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Trait-dependency of trophic interactions in zooplankton food webs

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22 **Declarations**

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34 applicable laws.

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.

72 Experimental setup

74 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^3) and, hence, realistically reproduced lake vertical heterogeneity. Enclosures were filled with lake water pumped from a 1.5-meter depth from June 29th to July 3rd 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_2PO_4) and ammonium nitrate (NH_4NO_3) with a N:P ratio of 20:1 by weight, for a load of phosphorus of $3.16 \text{ } \mu\text{g L}^{-1} \text{ d}^{-1}$. At the start of the experiment (July 13th), the zooplankton biomass was $> 200 \text{ } \mu\text{g dry weight L}^{-1}$. Additional details on the experimental setup can be found in Bertolo et al. (1999b) and in Bertolo et al. (2000).

84 On July 13th, three different triplicate treatments were applied randomly to the enclosures: (i) “Control”: fishless; (ii) “Perch”: 18 planktivorous perch (aged 0+), measuring $5.97 \text{ cm} \pm 0.55 \text{ cm}$ (fork length) and weighting $2.45 \pm 0.76 \text{ g}$ (fresh weight); (iii) “Roach”: six roach aged of at least a year (1+), measuring on average $8.13 \pm 0.37 \text{ cm}$ and weighting on average $7.50 \pm 0.50 \text{ g}$. The different numbers of roach and perch per enclosure were used to standardize fish biomass at 20 g.m^{-2} , 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 18th and 25th, August 1st 1995). All samples from the same
100 enclosure were pooled (24 L in total). Zooplankton of the pooled sample was filtered through a 50 μm
sieve and fixed in 4% formalin. One to two litres of 50 μm -sieved water (depending on filter clogging)
102 were filtered on a GF/C glass fiber filter (nominal cut-off: 1.2 μm) 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 μm 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 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

We identified five herbivorous cladoceran genera: *Ceriodaphnia* [*C. pulchella* Sars 1862 and *C. 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* (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 *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 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 only found very occasionally and were not taken into account in the subsequent analyses.

Assigning zooplankton taxa to trophic groups from prior knowledge

We assumed that the assembly of zooplankton trophic groups (and associated trophic interactions with 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 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 response to perch and roach (summarized in Table 1).

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 μm), which include mainly herbivores (Rotifers and nauplii of Copepods), and medium to large-bodied organisms (200-2000 μm , 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 μm (Rotifers and nauplii of Copepods), 200-600 μm (*Bosmina*, *Ceriodaphnia* and *Thermocyclops*) and 600-2000 μm 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
252 present or fish absent vs. perch present vs. roach present. We provide below more details on the
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 ϕ and number of failures r . The θ latent variable for absence of organisms was modelled as a Bernoulli process (B distribution) with probability ψ .

266

Line 4 in Eq. (1) shows that we modelled positive (non-zero) counts λ_i with a GLM including a natural logarithm (ln) link and a linear predictor in which α was an intercept, and β 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

γ 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). δ 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 (ϵ
effect, $n=9 \times 3=27$ levels), and of the low-level phylogenetic lumping T (ξ 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 ξ 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

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:

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

where subscript i indexes zooplankton individuals ($n = 3291$ individuals), N is the normal (Gaussian) distribution, and μ and σ^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 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 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).

Phytoplankton concentration

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 ($n = 2$ 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.

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
= three zooplankton trophic groups) in terms of both pseudo R^2 and parsimony (Table 2).

346

Interestingly, the combined body size- and behaviour-based zooplankton grouping structure, in which
348 “no escape” zooplankters were categorized as either large- or small-bodied (n = four zooplankton
trophic groups), was more parsimonious in terms of both AIC and BIC than the size-dependent model
350 (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”
352 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 (ζ 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 *Ancanthocyclops* 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, Chisq = 189.9, 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).

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,
respectively. Observed % effects sizes were computed as $100(\mu_F - \mu_C)/\mu_C$, where μ_F and μ_C are
494 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</i> , <i>Ceriodaphnia</i> , <i>Daphnia</i> , <i>Diaphanosoma</i> , <i>Bosmina</i> , <i>Acanthocyclops</i> , <i>Thermocyclops</i> , <i>Eudiaptomus</i>	- -	-81.3	-	-22.6	Higher cumulated attack rate in perch than roach.
	Small zooplankton	<i>Hexarthra</i> , <i>Polyarthra</i> , nauplii of Calanoids, nauplii of Cyclopoids	+ +	39.5	+	23.1	Depletion of large-bodied zooplankton favours competitively dominated smaller zooplankton.
	Phytoplankton		+ +	190.0	+	82.2	Trophic cascade driven by decreased grazing from large-bodied zooplankton.
Behaviour	Flee	<i>Bosmina</i> , <i>Diaphanosoma</i> , <i>Eudiaptomus</i>	- -	-91.2	=	6.7	Fleeing strategy by zooplankton inefficient against perch but efficient against roach.
	Hide	<i>Acanthocyclops</i> , <i>Thermocyclops</i> , nauplii of Cyclopoids	=	8.3	=	72.2	"Hide" strategy by zooplankton inefficient against both perch and roach.
	No escape	<i>Asplanchna</i> , <i>Ceriodaphnia</i> , <i>Daphnia</i> , <i>Hexarthra</i> , <i>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</i> , <i>Diaphanosoma</i> , <i>Eudiaptomus</i>	- -	-90.9	=	9.2	Response of "fleeing" zooplankton strategists is driven by behaviour only.
Body size and Behaviour	Hide	<i>Acanthocyclops</i> , <i>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</i> , <i>Daphnia</i> , <i>Ceriodaphnia</i>	- -	-92.1	-	-69.0	Response of "no escape" plankton strategists is driven by body size only.
	Small, no escape	<i>Hexarthra</i> , <i>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.

504

Candidate zooplankton grouping structure	Separate Perch-Roach effects	df	Deviance	Pseudo R^2	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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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 vs. roach present, and $Z = 12$ groups from a low-level lumping, high resolution phylogeny combined with copepod ontogeny, see Table 2).

Effect	Parameter	Model	Chisq	Df	P-value
Zooplankton group	β	1	676.0	33	<0.0001
		2	2772.1	18	<0.0001
Fish treatment	γ	1	205.9	24	<0.0001
		2	230.4	14	<0.0001
Fish treatment x Zooplankton group	δ	1	190.4	22	<0.0001
		2	189.9	12	<0.0001

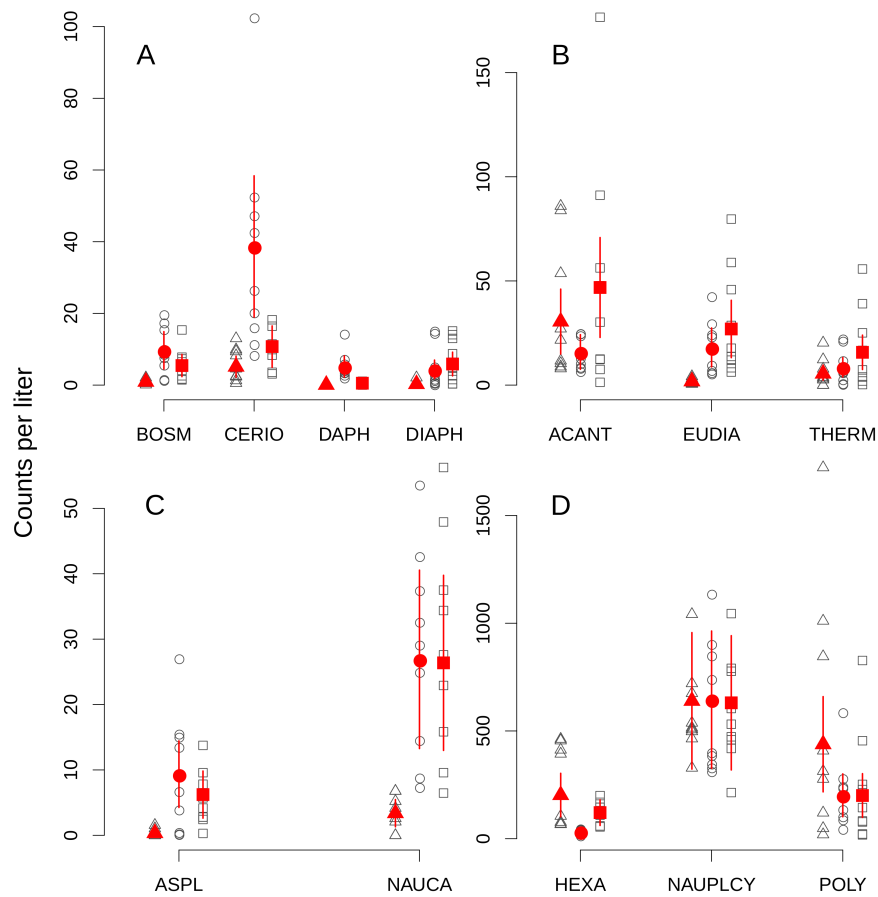
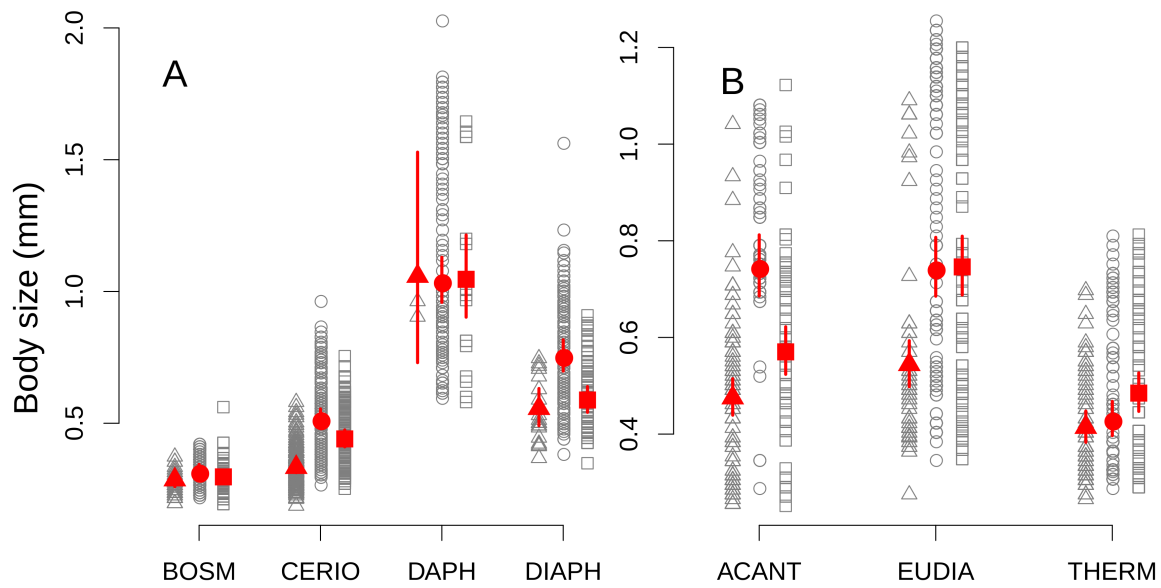
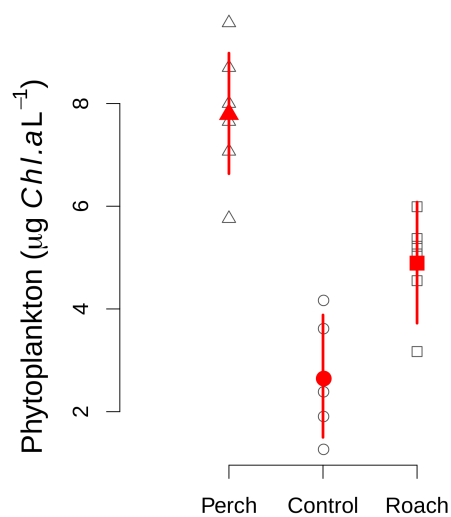


Fig. 1. Zooplankton numerical response to fish treatments. Filled, red symbols show mean predicted counts with 95% confidence intervals as predicted from model 1. For a sampling date-specific equivalent of this figure, see Fig. S1. Circles: fishless control treatment; triangles: perch treatment, squares: 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
mean predicted counts with 95% confidence intervals as predicted from model 2. Circles: fishless
534 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*,
536 CERIO = *Ceriodaphnia pulchella* and *C. quadrangula*, DAPH = *Daphnia longispina* complex, DIAPH
= *Diaphanosoma brachyurum*), B: Copepodite stages of copepods (ACANT = *Acanthocyclops*
538 *robustus*, EUDIA: *Eudiaptomus gracilis*, THERM = *Thermocyclops crassus* and *T. oithonoides*).



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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Candidate food-web Model	df	Deviance	Pseudo R ²	BIC	AIC	Delta AIC
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

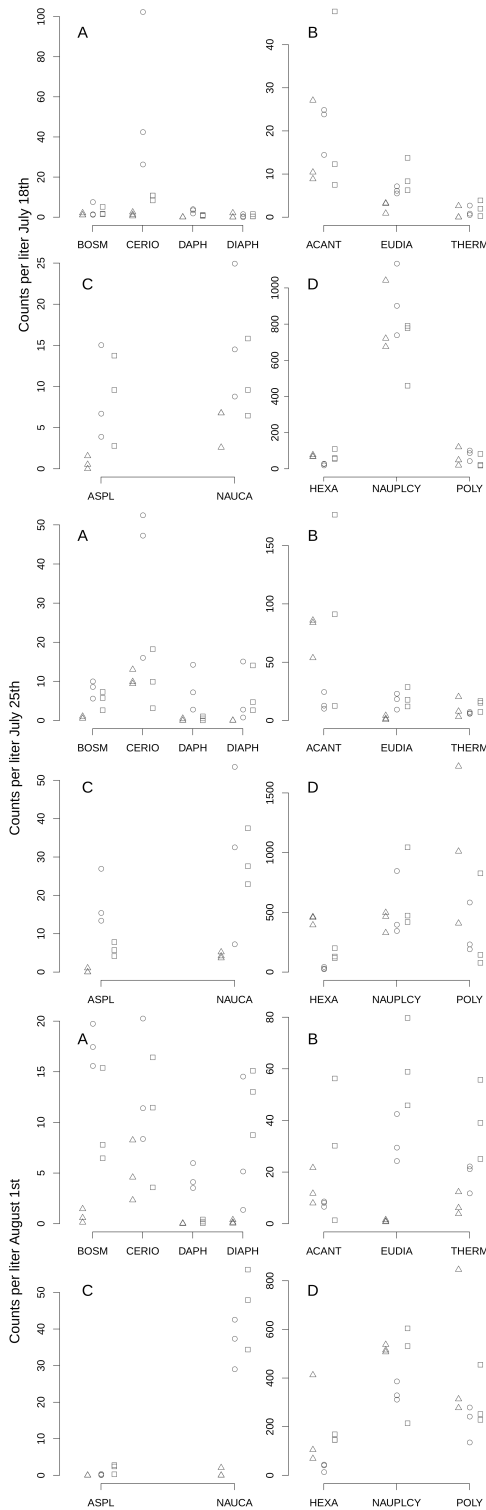


Fig. S1. Zooplankton numerical response to fish treatments at each sampling date. Circles: fishless control treatment; triangles: perch treatment, squares: roach treatment. Model predictions are not shown due to model over-parametrization resulting in inaccurate predictions. Zooplankton taxa were separated 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.