

Top-down effects of bighead carp (*Aristichthys nobilis*) and *Leptodora richardi* (Haplopoda, Leptodoridae) in a subtropical reservoir during the winter–spring transition: a mesocosm experiment

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Abstract To assess top-down effects of planktivorous fish and *Leptodora* in the freshwaters of southern China, a mesocosm experiment was conducted by manipulating bighead carp (*Aristichthys nobilis*) and *Leptodora richardi* within in situ enclosures installed in an oligo-mesotrophic reservoir. During the winter–spring transition, a low biomass (≈ 1 g wet weight m^{-3}) of fish significantly reduced *Daphnia* biomass and zooplankton clearance rates and markedly increased the biomass of total phytoplankton, small phytoplankton (GALD <30 μm), and large phytoplankton (GALD ≥ 30 μm). However, there was no significant effect of *Leptodora* and no interactive effect from fish and *Leptodora* on herbivorous zooplankton and phytoplankton. By contrast,

exclusion of fish from the enclosures triggered the outbreak of *Daphnia* and thus resulted in higher zooplankton clearance rates. Algal biomass decreased to a low level in the absence of fish relative to in their presence, particularly during the last 10 days (mean biomass ratio, 1:7–1:36). Our results indicate that fish play a more important role in top-down effects than *Leptodora*. This study, together with previous research, suggests that fish may prey heavily on large-bodied herbivores, especially *Daphnia*, in southern China and reduce the chances for top-down control of phytoplankton.

Keywords Tropical limnology · *Daphnia* · Filter-feeding fish · *Leptodora richardi* · Trophic cascade · Phytoplankton

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Introduction

Large-bodied zooplankton of the genus *Daphnia* are considered keystone herbivores in freshwater ecosystems, owing to their highly efficient grazing on phytoplankton (Mazumder, 1994). In temperate lakes, intense grazing of such large cladocerans on phytoplankton causes the well-known spring clear-water phase (Lampert et al., 1986; Sarnelle, 1993; Sommer et al., 2012) and even an extended period of water clarity that arises from a trophic cascade (e.g., Carpenter et al., 2001; Parker & Schindler, 2006). In contrast, the occurrence of an effective top-down

control over phytoplankton seems unusual in (sub)tropical regions (Jeppesen et al., 2005; van Leeuwen et al., 2007). As suggested by van Leeuwen et al. (2007), this may be because the genus *Daphnia* is typically absent or rare in warmer climates (e.g., Fernando, 1980; Arcifa, 1984; Dumont, 1994; Lin et al., 2003; Li et al., 2012), thereby reducing the top-down control of phytoplankton. Also, it has been suggested that the most likely cause for the pattern may be strong top-down control of large zooplankton by fish at lower latitudes (Jeppesen et al., 2005; van Leeuwen et al., 2007). Despite the lack of empirical evidence, this view is supported by the following studies at low latitudes: (1) piscivores are usually absent (Jeppesen et al., 2007); (2) omnivorous planktivorous fish often predominate fish community and attain high carrying capacity (Lazzaro, 1997); and (3) many fish species reproduce throughout the year (Fernando, 1994; Lazzaro, 1997), leading to the occurrence of abundant juvenile fish (Jeppesen et al., 2005). Therefore, planktivorous fish may exert a year-around, high predation pressure on zooplankton in warmer climates.

Like planktivorous fish, some large invertebrate predators, such as *Bythotrephes*, *Leptodora*, and *Chaoborus* larvae, can also prey heavily on herbivorous zooplankton (Hoffman et al., 2001; Wojtal et al., 2004; Castilho-Noll & Arcifa, 2007), and thus, by changing the grazing rate of zooplankton, indirectly impact phytoplankton biomass (Wojtal et al., 1999; Benndorf et al., 2000; Strecker & Arnott, 2005). The cladoceran *Leptodora*, a common invertebrate predator in the northern temperate zone (Rivier, 1998), was generally deemed to be absent from lower latitudes (Fernando et al., 1990; Dumont, 1994). Nevertheless, recent research has shown that it also exists in tropical and subtropical regions of the Northern Hemisphere (Lin et al., 2003; Xu et al., 2011, 2013). *Leptodora* can consume a wide range of prey organisms (Rivier, 1998) but appears to prefer herbivorous cladocerans, including *Bosmina*, *Diaphanosoma*, and small-bodied *Daphnia* (Herzig & Auer, 1990; Lunte & Luecke, 1990; Branstrator & Lehman, 1991). Numerous studies have demonstrated that *Leptodora* can have strong effects on the abundance, biomass, structure, and seasonal succession of herbivorous zooplankton community in the temperate zone (e.g., Herzig, 1995; Wojtal et al., 1999; Uusitalo et al., 2003; McNaught et al., 2004; Wojtal et al., 2004), yet little is known

about how it impacts the zooplankton community in the (sub)tropical zone. Moreover, its cascading effect on phytoplankton remains largely unexplored, though one study suggested that it may enhance algal biomass (Wojtal et al., 1999). On the other hand, *Leptodora* is a preferred prey item for many fish species (e.g., Herzig, 1995; Vogt et al., 2013; Wagner et al., 2013). Thus, its cascading effects on lower trophic levels would depend partly on predation pressure from fish.

In southern China, both fish and invertebrate predators may be able to play a major role in affecting plankton communities (Wang et al., 2011). Southern China, including mainly Guangdong Province and Hainan Island, lies at the northern margin of the tropical zone (18°10'N–25°31'N). Consistent with the observations in other tropical regions, large-bodied herbivorous zooplankton, especially *Daphnia*, are often absent from the freshwaters of this region or, although present in few deep reservoirs, have a low abundance (Lin et al., 2003; Li et al., 2012). These waters are extensively stocked with planktivorous filter-feeding fishes such as bighead carp (*Aristichthys nobilis* Richardson), silver carp (*Hypophthalmichthys molitrix* Val.), and tilapias for fish culture (Han & Dumont, 2011; Lin et al., 2014). Likewise, there has been speculation that fish may maintain a high predation pressure on zooplankton all year round, hence causing the absence or rarity of *Daphnia* (Lin et al., 2003; Zhao et al., 2013; Lin et al., 2014). *Leptodora richardi* (Korovchinsky), the major, large invertebrate predator in this region, exists in some deep reservoirs (Xu et al., 2011, 2013). In particular, unlike in temperate lakes where *Leptodora* is typically abundant in late summer (Herzig & Auer, 1990; Branstrator & Lehman, 1991; Vogt et al., 2013), it can attain high abundance in winter/early spring (e.g., 100–600 individuals m⁻³; Lin, 2007; Xu et al., 2013), suggesting a possible effect on the herbivorous zooplankton community. As a result, in different seasons and waters, the overall cascading effect on phytoplankton may result from either or both of planktivorous fish and *Leptodora*, which is as yet poorly understood.

Here we conducted an in situ mesocosm experiment in Liuxihe Reservoir, a reservoir containing *Leptodora* and *Daphnia* in southern China. In spite of lying in the tropical-subtropical transition zone, many lakes and reservoirs of southern China experience a brief period of relatively low temperature during winter and early

spring (e.g., Liuxihe Reservoir, see Fig. 1). This may reduce the feeding activity of fish and accordingly relieve their predation pressure on zooplankton. Besides, in the reservoirs with *Leptodora*, the above analyses suggest that it may also have the potential to affect plankton communities in this period. We, therefore, performed our experiment to examine the top-down effects of planktivorous fish and *Leptodora* on the plankton in southern China during the winter–spring transition. Our results may also improve our understanding of the absence/rarity of *Daphnia* in this region.

Materials and methods

Study site

Liuxihe Reservoir is a large (surface area, 15.25 km²; volume, 3.25 × 10⁸ m³), deep (maximum depth, 73 m; mean depth, 21.3 m), oligo-mesotrophic (total phosphorus, 6–27 µg l⁻¹; Zhao et al., 2013; chlorophyll *a*, 1.3–5.1 µg l⁻¹; Xiao et al., 2011) reservoir near the Tropic of Cancer (23°45'N, 113°46'E) in Guangdong Province, China. The annual mean surface water temperature is 23.6°C, with a minimum of 11–13°C in December/January (e.g., Fig. 1; Xiao et al., 2011). Bighead carp and silver carp, two main species stocked into the reservoir for fish culture, dominated the fish community. There were also a number of coexisting young-of-the-year (YOY) fish, which were largely reproduced by two tropical fishes, mud carp (*Cirrhinus chinensis* Günther) and tilapia, as well as other wild fishes. *Leptodora richardi* is the most important, large invertebrate predator, with a

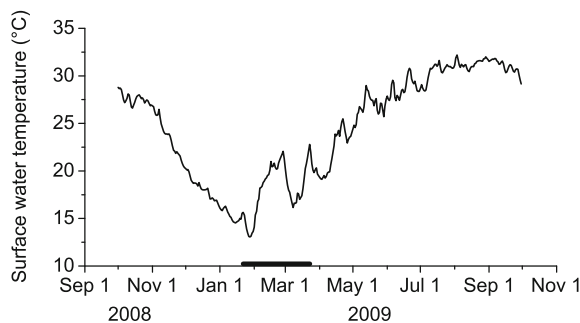


Fig. 1 Surface water temperature in the enclosures during October 1, 2008–October 1, 2009. The black bar denotes the experimental period (January 19–March 22, 2009)

high abundance in winter/early spring (Lin, 2007; Xu et al., 2013). The dominant herbivorous zooplankters include *Diaphanosoma orghidani transamurensis* (Korovchinsky), *Bosmina fatalis* (Burckhardt), *Bosminopsis deitersi* (Richard), *Phyllodiaptomus tunguidus* (Shen & Tai), and *Mesocyclops ogunnus* (Onabamiro). *Daphnia galeata* (Sars), the dominant *Daphnia* species, has a low abundance, biomass, and small body size. *Daphnia pulex* (Leydig), although present, is very rare (Lin, 2007).

Experimental design

The experiment was conducted in 12 in situ enclosures made of light blue, high-density polyethylene film (4 × 4 m² and 6 m deep). The enclosures, sealed at the bottom and open to the atmosphere at the top, were suspended from iron frames buoyed by iron bucket floats, which was fixed in a bay of Liuxihe Reservoir. The top of each enclosure was ≈0.5 m above the water surface so as to prevent fish jumping in or out of the enclosure. Therefore, the water column was 5.5 m or so deep in the enclosures, yielding a volume of ≈88 m³. Before the start of the experiment, all enclosures were filled by pumping water from the reservoir with two pumps within January 11–January 18, 2009. To exclude *L. richardi* from the enclosures as far as possible, each pump inlet was wrapped with stainless steel net (mesh size, 1 mm).

The enclosures were arranged in a 2 × 2 factorial design, in which bighead carp and *L. richardi* were independent factors. Four treatments were as follows: (1) Control: enclosures with no fish and no *L. richardi* addition; (2) Fish: enclosures with bighead carp addition; (3) *Leptodora*: enclosures with *L. richardi* addition; and (4) Fish + *Leptodora*: enclosures with bighead carp and *L. richardi* additions. Each treatment had three replicate enclosures. We selected bighead carp because it was the dominant fish species in Liuxihe Reservoir and most freshwaters of southern China. According to our design, *L. richardi* was intended to be absent from the enclosures served as the control treatment. Indeed, it occurred rarely and attained significantly lower abundance than in the *Leptodora* treatment (see results). Therefore, it seems reasonable to regard these enclosures as the control treatment.

Prior to the experiment, bighead carp, obtained from a fish hatchery, were stocked in a net enclosure

placed at the experimental site to acclimatize them to the reservoir. On January 19, 2009, three bighead carp, 11–13 cm total length, were stocked into each of six randomly chosen enclosures. The stocking biomass was 1.1 ± 0.05 g wet weight m^{-3} (60.5 ± 2.8 kg ha^{-1}) per enclosure. Although the actual fish biomass in Liuxihe Reservoir was unknown, 1–10 g wet weight m^{-3} has been estimated to be typical of the fish biomass in most reservoirs of Guangdong Province (B.-P. Han, unpublished data). Given that the depth was too shallow to shelter zooplankton from predation by fish in the enclosures, we selected the low stocking level. *L. richardi* were collected from the reservoir by towing plankton nets (1-mm mesh) and then immediately redistributed equally to the six *Leptodora* enclosures. Due to its fragility, quite a few individuals of *Leptodora* were damaged or dead before added into the enclosures. Therefore, we determined its addition abundance (≈ 2 individuals m^{-3}) by sampling the enclosures on January 22, when the dead individuals might have sunk to the enclosure bottom.

Sampling and analyses

Our previous studies have shown that surface water temperatures are very similar among the enclosures and to that of the reservoir in both warm and cold seasons, with a difference of basically $<0.5^\circ C$ (S.-Y. Zhao, unpublished data). Thus, one button data logger was placed at 0.5 m in one enclosure to monitor surface water temperature throughout the experiment.

The enclosures were first sampled on January 19, 2009 to assess pretreatment conditions before the additions of fish and *Leptodora*, and thereafter at 10- to 12-day intervals. The experiment ended on March 22, 2009. Water transparency was measured with a Secchi disk. Zooplankton except *Leptodora* were collected by vertically towing a plankton net with a 20-cm diameter and a 64- μm mesh from 5 m to the surface. Two hauls were made in each enclosure at each sampling date, yielding a 315-l sample volume. This sampling method may cause zooplankton abundance/biomass to be underestimated in our experiment. For instance, Sarnelle (1993) found that, compared with depth-integrated samples obtained with a sampler, net hauls only had a net efficiency of $\approx 60\%$ for crustaceans in his study. *L. richardi* was collected at night using a 51-cm-diameter, 1-mm-mesh net. 2–3 vertical hauls were taken from 5 m to the surface in each enclosure

and combined into one composite sample. At each sampling date, because the sample volume for *Leptodora* (2–3 m^3) and other taxa (315 l) just amounted to ≈ 2 –3 and 0.4% of total enclosure volume (88 m^3), respectively, zooplankton community may not be markedly altered. All zooplankton samples were preserved with sugar-formalin solution. Additionally, depth-integrated water samples were taken at 0.5, 1, 2, 3, and 4 m with a 5-l sampler. A 1 l portion of the sampled water was preserved in formalin solution for phytoplankton enumeration.

During the experiment, we took and froze water samples and algal cells filtered onto cellulose acetate membrane filters (pore size, 0.45 μm) for later analysis of total phosphorus, total nitrogen and chlorophyll *a*, but unfortunately all these samples were lost before analysis. Also, we collected zooplankton and phytoplankton samples from the reservoir nearby the experimental site, which lied in the littoral zone of the reservoir. Thus, the biomass/abundance of plankton in Liuxihe Reservoir (see results) used as reference may not be characteristic of its pelagic zone.

All individuals in *L. richardi* samples were counted and measured under a dissecting microscope with an ocular micrometer. For the other zooplankton species, 20–50 individuals of dominant species and a minimum of 400 individuals in total were examined in each sample. Biomass of each crustacean species was estimated from length-dry weight regressions (Dumont et al., 1975; McCauley, 1984; Culver et al., 1985). For rotifers, we calculated mean body volume of each species using geometric shapes provided by McCauley (1984). Mean dry weight for each rotifer species was obtained by assuming a specific gravity of 1 and a dry weight:wet weight ratio of 0.1 (McCauley, 1984). Population biomass of each zooplankton taxon was obtained through multiplying mean dry weight by population abundance. These were summed to yield the biomass of zooplankton community or taxonomic groups.

To assess the potential effect of zooplankton grazing on phytoplankton, we estimated zooplankton clearance rates using empirical models of Peters & Downing (1984). We used their “cladoceran” Eq. (1) for cladocerans, and their “all zooplankton” Eq. (2) for copepods and rotifers. V is individual clearance rate ($ml\ animal^{-1}\ d^{-1}$), W is animal dry weight (μg), S is algal biovolume ($10^6\ \mu m^3\ ml^{-1}$), and R is mean algal volume (μm^3). W , S , and R were obtained directly from our enclosure data. C , C_a , and M

represent volume of experimental vessel (ml), volume per animal (ml), and experiment duration (min) in laboratory feeding experiments, respectively, and do not apply to our enclosure experiment. Thus, we used the median values provided by Peters & Downing (1984: 768, Table 2). On each sampling date, population clearance rate for each taxon was obtained through multiplying mean individual clearance rate by population abundance. These were summed to yield a community clearance rate.

$$\log V = 0.173 + 0.750 \log W - 0.434 \log S - 0.0003 C + 0.014 C_a, \quad (1)$$

$$\log V = 0.110 + 0.546 \log W - 0.260 \log S + 0.121 \log R + 0.0001 C - 0.0002 M. \quad (2)$$

Phytoplankton were identified to species or genus, enumerated, and measured using a compound microscope with an ocular micrometer. All phytoplankton taxa were grouped into one of two size classes based on the greatest axial linear dimension (GALD): small algae (GALD <30 μm) and large algae (GALD $\geq 30 \mu\text{m}$). Phytoplankton biomass (wet weight) was obtained by considering algal cells as equivalent geometric shapes (Hillebrand et al., 1999) and assuming their specific gravity to be unity.

The effects of fish and *Leptodora* on each response variable (e.g., the groups of *Daphnia*, *Leptodora*, and small algae) over time were evaluated using two-way ANOVA with repeated measures. Fish and *Leptodora* were the between-subjects factors, and time was the within-subjects factor. One-way ANOVA was employed to assess whether pretreatment conditions were similar among treatments. Prior to ANOVA, all data were log-transformed to homogenize variances. All statistical analyses were performed with SPSS 19.0 (IBM SPSS Inc.).

Results

In the 2008–2009 winter, surface water temperature reached the minimum on January 28 and tended to rise thereafter, with a range of 13.1–22.8°C throughout the experiment (Fig. 1). During the first half of the study, in all enclosures Secchi depth equaled 5.5 m, the enclosure's maximum depth. During the second half

of the study, water looked more transparent in the treatments without fish, Secchi depth staying 5.5 m, whereas it decreased to less than 5.5 m in the treatments with fish (Fig. 2). Secchi depth of 5.5 m was a conservative measure of water transparency during the experiment because the disk was still clearly visible on enclosure bottom.

The zooplankton communities were temporally variable and showed responses to experimental treatments over time (Fig. 3; Table 1). Initially, *Tropocyclops bopingi* (Dumont), copepodites, and nauplii dominated the zooplankton communities. However, in the absence of fish, *Daphnia galeata* developed and became the dominant species towards the end of the experiment, eventually making up 71–92% of total zooplankton biomass and reaching 1.2–4.1 individuals l^{-1} in abundance. In contrast, *Daphnia* was almost absent in the treatments with fish in which the zooplankton community was dominated by *Phyllodiaptomus tunguidus* and small-bodied taxa, including nauplii and *Bosmina*. The large predatory cladoceran, *Leptodora richardi*, peaked in abundance (92–119 individuals m^{-3}) on February 20 or March 2 in the treatment with *Leptodora* addition but tended to disappear from the water column over the last 10 days (Fig. 3b). By contrast, *Leptodora* were scarcely observed in the other treatments (<8 individuals m^{-3}).

Similar to the variation pattern of total zooplankton biomass (Fig. 3a), clearance rates of herbivorous

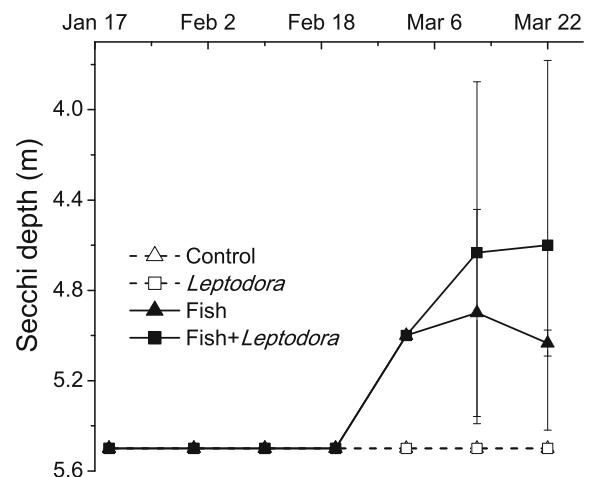
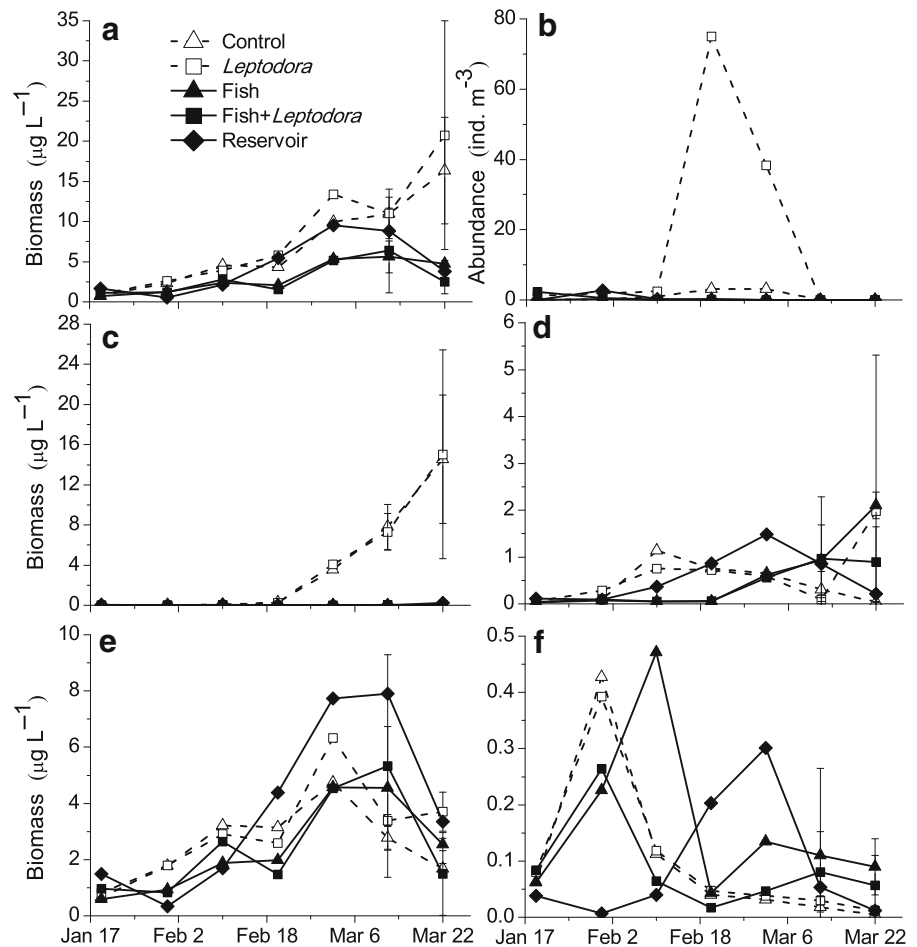


Fig. 2 Mean Secchi depth for each treatment during the experiment. Each point represents the mean value for all enclosures within a treatment on each date, with standard errors (\pm SE) on the last two sampling dates

Fig. 3 Total zooplankton biomass and biomass/abundance of each taxonomic group during the experiment. Each point represents the mean value for all enclosures within a treatment on each date, with standard errors (\pm SE) on the last two sampling dates. Results for Liuxihe Reservoir are also shown. **a** Total zooplankton; **b** *Leptodora richardi*; **c** *Daphnia galeata*; **d** smaller cladocerans; **e** copepods; **f** rotifers



zooplankton, representing their grazing pressure on algae, appeared to be higher in the absence of fish than in their presence, with a larger difference towards the end of the experiment (Fig. 4a). This trend was due mainly to the difference in *D. galeata*, the most efficient grazer in this study. In the enclosures with no fish, the community clearance rate greatly increased during the late period of the experiment, with *Daphnia* accounting for 69–89% of the total (Fig. 4). On average, clearance rates of herbivorous zooplankton were 2–24 times greater in the absence of fish than in their presence during the last 10 days.

The ANOVA indicated that fish markedly suppressed the biomass/abundance of zooplankton, *L. richardi* and *D. galeata* but had no significant influence on the biomass of other taxonomic groups (Table 1). Clearance rates of herbivorous zooplankton and *D. galeata* also showed negative responses to the

presence of fish. Nevertheless, neither *Leptodora* addition nor the fish \times *Leptodora* interaction significantly affected herbivorous zooplankton biomass or clearance rates (Table 1).

Bacillariophyta was the most important algal group in all enclosures, accounting for >70% of total algal biomass almost throughout the experiment. At the end of the experiment, Chlorophyta co-dominated the phytoplankton community in the treatments with fish (16–46% of total algal biomass). During the experiment, total algal biomass exhibited a peak in the treatments with fish, whereas it tended to decline in the treatments without fish (Fig. 5a). As a consequence, during the last 10 days total algal biomass, on average, was 7–36 \times higher in the presence of fish relative to in their absence. Small phytoplankton, which are vulnerable to zooplankton grazing, were significantly enhanced in biomass by the presence of fish (Table 1),

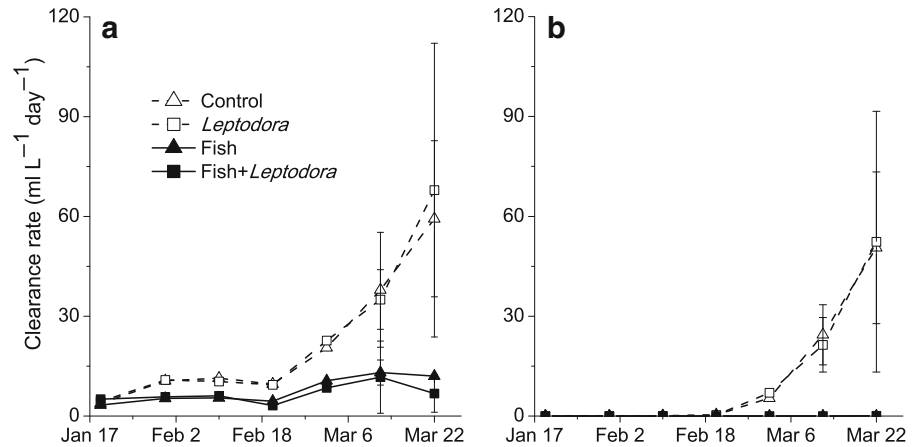
Table 1 P values of repeated measures ANOVA on the effects of fish and *Leptodora* on zooplankton and phytoplankton responses

| Response variables | Predictor variables | | | | | | | |
|-------------------------------------------|---------------------|----------------------|-------------------------|-----------------|-----------------|-------------------------|--------------------------------|--|
| | Fish | <i>Leptodora</i> | Fish × <i>Leptodora</i> | Time | Fish × time | <i>Leptodora</i> × time | Fish × <i>Leptodora</i> × time | |
| Total zooplankton biomass | 0.002 (–) | 0.984 | 0.638 | < 0.0001 | < 0.0001 | 0.747 | 0.400 | |
| <i>Leptodora richardi</i> abundance | < 0.0001 | (–) 0.001 (+) | 0.009 | 0.006 | 0.005 | 0.146 | 0.082 | |
| <i>Daphnia galeata</i> biomass | < 0.0001 | (–) 0.991 | 0.834 | < 0.0001 | < 0.0001 | 0.983 | 0.968 | |
| Smaller cladoceran biomass | 0.775 | 0.854 | 0.508 | 0.013 | 0.010 | 0.812 | 0.159 | |
| Copepod biomass | 0.202 | 0.919 | 0.660 | < 0.0001 | 0.172 | 0.724 | 0.166 | |
| Rotifer biomass | 0.657 | 0.568 | 0.490 | 0.002 | 0.205 | 0.439 | 0.413 | |
| Clearance rate of herbivorous zooplankton | 0.001 (–) | 0.537 | 0.641 | < 0.0001 | < 0.0001 | 0.638 | 0.588 | |
| Clearance rate of <i>D. galeata</i> | < 0.0001 | (–) 0.874 | 0.790 | < 0.0001 | < 0.0001 | 0.982 | 0.963 | |
| Total phytoplankton biomass | < 0.0001 | (+) 0.464 | 0.991 | < 0.0001 | < 0.0001 | 0.644 | 0.527 | |
| Small algae biomass | < 0.0001 | (+) 0.650 | 0.941 | < 0.0001 | < 0.0001 | 0.508 | 0.739 | |
| Large algae biomass | < 0.0001 | (+) 0.693 | 0.184 | < 0.0001 | < 0.0001 | 0.771 | 0.202 | |

Positive or negative signs in parentheses denote whether the effect significantly increased or decreased the response variable

Significant responses ($P < 0.05$) are in bold

Fig. 4 Clearance rates of total herbivorous zooplankton (a) and *Daphnia galeata* (b) during the experiment. Each point represents the mean value for all enclosures within a treatment on each date, with standard errors (\pm SE) on the last two sampling dates



corresponding to the suppressed clearance rates of herbivorous zooplankton. Biomass of large phytoplankton and total phytoplankton were also significantly increased with fish addition. However, there was no significant effect of *Leptodora* or interactive effect of fish and *Leptodora* on those algal variables (Table 1). The differences in total algal biomass among treatments were mainly associated with the difference in small algae, which constituted a relative biomass of 69–92% on average.

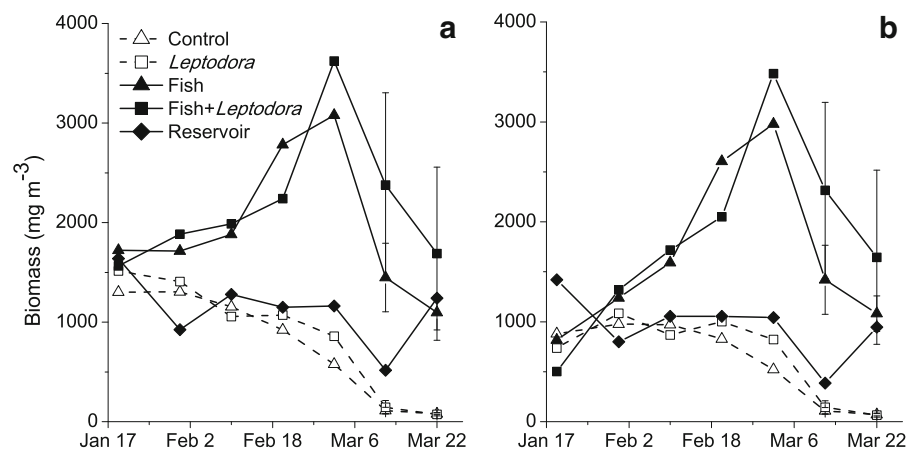
Discussion

The present study emphasizes the importance of planktivorous fish as a driver of top-down effects in a subtropical reservoir during the winter–spring transition. Under oligo-mesotrophic state, a low biomass

of bighead carp suppressed zooplankton biomass and clearance rates and enhanced phytoplankton biomass, indicating a strong trophic cascade. However, our results demonstrate that *Leptodora* shows no cascading effects on herbivorous zooplankton and phytoplankton. Also, no interactive effect of fish and *Leptodora* on herbivorous zooplankton and phytoplankton was observed. The development of *Daphnia* largely accounted for higher zooplankton biomass and clearance rates in the absence of fish than in their presence. This suggests that *Daphnia* is a keystone herbivore in Liuxihe Reservoir, as observed in typical temperate lakes.

Both direct and indirect effects of fish may play an important role in producing the strong cascading effects in our study. It has been suggested that fish can affect phytoplankton assemblages by modifying herbivory rates, by directly excreting nutrients, and by

Fig. 5 Biomass of total phytoplankton (a) and small algae (b) during the experiment. Each point represents the mean value for all enclosures within a treatment on each date, with standard errors (\pm SE) on the last two sampling dates. Results for Liuxihe Reservoir are also shown



modifying the rates and ratios at which herbivorous zooplankton recycle nutrients (Vanni & Layne, 1997). As a filter-feeding fish, bighead carp is known to feed primarily on zooplankton (Cremer & Smitherman, 1980; Spataru et al., 1983) and selectively prefer species with lower escape ability (Cooke et al., 2009). Similarly, probably owing to its slow swimming speed, *Daphnia*, the keystone species, was suppressed by bighead carp, thereby causing zooplankton clearance rates to be pronouncedly lower than in the absence of fish. Additionally, in the fish treatments, there was increase in biomass of large algae, which may be less susceptible to grazing. This suggests that bighead carp may change nutrient concentrations and ratios to impact phytoplankton through direct excretion and altering zooplankton composition (Fig. 3; Table 1). Taken together, fish significantly enhanced algal biomass and therefore reduced Secchi depth in the last period of the experiment. In contrast, the development of *Daphnia* partly explained the low biomass in the no-fish treatments. Given that in the later sampling period, algal biomass was strongly depressed in the absence of fish, water transparency may indeed be much higher than the conservative depth of 5.5 m.

In temperate lakes, the occurrence of spring clear-water phase is mainly attributable to the outbreak of *Daphnia* (Lampert et al., 1986; Sarnelle, 1993; Sommer et al., 2012), which coincides with low fish predation pressure (Luecke et al., 1990) and water temperature increasing above 15–16 °C (Schalau et al., 2008; Dröscher et al., 2009). In the present study, the *Daphnia* population showed a similar pattern in the absence of fish after water temperature rose to 15°C. However, a low biomass (1.1 g m⁻³/60.5 kg ha⁻¹) of bighead carp was enough to prevent the development of *Daphnia*. These findings have implications for understanding the distribution and dynamics of *Daphnia* in southern China. According to the surveys of Liuxihe Reservoir from 2001 to 2009 (Lin, 2007; Wang et al., 2011), *Daphnia* reaches its highest abundance in late winter/spring, suggesting that its population has the most potential to expand during the period of rising water temperature. However, zooplankton may often suffer heavy predation from bighead carp, silver carp, tilapia, as well as YOY fish in Liuxihe Reservoir. Therefore, no spring outbreak of *Daphnia* occurred except for in 2006 (Lin, 2007; Wang et al., 2011). Planktivorous fish

biomass may have been low in 2006 because of sustained fishing and the decline in fish stocking density (Wang et al., 2011); hence, *Daphnia* attained relatively high abundances (1.5 inds l⁻¹ or so) in spring, similar to the observation in our experiment. By contrast, there may be heavy fish predation on larger herbivores much of the year in most lakes and reservoirs of southern China, due to high water temperatures, fish stocking (typically >1 g m⁻³; B.-P. Han, unpublished data), and high abundances of YOY fish, as suggested by Lin et al. (2003) and Zhao et al. (2013). As a consequence, the genus *Daphnia* is often absent or rare (Lin et al., 2003; Li et al., 2012).

Although *Leptodora* addition markedly enhanced its own abundance in the absence of fish, it had no significant effect on herbivorous zooplankton community. This somewhat conflicts with our expectation and prior research from temperate regions, in which *Leptodora* can strongly reduce the population of herbivorous species under low fish predation pressure (e.g., Lunte & Luecke, 1990; Herzig, 1995; Wojtal et al., 1999, 2004). Two reasons may explain such a difference. First, *Leptodora* was rare during most of the experimental period (Fig. 3b), and its peak abundance of 92–119 inds m⁻³ was low in comparison with in previous studies (e.g., peak abundance >500 inds m⁻³; Herzig, 1995; Wojtal et al., 2004). Second, larger *Leptodora kindti* (Focke) appears to have a broader prey size range (Branstrator & Lehman, 1991). Thus, *L. richardi*, the taxon present in Liuxihe Reservoir and smaller in body size than *L. kindti* (Korovchinsky, 2009), may feed on a narrower size range of prey. Taken together, *L. richardi* did not affect herbivorous zooplankton as much as *L. kindti* in temperate regions. It in turn had no strong cascading effect on phytoplankton.

In Liuxihe Reservoir, *Leptodora* generally reaches its highest abundance (100–600 inds m⁻³) during winter/early spring (Lin, 2007; Xu et al., 2013), likely because relatively lower water temperatures minimize fish predation pressure. However, in other seasons, the *Leptodora* population tends to collapse or have a low abundance (Lin, 2007; Xu et al., 2013), perhaps due to intense fish predation. Thus, we suggest that only when *Leptodora* attains higher abundance in the reservoir during winter/early spring (e.g., peak abundance, ≈600 inds m⁻³; Xu et al., 2013), it may, to some degree, regulate some herbivore populations as in temperate lakes (e.g., Herzig, 1995; Wojtal et al.,

2004) and in turn cascade down to phytoplankton. These need to be further tested. With water temperature rising further in spring, fish may determine the top-down effects on plankton communities.

We recognize that some factors need to be considered carefully in using our results to reflect ecological dynamics of the reservoir. In Liuxihe Reservoir, *D. galeata* generally disappears from the water column in July/August and reappears in late autumn every year (Wang et al., 2011). Thus, the *Daphnia* population was likely re-established from resting eggs in reservoir sediments during the experiment. As we did not add sediments into the enclosures, a limited number of resting eggs may have delayed the development of *Daphnia* population and the timing of its peak abundance/biomass in our study. The fish biomass employed in our study, 1.1 g m^{-3} (60.5 kg ha^{-1}), was lower than that of typical reservoirs in southern China (B.-P. Han, unpublished data) but might be higher than that of Liuxihe Reservoir based on annual fish yield (Wang et al., 2011). In addition, the enclosure depth was insufficient to afford large-bodied zooplankton a refuge to escape predation by fish and invertebrate predators. Therefore, zooplankton would suffer a high predation risk in the enclosures. During the experiment, unlike the reservoir that can receive nutrients from surface runoff of its watershed and deep water, the enclosures received no external nutrients. Also, that enclosure bottom did not extend to the hypolimnion ($>10 \text{ m}$; Xiao et al., 2011) would make its thermal structure different from that of Liuxihe Reservoir. Thermal stratification or mixing, however, influences the composition and dynamics of phytoplankton community.

In conclusion, fish had a greater role than *Leptodora* in regulating plankton biomass in Liuxihe Reservoir during the winter–spring transition. Fish increased phytoplankton biomass via primarily depressing *Daphnia*, while *Daphnia* exerted top-down control on phytoplankton in the absence of fish. These trophic interactions are in accordance with the observations in many temperate lakes (e.g., Carpenter et al., 2001; Jeppesen et al., 2003; Parker & Schindler, 2006). As the biomass of fish we used in this experiment is typically lower than stocking biomass in the freshwaters of southern China, our results have implications for understanding the top-down effect of fish in these waters. Probably due to heavy fish predation, the top-down control of phytoplankton by

large-sized cladocerans, especially *Daphnia*, seems unusual in this low-latitude region, supporting the view of van Leeuwen et al. (2007). Overall, in spite of some defects, the present study provides useful information for exploring ecosystem structure and process in Liuxihe Reservoir. To better understand the mechanisms behind the plankton dynamics of freshwaters in southern China, enclosure experiments should be made as realistic as possible for future research that span a broad range of fish stocking levels, trophic states, enclosure depths, etc.

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