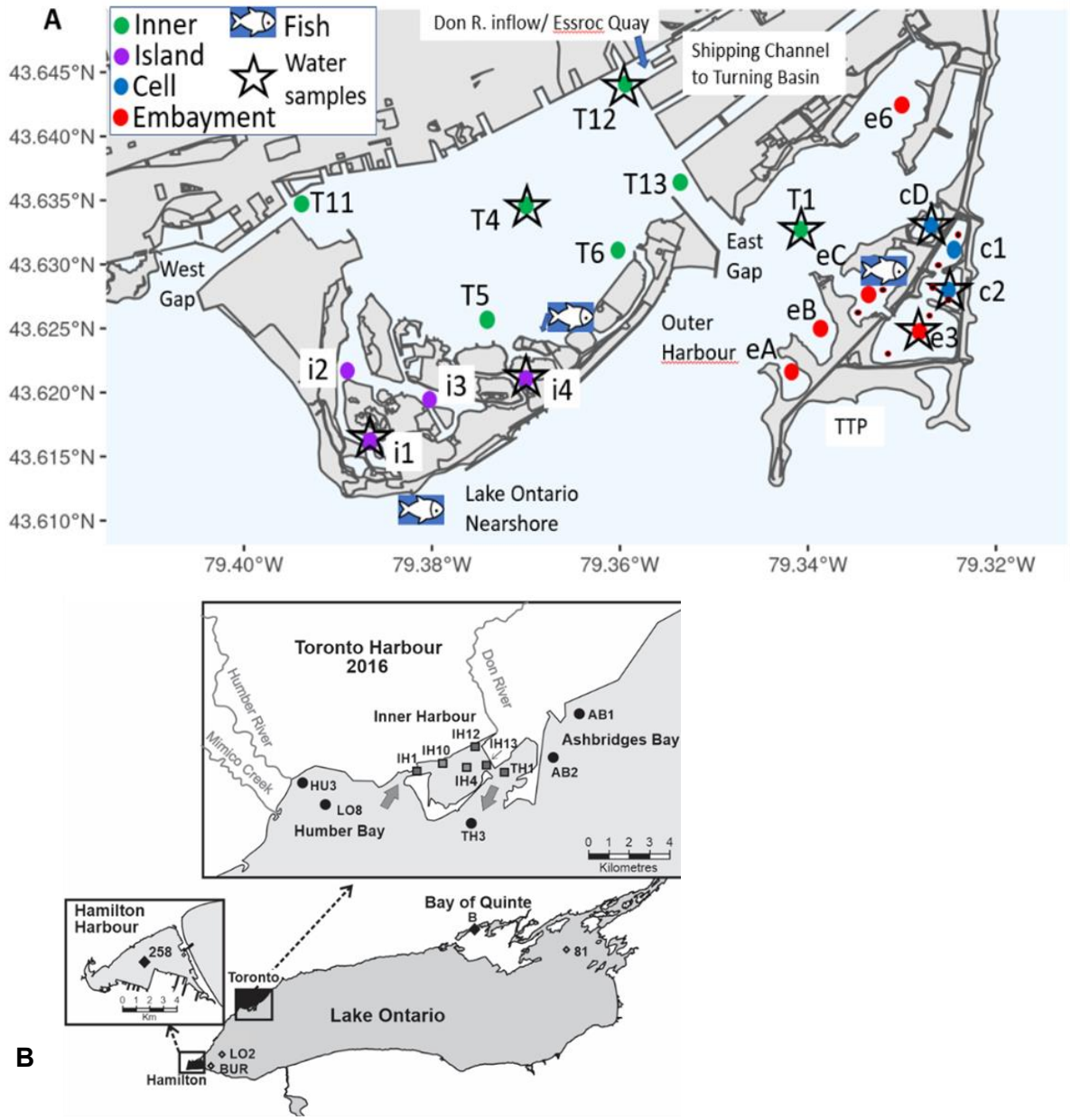

Figure 1: A) Sampling locations in Toronto Harbour 2019-2022, with Tommy Thompson Park (TTP) on right. All parameters, including nutrients and primary productivity, were measured at stations denoted with stars. Small dots within TTP indicate replicate stations two and three for zooplankton and sonde profiles. Alewife were collected for diet analyses at the three fish sites. B) Comparison study locations, including embayment AOCs (Bay of Quinte station Belleville and Hamilton Harbour 258 - filled diamonds) and main basin stations (open diamonds). The top inset map shows the Toronto Harbour 2016 sampling stations, including coastal stations (circles) and IH stations (squares). The wide arrows show the predominant direction of water flow through the IH.

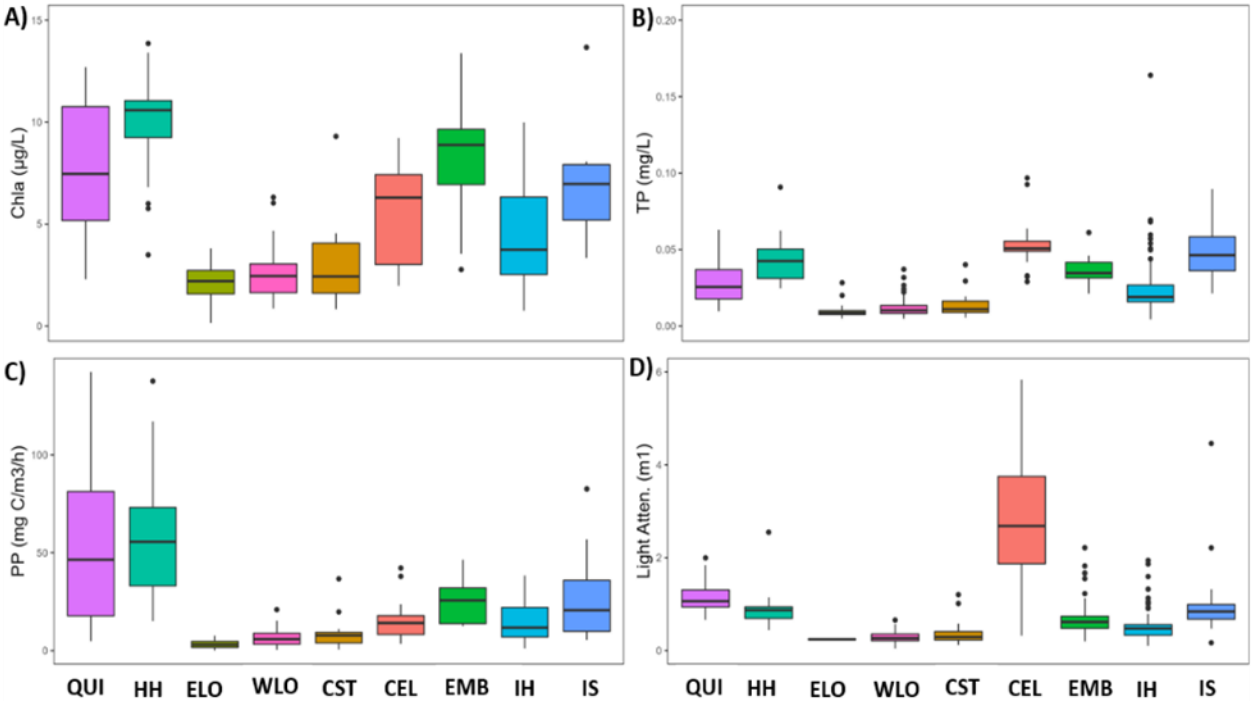

Figure 2: Boxplot comparison May to October averages of A) chlorophyll a, B) total phosphorus (TP), C) light attenuation, and D) primary productivity (PP). Shown are areas in the Lake Ontario basin sampled by DFO from 2014 to 2019, including upper Bay of Quinte at Belleville (QUI), Hamilton Harbour (HH), Kingston Basin of eastern Lake Ontario (ELO), western Lake Ontario (WLO) and 2016 coastal stations adjacent to Toronto (CST), and the four areas of the 2019 to 2022 Toronto study (Cells (CEL), Embayments (EMB), Toronto Islands (IS), and Inner Harbour (IH, including results from the 2016 survey).

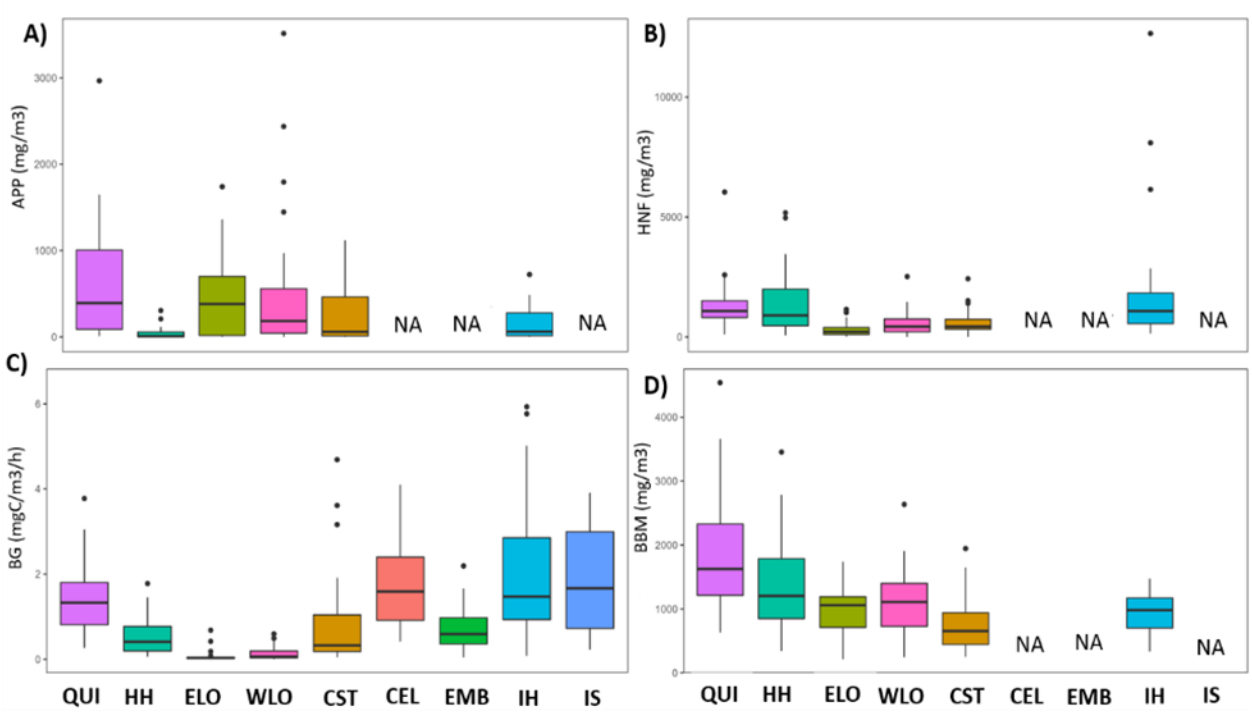

Figure 3: Boxplot comparison May to October averages of A) Autotrophic Picoplankton biomass (APP, mg m^{-3}), B) Heterotrophic Nanoflagellate biomass (HNF, mg m^{-3}), C) Bacterial productivity (BG, mg C m^{-3} hr^{-1}), and D) Bacterial Biomass (mg m-3). Shown are areas in the Lake Ontario basin sampled by DFO from 2014 to 2019, including upper Bay of Quinte at Belleville (QUI), Hamilton Harbour (HH), Kingston Basin of eastern Lake Ontario (ELO), western Lake Ontario (WLO) and 2016 coastal stations adjacent to Toronto (CST). Due to sample degradation, bacterial productivity were the only data available for the Cells (CEL), Embayments (EMB) and Island (IS) ecotypes for the 2019 to 2022 Toronto study. For the remaining parameters, only IH data from 2016 is available.

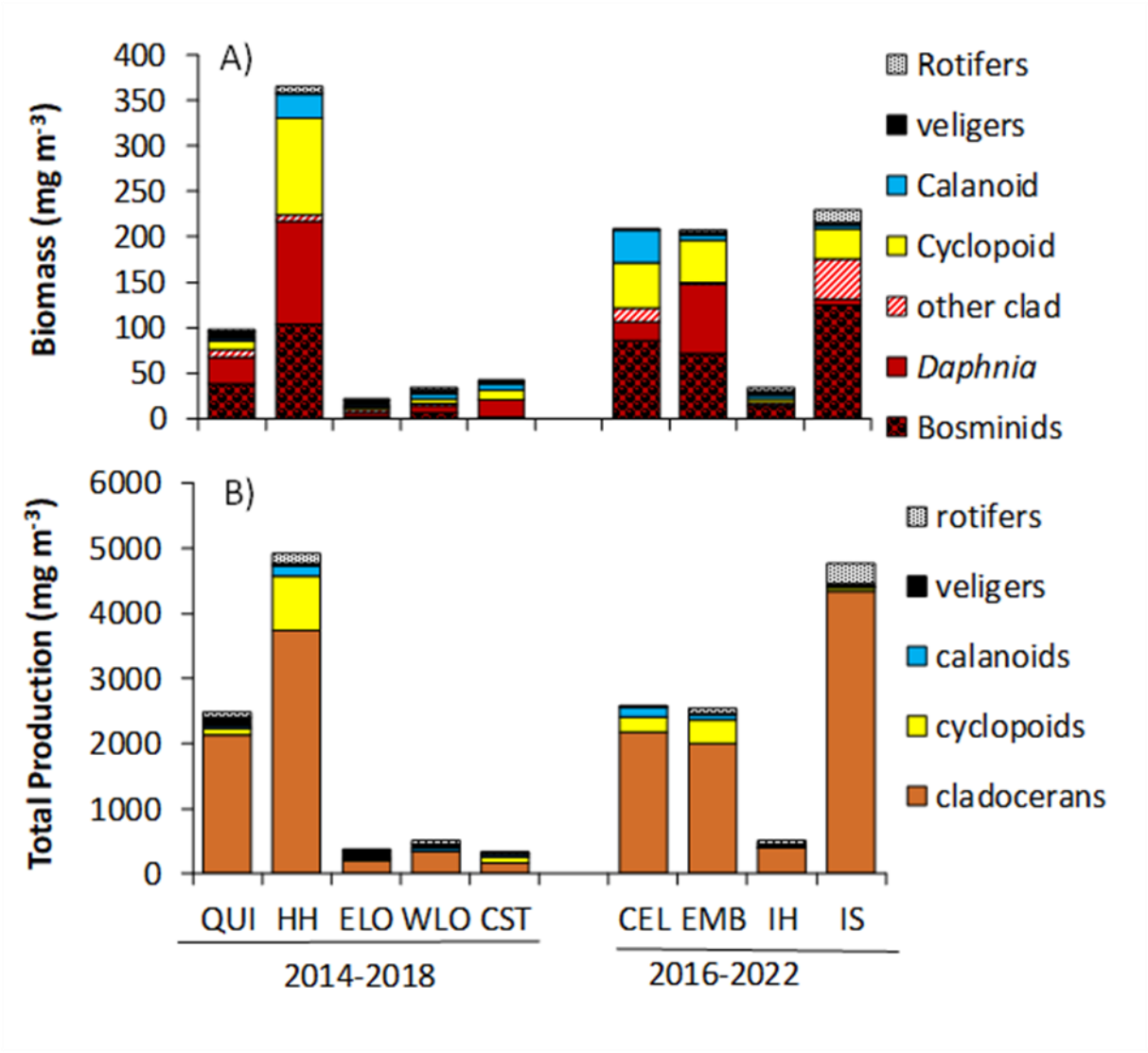

Figure 4: A) May to October mean biomass and B) May to October total production of dominant zooplankton groups in the Lake Ontario Basin. Shown are areas in the Lake Ontario basin sampled by DFO from 2014 to 2019, including upper Bay of Quinte at Belleville (QUI), Hamilton Harbour (HH), Kingston Basin of eastern Lake Ontario (ELO), western Lake Ontario (WLO) and 2016 coastal stations adjacent to Toronto (CST). Included in the IH are results from the 2016 survey.

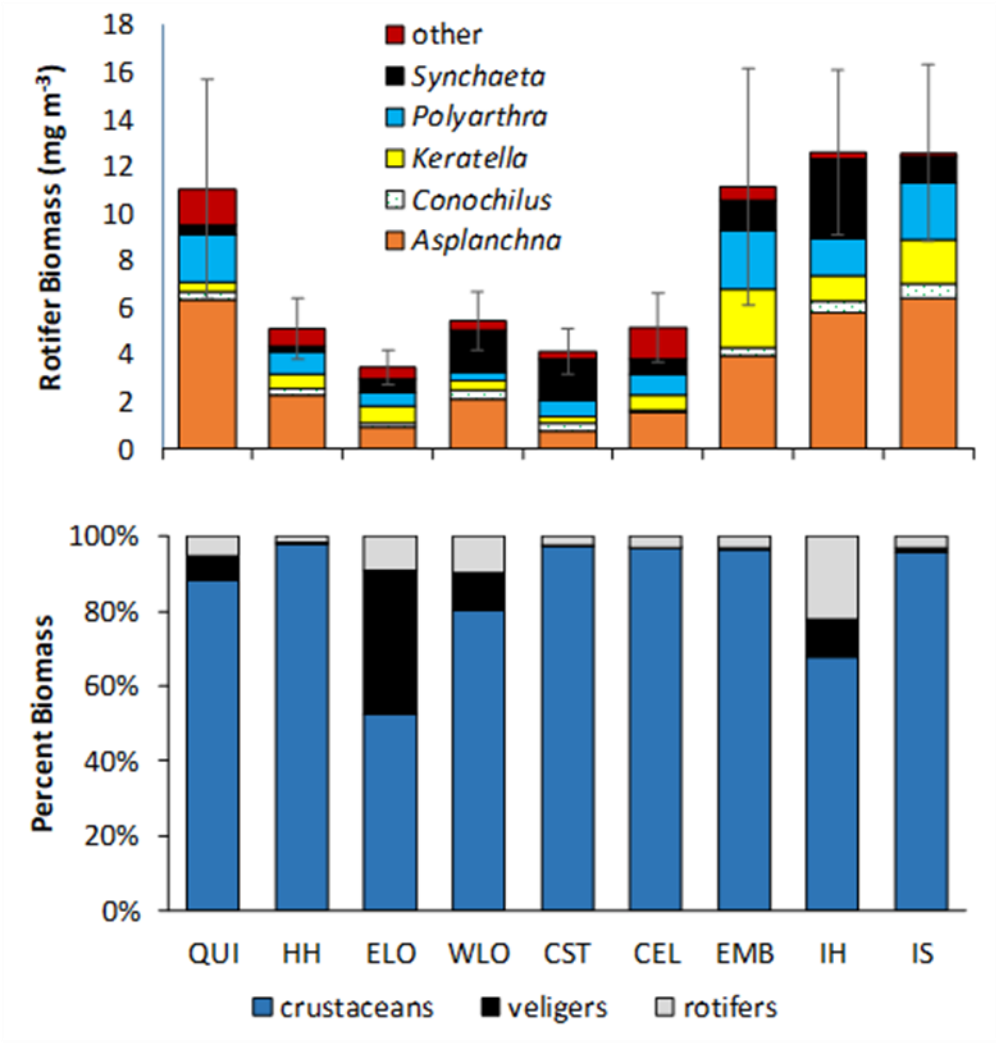

Figure 5: Mean summer biomass (top) of dominant rotifer groups, and percent composition by biomass of the total zooplankton community (bottom) at stations in the Lake Ontario Basin. These include the upper Bay of Quinte at Belleville (QUI), Hamilton Harbour (HH), Kingston Basin of eastern Lake Ontario (ELO), western Lake Ontario (WLO) and 2016 coastal stations adjacent to Toronto (CST), and the four areas of the 2019 to 2022 Toronto study (Cells (CEL), Embayments (EMB), Inner Harbour (IH) and Toronto Islands (IS)).

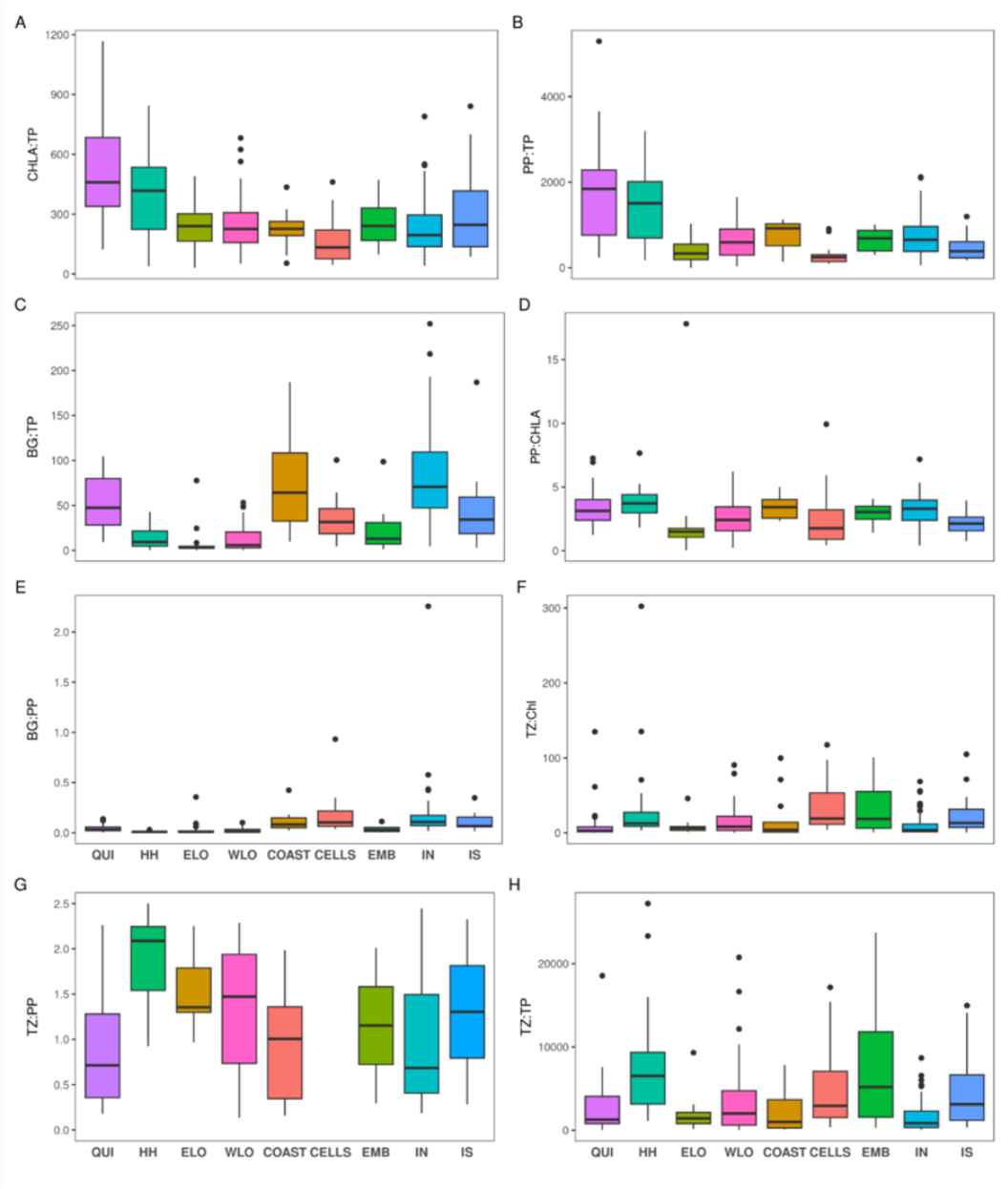

Figure 6: Boxplot comparison of May to October ratios of various parameters, including A) Chl a: TP, B) primary productivity (PP): TP, C) Bacterial Growth: TP, D) PP: Chl a, E) Bacterial Growth: PP, F) zooplankton: Chl a, G) zooplankton: PP, and H) zooplankton: TP. All zooplankton values represent total zooplankton biomass (TZ), and all Chl a values are from laboratory extractions. Shown are areas in the Lake Ontario basin sampled by DFO from 2014 to 2019, including upper Bay of Quinte at Belleville (QUI), Hamilton Harbour (HH), Kingston Basin of eastern Lake Ontario (ELO), western Lake Ontario (WLO) and 2016 coastal stations adjacent to Toronto (CST), and the four areas of the 2019 to 2022 Toronto study.

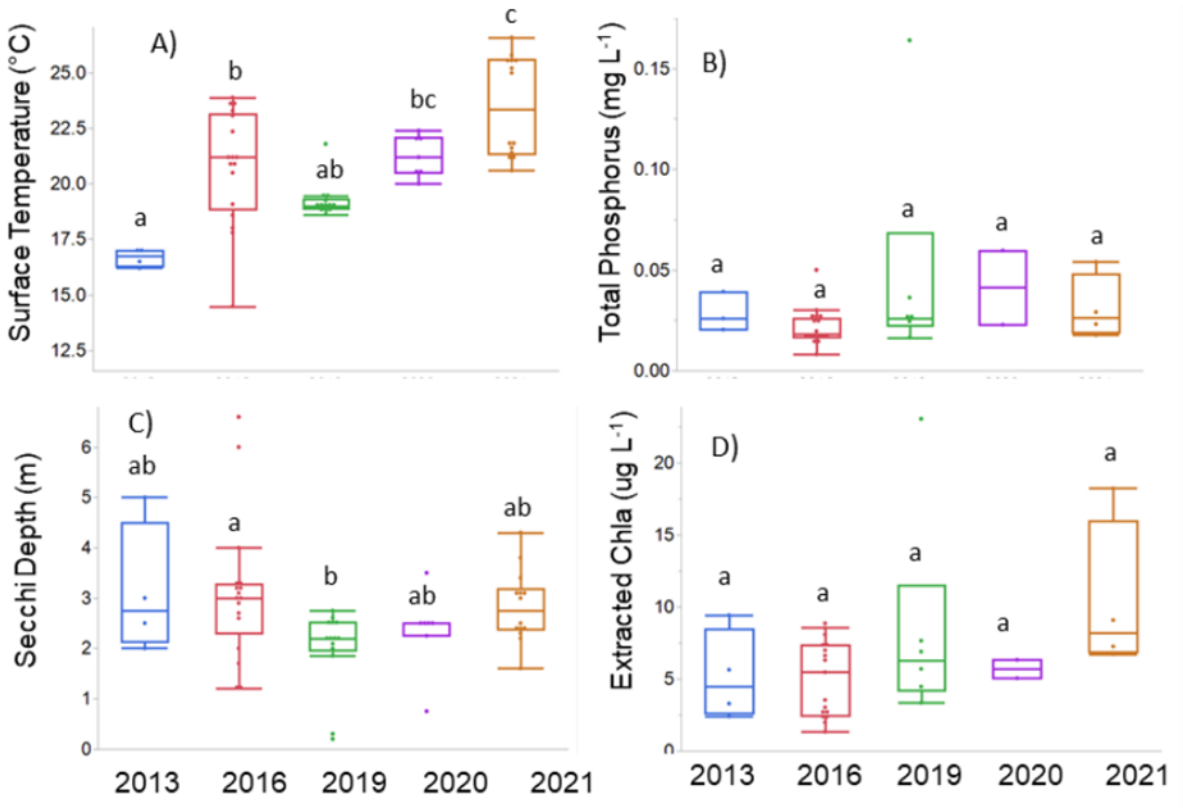

Figure 7: Inner Harbour mean summer A) surface temperature, B) total phosphorus, C) Secchi depth and D) extracted Chl a, averaged over the July to early Sept. period from 2013 to 2021. For a given parameter, bars with different letters are significantly different ($p<0.05$).

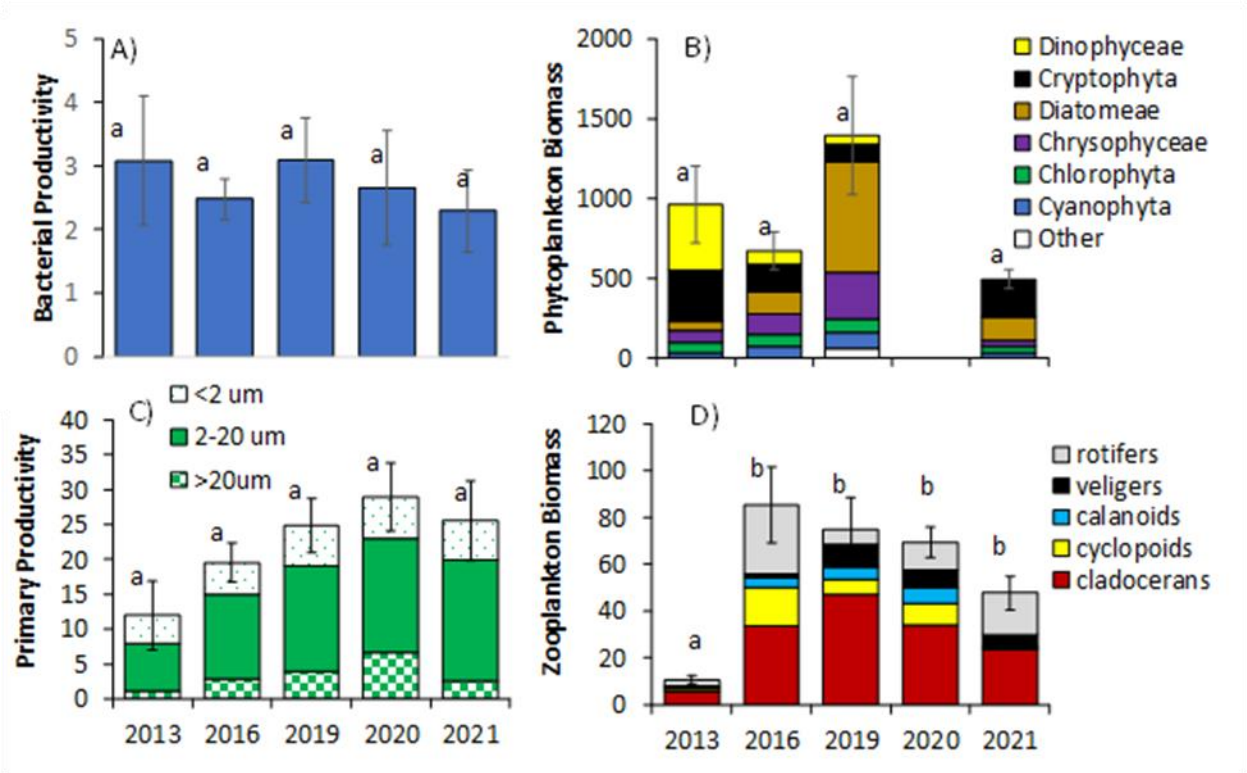

Figure 8: Inner Harbour mean summer A) bacterial growth rate (mg C m^{-3} hr^{-1}), B) phytoplankton wet biomass (mg m^{-3}), C) size-fractionated primary productivity (mg C m^{-3} hr^{-1}), and D) zooplankton dry biomass (mg m^{-3}) averaged over the July to early Sept. period from 2013 to 2021. Error bars and statistical results are for the total biomass and productivity values. For a given parameter, bars with different letters are significantly different ($p<0.05$).

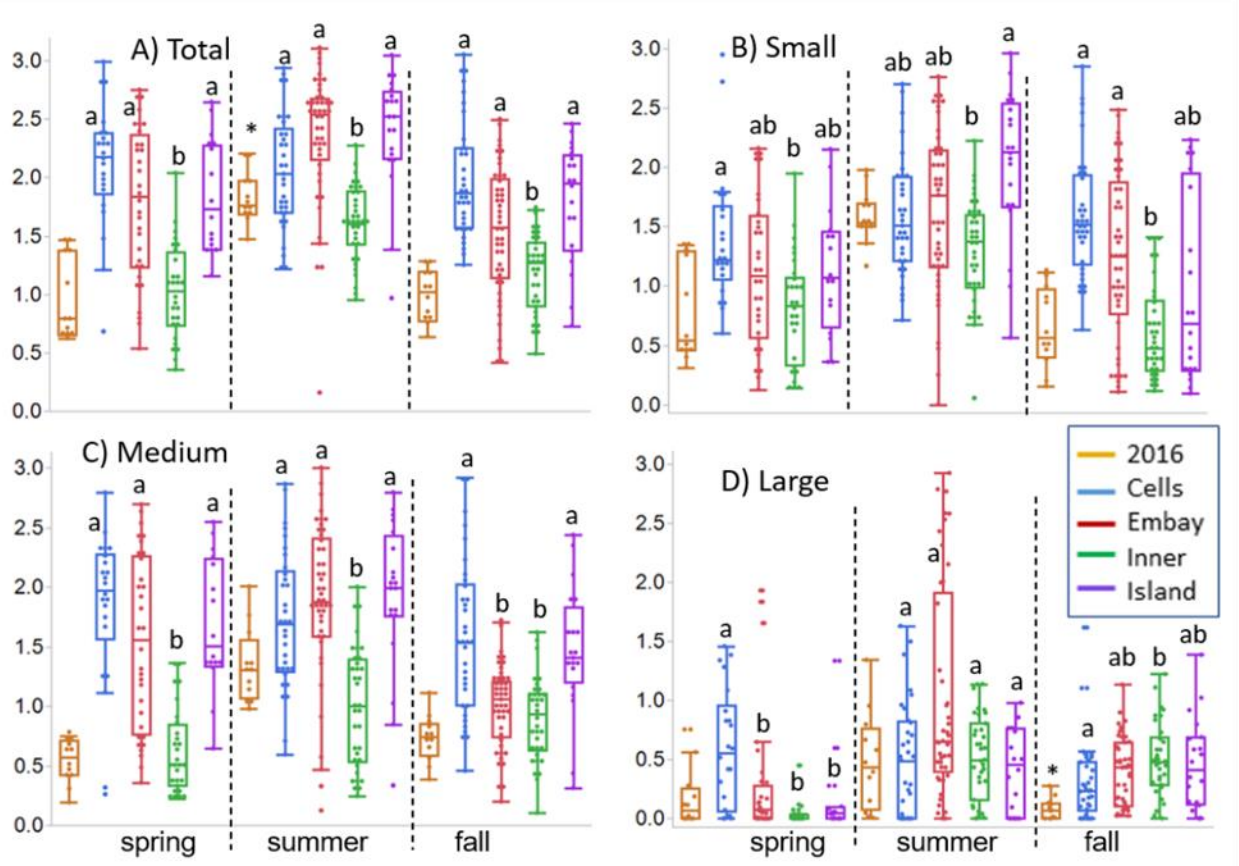

*Figure 9: Log-transformed total biomass of zooplankton divided into size bins, including A) total zooplankton, B) small (<0.35 mm mean length), C) medium (0.35-1.00 mm) and D) large (>1.0 mm). Shown are averages for the Inner Harbour in 2016, and the 2019 to 2022 study in the Cells, Embayments, Inner Harbour and Toronto Islands for the spring, summer and fall seasons. For a given season in the 2019 to 2022 study, bars with different letter codes are significantly different ($p<0.05$). When the two Inner Harbour values are significantly different, a * is placed over the 2016 bar.*

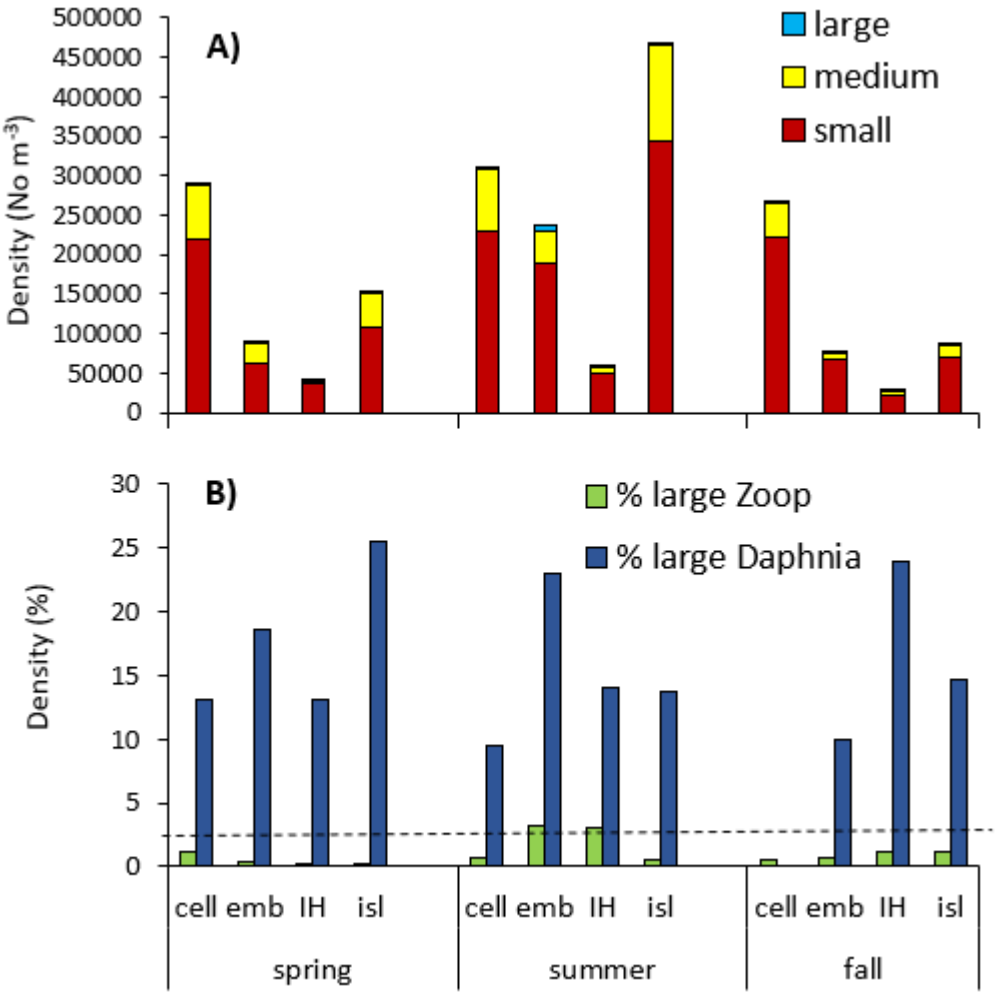

Figure 10: A) Mean density (# m^{-3}) of small (>0.35 mm), medium (0.35 to 1 mm) and large (>1 mm) zooplankton in Cells, Embayments (excluding e3), IH and Islands in spring, summer and fall (2019 to 2022). B) Percentage (by density) of large zooplankton and large Daphnia, with the dotted line representing 3% for large zooplankton.

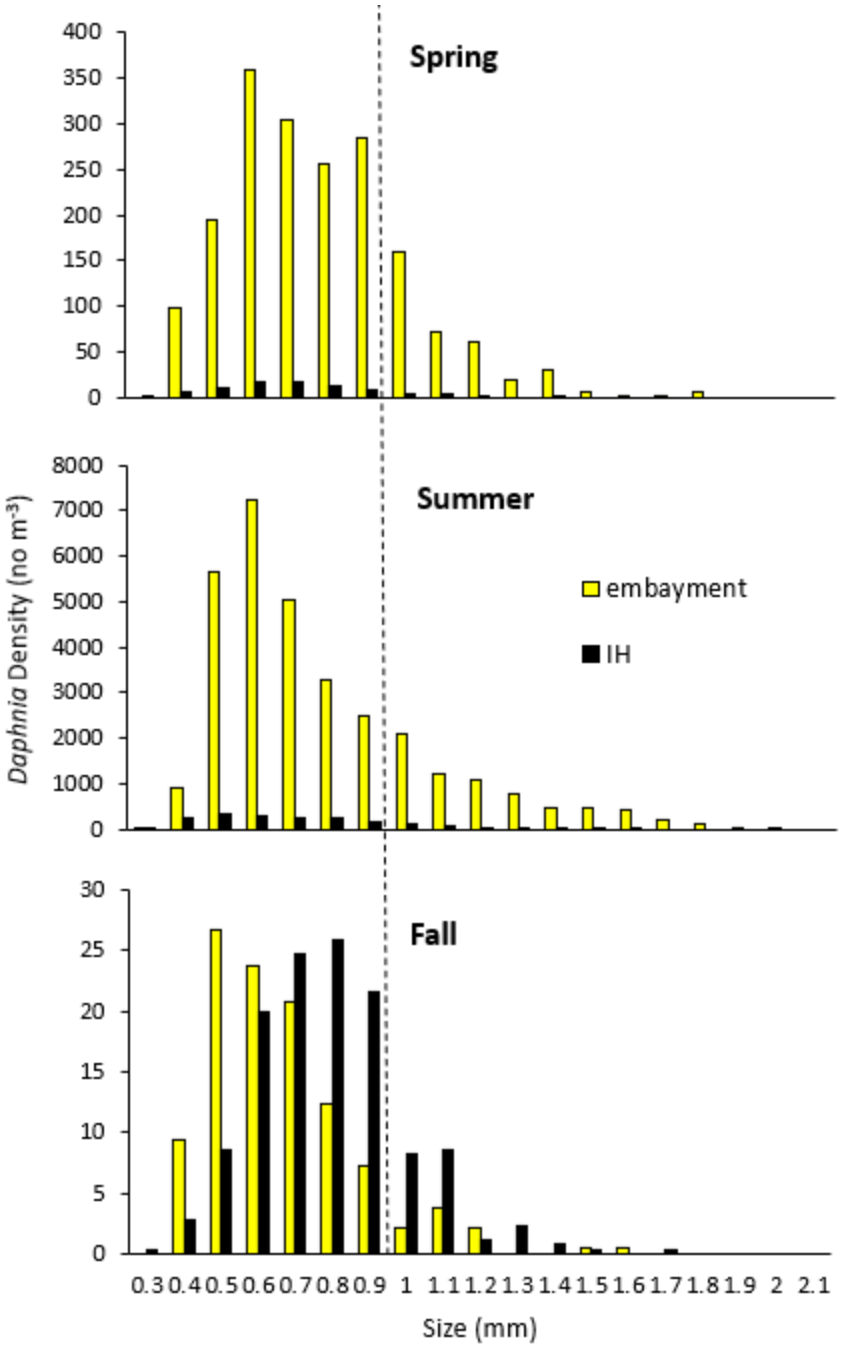

Figure 11: Mean density (# m^{-3}) of Daphnia in 0.1 mm size increments in Embayments (excluding e3) and IH in spring, summer and fall (2019 to 2022). The dotted line at 1 mm represents the division between medium and large Daphnia. The large individuals are preferred prey of forage fishes.

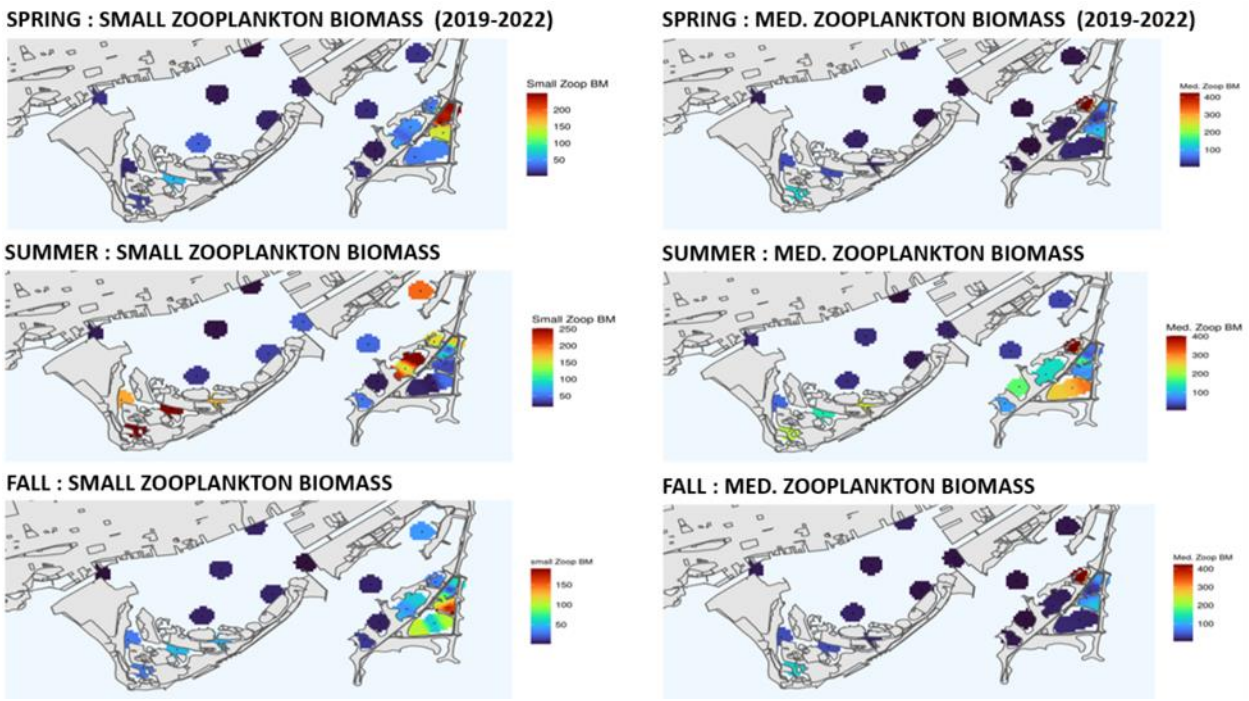

Figure 12: Spatial variability in zooplankton biomass (mg m^{-3}) at the Toronto area sampling stations during spring, summer and fall from 2019-2022. Shown are small zooplankton (<0.35 mm; left) and medium zooplankton (0.35-1.0 mm; right).

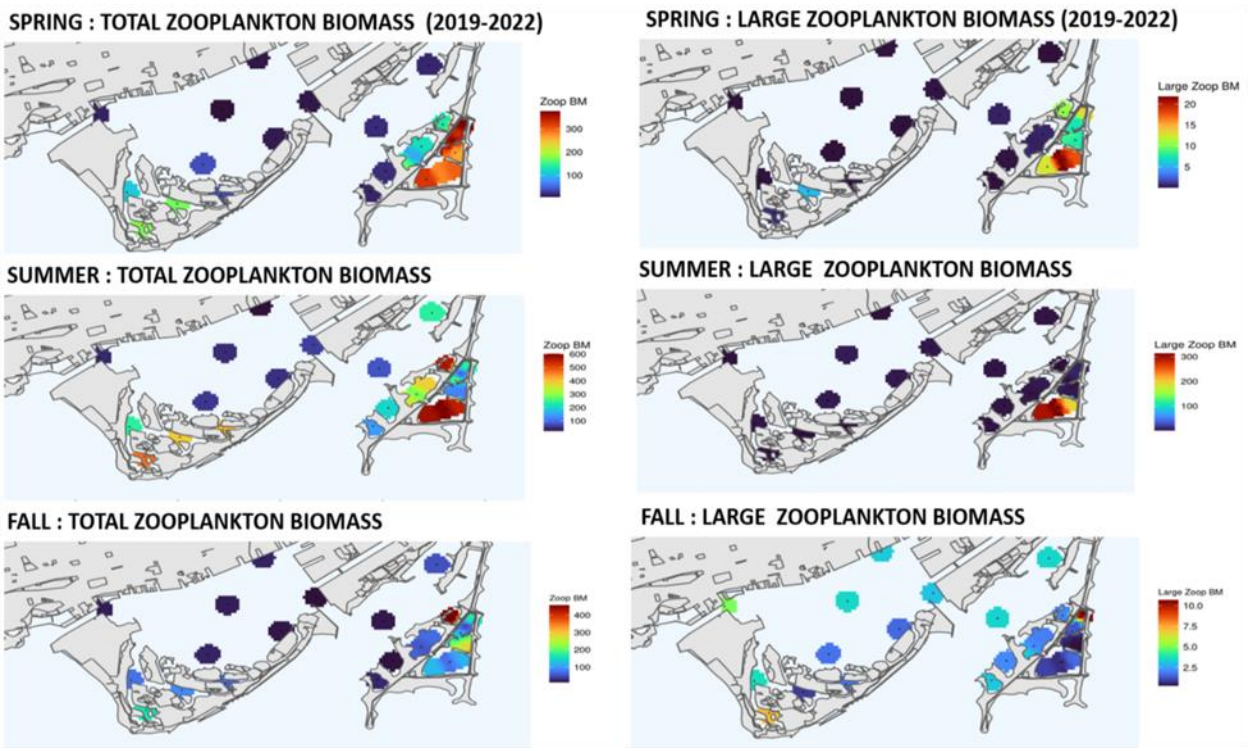

Figure 13: Spatial variability in zooplankton biomass (mg m^{-3}) at the Toronto area sampling stations during spring, summer and fall from 2019-2022. Shown are total zooplankton (left) and large zooplankton (>1.0 mm; right).

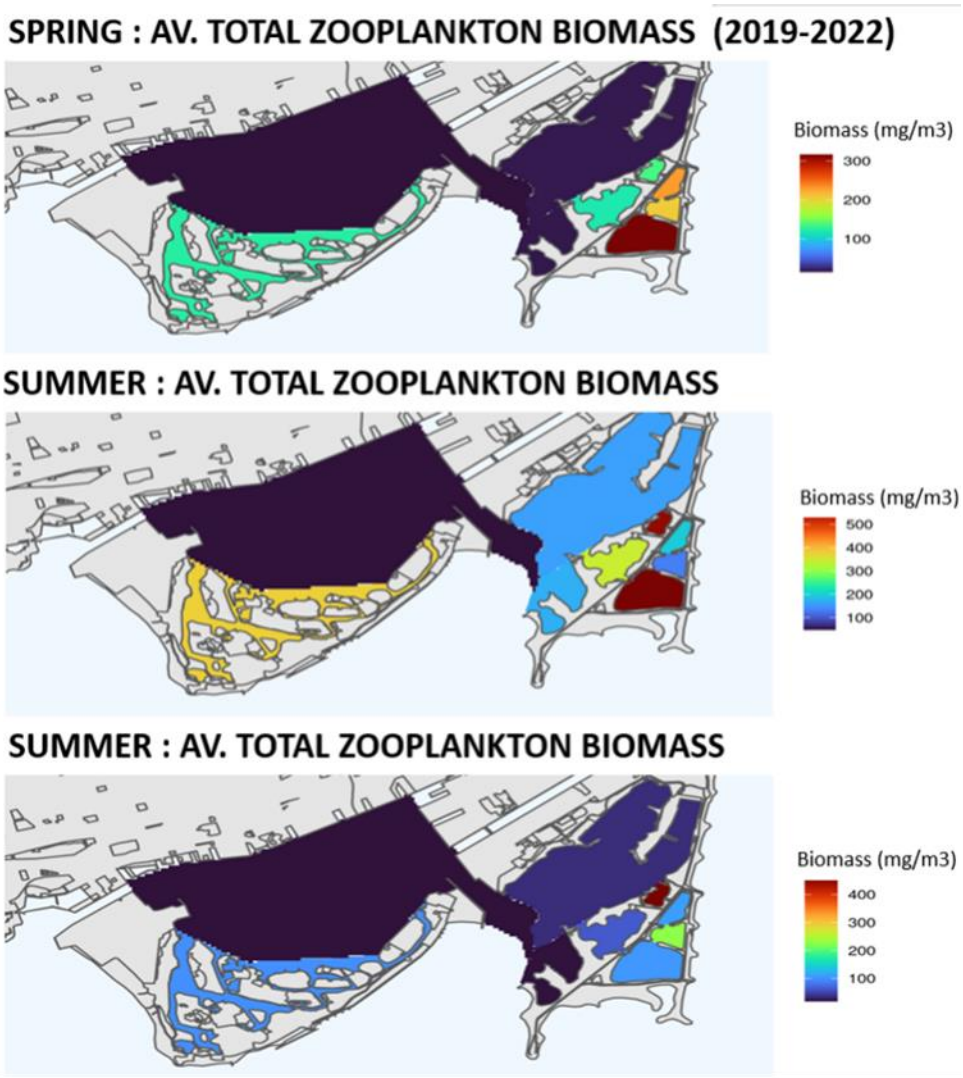

Figure 14: Biomass maps of total zooplankton (left) and large zooplankton (>1000 µm; right) of Toronto Region averaged across sampling zones in spring, summer and fall from 2019-2022.

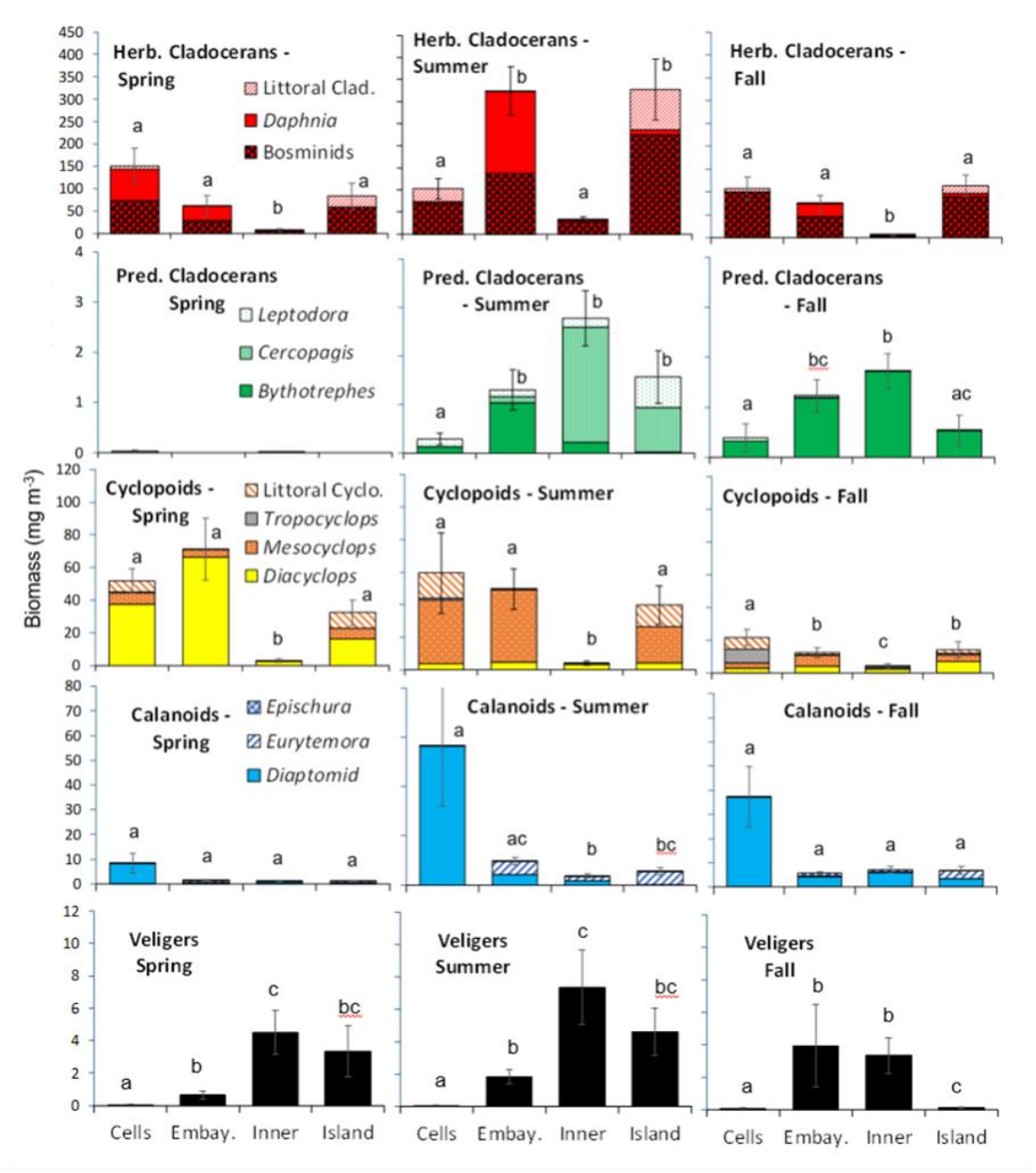

Figure 15: Mean biomass of dominant taxa of herbivorous cladocerans, predatory cladocerans, cyclopoids calanoids and veligers in spring, summer and fall, 2019 to 2022. Copepodids (juveniles) were allocated to genus for both cyclopoids and calanoids based on biomass of adults. Error bars represent standard errors for totals on each graph. For a given season, bars with different letter codes are significantly different ($p<0.05$). There were too few predatory cladocerans to test for differences in the spring.

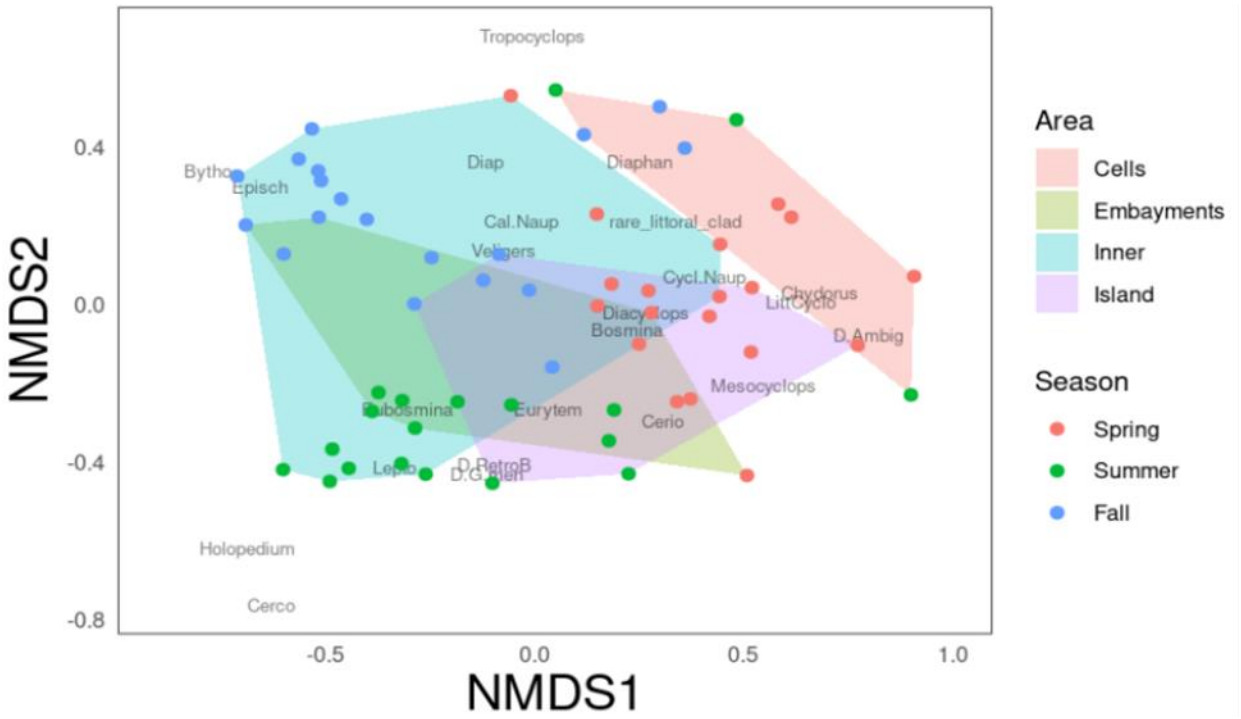

Figure 16: Nonmetric Multidimensional Scaling (NMDS) plot with shading by ecotype (Cells, Embayments, IH or Islands). Each dot represents a sampling station and are colored based on the season they were collected. The text labels represent the names of zooplankton taxa. Dots that are closer to a taxon name indicate that the corresponding station had a higher relative biomass of that taxon.

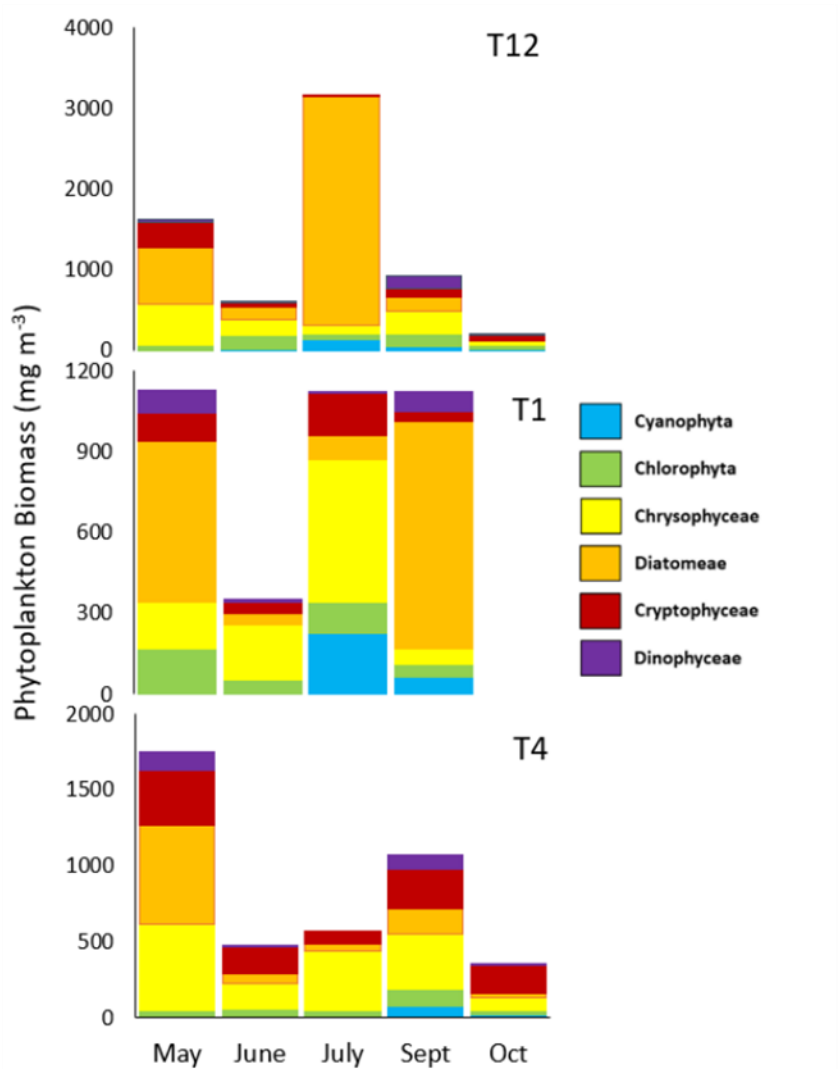

Figure 17: Phytoplankton biomass and composition by taxonomic group (mg m^{-3}) at IH stations, 2019.

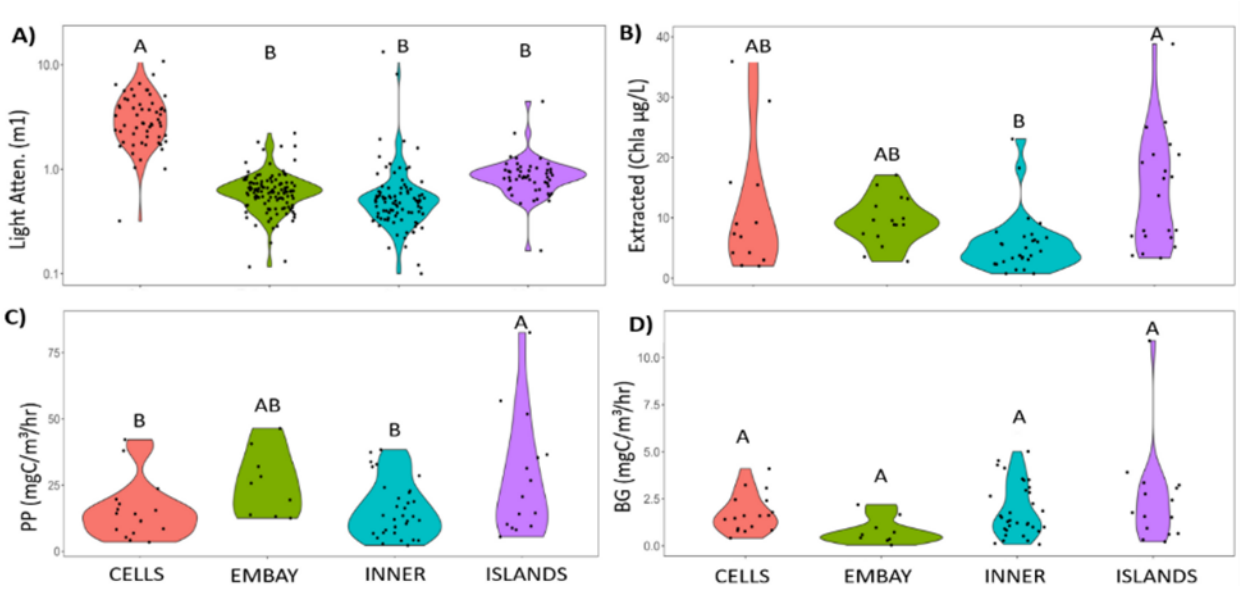

Figure 18: Plot by season of a) Light attenuation (Kd m^{-1}) and y-axis scale set to logarithmic, b) Total Primary Productivity (PP mg C m^{-3} hr^{-1}), c) Total chlorophyll a (extracted, µg L^{-1}) and d) Bacterial Productivity (BG mg C m^{-3} hr^{-1}). Ecotypes with the same letter code were not significantly different.

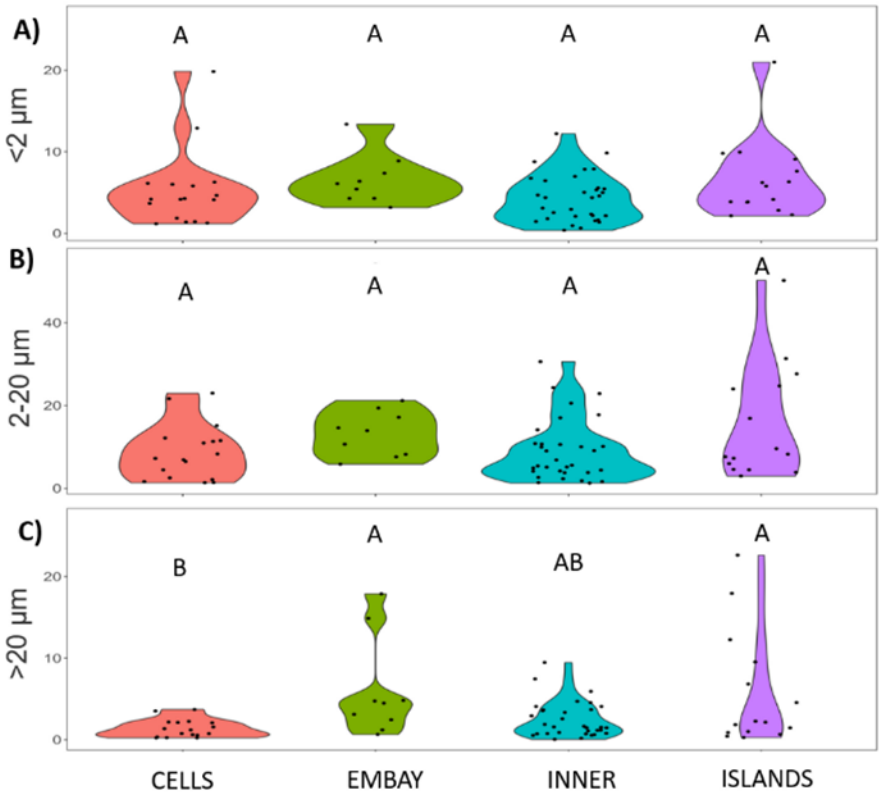

Figure 19: Size fractionated primary productivity (mg C m^{-3} hr^{-1}) by size class May to October, 2019-2022 A) less than 2 µm fraction B) 2-20 µm fraction C) over 20 µm fraction.

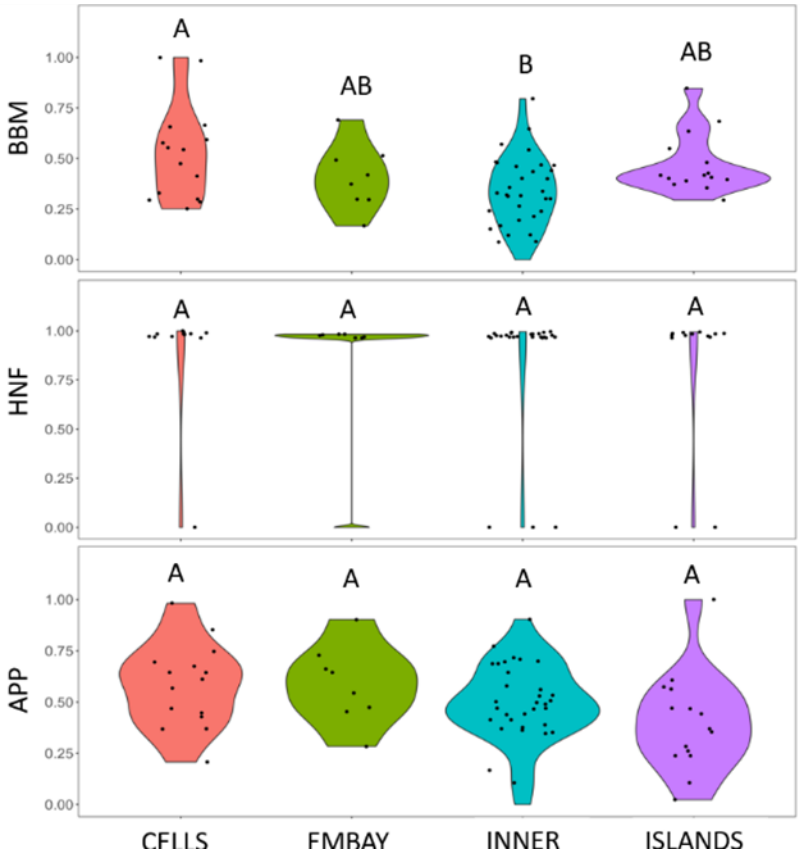

Figure 20: Plot by ecotype of top) Bacteria (BBM, ln transformed, normalized); middle) Heterotrophic Nanoflagellates (HNF, SHASH transformed, Normalized); and bottom) Autotrophic Picoplankton (APP SHASH transformed, normalized). Ecotypes with the same letter code were not significantly different.

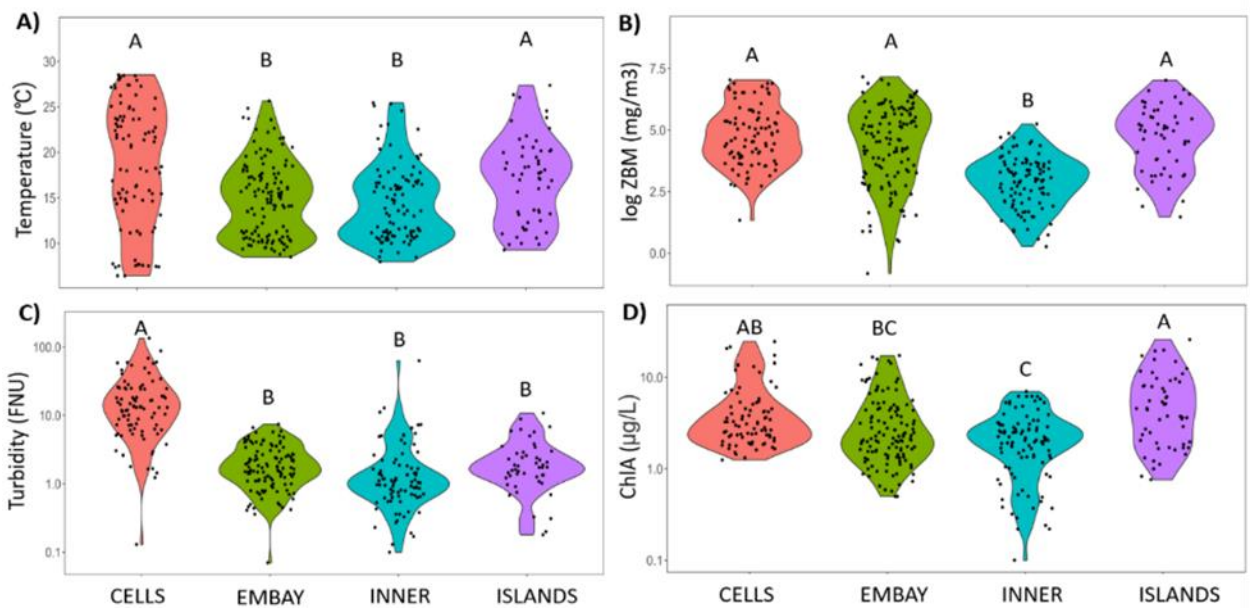

Figure 21: Violin plots of turbidity (FNU), temperature (°C), sonde chlorophyll a (µg L^{-1} using EXO sonde) and log zooplankton biomass (ZBM; mg m^{-3}) in Cells, Embay (Embayments), Inner Harbour and Island channel stations, Toronto Harbour. These are May to October averages of vertical profiles across the water column from 2019 to 2022. The Y axes for Chl a and Turbidity are shown on a logarithmic scale. Ecotypes with the same letter code were not significantly different.

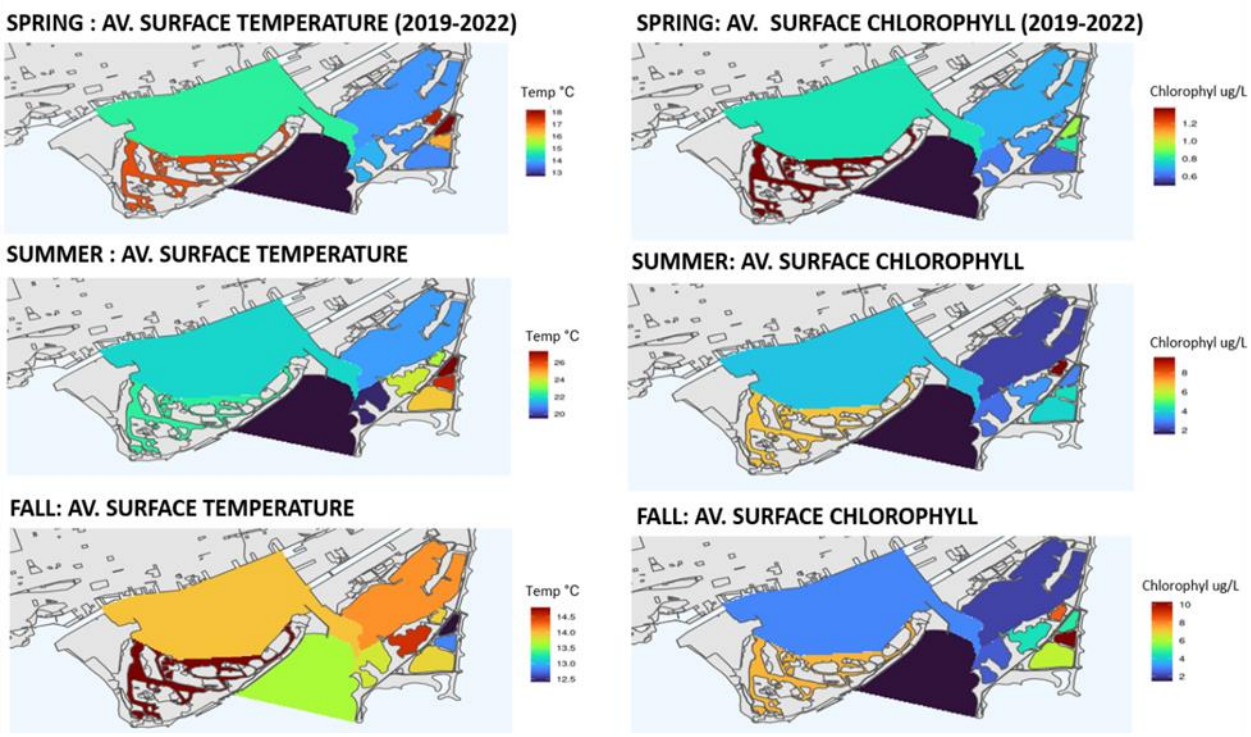

Figure 22: Spring, summer and fall maps of surface temperature (left) and chlorophyll a (right) averaged across each sampling area using the vessel flow-through system.

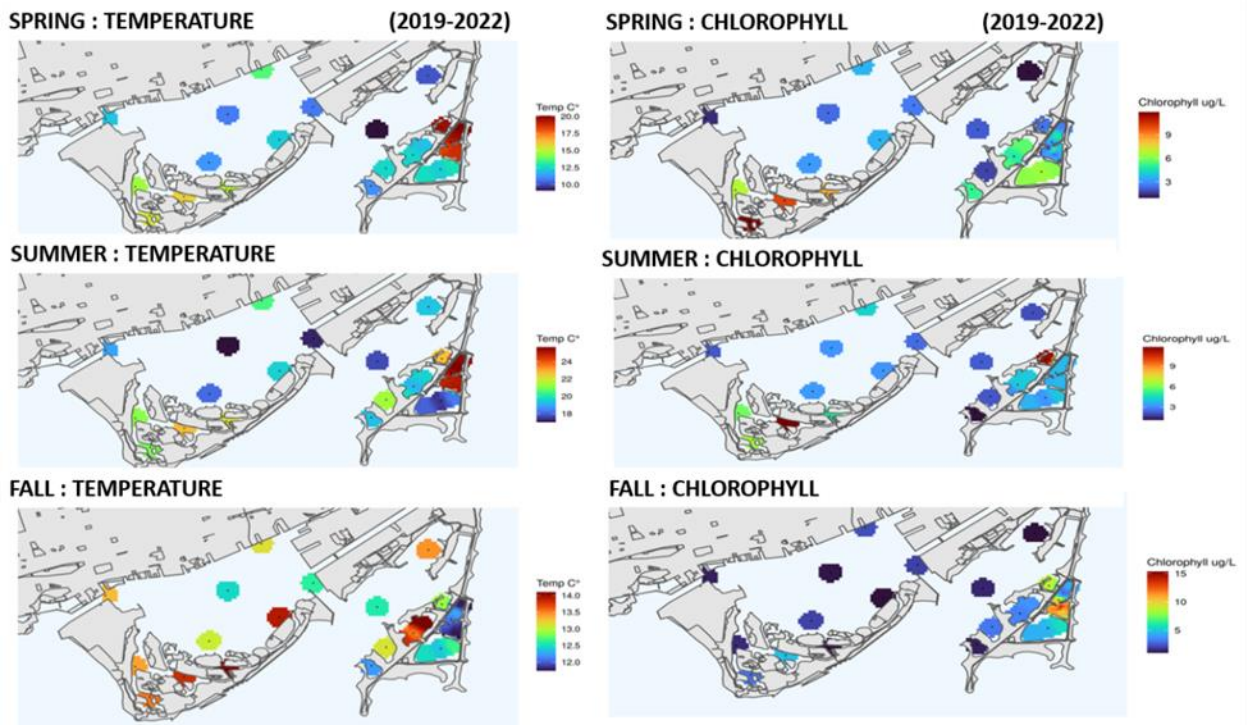

Figure 23: Maps of mean whole water column temperature (left) and sonde chlorophyll a (right) at discrete sampling stations in the Toronto area. Each season is shown separately, averaged across the 2019-2022 study period.

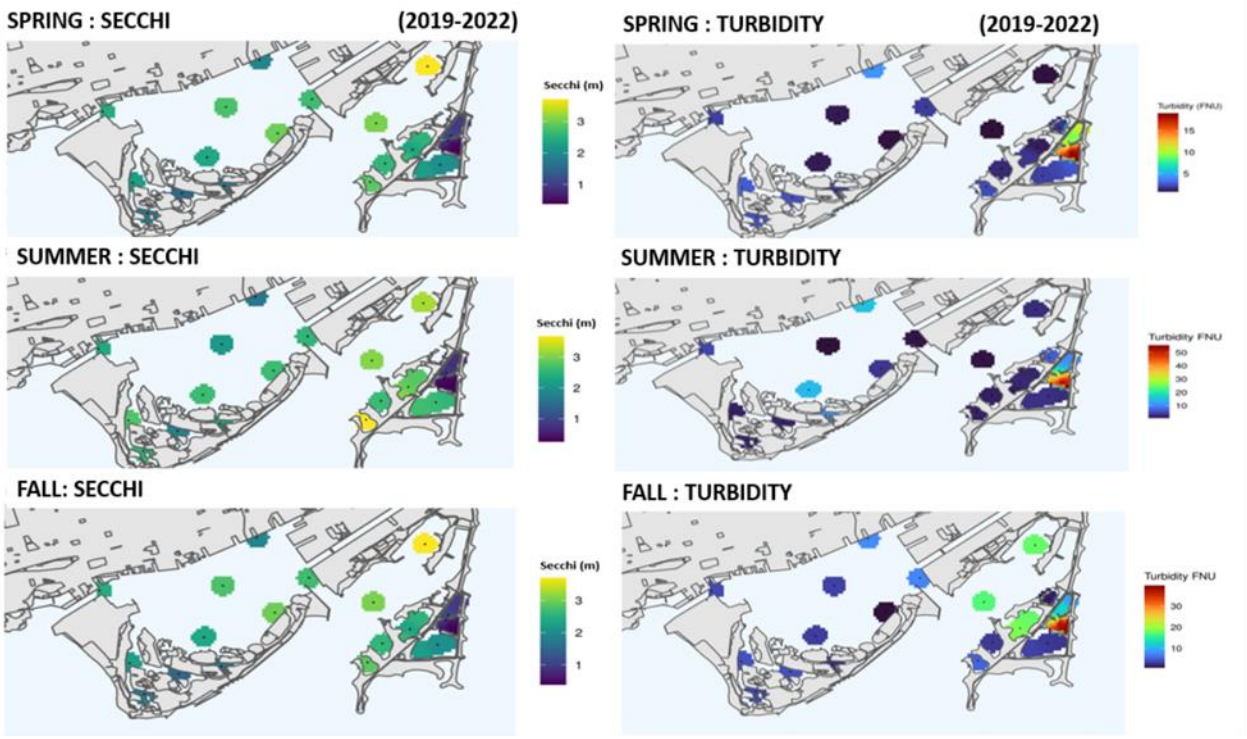

Figure 24: Maps of Secchi (left) and whole water column turbidity (right) at discrete sampling stations in the Toronto area. Each season is shown separately, averaged across the 2019-2022 study period.

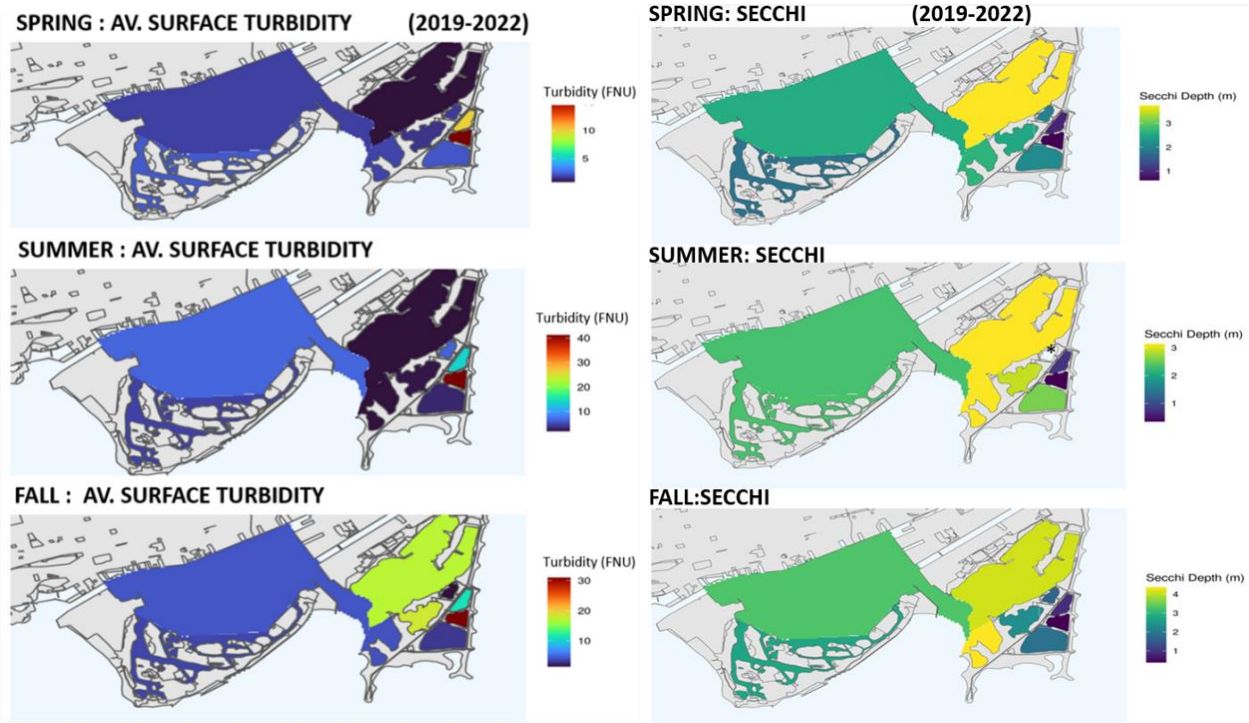

Figure 25: Spring, summer and fall maps of surface turbidity (left) averaged across each sampling area using the profile data and station Secchi depth (right) from 2019-2022. Secchi disk depth in Cell D was to bottom (>2m) in all summer samples.

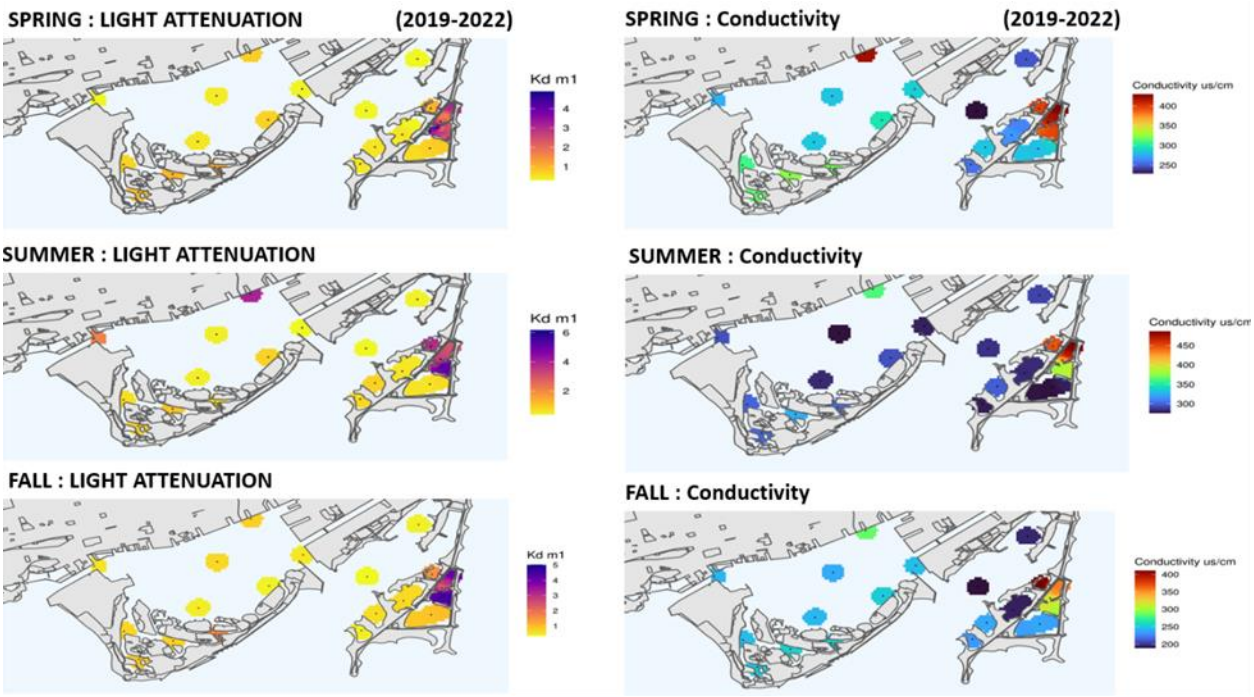

Figure 26: Maps of Light Attenuation (left) and Conductivity (right) at discrete sampling stations in the Toronto area. Each season is shown separately, averaged across the 2019-2022 study period.

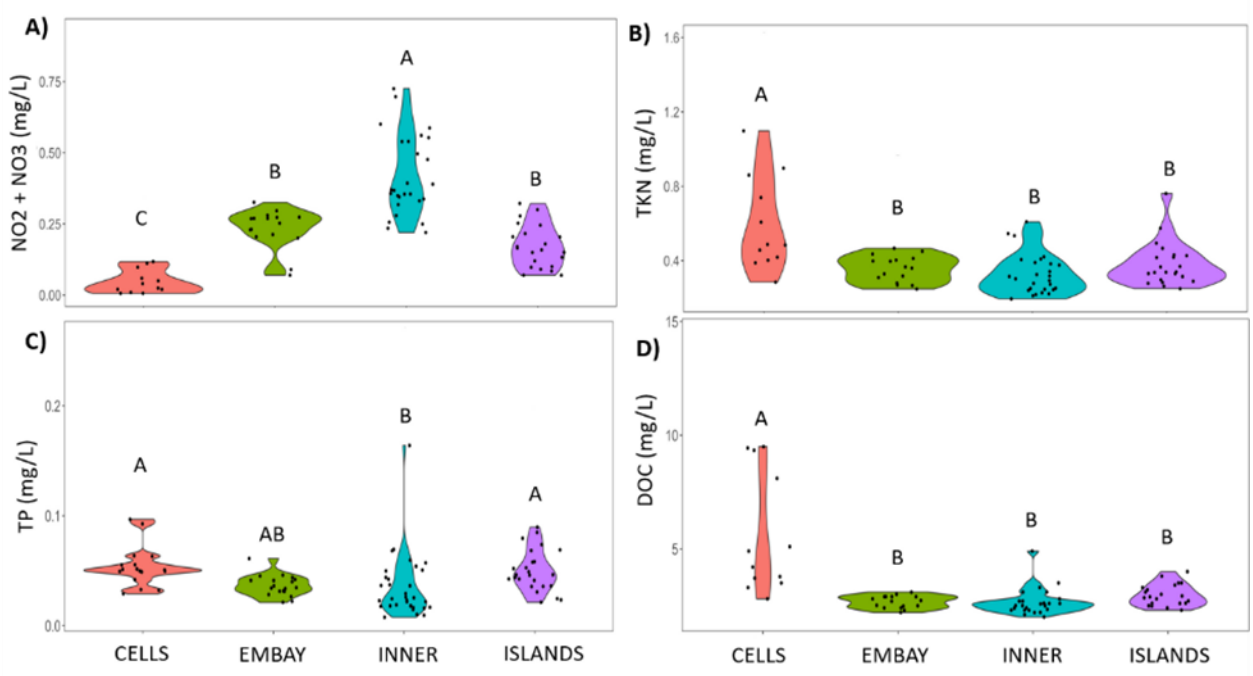

Figure 27: Plot by ecotype of A) Nitrite +Nitrate (mg L^{-1}) B) Total Kjeldahl Nitrogen (mg L^{-1}), C) Total Phosphorus (mg L^{-1}), and D) Dissolved Organic Carbon (mg L^{-1}). Letters indicate statistically significantly distinct groups.

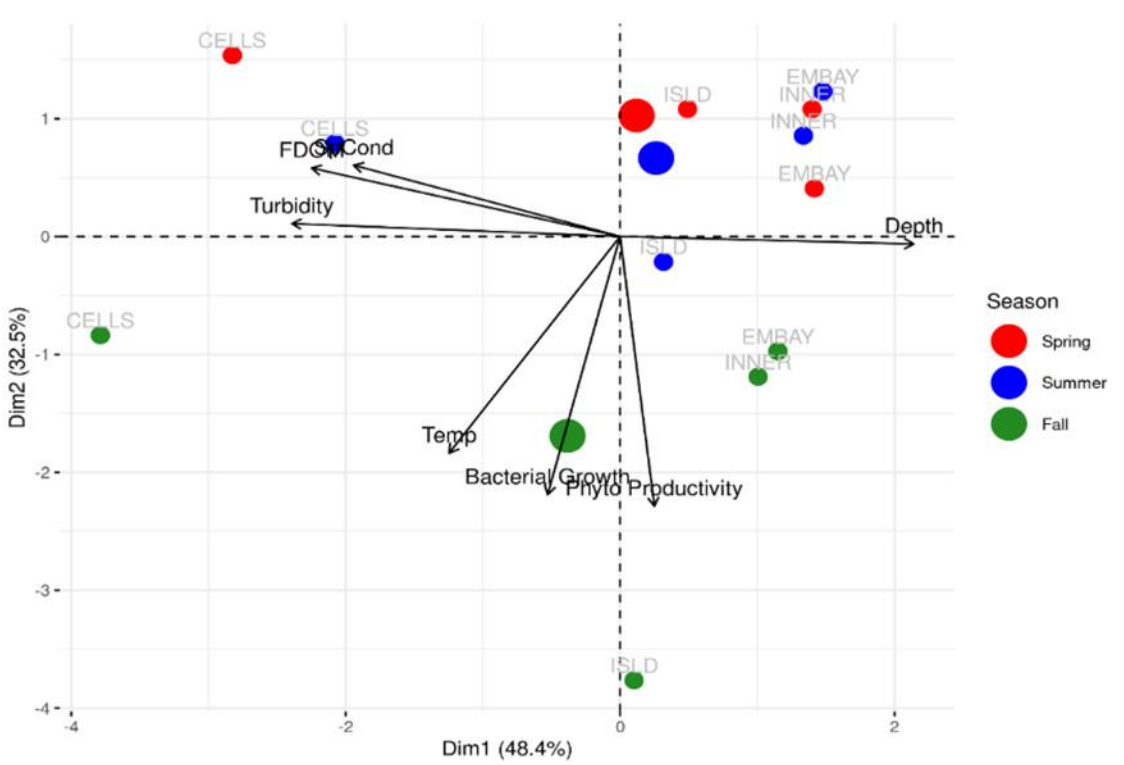

Figure 28: Principal component analysis (PCA) conducted with key water property variables for each of our seasons and sampling locations. Sites that are closer together tend to have similar water properties than sites that are farther apart.

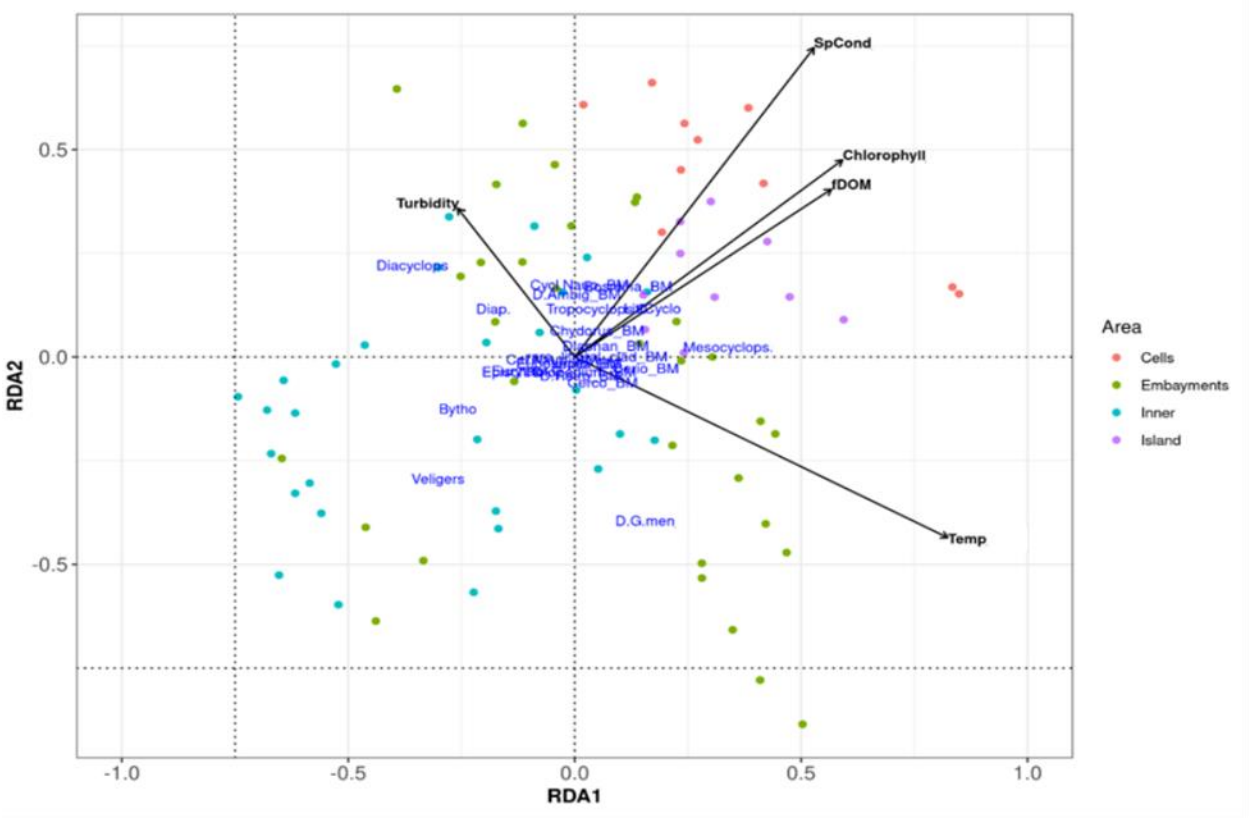

Figure 29: Results of redundancy analyses displaying water properties as predictor variables and zooplankton taxa as response variables. Circles represent sampling sites in each Toronto AOC ecotype. Taxa are represented by their text labels in blue. Water property variables are represented by arrows in black. Sites that are closer together in the ordination plot have more similar species composition than sites that are further apart. Chlorophyll measures are from YSI EXO sonde Chl *a.*

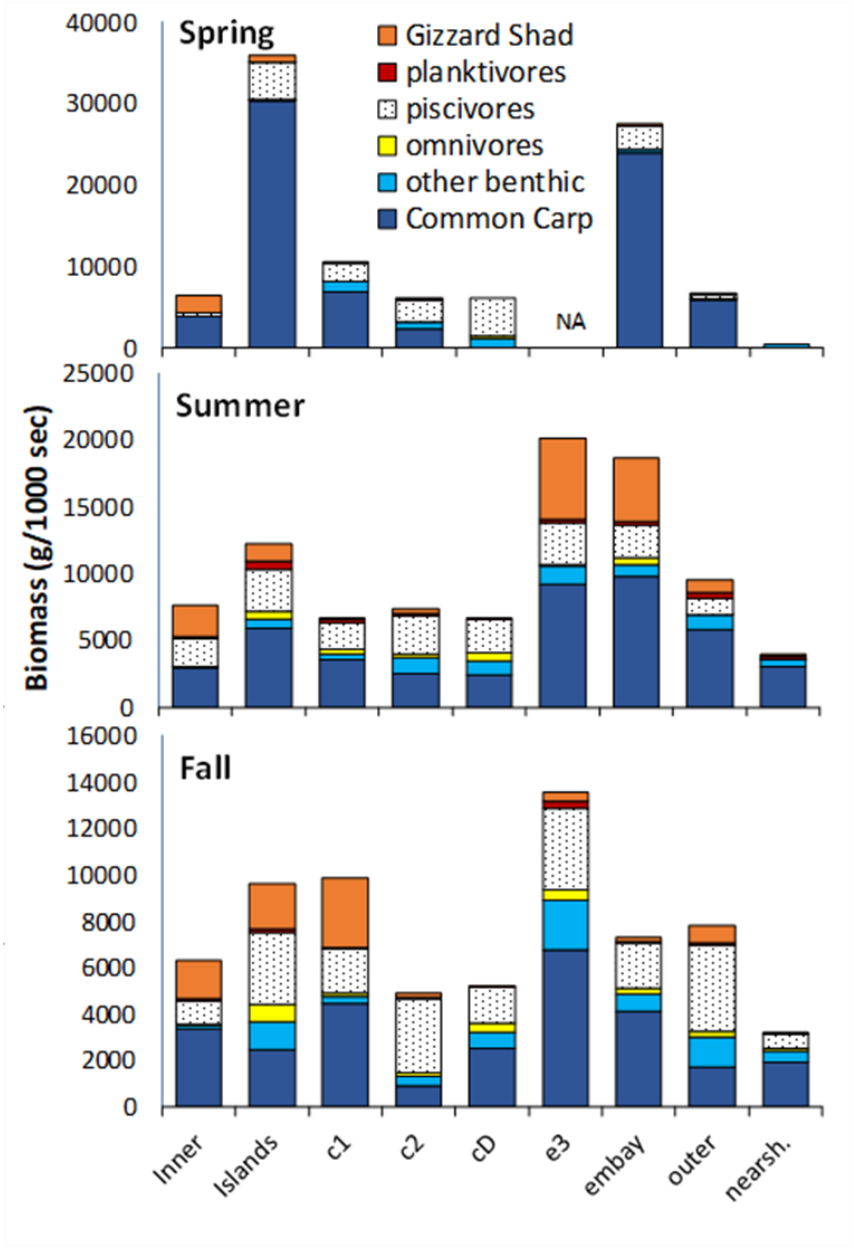

Figure 30: Mean biomass of fishes in the Toronto AOC in spring, summer and fall from 2013 to 2022. Nearshore fish were caught by boat electrofishing by TRCA, based on standardized run times of 1000 seconds. Feeding guilds are described in Appendix 1, Table A2. Embay represents TTP Embayments except e3, and nearsh. represents the nearshore of Lake Ontario on the outside of the Toronto Islands and TTP.

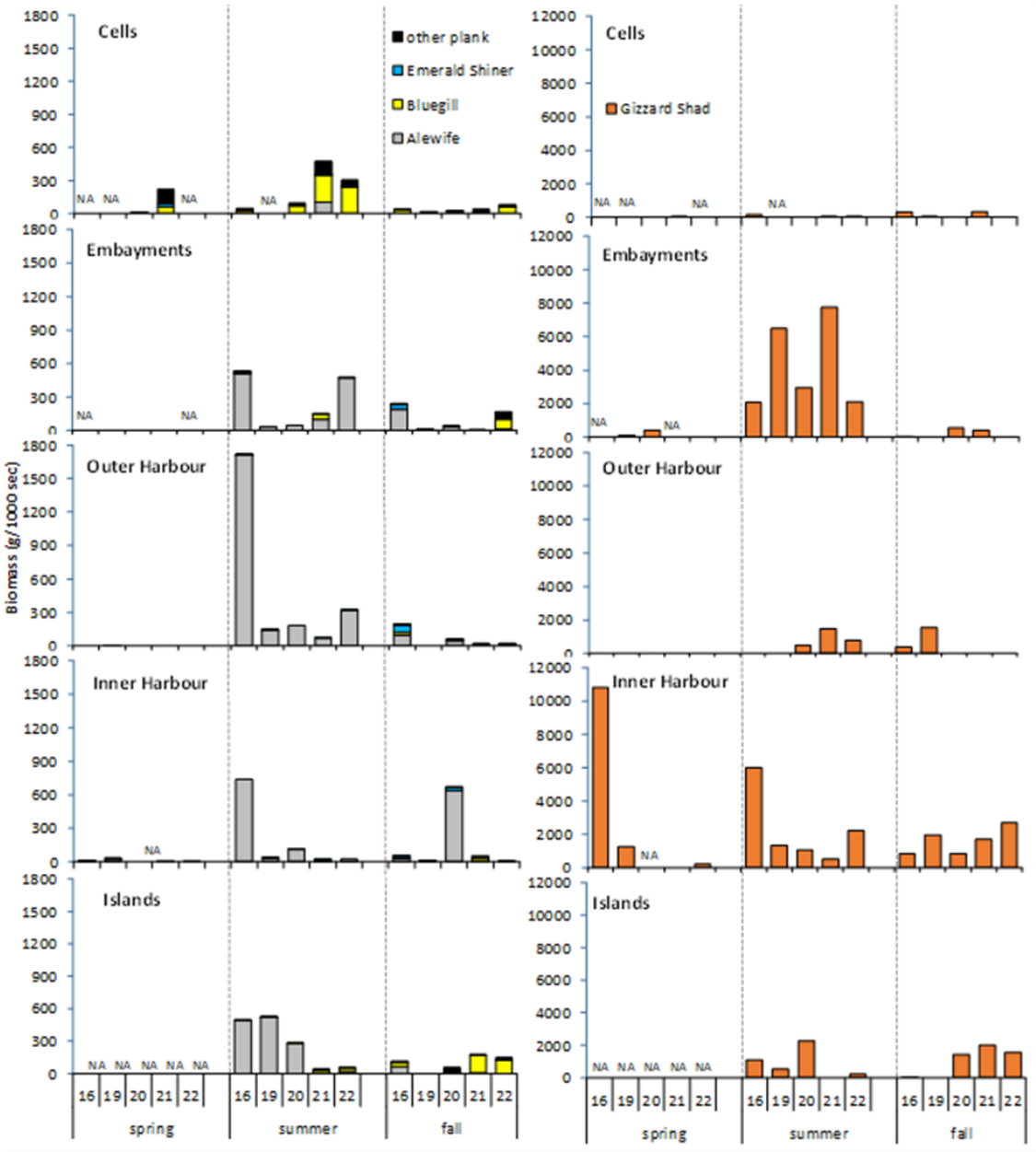

Figure 31: Mean biomass of dominant taxa of planktivorous fishes in areas corresponding to DFO's Toronto lower food web study in spring, summer and fall of 2016 and 2019 to 2022. Fish were caught by boat electrofishing by TRCA, based on standardized run times of 1000 seconds.

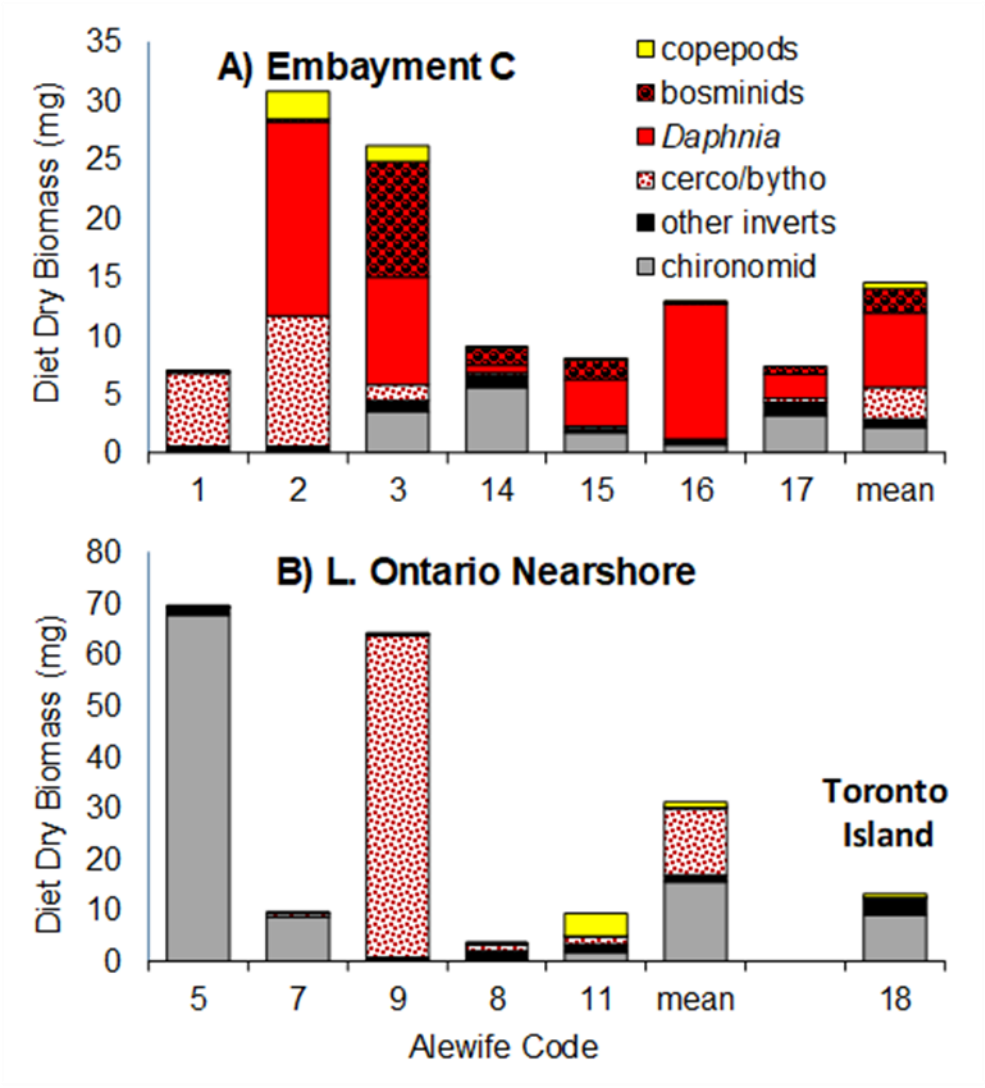

Figure 32: Estimated dry biomass of macroinvertebrate and zooplankton taxa in individual Alewife stomachs collected on 27 July 2021, from Embayment C in TTP (top) and the Lake Ontario nearshore near Gibraltar Point, along with one fish from the Toronto Island area (bottom).

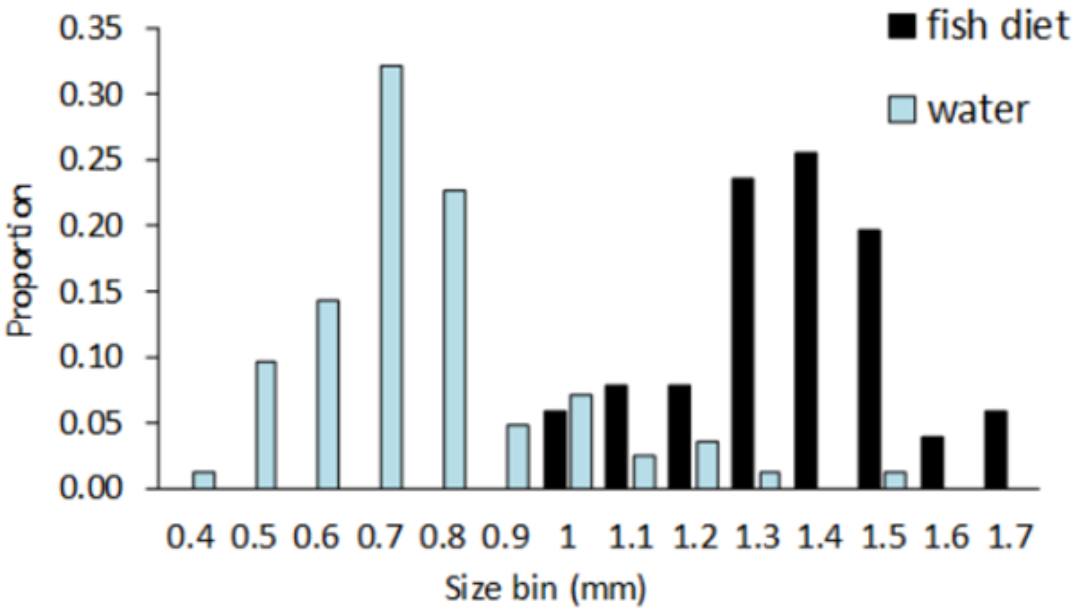

Figure 33: Embayment C size frequency distribution of Daphnia (by length) in fish diets on 27-July-2021, compared to Daphnia from the water column on July 13 and August 23, 2021.

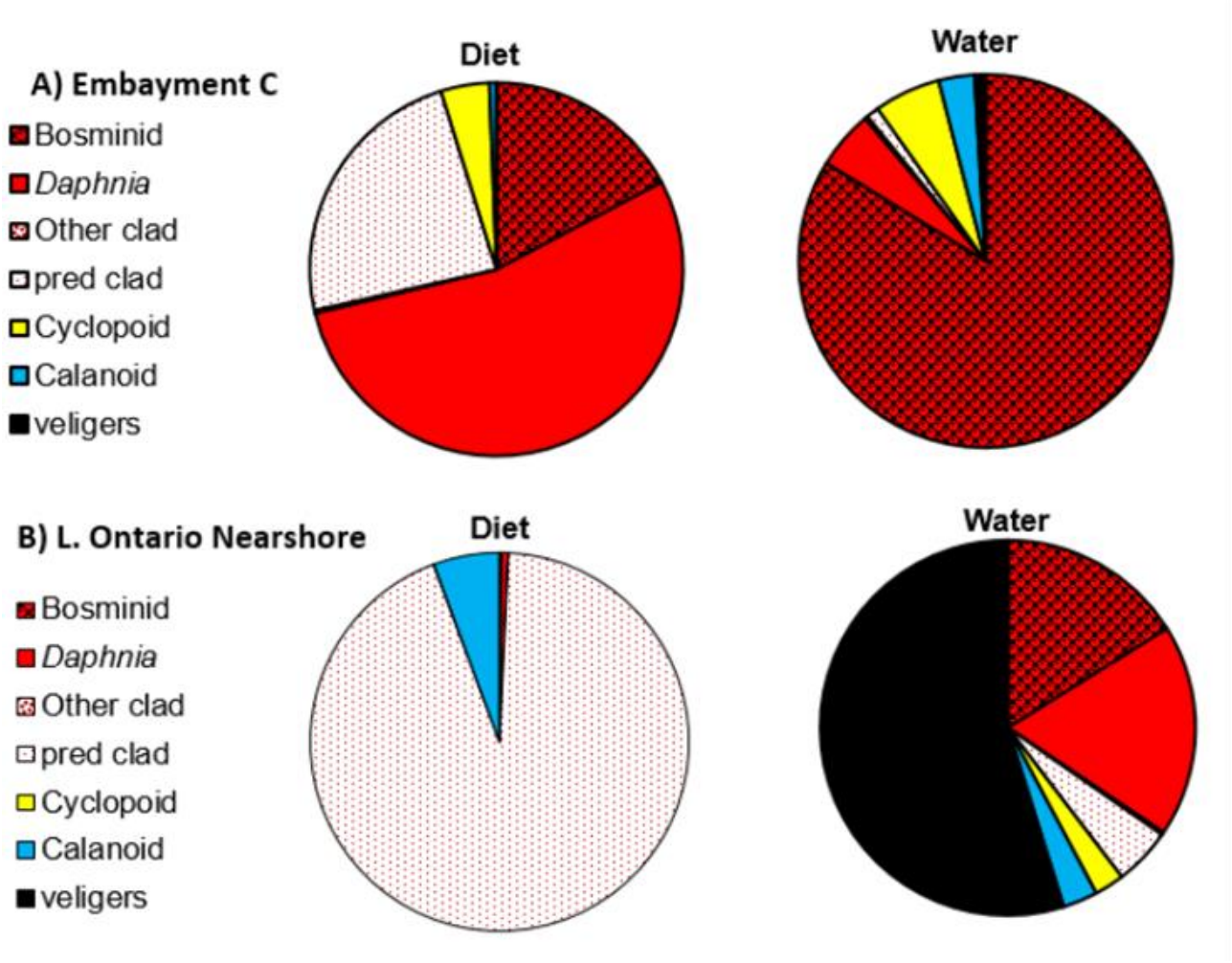

Figure 34: Proportion (by biomass) of zooplankton groups in Alewife diets compared to summer estimates in the water column in A) Embayment C in TTP, and B) Lake Ontario nearshore near Gibraltar Point.

Appendix 1

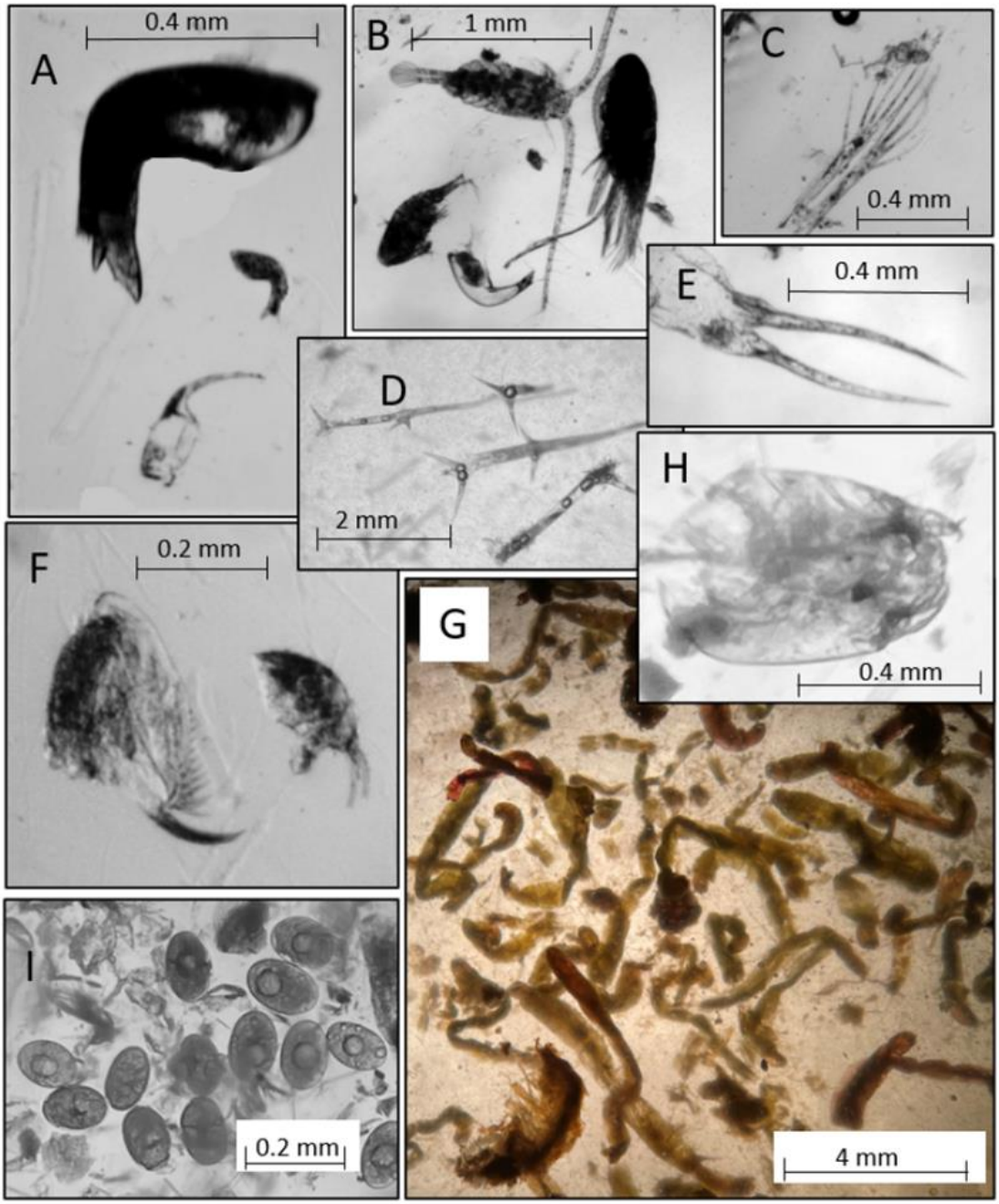

Figure A-1: Distinctive zooplankton and invertebrate parts in alewife stomachs used for counts, including A) mandibles of *Bythotrephes* (top), *Cercopagis* (middle) and *Leptodora* (bottom); B cyclopoid and calanoid copepods relative to size of *Bythotrephes* mandible; C) *Limnocalanus* caudal (tail) rami; D *Bythotrephes* caudal spines; E) *Leptodora* caudal spines; F) *Daphnia* post-abdominal spines (left) and *Bosmina* head showing antennae (right); G) chironomid larvae (midges) and amphipod; H) chironomid larva head capsule; and I) *Daphnia* eggs.

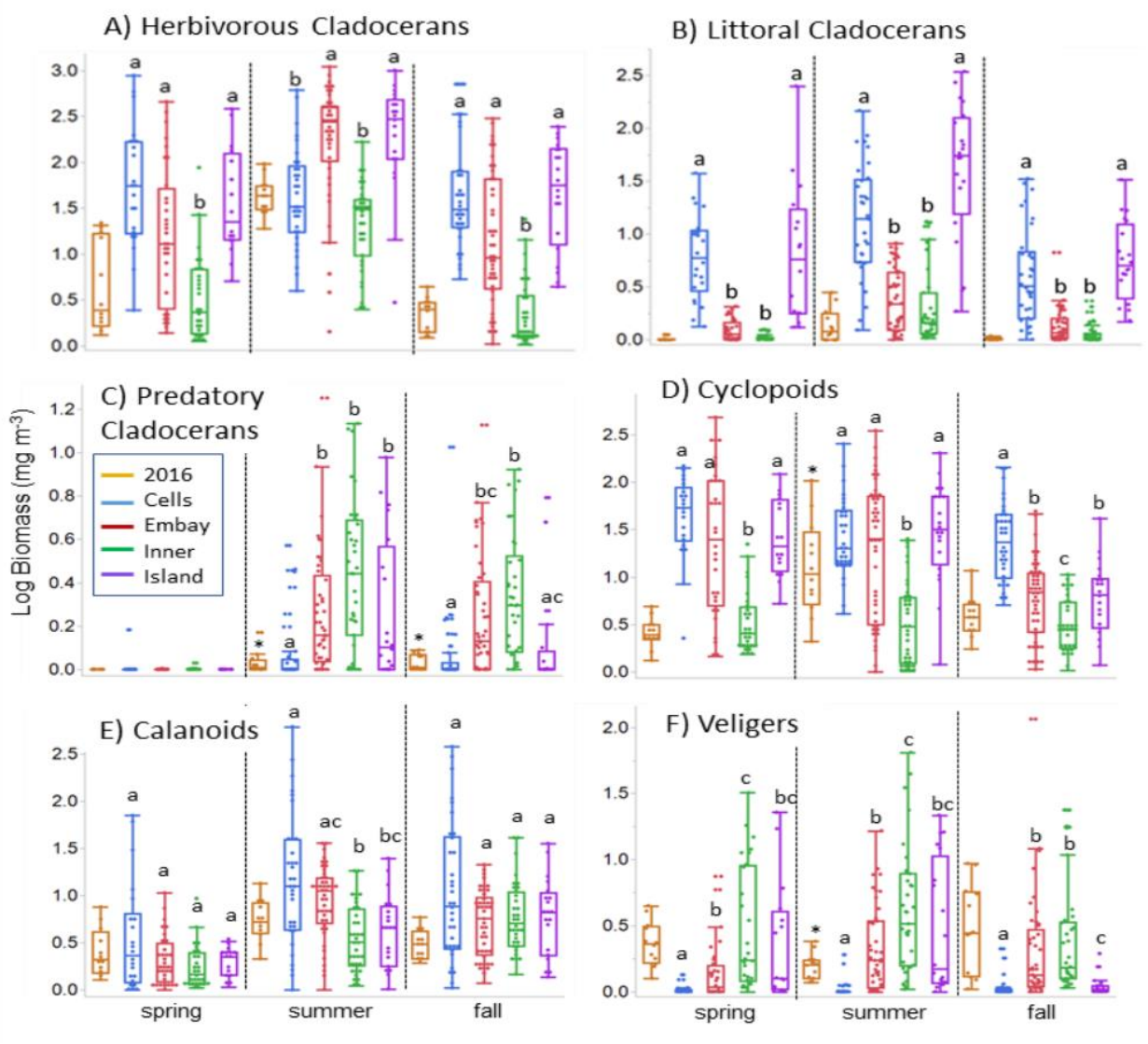

Figure A-2: Log-transformed total biomass of zooplankton divided into taxonomic groups, including A) herbivorous cladocerans, B) littoral cladocerans, C) predatory cladocerans, D) cyclopoids, E) calanoids and F) *Dreissena* veliger larvae. Shown are averages for the Inner Harbour in 2016, and the 2019 to 2022 study in the Cells, Embayments, Inner Harbour and Toronto Islands for the spring, summer and fall seasons. For a given season in the 2019 to 2022 study, bars with different letter codes are significantly different ($p<0.05$). When the two Inner Harbour values are significantly different, a * is placed over the 2016 bar.

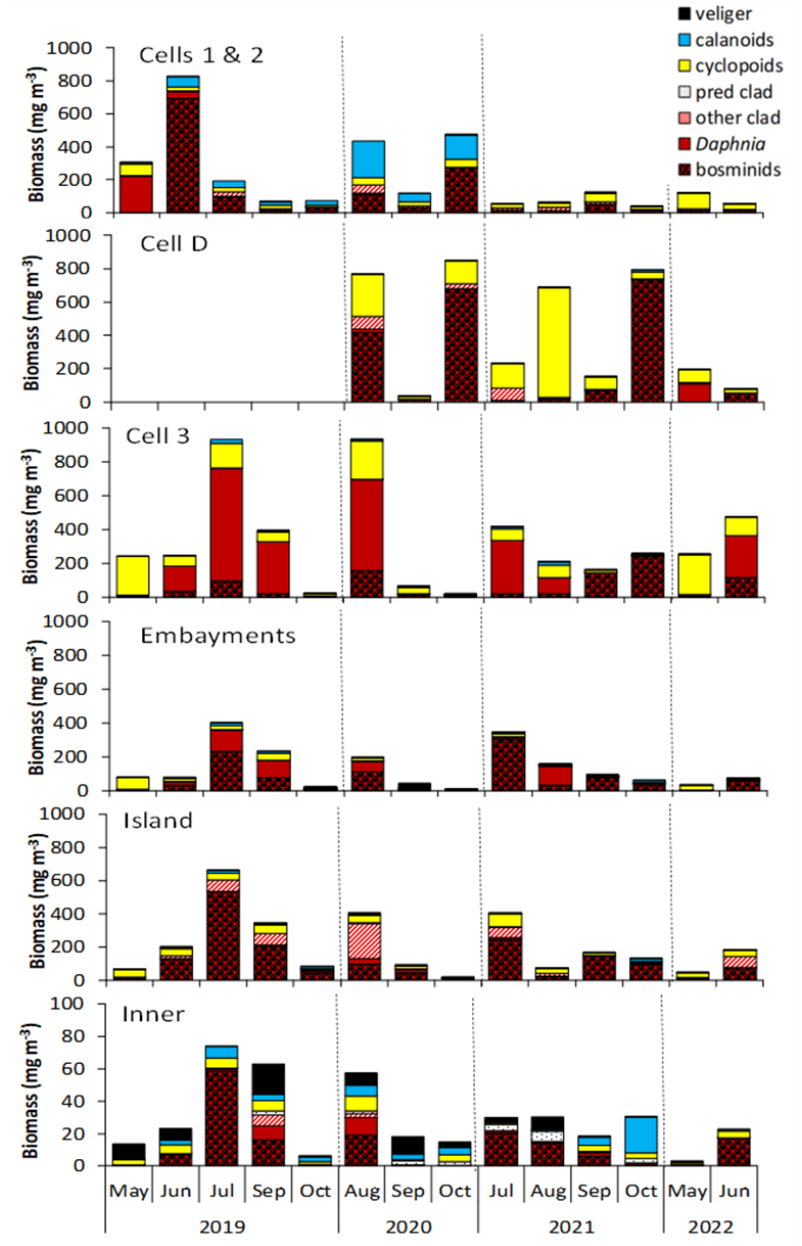

Figure A-3: Biomass of zooplankton groups in different study locations for each sampling event (May 2019 to June 2022). Note that Cell D was not sampled in 2019, and that the biomass scale in the Inner Harbour is 10 times lower than the other locations.

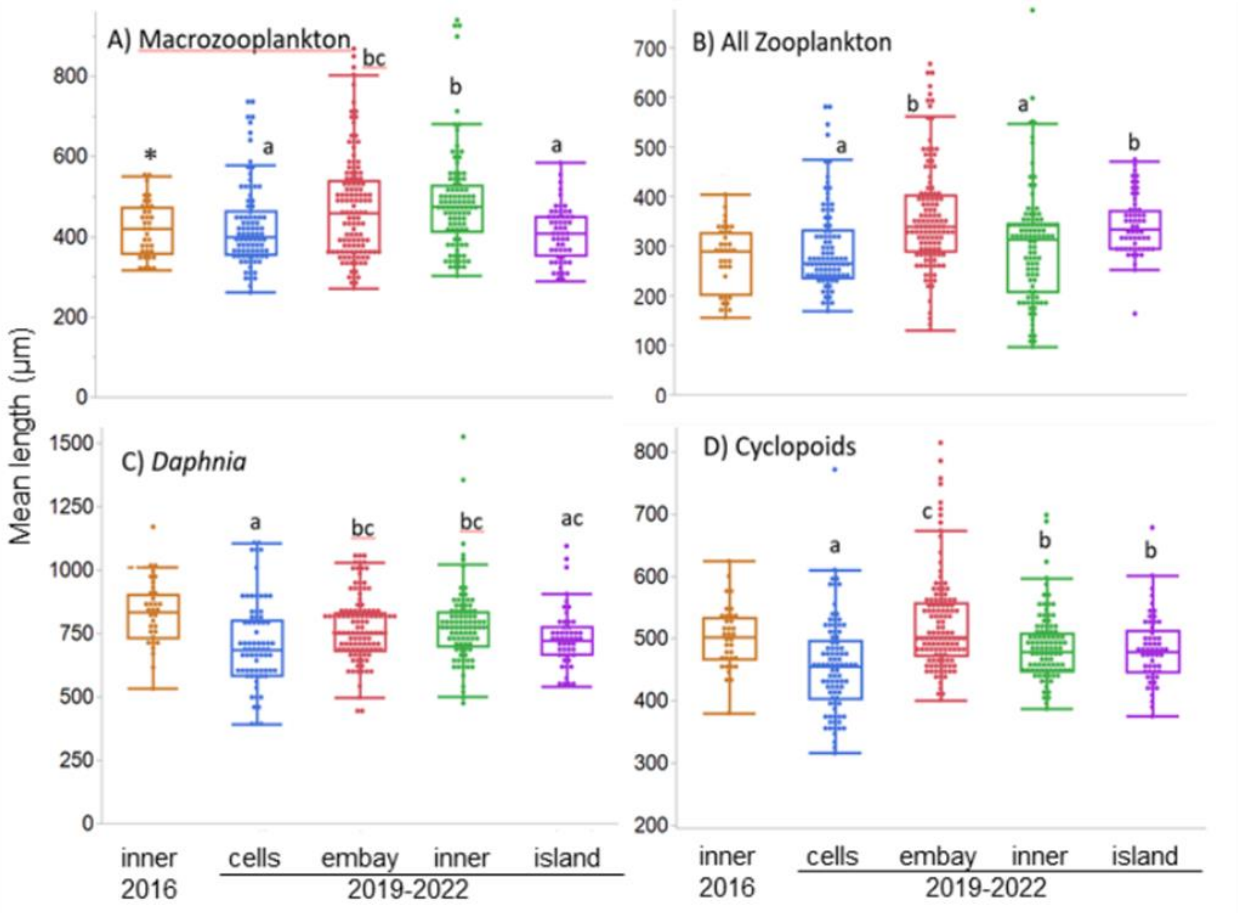

Figure A-4: Mean length (weighted for density) of A) macrozooplankton (*Dreissena* veligers and copepod nauplii excluded), B) all zooplankton, C) *Daphnia* and D) Cyclopoids (excluding nauplii) in the Inner Harbour in 2016, and the four study areas from 2019 to 2022. For a given taxon in the recent study, bars with the same letter code are not significantly different. An '*' indicates that the Inner Harbour values were significantly different between the two studies.

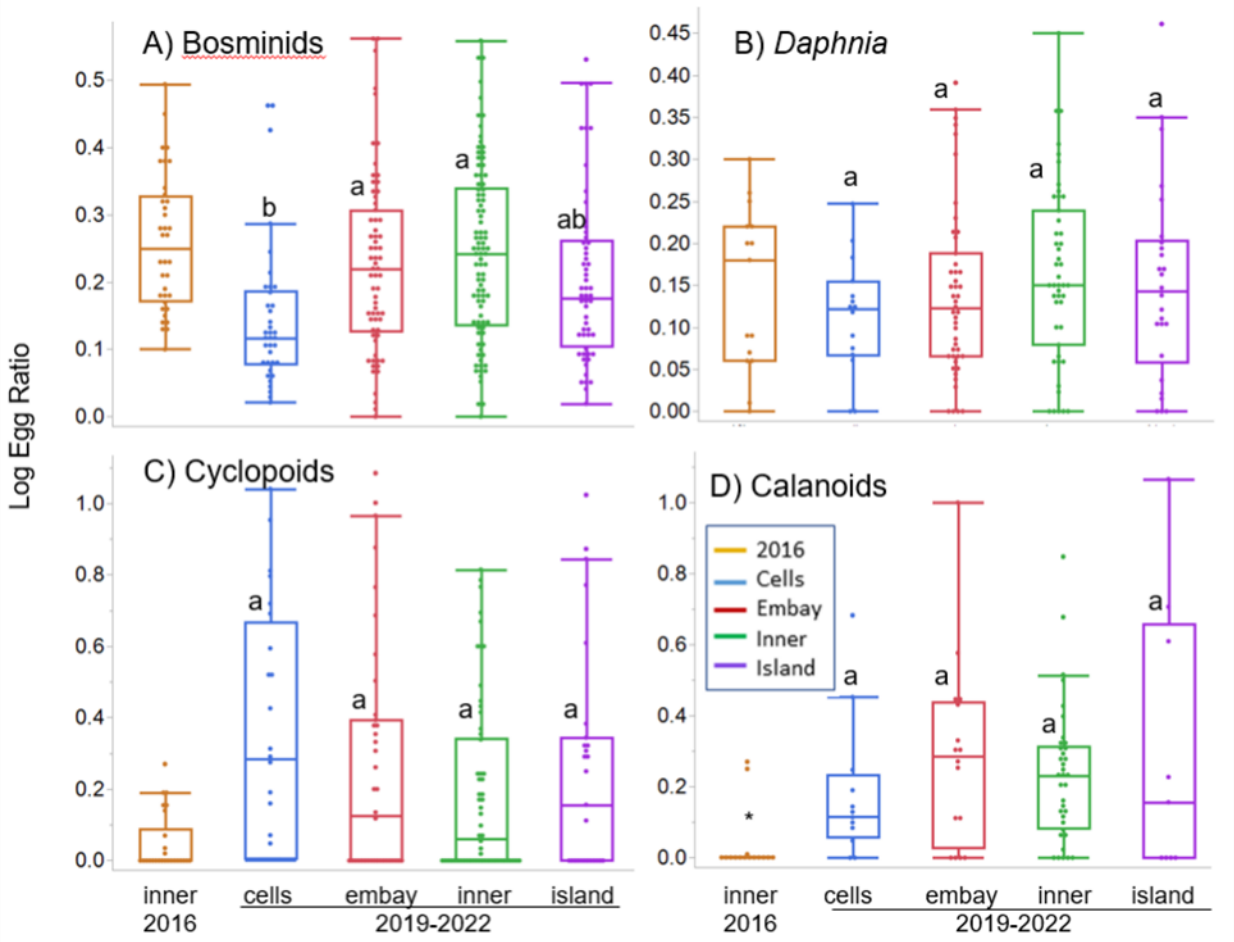

Figure A-5: Log transformed mean egg ratios (eggs per individual) of A) bosminids, B) *Daphnia*, C) adult cyclopoids and D) adult calanoids in the Inner Harbour in 2016, and the four study areas from 2019 to 2022. For a given taxon in the recent study, bars with the same letter code are not significantly different. An * indicates that the Inner Harbour values were significantly different between the two studies.

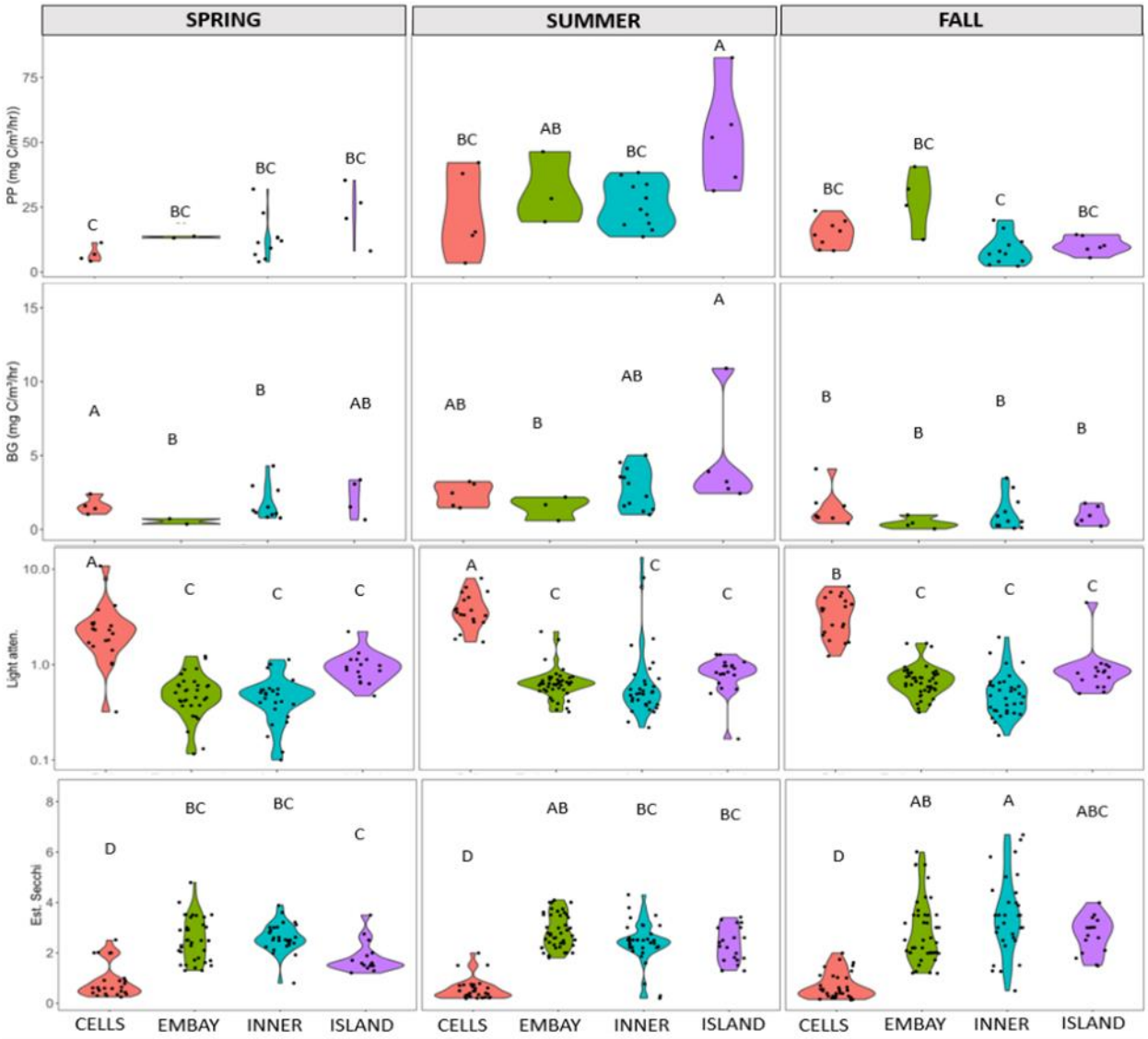

Figure A-6: Violin plots of Primary Productivity (mg C m^{-3} hr^{-1}), Bacteria Growth (mg C m^{-3} hr^{-1}), Light attenuation (m^{-1}; log10 axis) and Secchi Disc depth (m) at Cell, Embayment, Inner Harbour and Island Channel stations in the Toronto Harbour area. These are averages of vertical profiles in spring, summer and fall from 2019 to 2022.

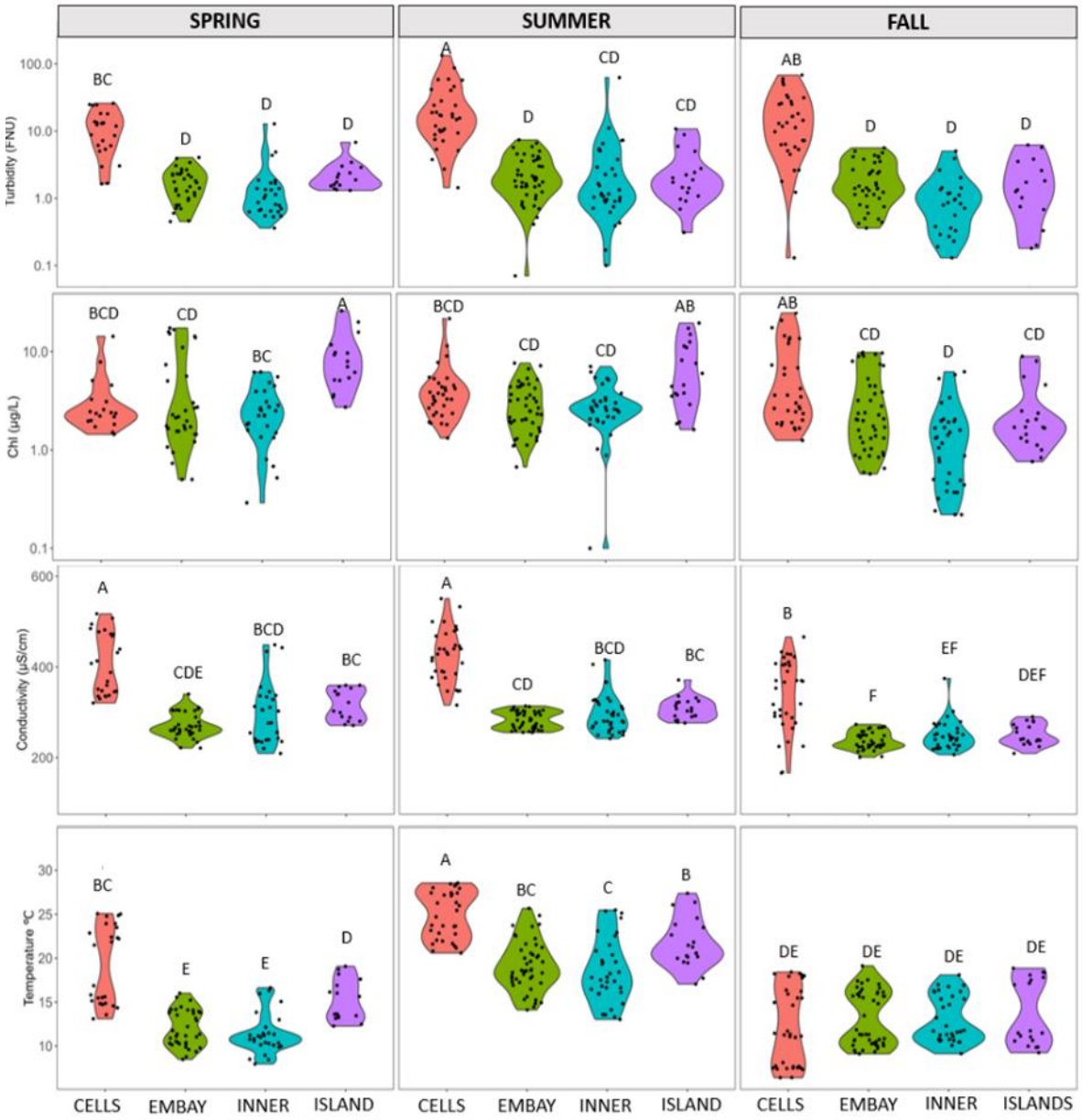

Figure A-7: Violin plots of turbidity, sonde chlorophyll *a,* conductivity and temperature measured using EXO sonde at Cell, Embayment, Inner Harbour and Island Channel stations in the Toronto Harbour. These are averages of full water column profiles in spring, summer and fall from 2019 to 2022.

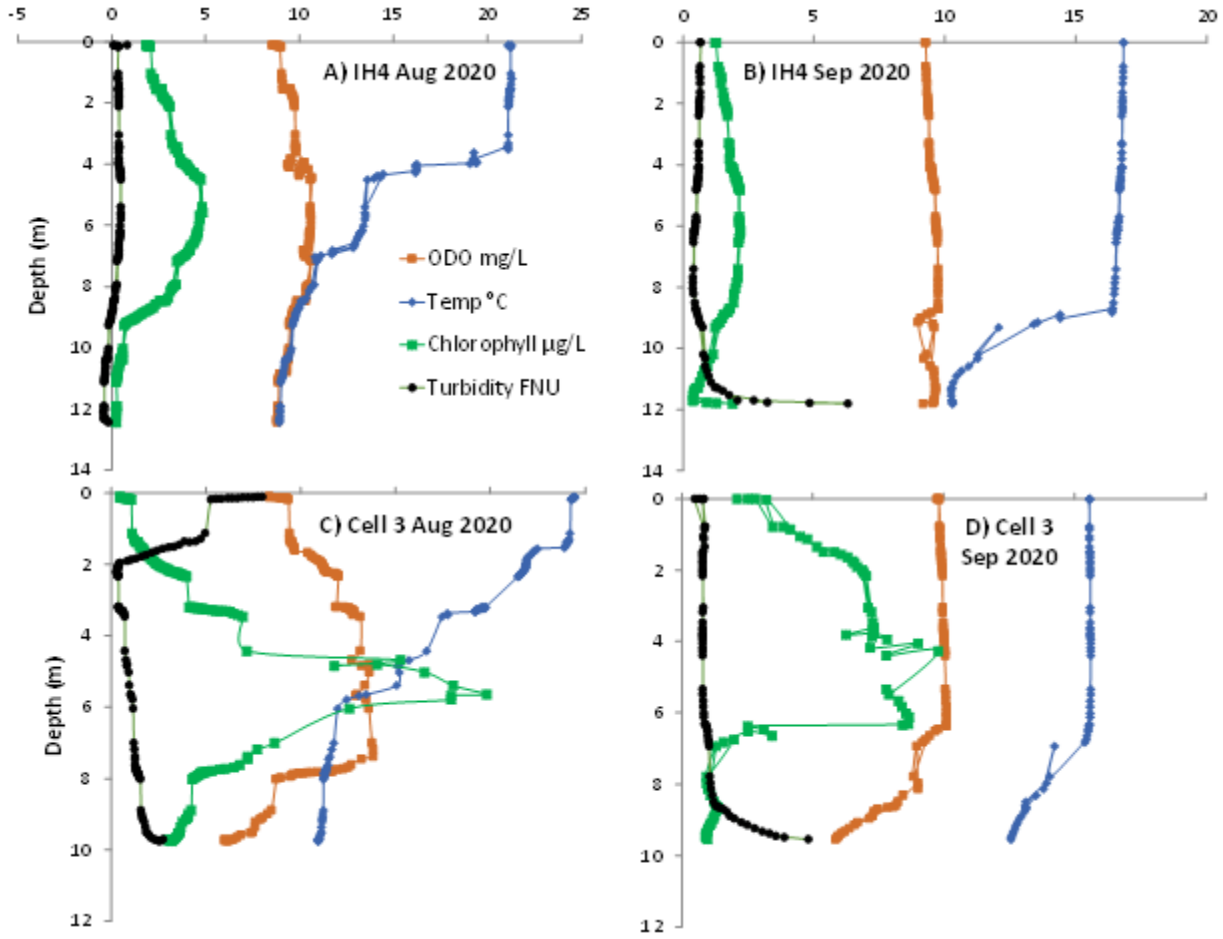

Figure A-8: Examples of vertical profiles taken with the EXO sonde at mid-Inner Harbour (A and B) and e3 (C and D) in August and Sept. 2020. Shown are dissolved oxygen (ODO), temperature, chlorophyll *a* and turbidity.

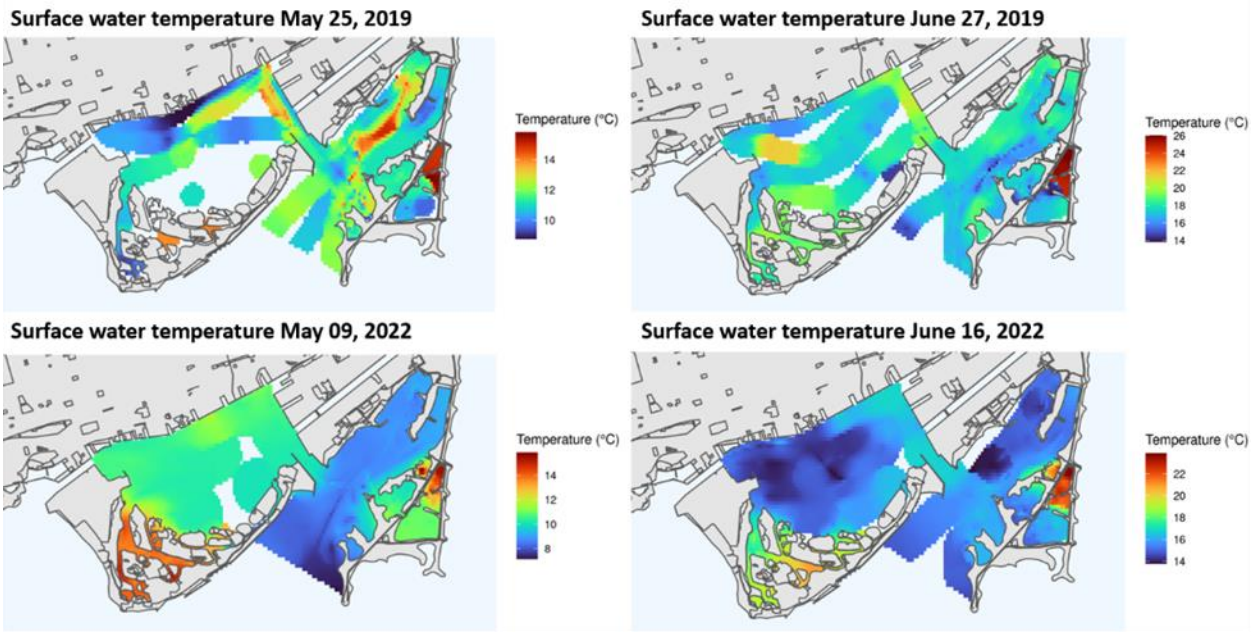

Figure A-9 Map of surface temperature on individual sampling days in the spring using the boat flow-through sonde in the Toronto AOC. Cell data were taken from the station profiles. Tows were not captured in full on May 25th, 2019 and June 27 2019 due to sonde malfunction.

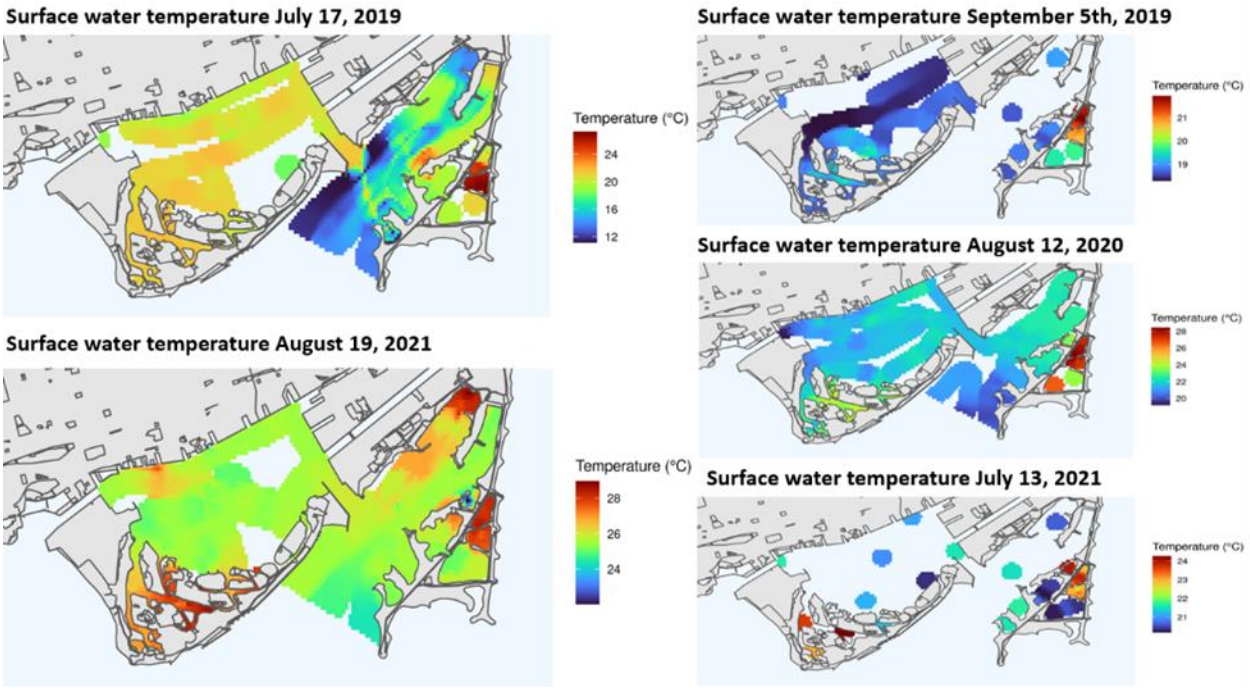

Figure A-10: Map of surface temperature on individual sampling days in the summer using the boat flow-through sonde in the Toronto AOC. Cell data were taken from the station profiles. Tows were not captured in full on September 5th, 2019, August 12, 2020, and July 13, 2021.

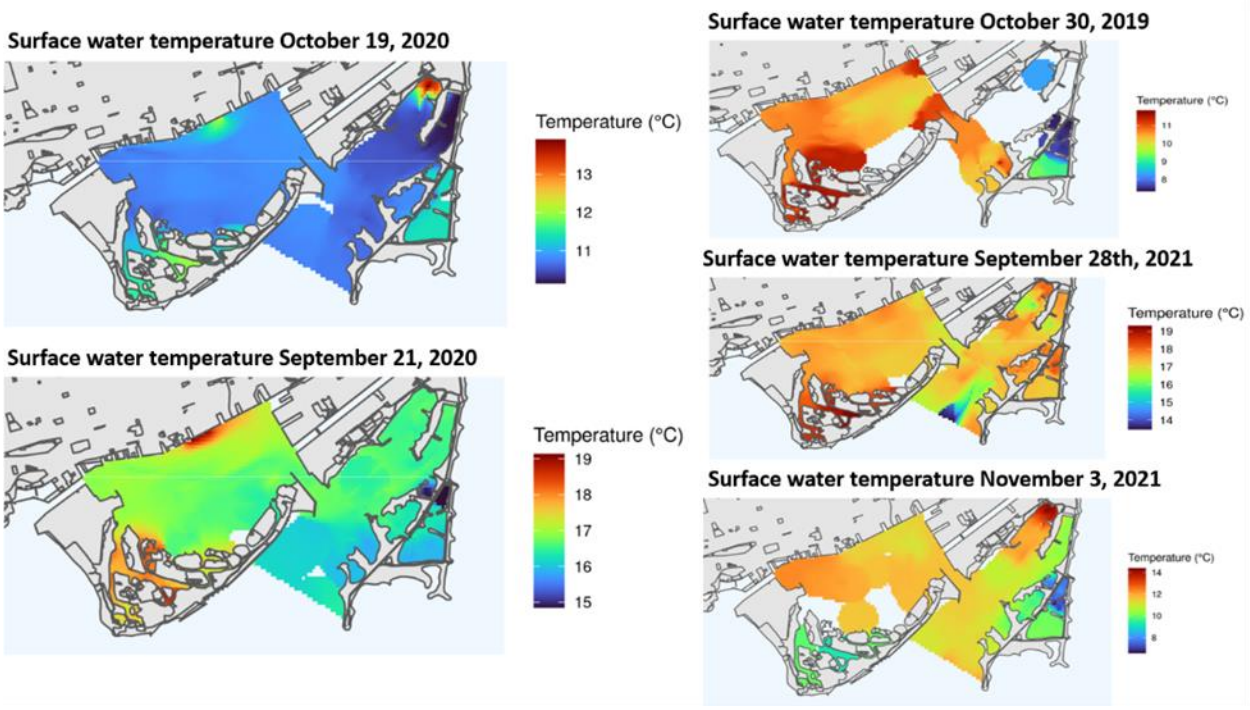

Figure A-11: Map of surface temperature on individual sampling days in the fall using the boat flow-through sonde in the Toronto AOC. Cell data were taken from the station profiles. Tows were not captured in full on October 30, 2019.

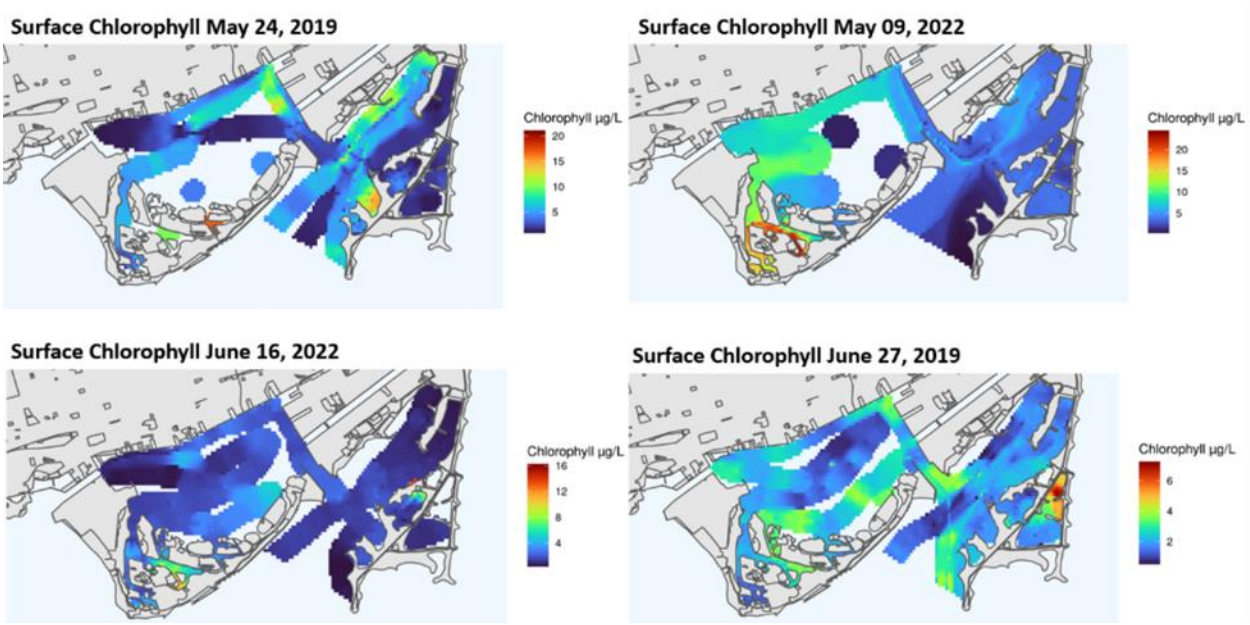

Figure A-12: Map of surface chlorophyll *a* on individual sampling days in the spring using the boat flow-through sonde in the Toronto AOC. Cell data were taken from the station profiles.

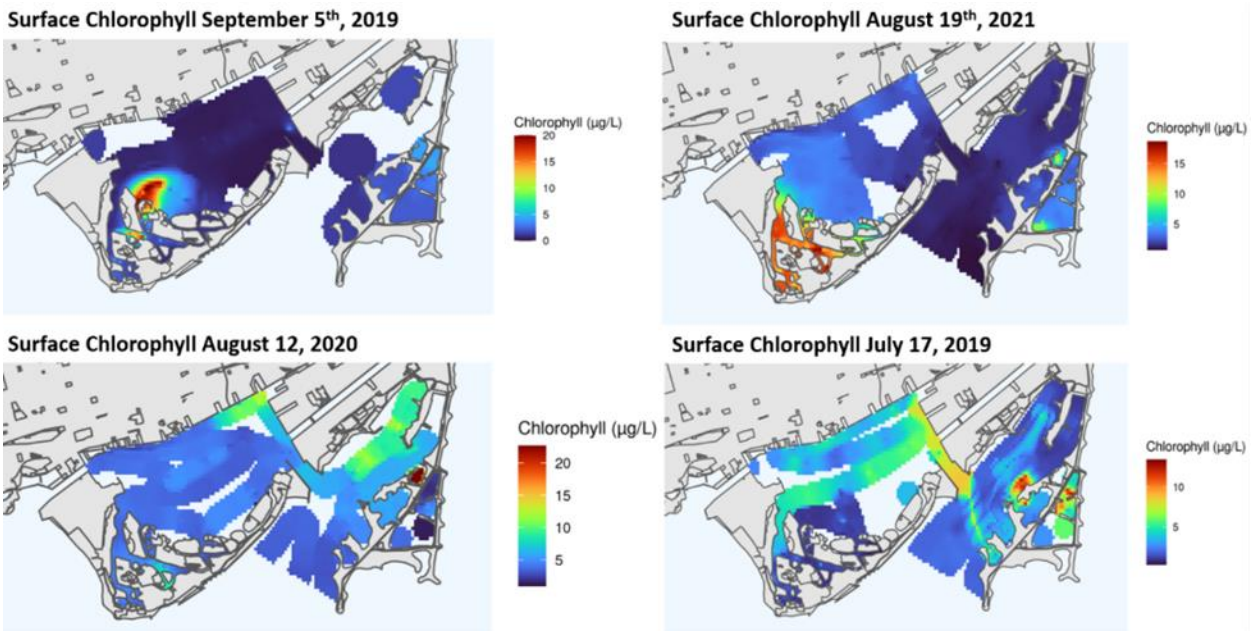

Figure A-13: Map of surface chlorophyll *a* on individual sampling days in the summer using the boat flow-through sonde in the Toronto AOC. Cell data were taken from the station profiles.

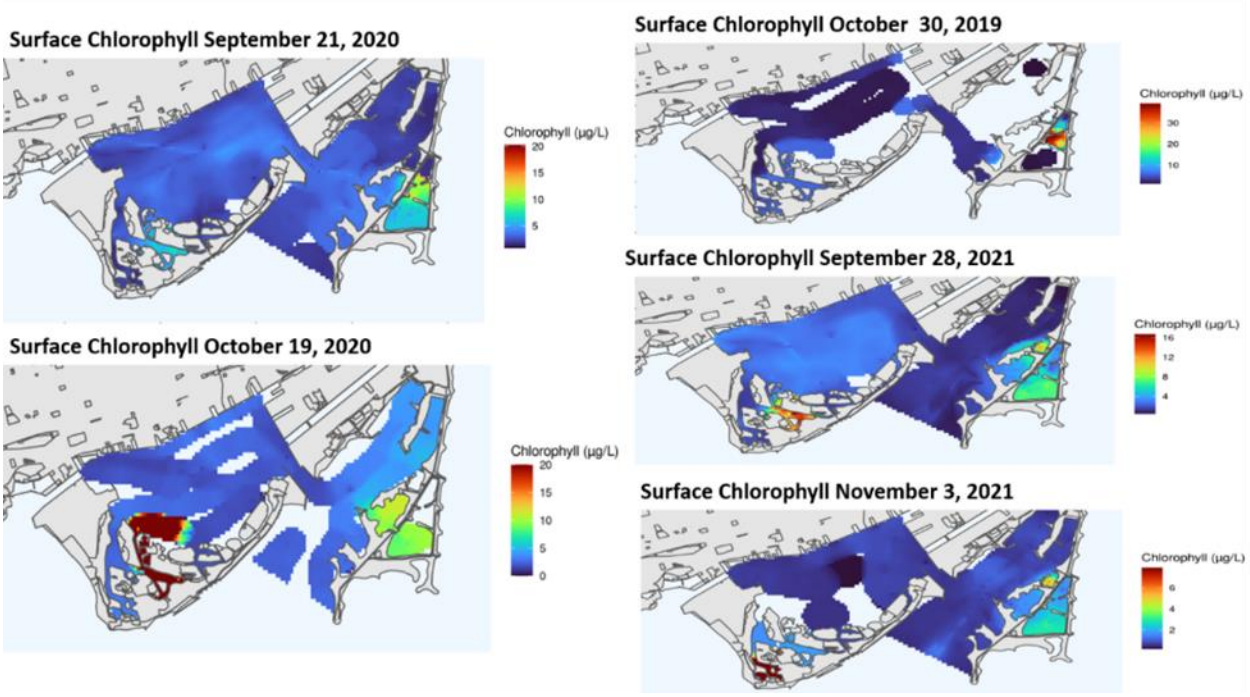

Figure A-14: Map of surface chlorophyll *a* on individual sampling days in the fall using the boat flow-through sonde in the Toronto AOC. Cell data were taken from the station profiles.

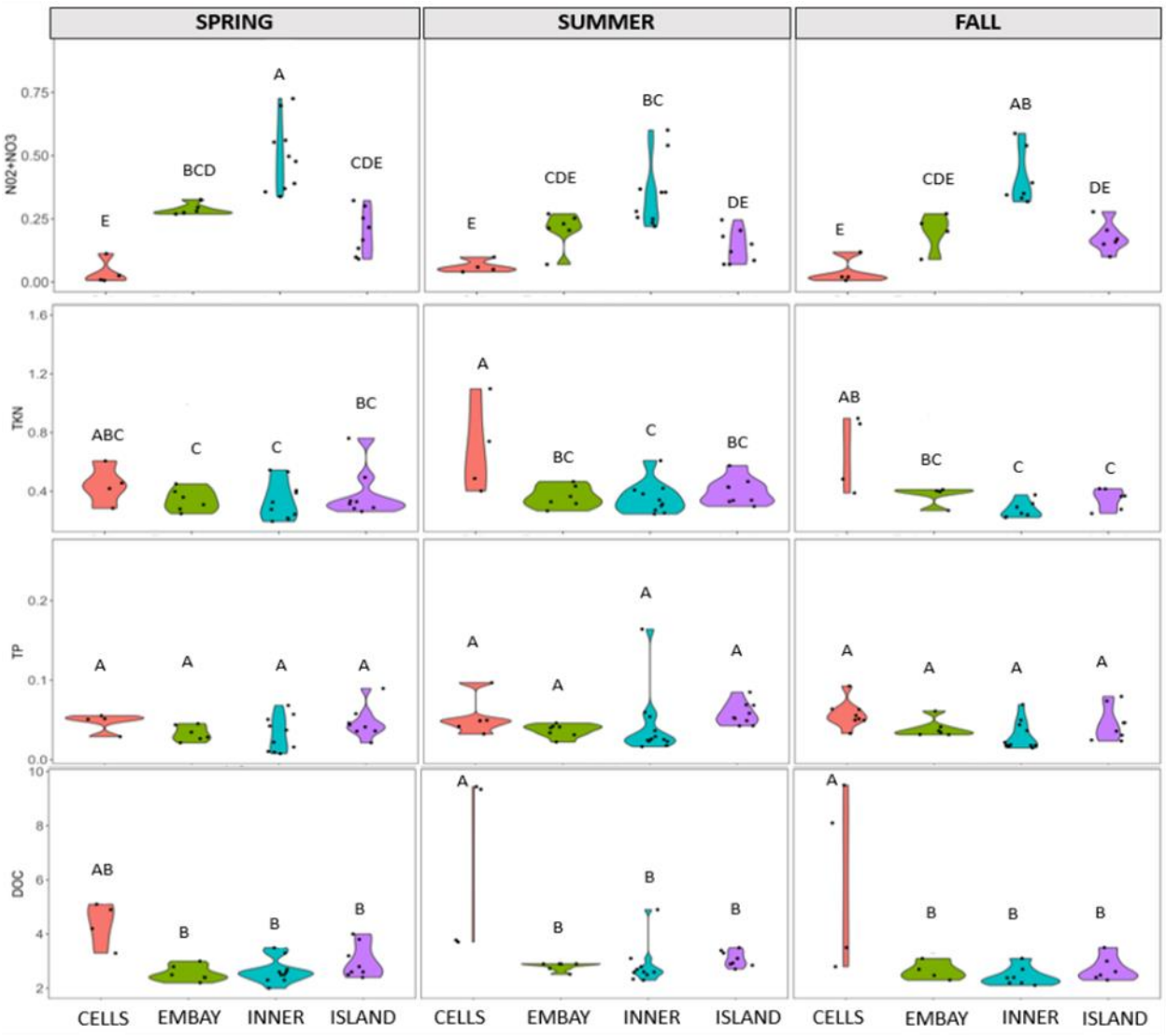

Figure A-15: Violin plots of A) Nitrite +Nitrate (mg L^{-1}) B) Total Kjeldahl Nitrogen (mg L^{-1}), C) Total Phosphorus (mg L^{-1}), and D) Dissolved Organic Carbon (mg L^{-1}) at Cell, Embayment, Inner Harbour and Island Channel stations in Toronto Harbour. These are from integrated water collections at a limited number of stations in spring, summer and fall from 2019 to 2022. Letters indicate statistically significantly distinct groups.

Table A-1: Additional study areas sampled by DFO between 2014 and 2019. E indicates epilimnetic sampling, and T indicates total water column sampling. Parameters measured include zooplankton (Z), water chemistry and primary production (C) and phytoplankton biomass and taxonomy (P).

		Depth		Parameters Measured					
Area	**Station**	**(m)**	**Strata**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**
Hamilton Harbour	centre (258)	24	E**	Z, C, P*	-	Z, C, P*	-	-	Z, C, P
Bay of Quinte	Belleville (B)	5	T	-	Z, C, P*	Z, C*	Z, C, P	Z, C, P	-
Lake Ontario	east (81)	35	E	-	Z, C, P*	Z, C*	Z, C, P*	C, P*	C*
Lake Ontario	West (BUR)	8	T	-	Z, C	Z, C	Z, C, P	Z, C, P	
Lake Ontario	West (LO2)	60	E	-	C, P	Z, C	Z, C, P	Z, C, P	C, P
Lake Ontario	West (BRO)	7	T	-	-	Z	-	-	-

* indicates approximate biweekly sampling. The rest were sampled monthly

** Total water column sampled for zooplankton

Table A-2: Feeding guilds of fishes caught in the Toronto area by boat electrofisher, 2013 to 2022. The * indicates the dominant fish species (by biomass) in each guild, and ** represents those with the highest biomass. Data provided by TRCA.

Benthivores	**Omnivores**
Brown Bullhead*	Banded Killifish
Common Carp**	Brook Stickleback
Freshwater Drum*	Central Stoneroller
Goldfish	Gasterosteidae
Goldfish x Common Carp hybrid	Hornyhead Chub
Logperch	Lake Chub
Round Goby	Pumpkinseed*
Rudd	Threespine Stickleback
White Sucker*	Yellow Perch*
	White Perch
Piscivores	
American Eel	**Planktivores**
Black Crappie (large)	Alewife*
Bowfin*	Black Crappie (small and med.)
Brown Trout	Blacknose Dace
Burbot	Bluegill*
Chinook Salmon**	Bluntnose Minnow
Coho Salmon	Brook Silverside
Largemouth Bass*	Common Shiner
Longnose Gar	Creek Chub
Northern Pike**	Emerald Shiner*
Rainbow Trout	Fathead Minnow
Rock Bass*	Gizzard Shad**
Sea Lamprey	Golden Shiner
Smallmouth Bass	Lepomis sp.
Walleye	Longnose Dace
White Bass	Rainbow Smelt
	Spotfin Shiner
	Spottail Shiner

Table A-3: Area designation and description of the TRCA electrofishing transects used in the DFO Toronto Harbour lower food web assessment.

Area	nearby DFO Stn	TRCA Description
Cells	c1	Tommy Thompson Park Cell 1 - East
Cells	c1	Tommy Thompson Park Cell 1 - West
Cells	c2	Tommy Thompson Park Cell 2
Cells	c2	Tommy Thompson Park Cell 2 - Fish Rescue
Cells	c2	Tommy Thompson Park Cell 2 East
Cells	c2	Tommy Thompson Park Cell 2 West
Cells	cD	Tommy Thompson Park Embayment D
Cells	cD	Tommy Thompson Park Embayment D Carp Removal
Cell 3	e3	Tommy Thompson Park Cell 3
Outer/Embay	T1	Cherry Beach North Shore
Outer/Embay	e6	Hearn Generating Station
Outer/Embay	e6	Rat Spit
Outer/Embay	T1	Tommy Thompson Park Embayment C- Outside
Outer/Embay	T1	Tommy Thompson Park Embayment D - Outside
Outer/Embay	e6	Tommy Thompson Park - West Shore
Outer/Embay	e6	Tommy Thompson Park Outer Harbour Marina
Outer/Embay	eA	Tommy Thompson Park Embayment A
Outer/Embay	eB	Tommy Thompson Park Embayment B
Outer/Embay	eC	Tommy Thompson Park Embayment C - Nw Footpad
Outer/Embay	eC	Tommy Thompson Park Embayment C - Se Footpad
Outer/Embay	eC	Tommy Thompson Park Embayment C Mouth- West Side
Outer/Embay	eC	Tommy Thompson Park Embayment C North East Shore
Outer/Embay	eC	Tommy Thompson Park Embayment C South Shore
Inner	T12	Cousins Quay
Inner	T12	Don River Keating Channel
Inner	T12	Inner Harbour Essroc Quay Stockpile Monitoring
Inner	T12	Inner Harbour Keating Channel West of Cherry St
Inner	T11	Spadina Quay Outer Breakwall
Inner	T11	Toronto Harbour - Billy Bishop Inner Exclusion Zone
Inner	T11	TORONTO HARBOUR BATHURST SLIP
Inner	T12	TORONTO HARBOUR BREAKWALL BETWEEN JARVIS AND PARLIAMENT SLIPS
Inner	T12	TORONTO HARBOUR JARVIS ST SLIP
Inner	T12	TORONTO HARBOUR PARLIAMENT SLIP
Inner	T4	Toronto Harbour Peter Street Slip
Inner	T4	TORONTO HARBOUR REESE SLIP
Inner	T4	TORONTO HARBOUR SIMCOE SLIP
Inner	T11	Toronto Harbour Spadina Quay
Inner	T11	TORONTO HARBOUR SPADINA SLIP
Inner	T4	Toronto Harbour York Quay
Inner	T13	Toronto Islands South Shore- look out point to Eastern gap
Islands	i3	Toronto Islands - Marina Southern Embayment
Islands		Toronto Islands - Seneca Avenue
Islands	i4	Toronto Islands- Centre Island
Islands	i2	Toronto Islands Donut Island
Islands	i2	Toronto Islands Donut Island Site 3
Islands	i2	Toronto Islands Donut Island Site 4
Islands	i3	Toronto Islands Forestry Island/radio Towers
Islands	i3	Toronto Islands Franklin Gardens
Islands	i1	Toronto Islands Lighthouse Bay
Islands	i2	Toronto Islands Long Pond
Islands	i4	Toronto Islands Sunfish Cut
Islands	i1	Toronto Islands Trout Pond
nearshore		Ontario Place Exposed Shore
nearshore		Tommy Thompson Park - Northeast Shore
nearshore		Tommy Thompson Park East Side
nearshore		Tommy Thompson Park Lighthouse Point
nearshore		Toronto Harbour - Billy Bishop Outer Exclusion Zone
nearshore		Toronto Islands between Gibraltar and Wards Island
nearshore		Toronto Islands Gibraltar Point
nearshore		Toronto Islands Wards Island

Table A-4: Average water chemistry parameter by station and season in Toronto Harbour 2019-2022.

		Spring									Summer									Fall								
		c2	c3	cD	eC	T1	T4	T12	i1	i4	c2	c3	cD	eC	T1	T4	T12	i1	i4	c2	c3	cD	eC	T1	T4	T12	i1	i4
Nitrate+ Nitrite filtered	Mean	0.07	0.28	0.01	0.30	0.36	0.43	0.63	0.15	0.24	0.07	0.19	0.05	0.23	0.30	0.28	0.44	0.12	0.16	0.07	0.17	0.01	0.27	0.32	0.34	0.51	0.15	0.20
(mg L^{-1})	Std Dev	0.06	0.01	0.00	0.03	0.04	0.07	0.09	0.05	0.10	0.04	0.09	0.01	0.03	0.08	0.06	0.16	0.06	0.07	0.07	0.07	0.01	.	.	0.01	0.10	0.05	0.07
	N	2	4	2	2	2	4	4	4	4	2	4	2	2	2	4	4	4	4	2	3	2	1	1	3	3	3	3
Total Kjeldahl Nitrogen	Mean	0.35	0.37	0.53	0.28	0.20	0.27	0.47	0.37	0.40	0.53	0.36	0.61	0.30	0.26	0.28	0.40	0.36	0.32	0.39	0.39	0.83	0.27	0.22	0.25	0.31	0.41	0.29
(mg L^{-1})	Std Dev	0.09	0.07	0.11	0.04	0.01	0.05	0.08	0.08	0.24	0.19	0.08	0.33	0.04	0.01	0.07	0.15	0.05	0.02	0.01	0.02	0.04	.	.	0.02	0.06	0.04	0.05
	N	2	4	2	2	2	4	4	4	4	2	4	2	2	2	4	4	4	4	2	3	2	1	1	3	3	3	3
Total Nitrogen filtered	Mean	0.407	0.634	0.482	0.583	0.567	0.675	1.055	0.564	0.571	0.595	0.552	0.669	0.531	0.558	0.558	0.843	0.482	0.484	0.452	0.556	0.817	0.540	0.540	0.587	0.811	0.599	0.492
(mg L^{-1})	Std Dev	0.030	0.071	0.137	0.012	0.025	0.070	0.166	0.158	0.028	0.233	0.135	0.327	0.072	0.100	0.132	0.306	0.080	0.089	0.045	0.089	0.004	.	.	0.038	0.159	0.052	0.073
	N	2	4	2	2	2	4	4	4	4	2	4	2	2	2	4	4	4	4	2	3	2	1	1	3	3	3	3
TP unfiltered mg L-1	Mean	0.042	0.034	0.051	0.031	0.009	0.022	0.053	0.056	0.037	0.047	0.035	0.065	0.038	0.025	0.021	0.069	0.063	0.052	0.065	0.040	0.049	0.034	0.018	0.018	0.044	0.056	0.026
(mg L^{-1})	Std Dev	0.019	0.012	0.001	0.004	0.002	0.014	0.013	0.024	0.011	0.004	0.010	0.046	0.006	0.001	0.004	0.055	0.015	0.012	0.019	0.012	0.013	.	.	0.003	0.018	0.019	0.004
	N	2	4	2	2	2	4	4	4	4	3	4	2	3	2	5	5	5	4	4	5	4	1	1	5	5	5	3
Soluble Reactive Phospho	Mean	0.005	0.003	0.001	0.005	0.000	0.000	0.008	0.001	0.003	0.013	0.008	0.011	0.005	0.001	0.004	0.013	0.012	0.012	0.005	0.004	0.005	0.006	0.001	0.007	0.014	0.017	0.005
(mg L^{-1})	Std Dev	0.006	0.003	0.001	0.006	0.000	0.000	0.007	0.001	0.005	0.005	0.004	0.003	0.005	0.001	0.003	0.011	0.011	0.008	0.002	0.003	0.005	.	.	0.000	0.008	0.013	0.004
	N	2	4	2	2	2	4	4	4	4	2	4	2	2	2	4	4	4	4	2	3	2	1	1	3	3	3	3
Dissolved Inorganic C	Mean	24.3	23.7	32.4	21.9	21.6	23.1	28.0	23.2	23.1	23.2	22.6	32.7	22.2	22.2	22.9	24.1	23.6	23.6	25.5	23.8	50.3	23.7	22.4	23.2	25.5	25.6	24.0
(mg L^{-1})	Std Dev	1.9	0.7	0.6	0.7	0.8	1.2	1.8	1.1	1.1	1.1	0.7	0.4	0.0	0.2	0.9	2.6	0.8	1.1	0.0	0.5	0.1	.	.	1.2	2.1	1.0	0.2
	N	2	4	2	2	2	4	4	4	4	2	4	2	2	2	4	4	4	4	2	3	2	1	1	3	3	3	3
Dissolved Organic C	Mean	3.75	2.53	5.00	2.60	2.45	2.38	2.98	3.05	2.93	3.75	2.76	9.39	2.90	2.70	2.53	3.23	3.13	3.04	3.15	2.63	8.80	2.70	2.40	2.34	2.56	2.84	2.60
(mg L^{-1})	Std Dev	0.64	0.34	0.14	0.28	0.21	0.30	0.50	0.72	0.60	0.05	0.18	0.07	0.00	0.14	0.17	1.16	0.26	0.35	0.49	0.42	0.99	.	.	0.32	0.47	0.58	0.36
	N	2	4	2	2	2	4	4	4	4	2	4	2	2	2	4	4	4	4	2	3	2	1	1	3	3	3	3
Extracted Chl_a_uncorrec	Mean	.	6.22	.	7.97	3.50	3.42	7.96	17.93	16.47	7.49	10.00	18.94	8.57	5.09	8.20	10.53	18.15	15.51	5.37	11.24	15.70	11.93	3.75	2.70	2.69	8.30	11.45
(µg L^{-1})	Std Dev	.	3.78	.	7.35	2.99	0.46	2.73	6.02	12.08	2.81	4.20	23.99	1.49	0.87	5.85	7.09	12.89	5.90	2.08	3.95	11.13	.	.	2.26	0.91	6.32	12.48
	N	0	2	0	2	2	2	2	2	2	3	4	2	3	2	5	5	5	4	4	5	4	1	1	5	5	5	3
Secchi depth	Mean	0.5	2.0	2.1	2.5	3.1	2.8	1.9	1.7	1.8	0.3	2.5	1.3	2.9	2.5	2.1	1.6	2.5	2.1	0.3	1.9	1.6	1.5	3.5	3.3	2.3	2.8	3.1
(m)	Std Dev	0.1	0.8	0.1	1.4	0.7	0.4	0.8	0.7	0.2	0.1	0.3	1.1	0.5	0.0	0.3	1.0	0.8	0.4	0.2	0.4	0.3	.	.	1.7	1.4	1.0	0.6
	N	2	4	2	2	2	4	4	4	4	4	4	2	3	2	5	5	5	4	4	5	4	1	1	5	5	5	2
Light attenuation	Mean	1.41	0.71	1.01	0.55	0.34	0.45	0.75	0.92	1.28	.	0.63	.	0.64	0.37	0.51	3.97	0.87	0.74	5.13	0.92	1.51	0.76	0.54	0.70	0.79	0.85	0.71
(m^{-1})	Std Dev	.	0.35	.	0.17	0.15	0.10	0.24	0.20	0.67	.	0.11	.	0.07	.	0.22	6.23	0.14	0.40	0.72	0.36	0.39	.	.	0.70	0.39	0.19	0.31
	N	1	4	1	2	2	4	3	4	4	0	3	0	2	1	5	4	5	4	2	5	2	1	1	5	5	4	2

Table A-5: The table presents the PCA results, showcasing the calculated values for PC1 and PC2. Each row corresponds to a specific category or group within the dataset; Cells, Embayments, Inner and Islands sites. PC1 and PC2 represent principal components from the data analysis showing the variance and relationships of the different study areas.

Area	PC1	PC2
CELLS	-2.82581	1.537125
CELLS	-2.07768	0.790049
CELLS	-3.78776	-0.83766
EMBAY	1.415628	0.407551
EMBAY	1.476999	1.230345
EMBAY	1.149612	-0.97396
INNER	1.398881	1.080126
INNER	1.335545	0.855522
INNER	1.005335	-1.18974
ISLD	0.491072	1.081371
ISLD	0.316556	-0.21486
ISLD	0.101629	-3.76587