

# Trait-dependency of trophic interactions in zooplankton food webs

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## Introduction

2 Trophic interactions determine population dynamics, evolutionary trajectories and the stocks and fluxes  
of energy in ecosystems (Polis and Winemiller 1996). Therefore, uncovering general rules that shape  
4 trophic interactions represents a central objective of ecological research. To reach this goal, researchers  
classically either use *a priori*-built models whose predictions are compared to observed food webs, or  
6 infer general rules *a posteriori* through mechanistic or statistical models fitted to observed networks  
(Rohr et al. 2017). Both approaches require high-quality, detailed food-web data built from direct  
8 observations. Such data are scarce and costly to acquire. A third, cost-effective but indirect approach to  
inferring trophic interactions is to experimentally measure community response to manipulating  
10 presence-absence of particular predators in the food web (Hulot et al. 2014).

12 Experimental manipulations of predator-prey systems have a long history in ecological research and, in  
particular, have proven powerful to reveal whether and how trophic interactions are predictable from  
14 single-trait phenotypes (Bolker et al. 2003, Werner and Peacor 2003, Schmitz 2008). Such studies have,  
for instance, confirmed the major role played by body size in structuring trophic interactions  
16 (Emmerson and Raffaelli 2004), a role that was already envisioned by Elton (1927) and robustly  
explored since then (Cohen et al. 1993, Woodward et al. 2005, Brose et al. 2006). Recently, such  
18 manipulative experiments have demonstrated that predator hunting mode interacts with prey  
antipredator behaviour in controlling predator-prey interactions and, from there, in determining both  
20 the strength of trophic cascades (Schmitz et al. 2004, Schmitz 2008) as well as the architecture of  
whole food webs (Lazzaro et al. 2009).

22

Besides body size, hunting mode and antipredator behaviour, other phenotypic traits may be involved  
24 in controlling the occurrence and strength of trophic links (e.g. palatability, chemical defences or  
mimicry). However, these other traits are often difficult to identify or measure, and are poorly reported  
26 in the literature. To tackle this complexity issue, phylogeny may be used as a “holistic trait”  
encapsulating the complex phenotypes that determine trophic niches. Accordingly, several studies  
28 suggest that phylogeny (using taxonomy as a proxy) has a higher power than body size in predicting  
trophic links in observed terrestrial and aquatic food webs (Cattin et al. 2004, Naisbit et al. 2012).  
30 Recently, some authors have further suggested that even a coarse phylogenetic resolution might still  
retain enough information to efficiently predict trophic links (Gauzens et al. 2013).

32

To our knowledge, however, no study has quantified the respective contributions of body size,  
34 antipredator behaviour, and phylogeny at different resolutions in driving the presence and strength of  
trophic interactions in complex food webs. To contribute bridging this knowledge gap, we measured  
36 the numerical response of a zooplankton community to the experimental manipulation of their fish  
predators in lake enclosures. We expected fish predation to have both direct effects through fish-  
38 zooplankton trophic interactions, and indirect effects through reorganizations of zooplankton-  
zooplankton and zooplankton-phytoplankton trophic interactions. To investigate the role of  
40 zooplankton traits in driving both these direct and indirect effects, we grouped zooplankters based on  
their body size, antipredator behaviour or on both their body size and antipredator behaviour, and we  
42 made qualitative predictions on whether and how each zooplankton group should numerically respond  
to fish. Specifically, we predicted fish to deplete large-bodied zooplankton, and to favour increased  
44 abundances of small-bodied zooplankton due to relaxed predation and competition from large-bodied  
zooplankton. We further predicted that, at a constant total fish biomass, smaller-bodied fish should have  
46 a higher cumulated attack rate on large-bodied zooplankton. Based on antipredator behaviour, we

predicted (i) “fleeing” zooplankton strategists to efficiently escape predation from cruising-foraging  
48 fish but not from visual-feeding fish, (ii) “hiding” zooplankton strategists to efficiently escape  
predation from both type of fish, and (iii) “no escape” zooplankton strategists to be equally depleted by  
50 both type of fish. Adding the effects of body size on top of the effects of antipredator behaviour  
changed (i) the predicted response of small-bodied, “no escape” zooplankton strategists to fish from  
52 negative to positive, and (ii) the predicted response of “hide” zooplankton strategists from nil to  
positive because they feed on small-bodied, “no escape” zooplankton. We formulate more detailed and  
54 referenced predictions in the Methods section.

56 We compared these qualitative predictions with observed effect sizes and, additionally, we adopted a  
model selection procedure that quantified the relative power of size- and behaviour-based zooplankton  
58 groups to predict zooplankton response to fish manipulation. We further grouped zooplankters based on  
phylogeny at a low, medium or high resolution, and included these phylogeny-based groups into the  
60 model selection procedure. In doing so, we combined phylogeny-based grouping with ontogeny-based  
grouping among Copepods (i.e., nauplii vs. copepodites and adults), as is usual when describing  
62 zooplankton communities. We show that body size-based zooplankton grouping outperformed  
behaviour-based zooplankton grouping in predicting zooplankton numerical response to fish, but  
64 performed less well than a grouping structure combining both zooplankton body size and behaviour. A  
phylogeny-based zooplankton grouping structure provided the overall best and most parsimonious fit to  
66 the data, and was therefore most powerful at predicting zooplankton numerical response to fish  
predators, but only at a high to medium phylogenetic resolution and when ontogeny was also accounted  
68 for.

## Materials and Methods

### 72 Experimental setup

Nine enclosures made of translucent polyethylene were installed on a floating pontoon on Lake Créteil, a 42-ha shallow lake of 4 m mean depth (max depth 6m). Whereas they did not include lake sediments, our enclosures were 1.5 m x 1.5 m x 4.5 m deep (9.5 m<sup>3</sup>) and, hence, realistically reproduced lake vertical heterogeneity. Enclosures were filled with lake water pumped from a 1.5-meter depth from June 29<sup>th</sup> to July 3<sup>rd</sup> 1995. After filling, enclosures were enriched with living zooplankton sampled from the pelagic area of the lake. In order to avoid nutrient limitation of phytoplankton, inorganic nutrients were added twice a week with a liquid mixture of potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) and ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) with a N:P ratio of 20:1 by weight, for a load of phosphorus of 3.16 µg L<sup>-1</sup> d<sup>-1</sup>. At the start of the experiment (July 13<sup>th</sup>), the zooplankton biomass was > 200 µg dry weight L<sup>-1</sup>. Additional details on the experimental setup can be found in Bertolo et al. (1999b) and in Bertolo et al. (2000).

On July 13<sup>th</sup>, three different triplicate treatments were applied randomly to the enclosures: (i) “Control”: fishless; (ii) “Perch”: 18 planktivorous perch (aged 0+), measuring 5.97cm ± 0.55 cm (fork length) and weighting 2.45 ± 0.76 g (fresh weight); (iii) “Roach”: six roach aged of at least a year (1+), measuring on average 8.13 ± 0.37 cm and weighting on average 7.50 ± 0.50 g. The different numbers of roach and perch per enclosure were used to standardize fish biomass at 20 g.m<sup>-2</sup>, which can be considered as a moderate stocking rate (Williams and Moss 2003). Hence, in our design fish identity is confounded with fish density, body size and hunting mode (see below), and we could thus not identify which fish traits would actually determine different zooplankton response when comparing perch to

92 roach. For this reason, our study is based on exploring zooplankton rather than fish traits and how these  
influence zooplankton response to fish.

94

### **Sampling**

96 In order to alleviate constraints inherent to mesocosms (higher fish biomass than in natural systems,  
limited movements of zooplankters, and severe prey depletion), we limited the duration of the  
98 experiments to 20 days. Water was sampled at every 30-cm depth in each enclosure using a 2-L  
Friedinger bottle on three dates (July 18<sup>th</sup> and 25<sup>th</sup>, August 1<sup>st</sup> 1995). All samples from the same  
100 enclosure were pooled (24 L in total). Zooplankton of the pooled sample was filtered through a 50  $\mu\text{m}$   
sieve and fixed in 4% formalin. One to two litres of 50  $\mu\text{m}$ -sieved water (depending on filter clogging)  
102 were filtered on a GF/C glass fiber filter (nominal cut-off: 1.2  $\mu\text{m}$ ) for estimation of chlorophyll *a*  
content. Algal cells were strongly dominated by small algae ( $< 25 \mu\text{m}$ ) mainly belonging to  
104 Chlorophyceae, Cryptophyceae and diatoms (Bertolo et al. 1999b, 2000), and a negligible fraction was  
retained on the 50  $\mu\text{m}$  sieve. Chlorophyll *a* was measured spectrophotometrically ( $\mu\text{g L}^{-1}$ ) after  
106 grinding the samples in 90% acetone and centrifugation (AFNOR 1990).

108 Before being analysed under a stereomicroscope, each zooplankton sample was subsampled and diluted  
so as to standardize abundances. Zooplankton counts from diluted subsamples were then transformed to  
110 zooplankton concentration (mean count  $\text{L}^{-1}$ ) in each enclosure. Zooplankton individuals were identified  
at the species level when only one species was present and at the genus level when two congeneric  
112 species co-occurred. Copepods were present in two development stages, specifically as nauplii vs.  
copepodites and adults (Reid and Williamson 2010). Copepod nauplii were segregated into nauplii of  
114 Calanoids and nauplii of Cyclopoids. Cladocera, and copepodites of Copepods were measured for body  
length (in mm, 40 individuals per zooplankton category and per sample).

**Zooplankton taxa**

118 We identified five herbivorous cladoceran genera: *Ceriodaphnia* [*C. pulchella* Sars 1862 and *C.*  
120 *quadrangula* (O.F. Müller 1785)], *Bosmina* [*B. longirostris* (O.F. Müller 1785)], *Diaphanosoma* [*D.*  
*brachyurum* (Liévin 1848)] and *Daphnia* (*D. longispina* complex), listed according to their relative  
abundance. The omnivorous copepods were represented by the Calanoid species *Eudiaptomus gracilis*  
122 (Sars 1863) and the Cyclopoid species *Acanthocyclops robustus* (Sars 1863), *Thermocyclops crassus*  
(Fischer 1853), and *Th. oithonoides* (Sars 1863). The herbivorous rotifers were dominated by  
124 *Polyarthra* sp. and *Hexarthra mira* (Hudson 1871), while the predatory genus *Asplanchna* (*A. girodi*  
De Guerne, 1888, *A. priodonta* Gosse, 1850) represented the carnivorous rotifers. Other zooplankton  
126 taxa, such as undetermined Chydorids, the calanoid Copepod *Eurytemora velox* (Lilljeborg 1853), and  
the rotifers *Keratella quadrata* (O.F. Müller 1786), *K. cochlearis* (Gosse 1851), and *Lecane* spp., were  
128 only found very occasionally and were not taken into account in the subsequent analyses.

**130 Assigning zooplankton taxa to trophic groups from prior knowledge**

We assumed that the assembly of zooplankton trophic groups (and associated trophic interactions with  
132 fish and other zooplankters) was controlled by zooplankton (i) body size only, (ii) antipredator  
behaviour only, (iii) body size and behaviour combined, and (iv) phylogeny (using taxonomy as a  
134 proxy) at different resolutions and combined with ontogeny for Copepod taxa. Below, we present the  
criteria used to assign zooplankton taxa into trophic groups and predict their associated numerical  
136 response to perch and roach (summarized in Table 1).

**138 Body size-based zooplankton trophic groups**

During summer, zooplankton in Lake Créteil include few large-bodied organisms and thus have a relatively narrow body-size range. They may be separated among small-bodied organisms (50-200  $\mu\text{m}$ ), which include mainly herbivores (Rotifers and nauplii of Copepods), and medium to large-bodied organisms (200-2000  $\mu\text{m}$ , among which 8.7 % were  $> 1000 \mu\text{m}$ ), which include both herbivorous or omnivorous/carnivorous microcrustaceans and carnivorous Rotifera (Bertolo et al. 1999a). In order to take into account other realistic ways of classifying zooplankton, we split it into three size classes: 50-200  $\mu\text{m}$  (Rotifers and nauplii of Copepods), 200-600  $\mu\text{m}$  (*Bosmina*, *Ceriodaphnia* and *Thermocyclops*) and 600-2000  $\mu\text{m}$  organisms (*Asplanchna*, *Daphnia*, *Diaphanosoma*, *Eudiaptomus*, *Acanthocyclops*). When fitting models to zooplankton count data (see below), however, this three size-class grouping structure did not perform better than the two size-class grouping structure (LR test: Chi-square = 2.5382, Df = 3, p = 0.4684), and was penalized in terms of Akaike's information criterion (AIC, 2674.1 vs. 2670.6). We therefore retained the two-size-classes grouping structure for further analyses.

Fish preferentially consume large-bodied zooplankters, while small-bodied zooplankters are both dominated competitors and prey for larger zooplankton (Brooks and Dodson 1965). Hence, we predicted that small-bodied zooplankton abundance should increase in the presence of fish (Table 1, Body size). Moreover, based on perch and roach differences in body size, we predicted perch to impose a stronger predation pressure than roach and thus, in turn, to have a larger positive effect on small-bodied zooplankton (Table 1, Body size). This prediction emerges from general body-mass scaling rules, where average attack rate for freshwater vertebrate ectotherms equals  $8.2 \cdot 10^{-6} m^{0.31}$  (where  $m$  is individual body mass, Rall et al. 2012). Using this relationship, cumulated attack rate on zooplankton from 18 perch weighing 2.45 g is predicted to be twice as large than that from six roach weighing 7.50 g. Finally, we predicted this higher cumulated attack rate from perch to result in a

162 stronger trophic cascade (i.e. increase in phytoplankton abundance) than in the presence of roach (Table  
1, Body size).

164

#### *Behaviour-based zooplankton trophic groups*

166 Possible behavioural responses of zooplankters facing predation from fish or from other invertebrates  
may be either (i) “fleeing” reaction, (ii) hiding in a spatial refuge or (iii) no escape. Whereas these  
168 behaviour-based zooplankton trophic groups are not mutually exclusive, we assigned each zooplankton  
organism to only one group based on prior literature knowledge about their predominant behaviour.

170

Specifically, nauplii of both Calanoid and Cyclopoid copepods have limited escape capabilities, while  
172 copepodite stages of are often equally categorized as “flee” strategists due to their jump-swimming  
behaviour. However, Calanoids occupy the upper water layers at both the nauplius and copepodite  
174 stages (Angeli et al. 1995, Lacroix and Lescher-Moutoué 1995, Lieschke and Closs 1999, Bertolo et al.  
1999a, Titelman and Fiksen 2004, Doulka and Kehayias 2011), and thus can not avoid encounters with  
176 fish. Hence, we categorized Calanoids as “no escape” strategists at the nauplius stage and as “flee”  
strategists at the copepodite stage. In contrast, Cyclopoids prefer the deep layers of the water column in  
178 Lake Créteil (Lacroix and Lescher-Moutoué 1995) and may even be regularly found in near-bottom  
habitats at both the nauplius and copepodite stages (Papińska and Prejs 1979, Papińska 1981). Hence,  
180 Cyclopoids may efficiently hide from fish predators by preferentially occupying deep water layers. We  
considered hiding as a more efficient antipredator strategy than fleeing, and we categorized Cyclopoids  
182 as “hide” strategists at both the nauplius and copepodite stages.

184 We also categorized as “flee” strategists *Diaphanosoma* spp., a jump-swimmer rapidly alternating  
sinking with powerful strokes of antennae (Williamson 1983, Chang and Hanazato 2003), but also

186 *Bosmina* spp., which use a “dead-man response” (in addition to having a hard carapace) to deter  
predation from copepods (Kerfoot 1978, Williamson 1983, Sakamoto and Hanazato 2008). Although  
188 jumping and a dead-man response are sharply different behaviours, we grouped them in a common  
“flee” category because their common purpose is to reduce mortality from unavoidable predator  
190 encounters. This choice was further justified *a posteriori* by fitting statistical models to zooplankton  
count data. A model considering *Bosmina* separately as a “passive escape” strategist did not fit the data  
better than a model grouping *Bosmina* together with *Diaphanosoma* and copepodites of Calanoids (LR  
test: Chi-square = 3.8594, Df = 3, p = 0.2771), and was penalized in terms of AIC (2688.7 vs. 2686.6).

194  
Finally, Daphnids have a reduced ability to escape by jumping. Additionally, in Lake Créteil both  
196 *Ceriodaphnia* spp. and *Daphnia* spp. preferentially use the upper layers of the water column (Lacroix  
and Lescher-Moutoué 1995), and thus are highly susceptible to predation by planktivorous fish. We  
198 thus categorized Daphnids as “no escape” strategists, together with Rotifers.

200 Perch and roach also have contrasted hunting modes. Planktivorous perch are typical visual feeders  
(Lacroix et al. 1996) able to feed on zooplankton only when light intensity is relatively high (Nurminen  
202 et al. 2010, Kestemont et al. 2015). They are stimulated by conspicuous prey, and prey movement can  
trigger an attack (Peterka and Matěna 2009). Perch are stop-and-burst predators that can repeatedly  
204 strike, particularly so when feeding on evasive copepods (Peterka and Matěna 2011). Hence, we  
predicted perch to efficiently deplete both “no escape” and “fleeing” zooplankton strategists, but to be  
206 less efficient on “hide” strategists (Table 1, Behaviour).

208 In contrast with perch, roach are cruising foragers that use continuous and rapid swimming, punctuated  
by slowdowns (Peterka and Matěna 2011, Helenius et al. 2015). They are able to switch between

210 particulate feeding and gulping, depending on zooplankton body size, abundance and light conditions  
(Van Den Berg et al. 1993, Helenius et al. 2015). Roach foraging efficiency is almost independent of  
212 the light intensity (Diehl 1988, Nurminen et al. 2010), indicating that they only optionally rely on  
visual cues to locate their prey. Moreover, even when light intensity is relatively high, roach are poorly  
214 capable to prey on “fleeing” zooplankton strategists like copepods (Lacroix et al. 1996, Peterka and  
Matěna 2009, 2011). Hence, we expected roach in our experiment to deplete “no escape” zooplankters,  
216 but not “fleeing” or “hide” strategists (Table 1, Behaviour).

#### 218 *Mixture of size- and behaviour-based zooplankton trophic groups*

Body size should be important to predict zooplankton-fish trophic links only for zooplankters that are  
220 unable to behaviourally avoid encounters with fish. Hence, we separated “no escape” zooplankton  
strategists into “large-bodied” and “small-bodied” zooplankton trophic groups. “Fleeing” strategists  
222 were similar in body sizes and were thus not partitioned into different body size-based zooplankton  
trophic groups. Finally, “hide” strategists, by efficiently avoiding predation from both perch and roach,  
224 were free from any size-dependency in their response to fish and were also not partitioned into different  
body size-based zooplankton trophic groups. Because they are omnivorous, however, we predicted  
226 “hide” strategists to increase in parallel with the increasing abundance of “small, no escape” strategists  
on which they feed. The predictions resulting from this zooplankton grouping structure are summarized  
228 in Table 1 (Body size and behaviour).

#### 230 *Phylogeny-based zooplankton trophic groups*

We identified 12 genera or species and two development stages for copepods (see above), yielding a  
232 total of 14 trophic groups. Reaching this taxonomic resolution in zooplankton counts, however, is a  
time-consuming task which is relieved if taxa may be lumped. It is therefore important to quantify the

234 effect of taxonomic lumping on information loss in predicting food-web structure (Gauzens et al.  
2013). Accordingly, we varied zooplankton lumping in our dataset from low to high to reach a (i) low-  
236 level lumping (12 trophic groups: *Ceriodaphnia*, *Bosmina*, *Diaphanosoma*, *Daphnia*, *Eudiaptomus*,  
*Acanthocyclops*, *Thermocyclops*, nauplii of Calanoids, nauplii of Cyclopoids, *Polyarthra*, *Hexarthra*,  
238 *Asplanchna*), (ii) medium-level lumping (10 trophic groups: *Daphnidae*, *Bosminidae*, *Sididae*,  
*Diaptomidae*, *Cyclopidae*, nauplii of copepods, *Hexarthridae*, *Synchaetidae*, *Asplanchnidae*) and (iii)  
240 high-level lumping (4 trophic groups: Cladocera, copepodite of copepods, nauplii of copepods, and  
Rotifera).

242

### **Data analysis**

#### 244 *Zooplankton counts*

Our analysis of zooplankton count data aimed at (i) quantifying the numerical response of the different  
246 zooplankton trophic groups to the fish treatments, (ii) identifying which zooplankton grouping structure  
provided the best and most parsimonious fit to the data, and (iii) quantifying, for each zooplankton  
248 grouping structure, the importance of considering perch and roach as having different effects on  
zooplankton numbers. We based our analysis on comparing the fit to zooplankton count data of the  
250 resulting 12 different generalized linear mixed models (Table 2), which corresponded to the six  
zooplankton grouping structures described above times two different fish effects: fish absent vs. fish  
present or fish absent vs. perch present vs. roach present. We provide below more details on the  
252 analyses.

254

Zooplankton counts  $C$  included a high proportion (25 %) of zeros, and positive counts were highly  
256 overdispersed. We thus modelled this data using a zero-inflated negative binomial model (Ntzoufras  
2009):

$$\begin{aligned}
C_i &\sim NB(\phi_i, r_i) \\
\phi_i &= \frac{r_i}{r_i + \lambda_i(1 - \theta_i)} \\
\theta_i &\sim B(\psi_i) \\
\ln(\lambda_i) &= \alpha + \beta_{Z[i]} + \gamma_{F[i]} + \delta_{Z[i], F[i]} + \epsilon_{E[i], D[i]} + \zeta_{T[i]} \\
\epsilon_{E[i], D[i]} &\sim N(0, \sigma_\epsilon^2) \\
\zeta_{T[i]} &\sim N(0, \sigma_\zeta^2)
\end{aligned} \tag{1},$$

260

where subscript  $i$  indexes sampling events corresponding to a taxon (low-level phylogenetic lumping, 12 groups) in a given enclosure on a given sampling day ( $n = 324$  sampling events),  $\sim$  means “follows distribution”, and  $NB$  is the negative binomial distribution with success probability  $\phi$  and number of failures  $r$ . The  $\theta$  latent variable for absence of organisms was modelled as a Bernoulli process (B distribution) with probability  $\psi$ .

266

Line 4 in Eq. (1) shows that we modelled positive (non-zero) counts  $\lambda_i$  with a GLM including a natural logarithm (ln) link and a linear predictor in which  $\alpha$  was an intercept, and  $\beta$  described deviations from the intercept associated with zooplankton trophic group  $Z$ , which corresponded to the six different grouping structures based on (i) body size (two  $Z$  groups), (ii) antipredator behaviour (four  $Z$  groups), (iii) body size and behaviour combined (five  $Z$  groups), (iv) low-level phylogenetic lumping (12  $Z$  groups), (v) medium-level phylogenetic lumping (10  $Z$  groups) and (vi) high-level phylogenetic lumping (four  $Z$  groups).

274

$\gamma$  described deviations from the intercept associated with fish treatment  $F$ , which took either two levels (fish absent vs. present) or 3 levels (fish absent vs. perch present vs. roach present).  $\delta$  described deviations from the intercept due to the interaction between  $Z$  and  $F$ . The model also

278 included the normally-distributed random effect of enclosures  $E$  on a given sampling date  $D$  ( $\epsilon$   
effect,  $n=9 \times 3=27$  levels), and of the low-level phylogenetic lumping  $T$  ( $\xi$  effect,  $n=12$   
280 levels), because these effects generated non-independence among observations but were not the  
primary focus of statistical inference (for further motivations to use random effects, see Kéry and  
282 Schaub 2012). Note that  $T$  was confounded with  $Z$  for the low-level (12-group) phylogenetic  
lumping model, and  $\xi$  was thus omitted from this particular model.

284

We assessed the relative fit of the 12 different candidate models (six different  $Z$  times two different  
286  $F$ ) to the count data using statistical model selection. Specifically, we ranked models based on their  
parsimony, as measured by AIC computed as  $AIC = -2 \ln(L) + 2k$  and Bayesian information criterion  
288 (BIC, also known as Schwarz's Bayesian criterion) computed as  $BIC = -2 \ln(L) + \ln(n_{obs})k$ , where  $L$   
is the likelihood of the estimated model,  $k$  is the total number of parameters that are estimated in the  
290 model and  $n_{obs}$  is the number of observations to which the model was fitted ( $n = 324$ ). The pseudo  $R^2$   
of each model was computed from the linear regression of observations on fitted model values. The  
292 statistical significance of fixed effect terms in the most parsimonious model (Table 3) was tested using  
likelihood ratio tests comparing the likelihood of the full model with that of a model omitting the focal  
294 term.

### 296 *Zooplankton body sizes*

In addition to zooplankton counts, our dataset included body size measurements for crustaceans (see  
298 above). We predicted fish predation to select for decreased body sizes in zooplankton, with the  
amplitude of body downsizing being proportional to the intensity of predation (Brooks and Dodson

300 1965). To test this prediction, we modelled the effect of fish on natural log-transformed, individual  
 zooplankton body sizes  $S$  using a linear mixed model:

302

$$\begin{aligned}
 S_i &\sim N(\mu_i, \sigma^2) \\
 \mu_i &= \alpha + \beta_{Z[i]} + \gamma_{F[i]} + \delta_{Z[i], F[i]} + \epsilon_{E[i], D[i]} \quad (\text{Eq. 2}), \\
 \epsilon_{E[i], D[i]} &\sim N(0, \sigma_\epsilon^2)
 \end{aligned}$$

304

where subscript  $i$  indexes zooplankton individuals ( $n = 3291$  individuals),  $N$  is the normal  
 306 (Gaussian) distribution, and  $\mu$  and  $\sigma^2$  are the mean and variance of  $S_i$ , respectively. Other  
 parameters are as described in Eq. 1 and the grouping structure for  $Z$  was that yielding the lowest  
 308 AIC in fitting model 1 to the count data. This structure was provided by the low-level taxonomic  
 lumping (see Results) which explains why, compared to Eq. 1, the  $\zeta_{T[i]}$  term is lacking in Eq. 2. We  
 310 modelled fish treatment  $F$  using three levels (fish absent vs. perch present vs. roach present), which  
 yielded a lower AIC (689 vs. 709) than using two levels (fish absent vs. present).

312

### *Phytoplankton concentration*

314 We modelled chlorophyll  $a$  concentration  $P$  ( $\mu\text{g L}^{-1}$ ), a proxy for phytoplankton concentration, using  
 a linear mixed model:

$$\begin{aligned}
 P_i &\sim N(\mu_i, \sigma^2) \\
 \mu_i &= \alpha + \beta_{F[i]} + \delta_{D[i]} \quad (\text{Eq. 3}), \\
 \delta_{D[i]} &\sim N(0, \sigma_\delta^2)
 \end{aligned}$$

where subscript  $i$  indexes a lake enclosure ( $n = 9$  enclosures) on a given sampling date ( $n = 2$   
 318 sampling dates). Here also, a three-level fish treatment  $F$  yielded a lower model AIC (61 vs. 74) than  
 a two-levels  $F$ . Other subscripts are as described in Eq. 1.

320

All statistics were performed in R version 3.6.0 (R Core Team 2019). We fitted model 1 using the  
322 `glmmTMB()` function from the `glmmTMB` package (Brooks et al. 2017). Predictions with confidence  
intervals for model 1 were obtained using the `predict.glmmTMB()` function with a `family =`  
324 `nbinom2` argument. We fitted models 2 and 3 using the `lmer()` function from the `lme4` package  
(Bates et al. 2015) and obtained predictions with confidence intervals for these models using the  
326 function `sim()` from the `arm` package (Gelman and Hill 2007). Significance tests of each term in the  
best-fitting version of model 1 (Table 3) were performed using the default `anova()` function.

328

## Results

### 330 *Zooplankton abundances and zooplankton trophic groups*

Overall, we found a relatively good agreement between our qualitative predictions and observed  
332 response of zooplankton abundance to perch and roach (Table 1), indicating that prior literature  
knowledge is a reliable source of information to roughly predict trophic interactions. In particular,  
334 perch and roach had remarkably different effects on zooplankton counts. For all of the six different  
zooplankton grouping structures, considering perch and roach as having different effects on  
336 zooplankton counts strongly improved model fit (Table 2).

338 Our qualitative predictions of the effects of perch and roach on body-size based and behaviour-based  
zooplankton trophic groups were also relatively well supported by observations, except for the “Hide”  
340 trophic group, which largely increased in response to roach when we predicted no response (Table 1,  
Behaviour-based – an increase which was mainly driven by *Acanthocyclops*, Fig. 1). This mismatch  
342 between predictions and observations suggests that body size was a more parsimonious predictor of  
zooplankton numerical response to fish than behaviour. Accordingly, the body size-based zooplankton

344 grouping structure ( $n =$  two zooplankton trophic groups) ranked better than the behaviour-based one ( $n$   
346  $=$  three zooplankton trophic groups) in terms of both pseudo  $R^2$  and parsimony (Table 2).

348 Interestingly, the combined body size- and behaviour-based zooplankton grouping structure, in which  
350 “no escape” zooplankters were categorized as either large- or small-bodied ( $n =$  four zooplankton  
352 trophic groups), was more parsimonious in terms of both AIC and BIC than the size-dependent model  
(Table 2), indicating that body size becomes a more important predictor of trophic interactions when  
behaviour can not deter predators. However, here also our predictions were poorly validated for “hide”  
zooplankton strategists, which abundances were mainly unchanged by perch when we predicted a  
strong increase, and were strongly increased by roach when we predicted a moderate increase (Table 1,  
354 Body size- and Behaviour-based).

356 Body size- or/and behaviour-based zooplankton grouping structures were largely outperformed by  
phylogeny-based structures in terms of both pseudo  $R^2$  and parsimony (Table 2). This was true for both  
358 the low (12 zooplankton trophic groups) and medium taxonomic-lumping models (10 zooplankton  
trophic groups), but not for the high taxonomic-lumping model (four zooplankton trophic groups),  
360 which was outperformed by the combined size- and behaviour-based zooplankton grouping structure  
(Table 2). Note that the lower parsimony of the low taxonomic lumping model (Table 2) was not due to  
362 the loss of a random-effect term ( $\zeta$  in Eq. 1), which penalized AIC and BIC through increasing the  
number of model parameters (a random intercept counts as one parameter when the fixed effect  
364 counted as 12 parameters).

366 A significant fish treatment-by-zooplankton group interaction (Table 3) indicated that the high  
performance of phylogeny in predicting zooplankton response to fish was explained by taxon-specific

368 patterns that could not accurately fit into body size- and behaviour-based trophic groups. Accordingly,  
among large-bodied zooplankters some decreased in response to fish (e.g., *Ceriodaphnia*, *Daphnia*),  
370 while others increased (e.g., *Acanthocyclops*) or were unaffected (e.g., *Diaphanosoma*, Fig. 1).  
Similarly, among “hide” strategists the strongly positive effect of fish on *Acanthocyclops* did not  
372 match with the absence of any effect on *Thermocyclops* (Fig. 1), indicating that similarly-behaving  
prey genera did not respond similarly to their joint predators.

374

Finally, we found that accounting for copepod ontogenetic stages was crucial to the performance of  
376 phylogeny at a high resolution, but not at a medium or low resolution (Table S1), indicating ontogeny-  
by-phylogeny interactions in copepod response to fish. Accordingly, Calanoids (i.e. *Eudiaptomus*) were  
378 similarly affected by fish at the nauplius and copepodite stages, while among Cyclopoids of either  
stages only copepodites of *Acanthocyclops* responded to fish (Fig. 1).

380

#### *Crustacean zooplankton body size*

382 There was a highly significant interaction between phylogeny and fish treatments on crustacean  
zooplankton body sizes (Fig. 2, LR-test,  $\text{Chisq} = 189.9$ ,  $\text{df} = 12$ ,  $p < 0.001$ ), which tended to mirror the  
384 phylogeny-fish interaction on zooplankton abundances described above. Specifically, pairwise *t*-tests  
(not shown) revealed that perch significantly decreased body sizes in all the seven crustacean genera  
386 but *Daphnia* (which were too few in fish treatments to draw statistical inference), while roach  
significantly reduced body sizes in *Bosmina*, *Ceriodaphnia*, *Diaphanosoma* and *Acanthocyclops* only  
388 (Fig. 2).

390 *Phytoplankton*

392 Fish induced a trophic cascade in which chlorophyll *a* concentration almost doubled in presence of  
394 roach and almost tripled in presence of perch (Table 1, Fig. 3). This result fits with the *a priori*  
predictions that both body size and hunting mode were making perch more efficient zooplankton  
predators than roach under the conditions of our experiment (Table 1).

396

## Discussion

398 To our knowledge, the relative contributions of body size, antipredator behaviour, and phylogeny in  
determining trophic interactions has not been previously assessed in a particular system. By using  
400 freshwater zooplankton communities as model, we showed that body size alone is a better predictor of  
trophic interactions than behaviour alone, but that prey body size and behaviour combined  
402 outperformed both. We further found that zooplankton phylogeny was a far better predictor of  
zooplankton trophic interactions than body size and behaviour combined when phylogeny was  
404 informed at a high (genus-level) to medium (family-level) resolution, but not at a low resolution (high-  
level taxonomic groups). These results have several general implications for food-web research.

406

Recent studies have highlighted that the interplay between predator hunting mode and prey antipredator  
408 behaviour is an important driver of trophic interactions (Schmitz 2008, Belgrad and Griffen 2016). Our  
results suggest, however, that zooplankton behaviour is less important than zooplankton body size in  
410 driving trophic interactions in food webs of shallow lakes. Our results are in line with the well-known  
importance of body size in driving trophic interactions in aquatic systems (Elton 1927, Cohen et al.  
412 2003, Emmerson and Raffaelli 2004, Woodward et al. 2005, Brose et al. 2006). Further studies would  
be needed to test whether our results hold true also in terrestrial systems, where body size is expected

414 to be less important than in aquatic communities to determining trophic interactions (Shurin et al.  
2006).

416

Our results testify for the importance of phenotypic traits in driving the strength of trophic interactions  
418 and ecosystem function at the plankton level. At the fish level also, traits were involved in driving the  
differential effects of perch and roach. Despite identical biomasses, perch more severely depleted  
420 zooplankters than roach, and induced a trophic cascade that was more than twice as large as the trophic  
cascade induced by roach (Table 1 effect sizes, Fig. 3). Our experimental design, however, varied fish  
422 identity in parallel with fish density and body size (owing to a constant fish biomass), but also in  
parallel with fish hunting mode. Hence, we could not rank the contributions of body size (and related  
424 fish density) and hunting modes in controlling the differential predatory effects of perch and roach on  
zooplankters. The strength of ecological interactions is classically considered as driven by the biomass  
426 of interacting species (Oksanen et al. 1981, Carpenter et al. 1985, Brett and Goldman 1996, Jeppesen et  
al. 2003), a rule dubbed “law of mass action”. Our result demonstrate that this paradigm is not  
428 sufficient to predict the strength of ecological interactions, in line with previous studies that  
demonstrated the importance of fish body size in determining the strength of their induced trophic  
430 cascades (Persson et al. 2003, Shackell et al. 2010, Renneville et al. 2016).

432 Despite the important role of body size and behaviour in determining zooplankton trophic groups, our  
results bring support to the previous contention that phylogeny is an even more powerful predictor of  
434 food-web structure (Cattin et al. 2004, Naisbit et al. 2012). Phylogenetic relatedness is a surrogate for a  
host of unknown or poorly documented traits that potentially influence trophic interactions, but that are  
436 not captured by body size or behavioural strategies such as, for instance, food preferences, diel activity  
cycles, body shape, mechanical defences (e.g. spines, helmets, carapaces), colouration or camouflages

438 (Boukal 2014). However, in our study phylogeny at a high resolution was the best predictor of the  
trophic structure only when combined with copepod ontogeny (i.e., nauplius *vs.* copepodite stages,  
440 Table S1), which is another synthetic proxy encapsulating information on body-size, behaviour or  
feeding preferences (Werner and Gilliam 1984). Ontogeny further accounts for the fact that juveniles  
442 are often more abundant than adults. Hence, phylogeny does not capture *all* the traits that determine  
trophic niches, which are probably best predicted by combining phylogeny with other, non-redundant  
444 and synthetic sources of information. We further found that the power of phylogeny in predicting  
trophic structure degraded at a low resolution, indicating that body size combined with behaviour (but  
446 not alone) should be preferred predictors of trophic niches when information on phylogenetic  
resolution is limited. However, information on hunting mode or antipredator behaviour may also be  
448 limited, or dependent on detailed phylogenetic information (e.g., species among the same genus may  
vary in behaviour), in which case the most readily implementable predictor of trophic niches remains  
450 body size.

452 Our study illustrates how food-web perturbation experiments may be used to infer trophic interactions.  
Our approach included two steps. First, we formed qualitative predictions from literature knowledge  
454 for the effects of fish manipulation on the abundance of planktonic organisms while assuming trophic  
interactions to be dependent on body size only, on antipredator behaviour only, or on body size and  
456 behaviour combined (Table 1). Second, we statistically modelled the effects of fish manipulation on  
plankton grouped into body size- and behavioural trophic groups (alone or mixed) and into  
458 phylogenetic trophic groups (combined with ontogeny). This modelling step allowed us to (i) compute  
effect sizes to which our qualitative predictions could be compared (Table 1), and (ii) to quantify the  
460 relative efficiencies of body size, behaviour and phylogeny in predicting plankton response to fish  
manipulation (Table 2).

462

Compared to classical approaches to study food-web structure, this experimental approach has the  
464 strong advantage not to require any *a priori* data on observed trophic networks. High-quality of such  
food-web data are costly to acquire and, often, only report presence or absence of pairwise trophic links  
466 with no information on the strength or potential transience of the links. In contrast, our approach  
allowed us to quantify the *realized* effects of controlled changes in the trophic structure. A drawback of  
468 our approach, however, is that realized effects do not separate direct from indirect effects of trophic  
interactions. Additionally, our approach still required prior knowledge to group organisms into separate  
470 body size and behavioural trophic groups. Body size data are readily available, but pertinent cutoffs for  
body size binning values are not necessarily easy to define. Here, we defined these cutoff values for  
472 zooplankton body sizes arbitrarily, based on the observed body-size distributions. Predator hunting  
mode and prey antipredator behaviour might also not be readily available from the literature, or simply  
474 for the taxonomic level at which organisms were determined. Therefore, just as approaches relying on  
*a priori* food-web data, our approach might be limited to organisms for which substantial prior  
476 knowledge is available.

478 To conclude, our results illustrate how experiments aiming at exploring community response to top-  
down perturbations may also be used to infer the rules that control food-web assembly. In taking this  
480 approach, we found that body size, behaviour, phylogeny and ontogeny were all pertinent predictors of  
trophic niches in zooplankton food webs of Lake Créteil, but that phylogeny combined with ontogeny  
482 was most powerful. This result highlights that, beyond the important roles of body size and behaviour,  
other traits are involved in structuring food webs. Uncovering these traits remains highly needed if we  
484 are to understand the ecological mechanisms that shape trophic networks and support biodiversity.

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488 **Table 1. Qualitative predictions and observed effect sizes for the numerical response to perch and**  
 490 **roach of zooplankton taxa assigned to trophic groups, based either on body size only, on**  
 492 **antipredator behaviour only, or on body size and behaviour combined.** Qualitative predictions  
 were formed based on prior literature knowledge. Predicted - (- -), + (+ +) or = represent a negative  
 (very negative), positive (very positive) or non-existent numerical response by zooplankton to fish,  
 494 respectively. Observed % effects sizes were computed as  $100(\mu_F - \mu_C)/\mu_C$ , where  $\mu_F$  and  $\mu_C$  are  
 mean plankton counts in fish and control treatments, respectively, as estimated from statistical models  
 described in Eq. 1 (zooplankton) and Eq. 3 (phytoplankton).

Basis for trophic groups	Zooplankton groups	Zooplankton Phyla	Predicted Perch effect	Observed Perch effect (%)	Predicted Roach effect	Observed Roach effect (%)	Explanation of predicted fish effects
Body size	Large zooplankton	<i>Asplanchna, Ceriodaphnia, Daphnia, Diaphanosoma, Bosmina, Acanthocyclops, Thermocyclops, Eudiaptomus</i>	- -	-81.3	-	-22.6	Higher cumulated attack rate in perch than roach.
	Small zooplankton	<i>Hexarthra, Polyarthra</i> , nauplii of Calanoids, nauplii of Cyclopoids	+ +	39.5	+	23.1	Depletion of large-bodied zooplankton favours competitively dominated smaller zooplankton.
Behaviour	Phytoplankton		+ +	190.0	+	82.2	Trophic cascade driven by decreased grazing from large-bodied zooplankton.
	Flee	<i>Bosmina, Diaphanosoma, Eudiaptomus</i>	- -	-91.2	=	6.7	Fleeing strategy by zooplankton inefficient against perch but efficient against roach.
	Hide	<i>Acanthocyclops, Thermocyclops</i> , nauplii of Cyclopoids	=	8.3	=	72.2	"Hide" strategy by zooplankton efficient against both perch and roach.
	No escape	<i>Asplanchna, Ceriodaphnia, Daphnia, Hexarthra, Polyarthra</i> , nauplii of Calanoids	- -	-58.8	- -	-42.1	"No escape" zooplankton strategists are similarly depleted by perch and roach.
	Phytoplankton		+ +	190.0	+	82.2	Stronger trophic cascade when both "no escape" and "fleeing" strategists are depleted.
	Flee	<i>Bosmina, Diaphanosoma, Eudiaptomus</i>	- -	-90.9	=	9.2	Response of "fleeing" zooplankton strategists is driven by behaviour only.
Body size and Behaviour	Hide	<i>Acanthocyclops, Thermocyclops</i> , nauplii of Cyclopoids	+ +	8.3	+	70.8	"Hide" zooplankton strategists are carnivorous and benefit from increasing abundances of small, no escape zooplankters on which they feed.
	Large, no escape	<i>Asplanchna, Daphnia, Ceriodaphnia</i>	- -	-92.1	-	-69.0	Response of "no escape" plankton strategists is driven by body size only.
	Small, no escape	<i>Hexarthra, Polyarthra</i> , nauplii of Calanoids	+ +	70.4	+	39.1	Response of "no escape" plankton strategists is driven by body size only.
	Phytoplankton		+ +	190.0	+	82.2	Trophic cascade driven by decreased grazing from large-bodied zooplankton and stronger when both "no escape" and "fleeing" zooplankters are depleted.

498 **Table 2. Fit of the 12 candidate models to zooplankton count data.** Columns “Candidate grouping  
 structure” and “Separate perch-roach effects” correspond to zooplankton trophic groups  $Z$  and fish  
 500 groups  $F$ , respectively, as described in Eq. 1. Non-shaded lines correspond to models that included a  
 separate effect for perch and roach (i.e., three-level  $F$ ). Shaded lines correspond to models that did  
 502 not include a separate effect for perch and roach (i.e., two-level  $F$ ). Df gives the number of  
 parameters in the model. Delta AIC compares each model to the lowest-AIC model.

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Candidate zooplankton grouping structure	Separate Perch-Roach effects	df	Deviance	Pseudo R <sup>2</sup>	BIC	AIC	Delta AIC
Phylogeny and ontogeny High resolution	Yes	39	2456.0	0.67	2681	2534	0
	No	27	2567.6	0.50	2724	2622	88
Phylogeny and ontogeny Medium resolution	Yes	31	2507.1	0.66	2704	2575	41
	No	24	2612.2	0.50	2751	2660	126
Body size x Behaviour	Yes	16	2597.2	0.59	2690	2629	95
	No	12	2660.9	0.50	2730	2685	151
Phylogeny and ontogeny Low resolution	Yes	16	2638.1	0.57	2731	2670	136
	No	12	2675.3	0.52	2745	2699	165
Body size	Yes	10	2650.6	0.59	2708	2671	137
	No	8	2691.2	0.50	2737	2707	173
Behaviour	Yes	13	2660.6	0.50	2736	2687	153
	No	10	2704.7	0.46	2762	2725	191

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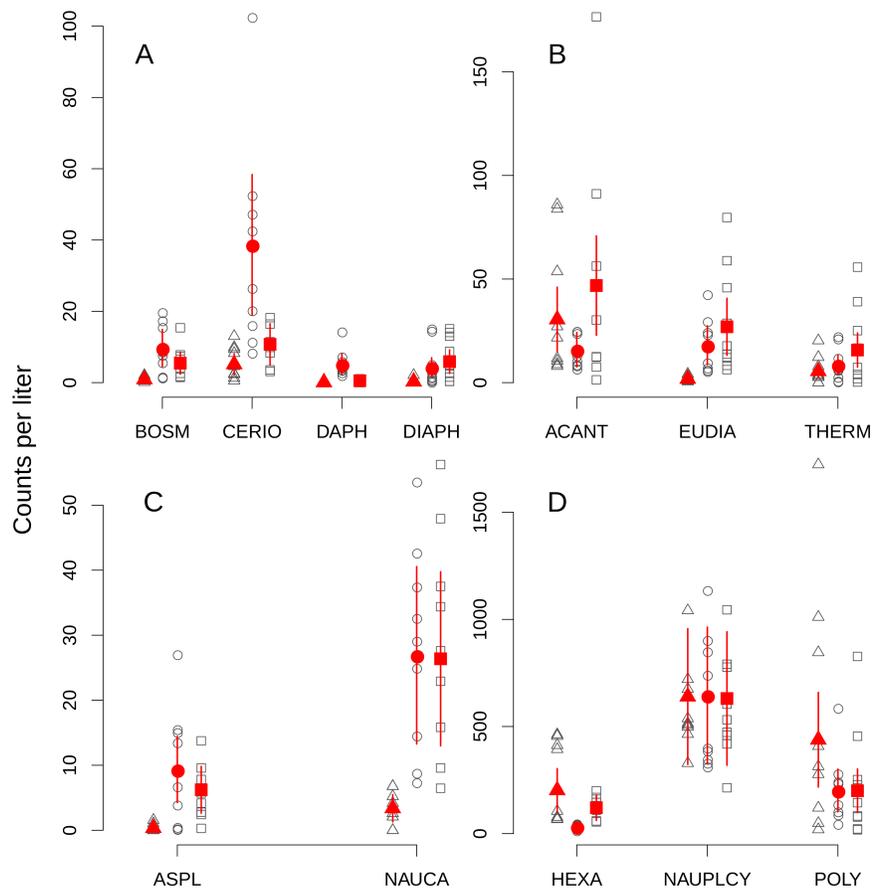
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510 **Table 3. Significance tests for focal terms in models 1 (zooplankton counts) and 2 (crustacean**  
**zooplankton body sizes).** In fitting models 1 and 2, we used the fish  $F$  and zooplankton  $Z$   
grouping structures that provided the best fit (i.e.,  $F = 3$  groups from fish absent vs. perch present  
512 vs. roach present, and  $Z = 12$  groups from a low-level lumping, high resolution phylogeny  
combined with copepod ontogeny, see Table 2).

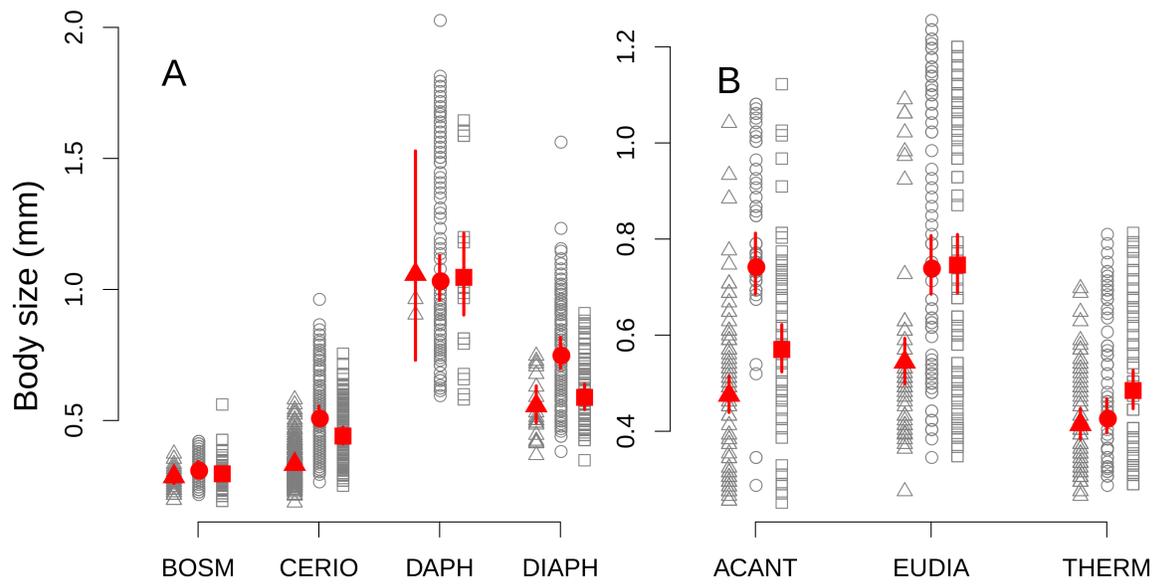
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Effect	Parameter	Model	Chisq	Df	P-value
Zooplankton group	$\beta$	1	676.0	33	<0.0001
		2	2772.1	18	<0.0001
Fish treatment	$\gamma$	1	205.9	24	<0.0001
		2	230.4	14	<0.0001
Fish treatment x Zooplankton group	$\delta$	1	190.4	22	<0.0001
		2	189.9	12	<0.0001

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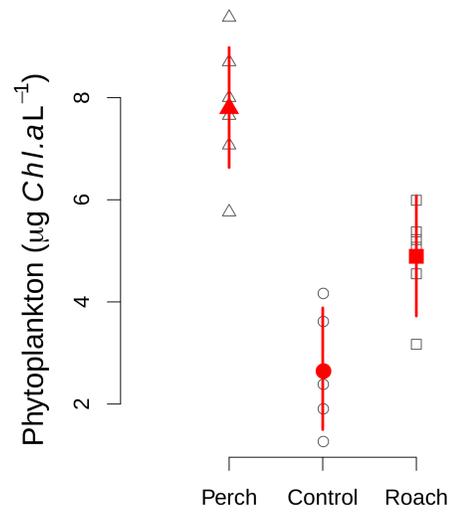


**Fig. 1. Zooplankton numerical response to fish treatments.** Filled, red symbols show mean predicted counts with 95% confidence intervals as predicted from model 1. For a sampling date-specific equivalent of this figure, see Fig. S1. Circles: fishless control treatment; triangles: perch treatment, squares: roach treatment. Open, gray symbols show the raw data. Zooplankton taxa were separated among A: Cladocerans (BOSM = *Bosmina longirostris*, CERIO = *Ceriodaphnia pulchella* and *C. quadrangula*, DAPH = *Daphnia longispina* complex, DIAPH = *Diaphanosoma brachyurum*), B: Copepodite stages (ACANT = *Acanthocyclops robustus*, EUDIA: *Eudiaptomus gracilis*, THERM = *Thermocyclops crassus* and *T. oithonoides*), C: NAUCA = nauplii of Calanoids, ASPL = *Asplanchna girodi* and *A. priodonta*, D: NAUPLCY = nauplii of Cyclopoids, HEXA = *Hexarthra mira*, POLY = *Polyarthra* sp.



532 **Fig. 2. Body-size response of crustacean zooplankton to fish treatments.** Filled, red symbols show  
 534 mean predicted counts with 95% confidence intervals as predicted from model 2. Circles: fishless  
 536 control treatment; triangles: perch treatment, squares: roach treatment. Open, gray symbols show the  
 538 raw data. Zooplankton taxa were separated among A: Cladocerans (BOSM = *Bosmina longirostris*,  
 CERIO = *Ceriodaphnia pulchella* and *C. quadrangula*, DAPH = *Daphnia longispina* complex, DIAPH  
 = *Diaphanosoma brachyurum*), B: Copepodite stages of copepods (ACANT = *Acanthocyclops*  
*robustus*, EUDIA: *Eudiaptomus gracilis*, THERM = *Thermocyclops crassus* and *T. oithonoides*).

540



544 **Fig. 3. Phytoplankton response to fish treatments.** Filled, red symbols show mean predicted counts  
 with 95% confidence intervals as predicted from model 2. Circles: fishless control treatment; triangles:  
 546 perch treatment, squares: roach treatment. Open, gray symbols show the raw data.

550 **Table S1. Fit of ontogeny-free phylogeny-based models to zooplankton count data.** Other models  
 appear shaded, are as in Table 2 and are provided to ease comparison. Models were ranked  
 552 according to their AIC score. All models included a 3-level fish treatment considering fish absent  
 vs. perch present vs. roach present.

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<b>Candidate food-web Model</b>	<b>df</b>	<b>Deviance</b>	<b>Pseudo R<sup>2</sup></b>	<b>BIC</b>	<b>AIC</b>	<b>Delta AIC</b>
Phylogeny and ontogeny High resolution	39	2456	0.67	2681	2534	0
Phylogeny and ontogeny Medium resolution	34	2507	0.66	2704	2575	41
Phylogeny alone Medium resolution	28	2528	0.62	2690	2584	50
Body size x Behaviour	16	2597	0.59	2690	2629	95
Phylogeny alone High resolution	33	2598	0.41	2789	2664	130
Phylogeny alone Low resolution	13	2644	0.54	2719	2670	136
Phylogeny and ontogeny Low resolution	16	2638	0.57	2731	2670	136
Body size	10	2651	0.59	2708	2671	137
Behaviour	13	2661	0.50	2736	2687	153

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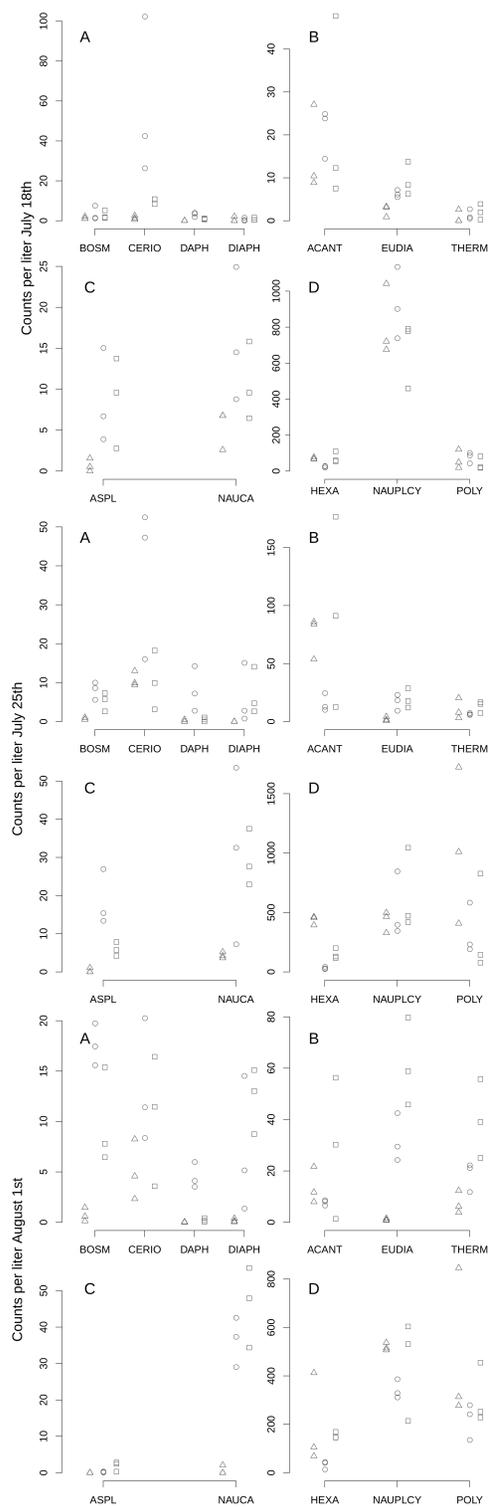
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**Fig. S1. Zooplankton numerical response to fish treatments at each sampling date.** Circles: fishless control treatment; triangles: perch treatment, squares: roach treatment. Model predictions are not shown due to model over-parametrization resulting in inaccurate predictions. Zooplankton taxa were separated among A: Cladocerans (BOSM = *Bosmina longirostris*, CERIO = *Ceriodaphnia pulchella* and *C. quadrangula*, DAPH = *Daphnia longispina* complex, DIAPH = *Diaphanosoma brachyurum*), B: Copepodite stages (ACANT = *Acanthocyclops robustus*, EUDIA: *Eudiaptomus gracilis*, THERM = *Thermocyclops crassus* and *T. oithonoides*), C: NAUCA = nauplii of Calanoids, ASPL = *Asplanchna girodi* and *A. priodonta*, D: NAUPLCY = nauplii of Cyclopoids, HEXA = *Hexarthra mira*, POLY = *Polyarthra* sp.