Running head: Diversity-stability trophic cascade

Title: Predator complementarity dampens variability of phytoplankton biomass in a diversity-stability trophic cascade

Authors: Chase J. Rakowski[1*], Caroline E. Farrior[1], Schonna R. Manning[2], Mathew A. Leibold[3]

[1]Department of Integrative Biology
University of Texas at Austin
Austin, Texas 78712

[2]Department of Molecular Biosciences
University of Texas at Austin
Austin, Texas 78712

[3]Department of Biology
University of Florida
Gainesville, Florida 32611

[*]Corresponding author: chaserak@utexas.edu

Abstract. Trophic cascades – indirect effects of predators that propagate down through food webs – have been extensively documented, especially in aquatic ecosystems. It has also been shown that predator diversity can mediate these trophic cascades, and, separately, that herbivore biomass can impact the stability of primary producers. However, whether predator diversity can cause cascading effects on the stability of lower trophic levels has not yet been studied. We conducted a laboratory microcosm experiment and a field mesocosm experiment manipulating the presence and coexistence of two heteropteran predators and measuring their effects on zooplankton herbivores and phytoplankton basal resources. We predicted that, if the predators partitioned their herbivore prey, for example by size, then co-presence of the predators would lead to 1) increased average values and 2) decreased temporal variability of phytoplankton basal resources. We present evidence that the predators partitioned their herbivore prey and found that their simultaneous suppression of herbivore groups reduced the variability of edible (smaller) phytoplankton biomass, without affecting mean phytoplankton biomass. We also found that phytoplankton that were more resistant to herbivory were not affected by our manipulations, indicating that the zooplankton herbivores played an important role in mediating this cascading diversity-stability effect. Our results demonstrate that predator diversity may indirectly stabilize basal resource biomass via a "diversity-stability trophic cascade," seemingly dependent on predator complementarity and the vulnerability of taxa to consumption, but independent of a classic trophic cascade in which average biomass is altered. Predator diversity, especially if correlated with diversity of prey use, may be important for regulating ecosystem stability, and this relationship suggests biological control methods for improving the reliability of microalgal yields.

Key words: *algae; biodiversity; biological control; ecosystem functioning; food web; niche*

complementarity; plankton; resource partitioning; species richness; stability; temporal variability; top-down control.

INTRODUCTION

A substantial body of work, generally motivated by global biodiversity loss, indicates that enhanced biodiversity of ecosystems often stabilizes community biomass (Jiang and Pu 2009, Loreau and de Mazancourt 2013, Gross et al. 2014). Most of these studies measured or modeled the effects of primary producer diversity on the variability of primary producer biomass (e.g. Hector et al. 2010, Loreau and de Mazancourt 2013, Gross et al. 2014). However, predators face greater extinction threats than do lower trophic levels, suggesting that predator diversity is more relevant to global biodiversity change than is primary producer diversity (Purvis et al. 2000). Furthermore, biodiversity often alters energy flow through food webs, and so manipulating diversity and measuring its effects within a single trophic level can give an incomplete picture of how biodiversity influences ecosystem functioning (Hines et al. 2015, Seabloom et al. 2017). While some literature addresses how predator diversity affects the average biomass of various other trophic groups in food webs (reviewed in Schmitz 2007), little is yet known about the influence of predator diversity on ecosystem stability. In particular, the existence of a link between predator diversity and the stability of non-adjacent lower trophic levels has not (to our knowledge) been tested. Yet, such a link would have critical implications for both the maintenance of stable natural ecosystems and for biological control to potentially stabilize crop yields.

Much existing research relating predator diversity to lower trophic level functioning was performed in the context of biological control, generally predicting that predator diversity would strengthen pest control and therefore enhance crop yields in a trophic cascade (Straub et al. 2008,

Greenop et al. 2018). Biological control is classically practiced by introducing a single specialist natural enemy to target a specific pest (van Driesche et al. 2008). This targeted method has a high failure rate, and in any case, agricultural plots are often affected by multiple pest species (van Driesche et al. 2008). Increasingly, scientists have advocated the use of multiple natural enemies as a way to improve pest control by conserving natural enemy biodiversity, a concept termed ‘conservation biological control’ (Snyder 2019). Meta-analyses have shown that the presence of more diverse assemblages of natural enemies generally leads to lower mean pest densities and higher mean crop yields, as long as the natural enemies exhibit complementarity in their feeding niches (Straub et al. 2008, Greenop et al. 2018). Following this reasoning, diversity of functional traits among predators related to prey use, such as body size, may play a key role in mediating trophic cascade strength (Straub et al. 2008). Besides average primary producer biomass, the variability of primary producer biomass may also be affected by functional predator diversity in a predictable way. While decreasing herbivore biomass generally increases primary producer biomass, it has also been shown to decrease the variability of (i.e., to stabilize) primary producer biomass (Thébault and Loreau 2005, Downing et al. 2014). Therefore, functional predator diversity may also indirectly reduce the variability of primary producer biomass, producing a “diversity-stability trophic cascade” (Table 1). Such an effect may be most likely seen when the primary producers are highly edible, such as in communities of small phytoplankton.

Culturing microalgae, especially phytoplankton, is a promising means of producing alternative fertilizers, animal feeds, and fuels with a lower environmental impact than current industrial standards (Benemann 2013). However, biomass yields are often low and unpredictable, preventing the production of algae-derived commodities from being economical (Benemann 2013). A major reason for algal crop failures is that algae ponds are quickly colonized by

zooplankton herbivores varying in size from ciliates <100 µm in length to crustaceans such as *Daphnia* >2 mm in length, triggering reductions and oscillations in algal biomass (Smith et al. 2010). Harnessing predator diversity may provide a useful means to control this range of aquatic "pests" and thereby improve algal crop yields and their reliability. We are aware of one case in which biological control was tested to improve algal yields, and this study used a single predator species (Sturm et al. 2012).

Here we report the results of a field mesocosm experiment and accompanying laboratory microcosm experiment to test for the existence of diversity-stability trophic cascades in which we manipulate the presence of two predator species (no predators, each predator alone, and both predators) and measure the resulting average biomass of herbivore groups as well as the average and variability of biomass of phytoplankton groups. We use the heteropterans *Notonecta* and *Neoplea* as the predators due to their substantial difference in body size and consequent likelihood of partitioning prey resources. Based on previous work (Murdoch et al. 1984) we predicted that *Notonecta* would mostly consume *Daphnia*, and we predicted that *Neoplea* would consume smaller zooplankton based on its smaller body size. We hypothesized that if *Notonecta* and *Neoplea* partition herbivore prey, then the addition of both species together would 1) increase the mean and 2) reduce the variability of biomass of the basal resources (edible phytoplankton), while the addition of a single predator species would have much weaker effects (Appendix S1: Fig. S1). We present evidence that these predators indeed partitioned herbivore prey, leading to an indirect effect on phytoplankton stability but we found no significant effect on mean phytoplankton biomass. While the presence of a relatively inedible phytoplankton strain dampened the effect on total phytoplankton, community biomass of smaller, more edible phytoplankton was more stable when both predators were present, demonstrating a diversity-stability trophic cascade within a compartment of the food web.

METHODS

Focal predators

We used two insect species in the suborder Heteroptera as the predators in both the field and laboratory experiments: the notonectid *Notonecta undulata* and the pleid *Neoplea striola*. Both species are mobile generalist predators that are widespread across North America. However, they differ starkly in size: *Notonecta undulata* adults measure ~11-13 mm and *Neoplea striola* adults measure ~1.5 mm in length. Studies have shown that while members of the genus *Notonecta* can take prey as small as microscopic rotifers, they strongly reduce large prey such as *Daphnia* and mosquito larvae (Leon 1998, Murdoch et al. 1984, Hampton and Gilbert 2001). *Neoplea* has been less studied; they have been documented to attack invertebrates ranging in size from rotifers to *Daphnia* (Hampton and Gilbert 2001, Gittelman 1977), but we predicted they would prefer smaller prey than *Notonecta*. Hereafter, we use the genus names (*Notonecta* and *Neoplea*) to refer to these two focal predator species.

Organism collection

We allowed communities of phytoplankton to assemble naturally in six outdoor tanks at the University of Texas' Brackenridge Field Laboratory, Austin, TX for ~six months. We then mixed a common inoculum from these tanks. The phytoplankton community became dominated by green algae (Chlorophyta), ranging in size from green picoplankton (~1 µm) in diameter to *Oocystis* with mother cell walls up to (~25 µm) in diameter and dominated by a few morphospecies, especially *Selenastrum* and *Oocystis* (Appendix S2: Table S1). We collected an array of zooplankton taxa from small water bodies nearby, including many rotifer species, *Spirostomum*, *Arctodiaptomus dorsalis*, *Mesocyclops edax*, and *Scapholeberis kingi*, and we ordered *Daphnia magna* from Sachs Systems Aquaculture (St. Augustine, FL) to extend the size range of the zooplankton to larger-bodied individuals (Appendix S2: Table S2). We similarly

mixed the zooplankton taxa together into a common inoculum. The predators *Notonecta* and *Neoplea* were collected from tertiary wastewater treatment ponds at Hornsby Bend, Austin, TX.

Laboratory experiment

To test whether *Notonecta* and *Neoplea* had different effects on zooplankton herbivore groups, we conducted a short time scale (5-day) experiment in the laboratory. Five days represents just under one generation for the dominant zooplankton with the shortest generation times, *Daphnia magna* and *Scapholeberis kingi*. Therefore we anticipated that five days would provide enough time for the predators to reduce zooplankton populations but would not provide enough time for the zooplankton populations to significantly recover, allowing us to better estimate the effects of the predators on mortality of different zooplankton species while minimizing the influence of zooplankton fecundity and adaptation. We mixed portions of the phytoplankton and zooplankton inocula into a common inoculum, half of which was filtered with a 100-μm sieve to concentrate the zooplankton to 2× density. Ten adults of each predator species were placed individually in microcosms with either the 1× or 2× zooplankton density mixture. *Notonecta* were placed in microcosms with 1.5 L plankton mixture, and *Neoplea* were placed in microcosms with 100 mL plankton mixture. Additionally, we established control microcosms with no predator. All treatments were replicated five times, yielding 40 total microcosms (2 predator species/microcosm sizes × predator presence or absence × 2 zooplankton concentrations × 5 replicates). The microcosms were randomized and placed in an environmental chamber maintained at 25 °C with fluorescent lights on a 16:8 h light:dark cycle. After five days, we filtered the contents of each microcosm using a 44-μm filter and preserved them in 10% Lugol's solution. We then estimated biomass of the zooplankton taxa as described in Appendix S3.

Field experiment

To evaluate the influence of predator diversity on phytoplankton biomass and stability, we established replicate pond communities in 200-L cattle tanks at Brackenridge Field Laboratory. Tanks were filled with well water and outfitted with a float valve to maintain constant water levels. Before beginning the experiment, we analyzed total N and P of the water following the methods of the American Public Health Association (APHA 1989). We then supplemented $NaNO_3$ and $NaH_2PO_4 \bullet H_2O$ to bring the total N and P to the concentrations found in COMBO medium (14 mg/L N and 1.55 mg/L P), a nutrient-rich medium commonly used for culturing plankton (Kilham et al. 1998). Immediately following weekly sampling (methods described below) we added both nutrient solutions to compensate for a 5% daily loss rate from the water column (as per Hall et al. 2004).

We distributed the phytoplankton inoculum equally among the tanks, allowed the phytoplankton to grow for 15 days, and then distributed the zooplankton inoculum equally among the experimental tanks in the same way. Finally, we added either no insect predators (controls), 6 adult *Notonecta*, 90 adult *Neoplea*, or 3 adult *Notonecta* with 45 adult *Neoplea* to the tanks. Each treatment was replicated five times, for a total of 20 tanks in a randomized design. The relative densities of *Notonecta* and *Neoplea* (1:15) were chosen to satisfy the null hypothesis that each tank with predators would experience the same total predation rate if the predators did not partition prey resources. This ratio was derived from the laboratory experiment, where individual *Notonecta* consumed 14.3× and 16.3× more animal mass than *Neoplea* in 1× and 2× zooplankton concentration microcosms, respectively.

Beginning a week after adding predators, we sampled plankton weekly for six weeks. To sample zooplankton, we used tube samplers to collect ~6 spatially-spread whole water column subsamples and pool them into a 12-L sample for each tank. We filtered this sample through 65-

μm mesh, returned any predators to the tank, and preserved the retained material in 10% Lugol's solution. To sample phytoplankton, we used 1-cm diameter PVC pipes (one per tank) to collect three spatially-spread whole water column subsamples and pool them into a 50-mL sample for each tank. We estimated biomass of zooplankton taxa as in the laboratory experiment, and additionally estimated biovolume of phytoplankton taxa (methods in Appendix S3).

Determining trophic groups

To test our hypothesis that *Notonecta* and *Neoplea* partitioned herbivorous prey based on body size, leading to differential cascading food web effects, it was necessary to divide plankton into trophic groups and size classes. We accomplished this using a combination of published literature and analysis of our data. Herbivorous zooplankton were defined as taxa that are primarily herbivorous over their life span and are not strictly benthic. Thus, *Mesocyclops* was included in analyses even though the adult stage is omnivorous (Adrian and Frost 1993). *Spirostomum* was also treated as herbivorous zooplankton despite characterization in the literature as a bacterivore, since preliminary analysis suggested it reduced the biomass of smaller phytoplankton. However, the dominant zooplankter in most tanks, the diaptomid copepod *Arctodiaptomus dorsalis*, was not included as herbivorous zooplankton. Diaptomid copepods are often omnivorous, and exhibit a much faster escape response and lower vulnerability to predators than other crustacean zooplankton (O'Brien 1979, Williamson 1987). Preliminary analysis showed that *Arctodiaptomus* had no effect on phytoplankton biomass or stability, and was not reduced by either predator, essentially acting as a bystander to the cascading food web effects. Due to the difficulty of distinguishing *Arctodiaptomus* nauplii from nauplii of the other copepod species, *Mesocyclops edax*, all nauplii were also excluded from analyses. *Mesocyclops* nauplii likely comprised a small fraction of herbivore biomass, since early nauplius instars do not feed,

and because *Arctodiaptomus* copepodites and adults were 5.35 times more numerous than *Mesocyclops* copepodites and adults on average.

Based on our prediction that *Notonecta* would selectively prey on *Daphnia* and *Neoplea* would prey on smaller zooplankton, we split herbivorous zooplankton into *Daphnia* and all other herbivores (hereafter, "smaller herbivores") for analysis. Phytoplankton were similarly grouped into two size classes representing the largest taxon and all smaller taxa. Large phytoplankton are only vulnerable to large filter feeders (i.e., *Daphnia*). The largest phytoplankter was an *Oocystis* sp. (hereafter, *Oocystis* 1); all smaller morphospecies were impacted more strongly by herbivores and were thus grouped together (hereafter, "smaller phytoplankton;" Appendix S2: Table S1). To check the sensitivity of the results to this arbitrary grouping, we re-ran the analyses grouping the next largest and the two next largest phytoplankton morphospecies with *Oocystis* 1 rather than with the smaller phytoplankton.

Data analysis

We analyzed the effects of predator treatment on mean biomass of *Daphnia* and smaller herbivores by fitting a generalized linear mixed model in the gamma family (gamma GLMM), using a dummy variable for each predator addition treatment to compare against the no-predator control treatment, and fit separately for each herbivore group. To analyze the effect of treatment on zooplankton biomass in the laboratory experiment, we used gamma GLMs with zooplankton concentration (1× or 2×) and predator presence as fixed effects, fit separately for each predator species and zooplankton group (four models). To assess whether changes in zooplankton biomass represented a mechanism mediating predation and phytoplankton stability, we tested the effects of mean biomass of the two focal zooplankton groups on the coefficient of variation (CV, a standard measure of variability) of biovolume for the three phytoplankton groupings. In this analysis we fit gamma GLMs with mean biomass of the two focal zooplankton groups as the two

fixed effects, again with a separate model for each phytoplankton grouping. This analysis was repeated using the temporal standard deviation of biomass of the zooplankton groups, instead of mean biomass, as predictors.

To analyze phytoplankton stability, we calculated the temporal CV of phytoplankton biovolume in each tank over the course of the experiment, measured separately for each of the three phytoplankton groupings (total phytoplankton, *Oocystis* 1, and smaller phytoplankton). Then we used gamma GLMs to test whether predator treatment affected the CV of phytoplankton biovolume, the same way we analyzed herbivore biomass. To compare mean phytoplankton biovolume by treatment, we used gamma GLMMs with a dummy variable for each predator addition treatment and tank as a random effect, using the *lme4* package (Bates et al. 2015). Again, we tested each phytoplankton grouping separately. In addition, we analyzed the CV and mean of phytoplankton biomass as estimated by absorbance (see Appendix S3) in the same way we analyzed phytoplankton biovolume. However, it is not possible to break these biomass proxies down by phytoplankton taxon, so they are comparable only to total phytoplankton biovolume. All analysis was conducted using R v. 3.5.3 (R Core Team 2017).

RESULTS

Predator populations and herbivore composition

Neither predator reproduced during the field experiment, and predator survival was estimated to be ~80% with no significant difference between the species or treatments. The herbivorous zooplankton composition in the laboratory slightly differed from the average composition in the field. However, in both experiments, *Scapholeberis kingi* dominated the smaller herbivores, and all zooplankton species were shared across the experiments except for a few rare rotifers found only in the field experiment (Appendix S2: Table S2). In the laboratory no-predator microcosms, *Daphnia* and the smaller herbivores comprised on average 57% and

43% of the total herbivore mass, respectively; in the field no-predator tanks, *Daphnia* and the smaller herbivores comprised 52% and 48%, respectively.

Effects of predators on herbivore groups

In the short-term laboratory experiment, the predators reduced opposing herbivore groups. *Notonecta* reduced *Daphnia* biomass by 97.1% (GLM, $P < 0.001$) averaged over both zooplankton concentrations, without affecting the smaller herbivores. On the other hand, *Neoplea* reduced the biomass of smaller herbivores by 68.1% (GLM, $P < 0.001$) averaged over both zooplankton concentrations, without affecting *Daphnia* biomass (Fig. 1a). In the longer-term field experiment, the two predators had similar effects on the herbivore groups, with one notable difference. *Daphnia* biomass was reduced by 99.6% in tanks with *Notonecta* and by 96.8% in tanks with both predators, but was unaffected in tanks with *Neoplea* (Fig. 1b, Appendix S4: Table S1). Deviating from the laboratory results, neither predator alone significantly affected the biomass of smaller herbivores; yet, there was a diversity effect such that adding both predators reduced the biomass of smaller herbivores by 84.6% (Fig. 1b, Appendix S4: Table S1).

Effects of herbivore groups on phytoplankton groups

Mean biomass of both *Daphnia* and the smaller herbivores was positively associated with variability of smaller phytoplankton biovolume, but only *Daphnia* biomass was positively associated with variability of *Oocystis* 1 biovolume (Appendix S4: Table S2). *Daphnia* biomass was also positively associated with variability of total phytoplankton biovolume, while the biomass of smaller herbivores was marginally positively associated with variability of total phytoplankton biovolume. For all three phytoplankton groupings, average biovolume was negatively associated with mean *Daphnia* biomass, but was not associated with mean biomass of smaller herbivores (Appendix S4: Table S2). Repeating these analyses using the temporal

standard deviations – rather than the means – of herbivore biomass as predictors yielded the same qualitative results.

Effects of predators on phytoplankton groups

The effect of predator diversity on the CV of total phytoplankton biomass depended on the measurement method. Absorbance *in vivo* at 680 nm, a crude proxy for total phytoplankton biomass, was significantly less variable only when both predators were present (GLM, P = 0.036). However, absorbance at 665 nm of extracted chlorophyll-*a*, a less crude proxy, was only marginally significantly less variable with both predators (GLM, P = 0.071), and variability of total phytoplankton biovolume was not significantly affected (GLM, P = 0.114, Fig. 2a). Similarly, variability of *Oocystis* 1 biovolume was not significantly affected by any predator treatment (Table 2, Fig. 3b). In contrast, the CV of smaller phytoplankton biovolume was reduced when both predators were present (Table 2, Fig. 2c). Decomposition of these CVs into their components suggests that predator diversity affected the CV of smaller phytoplankton biovolume primarily by increasing its mean without proportionally increasing its standard deviation (Appendix S4: Figure S1). However, average phytoplankton biomass, estimated in any way or for any of the phytoplankton groupings, was not significantly affected by predator treatment (Fig. 2d,e,f, Appendix S4: Table S3).

DISCUSSION

Our results show that the two focal predator species, *Notonecta* and *Neoplea*, partitioned their herbivorous prey (Figure 1), and that this complementarity likely indirectly reduced the variability of more edible (smaller) phytoplankton biomass. In the laboratory *Notonecta* reduced only *Daphnia* biomass while *Neoplea* reduced only smaller herbivore biomass; accordingly, only when both predators were present in the field were both herbivore groups simultaneously reduced. Meanwhile, lower biomass of each herbivore group was separately associated with

lower variability of smaller phytoplankton biomass. In turn, variability of smaller phytoplankton biomass was significantly lower than the no-predator control only when both predators were present. Thus the presence of both predators appeared to be necessary to suppress both *Daphnia* and the smaller herbivores to lower densities, thereby freeing the smaller phytoplankton of enough herbivory to stabilize their temporal dynamics (Fig. 3). Adding only one predator species failed to reduce both herbivore groups, allowing herbivore-induced variability to continue (Fig. 3b,c). On the other hand, the smaller herbivores did not affect the variability of *Oocystis* 1, which was the largest phytoplankton taxon and therefore expected to be least vulnerable to smaller herbivores. Unsurprisingly, there was no diversity effect on the variability of *Oocystis* 1 biomass; in fact, there was no difference across any predator treatments, perhaps because even *Daphnia* affected this large alga too weakly to cause an effect across treatments (Fig. 3). Because *Oocystis* 1 was not affected by predator treatment, total phytoplankton biomass was only marginally less variable when both predators were added, although this result depended on the method for estimating phytoplankton biomass. Average biomass of both phytoplankton groups was characterized by a similar pattern. That is, there were no differences in average phytoplankton biomass across predator treatments, and only *Daphnia* biomass was associated with average biomass of both phytoplankton groups.

There are several potential reasons for the differential effects of *Neoplea* on the smaller herbivores in the laboratory versus the field experiment. The laboratory experiment lasted only five days while the field experiment was carried out for seven weeks. We designed the laboratory experiment to estimate the prey preference of the predators without allowing time for the prey to recover appreciably; on the other hand, the field experiment allowed time for the prey populations to reproduce. This may have allowed the smaller herbivores to continually recover (at least partially) from predation by *Neoplea*, while the stronger effect of *Notonecta* on *Daphnia*

did not allow *Daphnia* to recover. With *Daphnia* virtually eliminated, *Notonecta* may have then switched to less preferred, smaller prey, increasing the total consumption of smaller herbivores (Fig. 3). Whatever the reason for the weaker effect of *Neoplea* in the field, both predators were still needed to suppress both herbivore groups as the laboratory results predicted.

The cascading diversity effect was not apparent in all tanks or taxa, but the observed pattern was consistent with background variation in the mesocosms and in edibility among the plankton taxa. In at least one mesocosm per treatment, variability of the smaller phytoplankton was at least as low as the average variability with both predators (Fig. 2f). This pattern can be explained by large background variation in the system: even in the absence of predators, variation in the density of *Daphnia* and of the smaller herbivores was very large, with some predator-free control tanks having consistently low herbivore densities. This meant that only some tanks within each treatment contained enough herbivores for a cascading predator effect to be detected. Similarly, some plankton taxa were relatively inedible and therefore were not involved in the cascading diversity effect. When such taxa were also dominant (i.e., *Arctodiaptomus*), they obscured the potential food web mechanism unless removed from analysis. The fact that the taxa which appeared not to be involved in the cascade stand out from the others in terms of lower capture probability only strengthens support for the proposed food web mechanism. While "edible taxa" were necessarily defined somewhat arbitrarily, defining them in several other ways did not change the qualitative results.

This study provides a simultaneous evaluation of effects of predator diversity on both the average and variability of primary producer biomass. Most previous predator diversity-ecosystem function experiments measured the mean but not the variability of trophic group biomass as dependent variables (Bruno and O'Connor 2005; Straub et al. 2008). This work indicates that if predators partition their resources, increasing predator diversity can lead to lower

mean herbivore biomass and higher mean autotroph biomass, i.e., a diversity-biomass trophic cascade (Table 1; Straub et al. 2008). In our field experiment, higher predator diversity decreased mean biomass of the focal herbivores but did not significantly increase mean phytoplankton biomass, although there was a weak, non-significant trend towards increasing biomass of smaller phytoplankton. Only *Daphnia* was associated with lower phytoplankton biomass, and not the smaller herbivores; overall, the effect of the herbivores on mean phytoplankton biomass was apparently too weak to complete a diversity-biomass trophic cascade. It is not uncommon for herbivores, and therefore changes in herbivore biomass, to have weak effects on plant biomass (Maron and Crone 2006). The strength of herbivory is often dampened by the variable food quality of plants, low encounter rates between herbivores and plants, indirect effects, or other factors (Leibold 1989, Borer et al. 2005, Maron and Crone 2006). On the other hand, both *Daphnia* and the smaller herbivores had de-stabilizing effects on smaller phytoplankton biomass, and so there was a stronger pathway from predator diversity to small phytoplankton stability, resulting in the completion of a diversity-stability trophic cascade. Thus, in this case, the temporal variability of primary producer biomass was more sensitive to changes in herbivore biomass than was average primary producer biomass, at least for the taxa more vulnerable to herbivory. Future studies will need to explore the generality of this result.

Previous studies have reported the effects of natural enemy diversity on the variability of food web interactions, and some have described theoretical mechanisms relevant to these studies. A few studies used surveys to relate parasitoid richness to the temporal variability of aggregate parasitism rates, finding either no relationship (Rodriguez and Hawkins 2000) or a negative relationship (Tylianakis et al. 2006, Macfadyen et al. 2011). Griffin and Silliman (2011) found that the combination of two predators which exhibited temporal complementarity in attack rates reduced the temporal variability of the total predation rate on a shared prey. However, our study

is the first (to our knowledge) to test whether predator diversity can reduce the temporal variability of basal resource biomass in a cascading effect. Theoretical work suggests several mechanisms linking diversity and stability in ecosystems, mostly focusing on single trophic levels (Loreau and de Mazancourt 2013). With a two-trophic level model, Thébault and Loreau (2005) showed that decreasing herbivore biomass stabilizes (and increases) plant biomass. Coupling this finding with the consensus from the literature that complementarity of resource use by predators tends to reduce herbivore biomass, it follows that predator complementarity may indirectly stabilize (and increase) plant biomass. This food web diversity-stability mechanism could have important implications for both ecosystem management and for biological control.

Our results suggest that adding multiple natural enemies to an agro-ecosystem can stabilize fluctuations in crop yields, provided the natural enemies partition pest resources. While a goal of farmers will always clearly be to achieve as high an average yield as possible, achieving consistent yields is often equally important. We showed that diversity of a predator trait known to correlate with prey preference, body size, can be a key factor leading to the cascading stabilizing effect on phytoplankton. Microalgae, especially phytoplankton, are increasingly cultured as a crop for a variety of purposes, from biomass production for biofuels or animal feed, to nutrient removal from wastewaters. However, algal cultivation is not yet widely practiced at the commercial scale, in large part because it has proven too difficult to achieve consistently high algal yields as open, raceway ponds are easily colonized by zooplankton pests ranging widely in size that are difficult or costly to control mechanically or chemically (Smith and Crews 2014, Montemezzani et al. 2015). Adding a functionally diverse array of predators to algal cultivation ponds may therefore be a feasible, economical, self-sustaining way to encourage more reliable algal yields. Terrestrial crops also can suffer yield instability associated with multiple pests, and thus may similarly benefit from addition or encouragement of a community

of natural enemy species varying in size (Gurr et al. 2012). Diversity of other functional traits related to prey choice may also encourage crop yield stability in a similar fashion and could also be managed in natural enemy communities when relevant information is available, including traits such as microhabitat preference, temporal patterns in predation strength, or variance in mode of prey suppression. For example, top-down control can be stabilized by adding some predators more active in warm temperatures and some more active in cold temperatures (Griffin and Silliman 2011), or by adding both parasites and predators (Ong and Vandermeer 2015), which encourages consistently low pest densities and stable crop yields. While our study emphasizes the importance of complementarity, redundancy is also likely important as temporal and spatial scales increase, to guard against periods of weakened control by, or extirpations of, natural enemies at certain times or locations (Peralta et al. 2014).

Here we have demonstrated that higher predator diversity, and accompanying complementarity of prey use, can cause a chain of effects that cascade down a food web to stabilize the temporal dynamics of basal resource biomass while leaving average basal resource biomass unchanged. This connection between predator diversity and primary producer stability is an important step towards joining biodiversity-ecosystem function theory with food web theory, as biodiversity and ecosystem functioning research has only recently begun incorporating energy flows in a food web context (Barnes et al. 2018). Future work is needed to further explore the simultaneous influence of predator diversity, in its various forms, on both the average and stability of community- and ecosystem-level functioning. It is important to uncover whether stability is generally more sensitive to cascading food web effects than is average biomass, or how the two effects are related in different contexts. Predator diversity may also have different effects on other measures of stability, such as resilience. Developing this field at the intersection of biodiversity-ecosystem functioning and food web ecology not only will improve our

understanding of the functioning of natural ecosystems and their vulnerability to anthropogenic biodiversity loss, but also will provide information that can be directly used to manage for more reliable crop production and other ecosystem services.

ACKNOWLEDGEMENTS

Thanks to L. A. Sekula and J. Earwood for help processing samples, to R. Deans for help with insect collection and experiment setup, to S. Duchicela for help with experimental setup, to D. Correa for help with nutrient analysis, and to D. Nobles for providing equipment. Thanks to A. Wolf, R. Decker, D. Cinoglu, S. Ortiz, E. Francis, D. Grobert, and A. Northup for feedback on an earlier version of the manuscript. This research was supported by the Department of Integrative Biology at the University of Texas at Austin and was made possible by the facilities at Brackenridge Field Laboratory.

LITERATURE CITED

Adrian, R., and T. M. Frost. 1993. Omnivory in cyclopoid copepods: comparisons of algae and invertebrates as food for three, differently sized species. Journal of Plankton Research 15:643–658.

APHA. 1989. Standard methods for the examination of water and wastewater, 17th edn. American Public Health Association, Washington, D.C.

Barnes, A. D., M. Jochum, J. S. Lefcheck, N. Eisenhauer, C. Scherber, M. I. O'Connor, P. de Ruiter, and U. Brose. 2018. Energy flux: the link between multitrophic biodiversity and ecosystem functioning. Trends in Ecology and Evolution 33:186–197.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Benemann, J. 2013. Microalgae for biofuels and animal feeds. Energies 6:5869–5886.

Borer, E. T., E. W. Seabloom, J. B. Shurin, K. E. Anderson, C. A. Blanchette, B. Broitman, S. D. Cooper, and B. S. Halpern. 2005. What determines the strength of a trophic cascade? Ecology 86:528–537.

Bruno, J. F., and M. I. O'Connor. 2005. Cascading effects of predator diversity and omnivory in a marine food web: cascading effects of predator diversity. Ecology Letters 8:1048–1056.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–67.

Cardinale, B. J., J. E. Duffy, D. Srivastava, M. Loreau, M. Thomas, and M. Emmerson. 2009. Towards a food-web perspective on biodiversity and ecosystem functioning. Pages 105–120 *in* Naeem S., D. Bunker, A. Hector, M. Loreau, and C. Perrings, editors. Biodiversity and human impacts. Oxford University Press, Oxford, UK.

Downing, A. L., B. L. Brown, and M. A. Leibold. 2014. Multiple diversity-stability mechanisms enhance population and community stability in aquatic food webs. Ecology 95:173–184.

Gittelman, S. H. 1977. Leg segment proportions, predatory strategy and growth in backswimmers (Hemiptera: Pleidae, Notonectidae). Journal of the Kansas Entomological Society 50:161–171.

Greenop, A., B. A. Woodcock, A. Wilby, S. M. Cook, and R. F. Pywell. 2018. Functional diversity positively affects prey suppression by invertebrate predators: a meta-analysis. Ecology 99:1771–1782.

Griffin, J. N., and B. R. Silliman. 2011. Predator diversity stabilizes and strengthens trophic control of a keystone grazer. Biology Letters 7:79–82.

Gross, K., B. J. Cardinale, J. W. Fox, A. Gonzalez, M. Loreau, H. Wayne Polley, P. B. Reich, and J. van Ruijven. 2014. Species richness and the temporal stability of biomass production: a new analysis of recent biodiversity experiments. The American Naturalist 183:1–12.

Gurr, G. M., S. D. Wratten, and W. E. Snyder. 2012. Biodiversity and insect pests: key issues for sustainable management. John Wiley and Sons, Inc., Hoboken, NJ.

Hall, S. R., M. A. Leibold, D. A. Lytle, and V. H. Smith. 2004. Stoichiometry and planktonic grazer composition over gradients of light, nutrients, and predation risk. Ecology 85:2291–2301.

Hampton, S. E., and J. J. Gilbert. 2001. Observations of insect predation on rotifers. Pages 115–121 *in* L. Sanoamuang, H. Segers, R. J. Shiel, and R. D. Gulati, editors. Rotifera IX. Springer, Dordrecht, Netherlands.

Hector, A., Y. Hautier, P. Saner, L. Wacker, R. Bagchi, J. Joshi, M. Scherer-Lorenzen, E. M. Spehn, E. Bazeley-White, M. Weilenmann, M. C. Caldeira, P. G. Dimitrakopoulos, J. A. Finn, K. Huss-Danell, A. Jumpponen, C. P. H. Mulder, C. Palmborg, J. S. Pereira, A. S. D. Siamantziouras, A. C. Terry, A. Y. Troumbis, B. Schmid, and M. Loreau. 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. Ecology 91:2213–2220.

Hines, J., W. H. van der Putten, G. B. De Deyn, C. Wagg, W. Voigt, C. Mulder, W. W. Weisser, J. Engel, C. Melian, S. Scheu, K. Birkhofer, A. Ebeling, C. Scherber, and N. Eisenhauer. 2015. Towards an integration of biodiversity-ecosystem functioning and food web theory to evaluate relationships between multiple ecosystem services. Pages 161–199 Advances in Ecological Research. Elsevier.

Jiang, L., and Z. Pu. 2009. Different effects of species diversity on temporal stability in single-trophic and multitrophic communities. The American Naturalist 174:651–659.

Kilham, S. S., D. A. Kreeger, S. G. Lynn, C. E. Goulden, and L. Herrera. 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377:147–159.

Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. The American Naturalist 134:922–949.

Leon, B. 1998. Influence of the predatory backswimmer, *Notonecta maculata*, on invertebrate community structure. Ecological Entomology 23:246–252.

Loreau, M., and C. de Mazancourt. 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. Ecology Letters 16:106–115.

Macfadyen, S., P. G. Craze, A. Polaszek, K. van Achterberg, and J. Memmott. 2011. Parasitoid diversity reduces the variability in pest control services across time on farms. Proceedings of the Royal Society B-Biological Sciences 278:3387–3394.

Rodriguez, M. A., and B. A. Hawkins. 2000. Diversity, function and stability in parasitoid communities. Ecology Letters 3:35–40.

Tylianakis, J. M., T. Tscharntke, and A.-M. Klein. 2006. Diversity, ecosystem function, and stability of parasitoid host interactions across a tropical habitat gradient. Ecology 87:3047–3057.

Maron, J. L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proceedings of the Royal Society B: Biological Sciences 273:2575–2584.

Montemezzani, V., I. C. Duggan, I. D. Hogg, and R. J. Craggs. 2015. A review of potential methods for zooplankton control in wastewater treatment high rate algal ponds and algal production raceways. Algal Research 11:211–226.

Murdoch, W. W., M. A. Scott, and P. Ebsworth. 1984. Effects of the general predator, *Notonecta* (Hemiptera) upon a freshwater community. The Journal of Animal Ecology 53:791–808.

O'Brien, W. J. 1979. The predator-prey interaction of planktivorous fish and zooplankton: recent research with planktivorous fish and their zooplankton prey shows the evolutionary thrust and parry of the predator-prey relationship. American Scientist 67:572–581.

Ong, T. W. Y., and J. H. Vandermeer. 2015. Coupling unstable agents in biological control. Nature Communications 6:5991.

Peralta, G., C. M. Frost, T. A. Rand, R. K. Didham, and J. M. Tylianakis. 2014. Complementarity and redundancy of interactions enhance attack rates and spatial stability in host-parasitoid food webs. Ecology 95:1888–1896.

Purvis, A., J. L. Gittleman, G. Cowlishaw, and G. M. Mace. 2000. Predicting extinction risk in declining species. Proceedings of the Royal Society of London. Series B: Biological Sciences 267:1947–1952.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ripple, W. J., J. A. Estes, O. J. Schmitz, V. Constant, M. J. Kaylor, A. Lenz, J. L. Motley, K. E. Self, D. S. Taylor, and C. Wolf. 2016. What is a trophic cascade? Trends in Ecology & Evolution 31:842–849.

Schmitz, O. J. 2007. Predator diversity and trophic interactions. Ecology 88:2415–2426.

Seabloom, E. W., L. Kinkel, E. T. Borer, Y. Hautier, R. A. Montgomery, and D. Tilman. 2017. Food webs obscure the strength of plant diversity effects on primary productivity. Ecology Letters 20:505–512.

Smith, V. H., and T. Crews. 2014. Applying ecological principles of crop cultivation in large-scale algal biomass production. Algal Research 4:23–34.

Smith, V. H., B. S. M. Sturm, F. J. deNoyelles, and S. A. Billings. 2010. The ecology of algal biodiesel production. Trends in Ecology and Evolution 25:301–309.

Snyder, W. E. 2019. Give predators a complement: conserving natural enemy biodiversity to improve biocontrol. Biological Control 135:73–82.

Steiner, C. F., T. L. Darcy-Hall, N. J. Dorn, E. A. Garcia, G. G. Mittelbach, and J. M. Wojdak. 2005. The influence of consumer diversity and indirect facilitation on trophic level biomass and stability. Oikos 110:556–566.

Straub, C. S., D. L. Finke, and W. E. Snyder. 2008. Are the conservation of natural enemy biodiversity and biological control compatible goals? Biological Control 45:225–237.

Sturm, B. S. M., E. Peltier, V. Smith, and F. deNoyelles. 2012. Controls of microalgal biomass and lipid production in municipal wastewater-fed bioreactors. Environmental Progress and Sustainable Energy 31:10–16.

Thébault, E., and M. Loreau. 2005. Trophic interactions and the relationship between species diversity and ecosystem stability. The American Naturalist 166:E95–E114.

van Driesche, R., M. Hoddle, and T. Center. 2008. Control of pests and weeds by natural enemies: an introduction to biological control. Blackwell Publishing, Malden, MA.

Williamson, C. E. 1987. Predator-prey interactions between omnivorous diaptomid copepods and rotifers: the role of prey morphology and behavior. Limnology and Oceanography 32:167–177.

Table 1. Trophic cascade terminology used in this paper.

Proposed term	Definition
trophic cascade	Indirect species interactions that originate with predators and spread downward through food webs (Ripple et al. 2016).
diversity-biomass trophic cascade	A change in *biomass* of a lower trophic level indirectly caused by a change in predator *diversity* and mediated by top-down effects on the intermediate trophic level(s).
diversity-stability trophic cascade	A change in the *stability* (defined as variability or perhaps otherwise) of the biomass of a lower trophic level, indirectly caused by a change in predator *diversity* and mediated by top-down effects on the intermediate trophic level(s).

Table 2. Results of gamma GLMs testing the effects of predator additions in the field experiment on the temporal CV of biovolume of a) total phytoplankton, b) *Oocystis* 1 (the largest morphospecies), and c) smaller phytoplankton (all but *Oocystis* 1).

Parameter	**Estimate**	**SE**	**t**	***P***
a) total phytoplankton				
intercept	-0.6181	0.3114	-1.985	0.065
Notonecta	-0.1808	0.4404	0.668	0.514
Neoplea	0.2940	0.4404	0.668	0.514
Both	-0.7366	0.4404	-1.673	0.114
b) *Oocystis* 1				
intercept	-0.4977	0.2399	-2.075	0.055
Notonecta	0.0010	0.3392	0.003	0.998
Neoplea	0.4843	0.3392	1.428	0.173
both predators	-0.1010	0.3392	-0.298	0.770
c) smaller phytoplankton				
intercept	-0.4310	0.3001	-1.436	0.170
Notonecta	-0.4497	0.4244	-1.060	0.305
Neoplea	0.0626	0.4244	0.148	0.885
both predators	-1.0622	0.4244	-2.503	**0.024**

Figure legends

Figure 1. Multiplicative effects of predator addition on biomass of *Daphnia* and on summed biomass of smaller herbivores (excludes *Arctodiaptomus* and nauplii) in the laboratory experiment (**a**) and the field experiment (**b**). Shapes represent coefficient estimates with 95% confidence intervals from GLMs (in **a**) and GLMMs (in **b**) modeling the effects of predator addition on the mass of each herbivore group. Note the log scale in **b** only. Dotted lines at 1 represent no effect; stars mark confidence intervals that do not overlap with this line. The dotted line at 0 (in **a**) represents total elimination of an herbivore group.

Figure 2. Time series of phytoplankton biovolume (means ±1 standard error of the mean, **a-c**) and temporal CV of phytoplankton biovolume (**d-f**) by predator treatment. Panels **a** and **d** show values for total phytoplankton, **b** and **e** show values for *Oocystis* 1 (the largest taxon), and **c** and **f** show values for smaller (edible) phytoplankton (all besides *Oocystis* 1). The star represents a significant effect of predator addition (GLMM comparing to the "neither" predator treatment, $P = 0.024$).

Figure 3. Diagrams depicting the food web present in each field experiment treatment and the apparent resulting mechanisms for food web effects on phytoplankton variability (CV). Panels represent predator treatments: no predators (**a**), *Notonecta* (**b**), *Neoplea* (**c**), and both (**d**). Arrows represent hypothesized energy flow, with thicker arrows indicating greater flow. Relative numbers of herbivore icons represent measured relative mean biomass. The time series at the bottom of each panel indicate relative temporal variability of each phytoplankton group (a cartoon version of the actual qualitative results, Figure 2). Stars indicate significant differences in the biomass of the group or in CV of phytoplankton biovolume compared with the no-predator controls (GLMs; $\alpha = 0.05$).

Figure 1

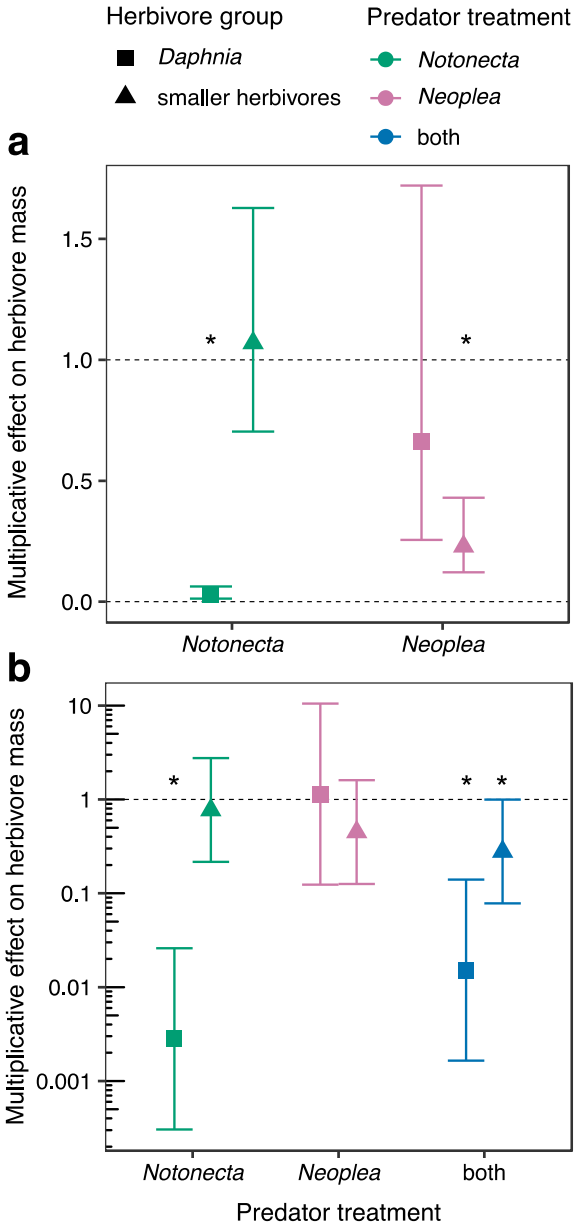

Herbivore group
Daphnia
smaller herbivores
Predator treatment
Notonecta
Neoplea
both
a
Multiplicative effect on herbivore mass
1.5
1.0
0.5
0.0
*
*
Notonecta
Neoplea
b
Multiplicative effect on herbivore mass
10
1
0.1
0.01
0.001
*
*
*
Notonecta
Neoplea
both
Predator treatment

Figure 2

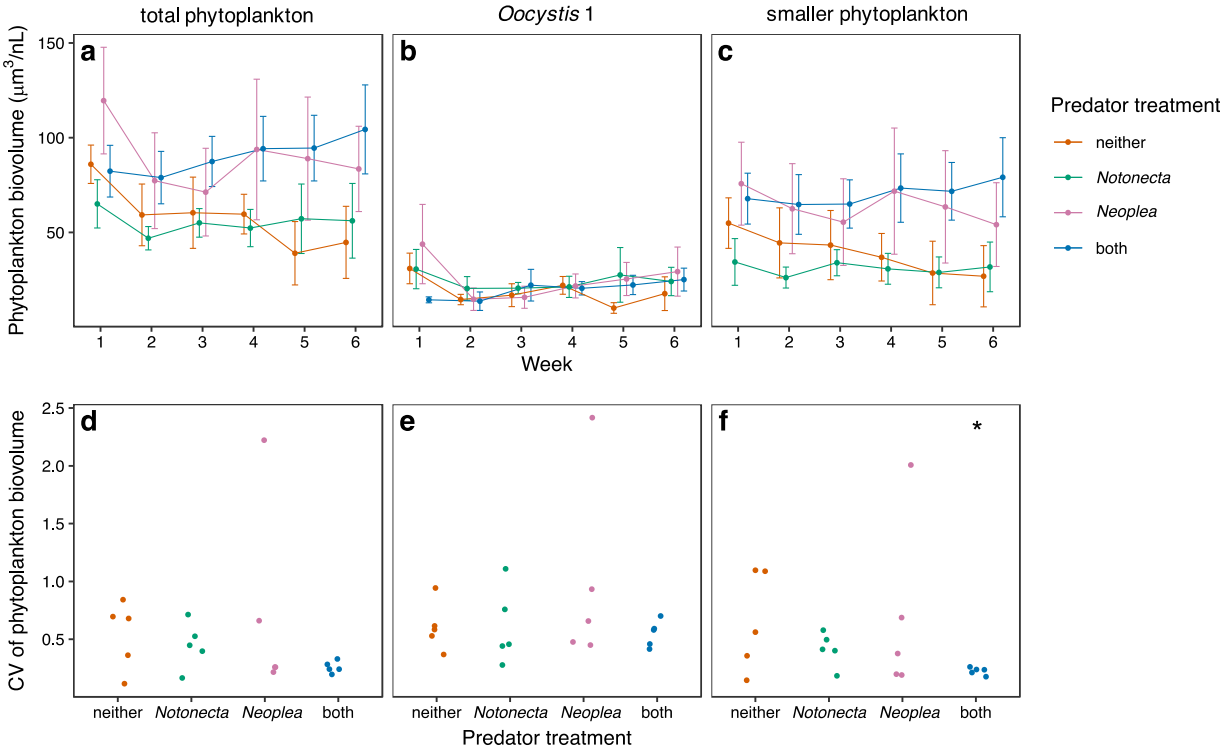
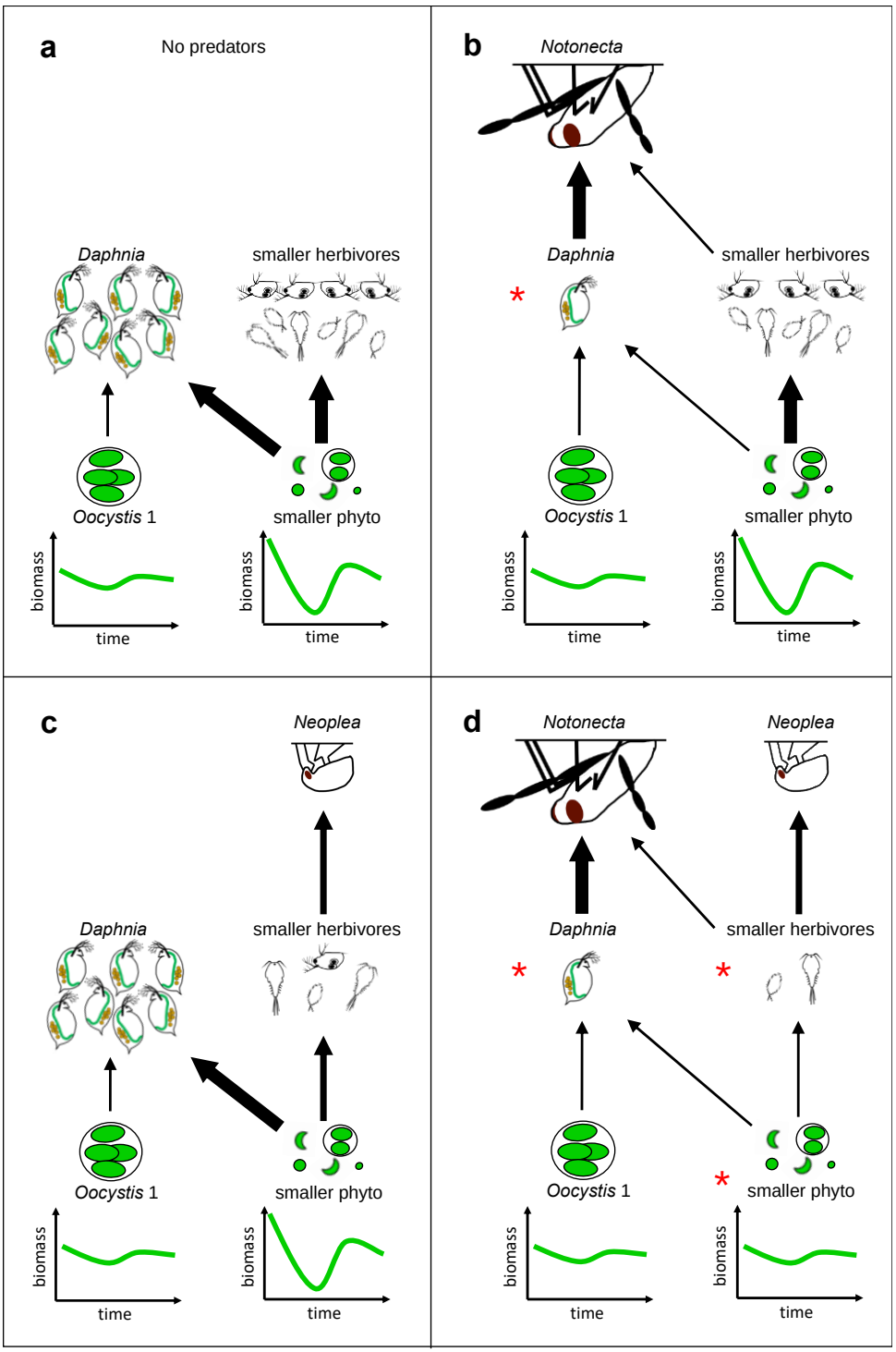

total phytoplankton
Oocystis 1
smaller phytoplankton
a
b
c
d
e
f
Phytoplankton biovolume (µm³/nL)
150
100
50
1
2
3
4
5
6
Week
CV of phytoplankton biovolume
2.5
2.0
1.5
1.0
0.5
*
neither
Notonecta
Neoplea
both
Predator treatment
Predator treatment
neither
Notonecta
Neoplea
both

Figure 3


a
No predators
Daphnia
smaller herbivores
Oocystis 1
smaller phyto
biomass
time
biomass
time
b
Notonecta
Daphnia
smaller herbivores
*
Oocystis 1
smaller phyto
biomass
time
biomass
time
c
Neoplea
Daphnia
smaller herbivores
Oocystis 1
smaller phyto
biomass
time
biomass
time
d
Notonecta
Neoplea
Daphnia
smaller herbivores
*
*
Oocystis 1
*
smaller phyto
biomass
time
biomass
time