



Large daphniids are keystone species that link fish predation and phytoplankton in trophic cascades

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With 5 figures and 2 tables

Abstract: Daphniids act as keystone species in lake ecosystems by controlling phytoplankton biomass and experiencing intense fish predation. However, the importance of single daphniid species as trophic links between phytoplankton and fish remains unclear, especially compared with other zooplankton taxa. To disentangle the role of individual zooplankton taxa in the food web of a large lake, we performed an in-situ mesocosm experiment with natural phytoplankton and zooplankton communities in three treatments with native, invasive or no fish predators, respectively. A large daphniid, *Daphnia longispina*, was the zooplankton most strongly preyed upon by both fish species, and also had the highest top-down effects on phytoplankton. All other zooplankton taxa, including a small daphniid species, had minor roles in terms of both predation by fish and grazing on phytoplankton. We suggest that daphniid species with large body sizes can strongly link higher and lower trophic levels in lake food webs, and thus function as keystone species in trophic cascades from fish to phytoplankton.

Keywords: Cladocera; *Daphnia*; food chain; ecosystem dynamics; oligotrophic lake; pelagic; trophic cascading

Introduction

Understanding the ecological roles of individual species and their interactions within food webs is essential for the characterisation of ecosystem dynamics. This is particularly true for keystone species, which have a strong influence on ecosystem functioning (Cottee-Jones & Whittaker 2012). For example, predator-mediated reductions in the abundance of keystone prey species can have indirect effects on lower trophic levels, resulting in trophic cascades (Carpenter et al. 1985; Moyle & Light 1996; Pagnucco et al. 2016). However, the influence of keystone prey species on food webs may change if the predation pressure they experience shifts, for example, due to the invasion of a new predator.

The development of strong trophic cascading effects depends on multiple factors, and disentangling top-down and bottom-up regulation within food webs may therefore be difficult. In general, bottom-up control is more pronounced at the base of food webs, whereas top-down control is stronger at higher trophic levels (Brett & Goldman 1997). Therefore, fish have a greater role in regulating zooplankton biomass and abundance than nutrients, but have less influence on phytoplankton (Post & McQueen 1987; Hansson et al. 2004; Vakkilainen et al. 2004). Multi-level cascades often occur in aquatic ecosystems (Jürgens 1994; Östman et al. 2016), especially when fish predation affects species with key roles in linking different trophic levels and transferring energy through food chains (Hansson et al. 2004).

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Although zooplankton represent a link between primary producers and fish in freshwater ecosystems (Brooks & Dodson 1965; Hansson et al. 2007), not all zooplankters are keystone species that link trophic levels. Substantial differences in functional traits between species lead to their different effects on phytoplankton (Cyr & Curtis 1999; Sommer et al. 2003; Barnett et al. 2007), and conceptualising zooplankton as a homogeneous functional group thus masks species-specific roles in within food webs. Hence, studies considering the effects of total zooplankton biomass on phytoplankton have typically found no or only weak top-down effects (McQueen et al. 1989; Mehner et al. 2008). More pronounced cascading effects have been observed for zooplankton of the genus *Daphnia* (McQueen et al. 1986; McQueen et al. 1989), which are considered as keystone taxa that link primary and tertiary production due to their effective grazing on a broad size spectrum of edible phytoplankton (Lampert 1987). As well as influencing phytoplankton in green food webs, *Daphnia* can cause complex cascading effects in detritivore-based brown food webs by consuming microorganisms through non-selective grazing (Zöllner et al. 2003).

Body size is among the most important traits influencing both zooplankton filtration rates and therefore top-down control of lower trophic levels (Gianuca et al. 2016). Many *Daphnia* species are large zooplankters and are therefore keystone grazers with a high capacity to reduce phytoplankton biomass (Vakkilainen et al. 2004). Larger-bodied daphniids can outcompete smaller species due to consuming wider range of phytoplankton, and the growth and reproduction of larger-bodied daphniids is greater when phytoplankton densities are low (Gliwicz 1990a; Gliwicz 1990b). Body size also influences fish predation, because most fish are size-selective predators that prefer larger and less evasive prey (Brooks & Dodson 1965). *Daphnia* densities can be strongly reduced by specialised planktivorous fish, which typically leads to an increase in phytoplankton biomass (Williams & Moss 2003).

Although many studies have considered fish effects on zooplankton and consequently on phytoplankton (Helminen & Sarvala 1997; Bertolo et al. 2000; Williams & Moss 2003; Vakkilainen et al. 2004), the importance of individual zooplankton species within trophic cascades is still poorly understood, even for potential keystone species such as daphniids. The role of individual zooplankton species in trophic cascades is challenging to investigate within natural ecosystems because species-specific effects are difficult to isolate. Equally, small-scale ex-situ top-down experiments de-

signed to compare the feeding impacts of individual species on phytoplankton (Adrian & Schneider-Olt 1999; Gianuca et al. 2016) lack a natural zooplankton community structure as well as predation by fish. In-situ mesocosm experiments, which allow examination of natural zooplankton communities exposed to natural predation, rarely focus on the effects of single zooplankton species on phytoplankton (Lynch 1979; Bertolo et al. 2000; Hansson et al. 2004). The role of individual zooplankton species in lake food webs thus remains unclear, especially in deeper pelagic and oligotrophic environments.

To investigate the trophic roles of individual zooplankton species, we performed a mesocosm experiment in an offshore area of Upper Lake Constance, central Europe. This area allowed us to represent the pelagic environment using natural phytoplankton and zooplankton communities and the two most abundant pelagic fish species, the native whitefish (*Coregonus wartmanni*; hereafter, whitefish), and the invasive three-spined stickleback (*Gasterosteus aculeatus*; hereafter, stickleback). Our aim was to determine which zooplankters function as keystone species that represent strong links between fish predation and phytoplankton biomass, and which zooplankters are susceptible to changes in top-down predation in natural environments. We tested four hypotheses: 1) smaller and more evasive zooplankton species and combined groups of multiple zooplankton species are less susceptible to fish predation than large daphniid species; 2) as specialised planktivores, native whitefish consume more zooplankton than sticklebacks, which are generalist feeders; 3) large daphniids cause greater reductions in edible and total phytoplankton biovolumes than other zooplankton taxa; and 4) fish stimulate increases in phytoplankton biovolumes by decreasing large daphniid densities.

Methods

Study sites

Lake Constance is a large (536 km²), deep (maximum 251 m) pre-Alpine lake situated at the border of Germany, Switzerland and Austria. It consists of two basins, the Lower Lake Constance and the larger Upper Lake Constance, in which our study was performed. Upper Lake Constance experienced several decades of anthropogenic eutrophication during the 20th century with phosphorus concentrations peaking in 1979, but has since returned to an oligotrophic state due to restoration efforts (IGKB 2013; Schotzko 2018). The most abundant zooplankton taxa in Upper Lake Constance are cyclopoid copepods, the calanoid copepod *Eudiaptomus gracilis*, and the cladocerans *Daphnophanes brachyurum*, *Bosmina* spp., *Daphnia longispina*

(formerly known as *D. hyalina*), *Daphnia galeata*, *Leptodora kindtii* and *Bythotrephes longimanus* (Straile & Geller 1998). Recently, a small daphniid species, *Daphnia cucullata*, has been recorded at high densities (IGKB 2018, Isanta Navarro et al. 2019). The fish fauna comprises approx. 30 species, of which three are common in the pelagic zone: the native lake char (*Salvelinus umbla*), the whitefish and the stickleback. The latter is an invasive, non-native species that has been occurring at high densities in the pelagic zone of Upper Lake Constance since 2012–2013 (Rösch et al. 2018; Eckmann & Engesser 2019), and in 2014 represented 96 % of the total abundance and 28 % of the biomass of pelagic fish (Alexander & Vonlanthen 2016).

Experimental design

The experiment was conducted in the Upper Guell bay of Upper Lake Constance between 27 June and 25 July 2019. It comprised 12 plastic cylindrical mesocosms made of transparent polyethylene, each with a 15-m depth, a 96-cm diameter and a 10 m³ volume. Mesocosms were heat-sealed at the base and open to the atmosphere above the water surface. Mesocosms were attached to pontoons, freely hanging in open water, with their bases approx. 2 m above the sediment. Each pontoon accommodated four mesocosms in a row at 25-cm intervals and in an east-west direction to prevent shading by adjacent mesocosms. Each mesocosm was covered with a transparent acrylic glass plate, to protect it from bird excrement and other external disturbances.

On June 19, the mesocosms were filled with filtered (<300 µm) lake water and then inoculated with lake zooplankton. Zooplankton were sampled using vertical net tows (300 µm) from a depth of 15 m to the surface, filtering the same volume of water as in the 12 mesocosms, to ensure inoculation of zooplankton at natural densities at these depths. Samples were filtered through a 630-µm sieve to remove predatory zooplankton such as *L. kindtii* and *B. longimanus* as well as fish larvae; this process also removed the largest adult daphniids and copepods. The sampled zooplankton volume was then divided into 12 equal parts and one part added to each mesocosm. Zooplankton were then left to acclimatise within the mesocosms for eight days, allowing conditions to stabilise and zooplankton to develop to adult sizes (Schwartz 1984; Peterson 2001). The experiment lasted four weeks, to provide enough time for phytoplankton and zooplankton reproduction, community development and response to experimental conditions (Riebesell et al. 2011). To enable characterisation of well-pronounced top-down effects, the experimental period coincided with the lake's phytoplankton spring peak, which occurred unusually late in the study year i.e. around 1 July (unpublished data).

Treatments were randomly assigned to mesocosms, ensuring that each pontoon had at least one replicate of each treatment and that the treatments were placed in different orders within the pontoons. After the acclimatisation period ended (i.e. day 0, 27 June), the first samples were collected from all mesocosms (see below), and predatory zooplankton and fish were then added to treatments. Due to the patchy distribution of larger predatory zooplankton species and their potentially important roles in pelagic food webs, 200 *L. kindtii* and 40 *B. longimanus* were counted and added separately to each mesocosm, representing 1/4 and 1/5 of their typical maximum Lake Constance densities, respectively. These densities were limited by unusually low numbers of these two species in the lake dur-

ing the study period. A treatment containing only zooplankton (including predatory taxa) acted as the control.

Two fish treatments received either four sticklebacks (mean ± SD, 0.31 ± 0.048 g) or four whitefish (0.32 ± 0.093 g). The body length of whitefish was generally greater than that of sticklebacks with the same biomass, although exact lengths were not measured. Total fish densities in mesocosms were 1.71 ± 0.096 g m⁻² SD for sticklebacks and 1.77 ± 0.094 g m⁻² SD for whitefish, representing natural densities in Lake Constance. Because fish densities in the lake are difficult to estimate precisely, annual fishery yields of adult whitefish were used as a reference, with 2007–2016 yields corresponding to 0.58 g m⁻² (Kugler & Friedl 2018). Because adult whitefish dominate pelagic fish biomass (Alexander & Vonlanthen 2016) and because they are usually harvested soon after reaching their adult size (Eckmann & Rösch 1998), densities 2–3 times higher than annual whitefish yields might adequately represent actual densities in the lake.

Sampling and sample processing

Starting on day 0, abiotic parameters were measured and both phytoplankton and zooplankton samples were taken from mesocosms. Sampling frequency was different for each parameter. Twice a week, dissolved oxygen concentrations, pH and temperature were measured in each mesocosm and in the lake with a multiparameter probe (CTD-90 M; Sea&Sun Technology), and chlorophyll concentrations were measured with a fluorescence probe (bbe Moldaenke, TS 17-09). These abiotic measurements were taken by lowering probes from the surface to a depth of 12 m, prior to disturbance of the water column during biotic sample collection. Zooplankton were sampled using vertical net hauls (16.5-cm opening, 100-cm length and 100-µm mesh) from a depth of 12 m to the mesocosm surface, on days 0, 14, 21 and 28. Phytoplankton were sampled on days 0, 14 and 28 by performing a vertical tow with an integrated water sampler across the same depth range, which collected 2 L of water, from which 100-mL subsamples were taken for analysis.

Collected zooplankton samples were preserved at –20 °C until processing. After thawing, samples were divided into aliquots of ≥300 zooplankters, identified to the lowest taxonomic resolution possible (order to species), counted, and values used to back-calculate densities of each taxon. Although hybrids of the three *Daphnia* species probably occurred in our samples (Isanta Navarro et al. 2019), we designated each as only one of three species based on its morphological features. All copepod life stages were included except nauplii, due to their inconsistent sampling by a 100-µm mesh and identification difficulties. The five most abundant taxa were used for further analyses: *Bosmina* spp. (3.6%), Cyclopoida (20.5%), *E. gracilis* (10.1%), *D. cucullata* (44.9%) and *D. longispina* (20.8%). In addition, *D. longispina* and *D. cucullata* were combined as a total daphniid fraction. All other zooplankton taxa (including *D. galeata*) represented <0.2 % of total zooplankton abundance and were therefore excluded from further analysis.

Phytoplankton samples were fixed in Lugol's acid solution + sodium acetate, identified to genus level using an inverted light microscope and counted following Utermöhl (1958). Biovolumes were calculated using taxon-specific body volumes established for Lake Constance (Pauli 1989; Kümmerlin & Bürgi 1989; Müller et al. 1991). Phytoplankton species were divided into two groups according to their edibility to large daphniids. Unicellular taxa < 40 µm in cell length were classified as edible,

whereas those $>40\ \mu\text{m}$ in cell length, colony-forming and mucilaginous species were classified as inedible (Lampert 1987); cell lengths were based on Rimet & Druart (2018).

Data analysis

Shapiro-Wilk tests confirmed that all zooplankton and phytoplankton data had normal distributions. Initial (day 0) total zooplankton densities were compared with a one-way ANOVA, to ensure comparability among treatments. To compare densities of different zooplankton species among treatments (hypothesis 1) including identification of differences between the whitefish and stickleback treatments (hypothesis 2), we performed a two-way repeated-measures (RM) ANOVA with treatment (control, whitefish, stickleback) as the between-subjects factor and day (0, 14, 21, 28) as the within-subjects factor. Mauchly's tests were used to test the RM ANOVA assumption of sphericity, with deviations from sphericity addressed using Greenhouse-Geisser corrections. Pairwise t-tests with Bonferroni corrections were used to identify differences between treatments on each day. Linear regression models (LM) were used to identify relationships between the densities of zooplankton taxa (as predictor variables) and total, edible and inedible fractions of phytoplankton biovolumes (as dependent variables; hypothesis 3). To test hypothesis 4, a second RM ANOVA and pairwise tests (as described above) were done to compare phytoplankton biovolumes in each treatment. All analyses were done in the statistical software R (R Core Development Team 2018), us-

ing the package *afex* (Singmann et al. 2020) to perform RM ANOVAs and Mauchly's tests, the basic R function *lm()* to run LM, and the package *ggplot2* (Wickham 2016) to plot graphics.

Results

Abiotic conditions were stable during the experiment: dissolved oxygen ranged from $10\text{--}12\ \text{mg L}^{-1}$ at the surface to $12\text{--}15\ \text{mg L}^{-1}$ at a depth of 12 m, pH varied between $8.5\text{--}9.5$ at all depths, and temperatures ranged from $22\text{--}25\ ^\circ\text{C}$ at the surface to $10\text{--}12\ ^\circ\text{C}$ at 12 m.

Zooplankton densities were comparable in all treatments at the start of the experiment (i.e. on day 0; one-way ANOVA $F_{2,6} = 0.03$, $p = 0.969$). Total zooplankton and total daphniid densities differed among treatments but not days, and no interactions were identified between day and treatment (RM ANOVA, Table 1). Both total zooplankton densities (pairwise t-test, $p = 0.012$) and total daphniid densities ($p = 0.004$) were higher in the control than in the whitefish treatment but not the stickleback treatment ($p > 0.05$). The large daphniid *D. longispina* was the only zooplankton taxon whose densities responded to fish, including a significant

Table 1. Results of two-way repeated-measures ANOVAs describing differences in zooplankton densities and total phytoplankton biovolumes among treatments, days and their interaction. Significant results ($p < 0.05$) are indicated in bold.

| Group | Effect | df | F | p |
|-----------------------------|--|----------|---------------|-------------------|
| <i>Bosmina</i> spp. | Treatment | 2 | 1.19 | 0.367 |
| | Day | 3 | 5.36 | 0.025 |
| | Treatment \times day | 6 | 0.96 | 0.462 |
| Cyclopoida | Treatment | 2 | 0.82 | 0.484 |
| | Day | 3 | 11.78 | < 0.001 |
| | Treatment \times day | 6 | 1.25 | 0.336 |
| <i>Daphnia cucullata</i> | Treatment | 2 | 1.64 | 0.26 |
| | Day | 3 | 5.17 | 0.036 |
| | Treatment \times day | 6 | 1.21 | 0.353 |
| <i>Daphnia longispina</i> | Treatment | 2 | 28.60 | < 0.001 |
| | Day | 3 | 2.46 | 0.133 |
| | Treatment \times day | 6 | 3.84 | 0.037 |
| <i>Eudiaptomus gracilis</i> | Treatment | 2 | 2.48 | 0.164 |
| | Day | 3 | 9.70 | 0.03 |
| | Treatment \times day | 6 | 2.06 | 0.152 |
| Total daphniids | Treatment | 2 | 11.39 | 0.006 |
| | Day | 3 | 1.28 | 0.306 |
| | Treatment \times day | 6 | 1.96 | 0.184 |
| Total zooplankton | Treatment | 2 | 6.69 | 0.024 |
| | Day | 3 | 0.22 | 0.751 |
| | Treatment \times day | 6 | 1.77 | 0.212 |
| Total phytoplankton | Treatment | 2 | 11.28 | 0.005 |
| | Day | 2 | 147.73 | < 0.001 |
| | Treatment \times day | 4 | 8.546 | 0.006 |

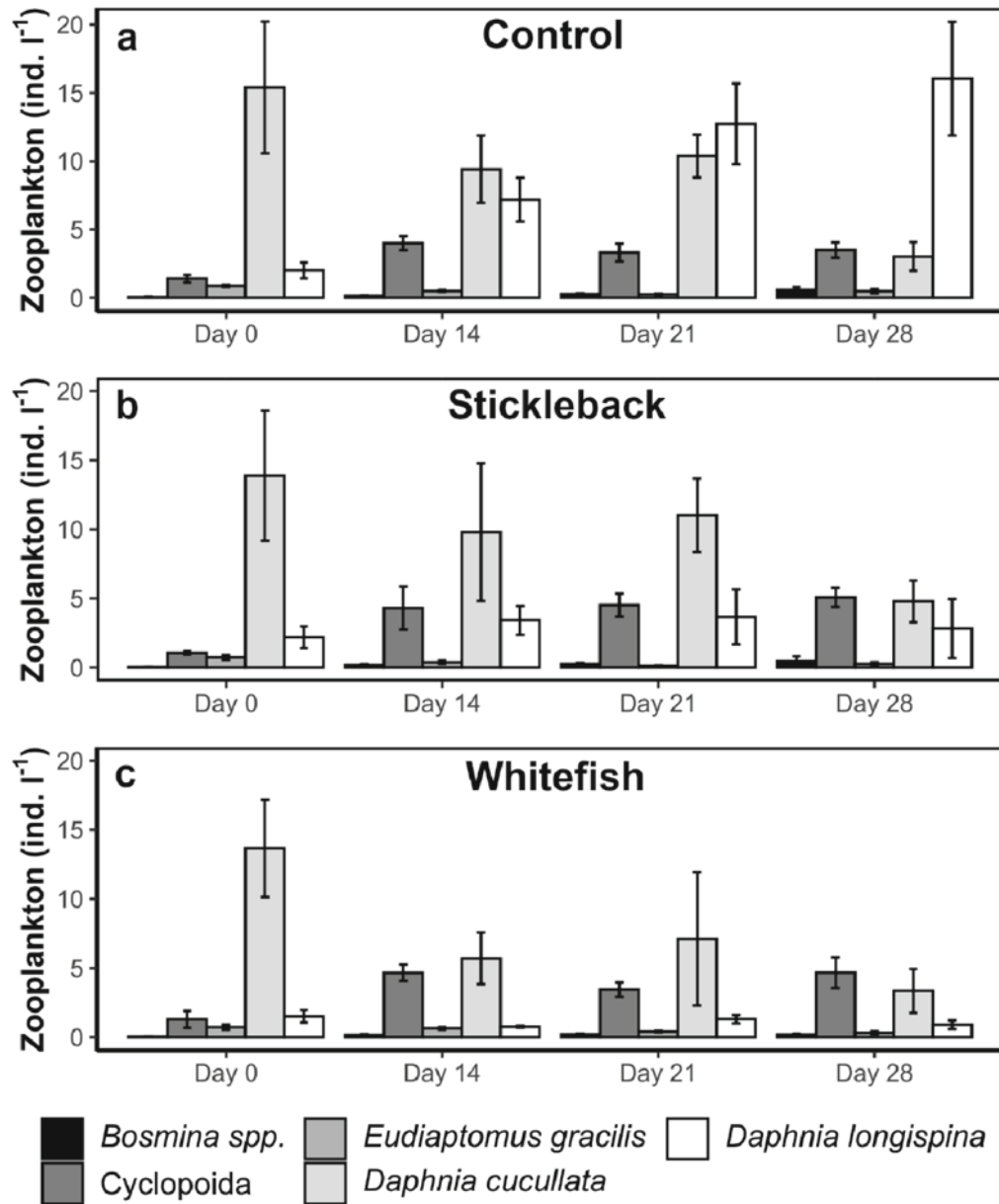


Fig. 1. Mean (± 1 SD) zooplankton taxa densities (individuals [ind.] l^{-1}) in control (a), stickleback (b) and whitefish (c) treatments on each sampling day.

treatment effect and a significant treatment–day interaction (Table 1, Fig. 1): while *D. longispina* densities were comparable on all days in both fish treatments, densities increased over time in the control treatment (Table 1, Fig. 2). *Daphnia longispina* densities were lower in the whitefish treatment compared to the control treatment on days 14 (pairwise t-test, $p=0.005$), 21 ($p=0.012$) and 28 ($p=0.009$). Densities of this taxon were also higher in the stickleback treatment compared to the control on days 21 ($p=0.038$) and 28 ($p=0.013$). Densities of all other zooplankton taxa

changed over time but did not differ among treatments, and no interactions were identified between treatment and time (Table 1, Fig. 1). *Bosmina* spp. (pairwise t-test, $p=0.013$) and Cyclopoida ($p<0.001$) densities increased whereas *E. gracilis* and *D. cucullata* densities decreased ($p<0.001$) between day 0 and day 28.

Total phytoplankton biovolumes were negatively related to *D. longispina* densities on both day 14 (LM; $p=0.049$) and day 28 ($p=0.01$), and also to total daphniid densities on day 28 ($p=0.03$; Table 2). Increasing *D. longispina* densities were related to a decline

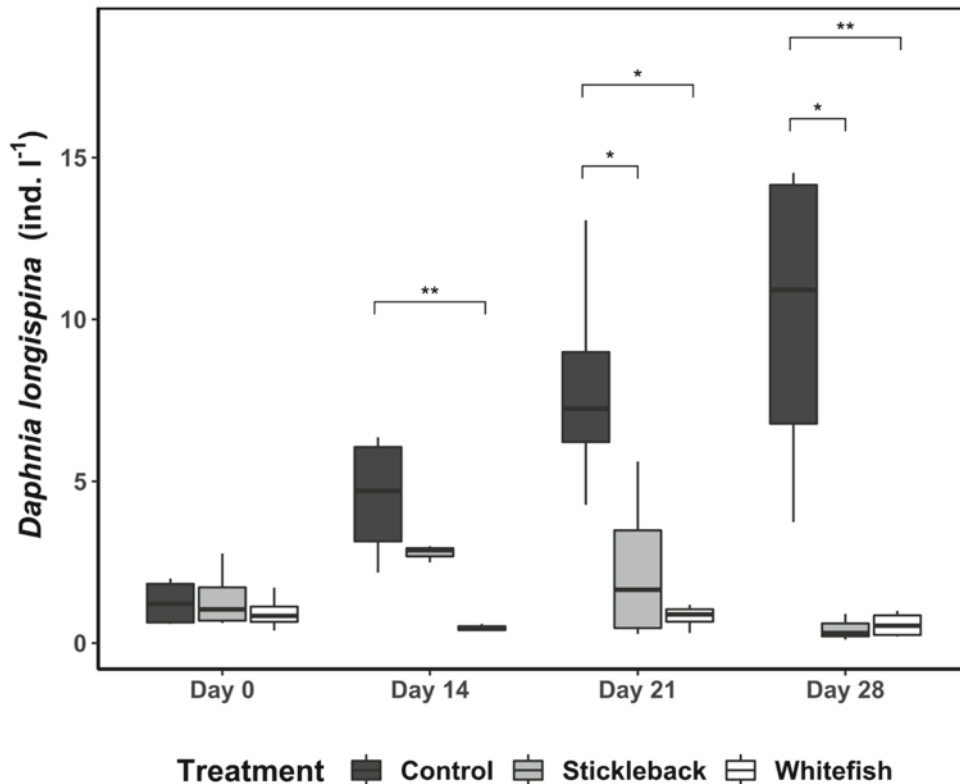


Fig. 2. *Daphnia longispina* densities (individuals [ind.] l⁻¹) in each treatment on each sampling day. The results of the pairwise t-tests comparing treatments are indicated by brackets and asterisks (* $p < 0.05$, ** $p < 0.01$).

Table 2. Linear model results describing relationships between total phytoplankton biovolumes and densities of different zooplankton taxa and groups. Significant results ($p < 0.05$) are indicated in bold.

| Zooplankton taxon/group | Day 14 | | Day 28 | |
|-----------------------------|----------------|--------------|----------------|-------------|
| | R ² | <i>p</i> | R ² | <i>p</i> |
| <i>Bosmina</i> spp. | 0.04 | 0.54 | 0.06 | 0.45 |
| Cyclopoida | 0.00 | 0.89 | 0.24 | 0.11 |
| <i>Daphnia cucullata</i> | 0.12 | 0.27 | 0.00 | 0.98 |
| <i>Daphnia longispina</i> | 0.33 | 0.049 | 0.51 | 0.01 |
| <i>Eudiaptomus gracilis</i> | 0.20 | 0.14 | 0.18 | 0.17 |
| Total daphniids | 0.14 | 0.23 | 0.39 | 0.03 |
| Total zooplankton | 0.12 | 0.28 | 0.28 | 0.07 |

in edible phytoplankton biovolumes on day 14 (LM, $R^2 = 0.41$, $p = 0.025$; Fig. 3a) and day 28 ($R^2 = 0.56$, $p = 0.005$; Fig. 3b), whereas biovolumes of the inedible fraction did not respond to *D. longispina* densities (Fig. 3c–d). Phytoplankton biovolumes were not related to densities of total zooplankton ($p = 0.07$) or of any other individual taxon ($p > 0.1$; Table 2).

Phytoplankton biovolumes differed between days and treatments and a significant interaction was observed between treatment and date (Table 1). In all

treatments, total phytoplankton biovolumes were higher on day 14 compared to day 0 (pairwise t-test, $p < 0.001$) then declined between days 14 and 28 ($p < 0.001$). Compared to the control, biovolumes were higher in the whitefish treatment on days 14 (pairwise t-test, $p = 0.001$) and 28 ($p < 0.001$) and in the stickleback treatment on day 28 ($p < 0.001$; Fig. 4). Biovolumes were higher in the whitefish treatment compared to the stickleback treatment on day 14 ($p = 0.002$; Fig. 4).

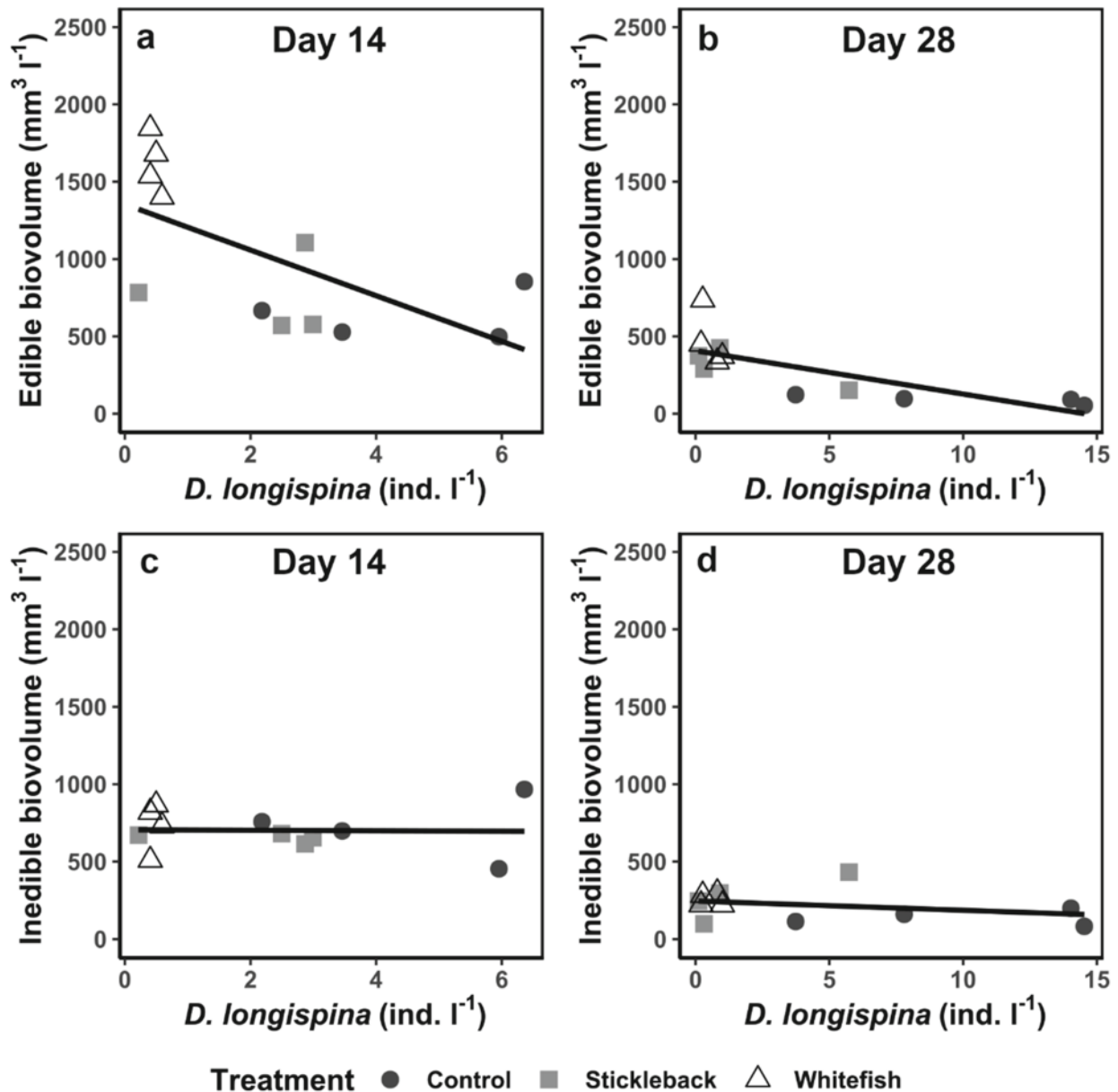


Fig. 3. Linear model ($y = b \cdot x$) showing the relationship between *Daphnia longispina* densities (individuals [ind. l^{-1}]) and biovolumes ($\text{mm}^3 \text{l}^{-1}$) of edible ($< 40 \mu\text{m}$) and inedible ($> 40 \mu\text{m}$) phytoplankton on day 14 (a, c) and day 28 (b, d).

Discussion

Zooplankton of the genus *Daphnia* are typically described as keystone grazers that link primary production and higher trophic levels in lake ecosystems, but the roles of single daphniid species remain poorly known. Supporting our first hypothesis, densities of the largest-sized daphniid studied, *D. longispina*, decreased in response to fish predation. In contrast, densities of other zooplankters, including the small daphniid *D. cucullata*, did not decline. These findings

demonstrate that considering the genus *Daphnia* as a keystone taxon is a generalisation that overlooks size-mediated differences between species. Whitefish and sticklebacks had comparable effects on zooplankton, which refutes our second hypothesis, that whitefish – as specialised planktivores – have a greater impact on zooplankton communities. Supporting our third hypothesis, phytoplankton biovolumes decreased primarily in response to *D. longispina* densities, likely their large body size enables filtration of a wider spectrum of phytoplankton taxa and also growth at lower

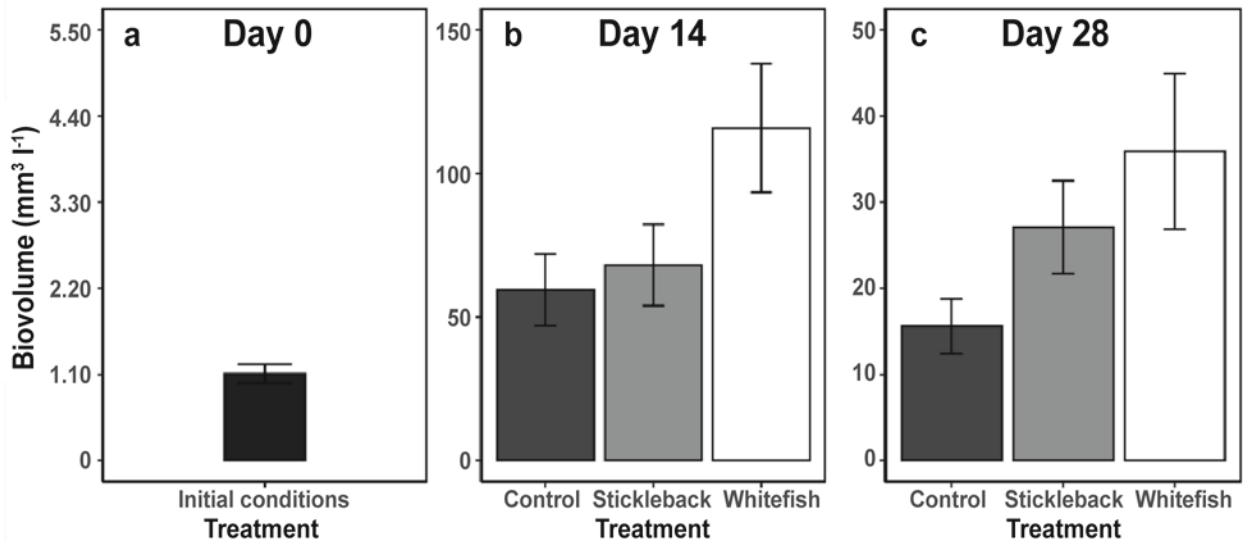


Fig. 4. Mean \pm 1 SD phytoplankton biovolumes ($\text{mm}^3 \text{l}^{-1}$) in control and fish treatments on sampling days 0 (a), 14 (b) and 28 (c). Identical phytoplankton biovolumes at day 0 are joined in one bar as initial conditions (a).

phytoplankton densities (Gliwicz 1990b). Therefore, evidence of cascading effects was seen as higher phytoplankton biovolumes in fish treatments, caused by fish-mediated decreases in the densities of large daphniids (Fig. 5), supporting our fourth hypothesis. Both fish treatments were linked to comparable increases in phytoplankton densities, which our results suggest were mediated by fish predation on zooplankton, in particular *D. longispina*. We thus suggest that *D. longispina* regulates trophic cascades by mediating top down effects in pelagic food webs including fish with both specialist and generalist feeding modes.

Fish predation on zooplankton

Compared to the initial lake conditions, *D. longispina* densities increased only in the fishless control treatments, reflecting release from vertebrate predation. Densities of other zooplankton taxa changed over time but not between treatments, suggesting that changes were not predation mediated. The increases in *Bosmina* spp. and cyclopoid copepods might be seasonal, because comparable increases were observed in the lake during the study. In addition, cyclopoid copepod densities might still have been increasing following inoculation, since their densities were lower on day 0 than on any other date. The drivers of temporal decreases in *D. cucullata* and *E. gracilis* are unclear but might reflect specific conditions within the closed mesocosm environment. Although densities of microcrustaceans other than large daphniids can be reduced when fish

predation is high (Post & McQueen 1987; Bertolo et al. 2000), we did not observe such reductions, probably because fish densities were low, as is natural in oligotrophic Lake Constance. Mehner et al. (2008) also found no negative relationship between the biomass of planktivorous coregonid fish and zooplankton species other than *Daphnia* in an oligotrophic lake.

Various factors contribute to reduced fish predation on zooplankton other than large daphniids. The contrasting movement pattern of cyclopoid and calanoid copepods makes them harder to capture compared to cladocerans such as *Daphnia* (Visser 2007; Peterka & Matěna 2011). Furthermore, due to their small size, taxa such as the genus *Bosmina* are less conspicuous and therefore less predated than larger zooplankters (Brooks & Dodson 1965). Although fish predate small and evasive zooplankton, densities of some zooplankters can increase at low fish densities due to the removal of their competitors by selective fish predation on larger and less evasive species (Brooks & Dodson 1965; Christoffersen et al. 1993). Comparable densities of the small daphniid *D. cucullata* in control and fish treatments may reflect its small size and also its morphology: it is slimmer and therefore less conspicuous than *D. longispina* of the same body length (Gliwicz 2001). The reactive distance of roach for detecting *D. longispina* is double that for *D. cucullata* of the same body length, resulting in a near-tenfold larger reactive field volume and thus higher fish predation on *D. longispina* (Gliwicz 2001). The dominance of small or large-sized zooplankton communities can thus

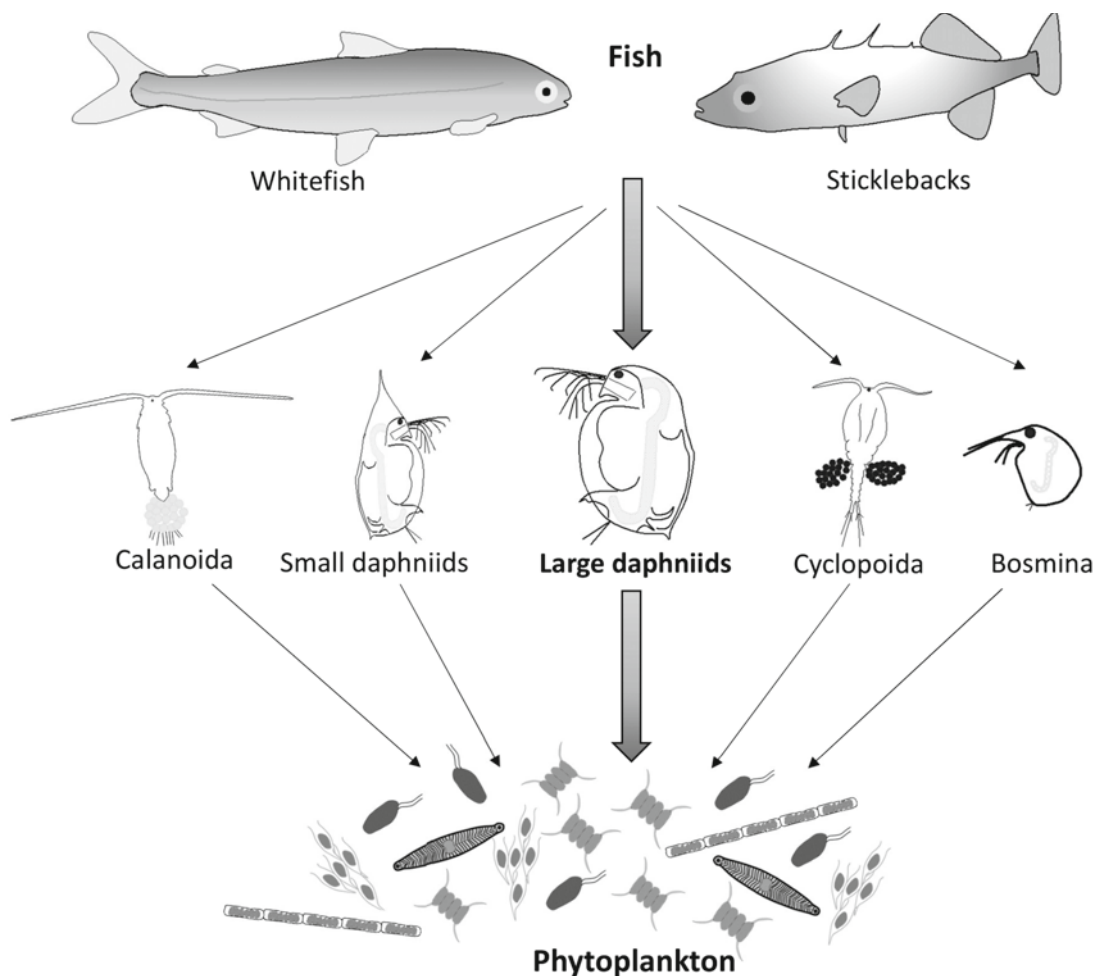


Fig. 5. A conceptual model of trophic cascading from fish (represented by a stickleback and a whitefish) to primary producers (phytoplankton) within a pelagic ecosystem. Thick arrows represent reductions in densities at lower trophic levels. Of all zooplankton taxa, only densities of the large daphniids were reduced by fish predation and thus caused declines in phytoplankton biovolumes, indicating their role as keystone species.

largely reflect predation pressure in lakes (Jeppesen et al. 1997; Iglesias et al. 2017; Ersoy et al. 2017), as indicated in our study by the dominance of *D. cucullata* and cyclopoid copepods in fish treatments and *D. longispina* in non-fish treatments.

The natural zooplankton community represented in our experiments included two large invertebrate predators, *B. longimanus* and *L. kindtii*. Although fish probably influenced their densities, we suggest that invertebrate predators did not cause pronounced differences in prey zooplankton composition between treatments. Invertebrate predation on zooplankton is typically much lower compared to vertebrate (fish) predation (Gliwicz & Pijanowska 1989; Šorf et al. 2014). In addition, due to their low densities, larger invertebrate predators were rarely observed in our zooplankton samples, and densities of smaller inverte-

brate predators (i.e. cyclopoid copepods) did not differ between treatments.

Cascading effects of fish species

Whitefish did not cause greater reductions in any zooplankton species compared to sticklebacks. However, cascading effects on phytoplankton via consumption of zooplankton (especially *D. longispina*) were initially more pronounced for whitefish than sticklebacks, as indicated by faster increases in phytoplankton biovolumes (i.e. by day 14). Whitefish are specialised zooplankton feeders with adaptations to pelagic environments whereas sticklebacks are generalist feeders (Morrow 1980; Becker & Eckmann 1992; Kotelat & Freyhof 2007), which probably enabled higher whitefish consumption of phytoplankton grazers. Al-

though differences in zooplankton densities between fish treatments were not significant, moderately lower densities in the whitefish treatment might have been enough to cause significant differences in phytoplankton biovolume. Over time, differences in zooplankton densities and phytoplankton biovolumes between whitefish and sticklebacks became less pronounced, and within 28 days, sticklebacks and whitefish triggered similar trophic cascades. Other mesocosm experiments have indicated greater species-specific differences between fish, with specialised planktivores affecting zooplankton and consequently phytoplankton communities more strongly than facultative planktivorous fish, at least in shallow (<1 m) eutrophic littoral waters (Williams & Moss 2003; Des Roches et al. 2013). However, we are not aware of any previous studies with similarly low fish densities and/or sufficiently deep mesocosms to develop a thermocline and thus to represent cascading in pelagic and oligotrophic environments; these factors may explain the contrasting patterns observed in our study.

The effects of large daphniids on phytoplankton biomass

Many studies indicate that the genus *Daphnia* causes greater reductions in edible phytoplankton biovolumes than other zooplankton taxa (McQueen & Post 1988; Adrian & Schneider-Olt 1999; Mehner et al. 2008). In particular, McQueen et al. (1986; 1989) studied how body size influences the effects of *Daphnia* on phytoplankton biomass and linked large-sized daphniids to reduced biomass in mesoeutrophic and eutrophic environments. Our results build on such research by comparing the effects of different-sized daphniid species, and suggest that larger daphniids such as *D. longispina* are the main zooplankters that reduce phytoplankton biovolumes in natural lake communities.

Although other zooplankton species, such as copepods, can have complementary effects to daphniids by grazing on different-sized phytoplankton species, thus contributing to greater overall reductions in biomass (Sommer et al. 2001; Sommer et al. 2003), they did not alter phytoplankton biovolumes at the natural densities represented in our communities. Total zooplankton densities had no significant effect on phytoplankton biovolume, due to the pronounced declines caused by *D. longispina* being offset by the limited effects of other, smaller taxa. Equally, the linear relationship between densities of the genus *Daphnia* and phytoplankton biovolumes on day 28 reflected only the pronounced effect of *D. longispina*, whereas *D. cucullata* had no effect on biovolumes. These results in-

dicate that previous research may have overstated the importance of small *Daphnia* species, if genus-level responses were caused solely by larger, co-occurring congeners. Larger *D. longispina* also have higher potential than *D. cucullata* to increase their population densities due to higher birth and biomass production rates (Vijverberg & Richter 1982). This greater fecundity might have contributed to increasing *D. longispina* dominance in our fishless mesocosms, where increasing densities enabled greater control of phytoplankton.

Cascading links from fish to phytoplankton

Our results indicate that *D. longispina* was a keystone species that linked both planktivorous and generalist fish predators to phytoplankton communities (Fig. 5). No other zooplankton or daphniid taxa or groups were so greatly predated by fish or showed such strong phytoplankton control, and multi-taxon groups therefore formed much weaker cascading links between fish and primary producers. Although smaller zooplankton species can also contribute to trophic cascades from fish to phytoplankton if large cladocerans are absent (Christoffersen et al. 1993; Helminen & Sarvala 1997), most studies indicate that the genus *Daphnia* – if present – is the taxon most negatively affected by fish predation, and has also the strongest effects on phytoplankton densities (Post & McQueen 1987; Mehner et al. 2008). However, variability in the roles of different *Daphnia* species in trophic cascades remains poorly studied.

Although fish can also have a positive effect on phytoplankton growth by remineralising nutrients, this effect is mostly limited to non-edible phytoplankton, whereas edible phytoplankton is more affected by zooplankton grazing (Vanni 2002). In our study, biovolumes of non-edible phytoplankton did not differ among treatments, whereas edible phytoplankton biovolumes decreased in response to *D. longispina* densities. These results suggest that phytoplankton biovolumes were strongly controlled by direct zooplankton (especially *D. longispina*) grazing, whereas fish effects such as remineralisation had a minor role.

Trophic cascades in a warming climate

Our results provide evidence that zooplankton taxa – including small *D. cucullata* – might have a more limited influence on phytoplankton biomass in large oligotrophic lakes than the larger *D. longispina*. Global warming is altering pelagic food webs by shrinking animal body sizes and shifting community compositions towards a higher proportion of small-sized spe-

cies and individuals (Daufresne et al. 2009; Geerts et al. 2015). As such, increasing temperatures that cause intraspecific or interspecific decreases in zooplankton body sizes could alter the functioning of green and brown food webs in oligotrophic freshwaters. Indeed, *D. cucullata*, which was rarely observed in Upper Lake Constance before 2015, became a summer-dominant cladoceran in 2017 (IGKB 2018). How recent and ongoing changes in *Daphnia* composition are altering ecosystem dynamics is unknown. Further research is needed to determine how body size and other functional traits interact to determine the roles of different daphniid species in trophic cascading and energy transfer from phytoplankton to fish.

Conclusions

Our study advances our understanding of species-specific roles of zooplankton in food webs and trophic cascades in oligotrophic pelagic environments. We demonstrate that considering both zooplankton and daphniids as uniform taxonomic groups, as it is common praxis, might limit understanding of links between multiple trophic levels in aquatic ecosystems. We suggest that the genus *Daphnia* is not a keystone taxon, but rather that large *Daphnia* species such as *D. longispina* can control food web structure at trophic levels from phytoplankton to fish. Our observation of comparable top-down predation on *D. longispina* by fish with specialist and generalist feeding modes suggests that the keystone role of large daphniids in oligotrophic lake food webs could extend across ecosystems with different fish predation regimes.

Authors' contributions

ŽO, CW, AJK and JIN designed the study. All authors conducted the experiment. CW analysed the data, drawings by JIN. ŽO and CW prepared the manuscript with contributions from all authors. ŽO and CW contributed equally to this work and share the first authorship.

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