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ABSTRACT

Invasive quagga mussels have recently replaced zebra mussels as the dominant filter-feeding bivalves in the Great Lakes. This study examined microzooplankton (i.e., grazers <200 μm) and their trophic interactions with phytoplankton, bacteria, and bivalve mussels in Saginaw Bay, Lake Huron, following the zebra to quagga mussel shift. Microzooplankton distribution displayed strong spatial and temporal variability (1.73–28.5 μg C/L) relative to phytoplankton distribution. Ciliates were the dominant component, especially in the spring and early summer. Rotifers and dinoflagellates increased toward late summer/fall in the inner and outer parts of the bay, respectively. Microzooplankton grazing matched bacterial growth rates and removed ca. 30% of the phytoplankton standing stock in the <100 μm size fraction per day. The greatest herbivory occurred at the site dominated by colonial cyanobacteria. Microzooplankton, which comprised <4% of the quagga mussels prey field (i.e. available prey), contributed 77% and 34% to the quagga carbon-based diet during *Microcystis* and diatom blooms, respectively. Feeding on microzooplankton could buffer mussels during lean periods, or supplement other consumed resources, particularly during noxious cyanobacterial blooms. The results of this study demonstrate that microzooplankton are a resilient and critical component of the Saginaw Bay ecosystem.

Introduction

The invasive Ponto–Caspian bivalve zebra mussel (*Dreissena polymorpha*) has had a profound effect on the Laurentian Great Lakes ecosystem since its inadvertent introduction with ship ballast water in the mid-1980s (Vanderploeg et al., 2002). After reaching its peak in the early 2000s, the abundance of the zebra mussel has declined, whereas its congener, the quagga mussel (*Dreissena rostriformis bugensis*), has continued to expand, first in shallow waters (Mills et al., 1999) and then at depths >50 m (Nalepa et al., 2009). The quagga mussel possesses a number of physiological adaptations, including high assimilation efficiency and growth rates (Baldwin et al., 2002; Ram et al., 2012; Stoeckmann, 2003), which have allowed it to displace the well-established and prolific zebra mussel (Ricciardi and Whoriskey, 2004). In Lake Michigan, quagga mussel expansion to mid-depth regions has caused the disappearance of the spring phytoplankton bloom (Vanderploeg et al., 2010) and produced conditions similar to the oligotrophic Lake Superior (Fahnenstiel et al., 2010).

In Saginaw Bay, after an initial decline related to *Dreissena* invasion (Fahnenstiel et al., 1995), phytoplankton biomass rebounded, with increasing dominance by colony-forming cyanobacteria, such as *Microcystis aeruginosa* (Millie et al., 2011; Vanderploeg et al., 2001, 2009). This species includes toxic (microcystin-producing) strains (Dyble et al., 2008; Vanderploeg et al., 2001, 2013) and now comprises a significant proportion of phytoplankton biomass during late summer in Saginaw Bay (Fishman et al., 2009; Vanderploeg et al., 2001, 2002, 2009). The success of *Microcystis* in Saginaw Bay and the ecologically similar western basin of Lake Erie has been linked to alteration of phytoplankton competitive dynamics by invasive mussels through selective grazing (Lavrentyev et al., 1995), rejection of *Microcystis* colonies as pseudo-feces (Vanderploeg et al., 2001, 2013), and to nutrient recycling (Conroy et al., 2005; Gardner et al., 1995; Lavrentyev et al., 2000) with recent evidence pointing to the greater importance of selective rejection rather than immediate recycling of nutrients from mussels (Johengen et al., 2013). The re-engineering of the aquatic environment by non-indigenous bivalves, including altering energy and nutrient fluxes and proliferation of noxious algal blooms and other invasive species, have received much attention in the Great Lakes (Fishman et al., 2010; Hecky et al., 2004; Vanderploeg et al., 2001, 2002). However, our knowledge of pelagic food web structure and dynamics in the Great Lakes after the recent zebra-quagga shift remains incomplete.

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Microzooplankton (*sensu lato* grazers 15–200 μm, including ciliates, rotifers, dinoflagellates, and sarcodines) are a critical component of the Great Lakes ecosystem. They possess biomass comparable to planktonic crustaceans (Fahnenstiel et al., 1998; Gardner, 2004; Vanderploeg et al., 2007) and high growth and herbivory rates (Carrick et al., 1992; Gobler et al., 2008; Lavrentyev et al., 2004). However, little is known about their trophic interactions with the quagga mussel. The impacts of zebra mussels on protists were examined experimentally in 1994–1995 in Saginaw Bay, where mussels preyed selectively on the larger and weak-swimming species of ciliates and flagellates (Lavrentyev et al., 1995, 2000). Zebra mussels have drastically reduced the abundance of planktonic rotifers in the shallow western basin of Lake Erie (MacIsaac et al., 1995) and Lake St. Clair (David et al., 2009).

Rotifers also are the only group of microzooplankton that has been examined post quagga invasion. Barbiero and Warren (2011) found a decline in the abundances of *Polyarthra* and *Keratella*, and a concomitant increase in the colonial *Conochilus unicornis*. To our knowledge, the quagga mussel impact on the entire microzooplankton community, including one of its key components, ciliates, has not been examined so far in the Great Lakes. Based on literature and our previous experiments with the zebra mussel, we hypothesized that the quagga mussel would have a similarly strong and selective impact on microzooplankton in Saginaw Bay. Thus, the objectives of our study were: (1) compare microzooplankton abundance and composition to those before the quagga mussel invasion, (2) examine the spatial distribution and seasonal dynamics of microzooplankton in Saginaw Bay in relation to major environmental factors, (3) measure microzooplankton grazing rates on phytoplankton and bacteria during a *Microcystis* bloom, and (4) determine microzooplankton contribution to the quagga mussel diet during different seasons.

Material and methods

The study sites and field sampling

Saginaw Bay is a large (82 km long and 42 km wide) bay extending from western Lake Huron. It can be divided into the shallow, eutrophic inner bay, which receives inflow from the Saginaw River, and the mesotrophic, deeper outer bay, which is open to Lake Huron (Millie et al., 2011; Nalepa et al., 2005). Sampling was conducted as part of the NOAA-sponsored Multiple Stressors project at established Great Lakes Environmental Research Laboratory (GLERL) sampling locations (Fig. 1), the same sites sampled in the 1990s. Microzooplankton spatial distribution was examined at five sites in May 2008 and at twelve sites in July 2008. Samples were collected at 1-m depth using a 5-L Niskin bottle. In July 2008, samples were also collected from the bottom layer (0.5 m from the bottom). In May–October 2009 and July–October 2010, microzooplankton seasonal dynamics were observed at three master stations: SB5, SB10, and SB20) representing the inner and outer bay (Fig. 1).

Microzooplankton grazing experiments

Phytoplankton and bacterial growth and grazing loss rates were determined in dilution experiments (Landry and Hassett, 1982). The general

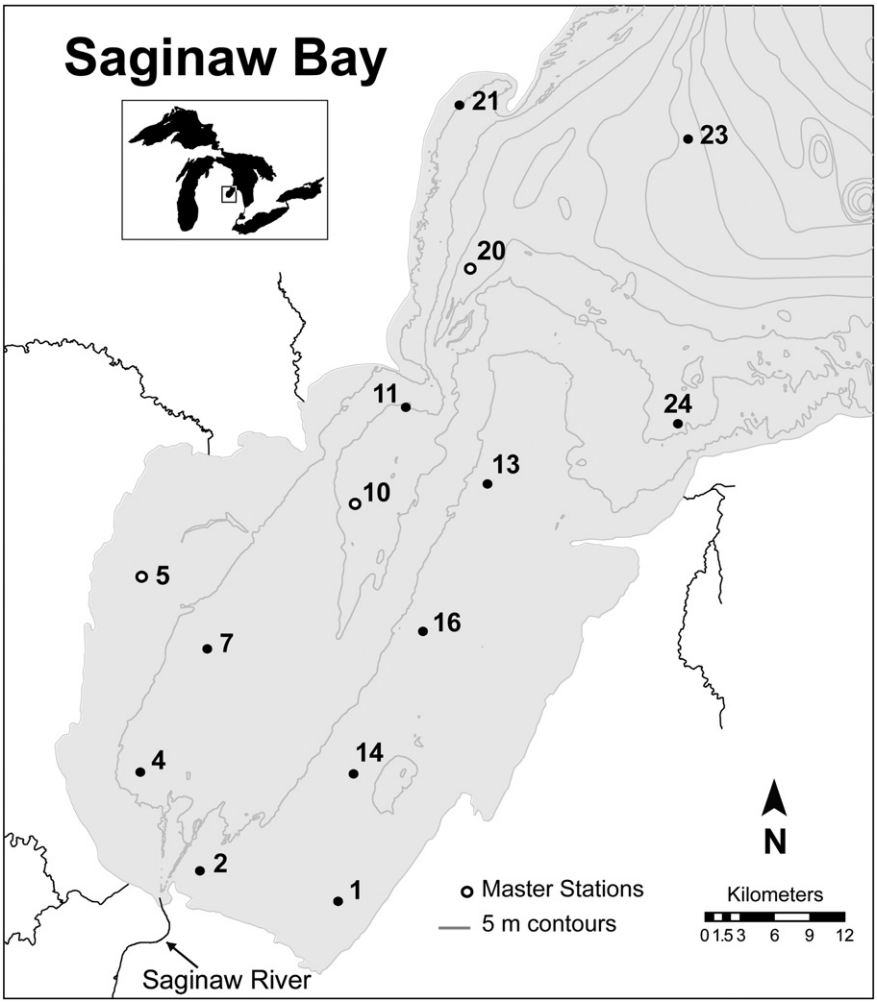

Fig. 1. Sampling locations in Saginaw Bay. Master stations (open circles: SB5, SB10, SB20) were sampled seasonally in 2008–9.

approach is to dilute whole lake water, containing natural microbial plankton assemblages, with 0.2-μm-filtered (almost particle-free) water from the same sample. Dilution reduces microzooplankton encounter rates with their prey so that phytoplankton and bacteria growth rates (μ) approach their intrinsic maximum at given temperature and light conditions in highly dilute treatments. To equalize prey growth conditions across the dilution series all treatments are amended with dissolved nutrients. The advantages and limitations of this popular method have been discussed (First et al., 2007; Landry and Calbet, 2004). In this study, we used the 2-point dilution assay (Landry et al., 1984). In this variation of the dilution technique, the grazing rate (g) is calculated as the difference in prey μ between undiluted and highly diluted treatments. This approach provides the same growth and grazing rate estimates as the original multi-point approach (Strom and Fredrickson, 2008) but eliminates the uncertainty related to changes in microzooplankton clearance and growth rates in dilution series (Dolan and McKeon, 2004; First et al., 2009).

Sub-surface lake water for microzooplankton grazing experiments was collected from three locations in July 2009: (1) SB2 (43 40′ 00″ N, 83 48′ 25″W) near the Saginaw River confluence; (2) SB5 (43 53′ 43″N, 83 51′ 38″W) in the inner bay near Au Gres, MI; and (3) SB20 (44 07′ 34″N, 83 30′ 00″W) in the outer bay (Fig. 1). The water column conditions during the experiments are shown in Table 3. The water was gently siphoned and pre-screened through a 153-μm mesh net using gravity reverse filtration to remove crustacean mesozooplankton. Samples were diluted to 10% with lake water filtered through 0.2 μm, large-volume pleated capsules (Pall Science) rinsed with copious amounts of deionized water. Diluted and undiluted samples were placed in duplicate, 1-L clear polycarbonate bottles and enriched with nutrients within the range reported from Saginaw Bay to equalize conditions in dilution treatments: 8 μM-N (final concentration, NH_4Cl) and 0.5 μM-P (KH_2PO_4). Control consisted of undiluted lake water without added nutrients. The bottles were individually shaded with a neutral density screen to mimic 50% incident radiation and incubated in the lake at ca. 0.25 m depth for 24 h. Sub-samples for bacteria, phytoplankton, and chlorophyll a determination were collected at 0 and 24 h and pre-processed immediately as described below.

Prey apparent growth rates were calculated assuming exponential growth:

$$\mu = \ln(N_t/N_0)/(t/24), \quad (1)$$

where:

μ growth rate (d),
N_0 and N_t prey abundance at the beginning and end of experiment, respectively, and
t time (hours).

Gross growth rates of phytoplankton (k) were estimated as the sum of their net growth rate in control and the grazing mortality rate (Caron, 2001).

Mussel feeding experiments

Collection, handling, and re-acclimation of mussels to natural seston followed closely methods described by Vanderploeg et al. (2009). Quagga mussels and sub-surface lake water were collected at SB5 in July and September 2011. On each sampling occasion, shallow-morph quagga mussels were collected by bottom trawl. Clusters of the mussels were wrapped in moist paper towels and placed in ice chests for transport back to an environmentally controlled room. A tray containing ice or cold gel packs was placed above (but not on) the mussels to keep them cool. Surface lake water from the same site was collected into 25-L polycarbonate carboys and transported to the laboratory in ice chests. Travel time from the collection site to GLERL was normally 4–5 h.

Upon arrival in the laboratory, carboys were placed under cool white fluorescent lights (40 μmol quanta/m^2/s) at ambient temperature and light/dark cycle in the same environmental room we carried out the experiments. We cut byssal threads of 100–120 medium-large mussels (16–22 mm) with a razor blade and gently brushed off periphyton and other attachments. Mussels were re-acclimated for ~ 17 h to 153-μm screened lake water at ambient lake temperature to re-establish natural feeding behaviors (and achieve digestive equilibrium with their food) in two steps: (1) cleaned mussels were immediately placed in a wide mesh (0.8 cm) polyethylene basket (7 cm H × 15 cm W × 34 cm L) on the bottom of a 100 L polypropylene drum filled with 85 L of water for ~14 h overnight; (2) the next morning all mussels were transferred to a 50-L aquarium filled with 153-μm screened water for 2–3 h before being used in the feeding experiments to insure that they received a fresh supply of water nearly identical to what they would experience in the feeding experiment. At the time of transfer to the aquarium, 20 mussels were sorted into each of four small baskets (2.5 cm H × 13 cm W × 15 cm L). An additional ~20–30 mussels, to be used related nutrient excretion experiment to be reported elsewhere, were also acclimated in the same aquarium with mussels to be used in the feeding experiments.

In broad terms, feeding experiments were similar to the methods described previously (Vanderploeg et al., 2001, 2009). Experiments were conducted in 20-L polyethylene buckets at in situ lake temperatures (25 °C and 17 °C in July and September, respectively). Light intensity during the experiments was ~8 μmol quanta/m^2/s. Gentle bubbling provided agitation to keep particles suspended during the 4.5–5 h experiments.

To set up the experiment 7 buckets were filled with 16 L of 153-μm screened water distributed from a stirred 130-L polyethylene drum with spigot to assure homogeneity of seston among buckets. After filling all buckets, 1 L of water was taken for analysis of initial conditions, using 200 mL of water for chlorophyll analysis and 100 mL for microzooplankton, the latter being preserved with 1% Lugol solution. Immediately after taking the initial water sample, the baskets, each containing the 20 mussels, were placed into four experimental buckets. Three additional buckets with empty baskets were used as controls. The experiment started once the bivalves opened and the inhalant and exhalant siphons were extended (typically within 10 min of mussel introduction) and lasted for 4.5 and 5 h in July and September, respectively. Average lengths and ash-free dry weights of mussels in experiments were: 19.4 mm and 13.9 mg in July; and 21.2 mm and 10.5 mg in September. To obtain final water column samples after incubation, we siphoned 14 L of the bucket contents into another bucket, from which samples were collected. The remaining 1 L at the bottom was mixed and sampled. Again 200 mL of water was taken for chlorophyll analysis, and 100 mL of water was preserved in 1% Lugol solution for microzooplankton analysis at each phase. Weighted average of microzooplankton biomass and chlorophyll a concentrations from water column and bottom water samples in each bucket was then calculated to give total concentrations in each bucket. Calculations of mussel net filtering (F_I) and ingestion rates (I), based on average microzooplankton biomass, followed the methods used in Vanderploeg et al. (2001, 2009):

$$F_I = (V/nt)\ln(C_{wt}/Z_{wt}), \quad (2)$$

where:

V volume of water in container (mL),
n mussel abundance in the experimental container
C_{wt} and Z_{wt} average final microzooplankton (μg C/L) and chlorophyll a (μg/L) in experimental and control containers at end of experiment, respectively, and
t time (hours).

$$I = F_I(Z_{wt}-C_0)/(\ln Z_{wt}-\ln C_0), \quad (3)$$

where:

C_0 microzooplankton biomass or chlorophyll a concentration in initial samples.

All clearance rates were normalized to ash-free dry weight (AFDW, mg) of the mussels used in the experiments. All analytical methods for chlorophyll a and ash-free dry weight measurement followed Vanderploeg et al. (2009). Microzooplankton species-specific growth rates in controls were determined using Eq. (1) but expressed in h.

Sample analyses

Chlorophyll a concentrations in dilution experiments were determined from water samples filtered onto 47 mm 0.2 μm nylon membrane filters. The filters were immediately frozen. At the laboratory, chlorophyll a was extracted in 90% acetone for 24 h at −20 °C and measured on a spectrophotometrically calibrated Turner Designs 700 fluorometer using a non-acidic method (Welschmeyer, 1994). In the mussel feeding experiments and field survey, chlorophyll a concentrations were determined as described in Vanderploeg et al. (2009). These values were converted to phytoplankton biomass assuming a carbon:chlorophyll a ratio of 35. This ratio was in turn based on phytoplankton composition (Vanderploeg, unpublished data), the chlorophyll–phytoplankton volume relationships established for Saginaw Bay (Dolan et al., 1978), and taxon-specific chlorophyll a and carbon contents (Menden-Deuer and Lessard, 2000; Montagnes et al., 1994).

Microzooplankton were preserved in 1% (final concentration) acid Lugol's iodine and stored at 4 °C. In the laboratory, the samples were settled in 50 to 100 ml Utermöhl chambers, and the entire chamber area was scanned under an Olympus IX70 inverted microscope equipped with differential interference contrast at 100–400×. Individual protists were identified to the species level whenever possible, sized (at least 30 individuals for each abundant taxon) using an eyepiece micrometer, and converted to carbon based on approximated geometric shapes and published volume-carbon conversions (Menden-Deuer and Lessard, 2000; Putt and Stoecker, 1989). In addition, live microzooplankton were examined in July 2009 to improve taxonomic resolution (Foissner et al., 1999). Rotifer biomass was determined as described in Fahnenstiel et al. (1998). Methods for determining mussel ash free dry weight followed those of Vanderploeg et al. (2001, 2009). The dry weight of bivalve veliger tissue was estimated based on their shell length according to Sprung (1984) and converted to carbon assuming 40% content (Brigolin et al., 2009).

Planktonic bacteria were preserved with 1% (final concentration) formaldehyde, concentrated onto 0.2 μm black polycarbonate membrane filters, stained with DAPI, mounted on microscopic slides, and stored frozen. In the laboratory, they were counted under an Olympus BX40 epifluorescent microscope at 1000× and with a high resolution digital camera. The obtained digital images were analyzed using Image Pro Plus software (Media Cybernetics). At least 2,000 bacterial cells were measured and counted per sample.

Phytoplankton were preserved with 1% formaldehyde and stored in the dark at 2 °C for three days until analysis at the laboratory. The cells were sized and enumerated using a FlowCAM. This instrument represents a combination of a flow-cytometer and a machine vision system (Sieracki et al., 1998). It has been used successfully in grazing experiments (Lavrentyev et al., 2004; Liu, 2005). Our instrument includes a blue (488 nm) 20 mW laser, apochromatic optics, a digital camera, a computer-aided syringe pump, a custom-designed flow chamber holder, and a vibration-free base. The FlowCAM was calibrated using microbeads of known size and a *Cryptomonas erosa* culture isolated from Lake Erie. Phytoplankton samples were processed in the fluorescence-triggered mode (emission >650 nm) at 200× and 100× magnification for cells between 3–15 μm and >15 μm in maximum linear dimension, respectively. The flow rate (0.1 to 0.5 ml/min) and particle concentrations were adjusted to capture one particle of interest per frame on average. When necessary, the samples were diluted with 0.2-μm filtered lake water.

Every particle passing through the flow chamber was measured, and its image was captured by a digital camera if it satisfied preset criteria. Ten optical characteristics of the captured particles, including linear dimensions, ratios, opacity, edge development, and chlorophyll a and phycoerythrin relative contents were recorded to the Visual Spreadsheet software. Visual examination and cleanup of the obtained raw data (to remove air bubbles, detritus, and other auto-fluorescing particles) to assure their quality was accomplished manually and via customized software filters. Biovolumes of single cells were calculated using their automatically measured linear dimensions. Based on our observations and published literature (Alvarez et al., 2011), the FlowCAM particle-per-chain algorithm does not always allow accurate determination of the number of cells within complex aggregates. Therefore, *Microcystis* colonies and chain-forming diatoms were counted as whole particles, and their volumes were determined automatically based on the ABD algorithm (areal based diameter), which provides more accurate volume estimates (Jakobsen and Carstensen, 2011) than the FlowCAM built-in ESD (equivalent spherical diameter) algorithm. The ESDs were then calculated manually, based on the above volumes, and used to describe the rates of phytoplankton size-dependent growth and grazing mortality.

Statistical treatment

The relationship between spatial distribution and seasonal dynamics of microzooplankton and environmental variables (temperature, chlorophyll a concentration) were examined using Pearson product–õmoment correlation. The biomasses of microzooplankton groups in controls and experimental containers in *Dreissena* feeding experiments were compared using a two-sample t-test. All statistical analyses were performed using Minitab 16 software.

Results

Specific composition

During the study period, the microzooplankton community consisted of 44 common taxa. Each of them occurred in >10% of processed samples (Table 1). Among ciliates, three representatives of the choreotrich genus *Rimostrombidium*, the tintinnids *Codonella cratera* and *Tintinnopsis* sp., and the prostomatids *Urotricha ristoi* and *Balanion planktonicum* were the most abundant across the bay. Many ciliates had either algal symbionts (e.g., *Askenasia vorax*, *Halteria chlorelligera*, *Didinium* sp., *Pelagodipletus trachelioides*) or sequestered chloroplasts in their cytoplasm (oligotrichs). The rotifers from the genera *Keratella* and *Polyarthra* were the most abundant in our samples. Athecate (unarmored) dinoflagellates from the genus *Gymnodinium* were the most common and included heterotrophic and plastid-bearing forms (mixotrophic). The thecate dinoflagellates *Ceratium* and *Peridinium* found in the bay were exclusively plastidic. The heliozoan *Actinophris sol* and the testate amoeba *Difflugia lobosoma* were the most common sarcodines.

Distribution

The abundance (0.98–21.5 × 10^3 ind./L) and biomass (1.73–28.5 μg C/L) of microzooplankton displayed strong spatial and temporal variability. Overall, ciliates were the most important group (45% of total biomass), followed by rotifers (35%). In May 2008, biomass was mostly composed of ciliates (70–100%, Fig. 2). They peaked in the middle section of the inner bay (SB5, SB10) and decreased toward the open lake and river mouth. Rotifers contributed substantially only at

Table 1
Common microzooplankton from Saginaw Bay.

Phylum	Phylum
CILIOPHORA	**DINOPHYTA**
Actinobolina sp.	*Ceratium hirundinella*
Askenasia volvox	*Gymnodinium helveticum*
Balanion planktonicum	*Gymnodinium* sp.
Codonella cratera	*Peridinium inconspicuum*
Cyclidium sp.	*Peridinium wisconsinense*
Cyclotrichium viride	*Peridnium cinctum*
Didinium chlorelligerum	
Halteria chlorelligera	**SARCODINA**
Halteria grandiniella	*Acanthoamoeba* sp.
Histiobalantion bodamicum	*Actinophrys sol*
Holophrya sp.	*Difflugia lobostoma*
Lacrymaria sp.	
Limnostrombidium pelagicum	
Limnostrombidium viride	**ROTIFERA**
Mesodinium pulex	*Ascomorpha* sp.
Monodinium vorax	*Asplanchna* sp.
Pelagodileptus trachelioides	*Brachionus caudatum*
Pelagostrombidium sp.	*Conochilus unicornis*
Pelagostrombidium mirabile	*Kellicotia longispina*
Rimostrobilidium brachykinetum	*Keratella cochlearis*
Rimostrobilidium humile	*Lophocharis salpina*
Rimostrobilidium lacustris	*Ploesoma truncatum*
Tintinnidium fluviatile	*Polyarthra major*
Tintinnopsis sp.	*Polyarthra remata*
Urotrica pelagicum	*Synchaeta kitina*
Urotrica ristoi (complex)	
Vorticella aquadulcis	

SB10. In contrast, they were an important part of microzooplankton in July, especially at the inner bay sites (Fig. 3a). Microzooplankton abundance varied between $3x10^3$ and $21x10^3$ ind./L, and total biomass peaked near the Saginaw River confluence (SB2), where it was augmented by testate amoeba. Microzooplankton biomass decreased gradually toward Lake Huron with a concomitant increase in dinoflagellate proportional biomass (up to 50% at SB23).

Four stations (SB2, SB14, SB5, SB20) were sampled for a surface-bottom comparison (Fig. 3b). At SB2, microzooplankton biomass in the bottom sample (26.7 μg C/L) exceeded that in the upper water column mostly due to testate amoeba, which contributed ca. 50%. The other two inner bay stations did not display vertical heterogeneity, and at the deeper outer bay site (SB20, 18 m), microzooplankton biomass was higher at the surface than near the bottom. Across Saginaw Bay, microzooplankton biomass, as well as that of ciliates and rotifers, increased with chlorophyll a concentration (Table 2).

The seasonal distribution of microzooplankton biomass in 2009 and 2010 at three stations (SB5, SB10, SB20) was characterized by early-mid summer and fall peaks of different magnitude (Fig. 4). Again, total

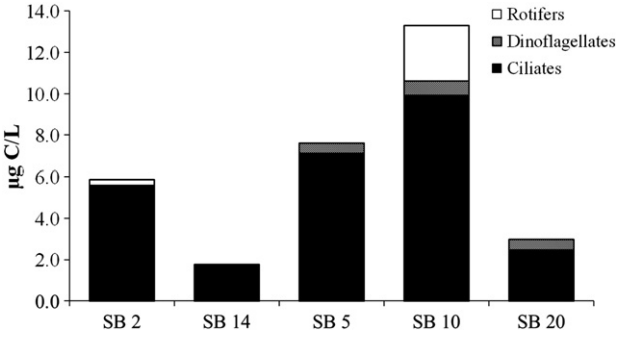

Fig. 2. Microzooplankton biomass composition in Saginaw Bay at five stations sampled in May 2008.

microzooplankton biomass was correlated with chlorophyll a concentration, but dinoflagellates were the only group that displayed this relationship seasonally (Table 2). Microzooplankton and, particularly, ciliates declined to their minimum in August 2009 at all study sites. This decline coincided with minimum water transparency (Johengen, unpublished data). The community in spring at the three stations was dominated by aloricated choreotrich and oligotrich ciliates from the genera *Rimostobidium* and *Pelagostrombidium*, respectively. Later in the season, they were joined by *Halteria* sp., prostomatids, and tintinnids. In summer and especially fall, the spatial differences were noticeable between the sites. In the shallow inner bay, rotifers comprised >75% of microzooplankton biomass in fall, whereas their contribution was <20% at SB20.

Microzooplankton grazing

In July 2009, chlorophyll a, microzooplankton biomass, and bacterial abundance all peaked near the river mouth (Table 3). The inner bay, particularly SB5, was in the initial stages of a *Microcystis* bloom. The relative reduction in chlorophyll a concentration after filtration through a 153-μm mesh net (SB2—9%, SB5—30%, SB20—16%) reflected the proportion of large colonies of cyanobacteria, including *Microcystis*, and diatoms. Nanoplankton-sized *Cyclotella* and chlorophytes were also abundant. Among microzooplankton, ciliates and rotifers formed 36, 63, and 37% and 49, 36, and 28% of microzooplankton biomass at SB2, SB5, and SB20, respectively. At the latter site, dinoflagellates contributed 33%.

Total chlorophyll *a* response to dilution was weak with the exception of SB20, with grazing losses at 0.3/d. In contrast, phytoplankton growth rates based on cell counts increased from 0.34/d (the average for all size classes) in the inner bay to 0.95/d at SB20. Overall, phytoplankton grew at 0.55/d, and microzooplankton consumed ca. 56% of their daily production (64% of the <50 μm ESD size class production). The highest average grazing rate was found at SB5 (0.65/d), where it matched or exceeded phytoplankton growth. On average, nanoplankton <10 μm ESD grew faster than larger phytoplankton (0.73/d vs. 0.47/d) but also sustained higher grazing mortality (88%) than other size groups (49% in 10–50 μm ESD and 27% in 50–100 μm ESD). Interestingly, the greatest grazing impact on larger phytoplankton (68%) was measured at SB5, where this size group was dominated by colonial cyanobacteria. At all three sites, grazers consumed ca. 50% of the cyanobacterium *Microcystis* daily production in the size fraction 30–100 μm, (Table 3).

Bacterial growth and bacterivory rates were nearly balanced at all three sites (average 0.36/d and 0.37/d, respectively) in the most abundant <1 μm size group. The larger bacteria grew faster (0.70/d), but their average grazing mortality was higher yet (1.12/d). The proportion of large cells in total bacterioplankton abundance and their growth rates were much higher at SB20 than at the two inner Bay sites (34.1% vs. 6.2–8.3% vs. and 1.13/d vs. 0.45–0.53/d, respectively).

Quagga mussel feeding

During the July 2011 experiment, phytoplankton biomass was composed mostly of cyanobacteria (ca. 80%, including *Anabaena*, *Chroococcus*, *Merismopedia*, and *Microcystis*; Vanderploeg, unpublished data). Aloricated ciliates and rotifers (mostly *Polyarthra* spp.) dominated microzooplankton and contributed nearly equally (46% and 39%, respectively) to total biomass (Fig. 5). In September, phytoplankton biomass more than doubled compared to July and consisted of ca. 60% diatoms (mostly *Aulocoseira* and *Fragilaria*). Microzooplankton biomass also increased, with rotifer contribution exceeding that of ciliates (46% vs. 28%). Dinoflagellate proportions increased from 13% in July to 21% in September.

In both experiments, final chlorophyll a concentrations and total microzooplankton and ciliate biomasses decreased significantly between

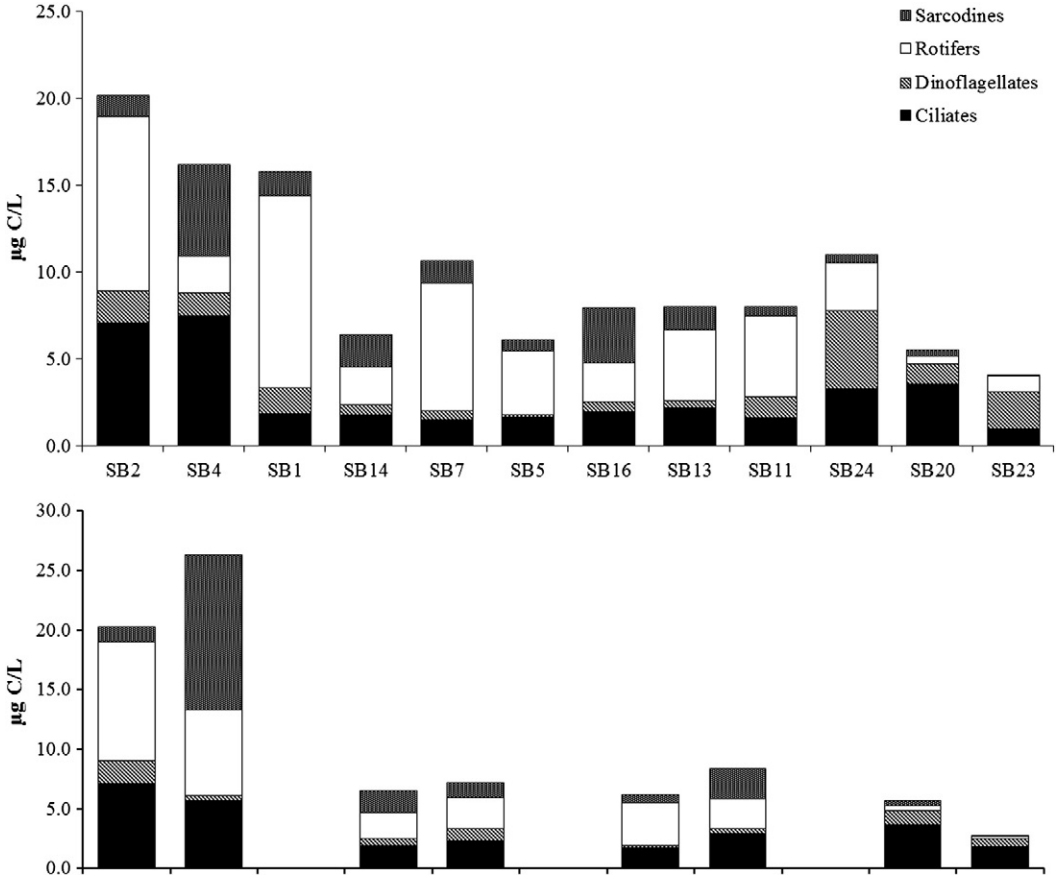

Fig. 3. Microzooplankton biomass composition in Saginaw Bay in July 2008 surface (top panel) and bottom layers (bottom panel) of sampled stations.

control and mussel treatments. Rotifer biomass also declined, but this difference was significant only in July. In this experiment, rotifers grew at 0.05/h in the control, and their biomass was reduced by 23% in the quagga mussel treatment (Table 4). Individual species of ciliates achieved very fast growth rates in the control (up to 0.174/h for *Rimostrombidium*), but their biomass was reduced by as much as 86% in the mussel containers. The resulting clearance rates (F_I) on ciliates varied from 3.56 to 23.1 mL/mg/h and exceeded those on rotifers. The abundance of bivalve larvae (mostly D-form and small veliconcha veligers), which were abundant at SB5 in July (148 ± 23 ind./L), was reduced by 51% in the quagga mussel containers in July. The biomasses of various armored microzooplankton (large thecate dinoflagellates, loricated ciliates and rotifers, testate amoebae) and *Gymnodinium* sp. and *Peridinium inconspicuum* also decreased in the mussel containers, but this decrease was not statistically significant.

Overall, the mussels ingested 0.083 μg C/mg/h (23.1 μg C/mussel/h) in July. The proportion of microzooplankton in the mussel diet was 77%, with phytoplankton and bivalve veligers contributing the remaining 13% and 10%, respectively. Ciliates alone contributed 52%. In September, the ingestion rate increased to 0.237 μg C/mg/h (49.6 μg/mussel/h), with phytoplankton being the largest fraction of the mussel diet (66%). Microzooplankton contribution remained substantial (34%), despite comprising only 2.5% of the mussel carbon-based prey field (3.5% in July).

Table 2
Pearson correlation coefficients between different microzooplankton groups and chlorophyll *a* concentrations in the mixed layer. ns = non-significant.

	Seasonal		Spatial	
	r	p value	r	p value
Ciliates	0.32	ns	0.42	<0.05
Dinoflagellates	0.40	<0.05	0.07	ns
Rotifers	0.48	ns	0.72	<0.01
Sarcodines	0.06	ns	0.34	ns
Microzooplankton	0.51	<0.01	0.71	<0.01

Discussion

Comparison of ciliate and rotifer assemblages between this and the previous study in Saginaw Bay (Lavrentyev et al., 1995) does not indicate that microzooplankton abundances have declined following colonization by quagga mussels. Ciliate abundance at SB5 in October 2009 and 2010 (1.27 and 2.67 cells × 10^3/L, respectively) was similar to that found at the same site in October 1994 (1.50 × cells 10^3/L). Rotifer abundance was actually higher in 2009–2010 than in 1994 (360 and 240 ind./L vs. 40 ind./L., respectively). The specific composition of ciliates did not change appreciably either. It should be noted that, by 1994, zebra mussels were well established in the bay (Nalepa et al., 1995). The previous study also did not include other microzooplankton, such as dinoflagellates and sarcodines. The average combined biomass of ciliates and rotifers in the outer bay in the present study (3.79 ± 0.87 μg C/L) was lower than that in open Lake Huron in the 1980s and early 1990s (ca. 7.5 μg C/L, Fahnenstiel et al., 1998 and references therein), but its maximum value of 12.6 μg C/L in October 2010 exceeded historical offshore biomass values.

Microzooplankton spatial and seasonal distribution in Saginaw Bay generally trended with chlorophyll a. Because microzooplankton typically grow as fast as their phytoplankton prey, there should be no time lag between their dynamics. However, a decline in total microzooplankton biomass toward late summer/early fall did not always coincide (SB 5 results) with the chlorophyll minima. This could be a result of poor food quality since the microzooplankton seasonal minimum coincided with that of low water transparency (as implied by high chlorophyll

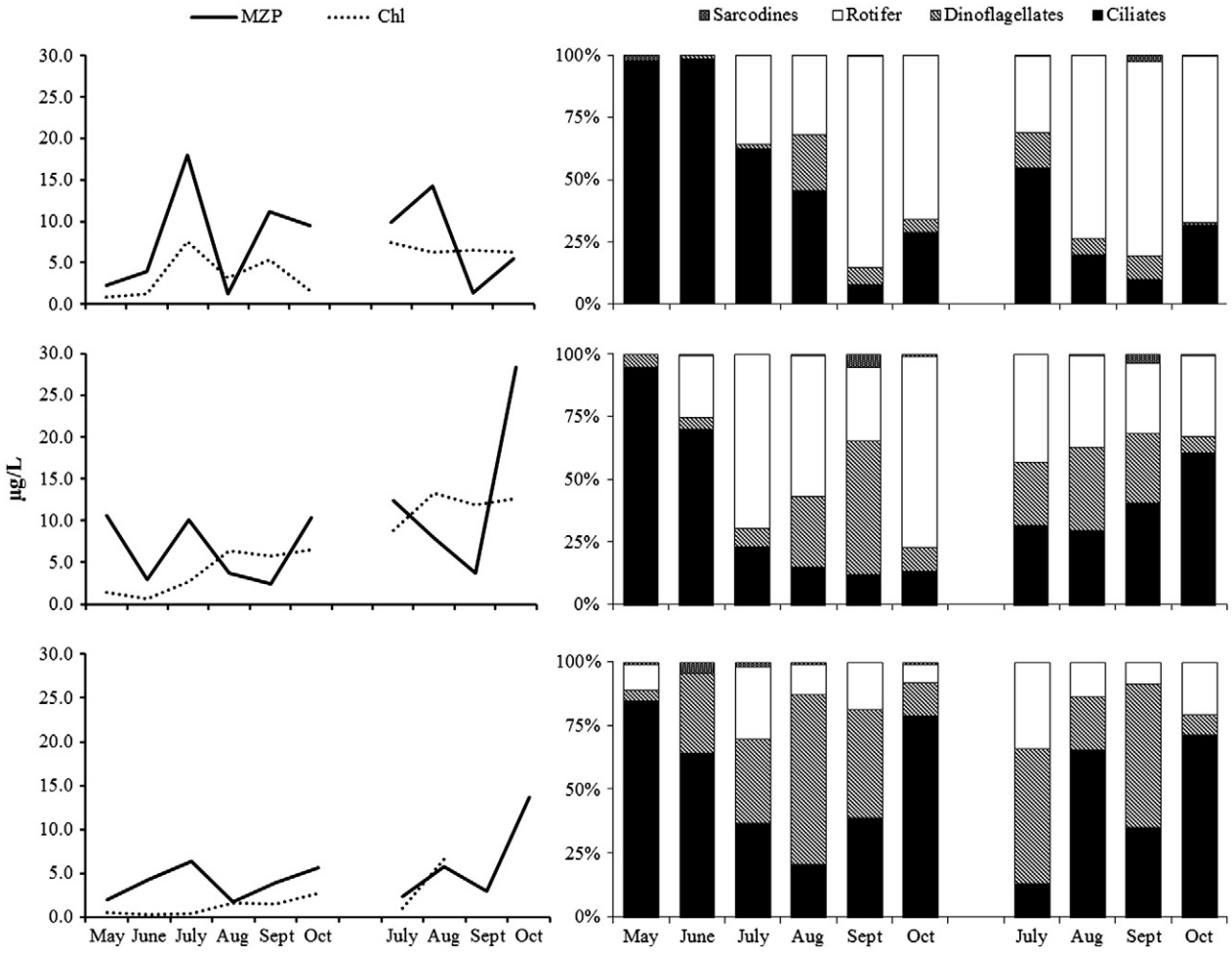

Fig. 4. Seasonal dynamics and relative biomass of microzooplankton at the master stations (see Fig. 1) in Saginaw Bay in 2009–2010.

concentration) driven by high concentration of *Microcystis*, which dominated the phytoplankton at this time. Also, predation pressure from mesozooplankton can partially explain microzooplankton dynamics at SB5 in both years and at SB2 in 2009. In Saginaw Bay, mesozooplankton abundance peak shifted from early summer (June) in the 1990s to early fall (September–October) in 2009–2010, with calanoid copepods, *Daphnia* spp., and *Bythotrephes* replacing cyclopoid copepods and Bosminidae (Pothoven et al., 2013). Ciliates are particularly vulnerable to planktonic crustacean predation – especially by copepods – and often are preferred food source for zooplankton in the Great Lakes (Bundy et al., 2005; Carrick et al., 1991; LeBlanc et al., 1997). In the outer bay (SB20), micro- and mesozooplankton both peaked in October.

Total microzooplankton biomass in the inner and outer bay (8.90 ± 1.06 µg C/L and 5.34 ± 0.93 µg C/L, respectively) was 25–30% of zooplankton post-invasion biomass, which also peaked in late spring and declined toward fall (Bridgeman et al. 1995). In open Lake Huron, microzooplankton biomass was ca. 10% of net zooplankton biomass prior to the quagga mussel invasion (Fahnenstiel et al., 1998). Given the rapid biomass turnover rates of microzooplankton, their production is likely to exceed that of net zooplankton in Saginaw Bay. Mixotrophic microzooplankton were as common in this study as they are in many other freshwater environments (Sanders, 2011), but their specific role in the Saginaw Bay ecosystem remains to be explored.

There is a paucity of data on pre- vs. post-quagga mussel invasion microzooplankton composition and abundance in the Great Lakes apart from the aforementioned rotifer survey by Barbiero and Warren (2011). In the Bay of Quinte, Lake Ontario, the abundance of mesozooplankton, including rotifers, declined following the invasion of zebra and quagga mussels (Bowen and Johannsson, 2011). The response of zooplankton in the latter study may have been confounded by another exotic species, the predatory cladoceran *Cercopagis pengoi*. In the Hudson River, microzooplankton, including tintinnids, rotifers, and

Table 3
Surface water temperature, chlorophyll *a*, and plankton distribution and the results of dilution experiments at three stations in Saginaw Bay in July 2009. k = gross growth rate, g = grazing rate.

	Stations	SB2	SB5	SB20
Temperature	°C	21.6	21.1	17.0
Chlorophyll *a*	(µg L^{-1})	7.75	3.82	0.58
Bacteria	(cells 10^9 L^{-1})	4.99	3.11	1.78
Microzooplankton	(µg L^{-1})	22.1	15.1	7.64
Chlorophyll a	k	−0.10	0.00	−0.05
	g	0.03	0.00	0.30
Phytoplankton: ESD 3–10	k	0.34	0.96	0.87
	g	0.07	1.34	0.51
ESD 10–20	k	0.26	0.10	1.42
	g	0.21	0.17	0.27
ESD 20–50	k	0.43	0.16	0.60
	g	0.33	0.40	0.00
ESD 50–100	k	0.32	0.16	0.91
	g	0.00	0.11	0.27
Microcystis <100 µm	k	0.50	0.18	1.34
	g	0.25	0.10	0.69
Bacteria < 1 µm				
	g	0.51	0.32	0.29
	k	0.53	0.45	1.13
Bacteria >1 µm				
	g	1.16	1.10	1.09

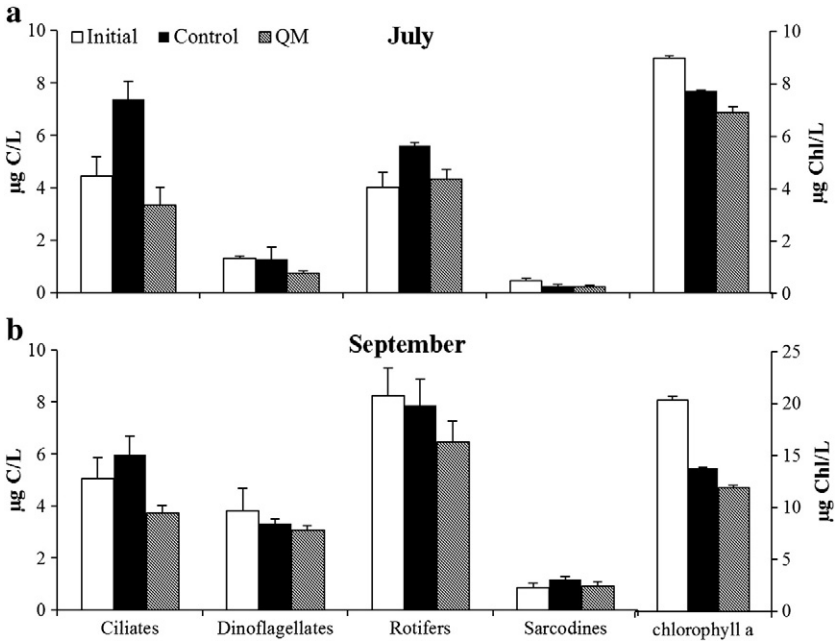

Fig. 5. Microzooplankton biomass and chlorophyll a concentrations in the quagga mussel feeding experiments.

nauplii all declined after zebra mussel invasion and have been scarce thereafter (Pace et al., 1998). Elsewhere, zebra mussels greatly reduced microzooplankton abundance and biomass in mesocosm experiments (MacIsaac et al., 1991; Miller and Watzin, 2007; Wilson, 2003). A survey of 50 small, thermally stratified lakes in Michigan has revealed a 44% decline in microzooplankton biomass in the presence of *Dreissena* (Kissman et al., 2010).

On the other hand, no significant difference was found between the water column abundance of naked amoebae and heterotrophic nanoflagellates in four lakes with and without zebra mussels (Bischoff and Horvath, 2011). These authors suggested that protist abundance reduction due to mussel filtration can be offset by re-suspension or migration of protists from the rich sediments around zebra mussel colonies. The sediment surface and interstitial spaces can provide a spatial refuge for micrograzers in the presence of filter-feeding bivalves. For example, the biomass of benthic ciliates did not change significantly in flow-through sediment core experiments from Saginaw Bay in the presence of zebra mussels, although their specific composition shifted toward the predominance of opportunistic scuticociliates (Lavrentyev et al., 2000). This scenario is also relevant to the survey part of this study because testate amoebae from the genus *Difflugia* are meroplanktonic. Some microzooplankton can attach themselves to larger particles to avoid predation (e.g., *Vorticella* sp. on colonies of cyanobacteria, Lavrentyev et al., 1995).

The results of this study suggest that various loricas, tests, and thecae may offer limited protection for plankton if the organisms that possess such armor get rejected by mussels. In contrast, aloricated ciliates may be destroyed upon contact with the bivalve feeding apparatus, although no direct evidence exists in the literature. The survival strategy of these microzooplankton apparently involves high population growth rates in addition to possible temporal separation from the mussel in the stratified waters of the outer bay. In this study, ciliates grew, on average, at nearly 100% their predicted intrinsic growth rates based on temperature and cell volume (Müller and Geller, 1993). The observed ciliate growth rates are similar to those measured in eutrophic coastal habitats of Lake Erie in summer (Lavrentyev et al., 2004). It should be noted that despite the removal of large zooplankton the growth rates of ciliates in field experiments are net rates becausee all microzooplankton components

Table 4
The results of quagga mussel feeding experiments in July (J) and September (S) 2011. μ = net growth rate (h^{-1}) of microzooplankton in control; SE = standard error, GL = grazing loss (%) based on biomass difference between the mussel treatments and control, p-values for this difference, F_I = mussel net clearance rate (mL/mg/h), I = ingestion rate (μg C/mg/h).

Species/Group	μ	SE	GL	p-value	F_I	SE	I	SE
Askenasia volvox (J)	0.017	0.014	26	<0.05	3.56	1.25	0.0005	0.0001
Askenasia volvox (S)	0.069	0.021	86	<0.05	20.2	2.50	0.0021	0.0002
Balanion planktonicum (J)	0.149	0.041	60	<0.05	10.7	2.47	0.0019	0.0003
Didinium sp. (J)	0.135	0.012	61	<0.01	10.3	0.90	0.0019	0.0001
Polyarthra remata (J)	0.038	0.004	25	<0.01	3.17	0.82	0.0131	0.0030
Rimostrombidium brachykinetum (J)	0.158	0.016	72	<0.01	14.6	2.38	0.0236	0.0016
Rimostrombidium brachykinetum (S)	−0.052	0.016	62	<0.01	16.3	2.72	0.0048	0.0006
Rimostrombdium lacustris (J)	0.174	0.049	82	<0.05	23.1	7.04	0.0139	0.0012
Chlorophyll *a* (J)	−0.024	0.001	11	<0.05	0.04	0.22	0.0003	0.0020
Chlorophyll *a* (S)	−0.068	0.001	13	<0.01	0.22	0.46	0.0045	0.0090
Ciliates (J)	0.084	0.015	55	<0.01	9.15	2.04	0.0435	0.0069
Ciliates (S)	0.025	0.019	37	<0.05	7.72	1.31	0.0399	0.0047
Rotifers (J)	0.055	0.004	23	<0.05	2.81	0.83	0.0134	0.0037
Microzooplankton (J)	0.058	0.005	40	<0.01	5.83	1.19	0.0640	0.0110
Microzooplankton (S)	0.003	0.002	23	<0.01	5.30	1.46	0.0800	0.0190

have been demonstrated to feed upon each other (Arndt, 1993; Han et al., 2011; Jeong et al., 2010). This mutual predation could partially explain the observed decrease in relative biomass of ciliates from spring to summer in addition to mesozooplankton and mussel predation.

Quagga mussel clearance rates on ciliates were higher than those by zebra mussels in the previous study in Saginaw Bay (Lavrentyev et al., 1995). Their clearance rates on choreotrich ciliates in this study were similar to those on the quagga mussel's preferred food in culture (*Cryptomonas*, 25 mL/mg/h, Vanderploeg et al., 2010). The fast-swimming choreotrich ciliate *R. lacustris*, which was cleared at 23 mL/mg/h by quagga mussels, escaped the inhalant currents of smaller (14–16 mm) zebra mussels in our previous study. At the same time, zebra mussels had high clearance rates on heterotrophic chrysophytes (up to 14 mL/mg/h). The impact of quagga and zebra mussels on *Polyarthra* in enclosures was equally strong (Thorp and Casper, 2002). Likewise, zebra and quagga mussels fed by green algae displayed similar per capita clearance rates; and, since quagga mussels have more biomass per unit shell, this resulted in their lower biomass-specific rates (Baldwin et al., 2002). It should be noted that the latter two studies did not involve *Microcystis*. Quagga mussels also removed more than half of veligers in less than 5 hours in July 2011. Cannibalism has been demonstrated previously in the zebra mussel (MacIsaac et al., 1991). Larviphagy is a common strategy among filter-feeding bivalves (Lehane and Davenport, 2004; Porri et al., 2008; Troost et al., 2008). In laboratory experiments, feeding on rotifers covered 16–23% of zebra mussel base metabolic rates (Wong et al., 2003). Despite representing a small part of the total potential mussel prey field in the present study (<4% based on the C:Chl of 35), microzooplankton, and especially ciliates, contributed disproportionately to the quagga mussel diet.

The low feeding rates on phytoplankton by quagga mussels in our July experiments coincided with high water temperature (25 °C). However, in concurrent feeding experiments with cultured *Cryptomonas* conducted at the same temperature, their clearance rates were much higher at 25 °C than at 17 °C (31.2 ± 3.61 mL/mg/h vs. 19.0 ± 0.69 mL/mg/h, Vanderploeg, unpublished data). Therefore, temperature by itself was not the issue. The quagga mussel herbivory could have been depressed in July because of food quality (*Microcystis* and other summer dominants). Likewise, the *Dreissena* mass/length ratios and feeding rates on phytoplankton are at their maximum in the spring and then decline during *Microcystis* blooms (Vanderploeg et al., 2009). The interactions between *Dreissena* and *Microcystis* cannot always be explained by microcystin concentrations, colony size, or nutrient content (Vanderploeg et al., 2013). For example, *Dreissena* and its veligers fed on toxic, unicellular *M. aeruginosa* in culture (Pires et al., 2004). However, under field conditions, it typically rejects microcystin-producing strains and mucilage covered macro-colonies as loosely consolidated pseudo-feces (Vanderploeg et al., 2001, 2009).

Microcystis and other colonial cyanobacteria usually have a negative effect on crustacean zooplankton feeding rates (Leonard and Paerl, 2005; Sellner et al., 1993). In contrast, various protists readily consume toxic cyanobacteria, including *Microcystis* (Kobayashi et al., 2013; Mizuta et al., 2011; Van Wichelen et al., 2010; Wilken et al., 2010). Microzooplankton grazing has been implicated as one of the factors influencing colony formation in *Microcystis* (Yang et al., 2006, 2008). However, it has been shown that amoebae can feed on *M. aeruginosa* colonies (Van Wichelen et al., 2012).

The abundance based growth and grazing rates indicate very dynamic microbial food web processes across Saginaw Bay during the *Microcystis* bloom. Dilution experiments measure community processes. In addition to microzooplankton, other grazers may have contributed to the observed herbivory and especially bacterivory rates. For example, a sizeable assemblage of heterotrophic nanoflagellates was found in Saginaw Bay in September and October 1994 (0.7 and 0.25 × 10^6 cells/L, respectively, Lavrentyev et al., 1995) and in May 2008 (0.37 to 2.11 × 10^6 cells/L, Lavrentyev unpublished data). These grazers prefer larger and actively growing bacteria (Jochem et al., 2004). Bivalve veligers are also known to feed upon small-sized phytoplankton (Sommer et al., 2000) and bacteria (Barnard et al., 2006).

The discrepancy between the chlorophyll and abundance based rates in dilution experiments in this study is not entirely surprising. The limitations of chlorophyll as a phytoplankton physiological rate proxy have been discussed in literature (Kruskopf and Flynn, 2006). Phytoplankton assemblages can undergo changes in cellular chlorophyll content due to the photoacclimation effect induced by changes of the external light environment within hours. For example, cyanobacteria acclimatize to changing irradiance by decreasing chlorophyll and phycocyanin concentrations with increasing light intensity, whereas their cellular carbon content does not change (Banares-Espana et al., 2013; Raps et al., 1983). Water transparency during the July 2009 experiments was <1.5 m following a wind-driven mixing event, but the samples were incubated near the surface in a sheltered harbor, where turbidity was much lower. If grazing rate estimates in dilution experiments rely on chlorophyll dynamics to represent phytoplankton growth, temporary decoupling between cellular division and photopigment synthesis rates will bias the results (Chen and Liu, 2011; Sherr et al., 2009). In addition, the chlorophyll based rates in our study reflect dynamics of the entire phytoplankton assemblage, including the groups that were and were not grazed, whereas the cell count based rates describe only its subsets. Large phytoplankton were present in the experimental containers despite 153-μm mesh screening, including *Microcystis* colonies. In the inner bay their concentration reached 30–35 particles/mL and 1–1.5 particles/mL in the size groups >100 μm and >300 μm, respectively. At SB20 these numbers declined to 2 and 0.2 particles/mL, respectively, but given the low phytoplankton abundance in the outer bay, they could have contributed substantially to total chlorophyll as well.

Consumption of large algae by microzooplankton in the inner bay corresponds to grazing on *Microcystis*. Overall, micrograzers removed ca. 60% of <100 μm ESD phytoplankton daily production, or 30% of the phytoplankton standing stock. In the western basin of Lake Erie, which is heavily infested by invasive bivalves and *Microcystis*, the combined grazing impact of *Dreissena* and crustacean zooplankton on the standing stock of "edible" (i.e., non-*Microcystis*) phytoplankton was estimated at about 30% per day (Zhang et al., 2011). In the latter ecosystem, microzooplankton alone removed 29% of phytoplankton standing stock per day (Davis et al., 2012). Microzooplankton herbivory exceeded that of mesozooplankton in the presence of another toxic cyanobacterium, *Cylindrospermopsis raciborskii*, in the St. Johns River system (Leonard and Paerl, 2005). It has been suggested that the shifting food preferences of herbivorous microzooplankton can create an opportunity for cyanobacterial blooms to occur (Boyer et al., 2011).

Because the *Microcystis* life cycle involves benthic recruitment, which in turn is driven by light, temperature, and re-suspension (Misson and Latour, 2012), it appears unlikely that microzooplankton can prevent their blooms from developing. However, microzooplankton grazing can influence *Microcystis* bloom dynamics directly by feeding on them and indirectly by removing its competitors. In addition, microzooplankton herbivory is a major source of recycled nutrients in the Great Lakes (Gardner et al., 2004). Further, large zooplankton can enhance the growth rates of *Microcystis* by decreasing microzooplankton abundance (Kagami et al., 2002). Mussels feeding voraciously on microzooplankton are likely to produce similar cascading effects.

Conclusions

The results of this study demonstrate that microzooplankton has remained an abundant and diverse component of the Saginaw Bay ecosystem over the last 15 years, despite quagga mussel proliferation. Microbial grazers consume a significant part of primary and bacterial

production and potentially are capable of influencing *Microcystis* dynamics. In turn, feeding on microzooplankton can be important for mussels during lean periods, or supplement other consumed resources, particularly during noxious cyanobacterial blooms.

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