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Title: Fish-mediated plankton responses to increased temperature in subtropical aquatic mesocosm ecosystems: implications for lake management

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Corresponding Author: Dr. Hu HE,

Corresponding Author's Institution: Nanjing institute of Geograph and Limnology

First Author: Hu HE

Order of Authors: Hu HE; Hui Jin; Erik Jeppesen; Kuanyi Li; zhengwen liu; Yongdong Zhang

Abstract: Although it is well established that climate warming can reinforce eutrophication in shallow lakes by altering top-down and bottom-up processes in the food web and biogeochemical cycling, recent studies in temperate zones have also shown that adverse effects of rising temperature are diminished in fishless systems. Whereas the removal of zooplanktivorous fish may be useful in attempts to mitigate eutrophication in temperate shallow lakes, it is uncertain whether similar mitigation might be achieved in warmer climates. We compared the responses of zooplankton and phytoplankton communities to climate warming in the presence and absence of fish (*Aristichthys nobilis*) in a 4-month mesocosm experiment at subtropical temperatures. We hypothesized that 1) fish and phytoplankton would benefit from warming, while zooplankton would suffer in fish-present mesocosms and 2) warming would favor zooplankton growth but reduce phytoplankton biomass in fish-absent mesocosms. Our results showed significant interacting effects of warming and fish presence on both phytoplankton and zooplankton. In mesocosms with fish, biomasses of fish and phytoplankton increased in heated treatments, while biomasses of *Daphnia* and total zooplankton declined. Warming reduced the proportion of large *Daphnia* in total zooplankton biomass, and reduced the zooplankton to phytoplankton biomass ratio, but increased the ratio of chlorophyll a to total phosphorus, indicating a relaxation of zooplankton grazing pressure on phytoplankton. Meanwhile, warming resulted in a 3-fold increase in TP concentrations in the mesocosms with fish present. The results suggest that climate warming has the potential to boost eutrophication in shallow lakes via both top-down (loss of herbivores) and bottom-up (elevated nutrient) effects. However, in the mesocosms without fish, there was no decline in large *Daphnia* or in total zooplankton biomass, supporting the conclusion that fish predation is the major driver of low large *Daphnia* abundance in warm lakes. In the fishless mesocosms, phytoplankton biomass and nutrient levels were not affected by temperature. Our study suggests that removing fish to mitigate warming effects on eutrophication may be potentially

beneficial in subtropical lakes, though the rapid recruitment of fish in such lakes may present a challenge to success in the long-term.

Dear Editors,

Although it is well established that climate warming can reinforce eutrophication symptoms in shallow lakes, recent studies in temperate zones have also shown that adverse effects of rising temperature are diminished in fishless systems. From the perspective of lake management, the removal of zooplanktivorous fish may therefore be useful in attempts to mitigate eutrophication in temperate shallow lakes, but whether similar mitigation might be achieved in warmer climates is uncertain. We compared the responses of zooplankton and phytoplankton communities to climate warming in the presence and absence of fish in a 4-month mesocosm experiment at subtropical temperatures. Our study showed a significant interplay between the effect of temperature and fish on lake plankton communities under subtropical temperature conditions. In fish-present systems, warming boost phytoplankton growth via a reduction in large-sized zooplankton (top-down) and to stimulate the release of sediment phosphorus (bottom-up). However, when fish were absent from these in warm waters, warming effects on both top-down and bottom-up processes of phytoplankton are diminished. Therefore, our findings suggest that the removal of fish may also be an effective means of mitigating warming effects on eutrophication. Our study have implication in management of inland waters. Thus, we think it is within the scope of Water Research and hope that our ms can be published in this journal.

Yours,

Hu He
and co-authors

Notes: The line numbers in our responses were based on the clear version of revised manuscript.

Reviewer #1

The manuscript entitled of "Fish-mediated plankton responses to increased temperature in subtropical aquatic mesocosm ecosystems: implications for lake management" tried to find out how fish determine the responses of plankton to increased temperature in subtropical shallow mesocosms. The manuscript looks very meaningful and the results help to gain a better strategy for combating eutrophication in shallow lakes in a warmer future. However, there are several aspects that must be revised before being considered for approval.

1. So many grammatical errors exist in this paper, please re-check carefully and modify them. It is highly recommended proofreading by a native English speaker to make this paper sound natural and professional.

Our response: We have invited an English speaker (Amy-Jane Beer) to polish the language.

2. The Highlights need to be reorganized.

Our response: We have now reorganized the highlights.

3. Graphical Abstract about fish absent confused me. Everything didn't change after temperature increasing? I think that both elevated herbivores from zooplankton and elevated nutrient for phytoplankton will occur. Rather than nothing happened.

Our response: The graphical abstract is entirely based on our experimental results. We found that in the fish-absent mesocosms, both zooplankton biomass and nutrient levels did not change significantly with warming. The former (no change in zooplankton) may be attributed to food limitation in our rather unproductive fishless mesocosms, while the latter (warming did not increase nutrient levels) may be due to the blocking by benthic algae, which usually grow well in high transparent waters and whose abundance may increase at elevated temperatures. Our results are in line with other mesocosm and field studies, for instance, Hansson et al., (2013) and Lin et al., (2017). Thus, as outlined in the discussion we find it reasonable that in fishless unproductive lakes, nutrient and herbivores are not significantly impacted by climate warming.

4. The introduction section, most important references about bighead carp (*Aristichthys nobilis*) controlling phytoplankton in subtropical shallow mesocosms were missing.

Our response: We have now mentioned that filter-feeding carp potentially could control phytoplankton in subtropical shallow lakes (e.g., Starling, 1993; Xie and Liu, 2001; Ke et al., 2009; Guo et al., 2015). See Line 114-128.

5. The experiment design is relatively simple. Please rewrite this section. There still exist some details need to be added, e.g., how to maintain these mesocosms? Need to be oxygenated? Feeding? And so on. Please give a brief description. I think the number of control is too little since there is only one fish in each tank. Besides, some related references should be added in this part.

Our response: Our experiment aimed to compare the responses of zooplankton, phytoplankton and nutrients to warming between fish-present and fish-absent systems. Temperature and fish were the treatments. Other factors such as dissolved oxygen,

zooplankton and algae were all dependent variables and their responses to heating and fish were studied. Accordingly, as in similar studies elsewhere, we did not feed the fish or oxygenate the water or made other disturbances that could violate our treatments. The fish had to feed on organisms inside the system as in real ecosystems. One would always like to have many replicates, but we disagree that the number of replicates is too few, and actually, it is the common setting for such type of experiments.

6. As we all know, filter-feeding fishes such as bighead carp (*Aristichthys nobilis*) used in this manuscript graze not only zooplankton but also phytoplankton. So, in fish-present mesocosms, why zooplankton would suffer because of fish grazing while phytoplankton would benefit from warming? Fish grazing didn't influence phytoplankton?

Our response: There are different opinion about the role of filter-feeding fish, e.g., silver carp and bighead carp, in controlling phytoplankton in lakes (Line 114-128). Filter-feeding fish can affect phytoplankton in various ways. For instance, they may reduce large-sized phytoplankton by direct filtering. However, they may also enhance phytoplankton by excretion, disturbance of sediments and reduction of large-sized zooplankton in shallow waterbodies (Jeppesen et al, 2012; Guo et al., 2015; Zhang et al., 2016). The net effect of filter-feeding fish depends on the status of the ecosystems in which they live. Stocking of silver carp may perhaps be appropriate in warm lakes that are highly eutrophic and naturally lack large-sized zooplankton. In those lakes, stocking of silver carp and bighead carp have been shown to reduced bloom-forming cyanobacteria (but not total phytoplankton) in summer seasons when blooms frequently occurred (Ke at al., 2009; Guo et al., 2015).

However, in less productive systems where phytoplankton growth are mainly inhibited by zooplankton and competed by benthic plants, the negative effects of stocking filter-feeding fish, e.g., disturbing sediment, grazing zooplankton and nutrient excretion, may overweigh their positive effects on lake eutrophication. We therefore hypothesized that zooplankton would suffer from fish predation in warm lakes and that phytoplankton would increase.

In the revised manuscript, we have also added extra information on this issue in the Introduction section (Line 114-128).

7. The part about "climate warming has the potential to boost eutrophication in subtropical shallow lakes via both top-down (loss of herbivores) and bottom-up (elevated nutrient) effects" needs to be more detailed.

Our response: This is a summary sentence of the effects of climate warming on phytoplankton (eutrophication) in fish-present mesocosms based on our detailed reporting of the findings that the abundance of zooplankton and large-sized *Daphnia* were lower in the heated mesocosms, indicating a difference in the top-down pressure (Line 36-41). In addition, we reported that TP increased 3-fold in consequence of heating, reflecting an increased bottom-up pressure (Line 41-42).

8. **Line 230:** Why "Warming and fish presence significantly increased TP concentrations"?

Our response: Fish may increase TP concentrations via both excretion and sediment disturbance, boosting sediment P released to water column (Zhang et al., 2016). Both mechanisms can be enhanced by temperature elevation.

9. Line 322-324, I didn't think this conclusion could be supported from this study. I would like to suggest that revise "temperature" to "temperature change".

Our response: We have changed "temperature" to "temperature change" (Line 358)

10. This paper emphasized that biomanipulation by fish removal may be beneficial in mitigating ongoing warming effects on eutrophication in subtropical shallow lakes. However, many studies found that biomanipulation by addition such as filter-feeding fishes could directly control phytoplankton. What is the conflict between the two viewpoints?

Our response: The studies finding that stocking of filter-feeding fishes reduce phytoplankton, e.g., Starling (1993) and Xie and Liu (2001), were both conducted in highly eutrophic lakes characterized by high algae biomass (frequent cyanobacterial blooms), lacking large-sized zooplankton and benthic plants. In their studies, stocking of filter-feeding fish was aimed to reduce the standing crops of bloom-forming cyanobacteria (mainly *Microcystis*) and indeed worked in summer months when blooms occurred frequently (Ke et al., 2009; Guo et al., 2015). However, from a long-term perspective, the total biomass of phytoplankton in most dates of their samplings was not obviously reduced or even higher by silver or bighead carp stocking (Guo et al., 2015). Moreover, field investigations in Yangtze shallow lakes where filter-feeding fish were heavily stocked have found that lakes with yields of filter-feeding fish $>100 \text{ kg ha}^{-1}$ often exhibited higher Chl-*a* and lower Secchi depth than those with yields $<100 \text{ kg ha}^{-1}$ (Wang et al., 2008). The effects of filter-feeding fish in controlling eutrophication, even in warm shallow lakes, remains in dispute.

In less productive waterbodies such as our mesocosms, phytoplankton growth was inhibited by zooplankton grazing and competition from benthic plants, e.g., submerged macrophytes and benthic algae. Stocking of filter-feeding fishes resulted in zooplankton loss and loss of benthic plants, which outweighed the positive effects of filter-feeding fish. Moreover, in less productive ecosystems, phytoplankton is often dominated by relatively small-sized algae species that cannot be efficiently removed by filter-feeding fish (Radke and Kahl, 2002). Therefore, the different consequences of filter-feeding fish on phytoplankton growth between our study (less productive, *Daphnia* dominated) and Xie and Liu (2001) (highly eutrophic, lacking *Daphnia*) may be largely attributed to the differences of both ecosystem status.

Reviewer #2

This study reports the plankton responses to climate warming in aquatic mesocosm ecosystems due to fish removal. The authors should carefully compare their finding with one recent publication "Responses of trophic structure and zooplankton community to salinity and temperature in Tibetan lakes: Implication for the effect of climate warming. Water Research. 2017". The knowledge gap should be clearly identified with sufficient literature. Another concern is how to practically remove fish from the lake? How to make it feasible for field application?

Our response: Yes, we acknowledge that there are many differences between Tibetan lakes in Lin et al., (2017) and our mesocosms. For instance, Tibetan lakes that they sampled were all highly oligotrophic ($\text{Chl-}a < 4 \mu\text{g/L}$), and many of them were deep and saline. Their results, that zooplankton to phytoplankton biomass ratios correlated negatively with temperature in lakes with zooplankton predators, while the opposite pattern was observed in lakes without those predators supported our sentence that top-down control is important in regulation of phytoplankton responses to climate warming in aquatic ecosystems. However, the pattern may not necessarily be transferred to shallow lakes that our study focused on. Therefore, in the revised manuscript, we have explicitly stated the status of the Tibetan lakes, e.g., oligotrophic, deep and saline, to avoid misunderstanding (Line 92-97).

Yes, it is very difficult to remove fish in warm lakes due to their small body size and fast reproduction. However, lake managers can help reducing the effects of fish. For instance, they can stop stocking of silver carp and bighead carp, which cannot naturally breed in lakes. Moreover, intensive harvesting may also be to some extent be helpful as seen in several studies of biomanipulation in Chinese lakes (i.e. Yu et al., 2016, Restoration of Shallow Lakes in Subtropical and Tropical China: Response of Nutrients and Water Clarity to Biomanipulation by Fish Removal and Submerged Plant Transplantation. Water, 8, 438).

Other comments:

Graphic abstract is not clear.

Our response: We have re-drawn the graphic abstract.

L379: please check the descriptions of "...that that favor..."

Our response: We have deleted the redundant "that" (Line 421)

The quality of Table 1 and Table 2 should be improved.

Our response: In Table 1 and Table 2, only *P* values were shown because there was not enough space to include the *F* and *DF* value in one Table. We have now provided detailed statistical data in the supplementary files.

Reviewer #3

This manuscript is an interesting study on the effect of warming on (sub-)tropical mesocosm in the presence and absence of fish. This study corroborates to some extent earlier findings that effect of warming on plankton dynamics is mediated by fish presence. For the most, the study is well written, although overly speculative in the discussion part. My main issues are with the experimental design and the analysis. In short, in order to make some of the statements on internal loading and increased presence of benthic primary producers, I feel crucial data on dissolved nutrients and benthic primary producers are missing. I would suggest to leave these very speculative parts out or make them much smaller. In addition, the different sampling intervals within the study duration may have affected the outcome of the rANOVA. I provide more detailed comments in the attached pdf.

Our response: We have added dissolved nutrient data to the revised manuscript (Fig. 2). Concerning the rANOVA, see our response below to the comments to Line 156-158.

Comments in the PDF

Abstract

Line 5: changed “The” into “Whereas the”

Our response: We have changed “The” into “Whereas the” (Line 26).

Line 6: Delete “therefore”

Our response: We have deleted “therefore” (Line 27).

Line 7: Changed “but” into “It is uncertain”

Our response: We have changed “but” into “it is uncertain” (Line 28).

Line 8: Delete “is certain”

Our response: We have deleted “is certain” (Line 29).

Line 10: “absence of fish”, Mention fish species here

Our response: We have added fish species “(*Aristichthys nobilis*)” (Line 30-31).

Line 22: changed “eu-trophication” to “eutrophication”

Our response: We have changed “eu-trophication” into “eutrophication” (Line 43).

Line 27: Changed “Phytoplankton” into “In the fishless mesocosms, phytoplankton”

Our response: We have changed “Phytoplankton” into “In the fishless mesocosms, phytoplankton” (Line 47).

Line 30: changed “subtrop-ical” to “subtropical”

Our response: We have changed “subtrop-ical” into “subtropical” (Line 50).

Highlights:

Line 1: Changed “Fish determine” into “The presence of fish determines”

Our response: We have re-written this sentence (See paragraph 1 in Highlights).

Line 2: Changed “boost” into “boosts”

Our response: We have re-written this sentence (Line paragraph 2 in Highlights).

Graphical Abstract

Font in this figure is blurred. Consider remaking

Our response: We have re-drawn the Graphical Abstract.

Abstract

See comments on abstract on earlier pages of the MS. I would recommend making hypothesis tested explicit

Our response: We have added the hypothesis to the Abstract section (Line 31-34).

Introduction

Line 60-61: Add a more appropriate reference for water quality. Water quality is more than the presence of blooms alone.

Our response: We have added a review (Delpla, I., et al., 2009) (Line 64), which focused on the impacts of climate change and water quality.

Line 62: excessive algal growth is a symptom of eutrophication. Consider using a better definition, e.g. nutrient over enrichment or something alike

Our response: Yes, excessive algal growth is one of the most objectionable symptoms of eutrophication. According to Hutson (1973), other symptoms such as increasing anoxia and fish kills were also included in the scope of eutrophication. Thus, in our revised manuscript, we have changed the definition of eutrophication to “a general term used to describe the suite of symptoms that a water body exhibits in response to nutrient over-enrichment” (Line 65-66).

Line 73: Added “in”

Our response: We have added “in” (Line 77).

Line 79: Changed “better” into “alternative”.

Our response: We have changed “better” into “alternative” (Line 82-83).

Line 84: changed “thanks” into “due”

Our response: We have changed “thanks” into “due” (Line 87).

Line 109-114: “We hypothesized ... phytoplankton biomass”, I recommend taking more explicit hypotheses up in the abstract section

Our response: We have added the hypotheses in the abstract (Line 31-34).

Line 126-128: “The water was screen (64- μ m size) to remove... to the tanks” Why was this done, if the response of the zooplankton community in warmer lakes was one of the prime response parameters? Please clarify the rationale behind this

Our response: At the beginning of experiment, we used a mesh to remove large-sized zooplankton. We did this just to make sure that the initial zooplankton biomass was similar in all mesocosms and that all the large-sized zooplankton had emerged from

the sediments late. This has also been done in other warming mesocosm experiments, e.g., Hansson et al., (2012) and Velthuis et al., (2017).

Line 133-134: “in the concrete pond” Unclear what is meant here. Was the concrete pond an inoculum source or just a water bath or? Clarify

Our response: The concrete pond was just a water bath. The main reason why we placed all tanks in the pond was to reduce the daily temperature fluctuation. If the tanks were placed on the ground, the daily changes in water temperature could be very large, which is not the case in lakes with large volumes of water. We have now clarified this in the text (Line 152-153).

Line 149: “simulating external loading in the lake” add reference of this. And external loading of the reference lake was based on a nutrient budget?

Our response: The daily nutrient doses added were slightly higher than the annual external nutrient loading in Lake Taihu, but resembled nutrient levels occasionally recorded in peak seasons. We have added this information and a reference “Paerl et al., 2011”, to the revised manuscript (Line 173-175). The external loading of Lake Taihu was based on nutrient inputs from tributaries of Lake Taihu.

Line 156-158: “carried out at 5-day intervals (day 154 to day 158)” How did you deal with this difference in sampling interval in your statistical analyses?

Our response: The statistical method – rANOVA - can also treat unequal-space data if the assumptions of normality or homogeneity of variance were met. This requires, however, re-programing. We have added a sentence explaining this, “WSFACTOR= =time k Polynomial(t1 ...tk)” in the “Syntax window” to take the unequal time interval into consideration. The reference is below:

Yunming Li, Wenjun Cao, et al., 2007. Comparison of unequally-spaced repeated measures design ANOVA using SAS and SPSS. Chinese Journal of Health Statistics, 24: 352-355. (in Chinese).

Line 159: “(8 cm diameter, 64 cm length)” Could it have been possible to miss the zooplankton population through DVM, given that your tube is much smaller in length than the depth of your mesocosm?

Our response: To avoid touching the sediment, the length of the sampler that we used was nearly 15-cm shorter than the depth of our mesocosms. At each sampling event, water was collected 4 times to reach a total volume of 12 litres. On two of sampling occasions, deep water samples were collected by placing the sampler somewhat deep (almost 5 cm above sediment), followed by thorough mixing with samples of relatively shallower water derived from another 2 sampling occasions. We used this method to get depth-integrated water samples in each mesocosm and thus avoid, as far as possible, the effects of DVM. We have now also give further details about the sampling methods in the revised manuscript (Line 183-187).

Line 159-160: “In the laboratory ... (TP)” What about dissolved nutrients? I think it is necessary to add these data, also to get more insight on bottom up processes

Our response: We have added the data on dissolved nitrogen and phosphorus (See Fig. 2).

Line 166: “1-L depth-integrated” Same sampler as above? Please state so

Our response: Yes, we directly collected 1-L water from 12-L depth-integrated water samples for phytoplankton identification. The word “subsample” we used in the manuscript means that this sample were rooted from the total water samples (Line 195).

Line 172: “of at least 30 cells” Once in the experiment? Or on multiple time occasions. It is know that phytoplankton size changes with resource availability

Our response: We measured algal size several times. We did not find significant size differences between time and treatments for any of the phytoplankton genera.

Line 200: “was statistically test for ... time” See earlier comments, how did you handle the different sampling intervals with your rANOVA. This type of analysis is pretty sensitive to that. Pleas clarify

Our response: Again, we used rANOVA method with re-programing to deal with all unequally-spaced data in our experiment.

Line 201: “ANOVA (rANOVA)” package used?

Our response: All comparisons were conducted using the statistical package SPSS, version 23.0 (IBM Corporation, Somers, NY, USA). We have mentioned this at the end of the session of data analysis (Line 249-250).

Line 217-218: “The effects of ... rANOVA” See comments above: how did you handle the different sampling intervals

Our response: Again, we used rANOVA method with re-programing to deal with all unequally-spaced data in our experiment.

Line 225: “on TN” What about dissolved nutrients? Warming causes faster demineralization, so the effects should perhaps be detectable through Nitrate, Nitrate and Ammonium

Our response: The TDN concentrations were significantly lower in the heated than in the unheated mesocosms at both fish regimes. We have included TDN data in the revised manuscript (Table 1; Fig. 2). However, we did not measure nitrate, nitrate and ammonium.

Line 230: “increased TP concentrations” Again, I think it is crucial to also show the dissolved nutrients here.

Our response: We have included TDP data in the manuscript (Table 1; Fig. 2).

Line 243: changed “*Phomidium*” into “*Phormidium*”

Our response: We have changed “*Phomidium*” to “*Phormidium*” (Line 278)

Line 245: “biomass of phytoplankton” They way you assessed biomass might be a bit tricky though. What was the correlation between Chl-a and phytoplankton biomass? Given that the majority of your phytoplankton seem to be colonial and filamentous species, you biomass estimate might be off.

Our response: In our study, there was a significant positive correlation between Chl-*a* and phytoplankton biomass, see graph below.

Line 257: “zooplankton” How did your lack of inoculum (as I understood from meshing a size fraction under 63 micron) affect your community? These are the species that hatched from a 10 cm sediment (?) or that came there through dispersal? How representative is this community for your reference lake?

Our response: At the beginning of the experiment, the water was filtered through a 64- μm mesh. This was done to ensure initial homogeneity of zooplankton among the mesocosms. Thus, in our experiment, most of the cladocerans were hatched from 10-cm sediment, and copepods were developed from the small nauplii passing through our net.

Our study was aimed to explore the potential effects of warming and planktivorous fish on plankton dynamics in subtropical climates. Although all materials such as sediments and water were collected from Lake Taihu, we did not intend to mimic Lake Taihu, which is a much more complicated system than our mesocosms. For instance, the nutrient levels and hydrodynamic conditions in our mesocosms both differ from those found in large Lake Taihu. Therefore, our mesocosms do not represent the conditions of Lake Taihu. For this reason, we do not focus on Lake Taihu except in the Method and Material section.

The initial zooplankton community in our mesocosms with rotifer and copepod nauplii dominance is commonly dominating in small subtropical or tropical lakes with high fish densities, and cladocerans could hatch from the sediment. Therefore, our experiment was, in our opinion, well designed for the purpose of exploring effects of warming and fish on plankton dynamics in subtropical shallow lakes despite the community difference from Lake Taihu.

Line 291: “chlorophyll *a* to TP ratio” I think SRP would be a better metric here, as it would better reflect resource availability

Our response: Here, we focused on the differences in top-down control pressure between unheated and heated mesocosms in fish-present regimes (Line 326). The metrics zooplankton: phytoplankton biomass ratio and chlorophyll *a*: TP ratio have been used as indicators for top-down control many studies (Jeppesen et al., 2003; Jackson et al., 2007). We agree that SRP may, to some extent, reflect the resource availability of phytoplankton. However, we do not find it to be better than Chl:TP as the main topic of our study is top-down control.

Line 325: “Chl : TP” See comments above, I do not think this is the right metric to use here.

Our response: Again, for top-down control, we believe that Chl:TP combined with the zooplankton to phytoplankton ratio is a much better variable than SRP (Line 360).

Line 335: “bottom-up (nutrient elevation)” Again, show dissolved nutrient as a proof of higher phosphorus mobilization. Technically, temperature induced higher activity of bottom dwelling organisms could also lead to higher TP presence.

Our response: We have added data on dissolved nutrients (Fig. 2). However, the TDP concentrations did not differ significantly between unheated and heated mesocosms (Table 1). This may be attributed to the combined results of sediment release, fish excretion and phytoplankton uptake. The pattern that high Chl_a yields to TP are closely related to greater contributions of TDP to TP is commonly found in meso- to hypereutrophic lakes (Mazumder, 1994). We have also explained this in the revised manuscript (Line 368-372).

The increment in water TP by heating indicated that more phosphorus entered the water column from the sediments. In consideration of the simultaneous sharp increase of Chl-*a* in water column, we therefore concluded that phytoplankton were benefited from TP increases (bottom-up).

Line 338-340: “This hypothesis ... (Fig.4d)” As the different prediction follow from each other, how does that effect your first prediction?

Our response: The prediction was made based on studies in cold areas, for instance Europe and Tibet, due to lack of studies in subtropical and tropical lakes. However, our results did not confirm this prediction. We have now better explained the reason for this difference in the text (Line 389-417).

Line 350: “(Fig. 3b)” How often did this occur? Judging from figure 3b, this did not happen too often.

Our response: In fish absent mesocosms, only 6 points (36 points in total) significantly exceed $10 \mu\text{g L}^{-1}$, so we use the term “often”. We have changed this sentence into “the average water Chl-*a* concentration only exceeded $10 \mu\text{g L}^{-1}$ on 6 sampling occasions (36 in total) (Line 393-394)” to make it clear. Meanwhile, we have also added a horizontal dash line at $10 \mu\text{g L}^{-1}$ in Fig. 3b to clarify this.

Line 356-359: “Another ... competition” I think this line of reasoning is not very strong. With crowding, you would see an increase at some point, followed by a decrease. You cannot crowd when densities are low.

Our response: We have deleted this sentence and exclude the potential effect of crowding (Line 400).

Line 361: “50” Being 40 percent smaller, so not a very convincing argument

Our response: We have excluded the possibility of crowding (Line 400).

Line 368: “TP”, what about SRP?

Our response: We have added TDP in the revised manuscript (Line 406). We did not observe significant elevation in TP and TDP concentrations induced by warming (Table 1; Fig. 2).

Line 371: “allowed benthic algae to grow ... surface” Did you quantify epipelton to

substantiate this argument

Our response: We did not quantify epipelon in our experiment. We just consider benthic algae to be a possible factor explaining why P was not released from the sediment in the heated compared with unheated mesocosms (in the fish-absent regime). We have also rewritten this part in the revised manuscript to enhance clarity (Line 408-417).

Line 375: “dominance of benthic primary producers” But you have to provide data for this argument in order to make a case when stating: "Our study suggests"

Our response: This sentence has been deleted in the revised manuscript as we did not measure epipelon (Line 417).

Fig.3: I do not see in figure 3b that Chl-a is most of the time <10 micrograms. Please use more careful wording

Our response: In the fish-absent mesocosms, only 6 points (36 points in total) significantly exceeded $10 \mu\text{g L}^{-1}$. We have added a dash line at $10 \mu\text{g L}^{-1}$ in Fig. 3b for clarification.

Highlights:

Plankton responses to warming were mediated by fish in subtropical shallow mesocosms

Phytoplankton benefited, while zooplankton suffered from warming when fish existed

Warming effects on plankton dynamics were low in fishless mesocosms

Fish-mediated plankton responses to increased temperature in subtropical aquatic mesocosm
ecosystems: implications for lake management

Hu He^{1,*}, Hui Jin², Erik Jeppesen^{3,4}, Kuanyi Li^{1,4}, Zhengwen Liu^{1,4,5}, Yongdong Zhang¹

¹ State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing, 210008, China

² Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

³ Department of Bioscience, Aarhus University, Aarhus, Denmark

⁴ Sino-Danish Centre for Education and Research, Beijing 100049, China

⁵ Department of Ecology and Institute of Hydrobiology, Jinan University, Guangzhou 510632, China

*Corresponding author: Dr. Hu He

E-mail: hehu@niglas.ac.cn

Postal address: 73 East Beijing Road, Nanjing, 210008, China

Phone number: +86-025-86882237

ABSTRACT

Although it is well established that climate warming can reinforce eutrophication in shallow lakes by altering top-down and bottom-up processes in the food web and biogeochemical cycling, recent studies in temperate zones have also shown that adverse effects of rising temperature are diminished in fishless systems. ~~Whereas t~~The removal of zooplanktivorous fish may ~~therefore~~ be useful in attempts to mitigate eutrophication in temperate shallow lakes, ~~but it is uncertain~~ whether similar mitigation might be achieved in warmer climates ~~is uncertain~~. We compared the responses of zooplankton and phytoplankton communities to climate warming in the presence and absence of fish (*Aristichthys nobilis*) in a 4-month mesocosm experiment at subtropical temperatures. We hypothesized that 1) fish and phytoplankton would benefit from warming, while zooplankton would suffer in fish-present mesocosms and 2) warming would favor zooplankton growth but reduce phytoplankton biomass in fish-absent mesocosms. Our results showed significant interacting effects of warming and fish presence on both phytoplankton and zooplankton. In mesocosms with fish, biomasses of fish and phytoplankton increased in heated treatments, while biomasses of *Daphnia* and total zooplankton declined. Warming reduced the proportion of large *Daphnia* in total zooplankton biomass, and reduced the zooplankton to phytoplankton biomass ratio, but increased the ratio of chlorophyll *a* to total phosphorus, indicating a relaxation of zooplankton grazing pressure on phytoplankton ~~under the experimental warm-~~

~~ing-scenario~~. Meanwhile, warming resulted in a 3-fold increase in TP concentrations in the mesocosms with fish present. The results suggest that climate warming has the potential to boost eutrophication in shallow lakes via both top-down (loss of herbivores) and bottom-up (elevated nutrient) effects. However, in the mesocosms without fish, there was no decline in large *Daphnia* or in total zooplankton biomass, supporting the conclusion that fish predation~~;~~ is the major driver of low large *Daphnia* abundance declines in large *Daphnia* in subtropical warm lakes. In the fishless mesocosms, ~~p~~Phytoplankton biomass and nutrient levels ~~appeared-were~~ not ~~to be~~ affected by temperature. Our study suggests that removing fish to mitigate warming effects on eutrophication may be potentially beneficial in subtropical lakes, though the rapid recruitment of fish in such lakes may present a challenge to success in the long-term.

Key words: climate warming; fish removal; eutrophication; top-down control; shallow lakes

INTRODUCTION

Global temperatures have been rising for decades and are predicted to rise by an additional 3–5°C over the next century (IPCC 2014). Ongoing climate warming is predicted to bring a range of undesirable changes in biodiversity, ecosystems and ecosystem services, including the reduced distribution of stenothermic species (Jeppesen et al., 2012), expansion of alien species (Walther et al., 2009), disruption of seasonal clockwork of organisms (Winder and Schindler, 2004), exacerbation of algal blooms and degradation water quality in freshwater ecosystems (Paerl and Huisman, 2008; [Delpla et al., 2009](#); Kosten et al., 2012).

Eutrophication ([a general term used to describe the suite of symptoms that a water body exhibits in response to nutrient over-enrichment](#)) (~~excessive algal growth resulting from nutrient enrichment~~) has been considered a primary problem [for a large number of freshwaters facing most surface waters in recent times](#) (Smith and Schindler, 2009), and climate warming is predicted to reinforce the ~~symptoms-issues~~ in affected aquatic ecosystems in a variety of direct and indirect ways (Paerl and Huisman, 2008; [Jeppesen et al., 2010](#); Moss et al., 2011). For instance, warming can enhance the growth rate of an entire phytoplankton community by increasing the efficiency of nutrient use (De Senerpont Domis et al., 2013). Meanwhile, cyanobacteria with relatively high [optimum niche optimal](#) temperatures may gain significant competitive advantage (Paerl and Huisman, 2008; Kosten et al., 2012). ~~and In~~ shallow lakes, warming-induced increases in nutrient release from fertile sediments

(Søndergaard et al., 2013) and in temperate lakes, external loading from catchments (Jeppesen et al., 2009) may boost plankton growth ~~via elevated internal and external nutrient loading~~. Changes in food web structures ~~in shallow lakes~~ prompted by warming ~~process often~~ also favor phytoplankton growth in shallow lakes (Gyllström and Hansson, 2005; Meerhoff et al., 2012). For example, an increasing proportion of small, omnivorous fish in warmer lakes (Jeppesen et al., 2010; González-Bergonzoni et al., 2012) may reduce herbivorous zooplankton, and thereby release grazing pressure on phytoplankton (Gyllström and Hansson, 2005). Thus, from the perspective of lake management, a warmer future will demand alternative ~~better~~ strategies for combating eutrophication ~~in shallow lakes~~.

Hansson et al. (2013) emphasized the importance of fish in top-down regulation of phytoplankton responses to climate warming in shallow aquatic ecosystems. They found that when fish were present in mesocosms maintained under warming conditions, phytoplankton biomass was significantly increased ~~thanks due~~ to a release from zooplankton grazing pressure. In fish-free mesocosms, however, zooplankton herbivory increased under warming and pressured a significant reduction in phytoplankton. These results are supported by other mesocosm climate studies in the north temperate zone, e.g., Yvon-Durocher et al., (2011) and Velthuis et al., (2017) (fish absent), and Šorf et al., (2015) (fish present), ~~and by F~~ field investigations in 45 high altitude Tibetan lakes, although most of them were highly oligotrophic and varied in water depth and salinity, also shown that ~~where~~ zooplankton to phytoplankton bio-

mass ratios correlated negatively with temperature in lakes with zooplankton predators (included fish and the amphipod *Gammarus*)~~fish present~~, while the opposite pattern was observed in lakes without those predators~~fishless lakes~~ (Lin et al., 2017).

The interplay between warming and fish presence suggest that food web manipulation, in the form of fish removal, is a practice worth considering~~may be a useful practice~~ in lake management in future warmer climates. There are, h~~However~~, three concerns for applying this method to warm lakes. Firstly, previous mesocosms and field given that~~these~~ studies ~~were~~have all been conducted in cool regions such as northern Europe (Hansson et al., 2013; Šorš et al., 2015) ~~and or at~~ high attitude, as in Tibet (Lin et al., 2017); and it is known that trophic dynamics and metabolism change with temperature (Brown et al., 2004; Gyllström and Hansson, 2005); Meanwhile the evidence from ~~warm~~ subtropical and tropical shallow lakes remains~~is also~~ needed. ~~Furthermore~~Secondly, since temperate climate mesocosms used in previous studies contained only small cladocerans~~only small cladocerans existed in previous temperate climate mesocosm studies~~ (Hansson et al., 2013; Velthuis et al., 2017), further studies are necessary to examine the effects of rising temperature on larger grazers of phytoplankton such as *Daphnia* spp. Latitudinal comparisons have suggested that large-sized *Daphnia* will decline quickly when water temperatures exceed 20°C (Gillooly and Dodson, 2000), but recent studies in subtropical lakes revealed that *Daphnia* can maintain population size in temperatures exceeding 30°C in the absence of fish predation (Iglesias et al., 2011; Havens et al., 2015). It may be that, fish preda-

tion in warming scenarios is a more important structuring force on zooplankton than temperature itself (Meerhoff et al, 2012). Thirdly, several researchers have suggested that stocked species of filter-feeding fish such as silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), are useful in maintaining good water quality in subtropical and tropical shallow lakes, as they may filter phytoplankton (Starling, 1993; Xie and Liu, 2001; Ke et al., 2009; Guo et al., 2015). Others have the complete opposite opinion, arguing that such species also filter zooplankton and disturb the sediment, resulting in further release of the nutrients that stimulate phytoplankton growth (Wang et al., 2008; Jeppesen et al, 2012b; Zhang et al., 2016). Others again argue that stocking with filter feeders will only be useful in highly eutrophic systems that naturally lack large cladoceran zooplankton (Radke & Kahl, 2002), while the effect would be negative in less productive lakes as predation would reduce zooplankton and boost small-sized algae (Radke and Kahl, 2002) or enhance nutrient levels by sediment disturbance (Zhang et al., 2016). These mixed opinions present an overall rather ambiguous picture regarding the likely effects of filter-feeding fish.

In this study, mesocosms operating at a subtropical temperature setting but within the thermal tolerance of *Daphnia* were used to compare the responses of zooplankton, phytoplankton and nutrients to warming in fish-present and fish-absent scenarios. We hypothesized that under subtropical conditions: (1) fish and phytoplankton in fish-present mesocosms would benefit from warming, while zooplankton (especially *Daphnia* spp.) would suffer; and (2) warming would favor zooplankton growth and

enhanced herbivory in mesocosms without fish, ~~and thereby leading to~~ reduced phytoplankton biomass.

MATERIALS AND METHODS

Experimental design

The outdoor mesocosm experiment was conducted from 7 March (day 67 in Year 2016) to 3 July 2016 (day 184 in Year 2016) at Taihu Laboratory for Lake Ecosystem Research (31°25'3"N, 120°13'13"E), located in Meiliang Bay, on the northern edge of Lake Taihu, China. The selected period represents the time when phytoplankton abundance is controlled ~~mainly to some extent~~ by zooplankton grazing (Chen et al., 2011). The mesocosms ~~consisted of~~ were twelve 400 L fiberglass-reinforced plastic tanks (90-cm high; 80-cm inside diameter at the top; 70-cm inside diameter at the base). All ~~tanks-mesocosms~~ were filled with 370 L lake water with a 10-cm layer of pre-sieved sediment (1.7 mm mesh size). The water was screened (64-μm mesh size) to remove crustacean zooplankton and large inorganic particles, and mixed to ensure homogeneity before being added to the tanks. All mesocosms were open during the experimental period, and suspended in a concrete pond (7 m long × 6 m wide × 2 m deep) filled with lake water. The concrete pond served as a water bath used to reduce the daily temperature fluctuation.

A two-way factorial experiment was carried out under two temperature settings (ambient and warming) and two fish levels (fish present/absent). Each treatment had three

replicates, making a total of 12 experimental units. In the unheated ambient scenario, the temperature of the tank water was allowed to equilibrate with that in the concrete pond, which ranged between 8 °C and 30 °C (Fig. 1). In the warming scenario, temperatures were maintained consistently at 3°C warmer than the pond water (Fig. 1) using a customized intelligent temperature-control device with two 200-W heating rods. Real time temperatures in the heated and unheated tanks were measured/logged automatically every ten minutes.

The fish used in the experiment were bighead carp (*Aristichthys nobilis*), a common zooplanktivore-filter-feeding fish in Lake Taihu and other shallow lakes. Carp with an average length of 9 ± 0.4 cm and mean wet weight of 7.9 ± 0.6 g was obtained from a local aquaculture facility and acclimatized in lake water for a week before the experiment began. Six mesocosm tanks were chosen to receive fishes, with one fish per tank, amounting to ~ 208 kg ha⁻¹. The fish density approximated to a biomass of 208 kg ha⁻¹, which is comparable to the biomass observed naturally in Lake Taihu (217 kg ha⁻¹ in Year 2015). After fish were added to their assigned mesocosm, the experimental temperature settings were maintained uninterrupted until the end of the experiment.

Nutrients (5 µg P L⁻¹ d⁻¹; 130 µg N L⁻¹ d⁻¹) were supplied daily to each microcosm throughout the experiment, simulating external loading in the lake. These loadings were slightly higher than the annual average for external loading in Lake Taihu but do occur in peak seasons (Paerl et al., 2011). Phosphorus and nitrogen were added separately as aqueous solutions of potassium dihydrogen phosphate (KH₂PO₄) and potas-

sium nitrate (KNO₃), the dominant inorganic nutrient forms in Lake Taihu. The enclosure walls were scrubbed with banister brushes every evening to minimize the growth of periphytic algae.

Sampling and processing

Water chemicals and chlorophyll *a*

Sampling for monitoring of nutrients and chlorophyll *a* levels was ~~carried out~~collected at 5-day intervals during the spring months (day 67 to day 154), and at 10-day interval in the last ~~summer~~ month of the study, as it ran into summer (day 154 to day 184). A tube sampler (8 cm diameter, 64 cm length) was used to collect depth-integrated water samples. In order to reach a total volume of 12 litres, the sampler was deployed four times in each mesocosm on each sampling occasion, twice at depth (around 5 cm above sediment to minimise disturbance), and twice from the surface down. These four samples were then well mixed. ~~On each occasion, we used a tube sampler (8 cm diameter, 64 cm length) to collect depth-integrated water.~~ In the laboratory, total nitrogen (TN), ~~and~~ total phosphorus (TP), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were measured according to Chinese standard methods (Jin and Tu 1990). Chl-*a* concentrations were measured spectrophotometrically from matter retained on a GF/C filter and extracted in a 90% (v/v) acetone/water solution for 24 h. No correction was carried out for pheophytin interference (SEPA 2002).

Phytoplankton community

205 At each 10-day sampling interval, a 1-L depth-integrated subsample taken from each
206 mesocosm was treated with 10 mL Lugol's iodine solution and sedimented for 48 h.
207 The supernatant was removed, and the residue was collected and examined under
208 $\times 100 - \times 400$ magnification for enumeration of phytoplankton. Phytoplankton was
209 identified to genus level according to Hu (2006) and following recent taxonomic revisions
210 as far as possible (Guiry and Guiry 2014). The biomass of common phytoplankton
211 taxa was calculated based on cell size measurements of at least 30 cells of each
212 taxon and using formulae for geometric shapes approximating cell forms (Zhang and
213 Huang 1991). Where possible, at least 1000 cells of each taxon were counted per
214 sample, but for less common taxa, calculations were based on fewer measurements.
215 Phytoplankton biovolume was multiplied by 0.29 to obtain an approximation of dry
216 weight (Reynolds 1984).

217 Zooplankton community

218 Zooplankton were sampled at 10-day intervals in the first (March) and last (June)
219 months of the experiment, and at 5-day intervals during April and May, when large
220 populations of *Daphnia* develop quickly (Chen et al., 2011). At each sampling, a 10-L
221 depth-integrated water sample was filtered through a 64- μm net and the retained material
222 was preserved in 4% formaldehyde. Crustacean zooplankton (cladocerans, copepodite
223 and adult copepods) were counted at magnifications between $\times 10$ and $\times 40$.
224 Species represented by more than 100 individuals were deemed to be dominant and
225 were identified according to Chiang and Du (1979) and Shen and Du (1979). Cope-

pod nauplii were counted without further taxonomic distinction. Zooplankton biomass (dry weight) was estimated using equations from Dumont et al. (1975) and Huang (1999). Where possible, up to 20 individuals of each taxon were measured. Rotifers were counted directly from phytoplankton samples at $\times 100$ magnification (at least 100 individuals of the most abundant taxa), and biomass of rotifers was calculated using standard dry weights obtained from multiple lakes in the middle and lower Yangtze river basin.

Fish

At the end of the experiment (day 184), fish were collected from the fish mesocosms (6 tanks) and their final length and body weight were measured.

Data analysis

Nutrient and plankton dynamics

The time series data (including values for nutrients, zooplankton and phytoplankton biomass) were statistically tested for effect of treatment, time and their interaction by repeated-measurements ANOVA (rANOVA). Prior to analysis, data were $\log_{10}x$ or $\log_{10}(x+1)$ (if a value of zero existed) transformed to meet assumptions of normality or homogeneity of variance. If the assumption of sphericity of the variance-covariance matrices of the rANOVA analyses was violated, the degrees of freedom (df) were Huyn-Feldt corrected, resulting in an adjustment of the significance of the F ratio (Huyn and Feldt 1976). In the event of significant interaction terms, a post-hoc pairwise comparison was performed (Holm-Sidak method) to determine where the

differences occurred, using fish as a categorical factor and temperature as a quantitative factor.

~~Fish and T~~op-down control

To compare warming-induced differences in top-down pressure between contrasting fish treatments, we computed three biological metrics indicative of food web interactions (Jeppesen et al., 2003; Jackson et al., 2007). The proportion of large-sized cladocerans (species sensitive to fish grazing) within the total zooplankton biomass was used to reflect the predation pressure exerted on zooplankton by fish. Ratios of zooplankton to phytoplankton biomass and chlorophyll *a* to TP were used to reflect grazing pressure of zooplankton on phytoplankton (Jeppesen et al., 2003). The effects of warming and fish presence on the three metrics were also compared using rANOVA, pre-treated as above. Student's *t* tests were used to reveal the effects of warming on fish body length and weight. All comparisons were conducted using the statistical package SPSS, version 16.0 (IBM Corporation, Somers, NY, USA).

RESULTS

Nutrients

No significant effect of warming or fish presence was apparent on TN and no interaction was detected (Table 1). In both fish-present and fish-absent scenarios, TN showed a steady decline from day 67 to day 83. After that, TN values changed

unimodally with time, peaking at day 124 in the fish-present mesocosms (Fig. 2a) and at day 129 in fish-absent treatments (Fig. 2b).

Warming and fish presence significantly increased TP concentrations, and their interaction was significant (Table 1). In fish-present, unheated treatments, TP concentrations did not alter significantly with time, while in heated mesocosms with fish present, TP increased sharply from day 114, reaching a final value three times higher than that of the unheated controls (Fig. 2c; Holm-Sidak method, $F = 25.08$, $P = 0.001$). In fish-absent mesocosms, TP concentrations in both unheated and heated mesocosms fluctuated slightly throughout the experiment (Fig. 2d) and did not differ significantly (Holm-Sidak method, $F = 0.0018$, $P = 0.898984$).

No interactive effects of warming and fish presence were observed for TDN and TDP concentrations (Table 1). The TDN in both fish-present and fish-absent regimes was, however, significantly lower in heated mesocosms than in unheated ones (Fig. 2e-f; Table 1). Warming did not obviously affect TDP concentrations, whilst the fish effects were significant, with slightly lower TDP concentrations in fish-present mesocosms than in fish-absent ones (Fig. 2g-h; Table 1).

Phytoplankton

The phytoplankton community followed a succession from euglenophyte dominated (mainly *Euglena* spp.) to diatom dominated (mainly *Synedra* spp.), and finally to green alga dominated (mainly *Schroederia*, *Scenedesmus* and *Arthrodesmus*) in all mesocosms, except for the heated fish-present treatment, where cyanobacteria

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(*Phormidium* spp.) dominated the community at the end of experiment. However, rANOVA showed no significant effect of fish and warming on either percentage or biomass of phytoplankton at phylum level.

The effects of time, warming and fish, and their interactions on Chl-*a* concentration and phytoplankton biomass were all significant (Fig. 3a-d, Table 1). With fish present, Chl-*a* concentrations in heated mesocosms increased sharply from day 109, and were 10 times higher than in unheated mesocosms at the end of experiment (Fig. 3a; Holm-Sidak method, $F = 56.212$, $P = 0.000$). Similarly, phytoplankton biomass in heated mesocosms was ~~also~~ significantly higher than in unheated mesocosms (Fig. 3c; Holm-Sidak method, $F = 11.831$, $P = 0.009$). By contrast, ~~in under~~ the fish-absent regime, Chl-*a* concentrations (Fig. 3b) and phytoplankton biomass (Fig. 3d) remained mostly low in both temperature treatments and no significant difference was observed (Holm-Sidak method, $F = 0.347$, $P = 0.347$ for Chl-*a*; $F = 0.000$, $P = 0.999$ for phytoplankton biomass).

Zooplankton

A total of twenty-two zooplankton taxa were recorded in all treatments across the entire experimental period, including five cladoceran genera (*Daphnia*, *Bosmina*, *Chydorus*, *Alona* and *Spapholeberis*), two copepods (*Mesocyclops leuckarti* and *Sinocalanus dorrii*) and 15 rotifers (dominated by *Brachioums angularis*, *Keratella cochlearis*, *Polyarthra trigla* and *Filinia longistea*). At the end of experiment, *Daph-*

nia were dominant in all mesocosms except for the fish plus warming treatments, where small cladocerans (*Spapholeberis* spp.) and copepods prevailed.

Warming and fish presence had a significant interactive effect on *Daphnia* biomass (Table 2). In fish-present regimes, *Daphnia* changed unimodally over time and peaked at day 109 in both heated and unheated mesocosms (Fig. 4a). After that, *Daphnia* declined in both temperature scenarios but the magnitude of decline was significantly greater in heated mesocosms than in unheated ones (Fig. 4a), leading to a significant warming-induced reduction in *Daphnia* biomass by the end of the experiment (Holm-Sidak method, $F = 59.91965.789$, $P = 0.000$; Fig. 4a). In fish-absent regimes, no significant effect of warming on *Daphnia* was apparent (Holm-Sidak method, $F = 0.01079$, $P = 0.90027$; Fig. 4b), and *Daphnia* biomass in both temperature treatments remained high from day 109 to the end of experiment (Fig. 4b).

Warming and fish had significant interactive effects on total zooplankton (Table 2). In the fish-present regimes, warming significantly reduced total zooplankton biomass (Fig. 4e) (Holm-Sidak method, $F = 32.066$, $P = 0.000$; Fig. 4c), while in the fish-absent regimes warming effects on total biomasses were not significant (Holm-Sidak method, $F = 1.817$, $P = 0.215$; Fig. 4d).

Fish and top-down control

In the fish-present regimes, both lengths and body weights of fish at the end of experiment were higher in heated mesocosms than in unheated mesocosms (t-test, $P = 0.031$ for body length; $P = 0.003$ for weight; Fig. 5).

Warming and fish had significant interactions on the percentage of *Daphnia* in total zooplankton biomass (Table 2). In fish-present treatments, warming significantly reduced the percentage *Daphnia* biomass (Holm-Sidak method, $F = 75.815$, $P = 0.000$; Fig. 6a), while in the absence of fish no significant difference in *Daphnia* biomass percentage was detected between unheated and heated mesocosms (Holm-Sidak method, $F = 3.356$, $P = 0.104$; Fig. 6b).

Significant interactions were detected between warming and fish on the ratios of zooplankton to phytoplankton biomass (Zoop : Phyt) and chlorophyll *a* to TP ratio (Chl : TP) (Table 2). In fish-present regimes, warming significantly reduced both Zoop : Phyt (Holm-Sidak method, $F = 21.467$, $P = 0.002$; Fig. 6c) and Chl : TP (Holm-Sidak method, $F = 41.554$, $P = 0.000$; Fig. 6e), while in fish-absent regimes, neither metric was found to differ significantly between unheated and heated treatments (Holm-Sidak method, $F = 0.967$, $P = 0.354$ for Zoop : Phyt; Fig. 6d; $F = 0.409$, $P = 0.543$ for Chl : TP; Fig. 6f).

DISCUSSION

Having tested the interactive effects of warming and fish on plankton dynamics and the strength of top-down effects under subtropical conditions, we will evaluate each of our predictions below.

Prediction 1: In fish-present mesocosms, phytoplankton would benefit from warming, while zooplankton (especially large Daphnia) would suffer.

This prediction is fully supported. In our fish-present mesocosms, phytoplankton biomass and fish weight were both higher in heated mesocosms than in unheated controls (Fig. 3a; 3c; Fig. 5), while biomasses of both large-sized *Daphnia* and total zooplankton were reduced by warming (Fig. 4a; 4c). Our results echo previous climate mesocosm (fish-present) experiments carried out in colder regions (Hansson et al., 2013; Šorf et al., 2015), and support the conclusion that climate warming will alter the food web structure of shallow lakes in favor of phytoplankton growth (Jeppesen et al., 2009, 2010).

Zooplankton losses in heated mesocosms were largely due to the enhanced top-down control exerted by fish, as evidenced by the reduced proportion of *Daphnia* amongst zooplankton (Fig. 6a) and rapid fish growth (Fig. 5). Moreover, changes in zooplankton community structure under warming, such as the rise to dominance of small cladocerans and copepods in heated mesocosms while *Daphnia* prevailed in unheated treatments (data not shown), also corresponded well with the effects of fish predation on zooplankton assemblages (Vakkilainen et al., 2004). Thermal stress could not explain the loss of large *Daphnia* and total zooplankton in heated mesocosms (Fig. 4a), as these species maintained their population sizes in heated mesocosms without fish (Fig. 4b). Our studies therefore support the conclusion that fish predation, rather than temperature change, is the major driver of large *Daphnia* decline in subtropical lakes (Iglesias et al., 2011; Havens et al., 2015).

In fish-present regimes, the significantly reduced Zoop : Phyt and elevated Chl : TP ratios observed in heated mesocosms emphasized the importance of top-down pressure in regulating phytoplankton biomass (Fig. 6c; 7e). However, our results also indicate enhanced bottom-up effects under warming scenarios, with significant increases in TP concentrations in heated mesocosms (Fig. 2c-a). Interestingly, although the importance of temperature on internal P loading in shallow lakes has been long recognized (Jensen and Andersen, 1992; Søndergaard et al., 1999), definite evidence has not been recorded in temperate climate mesocosm studies before (Hansson et al., 2013; Özen et al., 2013). In fish-present mesocosms, TDP concentrations were not enhanced by temperature increase as seen for TP (Fig. 2g), maybe as a result of the greater sequestering of P by phytoplankton under warmer temperatures. A pattern whereby Chl yields to TP increase with decline in contributions of TDP to TP has been commonly found in mesoeutrophic to hypereutrophic lakes (Mazumder et al., 1994). Our study, based on a subtropical temperature scenario, demonstrates that climate warming has the potential to boost eutrophication in shallow lakes via both top-down (herbivores loss) and bottom-up (~~TP nutrient~~ elevation) effects. Moreover, with respect to the debate on the impact of filter-feeding fishes on eutrophication in subtropical and tropical shallow lakes, our results, e.g., the high Chl-a and TP concentrations in mesocosms with fish (Fig. 2c-d; 3a-b), support the view bighead carp have a exacerbating effect on eutrophication in low productivity warm lakes, as they reduce

zooplankton (Fig. 4a-b) and enhance phytoplankton growth (Fig. 3a-b) and nutrient levels (Fig. 2c-d).

Prediction 2: In fish-absent mesocosms, warming would favor zooplankton growth and enhanced herbivory and thereby reduce phytoplankton biomass

This hypothesis is not supported, as no significant difference was observed between ~~in neither phytoplankton nor zooplankton biomass was observed between~~ unheated and heated fishless treatments in terms of either phytoplankton or zooplankton biomass without fish (Fig. 3b; 3d; Fig. 4d). However, our study did support earlier ~~sug-~~ gestions indications from temperate regions that fish removal might be a useful means of mitigating the effects of warming on eutrophic shallow lakes (Hansson et al., 2013), as our fish-free heated mesocosms maintained ~~clear water conditions~~ low Chl-*a* until the end of experiment (Fig. 3b).

In fish-absent regimes, warming did not increase zooplankton (*Daphnia* spp. dominated) biomass as it did in similar climate mesocosms of Hansson et al. (2013), where small cladoceran genera such as *Bosmina* and *Chydorus* dominated. ~~The latter were dominated by small cladoceran genera such as *Bosmina* and *Chydorus*.~~ Population development for the large *Daphnia* present in our mesocosms may have been constrained by low food availability, as the average water Chl-*a* concentration only exceeded 10 $\mu\text{g L}^{-1}$ on 6 sampling occasions (36 in total) fish-absent treatments by low food availability, as water Chl-*a* concentrations were often $< 10 \mu\text{g L}^{-1}$ (Fig. 3b).

A similar trade-off between food and temperature in *Daphnia* growth has previously

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411 been recorded in ~~other~~ mesocosm and laboratory studies of unproductive ecosystems.
412 For instance, Strecker et al., (2004) found that experimental warming (+3.6°C) signif-
413 icantly reduced abundance of *Daphnia pulex* in fishless oligotrophic mesocosms,
414 ~~while~~ Heugens et al., (2016) found that the positive effect of temperature on popula-
415 tion growth of *Daphnia magna* was diminished under limited food constraints. ~~An-~~
416 ~~other explanation for the lack of thermal effect on *Daphnia* abundance may be crowd-~~
417 ~~ing, a density dependent mechanism controlled by chemical substances released by~~
418 ~~the organisms or by physical contact, but independent of food competition. A previ-~~
419 ~~ous laboratory study suggests that at 20 °C, *Daphnia* population development would~~
420 ~~be limited by crowding when abundance exceeds 85 ind L⁻¹ (Burns, 2000). In our~~
421 ~~fish-free mesocosms, *Daphnia* densities were around 50 ind L⁻¹ when water tempera-~~
422 ~~tures were above 25 °C (Fig. 4b).~~

423 In a further contrast to the results of the temperate climate studies of Hansson et al.,
424 (2013) and Velthuis et al., (2017), ~~we found that neither~~ Chl-*a* concentration ~~nor~~ and
425 phytoplankton biomass were not reduced by warming in the fish-absent mesocosms
426 (Fig. 3b; 3d) and Zoop : Phyt and Chl : TP ratios remained unchanged (Fig. 6d; 7f),
427 suggesting that top-down control of phytoplankton by zooplankton ~~was~~ not strength-
428 ened by warming. Furthermore, in the absence of fish, water TP and TDP concentra-
429 tions did not increase with rising temperature (Fig. 2c-d), indicating no stimulation of
430 phytoplankton growth by warming. A similar pattern was found in the warming ex-
431 periment of Velthuis et al., (2017) who attributed the low and unchanged phosphorus

432 levels between heated and unheated mesocosms to the development of periphyton,
433 benthic algae and filamentous algae in their unproductive, fishless mesocosms. This
434 may also be the case for our fishless mesocosms. High water transparency owing to
435 the facts that lacking sediment disturbance by fish and low Chl-*a* concentrations (Fig.
436 3b) may have promoted the growth of benthic algae, thereby immobilizing nutrients
437 within sediments in fishless mesocosms (Zhang et al., 2013). Unfortunately, direct
438 evidence of the abundance of e.g., benthic algae and periphyton, are lacking in our
439 experiment, but would be well worth pursuing future.

440 ~~The result may be attributed to the high water transparency of our fishless~~
441 ~~mesocosms, which allowed benthic algae to grow well at the sediment surface, there-~~
442 ~~by immobilizing nutrients within sediments (Zhang et al., 2013). Our studies therefore~~
443 ~~suggest that under warming scenarios, in addition to top-down effects, fish removal~~
444 ~~can also contribute to maintenance of clear water lake conditions via a bottom-up~~
445 ~~pathway, supporting the dominance of benthic primary producers.~~

446 *Implications for lake management in future warmer climate*

447 IPCC (2014) predicts that air temperatures will likely increase by 1-4°C (relative to
448 1986-2005) in many parts of the world in this century. ~~An overwhelming body of sci-~~
449 ~~entific evidences suggests that air temperatures will increase by 2-5°C in many parts~~
450 ~~of the world during the coming generation (IPCC, 2014).~~ Under this scenario, fish
451 communities are expected to develop structures that ~~that~~ favor phytoplankton growth
452 via cascading effects (Jeppesen et al., 2010), amplifying the existing eutrophication

~~problems in shallow lakes. The superposition of warming and changing fish community structure is likely to amplify existing eutrophication problems in shallow lakes~~ (Jeppesen et al., 2009, Moss et al., 2011). Our study showed a significant interplay between the effect of temperature and fish on lake plankton communities under subtropical temperature conditions. In fish-present systems, warming ~~is seen to~~ benefited phytoplankton growth via a reduction in large *Daphnia* (Fig. 4a) and ~~elevated TP levels (Fig. 2c) are likely as result of increased release from the sediment to stimulate the release of sediment P (Fig. 2a).~~ However, when fish were absent from these ~~systems in warm waters,~~ abundances of large *Daphnia* ~~are were~~ maintained (Fig. 4b), and warming effects on both top-down and bottom-up processes ~~are were~~ diminished ~~under the given nutrient scenario. Thus, F~~ from the perspective of lake management, our findings suggest that ~~the~~ removal of fish may ~~also~~ be an effective means of mitigating warming effects on eutrophication in subtropical lakes ~~(as in temperate lakes),~~ though the rapid recruitment of fish in warm lakes may present a challenge to the success of such measures in the long-term (Jeppesen et al., 2012b).

CONCLUSION

Our study showed significant interactive effects between warming and fish presence on plankton dynamics and nutrient levels in subtropical aquatic mesocosms. In mesocosms with fish, biomasses of fish and phytoplankton increased in heated treatments, while biomasses of *Daphnia* and total zooplankton declined. Meanwhile,

warming resulted in a 3-fold increase in TP concentrations. The results suggest that climate warming has the potential to boost eutrophication in subtropical shallow lakes via both top-down (loss of herbivores) and bottom-up (elevated nutrient) effects. However, in the mesocosms without fish, no decline in large *Daphnia* or in total zooplankton biomass was observed in heated mesocosms, and phytoplankton biomass and nutrient levels were not affected by warming, ~~either~~. From a lake management perspective, our results emphasize that removing fish will be beneficial in mitigating ongoing warming effects on eutrophication in subtropical shallow lakes, though the rapid recruitment of fish in warm lakes may present a challenge.

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Table 1. Summary of rANOVA results on the effects of different temperature (Heating) and planktivorous fish (Fish) scenarios on the concentrations (mg L⁻¹) of total nitrogen (TN) and total phosphorus (TP), and on phytoplankton biomass as indicated by chlorophyll *a* (Chl-*a*: µg L⁻¹) concentration during the course of the experiment (Time).

<u>Effects</u>	<u>TN</u>	<u>TP</u>	<u>TDN</u>	<u>TDP</u>	<u>Chl-<i>a</i></u>
<u>Heating</u>	<u>0.877</u>	<u>0.009</u>	<u>0.006</u>	<u>0.856</u>	<u>0.002</u>
<u>Fish</u>	<u>0.093</u>	<u>0.012</u>	<u>0.176</u>	<u><0.001</u>	<u><0.001</u>
<u>Heating × Fish</u>	<u>0.226</u>	<u>0.010</u>	<u>0.077</u>	<u>0.137</u>	<u><0.001</u>
<u>Time</u>	<u><0.001</u>	<u>0.001</u>	<u><0.001</u>	<u><0.001</u>	<u><0.001</u>
<u>Time × Heating</u>	<u>0.101</u>	<u>0.020</u>	<u>0.002</u>	<u><0.001</u>	<u><0.001</u>
<u>Time × Fish</u>	<u>0.049</u>	<u>0.135</u>	<u>0.002</u>	<u>0.006</u>	<u><0.001</u>
<u>Time × Heating × Fish</u>	<u>0.195</u>	<u>0.084</u>	<u>0.065</u>	<u>0.005</u>	<u>0.015</u>

<u>Effects</u>	<u>TN</u>	<u>TP</u>	<u>Chl-<i>a</i></u>
<u>Heating</u>	<u>0.905</u>	<u>0.008</u>	<u>0.002</u>
<u>Fish</u>	<u>0.067</u>	<u>0.012</u>	<u><0.001</u>
<u>Heating × Fish</u>	<u>0.126</u>	<u>0.009</u>	<u><0.001</u>
<u>Time</u>	<u><0.001</u>	<u><0.001</u>	<u>0.002</u>
<u>Time × Heating</u>	<u>0.263</u>	<u>0.001</u>	<u><0.001</u>
<u>Time × Fish</u>	<u>0.108</u>	<u>0.079</u>	<u><0.001</u>
<u>Time × Heating × Fish</u>	<u>0.362</u>	<u>0.038</u>	<u>0.002</u>

Notes: Values indicate probability levels; values in bold are below significance level
| (0.05).

Table 2. Summary of rANOVA results on the effect of different temperature (Heating) and planktivorous fish (Fish) scenarios on biomass ($\mu\text{g L}^{-1}$) of *Daphnia*, total zooplankton and food web metrics during the course of the experiment (Time).

Effects	<i>Daphnia</i>	Total	<i>Daphnia</i> %	Zoop.: Phyt	Chl : TP
Heating	0.001	0.001	≤ 0.001	0.033	0.0067
Fish	<0.001	0.001	<0.001	<0.001	<0.001
Heating $\times$ Fish	0.001	0.016	<0.001	0.004	0.002
Time	<0.001	<0.001	<0.001	<0.001	<0.001
Time $\times$ Heating	<0.001	0.03018	<0.0019	0.04206	<0.001
Time $\times$ Fish	<0.0021	<0.0021	<0.05701	<0.0010	<0.001
Time $\times$ Heating $\times$ Fish	0.4205	<0.0016	0.044228	0.05209	0.05543
<i>Notes:</i> Values indicate probability levels; values in bold are below significance level 0.05.					

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1 Fish-mediated plankton responses to increased temperature in subtropical aquatic mesocosm
2 ecosystems: implications for lake management

3

4 Hu He^{1, *}, Hui Jin², Erik Jeppesen^{3, 4}, Kuanyi Li^{1, 4}, Zhengwen Liu^{1, 4, 5}, Yongdong
5 Zhang¹

6

7 ¹ State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geog-
8 raphy and Limnology, Chinese Academy of Sciences, Nanjing, 210008, China

9 ² Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
10 Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

11 ³ Department of Bioscience, Aarhus University, Aarhus, Denmark

12 ⁴ Sino-Danish Centre for Education and Research, Beijing 100049, China

13 ⁵ Department of Ecology and Institute of Hydrobiology, Jinan University, Guangzhou
14 510632, China

15

16 *Corresponding author: Dr. Hu He

17 E-mail: hehu@niglas.ac.cn

18 Postal address: 73 East Beijing Road, Nanjing, 210008, China

19 Phone number: +86-025-86882237

20

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ABSTRACT

Although it is well established that climate warming can reinforce eutrophication in shallow lakes by altering top-down and bottom-up processes in the food web and biogeochemical cycling, recent studies in temperate zones have also shown that adverse effects of rising temperature are diminished in fishless systems. Whereas the removal of zooplanktivorous fish may be useful in attempts to mitigate eutrophication in temperate shallow lakes, it is uncertain whether similar mitigation might be achieved in warmer climates. We compared the responses of zooplankton and phytoplankton communities to climate warming in the presence and absence of fish (*Aristichthys nobilis*) in a 4-month mesocosm experiment at subtropical temperatures. We hypothesized that 1) fish and phytoplankton would benefit from warming, while zooplankton would suffer in fish-present mesocosms and 2) warming would favor zooplankton growth but reduce phytoplankton biomass in fish-absent mesocosms. Our results showed significant interacting effects of warming and fish presence on both phytoplankton and zooplankton. In mesocosms with fish, biomasses of fish and phytoplankton increased in heated treatments, while biomasses of *Daphnia* and total zooplankton declined. Warming reduced the proportion of large *Daphnia* in total zooplankton biomass, and reduced the zooplankton to phytoplankton biomass ratio, but increased the ratio of chlorophyll *a* to total phosphorus, indicating a relaxation of zooplankton grazing pressure on phytoplankton. Meanwhile, warming resulted in a 3-fold increase in TP concentrations in the mesocosms with fish present. The results suggest that cli-

mate warming has the potential to boost eutrophication in shallow lakes via both top-down (loss of herbivores) and bottom-up (elevated nutrient) effects. However, in the mesocosms without fish, there was no decline in large *Daphnia* or in total zooplankton biomass, supporting the conclusion that fish predation is the major driver of low large *Daphnia* abundance in warm lakes. In the fishless mesocosms, phytoplankton biomass and nutrient levels were not affected by temperature. Our study suggests that removing fish to mitigate warming effects on eutrophication may be potentially beneficial in subtropical lakes, though the rapid recruitment of fish in such lakes may present a challenge to success in the long-term.

Key words: climate warming; fish removal; eutrophication; top-down control; shallow lakes

INTRODUCTION

Global temperatures have been rising for decades and are predicted to rise by an additional 3–5°C over the next century (IPCC 2014). Ongoing climate warming is predicted to bring a range of undesirable changes in biodiversity, ecosystems and ecosystem services, including the reduced distribution of stenothermic species (Jeppesen et al., 2012), expansion of alien species (Walther et al., 2009), disruption of seasonal clockwork of organisms (Winder and Schindler, 2004), exacerbation of algal blooms and degradation water quality in freshwater ecosystems (Paerl and Huisman, 2008; Delpla et al., 2009; Kosten et al., 2012).

Eutrophication (a general term used to describe the suite of symptoms that a water body exhibits in response to nutrient over-enrichment) has been considered a primary problem for a large number of freshwaters (Smith and Schindler, 2009), and climate warming is predicted to reinforce the issues in affected aquatic ecosystems in a variety of direct and indirect ways (Paerl and Huisman, 2008; Jeppesen et al., 2010; Moss et al., 2011). For instance, warming can enhance the growth rate of an entire phytoplankton community by increasing the efficiency of nutrient use (De Senerpont Domis et al., 2013). Meanwhile, cyanobacteria with relatively high optimum niche temperatures may gain significant competitive advantage (Paerl and Huisman, 2008; Kosten et al., 2012). In shallow lakes, warming-induced increases in nutrient release from fertile sediments (Søndergaard et al., 2013) and in temperate lakes, external loading from catchments (Jeppesen et al., 2009) may boost plankton growth. Changes

in food web structures prompted by warming also favor phytoplankton growth in shallow lakes (Gyllström and Hansson, 2005; Meerhoff et al., 2012). For example, an increasing proportion of small, omnivorous fish in warmer lakes (Jeppesen et al., 2010; González-Bergonzoni et al., 2012) may reduce herbivorous zooplankton, and thereby release grazing pressure on phytoplankton (Gyllström and Hansson, 2005). Thus, from the perspective of lake management, a warmer future will demand alternative strategies for combating eutrophication.

Hansson et al. (2013) emphasized the importance of fish in top-down regulation of phytoplankton responses to climate warming in shallow aquatic ecosystems. They found that when fish were present in mesocosms maintained under warming conditions, phytoplankton biomass was significantly increased due to a release from zooplankton grazing pressure. In fish-free mesocosms, however, zooplankton herbivory increased under warming and pressured a significant reduction in phytoplankton. These results are supported by other mesocosm climate studies in the north temperate zone, e.g., Yvon-Durocher et al., (2011) and Velthuis et al., (2017) (fish absent), and Šorf et al., (2015) (fish present). Field investigations in 45 high altitude Tibetan lakes, although most of them were highly oligotrophic and varied in water depth and salinity, also shown that zooplankton to phytoplankton biomass ratios correlated negatively with temperature in lakes with zooplankton predators (included fish and the amphipod *Gammarus*), while the opposite pattern was observed in lakes without those predators (Lin et al., 2017). The interplay between warming and fish presence suggest that food

web manipulation, in the form of fish removal, is a practice worth considering in lake management in future warmer climates. There are, however, three concerns for applying this method to warm lakes. Firstly, previous mesocosms and field studies have all been conducted in cool regions such as northern Europe (Hansson et al., 2013; Šorfi et al., 2015) or at high altitude, as in Tibet (Lin et al., 2017) and it is known that trophic dynamics and metabolism change with temperature (Brown et al., 2004; Gyllström and Hansson, 2005). Meanwhile the evidence from subtropical and tropical shallow lakes remains needed. Secondly, since temperate climate mesocosms used in previous studies contained only small cladocerans (Hansson et al., 2013; Velthuis et al., 2017), further studies are necessary to examine the effects of rising temperature on larger grazers of phytoplankton such as *Daphnia* spp. Latitudinal comparisons have suggested that large-sized *Daphnia* will decline quickly when water temperatures exceed 20°C (Gillooly and Dodson, 2000), but recent studies in subtropical lakes revealed that *Daphnia* can maintain population size in temperatures exceeding 30°C in the absence of fish predation (Iglesias et al., 2011; Havens et al., 2015). It may be that, fish predation in warming scenarios is a more important structuring force on zooplankton than temperature itself (Meerhoff et al., 2012). Thirdly, several researchers have suggested that stocked species of filter-feeding fish such as silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), are useful in maintaining good water quality in subtropical and tropical shallow lakes, as they may filter phytoplankton (Starling, 1993; Xie and Liu, 2001; Ke et al., 2009; Guo et al.,

2015). Others have the complete opposite opinion, arguing that such species also filter zooplankton and disturb the sediment, resulting in further release of the nutrients that stimulate phytoplankton growth (Wang et al., 2008; Jeppesen et al., 2012b; Zhang et al., 2016). Others again argue that stocking with filter feeders will only be useful in highly eutrophic systems that naturally lack large cladoceran zooplankton (Radke & Kahl, 2002), while the effect would be negative in less productive lakes as predation would reduce zooplankton and boost small-sized algae (Radke and Kahl, 2002) or enhance nutrient levels by sediment disturbance (Zhang et al., 2016). These mixed opinions present an overall rather ambiguous picture regarding the likely effects of filter-feeding fish.

In this study, mesocosms operating at a subtropical temperature setting but within the thermal tolerance of *Daphnia* were used to compare the responses of zooplankton, phytoplankton and nutrients to warming in fish-present and fish-absent scenarios. We hypothesized that under subtropical conditions: (1) fish and phytoplankton in fish-present mesocosms would benefit from warming, while zooplankton (especially *Daphnia* spp.) would suffer; and (2) warming would favor zooplankton growth and enhance herbivory in mesocosms without fish, leading to reduced phytoplankton biomass.

MATERIALS AND METHODS

Experimental design

The outdoor mesocosm experiment was conducted from 7 March (day 67 in Year 2016) to 3 July 2016 (day 184 in Year 2016) at Taihu Laboratory for Lake Ecosystem Research (31°25'3"N, 120°13'13"E), located in Meiliang Bay, on the northern edge of Lake Taihu, China. The selected period represents the time when phytoplankton abundance is controlled to some extent by zooplankton grazing (Chen et al., 2011). The mesocosms consisted of twelve 400 L fiberglass-reinforced plastic tanks (90-cm high; 80-cm inside diameter at the top; 70-cm inside diameter at the base). All mesocosms were filled with 370 L lake water with a 10-cm layer of pre-sieved sediment (1.7 mm mesh size). The water was screened (64- μ m mesh size) to remove crustacean zooplankton and large inorganic particles, and mixed to ensure homogeneity before being added to the tanks. All mesocosms were open during the experimental period and suspended in a concrete pond (7 m long $\times$ 6 m wide $\times$ 2 m deep) filled with lake water. The concrete pond served as a water bath used to reduce the daily temperature fluctuation.

A two-way factorial experiment was carried out under two temperature settings (ambient and warming) and two fish levels (fish present/absent). Each treatment had three replicates, making a total of 12 experimental units. In the unheated ambient scenario, the temperature of the tank water was allowed to equilibrate with that in the concrete pond, which ranged between 8 °C and 30 °C (Fig. 1). In the warming scenario, temperatures were maintained consistently at 3°C warmer than the pond water (Fig. 1) using a customized intelligent temperature-control device with two 200-W heating

rods. Real time temperatures in the heated and unheated tanks were measured/logged automatically every ten minutes.

The fish used in the experiment were bighead carp (*A. nobilis*), a common filter-feeding fish in Lake Taihu and other shallow lakes. Carp with an average length of 9 ± 0.4 cm and mean wet weight of 7.9 ± 0.6 g was obtained from a local aquaculture facility and acclimatized in lake water for a week before the experiment began. Six mesocosms were chosen to receive fishes, with one fish per tank, amounting to approximated 208 kg ha^{-1} , which is comparable to the biomass observed naturally in Lake Taihu (217 kg ha^{-1} in Year 2015). After fish were added to their assigned mesocosm, the experimental temperature settings were maintained uninterrupted until the end of the experiment.

Nutrients ($5 \text{ } \mu\text{g P L}^{-1} \text{ d}^{-1}$; $130 \text{ } \mu\text{g N L}^{-1} \text{ d}^{-1}$) were supplied daily to each microcosm throughout the experiment. These loadings were slightly higher than the annual average for external loading in Lake Taihu but do occur in peak seasons (Paerl et al., 2011). Phosphorus and nitrogen were added separately as aqueous solutions of potassium dihydrogen phosphate (KH_2PO_4) and potassium nitrate (KNO_3), the dominant inorganic nutrient forms in Lake Taihu. The enclosure walls were scrubbed with banister brushes every evening to minimize the growth of periphytic algae.

Sampling and processing

Water chemicals and chlorophyll *a*

Sampling for monitoring of nutrients and chlorophyll *a* levels was collected at 5-day intervals during the spring months (day 67 to day 154), and at 10-day interval in the last month of the study, as it ran into summer (day 154 to day 184). A tube sampler (8 cm diameter, 64 cm length) was used to collect depth-integrated water samples. In order to reach a total volume of 12 litres, the sampler was deployed four times in each mesocosm on each sampling occasion, twice at depth (around 5 cm above sediment to minimise disturbance), and twice from the surface down. These four samples were then well mixed. In the laboratory, total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were measured according to Chinese standard methods (Jin and Tu 1990). Chl-*a* concentrations were measured spectrophotometrically from matter retained on a GF/C filter and extracted in a 90% (v/v) acetone/water solution for 24 h. No correction was carried out for pheophytin interference (SEPA 2002).

Phytoplankton community

At each 10-day sampling interval, a 1-L depth-integrated subsample taken from each mesocosm was treated with 10 mL Lugol's iodine solution and sedimented for 48 h. The supernatant was removed, and the residue was collected and examined under $\times 100 - \times 400$ magnification for enumeration of phytoplankton. Phytoplankton was identified to genus level according to Hu (2006) and following recent taxonomic revisions as far as possible (Guiry and Guiry 2014). The biomass of common phytoplankton taxa was calculated based on cell size measurements of at least 30 cells of each

taxon and using formulae for geometric shapes approximating cell forms (Zhang and Huang 1991). Where possible, at least 1000 cells of each taxon were counted per sample, but for less common taxa, calculations were based on fewer measurements. Phytoplankton biovolume was multiplied by 0.29 to obtain an approximation of dry weight (Reynolds 1984).

Zooplankton community

Zooplankton were sampled at 10-day intervals in the first (March) and last (June) months of the experiment, and at 5-day intervals during April and May, when large populations of *Daphnia* develop quickly (Chen et al., 2011). At each sampling, a 10-L depth-integrated water sample was filtered through a 64- μ m net and the retained material was preserved in 4% formaldehyde. Crustacean zooplankton (cladocerans, copepodite and adult copepods) were counted at magnifications between $\times 10$ and $\times 40$. Species represented by more than 100 individuals were deemed to be dominant and were identified according to Chiang and Du (1979) and Shen and Du (1979). Copepod nauplii were counted without further taxonomic distinction. Zooplankton biomass (dry weight) was estimated using equations from Dumont et al. (1975) and Huang (1999). Where possible, up to 20 individuals of each taxon were measured. Rotifers were counted directly from phytoplankton samples at $\times 100$ magnification (at least 100 individuals of the most abundant taxa), and biomass of rotifers was calculated using standard dry weights obtained from multiple lakes in the middle and lower Yangtze river basin.

223 Fish

224 At the end of the experiment (day 184), fish were collected from the fish mesocosms
225 (6 tanks) and their final length and body weight were measured.

226 **Data analysis**

227 Nutrient and plankton dynamics

228 The time series data (including values for nutrients, zooplankton and phytoplankton
229 biomass) were statistically tested for effect of treatment, time and their interaction by
230 repeated-measurements ANOVA (rANOVA). Prior to analysis, data were $\log_{10}x$ or
231 $\log_{10}(x+1)$ (if a value of zero existed) transformed to meet assumptions of normality
232 or homogeneity of variance. If the assumption of sphericity of the variance–
233 covariance matrices of the rANOVA analyses was violated, the degrees of freedom
234 (df) were Huyn-Feldt corrected, resulting in an adjustment of the significance of the F
235 ratio (Huyn and Feldt 1976). In the event of significant interaction terms, a post-hoc
236 pairwise comparison was performed (Holm-Sidak method) to determine where the
237 differences occurred, using fish as a categorical factor and temperature as a quantita-
238 tive factor.

239 Top-down control

240 To compare warming-induced differences in top-down pressure between con-
241 trasting fish treatments, we computed three biological metrics indicative of food web
242 interactions (Jeppesen et al., 2003; Jackson et al., 2007). The proportion of large-sized
243 cladocerans (species sensitive to fish grazing) within the total zooplankton biomass

was used to reflect the predation pressure exerted on zooplankton by fish. Ratios of zooplankton to phytoplankton biomass and chlorophyll *a* to TP were used to reflect grazing pressure of zooplankton on phytoplankton (Jeppesen et al., 2003). The effects of warming and fish presence on the three metrics were also compared using rANOVA, pre-treated as above. Student's *t* tests were used to reveal the effects of warming on fish body length and weight. All comparisons were conducted using the statistical package SPSS, version 16.0 (IBM Corporation, Somers, NY, USA).

RESULTS

Nutrients

No significant effect of warming or fish presence was apparent on TN and no interaction was detected (Table 1). In both fish-present and fish-absent scenarios, TN showed a steady decline from day 67 to day 83. After that, TN values changed unimodally with time, peaking at day 124 in the fish-present mesocosms (Fig. 2a) and at day 129 in fish-absent treatments (Fig. 2b).

Warming and fish presence significantly increased TP concentrations, and their interaction was significant (Table 1). In fish-present, unheated treatments, TP concentrations did not alter significantly with time, while in heated mesocosms with fish present, TP increased sharply from day 114, reaching a final value three times higher than that of the unheated controls (Fig. 2c; Holm-Sidak method, $F = 25.08$, $P = 0.001$). In fish-absent mesocosms, TP concentrations in both unheated and heated mesocosms

fluctuated slightly throughout the experiment (Fig. 2d) and did not differ significantly (Holm-Sidak method, $F = 0.018$, $P = 0.898$).

No interactive effects of warming and fish presence were observed for TDN and TDP concentrations (Table 1). The TDN in both fish-present and fish-absent regimes was, however, significantly lower in heated mesocosms than in unheated ones (Fig. 2e-f; Table 1). Warming did not obviously affect TDP concentrations, whilst the fish effects were significant, with slightly lower TDP concentrations in fish-present mesocosms than in fish-absent ones (Fig. 2g-h; Table 1).

Phytoplankton

The phytoplankton community followed a succession from euglenophyte dominated (mainly *Euglena* spp.) to diatom dominated (mainly *Synedra* spp.), and finally to green alga dominated (mainly *Schroederia*, *Scenedesmus* and *Arthrodesmus*) in all mesocosms, except for the heated fish-present treatment, where cyanobacteria (*Phormidium* spp.) dominated the community at the end of experiment. However, rANOVA showed no significant effect of fish and warming on either percentage or biomass of phytoplankton at phylum level.

The effects of time, warming and fish, and their interactions on Chl-*a* concentration and phytoplankton biomass were all significant (Fig. 3a-d, Table 1). With fish present, Chl-*a* concentrations in heated mesocosms increased sharply from day 109, and were 10 times higher than in unheated mesocosms at the end of experiment (Fig. 3a; Holm-Sidak method, $F = 56.212$, $P = 0.000$). Similarly, phytoplankton biomass in heated

mesocosms was significantly higher than in unheated mesocosms (Fig. 3c; Holm-Sidak method, $F = 11.831$, $P = 0.009$). By contrast, in the fish-absent regime, Chl-*a* concentrations (Fig. 3b) and phytoplankton biomass (Fig. 3d) remained mostly low in both temperature treatments and no significant difference was observed (Holm-Sidak method, $F = 0.347$, $P = 0.347$ for Chl-*a*; $F = 0.000$, $P = 0.999$ for phytoplankton biomass).

Zooplankton

A total of twenty-two zooplankton taxa were recorded in all treatments across the entire experimental period, including five cladoceran genera (*Daphnia*, *Bosmina*, *Chydorus*, *Alona* and *Spapholeberis*), two copepods (*Mesocyclops leuckarti* and *Sinocalanus dorrii*) and 15 rotifers (dominated by *Brachionus angularis*, *Keratella cochlearis*, *Polyarthra trigla* and *Filinia longistea*). At the end of experiment, *Daphnia* were dominant in all mesocosms except for the fish plus warming treatments, where small cladocerans (*Spapholeberis* spp.) and copepods prevailed.

Warming and fish presence had a significant interactive effect on *Daphnia* biomass (Table 2). In fish-present regimes, *Daphnia* changed unimodally over time and peaked at day 109 in both heated and unheated mesocosms (Fig. 4a). After that, *Daphnia* declined in both temperature scenarios but the magnitude of decline was significantly greater in heated mesocosms than in unheated ones (Fig. 4a), leading to a significant warming-induced reduction in *Daphnia* biomass by the end of the experiment (Holm-Sidak method, $F = 65.789$, $P = 0.000$; Fig. 4a). In fish-absent regimes, no

significant effect of warming on *Daphnia* was apparent (Holm-Sidak method, $F = 0.017$, $P = 0.900$; Fig. 4b), and *Daphnia* biomass in both temperature treatments remained high from day 109 to the end of experiment (Fig. 4b).

Warming and fish had significant interactive effects on total zooplankton (Table 2). In the fish-present regimes, warming significantly reduced total zooplankton biomass (Holm-Sidak method, $F = 32.066$, $P = 0.000$; Fig. 4c), while in the fish-absent regimes warming effects on total biomasses were not significant (Holm-Sidak method, $F = 1.817$, $P = 0.215$; Fig. 4d).

Top-down control

In the fish-present regimes, both lengths and body weights of fish at the end of experiment were higher in heated mesocosms than in unheated mesocosms (t-test, $P = 0.031$ for body length; $P = 0.003$ for weight; Fig. 5).

Warming and fish had significant interactions on the percentage of *Daphnia* in total zooplankton biomass (Table 2). In fish-present treatments, warming significantly reduced the percentage *Daphnia* biomass (Holm-Sidak method, $F = 75.815$, $P = 0.000$; Fig. 6a), while in the absence of fish no significant difference in *Daphnia* biomass percentage was detected between unheated and heated mesocosms (Holm-Sidak method, $F = 3.356$, $P = 0.104$; Fig. 6b).

Significant interactions were detected between warming and fish on the ratios of zooplankton to phytoplankton biomass (Zoop : Phyt) and chlorophyll *a* to TP ratio (Chl : TP) (Table 2). In fish-present regimes, warming significantly reduced both

Zoop : Phyt (Holm-Sidak method, $F = 21.467$, $P = 0.002$; Fig. 6c) and Chl : TP (Holm-Sidak method, $F = 41.554$, $P = 0.000$; Fig. 6e), while in fish-absent regimes, neither metric was found to differ significantly between unheated and heated treatments (Holm-Sidak method, $F = 0.967$, $P = 0.354$ for Zoop : Phyt; Fig. 6d; $F = 0.409$, $P = 0.543$ for Chl : TP; Fig. 6f).

DISCUSSION

Having tested the interactive effects of warming and fish on plankton dynamics and the strength of top-down effects under subtropical conditions, we will evaluate each of our predictions below.

Prediction 1: In fish-present mesocosms, phytoplankton would benefit from warming, while zooplankton (especially large Daphnia) would suffer.

This prediction is fully supported. In our fish-present mesocosms, phytoplankton biomass and fish weight were both higher in heated mesocosms than in unheated controls (Fig. 3a; 3c; Fig. 5), while biomasses of both large-sized *Daphnia* and total zooplankton were reduced by warming (Fig. 4a; 4c). Our results echo previous climate mesocosm (fish-present) experiments carried out in colder regions (Hansson et al., 2013; Šorš et al., 2015), and support the conclusion that climate warming will alter the food web structure of shallow lakes in favor of phytoplankton growth (Jeppesen et al., 2009, 2010).

Zooplankton losses in heated mesocosms were largely due to the enhanced top-down control exerted by fish, as evidenced by the reduced proportion of *Daphnia* amongst zooplankton (Fig. 6a) and rapid fish growth (Fig. 5). Moreover, changes in zooplankton community structure under warming, such as the rise to dominance of small cladocerans and copepods in heated mesocosms while *Daphnia* prevailed in unheated treatments (data not shown), also corresponded well with the effects of fish predation on zooplankton assemblages (Vakkilainen et al., 2004). Thermal stress could not explain the loss of large *Daphnia* and total zooplankton in heated mesocosms (Fig. 4a), as these species maintained their population sizes in heated mesocosms without fish (Fig. 4b). Our studies therefore support the conclusion that fish predation, rather than temperature change, is the major driver of large *Daphnia* decline in subtropical lakes (Iglesias et al., 2011; Havens et al., 2015).

In fish-present regimes, the significantly reduced Zoop : Phyt and elevated Chl : TP ratios observed in heated mesocosms emphasized the importance of top-down pressure in regulating phytoplankton biomass (Fig. 6c; 7e). However, our results also indicate enhanced bottom-up effects under warming scenarios, with significant increases in TP concentrations in heated mesocosms (Fig. 2c). Interestingly, although the importance of temperature on internal P loading in shallow lakes has been long recognized (Jensen and Andersen, 1992), definite evidence has not been recorded in temperate climate mesocosm studies before (Hansson et al., 2013; Özen et al., 2013). In fish-present mesocosms, TDP concentrations were not enhanced by temperature

increase as seen for TP (Fig. 2g), maybe as a result of the greater sequestering of P by phytoplankton under warmer temperatures. A pattern whereby Chl yields to TP increase with decline in contributions of TDP to TP has been commonly found in mesoeutrophic to hypereutrophic lakes (Mazumder et al., 1994). Our study, based on a subtropical temperature scenario, demonstrates that climate warming has the potential to boost eutrophication in shallow lakes via both top-down (herbivores loss) and bottom-up (TP elevation) effects. Moreover, with respect to the debate on the impact of filter-feeding fishes on eutrophication in subtropical and tropical shallow lakes, our results, e.g., the high Chl-*a* and TP concentrations in mesocosms with fish (Fig. 2c-d; 3a-b), support the view bighead carp have a exacerbating effect on eutrophication in low productivity warm lakes, as they reduce zooplankton (Fig. 4a-b) and enhance phytoplankton growth (Fig. 3a-b) and nutrient levels (Fig. 2c-d).

Prediction 2: In fish-absent mesocosms, warming would favor zooplankton growth and enhanced herbivory and thereby reduce phytoplankton biomass

This hypothesis is not supported, as no significant difference was observed between unheated and heated fishless treatments in terms of either phytoplankton or zooplankton biomass (Fig. 3b; 3d; Fig. 4d). However, our study did support earlier indications from temperate regions that fish removal might be a useful means of mitigating the effects of warming on eutrophic shallow lakes (Hansson et al., 2013), as our fish-free heated mesocosms maintained low Chl-*a* until the end of experiment (Fig. 3b).

In fish-absent regimes, warming did not increase zooplankton (*Daphnia* spp. dominated) biomass as it did in similar climate mesocosms of Hansson et al. (2013), where small cladoceran genera such as *Bosmina* and *Chydorus* dominated. Population development for the large *Daphnia* present in our mesocosms may have been constrained by low food availability, as the average water Chl-*a* concentration only exceeded 10 µg L⁻¹ on 6 sampling occasions (36 in total) (Fig. 3b). A similar trade-off between food and temperature in *Daphnia* growth has previously been recorded in mesocosm and laboratory studies of unproductive ecosystems. For instance, Strecker et al., (2004) found that experimental warming (+3.6°C) significantly reduced abundance of *Daphnia pulex* in fishless oligotrophic mesocosms, while Heugens et al., (2016) found that the positive effect of temperature on population growth of *Daphnia magna* was diminished under limited food constraints.

In a further contrast to the results of the temperate climate studies of Hansson et al., (2013) and Velthuis et al., (2017), Chl-*a* concentration and phytoplankton biomass were not reduced by warming in the fish-absent mesocosms (Fig. 3b; 3d) and Zoop : Phyt and Chl : TP ratios remained unchanged (Fig. 6d; 7f), suggesting that top-down control of phytoplankton by zooplankton was not strengthened by warming. Furthermore, in the absence of fish, water TP and TDP concentrations did not increase with rising temperature (Fig. 2c-d), indicating no stimulation of phytoplankton growth by warming. A similar pattern was found in the warming experiment of Velthuis et al., (2017) who attributed the low and unchanged phosphorus levels between heated and

unheated mesocosms to the development of periphyton, benthic algae and filamentous algae in their unproductive, fishless mesocosms. This may also be the case for our fishless mesocosms. High water transparency owing to the facts that lacking sediment disturbance by fish and low Chl-*a* concentrations (Fig. 3b) may have promoted the growth of benthic algae, thereby immobilizing nutrients within sediments in fishless mesocosms (Zhang et al., 2013). Unfortunately, direct evidence of the abundance of e.g., benthic algae and periphyton, are lacking in our experiment, but would be well worth pursuing future.

Implications for lake management in future warmer climate

IPCC (2014) predicts that air temperatures will likely increase by 1-4°C (relative to 1986-2005) in many parts of the world in this century. Under this scenario, fish communities are expected to develop structures that favor phytoplankton growth via cascading effects (Jeppesen et al., 2010), amplifying the existing eutrophication problems in shallow lakes (Jeppesen et al., 2009, Moss et al., 2011). Our study showed a significant interplay between the effect of temperature and fish on lake plankton communities under subtropical temperature conditions. In fish-present systems, warming benefited phytoplankton growth via a reduction in large *Daphnia* (Fig. 4a) and elevated TP levels (Fig. 2c) are likely as result of increased release from the sediment. However, when fish were absent from these systems, abundances of large *Daphnia* were maintained (Fig. 4b), and warming effects on both top-down and bottom-up processes were diminished under the given nutrient scenario. From the perspective of lake man-

agement, our findings suggest that removal of fish may be an effective means of mitigating warming effects on eutrophication in subtropical lakes (as in temperate lakes), though the rapid recruitment of fish in warm lakes may present a challenge to the success of such measures in the long-term (Jeppesen et al., 2012b).

CONCLUSION

Our study showed significant interactive effects between warming and fish presence on plankton dynamics and nutrient levels in subtropical aquatic mesocosms. In mesocosms with fish, biomasses of fish and phytoplankton increased in heated treatments, while biomasses of *Daphnia* and total zooplankton declined. Meanwhile, warming resulted in a 3-fold increase in TP concentrations. The results suggest that climate warming has the potential to boost eutrophication in subtropical shallow lakes via both top-down (loss of herbivores) and bottom-up (elevated nutrient) effects. However, in the mesocosms without fish, no decline in large *Daphnia* or in total zooplankton biomass was observed in heated mesocosms, and phytoplankton biomass and nutrient levels were not affected by warming. From a lake management perspective, our results emphasize that removing fish will be beneficial in mitigating ongoing warming effects on eutrophication in subtropical shallow lakes, though the rapid recruitment of fish in warm lakes may present a challenge.

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Table 1. Summary of rANOVA results on the effects of different temperature (Heating) and planktivorous fish (Fish) scenarios on the concentrations (mg L⁻¹) of total nitrogen (TN) and total phosphorus (TP), and on phytoplankton biomass as indicated by chlorophyll *a* (Chl-*a*: µg L⁻¹) concentration during the course of the experiment (Time).

Effects	TN	TP	TDN	TDP	Chl- <i>a</i>
Heating	0.877	0.009	0.006	0.856	0.002
Fish	0.093	0.012	0.176	<0.001	<0.001
Heating × Fish	0.226	0.010	0.077	0.137	<0.001
Time	<0.001	0.001	<0.001	<0.001	<0.001
Time × Heating	0.101	0.020	0.002	<0.001	<0.001
Time × Fish	0.049	0.135	0.002	0.006	<0.001
Time × Heating × Fish	0.195	0.084	0.065	0.005	0.015

Notes: Values indicate probability levels; values in bold are below significance level (0.05).

Table 2. Summary of rANOVA results on the effect of different temperature (Heating) and planktivorous fish (Fish) scenarios on biomass ($\mu\text{g L}^{-1}$) of *Daphnia*, total zooplankton and food web metrics during the course of the experiment (Time).

Effects	<i>Daphnia</i>	Total	<i>Daphnia</i> %	Zoop : Phyt	Chl : TP
Heating	0.001	0.001	<0.001	0.033	0.006
Fish	<0.001	0.001	<0.001	<0.001	<0.001
Heating $\times$ Fish	0.001	0.016	<0.001	0.004	0.002
Time	<0.001	<0.001	<0.001	<0.001	<0.001
Time $\times$ Heating	0.001	0.038	0.019	0.042	0.001
Time $\times$ Fish	0.002	0.002	0.057	0.010	0.001
Time $\times$ Heating $\times$ Fish	0.205	0.016	0.228	0.052	0.055

Notes: Values indicate probability levels; values in bold are below significance level 0.05.

Table S1. Detail information of rANOVA results on the effects of different temperature (Heating) and planktivorous fish (Fish) scenarios on nutrient and Chlorophyll *a* concentrations, *Daphnia* and total zooplankton biomass, as well as top-down metrics during the course of the experiment (Time)

Variables	Effects	Statistical parameters			
		F	DF1	DF2	<i>P</i>
TN	Heating	0.026	1	8	0.877
	Fish	3.629	1	8	0.093
	Heating × Fish	1.72	1	8	0.226
	Time	69.472	5.503	44.026	<0.001
	Time × Heating	2.421	5.503	44.026	0.046
	Time × Fish	3.215	5.503	44.026	0.012
	Time × Heating × Fish	1.734	5.503	44.026	0.141
TP	Heating	13.216	1	8	0.009
	Fish	10.605	1	8	0.012
	Heating × Fish	11.886	1	8	0.010
	Time	6.633	7.622	60.977	<0.001
	Time × Heating	3.947	7.622	60.977	0.001
	Time × Fish	1.862	7.622	60.977	0.086
	Time × Heating × Fish	2.17	7.622	60.977	0.045
TDN	Heating	13.73	1	8	0.006
	Fish	2.203	1	8	0.176
	Heating × Fish	4.111	1	8	0.077
	Time	74.835	7.029	56.234	<0.001
	Time × Heating	6.424	7.029	56.234	<0.001
	Time × Fish	6.365	7.029	56.234	<0.001
	Time × Heating × Fish	2.719	7.029	56.234	0.017
TDP	Heating	0.035	1	8	0.856
	Fish	46.746	1	8	<0.001
	Heating × Fish	2.725	1	8	0.137
	Time	42.358	8.229	65.831	<0.001
	Time × Heating	8.468	8.229	65.831	<0.001
	Time × Fish	4.878	8.229	65.831	<0.001
	Time × Heating × Fish	5.034	8.229	65.831	<0.001
Chl- <i>a</i>	Heating	21.067	1	8	0.002
	Fish	164.651	1	8	<0.001
	Heating × Fish	34.949	1	8	<0.001
	Time	19.115	7.97	63.762	<0.001
	Time × Heating	8.498	7.97	63.762	<0.001
	Time × Fish	8.212	7.97	63.762	<0.001
	Time × Heating × Fish	4.032	7.97	63.762	0.015
<i>Daphnia</i>	Heating	30.693	1	8	0.001
	Fish	88.214	1	8	<0.001

Total zoop	Heating × Fish	29.234	1	8	0.001
	Time	32.364	9.95	79.602	<0.001
	Time × Heating	6.601	9.95	79.602	0.001
	Time × Fish	5.742	9.95	79.602	0.002
	Time × Heating × Fish	1.592	9.95	79.602	0.205
	Heating	24.574	1	8	0.001
	Fish	31.399	1	8	0.001
	Heating × Fish	9.308	1	8	0.016
	Time	21.976	16.845	131.88	<0.001
	Time × Heating	2.702	16.845	131.88	0.038
<i>Daphnia</i> %	Time × Fish	5.002	16.845	131.88	0.002
	Time × Heating × Fish	3.287	16.845	131.88	0.016
	Heating	44.346	1	8	<0.001
	Fish	160.79	1	8	<0.001
	Heating × Fish	36.704	1	8	<0.001
	Time	19.215	9.268	74.147	<0.001
	Time × Heating	3.631	9.268	74.147	0.001
	Time × Fish	2.672	9.268	74.147	0.009
	Time × Heating × Fish	1.515	9.268	74.147	0.157
Zoop : Phyt	Heating	6.66	1	8	0.033
	Fish	70.452	1	8	<0.001
	Heating × Fish	15.773	1	8	0.004
	Time	16.608	8.076	64.608	<0.001
	Time × Heating	3.044	8.076	64.608	0.006
	Time × Fish	4.473	8.076	64.608	<0.001
	Time × Heating × Fish	2.838	8.076	64.608	0.009
Chl : TP	Heating	14.638	1	8	0.006
	Fish	183.236	1	8	<0.001
	Heating × Fish	22.797	1	8	0.002
	Time	19.764	8.645	60.516	<0.001
	Time × Heating	7.439	8.645	60.516	0.001
	Time × Fish	7.934	8.645	60.516	0.001
	Time × Heating × Fish	2.903	8.645	60.516	0.055

DF1, DF2, degree of freedom of numerator and denominator, respectively

Fig. 1. Time series of temperature development (°C) in both temperature treatments.

Unheated = surface water temperature of ponds, Heated = +3 °C increase above unheated temperature.

Fig. 2. Time series of nutrient concentrations in both fish scenarios, with TN (a-b), and TP (c-d). Values represent mean $\pm$ SE.

Fig. 3. Time-series development of chlorophyll *a* concentrations (*a-b*) and phytoplankton biomass (*c-d*) in both fish scenarios. Values represent mean $\pm$ SE.

Fig. 4. Time-series development of biomass of *Daphnia* spp. (a-b) and total zooplankton (c-d) in fish-present (left panel) and fish-absent (right panel) treatments. Values represent mean $\pm$ SE.

Fig. 5. Fish body length and weight at the end of experiment in both temperature scenarios. Values represent mean $\pm$ SE.

Fig. 6. Time-series development of food web metrics in both fish scenarios, with *Daphnia* biomass percentage (*Daphnia* %; *a-b*), zooplankton to phytoplankton ratio (Zoop : Phyt; *c-d*) and Chl-*a* to TP ratio (Chl : TP; *e-f*). Values represent mean $\pm$ SE.