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Abundances of Naked Amoebae and Macroflagellates in Central New York Lakes: Possible Effects by Zebra Mussels

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Summary. Zebra mussels (*Dreissena polymorpha*) are bivalve mollusks that have invaded and altered the ecology of many North American lakes and rivers. To identify possible ecological effects of zebra mussels on naked amoebae, this study compared abundances of sediment and water column naked amoebae in shallow water zones of four lakes with and four lakes without zebra mussels. Additional data was collected on the density of macroflagellates. Although no statistically significant difference in naked amoebae density was found, higher ratios of sediment to water column naked amoebae abundances in zebra mussel lakes were observed due to increased naked amoebae abundances in the sediments. However, we did not observe a concomitant decrease in water column naked amoebae abundances. Flagellate abundances revealed no significant differences between the two lake types. Taken together, the data show that naked amoebae and flagellates thrive in shallow water zones of zebra mussel lakes and that the filter feeding activities of zebra mussels and reported reduced water column protists abundances may be offset by the flocculation of protists from the rich zebra mussel colonies.

Key words: Flagellates, invasive species, naked amoebae, nearshore zone, protists, zebra mussels.

INTRODUCTION

Naked amoebae and heterotrophic flagellates play important roles in aquatic systems. In addition to bacteria (e.g., Huws *et al*. 2005, Weitere *et al*. 2005), varied protist species feed on algae and fungi (e.g. Adl and Gupta 2006) and their respiratory activity returns CO_2 to the atmosphere. Egested residues of heterotrophic protists, rich in carbon, nitrogen, phosphorus and sulfur supports plant growth and maintain bacterial densities, the so called "microbial loop" (Azam *et al*. 1983, Clairholm 1985, Coleman 1994). Naked amoebae and heterotrophic flagellates are fed upon by metazoans including nematodes and rotifers, and thus provide a key food web link to higher trophic levels.

Vast differences in modes of locomotion, feeding structures, and dietary requirements largely regulate and differentiate the ecological niches exploited by naked amoebae compared to heterotrophic flagellates. Studies of naked amoebae ecology within aquatic systems show that most are substrate attached (e.g. Rogerson and Gwaltney 2000, Rogerson *et al*. 2003). Amoeboid movement by definition necessitates substrate attached

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locomotion and studies of the feeding behavior of naked amoebae demonstrate the high levels of efficiency in which naked amoebae are able to chemotactically detect and use pseudopodia in the capture of even well-concealed bacterial prey (e.g. Anderson 1994, Darbyshire 2005). Observations of naked amoebae feeding on phototrophic components of biofilms (e.g. Jahnke *et al*. 2007) demonstrate the range of substrate-attached prey available to naked amoebae. The work of flagellate ecologists, especially Boenigk and Arndt (2002), make it clear that flagellate feeding behaviors are very different than naked amoebae. Even among flagellates, differences exist in the food selected and captured as prey. For example, pelagic flagellates are most often not attached to substrates and utilize direct grasping and interception feeding strategies. In contrast, most benthic flagellates glide along substrates in search of bacterial prey. Broadly classified, naked amoebae and heterotrophic flagellates are adapted to, and largely occupy, separate niches within aquatic ecosystems. Their contributions, separately and together are essential in maintaining the ecological stability within these systems.

The invasive zebra mussel *Dreissena polymorpha* (Pallas) has only recently colonized lakes in the Upper Susquehanna River basin (Horvath 2008), having been in the Great Lakes since 1986 (Hebert *et al*. 1988). Although zebra mussel impacts on the ecology of aquatic systems are well documented, data are mostly from studies of suspended algal and diatom cells (Caraco *et al*. 1997), blooms of toxic microcystis bacteria (Vanderploeg *et al*. 2001), and on populations of aquatic plants and fish (e.g. Leach 1993). Only a few researchers have studied the effects of zebra mussels on pelagic and benthic heterotrophic protists (Laventyev *et al*. 1995, Findlay *et al*. 1998, Bischoff and Wetmore 2009).

This study investigates the potential impact that zebra mussels have on the heterotrophic protist communities in the pelagic and benthic habitats of lakes.

MATERIALS AND METHODS

Sample sites

All of the lakes used in this study are located in the same geographical area of central upstate New York U.S.A. and all are within a 50 km radius of each other. They were selected based on previous knowledge of their limnological properties, ease of access and presence (or absence) of zebra mussels. Lakes with zebra mussels include Eaton Brook Reservoir (EBR: 42.86414 N, –75.68756 W), Canadarago Lake (CAN: 42.81077 N, –74.99748 W), Otsego Lake (OTL: 42.79105 N, –74.89766 W) and Goodyear Lake (GYL: 42.50583 N, –74.98650 W). Lakes without zebra mussels include Oneonta Reservoir (ONR: 42.51191 N, –75.05447 W), Arnold Lake (ARN: 42.61236 N, –75.00864 W), Upper Leland Pond (ULP: 42.87890 N, –75.57564 W), and Chenango Lake (CHE: 42.57818 N, –75.43814 W). The lakes vary in their colonization history – EBR since 2000, CAN since 2003, GYL since 2004, and OTL since 2006 (Horvath 2008). All the lakes are considered eutrophic with the exception of the mesotrophic Otsego Lake. Regardless, we measured basic limnological variables at collection site using a Eureka Manta multiprobe (Table 1).

Naked amoebae sampling

Each lake was sampled once between 24 August and 28 September 2009. Samples were collected in a tight time frame so that abundance patterns could be analyzed with minimum interference from seasonal fluctuation patterns. In all assays, sub-sampling from a single sample, homogenized by vortexing during assay preparation, was used to obtain abundance data void of spatial heterogeneity that may have been present due to patchy distribution of protists.

Sediment samples were collected with a 50-ml syringe with the tip bored off. Multiple pulls of the syringe was necessary to collect approximately 100 ml of a sediment-rich suspension. In zebra mussel lakes, the syringe was held within a bed of zebra mussels

Table 1. Basic limnological variables for each site EBR – Eaton Brook Reservoir, CAN – Canadarago Lake, OTL – Otsego Lake, GYL – Goodyear Lake, ONR – Oneonta Reservoir, ULP – Upper LeLand Pond, ARN – Arnold Lake, CHE – Chenango Lake.

Sites	Temperature (°C)	pH	Conductivity (µmhos/cm)	Dissolved oxygen (mg/L)	Sediment Organic Matter (% Dry Mass)
EBR	20	8.4	150	9.6	2.09
CAN	21	8.6	320	10.0	0.57
OTL	22	8.5	300	9.9	0.29
GYL	24	8.1	270	9.9	1.99
ONR	24	8.1	70	9.9	0.81
ULP	20	8.6	580	9.8	0.87
ARN	19	8.7	60	9.9	0.67
CHE	19	8.6	200	9.7	0.54

and slowly moved around the colony as the plunger was pulled. Care was taken to collect the sediment sample within zebra mussel colonies. The sample was immediately transferred to a sterile plastic bottle. Water column samples were collected in 100-ml plastic bottles held approximately 25 cm above zebra mussel colonies. Sediment and water column samples in non-zebra lakes were collected from nearshore sites in the same manner.

Flagellate sampling

Sediment samples were collected in the same manner described for amoebae, transferred to a collection bottle and immediately fixed with Lugol's iodine. Water column samples were collecting by inverting a 1,000-ml flask in the water column at about knee-deep water at each site. Samples were again immediately fixed with Lugol's iodine.

Enumeration of naked amoebae

The number of naked amoebae/ml of sediment and number/ml in the water column was determined using a standard culture-enrichment method previously described (e.g., Anderson and Rogerson 1995). Because many naked amoebae are floc-associated the plastic sample collection bottle was vortexed for 1 min to disrupt the floc material and release naked amoebae. Rogerson and Gwaltney (2000) compared abundance counts of planktonic naked amoebae from vigorously shaken and 1-min vortexed samples and reported a three-fold increase in vortexed samples. Following culture enrichment protocols, after waiting ~30 s for heavy inorganic particles to settle, 1-ml aliquots were removed and diluted (1–100 ml for sediments and 1–10 ml for water column) in 0.45 µm filtered lake water (MFLW). Diluted subsamples were briefly vortexed on slow speed to disperse the amoebae and 10-µl aliquots were inoculated into each well of a 24-well Falcon culture dish already supplied 2 ml MFLW and a small piece of malt yeast agar to support bacterial growth (Page 1983). The culture dishes were incubated in the dark at room temperature and observed after ~ 10 days and then again after about ~20 day to fully capture naked amoebae that may have bloomed up due to succession (Smirnov and Brown 2004). The naked amoebae were observed at 400X with a Zeiss Axiovert 40C inverted microscope equipped with phase contrast, camera and software, and an ocular micrometer. Observed amoebae were measured for length and width and attempts to identify most were made by comparison to the sixteen morphological sketches and photo plates provided by Smirnov and Brown (2004) and with Page's (1988) soil and freshwater gymnamoebae identification key. Because the identification of naked amoebae to species level requires morphologicial and ultrastructural data beyond the intent of this project designed to identify similarities and differences in naked amoebae abundances in zebra mussel and comparison lakes, observations were tallied using a conservative four morphotype classification scheme (e.g. Anderson and Rogerson 1995, Bass and Bischoff 2001, Bischoff 2002) that allows documentation of abundance patterns without making dubious taxonomic errors. Type 1 Mts exhibit lobose or fine sub-pseudopodia usually emerging from the anterior hyaline edge during locomotion. Genera *Acanthamoeba*, *Vexillifera* and *Korotnevella* are examples. Type 2 Mts are limax, sometimes with a distinct hyaline cap. Type 2 examples are genera *Hartmannella* and *Saccamoeba*. Type 3 Mts are limax and distinctly eruptive during locomotion. An example genus is *Vahlkampfia*. Type 4 Mts are flattened or discoidal and examples genera are *Platyamoeba* and *Vannella*. The density of naked amoebae suspended in the water column expressed as number/ml water, and density of naked amoebae in the sediment rich samples expressed as number/ml of sediment water, was then calculated using proportional analyses.

Abundances of Naked Amoebae Relative to Sediment Volume

Most studies involving sediment abundances of naked amoebae (e.g. Butler and Rogerson 1995, Smirnov and Thar 2003) use core samples and the counts are reported as abundances/ml of core sample. Zebra mussel colonies are most typically attached to artificial and natural hard substrates (i.e. boat hulls and rocks) making it impossible to extract cores for analyses. At the same time we recognized that our method of extracting sediments with the bored out syringe yielded variable concentrations of sediment material to water and that our abundance calculations should also be reported relative to the total amount of suspended particulates available to amoebae. Therefore, in addition to reporting naked amoebae abundances as number/ml of water and number/ml of sediment water, we also report abundances as number/ml of sediment. To do this, we centrifuged, at high speed for one minute, the sample remaining after withdrawing 3 ml for the culture analyses (~ 97 ml as 1 ml was withdrawn for each of three assays) and fractions of sediment to total volume recorded. Abundances of naked amoebae/ml sediment water, determined from the culture enrichment method described above, were converted to number/ml sediment using the following equation: $N = (n / ml) \times (sv / tv)$, where N is the abundance of naked amoebae /ml of sediment, n/ml is the abundance in the sediment water calculated from the culture enrichment method, sv is the sediment volume, and tv is the total volume of the sample.

Enumeration of flagellates

Each Lugol's stained sediment sample was transferred to a 100-ml graduated cylinder and allowed to settle for at least 10 days in the lab. Immediately before microscope analysis, overlying water was carefully removed to reduce a sample to 10 ml. This was then homogenized by vortexing at high speed for 60 s. The vortexed sample was again allowed to settle for about 2–3 min. to allow the larger particles to drop out of suspension, and then 10 µl was pipetted to a Neubauer hemocytometer. Using 400 × bright field magnification, flagellates were enumerated using standard procedures for a hemocytometer. All the cells of the hemocytometer grid were counted unless the samples were too dense, then cells in every other column were counted.

Each water column sample was transferred to a 1,000-ml graduated cylinder and sealed with parafilm in the lab. Samples were allowed to settle for at least 10 days, after which the overlying water was carefully removed to reduce each sample to 100 ml. The remaining 100 ml sample was vortexed at high speed for 60 s to homogenize it before transferring it to a 100-ml graduated cylinder and sealed. This was allowed to settle again for at least 10 days. Immediately prior to microscopic analysis, each sample was reduced to 10 ml, which was then homogenized by vortexing at high speed for 60 s. The homogenized sample was transferred to a graduated centrifuge tube and spun at high speed for 3 min. The overlying water was carefully removed to reduce the sample to 0.5 ml, which was then vortexed again to homogenize it. The homogenized sample was

allowed to settle for 2--3 min to allow the larger particles to drop out of suspension, and then 10 µl was pipetted to the hemocytometer. Using 400x bright field magnification, flagellates were enumerated using standard procedures for a hemocytometer. Only the central 25 cells of the hemocytometer were counted because the concentration processes effectively reduced the samples to dense yields.

In both the sediment and water column samples, we were only able to enumerate the macroflagellates. Microflagellates, defined by size of 2-10 µm, require epifluorescence microscopy (e.g., Fenchel 1982), which we did not use.

Organic matter content

Organic Matter content of the sediments (OM) was measured at each site within a meter of the sample for the protists. The same sampling technique for protists, aspirating about 100 ml of sediment and water using a syringe, was used to collect OM samples. Samples were transported to the laboratory in a cooler and stored in a refrigerator for one day before processing. Stored samples were filtered onto pre-ashed and weighed glass-fiber filters (0.7 µm). Filters and sediment were dried to consistent weight at 105°C and then reweighed. Dry mass of sediments was calculated by subtracting the filter weight from the filter plus sediment weight. Samples were then combusted to remove OM in a muffle furnace at 550°C for 1 h. Samples were moved to a desicator to cool, and then weighed again. Ash-free dry mass (AFDM) was calculated by subtracting the dry mass from the remaining post-ashed weight. OM is reported as %OM (100*AFDM/dry mass).

Statistical analyses

In addition to describing the protist communities in these lakes, we were also interested in exploring any potential differences in amoeba communities between lakes with and without zebra mussel colonies. The microscopy techniques are labor and time intensive, therefore we could not both characterize the amoeba communities in any single lake at the same time keeping a reasonable sample size (lakes were considered as a sample unit to avoid pseudoreplication). Because of the lack of detailed sampling in any lake, we strongly temper any of the interpretations of statistical analyses and are conservative in our conclusions. However, the design does allow for comparisons between the lake type (with or without zebra mussels). Either a general linear model ANOVA or a two-sample t-tests was used to test for differences in protist abundances between the zebra mussel and non-zebra mussel lakes ($\alpha = 0.05$). In all cases, assumptions of normality and equality of variance were checked with a Kolmogorov-Smirnoff one-sample test and a Levene's test, respectively. The percent organic matter data were arcsin-square root transformed before analysis. Relationships between variables were analyzed by a rank correlation analysis because of the presence of a few extreme values in the data. Data were first ranked and then a Pearson correlation was performed. All analyses were performed using Minitab® statistical software (version 15).

RESULTS

Protist abundances from sediment samples could be calculated based on either the total volume of the sample, which would include sediments aspirated as well as the overlying waters, or based just on the volume of the sediment alone (Tables 2 and 3). We prefer to use the data from the sediment slurry because we feel it best represents the actual habitat sampled. However, we were concerned that the volume of actual sediment in each of the sediment samples would influence the analyses. Although we did not detect a difference in sediment volume collected between the two lake types ($t = 1.40$; $P \leq 0.24$), we added the sediment volume to a general linear model as a covariate as a first step to analyzing sediment samples (both amoebae and flagellate abundances).

Table 2. Amoebae abundances in the two habitats sampled (water column and sediments). Abundances in sediment samples were calculated from both the entire volume of the sediment slurry (sediment and water) and the volume of the sediment alone. Means and standard deviations (SD) are calculated from the 3 subsamples processed. The ratios are abundances in the sediments/water column and are also shown for each of the methods for calculating final abundances.

	Sediment samples		Water column	Ratios	
Lakes	Mean /ml Sediment Slurry (SD)	Mean/ml Sediment (SD)103	Mean /ml (SD)	N/ml Sediment Slurry	N/ml Sediment volume
GYL	1,991 (1,388)	264 (184)	339 (587)	5.9	778
CAN	3,760 (1,799)	249 (193)	1,080 (70)	3.5	230
EBR	4,242 (1,164)	128 (35)	848 (187)	5.0	151
OTL	736 (613)	40 (33)	987 (347)	0.7	44
ONR	802 (298)	4 (2)	786 (515)	1.0	5
ARN	1,600 (400)	11 (3)	1,110 (441)	1.5	10
ULP	1,515 (921)	11 (7)	848 (97)	1.8	13
CHE	2,986 (129)	125 (5)	1,126 (163)	2.7	111

Table 3. Flagellate abundances in the two habitats sampled (water column and sediments). Abundances in sediment samples were calculated from both the entire volume of the sediment slurry (sediment and water) and the volume of the sediment alone. The ratios are abundances in the sediments/water column and are also shown for each of the methods for calculating final abundances.

	Sediment samples		Water Column	Ratios	
	Per ml Sediment Slurry	Per ml Sediment 10^3	Per ml water	N/ml Sediment Slurry	N/ml Sediment volume
GYL	18,666	2,667	107	174	24,806
CAN	4,667	101	245	19	414
EBR	889	296	835	1	355
OTL	1,333	156	120	11	1,307
ONR	4,444	222	243	18	916
ARN	2,444	27	45	54	604
ULP	1,333	26	113	12	232
CHE	17,778	1,185	148	121	8,035

Sediment volume was not a significant factor in the one-way ANOVA model for the amoebae ($F < 0.01$; $P \leq 0.99$). Sediment volume also did not contribute significantly to the model for the flagellate data ($F = 0.26$; $P \leq 0.63$). Thus, we subsequently removed the sediment volume as a covariate from all other analyses on amoebae and flagellate abundances and used only the values of abundances calculated using the total volume of the sediment slurry. Within the sediments, organic matter content did not differ significantly between the two lake types ($P \leq 0.47$). We detected no significant relationship between the organic matter content of the sediments and the amoebae ($r = 0.41$; $P \leq 0.32$) or the flagellate ($r = 0.22$; $P \leq 0.61$) abundances. Even when we split the two lake types, no relationships were significant.

When we pooled all 8 lakes, mean amoebae abundances in the sediment slurries (= 2204; S.D. 1512) were generally 2.5 times higher than in the water column (= 890; S.D. 385). The relatively high standard deviation values reveal a wide distribution of abundances around the reported means.

Within the sediment samples (Table 2), both the highest (EBR, = 4242/ml; S.D. = 1164) and the lowest (OTL, = 736; S.D. = 613) naked amoebae abundances were found in the lakes with zebra mussels. Although the mean abundance was higher in the zebra mussel lakes (= 2682/ml; S.E. = 809) compared to the non-zebra mussel lakes (= 1725/ml; S.E. = 456), the difference was not significant ($t = 1.03$; $P \leq 0.34$). Likewise, mean naked amoebae abundances in the zebra mussel lake water columns (= 813; S.E. = 165) were very similar to the non-zebra mussel lakes (= 967; S.E. = 88) and not significantly different ($t = 0.82$; $P \leq 0.44$).

The ratio of amoebae abundances in the sediment slurry to water column was higher in the zebra mussel lakes compared to the non-zebra mussel lakes (Fig. 1); however, the difference was not significant ($t = 1.74$; $P \leq 0.13$). Although not statistically identified as an outlier, likely due to the low sample number, the amoebae abundance in the sediment sample from OTL was almost five-times lower than the other zebra mussel lakes. If we remove the OTL data, the difference in the ratio between the two lake groups becomes significant ($t = 4.29$; $P \leq 0.01$).

Some interesting patterns in the naked amoebae abundances are revealed when the data are distributed by morphotypes (Table 4). In the zebra mussel lakes, the morphotype abundances in the sediments are mostly a magnification of what is in the water. The extent of the magnification varies considerably generating ratio scores as low as 0.4 in OTL for morphotypes 1 and 2, and one ratio as high as 34.6 for mt-4 in GDY. As indicated by the dashes, morphotypes undetectable in the water were mostly undetectable in the sediments as well.

The comparatively low ratio scores in the non-zebra mussel lakes indicate the magnification of water column morphotypes in the sediments is less extreme. Two exceptions were relatively high ratios of 7.0 and 6.6 for mt 4 and 3 respectively in ARN and ULP. Similar to the zebra mussel lakes, morphotypes undetectable in the water column were usually undetectable in the sediments.

The sediment flagellate data were highly variable within and among the two lake types. The highest (18,666/ml) and lowest (889/ml) zebra mussel sediment

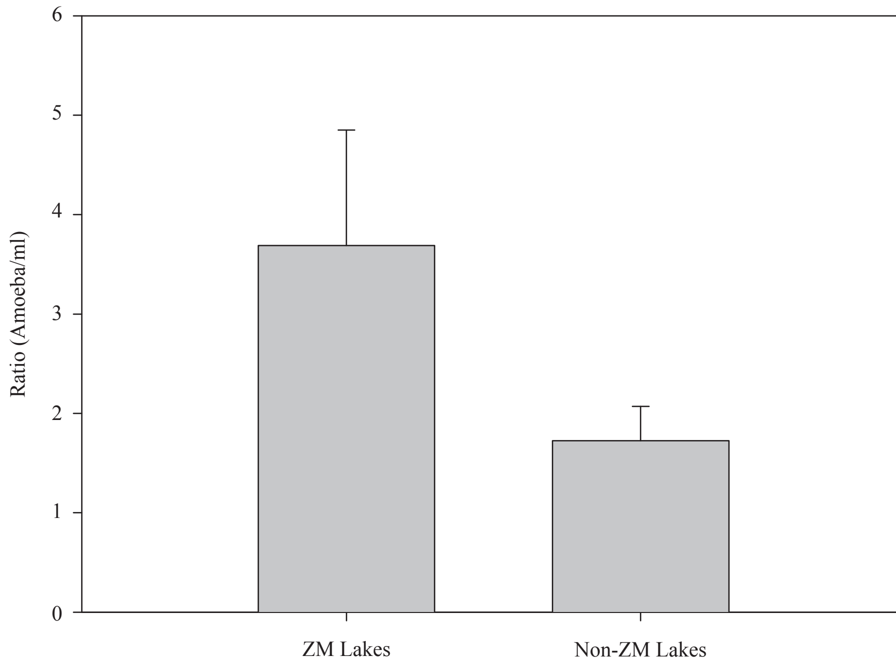

Fig. 1. Amoebae abundance (amoebae ml^{-1}) shown as a ratio of sediment to water column abundance. Error bars are + 1 standard error of four lakes for each category. ZM – zebra mussel.

Table 4. Mean morphotype abundances followed by standard deviation in parentheses and ratio scores. S – sediments, W – water column, R – ratio. Dashed line divides zebra mussel and non-zebra mussel lakes.

	Morphotypes			
	Mt-1	Mt-2	Mt-3	Mt-4
GDY S	1,341 (1,227)	43 (75)	86 (75)	519 (259)
W	293 (507)	–	31 (53)	15 (26)
R	4.6		2.7	34.6
CAN S	3,549 (1,728)	–	130 (225)	87 (75)
W	1,034 (70)	–	30 (27)	15 (26)
R	3.4	–	4.3	5.8
EBR S	3,549 (585)	–	–	693 (666)
W	848 (187)	–	–	–
R	4.1	–	–	–
OTL S	216 (149)	87 (150)	–	433(326)
W	555 (361)	200 (347)	31 (53)	200 (271)
R	0.4	0.4	--	2.1
ONR S	460 (349)	–	21 (35)	321 (175)
W	508 (395)	–	–	277 (122)
R	0.9	–	–	1.1
ARN S	1,167 (130)	130 (130)	86 (150)	216 (270)
W	802 (174)	46 (80)	231 (245)	31 (54)
R	1.5	2.8	0.4	7.0
ULP S	1,125 (738)	–	303 (270)	86 (150)
W	679 (53)	31 53	46 (80)	92 (160)
R	1.7	–	6.6	0.9
CHE S	2,813 (326)	87 (150)	87 (150)	–
W	1,034 (70)	–	93 (160)	–
R	2.7	–	0.9	–

flagellate data were found in GYL and EBR, respectively. Among the non-zebra mussel lakes, the highest sediment flagellate density (17,778/ml) was found in CHE and the lowest (1,333/ml) in ULP. Comparisons of the two lake types identified no significant differences (t = 0.02; $P \leq 0.99$) in the sediment abundances. Likewise, water column flagellate abundances were quite variable within and among the two lake types for both habitats sampled. In the zebra mussel lakes, the highest (835/ml) and lowest (107/ml) water column flagellate densities were found in EBR and GYL, respectively. For the non-zebra mussel lakes, the highest water column flagellate density (243/ml) was found in ONR and the lowest (45/ml) in ARN Lake. Statistically, we detected no differences (t = 1.07; $P \leq 0.32$) in the water column flagellate abundances.

DISCUSSION

Zebra mussels typically occur in very high numbers in newly colonized ecosystems, often dominating the benthic animal biomass (Leach 1993). Their filter-feeding activities coupled with this high biomass results in significant changes in the location of available energy resources (Horgan and Mills 1997, Roditi *et al*. 1997). This change is primarily in the direction of pelagic to

benthic transfers, as zebra mussels deposit previously suspended materials to the sediments in the form of feces and pseudofeces (Low and Pillsbury 1995). The effects of this "benthification" have been reported on primary producers (Caraco *et al*. 1997, Zhu *et al*. 2006) and secondary producers (Stewart *et al*. 1998). It is reasonable to predict that zebra mussels are having an impact on the heterotrophic communities in both the pelagic and benthic zones of lakes. The enriched benthic zone may increase protist production there, while at the same time reducing protist numbers in the pelagic zone. We were particularly interested in the naked amoebae community because their morphology, modes of locomotion and tactics of predation make them dependent on substrate attachment in both pelagic and benthic systems. As a consequence, we anticipated the naked amoebae data would be most indicative on any zebra mussel effects on water column and sediment protist distributions.

We were unable to detect any statistically significant differences between the two lake types for abundances of either naked amoebae or flagellates. Our hypotheses that the active feeding by zebra mussels would result in reduced numbers of protists in the water columns of zebra mussel lakes compared to lakes lacking zebra mussels was not statistically supported. Small sample sizes coupled with the inherent variability in protist abundances among lakes in general may have masked any true differences among the two lake types. For example, Butler and Rogerson (1995) report highly variable and unpredictable flagellate abundances in marine benthic sediments and Kiss *et al*. (2009) reported high variability among flagellates and naked amoebae in the Danube River of Hungary. The standard deviations around our reported mean abundances also indicate that variability among lakes is also very high. We were only able to take a single snapshot of the protist community in a single site which greatly limits our abilities to describe the community both temporally and spatially in these lakes. However, Bischoff and Wetmore (2009) reported that the naked amoebae community structure and density/gram matter within the biofilms on zebra mussels in Goodyear Lake followed seasonal patterns very similar to those within nearby rock biofilms. Although no significant differences in densities between the two substrates were found, the increased surface area formed when zebra mussel colonies attach to substrates would in effect vastly increase the overall abundances of benthic naked amoebae. That study, utilizing intensive sampling protocols within a single lake, raised several questions about the possible impacts of zebra mussels on naked amoebae (and other protists) abundances. For example, that study did not examine simultaneous water column abundances from zebra mussel and non zebra mussel zones within Goodyear Lake, nor did it compare data from several lakes (zebra mussel and non zebra mussel invaded) to see if the observed zebra mussel and nearby rock biofilm abundance patterns were connected to zebra mussels.

The lakes used in the study are representative of many of the lakes in the area. Lakes in central New York are predominately of glacial origin. A few lakes are considered large finger lakes (long, narrow, deep) such as Otsego Lake, but most are smaller (1–120 hectares), shallower lakes. Nearly all lakes in the region are in advanced stages of Eutrophication, with nutrient additions from agriculture or domestic waste water causing most problems. Our sample lakes are all relatively close together and are quite similar in terms of their water chemistry. Even though protist communities are very likely spatially variable, we tried to minimize among-lake variation as much as possible.

Despite the limitations in the sampling design, several patterns in the naked amoebae data are indicative of zebra mussel effects. The ratio (Fig. 1) of amoebae in the two habitat types (sediment:water column) compared between the two lake types may be the most instructive. Calculating this ratio likely strengthened the signal of a zebra mussel effect. In fact, if we remove Otsego Lake from the data, we did detect a significant difference. Otsego Lake, of the four lakes with zebra mussels, is the most unique for a number of reasons, and removing it from this analysis for the purposes of this discussion is defendable. It is the only lake in the entire set that is not truly eutrophic, but is best described as mesotrophic to oligotrophic. It is the latest of the lakes to be colonized, so the population of mussels may not have had enough time to exert an effect on the protists. It also has the lowest concentration of zebra mussels, especially given its large volume, again likely reducing the influence of zebra mussels. With the removal of Otsego Lake from the data, the significantly higher naked amoebae ratio in zebra mussel lakes indicates that the mussels are very likely contributing to naked amoebae abundance distribution patterns.

The lack of a significant difference in the flagellate abundance data between the zebra mussel and non-zebra mussel lakes is important because it tells us that zebra mussels are not destroying flagellate habitat in shallow water regions of lakes. Pelagic populations, most

susceptible to filtration, are likely sustained by wave action sloughing from the benthic rich populations.

In summary, even though zebra mussels filter vast numbers of pelagic protists, shallow water zebra mussel colonies are attractive habitats for naked amoebae and flagellates. These rich benthic populations seem sufficient to largely replenish filter-reduced pelagic population. This is particularly evident in the naked amoebae data where there is evidence of high densities within zebra mussel colonies. Impacts of the increased densities of naked amoebae on high level trophic organisms such as nematodes, copepods, rotifers and other meiofauna would be an interesting follow up study and would shed light on the dynamic trophic shifts that occur when zebra mussels invade.

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