Effects of small-sized crucian carp (*Carassius carassius*) on the growth of submerged macrophytes: implications for shallow lake restoration

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Abstract

Reestablishment of submerged macrophytes is considered important when restoring shallow eutrophic lakes. To improve water clarity and consequently the growth conditions of macrophytes, removal of plankti-benthivorous fish has been used. In sub-tropical shallow lakes, however, rapid recruitment of small fish, especially benthivores during restoration, may hamper early reestablishment of submerged macrophytes. Crucian carp (*Carassius carassius*) and *Vallisneria natans* are common species dominated in sub-tropical shallow lakes. To investigate the effect of small benthivorous fish on the growth of *Vallisneria natans*, a 28-day outdoor controlled experiment was undertaken in 12 mesocosms with three densities of *Carassius carassius* – low (10 g m⁻²), high (40 g m⁻²) and no fish (all in four replicates). The results showed that the fish significantly increased chlorophyll-*a* concentrations and periphyton biomass in both fish treatments, most significantly at high density for Chl *a*. This concurs with an increase in nutrient concentrations, likely mediated by fish sediment disturbance and excretion, and a reduction of zooplankton biomass (less algal grazing). Increased concentrations of inorganic suspended solids with increasing fish density further enhanced turbidity, causing shading of the macrophytes. Accordingly, the relative growth rate, ramet number and root/leaf ratio of *V. natans* decreased significantly at low and high fish density compared with the controls, but the effects did not depend on fish density. However, mean leaf length rose with increasing fish density, likely to allow the plants to obtain more light. Overall, our results show that not only

large-bodied carp, as demonstrated frequently, but also small-sized crucian carp posed a constraint on submerged macrophyte reestablishment, and we conclude that crucian carp may hamper restoration efforts in sub-tropical shallow lakes. Restoration by biomanipulation should therefore target also small-sized crucian carp.

Keywords: Small-sized fish; Crucian carp; *Vallisneria natans*; Restoration

Introduction

Submerged macrophytes play an important role in shallow lake ecosystems and help to maintain a clear water state (Scheffer et al., 1993; Jeppesen et al., 2005; Vanderstukken et al., 2011) by reducing sediment resuspension induced by wind and fish (Nurminen and Horppila, 2009), storing substantial amounts of nutrients, producing allelopathic substances against phytoplankton (Donk and Bund, 2002) and providing crucial habitats and food for organisms, thereby promoting species diversity (Declerck et al., 2007). Thus, reestablishment of submerged macrophyte communities has been considered a key measure to obtain long-term recovery of shallow lakes.

To speed up recovery and facilitate growth of submerged macrophytes, biomanipulation, including removal of coarse fish and sometimes also planting of macrophytes, has been used (Coveney et al., 2002; Søndergaard et al., 2008; Jeppesen et al., 2012). Following biomanipulation, the abundance of small fish generally increases, which leads to reduced water transparency, potentially impeding the progress of restoration (Gulati et al., 2008; Lorenz et al., 2013). While it is well established that small fish, when abundant, impoverish the water quality by reducing top-down control on phytoplankton via their high predation pressure on zooplankton (Iglesias et al., 2011; Meerhoff et al., 2012) and disturbance of the sediment (Persson and Svensson, 2006), their effects on submerged plants so far remain poorly elucidated.

In warm lakes, fish communities are characterised by a higher proportion of benthivorous species and small sizes than in temperate and cold lakes (Brucet et al.,

2010; Jeppesen et al., 2010). Benthivores may affect macrophyte abundance, both directly by feeding or uprooting and indirectly by reducing the light conditions via sediment resuspension when foraging on the lake bottom, concurrently with proliferation of phytoplankton in water enriched with excreted nutrients (Rodrigo et al., 2013; Badiou and Goldsborough, 2015). For instance, common carp (*Cyprinus carpio*), feeds mainly on benthic invertebrates with an inferior mouth that easily dig into the sediment, hampering sediment consolidation with lower water transparency as a result (Fischer et al., 2013). Crucian carp (*Carassius carassius*) is also benthivorous, commonly occurring and abundant in shallow lakes. Although crucian carp is taxonomically and somehow functionally similar to common carp (Chen et al., 2009), its influence on the growth of submerged macrophytes is less certain. Unlike common carp, crucian carp has a terminal mouth, and the limited gape of this species prevents easy uprooting of aquatic macrophytes, potentially preventing strong resuspension effects. Additionally, crucian carp is more omnivorous and feeds on zooplankton and small benthic animals, but also on aquatic plants and filamentous algae (FAO, 2004-2015).

During a restoration carried out in Wuli Bay (in the northern part of Lake Taihu, China) (Chen et al., 2013), fish eggs adhering to plants were inevitably carried into the lake when transplanting the submerged macrophytes in May 2010. After two months, common carp and crucian carp (the latter accounting for more than 80% of the biomass of the fish stock) recruited rapidly inside the restored area, exhibiting biomasses up to

10 g m⁻² (Liu, unpublished data). Soon after, the water became turbid and recovery of submerged plants was poor (Ye et al., 2011; Chen et al., 2013). Whether this was related to the expansion of small crucian carp is uncertain. The focus of the present study was therefore to conduct an outdoor mesocosm experiment to elucidate the effects of small crucian carp on the growth of *Vallisneria natans*, an abundant plant in Wuli Bay after restoration, by simulating the natural fish density in Wuli Bay. We hypothesised (1) that small-sized fish through removal of zooplankton grazers, sediment disturbance and promotion of nutrient release would stimulate phytoplankton and epiphyte growth, resulting in reduced growth of plant, and (2) that these effects would grow with increasing fish density. However, direct plant ingestion may be of minor importance due to the small size of the fish.

Materials and methods

Experimental material

In the experiment, young seedlings of *V. natans* germinating from tubers were collected from Lake Taihu (31°30' N, 120°30' E) and pre-cultivated in outdoor polyethylene tanks (length 80 cm, width 50 cm, height 55 cm) filled with 10 cm lake sediments and 30 cm lake water (total nitrogen: 1.32 ± 0.11 mg L⁻¹, total phosphorus: 0.05 ± 0.004 mg L⁻¹) for a month before the experiment began. Small *C. carassius*, a benthivorous fish commonly found in Lake Taihu, were obtained from Wuli Bay.

Sediment and water were collected from Lake Taihu. Prior to the experiment, the sediment was sieved through a 0.5 cm sieve to remove coarse debris and the water was

filtered through a 112 μm mesh plankton net to remove larger organisms and inorganic particles. The sediments and water were stirred separately to ensure homogeneity and to secure a standardised zooplankton inoculum in the tanks before the start of experiment.

Experimental design

The mesocosm experiment was conducted in the vicinity of the Taihu Laboratory for Lake Ecosystem Research (TLER) from 10 August to 14 September 2014. A total of 12 mesocosms (height 95 cm, upper diameter 97 cm, bottom diameter 77 cm, area of each mesocosm approximately 0.5 m^2), each filled with 10 cm sediment and 500 L pre-filtered lake water, were placed on the ground near the shore of the lake. Fifteen separate young plant individuals (each having approximately 5-7 leaves, being 26 ± 0.28 cm high and having a wet weight of 24 ± 0.05 g) were transplanted into each mesocosm one week before the start of the experiment, a density consistent with the mean density of transplanted *V. natans* in an eco-demonstration site of Wuli Bay, Lake Taihu (Liu et al., unpublished data). Next, four 8×10 cm sized polyethylene (PE) boards were placed at a depth of 35 cm in each tank to allow estimation of the amount of periphyton (in the present study defined as a mixture of algae, bacteria and detritus attached to a substratum) on the leaf surface of *V. natans*. The PE boards were vertically suspended in the water column using rope and weights.

The experiment followed a single factor design in which there were three fish densities, including four replicates of each treatment. The mesocosms were stocked accordingly: no fish (control), 2 individuals (low density) and 8 individuals (high

density), corresponding to densities of 0, 10 and 40 g m⁻², respectively. The average total length of *C. carassius* was 6.5±0.11 cm and mean wet weight was 2.5 ± 0.02 g. The densities used were determined from observations undertaken during a field campaign in some restored areas of Wuli Bay (Liu, unpublished data). To prevent fish from jumping out of the mesocosms, the top of each tank was covered with thin gauze (5 mm mesh size). Dead fish were removed and replaced. During the experimental period, water temperatures were maintained between 26 and 30°C, and the temperature differences among the mesocosms varied at maximum no more than 2°C.

In the morning on day 0, 7, 14, 21 and 28 of the experiment, turbidity was recorded *in situ* using a portable instrument (YSI 9500) placed in the middle of the water column at about 30 cm water depth. Thereafter, a 2 L mixed water sample was collected from each mesocosm for analysis of water chemistry, chlorophyll *a* (Chl *a*) concentrations and suspended solids (SS). Total nitrogen (TN), total dissolved nitrogen (TDN), total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were determined according to Jin & Tu (1990). Chl *a* was measured spectrophotometrically from matter retained on a GF/C (Whatman) filter extracted over 24 h in a 90% (v/v) acetone/water solution, and values were calculated without correction for phaeophytin interference (SEPA, 2002). To determine total suspended solids (TSS), 100-200 ml water subsamples were filtered through a pre-weighed glass microfiber filter (GF/C), which was oven dried at 105 °C for 4 h, after which the filter was weighed again to assess the concentration of TSS. Subsequently, the filter was burnt in a muffle furnace at 550 °C

for 2 h to combust all organic matter and cooled in a desiccator to calculate the concentration of inorganic suspended solids (ISS).

Light intensity was measured in the morning on day 0, 14 and 28 of our experiment before collection of water samples using a digital luxmeter (ZDS-10W, Shanghai, China). Ratios of light intensity at 30 cm depth relative to the water surface (0 cm) were used to calculate the changes in light over time and between treatments.

During each sampling event, one PE board from each mesocosm was removed and enclosed in a zip-lock bag. The attachments (mostly periphyton) on the PE board were carefully brushed into a beaker with distilled water, and 100–200 ml of well-mixed subsamples were filtered through pre-weighed, pre-combusted (4 h at 450 °C) glass fiber filters (GF/C) and dried at 105 °C for 2 h in order to determine periphyton dry weight. Filamentous algae occurred in low densities in one of the control mesocosms and in one low fish density treatment, but were not collected separately.

Zooplankton was sampled at the start and at the end of the experiment. Five litres of depth-integrated water were taken from each mesocosm, filtered through a plankton net (64 µm mesh size) and then preserved with 4% formaldehyde. Rotifers were counted directly from phytoplankton samples at 100× magnification. Crustacean zooplankton (cladocerans and copepods) was counted at 40× magnification. Species identification was made according to Wang (1961), Chiang & Du (1979) and Shen & Du (1979). Zooplankton biomass (dry weight) was estimated using equations from Huang (1999).

At the end of the experiment, all *V. natans* were harvested by hand after emptying the

mesocosms. Total wet weight and the wet weight of roots (including tubers) and leaves (excluding stems) of *V. natans* were determined for each mesocosm. The number of ramets and the mean leaf length per plant were also recorded. The relative growth rate (RGR) was calculated as:

$$\text{RGR (mg g}^{-1} \text{ d}^{-1}) = 1000 \times \ln (W_f / W_i) / \text{days}$$

where W_f (g) and W_i (g) were the final and initial total wet weight of plants per tank, respectively. The root/leaf ratio was calculated as root biomass (referring to all biomass below the ground surface) divided by shoot biomass (referring to all biomass above the ground surface). *C. carassius* were collected from each mesocosm by electrofishing and their guts were subsequently removed and preserved with 4% formalin to conduct microscope analysis to determine the stomach content. Frequency of occurrence (FO%) of each prey item was calculated as: $\text{FO\%} = \text{number of stomachs containing prey item } i / \text{total number of stomachs} \times 100$. All (forty) fish guts were analysed to determine the diet compositions.

Statistical analysis

All statistical analyses were performed using SPSS 16.0. Repeated measures analysis of variance (rANOVA) was applied to analyse time-series data, including nutrient concentrations of water, Chl *a*, periphyton, TSS, ISS, turbidity and light intensity. One-way analysis of variance (one-way ANOVA) was used to analyse zooplankton abundances between treatments at the start and at the end of the experiment and the effects of *C. carassius* densities on *V. natans*, with post hoc multiple comparisons

carried out by applying Tukey's least significant difference procedure. Where necessary, data were $\log_{10}$ transformed to stabilise normality and homogeneity. Results with $p < 0.05$ were reported as significant in the ANOVA.

Results

Dynamics of nutrient concentrations

The treatment with small crucian carp had significant effects on several physico-chemical and plankton variables (Fig. 1). The mean concentrations of TN and TP significantly increased over time in the fish treatments compared with the control group ($p < 0.01$) and were significantly higher at high fish density than at low fish density. However, mean TDN and TDP concentrations in the fish treatments were significantly lower than in the control group ($p < 0.05$) but did not differ significantly between the two fish densities, the trend revealing a notable decrease in TDN in the fish treatments over time and a slightly reduced TDP at the start of experiment after an initial significant increase ($p < 0.05$).

Light intensity, TSS, ISS and turbidity

The presence of small fish decreased the light penetration via their increased density; thus, the ratios of light intensity at 30 cm depth relative to the surface were significantly lower in the fish-holding mesocosms than in the controls ($p < 0.05$) (Tab.1). Small crucian carp also had a significant effect on TSS, ISS and turbidity ($p < 0.01$) (Fig. 2), and these three variables were much higher in the fish treatments than in the controls from day 7 of the experiment ($p < 0.01$). TSS and turbidity significantly increased with

increasing density of fish over time ($p < 0.01$). Mean TSS values in the low and high density treatments were 2.9 and 3.4 times higher than in the controls. Turbidity was also higher, 271% and 413% in the low and high density treatments, respectively, than in the controls. Mean ISS increased by 355% and 594% in the low and high density treatments, respectively, compared with the controls despite a slight decrease in the fourth week with fish treatment.

Zooplankton, phytoplankton and periphyton

A one-way ANOVA analysis did not reveal any significant differences in zooplankton biomasses and communities between treatments ($p > 0.05$) at the experimental start-up, indicating homogeneity prior to fish addition (Fig. 3). The post-experiment zooplankton biomass was significantly higher than the pre-experiment biomass in the controls ($p < 0.05$) but significantly lower in fish treatments ($p < 0.05$). At the end of the experiment, the biomass of zooplankton had decreased significantly with fish density. In the low and high density treatment, zooplankton biomass had decreased by 63% and 87%, respectively, compared with the control group ($p < 0.01$). Specifically, the biomasses of both cladocerans (*Ceriodaphnia* and *Moina*) and copepods (*Mesocyclops*) were affected by fish density in the fish treatments, resulting in a reduced zooplankton biomass compared with the controls. *Daphnia* and *Sinocalanus* were absent in all treatments with fish. In contrast, rotifer biomass was not affected by fish density. The zooplankton biomass in the mesocosms with fish was dominated by copepods (*Mesocyclops*) and rotifers (*Brachionus*).

During the experiment, Chl *a* concentrations were also significantly affected by crucian carp density ($p < 0.01$) (Fig. 4) and had increased by 108% and 236% in the low and high density mesocosms compared with the controls ($p < 0.01$). In addition, the accumulation of periphyton was found to be significantly lower in the controls than in the mesocosms with fish ($p < 0.01$ for both low and high fish densities), and it was significantly higher at low than at high density ($p < 0.05$).

Fish guts

Macrophytes and zooplankton were frequently found in the fish guts of crucian carp at both low and high density, with a percentage occurrence $\geq 85\%$ (Tab. 2). Phytoplankton and filamentous algae were seldom found (percentage occurrence $\leq 15\%$). Macrophytes and zooplankton constituted the main food items at both low and high crucian carp densities.

Macrophytes

The presence of small fish negatively affected the growth of *V. natans* (Fig. 5). The relative growth rate, ramet number and root/leaf ratio were significantly lower in the crucian carp treatments than in the controls ($p < 0.01$). Compared with the control plants, the relative growth rate of *V. natans* in the low and high density treatments decreased by 30% and 25%, the ramet number decreased by 69% and 55% and the root/leaf ratio decreased by 41% and 32%. However, the three parameters did not differ significantly between the two fish treatments ($p > 0.05$) and were only slightly higher in the high

density than in the low density treatment. In contrast, the mean leaf length of *V. natans* increased significantly in the fish treatment ($p < 0.01$), averaging 50 cm and 62 cm in the low and high density treatments, respectively, which is 22% and 51% longer than in the controls.

Discussion

As hypothesised, small-sized crucian carp hampered submerged macrophyte growth, even at low fish biomass (10 g m^{-2}). This was evidenced by the significantly lower relative growth rate, ramet number and root/leaf ratio of *V. natans* in the mesocosms with fish than in the fishless controls. However, the hypothesis of decreased *V. natans* growth with increased crucian carp density could not be confirmed.

The presence of small-sized crucian carp strongly stimulated the growth of phytoplankton and periphyton, partly resulting in higher turbidity. Also Holopainen (1992) found increasing concentrations of chlorophyll *a*, biomass and species richness of phytoplankton with an increasing density of small-sized crucian carp. Enhanced nutrient concentrations caused by physical disruption of sediment and excretion by small-sized crucian carp (Liu et al., 2014) may ultimately augment chlorophyll *a* and periphyton levels as reported in studies with common carp transporting nutrients from sediments to the overlying water when foraging (Roozen et al., 2007). In the present study, treatments with small-sized crucian carp led to an increase in TN and TP concentrations with increasing density. TDP and TDN, however, decreased and were lower in the fish treatments than in the controls, suggesting uptake by phytoplankton

and periphyton. Fish predation on zooplankton may further stimulate the growth of these primary producers (e.g. (Meerhoff et al., 2006; Meerhoff et al., 2007)). In this study, zooplankton biomass decreased with fish density. The biomass of large-bodied cladocerans was lower in the fish treatments compared with the fishless controls, suggesting that even low densities of these fish can dramatically reduce large-bodied zooplankton, such as *Daphnia* spp. and that zooplankton communities tend to be dominated by copepods and rotifers. This may be attributed to a higher predation pressure by small-sized crucian carp and, consequently, a reduction in the grazing on phytoplankton.

The turbidity increase and shading of the macrophytes may be further reinforced by small-sized crucian carp as these normally dwell in the bottom layer of the water column and forage in the sediment (Lorenzoni et al., 2007), as is the case for common carp and bream (*Abramis brama*) (Williams et al., 2002). We found an increase in TSS, primarily ISS, proportionally with increasing fish density. High ISS concentrations in the water column, indicative of sediment resuspension, have a marked effect on water turbidity and thus light availability for plant photosynthesis (Roozen et al., 2003). In a study evaluating the relationship between water clarity and macrophytes in shallow wetlands, Lougheed (1998) observed a threshold of 20 NTU beyond which conditions become unsuitable for plant growth. In this study, mesocosms with crucian carp typically had turbidity levels > 20 NTU, further lowering the ratio of light intensity and, accordingly, we found lower relative growth rates, lower tiller number and a lower

root/leaf ratio of *V. natans* in fish treatments than in the fishless controls.

Under conditions with high turbidity and low photosynthesis, there is no translocation of organic compounds to roots and plants to shift resources away from below-ground production to above-ground elongation (Greg and David, 2003), resulting in a lower root/leaf ratio and longer leaf length of *V. natans* in the fish treatments than in controls. Some studies claim that longer leaf length may also be viewed as shade adaptation – the leaves extend higher upwards in the water column where the light intensity is greater, thus enlarging the area of photosynthesis to accelerate light harvesting and/or to minimise self-shading by increasing the vertical separation of leaves (Barko et al., 1991; Doyle and Smart, 2001). Hence, it may be this adaptive ability of *V. natans* to high turbidity that explains the absence of difference in plant growth at the two fish densities by the end of our study. It should be noted, though, that our experiment ran for 1 month only, which may be too short a period to detect clear differences in macrophytes growth, despite strong effect on water clarity.

Direct consumption by crucian carp can affect plant growth in water systems and may further hamper the recovery of submerged macrophytes. Yu et al. (2016) investigated the food web structure and energy pathways of fish in the restored part of subtropical Lake Wuli, China, and found that macrophytes constituted an important food source for adult crucian carp (approximately 99.4%) but were of minor importance in the diet of juvenile fish. In contrast, in our study, macrophytes were frequently found in the fish guts of the juvenile, with a percentage occurrence above 85%. Despite the fact that

small fish predominantly feed on zooplankton (Dorenbosch and Bakker, 2012), animal food sources may not be sufficient if fish densities are high in a limited space, leading to consumption of plants that may be more easily assimilated by fish at warmer temperatures (like in our experiment), as suggested by Behrens & Lafferty (2007). In our study, it is, however, difficult fully to determine the percentage contribution of macrophytes to the diet of small-sized crucian carp as we unfortunately do not have quantitative data on fish gut contents.

It is well established that in a warming climate, a shift in fish community structure towards high abundances of small fish is highly likely due to increasing growth and reproduction rates and reduced control by predatory fish (Jeppesen et al., 2012). Apart from weak control of phytoplankton by zooplankton due to high abundance of small-sized fish, our study also suggested that small-sized fish like crucian carp can hamper the growth of submerged macrophytes through disturbance, and perhaps also consumption, and this may lead to reduced transparency, further delaying or hampering the recovery of submerged macrophytes following restoration (Gao et al., 2014). A potential limitation of our experiment is that the fish-plant interactions might be stronger in this small-scale experiments than in natural lakes, although even higher fish biomasses per unit of volume have been found in turbid shallow lakes (Fischer et al., 2013; Gao et al., 2014) with potential stronger interactions than in our experiment. Therefore, how to control the expansion of small fish after biomanipulation is a key issue that needs to be addressed if long-term effective restoration of submerged

macrophytes is to be obtained. Adjusting the timing of the transplantation to avoid the adhering eggs may be a valid measure, but this needs to be tested further.

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Fig. 1 Variations in concentrations of TN, TP, TDN and TDP during the 28-day experiment. Values are means of the four replicates; error bars represent standard deviation. Treatments labeled with the same letters do not differ significantly at $p > 0.05$.

Fig. 2 Time series of TSS, ISS and turbidity in the different treatments during the experiment. TSS, total suspended solids; ISS, inorganic suspended solids. Values are means of the four replicates; error bars represent standard deviation. Treatments labeled with the same letters do not differ significantly at $p > 0.05$.

Fig. 3 Total zooplankton biomass (values are means of the four replicates; error bars represent standard deviation) in the different treatments. S means start of the experiment and E means end of the experiment. Treatments labeled with the same letters do not differ significantly at $p > 0.05$.

Fig. 4 Variations in chlorophyll *a* (Chl *a*) concentrations and periphyton biomass in the different treatments during the experiment. Values are means of the four replicates; error bars represent standard deviation. Treatments labeled with the same letters do not differ significantly at $p > 0.05$.

Fig. 5 Relative growth rate, ramet number, root/shoot ratio and mean length of *V. natans* in the different treatments. Values are means of the four replicates; error bars represent standard deviation. Treatments labeled with the same letters do not differ significantly at $p > 0.05$.

Table 1 The ratio of light intensity at 30 cm water depth relative to the water surface and significance level (rANOVA) of differences between the treatments (means $\pm$ SD, n=4)

	0 d	14 d	28 d
Controls	0.46 $\pm$ 0.005	0.45 $\pm$ 0.032	0.40 $\pm$ 0.008
Low density	0.45 $\pm$ 0.010	0.35 $\pm$ 0.026	0.19 $\pm$ 0.018
High density	0.48 $\pm$ 0.007	0.26 $\pm$ 0.030	0.13 $\pm$ 0.009
<i>p</i>	> 0.05	< 0.01	< 0.05

Table 2 Frequency of occurrence (%) of each prey item in the fish diet

Prey items	Macrophytes	Zooplankton	Phytoplankton	Filamentous algae
Low density	87.5	100	12.5	12.5
High density	93.8	90.6	3.1	0