The host mussel *Sinanodonta woodiana* alleviates negative effects of a small omnivorous fish (*Acheilognathus macropterus*) on water quality: A mesocosm experiment

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Abstract: Omnivorous fishes are prevalent in warm waters and may have strong impacts on water quality by excreting nutrients and reducing periphyton biomass. However, most studies have focused on large-sized species and overlooked the role of small omnivores. Filter-feeding mussels may modulate the negative effects of small omnivorous fishes on water quality, and stocking of mussels has been frequently used in shallow eutrophic freshwaters in China to improve the water clarity. However, the mechanisms behind such management practices are poorly studied. We conducted a mesocosm experiment to examine the ecosystem effects of the bitterling *Acheilognathus macropterus* as modulated by the mussel *Sinanodonta woodiana*, one of the mussels upon which it relies for breeding. We hypothesized that bitterling would exert negative effects on the lake environment, specifically higher phytoplankton biomass and lower water clarity, but that these effects might be alleviated by the filter-feeding activities of *S. woodiana*. In a 56-d mesocosm experiment with and without bitterling in the presence and absence of mussels, we found interactive effects of bitterling and mussels. In mesocosms with bitterling, nutrient concentrations, phytoplankton biomass, and total suspended solids (TSS) increased, but there were no changes in periphyton biomass in the mussel-free treatments. In contrast to the effects of large-sized omnivorous fishes reported from the literature, bitterling mainly affected TSS levels by increasing organic suspended solids rather than inorganic solids, indicating weak effects on sediment resuspension. However, the presence of mussels alleviated the negative effects of bitterling by decreasing nutrient levels, phytoplankton biomass, and TSS concentrations. Mussels alone had no effects on periphyton biomass, but the mussel–bitterling interactions boosted the growth of periphyton. Our study suggests that the negative effects of bitterling on water quality (e.g., increased nutrient concentrations and phytoplankton biomass) are alleviated by the presence of filter-feeding mussels, but the stimulatory interactive effects of mussels and bitterling on periphyton may impair the recovery of submerged macrophytes.

Keywords: lake restoration, omnivore, mussel stocking, pelagic–benthic coupling, periphyton
Omnivorous fishes are prevalent in freshwaters and their proportion in fish assemblages increases from high to low latitudes (Meerhoff et al. 2007, Moss 2010, González-Bergonzoni et al. 2012). Because they feed on at least 2 trophic levels, omnivorous fishes may affect lake ecosystems through various pathways. The presence of omnivorous fishes can increase nutrient concentrations, turbidity (total suspended solids [TSS], especially inorganic suspended solids [ISS]), and the biomass of both phytoplankton (Drenner et al. 1998, Gu et al. 2016, Zhang et al. 2017, He et al. 2019) and periphyton (Gu et al. 2016), thereby suppressing the growth of submerged macrophytes (Miller and Crowl 2006, Qiu et al. 2019). In a study including both the omnivorous Common Carp (Cyprinus carpio Linnaeus, 1758) and the omni-planktivorous filter-feeding Silver Carp (Hypophthalmichthys molitrix Valenciennes, 1844), Zhang et al. (2016) found that Common Carp substantially increased the biomass of pelagic algae but decreased the biomass of benthic algae (periphyton), whereas Silver Carp did not influence the pelagic or benthic algal biomass. In experiments manipulating fish assemblage composition in Chinese subtropical and tropical shallow eutrophic lakes (e.g., Lake Wuli and Huizhou West Lake, China), re-oligotrophication was observed after removal of omnivorous fishes combined with macrophyte transplantation (Yu et al. 2016a, b, Liu et al. 2018). Fish biomass quickly returned to its pre-restoration levels in Huizhou West Lake (Gao et al. 2014). In Lake Wuli, the new assemblage was dominated by small omnivorous fishes (77% by abundance and 43% by biomass of the fish assemblages; Yu et al. 2016a). These small omnivores feed mainly on submerged macrophytes (Yu et al. 2016a), which may inhibit macrophyte growth, potentially shifting the lake back to a turbid water state in the long term. However, most studies to date have focused on large fish species and have not documented the role of small omnivores. This lack of data is unfortunate as the effects of small omnivorous fishes on shallow lake ecosystems may differ from those of large species.

Like large-sized omnivores, bitterling and other small omnivorous fishes have the potential to influence water quality. Bitterling, comprising fishes from the Rhodius and Acheilognathus genera and belonging to the subfamily Acheilognathinae of the family Cyprinidae, are small-bodied fishes (maximum body length ~11 cm; Froese and Pauly 2019) with high diet plasticity (Koutrakis et al. 2003). Previous research conducted in reed belts of Lake Kitaura, Japan, indicated that 2 species of bitterling (Rhodius ocellatus Kner, 1866 and Acheilognathus macropertus Bleeker, 1871) consumed mainly diatoms, filamentous algae, and decomposing reeds (Usui et al. 2018), whereas in shallow, macrophyte-dominated Lake Wuli, both small- and large-sized A. macropertus fed mainly on submerged macrophytes (Yu et al. 2016a). There is evidence that zooplankton are important food for bitterling (Yu et al. 2016a), yet Usui et al. (2018) found that zooplankton made up only a small proportion of the diet. These differing results indicate that the diet composition of bitterling may depend on food availability. Thus, bitterling have the potential to influence water quality through both top-down (zooplankton consumption) and bottom-up forces (mobilization of benthic nutrients and consumption of benthic primary producers, such as periphyton and submerged macrophytes). However, the impacts of bitterling on important lake-ecosystem parameters (e.g., nutrient concentrations, biomass of pelagic and benthic primary producers) are poorly documented.

Bitterling are totally reliant on freshwater mussels of the genera Unio and Anodonta for spawning, as the female fish oviposit inside the mussel branchial cavity (Reynolds et al. 1997, Smith et al. 2000, 2004). Fertilization takes place in the gills of the mussel (Aldridge 1999), where a few large elliptical eggs are produced (Blaxter 1969). After completing embryonic development inside the mussel, the juvenile bitterling actively swim out of their host. Bitterling–mussel interactions have been intensively studied from the perspective of co-evolution (Thompson 1999, Mills and Reynolds 2003, Reichard et al. 2007, 2010, 2015) and with respect to the species and size preference of breeding substrates (Smith et al. 2000, 2001, Przybylski et al. 2007, Wang et al. 2011). However, the effects of the bitterling–mussel system on aquatic ecosystems are poorly understood.

Filter-feeding mussels improve water clarity by reducing phytoplankton biomass (He et al. 2014, Zhang et al. 2014, Wu et al. 2018), consequently promoting the development of macrophytes and benthic primary production (He et al. 2014, Zhang et al. 2014). In recent years, mussel stocking has been considered a useful supplementary tool in the restoration of eutrophic shallow freshwaters in China (Chen et al. 2009, Qin 2013, Gao et al. 2017, Ke et al. 2019). Indeed, mussels (e.g., Cristaria plicata Leach, 1814; Sinanodonta woodiana Lea, 1834; and Nodularia douglasiae Griffith and Pidgeon, 1833) have been used in lake restoration projects in China (Chen et al. 2009, Gao et al. 2017, Ke et al. 2019) where they also act as important breeding substrate for bitterling (Rhodeus sinensis Günther, 1868) (Shen 2000). However, the stocked mussels may hold bitterling embryos and can thereby increase bitterling populations, both by transferring embryos and by offering extra spawning substrate for existing bitterling populations (Shen 2000). It is not known if the balance of the effects of the bitterling–mussel system is positive or negative for water quality. For example, water clarity may decrease if bitterling predation on zooplankton or mobilization of nutrients from the sediment leads to higher phytoplankton biomass. However, if mussel filtration of phytoplankton outweighs these effects of bitterling, phytoplankton biomass will decrease and water clarity will increase.

We conducted a mesocosm experiment to separate the dual effects of bitterling–mussel interactions on the physicochemical aquatic environment and on pelagic–benthic
coupling. We established 4 treatments with 3 replicates each: control (without bitterling and mussel), only bitterling (A. macropterus), only mussel (S. woodiana), and both bitterling and mussels. We predicted, based on the evidence for large-sized omnivores, that the presence of bitterling alone would: 1) increase nutrient concentrations (particularly for dissolved forms) and, thus, stimulate phytoplankton growth; and 2) increase the concentrations of TSS but not those of ISS. We also predicted that the filter-feeding activities of mussels would alleviate the negative effects of bitterling on water quality and that bitterling–mussel interactions would reduce both phytoplankton and periphyton biomass.

**METHODS**

Experimental design

We conducted an 8-wk outdoor mesocosm (uncovered) experiment starting in July 2018 at Eastern Lake Taihu Field Station, situated on the southeast shore of Lake Taihu, China. We constructed 12 high-density polyethylene mesocosms (height 66 cm, top diameter 50 cm, bottom diameter 41 cm, volume ~130 L), which we subsequently filled with 15 cm sediment and 90 L lake water pre-filtered through a plankton net (mesh size 64 μm). We established 4 treatments (control, mussel, bitterling fish [BF], mussels and bitterling fish [Mu-BF]) with 3 replicates each. For the control group, we added only water and sediment to the mesocosms, whereas we added 2 S. woodiana with a biomass of 121.8 ± 4.0 standard deviance (SD) g/m² to the mussel treatment mesocosms. We stocked the BF mesocosms with 4 bitterling (A. macropterus) with a biomass of 23.3 ± 1.2 SD g/m² (7.6 ind./m², mean total length: 6.2 ± 0.1 SD cm/ind., mean wet mass: 3.0 ± 0.2 SD g/ind.). In the Mu-BF treatment, we added 2 mussels and 4 fish (similar biomass as in the mussel and bitterling treatments). The density of fish used was intermediate between the densities used in previous studies with omni-benthivorous fishes (He et al. 2017, 2019, Gu et al. 2016) and was close to the natural density (8.7 ind./m²) of small fishes (e.g., A. macropterus, R. ocellatus, and Pseudorasbora parva Temminck and Schlegel, 1846) found in a natural lake (Ye et al. 2006). We collected bitterling from Lake Taihu and only used female adults of similar size in our experiment. The mussel-to-fish biomass ratio used in our experiment was 10.0 ± 0.2 SD.

Sampling and analytical methods

We estimated nutrient concentrations, phytoplankton biomass as chlorophyll a (Chl a), TSS, ISS, and organic suspended solids (OSS) at 7-d intervals. We measured light intensity (μmol m⁻² s⁻¹) in each mesocosm at 2 depths (0 and 30 cm) with a digital lux meter (Apogee MQ-510; Apogee Instruments, Logan, Utah) on d 6, 14, 28, 44, and 56 during the experiment. To allow periphyton colonization on a standardized substrate, we suspended a polyethylene (PE) board (10 × 10 cm) vertically at 40-cm depth (close to the bottom) in each mesocosm. We assessed the Chl a content of periphyton from the PE board of each mesocosm only at the end of the experiment.

To measure water column nutrients, Chl a, and suspended solid concentrations (i.e., TSS, ISS, OSS), we collected 2-L water samples from 2 different layers (surface and ~40 cm) and mixed them for each mesocosm. In the laboratory, we measured nutrients and Chl a in a 500-mL subsample of the water collected from each mesocosm. We determined concentrations of total nitrogen (TN), total dissolved nitrogen (TDN), total phosphorus (TP), and total dissolved phosphorus (TDP) via colorimetry after digestion with K₂S₂O₈ and NaOH solution. We directly measured TN and TDP concentrations at 210 nm in a spectrophotometer (BioMate™ 3S; Thermo Fisher Scientific, Waltham, Massachusetts), and we used the ascorbic acid method (Jin and Tu 1990) to detect TP and TDP at 700 nm. We measured Chl a concentration spectrophotometrically from the matter retained on a GF/C filter by filtering 300- to 500-mL subsamples and extracting in a 90% (volume/volume) acetone/water solution for 24 h. We did not correct for pheophytin interference (SEPA 2002).

To determine TSS concentrations, we passed 500- to 1000-mL subsamples through pre-ashed and pre-weighed GF/C filters, which we then oven-dried at 105°C for 4 h and re-weighed. Subsequently, we burned the filters in a muffle furnace at 550°C for 2 h to combust all organic matter and then cooled them in a desiccator to calculate the concentrations of ISS and OSS.

At the end of the experiment, we carefully removed the periphyton substrate (i.e., PE board) from each mesocosm and placed it in a plastic bag for laboratory analysis of periphyton biomass. We rinsed the periphyton off the board with distilled water and used 50- to 100-mL subsamples to assess Chl a biomass using the same methods described above.

Statistical analysis

We used repeated measurements analysis of variance (rmANOVA) to test the effects of fish, mussel, time, and their interactions on nutrients, Chl a in phytoplankton, TSS, ISS, OSS, and light intensity. Prior to analysis, if needed, we log₁₀ transformed data (e.g. TN, TSS, OSS, and periphyton biomass) to meet the assumption of normality (Kolmogorov–Smirnov test) or homogeneity (Levene’s test) of variance. Sphericity was accounted for in all rmANOVAs by modifying the degrees of freedom using the Greenhouse–Geisser approximation (Stevens 2012). In instances where the rmANOVA detected substantial treatment or time effects, we subsequently conducted Tukey’s post-hoc multiple comparisons. We used a 2-way ANOVA to detect the effects of fish, mussels, and their interactive effects on
Results

Nutrients

Bitterling and mussels had interactive effects on TN concentration ($F_{1,8} = 32.4$; Tables 1, S1). In the BF treatment, TN varied substantially with time ($F_{2,18} = 13.8$; Tables 1, S1), peaking on d 28 but remaining high until the end of the experiment (Fig. 1A). In the Mu-BF treatment, TN was lower compared with the BF treatment (post-hoc test $F_{1,8} = 69.1, p < 0.0001$; Fig. 1A) and similar to TN in the mussel treatment (post-hoc test $F_{1,8} = 1.8, p = 0.22$; Fig. 1A, B). However, in the bitterling-absent treatments, there was no difference in TN between the mussel treatment and the control (post-hoc test $F_{1,8} = 0.07, p = 0.80$; Fig. 1B).

Concentrations of TP were affected by a substantial mussel–bitterling interaction ($F_{1,8} = 67.0$; Tables 1, S1), and the effect varied substantially over time ($F_{2,16} = 6.6$; Tables 1 and S1). In the bitterling-present treatments (i.e., BF and Mu-BF), the presence of mussels substantially decreased TP concentration compared with the BF treatment (post-hoc test $F_{1,8} = 158.7, p < 0.0001$; Fig. 1A). In the bitterling-absent treatments, TP in the mussel and control mesocosms did not differ throughout the experiment (post-hoc test $F_{1,8} = 1.0, p = 0.34$; Fig. 1D).

There were interactive effects of mussels and bitterling on both TDN and TDP ($F_{1,8} = 6.4$ for TDN, $F_{1,8} = 22.7$ for TDP; Tables 1 and S1). In the BF treatment, TDN was much higher than in the Mu-BF treatment (post-hoc test $F_{1,8} = 9.8, p = 0.014$; Fig. 1E). In the bitterling-absent treatments, no differences were found for TDN between mesocosms with and without mussels (post-hoc test $F_{1,8} = 0.2, p = 0.66$; Fig. 1F). Moreover, TDP in the BF mesocosms was substantially higher than in the controls (post-hoc test $F_{1,8} = 96.9, p < 0.0001$; Fig. 1G, H), but the effect was only notable on d 14 and 21. In the bitterling-absent treatments, TDP did not differ between the mussel and control mesocosms at any time (post-hoc test $F_{1,8} = 0.05, p = 0.84$; Fig. 1H).

Phytoplankton

The effects of time, mussels, bitterling, and mussel–bitterling interactions on phytoplankton biomass (as indicated by Chl $a$ concentration) were substantial (Tables 1, S1; Fig. 2A, B). The biomass of phytoplankton in the BF mesocosms was higher than in the controls (post-hoc test $F_{1,8} = 176.8, p < 0.0001$) and the Mu-BF treatment (post-hoc test $F_{1,8} = 151.7, p < 0.0001$; Fig. 2A). In the bitterling-absent treatments, there were no differences in the phytoplankton biomass between the 2 groups (mussels vs control; post-hoc test $F_{1,8} = 0.1, p = 0.72$; Fig. 2B).

Suspended solids

The effects of time, mussels, and bitterling on TSS were substantial (Tables 1, S1; Fig. 3A). In the bitterling-present treatments, TSS peaked in wk 4 in the BF treatment but peaked in wk 2 in the Mu-BF treatment. These peaks were followed by a gradual decrease in TSS until the end of the experiment (Fig. 3A). However, TSS levels in the Mu-BF treatment were markedly lower than in the BF mesocosms (post-hoc test $F_{1,8} = 115.9, p < 0.0001$; Fig. 3A). In the bitterling-absent treatments, TSS concentrations in the mussel mesocosms did not differ from those in the controls (post-hoc test $F_{1,8} = 0.2, p = 0.84$; Fig. 3B).

The effects of mussels, bitterling, and their interactions on OSS concentrations varied with time (Tables 1, S1). OSS was much higher in the BF treatment than in the controls (post-hoc test $F_{1,8} = 331.5, p < 0.0001$), and the presence of mussels counteracted the OSS increase induced

Table 1. Summary of repeated measures analysis of variance results on the effects of bitterling and mussel treatments (Control: no mussels or fish, Mussel: only with mussels, BF: only with bitterling fish, Mu-BF: with both mussels and fish) on the concentrations (mg/L) of total nitrogen (TN), total phosphorus (TP), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total suspended solids (TSS), total suspended organic solids (OSS), and total suspended inorganic solids (ISS) as well as phytoplankton biomass (chlorophyll $a$ [Chl $a$]) concentrations (mg/L), and light intensity ($\mu$mol m$^{-2}$ s$^{-1}$ [Light: ratio of light intensity at 30-cm water depth relative to the water surface]). The experiment was conducted in mesocosms for 8 wk (Time).

| Effects          | TN   | TP   | TDN  | TDP  | TSS  | OSS  | ISS  | Chl $a$ | Light |
|------------------|------|------|------|------|------|------|------|---------|-------|
| Mussel           | <0.0001 | <0.0001 | 0.096 | 0.002 | <0.0001 | <0.0001 | 0.14 | 0.002 | <0.0001 |
| BF               | <0.0001 | <0.0001 | 0.011 | <0.0001 | <0.0001 | <0.0001 | 0.007 | <0.0001 | <0.0001 |
| Mussel $\times$ BF | <0.0001 | <0.0001 | 0.035 | 0.001 | <0.0001 | <0.0001 | 0.55 | 0.001 | 0.001 |
| Time             | <0.0001 | <0.0001 | 0.01 | <0.0001 | <0.0001 | <0.0001 | 0.001 | <0.0001 | <0.0001 |
| Time $\times$ Mussel | <0.0001 | 0.003 | 0.024 | 0.003 | <0.0001 | <0.0001 | 0.37 | 0.003 | <0.0001 |
| Time $\times$ BF | <0.0001 | <0.0001 | 0.017 | <0.0001 | <0.0001 | <0.0001 | 0.13 | <0.0001 | <0.0001 |
| Time $\times$ Mussel $\times$ BF | 0.008 | 0.008 | 0.054 | 0.001 | <0.0001 | 0.001 | 0.72 | 0.001 | 0.013 |
by bitterling, as shown by the low OSS in the Mu-BF treatment (post-hoc test $F_{1,8} = 291.9$, $p < 0.0001$; Fig. 3C). In the bitterling-absent treatments, there was no difference in OSS between the 2 groups (post-hoc test $F_{1,8} = 1.2$, $p = 0.3$; Fig. 3D). For ISS, no substantial differences were found between the 2 bitterling-present treatments (post-hoc test $F_{1,8} = 2.6$, $p = 0.15$; Fig. 3E). ISS concentrations in the bitterling-absent treatments did not differ between the mesocosms with and without mussels throughout the experiment (post-hoc test $F_{1,8} = 0.5$, $p = 0.49$; Fig. 3F). In addition, no substantial differences were found for ISS between the BF and the control mesocosms ($F_{1,8} = 4.4$, $p = 0.07$).

Light intensity

The effects of mussels, bitterling, and their interactions on the light intensity at 30 cm varied over time (Tables 1, S1). Light intensities in the BF treatment were lower than in the controls (post-hoc test $F_{1,8} = 341.9$, $p < 0.0001$; Fig. 4A). However, in the bitterling-present treatments, the presence of mussels (Mu-BF treatment) improved light intensity (post-hoc test $F_{1,8} = 109.7$, $p < 0.0001$; Fig. 4A), but light intensity in the bitterling-absent treatments did not differ between the mussel treatment and control mesocosms (post-hoc test $F_{1,8} = 1.8$, $p = 0.21$, Fig. 4B).

Periphyton

We found interactive effects of mussels and bitterling on periphyton biomass at the end of the experiment (post-hoc test $F_{1,8} = 6.2$, $p = 0.037$). Periphyton biomass was higher in the Mu-BF treatment than in the BF (post-hoc test $F_{1,8} = 33.6$, $p < 0.0001$) and mussel (post-hoc test $F_{1,8} = 12.1$, $p = 0.008$) treatments (Fig. 5A, B), whereas there was no difference between the control and the BF treatment (post-hoc test $F_{1,8} = 0.003$, $p = 0.96$; Fig. 5A, B). Furthermore, in the bitterling-absent treatments, no difference emerged between the mussel treatment and the control (post-hoc test $F_{1,8} = 5.2$, $p = 0.053$; Fig. 5B).

DISCUSSION

Our study sought to determine whether the impacts of host mussels offset possible impacts of small omnivorous bitterling on water quality parameters. In particular, we were interested in the effects of bitterling and the interactive effects of bitterling and mussels on the concentrations of nutrients and suspended solids and the biomass of pelagic (phytoplankton) and benthic (periphyton) algae. The results of our 56-d mesocosm experiment supported our prediction that the presence of bitterling would increase nutrient concentrations, resulting in higher phytoplankton biomass. Moreover, bitterling increased the concentration of TSS, thereby reducing the light intensity. As predicted, the presence of mussels substantially alleviated the negative impact of bitterling by reducing the concentrations of nutrients and TSS and increasing light intensity. Furthermore, our results indicate interactive effects of mussels and bitterling on pelagic–benthic coupling, as pelagic algae (phytoplankton) biomass was reduced when both bitterling and mussels were present, whereas benthic algae (periphyton) biomass increased in these conditions.

Effects of bitterling on water physicochemical parameters and pelagic–benthic coupling

We found nutrient concentrations to be higher in the bitterling-only (BF) treatments than in the treatments without fish, which is consistent with results from other studies of omnivorous fishes (Zhang et al. 2016, 2017, He et al. 2017, 2018). Fish excretion (Brabrand et al. 1990, 2000, 2005).
Attayde and Hansson 2001) and disturbance of sediment (Zhang et al. 2016, He et al. 2017, 2019) can have significant effects on water-column nutrient concentrations. In general, ISS concentrations are used to determine the magnitude of sediment resuspension. In our experiment, however, concentrations of ISS were low in the BF mesocosms. This result, which indicates that bitterling did not enhance sediment resuspension, contrasts with most findings for larger benthivorous fishes, such as carp (Gu et al. 2016, Zhang et al. 2016, He et al. 2019, Qiu et al. 2019). Bitterling excretion may, therefore, be the key factor affecting nutrient levels (higher TDN and TDP) in the BF mesocosms. This conclusion is also supported by a bitterling nutrient excretion experiment showing 9.6- and 19.6-fold higher concentrations of TDN and TDP, respectively, in fish-present treatments compared with controls (JY, unpublished data). The results of the current study may also be explained by the omnivorous bitterling’s highly diverse and plastic diet (Frankiewicz et al. 1991, Koutrakis et al. 2003, Yu et al. 2016a). In our mesocosm experiment, bitterling likely increased nutrient cycling through feeding on zooplankton and macroinvertebrates, as indicated by the fact that bitterling presence led to the absence of large-sized cladocerans and chironomids in our study (Yu et al. 2020). Bitterling may also feed on periphyton (Koutrakis et al. 2003, Usui et al. 2018) and, thus, mobilize periphyton-bound nutrients into the water column. By feeding on at least 2 trophic levels, omnivores may greatly amplify the recycling of nutrients and thereby increase nutrient concentrations, as shown for other species (Zhang et al. 2016).

The increased nutrient levels in the BF treatment stimulated pelagic algae (phytoplankton) growth and led to higher OSS and TSS concentrations. Accordingly, light penetration was reduced in the BF treatment. This result may explain the lack of stimulation of benthic algae (periphyton) growth in the BF treatment (relative to the controls) despite much higher nutrient concentrations in the BF mesocosms than in...

Figure 2. Comparison of phytoplankton biomass chlorophyll a (Chl a) concentrations between treatments. Values represent mean ± standard error. Treatments: Control = no mussels or fish, Mussel = only with mussels, BF = only with bitterling fish, Mu-BF = with both mussels and fish.

Figure 3. Comparisons of total suspended solids (TSS; A, B), organic suspended solids (OSS; C, D), and inorganic suspended solids (ISS; E, F) concentrations between treatments. Values represent mean ± standard error. Treatments: Control = no mussels or fish, Mussel = only with mussels, BF = only with bitterling fish, Mu-BF = with both mussels and fish.
the controls. Based on these findings, we conclude that bitterling can have strong effects on lake ecosystems by enhancing the growth of phytoplankton in the water column.

**Effects of mussels on water physicochemical parameters and pelagic–benthic coupling**

Mussels can affect nutrient concentrations by excretion (Johengen et al. 1995, Coelho et al. 2018, Parr et al. 2019). However, our results showed no difference in nutrient concentrations between the mesocosms with and without mussels in the bitterling-absent treatments, indicating that the effects of mussel excretion on nutrient concentrations were negligible within the time frame of our experiment. Mussels can reduce the biomass of pelagic algae through filter feeding, subsequently leading to increases in benthic algae (Spooner and Vaughn 2006, Zhang et al. 2014). However, in the bitterling-absent treatments, the presence of mussels did not affect the biomass of pelagic or benthic algae compared with the controls. This result may be attributable to the low concentrations of nutrients in the 2 treatments producing a similar biomass of both benthic and pelagic algae. Thus, our short-term experiment suggests that the effects of mussels on benthic–pelagic coupling were inconsequential in this low-nutrient system. This conclusion is consistent with former studies (e.g., Wu et al. 2018) showing that the presence of mussels did not substantially affect the biomass of pelagic algae in a clear lake. In contrast, mussels significantly reduced the phytoplankton biomass (Chl a) in a turbid lake (Wu et al. 2018), emphasizing that the mussel effect likely depends on the trophic state of the lake.

**Interactive effects of mussels and bitterling on benthic–pelagic coupling**

Both omnivorous fishes and filter-feeding mussels can affect benthic–pelagic coupling. Omnivorous fishes might depress benthic algae growth through shading effects caused by increasing sediment resuspension and enhanced phytoplankton biomass (Zhang et al. 2016, 2017). In contrast, filter-feeding mussels may boost the biomass of benthic algae by increasing water transparency (Spooner and Vaughn 2006, Zhang et al. 2014). In our experiment, the presence of both mussels and bitterling showed substantial interactive effects on benthic–pelagic coupling.

![Figure 4](image.png)

**Figure 4.** Light penetration (indicated by the ratio of light intensity [μmol m\(^{-2}\) s\(^{-1}\)] at 30-cm water depth relative to the water surface) in the different treatments. Values represent mean ± standard error. Treatments: Control = no mussels or fish, Mussel = only with mussels, BF = only with bitterling fish, Mu-BF = with both mussels and fish.

![Figure 5](image.png)

**Figure 5.** Periphyton biomass in treatments with presence (A) and absence (B) of bitterling at the end of the experiment as estimated from the polyethylene board placed at 40-cm depth in each treatment. Values represent mean ± standard error. Treatments: Control = no mussels or fish, Mussel = only with mussels, BF = only with bitterling fish, Mu-BF = with both mussels and fish.
effects on benthic–pelagic coupling. In the bitterling-present treatments, the presence of mussels reduced TSS levels and pelagic algae biomass compared with the mussel-absent but fish-present (BF) treatment, as has been seen in some previous studies (Johengen et al. 1995, He et al. 2014, Zhang et al. 2014). Light intensity was substantially enhanced by the presence of mussels and led to Mu-BF mesocosms having higher benthic primary periphyton biomass compared with the BF treatment. This result was contrary to our prediction of low biomass of both pelagic algae (because of filtration by mussels) and benthic algae (because of consumption by bitterling) in the Mu-BF treatment. In freshwater ecosystems, periphyton growth can be strongly determined by nutrient availability (Özkan et al. 2010, Cao et al. 2019), light (Sand-Jensen and Borum 1991), and grazing (Jones et al. 2002). The high biomass of periphyton in the Mu-BF mesocosms indicates that improved water clarity (increased light penetration) caused by mussel filtering of phytoplankton stimulated the accrual of periphyton, which increased to such an extent that grazing by the limited number of fish in the mesocosms had little impact on periphyton under our experimental conditions.

**Temporal variation in treatment effects**

The effects of bitterling and mussels on many variables varied with time in our experiment (Table 1), indicating that potentially transient conditions in our batch experimental approach may have affected several variables. For example, changes in potential food sources (e.g., zooplankton or periphyton) for the fishes over the course of the experiment may have directly or indirectly produced the substantial time × treatment interaction effects for Chl a and the various nutrient variables. However, we have focused the discussion on the main effects rather than time interactions because the main effects were most clear.

**Implications for lake restoration**

Foodweb biomanipulation involving control of fish assemblages and recovery of submerged macrophytes has proven an effective technique for restoring warm, shallow eutrophic lakes in China during the last 15 y (Gao et al. 2014, Yu et al. 2016b, Liu et al. 2018). Bottom-up effects of submerged macrophytes on nutrients are considered an important mechanism for maintaining clear water conditions in shallow tropical lakes (Liu et al. 2018). Thus, successful recovery of submerged vegetation is essential for restoring water quality in eutrophic lakes. To improve and maintain high water clarity to meet the light requirements for submerged macrophyte growth, stocking of filter-feeding mussels has become a supplementary method used in the restoration of many Chinese lakes (Chen et al. 2009, Qin 2013, Gao et al. 2017). However, increased mussel abundance may also enhance the growth of bitterling populations. Indeed, we found that bitterling presence impairs water quality, but the negative effects of bitterling were alleviated by filter-feeding mussels, and the positive interactive effects of bitterling and mussels facilitated increases in benthic algae biomass. Previous studies have shown that periphyton proliferation negatively affects the coverage and biomass of submerged macrophytes (Jones et al. 2002, Jones and Sayer 2003, Hilt et al. 2010), mainly through shading (Roberts et al. 2003). Moreover, in a subtropical shallow lake, bitterling have been shown to feed on submerged macrophytes (Yu et al. 2016a). Thus, the presence of bitterling may inhibit the growth of submerged macrophytes through both shading (caused by increased phytoplankton biomass, as shown in BF treatment, and periphyton biomass, as shown in Mu-BF treatment) and grazing effects.

In our study, the combined effects of bitterling and mussels appeared to create high water clarity. However, the longer-term effects of high periphyton biomass induced by the bitterling–mussel combination and the potential pressure from bitterling feeding on submerged macrophytes may negatively affect the recovery of macrophytes. Furthermore, many studies have reported that mussels enhance nutrient concentrations through excretion (Johengen et al. 1995, Coelho et al. 2018, Parr et al. 2019). In restored lakes with transplantation of submerged macrophytes and stocking of mussels, mussel-derived nutrients may support the growth of submerged macrophytes, provided that the mussels can control the development of phytoplankton biomass stimulated by these nutrients so that the macrophytes get sufficient light to grow. Submerged macrophytes and associated periphyton may, in turn, take up nutrients from the water (Jeppesen et al. 2012, Liu et al. 2018) and thereby alleviate the potential negative effect of nutrients released by the mussels. Ultimately, these nutrients may make important contributions to the aquatic food web, as seen in riverine systems (Atkinson et al. 2014). These topics are important for future studies over longer time periods.

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