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The Effects of Zebra Mussels on the Lower Planktonic Foodweb in Lake Champlain

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ABSTRACT. *Selective grazing by zebra mussels has altered phytoplankton communities in many North American lakes, but the specific changes are not the same in each ecosystem. Because of this variation in response, we investigated the impacts of zebra mussels on the plankton community of Lake Champlain with two objectives: first to determine whether zebra mussels increased the dominance of potentially toxic cyanobacteria in the phytoplankton, and second to explore the impact of zebra mussels on protozoans, rotifers, copepod nauplii, and other microzooplankton in the lower food web. Experiments were conducted in 200-L mesocosms filled with Lake Champlain water filtered through a 150-μm sieve to remove macrozooplankton. Zebra mussels were added to half of the mesocosms while the others were maintained as controls. Over a 96-hour experimental period, we tracked nitrogen and phosphorus concentration, chlorophyll a, microcystin concentration, and both phytoplankton and microzooplankton composition and abundance. We found an increase in SRP and total nitrogen concentration and a decrease in the ratio of TN:TP in the zebra mussel treatments over time. Microcystin was undetectable throughout the experiment using the ELISA assay. Phytoplankton biovolume, including cyanobacteria biovolume, declined significantly in the zebra mussel treatments, as did rotifer, protozoan and nauplii abundance. By both direct (consumption) and indirect (altered nutrient availabilities and increased competition) means, zebra mussels clearly seem capable of strongly influencing the lower planktonic foodweb in the many shallow water habitats of Lake Champlain.*

INDEX WORDS: *Zebra mussels, Lake Champlain, cyanobacteria, rotifers, nauplii, TN:TP.*

INTRODUCTION

Zebra mussels, *Dresseina polymorpha* Pallas, became established in North America in the late 1980s (Turner 1990, Smirnova *et al.* 1992). Because they are voracious filter feeders, processing water at rates of one liter (Reeders *et al.* 1989) to more than five liters (Horgan and Mills 1997) per day per mussel, the introduction of zebra mussels has resulted in measurable declines in chlorophyll *a* concentrations and phytoplankton biomass in a variety of freshwater ecosystems (Heath *et al.* 1995, Baker *et al.* 1998, Pace *et al.* 1998, Nicholls 2001, Idrisi *et al.* 2001). While at first glance these changes might appear beneficial because eutrophication is a major concern in many of these systems, the response of the various planktonic groups has not been consistent in all places. Such a view does not take into account which algal species are being reduced or what changes in established foodwebs might occur along with such intense filtration.

For example, in Lake Erie, the introduction of zebra mussels was followed by increases in cyanobacteria even as other phytoplankton groups were drastically reduced (Makarewicz *et al.* 1999, Vanderploeg *et al.* 2001, Nicholls *et al.* 2002). Because cyanobacteria can form noxious blooms, and some species produce toxins (Chorus and Bartram 1999), these changes are generally considered undesirable. In enclosure experiments in Saginaw Bay, Lake Huron, total phytoplankton biomass decreased, but cyanobacteria biomass (dominated by *Aphanocapsa* sp.) was not affected by zebra mussel introduction (Heath *et al.* 1995). In contrast, in the Hudson River, the cyanobacterium *Microcystis*

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aeruginosa almost completely disappeared following zebra mussel establishment (Smith *et al.* 1998).

The mechanisms behind any zebra mussel promotion of cyanobacteria are unclear and several possibilities have been suggested. First, the preferential filtration of some algal groups over cyanobacteria may cause a shift in the species composition of the phytoplankton assemblage. Clearance rates have been shown to vary depending on food source (Baker *et al.* 1998, Dionisio Pires and van Donk 2002). Second, cyanobacteria may be preferentially rejected in pseudofeces (possibly related to the presence of toxins in some cells) and resuspended in the water column where they can continue to grow (Vanderploeg *et al.* 2001). Finally, changes in nutrient availability mediated by zebra mussels may favor the development of cyanobacteria (Heath *et al.* 1995, Arnott and Vanni 1996). Changes in both phosphorus and nitrogen concentrations following zebra mussel introduction have been documented (Heath *et al.* 1995, Holland *et al.* 1995, Johengen *et al.* 1995, Arnott and Vanni 1996, Makarewicz *et al.* 2000, James *et al.* 2001, Hall *et al.* 2003), however, these changes are not all consistent.

There is a large literature on the effects of ambient concentrations of nitrogen and phosphorus on cyanobacteria (Smith 1983, Trimbee and Prepas 1987, Huszar and Caraco 1998, Elser 1999, Hyenstrand *et al.* 1998). In general, cyanobacteria densities and total phosphorus concentrations are positively correlated (e.g., Trimbee and Prepas 1987, Huszar and Caraco 1998, Elser 1999); however, Raikow *et al.* (2004) did not find this relationship in the presence of zebra mussels. In laboratory studies, zebra mussels excrete at lower N:P ratios than the ratios found in the seston they ingest (Arnott and Vanni 1996), presumably because their elemental stoichiometry requires proportionally more nitrogen than phosphorus (Frost *et al.* 2002). Cyanobacteria seem to be more common in systems where the molar ratio of N:P falls below 64:1 (Smith 1983).

Heterotrophic and mixotrophic protozoa, such as small flagellates and ciliates, have also been shown to decrease in the presence of zebra mussels (Lavrentyev *et al.* 1995, Pace *et al.* 1998, Bastviken *et al.* 1998, Wilson 2003). This decrease has consequences not only for those rotifers and macrozooplankton that may feed on protozoa (Carrick *et al.* 1991) but also for bacterial abundances and the recycling of nutrients within the microbial loop. Large reductions in rotifer populations have been documented following invasion by zebra mussels (MacIsaac *et al.* 1995, Pace *et al.* 1998, Jack and Thorp 2000). These reductions might be the result of both starvation, as protozoan food resources become limiting (Cordova *et al.* 2001), as well as direct removal by zebra mussels (MacIsaac *et al.* 1995).

The effects of zebra mussels on macrozooplankton are still not clear. Although no significant impacts on copepods and copepod nauplii density were found in experimental manipulations in the lab (MacIsaac *et al.* 1995) and field mesocosms (Jack and Thorp 2000), a decline in nauplii following zebra mussel invasion of the Hudson River has been documented (Pace *et al.* 1998). A decline in adult copepod and cladoceran densities in Lake Erie may have occurred since zebra mussel invasion (MacIsaac *et al.* 1995), and a decline in the population growth rates of all crustacean zooplankton occurred in the zebra mussel treatments of *in situ* experiments in the Ohio River (Jack and Thorp 2000). A decline in macrozooplankton abundance could potentially have serious consequences for both phytoplankton and the higher trophic levels that larger zooplankton support (MacIsaac *et al.* 1992).

Because existing data provided no clear predictions of the implications of the zebra mussel invasion in Lake Champlain, the objective of this study was to experimentally test the effects of zebra mussels on a natural plankton community from Lake Champlain. Using laboratory mesocosms, we tested the effects of adding zebra mussels on nutrient concentrations, N:P ratios, phytoplankton composition, and the microzooplankton. Our specific hypotheses were that: (1) cyanobacteria biovolume would remain the same or increase in the presence of zebra mussels; (2) N:P ratios would decrease in the presence of zebra mussels; and (3) protozoa, rotifers and nauplii would decrease in the presence of zebra mussels.

MATERIALS AND METHODS

Lake Champlain, bordered by Vermont, New York, and Quebec, Canada, has a similar origin to the Laurentian Great Lakes and also drains to the St. Lawrence River. It is 170 km long and at its broadest point, known as the Main Lake, it is 20 km wide. It has a maximum depth of 122 m in the Main Lake and a mean depth of 23 m. The Main Lake is generally considered oligotrophic to mesotrophic (VTDEC 2004), and despite the depth of the central

trough, it contains an extensive littoral zone, with water depths < 15 m. Zebra mussels were confirmed in southern Lake Champlain in 1993 (VTDEC 2004) and they can now be found throughout the Main Lake at high densities. Densities of adult zebra mussels (5.8–24.6 mm in length) in the littoral zone in the Main Lake just north of Burlington, Vermont averaged 31,312 individuals/m^2 in 2001 and 45,656 individuals/m^2 in 2002 (Beekey *et al.* 2004). The late summer phytoplankton community biomass in the Main Lake is often dominated by cyanobacteria, though cryptophytes are also numerically abundant (Shambaugh *et al.* 1999). Rotifer densities in the Main Lake in the late summer of 2002 ranged from less than 50 to 200 individuals/L (Watzin *et al.* 2005). The most abundant rotifer genera present in late summer are *Polyarthra, Synchaeta,* and *Keratella*.

Study Design and Sampling

Two runs of a manipulative experiment were conducted in the late summer of 2002. For each run, water was collected from a deep water section of the Main Lake (about 75 m), offshore from Burlington, Vermont using the Jabsco centrifugal pump onboard the R/V *Melosira*. The pump draws water at a depth of about 0.5 m and previous analysis showed no damage to plankton resulting from collection with this pump. About 2,000 L of water were collected, filtered using a 150-µm mesh sieve to remove the macrozooplankton, and stored in three large tanks. On return to shore, the sieved lake water was pumped from the R/V *Melosira* into the laboratory (in virgin polypropylene pipes), filling eight clean polypropylene barrels (0.83 m deep and 0.5-m diameter) with 200 L of water. Barrels were continuously aerated and kept at 20°C throughout the duration of the experiment. New full spectrum lights were placed directly on top of the barrels. These lights delivered approximately 80 µE/m^2s (measured just beneath the surface of the water) on a 16/8 hrs light/dark regime. After filling with lake water, barrels were allowed roughly 1 day to acclimate to laboratory conditions before the treatments were established and the experiments were started.

Two days prior to the start of the experiments, zebra mussels were collected from Lake Champlain and divided into roughly equal clumps of about 50 variously sized individuals in Run 1 and 75 individuals in Run 2. These densities were chosen to represent about 10% of natural densities observed in the nearby littoral zone of Lake Champlain (~10 m water depth) (Beekey *et al.* 2004) because our experimental barrels held a 1-m column of water. Mussels were gently brushed clean of periphyton using a toothbrush and placed in 20-L glass aquaria where they were maintained in the lab without food until the start of the experiment.

Treatments consisted of: 1) the addition of zebra mussels, and 2) a control which received no additions. For the zebra mussel treatments, zebra mussel clumps were gently lowered onto Vexar mesh (1.2 cm) platforms. These platforms were constructed roughly 8 cm from the bottom to allow zebra mussel waste to fall below the colonies where it would not be as easily resuspended by aeration-induced currents. For each run, the four replicates of each treatment were assigned randomly within rows of four barrels each. One run was conducted in mid-August and the other in early September 2002. The late summer season was selected because of the typically high densities of cyanobacteria at that time of year.

Water samples were collected for total nitrogen (TN), total phosphorus (TP), soluble reactive phosphorus (SRP), chlorophyll *a*, microcystin and plankton analysis prior to zebra mussel addition (time 0), and again at 12 hrs, 24 hrs, 48 hrs, 72 hrs, and 96 hrs. Barrels were stirred well before bottles were submerged and inverted to obtain samples. Approximately 3.3 L were removed at each sampling time for all analyses, which amounted to the removal of less than 10% of the original water over the course of the experiment. At the conclusion of the entire experimental run, zebra mussels from each barrel were counted and sized to the nearest 0.01 mm using calipers.

Analysis of Samples

Samples for SRP were immediately filtered through pre-soaked 0.45-µm membrane filters and analyzed within 24 hrs using the ascorbic acid colorimetric method (APHA 1995). Samples for TP analysis were immediately frozen after collection in acid-washed polypropylene sample bottles. Later, thawed samples underwent a persulfate digestion prior to analysis for TP using the same ascorbic acid colorimetric method. Both the TP samples and SRP samples were analyzed on a Shimadzu UV-1601 spectrophotometer. Total nitrogen samples were acidified to a pH of less than 2 using concentrated sulfuric acid and stored in new polypropylene centrifuge tubes (blanks revealed acid-washing was unnecessary) at 4°C. Analysis for TN also em-

ployed a persulfate digestion (APHA 1995) and was performed by the Larosa Environmental Laboratory, Vermont Department of Environmental Conservation.

For microcystin analysis, water was filtered through Whatman 934/AH filters (1.5 µm retention) immediately following collection and stored in 50% methanol at –80°C until analysis. Before analysis, filters underwent three freeze-thaw cycles in order to ensure cell lysis. An EnviroLogix Microcystin Plate Kit, a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA), was used to determine microcystin levels.

Chlorophyll *a* concentration of samples was determined using the hot ethanol extraction method of Sartory and Grobbelaar (1984) with the modifications of Levine *et al.* (1997). The sample was filtered through a GF/F filter, placed in a pre-labeled centrifuge tube and kept frozen and wrapped in foil until analysis. Chlorophyll concentration was determined from absorbance read on a Shimadzu UV-1601 spectrophotometer using the calculations of Lorenzen (1967) and values of Sartory and Grobbelaar (1984).

Each sample collected for microzooplankton (including rotifer, nauplii, and *Difflugia*) analysis was sieved through 63-µm mesh, transferred to an appropriate bottle, and narcotized with carbonated water prior to preservation with 70% alcohol. All organisms present were identified and counted using an Olympus CK2 inverted microscope at 100× magnification.

Phytoplankton samples were preserved with Lugol's preservative with silica beads to prevent diatom dissolution. Samples were concentrated using settling chambers prior to identification to genus and enumeration of cells using an Olympus inverted microscope at 200 to 400× magnification in the style of Utermöhl (Wetzel and Likens 1991). Count data were converted into biovolume using the equations of Wetzel and Likens (1991) and measurements of the cell geometry of at least 10 but generally 20 individuals of each genus.

Statistical Analyses

Two-way, repeated measures analysis of variance (RM-ANOVA) was used to assess the impact of the treatment on nutrient concentrations, phytoplankton density, phytoplankton composition, chlorophyll *a* concentration, and rotifer, *Difflugia*, ciliate, and nauplii abundances for each run. Runs were not combined because of differences in the natural phytoplankton communities at each time. To achieve homogeneity of variance and normality, log and square root transformations were applied to raw data when necessary. Ranked data were used in the RM-ANOVA if homogeneity of variance could not be achieved. If the interaction term was significant, one-way ANOVAs were run separately at each sampling time to find significant differences between treatments (Sokal and Rohlf 1981). Similarly, the effect of time within treatments was further explored using one-way ANOVAs by treatment. Statistical analyses were conducted using SAS Systems software (SAS for Windows version 8.02 2001) using the mixed procedure for RM-ANOVA, and ANOVA procedure for one-way ANOVAs. Differences were considered significant at $p < 0.05$.

RESULTS

Zebra mussels survived well in both runs, but were larger and somewhat more abundant in barrels during the second experiment. Mean zebra mussel density used in Run 1 was 3,106 individuals/m^2, and in Run 2 to 3,870 individuals/m^2, or about 10% of the density documented in nearby Lake Champlain in about 10 m water depth (Beekey *et al.* 2004), a depth that is about 10 times greater than our experimental barrels. Although there was some variability between the average size and number of zebra mussels added to each treatment in each run, the difference in number was offset by difference in size when treatment replicates were established. The sum of lengths per barrel showed similarity in treatments across the barrels; individual barrel sums were all within 25% of each Run mean.

Nutrients

Initial concentrations of nitrogen, phosphorus, and soluble reactive phosphorus were similar across treatments and fell within the range of concentrations commonly found within the Main Lake in late summer (Watzin *et al.* 2005). For TN, time, treatment and the time × treatment interaction were all significant in the RM-ANOVA for both runs (Table 1). TN in zebra mussel treatments increased markedly over time in both runs (Fig. 1). One-way ANOVA by time indicated that in Run 1, significant differences between the zebra mussel treatment and control were apparent by 12 hrs and continued through the end of the experiment (except at 48 hrs), whereas in Run 2 no significant differences were seen until 72 hrs.

TABLE 1. Results of repeated measures analysis of variance for nutrients in the 96 hours following initiation of the experiment. Ranked data were used where indicated by *.

	Run 1			Run 2		
	F	p	d.f.	F	p	d.f.
Total Nitrogen						
Treatment	31.52	0.0014	1,6	6.41	0.0446	1,6
Time	5.5	0.0012	5,28	28.74	< 0.0001	5,30
Interaction	4.6	0.0035	5,28	12.49	< 0.0001	5,30
Total Phosphorus						
Treatment	3.84	0.0978	1,6	0.63	0.4584	1,6
Time	7.77	0.0001	5,28	9.28	< 0.0001	5,24
Interaction	8.79	< 0.0001	5,28	9.45	< 0.0001	5,24
Soluble Reactive Phosphorus						
Treatment	9.81*	0.0203	1,6	45.12	0.0005	1,6
Time	97.6*	< 0.0001	4,23	99.63	< 0.0001	5,30
Interaction	3.5*	0.0226	4,23	5.11	< 0.0001	5,30
TN:TP Ratio						
Treatment	1.86	0.2219	1,6	0.06	0.8216	1,6
Time	4.67	0.0032	5,28	2.13	0.096	5,24
Interaction	7.04	0.0002	5,29	5.41	0.0018	5,24

In the overall RM-ANOVA for TP, time and the interaction term were highly significant, but treatment was only marginally significant in Run 1 and not significant in Run 2 (Table 1). Separate one-way ANOVAs did reveal significant treatment effects at 96 hrs in Run 1 and 72 and 96 hrs in Run 2 (Fig. 1), with zebra mussel treatments having significantly higher TP concentrations than the controls.

Soluble reactive phosphorus concentrations were clearly affected by the introduction of zebra mussels; time, treatment, and the interaction were all significant in the RM-ANOVA (Table 1). In both runs, SRP concentrations increased substantially and significantly after 48 hours. In Run 2, SRP concentrations in the zebra mussel treatment increased significantly at 48 hrs while the SRP level in the control remained nearly constant for the duration of the run (Fig. 1).

There was no significant effect of treatment on the molar ratio of TN to TP (TN:TP); however, both time in Run 1 and the interaction term in both runs were significant (Table 1). In the zebra mussel treatment of Run 2, there was an initial increase in TN:TP followed by a decline in the ratio. One-way ANOVAs at 96 hrs showed significantly lower TN:TP ratios in the zebra mussel treatment compared to the control in both runs (mean $\pm$ 1 SE in zebra mussel treatment = 46.6 Run 1, 43.6 Run 2; mean $\pm$ 1 SE in controls = 74.3 Run 1, 72.2 Run 2).

Phytoplankton

In both runs, time, treatment and the interaction were all highly significant in the RM-ANOVA for chlorophyll *a*, a widely used measure of phytoplankton abundance (Table 2). Although chlorophyll *a* decreased significantly across both the zebra mussel treatments and the controls, the reductions in the zebra mussel treatments were more rapid and extreme in both experimental runs. Similarly, time, treatment and the interaction were also all significant for total phytoplankton biovolume in both runs (Table 2). As was expected, phytoplankton biovolume declined rapidly in the zebra mussel treatments in both runs (Fig. 2). There was also a decline, but a much more gradual one, in phytoplankton biovolume in control treatments which was probably a laboratory side-effect resulting from changes in light, temperature, and mixing regimes. In Run 1, the significant differences in total phytoplankton biovolume between treatments appear at 48 hrs and continue until the end of the experiment. The differences at 24 hrs ($p = 0.06$) and at 96 hrs ($p = 0.07$) were marginally significant. In Run 2, total phytoplankton biovolume dropped almost to zero at 24 hrs in the zebra mussel treatment and remained very low throughout the remainder of the experiment (Fig. 2).

The phytoplankton assemblage was comprised of many groups. Overall, large diatoms dominated phytoplankton biovolume, but smaller flagellated

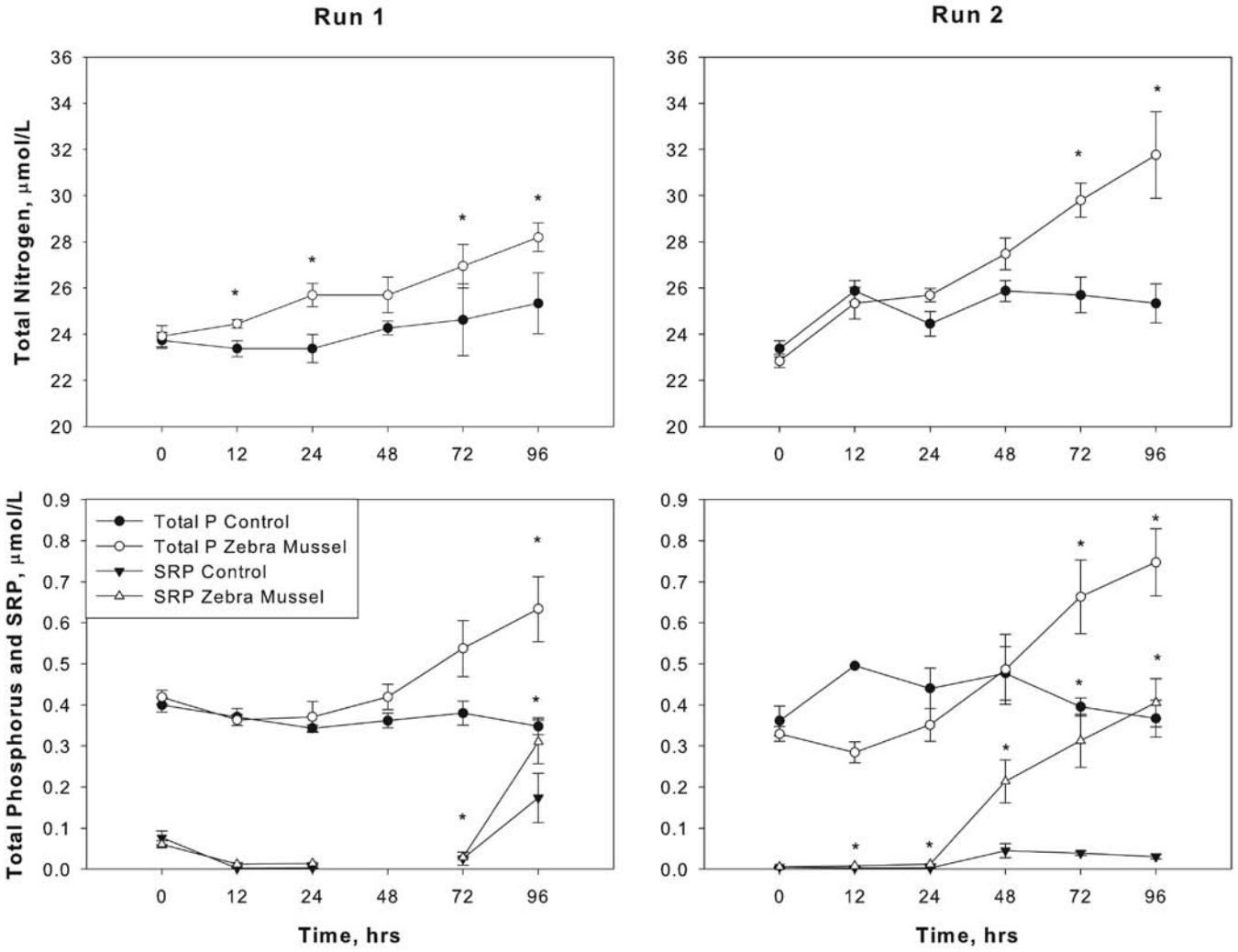

FIG. 1. Mean nutrient concentrations (±1SE): total nitrogen, µmol/L, total phosphorus, µmol/L, and soluble reactive phosphorus (SRP), µmol/L (Note: 25 µmol/L N = 0.35 mg/L; 0.5 µmol/L P = 15.46 µg/L), in both treatments over time for Run 1 and Run 2. Significant differences between treatments indicated by *. SRP data from Run 1 at 48 hrs was not included in statistical analyses because the samples may have been contaminated in processing.

cryptophytes were also numerous. Cyanobacteria, chrysophytes, chlorophytes and pyrrophytes were also present. The addition of zebra mussels caused significant declines in all phytoplankton groups analyzed statistically, including diatoms, cryptophytes, and cyanobacteria (Table 2). Diatom genera representing the most biovolume in both runs included *Fragilaria, Asterionella,* and *Aulacoseira*, with *Tabellaria* and *Cyclotella* also present. *Aulacoseira* was more abundant in the second run than in the first.

Cyanobacteria clearly decreased following zebra mussel introduction. Small *Coelosphaerium* colonies and small *Microcystis* colonies represented the largest portion of cyanobacteria biovolume in both runs, but *Anabaena flos-aquae, Anabaena planktonica* and *Aphanizomenon flos-aquae* were also present in low numbers. In both runs, there was a significant effect of treatment and interaction on cyanobacteria biovolume (Table 2), with significant differences between the control and zebra mussel treatment occurring at 12 hrs and continuing through 96 hrs (Fig. 2). There was no evidence of the toxin microcystin at any time during the experi-

TABLE 2. Results of repeated measures analysis of variance for mean chlorophyll a, and mean total phytoplankton biovolume, diatom biovolume, cryptophyte biovolume and cyanobacteria biovolume in the 96 hours following initiation of experiments. Ranked data were used where indicated by *.

	Run 1			Run 2		
	F	p	d.f.	F	p	d.f.
Chlorophyll *a*						
Treatment	75.43	0.0001	1,6	93.95	< 0.0001	1,6
Time	152.38	< 0.0001	5,30	38.88	< 0.0001	4,24
Interaction	15.52	< 0.0001	5,30	11.04	< 0.0001	4,24
Total Phytoplankton						
Treatment	16.2	0.0069	1,6	18.57	0.005	1,6
Time	20.62	< 0.0001	5,30	7.38	0.0039	5,10
Interaction	5.21	0.0015	5,30	4.42	0.042	2,10
Diatoms						
Treatment	14.16	0.0094	1,6	11.73*	0.0141	1,6
Time	22.19	< 0.0001	5,30	9.2*	0.0001	5,19
Interaction	5.1	0.0017	5,30	0.75*	0.5342	3,19
Cryptophytes						
Treatment	78.89*	0.0001	1,6	26.09	0.0022	1,6
Time	48.72*	< 0.0001	4,23	15	< 0.0001	5,19
Interaction	5.44*	0.0011	4,23	11.87	0.0001	3,19
Cyanobacteria						
Treatment	17.67	0.0057	1,6	81.93	0.0001	1,6
Time	0.9	0.4914	5,30	11.56	< 0.0001	5,19
Interaction	3.95	0.0072	5,30	8.84	0.0007	3,19

ments; all analyses showed concentrations below the detection level of the ELISA.

Protozoa

Heterotrophic and mixotrophic protozoa enumerated included the sarcodine protozoa *Difflugia* and ciliates. Protozoan data from 0 hrs of Run 1 were excluded from statistical analysis because of problems with the integrity of these samples. The RM-ANOVA showed a significant effect of treatment, time and their interaction in both runs for both *Difflugia* and ciliates (Table 3). The abundance of *Difflugia* decreased rapidly following zebra mussel introduction (Fig. 3); significant differences between the zebra mussel treatment and control were apparent beginning at 12 hrs in Run 1 and 24 hrs in Run 2; however, abundances in the controls showed a different pattern over time in the two runs. In Run 1 *Difflugia* abundance in the control decreased almost linearly from 12 hrs and ended at approximately 1 individual/L at 96 hrs. In Run 2, *Difflugia* abundances in the control did not show a significant change over time, although there was the suggestion of a decline and recovery.

For the ciliates, again, there were fewer in the zebra mussel treatment than in the control beginning at 12 hrs in Run 1 and at 24 hrs in Run 2 (Fig. 3). As with *Difflugia*, the pattern of the controls was different between runs. In Run 1 there was an increase to a clear maximum biovolume of ciliates at 48 hrs; this time was significantly different from all other times. In Run 2, there was the suggestion of peak abundance at 72 hrs; however, abundance at this time was not statistically different from other times.

Rotifers

The RM-ANOVA for rotifers showed highly significant treatment effects in both runs and a significant effect of time in Run 2 (Table 3). Rotifers were rapidly and significantly reduced in the zebra mussel treatment in both runs (Fig. 3). In Run 1 *Polyarthra* was the most abundant genus, followed closely by *Keratella* and then *Ascomorpha* and *Synchaeta*. Other genera such as *Kellicottia*, *Asplanchna,* and *Lecane* were only rarely observed. In Run 2 *Keratella* was equally dominant with *Polyarthra*, but otherwise species composition was similar. All genera present appeared equally affected by zebra mussels. One-way ANOVAs re-

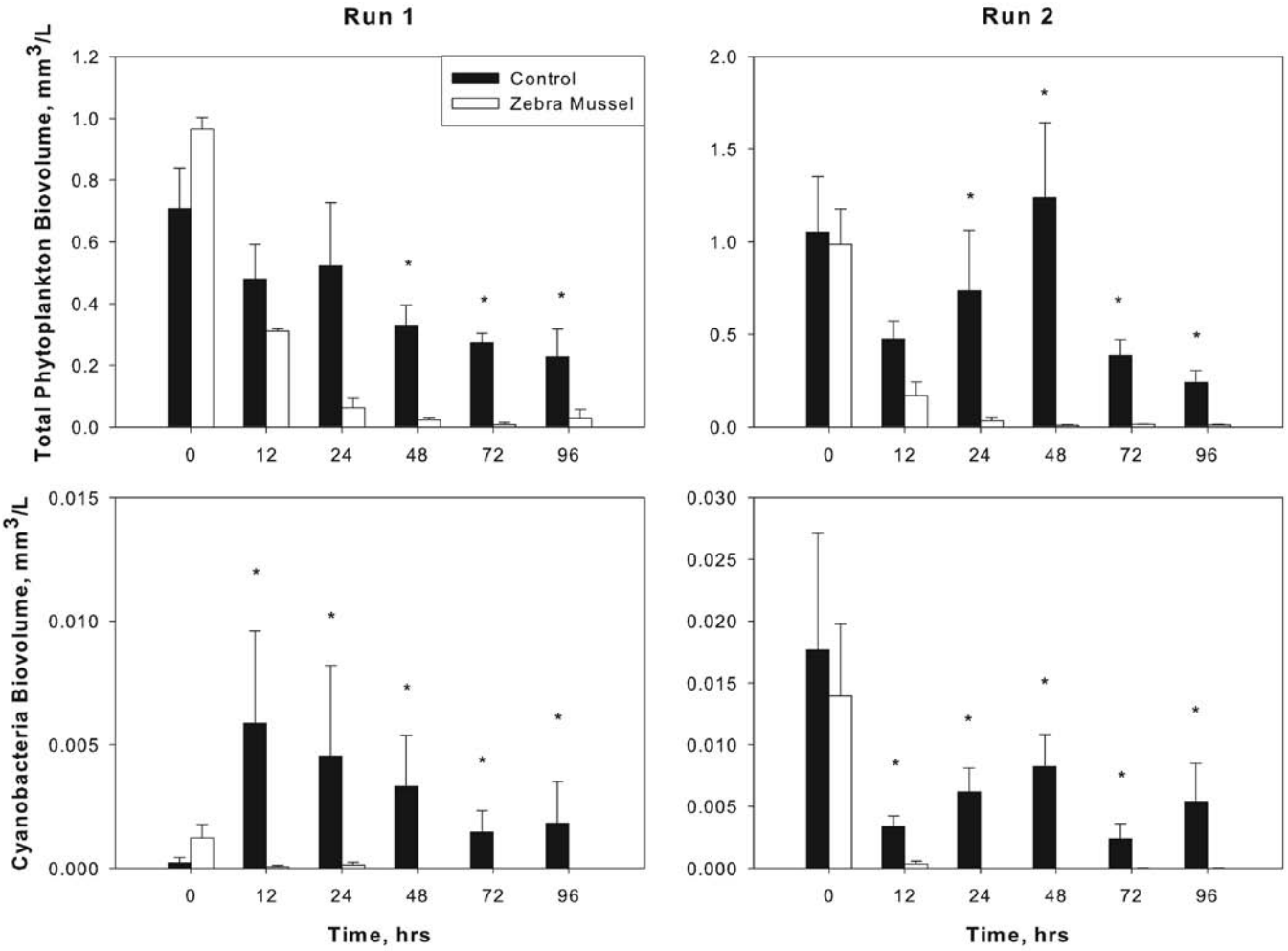

FIG. 2. Mean total phytoplankton biovolume (+1SE) and mean cyanobacteria biovolume (+1SE), mm^3/L, in both treatments for Run 1 and Run 2. Significant differences between treatments indicated by *.

vealed that rotifer abundance in the control of Run 1 was higher from 24 to 96 hrs than at 12 hrs; likewise the control of Run 2 increased significantly at 72 and 96 hrs.

Nauplii

Copepod nauplii, which were small enough to pass through the 150-µm sieve used in the initial filtration, were relatively abundant in the samples, and RM-ANOVA revealed a significant effect of treatment in both runs (Table 3) and significant time and interaction effects for Run 2. Nauplii abundance was reduced by the zebra mussel treatment (Fig. 3), although not as quickly as for rotifers. Trends were not identical between runs. In Run 2, no significant difference between treatments was seen until 48 hrs, and nauplii abundance in the zebra mussel treatment at 0 hrs was significantly higher than at all other times.

DISCUSSION

Phytoplankton

In these mesocosm experiments, the phytoplankton community as a whole was clearly impacted by the introduction of zebra mussels. A decline in overall phytoplankton abundance, measured as chlorophyll *a* and phytoplankton biovolume, was obvious in the zebra mussel treatments, a result consistent with several other studies (Fahnenstiel *et al.* 1995, Bastviken *et al.*1998, Nicholls 2001, Idrisi *et al.* 2001, Hall *et al.* 2003). All three of the main groups present, diatoms, cryptophytes, and

TABLE 3. Results of repeated measures analysis of variance for mean Difflugia, ciliate, total rotifer and nauplii abundance in the 96 hours following initiation of experiments. Ranked data were used where indicated by *.

	Run 1			Run 2		
	F	p	d.f.	F	p	d.f.
Difflugia						
Treatment	50.75	0.0004	1,6	103.99	< 0.0001	1,6
Time	9.04	0.0001	4,24	8.35	< 0.0001	5,30
Interaction	3.09	0.0347	4,24	2.68	0.0407	5,30
Ciliates						
Treatment	308.16	< 0.0001	1,6	210.39	< 0.0001	1,6
Time	21.56	< 0.0001	4,24	3.12	0.0224	5,29
Interaction	25.46	< 0.0001	4,24	5.43	0.0012	5,29
Total Rotifers						
Treatment	138.95*	< 0.0001	1,6	44.26	0.0006	1,6
Time	1.88*	0.1465	4,24	13.86	< 0.0001	5,30
Interaction	3.47*	0.0227	4,24	19.69	< 0.0001	5,30
Nauplii						
Treatment	24.98	0.0025	1,6	27.6	0.0019	1,6
Time	1.9	0.1439	4,24	5.54	0.001	5,30
Interaction	1.35	0.2812	4,24	4.31	0.0045	5,30

cyanobacteria, were very significantly reduced following the addition of zebra mussels.

The first objective of this study was to test the hypothesis that zebra mussel grazing can lead to blooms of potentially toxic cyanobacteria (Vanderploeg *et al.* 2001, Heath *et al.* 1995). Clearly zebra mussels were capable of grazing cyanobacteria in our mesocosms. Two factors are likely important in thinking about what this may mean for Lake Champlain. First, colony size and form probably affect how zebra mussels graze cyanobacteria. Dionisio Pires and van Donk (2002) found no significant differences in the clearance rates for non-toxic unicellular *Microcystis* and comparably sized green algae. Vanderploeg *et al.* (2001) found that when phytoplankton sizes are similar, zebra mussels appear unable to sort between them. The cyanobacteria present in our study were all small non-toxic colonies without excessive mucilage, and they were similar in size to many of the diatoms present. The second consideration is the well mixed conditions of our mesocosms. The impact of zebra mussel filtration can be affected by basin mixing rates (Noonburg *et al.* 2003), something that was not considered in this study. Although there are many shallow well mixed sections of Lake Champlain, in the stratified deep waters of Lake Champlain and other large lakes, cyanobacteria may be able to avoid zebra mussel filtration by staying in the surface waters. Based on our results, it is unclear whether zebra mussels can affect cyanobacteria bloom formation directly through grazing.

Zebra mussels may also be affecting cyanobacteria indirectly through changes in nutrient availability. Our results demonstrate that zebra mussels can have a profound effect on nitrogen and phosphorus concentrations (Fig. 1). TN and SRP in zebra mussel treatments were elevated throughout the experimental period, and TP concentrations were higher in the zebra mussel treatment than in the control at the conclusion of each run of the experiment (Fig. 1). Similar increases in soluble phosphorus have been observed following zebra mussel introduction in other studies in both the field and laboratory (Heath *et al.* 1995, Holland *et al.* 1995, Arnott and Vanni 1996, Makarewicz *et al.* 2000, James *et al.* 2001). Observations of changes in TP and TN have been more variable (Heath *et al.* 1995, Gardner *et al.* 1995, Makarewicz *et al.* 2000, Idrisi *et al.* 2001).

Although the effect of treatment on TN:TP was not significant, the interaction of treatment and time was significant, and lower N:P ratios were found in zebra mussel treatments at 96 hrs. Nutrient ratios in zebra mussel treatments dropped from 58-68 (by moles) at the start of the experiment to approximately 47 at the conclusion of each run, within the range that Smith (1983) has suggested favored cyanobacteria. Our findings support the theory that zebra mussels may create lower ambient N:P ratios,

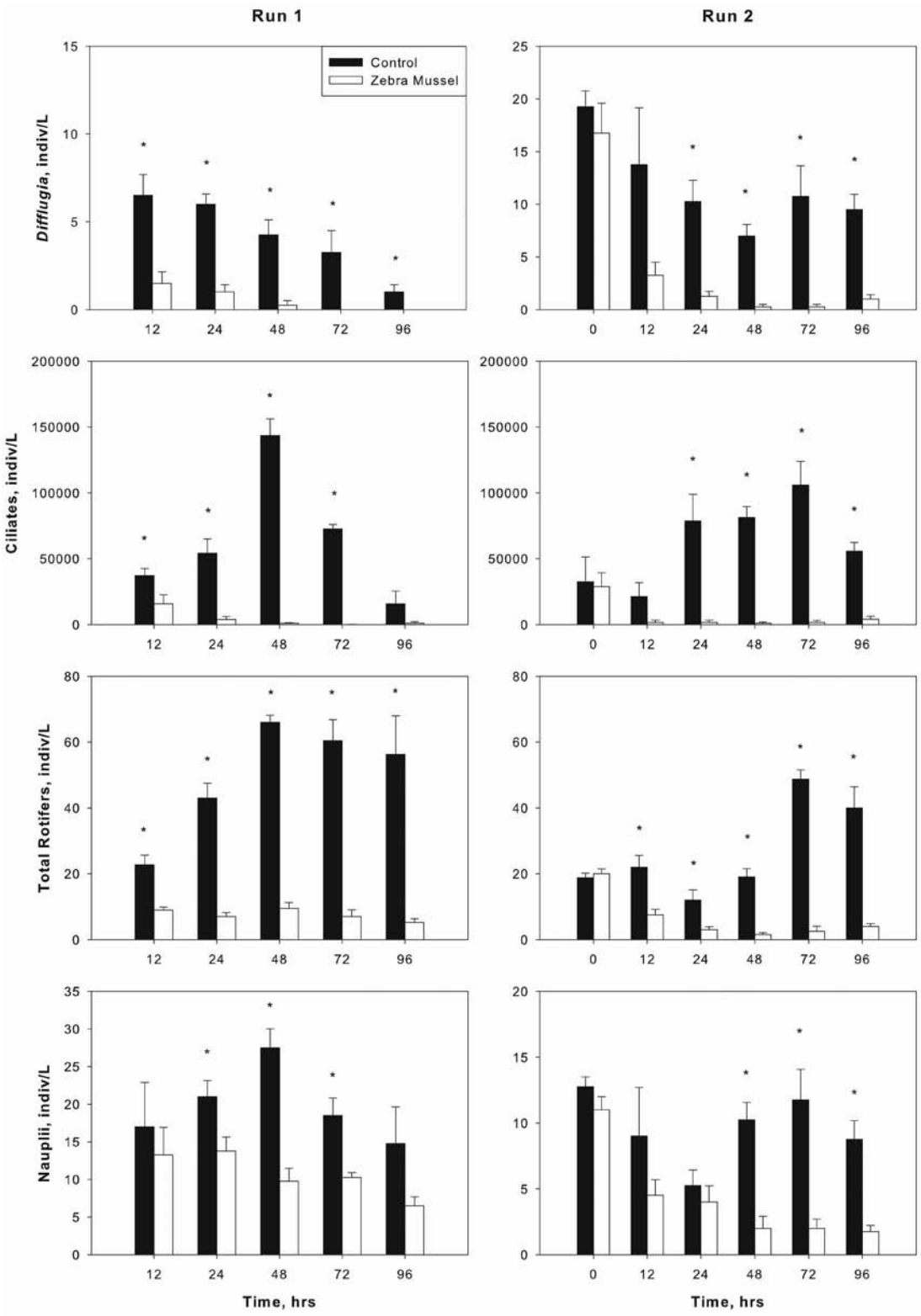

FIG. 3. Changes in mean **Difflugia**, *ciliate, total rotifer and nauplii abundance (+1SE), individuals/L, in both treatments for Run 1 and Run 2. Data from 0 hrs of Run 1 were not included because sample integrity was compromised. Note the difference in scale between the two runs for* **Difflugia** *and nauplii. Significant differences between treatments indicated by *.*

which may be a contributing factor in the development of cyanobacteria blooms (Arnott and Vanni 1996).

Zebra mussels might also change the availability of nutrients by disrupting nutrient recycling within the microbial loop. In our experiments, zebra mussels significantly decreased ciliate and *Difflugia* abundances. Declines in protozoans have been observed in the presence of zebra mussels in other studies as well (Lavrentyev *et al.* 1995, Pace *et al.* 1998, Wilson 2003). By decreasing protozoan abundance, zebra mussels may be changing the amount or rate of nutrient remineralization that occurs and thus changing the availability of soluble nutrients for uptake by phytoplankton (Azam *et al.* 1983).

One way that remineralization might be affected is through an increase in bacterial abundance following the removal of bactivorous protozoans. We did not measure bacteria in this study, however, increases in bacterial abundances following zebra mussel invasion have generally not been observed in other studies, in part because zebra mussels can clear bacteria relatively effectively (Cotner *et al.* 1995, Dionisio Pires *et al.* 2004). More study is needed on the impacts of zebra mussels on both protozoans and bacteria before any conclusive statements can be made about their net impact on the microbial loop.

Rotifers and Macrozooplankton

Rotifers were quickly and markedly reduced in the presence of zebra mussels in our study, as others have also observed (MacIsaac *et al.* 1991, MacIsaac *et al.* 1995, Jack and Thorp 2000). All rotifer genera present appeared to decline equally. Zebra mussel clearance rates with rotifers can be similar to those for phytoplankton (MacIsaac *et al.* 1995); thus it may be that the decrease in rotifer abundance in our study was the result of direct filtration or injuries sustained during filtration. It is also possible that the decline in rotifers occurred because of starvation resulting from competition with zebra mussels for phytoplankton food resources; however, we observed significant decreases in rotifer abundance coincident with, rather than subsequent to, the decreases in phytoplankton at 12 hrs. Therefore, we conclude that direct consumption by zebra mussels is the more likely explanation.

The decrease in nauplii densities clearly demonstrates that zebra mussels can impact macrozooplankton assemblages. Our results are consistent with the decline in nauplii seen following the zebra mussel invasion of the Hudson River (Pace *et al.* 1998) and stand in contrast to previous studies which have found no effect of zebra mussels on nauplii abundance (MacIsaac *et al.* 1995, Jack and Thorp 2000). Increasing mortality of nauplii is likely to result in lower copepod abundance over time. Presumably juvenile cladocerans would also be vulnerable to zebra mussel filtration, though too few were present in our study to document this. Decreases in nauplii were most likely the result of mortality due to filtration or injuries sustained in filtration by zebra mussels, although as with rotifers, sparser food resources may also have been a factor.

By whatever mechanism, zebra mussels may impact the zooplankton by favoring a shift to those species that are more resistant to zebra mussel filtration (Horgan and Mills 1999, Idrisi *et al.* 2001). Nevertheless, because zooplankton directly support planktivorous fish, changes within the zooplankton may have serious implications for the higher trophic levels within aquatic ecosystems (MacIsaac *et al.* 1992).

Implications for Nutrient Limitation in Lake Champlain

Prior to the establishment of zebra mussels throughout Lake Champlain, nutrients generally influenced phytoplankton more than grazing by macrozooplankton (Levine *et al.* 1999). When nutrients were limiting to phytoplankton, it was usually phosphorus that controlled phytoplankton densities, although nitrogen also appeared to play an important limiting role at times (Levine *et al.* 1997).

The results of our experiments indicate that zebra mussels have the potential to significantly alter nutrient cycling in addition to exerting substantial grazing pressure on phytoplankton. We saw phosphorus concentrations increase and N:P ratios decrease following the addition of zebra mussels to our experimental systems. Although we only measured nutrient concentration and not uptake rate or indicators of phosphorus status, it is possible that the zebra mussels increased phosphorus availability to phytoplankton, especially relative to nitrogen. If more phosphorus is available in Lake Champlain as the result of the zebra mussel invasion, a shift away from phosphorus limitation may occur, which could favor cyanobacteria dominance. The assumption

that phosphorus is the limiting factor for algal growth in Lake Champlain is the basis for a multimillion dollar phosphorus reduction strategy (VTDEC and NYSDEC 2002, LCSC 2003). Once the zebra mussel population stabilizes, it will be important to reassess the phosphorus status of Lake Champlain phytoplankton to determine whether increasingly difficult and costly reductions in phosphorus loading are still likely to result in the decreases in algal growth that are expected and to consider any increases in the frequency of cyanobacteria dominance resulting from increased nitrogen limitation that may occur. Although it is impossible to predict how the planktonic foodweb in Lake Champlain might adjust to the continuous presence of zebra mussels, the changes we observed demonstrate that zebra mussels have the potential to strongly influence the plankton community and possibly those higher trophic levels that depend on the plankton.

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