

1 **Beyond the fish-*Daphnia* paradigm: testing the**
2 **potential for *Neoplea striola* (Hemiptera: Pleidae) to**
3 **cause a trophic cascade in subtropical ponds**

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14

15 **Abstract**

16
17 Trophic cascades, or indirect effects of predators on non-adjacent lower trophic levels, are
18 thought to pervade diverse ecosystems, though they tend to be stronger in aquatic ecosystems.
19 Most research on freshwater trophic cascades focused on temperate lakes where *Daphnia* tend to
20 dominate the zooplankton community, and these studies identified that *Daphnia* plays a key role
21 in facilitating trophic cascades by linking fish to algae with strong food web interactions.
22 However, *Daphnia* are rare or absent in most tropical and subtropical lowland freshwaters, and
23 many invertebrate predators have received little attention in food web research despite being
24 common and widespread. Therefore, we aimed to test whether trophic cascades are possible in
25 small warmwater ponds where small invertebrates are the top predators and *Daphnia* are absent.
26 We collected naturally occurring plankton communities from small fishless water bodies in
27 central Texas and propagated them in replicate pond mesocosms. We removed zooplankton from
28 some mesocosms, left the plankton community intact in others, and added one of two densities of
29 the predaceous insect *Neoplea striola* to others. Following an incubation period we then
30 compared biomasses of plankton groups to assess food web effects between the trophic levels
31 including whether *Neoplea* caused a trophic cascade by reducing zooplankton. The zooplankton
32 community became dominated by copepods which prefer large phytoplankton and exhibit a fast
33 escape response. Perhaps due to these qualities of the copepods and perhaps due to slow
34 consumption rates by *Neoplea* on key grazers, no food web effects were found other than
35 zooplankton marginally reducing large phytoplankton. More research is needed to understand the
36 behavior and ecology of *Neoplea*, but trophic cascades may generally be weak or absent in
37 subtropical and tropical lowland freshwaters where *Daphnia* is rare.

38 39 **Introduction**

40
41 An extensive body of literature has demonstrated the importance of direct food web
42 effects, as well as indirect food web effects, or trophic cascades, with much of this work focusing
43 on freshwater ecosystems from an early time. Many of the studies on trophic cascades in
44 freshwater pelagic ecosystems have focused on large-bodied cladocerans, especially *Daphnia*, as
45 the herbivorous prey linking predators to autotrophs (e.g. Carpenter et al. 2001). However, a
46 relative paucity of such studies were performed in tropical and subtropical lowlands, where
47 *Daphnia* is rare (Dumont 1994). Furthermore, the predators that most of these studies focused on
48 were fish, with certain invertebrate taxa such as *Chaoborus* or *Notonecta* receiving some
49 attention while the food web effects of other common and widespread invertebrate predators
50 remain little known (Carpenter et al. 1992, 2001). Understanding the effects of these
51 understudied predaceous invertebrate species is important for understanding their role in
52 ecosystems and for conservation planning, as predators are more threatened with extinction than
53 lower trophic levels (Purvis et al. 2000).

54

55 Members of the family Pleidae are small but common heteropteran insects which are
56 closely related to the family Notonectidae which includes *Notonecta* (backswimmers). Pleids
57 have been much less studied than their larger cousins the notonectids (especially *Notonecta* and
58 *Buenoa*). In this paper we specifically study the pleid *Neoplea striola* (hereafter, *Neoplea*) as our
59 manipulated zooplanktivorous predator. *Neoplea* is a widespread inhabitant of lentic freshwater
60 in Central and North America, and is known to tolerate low oxygen conditions (Gittelman 1975).
61 It is a small insect, with adults measuring 1.5 mm in length. *Neoplea* is an active hunter which
62 uses sight, vibrations, and possibly chemicals to sense its prey (Papacek 2001). They have been
63 shown to attack and consume small zooplankton such as rotifers, mesozooplankton such as
64 *Daphnia*, and even prey as large as dipteran larvae (Gittelman 1974, Hampton and Gilbert 2001,
65 Papacek 2001). However, the effects of *Neoplea* on pond communities are still not fully
66 understood, including whether they can cause trophic cascades.

67
68 Here we report the results of a field mesocosm experiment in which we manipulated
69 densities of *Neoplea* and zooplankton to test the effects of both of these trophic groups on
70 plankton composition and biomass, including direct effects on the next trophic level and indirect
71 (trophic cascade) effects of *Neoplea* on phytoplankton. Our plankton communities were
72 composed of freshwater plankton collected locally in central Texas, with no *Daphnia* or other
73 large-bodied cladocerans present. Due to the fast escape response of copepods, we expected
74 *Neoplea* would reduce the biomass of non-copepod zooplankton, especially cladocerans and
75 ostracods. We further predicted that zooplankton would reduce the biovolume of total
76 phytoplankton, and that *Neoplea* would cause a trophic cascade, i.e. indirectly increase total
77 phytoplankton biovolume via its suppression of herbivorous zooplankton. However, we found no
78 evidence for food web effects of *Neoplea* and only weak evidence for effects of zooplankton on
79 phytoplankton in the experiment.

80

81 **Materials & Methods**

82

83 *Organism collection*

84

85 We allowed phytoplankton communities to naturally assemble in two plastic tanks at the
86 University of Texas' Brackenridge Field Laboratory in Austin, TX for ~3 months. We then
87 mixed 10 L from each tank with 12 L carbonated mineral water to narcotize any zooplankton,
88 filtered this mixture through 45 μ m mesh to remove the zooplankton, and mixed well to produce
89 a phytoplankton inoculum. To create a zooplankton inoculum, we collected and mixed
90 zooplankton-rich water from several small fishless water bodies nearby, and concentrated the
91 mixture with a 45 μ m mesh. We collected *Neoplea* from small water bodies in Austin, TX.

92

93 *Experiment setup and design*

94

95 We established 20 pond communities in 200 L cattle tanks in an unshaded field at
96 Brackenridge Field Laboratory. We fit the tanks with float valves to maintain constant water
97 levels and covered them in 1 mm² screens to prevent insect immigration. Prior to the experiment,
98 we analyzed total N and P in the water following standard American Public Health Association
99 methods (APHA 1989). We then added NaNO₃ to bring total N to 14 mg/L N and added
100 NaH₂PO₄•H₂O to bring total P to 1.55 mg/L P. These are the total N and P concentrations in
101 COMBO medium, a eutrophic medium commonly used for culturing plankton (Kilham et al.
102 1998). Every five or six days thereafter for six weeks, enough of both nutrients were added to
103 each tank to compensate for a 5% daily loss rate from the water column (as per Hall et al. 2004);
104 this same amount of both nutrients was also added immediately following the first sampling
105 (methods described below) after a 22-day pause.

106
107 We inoculated each tank with 600 mL of the phytoplankton inoculum. After allowing
108 phytoplankton to reproduce for five days, we added an equal volume of zooplankton inoculum to
109 15 of the tanks. To the other five tanks we added some of the filtrate left from concentrating the
110 zooplankton inoculum, to ensure all tanks received the same phytoplankton and picoplankton
111 species, as there may have been strains in the zooplankton inoculum not represented in the
112 phytoplankton inoculum. Lastly, after allowing the zooplankton to reproduce for 15 days we
113 added 20 *Neoplea* adults to five tanks with zooplankton and 40 *Neoplea* adults to another five,
114 then added the same amount to the same tanks two days later to bring the totals to 40 and 80.
115 Thus there were four treatments: no zooplankton added (“no zoop.”), zooplankton but no
116 *Neoplea* added (“no *Neoplea*”), zooplankton and 40 *Neoplea* added (“40 *Neoplea*”), and
117 zooplankton and 80 *Neoplea* added (“80 *Neoplea*”). Each treatment was replicated five times for
118 a total of 20 mesocosms arranged randomly.

119 120 ***Sampling and biomass estimation***

121
122 We sampled zooplankton and phytoplankton 40 days after adding the *Neoplea*, and then
123 again six days later. To sample zooplankton, we used tube samplers to collect ten whole water
124 column subsamples spread across each tank, and pooled them into a 10 L sample for each tank.
125 We filtered this sample through 65 µm mesh, returning any predators to the tank, and preserved
126 the retained organisms in 10% Lugol’s solution. To sample phytoplankton, we used PVC pipes
127 with 1 cm diameter (one per tank) to collect three whole water column subsamples spread over
128 the tank and pooled them into a 50 mL sample for each tank. We preserved these phytoplankton
129 samples in 10% Lugol’s solution. We additionally estimated surviving *Neoplea* populations after
130 the second plankton sampling by using a dipnet to count individuals until we returned three
131 successive empty sweeps.

132
133 To estimate biomass of zooplankton taxa, we identified, counted, and measured
134 zooplankton in subsamples such that for each taxon, at least 25 individuals or 10% of the sample

135 was counted – whichever came first – and at least 50 total individuals were counted. We used an
136 ocular micrometer to measure the length of each crustacean and individual *Spirostomum* to the
137 nearest half increment (0.24 mm), and to measure the length and width of each rotifer and width
138 of *Spirostomum* to the nearest 0.05 increment (0.024 mm). We converted crustacean length to
139 dry mass using length-mass regressions (Culver et al. [1985] for copepods, McCauley [1984] for
140 *Scapholeberis*, and Anderson et al. [1998] for ostracods). When rotifers were identified to
141 species, we converted rotifer dimensions to dry mass using species-specific equations from the
142 EPA protocol (EPA 2016). Otherwise, we used biovolume equations (McCauley 1984) and
143 converted to dry mass assuming a 10:1 biovolume:dry mass ratio. We converted *Spirostomum*
144 dimensions to dry mass by approximating cells as cylinders and assuming the same 10:1
145 biovolume:dry mass ratio.

146
147 To estimate biovolume of phytoplankton taxa, we calculated phytoplankton densities
148 with a hemocytometer. We counted 25 nL or 50 cells of the most common morphospecies –
149 whichever came first – and we counted at least 100 nL for the other taxa. We also took several
150 micrographs of each morphospecies from various tanks and sampling dates and used ImageJ to
151 measure the cell dimensions (Schneider et al. 2012), measuring at least 15 cells of all but the
152 rarest morphospecies. We then used geometric approximations to calculate the biovolume of
153 each cell.

154 155 ***Data analysis***

156
157 Based on previous research (Rakowski et al. *under review*), we expected *Neoplea* to have
158 differential effects on zooplankton groups based on taxonomy/behavior and size. Copepods have
159 been shown to have faster escape responses than other zooplankton, so we analyzed their
160 biomass separately. Since copepods dominated the zooplankton community, we analyzed the
161 sum of all other zooplankton biomass (“non-copepods”) as we predicted *Neoplea* would
162 primarily affect these other taxa. We also separately analyzed the major groups of non-copepod
163 zooplankton, including cladocerans and ostracods as one group (grouped for similarity in size,
164 morphology, and slow swimming speed), *Spirostomum* as its own group as it is quite distinct
165 from any other taxon in the community, and lastly the rotifers. We expected zooplankton to
166 either affect the whole phytoplankton community, or alternatively to affect only a certain size
167 class, either large or small morphospecies. Therefore we analyzed total phytoplankton biovolume
168 as well as the summed biovolume of larger morphospecies and of smaller morphospecies.

169
170 We tested for a difference in *Neoplea* survival between the two densities using a t test. To
171 analyze the effects of zooplankton and *Neoplea* additions on the biomass of plankton groups, we
172 fit a generalized linear mixed model in the gamma family (gamma GLMM) for each plankton
173 grouping using the *lme4* package (Bates et al. 2015). We included tank as a random effect to
174 account for the repeated measures, and fixed effects for zooplankton addition and initial *Neoplea*

175 density. We also fit the nested models excluding *Neoplea* density as well as the null models with
176 neither *Neoplea* density nor zooplankton addition, and used likelihood ratio tests to assess
177 whether including either addition significantly improved model fit. All analyses were performed
178 in R v. 3.5.3 (R Core Team 2017).

179

180 Results

181

182 On average *Neoplea* survived at a rate of 74%, with no significant difference in survival
183 between the two densities (d.f. = 7.98, $t = -0.518$, $P = 0.618$). No evidence was found of *Neoplea*
184 reproduction during the experiment.

185

186 By the sampling dates, the zooplankton community had become dominated by copepods.
187 However, cladocerans, *Spirostomum*, and rotifers all individually composed at least 15% of the
188 zooplankton mass on average in one or more treatments (Fig. 1). A small amount of zooplankton,
189 mostly copepods, became established in the control tanks receiving no zooplankton (Fig. 1, Fig.
190 2). The zooplankton additions were successful in sustaining a significant increase in biomass of
191 all zooplankton groups (Table 1, Fig. 2). However, *Neoplea* additions had no significant effects
192 on the biomass of any zooplankton group (Fig. 2, Table 1). Mean copepod mass was higher and
193 the mean proportion of rotifers was lower the more *Neoplea* were added (Fig. 1), but neither
194 copepod nor rotifer mass was significantly affected by *Neoplea* (Fig. 2, Table 1).

195

196 The phytoplankton community became dominated by ovoid single-celled green algae,
197 with pennate diatoms contributing the next most biovolume in tanks with no zooplankton added
198 and *Oocystis* contributing the next most biovolume in tanks with 80 *Neoplea* added (Fig. 3).
199 While the average total phytoplankton biovolume with zooplankton was less than half of the
200 average without zooplankton, there was no significant effect of zooplankton addition on total
201 phytoplankton biovolume due to large variation within treatments (Fig. 3, Fig. 4A, Table 3A,
202 Table 4A). When the largest morphospecies were taken together, including the larger ovoid
203 chlorophytes, pennate diatoms, and *Oocystis*, zooplankton reduced their summed biovolume
204 marginally significantly by 77.9% (Fig. 4B, Table 3B, Table 4B). On the other hand, summed
205 biovolume of the smaller morphospecies (small ovoid chlorophytes, *Chlorella*, *Selenastrum*, and
206 photosynthetic picoplankton) was not significantly impacted by zooplankton addition (Fig. 4C,
207 Table 3C, Table 4C). Similarly, *Neoplea* addition had no significant effects on the biovolume of
208 any phytoplankton grouping (Fig. 4, Table 3).

209

210 Discussion

211

212 Our results show that *Neoplea* had no demonstrable effect on the plankton in this
213 experiment. Furthermore, the copepod-dominated zooplankton only caused a marginal reduction
214 of larger phytoplankton taxa. Any effect of *Neoplea* on phytoplankton composition or biomass

215 would have likely been mediated by an effect on zooplankton composition or biomass, as
216 *Neoplea* are carnivorous. Therefore it is unsurprising there was no indirect effect of *Neoplea* on
217 phytoplankton, or trophic cascade, considering there were only weak or nonexistent direct effects
218 between the three trophic levels.

219
220 There are several potential reasons why *Neoplea* did not reduce the biomass of any
221 zooplankton groups in our experiment. First, it is possible *Neoplea*'s predation rate was similar
222 enough to the reproduction rate of its prey that prey populations did not significantly change over
223 the course of the 46-day experiment, a scenario most likely if *Neoplea* preferred prey with short
224 generation times such as cladocerans. While a few studies observed predation by *Neoplea* on
225 various prey taxa, we are aware of only one study that measured the effect of *Neoplea* on the
226 biomass of prey taxa over time (Rakowski et al. *under revision*). In this study *Neoplea* had strong
227 effects on non-copepod zooplankton, which were dominated by fast-reproducing cladocerans, on
228 the short term (under one cladoceran generation). In contrast, *Neoplea* had weak effects on a
229 longer term (~six cladoceran generations) in the absence of other predators. Interestingly
230 however, when the larger predator *Notonecta* was also present, *Neoplea* acted synergistically
231 with *Notonecta* to suppress the non-copepod zooplankton. Therefore it may be that *Neoplea*
232 exhibits a slow predation rate and prefers prey with short generation times, with the effect that
233 over the long term it can have a cryptic food web effect not apparent unless other predators are
234 present.

235
236 Other potential reasons for the lack of an effect of *Neoplea* on zooplankton relate to other
237 aspects of the predaceous insect's behavior and to the composition of potential prey. It is
238 possible that *Neoplea* consumed chironomid larvae, which were common in the mesocosms but
239 not effectively sampled due to their benthic nature and our sampling methods targeting plankton.
240 *Neoplea* is known to attack and consume chironomid larvae (Papacek 2001). Indeed, the *Neoplea*
241 were most commonly observed clinging to the sides and bottom of the mesocosms, putting them
242 in close proximity to the chironomids. In nature, *Neoplea* is normally found clinging to
243 submerged vegetation (Gittelman 1974). However, it was impossible to observe the *Neoplea*
244 throughout most of the experiment due to the high density of phytoplankton. This high turbidity
245 may have made it difficult for the *Neoplea* to hunt effectively, as they can detect prey visually
246 and are most commonly found in clear waters, although they can also detect prey by tactile and
247 possibly chemosensory methods (Gittelman 1974, Papacek 2001). When the *Neoplea* did swim
248 through the water column, they would have mostly encountered copepods, the largest and
249 dominant group of zooplankton. However, even in clear water copepods are relatively resistant
250 to predation due to their fast escape response. Among copepods, diaptomids have an especially
251 fast escape response, and the diaptomid *Arctodiaptomus* dominated our mesocosms. Therefore it
252 may not be surprising that the *Neoplea* were unsuccessful in suppressing the zooplankton in the
253 experiment. While other more easily captured prey were also present, it may have been difficult
254 for the *Neoplea* to encounter these rare prey among all the copepods in a turbid environment.

255

256 The dominance of copepods in the zooplankton community may also explain the weak
257 effects of zooplankton on phytoplankton in the experiment. Unlike *Daphnia*, copepods do not
258 generally impose strong top-down control on community phytoplankton biomass, due in part to
259 their selective grazing on larger phytoplankton (Sommer and Sommer 2006). Indeed, the
260 copepod-dominated zooplankton only reduced larger phytoplankton, and even this effect was
261 marginal, fitting the general understanding of copepods' top-down effects on phytoplankton in
262 ecosystems with copepod-dominated herbivore communities such as open oceans (Sommer and
263 Sommer 2006). Such weak top-down control of crustacean zooplankton on phytoplankton may
264 be more pervasive at low latitudes, as *Daphnia* is largely absent from tropical and subtropical
265 lowland freshwaters and copepods are instead more likely to dominate (Dumont 1994, Havens
266 and Beaver 2011). In combination with the ability of copepods to evade predation much more
267 easily than *Daphnia*, it appears likely that trophic cascades mediated by zooplankton are less
268 common in warm, low-latitude lakes and ponds than in their colder counterparts (Rejas et al.
269 2005).

270

271 The food web ecology of *Neoplea* and pleids generally will remain unclear without
272 further research. More behavioral research on these diminutive predators is needed to better
273 understand their hunting habits, such as where in the habitat they make most of their captures,
274 their relative dependence on different senses for prey detection, and their relative preference for
275 various prey. Experimental work is needed to better quantify the ecology of pleids, and these
276 experiments will likely benefit from better replicating their preferred habitat of clear, still water
277 with submerged vegetation. A higher population density may be necessary for their food web
278 effects to be apparent when they are the sole predator.

279

280 **Conclusions**

281

282 We predicted that *Neoplea* would suppress non-copepod zooplankton, and that
283 zooplankton would suppress phytoplankton, resulting in a trophic cascade where *Neoplea*
284 indirectly increased phytoplankton biomass. Instead, *Neoplea* had no effect on plankton biomass
285 or composition in this field mesocosm experiment. The zooplankton, dominated by copepods and
286 lacking *Daphnia* as is typical of lowland tropical and subtropical lakes and ponds, only weakly
287 reduced larger phytoplankton. While our data cannot definitively explain these weak effects, they
288 could have resulted from *Neoplea* consuming prey able to reproduce quickly enough to make up
289 for losses to predation, from *Neoplea* consuming benthic prey which was not effectively
290 sampled, or from the dominance of copepods which are adept at evading capture and which
291 selectively feed on larger phytoplankton. This study suggests that lentic ecosystems dominated
292 by *Neoplea* and copepods may be characterized by weak top-down control. This represents
293 another example of a weak or non-existent trophic cascade in lowland tropical or subtropical
294 lentic freshwater, which appears to be much more common in these systems than in cooler lakes

295 and ponds. However, more research will be needed to achieve a clearer understanding of the
296 ecological impacts of pleids. A better understanding of the ecology of these and other
297 understudied invertebrate predators will be important for conservation planning, as predators are
298 more threatened with extinction than lower trophic levels, a pattern that does not only apply to
299 charismatic megafauna.

300

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302

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308

309 **References**

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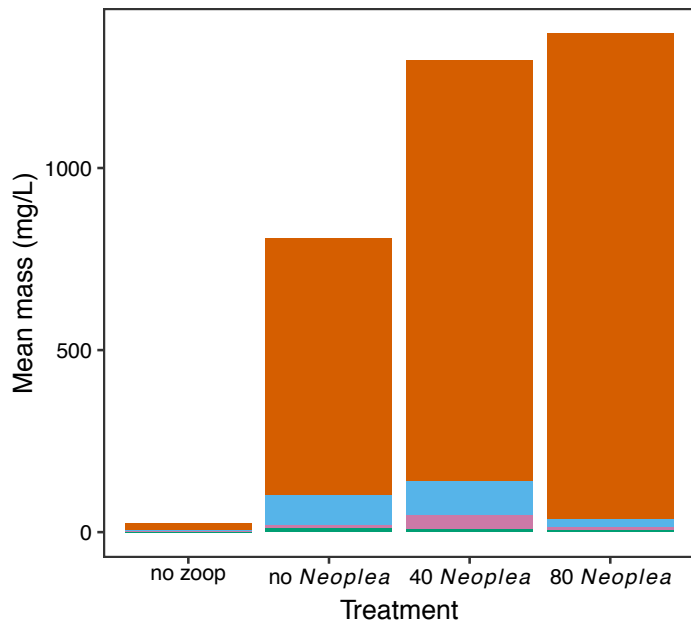
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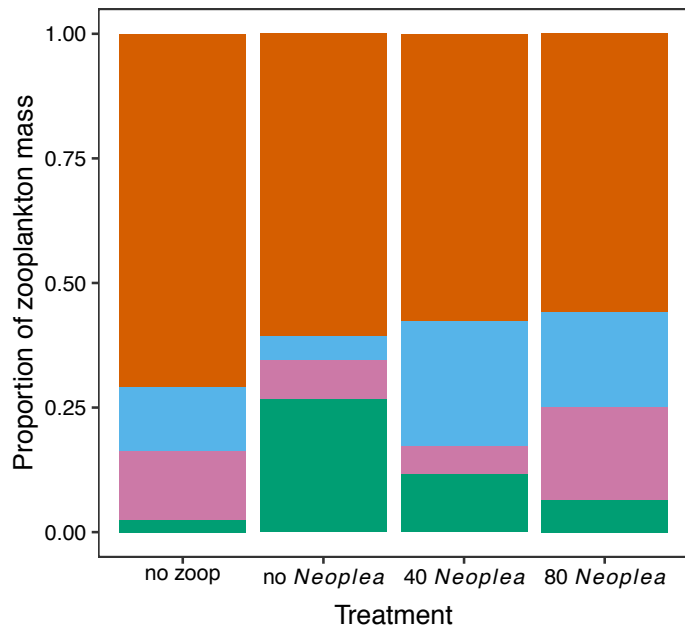
Zooplankton group

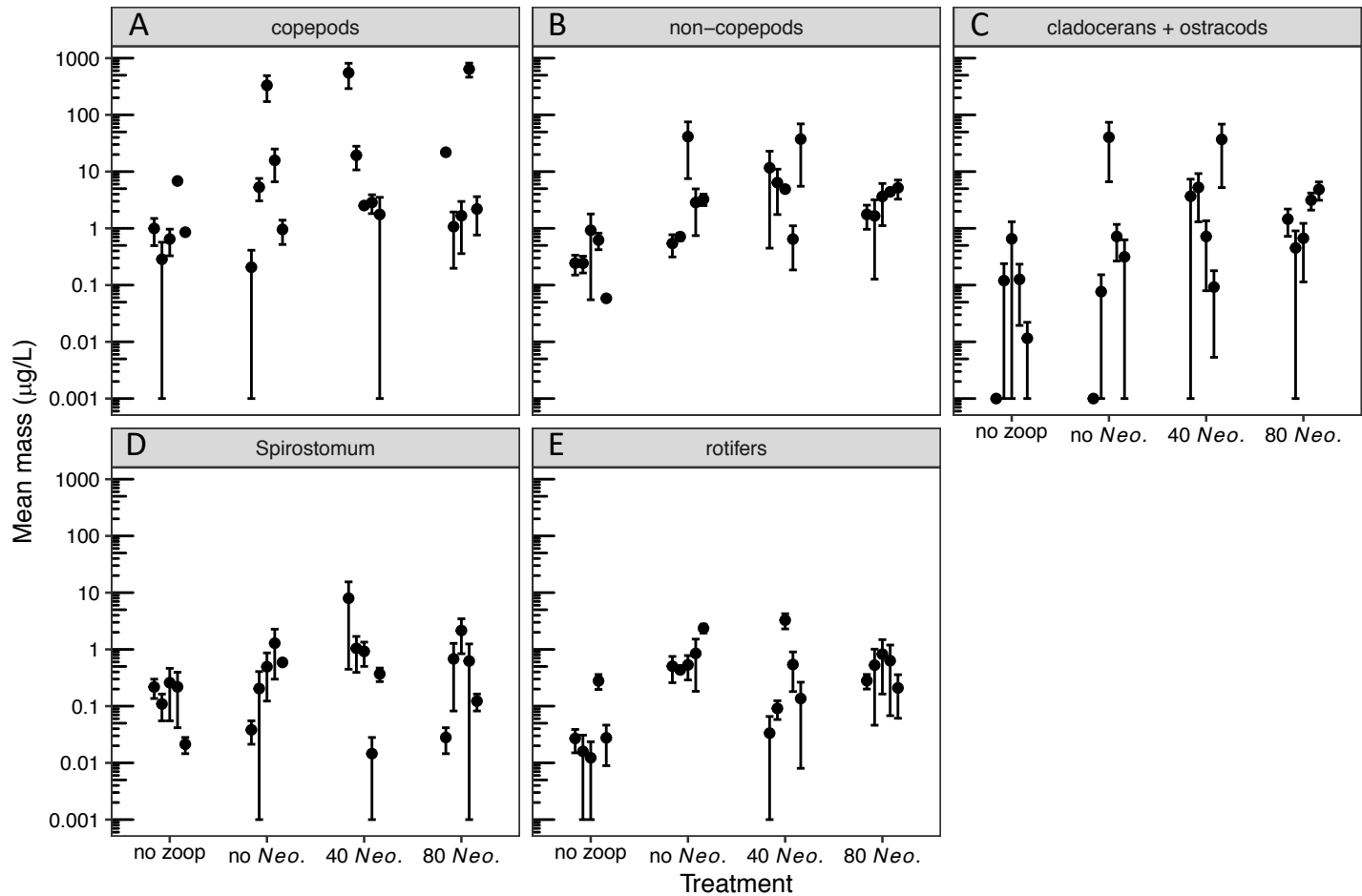
- copepods
- cladocerans + ostracods
- *Spirostomum*
- rotifers

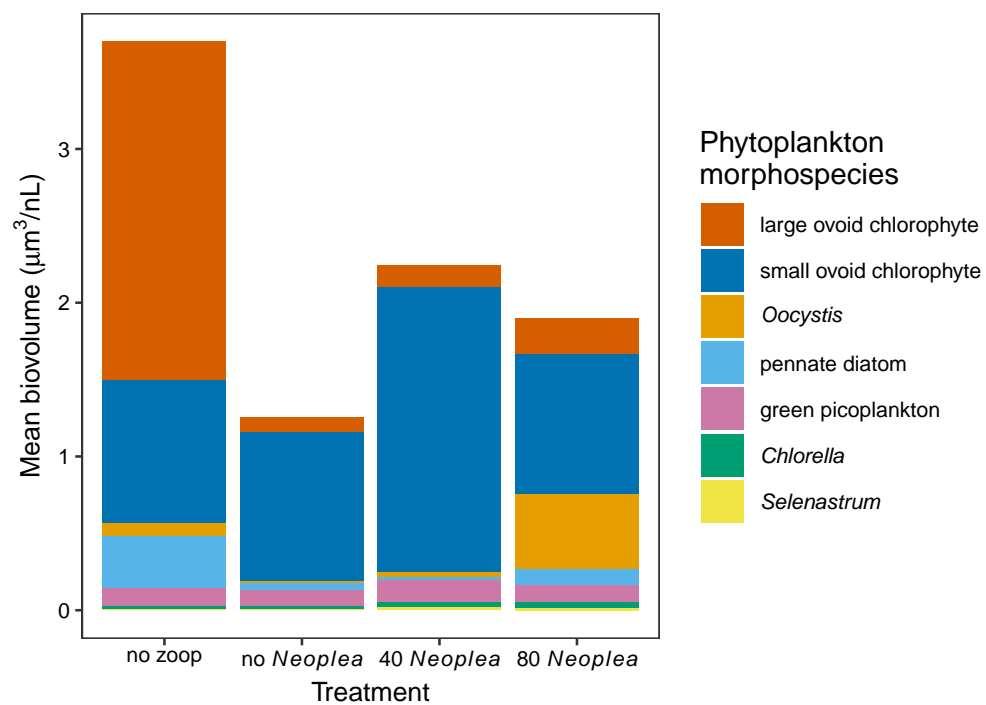
A



B







Mean phytoplankton biovolume ($\mu\text{m}^3/\text{nL}$)

