

STATUS REPORT

Assessment of the Degradation of Phytoplankton and Zooplankton Populations (BUI #13) in the Detroit River Canadian Area of Concern

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Introduction

Plankton are small (usually microscopic), floating organisms that live in freshwater and marine ecosystems. Phytoplankton include diatoms, desmids, and algae that require photosynthesis to live, while zooplankton are small animals that feed on the tiny phytoplankton. Zooplankton become food for fish and other organisms, some of which are consumed by people. For a healthy food web, there must be enough plankton to form a strong base of the food web, or else, other organisms that depend on plankton for food may be negatively impacted. Together, phytoplankton and zooplankton make up an important part of the aquatic food web and are sensitive to changes in water quality; which makes them important indicators of environmental quality.

The Detroit River was listed as a Great Lakes Area of Concern (AOC) in 1987 under the Great Lakes Water Quality Agreement (GLWQA) due to the degradation of water quality that was sufficient to impair one or more of 14 beneficial uses specified under the Agreement for AOCs. To guide the restoration of beneficial use impairments (BUI) in each AOC, Remedial Action Plans (RAPs) and restoration targets for each BUI were developed by local RAP Teams. Once all necessary actions are completed and the restoration targets, commonly referred to as BUI delisting criteria, are met for all impairments, the AOC can be removed from the list of AOCs under the GLWQA. The removal of an AOC from the list, also known as delisting an AOC, is the ultimate goal of the RAP Team.

The RAP Stage I report (MDNR and OMOE 1991), published in 1991, described the environmental condition of the AOC and using the best available science, designated each BUI with a status of impaired or not impaired. When there was insufficient or inconclusive data, a BUI could be deemed requires further assessment (RFA). Generally, an impaired plankton population may be evident when there is not enough plankton available to form the basis of a healthy food web, or when there is too much plankton and algal blooms form (algae are a form of plankton). For the Degradation of Phytoplankton and Zooplankton Populations BUI (BUI #13), the BUI status was deemed not impaired based on the density, diversity, and species composition of phytoplankton and zooplankton in the Detroit River. However, the composition of permanent, nearshore zooplankton populations was not examined at the time, thus it was noted that further assessment of the nearshore zooplankton communities was needed (MDNR and OMOE 1991). The status remained not impaired in the 1996 RAP Update Report, but one reviewer commented that phytoplankton bioassays suggested impairment (MDEQ 1996) based on Trenton Channel sediment, located on the U.S. side of the river. In 2006, Leney and Haffner (2006) proposed that the status be changed to Requires Further Assessment (RFA) due to limited information about the composition of Detroit River phytoplankton and zooplankton.

In the 2010 Stage II RAP report (Green et al. 2010), the status of the Degradation of Phytoplankton and Zooplankton BUI was changed to RFA and delisting criteria was developed. The delisting criteria stated that this BUI will be considered not impaired *“when the composition and relative abundance of phytoplankton and zooplankton of the Detroit River reflect that of Lake Huron, and therefore represent primarily oligotrophic/mesotrophic conditions.”*

The purpose of this report is to provide a summary of the studies completed since 2006, all of which indicate that the Degradation of Phytoplankton and Zooplankton BUI is not impaired for the Detroit River AOC. The results of the most recent and comprehensive study conducted by the Department of

Fisheries and Oceans in 2019 are appended, and ultimately provide the rigorous scientific evidence required to support a not impaired status for this BUI in the Detroit River AOC.

Studies to Date

Great Lakes Institute for Environmental Research (Haffner et al. 2008)

A study was conducted by the University of Windsor Great Lakes Institute for Environmental Research between August and November 2007 to determine if the composition and relative abundance of species of phytoplankton and zooplankton in the waters of the Detroit River changed from upstream to downstream. Samples were taken in the Detroit River at the headwaters (just below Peche Island), mid-section (at Zug Island) and at the discharge to Lake Erie (Celeron Island). Three stations were sampled at each location representing 20 m from the Canadian shore, mid-stream, and 20 m from the American shore. Sub-samples were taken and analyzed for biological parameters such as phytoplankton biomass and chlorophyll-*a*.

The concentration of chlorophyll-*a* in the Detroit River was found to be very low (1 - 10 µg/L) from upstream to downstream sampling locations as the water moved through the connecting channel. The very low chlorophyll-*a* data were representative of low phytoplankton populations indicative of oligotrophic conditions which would be expected considering over 99% of the water mass originated from the more oligotrophic Lake Huron only 21-24 hours previously. The abundance of phytoplankton communities did not change significantly from upstream to downstream locations. The community assemblages in the Detroit River were comprised mostly of oligotrophic and mesotrophic species (e.g., *Rhodomonas*, *Chlamydomonas*, *Dinobryon*, green algae in a chain, diatoms) originating from Lake Huron. Similar to the phytoplankton community, the zooplankton community assemblage was quite typical of the oligotrophic-mesotrophic Lake Huron community composition (e.g., copepods (calanoid, cyclopoid), rotifers, cladocerans (*Bosmina*)). There was no indication of a change in composition and relative abundance of the overall Detroit River zooplankton community from upstream to downstream sampling locations.

Overall, phytoplankton abundance in the Detroit River was low; however, this result is not surprising considering waters in the Detroit River is replaced in approximately 21 hours and is representative of Lake Huron waters. **The phytoplankton and zooplankton community assemblages were shown to be indicative of oligotrophic-mesotrophic conditions that are representative of the upstream waters of Lake Huron.** There were no obvious signs of deformities in zooplankton from the Detroit River.

Canada Centre for Inland Waters (S. Watson, unpublished data)

Sixty-five archived plankton samples collected during different surveys were analyzed to compare plankton conditions within the Detroit River with reference conditions at the Lake Huron outflow. Samples were collected in 2006, 2010, and 2013 from the Detroit River. The results of the analysis indicate that there was low-moderate total biomass and chlorophyll-*a*, indicating oligo-mesotrophic conditions within the Detroit River. There was moderate taxonomic diversity and a slight change in species and biomass from Lake Huron and St. Clair River sites. There was also a similar abundance of heterotrophs and microzooplankton and the Detroit River had lower average biomass than Lake St. Clair. In the Detroit River, there was also a predominance of small-celled plankton and typical river/benthic taxa. **In summary, this analysis of Detroit River surface samples showed no evidence of any significant degradation of phytoplankton and zooplankton communities.**

Great Lakes Institute for Environmental Research (Drouillard et al., 2017)

A survey was conducted between June and November 2015 to cover spatial and seasonal changes in the zooplankton community structure in both nearshore and offshore waters of the Detroit River AOC and in Lake St. Clair (reference site).

Twelve locations were sampled consisting of triplicate sites in nearshore and offshore waters of the upper Detroit River and lower Detroit River. There were three offshore locations and three nearshore locations sampled for the reference area Lake St. Clair. Samples from each location were collected in June, August, and November to capture a spring, summer, and fall seasonal time series. Preserved net samples were submitted for zooplankton characterization, including identification of zooplankton species (to nearest practical taxonomic unit), abundance, mean body length, and biomass.

Data analysis compared zooplankton quality in the AOC relative to the appropriate habitat reference across 8 quality metrics previously identified in published plankton biological integrity indices. For each quality metric a zooplankton quality score was assigned a value of 0, 1, or 2, whereby 0 indicates lower quality compared to the reference, 1 indicates equivalent quality relative to the reference, and 2 represents higher quality compared to the reference. A final percentage Quality Score was then compiled by summing the individual quality scores across the 8 metrics and referencing to an unimpaired score of 8 in percentage points. Thus, a weight of evidence score of 100% would be indicative of an unimpaired status. Scores exceeding 100% would indicate that zooplankton quality in the AOC exceeds that of the reference while scores below 100% would be interpreted as evidence for impairment. The overall mean±standard error zooplankton quality score was 100.8 ±4.3% for the AOC, indicative of an unimpaired status. The mean±standard error zooplankton quality scores in the nearshore zone was 103.1±3.1% and in the offshore zone it was 98.4±8.3%. Although slightly less than 100%, the offshore zooplankton percentage Quality score was not significantly different than 100% nor was it significantly different from the nearshore score. The combined zooplankton percentage Quality Score for the Upper Detroit River was 100.0±7.8% and for the lower Detroit River it was 101.6±4.4%.

It was concluded that the overall quality of zooplankton communities in the Canadian waters of the Detroit River AOC does not differ from its nearest upstream water body, Lake St. Clair. As such, **it was recommended that the zooplankton community component of BUI #13 be considered unimpaired for the Canadian waters of the AOC.**

Department of Fisheries and Oceans (DFO; Fitzpatrick et al., 2020)

The most recent study of phytoplankton, zooplankton, and microbial communities in the Detroit River was conducted by the Department of Fisheries and Oceans during May, July, September, and November of 2019. During each survey, 8 sites from the head of the Detroit River at Lake St Clair to Amherstburg were sampled capturing both upstream – downstream as well as nearshore – offshore gradients. This is the most comprehensive study of the microbial – planktonic communities undertaken within the AOC to date. The results of this assessment indicate that there is **no evidence of anthropogenic impairment of the phytoplankton and zooplankton populations and microbial loop of the Detroit River.** The DFO technical report is included as Appendix 1 of this report.

Key findings from this assessment include:

- Phytoplankton biomass was low in the river and the composition was generally variable including species of Diatomeae, Chlorophyta, Cyanophyta, Chrysophyceae and Cryptophyceae.

- Despite the low phytoplankton biomass, primary production rates were moderately high indicating that the river supports viable phytoplankton populations.
- Heterotrophic microbial organisms are important contributors to the lower food web of the Detroit River.
- Approximately 80% of the organic carbon pool was autotrophic (meaning it is able to photosynthesize to produce its own food; phytoplankton and autotrophic picoplankton) and not likely being sequestered by heterotrophic nanoflagellates as observed in impaired systems like the Bay of Quinte.
- Phytoplankton biomass and primary productivity were significantly higher in the lower flow nearshore than offshore areas as expected in riverine environments.
- Zooplankton biomass in the Canadian waters of the Detroit River was typically very low and attenuates with increasing distance downstream.
- Zooplankton communities are comprised primarily of dreissenid veliger larvae, the small cladoceran *Bosmina*, and both calanoid and cyclopoid copepods. Aside from some adult copepods, the zooplankton community tends to be comprised of small taxa. Rotifers in the river also appear to be relatively unimportant
- The extremely low biomass, and loss of zooplankton with increasing distance downstream in the Detroit River is an expected consequence of riverine conditions, and not necessarily indicative of anthropogenic stresses.
- Approximately 80% of the organic carbon pool was autotrophic, indicating a lack of anthropogenic stress on phytoplankton and zooplankton populations in the river. When comparing the organic carbon pool composition from the Detroit River to other Areas of Concern, the organic carbon pool in the Detroit River has the highest percentage of phytoplankton (Table 1).

Table 1 Percent phytoplankton in the organic carbon pool in various AOCs

AOC	Beneficial Use Status	Phytoplankton
Detroit River	RFA	83.2%
Bay of Quinte	Impaired	63.5%
Hamilton Harbour	RFA	62.4%
Inner Toronto Harbour	RFA	44.8%
Outer Toronto Harbour	RFA	39.1%

- Overall, **the planktonic communities were found to behave as expected in a high flow connecting channel, responding more to seasonality and the physical regime than to factors attributable to human impairments.**

Status Recommendation

The status of this BUI has changed from not impaired in the 1991 Stage I report to requires further assessment in the 2010 Stage II report as there was limited data on the nearshore plankton community composition. Over the past decade, several studies have been undertaken to determine the status of the BUI with the most comprehensive completed by the Department of Fisheries and Oceans in 2019. In this study, the changes in biomass and community between upstream and downstream and the offshore

and nearshore, were consistent with a riverine environment and there was no evidence of impairment attributed to human activities within the AOC.

As indicated in the Stage II RAP report, this BUI was deemed RFA and the delisting criteria for this BUI stated it will be considered not impaired *“when the composition and relative abundance of phytoplankton and zooplankton of the Detroit River reflect that of Lake Huron, and therefore represent primarily oligotrophic-mesotrophic conditions”*.

While the delisting criteria specifically identified Lake Huron as a reference site, the results of the most recent DFO study clearly show that this is not possible due to the changes in the phytoplankton and zooplankton communities that occur as the water flows from Lake Huron to the Detroit River. Although the phytoplankton and zooplankton communities are not reflective of Lake Huron, the DFO study found the phytoplankton and zooplankton populations to be consistent with a riverine environment with no evidence of an impairment attributed to human activities within the AOC. The DFO study findings, in addition to findings of earlier studies completed between 2008 and 2017, provide ample evidence to support a not impaired status for this BUI.

Based on the body of evidence collected and considered by the DRCC, the DRCC is pleased to recommend the status of the *Degradation of Phytoplankton and Zooplankton Populations* BUI be designated as not impaired for the Detroit River AOC.

Work Group, Four Agency Managers Committee, Indigenous, and Public Engagement

See Appendix 2 for details on engagement with various groups on this BUI status assessment.

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APPENDIX 1: Assessment of BUI #13 Degradation of Phytoplankton and Zooplankton Populations in the Canadian Detroit River Area of Concern

Assessment of BUI #13 Degradation of Phytoplankton and Zooplankton Populations in the Canadian Detroit River Area of Concern

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Executive Summary

Phytoplankton, zooplankton and microbial communities of the Detroit River were sampled during May, July, September and November of 2019 in order to provide guidance regarding the status of Beneficial Use Impairment 13: Degradation of Phytoplankton and Zooplankton Populations. During each survey, 8 sites from the Detroit River entry at Lake St Clair to Amherstburg were sampled capturing both upstream – downstream as well as nearshore – offshore gradients. To our knowledge, this is the most comprehensive study of the microbial – planktonic communities undertaken within the AOC. Ideally, for due diligence we would recommend that this type of survey be conducted over multiple years in order to confirm the results. Biomass of each of the components including: phytoplankton, autotrophic picoplankton, heterotrophic nanoflagellates, ciliates, rotifers and zooplankton, was generally low with zooplankton biomass attenuating significantly downstream. Phytoplankton biomass was observed to be significantly, though modestly, higher nearshore than offshore. Primary (phytoplankton) productivity was moderately high, given the low phytoplankton biomass, and demonstrates the river supports viable phytoplankton assemblages. In general, the observed differences in the planktonic communities occurring within the AOC could be attributed directly to riverine ecology. Likewise, our assessment of the organic carbon pool showed that, despite the low overall amount ($50 - 100 \text{ mg C m}^{-3}$ on average), the food web was still predominantly autotrophic and not bound up in heterotrophic microbes. Our survey did not find any evidence of anthropogenic impairment of the phytoplankton, zooplankton and microbial loop populations of the Detroit River.

Introduction

The Detroit River is a 45.3 km long connecting channel receiving waters from Lake St. Clair and terminates at Lake Erie (Manny and Kenaga 1991; Derecki 1984). It represents an important hydrological and ecological link between the upper and the lower Great Lakes (Hartig et al. 2018). This urbanized watershed supports 5.5 million people across Canada and the US and has sustained rapid development since early European settlement (Hartig et al. 2009). The Detroit River has been subject to an extensive history of anthropogenic alterations in support of commercial industry and shipping, such as the creation of shipping channels, dredging, and shoreline hardening (Bennion and Manny 2011, Keeler et al. 2019). Other negative ecological impacts associated with this urban area include population growth, land use alteration, habitat loss/degradation, exotic species introduction, phosphorous discharges, and climate change (Hartig et al. 2009). Known as the automobile production capital of the world and a major producer of steel, Detroit's industry has deposited toxic substances and contaminants in the river (Manny and Kenaga 1991). Despite a long history of pollution and industry, the Detroit River supports biodiversity and remains an ecologically important Great Lakes ecosystem (McDonald et al. 2014).

As a result of extensive and long-standing environmental issues, the Detroit River was identified by the International Joint Committee (IJC) in 1985 as one of 42 Great Lakes Areas of Concerns (AOC). Remedial Action Plans (RAPs) were developed to identify specific measures aimed at restoring beneficial use impairments (BUIs). Within the Detroit River, BUI #13: *Degradation of Phytoplankton and Zooplankton Populations* delisting criteria and status have changed several times over the life of the RAP. In the 1990 Stage I RAP report, phytoplankton were stated to be similar to the upper Great Lakes and Lake St. Clair and not impaired, whereas further assessment of the nearshore zooplankton community was recommended (MDNR and MOE 1990). In 2005, the new delisting criteria for BUI #13 recommended the 'identification of 3 successive years data showing phytoplankton and zooplankton community structures are seasonally and spatially identified as high quality based on an objective and quantitative community analysis, an index of biological integrity, and/or a comparison to an appropriate control site in addition to bioassays and analysis of persistent, bioaccumulative substances' (DRCC 2005). In 2010 the recommendations from the Stage II RAP report for 'bioassays' and analyses for 'bioaccumulative substances' were dropped; the revised criteria once again called for composition and relative abundance of both phytoplankton and zooplankton populations reflecting that of Lake Huron. To date BUI #13 is listed as "Requires Further Assessment" and there remains a paucity of studies to provide direction on the delisting this action item (DRCC 2013).

Addressing BUI #13 is imperative because phytoplankton and zooplankton support the whole ecosystem, acting as vectors transferring energy from primary production to higher trophic levels (Wetzel and Likens 2000). In addition to the anthropogenic degradation within the Detroit River, other factors due to the riverine environment affect distribution and composition of plankton communities (Walks 2007). Specifically, in contrast to lakes, rivers exhibit high flow rates and shorter residency times resulting in dynamic hydrology (Edwards et al. 1989). A holistic approach is required to understand these planktonic communities because physical conditions (i.e. light, temperature, current) and nutrient levels (i.e. phosphorus and nitrogen) directly affect the amount of energy that will be transferred up the food web. It is important to have seasonal data to be able to estimate zooplankton growth and to have direct measures of growth of the primary producers. Additionally, these very tiny organisms exhibit complex interactions with the extended planktonic community, specifically bacteria, nanoflagellates, rotifers and ciliates, and as such these components are also important in understanding the overall health of the system (Fitzpatrick and Munawar 2019).

To date, studies examining zooplankton and phytoplankton communities in the Detroit River have been limited both temporally and spatially, resulting in insufficient information to assess BUI #13. Previous work has focused on other aspects on the Detroit River ecosystem, while incorporating plankton data (McDonald et al. 2014; Russell et al. 1999). For example, McDonald et al. (2014) examined several factors, including zooplankton densities, on distribution of larval fishes in the Detroit River. Additionally, there have been studies assessing bioaccumulation that have also included zooplankton and phytoplankton data (i.e. Russell et al. 1999). Keeler et al. (2019) found that the zooplankton community within the U.S. side of the Detroit River was dominated by cyclopoids and cladocerans. The authors also determined that zooplankton densities and biomass in the Detroit River were usually low compared to the rest of the St. Clair-Detroit River System, but they did not assess veligers of *Dreissena* or rotifers. Their taxa richness and diversity values were similar or higher than the rest of the system, likely due to the production associated with wetland outflow from Lake St. Clair. One major attempt to collect information specifically pertaining to the assessment of BUI #13, was conducted in 2015 (Drouillard 2017). However, the robustness of this study was compromised due to unrepresentative sampling, a lack of temporal resolution, issues with sampling gear and concern with assessment metrics and analyses (Rozon et al. 2019). Other work on Detroit River phytoplankton populations was conducted by Environment Canada between 2006 and 2013 (S. Watson unpublished data, Davis et al. 2014); however, these data were limited both temporally and spatially. In addition, there were issues including poor taxonomic identifications, sample degradation and lack of seasonal coverage. Broadly, previous plankton work has been conducted over short sampling periods, targeting other specific scientific questions (i.e. larval fish distribution or water contamination) or involved broad taxa identification. Most importantly, both of these studies attempted to determine the status of phytoplankton and zooplankton populations independent of each other and to the environment they were a part of.

In previous reports to Environment Canada (Fitzpatrick and Munawar 2019; Rozon et al. 2019) we documented what we felt were the major shortcomings with previous attempts to assess BUI 13 in the Detroit River AOC. These included: constantly shifting delisting criteria, a lack of phytoplankton sampling within the AOC, a separation of the phytoplankton component of the BUI from the zooplankton component, and confounding with BUI 8: *Eutrophication or undesirable algae* among others.

Our key recommendations were:

1. Be holistic: phytoplankton and zooplankton populations are inextricably linked and should not be assessed independently.
2. A clear, integrative, delisting criteria needs to encompass not only phytoplankton and zooplankton populations *per se* but also their interactions within the broader microbial-planktonic food web and ultimately their role in supporting and sustaining fish populations.
3. Delisting criteria should not be linked to trophic state.
4. In order to make a sound, scientifically defensible assessment of BUI 13, a spatially and temporally comprehensive food web study of the lower trophic levels that encompasses the structure and function of phytoplankton, zooplankton and microbial communities is called for.

To achieve a holistic understanding of the state of the planktonic food web, sampling needs to be conducted at appropriate spatial and temporal resolutions to ensure the data were representative of the Detroit River AOC phytoplankton and zooplankton populations. It is also crucial to include sampling of microbial loop (heterotrophic nanoflagellates, bacteria, autotrophic picoplankton and ciliates), rotifers, phytoplankton and zooplankton, in addition to instantaneous productivity rates along with nutrients and standard water quality parameters. Our objectives were: 1) determine if there was a

significant change in the condition of the planktonic communities occurring within the Area of Concern (assessed along nearshore – offshore and upstream – downstream gradients), and 2) if so, does it constitute a Beneficial Use Impairment? The overall intent of this study was to provide science-based and defensible recommendations regarding the delisting BUI #13.

Materials and Methods

Site Description and Study Design

We conducted 4 research cruises during 2019 on May 28, July 23, September 24 and November 6 to capture the seasonality of spring, summer and fall conditions. Eight sites were sampled from the Canadian waters during each cruise from Lake St. Clair to Amherstburg, ON (Figure 1, Table 1). Within this study area, site depths ranged from $\approx 2 - 15$ m (Table 1). In general, sites were chosen to capture upstream / downstream and nearshore / offshore gradients. We also attempted to include sites near habitat restoration projects, including offshore spawning shoals near Fighting Island, shoreline restorations at LaSalle and shoreline and watershed restoration at River Canard (Hartig et al 2018b). We also sampled downstream of Windsor's water treatment plant and the River Canard inflow, the main tributary to Detroit River. We did not sample downstream (south) of Boblo Island in order to avoid the stretch where the Detroit River mixes with Lake Erie.

Sample Collection and Analyses

At each site, a YSI Exo multiparameter sonde was used to measure temperature, dissolved oxygen, pH, turbidity, fDOM, chlorophyll *a*, phycocyanin, and specific conductivity. Light attenuation was measured with an RBR Duo PAR-D (photosynthetically active radiation-Depth) sensor. Surface flow rates were determined for the July, September and November cruises. Large navel oranges (citrus) were deployed in the river at each site over 4 – 5 minute intervals (Wetzel and Likens 2000). Start/Stop positions were tracked using the vessel's Raymarine GPS and plotted to determine surface flow rates at each site. Integrated water samples were collected using a 4 L bottle integrator, generally from 0 – 6 m depth at offshore sites and 0 – 2 m at nearshore sites. Water was stored in darkened, insulated carbuoys and kept on ice and out of direct sunlight. Subsamples were drawn for nutrients, chlorophyll *a*, size-fractionated primary productivity and bacterial growth assays and preserved for microscopic analyses of phytoplankton, microbial loop, and ciliates. All nutrient samples were filtered within 15 minutes of collection using 0.45 μm cellulose acetate inline syringe filters and stored on ice. Nutrient analysis, including total phosphorus (TP), nitrate + nitrite, and silica followed the standard protocol of the National Laboratory for Environmental Testing (NLET 1997). Chlorophyll *a* was determined by acetone pigment extraction (Strickland and Parsons 1972). For rotifers, an additional 8 L of water was filtered through 20 μm mesh and the rotifers remaining on the sieve were narcotized with carbonated water then preserved in 4% sugar buffered formalin (see Bowen 2017).

Size fractionated primary productivity and bacterial growth measurements were run the next day using water transported back to the Bayfield Institute. Size-fractionated primary productivity was estimated for three size categories of phytoplankton ($<2 \mu\text{m}$, $2 - 20 \mu\text{m}$ and $>20 \mu\text{m}$) by the ^{14}C Carbon technique following the standard protocol of Munawar and Munawar (1996). Whole water samples were spiked with $\text{Na}^{14}\text{CO}_3$, incubated for 4 hours at surface temperature and exposed to a constant light level of $240 \mu\text{E s}^{-1} \text{m}^{-2}$. Because light and temperature levels were constant, the results should be interpreted as potential rather than actual production. After incubation, size classes were determined by filtration of the sample through polycarbonate filters. All filters were rinsed with hydrochloric acid (0.5 N) in order to remove excess ^{14}C - CO_2 . Radioactivity was determined by liquid scintillation. Bacterial growth rates were

estimated by ^3H -Leucine incorporation into bacterial proteins following the protocol of Jørgensen (1992) and radioactivity was determined by liquid scintillation. Detailed procedures are available in Heath and Munawar (2004).

Phytoplankton, microbial loop and ciliate samples were preserved on the day of sampling. Phytoplankton samples were fixed with acidified Lugol's iodine upon collection. Identification and enumeration were carried out by the Utermöhl (1958) inverted microscope technique as described by Munawar and Munawar (1996). A minimum of 200 units were counted to achieve an acceptable counting efficiency (Lund et al. 1958). Within each sample, cell dimensions were measured directly and the average cell volume for each species was determined by applying the average cell dimensions to a standard geometric shape that most closely resembled the species. In the case of colonial forms, the average number of cells per colony was determined. Cell volume was converted to wet biomass assuming a specific gravity of 1.0 (Strickland 1960). Microbial loop samples, including bacteria, autotrophic picoplankton and heterotrophic nanoflagellates, were fixed with 1.6% formaldehyde and enumerated using DAPI staining (Porter and Feig 1980) under epi-fluorescence microscopy (Munawar and Weisse 1989). Wet weight biomass was estimated as $2000 \text{ fg cell}^{-1}$ for APP, 100 fg cell^{-1} for bacteria and 140 pg cell^{-1} for HNF (Sprules et al. 1999). Ciliate samples were preserved in acidified Lugol's iodine upon collection. Within 6 months, the samples were post-fixed by adding concentrated Bouin's fluid to a final concentration of 5% and stained using the Quantitative Protargol Stain (Montagnes and Lynn 1993). Abundance and bio-volume were calculated using standard geometric shapes and the Microbiota software developed by Roff and Hopcroft (1986). Bio-volume was converted to biomass assuming a specific gravity of 1 and 20% shrinkage after preservation and staining (Jerome et al. 1993).

Zooplankton (crustaceans + veligers of *Dreissena*) were collected by taking a vertical total water column net haul from 1 m off bottom to the surface, using a metered, $64 \mu\text{m}$ mesh, 40 cm diameter Wisconsin net. About 3 kg of dive weights were added to the net and samples were collected while the boat was drifting to better ensure vertical hauls. Maximum sampling depth was recorded with a Wildlife Computers MK9 tag. Zooplankton samples were preserved in 4% sugar buffered formalin. Zooplankton samples were counted and identified to the species or genus level whenever possible, as described in Appendix 1 of Bowen (2017). Calanoids in the genera *Leptodiaptomus* and *Skistodiaptomus* were only classified as diaptomids. Loose eggs were also enumerated and identified as cladoceran, cyclopoid or calanoid where possible. Animal lengths were used to estimate biomass based on the length-weight regressions summarized in Bowen (2017). Length measurements and egg counts were taken for 25 to 50 animals within each taxon at each station. For copepods, measurements were taken on the first 30 to 40 animals encountered in each order (calanoid or cyclopoid), and nauplii were measured separately. Total zooplankton lengths were weighted according to taxa density each cruise. This was done by adding up the length * density values for all the taxa within each sample, then dividing the sum by the total density in the sample. Rotifer samples from all four cruises were combined to create a May to November seasonal composite sample for each station. It was decided after a qualitative examination indicated these animals were relatively sparse, that rotifers would only be enumerated in the two upper and two lower river seasonal composite samples (DR1, DR2, DR7 and DR8). Rotifers were counted, measured and identified to the lowest possible taxonomic level by an experienced taxonomist (Bowen 2017).

Statistical Analyses

The statistical approaches used here were consistent with the methods used in other assessments of BUI 13 from this lab (Currie et al. 2017; 2018; Rozon et al. 2017). These included paired sample t-tests to assess nearshore vs offshore differences and exponential regressions to test for upstream vs

downstream differences. Since neither of these properly account for seasonal variation within the microbial and planktonic communities we also employed ANOVAs using “habitat” (nearshore/offshore) and “location” (upstream/downstream) as primary factors and “month” as the secondary factor. Differences between sites could then be evaluated through post-hoc testing, if applicable. Stepwise regressions were used to determine which of the physical and chemical factors were affecting the planktonic communities. All statistical analysis was completed using JMP v14.2 and Systat v11.

Results

Water Level and Site Conditions

Precipitation in 2019 was above average in the United States, with Michigan and other northern states experiencing the wettest year on record (NOAA 2020a). Water levels in lakes Michigan and Huron were within 2 cm of record high values for June, July and December of 2019, and the July peak was the second highest since 1918 (US Army Corps of Engineers 2020). These extremely high water levels resulted in extensive shoreline flooding and increased erosion along the Detroit River in 2020 (e.g., Battagello 2019, Williams 2019). Our 2019 estimates of surface current velocities at Windsor were 0.92 m s^{-1} in June, 0.83 m s^{-1} in September, and 1.25 m s^{-1} in November. In 2019, provisional current velocities just south of the Ambassador Bridge were similarly elevated, averaging around 0.95 m s^{-1} in May, July and September, and 1.03 m s^{-1} in November (USGS 2020). Over the 2015 to 2018 period, USGS means for these same months ranged from 0.87 m s^{-1} to 0.91 m s^{-1} . In comparison, NOAA (2020b) reports that under the typical high discharge rate of $5947 \text{ m}^3 \text{ s}^{-1}$, the average high current velocity just south of the Ambassador Bridge is only 0.72 m s^{-1} . Discharge rates at this site in 2019 ranged from $6826 \text{ m}^3 \text{ s}^{-1}$ in September to $7198 \text{ m}^3 \text{ s}^{-1}$ in November (USGS 2020).

Seasonal Water Chemistry and Microplankton

Spring - May 28, 2016

During the May survey, surface temperatures ranged from $13.9 - 15.8 \text{ }^{\circ}\text{C}$; light attenuation, calculated as the vertical PAR attenuation coefficient (k_d) ranged from $0.5 - 0.8 \text{ m}^{-1}$; total phosphorus concentrations ranged from $8.2 - 16.4 \text{ } \mu\text{g l}^{-1}$; nitrate + nitrite from $0.7 - 0.7 \text{ mg l}^{-1}$, and silica from $1.0 - 1.3 \text{ mg l}^{-1}$. Chlorophyll *a* over this period was low, ranging between 1.2 and $2.1 \text{ } \mu\text{g l}^{-1}$ (Appendix 1).

For primary productivity, total productivity ranged from $0.8 - 7.1 \text{ mg C m}^{-3} \text{ h}^{-1}$ in May, the nanoplankton ($2 - 20 \text{ } \mu\text{m}$) size class was most active accounting for $50.8 - 82.5\%$ of the total carbon uptake followed by picoplankton ($14.3 - 28.2\%$; Figure 2 a). Larger net plankton typically accounted for less than 8% of the total primary productivity. Bacterial productivity ranged from $0.06 - 0.9 \text{ mg C m}^{-3} \text{ h}^{-1}$ at this time.

Phytoplankton biomass in May ranged from $254.6 - 410.6 \text{ mg m}^{-3}$ with diatoms (Diatomeae) accounting for roughly $50 - 80\%$ of the total biomass, followed by Chlorophyta ($7 - 26\%$) and Chrysophyceae ($5 - 24\%$; Figure 2 b). Dominant species (contributing $>5\%$ to total biomass) observed during this time included: *Cyclotella ocellata* (Diatomeae), very small ($<2 \text{ } \mu\text{m}$) Chlorococcales (Chlorophyta) and species of *Mallomonas*, *Gyromitus* and *Ochramonas* (Chrysophyceae; Table 2a).

Regarding the microbial loop, autotrophic picoplankton (APP) biomass ranged from $0.1 - 0.6 \text{ mg m}^{-3}$, Bacteria biomass ranged from $28.2 - 56.2 \text{ mg m}^{-3}$, and heterotrophic nanoflagellates (HNF) biomass ranged from $3.2 - 151.2 \text{ mg m}^{-3}$ (Figure 2 c). Ciliate biomass ranged from $5.3 - 20.9 \text{ mg m}^{-3}$ with *Strombidium* spp. representing the largest identifiable taxonomic group.

Total zooplankton biomass ranged from 0.4 – 5.8 mg m⁻³ (dry weight, Figure 2 d). Dreissenid veligers were the largest component of the zooplankton biomass (0.1 – 4.0 mg m⁻³) followed by Calanoids (0.2 – 1.3 mg m⁻³) and Cyclopoids (0.02 – 0.5 mg m⁻³).

Summer - July 23, 2019

The July cruise was characterized by surface temperatures ranging from 23.6 – 24.6 °C, and light attenuation (k_d) from 0.2 – 1.2 m⁻¹ (Appendix 1). Current speeds ranged from 0.1 – 0.3 m s⁻¹ nearshore and 0.6 – 0.9 m s⁻¹ offshore. Total phosphorus throughout the river ranged from 4.9 – 19.3 µg l⁻¹; nitrate + nitrite showed little variability: 0.25 – 0.29 mg l⁻¹, and silica ranged from 1.4 – 1.6 mg l⁻¹. Chlorophyll *a* during July ranged from 0.5 – 3.8 µg l⁻¹ in the Detroit River.

Primary productivity in July ranged from 1.7 – 15.9 mg C m⁻³ h⁻¹ (Figure 3 a). Nanoplankton (2 – 20 µm) was generally the most active size class, accounting for 32.6 – 57.0% of the total productivity followed by picoplankton (18.5 – 51.9%) and net plankton (12.6 – 33.8%). Bacterial productivity ranged from 0.6 – 1.7 mg C m⁻³ h⁻¹.

Phytoplankton biomass during July ranged from a low of 141.9 mg m⁻³ to a high of 868.0 mg m⁻³ (Figure 3 b). Taxonomic composition showed more variety in July compared to May. The most prevalent taxonomic groups, based on contribution to total biomass, were: Chlorophyta (11.3 – 37%); Cyanophyta (10.0 – 42.3%), Dinophyceae (2.4 – 30.0%) and Diatomeae (1.1 – 50.6%). During this period, the phytoplankton community included: very small (<2 µm) species of Chlorococcales (Chlorophyta); *Aphanocapsa holsatica*, *Chroococcus minimus* and *Merismopedia glauca* (Cyanophyta), and *Gymnodinium mirabilis*, *Ceratium hirundinella*, *Peridinium elpatiewskyi* (Dinophyceae; Table 2b).

With respect to the microbial loop, bacteria biomass ranged from 48.1 – 88.9 mg m⁻³, APP from 0.2 – 5.6 mg m⁻³ and HNF from 8.5 – 46.5 mg m⁻³ (Figure 3 c). Ciliate biomass ranged from 7.6 – 37.8 mg m⁻³ including mostly species of *Strobilidium* and *Strombidium*.

Total zooplankton biomass in July ranged from 2.2 – 29.4 mg m⁻³ (dry weight Figure 3 d). Dreissenid veligers were the largest group (0.9 – 16.0 mg m⁻³), followed by Calanoids (0.1 – 15.1 mg m⁻³) and Cyclopoids (0.1 – 5.0 mg m⁻³).

Late Summer - September 24, 2019

During late September, surface temperatures in the Detroit River ranged from 19.0 – 20.1 °C and light attenuation (k_d) varied from 0.5 – 1.0 m⁻¹ (Appendix 1). Water velocity ranged from 0.1 – 0.4 m s⁻¹ nearshore compared to 0.4 – 0.9 m s⁻¹ at the offshore sites. With respect to nutrients, total phosphorus ranged from 10.5 – 17.5 µg l⁻¹; both nitrate + nitrite (0.25 – 0.29 mg l⁻¹) and silica (1.58 – 1.66 mg l⁻¹) showed little variability. Chlorophyll *a* concentrations ranged from 0.9 – 1.6 µg l⁻¹.

Primary productivity in September ranged from 8.2 – 12.4 mg C m⁻³ h⁻¹ with nanoplankton being the most photosynthetically active fraction, accounting for 57.3 – 69.1% of the total productivity (Figure 4 a). By comparison, picoplankton represented 13.7 – 24.6% and net plankton 12.6 – 22.2% of the total productivity. Bacterial productivity in September ranged from 0.6 – 1.1 mg C m⁻³ h⁻¹.

Phytoplankton biomass ranged from 74.6 – 279.9 mg m⁻³ in September and contained a mixture of Chrysophyceae (8.1 – 56.4% of total biomass), Cyanophyta (3.8 – 64.9%), Diatomeae (9.0 – 32.7%) and Chlorophyta (3.8 – 64.9%; Figure 4 b). The phytoplankton community included: *Ochromonas* spp. (Chrysophyceae); *Microcystis botrys*, *M. novackii* (Cyanophyta); *Cocconeis placentula*, *Cyclotella ocellata* (Diatomeae), and small Chlorococcales (Chlorophyta) (Table 2c).

Microbial loop biomass was variable during September. Bacteria ranged from 41.0 – 79.0 mg m⁻³, APP from 0.9 – 12.1 mg m⁻³, and HNF from 13.7 – 44.1 mg m⁻³ (Figure 4 c). Ciliate biomass in this period ranged from 8.0 – 36.1 mg m⁻³ and composed of species of *Strombidium* and *Strobilidium*.

Zooplankton biomass ranged from 2.1 - 8.3 mg m⁻³ (dry weight) in September and was composed of Bosminids (0.5 – 3.2 mg m⁻³), Dreissenid veligers (0.8 – 2.5 mg m⁻³) and calanoids (0.3 – 3.2 mg m⁻³) (Figure 4 d).

Fall - November 6, 2019

In November, surface temperatures along the Detroit River ranged from 7.6 – 8.6 °C and light attenuation (k_d) from 0.6 – 1.0 m⁻¹ (Appendix 1). Water velocities ranged from 0.05 – 0.5 m s⁻¹ nearshore and from 0.7 – 1.3 m s⁻¹ offshore. Total phosphorus concentrations ranged from 7.2 – 39.8 µg l⁻¹, nitrate+nitrite from 0.27 – 0.3 mg l⁻¹ and silica from 2.0 – 2.2 mg l⁻¹. Chlorophyll *a* concentrations at this time ranged from 0.7 – 1.5 µg l⁻¹.

Primary productivity during November ranged from 9.1 – 15.6 mg C m⁻³ h⁻¹ with nanoplankton being the most productive fraction (55.7 – 84.0% of the total productivity), followed by picoplankton (11.9 – 39.0%) and net plankton (2.9 – 6.0% Figure 5 a). Bacterial productivity ranged from 0.1 – 0.3 C m⁻³ h⁻¹ at this time.

Phytoplankton biomass ranged from 33.3 – 174.0 mg m⁻³ with the dominant taxa being Chlorophyta (5.7 – 80.7% of total biomass), Cryptophyceae (4.8 – 38.6%), and Chrysophyceae (4.1 – 24.3%; Figure 5 b). Common species observed during November included: *Botryococcus braunii* and small Chlorococcales (Chlorophyta); *Plagioselmis nanoplanktica* (Cryptophyceae), and medium to large sized species of *Ochromonas* (Table 2d).

Regarding microbial loop, bacteria biomass ranged from 19.7 – 71.6 mg m⁻³, APP from 0.5 – 4.8 mg m⁻³, and HNF from 4.2 – 46.5 mg m⁻³ (Figure 5 c). Ciliate biomass was in the range of 4.8 – 59.1 mg m⁻³ with species of *Strombidium*, *Strobilidium* and Haptorida collectively accounting for about 75% of the total biomass.

Zooplankton biomass during November ranged from 0.4 – 5.8 mg m⁻³, and was dominated by Calanoids (0.1 - 3.3 mg m⁻³) and bosminids (0.2 – 2.5 mg m⁻³; Figure 5 d).

Water Chemistry, Physical Parameters and Lower Food Web

Water chemistry and physical parameters were averaged by month (Table 3) and plotted to visualize seasonality (Figure 6). Light attenuation coefficients of photosynthetically active radiation (K_d) ranged from 0.15 to 1.18 m⁻¹ with an average of 0.67 ± 0.04 m⁻¹ (Table 3). Calcium and magnesium have little variation between stations but show some seasonality with a decrease in the mid season (Figure 6). Other parameters (sodium, nitrate+nitrite, Ammonia, TP, potassium and DOC) also appear to be higher on either one end or the other and lower in the summer. The mean total phosphorus concentration in the Detroit River was 13.8 ± 1.3 µg l⁻¹ and the highest TP values are observed in November (e.g. 39.8 µg l⁻¹ at DR 4) when all forms of phosphorus were at their peak and showed the greatest variation (Figure 6). Mean nitrite+nitrate was 0.34 ± 0.02 mg l⁻¹ and nitrogen forms were low in November. Mean silica was 1.58 ± 0.02 mg l⁻¹ and showed an increasing trend over the year. Water chemistry parameters were also averaged by station to visualize upstream downstream or nearshore offshore patterns (Figure 7). For most parameters there was no obvious difference between stations. Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON) along with sodium and potassium showed higher values at DR2 and DR5 but not at other stations. Soluble Reactive Phosphorus (SRP) and dissolved TP both

increased moving downstream from a low at DR1 (near Lake St Clair) to a peak at station DR 3 (downtown Windsor) and then begin to drop while moving downstream. Dissolved TP showed a second peak at DR8 (nearshore Boise Island; Figure 7).

A series of paired sample t-tests and 2-way ANOVAs were run in order to assess potential differences along nearshore-offshore and upstream-downstream gradients while accounting for seasonal variability. First, Paired sample t-tests were used to assess differences between nearshore and offshore at Flemming Channel (DR1, DR2), Fighting Island (DR 4, DR5) and Boise Island (DR 7, DR 8) (see Figure 1). Sites located at Windsor (DR 3, offshore) and River Canard (DR 6, nearshore) were not included because they lacked immediate geographic pairings. Second, a 2-way ANOVA was run with Location (upstream/downstream) and Month (May, July, September, November) to differentiate potential geographical impacts from seasonal variability. In order to maintain parity, sites DR1, DR2, DR3 and DR4 were included as upstream and sites DR5, DR6, DR7 and DR8 were included as downstream. The division is somewhat arbitrary but close to the boundary established by Derecki (1984). Third, an additional 2-way ANOVA was run with Habitat (Nearshore, Offshore) and Month as variables. All nearshore and offshore sites were included in this analysis.

Each of these tests were applied to a suite of 24 chemical and biological parameters, specifically: flow rates, light attenuation (k_d), surface temperature, total phosphorus, nitrate+nitrite, silica, chlorophyll a , total phytoplankton biomass, Cyanophyta biomass, Chlorophyta biomass, Chrysophyceae biomass, Cryptophyceae biomass, Diatomeae biomass, Dinophyceae biomass, total primary productivity, net plankton ($>20\ \mu\text{m}$) productivity, nano-plankton ($2 - 20\ \mu\text{m}$) productivity, picoplankton ($<2\ \mu\text{m}$) productivity, bacterial productivity, autotrophic picoplankton (APP) biomass, bacteria biomass, heterotrophic nanoflagellates (HNF) biomass, total ciliate biomass and total zooplankton biomass.

The results of the paired sample t-test showed significant differences ($P < 0.05$) in flow rates, temperature, light attenuation, primary productivity, chlorophyll a and Cryptophyceae biomass among nearshore and offshore habitats (Table 4). With respect to upstream and downstream differences, a 2-way ANOVA found that only silica, HNF biomass and zooplankton biomass had a significant effect due to location, but seasonal effects were observed for these as well as other parameters (Table 6). For nearshore and offshore habitats, a 2-way ANOVA with Month as the second factor, showed significant differences between habitats for flow, light attenuation, chlorophyll a , that were not affected by season (Table 5). Significant differences for phytoplankton biomass, Diatomeae biomass and primary productivity (total), nanoplankton productivity and picoplankton productivity were also observed but affected by season. All other significant differences were related to season.

A series of linear regressions were run to test for significant relationships among total phytoplankton biomass and biomass of individual taxonomic groups, chlorophyll a and primary productivity with known drivers of algal growth including temperature, flow, Julian day (JDay), total phosphorus (TP), silica (SiO_2) and nitrite+nitrate. In Detroit River during 2019 there was no significant relationship between phytoplankton biomass (or any of the individual phytoplankton groups) with either light attenuation, temperature, total phosphorus, or nitrate+nitrite. There was a significant but weak decline in phytoplankton biomass with increasing silica but this is explained by the strong increase in silica over the season ($\text{SiO}_2 = -241 + 6.66\text{e}^{-8}\text{JDay}$, $R^2 = 0.901$, $P < 0.0001$) and a similar decrease in Diatom biomass with increasing silica ($\text{Diatom} = 325.3 - 159.3\text{SiO}_2$, $R^2 = 0.46$, $P < 0.001$).

Overall there was a strong seasonal pattern to phytoplankton biomass and primary productivity rates in 2019 (Figure 8). Phytoplankton biomass declined over the season ($\text{Phyto} = 61405 - 1.68\text{e}^{-5}\text{JDay}$,

$R^2 = 0.309$, $P = 0.0009$) while productivity increased ($PP_{Tot} = -1843 + 5.07 e^{-7*JDay}$, $R^2 = 0.45$, $P < 0.0001$; Figure 8). This observed seasonal change in phytoplankton biomass is not a direct reflection of a relationship with temperature (Figure 8 b).

Both flow and depth were better linearly correlated with measurements of phytoplankton than were nutrients (Figure 9). Chlorophyll *a* was more strongly related than phytoplankton biomass ($Chl\ a = 1.583 - 0.762*flow$, $R^2 = 0.21$; $P = 0.025$; $Chl\ a = 1.70 - 0.06*Depth$; $R^2 = 0.17$; $P = 0.020$) and declined with increased depth and flow (Figure 9). Although there was no relationship of standing stock with nutrients, primary productivity did show a statistically significant nonlinear relationship with total phosphorus ($R^2 = 0.468$, $P = 0.018$; $P_{tot} = \frac{c}{(1 + \exp(-a \cdot (TP - b)))}$, where $a = 0.27$, $b = 9.14$, $c = 11.63$) as shown in Figure 10.

A stepwise regression found that bacterial growth rates were most highly related to chl *a* levels, followed by temperature and then depth ($R^2 = 0.94$, $P > 0.001$, Figure 11). Nutrient levels and flow were not significant in the stepwise regression.

To visualize the proportion of the food web belonging to heterotrophic and autotrophic groups the organic carbon of each group was calculated following the method of Munawar et al. (2011) and plotted for each cruise (Figure 12). The size of the organic carbon pool ranged from a low of $48.6 \pm 8.8\ mg\ C\ m^{-3}$ in November to a high of $109.2 \pm 6.7\ mg\ C\ m^{-3}$ in July. On each date $\approx 80\%$ of the organic carbon pool was autotrophic, that is bound up in various taxa of phytoplankton with relatively little observable change in either the amount or proportion of organic carbon among components (Figure 12). It is also worth noting that among the heterotrophs, bacteria and HNF each account for about twice as much organic carbon than does zooplankton.

Zooplankton Density and Biomass

In 2019, total zooplankton density in the Detroit River was very low, with values usually $< 20\ ind.\ l^{-1}$ (Figure 13). The highest densities overall were observed at DR1 ($47.0\ ind.\ l^{-1}$) and DR2 ($38.5\ ind.\ l^{-1}$) in July. Small taxa such as dreissenid veliger larvae, copepod nauplii larvae and the cladoceran *Bosmina* were numerically dominant in almost all samples. On average, veligers comprised 85% of the zooplankton community by density in May, 69% in July, 61% in September, and only 39% in November. Nauplii comprised around 10% of the total during the spring and early summer, but only 3 to 5% in the fall. Conversely, *Bosmina* made up about 1% of the community by density during the first two cruises, but averaged around 30% on the latter two dates. Adult and juvenile (copepodid) copepods and other cladoceran taxa made up the remainder. The very low densities of the herbivorous cladoceran *Daphnia* ($< 0.01\ ind.\ l^{-1}$) in the Detroit River were noteworthy, along with the absence of the invasive predatory cladocerans *Bythotrephes* and *Cercopagis*. A single *Holopedium gibberum* was found all season.

Total zooplankton biomass in the Detroit River was similarly very low, with values rarely over $9\ mg\ m^{-3}$ (Figure 14). Biomass showed a seasonal pattern typical of the Great Lakes, with the lowest means (2.6 to $2.8 \pm 0.7\ mg\ m^{-3}$) in spring and late fall, respectively, the highest value in mid-summer ($11.2 \pm 3.6\ mg\ m^{-3}$) and intermediate biomass in early fall ($5.4 \pm 0.9\ mg\ m^{-3}$). Veligers were important in terms of biomass in May (55%), July (54%) and September (30%), but they comprised only 10% in November. Calanoids also dominated biomass due to their large size, averaging between 20% and 43% of the total. Although they varied across stations, calanoids were usually less important in July. *Bosmina* were also noteworthy (about 37%) in the fall. Cyclopoids comprised from 5 to 30% of biomass at many stations, but they generally declined in importance downstream of Windsor.

On each of the four cruises we observed exponential declines in zooplankton density and biomass with increasing distance downriver. The biomass relationships were very strong in May ($R^2 = 0.92$, $P < 0.001$) and November ($R^2 = 0.85$, $P < 0.001$); but less so in July ($R^2 = 0.57$; $P = 0.031$) (Figure 14). In September the relationship was not significant ($R^2 = 0.44$; $P = 0.073$). Cyclopoids (especially adults), had almost disappeared in the lower river. Biomass declines with increasing distance were also seen in veligers in May ($R^2 = 0.90$, $P < 0.001$) and September ($R^2 = 0.62$, $P = 0.020$), but the relationships were not significant in July ($R^2 = 0.10$, $P = 0.439$) and November ($R^2 = 0.30$, $P = 0.146$). Using geometric mean values for the May to November period at each of the stations, the zooplankton relationship was $ZBM = 8.523 e^{-0.046d}$, where ZBM is total zooplankton biomass in mg m^{-3} , and d is distance downstream in km ($R^2 = 0.93$, $P < 0.001$; Figure 15). We observed a similar biomass decline the Niagara River in 2014 (Rozon et al. 2018), although the initial biomass value in at the mouth of the river in eastern Lake Erie was higher and the rate of decline greater ($Y = 77.181e^{-0.061d}$, $R^2 = 0.81$; $P = 0.014$) (Figure 15). In contrast, phytoplankton biomass did not show a significant change with increasing distance downriver in either the Detroit River ($R^2 = 0.01$; $P = 0.840$) or the Niagara River ($R^2 = 0.06$, $P = 0.846$).

Except for the loss of cyclopoids, zooplankton composition was fairly consistent down the length of the river in the spring and fall (Figures 13, 14). The main seasonal changes were the emergence of *Bosmina* as a dominant taxon in the fall, and the decline in veligers by November. In both May and November, the dominant copepods were the cyclopoid *Diacyclops thomasi* and diaptomid calanoids. The influence of Lake St. Clair and the nearshore macrophyte beds was most evident during the summer and early fall. In July, there were few adult crustaceans ($<2 \text{ ind. l}^{-1}$) entering the river system at the main channel site DR1, and biomass was comprised mostly of veligers, nauplii and juveniles of the calanoid *Epischura*. This veliger-dominated community persisted downstream at mid-channel DR4 and in the lower river. In contrast, the July zooplankton community at the upper nearshore station DR2 had a high proportion of littoral taxa, including the cyclopoid *Acanthocyclops vernalis*, the calanoid *Eurytemora*, and the cladoceran genera *Chydorus*, *Alona*, *Ceriodaphnia*, *Pleuroxus* and *Sida*. This sample contained the highest crustacean density (23.7 ind. l^{-1}) and biomass (28.7 mg m^{-3}) of the 2019 survey (Figures 13, 14). Likely originating from Lake St. Clair, these littoral crustaceans were still evident by Windsor (DR3), although the populations of most taxa had declined. Proportionally, cyclopoids and veligers became more important at DR3 relative to DR2, but the percentage of cladocerans was lower. Community composition of the dominant groups was very similar at the nearshore stations DR2 and DR5, despite an overall decline in biomass downstream. Cyclopoid biomass (*A. vernalis*, *Eucyclops agilis* and *Mesocyclops edax*) remained elevated at DR5, but these taxa were nearly absent further downstream. Except for a small increase at nearshore station DR5, littoral cladocerans had largely disappeared at stations downstream of DR3.

A similar composition change was seen at DR2 September (Figures 14, 16), with increases in cyclopoids (*A. vernalis* and *D. thomasi*), *Eurytemora*, *Daphnia* and the predatory cladoceran *Leptodora*, although total biomass at this site was similar to the other upstream locations. These taxa again showed rapid attenuation downstream. Adult cyclopoids or *Eurytemora* were not detected in the main channel DR1.

During both the July and September surveys, biomass was lowest (around 2 mg m^{-3}) at DR6 near the outlet of the River Canard (Figure 14). Biomass at DR5 was similarly low in September, although it supported more littoral cladocerans than the other middle and lower river sites. Adult copepods were rare in all three samples, and *Bosmina* were depressed in September. Composition and biomass at these two nearshore stations were similar to adjacent stations in May and November.

Bottom-Up Correlations with Zooplankton

We examined bottom-up food web relationships in the Detroit River by performing least-squares linear regression analyses using annual mean zooplankton biomass at each of the 8 stations and total phytoplankton biomass, chlorophyll *a*, primary productivity of the most edible algal size fraction (2 – 20 µm) and total phosphorus (Figure 16 - black dots). While not included in the regression analyses, we also plotted stations from the Bay of Quinte, Lake Ontario (nearshore BUR, offshore LO2 and Kingston Basin LO81) and inner Toronto Harbour sampled between 2014 and 2018 (station locations are given in Appendix 3 of Bowen 2017). In general, zooplankton biomass appeared to be fairly insensitive to bottom-up forces in the Detroit River, as there were no significant ($P < 0.05$) regressions with any of the parameters listed above (Figure 16). This means that zooplankton biomass does not linearly shift with changes in the bottom-up variables. However, there were likely too few sampling events to conclusively determine any relationships. When all of the water bodies are examined together, giving a wider range of trophic conditions, we tend to see positive relationships between zooplankton biomass and phytoplankton biomass, chlorophyll *a* and total phosphorus, but not with primary production. It is also noteworthy that Detroit River zooplankton biomass was very low compared to Lake Ontario sites of a similar trophic status.

Zooplankton Size

Patterns in mean length were examined for total zooplankton, *Bosmina*, cyclopoids, calanoids and veligers. Nauplii, the larval stage of copepods, were excluded. Generally no consistent trends (nearshore to offshore or upper to lower river) were observed across the sampling season for any group (Figure 17). When weighted for density, zooplankton were consistently small in May due to the high proportion of veligers and nauplii. They were similarly small in July at DR1 and the lower river, but larger at the nearshore stations DR2, DR3 and DR5 due to the presence of littoral cladocerans and adult copepods. The nearshore influence was less evident in September, although animals were smallest at DR6. In November, there was an increase in mean size from DR1 to DR2, and a gradual decline with increasing distance downriver.

There were no nearshore to offshore size differences for *Bosmina* or Calanoids. *Bosmina* are typically small, with mean sizes around 300 µm or less. They were usually smallest in September. Cyclopoids tended to show higher size variability among samples, with means usually ranging from 400 to 550 µm. In July and September, cyclopoids tended to be smallest entering the river at DR1 and at DR6. Calanoids were similar in size at all stations in July and September, averaging 650 µm or less. In May, lower river calanoids were bigger than those at the first four stations, whereas in November, calanoids tended to drop in size down the length of the river. Veligers found in the river were usually small, averaging 120 µm or less in most samples. Few individuals approached the size required for settlement (>200 µm). Veligers tended to be larger in July and at the nearshore sites DR2 and DR8 in November.

Zooplankton Egg Ratios

Egg ratios (the mean number of eggs per adult) were examined for the main egg-bearing groups [*Bosmina*, cyclopoids and calanoids (Diaptomids + *Eurytemora*)] to provide an indication of reproductive potential (Figure 18). *Bosmina* egg ratios were highly variable from station to station in May and July when animal densities were low, ranging from 0.3 to 2.0 eggs ind.⁻¹. In September and November when *Bosmina* densities were much higher, egg ratios were lower and more consistent, ranging from 0.1 to 0.4 eggs ind.⁻¹. Cyclopoid egg ratios were also variable in the spring and early summer, with the highest values in July at the nearshore stations DR2 (9.4 eggs ind.⁻¹) and DR5 (5.7 eggs ind.⁻¹). Except for DR7 in May, there were no cyclopoid eggs below DR5 throughout the season, and almost none at any station in

the fall. Calanoid egg ratios were usually below 0.5 eggs ind.⁻¹ in May, September and November, and in July ranged from 0 eggs ind.⁻¹ at DR1 to 11.3 eggs ind.⁻¹ at DR3. They tended to be highest in the middle section of the river.

Rotifers

In the Detroit River, rotifers were effectively unimportant, and comprised only about 1% of rotifer plus zooplankton biomass, in part due to their very low densities and small size. In terms of density, rotifers represented between 17% (DR1) and 33% (DR2) of the zooplankton plus rotifer community. May to November rotifer density and biomass values in the Detroit River averaged 4.7 ind. l⁻¹ and 0.07 mg m⁻³, respectively; values 1 to 2 orders of magnitude lower than Lake Ontario systems sampled by DFO (Figure 19 a). Niagara R. biomass in 2014 was more similar, averaging 0.11 mg m⁻³. Rotifers showed the same spatial pattern of downstream attenuation as zooplankton in both connecting channels. Annual biomass was 0.07 mg m⁻³ at DR1 in the main channel, 0.15 mg m⁻³ at the upstream nearshore site DR2, and only 0.03 mg m⁻³ at DR7 and 0.04 mg m⁻³ at DR8 (Figure 19 b).

The upstream rotifer community was comprised mostly of *Keratella cochlearis*, *K. quadrata*, *Ploesoma truncatum*, *Polyarthra vulgaris* and *Synchaeta stylata*. *Pompholyx sulcata* was also common at DR1 and *Trichocerca multicrinis* and *Gastropus stylifer* at DR2. All of these except *Keratella* are soft-bodied forms, and all but *K. quadrata* and *G. stylifer* were largely eliminated by downstream mid-channel station DR7. The community at nearshore DR8 was slightly more diverse, also containing *S. stylata* and *P. vulgaris*.

Discussion

Nutrients and Chlorophyll *a*

Phosphorus, nitrogen and silica are important nutrients that affect phytoplankton growth; chlorophyll *a* is pigment found in phytoplankton that is relatively easy to measure and often used as proxy for the algal standing crop. Total Phosphorus and chlorophyll *a* in particular are often used to assess trophic state (e.g. Vollenweider et al. 1974; Carlson 1977). The mean TP concentration in the Detroit River of $13.8 \pm 1.3 \mu\text{g l}^{-1}$ is generally associated with a mesotrophic environment however the chlorophyll *a* concentration ($1.3 \pm 0.1 \mu\text{g l}^{-1}$) is characteristic of ultra-oligotrophic ones (Table 3). This discrepancy is not surprising in a river due to the resuspension of sediment-bound phosphorus although this fraction is generally not bioavailable. The highest total phosphorus values in the current study (e.g. $39.8 \mu\text{g l}^{-1}$ at DR 4) are observed in the fall and are related to the increase in dissolved phosphorus fraction (Appendix 1).

The depletion of bioavailable nitrogen (nitrate+nitrite) in a phosphorus enriched environment can affect the composition of the phytoplankton community by promoting the growth of nitrogen fixing cyanobacteria (blue-green algae). However, the values of nitrate+nitrite observed in the Detroit River (Table 3, Appendix 1) were well above limiting thresholds (e.g. Bode and Dortch 1996; Munawar and Fitzpatrick 2018). Likewise, silica is important for diatom growth and its presence or absence can have a major impact on the composition of the phytoplankton community (Schelske et al. 1986). All observations of silica (Appendix 1) were well above the limiting threshold of 0.8 mg l^{-1} (Lund 1954).

Despite the moderately high TP concentration, there is little evidence of it having a strong effect on the algal standing crop given the low chlorophyll *a* concentration as well as concentrations of nitrate and silica remaining above limiting thresholds. However, we discuss the algal standing crop, in particular phytoplankton biomass and composition, in more detail below as part of the broader food web analysis.

Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON) along with sodium and potassium showed higher values at DR2 and DR5 but not at the other stations including those

considered nearshore (Figure 7). POC and PON are associated with higher planktonic biomass or with increased sediment load. These stations are the furthest upstream nearshore stations where the highest zooplankton and higher chlorophyll was seen (Figures 2 – 5; Appendix 1). Another interesting observation is the increase in Soluble Reactive Phosphorus (SRP) and dissolved TP from a low at DR1 to a peak at the station off of downtown Windsor (DR3) then a drop while continuing downstream. Given the high variability this is not a significant trend but it is interesting in that it coincides with the zone of greatest decrease in zooplankton populations. Dissolved TP showed a second high peak at DR8 the nearshore station.

Autotrophs: APP, Phytoplankton Biomass, Composition and Primary Productivity

Phytoplankton biomass and taxonomic composition are a direct measure of the pelagic algal standing crop. On average, phytoplankton biomass of $217.3 \pm 28.3 \text{ mg m}^{-3}$ is rather low, more consistent with the deep offshore waters of Lake Ontario for example than the nutrient enriched embayments of Hamilton Harbour and the Bay of Quinte. Autotrophic Picoplankton (APP) are also part of the phytoplankton community but due to their small size ($<2 \text{ }\mu\text{m}$) are enumerated independently using epifluorescence microscopy. APP can be an important food resource for zooplankton (Brett et al. 2009), but with a mean value of $2.1 \pm 0.4 \text{ mg m}^{-3}$, there is a very limited supply. In general, the standing crop of phytoplankton (including APP) is low which in turn limits the food resources available to zooplankton. This is discussed later in this report along with the potential of the microbial loop (bacteria, HNF and ciliates) as an alternate source of energy to zooplankton.

Apart from the May survey, where the phytoplankton community was mostly composed of centric diatoms (61% on average) followed by Chlorophyta (14%), community composition was highly variable (see Figures 2 – 5; Table 2 a – d). During the more productive summer season for example, phytoplankton biomass ranged from 136 to 299 mg m^{-3} dominated by a mixture of eutrophic to oligotrophic species belonging to Chlorophyta, Cyanophyta and Chrysophyta (Table 2 b, c). Phytoplankton can respond quickly to rapid environmental changes (i.e. ‘the paradox of the plankton’ Hutchinson 1961) and maintain a certain level of diversity despite what appear to be broadly similar physical and chemical regimes.

Primary productivity refers the assimilation of dissolved inorganic carbon by phytoplankton through photosynthesis. It is a direct measure of the energy produced within an ecosystem and available for transfer to higher trophic levels. Primary productivity averaged $8.1 \pm 0.7 \text{ mg C m}^{-3} \text{ h}^{-1}$ but observations from September and October ($10 - 11 \text{ mg C m}^{-3} \text{ h}^{-1}$) were essentially double those observed in May and July ($5 - 6 \text{ mg C m}^{-3} \text{ h}^{-1}$). Throughout the study, the most productive size classes were generally the smaller ones, specifically nanoplankton ($2 - 20 \text{ }\mu\text{m}$) and picoplankton ($<2 \text{ }\mu\text{m}$) as shown in Figures 2 – 5. These results are higher than expected given the relatively low biomass of phytoplankton and may suggest an efficient photosynthetic capacity with fairly rapid turnover rates. However, it is important to note that these measurements were conducted in an incubator and phytoplankton samples were exposed to optimum light and temperature levels; furthermore, they were also not affected by the river current. All of which is to say that physical conditions in the river probably restrict the ability of the larger phytoplankton community to photosynthesize and limit the overall size of the standing crop. At the same time, the primary productivity experiments show that these are viable phytoplankton populations responding quickly to improved environmental conditions.

Heterotrophs: Microbial Loop, Bacterial Productivity and Zooplankton

It is well established that the microbial food web plays an important role in recycling organic matter and regenerating energy for transfer to higher trophic levels. Within the Detroit River AOC, bacterial biomass

($54.2 \pm 3.0 \text{ mg m}^{-3}$), HNF biomass ($37.4 \pm 5.8 \text{ mg m}^{-3}$) and ciliate biomass ($17.4 \pm 1.9 \text{ mg m}^{-3}$) were generally low during the May – November period. By way of comparison, crustacean zooplankton biomass was also very low at $53.3 \pm 9.9 \text{ mg m}^{-3}$, essentially the same as bacteria, during that time (Figures 2 – 5). There is considerable debate in the literature as what role each of these organisms play, but zooplankton may feed on bacteria (Sanders and Wickham 1993; Hwang and Heath 1997; Pace et al, 2004) and may also have to compete with HNF and ciliates for both autotrophic and heterotrophic food resources (Jürgens and Stolpe 1995; Tadonl     et al. 2004). High turnover rates of bacteria could provide an additional vector of energy transfer from bacteria to zooplankton. Overall, these results demonstrate the importance of the microbial loop to the lower food web of the Detroit River.

On that note, bacterial productivity in the Detroit River averaged $0.6 \pm 0.07 \text{ mg C m}^{-3} \text{ h}^{-1}$ (Figures 2 – 5) which is somewhat high compared to the offshore waters of Lake Ontario ($\approx 0.1 \text{ mg C m}^{-3} \text{ h}^{-1}$) but similar to observations from Hamilton Harbour ($\approx 0.5 \text{ mg C m}^{-3} \text{ h}^{-1}$) and considerably less than inner Toronto harbour ($\approx 1.8 \text{ mg C m}^{-3} \text{ h}^{-1}$) (Munawar et al. 2018). Urban (and agricultural) runoff can influence bacterial dynamics and in that regard, the Detroit River and Hamilton Harbour support similar sized populations (500 000 – 600 000). While bacterial production may represent a source of energy for higher trophic levels, excess bacterial production can also be a public health concern because it may spread disease. We did not observe the accumulation of large standing crops of bacteria nor organisms which may feed on them including HNF, ciliates and zooplankton. We would also expect considerable dispersion, given the flow rates.

Spatial Gradients within the AOC

This study of the planktonic communities of the Detroit River AOC specifically considered the potential for nearshore-offshore, upstream-downstream and seasonal variability. Significant differences were assessed using a paired sample t-test (nearshore vs offshore), followed by a 2-way ANOVA with upstream/downstream and month (May, July, Sept, Nov) as factors, and an additional 2-way ANOVA using nearshore/offshore and month (May, July, Sept, Nov) as factors. With respect to the nearshore – offshore gradient, our expectation was that a potential impairment would most likely be observed at nearshore sites due to the multitude of anthropogenic stresses (e.g. urban and agricultural runoff, municipal and industrial effluent). The main physical differences observed were significantly ($P < 0.05$) higher flow rates and reduced light attenuation (i.e. clearer water) offshore compared to nearshore (both t-test and ANOVA, see Table 4, 5) which were not affected by any seasonal variability. Chlorophyll *a* was also significantly lower offshore compared to nearshore (t-test, ANOVA) and did not show a seasonal effect (Table 4, 5). Primary productivity for both the nanoplankton (2 – 20 μm) and picoplankton (<2 μm) size fractions were significantly lower offshore than nearshore using both the t-test and ANOVA however the ANOVA also revealed a significant seasonal effect (Table 4, 5). There were some mixed results with respect to the phytoplankton community. The t-test showed that Cryptophyceae biomass was significantly higher nearshore than offshore (Table 4) whereas the ANOVA showed that total phytoplankton biomass and Diatomeae biomass were significantly higher nearshore than offshore with a strong seasonal effect as well (Table 5). Habitat preferences of individual phytoplankton species are highly variable (e.g. Reynolds et al. 2002; Padisak et al. 2009), but these results suggest the dominant species of Cryptophyceae (*Plagioselmis nanoplanktica*) and Diatomeae (*Cyclotella ocellata*, *Cocconeis placentula*) found more favourable conditions in the nearshore habitats.

Potential upstream vs. downstream differences were also tested for using a 2-way ANOVA again with month as the second factor to account for seasonal differences. Silica was observed to be significantly higher downstream when compared to upstream, HNF biomass was also significantly higher

downstream whereas zooplankton biomass was significantly lower downstream, but all showed a significant seasonal effect as well (Table 6). The observed decline in zooplankton biomass from upstream ($8.0 \pm 1.7 \text{ mg m}^{-3}$) to downstream ($2.6 \pm 0.5 \text{ mg m}^{-3}$) is characteristic of lotic (riverine) environments (e.g. Rozon et al. 2016) and may in fact help to explain the increased HNF biomass since zooplankton and HNF may compete with each other for food resources (e.g. Tadonl     et al. 2004).

For all of the other biological and chemical parameters tested using 2-way ANOVAs (Table 5, 6), significant effects were either seasonal or not observed at all. Taken together, these findings suggest that seasonal variability is likely the biggest factor affecting the phytoplankton and microbial communities in the Detroit River AOC; differences between sites, either upstream/downstream or nearshore/offshore, are comparatively few.

Bottom Up Effects on Phytoplankton and the Microbial loop

In typical riverine systems, primary producers are regulated by the influences of hydrological (e.g. water discharge, residence time and turbulence), physical (e.g. temperature and turbidity) and chemical (e.g. nutrient and mineral content) factors as well as top down biological factors (e.g. viruses or grazing pressure). River flow may determine physical habitat conditions and directly or indirectly affect many other key physiochemical variables affecting ecological processes. Unfortunately, there is no general consensus as to which factors regulate phytoplankton communities in lotic habitats (Basu and Pick 1995). A study by Wu et al. (2011), for example, found that both hydrological factors and major nutrients were of equal importance in the determination of phytoplankton assemblages in the Kielstau River. Desortova and Punochar (2011) found that flow and temperature were more important than nutrient level and Reynolds and Descy (1996) state the nutrients and grazing rarely exert critical control except where physical constraints are alleviated. Linear regressions found there was no significant relationship between phytoplankton biomass (or any of the individual phytoplankton groups) with either light attenuation, temperature, total phosphorus, or nitrate+nitrite. There was a significant but weak decline in phytoplankton biomass with increasing silica but this is explained by the strong increase in silica over the season and a similar decrease in Diatom biomass with increasing silica. This type of relationship is expected since diatoms require silica for growth. The lack of correlation with total phosphorus and nitrate+nitrite, suggests that the nutrient regime was not likely affecting the size of the algal standing crop.

Like Desortova and Punochar (2011), we found that both flow and depth are better linearly correlated with measurements of phytoplankton (specifically chlorophyll *a* and, more weakly, phytoplankton biomass) than are nutrients. Chlorophyll *a* and phytoplankton biomass declined with increased depth and flow, which in our study are represented by the offshore stations (Figure 9). This is an expected response by phytoplankton in turbulent rivers because they spend more time out of the euphotic zone in deeper waters and would experience less growth. However, light attenuation coefficients of photosynthetically active radiation (K_d) were indicative of clear water with the euphotic zone extending to the river bed at all stations on most days and therefore not limiting phytoplankton growth. The high light penetration combined with the very short residence time in the river leads us to suspect that the weak but significant relationship between chlorophyll *a* and flow can be attributed to growth at the nearshore sites.

Primary productivity shows a statistically significant nonlinear relationship with total phosphorus (Figure 10). These instantaneous rates of primary production are measured in ‘idealized’ laboratory conditions and removed from the turbulence of the river. These measurements are expected to be more responsive to nutrients than phytoplankton biomass in general, but we would anticipate a more

pronounced response in a riverine environment. Having said that, the observed increase in primary productivity with total phosphorus concentrations is well established and the Detroit River appears to meet that expectation.

The source of plankton found in rivers is typically thought to be either upstream lentic waterbodies, potamoplankton capable of reproducing in flowing water or suspended periphyton. True river potamoplankton is thought to attain high biomass in large rivers with low flow rates and longer residence times (Basu and Pick 1995). Having said that, the Detroit River is a connecting channel discharging between 3003-9000 m³ of water per second (USGS 2020) with a high flow rate of 0.5 m s⁻¹, and faster in the channel (0.8 m s⁻¹), with an residence time of 14 hours in the channel and up to 22 hours in the lower flow areas. It is likely that almost all plankton found in the Detroit River are from upstream sources including Lakes Huron and St. Clair, although there may be some reproduction in areas of low flow (Centis et al. 2010, Desortova and Puncochar 2011). Our finding that there is increased chl a in areas with lower current velocities supports this idea and indicates a normally functioning ecosystem.

Overall there was a strong seasonal pattern to phytoplankton biomass and primary productivity rates in 2019 (Figure 8). Phytoplankton biomass declined over the season while productivity increased (Figure 8). The decline in biomass is opposite to the patterns seen recently in the open waters of Lake Ontario where there is an increase in both biomass and productivity rates from May to late October (Bowen et al. in prep). Recent seasonal trends in phytoplankton biomass of Lakes Huron or St. Clair are not available. This observed seasonal change in phytoplankton biomass is not a direct reflection of a relationship with temperature (Figure 8 b), but rather suggests that multiple influences are at work.

When compared to other habitats in the Great Lakes we observe that phytoplankton biomass is very low overall in the Detroit River (Figure 16 a, b). The expected bottom up control of total phosphorus on phytoplankton biomass is seen to be much less influential in the Detroit River compared to the lacustrine systems (Figure 16 b). In rivers, nutrients are not a predictor of phytoplankton biomass because physical conditions are sub optimal. Additionally, in the Detroit River, primary productivity is higher for a lower phytoplankton biomass than in Lake Ontario or Toronto Harbour (Figure 16 a).

Bacterial production has been shown to be strongly affected by regulators such as water temperature (e.g., Shiah and Ducklow 1994; Coveney and Wetzel 1995), phosphorus and primary productivity (Tsuchiya et al. 2019). Nearly all rates of biological activity increase exponentially with temperature (Brown et al. 2004) but high variability of bacterial productivity at high temperatures has also been observed (Coveney and Wetzel 1995). Possible explanations include gradients of algal biomass (White et al. 1991) and/or phosphorus concentrations (Gurung and Urabe 1999). In the Detroit River during 2019, we found that the bacterial growth rate had a strong peaked seasonal pattern (i.e. highest in summer) and was most highly related to chlorophyll *a* levels, then temperature and then depth (Figure 11). Nutrient levels and flow were not significant in the stepwise regression. We found that bacterial productivity had no significant relationship with number of bacteria found in the sample which is suggested as strong top down regulation by Tsuchiya et al. (2019) but, in our study, there were no strong correlations found between bacteria and any of the micro grazers (HNF, ciliates, nauplii, any zooplankton group) which all had very low biomasses in the Detroit River. Again, this is almost certainly influenced by the very short residence time within the river.

Organic Carbon Resources

The organic carbon pool represents the amount of energy in the system available to higher trophic levels. In general, a large proportion of heterotrophs (bacteria, HNF, ciliates, zooplankton) relative to autotrophs is typically associated with anthropogenic stress, but in less disturbed systems may be indicative of a tightly coupled food web (see McCauley et al. 2018, for a complete discussion). Having said that, our experience in Great Lakes Areas of Concern has been more consistent with the former i.e. that a large proportion of heterotrophic microbes especially bacteria and HNF should be interpreted as a sign of disturbance (Munawar and Fitzpatrick 2017; Munawar et al. 2013). During 2019, we observed that $\approx 80\%$ of the organic carbon pool was autotrophic, that is bound up in various taxa of phytoplankton, during all 4 cruises with relatively little observable change in either the amount or proportion of organic carbon among components (Figure 12). It is also worth noting that among the heterotrophs, bacteria and HNF each account for about twice as much organic carbon than does zooplankton which suggests that they have an important role in food web dynamics. This suggests that while the pelagic food web may only supply a small amount of energy, there is no evidence it is functioning improperly. By way of comparison, in Hamilton Harbour and the Bay of Quinte (both of which are impaired under BUI 13), we have at times observed 40 – 60% of the organic carbon being bound up in HNF (Munawar et al. 2011; Munawar and Fitzpatrick 2017). It should be noted however that we have also observed considerable inter-annual variability in the structure of the organic carbon pool and one study alone may not provide enough information about the dynamics of the system.

Zooplankton Populations

Although zooplankton population dynamics and ecology have been widely studied in the lacustrine waters of the Laurentian Great Lakes (e.g., Barbiero et al. 2019; Makarawicz et al. 1989; Rudstam et al. 2015), there have been few studies in the connecting channels linking the lakes together (e.g., Edwards et al., 1989; Munawar et al. 2014; Rozon et al. 2018). Prior to our 2019 work, the most comprehensive study of zooplankton in the Detroit River was carried out in the US waters of the river in 2014 by Keeler et al. (2019). Two unpublished zooplankton studies were also carried out in the river by researchers at the University of Windsor in 2007 (DRCC 2012) and 2015 (Drouillard 2017).

Crustacean zooplankton biomass is relatively low in the oligotrophic waters of Lake Huron that supply most of the mid-channel flow into the Detroit River (Figure 20), with summer values averaging around 23 mg m^{-3} (Barbiero et al. 2019). Summer crustacean biomass in the Detroit River was considerably lower in both 2014 (Keeler et al. 2019) and 2019, with values of 2.4 and 4.6 mg m^{-3} , respectively. Productivity declines in Lake Huron since 2003 (Barbiero et al. 2009) have resulted in lower zooplankton densities in the St. Clair – Detroit River corridor in recent years (Keeler et al. 2019). For example, in the upper Detroit River in 2007, densities of *Bosmina* (summer and fall) and copepods (fall only) were about an order of magnitude higher than during the two recent surveys (DRCC 2012). In summer of 1984 prior to dreissenid invasion, crustacean biomass values in the main channel and outlet of Lake St. Clair were one to two orders of magnitude higher than in either the 2014 or 2019 surveys (Sprules and Munawar 1991). Similarly, David et al. (2009) reported a drop in cladocerans by 69% and copepods by 66% in Lake St. Clair relative to the 1970s.

Biomass of juvenile cyclopoids, *Bosmina*, *Chydorus* and other littoral cladocerans were higher in our 2019 survey than in 2014, in part because the Keeler study did not include slower velocity, nearshore sites where some of these taxa were more common. Furthermore, the Keeler survey used $153 \mu\text{m}$ mesh nets, and as these organisms are often small, they may have been better retained by our $64 \mu\text{m}$ nets (Pace et al. 1992; Thomas et al. 2017). Veligers, rotifers and copepod nauplii were not counted in the

2014 study because they used larger mesh. The 2007 survey used 120 µm mesh nets and also did not include veligers (DRCC 2012). Our work and the Rozon et al. (2018) survey of the Niagara R. illustrate the importance of using 64 µm nets in these Great Lakes connecting channels, as veligers often comprise a substantial portion of zooplankton biomass. When averaged across the season, they made up 43% of total biomass in the Detroit River and 24% in the Niagara River; a component that would have been largely missed had 153 µm nets been used (Bowen et al. 2018). In fact, we found veligers to be the most abundant taxon in most of our 2019 spring and summer samples. David et al. (2009) also found veligers to be the most abundant group in Lake St. Clair. Although they are small and easily swept along in the current from upstream sources, it is likely that spawning dreissenid mussels attached to rocks, hardened shorelines, structures and macrophytes along the length of the Detroit River also contribute to its large veliger population. This may help explain the persistence of veligers down the length of the river.

Many studies show that rotifers are numerically prevalent in riverine systems, especially in fast flowing channels (Burger et al. 2002; Pace et al. 1992; Saunders and Lewis 1989; Thorp et al. 1994). Rotifers were also a dominant group by density (17 to 42%) in both the Detroit River and the Niagara River (Rozon et al. 2018). Many of these rivers were dominated by the same resilient taxa found in the Detroit River (e.g. *Keratella* sp. and *Polyarthra* sp.). However, in terms of biomass, rotifers in the connecting channels were usually less important ($\leq 1\%$) than in the riverine studies listed above, and were at least two orders of magnitude lower than values at the Detroit River inlet in 1984 (Sprules and Munawar 1991). David et al. (2009) also reported a 90% drop in Lake St. Clair rotifers since the early 1970s, probably due to dreissenid predation.

The loss of zooplankton with increasing distance downstream has been consistently observed in all studies of the Detroit River (ours, Keeler et al. 2019 and DRCC 2012), as well as in the Niagara River (Rozon et al. 2018). The very low zooplankton biomass observed in the Detroit River (Figure 20) is typical of high-flow riverine environments, and rivers often show significant declines in zooplankton as compared to upstream lakes (Pace et al. 1992; Thorp et al. 1994). Water entering the Detroit River has already experienced attenuation of zooplankton biomass as it passed through the St. Clair River, as shown by Keeler et al. (2019). Taxa more vulnerable to stresses in riverine systems, including large cladocerans such as *Daphnia* sp., *Holopedium gibberum* and *Bythotrephes* have already been largely eliminated prior to reaching the Detroit River. These taxa are relatively common in southern Lake Huron, especially during the summer and early fall (Barbiero et al. 2019; Keeler et al. 2019).

These studies indicate that zooplankton community composition in Lake Huron (more calanoids, *Daphnia*, *Holopedium* and *Bythotrephes*, and fewer cyclopoids and littoral cladocerans) is not equivalent to the Detroit River, especially in the summer. The *Degradation of Phytoplankton and Zooplankton Populations* BUI for the Canadian waters of the Detroit River states that zooplankton will no longer be considered impaired “when the composition and relative abundance of phytoplankton and zooplankton of the Detroit River reflect that of Lake Huron, and therefore represent primarily oligotrophic-mesotrophic conditions” (Green et al. 2010). However, Lake Huron is not an appropriate reference area for the Detroit River, as the zooplankton community has already undergone substantial changes as it has passed through both the St. Clair River and Lake St. Clair, each of which is likely to reduce the biomass and change species composition. Furthermore, composition of the zooplankton community, particularly in upstream and mid-river nearshore areas, does not indicate that it is simply a Lake Huron community washed downstream, as the Stage 2 RAP Report suggests (Green et al. 2010). An examination of the nearshore sites shows that Lake St. Clair and nearshore wetlands of the Detroit River contribute littoral cladocerans, calanoids and cyclopoids typically not abundant in Lake Huron, although this addition tends to be lost by the lower reaches of the river. We recommend that this wording be changed because it is highly unlikely that the zooplankton community in the Detroit River would ever be similar to Lake Huron.

Because of these issues, we used upstream Detroit River stations at the outlet of Lake St. Clair for comparison in our study.

The reason for the particularly low crustacean biomass at DR6 in the plume of the River Canard in July and September is not known. This may simply be a dilution effect caused by turbid River Canard water that contains few crustaceans, or possibly that poor water quality in the tributary is leading to mortality. There were also fewer crustaceans than expected at nearshore site DR5 in September, again for unknown reasons. This area is quite heavily vegetated, and predation pressures from wetland dwelling fishes may be high.

There appear to be resilient zooplankton species which are more suited to riverine environments (Reif 1939). The small cladoceran *Bosmina* is one of the most abundant crustaceans in the Detroit River. It is a highly resilient, ubiquitous taxon that often dominates the lower Great Lakes (Barbiero et al. 2019; Bowen and Johannsson 2011; Bowen and Currie 2017; Rozon et al. 2018), Lake St. Clair (David et al. 2009) and southern Lake Huron (Keeler et al., 2019), as well as other riverine systems (Pace et al., 1992; Thorp et al. 1994; Wahl et al. 2008). Calanoid copepods, highly effective swimmers compared to cladocerans also dominate the Detroit River. They are also important in the Ohio River (Thorp et al. 1994) and in Lake Huron, despite recent declines in other crustaceans in the latter (Barbiero et al. 2019). Many of the diaptomids and *Epischura* in the upper Detroit River likely originated in Lake Huron. Cyclopoid copepods (especially juveniles) also made up an important part of the zooplankton community in our study, but these have been less abundant in Lake Huron in recent years (Barbiero et al. 2019; Keeler et al. 2019).

The shallow nearshore sites support aquatic macrophytes and tend to have more littoral cladocerans and cyclopoids than the main channel stations, especially during the summer. The upstream site DR2 appears to be heavily influenced by zooplankton washing out of the shallow wetland habitats of Lake St. Clair. This site receives water from the highly productive southeastern part of the lake, which is influenced by nutrient-enriched plumes of the Thames and Sydenham rivers (David et al. 2009; Sprules and Munawar 1991). This part of Lake St. Clair was not sampled by Keeler et al. (2019). The July and September DR2 samples contained more littoral warm water taxa, particularly those adapted to more eutrophic conditions, including the small cladoceran *Chydorus sphaericus*, the cyclopoid *Acanthocyclops vernalis* and the calanoid *Eurytemora*. These taxa are rare or absent in Lake Huron (Barbiero et al. 2019; Keeler et al. 2019). Both *A. vernalis* and *C. sphaericus* are good indicators of eutrophic conditions in the Great Lakes (Gannon and Stemberger 1978; Pejler 1983). These copepod taxa persisted at DR3 (adjacent to Windsor and Detroit) where nearshore and offshore waters are mixed due to channelization. Farther downstream, nearshore DR5 showed a small resurgence in both littoral cladocerans and copepods. This pattern suggests that both Lake St. Clair and Detroit River nearshore habitats are important sources of zooplankton for the upper and mid-sections of the Detroit River, particularly for warm water littoral taxa not common in the cool, oligotrophic waters of Lake Huron. Other studies have shown that nearshore and side channel refugia can be important sources of copepods and cladocerans to the main river channel (Furst et al. 2014; Saunders and Lewis 1988; Thorp et al. 1994).

Other than the changes noted above in the summer nearshore samples, zooplankton composition down the length of the river was fairly consistent in 2019. The 2007 study also noted that the Detroit River zooplankton were typical of the oligotrophic-mesotrophic community in Lake Huron, and there were no obvious changes in composition from upstream to downstream (Green et al. 2010).

There are likely a number of causes leading to the loss of zooplankton in the Detroit River that are unrelated to the Area of Concern. Lentic zooplankton carried into rivers are subject to the advective

losses of food and individuals downstream by water currents (Hynes 1970; Wahl et al. 2008), although in the Detroit River they are constantly replenished by upstream populations in Lake Huron and Lake St. Clair. This is important because residence time in the main channel of the river is thought to be less than a day (Derecki 1984); far shorter than the generation times for macrozooplankton. Rivers are not ideal habitats for most zooplankton, and causes of mortality may include physical stress caused by turbulence (Bickel et al. 2011; Horvath and Lamberti 1999), increased turbidity, high concentrations of suspended particles (Arruda et al. 1983; Levine et al. 2005), and limited high-quality food (Pace et al. 1992). However, turbidity in the main channel of the Detroit River is not particularly high, with Secchi depths averaging 2.3 ± 0.1 m in 2019. While the nearshore sites are significantly more turbid (Secchi = 1.4 ± 0.1 m), their biomass is similar or higher to the offshore waters.

The strong currents in main channel of the Detroit River (averaging 0.7 to 1.0 m s^{-1}) are likely more detrimental to zooplankton. Increased current velocities have been documented to increase mortality up to 20% among cladocerans, 40% in rotifers and 50% in copepods (Telesh 1986) and it has been estimated that current speeds greater than 0.25 m s^{-1} may lead to the death of lentic zooplankton (Tang et al. 2014). However, calanoids are powerful swimmers and may be better able to survive in fast-flowing systems (Jack et al. 2006; Tóth et al. 2011; Visser et al. 2009). Riverine zooplankton may be better able to survive in refugia from the current, such as eddies, embayments, and low flow nearshore areas (Walks 2007; Genin et al. 2005; Pace et al. 1992; Reynolds et al. 1994; Thorp and Casper 2003). Current speeds at the Detroit River nearshore sites were slower (0.1 to 0.4 m s^{-1}), providing conditions more favorable to lentic zooplankton.

Predation of zooplankton by planktivorous and larval fishes is thought to be a key source of mortality in riverine systems (Thorp and Casper 2003; Walks and Cyr 2004), and this is likely true in both the Detroit River and Lake St. Clair. Benthic animals such as dreissenid mussels that filter out phytoplankton and microzooplankton (e.g., rotifers) are another source of mortality (David et al. 2009; Thorp and Casper 2003; Twiss et al. 2010). Despite extensive fish habitat degradation in the Detroit River, recovery efforts such as the construction of spawning reefs continue (Hartig et al. 2018a). Over thirty species of fish use the river to spawn, including Lake Whitefish and Walleye (Green et al. 2010). The larvae of most fish species, and many juveniles, depend on zooplankton prey for at least a portion of their life cycle. The fish community in the wetlands and littoral edges of the main channel is comprised of shiners, Bluntnose Minnows, Brook Silverside and small centrarchids (Francis et al. 2014; Lapointe et al. 2007), many of which will consume zooplankton.

Larger zooplankton, particularly *Daphnia* sp., *Leptodora*, *Bythotrephes* and adult copepods, are preferentially consumed by fishes such as young Yellow Perch and Emerald Shiners (Brooks and Dodson 1965; Mills et al. 1987; Pothoven et al. 2009). These large cladocerans were rare even in the upper reaches of the Detroit River in 2019, after having run the gauntlet of feeding fishes farther upstream. We also saw a loss of adult copepods, and cyclopoids in particular, downstream of DR5 in our study. There were also fewer egg-bearing female calanoids in the lower river, possibly because these large animals are more visible, and therefore vulnerable to visual feeding predators. Small taxa such as *Bosmina* are generally not impacted to the same extent by planktivorous fishes (Thorp and Casper 2003).

Finally, the zooplankton community in the Detroit River does not appear to be strongly influenced by bottom-up forces such as phytoplankton biomass or primary production rates. This is not unexpected as a literature review of riverine systems by Pace et al. (1992) also found that zooplankton biomass was not usually correlated with chlorophyll concentrations.

Summary and Conclusions

This study included a comprehensive survey of the phytoplankton, microbial loop (including autotrophic picoplankton, bacteria, heterotrophic nanoflagellates, ciliates) and zooplankton communities of the Detroit River during 2019 in support of the assessment of Beneficial Use Impairment 13: “Degradations of phytoplankton and zooplankton populations”. This is the first study in which all of these components have been examined simultaneously at in an integrative, holistic manner. On the whole, phytoplankton biomass was rather low in the river ($\approx 220 \text{ mg m}^{-3}$ on average) and the composition was generally variable including species of Diatomeae, Chlorophyta, Cyanophyta, Chrysophyceae and Cryptophyceae. Chlorophyll *a*, a common indicator of the algal standing crop, was also similarly low. Despite the low phytoplankton biomass, primary production rates were moderately high ($\approx 10 \text{ mg C m}^{-3} \text{ h}^{-1}$) indicating that the river supports viable phytoplankton populations. With respect to the microbial and planktonic food web, bacteria contributed as much biomass on average as crustacean zooplankton (both $\approx 50 \text{ mg m}^{-3}$) followed by HNF (35 mg m^{-3}). Coupled with bacterial production rates of $\approx 0.6 \text{ mg C m}^{-3} \text{ h}^{-1}$, our findings show that these heterotrophic microbial organisms are important contributors to the lower food web of the Detroit River. We also analysed the planktonic and microbial communities in terms of organic carbon composition and found that approximately 80% of the organic carbon pool was autotrophic (phytoplankton and autotrophic picoplankton) and not likely being sequestered by heterotrophic nanoflagellates as observed in other impaired systems like the Bay of Quinte. As part of the study objectives, we considered variability within the system along nearshore/offshore and upstream/downstream gradients to test whether or not microbial and planktonic communities in certain parts of the river were affected by anthropogenic stressors. We observed phytoplankton biomass and primary productivity to be significantly higher in the lower flow nearshore than offshore as expected in riverine environments. Likewise, most of the observed differences in the microbial and planktonic communities could be attributed to seasonality and the physical regime. On the whole, our study does not provide evidence of impairment within the phytoplankton and microbial communities of the Detroit River Area of Concern

Zooplankton biomass in the Canadian waters of the Detroit River is typically very low and attenuates with increasing distance downstream. It is comprised primarily of dreissenid veliger larvae, the small cladoceran *Bosmina*, and both calanoid and cyclopoid copepods. Aside from some adult copepods, the zooplankton community tends to be comprised of small taxa, and there are very few large cladocerans such as *Daphnia* throughout the season. Rotifers in the river also appear to be relatively unimportant. There are usually fewer adult copepods, particularly cyclopoids and egg-bearing females in the lower river. The extremely low biomass, and loss of zooplankton with increasing distance downstream in the Detroit River is an expected consequence of riverine conditions, and not necessarily indicative of anthropogenic stresses. Similar patterns were observed in the Niagara River AOC, for example. Physical stresses in the river such as strong currents and predation by larval, juvenile and planktivorous fishes are likely the factors most responsible for loss of zooplankton. As zooplankton play a key role in transferring energy and nutrients from phytoplankton and the microbial community to fishes, this predation effect should not be considered negative since the biomass is incorporated into the food web. Overall, there is no compelling evidence to suggest impairment of zooplankton in the Canadian waters of the Detroit River AOC that can be attributed to human activities within the AOC (e.g., cultural eutrophication) and the residence time within the connecting channel is very short (likely < 1 day). Larger factors such as conditions in the upstream waters of Lake Huron, the St. Clair River and Lake St. Clair, along with

invasive species (e.g., *Dreissena* producing veligers) are probably more critical in shaping the Detroit River zooplankton community.

Given the knowledge of changes in plankton communities with distance down river, Lake Huron is not an appropriate reference area for the Detroit River. We recommend that meeting the listed criteria “when the composition and relative abundance of phytoplankton and zooplankton of the Detroit River reflect that of Lake Huron, and therefore represent primarily oligotrophic-mesotrophic conditions” is not necessary to reach a finding of unimpaired for BUI 13 because there is no reason to expect the planktonic communities to resemble those of Lake Huron approximately 100 km downstream. Instead, we focused on assessing potential changes occurring within the AOC and determining whether or not they were consistent with a riverine environment.

Overall, the planktonic communities were found to behave as expected in a high flow connecting channel, responding more to seasonality and the physical regime than to factors attributable to human impairments. We did not find evidence of impairment within any of the planktonic communities of the Detroit River Area of Concern.

Recommendations for Future Work and Analyses

The 2019 survey of the planktonic communities of the Detroit River AOC generated a robust data set of physical, chemical and biological parameters which could be used in future analyses to support the Remedial Action Plan. Such analyses could include: trophic ratios, phytoplankton edibility, and application of food web indices as done in other AOCs. Likewise, the data could be used to help inform habitat restoration efforts. We would also recommend multi-year sampling of the planktonic communities in order to establish true baseline conditions for the Detroit River under different water heights and flow regimes.

Response to Comments from the Detroit River Cleanup Committee

Assessment Criteria

The fundamental question is: what is happening to the planktonic communities within the AoC?

This is addressed by sampling the planktonic communities and related measures along the nearshore/offshore and upstream/downstream gradients and testing for significant differences using statistical approaches.

Are the observed changes (if any) consistent with expectations for a riverine environment? If this is the case, then there is no impairment.

By way of example, there are expectations of reduced zooplankton biomass downstream due to the flow regime, and elevated phytoplankton biomass in nearshore habitats given the dynamics of a lotic ecosystem. This is not exhaustive, but it gives a sense of how ecosystem function must be carefully considered in any assessment of the BUI.

Alternatively, are the observed changes of a nature that can only be explained by anthropogenic impairment?

This also requires careful consideration, but some hypothetical examples could include: 1) bacteria-laced urban runoff contaminating the nearshore waters of the AoC resulting in elevated microbial activity (e.g. bacterial productivity, heterotrophic nanoflagellate biomass, ciliate biomass) and suppressed zooplankton biomass; 2) sewage effluent (Lou Romano Sewage Treatment Plant)

resulting in an uptick of phytoplankton and bacterial productivity coupled with increased phytoplankton and microbial biomass downstream, and/or 3) high turbidity and agricultural runoff (River Canard) leading to increased microbial activity downstream given the expected reduction in zooplankton biomass.

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Table 1. Location and physical description of study sites on the Detroit River during the 2019 planktonic food web survey. *Depth is the mean observed over 4 surveys.

Station	Name/Description	Latitude	Longitude	Position	Depth (m)*
DR 1	Fleming Channel offshore	42°20'45.31"N	82°56'41.64"W	Upstream	10.17
DR 2	Fleming Channel nearshore	42°20'33.18"N	82°56'35.11"W	Upstream	2.10
DR 3	Windsor-Detroit offshore	42°19'26.39"N	83° 2'27.14"W	Central	13.50
DR 4	Fighting island offshore (downstream Windsor-Detroit)	42°14'51.99"N	83° 7'4.79"W	Central	9.32
DR 5	LaSalle nearshore	42°14'6.60"N	83° 6'26.90"W	Central	5.02
DR 6	River Canard nearshore	42° 9'32.12"N	83° 6'44.79"W	Downstream	2.10
DR 7	Boise Island offshore	42° 6'25.94"N	83° 7'24.64"W	Downstream	10.00
DR 8	Boise Island nearshore	42° 6'9.13"N	83° 7'31.20"W	Downstream	3.05

Table 2a. Dominant (top 5) phytoplankton taxa observed in the Detroit river Area of Concern on May 28, 2019. Taxa listed contributed >5% to total phytoplankton biomass. Stations are listed from upstream to downstream with nearshore stations indicated with (N).

Group	Category	DR1	DR2 (N)	DR3	DR4	DR5 (N)	DR6 (N)	DR7	DR8 (N)
Chlorophyta	<i>Chlorococcales</i>	8.12	14.85	5.87	5.70	12.55	13.49	13.18	14.50
	<i>Spermatozopsis exaltans</i>		9.82						
Chrysophyceae	Flagellates	5.63							
	<i>Gyromitus cordiformis</i>			9.09					
	<i>Mallomonas</i> sp.	11.82							
	<i>Ochromonas</i> sp.			6.01					7.28
Cryptophyceae	<i>Plagioselmis nanoplanktica</i>		10.92	13.01		6.16			
Cyanophyta	<i>Synechoccales</i>		9.82			6.12	6.55	8.27	6.36
Diatomeae	<i>Aulacoseira granulata</i>							7.43	
	<i>Cyclotella ocellata</i>	50.18	45.29	50.91	76.30	61.48	66.98	56.57	60.37
Dinophyceae	<i>Parvodinium inconspicuum</i>	5.19							

Table 2b. Dominant (top 5) phytoplankton taxa observed in the Detroit river Area of Concern on July 22, 2019. Taxa listed contributed >5% to total phytoplankton biomass. Stations are listed from upstream to downstream with nearshore stations indicated with (N).

Group	Category	DR1	DR2 (N)	DR3	DR4	DR5 (N)	DR6 (N)	DR7	DR8 (N)
Chlorophyta	<i>Chlamydomonas</i> sp.					6.22			
	Chlorococcales	22.79			15.01	15.35	7.93		
	<i>Golenkenia brevispina</i>	5.70							6.49
	<i>Gomphosphaeria vireiuxii</i>		5.32						
	<i>Mougeotia</i> sp.			9.95		5.00			
	<i>Pediastrum boryanum</i>		5.28						
	Synechoccales								12.97
Chrysophyceae	Chlorococcales								17.57
	Flagellates			5.66	7.01			10.36	
	Ochromonads	21.54		12.58	6.15			18.25	
Cryptophyceae	<i>Plagioselmis nanoplanktica</i>	8.76		11.55		9.53		5.71	7.04
Cyanophyta	<i>Anathece</i> sp.			6.92					
	<i>Aphanocapsa delicatissima</i>			5.43					
	<i>Aphanocapsa holsatica</i>		15.92						
	<i>Chroococcus limneticus</i>				7.28				
	<i>Chroococcus minimus</i>			7.55					
	<i>Merismopedia glauca</i>		14.75						
	Synechoccales	14.25		11.72	18.65	17.31	8.33	10.36	17.43
Diatomeae	<i>Cocconeis placentula</i>					11.05			6.14
	<i>Cocconeis</i> sp.						42.80		
	Small centrics					6.12			
Dinophyceae	<i>Ceratium hirundenella</i>						10.56		
	<i>Gymnodinium mirabile</i>							28.40	
	<i>Gymnodinium</i> sp.				10.78				
	<i>Peridinium elpatiewskyi</i>		5.14						9.00

Table 2c. Dominant (top 5 phytoplankton taxa observed in the Detroit river Area of Concern on September 24, 2019. Taxa listed contributed >5% to total phytoplankton biomass. Stations are listed from upstream to downstream with nearshore stations indicated with (N).

Group	Category	DR1	DR2 (N)	DR3	DR4	DR5 (N)	DR6 (N)	DR7	DR8 (N)
Chlorophyta	Chlorococcales	12.30	7.33	6.35	20.24	25.22		6.33	11.99
	<i>Coelastrum microporum</i>		7.87						
	<i>Oedogonium</i> sp.			5.58					
Chrysophyceae	Flagellates	6.25			9.38			5.01	6.97
	Ochromonads	10.86		6.73	5.63	15.20		35.35	16.29
	<i>Ochromonas</i> sp.	14.14		11.80	7.24			9.60	8.40
Cryptophyceae	<i>Plagioselmis nanoplanktica</i>				16.22	5.95			
Cyanophyta	<i>Anathece</i> sp		28.27					8.07	
	<i>Aphanocapsa holsatica</i>			6.73					
	<i>Chroococcus</i> sp.		6.87						
	<i>Microcystis botrys</i>						60.49		
	<i>Microcystis novacekii</i>		13.27						
	<i>Radiocystis geminata</i>			6.22					
Diatomeae	<i>Cocconeis placentula</i>					6.54			
	<i>Cyclotella ocellata</i>	6.25							
	<i>Cyclotella</i> sp.		11.73	31.35	25.20	17.32	7.15	10.51	15.06
Dinophyceae	<i>Gymnodinium helveticum</i>							5.57	
	<i>Gymnodinium</i> sp.	18.55							
	<i>Parvodinium inconspicuum</i>	9.22		8.50					
	<i>Peridinium elpatiewskyi</i>	9.02							

Table 2d. Dominant (top 5) phytoplankton taxa observed in the Detroit River Area of Concern on November 6, 2019. Taxa listed contributed >5% to total phytoplankton biomass. Stations are listed from upstream to downstream with nearshore stations indicated with (N).

Group	Category	DR1	DR2 (N)	DR3	DR4	DR5 (N)	DR6 (N)	DR7	DR8 (N)
Chlorophyta	<i>Botryococcus braunii</i>				70.79				
	Chlorococcales	22.13	28.15		7.63	23.69	21.72	52.75	
	<i>Desmodesmus communis</i>			5.10					
	<i>Pediastrum boryanum</i>			10.70					
Chrysophyceae	Flagellates							6.28	
	Ochromonads		5.77			12.42	5.98	6.02	17.42
	<i>Ochromonas</i> sp.							5.37	
Cryptophyceae	<i>Plagioselmis nanoplanktica</i>	24.19	34.97	37.56		23.15	30.11	7.33	24.32
Cyanophyta	<i>Aphanocapsa holsatica</i>								11.11
	Synechococcales	8.58	5.70	5.10				6.54	16.82
Diatomeae	<i>Achnantheidium</i> sp.	9.09							
	<i>Cocconeis placentula</i>					6.78			
	<i>Cyclotella</i> sp.	5.15						6.15	8.71
	<i>Navicula</i> sp.	7.38				19.33			
	<i>Stephanodiscus niagarae</i>						14.43		
Dinophyceae	<i>Peridinium</i> sp.		11.97	22.51			5.57		

Table 3. Average physical and chemical properties of the Detroit River observed on May 28; July 23; September 24; and November 6, 2019. Temp = surface temperature (°C), k_d = vertical (light) attenuation coefficient (m^{-1}), Flow = flow rate / water velocity ($m\ s^{-1}$), TP = total phosphorus concentration ($\mu g\ l^{-1}$), $NO_{3/2}$ = nitrate + nitrite concentration ($mg\ l^{-1}$), SiO_2 = silica concentration ($\mu g\ l^{-1}$), and Chl a = chlorophyll a concentration ($\mu g\ l^{-1}$).

Cruise		Temp	k_d	Flow	TP	$NO_{3/2}$	SiO_2	Chl a
28 May	Mean	14.6	0.65		11.4	0.52	1.11	1.61
	N	7	8	0	8	8	8	8
	SE	0.2	0.03		1.1	0.02	0.04	0.12
23 Jul	Mean	24.1	0.59	0.51	9.5	0.27	1.47	1.27
	N	8	8	8	8	8	8	8
	SE	0.1	0.12	0.12	1.7	0.004	0.03	0.38
24 Sep	Mean	20.1	0.67	0.48	13.4	0.26	1.63	1.22
	N	8	8	8	8	8	8	8
	SE	0.3	0.06	0.12	0.7	0.003	0.01	0.09
6 Nov	Mean	8.1	0.75	0.64	20.9	0.29	2.13	1.02
	N	8	8	8	8	8	8	8
	SE	0.1	0.04	0.18	3.9	0.004	0.02	0.10
All dates	Mean	16.8	0.67	0.55	13.8	0.34	1.58	1.28
	N	31	32	24	32	32	32	32
	SE	1.1	0.04	0.08	1.3	0.020	0.07	0.11

Table 4. Results of Paired sample t-test (2 tail) comparing nearshore and offshore means of multiple physical, chemical and biological parameters in the Detroit River AOC conducted during 2019. Only significant ($P < 0.05$) results are reported. Notes: *Flow rates not measured during May; **Temperature data from Fighting Island was not available for May.

Parameter	Mean (off)	Mean (Near)	t	DF	$P > t $
Flow*	0.84	0.20	-8.34	8	0.0001
Light Atten. (K_d)	0.53	0.75	-3.59	11	0.0042
Temperature**	16.61	17.01	-3.00	10	0.01
Chlorophyll a	0.93	1.61	-2.64	11	0.02
Cryptophyceae	7.23	19.42	-2.72	11	0.02
Primary productivity (total)	6.08	9.81	-2.93	11	0.01
2 – 20 μm productivity	4.12	5.73	-2.48	11	0.03
<2 μm productivity	1.38	2.71	-2.85	11	0.02

Table 5. Results of the 2-Way ANOVA with Habitat (Nearshore or Offshore) and Month (May, July, September, November) as Factors. Only results that are significant ($P < 0.05$) overall are reported. For factors, P values > 0.05 are listed as not significant, “n.s.”.

	Overall		Effect: Habitat		Effect: Month		Effect: Habitat*Month	
Parameter	F (7,24)	P	F (1,30)	P	F (3,28)	P	F(3,28)	P
Flow	18.902 F (5,18)	<.0001	87.395 F (1,22)	<.0001	1.857 F (2,21)	n.s.	1.700 F(2,21)	n.s.
Light Atten. (K_d)	8.837	<.0001	34.443	<.0001	2.323	n.s.	6.815	0.002
Temperature	327.084	<.0001	2.922	n.s.	761.612	<.0001	0.609	n.s.
Chlorophyll <i>a</i>	2.458	0.047	9.648	0.005	1.736	n.s.	0.784	n.s.
Nitrate+Nitrite	41.413	<.0001	0.818	n.s.	96.257	<.0001	0.101	n.s.
Silica	130.979	<.0001	0.809	n.s.	304.173	<.0001	1.175	n.s.
Total Phytoplankton	4.455	0.003	5.793	0.024	6.239	0.003	2.224	n.s.
Diatomeae	13.167	<.0001	5.240	0.031	26.866	<.0001	2.111	n.s.
Dinophyceae	3.333	0.013	0.003	n.s.	6.554	0.002	1.223	n.s.
Primary productivity	7.183	<.0001	11.025	0.003	12.047	<.0001	1.038	n.s.
>20 μ m productivity	3.717	0.007	3.948	n.s.	6.280	0.003	1.077	n.s.
2 – 20 μ m productivity	12.629	<.0001	8.475	0.008	25.162	<.0001	1.480	n.s.
<2 μ m productivity	3.808	0.006	8.218	0.008	5.020	0.008	1.126	n.s.
Bacterial Productivity	8.019	<.0001	1.626	n.s.	17.441	<.0001	0.729	n.s.
APP	3.118	0.017	2.419	n.s.	4.415	0.013	2.05	n.s.
Bacteria	2.495	0.045	0.002	n.s.	5.013	0.008	0.808	n.s.
HNF	3.113	0.018	0.493	n.s.	6.732	0.002	0.368	n.s.

Table 6. Results of the 2-Way ANOVA with Location (Upstream or Downstream) and Month (May, July, September, November) as Factors. Only results that are significant ($P < 0.05$) overall are reported. For factors, P values > 0.05 are listed as not significant, “n.s.”. Note that for temperature, degrees of freedom (df) are reported in the cell.

	Overall		Effect: Location		Effect: Month		Effect: Location*Month	
Parameter	F(7,24)	P	F(1,30)	P	F(3,28)	P	F(F3,28)	P
Temperature	497.262 (df=16)	<.0001	1.091 (df=22)	n.s.	1155.120 (df=20)	<.0001	4.794 (df=20)	0.014
Total phosphorus	2.482	0.0456	0.582	n.s.	4.890	0.009	0.708	n.s.
Nitrate+Nitrite	74.544	<.0001	2.306	n.s.	167.376	<.0001	5.791	0.004
Silica	163.452	<.0001	5.924	0.023	377.660	<.0001	1.752	n.s.
Diatomeae	9.207	<.0001	2.279	n.s.	20.455	<.0001	0.268	n.s.
Dinophyceae	3.169	0.0161	0.591	n.s.	6.394	0.002	0.802	n.s.
Primary productivity (total)	3.428	0.011	0.008	n.s.	7.784	0.001	0.211	n.s.
>20 μm productivity	2.619	0.0369	0.689	n.s.	5.315	0.006	0.567	n.s.
2-20 μm productivity	8.190	<.0001	0.335	n.s.	18.206	<.0001	0.792	n.s.
Bacterial productivity	6.926	<.0001	0.059	n.s.	15.775	<.0001	0.365	n.s.
HNF	7.421	<.0001	4.837	0.038	11.165	<.0001	4.539	0.012
Zooplankton	6.174	0.0003	15.497	0.001	7.231	0.001	2.011	n.s.

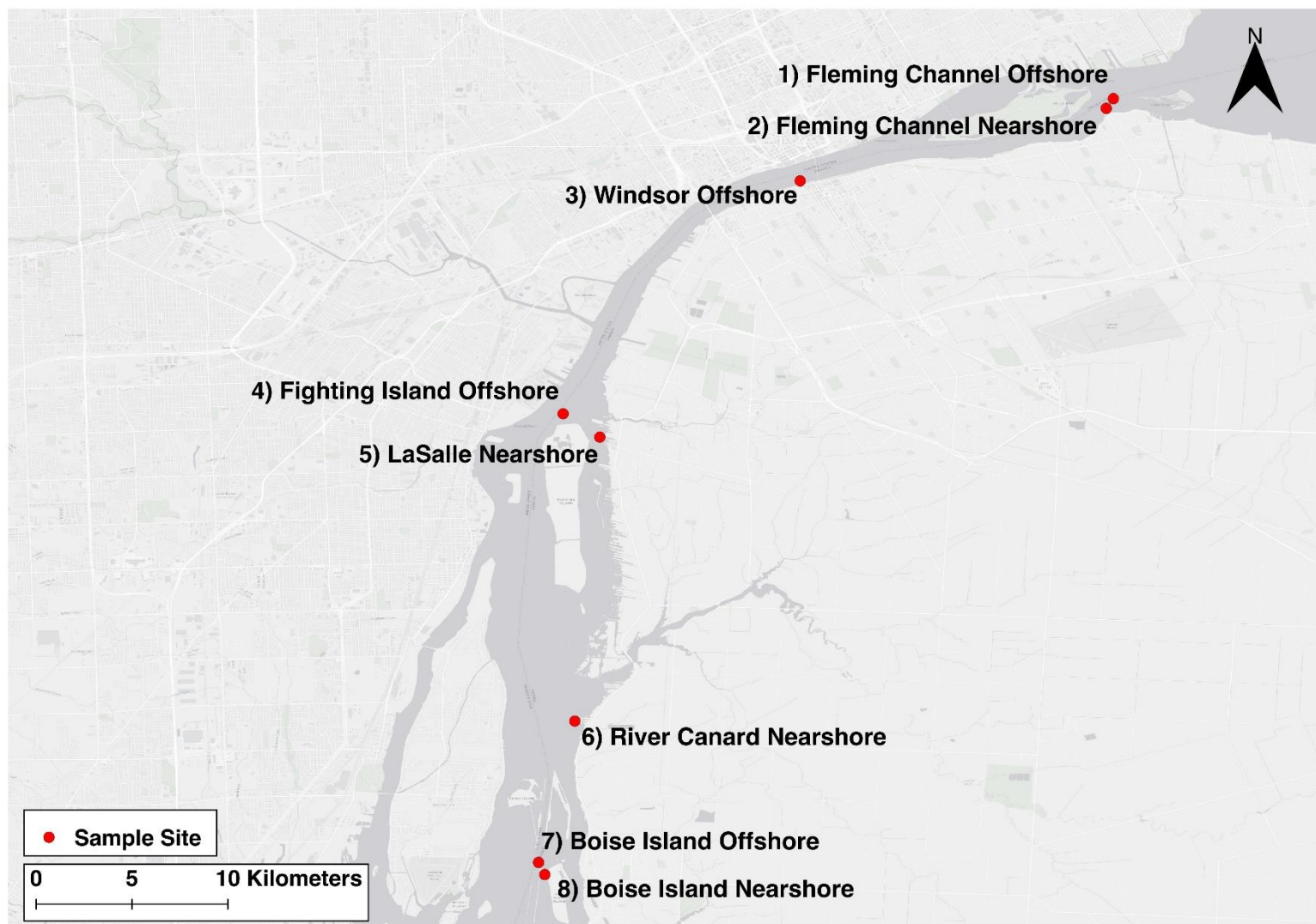
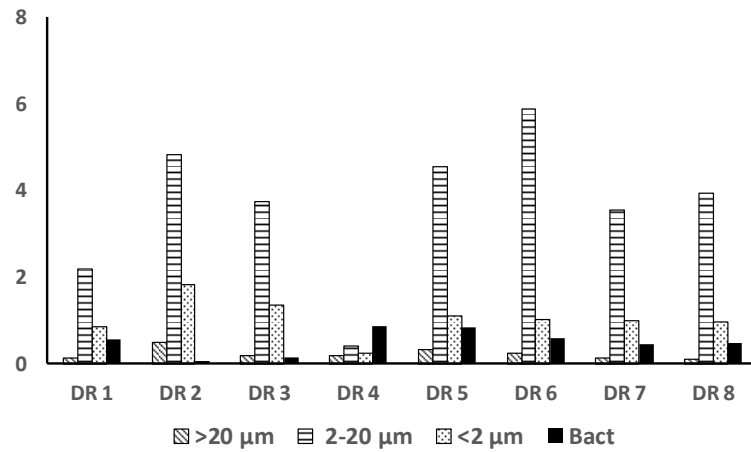
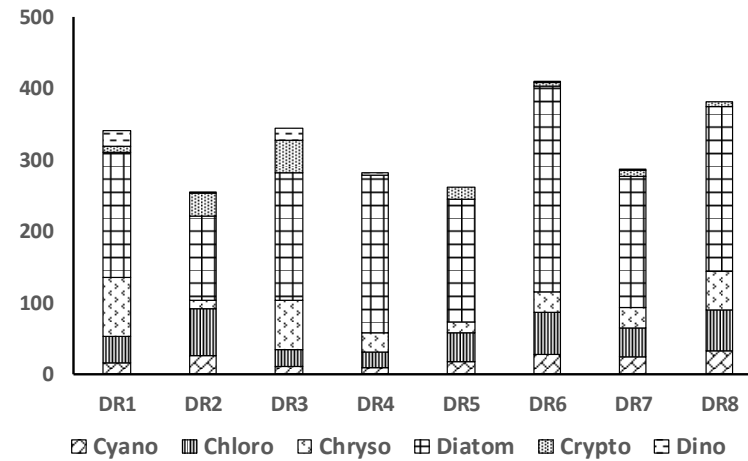


Figure 1. Locations sampled in 2019 lower food web study.

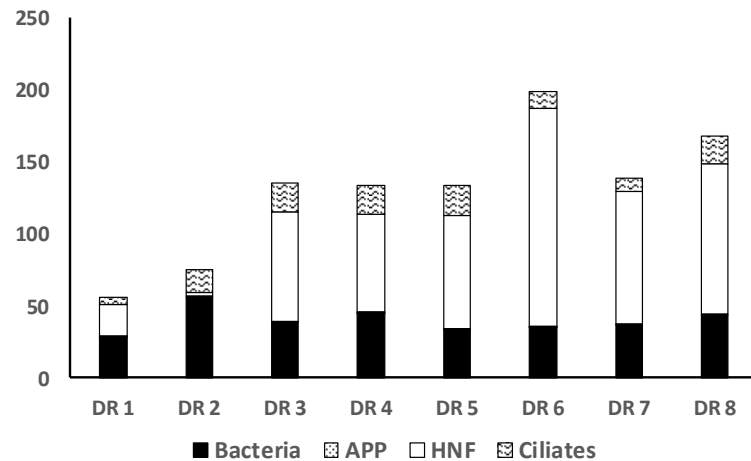
a) Primary Productivity and Bacterial Productivity ($\text{mg C m}^{-3} \text{ h}^{-1}$)



c) Phytoplankton Biomass (mg m^{-3}) and Composition



b) Microbial Loop (APP, HNF, Bacteria) and Ciliate Biomass (mg m^{-3})



d) Zooplankton Biomass (mg m^{-3} dry weight) and Composition

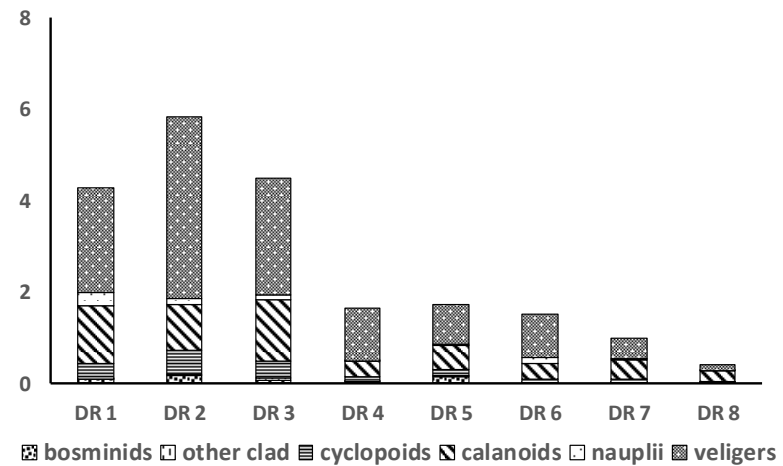


Figure 2. May 2019 planktonic food web assessment of the Detroit River Area of Concern: a) Size fractionated primary (phytoplankton) productivity including net plankton ($>20 \mu\text{m}$), nano-plankton ($2 - 20 \mu\text{m}$) and pico-plankton ($<2 \mu\text{m}$) as well as bacterial (Bact) productivity; b) Microbial Loop and ciliate biomass (APP = autotrophic picoplankton, HNF = heterotrophic nanoflagellates, others as indicated); c) phytoplankton biomass and composition (Cyano = Cyanophyta, Chloro = Chlorophyta, Chryso = Chrysophyceae, Diatom = Diatomeae, Crypto = Cryptophyceae, Dino = Dinophyceae), and d) Zooplankton biomass and composition (clad = cladoceran, all others as indicated).

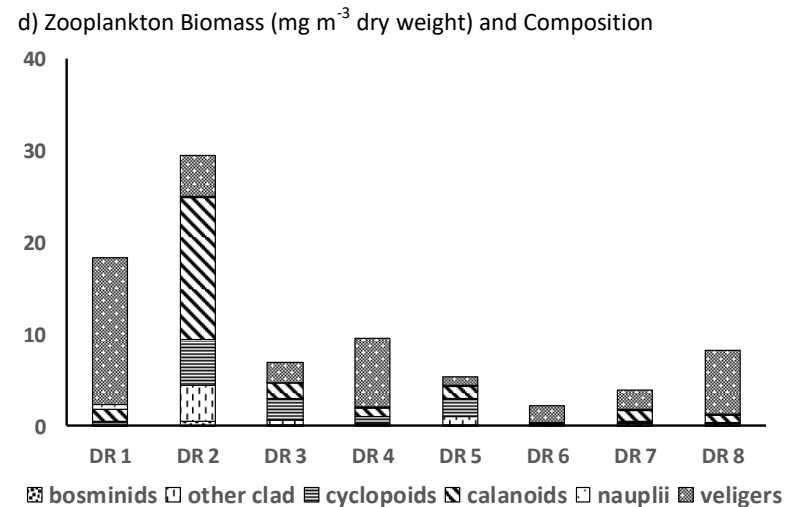
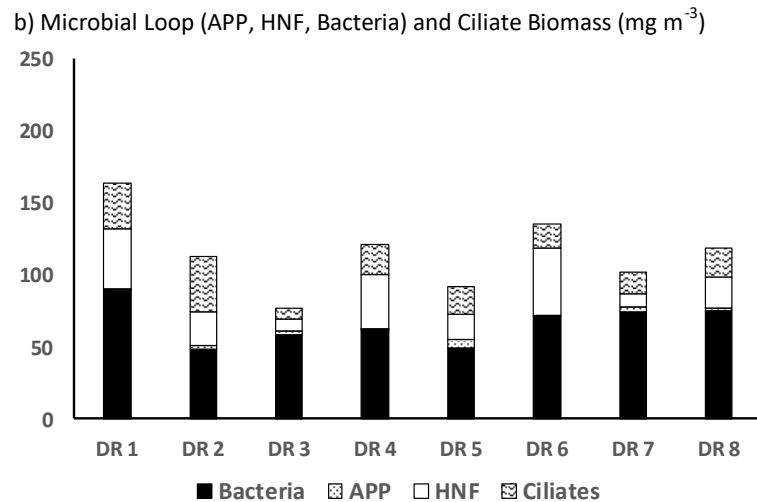
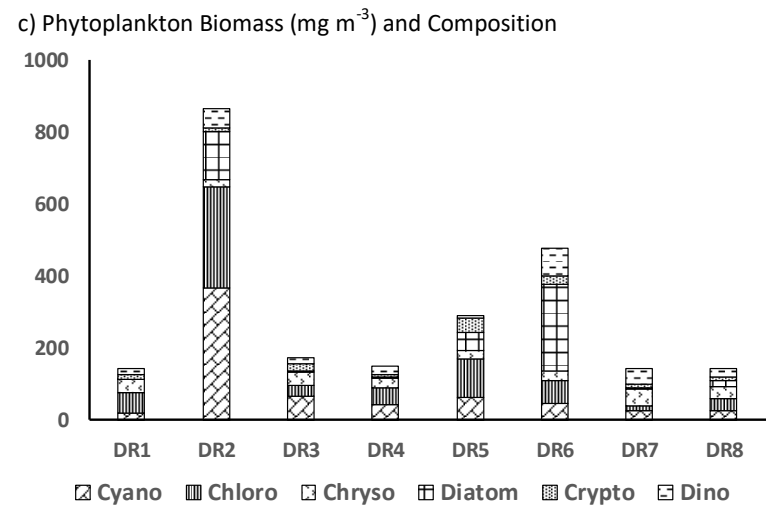
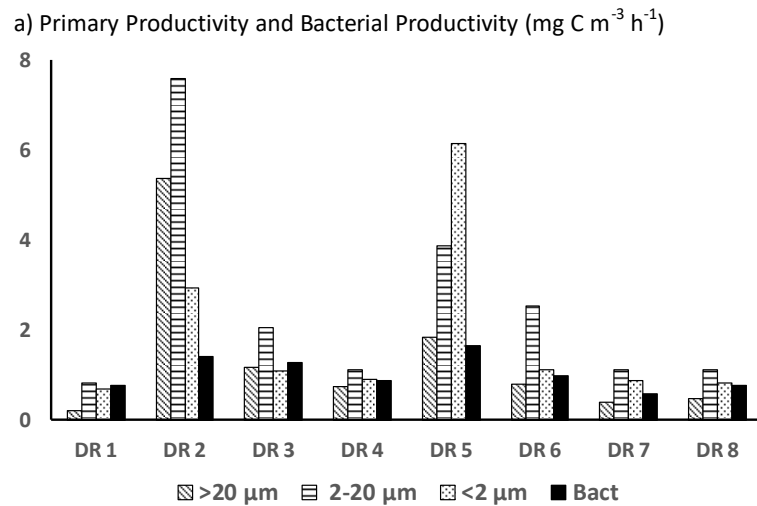
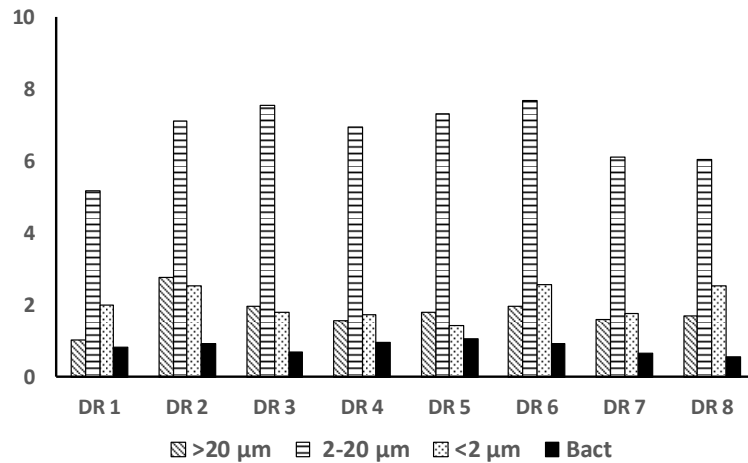
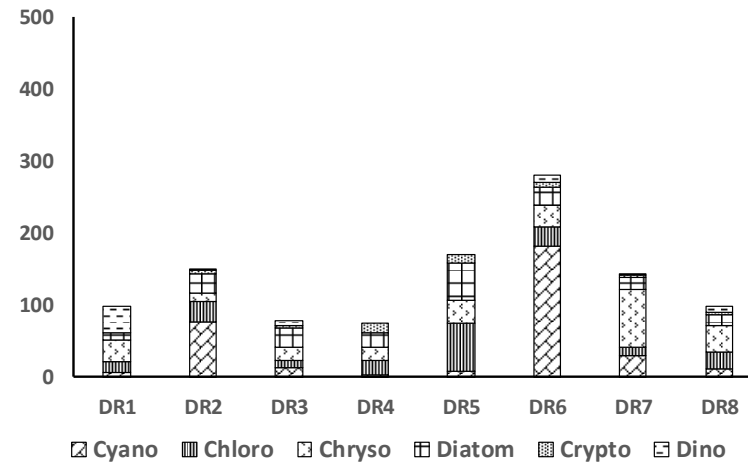


Figure 3. July 2019 planktonic food web assessment of the Detroit River Area of Concern: a) Size fractionated primary (phytoplankton) productivity including net plankton ($>20 \mu\text{m}$), nano-plankton ($2 - 20 \mu\text{m}$) and pico-plankton ($<2 \mu\text{m}$) as well as bacterial (Bact) productivity; b) Microbial Loop and ciliate biomass (APP = autotrophic picoplankton, HNF = heterotrophic nanoflagellates, others as indicated); c) phytoplankton biomass and composition (Cyano = Cyanophyta, Chloro = Chlorophyta, Chryso = Chrysophyceae, Diatom = Diatomeae, Crypto = Cryptophyceae, Dino = Dinophyceae), and d) Zooplankton biomass and composition (clad = cladoceran, all others as indicated).

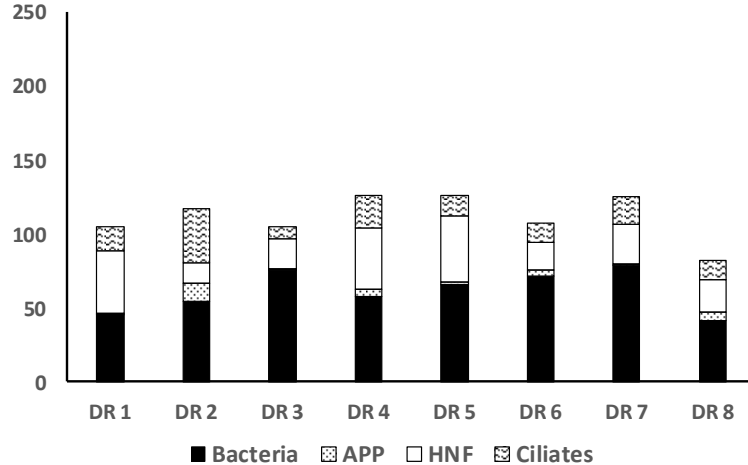
a) Primary Productivity and Bacterial Productivity ($\text{mg C m}^{-3} \text{ h}^{-1}$)



c) Phytoplankton Biomass (mg m^{-3}) and Composition



b) Microbial Loop (APP, HNF, Bacteria) and Ciliate Biomass (mg m^{-3})



d) Zooplankton Biomass (mg m^{-3} dry weight) and Composition

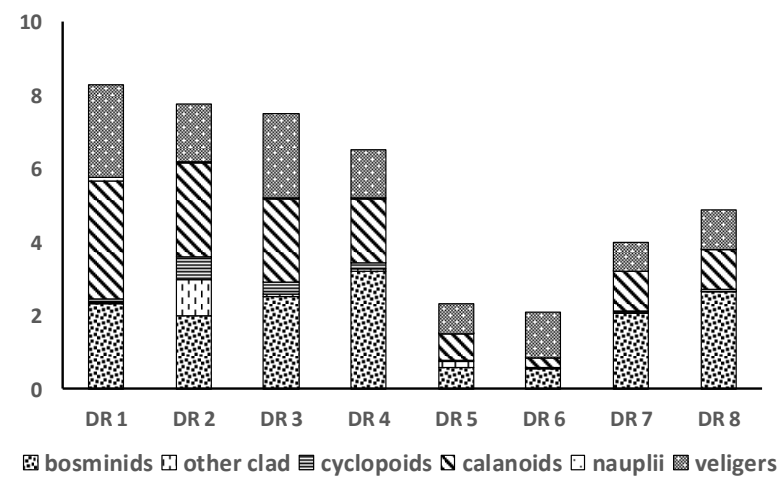


Figure 4. September 2019 planktonic food web assessment of the Detroit River Area of Concern: a) Size fractionated primary (phytoplankton) productivity including net plankton ($>20 \mu\text{m}$), nano-plankton ($2 - 20 \mu\text{m}$) and pico-plankton ($<2 \mu\text{m}$) as well as bacterial (Bact) productivity; b) Microbial Loop and ciliate biomass (APP = autotrophic picoplankton, HNF = heterotrophic nanoflagellates, others as indicated); c) phytoplankton biomass and composition (Cyano = Cyanophyta, Chloro = Chlorophyta, Chryso = Chrysophyceae, Diatom = Diatomeae, Crypto = Cryptophyceae, Dino = Dinophyceae), and d) Zooplankton biomass and composition (clad = cladoceran, all others as indicated).

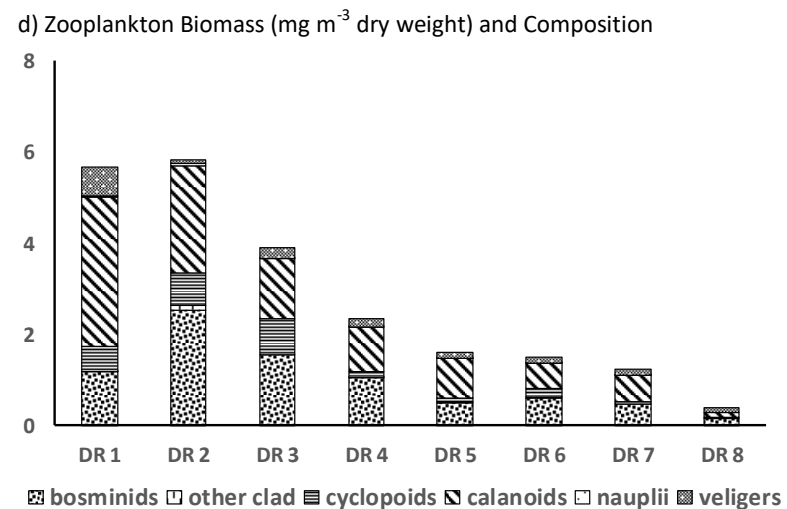
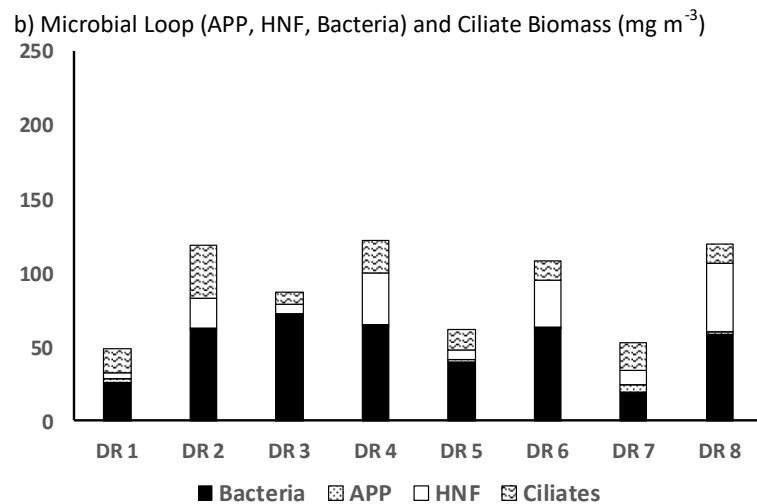
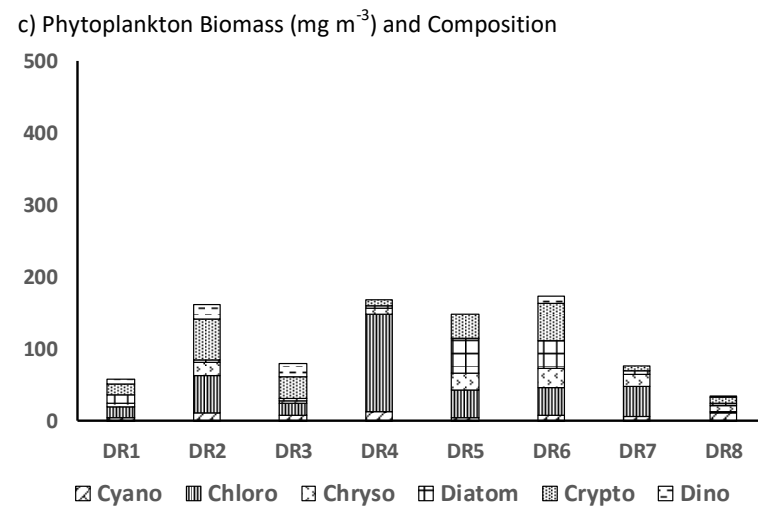
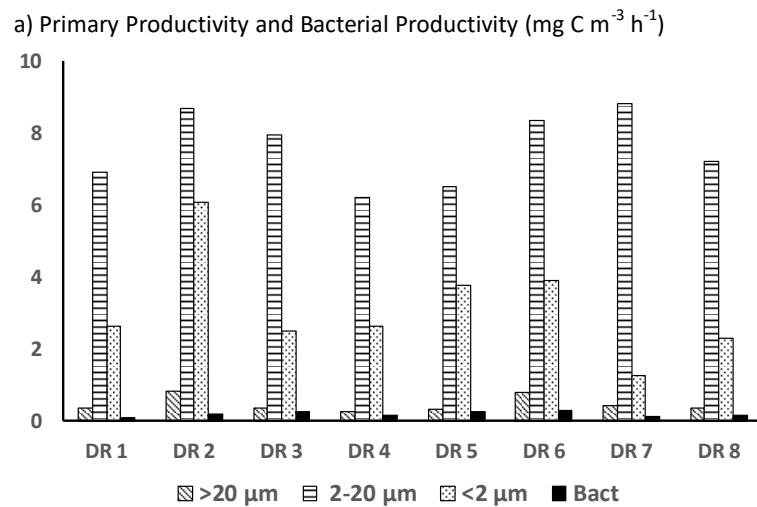


Figure 5. November 2019 planktonic food web assessment of the Detroit River Area of Concern: a) Size fractionated primary (phytoplankton) productivity including net plankton (>20 µm), nano-plankton (2 - 20 µm) and pico-plankton (<2 µm) as well as bacterial (Bact) productivity; b) Microbial Loop and ciliate biomass (APP = autotrophic picoplankton, HNF = heterotrophic nanoflagellates, others as indicated); c) phytoplankton biomass and composition (Cyano = Cyanophyta, Chloro = Chlorophyta, Chryso = Chrysophyceae, Diatom = Diatomeae, Crypto = Cryptophyceae, Dino = Dinophyceae), and d) Zooplankton biomass and composition (clad = cladoceran, all others as indicated).

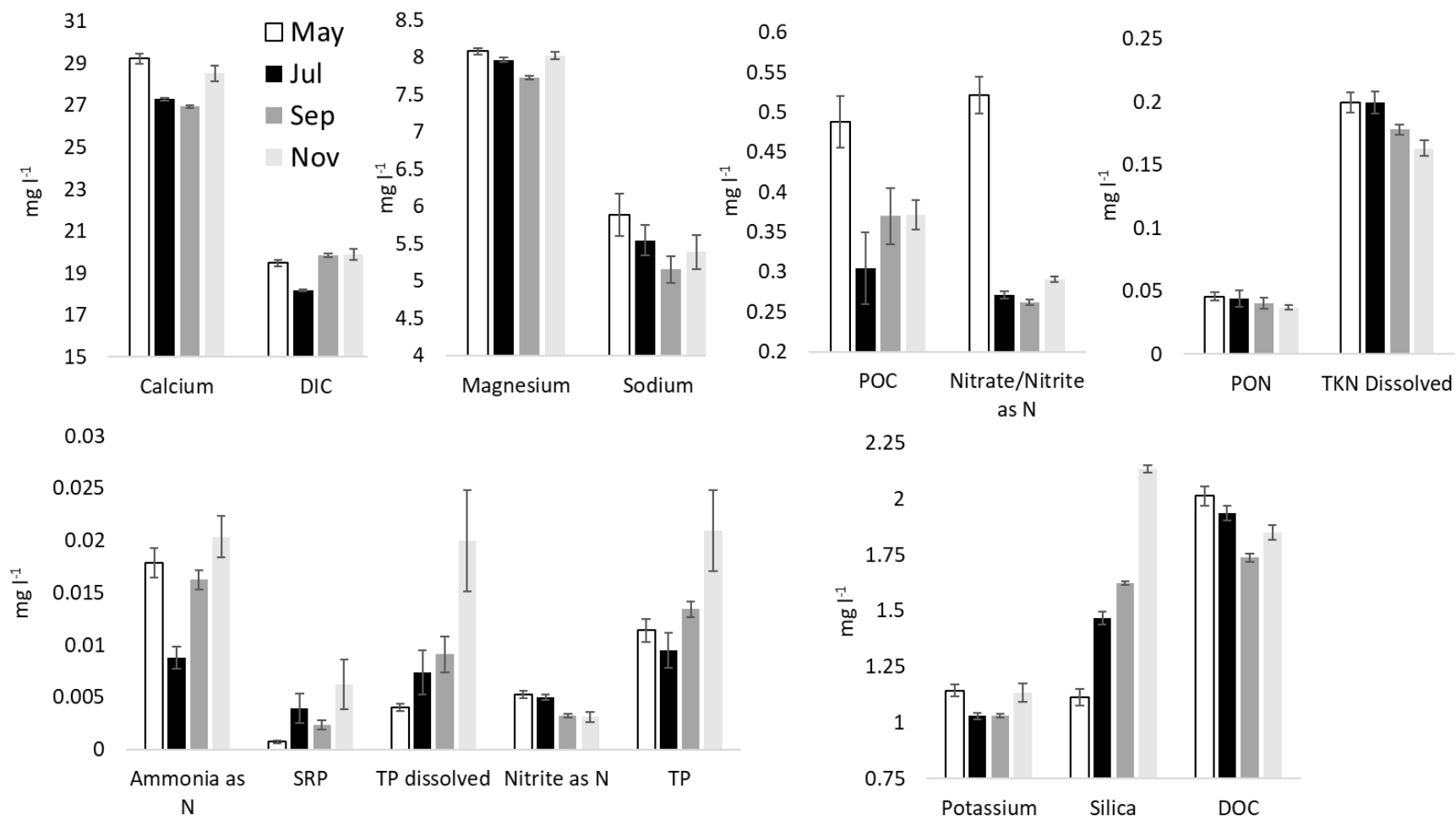


Figure 6. Average Detroit River water chemistry parameters by month in May, July, September and November 2019

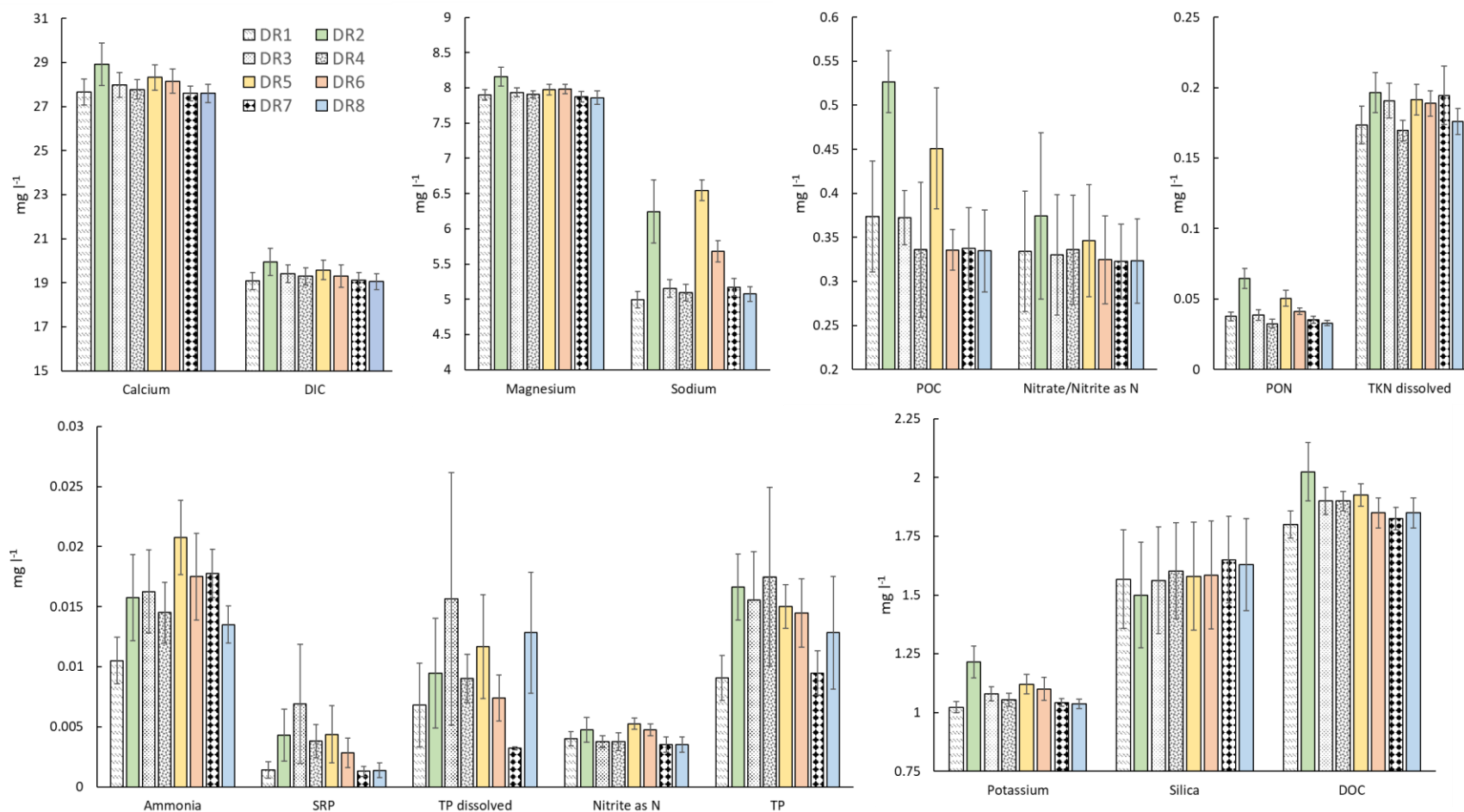


Figure 7. Average Detroit River water chemistry parameters by station. Samples collected in May, July, September and November 2019. Nearshore stations are solid shading, offshore stations are patterned.

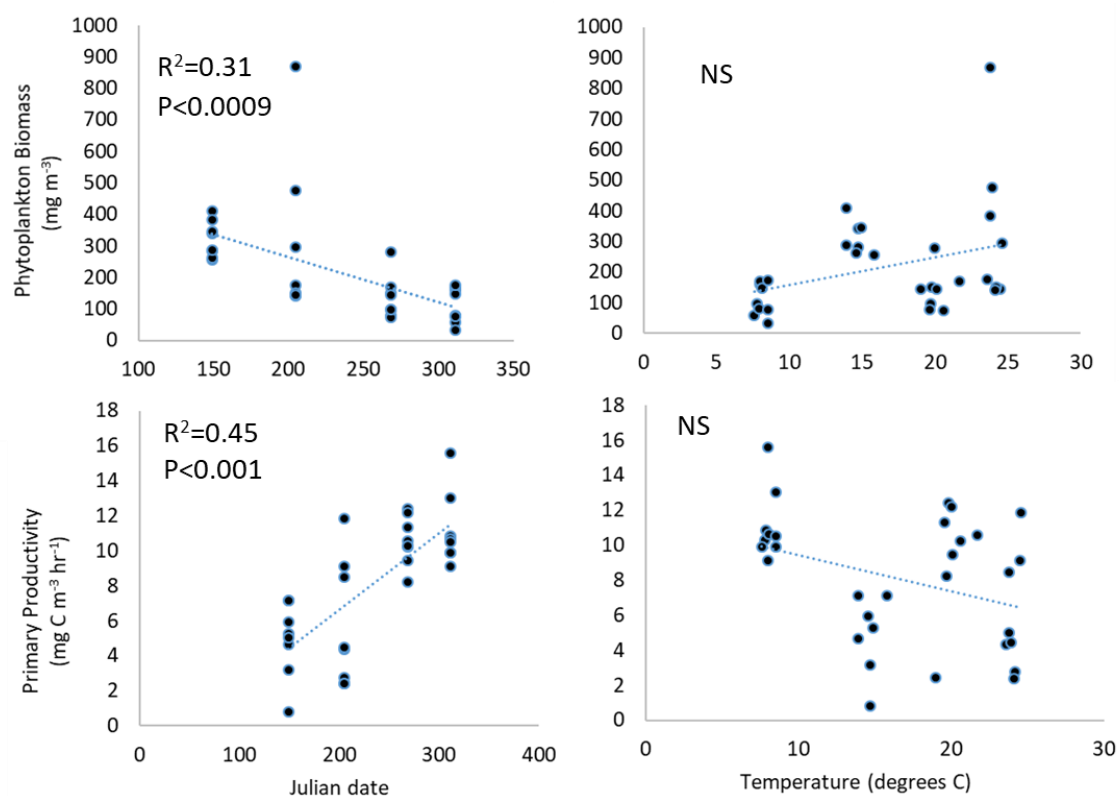


Figure 8. Relationship between a) Phytoplankton Biomass (mg m^{-3}) and Julian Date b) Phytoplankton Biomass (mg m^{-3}) and temperature ($^{\circ}\text{C}$) c) Primary Productivity ($\text{mg C m}^{-3} \text{ hr}^{-1}$) and Julian Date and d) Primary Productivity ($\text{mg C m}^{-3} \text{ hr}^{-1}$) and temperature ($^{\circ}\text{C}$).

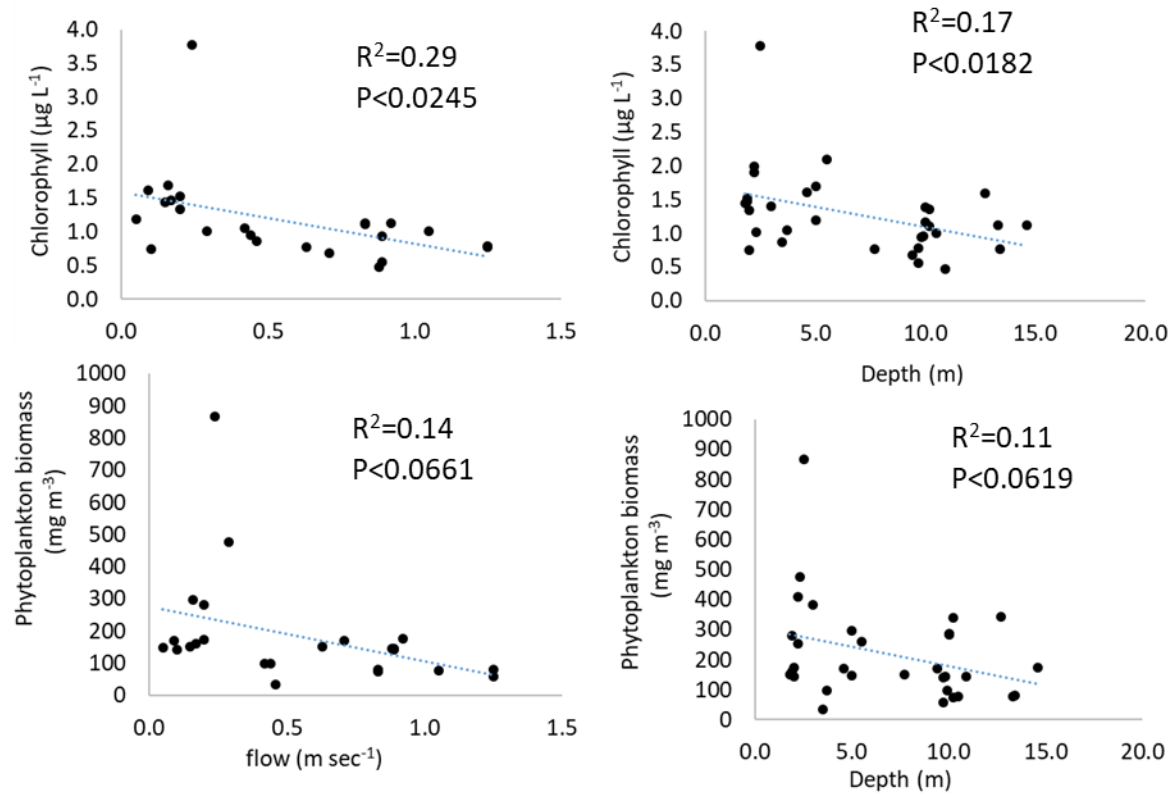


Figure 9. Relationship between) and a) chlorophyll a ($\mu\text{g l}^{-1}$) and flow rates (m sec^{-1}) b) chlorophyll a ($\mu\text{g l}^{-1}$) and depth (m) c) phytoplankton biomass (mg m^{-3}) and flow rates (m sec^{-1}) d) phytoplankton biomass (mg m^{-3}) and depth (m).

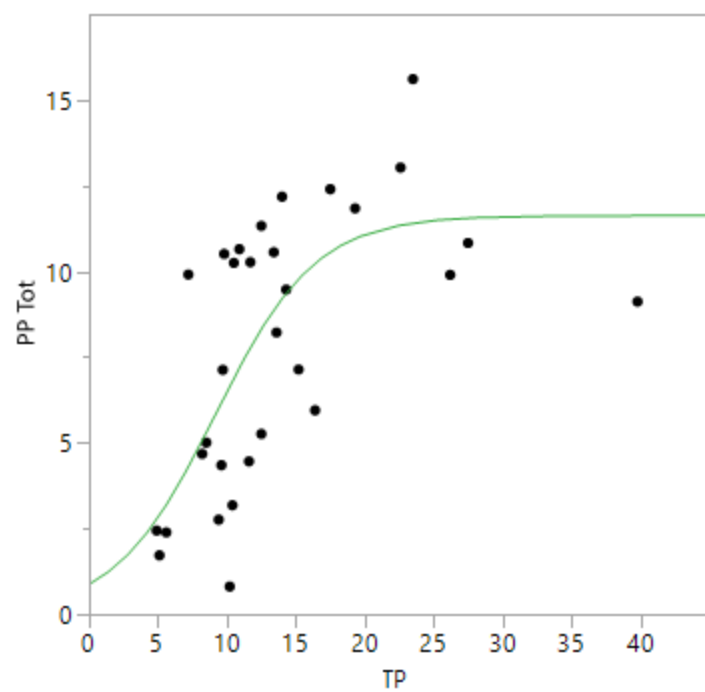
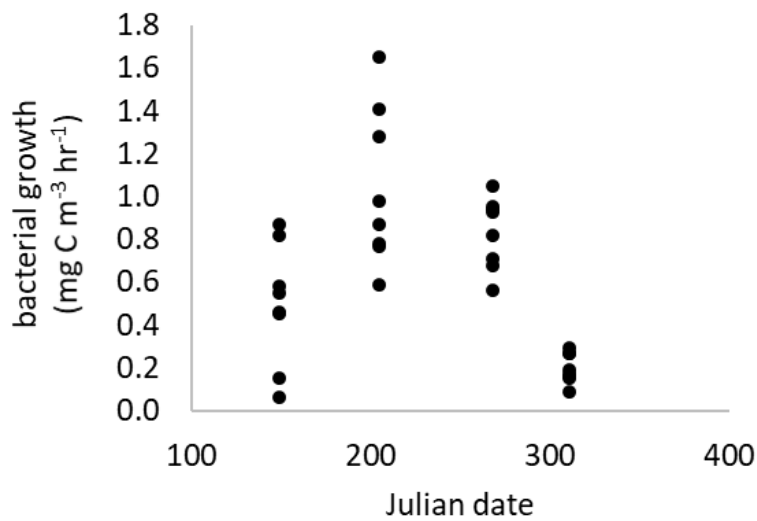


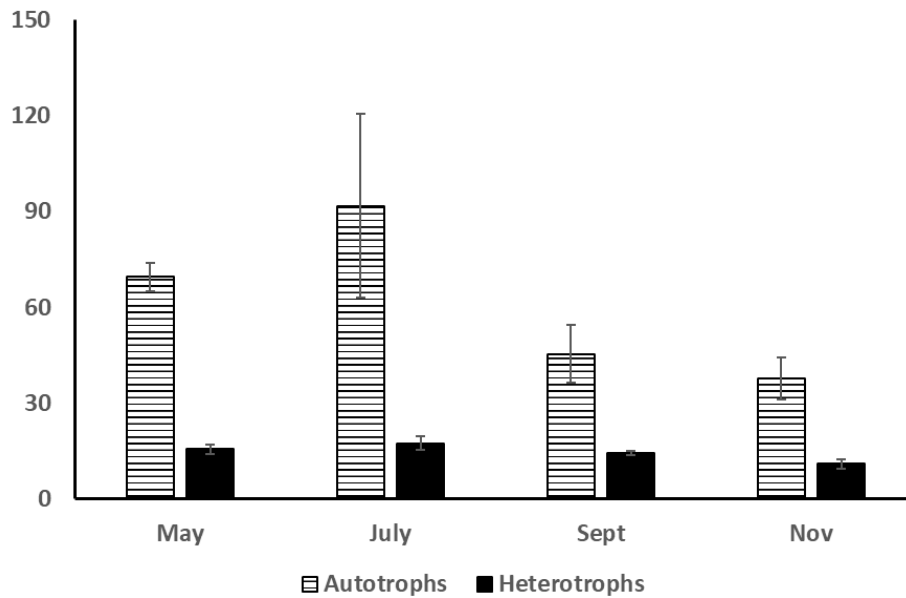
Figure 10. Relationship of total primary productivity rates (PP Tot in $\text{mg C m}^{-3} \text{ hr}^{-1}$) with total phosphorus (TP in mg l^{-1}). Line is logistic best of fit.



Source	LogWorth		PValue
Chl a	2.689	<div></div>	0.00205
Temp	1.448	<div></div>	0.03564
Depth	1.407	<div></div>	0.03919
TP	1.100	<div></div>	0.07938
NO3/2	0.627	<div></div>	0.23627
Bact	0.398	<div></div>	0.39956
Epar	0.343	<div></div>	0.45379
Flow	0.250	<div></div>	0.56286
SiO2	0.138	<div></div>	0.72800

Figure 11. Relationship of bacterial growth rates (mg C m⁻³ hr⁻¹) with sampling date, and results of step wise regression of bacterial growth rate and physical and nutrient parameters.

a) Mean Organic Carbon (mg m^{-3})



b) Relative contribution to organic carbon (% organic carbon)

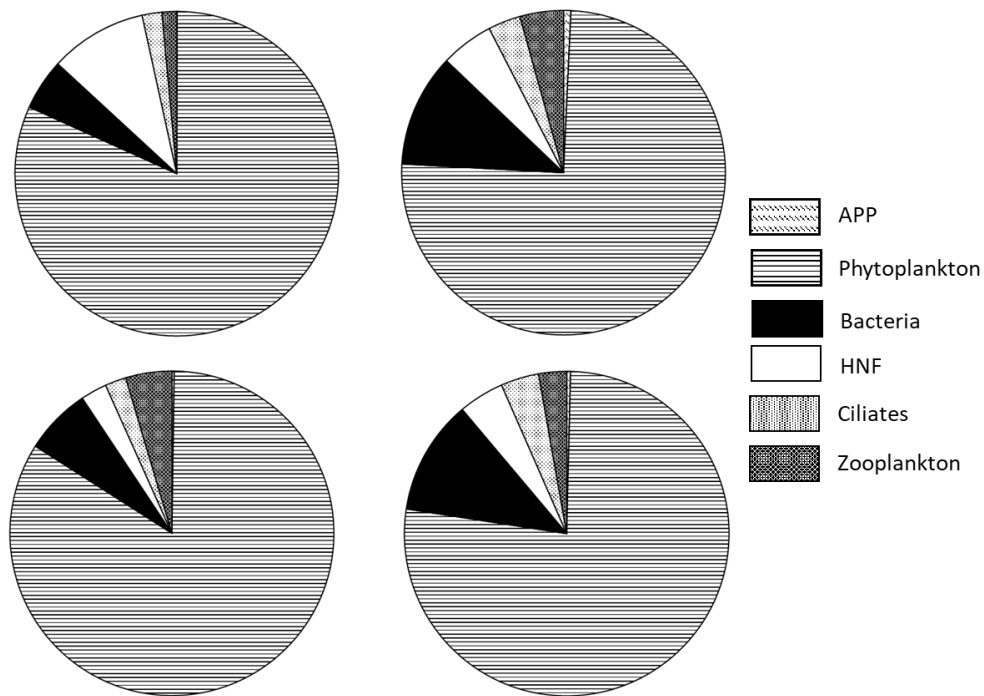


Figure 12. a) Organic carbon resources in the Detroit River for autotrophic and heterotrophic components of the microbial planktonic food web. b) Relative contribution of each component during May, July, September and November. Autotrophs: Phytoplankton and autotrophic picoplankton (APP); Heterotrophs: bacteria, heterotrophic nanoflagellates (HNF), ciliates and zooplankton.

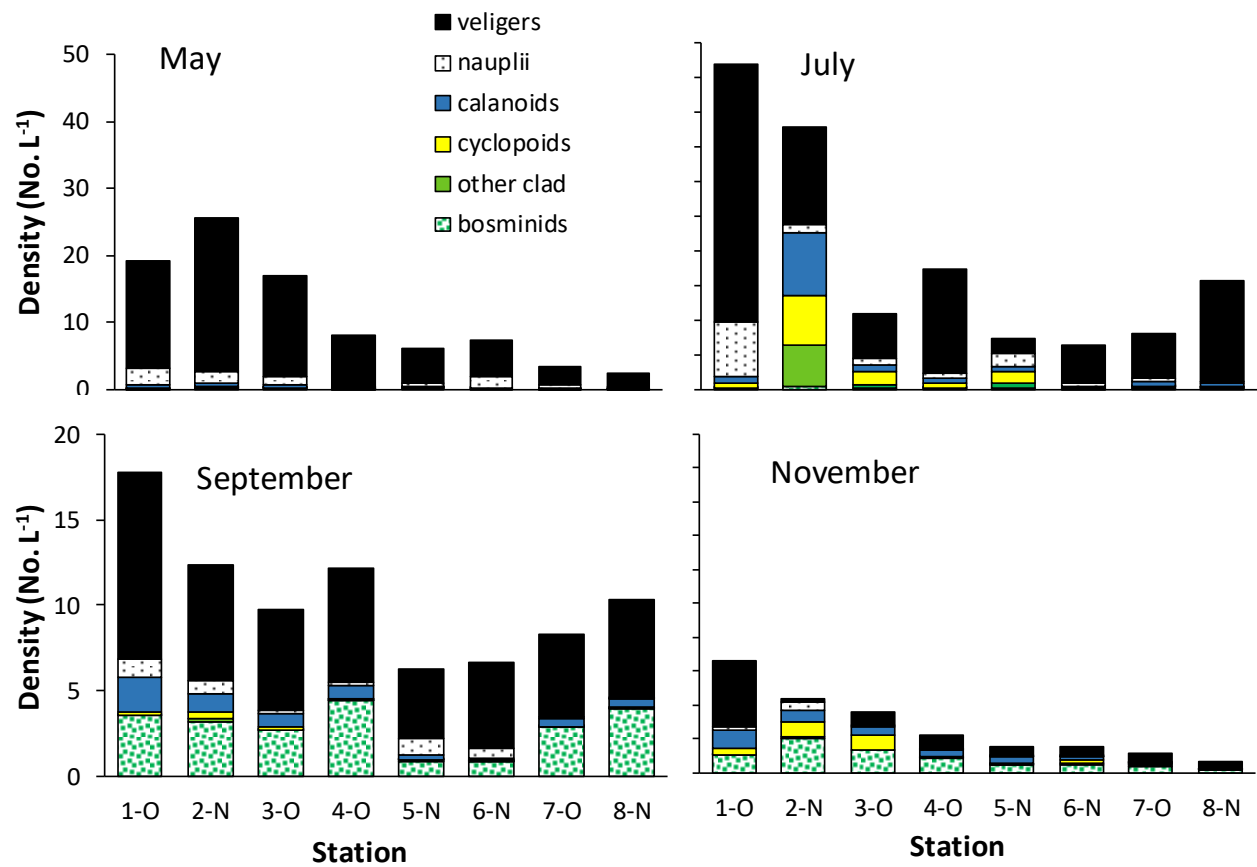


Figure 13. Density of the dominant zooplankton groups in the Detroit River during 4 surveys from May to November 2019. Offshore (O) and nearshore (N) stations are ordered from upstream (left) to downstream (right).

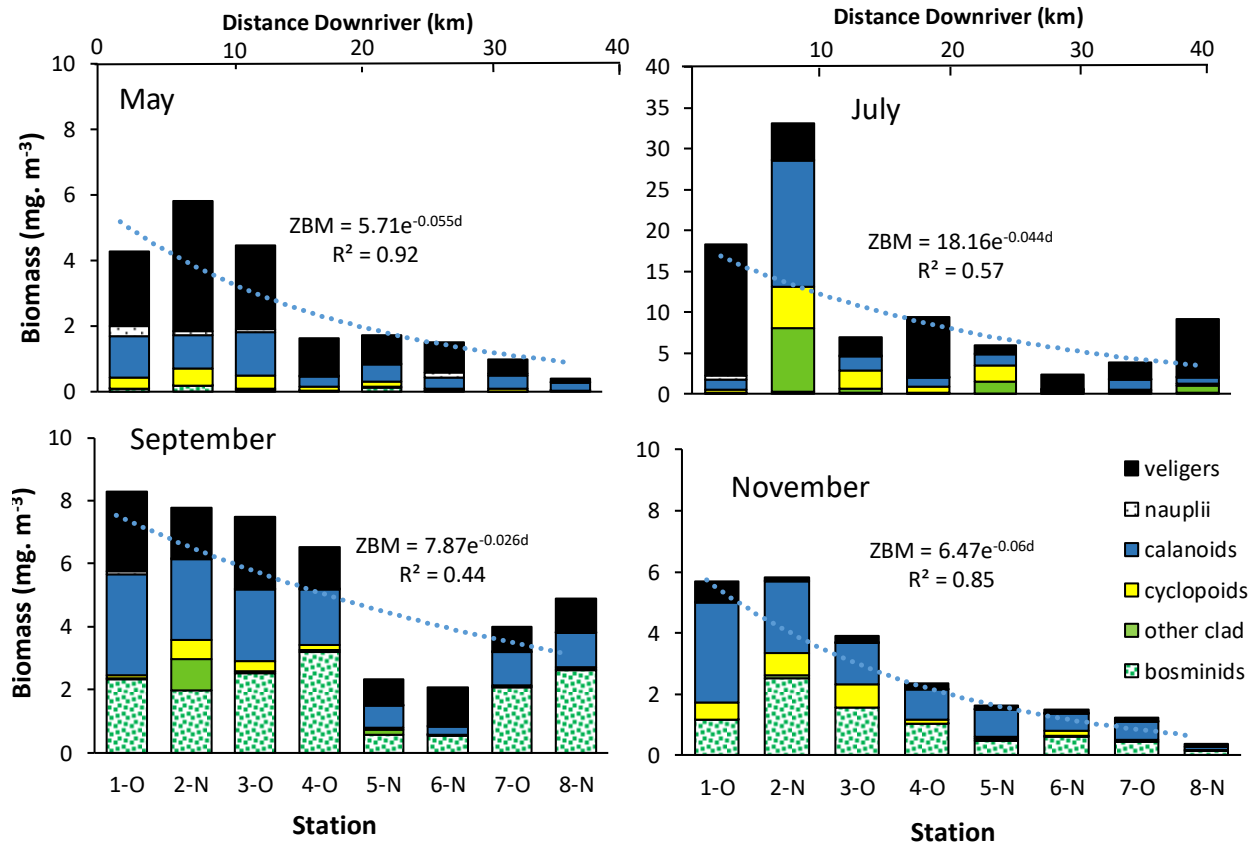


Figure 14. Dry-weight biomass of the dominant zooplankton groups in the Detroit River from May to November 2019. Offshore (O) and nearshore (N) stations are ordered from upstream (left) to downstream (right). Also shown are the fitted exponential curves and equations for total zooplankton biomass (ZBM) attenuation with increasing distance downstream (d), plotted on the top axis.

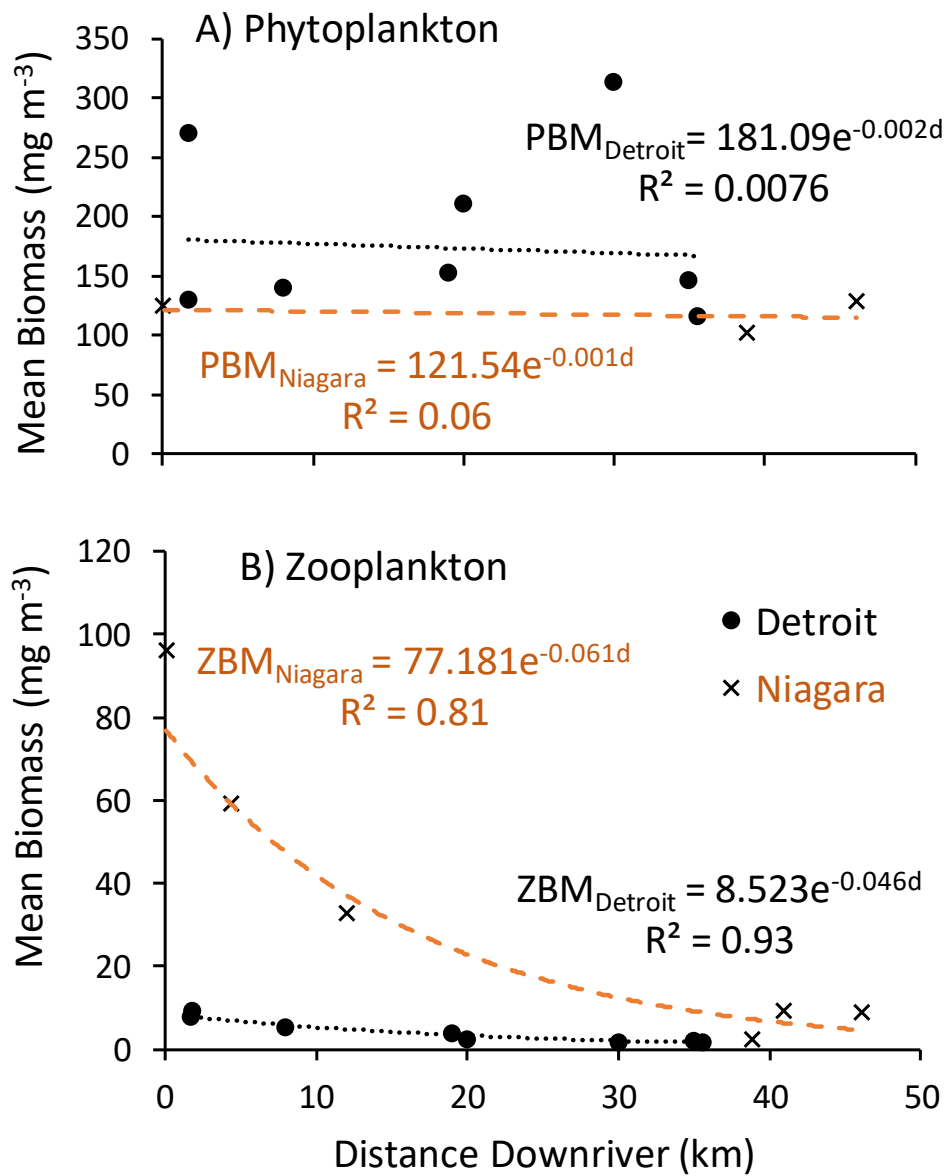


Figure 15. Exponential attenuation of A) total phytoplankton biomass (PBM) and B) total dry zooplankton biomass (ZBM) in the Detroit R. and Niagara R. with increasing distance downstream (d). Values are the geometric means of the four late May early November 2019 surveys in the Detroit River, and six June to October 2014 surveys in the Niagara River (see Rozon et al. 2018).

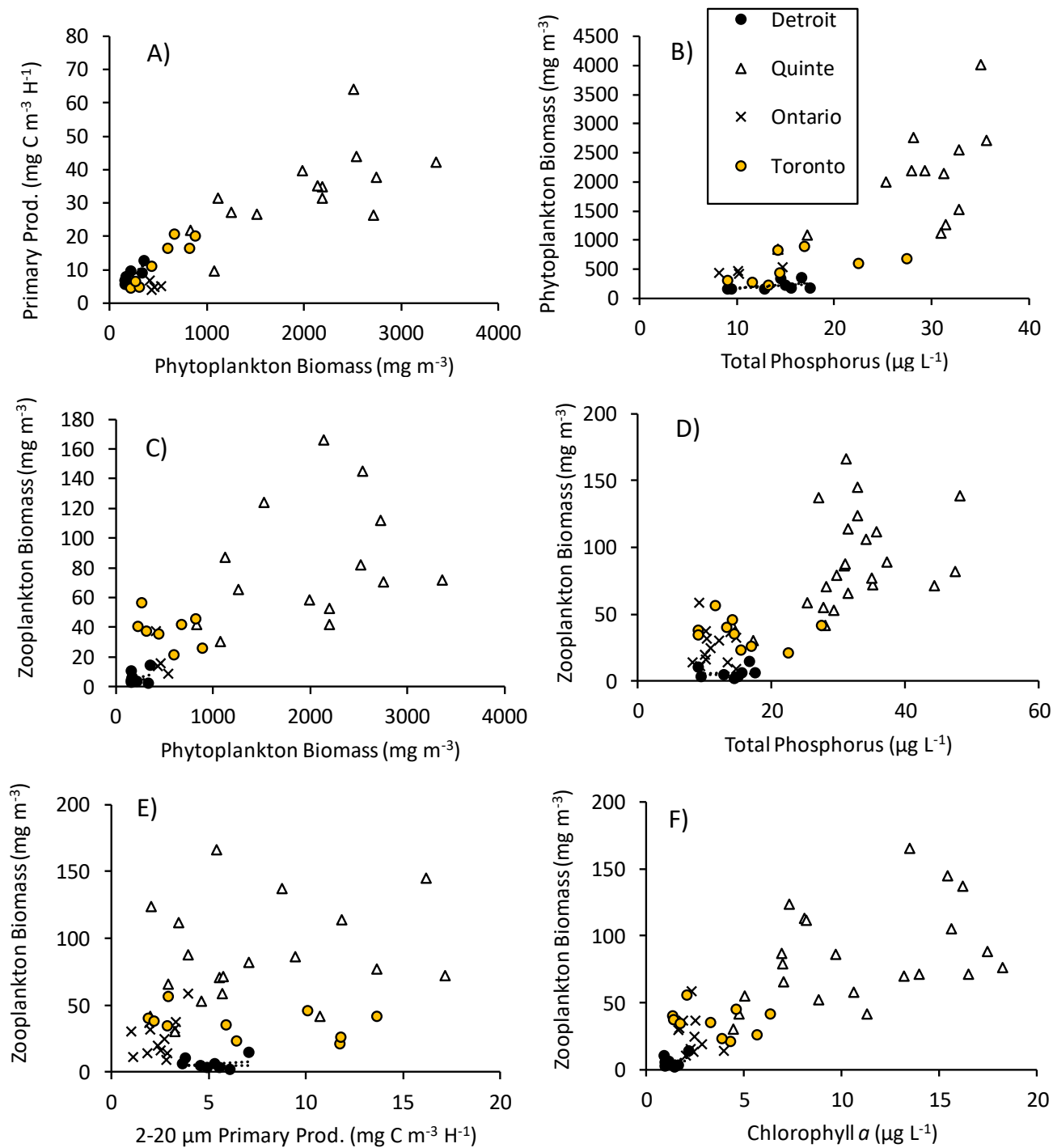


Figure 16. Mean May to October A) total primary production relative to total phytoplankton biomass, B) total phytoplankton biomass relative to total phosphorus (TP), and total zooplankton biomass relative to C) total phytoplankton biomass, D) TP, E) primary production (2 to 20 μm size fraction) and F) chlorophyll *a*. Detroit River 2019 sites are plotted with sites from the Bay of Quinte, Lake Ontario and Toronto Harbour. Samples were collected between 2014 and 2019 at these sites

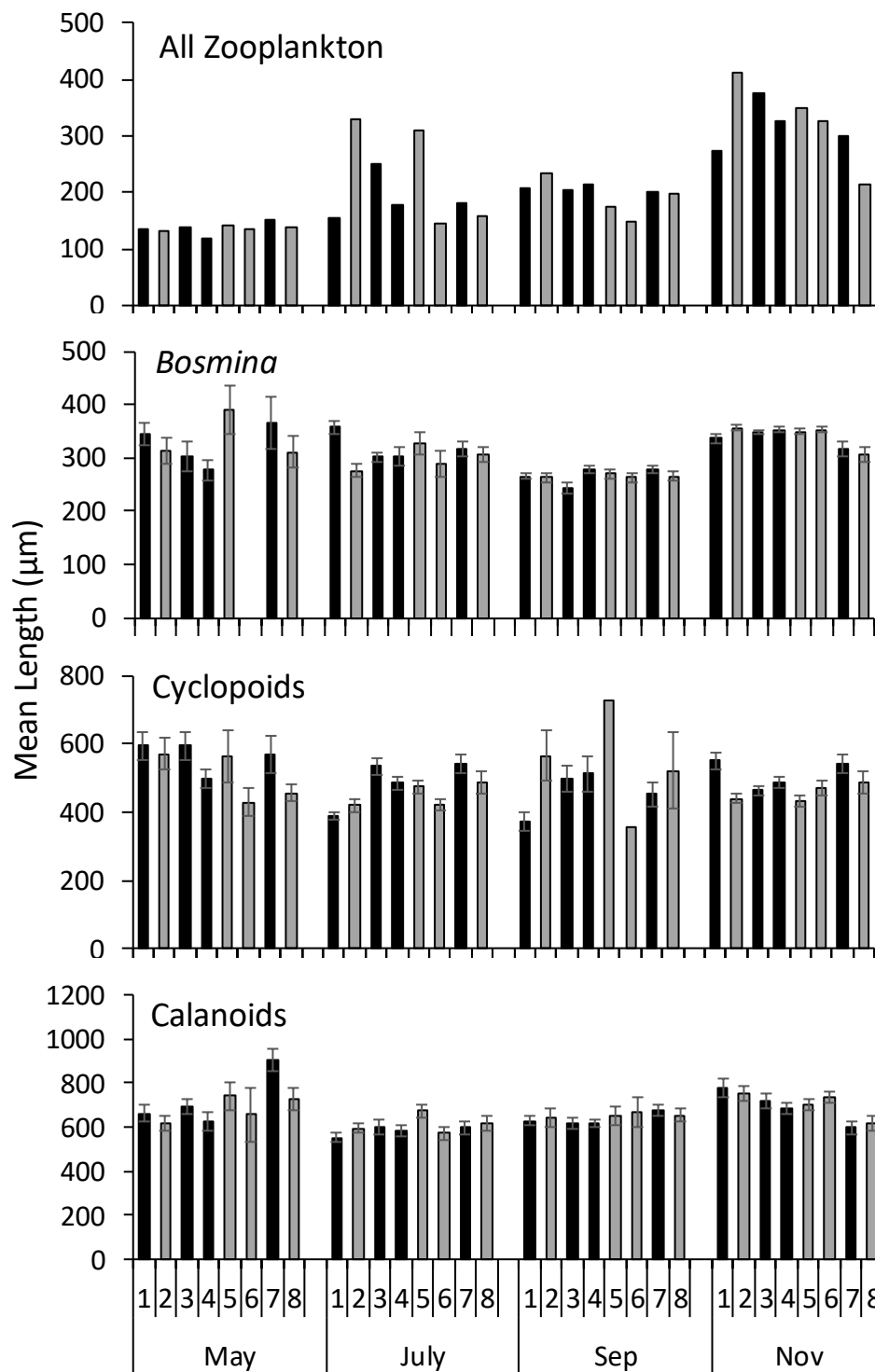


Figure 17. Mean Lengths of total zooplankton, *Bosmina*, cyclopoids and calanoids in the Detroit River, May to November. Stations are shown upstream (1) to downstream (8), with the offshore sites shown in black. Standard errors are shown for the latter three groups as these are based on individual measurements, whereas total zooplankton lengths are weighted for density.

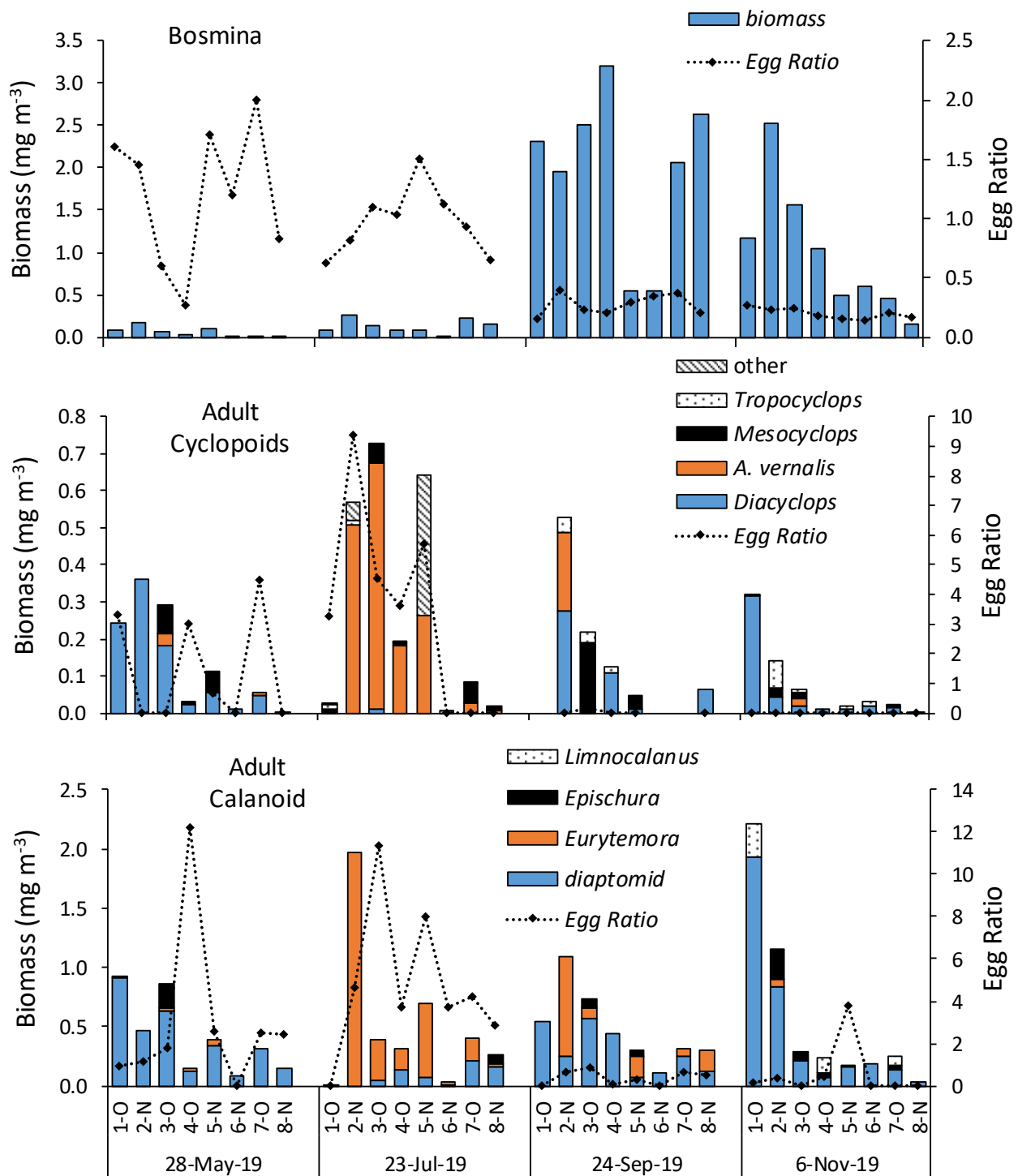


Figure 18. Biomass of *Bosmina* and dominant taxa of adult cyclopoid and calanoid copepods in the Detroit River from May to November 2019. Offshore (O) and nearshore (N) stations are ordered from upstream (left) to downstream (right). Also shown are the mean egg ratios (number of eggs per adult – dotted line) plotted on the secondary y axis.

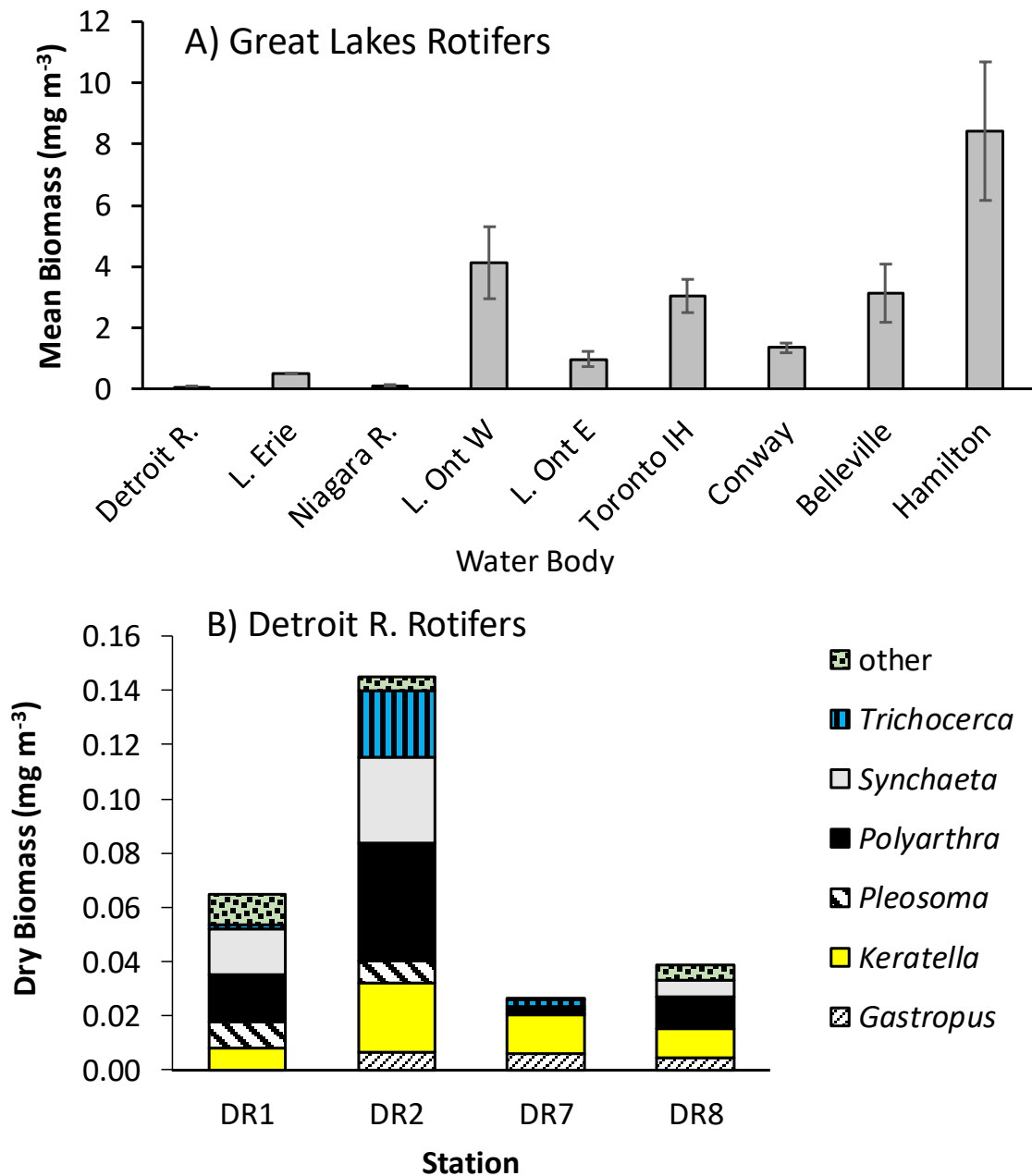


Figure 19. A) Comparison of annual mean Detroit River rotifer biomass to other sites in the Great Lakes sampled by DFO between 2014 and 2018 (see Fig. 20 for site details). B) May to November 2019 annual mean dry biomass of dominant rotifer taxa at selected stations in the Detroit River. DR1 (offshore) and DR2 (nearshore) are in the upper river, and DR7 (offshore) and DR8 (nearshore) are in the lower river.

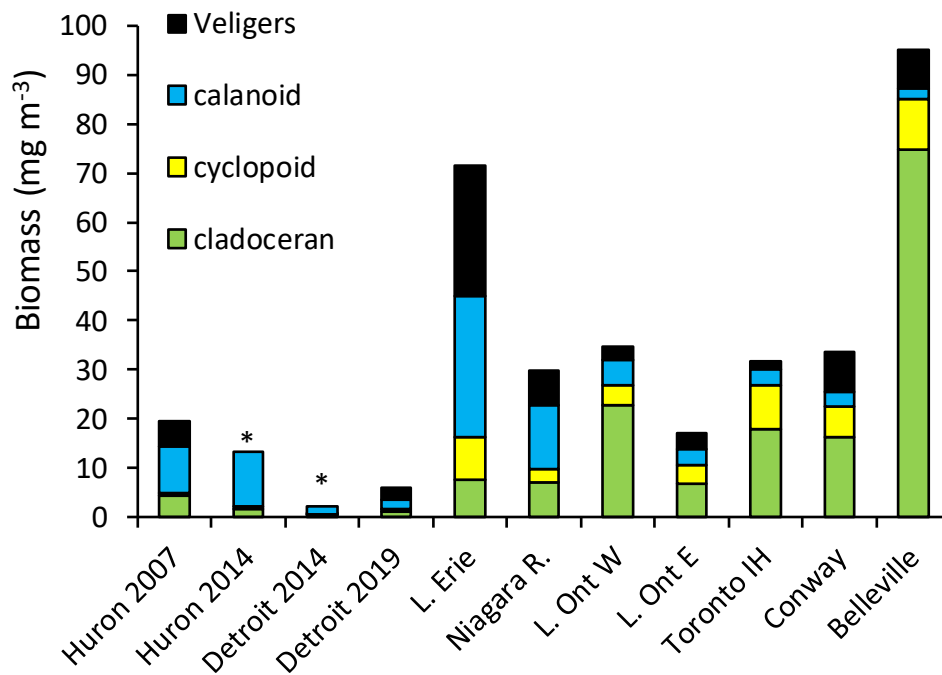


Figure 20. Mean May to October zooplankton biomass in the Detroit River in 2019 compared to other sites. Sites marked with (*) are 2014 sampling in Lake Huron and Detroit River conducted by Keeler et al. (2019), which did not enumerate veligers. The remaining samples were collected by DFO as part of routine monitoring programs. Huron represents a single station sampled near the outlet to the St. Clair River. Lake Erie is represented by an eastern basin station at the mouth of Niagara River in 2014, and Niagara River is based on five riverine stations in 2014 (Rozon et al. 2018). Lake Ontario stations included a western nearshore station (L. Ont W) and a Kingston Basin station (L. Ont E). Bay of Quinte stations include Conway in the lower bay and Belleville in the upper bay. Ontario and Quinte stations represent averages of the 2014 to 2018 period and Toronto Inner Harbour (IH) was sampled in 2016.

Appendices

Appendix 1 Physical and Chemical Properties of the Detroit River observed on May 28; July 23; September 24; and November 6, 2019. Temp = surface temperature (°C), k_d = vertical (light) attenuation coefficient (m^{-1}), Flow = flow rate / water velocity ($m\ s^{-1}$), TP = total phosphorus concentration ($\mu g\ l^{-1}$), $NO_{3/2}$ = nitrate + nitrite concentration ($mg\ l^{-1}$), SiO_2 = silica concentration ($\mu g\ l^{-1}$), and Chl a = chlorophyll a concentration ($\mu g\ l^{-1}$).

Date	Station	Temp	k_d	Flow	TP	$NO_{3/2}$	SiO_2	Chl a
28 May	DR 1	14.7	0.64	n/a	10.4	0.54	1.16	1.35
28 May	DR 2	15.8	0.76	n/a	15.2	0.66	0.97	1.99
28 May	DR 3	14.9	0.64	n/a	12.5	0.54	1.06	1.60
28 May	DR 4	14.7	0.66	n/a	10.2	0.52	1.16	1.17
28 May	DR 5	n/a	0.64	n/a	16.4	0.54	1.05	2.10
28 May	DR 6	14.6	0.75	n/a	9.7	0.47	1.02	1.90
28 May	DR 7	13.9	0.55	n/a	8.2	0.45	1.26	1.38
28 May	DR 8	13.9	0.54	n/a	8.5	0.47	1.22	1.40
23 July	DR 1	23.8	0.29	0.88	5.1	0.25	1.36	0.47
23 July	DR 2	24.5	0.67	0.24	10.3	0.27	1.36	3.78
23 July	DR 3	23.8	0.35	0.92	9.6	0.26	1.42	1.12
23 July	DR 4	23.6	0.41	0.63	9.4	0.27	1.47	0.77
23 July	DR 5	24.2	0.80	0.16	19.3	0.29	1.52	1.69
23 July	DR 6	24.6	1.18	0.29	11.6	0.28	1.52	1.01
23 July	DR 7	23.9	0.15	0.89	5.6	0.28	1.56	0.55
23 July	DR 8	24.1	0.88	0.10	4.9	0.28	1.53	0.75
24 Sep	DR 1	19.0	0.61	0.44	13.6	0.26	1.62	0.95
24 Sep	DR 2	19.7	0.60	0.15	17.5	0.29	1.64	1.44
24 Sep	DR 3	19.8	0.63	0.83	12.5	0.26	1.62	1.13
24 Sep	DR 4	19.6	0.52	0.83	10.5	0.26	1.64	1.11
24 Sep	DR 5	20.6	0.93	0.09	13.4	0.26	1.58	1.61
24 Sep	DR 6	21.7	0.96	0.20	14	0.25	1.66	1.52
24 Sep	DR 7	20.0	0.49	0.89	14.3	0.26	1.63	0.94
24 Sep	DR 8	20.1	0.67	0.42	11.7	0.26	1.61	1.05
6 Nov	DR 1	7.8	0.61	1.25	7.2	0.29	2.13	0.78
6 Nov	DR 2	7.6	0.87	0.17	23.5	0.29	2.03	1.47
6 Nov	DR 3	8.0	0.68	1.25	27.5	0.27	2.15	0.77
6 Nov	DR 4	7.9	0.65	0.71	39.8	0.29	2.14	0.69
6 Nov	DR 5	8.0	0.75	0.05	10.9	0.30	2.17	1.19
6 Nov	DR 6	8.1	0.76	0.20	22.6	0.30	2.14	1.34
6 Nov	DR 7	8.5	0.73	1.05	9.8	0.30	2.15	1.01
6 Nov	DR 8	8.6	0.96	0.46	26.2	0.29	2.16	0.87

APPENDIX 2: Phytoplankton and Zooplankton Populations BUI Assessment Report Tracking

The DFO 2020 Assessment Report (Appendix 1) was presented to the Monitoring and Research Work Group, Steering and Implementation Committee, and Public Advisory Council. The report was also sent to the Four Agency Managers for review and comment by the U.S. Environmental Protection Agency and the Michigan Department of Environment, Great Lakes, and Energy. The DRCC also sent the report to Aamjiwnaang and Caldwell First Nations and the Métis Nation of Ontario for review and comment. All comments received were acknowledged and/or addressed. The responses from all parties engaged in the potential designation of this BUI were positive. See below table for details regarding U.S. Agency, First Nation, Métis, and public engagement on this BUI re-designation.

DRCC Monitoring and Research Work Group	Draft assessment presented on June 8, 2020; comments addressed. Decision to move assessment forward to DRCC Steering and Implementation Committee.
DRCC Steering and Implementation Committee (SIC)	Draft assessment presented to SIC August 17, 2020; comments addressed. Decision to move forward with the status designation of 'not impaired'.
Public Review	<p>Presented at an online virtual open house on December 3, 2020. Attendees of the online virtual open house were polled as to whether they agreed with the DRCC's status assessment of this BUI. Two participants agreed with the status assessment of 'not impaired' and no participants identified they did not agree.</p> <p>Assessment and associated fact sheets were posted on DRCC website for public comment from November 6, 2020 – December 31, 2020 (periodic Facebook, Twitter, and Instagram reminders to comment were posted on social media; notice in December 2020 newsletter). No comments received.</p>
Indigenous Review	<p>Reports and fact sheets were sent to Aamjiwnaang and Caldwell First Nations on November 23, 2020. Comments were received indicating support for the recommendation of changing the status of this BUI to 'not impaired'.</p> <p>Reports and fact sheets were also sent to the Métis Nation of Ontario on January 20, 2021. The Region 9 Consultation Committee did not have any comments on the report.</p>
Four Agency Management Committee	The status assessment report was sent to Michigan Environment, Great Lakes, and Energy and the US Environmental Protection Agency on December 17, 2020.

	<p>Comments were received from Michigan Environment, Great Lakes, and Energy on February 8, 2021; no concerns identified and comments were addressed.</p> <p>Comments received from US EPA on March 10, 2021; no concerns identified and comments were addressed.</p>
COA AOC Annex Leads	Submitted for formal re-designation July 14, 2021.