Running head: Diversity-stability trophic cascade

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

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Abstract

The indirect effects of top predators on the biomass of lower trophic levels have been extensively documented, especially in aquatic ecosystems and are commonly characterized as "trophic cascades." There have also been studies showing that predator diversity can play an important role in mediating these trophic cascades. In addition, some studies have demonstrated that the diversity of consumers can impact the stability of their resources. However, the effects of predator diversity on the stability of lower trophic levels (cascading effects of predator diversity) have not yet been studied. We conducted a field pond mesocosm experiment and accompanying laboratory microcosm experiment in which we grew phytoplankton with zooplankton herbivores, manipulating the presence and co-habitation of two heteropteran predators while controlling for total expected predation. We hypothesized 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 biomass. We present evidence that the predators partitioned their herbivore prey, and found that this simultaneous suppression of different herbivore groups reduced the variability of edible (smaller) phytoplankton biomass, without affecting mean phytoplankton biomass. We also found that phytoplankton 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 can indirectly stabilize primary producer biomass via a "diversity-stability cascade" in the manner of a trophic cascade, seemingly dependent on predator complementarity and the vulnerability of taxa to consumption, and independent of a more traditional trophic cascade in which average biomass is enhanced. Predator diversity, especially in terms of functional traits relating to differential prey use such as body size, may be

important for regulating ecosystem stability, and may provide a way to improve the reliability of algal crop yields via biological control.

Key words: biodiversity, species richness, ecosystem functioning, stability, temporal variability, resource partitioning, niche complementarity, food web, top-down control, plankton, algae, biological control

Introduction

A substantial body of work indicates that enhanced biodiversity of ecosystems often stabilizes community biomass (Jiang and Pu 2009, Cardinale et al. 2012, Loreau and de Mazancourt 2013). Most of these studies were motivated by global biodiversity loss and measured the effects of primary producer diversity on primary producer biomass (Cardinale et al. 2009). However, predators face greater extinction threats than lower trophic levels, suggesting the implications of changing predator diversity are more relevant to global biodiversity change (Purvis et al. 2000). While a moderate body of literature has asked how predator diversity affects the average biomass of trophic groups (Schmitz 2007), ecologists have rarely experimentally tested the influence of predator diversity on any form of ecosystem stability. Yet, predator diversity-stability effects have important implications for both the maintenance of stable natural ecosystems and also for biological control to potentially stabilize crop yields.

Much existing research on the effects of predator diversity on lower trophic levels has been performed in the context of biological control. Biological control is classically practiced by releasing a specialist natural enemy to target a specific pest (van Driesche et al. 2008). However, 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 while conserving natural enemy biodiversity, a concept termed ‘conservation biological control’ (Snyder 2019). A meta-analysis showed that harnessing 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). Following this reasoning, diversity of functional traits among predators related to prey use, such as body size, may play a key role in mediating the effects of predator diversity on ecosystem function (Straub et al. 2008). In theory, decreases in herbivore mass have been shown to decrease the variability of primary producer biomass (Thébault and Loreau 2005), suggesting that functional predator diversity may also indirectly reduce the variability of primary producer biomass and thus produce a “diversity-stability cascade.” Such an effect may be most likely seen when the primary producers are highly edible, such as in communities of small phytoplankton.

Culturing algae, 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, algal yields are often low and unpredictable, preventing the production of algae-derived commodities from being economical (Benemann 2013). A major reason for these algal crop failures is that algae ponds are quickly colonized by zooplankton herbivores varying in size from ciliates <100 um 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. However, we are only 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 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 consume *Daphnia*, and we predicted that *Neoplea* would consume smaller zooplankton based on its smaller body size. We hypothesized that, given *Notonecta* and *Neoplea* partition herbivore prey, the addition of both species together would 1) increase the mean and 2) reduce the variability of phytoplankton biomass, while addition of a single predator species would have either no such effect or much weaker effects. We present evidence that these predators indeed partitioned herbivore prey, leading to an indirect effect on phytoplankton stability without affecting mean phytoplankton biomass. While the unexpected presence of a relatively inedible phytoplankton strain dampened the effect on total phytoplankton, we show that predator diversity stabilized community biomass of smaller, more edible phytoplankton and thus demonstrate a diversity-stability cascade.

Methods

Focal predators

We used two insect species in the suborder Heteroptera as the predators in both the field and laboratory experiments: the notonectid (backswimmer) *Notonecta undulata* and the pleid (pygmy backswimmer) *Neoplea striola*. Both species are mobile generalist predators and are widespread across North America. Numerous studies have investigated the ecology of members of the genus *Notonecta*, showing they strongly reduce large crustacean zooplankton such as *Daphnia* and insects such as mosquito larvae, although they can take prey as small as microscopic rotifers (Leon 1998, Murdoch et al. 1984, Hampton and Gilbert 2001). *N. undulata* adults measure ~11-13 mm in length. In contrast, *N. striola* is much smaller, with adults measuring ~1.5 mm. *N. striola* has been documented to prey on invertebrates ranging in size from rotifers to *Daphnia* (Hampton and Gilbert 2001, Gittelman 1977). Hereafter, we use the genus names (*Notonecta* and *Neoplea*) to refer to these two focal predator species.

Field experiment

We established replicate experimental pond communities in 200-L cattle tanks at Brackenridge Field Laboratory, Austin, TX. Tanks were filled with well water and outfitted with a float valve to maintain constant water levels. We analyzed total N and P of the water before beginning the experiment following the methods of the APHA (1989). We added enough $NaNO_3$ and $NaH_2PO_4 \bullet H_2O$ to bring total N and P to the same concentrations as 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 enough of both nutrients to compensate for a 5% daily loss rate from the water column (as per Hall et al. 2004).

We inoculated each tank with a common inoculum of phytoplankton collected from nearby tanks which had been colonized by local phytoplankton strains for several months. The phytoplankton community became dominated by green algae (Chlorophyta), ranging in size from green picoplankton ~1 um in diameter to *Oocystis* with mother cell walls up to ~25 um in diameter and dominated by a few morphospecies, especially *Selenastrum* and *Oocystis* (Appendix 1). We allowed the phytoplankton to grow for 15 days before adding zooplankton. 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 ordered *Daphnia magna* from Sachs Systems Aquaculture (St. Augustine, FL) to extend the size range of the zooplankton to include larger-bodied individuals (Appendix 1). We mixed the zooplankton taxa together and added an equal volume to each tank.

Notonecta and *Neoplea* were collected from ponds at the Austin Water Utility at Hornsby Bend, Austin, TX and added after the zooplankton. 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 chosen relative densities of *Notonecta* and *Neoplea* (1:15) were determined to satisfy the null hypothesis that each treatment with predators would experience the same total predation rate if the predators did not partition prey resources. We determined this ratio by measuring the dry masses of several adults of each taxon and then using the metabolic scaling law reported for insects (Glazier 2010) to estimate the number of *Neoplea* that would be expected to consume the same prey mass per day as 3 *Notonecta*. Additionally, in the laboratory experiment (methods described below) individual *Notonecta* consumed 14.3x more animal mass than *Neoplea* in the 1x zooplankton concentration microcosms and 16.3x more animal mass than *Neoplea* in the 2x

zooplankton concentration microcosms, supporting the 15:1 ratio. Neither predator reproduced during the experiment. Survival of both species was estimated by sampling tanks with a D-net, and was estimated to be ~80% with no significant difference between the species or treatments.

Beginning a week after adding predators, we sampled zooplankton and phytoplankton weekly for six weeks. To sample zooplankton, we used iterated spatially-spread subsamples to collect 12L of water from each tank using tube samplers. We filtered this water using 65 μm mesh, returned any predators to the tank, and preserved the retained material in 10% Lugol's solution. We then identified and counted zooplankton in subsamples of this collection such that for each taxon, at least 25 individuals or 10% of the sample was counted, whichever came first, and at least 50 total individuals were counted. We measured the length of each crustacean and *Spirostomum* with a micrometer to the nearest half increment (0.24 mm) and measured the length and width of rotifers and width of *Spirostomum* to the nearest 0.05 increment (0.024 mm). We converted crustacean length to biomass using length-mass regressions (Culver et al. [1985] for copepods, McCauley [1984] for *Scapholeberis*, Dumont et al. [1975] for *Daphnia magna* and *Chydorus*, and Anderson et al. [1998] for ostracods). When rotifers were identifiable to species, we converted rotifer dimensions to biomass using species-specific equations from the EPA protocol (EPA 2016); otherwise, we used biovolume equations (McCauley 1984) and converted to biomass assuming a 10:1 biovolume:dry mass ratio. We converted *Spirostomum* dimensions to biomass approximating cells as cylinders and assuming the same 10:1 biovolume:dry mass ratio.

To sample phytoplankton, we used iterated spatially-spread subsamples to collect 50 mL water column samples from each tank using 1-cm diameter PVC pipes. After storing the phytoplankton samples for ~24 hours in darkness on ice, we measured the absorbance of each sample at 680 *in vivo* using a spectrophotometer. We then extracted chlorophyll-*a* and measured

chlorophyll-*a* fluorescence accounting for turbidity, following standard methods (APHA 1989). We also preserved subsamples in 10% formaldehyde and enumerated phytoplankton densities using a hemocytometer. We counted 25 nL or 50 cells of the most common morphospecies, whichever came first, and counted at least 100 nL for the other taxa. Two individuals separately counted each sample using these methods and then the counts were averaged. We also took several micrographs of each morphospecies across tanks and dates and measured the cell dimensions using ImageJ (Schneider et al. 2012), measuring >15 cells of all but the rarest morphospecies. Then we used geometric approximations to calculate the biovolume of each cell (Appendix 1).

Laboratory experiment

We conducted an accompanying laboratory experiment to test whether *Notonecta* and *Neoplea* had different effects on zooplankton herbivore groups on a short time scale. Following completion of the field experiment, we collected plankton samples from several of the field mesocosms and mixed them together. Half of the plankton mixture was filtered with a 100 μm filter to concentrate the zooplankton to 2x ambient density. Additionally, ten adults of both insect predator species were collected from the tanks. Each predator was placed individually in a microcosm with either the 1x or 2x zooplankton density. *Notonecta* were placed in microcosms filled with 1.5 L plankton mixture, and *Neoplea* were placed in microcosms with 100 mL plankton mixture, such that the volumes were proportional to the 15:1 ratio of expected prey consumption by the two species. Additionally, we established control microcosms with no predator. All treatments were replicated five times, yielding 40 total microcosms (2 predator species/microcosm sizes X predator presence or absence X 2 zooplankton concentrations X 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. Five days represents just under one generation for the dominant zooplankton with the shortest generation time, *Daphnia magna* and *Scapholeberis kingi*. Therefore we anticipated that five days would provide enough time for the predators to impact their prey 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 plankton species while minimizing the influence of plankton fecundity and adaptation.

Grouping phytoplankton and zooplankton for analysis

An herbivorous zooplankter was defined as any taxon that, according to the literature and our data, is primarily herbivorous over its life span and is not strictly benthic. Thus, *Mesocyclops* was included in analyses even though the adult stage is omnivorous (Adrian and Frost 1993). *Spirostomum*, a filter-feeding ciliate, was also included in the herbivorous zooplankton despite characterization in the literature as a bacterivore, since preliminary analysis also suggested that it reduced the biomass of smaller algae. However, the most dominant zooplankter in most tanks, the diaptomid copepod *Arctodiaptomus dorsalis*, was not included. Diaptomid copepods exhibit a much faster escape response and lower vulnerability to predators than other crustacean zooplankton, and are often omnivorous (O'Brien 1979, Williamson 1987). Preliminary analysis showed that *Arctodiaptomus* biomass was unaffected by either predator, and also had no effect on algal biomass or stability, 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 analysis. *Arctodiaptomus* copepodites and adults were 5.35 times as numerous as *Mesocyclops* copepodites and adults on

average, so biomass of *Mesocyclops* nauplii likely represented a small fraction of herbivore biomass.

To test our hypothesis that *Notonecta* and *Neoplea* partitioned their resources based on body size, leading to differential size-based cascading food web effects, it was necessary to divide plankton into groups based on size. 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. *Daphnia* was the largest and the second most dominant herbivore taxon, comprising 28% of herbivore biomass on average in control tanks, while the smaller herbivores comprised a similar 26% of herbivore biomass in control tanks (*Arctodiaptomus* comprised 43%). The smaller herbivores were dominated by *Scapholeberis kingi*, a hyponeustonic daphniid (Appendix 1). Zooplankton were grouped in the same way for analysis of the laboratory experiment. Since the zooplankton used in the laboratory experiment were sampled from a subset of tanks after the field experiment had ended, the zooplankton composition slightly differed from the average composition in the field experiment. But as in the field experiment, *Scapholeberis* was by far the most dominant species among the smaller herbivores; no new species appeared in the laboratory experiment, and all the species found in the field experiment were included in the laboratory experiment except for a few of the rarest rotifers (Appendix 1). In the predator-free laboratory microcosms, *Daphnia* and the smaller herbivores composed 51% and 38% of the total herbivore mass on average, respectively.

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 (Appendix 1). We also ran analyses using alternative groupings including the two largest and the three largest morphospecies.

Data analysis

We analyzed the effects of predator treatment on mean biomass of *Daphnia* and smaller herbivores by fitting a gamma GLMM (generalized linear mixed model in the gamma family), 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 (1x or 2x) 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 algal stability, we tested the effects of mean biomass of the two focal zooplankton groups on the CV of biovolume of the three algal groupings. For 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 algal grouping. This analysis was repeated using the within-tank standard deviation of biomass of the zooplankton groups, instead of mean biomass, as predictors.

To analyze algal stability, we calculated the temporal coefficient of variation (CV, a standard measure of variability) of algal biovolume in each cattle tank over the course of the experiment, measured separately for each of the three algal groupings (total, *Oocystis* 1, and all but *Oocystis* 1). Then we used gamma GLMs to test whether predator treatment affected the CV of algal biovolume, the same way we analyzed biomass of herbivore groups. To compare mean algal biovolume by treatment, we used gamma GLMMs (generalized linear mixed models in the

gamma family) 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 algal grouping separately. In addition, we compared the CV and mean of algal biomass as estimated by chlorophyll-*a* extraction and by absorbance at 750 nm and 680 nm in the same way we analyzed algal biovolume; however, it is not possible to break these measurements down by algal taxon, and so these proxies are comparable only to total algal biovolume. All data analysis was conducted using version 3.5.3 of the R software package (R Core Team 2017).

Results

While the addition of one or the other heteropteran predator by themselves did not significantly affect the variability of phytoplankton biomass, addition of both predators stabilized the community biomass of edible phytoplankton by reducing the biomass of both the largest herbivore, *Daphnia*, and of the smaller herbivores. In the short-term laboratory experiment, the predators reduced opposing herbivore groups: *Notonecta* reduced *Daphnia* biomass (GLM, $P<0.001$) by 97.1% averaged over both zooplankton concentrations without affecting the smaller herbivores, while *Neoplea* reduced the biomass of smaller herbivores (GLM, $P<0.001$) by 68.1% averaged over both zooplankton concentrations without affecting *Daphnia* biomass (Fig. 1). 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* (Table 1, Fig. 2). 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% (Table 1, Fig. 2).

Mean biomass of both *Daphnia* and the smaller herbivores was associated with increased variability of biovolume of smaller phytoplankton, but only *Daphnia* biomass was associated with increased variability of *Oocystis* 1 biovolume (Table 2). Not surprisingly, *Daphnia* biomass was also associated with increased variability of total phytoplankton biovolume, while the biomass of smaller herbivores was only marginally associated with increased variability of total phytoplankton biovolume. For all three algal groupings, mean biovolume was negatively associated with mean *Daphnia* biomass, but was not affected by mean biomass of smaller herbivores (Table 2).

Variability of the biovolume of smaller phytoplankton was not affected by adding either predator alone, but was reduced when both predators were added (Table 3, Fig. 3C). In contrast, variability of *Oocystis* 1 biovolume was not significantly affected by any predator treatment (Table 3, Fig. 3B). When both groups of phytoplankton were summed together as total community biomass, it was unclear whether predator diversity affected variability because different methods of biomass estimation yielded different conclusions. Absorbance *in vivo* at 680 nm, a crude proxy for algal biomass, was significantly less variable only when both predators were added (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). Mean phytoplankton biomass, estimated in any way or for any of the phytoplankton groupings, was not significantly affected by predator treatment (Table 3, Fig. 3D,E,F).

Discussion

Our results show that the two focal predator species, *Notonecta* and *Neoplea*, partitioned herbivorous prey, and that this complementarity led to a reduction in the variability of smaller, more edible phytoplankton biomass. *Notonecta* reduced *Daphnia* biomass and *Neoplea* reduced biomass of a group of smaller herbivores in the laboratory; accordingly, both herbivore groups were simultaneously reduced in the field only when both predators were added. Biomass of both herbivore groups was separately and positively associated with variability of biomass of smaller phytoplankton. In turn, variability of biomass of the smaller phytoplankton was only reduced in the treatment with both predators. Thus, as represented in Figure 4, the presence of both predators appeared to be necessary to suppress both *Daphnia* and the smaller herbivores at low densities, thereby freeing the smaller phytoplankton of enough herbivory to stabilize its temporal dynamics (Fig. 4d). Adding only one or the other predator failed to reduce both herbivore groups, allowing herbivore-induced variability to continue (Fig. 4b-c). On the other hand, the smaller herbivores did not affect the biomass variability of *Oocystis* 1, which was the largest phytoplankton taxon and therefore expected to be least vulnerable to small herbivores. Unsurprisingly, then, 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. 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 mean phytoplankton

biomass across predator treatments, and higher biomass of *Daphnia* – but not of smaller herbivores – was associated with lower mean biomass of both phytoplankton groups.

There are several potential reasons for the difference in the effect of *Neoplea* on the smaller herbivores in the laboratory versus the field experiment. One of the largest differences between the experiments is the length of time the predators were added: five days in the laboratory versus seven weeks in the field. The laboratory experiment attempted 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. 4). Whatever the reason for the weaker effect of *Neoplea* in the field, both predators were still needed to suppress both herbivore groups just as the laboratory results suggested.

The cascading diversity effect was not apparent in all tanks or taxa, but this 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. 3). 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 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. Still, the diversity-stability effect remained significant when the smaller ("edible") phytoplankton were defined in

every reasonable way, and is further supported by an intuitive mechanism linking complementarity to stability. The experiments were not set up to prove this mechanism, so our description of this mechanism amounts to our hypothesis for how predator complementarity led to lower algal variability. We did not know *a priori* which planktonic species would establish in the mesocosms, nor how they would interact, and so it was necessary to explore the data to find a hypothesized mechanism. Dominant species (i.e., *Arctodiaptomus*) that were not affected by the treatments obscured the potential food web mechanism unless removed from analysis. It is not surprising that some taxa were not involved in the cascading food web effect, since species within trophic levels commonly differ greatly in their capture probability and edibility. In 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.

This study provides the first 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 only mean biomass of trophic groups as dependent variables (Bruno and O'Connor 2005; Straub et al. 2008). This work tended to show that increasing predator diversity, as long as the predators partitioned resources, led to lower mean herbivore biomass and higher mean autotroph biomass – i.e., a diversity-biomass trophic cascade (Straub et al. 2008). In our field experiment, 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 higher 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 seemed to be 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 (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 autotroph biomass was more sensitive to changes in herbivore biomass than was average autotroph biomass, at least for the taxa more vulnerable to herbivory. Future studies will need to explore the generality of this result.

A few studies have reported the effects of predator diversity on the variability of predation or adjacent trophic levels, and some have described theoretical mechanisms relevant to these studies. 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. Ong and Vandermeer (2015) showed theoretically that the combination of a predator and a parasite can suppress a pest to a low, stable population. However, our study is the first (to our knowledge) to test whether predator diversity can cascade down a food web to reduce temporal variability of primary producer biomass. 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 should indirectly stabilize (and increase) plant biomass. However, we are not aware of a theoretical study making this argument; thus, our results suggest a new diversity-stability mechanism that could be important in 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 just as important. We showed that combining predators differing in a trait known to correlate with prey preference, body size, can be a key factor leading to the cascading stabilizing effect on phytoplankton. Phytoplankton are increasingly cultured as a crop for a variety of purposes from biomass production for biofuels, animal feed, or fertilizer, to nutrient removal from wastewaters. However, algal culturing is not yet widely practiced on large scales, in large part because it has so far proven too difficult to achieve consistently high algal yields as algae ponds are colonized by pests that are difficult or costly to control by mechanical or chemical means. Adding a functionally diverse array of predators to algae ponds may be a feasible, economical, self-sustaining way to encourage more reliable algal yields.

The ecological principles that seemed to govern the diversity-stability trophic cascade effect in our experiment are general and therefore may apply to terrestrial agriculture as well. Our results suggest a simple biological control technique wherein consortia of natural enemy species varying in size are added or encouraged to an agro-ecosystem to promote crop stability. Diversity of other functional traits related to prey choice, microhabitat preference, temporal patterns in control strength, or variance in mode of prey suppression may also encourage crop yield stability in a similar fashion and could also be managed in natural enemy consortia when relevant information is available. 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 both parasites and predators (Ong and Vandermeer 2015), leading to consistently low pest densities and stabilizing 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 higher complementarity of prey resource use, can cascade down to stabilize temporal dynamics of algal biomass without affecting mean algal biomass. 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. 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 spatial variability and 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.

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Literature cited

Adrian, Rita, and Thomas M Frost. 2018. "Omnivory in Cyclopoid Copepods: Comparisons of Algae and Invertebrates as Food for Three, Differently Sized Species," 16.

Anderson, David H., Stephen Darring, and Arthur C. Benke. 1998. "Growth of Crustacean Meiofauna in a Forested Floodplain Swamp: Implications for Biomass Turnover." *Journal of the North American Benthological Society* 17 (1): 21–36. https://doi.org/10.2307/1468049.

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

Douglas Bates, Martin Maechler, Ben Bolker, Steve Walker (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1), 1-48.<doi:10.18637/jss.v067.i01>.

Benemann, John. 2013. "Microalgae for Biofuels and Animal Feeds." *Energies* 6 (11): 5869–86. https://doi.org/10.3390/en6115869.

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 (2): 528–37. https://doi.org/10.1890/03-0816.

Bruno, John F., and Mary I. O'Connor. 2005. "Cascading Effects of Predator Diversity and Omnivory in a Marine Food Web: Cascading Effects of Predator Diversity." *Ecology Letters* 8 (10): 1048–56. https://doi.org/10.1111/j.1461-0248.2005.00808.x.

Cardinale, Bradley J., J. Emmett Duffy, Andrew Gonzalez, David U. Hooper, Charles Perrings, Patrick Venail, Anita Narwani, et al. 2012. "Biodiversity Loss and Its Impact on Humanity." *Nature* 486 (7401): 59–67. https://doi.org/10.1038/nature11148.

Cardinale, B. J., Emmett Duffy, Diane Srivastava, Michel Loreau, Matt Thomas, and Mark Emmerson. 2009. Towards a food-web perspective on biodiversity and ecosystem functioning. – In: Naeem, S. et al., Biodiversity and Human Impacts. Oxford University Press, pp. 105–120.

Culver, David A., Mary M. Boucherle, David J. Bean, and James W. Fletcher. 1985. "Biomass of Freshwater Crustacean Zooplankton from Length–Weight Regressions." *Canadian Journal of Fisheries and Aquatic Sciences* 42 (8): 1380–90. https://doi.org/10.1139/f85-173.

Dumont, Henri J., Isabella Van de Velde, and Simonne Dumont. 1975. "The Dry Weight Estimate of Biomass in a Selection of Cladocera, Copepoda and Rotifera from the Plankton, Periphyton and Benthos of Continental Waters." *Oecologia* 19 (1): 75–97. https://doi.org/10.1007/BF00377592.

EPA. 2016. “Standard Operating Procedure for Zooplankton Analysis” (LG403, Revision 07). Environmental Protection Agency, Washington, D.C.

Glazier, Douglas S. 2010. “A Unifying Explanation for Diverse Metabolic Scaling in Animals and Plants.” *Biological Reviews* 85 (1): 111–38. https://doi.org/10.1111/j.1469-185X.2009.00095.x.

Griffin, John N., and Brian R. Silliman. 2011. “Predator Diversity Stabilizes and Strengthens Trophic Control of a Keystone Grazer.” *Biology Letters* 7 (1): 79–82. https://doi.org/10.1098/rsbl.2010.0626.

Hall, Spencer R., Mathew A. Leibold, David A. Lytle, and Val H. Smith. 2004. “Stoichiometry and planktonic grazer composition over gradients of light, nutrients, and predation risk.” *Ecology* 85 (8): 2291–2301. https://doi.org/10.1890/03-0471.

Hampton, Stephanie E., and John J. Gilbert. 2001. “Observations of Insect Predation on Rotifers.” In *Rotifera IX*, edited by L. Sanoamuang, H. Segers, R. J. Shiel, and R. D. Gulati, 115–21. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-010-0756-6_17.

Jiang, Lin, and Zhichao Pu. 2009. “Different Effects of Species Diversity on Temporal Stability in Single□Trophic and Multitrophic Communities.” *The American Naturalist* 174 (5): 651–59. https://doi.org/10.1086/605961.

Kilham, Susan S, Daniel A Kreeger, Scott G Lynn, Clyde E Goulden, and Lazaro Herrera. 1998. “COMBO: A Defined Freshwater Culture Medium for Algae and Zooplankton.” *Hydrobiologia* 377: 147–59.

Leon, Blaustein. 1998. “Influence of the Predatory Backswimmer, Notonecta Maculata, on Invertebrate Community Structure.” *Ecological Entomology* 23 (3): 246–52. https://doi.org/10.1046/j.1365-2311.1998.00138.x.

Loreau, Michel, and Claire de Mazancourt. 2013. “Biodiversity and Ecosystem Stability: A Synthesis of Underlying Mechanisms.” Edited by Emmett Duffy. *Ecology Letters* 16 (May): 106–15. https://doi.org/10.1111/ele.12073.

Maron, John L, and Elizabeth Crone. 2006. “Herbivory: Effects on Plant Abundance, Distribution and Population Growth.” *Proceedings of the Royal Society B: Biological Sciences* 273 (1601): 2575–84. https://doi.org/10.1098/rspb.2006.3587.

McCauley, Edward. 1984. “The Estimation of the Abundance and Biomass of Zooplankton in Samples.” In *A Manual for the Assessment of Secondary Productivity in Fresh Waters*, edited by John A Downing and F.H. Rigler, 228–65. Blackwell Scientific Publishers.

Murdoch, William W., Mary Ann Scott, and Peter Ebsworth. 1984. “Effects of the General Predator, Notonecta (Hemiptera) Upon a Freshwater Community.” *The Journal of Animal Ecology* 53 (3): 791. https://doi.org/10.2307/4660.

Ong, Theresa Wei Ying, and John H. Vandermeer. 2015. “Coupling Unstable Agents in Biological Control.” *Nature Communications* 6 (1). https://doi.org/10.1038/ncomms6991.

Peralta, Guadalupe, Carol M. Frost, Tatyana A. Rand, Raphael K. Didham, and Jason M. Tylianakis. 2014. “Complementarity and Redundancy of Interactions Enhance Attack Rates and Spatial Stability in Host–Parasitoid Food Webs.” *Ecology* 95 (7): 1888–96. https://doi.org/10.1890/13-1569.1.

Purvis, Andy, John L. Gittleman, Guy Cowlishaw, and Georgina M. Mace. 2000. “Predicting Extinction Risk in Declining Species.” *Proceedings of the Royal Society of London.*

Series B: Biological Sciences 267 (1456): 1947–52. https://doi.org/10.1098/rspb.2000.1234.

R Core Team. 2017. "R: A language and environment for statistical computing." R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Schmitz, Oswald J. 2007. “Predator Diversity and Trophic Interactions.” *Ecology* 88 (10): 2415–26. https://doi.org/10.1890/06-0937.1.

Schneider, Caroline A, Wayne S Rasband, and Kevin W Eliceiri. 2012. “NIH Image to ImageJ: 25 Years of Image Analysis.” *Nature Methods* 9 (7): 671–75. https://doi.org/10.1038/nmeth.2089.

Smith, Val H., Belinda S.M. Sturm, Frank J. deNoyelles, and Sharon A. Billings. 2010. “The Ecology of Algal Biodiesel Production.” *Trends in Ecology & Evolution* 25 (5): 301–9. https://doi.org/10.1016/j.tree.2009.11.007.

Snyder, William E. 2019. “Give Predators a Complement: Conserving Natural Enemy Biodiversity to Improve Biocontrol.” *Biological Control* 135 (August): 73–82. https://doi.org/10.1016/j.biocontrol.2019.04.017.

Straub, Cory S., Deborah L. Finke, and William E. Snyder. 2008. “Are the Conservation of Natural Enemy Biodiversity and Biological Control Compatible Goals?” *Biological Control* 45 (2): 225–37. https://doi.org/10.1016/j.biocontrol.2007.05.013.

Sturm, Belinda S.M., Edward Peltier, Val Smith, and Frank deNoyelles. 2012. “Controls of Microalgal Biomass and Lipid Production in Municipal Wastewater-Fed Bioreactors.” *Environmental Progress & Sustainable Energy* 31 (1): 10–16. https://doi.org/10.1002/ep.10586.

Thébault, Elisa, and Michel Loreau. 2005. “Trophic Interactions and the Relationship between Species Diversity and Ecosystem Stability.” *The American Naturalist* 166 (4): E95–114. https://doi.org/10.1086/444403.

van Driesche, Roy, Mark Hoddle, and Ted Center. 2008. “Control of pests and weeds by natural enemies: an introduction to biological control.” Blackwell Publishing: Malden, MA.

Table 1. Results of gamma GLMMs testing the effects of predator additions in the field experiment on biomass of a) *Daphnia*, b) smaller herbivores, and c) both of these herbivore groups combined.

Parameter	**Estimate**	**SE**	**t**	***P***
a) *Daphnia*				
intercept	7.466	0.802	9.315	**<0.001**
Notonecta	-5.874	1.134	-5.183	**<0.001**
Neoplea	0.123	1.134	0.114	0.909
both predators	-4.186	1.134	-3.693	**<0.001**
b) smaller herbivores				
intercept	6.483	0.460	14.106	**<0.001**
Notonecta	-0.259	0.650	-0.399	0.690
Neoplea	-0.801	0.650	-1.233	0.218
both predators	-1.278	0.650	-1.966	**0.049**
c) both herbivore groups				
intercept	7.914	0.531	14.913	**<0.001**
Notonecta	-1.662	0.750	-2.216	**0.027**
Neoplea	-0.018	0.751	-0.024	0.981
both	-2.370	0.750	-3.159	**0.002**

Table 2. Results of gamma GLMs testing the effects of mean *Daphnia* biomass, and of summed biomass of smaller herbivorous zooplankton, on the CV of biovolume of a) total phytoplankton, b) *Oocystis* 1, and c) smaller phytoplankton.

Parameter	**Estimate**	**SE**	**t**	***P***
a) Total phytoplankton				
intercept	-1.171	0.1404	-8.339	<0.001
Daphnia	3.578×10^{-5}	9.861×10^{-6}	3.629	**0.0021**
smaller herbivores	2.434×10^{-4}	1.305×10^{-4}	1.865	0.0795
b) *Oocystis* 1				
intercept	-0.5875	0.1077	-5.458	<0.001
Daphnia	2.703×10^{-5}	7.562×10^{-6}	3.575	**0.0023**
smaller herbivores	5.127×10^{-5}	1.001×10^{-4}	0.512	0.6150
c) Smaller phytoplankton				
intercept	-1.239	0.1630	-7.600	<0.001
Daphnia	3.449×10^{-5}	1.145×10^{-5}	3.012	**0.0079**
smaller herbivores	3.613×10^{-4}	1.515×10^{-4}	2.385	**0.0290**

Table 3. Results of gamma GLMs testing the effects of predator additions in the field experiment on the temporal CV of biovolume of a) smaller phytoplankton (all but the largest morphospecies, *Oocystis* 1), b) *Oocystis* 1, and c) total phytoplankton.

Parameter	**Estimate**	**SE**	**t**	***P***
a) 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**
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) 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

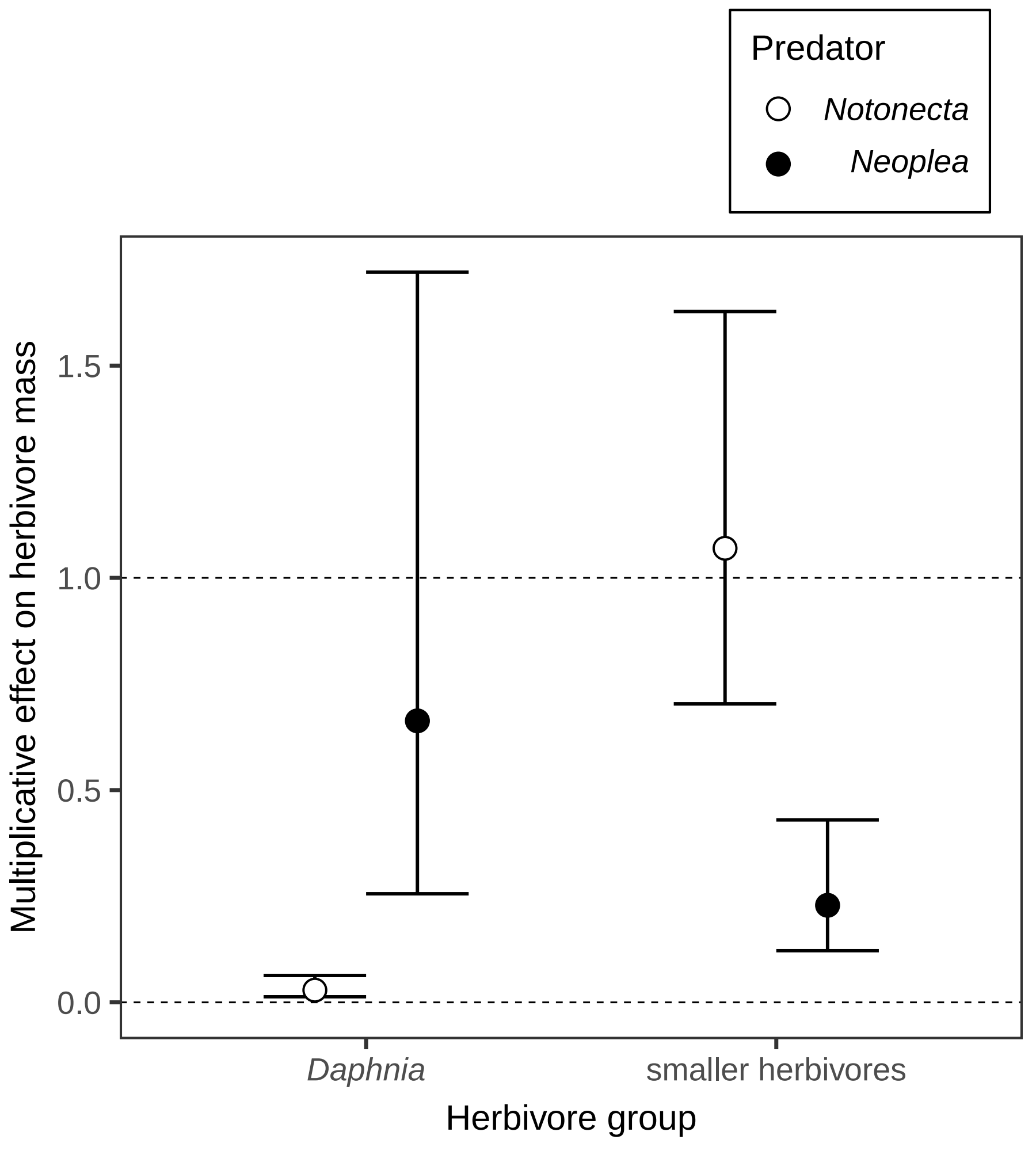
Figure legends

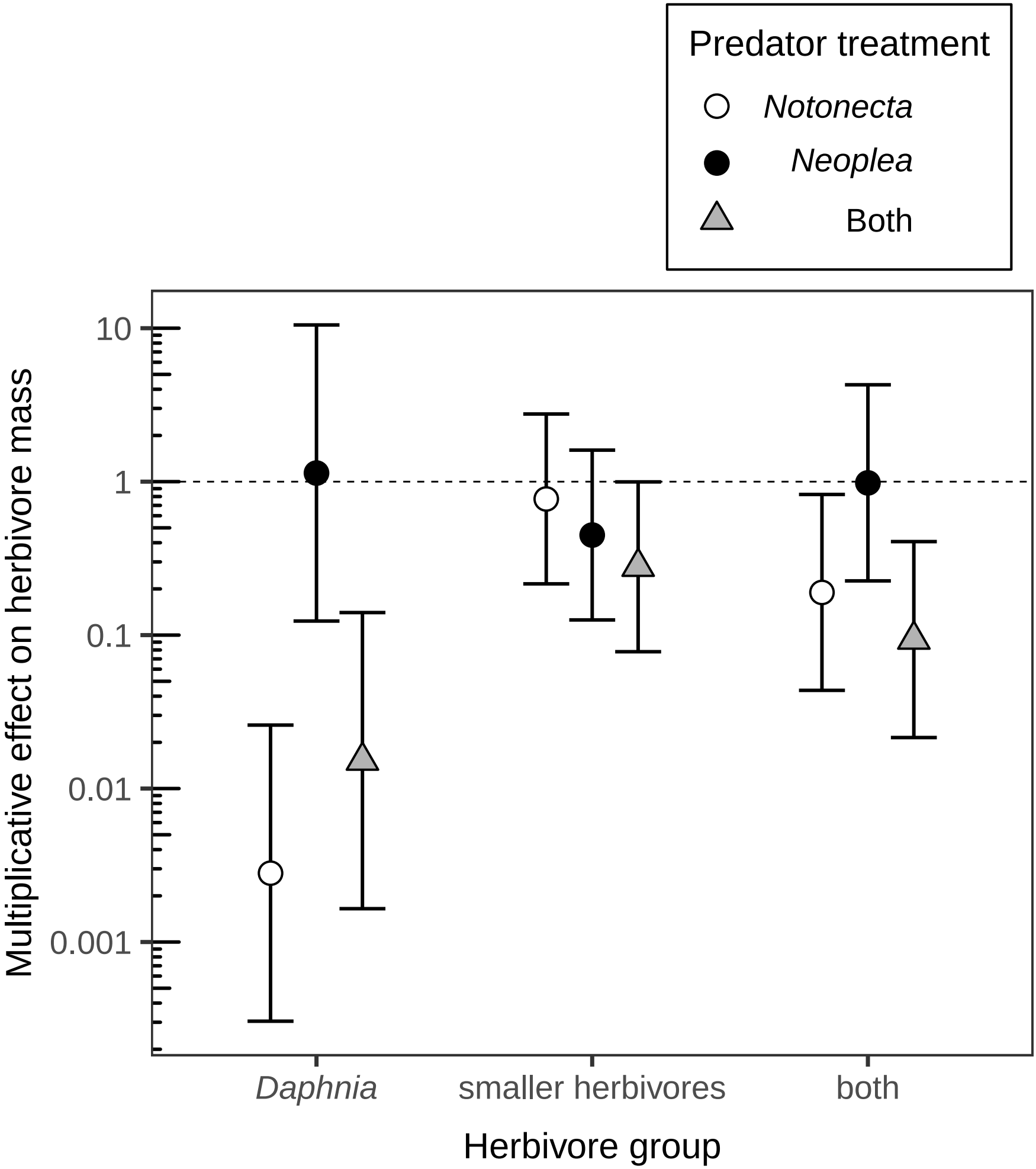
Figure 1. Multiplicative effects of addition of *Notonecta* and of *Neoplea* on biomass of *Daphnia* and on summed biomass of smaller herbivores in the laboratory experiment. Circles represent coefficient estimates from GLMs modeling the effects of predator addition on the mass of each herbivore group, and error bars represent 95% confidence intervals about the estimates. The dotted line at 1 represents no effect, and the dotted line at 0 represents total elimination of an herbivore group.

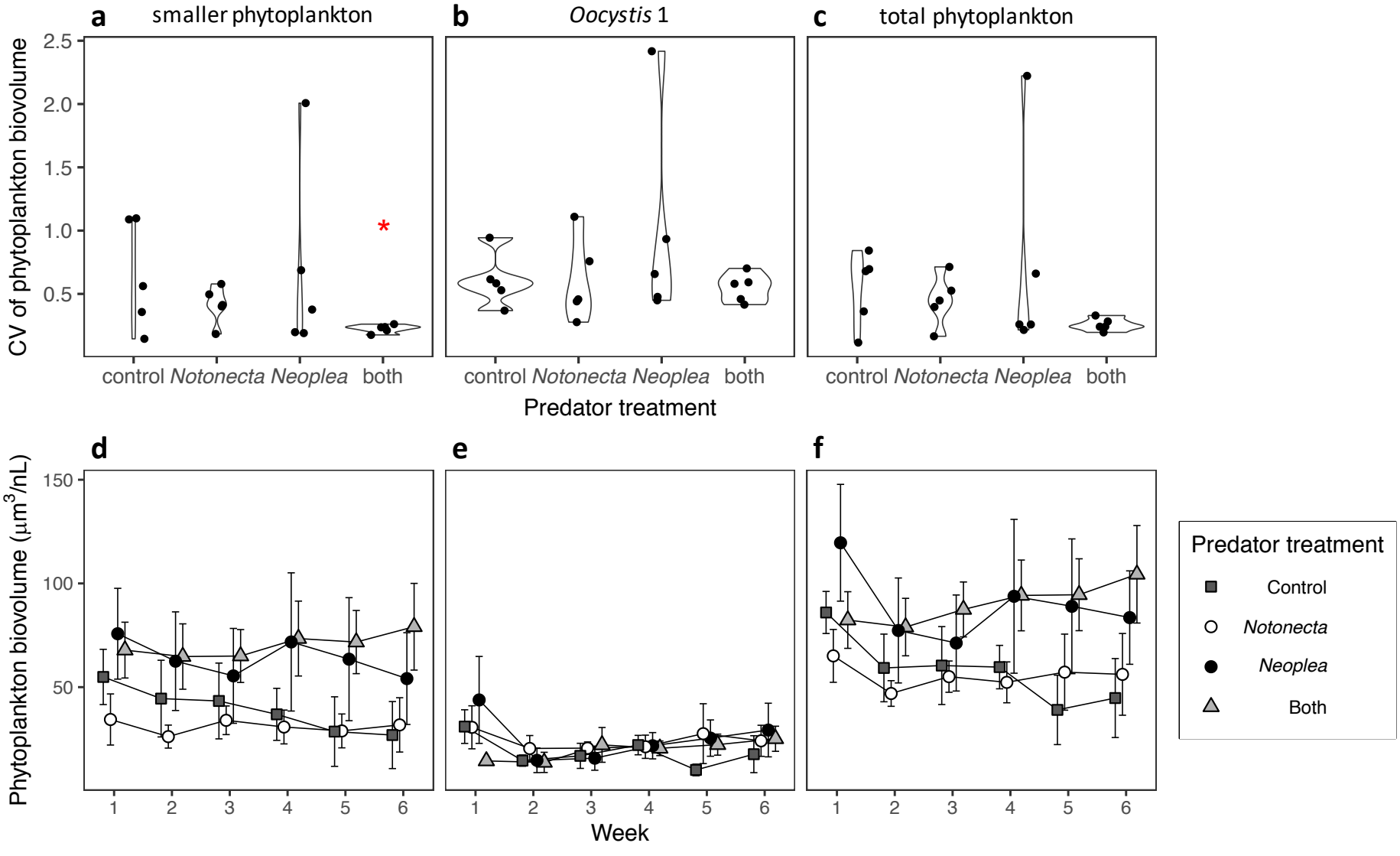
Figure 2. Multiplicative effects of addition of *Notonecta*, *Neoplea*, and of both predators together on biomass of *Daphnia*, summed biomass of smaller herbivores (excludes *Arctodiaptomus* and nauplii), and biomass of both herbivore groups summed together in the field experiment. Shapes represent coefficient estimates from GLMMs modeling the effects of predator addition on the mass of each herbivore group, and error bars represent 95% confidence intervals about the estimates, plotted on a natural log scale. The dotted line represents no effect.

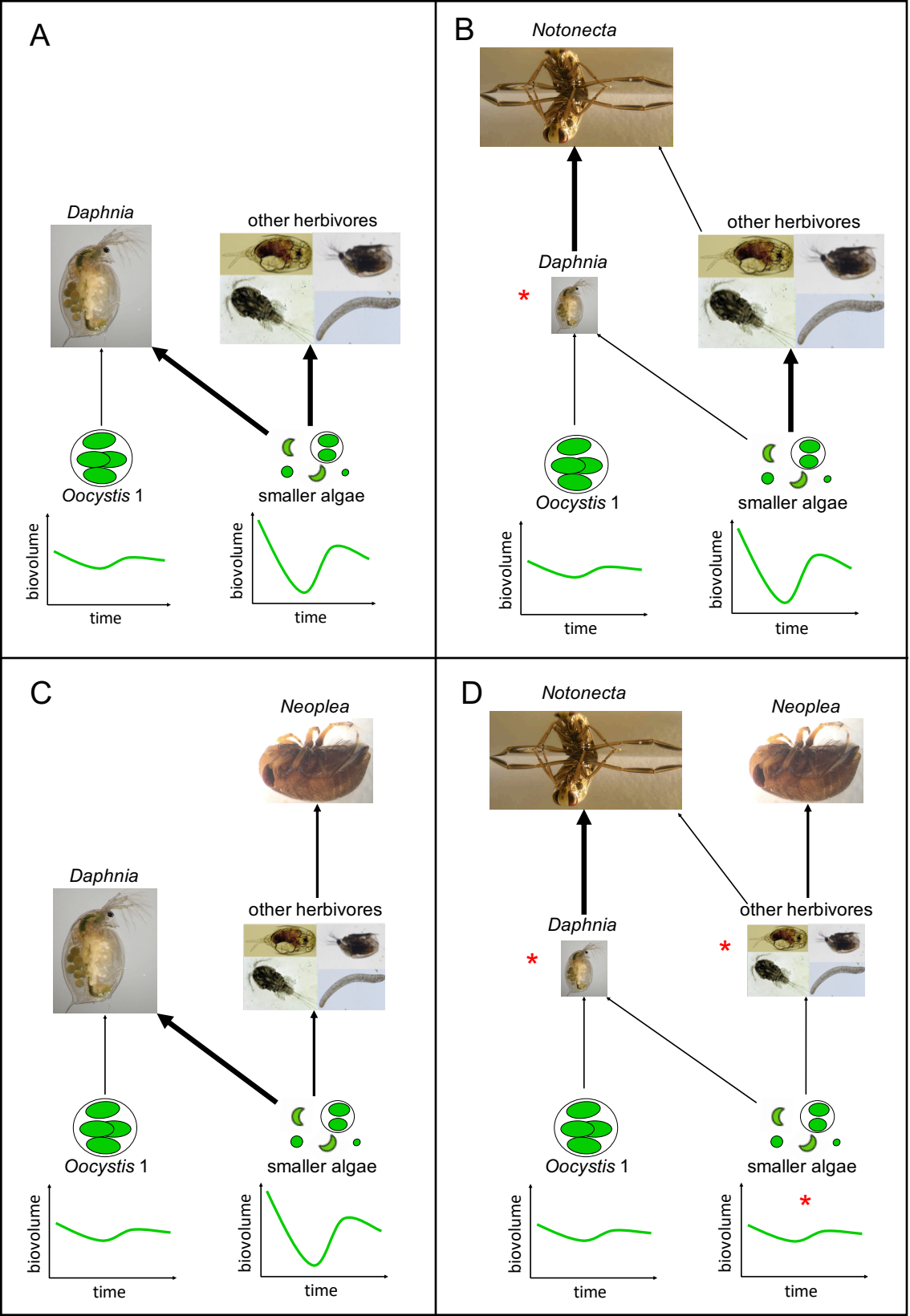
Figure 3. Temporal coefficient of variation (CV) of phytoplankton biovolume, a-c, and time series of mean phytoplankton biovolume, d-f, by predator treatment. Symbols in d-f are means ±1 SEM. Panels a and d show values for smaller phytoplankton (all besides the largest morphospecies, *Oocystis* 1), b and e show values for *Oocystis* 1, and c and f show values for total phytoplankton. The red star represents a significant effect of that predator treatment (GLMM, p=0.024) compared against the control.

Figure 4. Diagrams depicting the hypothesized food web and mechanism for food web effects on phytoplankton stability in the field experiment under each predator treatment. A represents the controls (no predators added), B represents addition of *Notonecta* only, C represents addition of *Neoplea* only, and D represents addition of both predators. Arrows represent hypothesized energy flow, with thicker arrows indicating faster consumption rates. Relative size of the *Daphnia* and "other herbivores" represent relative mean biomass. The time series at the bottom of each panel indicate relative temporal variability of each algal group. Red stars indicate significant differences compared against the control treatment (either in mean herbivore biomass with GLMM, or in CV of algal biovolume with GLM; α=0.05).


Predator
Notonecta
Neoplea
Multiplicative effect on herbivore mass
1.5
1.0
0.5
0.0
Daphnia
smaller herbivores
Herbivore group


Predator treatment
Notonecta
Neoplea
Both
10
1
0.1
0.01
0.001
Multiplicative effect on herbivore mass
Daphnia
smaller herbivores
both
Herbivore group


a
smaller phytoplankton
b
Oocystis 1
c
total phytoplankton
CV of phytoplankton biovolume
2.5
2.0
1.5
1.0
0.5
*
control Notonecta Neoplea both
Predator treatment
d
e
f
Phytoplankton biovolume (μm3/nL)
150
100
50
1 2 3 4 5 6
Week
Predator treatment
Control
Notonecta
Neoplea
Both


A
Daphnia
other herbivores
Oocystis 1
smaller algae
biovolume
time
biovolume
time
B
Notonecta
Daphnia
*
other herbivores
Oocystis 1
smaller algae
biovolume
time
biovolume
time
C
Neoplea
Daphnia
other herbivores
Oocystis 1
smaller algae
biovolume
time
biovolume
time
D
Notonecta
Neoplea
Daphnia
*
other herbivores
*
Oocystis 1
smaller algae
*
biovolume
time
biovolume
time