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**Research Article**

# Antagonistic effects of invasive zebra mussels and nutrient enrichment on algal and rotifer biomass

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## Abstract

We conducted a mesocosm experiment to determine how multiple stressors including invasive zebra mussels (ZM), a nutrient pulse (NP), and the addition of herbivorous large-bodied zooplankton (*Daphna pulex* and *Simocephalus vetulus*; LBZ) affected resident plankton (phytoplankton and zooplankton) in both single- and multiple-factor treatments. We also assessed whether the establishment of LBZ was influenced by ZM and/or NP. Algal and rotifer biomass was significantly greater in treatments with nutrients than in treatments with both nutrients and zebra mussels indicating antagonistic effects of the two stressors. Zebra mussels also had several effects on zooplankton that were independent of the other stressors: zebra mussels reduced the biomass of the invertebrate predator *Chaoborus* sp., while the biomass of resident cladocerans and large-bodied zooplankton tended to be higher in at least some of the treatments with zebra mussels. Large-bodied zooplankton did not affect algal biomass or resident zooplankton in any of the treatments. Our results contribute to a growing body of research showing that invasive zebra mussels can be the primary drivers of plankton dynamics when multiple stressors are present in aquatic ecosystems.

**Key words:** eutrophication, multiple stressors, cumulative effects, *Chaoborus*

## Introduction

Freshwater habitats are increasingly being invaded by non-native species and an important goal of ecologists is to document how these invaders impact native ecosystems (Strayer 2010). However, invasive species represent only one of many possible stressors (Olden et al. 2010; Ormerod et al. 2010). Multiple stressors often affect ecosystems differently than single stressors (Crain et al. 2008): effects can be additive, where the combined effects are predicted from the sum of the effects of individual stressors; or non-additive where the effects are more (synergistic) or less (antagonistic) than those predicted from summing individual stressors (Crain et al. 2008; Darling and Côté 2008). While a large body of research has focused on how invasive species impact native systems, much less is known about how invaders interact with other stressors (Vye et al. 2015, 2017).

The zebra mussel (*Dreissena polymorpha* Pallas, 1771), which invaded parts of Europe in the late 18th and early 19th centuries (Van der Velde et al. 2010) and North America more recently in the 1980s (Carlton 2008), has affected phytoplankton and zooplankton in invaded ecosystems. Zebra mussels are efficient filter feeders that can reduce algal biomass (Basu and Pick 1997; Caraco et al. 1997; Ietswaart et al. 1999); promote cyanobacteria through

selected consumption and the rejection of cyanobacteria as pseudofeces (Dionisio Pires and Van Donk 2002; Bierman et al. 2005; Wojtal-Frankiewicz et al. 2010); alter nutrient concentrations and ratios (Arnott and Vanni 1996; Wojtal-Frankiewicz and Frankiewicz 2011); consume small zooplankton including rotifers (MacIsaac 1996; Jack and Thorp 2000; Thorp and Casper 2002); and outcompete large-bodied zooplankton for algal resources (Wojtal-Frankiewicz et al. 2010; Kissman et al. 2010). Another stressor that impacts plankton is eutrophication, or nutrient enrichment (Smith 2003). In contrast to zebra mussels, however, nutrient enrichment generally leads to increases in phytoplankton and zooplankton biomass. Several mesocosm experiments have shown that zebra mussels and nutrient enrichment act antagonistically: plankton biomass (both total phytoplankton and some zooplankton taxa) increased with nutrient enrichment but zebra mussels prevented these increases when they were also present (Dzialowski and Jessie 2009; Dzialowski 2013; Sinclair and Arnott 2015).

Another factor that has the potential to interact with zebra mussels and nutrient enrichment is large-bodied herbivorous zooplankton. Although large-bodied zooplankton are not a stressor in the same context as invasive species and nutrients (e.g., they are not human caused stressors), they do play an important role in structuring plankton communities (Brooks and Dodson 1965; Persson et al. 2007). Large-bodied zooplankton may outcompete smaller zooplankton for algal resources because they have lower threshold food concentrations (food concentrations at which population growth equals zero, Gliwicz 1990, 2003), they can reduce algal biomass through strong grazing pressure (Lampert et al. 1986), and they can buffer against increases in algae that may result from external nutrient pulses (Cottingham and Schindler 2000). Because zebra mussels and large-bodied zooplankton both consume phytoplankton, they may have an additive effect on phytoplankton biomass, especially if they differentially consume algal resources. However, the relationship between zebra mussels and large-bodied zooplankton is not well understood. Zebra mussels can outcompete large-bodied zooplankton, and the biomass of large-bodied zooplankton is often lower in invaded systems (Higgins and Vander Zanden 2010). In contrast, zebra mussels promoted the success of large-bodied *Daphnia* in mesotrophic mesocosms, which may have resulted from zebra mussel mediated changes in algal quality (e.g., lower C:P content of seston; Feniova et al. 2015). Therefore, a better understanding of how zebra mussels affect large-bodied zooplankton will help to determine the combined effects of these two important consumers on resident plankton.

The purpose of this mesocosm study was to determine how plankton (both phytoplankton and zooplankton) responded to zebra mussels, a pulse of nutrients, and large-bodied zooplankton in single factor treatments; and then if zebra mussels modified how plankton responded to the nutrient pulse and large-bodied zooplankton treatments. We predicted that zebra mussels would interact antagonistically with nutrients by preventing plankton biomass from increasing in response to nutrient enrichment due to strong filter feeding by zebra mussels. We also assessed how zebra mussels affected large-bodied zooplankton in the mesocosms and whether there were interactive effects of zebra mussels and large-bodied zooplankton on resident plankton.

## Methods

### *Experimental set-up*

We conducted a 44-day experiment, starting June 2012, using rectangular mesocosms (940 × 640 × 500 mm; 300 L; food safe, High Density Polyethylene containers) that were situated on the shore of Lake Mikołajskie (Mazurian Lakeland, Poland). We filled the mesocosms with approximately 270 L of Lake Mikołajskie water, which to our knowledge did not contain zebra mussels nor were any settling larvae observed in any of the mesocosms over the course of the experiment. This unmanipulated lake water with natural phytoplankton and zooplankton (i.e., resident plankton) served as our control (C). We established the experimental treatments by manipulating nutrient concentrations, the presence of large-bodied zooplankton, and zebra mussels in a 2 × 2 × 2 factorial design (Figure 1).

The nutrient pulse (NP) treatment was established by adding nitrogen (N: 0.192 mg $L^{-1}$ of $NH_4$-N and 1.728 mg $L^{-1}$ of $NO_3$-N) and phosphorus (P: 0.12 mg $L^{-1}$ of $PO_4$-P) at a ratio of 16:1 based on the Redfield Ratio. The nutrients were added on a single date to simulate a large pulse of nutrients that was consistent with nutrient concentrations in a hypereutrophic waterbody. The large bodied zooplankton (LBZ) treatment was established by adding two cladocerans, that are not present in Lake Mikołajskie but occur in several nearby waterbodies, at densities similar to those in the surrounding area (personnel observation): we added *Daphnia pulex* Leydig, 1860 at a density of 3.5 ind. $L^{-1}$ and *Simocephalus vetulus* Muller, 1776 at a density of 1.7 ind. $L^{-1}$. These two taxa were used as large-bodied zooplankton in the experiment because they are larger than the other taxa that were included as the resident zooplankton in the mesocosms. For example, the average individual biomasses

of *D. pulex* and *S. vetulus* were 0.18 ± 0.005 mg and 0.34 ± 0.003 mg, respectively. In contrast, the average biomasses of the other zooplankton taxa ranged from 0.024 mg to 0.0037 mg. The zebra mussel (ZM) treatment was established by adding zebra mussels at a biomass of 250 g $m^{-2}$ wet weight, which was approximately 200 individuals per mesocosm or 0.74 inds $L^{-1}$; this stocking biomass is similar to biomasses reported in nature (Mellina et al. 1995) and used in previous mesocosm experiments (e.g., Sinclair et al. 2015). Zebra mussels (10–20 mm) were collected from nearby Lake Boczne and gently brushed to remove attached algae before they were added to the mesocosms.

In total there were eight treatments (C, NP, ZM, LBZ, NP+ZM, ZM+LBZ, NP+LBZ, and NP+ZM+LBZ), each replicated in triplicate mesocosms giving 24 mesocosms (Figure 1). The treatments were established three days after the mesocosms were filled with Lake Mikołajskie water (day 0), and the first samples were retrieved on day 13. Thereafter, phytoplankton and zooplankton samples were collected approximately every 10 days (days 22, 35, 46). The water temperature during the experiment varied around 17–18 °C.

We estimated chlorophyll concentrations using a PHYTOPAM fluorometer (Walz, Germany). We used the PHYTOPAM to measure relative fluorescence (unit less) on whole water samples and used these data as estimates for total algal biomass in the mesocosms. Water samples were collected from the middle of each mesocosm using a Limnos (Hydro-Bios) water sampler (2.6 L) and returned to the laboratory for fluorescence measurements.

We enumerated resident zooplankton (e.g., rotifers, copepods, and cladocerans) separately from the large-bodied zooplankton that we added to the mesocosms. We identified rotifers from 1 L water samples that were collected from the center of each mesocosm by submerging a water bottle under the surface, concentrated using a 30 µm mesh net, and preserved in Lugol's solution and 4% formalin. We collected water samples for the enumeration and identification of the remaining resident cladocerans and the manipulated large-bodied zooplankton from the surface and 0.5 m below the surface of each mesocosm and combined them into a single sample (5.2 L) that was filtered through a 100 µm mesh net. A larger (60 L) sample was collected from each mesocosm on the final sample date. These samples were preserved in 4% formalin for the identification of copepods, cladocerans, and the invertebrate predator *Chaoborus flavicans* Meigen, 1830. We used length measurements (~ 10–25 inds. per species) to estimate dry weight biomass using length:weight relationships for rotifers (Ejsmont-Karabin 1998), crustaceans (Balushkina and Vinberg 1978), and *Chaoborus* (Dumont and Balvay 1979).

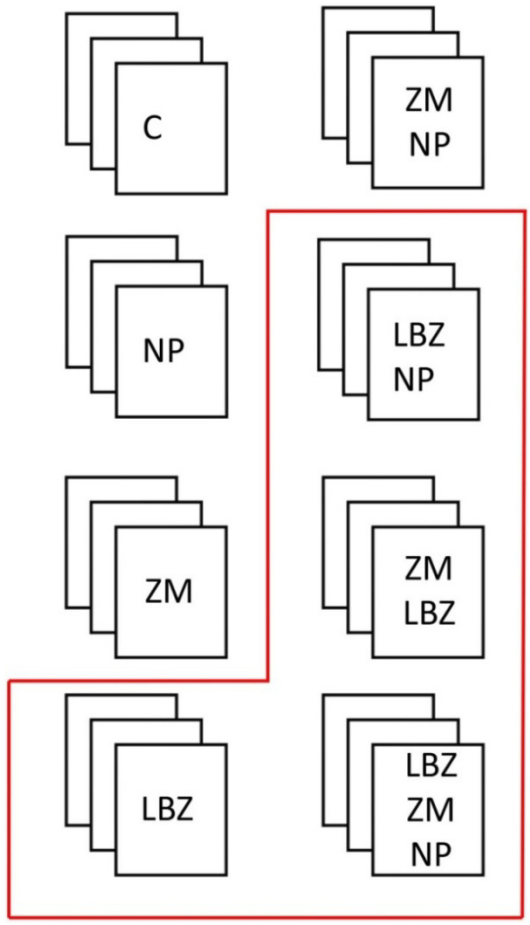


**Figure 1.** Experiment set-up. Each rectangle represents a mesocosm. C = control, ZM = zebra mussel, NP = nutrient pulse; LBZ = large-bodied zooplankton. The treatments outlined in red were used to determine how zebra mussels affected the biomass of the added large-bodied zooplankton. We measured algal (relative fluorescence), rotifer, copepod, resident cladoceran, large-bodied zooplankton, and *Chaoborus* biomass on days 13, 22, 35, 46.

### *Statistical analyses*

We constructed mixed General Linear Models (GLM) to compare the treatments (C, ZM, NP, LBZ, ZM+NP, ZM+LBZ, NP+LBZ, and ZM+NP+LBZ), interactions between treatment and Time, and the effects of mesocosm. Treatment and time (repeated measure) were specified as fixed factors, while mesocosm was randomly nested within treatments. Dependent variables that we measured on each date included algal, rotifer, copepod, cladoceran and *Chaoborus* biomass. We also used a subset of the treatments (LBZ, ZM+LBZ, NP+LBZ, ZM+NP+LBZ) to determine how the zebra mussel and nutrient pulse treatments affect the biomass of the large-bodied zooplankton (*D. pulex* and *S. vetulus)* that were added to the mesocosms. Where data did not show sphericity,

assessed using Mauchley's criterion, degrees of freedom were adjusted using Greenhouse-Geisser epsilon (Time and Time × Treatment factors). If significant treatment effects were detected, we used Fisher's LSD Post hoc test ($P < 0.05$) to determine which means differed. We used sequential Bonferroni Post hoc tests ($P < 0.05$) to establish significant differences within each treatment on different sample dates, and for the comparison of each dependent variable at each individual sample date. Interaction plots for the three stressors over time and the results from sequential Bonferroni Post hoc comparisons are present in the supplementary material.

All data were $\log_{10}$-transformed prior the analyses to achieve normality of residuals and improve homoscedasticity of variance. All statistical analyses and figure construction were performed in R 3.3 (R Core Team 2017) and in NCSS v. 7.

## Results

Algal biomass (measured as relative fluorescence) increased in response to the nutrient pulse (NP and NP+LBZ treatments > all other treatments). However, algal biomass decreased in response to zebra mussels, including in the ZM+NP and ZM+NP+LBZ treatments (Fishers LSD, $P < 0.05$, Figure 2), which suggests an antagonistic effect of the multiple stressors on algal biomass (ZM × NP interaction, $P < 0.01$; Table 1, Supplementary material Table S1). The effects of the nutrient pulse became less prominent over the course of the experiment and there were no longer significant differences between treatments by day 35 (sequential Bonferroni, $P < 0.05$, Table S1). The large-bodied zooplankton did not significantly influence algal biomass, or any of the response variables presented below (Table 1 and Figure 2).

Rotifer biomass did not differ between the ZM treatment and the control (Figure 2). However, there was an antagonistic ZM × NP interaction on rotifers where treatments with nutrients were significantly higher than treatments with both zebra mussels and nutrients: the NP and NP+LBZ treatments were significantly greater than the ZM+NP and ZM+NP+LBZ treatments (ZM × NP interaction $P < 0.01$, Table 1, Figure 2). Most differences between treatments were observed on days 22 and 35 (ZM × Time interaction, $P = 0.02$; Table S2).

Copepod biomass increased in response to the nutrient pulse: biomass was greater in the NP and NP+LBZ treatments than it was in the control (Fisher's LSD, $P < 0.05$, Figure 2). However, the only differences observed between treatments based on the post hoc comparisons for the individual dates occurred between the C and NP treatments on day 46 (sequential Bonferroni, $P < 0.05$, Table S3). Copepod biomass did not increase in treatments that had both zebra mussels and nutrients relative to the control (ZM × NP interaction, $P = 0.01$; Table 1). In several treatments, copepod biomass increased from day 13 to day 22, and then decreased from day 35 to 46 (Time effect, $P < 0.01$, Figure 2, Table S3).

Resident cladoceran biomass was higher in most treatments with zebra mussels than it was in the control (Fisher's LSD, $P < 0.05$, Figure 2). There was also a significant ZM × LBZ interaction ($P = 0.02$, Table 1) where the biomass of resident cladocerans was greater in the ZM+LBZ and ZM+NP+LBZ than it was in the LBZ single factor treatment. However, when looking at the post hoc comparisons on the individual sample dates, the only significant differences occurred on day 35 and there were few differences between the actual treatments (sequential Bonferroni, $P < 0.05$, Table S4).

*Chaoborus* biomass was significantly lower in all of the treatments with zebra mussels than it was in the control (Fisher's LSD, $P < 0.05$, Figure 2). However, there was also a significant ZM × Time interaction for *Chaoborus* showing that differences between treatments with and without zebra mussels were only observed on days 35 and 46 of the experiment (Table S5). *Chaoborus* biomass also tended to increase over time except in most treatments with zebra mussels (sequential Bonferroni, $P < 0.05$, Table S5).

*Daphnia pulex* biomass was significantly greater in the ZM+LBZ treatment than it was in the LBZ and NP+LBZ treatments (ZM × NP effect, $P = 0.04$; Table 1, Figure 2). However, there were no post hoc differences between treatments on any of the individual sampling dates nor were there differences over time in each treatment (sequential Bonferroni, $P > 0.05$, Table S6). With respect to *S. vetulus* biomass, there was a significant ZM effect (ZM effect, $P < 0.01$; Table 1) and biomass was greater in the ZM+NP+LBZ treatment than it was in the control (Fisher's LSD, Figure 2). The individual post hoc tests showed that *S. vetulus* biomass was greater in the two treatments with zebra mussels (ZM+LBZ and ZM+NP+LBZ) than it was in the two treatments without zebra mussels (LBZ and NP+LBZ) on day 22 and between the LBZ treatment and the ZM+NP+LBZ treatment on days 35 and 46 (sequential Bonferroni, $P < 0.05$, Table S7).

## Discussion

Zebra mussels and the nutrient pulse had contrasting effects on several of the plankton response variables. The biomass of algae, rotifers, and copepods increased in response to nutrients, which is consistent with previous eutrophication research (Smith 2003).

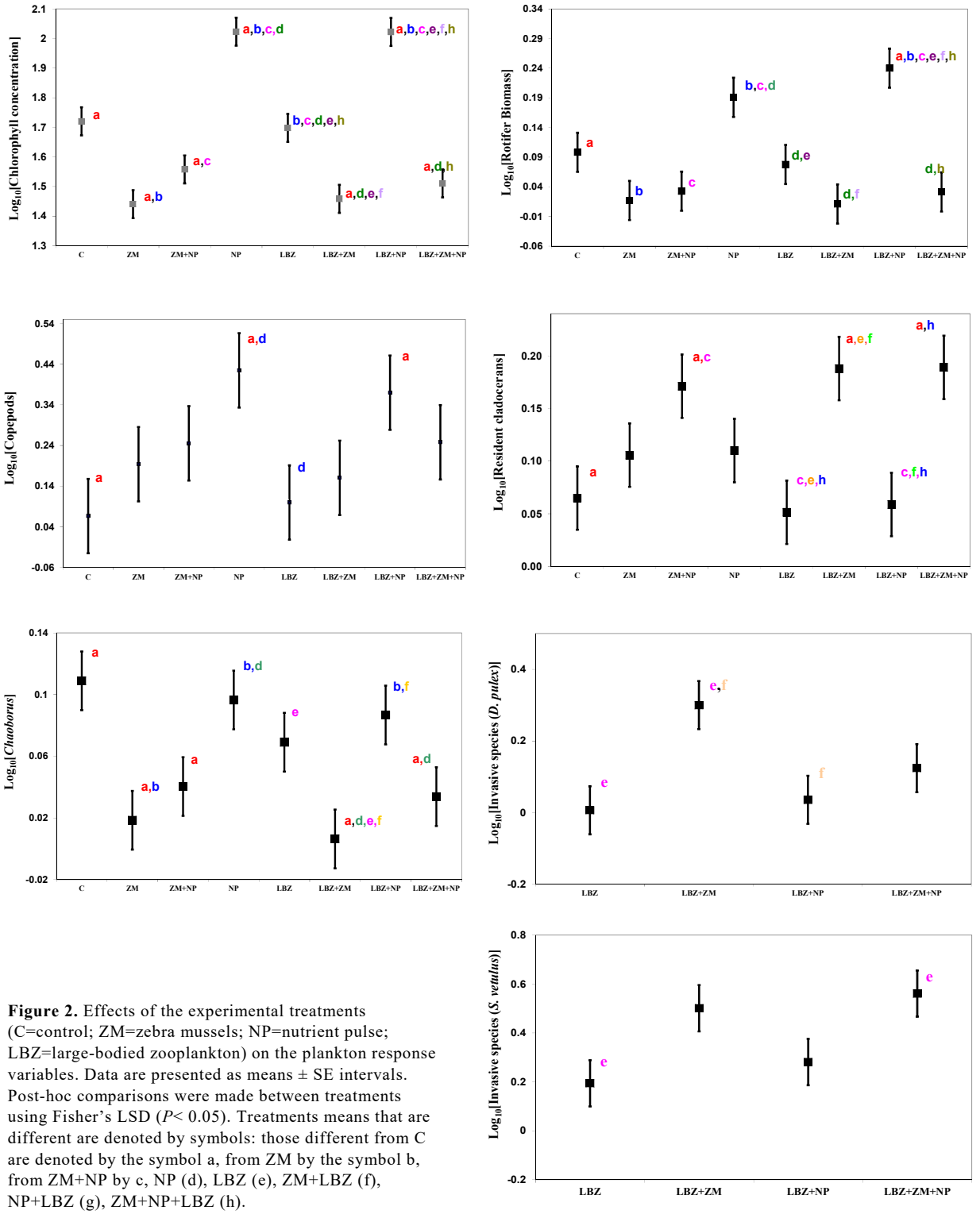


**Figure 2.** Effects of the experimental treatments (C=control; ZM=zebra mussels; NP=nutrient pulse; LBZ=large-bodied zooplankton) on the plankton response variables. Data are presented as means ± SE intervals. Post-hoc comparisons were made between treatments using Fisher's LSD ($P< 0.05$). Treatments means that are different are denoted by symbols: those different from C are denoted by the symbol a, from ZM by the symbol b, from ZM+NP by c, NP (d), LBZ (e), ZM+LBZ (f), NP+LBZ (g), ZM+NP+LBZ (h).

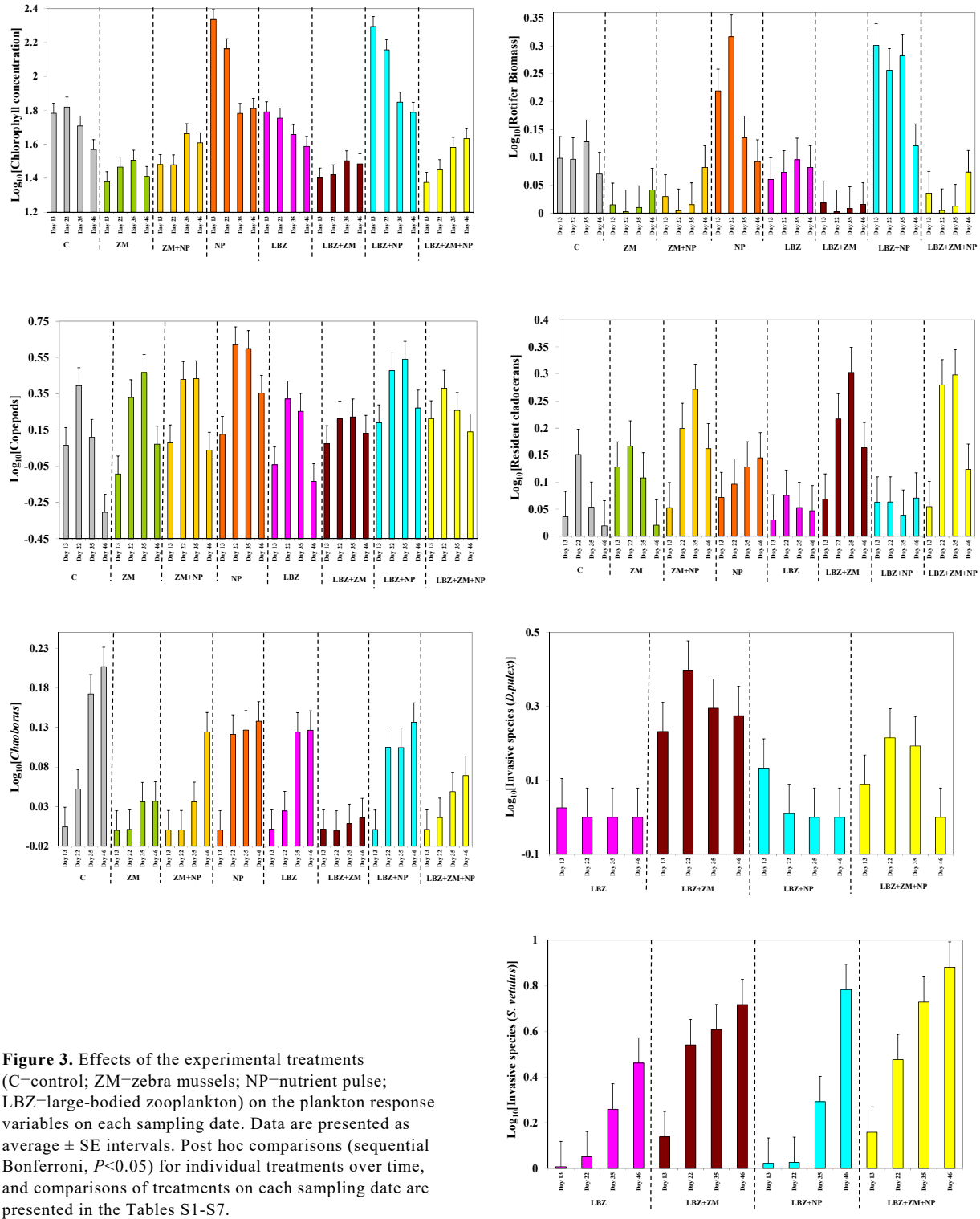


**Figure 3.** Effects of the experimental treatments (C=control; ZM=zebra mussels; NP=nutrient pulse; LBZ=large-bodied zooplankton) on the plankton response variables on each sampling date. Data are presented as average ± SE intervals. Post hoc comparisons (sequential Bonferroni, $P<0.05$) for individual treatments over time, and comparisons of treatments on each sampling date are presented in the Tables S1-S7.

**Table 1.** RM-ANOVA table summarizing the effects of the zebra mussel (ZM), the nutrient pulse (NP), and large-bodied zooplankton (LBZ) treatments and their interactions with time on algal (relative fluorescence), rotifer, copepod, resident cladoceran, *Chaoborus*, and large bodied zooplankton (*D. pulex* and *S. vetulus*) biomass. The analyses were carried out using mixed GLMs, where *F* is Fisher's test, DF is degrees of freedom, and the associated P-value. Significant effects are highlighted in bold (P<0.05). Results of post hoc comparisons between treatments are presented in Tables S1-S7.

| Effect | DF | Algal Biomass | | Rotifer Biomass | | Copepod Biomass | | Resident cladocera | | *Chaoborus* Biomass | | DF | (*D. pulex*) | | (*S. vetulus*) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P | F | P | F | P | | F | P | F | P |
| ZM | 1 | 233.2 | **<<0.01** | 59.2 | **<<0.01** | 0.41 | 0.53 | 26.7 | **<<0.01** | 42.8 | **<<0.01** | 1 | 15.8 | **<<0.01** | 18.8 | **<<0.01** |
| NP | 1 | 66.6 | **<<0.01** | 18.9 | **<<0.01** | 18.7 | **<<0.01** | 2.89 | 0.1 | 1.85 | 0.18 | 1 | 2.3 | 0.14 | 1.17 | 0.29 |
| LBZ | 1 | 0.36 | 0.55 | 0.1 | 0.75 | 0.09 | 0.77 | 0.25 | 0.62 | 2.9 | 0.09 | | | | | |
| ZM×NP | 1 | 21.08 | **<<0.01** | 10.7 | **<<0.01** | 7.7 | **0.01** | 0.04 | 0.84 | 1.21 | 0.28 | 1 | 4.6 | **0.04** | 0.04 | 0.88 |
| ZM×LBZ | 1 | 0.02 | 0.9 | 0.29 | 0.59 | 0.01 | 0.96 | 5.34 | **0.02** | 0.60 | 0.44 | | | | | |
| NP×LBZ | 1 | 0.15 | 0.7 | 1.24 | 0.27 | 0.09 | 0.77 | 2.05 | 0.16 | 0.79 | 0.38 | | | | | |
| ZM×NP×LBZ | 1 | 0.67 | 0.42 | 0.94 | 0.34 | 0.49 | 0.49 | 0.14 | 0.71 | 0.37 | 0.54 | | | | | |
| Time | 3 | 4.87 | **<<0.01** | 0.45 | 0.72 | 15.8 | **<<0.01** | 6.88 | **<<0.01** | 21.5 | **<<0.01** | 3 | 0.56 | 0.65 | 15.9 | **<<0.01** |
| ZM×Time | 3 | 24.31 | **<<0.01** | 3.49 | **0.02** | 0.59 | 0.62 | 3.75 | **0.02** | 4.96 | **<<0.01** | 3 | 1.08 | 0.37 | 1.46 | 0.24 |
| NP×Time | 3 | 1.72 | 0.17 | 0.91 | 0.44 | 0.3 | 0.83 | 0.91 | 0.44 | 1.17 | 0.33 | 3 | 0.28 | 0.84 | 0.84 | 0.48 |
| LBZ×Time | 3 | 0.30 | 0.83 | 0.37 | 0.77 | 1.0 | 0.4 | 0.35 | 0.79 | 0.75 | 0.53 | | | | | |
| ZM×NP×Time | 3 | 5.58 | **<<0.01** | 2.08 | 0.11 | 2.23 | 0.09 | 1.03 | 0.39 | 3.18 | **0.03** | 3 | 0.16 | 0.92 | 0.14 | 0.93 |
| ZM×LBZ×Time | 3 | 0.19 | 0.9 | 0.42 | 0.74 | 1.01 | 0.39 | 1.16 | 0.33 | 0.14 | 0.94 | | | | | |
| NP×LBZ×Time | 3 | 0.35 | 0.79 | 0.5 | 0.68 | 0.19 | 0.91 | 1.85 | 0.15 | 0.11 | 0.95 | | | | | |
| ZM×NP×LBZ×Time | 3 | 0.17 | 0.92 | 0.58 | 0.63 | 0.42 | 0.74 | 0.23 | 0.88 | 0.54 | 0.66 | | | | | |
| *Error* | 64 | Model $R^2$=87% | | Model $R^2$=65% | | Model $R^2$=59% | | Model $R^2$=79% | | Model $R^2$=69% | | 32 | Model $R^2$=48% | | Model $R^2$=70% | |

However, when both zebra mussels and nutrients were present in the multiple stressor treatments, the biomass of algae and rotifers did not increase with nutrient enrichment. Zebra mussels filter a wide range of particles (up to 1000 µm as reviewed by Wong and Levinton 2005) that overlaps with the body size of most rotifers (between 100 µm and 500 µm; Smith 2001). Therefore, zebra mussels likely directly consumed algal and rotifers preventing them from increasing in biomass in response to the nutrient pulse. Our results support previous research showing that zebra mussels and nutrients have antagonistic effects on algal biomass and at least some groups of zooplankton (Dzialowski and Jessie 2009; Dzialowski 2013; Sinclair and Arnott 2015).

The observed antagonistic effects of zebra mussels and nutrients on algal biomass have important implications for invaded lakes. While grazing studies show that zebra mussels are effective at removing algal biomass, our results show a direct interaction between zebra mussels, nutrient enrichment, and reductions in algal biomass that are consistent with a decoupling of chlorophyll *a* – TP relationships in invaded lakes (Nicholls et al. 1999; Higgins et al. 2011; Cha et al. 2013; Greene et al. 2014). For example, less chlorophyll *a* is often produced than predicted from total phosphorus concentrations in lakes that are invaded by zebra mussels (Higgins et al. 2011). This "masking" of nutrient enrichment by zebra mussels will likely occur in invaded lakes even if water column nutrient concentrations are high (Dzialowski and Jessie 2009).

Zebra mussels also affected several of the plankton response variables independently of the other stressor treatments. For example, the biomass of *Chaoborus* was generally lower in most treatments with zebra mussels, which may be partly explained by the direct effects of zebra mussels on algal and rotifer biomass. Early instar *Chaoborus* larvae can feed on algae and small zooplankton including rotifers (Moore 1988; Persaud and Dillon 2010). Zebra mussels may therefore have had a negative competitive effect on *Chaoborus* by reducing potential prey items in the mesocosms. However, additional research is needed to better understand why biomass of *Chaoborus* was lower in mesocosms with zebra mussel.

Zebra mussels tended to have a positive effect on both the biomass of resident cladocerans and the large-bodied zooplankton that we added to the mesocosms. While it is generally assumed that zebra mussels reduce the biomass of cladocerans through strong competitive interactions (Higgins and Vander Zanden 2010), cladocerans may also increase in response to zebra mussels (Sinclair and Arnott 2015; Feniova et al. 2015). Zebra mussels in mesocosms can shift zooplankton communities towards dominance by cladocerans and copepods (Sinclair and Arnott 2015), and can promote the establishment of large-bodied zooplankton (Feniova et al. 2015). Several hypotheses may help to explain why cladoceran biomass increased in some mesocosms with zebra mussels. First, zebra mussels recycle nutrients back into the water column (Arnott and Vanni 1996;

Wojtal-Frankiewicz and Frankiewicz 2011) potentially changing the nutritional quality of algae. In support, Feniova et al. (2015) found that seston in mesocosms with zebra mussels had lower C:P ratios than mesocosms without zebra mussels, which may favor zooplankton with higher P requirements including large-bodied cladocerans. Second, as summarized by Sinclair and Arnott (2015), zebra mussels may cause some zooplankton to shift their diet towards bacteria, protozoans, and/or allochthonous material so that competition between zebra mussels and zooplankton is reduced (Pace et al. 1998; Maguire and Grey 2006).

The large-bodied zooplankton did not affect any of the plankton response variables in the mesocosms. Both zebra mussels and large-bodied zooplankton are effective filter feeders and we hypothesized that the effects of these two stressors on algae could be additive. However, this did not appear to be the case as algal biomass did not differ between treatments with zebra mussels and treatments with both zebra mussels and large-bodied zooplankton. As noted above, zooplankton may shift their diet to non-algal resources in the presence of zebra mussels and the bulk measurement of algal biomass that we used (relative fluorescence) may not have detected finer scale differences in algae or resource use that might occur from the combined filtering pressures of these two consumers.

It is important to note that the effects of zebra mussels on the plankton response variables (both in single stressor and multiple stressor treatments) tended to be greater for those variables that zebra mussels directly consumed (algae and to a lesser degree rotifers) compared to the zooplankton that were competitors. For many of the zooplankton response variables the effects of zebra mussels were not consistent across all treatments that contained a stressor, and/or differences between treatments were only observed on a single sample date over the course of the experiment. Pace et al. (1998) found that the effects of zebra mussels on zooplankton were not as strong in the Hudson River as they were for algae and microzooplankton (including rotifers): while copepod and cladoceran biomass tended to be lower, they did not exhibit a significant decrease following invasion. This was attributed to variability in the zooplankton populations, potential changes in algal food resources, and/or zooplankton shifting to feed on other food resources (e.g., bacteria, protozoans, and detritus) (Pace et al. 1998). In our study, we were also limited by the number of mesocosms available and it is possible that the low sample size per treatment (3 replicate mesocosms per treatment) combined with the inherent variability that can exist between mesocosms kept us from detecting stronger impacts of zebra mussels on resident cladocerans and copepods. Furthermore, our mesocosm experiment was relatively short compared to other mesocosm experiments such that the impacts of zebra mussels may have become stronger over a longer time due to consistent grazing pressure and development of mussels (e.g., Sinclair and Arnott 2015).

Antagonistic effects of multiple stressors are common in freshwater ecosystems (Jackson et al. 2016). In our study, zebra mussels and nutrients generally had contrasting effects on several of the plankton response variables in single factor treatments. However, the combined effect of these two stressors was antagonistic and zebra mussels were the main drivers of algal and rotifer biomass in mesocosms with multiple stressors: zebra mussels prevented algal and rotifer biomass from increasing in response to nutrient enrichment. Zebra mussels also generally had a negative effect on *Chaoborus* biomass that was independent of the other stressor treatments, and a positive effect on resident cladocerans and the biomass of the added large-bodied zooplankton. In conclusion, our results show that the presence of zebra mussels was the primary factor dictating the strength of plankton responses in the presence of multiple stressors, highlighting the importance of considering how multiple stressors interact to influence aquatic ecosystems.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** Post hoc comparisons of $Log_{10}$[Algal Fluorescence] in each treatment over time and between treatments on each sampling date.

**Table S2.** Post hoc comparisons of $Log_{10}$[Rotifer Biomass] in each treatment over time and between treatments on each sampling date.

**Table S3.** Post hoc comparisons of $Log_{10}$[Copepod Biomass] in each treatment over time and between treatments on each sampling date.

**Table S4.** Post hoc comparisons of $Log_{10}$[Resident Cladoceran Biomass] in each treatment over time and between treatments on each sampling date.

**Table S5.** Post hoc comparisons of $Log_{10}$[*Chaoborus* Biomass] in each treatment over time (top) and between treatments on each sampling date (bottom).

**Table S6.** Post hoc comparisons of $Log_{10}$[*Daphnia pulex* Biomass] in each treatment over time and between treatments on each sampling date.

**Table S7.** Post hoc comparisons of $Log_{10}$[*Simocephalus vetulus* Biomass] in each treatment over time and between treatments on each sampling date.

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