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Outcome of competition between two Daphnia species in the absence of predators: laboratory experiment findings support field observations

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Corresponding Author:	Leif Kristian Hembre, Ph.D. Hamline University St. Paul, MN UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Hamline University
Corresponding Author's Secondary Institution:	
First Author:	Leif Kristian Hembre, Ph.D.
First Author Secondary Information:	
Order of Authors:	Leif Kristian Hembre, Ph.D.
	August Henneck, B.S. in Biology
	Gardea Simoke, B.A. in Biology
Order of Authors Secondary Information:	
Abstract:	Zooplankton communities are typically comprised of smaller-bodied species when size-selective fish predators are abundant, but become dominated by large-bodied species when fish predators are scarce. Superiority by larger-bodied grazers over smaller-bodied species in competition for algal resources has been proposed to be the mechanism responsible for this observed pattern. To investigate this mechanism, we performed a laboratory experiment with two freshwater zooplankton species, the larger-bodied Daphnia pulicaria and smaller-bodied D. mendotae, obtained from Square Lake (Washington County, Minnesota). The Daphnia species were grown in monoculture and in combination over a 24 day period to assess the outcome of competition between the two species and their effect on algae cell densities. We hypothesized that the larger-bodied D. pulicaria species would outcompete D. mendotae, and that D. pulicaria would exert greater control on algae levels than would D. mendotae. Results of the experiment strongly supported these hypotheses, and were consistent with findings of a recently completed field study of Square Lake that discovered that terminating the program of stocking rainbow trout (a zooplanktivorous predator) in the lake resulted in D. pulicaria replacing D. mendotae as the dominant Daphnia species and in the reduction of algae levels in the lake's surface waters.

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Title: Outcome of competition between two *Daphnia* species in the absence of predators: laboratory experiment findings support field observations

Authors: Leif K. Hembre, August T. Henneck, and Gardea R. Simoke

Affiliation: Biology Department, Hamline University, Saint Paul, MN 55104

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Correspondence to: lhembre@hamline.edu

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ABSTRACT

Zooplankton communities are typically comprised of smaller-bodied species when size-selective fish predators are abundant, but become dominated by large-bodied species when fish predators are scarce. Superiority by larger-bodied grazers over smaller-bodied species in competition for algal resources has been proposed to be the mechanism responsible for this observed pattern. To investigate this mechanism, we performed a laboratory experiment with two freshwater zooplankton species, the larger-bodied *Daphnia pulicaria* and smaller-bodied *D. mendotae*, obtained from Square Lake (Washington County, Minnesota). The *Daphnia* species were grown in monoculture and in combination over a 24 day period to assess the outcome of competition between the two species and their effect on algae cell densities. We hypothesized that the larger-bodied *D. pulicaria* species would outcompete *D. mendotae*, and that *D. pulicaria* would exert greater control on algae levels than would *D. mendotae*. Results of the experiment strongly supported these hypotheses, and were consistent with findings of a recently completed field study of Square Lake that discovered that terminating the program of stocking rainbow trout (a zooplanktivorous predator) in the lake resulted in *D. pulicaria* replacing *D. mendotae* as the dominant *Daphnia* species and in the reduction of algae levels in the lake's surface waters.

INTRODUCTION

Predation on zooplankton by visually-orienting fish is known to be size-selective (Gliwicz & Pijanowska, 1989), and when these zooplanktivores become abundant they typically cause the composition of the zooplankton community to shift from a dominance of larger-bodied species (e.g., large-bodied *Daphnia* species, large copepods, invertebrate predators) to smaller-bodied species such as small-bodied *Daphnia* species and other small cladocerans (e.g., *Chydorus*, *Bosmina*), small copepods, and rotifers (e.g., Galbraith, 1967; Hembre & Megard, 2005). Though the compositional changes that occur in zooplankton communities after an increase in predation by zooplanktivorous fish are well documented, the mechanism that promotes dominance by large-bodied zooplankton grazers (e.g., large *Daphnia*) when zooplanktivory by fish is low is less clear.

One long-held explanation, the *size-efficiency hypothesis* (Brooks & Dodson, 1965), posits that larger-bodied grazers outcompete smaller-bodied grazers because they are able to consume a wider size range of fine particulate matter and therefore competitively exclude smaller-bodied zooplankton when size-selective fish predators are scarce. However, studies that have tested this hypothesis have had conflicting results, with some supporting the size-efficiency hypothesis (e.g., Kreutzer and Lampert, 1999; Gliwicz, 1990; Vanni, 1986) and others not (e.g., Dodson, 1974). A study that supported the size-efficiency hypothesis was a laboratory experiment (Kreutzer and Lampert, 1999) with two differently sized *Daphnia* species that found that the larger-bodied species, *D. pulicaria*, had a lower threshold food concentration, C^* (analogous to Tilman's R^*, Tilman, 1982) than that of a smaller-bodied species, *D. galeata*. When cultured together, *D. pulicaria* competitively excluded *D. galeata* when resource (algae) levels fell below the C^* required by *D. galeata*. An alternative, but not mutually exclusive, hypothesis proposed to explain the prevalence of large-bodied grazers when zooplanktivory by fish is low, is that invertebrate predators (e.g., *Chaoborus*, *Leptodora*) become more abundant and prey on smaller-bodied zooplankton, leaving large-bodied grazers as the dominant constituents of the zooplankton community (e.g., Hanazato & Yasuno, 1989; Elser et al., 1987).

Fisheries management practices such as harvest limits and the stocking of particular fish species may affect the intensity of zooplanktivory occurring in lakes. These practices can therefore alter and regulate the species composition of zooplankton communities. For example, rainbow trout (*Oncohrynchus mykiss*), a species commonly stocked in lakes throughout the world (Stankovic et al., 2015), has been shown to selectively prey on large *Daphnia* in lakes to which they are stocked (Geist et al., 1993; Wang et al., 1996; Hembre & Megard, 2005). The effect of zooplanktivory by rainbow trout on zooplankton community composition and water quality was examined in a recent multi-year monitoring study (Hembre, 2019) of Square Lake (Washington County, MN). This study found that rainbow trout selectively preyed on the large-bodied species of *Daphnia* in the lake (*D. pulicaria*), and that in years when trout were stocked to the lake by the Minnesota Department of Natural Resources (MNDNR), the dominant *Daphnia* species in the lake was the smaller-bodied *D. mendotae*. After the stocking of trout in the lake was discontinued by the MNDNR, the larger-bodied *D. pulicaria* replaced *D. mendotae* as the dominant *Daphnia* species in the lake and levels of algae biomass (measured as Chl a concentrations) in the surface water of the lake decreased. Levels of predatory invertebrates (*Chaoborus*, *Leptodora*, Hydracarina water mites) observed after the trout stocking was discontinued did not differ significantly from levels in years that trout were stocked, suggesting that the shift to dominance by the *D. pulicaria* may have been the result of competitive superiority of that species over *D. mendotae*.

To evaluate whether competition could explain the shift in *Daphnia* species composition that was observed in the monitoring study (Hembre, 2019), we performed a controlled experiment in the laboratory with animals of these two *Daphnia* species that were obtained from Square Lake. Both species were grown in monocultures and in combination over 24 days, and we hypothesized that *D. pulicaria* would grow and reproduce better than *D. mendotae* when the two species were cultured together. A secondary aim of this experiment was to assess how algae levels were affected in the zooplankton treatments compared to an algae-only control treatment, with the expectation that higher levels of *Daphnia* grazers would depress algae levels, and that *D. pulicaria* would exert greater control on algae levels than would *D. mendotae*.

MATERIALS AND METHODS

Sampling and culturing of *Daphnia* to be used in experiment

Zooplankton samples were collected from Square Lake on 17 May, 2014 with a closing-style zooplankton net (diameter = 30 cm, mesh size = 80 µm). *D. mendotae* and *D. pulicaria* were separated from the samples and animals of each species were maintained in separate bulk cultures at densities of 20 L^{-1} in a *Conviron*© model E15 growth chamber at 20 ^{o}C on a 16:8 Light:Dark cycle. These cultures were maintained in the growth chamber for four weeks and fed an algae mixture comprised of three types of green algae: *Closterium*, *Chorella*, and *Scenedesmus*. After two weeks of culturing in the growth chamber, 20 gravid females of each *Daphnia* species were placed individually in 25 mL Ehrlenmeyer flasks. These animals were monitored over a 3 d period for the release of young, and neonates produced from these gravid females were used to initiate the experiment.

Experimental design

The experiment included four treatments: an algae-only treatment (treatment A), and three zooplankton treatments. Two of the zooplankton treatments were monocultures of *D. pulicaria* (treatment P) and *D. mendotae* (treatment M), and the third was a combination treatment with both *Daphnia* species (treatment PM). Replicates of each experimental treatment were established in 125 mL Ehrlenmeyer flasks containing 100 mL of lake water (filtered with 0.45 µm pore size glass-fiber filter) from Square Lake. The green algae mixture was added to all flasks to establish initial concentrations of 250,000 cells mL^{-1}, a food level sufficient to promote asexual reproduction for *Daphnia* (Schaack et al., 2013). In addition to the algae, replicates for the zooplankton treatments were initiated with *Daphnia* neonates obtained from the isolated gravid females. Monocultures were initiated with 10 neonates of either *D. pulicaria* (treatment P) or *D. mendotae* (treatment M) and replicates for the combination treatment (treatment PM) were initiated with 5 neonates of each species. Neonates obtained from the isolated gravid females were randomly assigned to the appropriate experimental flasks for the various treatments. Five replicates were initiated for the algae-only (A) treatment and the combination treatment (PM). However, the monoculture treatments (P and M) had four replicates instead of five because of insufficient numbers of available neonates when the experiment was initiated.

Experimental procedures and measured variables

Body sizes of neonates were measured, from top of head to base of tail spine, to determine biomass (using species-specific length-weight regression equations from Bottrell et al. 1976) on the initial day of the 24 d experiment. Culture flasks were gently swirled at least once each day of the experiment to keep algae in suspension. Every third day of the experiment thereafter, the cultures were monitored to assess somatic and population growth of the *Daphnia*, and to determine algae abundance. *Daphnia* in the experimental cultures were assessed by pipetting the animals out of the cultures into a petri dish and counting the number of live individuals of each species. After the animals were removed from the experimental cultures, the flasks were swirled to mix the water. Algae in three 100 µL subsamples were enumerated using a hemocytometer to determine algal cell density. Hemocytometer counts of algae were also done for the algae-only controls every third day of the experiment. *Daphnia* from the zooplankton treatments that were held in the petri dishes were then transferred individually onto a flat microscope slide in a small drop of water and observed at either 40x or 100x magnification with a compound microscope to measure body length for calculation of biomass. After individual *Daphnia* were examined under the compound microscope they were promptly rinsed from the slide with a small volume of filtered lake water back into the appropriate experimental flask. To account for evaporation, experimental cultures were topped off with filtered lake water as needed to restore cultures to their original volumes (100 mL). No new algae was added after the initial set up to assess how algae abundance changed over the course of the experiment, and so that competition for food could play out in the *Daphnia* treatments.

Data analysis

Analyses using repeated measures ANOVA with the general linear models (GLM) routine in SPSS (IBM© SPSS Statistics version 25.0) were used to evaluate how algae concentrations and *Daphnia* population size and total biomass changed over the 24 d experiment. For the algae concentration analysis, all four treatments (A, P, M, and PM) were analyzed and the main effects of treatment and time, as well as the treatment x time interaction effect were analyzed. For *Daphnia* abundance and *Daphnia* biomass, each species was compared between the monoculture treatment (P or M) and the competition treatment (PM), and analyzed for the main effects of treatment and time and the treatment x time interaction effect. To normalize the data for the repeated measures ANOVA analyses, algae concentration data were square root-transformed, and *Daphnia* abundance and biomass levels were Log_{10} (x + 1)-transformed.

RESULTS

Consistent with expectations, the larger-bodied species, *D. pulicaria*, outcompeted the smaller-bodied *D. mendotae* over the 24 d experiment (Fig. 1). The smaller-bodied species (*D. mendotae*) performed significantly worse in competition with the larger-bodied *D. pulicaria* (PM treatment) than it did in monoculture (M treatment) with respect to population growth (Fig. 1a) and biomass (Fig. 1b). In competition with *D. pulicaria*, *D. mendotae* abundance and biomass decreased significantly after day 6 of the experiment (Fig. 1a and 1b). By the end of the experiment, *D. mendotae* were reduced to an average of 1.20 $\pm$ 0.89 individuals and were eliminated in three of the five PM replicates (by day 9 for one replicate and by day 21 for two other replicates). In monoculture, however, the average *D. mendotae* abundance (Fig. 1a) approximately tripled over the experimental period (from 10 per replicate to a final average level across all replicates of 32 ± 12 individuals). This corresponded to a more than 3-fold increase in the average total biomass of *D. mendotae* (Fig. 1b) in the monocultures by the end of the experiment (from 24.5 ± 7.3 μg to 210 ± 77 μg). Repeated measures ANOVAs assessing the average abundance and biomass of *D. mendotae* over time (Table 1) revealed highly significant effects of treatment ($F_{1,7} = 48.47$, $p < 0.001$ for abundance, and $F_{1,7} = 23.63$, $p < 0.001$ for biomass), and the treatment x time interaction ($F_{8, 56} = 12.98$, $p < 0.001$ for abundance, and $F_{8, 56} = 10.35$, $p < 0.001$ for biomass). The effect of time was highly significant for the abundance analysis ($F_{8, 56} = 4.08$, $p = 0.001$), but was only marginally significant for biomass ($F_{8, 56} = 1.93$, $p = 0.076$).

In contrast to the results for *D. mendotae*, *D. pulicaria* abundance and biomass increased over the 24 d experiment for both the monoculture (P) and competition (PM) treatments (Fig. 1c and 1d), and by the end of the experiment the average abundance and biomass of *D. pulicaria* was similar between the two treatments (~ 50 animals, Fig. 1c; and ~ 560 μg, Fig. 1d). Statistical results for the repeated measures ANOVAs show that the average abundance and biomass of *D. pulicaria* (Table 2) did not differ significantly between the monoculture and competition treatments ($F_{1,7} = 1.10$, $p < 0.328$ for abundance, and $F_{1,7} = 5.12$, $p = 0.058$ for biomass), but that the time effect ($F_{8, 56} = 68.12$, $p < 0.001$ for abundance, and $F_{8, 56} = 60.32$, $p < 0.001$ for biomass) and the time x treatment interaction effect were highly significant ($F_{8, 56} = 3.76$, $p = 0.001$ for abundance, and $F_{8, 56} = 3.02$, $p = 0.007$ for biomass).

Algae concentrations increased from initial levels of 250,000 cells mL^{-1} over the first few days of the experiment for all treatments, but then fluctuated in different ways among treatments thereafter (Fig. 2). In the algae-only control treatment, cell concentrations varied the least over time and were the highest for any treatment by the end of the experiment (594,000 ± 84,000 cells mL^{-1}). Results for the *Daphnia* treatments were largely consistent with expectations in that algae levels in the treatments that became heavily populated with *D. pulicaria* (Fig. 1) declined to levels near (211,000 ± 54,000 cells mL^{-1} for the PM treatment) or below (91,000 ± 28,000 cells mL^{-1} for the P treatment) the algae concentration at which the experiment was initiated. Interestingly, algae concentrations reached their highest levels for any treatment in the *D. mendotae* monoculture treatment (M) on day 6 (1,132,000 ± 207,000 cells mL^{-1}). After that maximum, algae concentrations gradually decreased as *D. mendotae* abundance and biomass increased (Fig. 1a and 1b). By the end of the experiment, algae levels in the M treatment were somewhat lower (403,000 ± 143,000 cells mL^{-1}) than those in the algae-only control (Fig. 2). Statistical results of the repeated measures ANOVA for algae concentration showed highly significant effects for treatment ($F_{3,14} = 7.62$, $p = 0.003$), time ($F_{8,112} = 10.60$, $p < 0.001$), and the time x treatment interaction ($F_{24,112} = 2.34$, $p = 0.002$).

DISCUSSION

The hypothesis that the larger-bodied *D. pulicaria* would outcompete the smaller-bodied *D. mendotae* in the absence of fish predators was strongly supported by this experiment (Fig. 1, Tables 1 and 2). This result is consistent with expectations of the size efficiency hypothesis (Brooks and Dodson, 1965) and the findings of others (e.g., Kreutzer and Lampert, 1999) that large-bodied *Daphnia* are better competitors than smaller zooplankton species. The competitive superiority of *D. pulicaria* over *D. mendotae* observed in this experiment also provides the likely explanation for why *D. pulicaria* became the dominant *Daphnia* species in years after the stocking of rainbow trout (a size-selective zooplanktivore) was discontinued in Square Lake (Hembre, 2019). While the outcome of this controlled laboratory experiment resulted in *D. mendotae* becoming eliminated from three of the five PM treatment replicates and being driven to low levels in the other two replicates (final abundances of 2 and 4 individuals), circumstances in nature may allow the two *Daphnia* species to coexist. In this experiment, the *Daphnia* were maintained in conditions that were spatially uniform and absent of predation. In nature though, seasonal stratification of the water column creates spatial heterogeneity in several environmental conditions (e.g., light, temperature, dissolved oxygen levels) that may promote coexistence via habitat partitioning by the species (Schulz et al., 2012; Havel and Lampert, 2006). For example, smaller-bodied species less vulnerable to visual predators are likely to more abundant than larger-bodied species in well-lit surface waters (Leibold & Tessier, 1991), and hemoglobin production by some *Daphnia* species enables them to inhabit deep water with low oxygen levels that other species cannot tolerate (Sell, 1998).

The secondary hypothesis that *D. pulicaria* would exert greater control on algae than would *D. mendotae* was also supported by the results of this experiment (Fig. 2, Table 3). Algae concentrations in the *D. pulicaria* monocultures (treatment P) and in the competition treatment (treatment PM) that became dominated by *D. pulicaria* as the experiment progressed (Fig. 1c and 1d) became significantly lower compared to the algae-only control treatment (treatment A) and the *D. mendotae* monoculture treatment

(treatment M). The finding of this experiment that algae levels decreased as *D. pulicaria* abundance and biomass increased is consistent with field observations of Square Lake in which surface water algae biomass levels were lower in years that trout were not stocked to the lake and *D. pulicaria* biomass concentrations were high, compared to years when trout were stocked and *D. pulicaria* biomass concentrations were relatively low (Hembre, 2019). High abundances of *Daphnia* can reduce algae levels directly via grazing pressure, but may also limit algae growth indirectly through nutrient limitation because *Daphnia* homeostatically maintain higher levels of phosphorus in their bodies than do other zooplankton taxa (Elser et al., 1996; Sterner and Elser, 2002). Therefore, in cases when phosphorus is the nutrient that limits algae growth, sequestration of phosphorus in the bodies of *Daphnia* can strengthen their control of algae levels. In the field study of Square Lake, Hembre (2019) found that total phosphorus concentrations in surface waters were significantly lower in the summer in years that trout were not stocked and that the increased *D. pulicaria* standing biomass in those years accounted for a large percentage (> 50% in April-June) of the decrease in phosphorus in the water. So, it is possible that that the high standing biomass of *Daphnia* in the P and PM treatments in the latter half of this experiment may have depressed algae levels by decreasing the availability of phosphorus. However, because phosphorus levels in the water were not monitored in this experiment, it is not possible to conclude whether phosphorus limitation of the algae occurred. To investigate this mechanism, the design of this experiment could be modified in future research to assess the relative importance of nutrient availability and grazing intensity on algae levels.

In summary, the findings of this laboratory experiment supported the hypotheses that the larger-bodied *D. pulicaria* would outcompete the smaller-bodied *D. mendotae* in the absence of predators (Fig. 1), and that *D. pulicaria* would cause algae levels to decline as they became abundant (Fig. 2). These results are in line with observations from the field study of Square Lake (Hembre, 2019) that found after the stocking of zooplanktivorous rainbow trout was terminated, *D. pulicaria* replaced *D. mendotae* as the dominant *Daphnia* species, and that surface water algae biomass in the lake decreased.

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Table legends

Table 1. Analyses of variance with repeated measures assessing the effects of the *Daphnia* treatments (D*. mendotae* monoculture = M, and competition treatment with both *Daphnia* species = PM) on Log10 (x+1) transformed abundance and biomass (mg) of *D. mendotae* over time in the 24-day experiment.

Table 2. Analyses of variance with repeated measures assessing the effects of the *Daphnia* treatments (D*. pulicaria* monoculture = P, and competition treatment with both *Daphnia* species = PM) on Log10 (x+1) transformed abundance and biomass (mg) of *D. pulicaria* over time in the 24-day experiment.

Table 3. Analysis of variance with repeated measures assessing the effects of the treatments (Algae only control = A, *D. pulicaria* monoculture = P, *D. mendotae* monoculture = M, and competition treatment with both *Daphnia* species = PM) on square root-transformed algae cell concentrations (cells mL^{-1}) over time in the 24-day experiment.

Figure legends

Figure 1. Mean (+/- SE) abundances and biomass levels of *D. mendotae* and *D. pulicaria* over the 24-day experiment in monoculture and competition treatments. Results for *D. mendotae* are shown in panels A and B, and results for *D. pulicaria*

shown in panels C & D. Solid grey circles with grey lines indicate results for the *D. mendotae* monoculture treatment, solid black circles with black lines indicate results for the *D. pulicaria* monoculture treatment, and open circles with blacked dashed lines indicate results for the interspecific competition treatment. Note that abundance and biomass results are shown on a log scale.

Figure 2. Mean (+/- SE) algae cell concentrations over the 24-day experiment in the algae-only control treatment (A), monocultures of *D. mendotae* (M) and *D. pulicaria* (P) and the competition treatment with both *Daphnia* species (PM). Black triangles and the dotted line indicate results for the algae-only control treatment, and the symbols and lines for the *Daphnia* treatments are as described in the legend for Fig. 1.

Table 1. Analyses of variance with repeated measures assessing the effects of the *Daphnia* treatments (D. *mendotae* monoculture = M, and competition treatment with both *Daphnia* species = PM) on Log10 (x+1) transformed abundance and biomass (μg) of *D. mendotae* over time in the 24-day experiment.

	Abundance				**Biomass**		
Effects	df	MS	*F*	*P*	MS	*F*	*P*
Treatment	1	11.33	48.47	< 0.001	21.76	23.63	0.002
Error	7	0.23			0.92		
Time	8	0.16	4.08	0.001	0.17	1.93	0.076
Treatment x time	8	0.05	12.98	< 0.001	0.90	10.35	< 0.001
Error	56	0.04			0.09		

Table 2. Analyses of variance with repeated measures assessing the effects of the *Daphnia* treatments (D. *pulicaria* monoculture = P, and competition treatment with both *Daphnia* species = PM) on Log10 (x+1) transformed abundance and biomass (μg) of *D. pulicaria* over time in the 24-day experiment.

	Abundance				**Biomass**		
Effects	df	MS	*F*	*P*	MS	*F*	*P*
Treatment	1	0.05	1.10	0.328	0.62	5.12	0.058
Error	7	0.04			0.11		
Time	8	1.36	68.12	<0.001	2.04	60.32	< 0.001
Treatment x time	8	0.075	3.76	0.001	0.10	3.02	0.007
Error	56	0.02			0.34		

Table 3. Analysis of variance with repeated measures assessing the effects of the treatments (Algae only control = A, *D. pulicaria* monoculture = P, *D. mendotae* monoculture = M, and competition treatment with both *Daphnia* species = PM) on square root-transformed algae cell concentrations (cells mL^{-1}) over time in the 24-day experiment.

Effects	df	MS	*F*	*P*
Treatment	3	553.85	7.62	0.003
Error	14	72.67		
Time	8	290.23	10.60	<0.001
Treatment x time	24	64.05	2.34	0.002
Error	112	27.37		

Fig. 1. Mean (+ SE) abundances and biomass levels of *D. mendotae* and *D. pulicaria* over the 24-day experiment in monoculture and competition treatments.

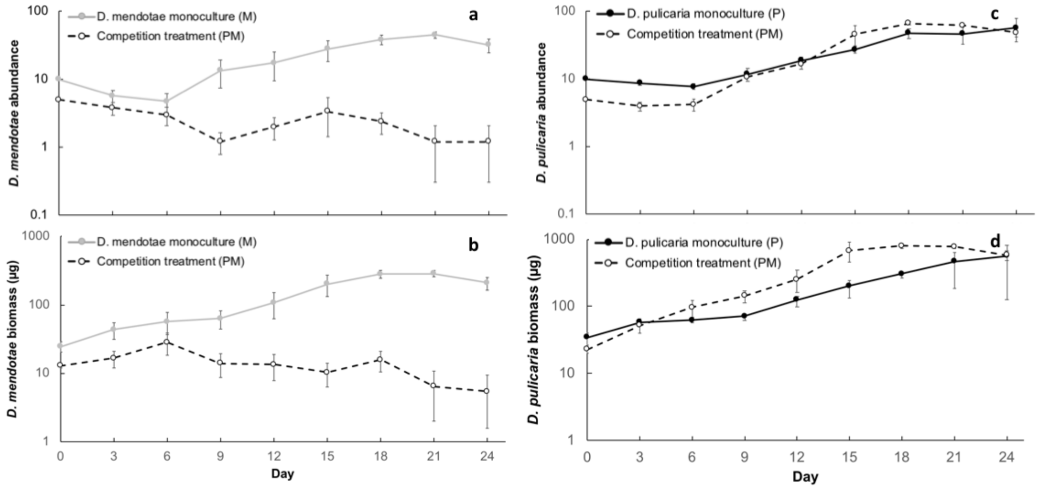

Fig. 2. Mean (+ SE) algae cell concentrations over the 24-day experiment in the algae-only control treatment (A), monocultures of *D. mendotae* (M) and *D. pulicaria* (P) and the competition treatment with both *Daphnia* species (PM).