Limnological changes in a pond ecosystem caused by grass carp (*Ctenopharyngodon idella* Val.) low stocking density

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ABSTRACT: Grass carp (*Ctenopharyngodon idella* Val.) stocked (29 kg/ha) in a small pond reduced the biomass of aquatic macrophytes from 109 g/m^2 to 33 g/m^2 during one growing season. The only changes in hydrochemical parameters (pH, alkalinity, acidity, BOD_5, COD_{Mn}, NH_4-N, NO_2-N, NO_3-N, TN, PO_4-P and TP) associated with the grass carp stocking were a decrease in pH (from 8.43 to 7.57) and in NO_3-N concentration (from 0.99 mg/l to 0.56 mg/l). The increases in organic matter content and NO_3-N concentration in the surface sediment layer were higher in the control pond than in the pond stocked with grass carp. No changes were detected in the other parameters (NH_4-N, PO_4-P and TP) in the upper sediment layer and between all parameters measured in the lower inorganic layer. The grass carp grazing had no impact on phytoplankton biomass (concentration of chlorophyll-a) or species composition. There were no changes either in the abundance or in the species composition of zooplankton and zoobenthos induced by grass carp. Statistically significant indirect changes (in water and sediment chemistry) following the grass carp stocking were connected especially with a reduction in the biomass of the filamentous alga (*Cladophora globulina*) or rather with its maintenance in the control pond.

Keywords: aquatic macrophytes; water and sediment chemistry; phytoplankton; zooplankton; zoobenthos

The direct effect of grass carp (*Ctenopharyngodon idella* Val.) on a water ecosystem is caused by its grazing on aquatic macrophytes. The indirect effects are associated with grass carp excrements which are rich in nutrients because of the poor utilization of plant material by grass carp and thus depend on the amount of consumed plant biomass. About 50% of ingested phosphorus and nitrogen are released in grass carp excrements (Lembi et al., 1978). Nutrients can either remain in the water or be utilized by phytoplankton (Bettoli et al., 1990; Maceina et al., 1992) or accumulate in the sediment (Terrell, 1975). Animal communities (zooplankton and zoobenthos) could be influenced not only by

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changes in bottom links of food chains (bottom-up effect) by the increased phytoplankton abundance but also negatively by the changed habitat structure (Zweerde van der, 1982; Richard et al., 1985).

In spite of the extensive use of grass carp for controlling the aquatic macrophytes, most of the experiments studying indirect consequences following from grass carp stocking compared a few ponds/lakes stocked with grass carp with ponds/lakes without fish before and after they were stocked with grass carp (Fowler and Robson, 1978; Lembi et al., 1978; Mitzner, 1978; Small et al., 1985; Maceina et al., 1992; Kirkagac and Demir, 2004). None of the studies used real control pond(s) which would indicate changes both in space (the initial difference between the ponds chosen for stocking with grass carp and control pond(s)) and in time, caused especially by weather, water level fluctuations or fish stock of other fish in all ponds.

The purpose of this experiment was to identify: (1) changes in nutrient concentrations particularly of phosphorous and nitrogen in the water and sediment, (2) changes in phytoplankton, zooplankton and zoobenthos biomass and abundance after a moderate stocking rate of grass carp in a small and shallow pond.

MATERIAL AND METHODS

Experimental design. The experiment was carried out in two small eutrophic ponds (40 × 20 m, average depth 0.5 m), located in the experimental facility of the Research Institute of Fish Culture and Hydrobiology (Vodňany: latitude 49°10'; longitude 14°10', Czech Republic) in 1998–2000. Average air temperature was 14.6°C during the growing season (June–September) in 1998 and 1999. A BACI (Before and After Control Impact) design was applied. It separates both the space (control and impacted ponds) and the time variability (April 1998–March 1999 and April 1999–March 2000) from changes caused by the grass carp stocking. In 1998, both ponds were without fish. In mid-April 1999, one of the ponds was stocked with 10 individuals of two-years-old grass carp (29 kg/ha) while the control pond remained unstocked. The stocked pond was drained at the beginning of April 2000.

Sampling of aquatic macrophytes. Macrophyte biomass was harvested monthly from 10 randomly selected quadrates (0.5 m × 0.5 m) from both ponds from May to September in 1998 and 1999. The macrophyte samples were divided into species and dried at 105°C to constant weight to determine the dry weight of each constituent species.

Chemical analysis of water. Alkalinity, acidity, biochemical oxygen demand (BOD_5), chemical oxygen demand (COD_{Mn}) and concentrations of NO_3-N, NO_2-N, NH_4-N, total nitrogen (TN), PO_4-P and total phosphorus (TP) were measured at monthly intervals using the methods described by Horáková et al. (1989). The concentration of TN was assessed by the Kjeldahl method, NO_3-N colorimetrically with sodium salicylate, NO_2-N colorimetrically with sulphanyl acid and N-(1-naphthyl)-ethylene-diamine dihydrochloride, and NH_4-N using the Nessler method after distillation. The concentrations of PO_4-P and TP (after persulphate mineralization) were determined using the phosphomolybdenum method. Vertical profiles of oxygen concentration, pH and temperature were measured once a month (at 07:00, 13:00 and 16:30 hours) four times per growing season using WTW Multiline P4. Secchi disc transparency was measured monthly at the deepest site nearby the pond outlets.

Chemical analysis of sediment. Four sediment samples were taken with a core sampler from each pond three times during the growing seasons. In the 20 cm deep sediment profile, two different layers were distinguished: the upper "organic layer" (dark in colour), overlaying the "inorganic layer". The pH, dry mass (105°C, to constant weight) and loss on ignition (550°C, 2 h) were determined. Total carbon (TC) and total phosphorus (TP) contents were assessed in the samples sieved through 1 µm mesh and lyophilized. TC was analysed using a LiquiTOC analyser (Foss/Heraeus, Germany). The standard phosphomolybdenum complex method (Murphy and Riley, 1962) was used for TP estimation after HNO_3 and $HClO_4$ mineralization (Kopáček and Hejzlar, 1995). Prior to the following analyses, the samples were extracted and then filtered through a Whatmann GF-MN-5 glass fibre filter of 0.4 µm mesh size. PO_4-P was determined colorimetrically in the aqueous extract and in the extract with 0.5M $NaHCO_3$ (Olsen et al., 1954). The bis-pyrazolone method (Procházková, 1964) was used to assess the concentration of NH_4-N in the extract with 2M KCl (Bremner, 1968). Nitrate (NO_3-N) concentration was determined in the aqueous extract by ion chromatography.

Sampling of phytoplankton, zooplankton and zoobenthos. Qualitative and quantitative samples of phytoplankton, zooplankton and zoobenthos were collected at monthly intervals between April and November during all years (1998–2000) except for several sampling campaigns which could not be performed regularly on monthly basis due to thick ice cover or other reasons. Chlorophyll-a determination was done monthly by the spectrophotometric method (Lorenzen, 1966). Pooled samples of phytoplankton and zooplankton were taken randomly 5 times from the whole water column in both ponds using the Patalas sampler (2 l). From 10-litre pooled samples, 100 ml were preserved with Utermöhl's solution for future phytoplankton determination and the remaining volume was filtered through a 53-µm mesh net and preserved in a 4% formaldehyde solution as zooplankton samples. Zoobenthos was sampled using the Ekman grab sampler with the area of 75 cm^2 (2 pooled samples) and preserved with 4% formaldehyde solution. Zoobenthos biomass was determined after 4-month stabilisation.

Statistical analysis. The data were analysed using three-way analysis of variance (ANOVA) with pond and year as fixed factors and month as a random factor. The impact of the grass carp was considered significant when the interaction of space, year and month had $P < 0.05$ (Ter Braak and Šmilauer, 1998). Normality was tested using the Kolmogorov-Smirnov test and homogeneity of variance by Bartlett's test (Zar, 1984) in the Statistica for Windows (5.1) programme. The Bonferroni correction was applied to interpret the impact of grass carp on the biomass of each aquatic plant species or on the abundance of phytoplankton, zooplankton and zoobenthos species (Scheiner, 1993).

RESULTS

Grass carp stock

The biomass of grass carp stock increased from the initial 29 kg/ha in 1999 (mean individual weight ± SD: 230 ± 101 g) to 92 kg/ha in 2000 (mean individual weight 730 ± 240 g). No mortality was recorded.

Direct consequence of grass carp stocking Biomass of aquatic macrophytes

Significantly lower values of total biomass of aquatic macrophytes (F(1,176) = 16.5, $P = 10^{-4}$), vascular aquatic plants (F(1,176) = 4.5, $P = 0.04$)

Table 1. Hydrochemical parameters (mean ± SD; $n = 11$) in the pond stocked with grass carp and in the control pond before and after grass carp stocking

Hydrochemical parameters	Before grass carp April 1998–March 1999	Stocked with grass carp April 1999–March 2000	Control	
			April 1998–March 1999	April 1999–March 2000
pH	8.43 ± 1.10	7.57 ± 0.69	8.40 ± 1.03	7.87 ± 1.33
ANC4.5 (mmol/l)	1.15 ± 0.39	1.07 ± 0.29	1.14 ± 0.23	1.10 ± 0.27
ANC8.3 (mmol/l)	0.15 ± 0.13	0.02 ± 0.04	0.15 ± 0.16	0.13 ± 0.22
BNC8.3 (mmol/l)	0.01 ± 0.03	0.06 ± 0.04	0.03 ± 0.05	0.06 ±0.05
BOD5 (mg/l)	4.3 ± 1.8	4.0 ± 2.1	3.6 ± 3.0	1.5 ± 1.8
CODMn (mg/l)	11.1 ± 2.9	11.9 ± 4.6	10.3 ± 2.9	10.5 ± 2.6
PO_4-P (mg/l)	0.027 ± 0.018	0.052 ± 0.051	0.028 ± 0.017	0.054 ± 0.027
TP (mg/l)	0.093 ± 0.044	0.132 ± 0.083	0.080 ± 0.041	0.131 ± 0.064
NH_4-N (mg/l)	0.09 ± 0.07	0.09 ± 0.08	0.14 ± 0.13	0.17 ± 0.31
NO_2-N (mg/l)	0.009 ± 0.010	0.008 ± 0.004	0.009 ± 0.005	0.014 ± 0.012
NO_3-N (mg/l)	0.99 ± 1.48	0.56 ± 0.52	0.67 ± 0.76	1.06 ± 0.75
TN (mg/l)	2.64 ± 0.83	2.23 ± 0.34	2.20 ± 0.77	2.17 ± 0.70

pH: $n = 80$; BOD_5: $n = 5$; TN: before ($n = 8$) and after grass carp stocking ($n = 3$)

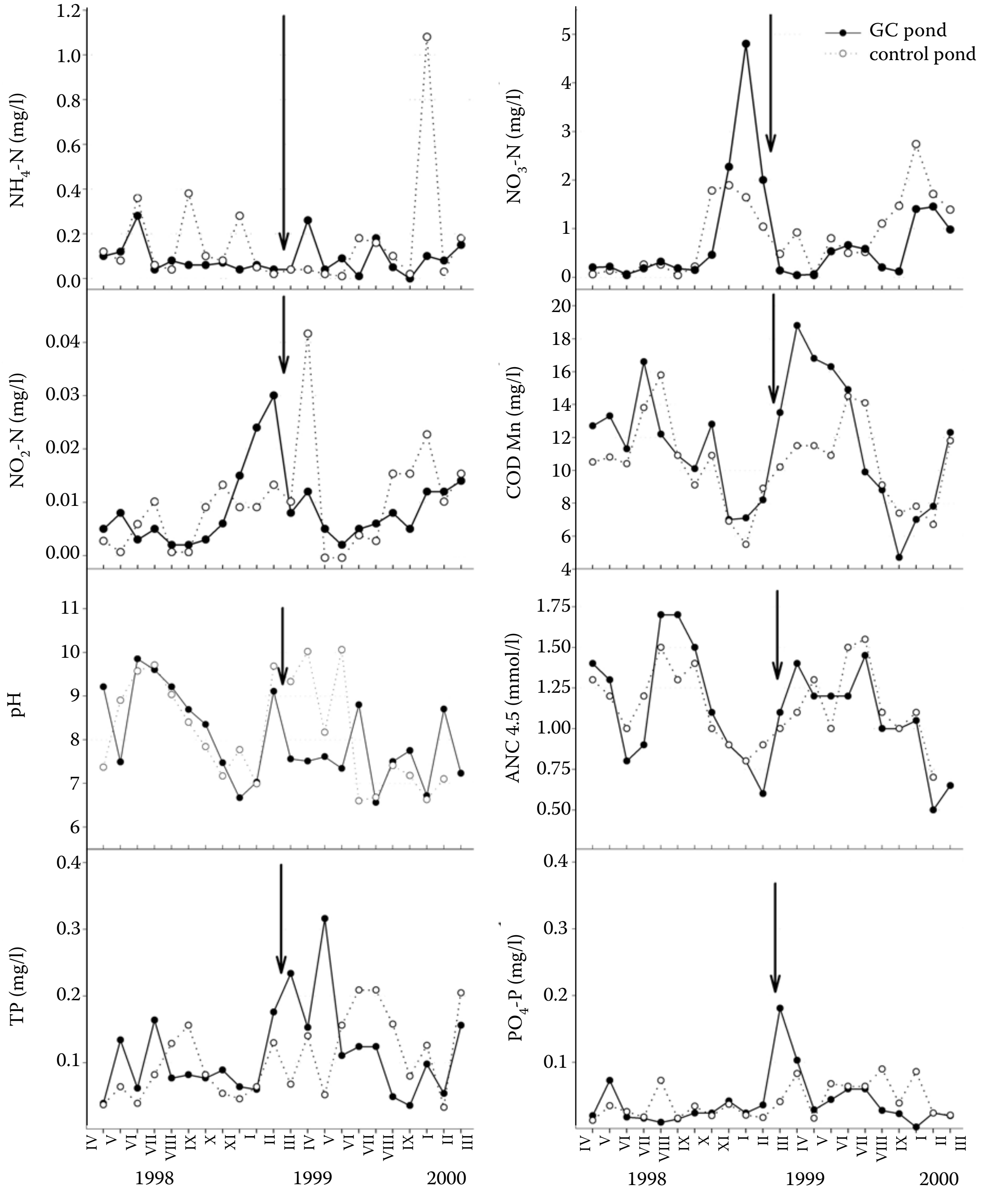

Figure 1. Hydrochemical parameters in the pond stocked with grass carp (GC pond) and in the control pond before (April 1998–March 1999) and after grass carp stocking (April 1999–March 2000); ↓ grass carp stocking

and filamentous algae (F(1,176) = 39.45, $P < 10^{-6}$) were recorded in the pond stocked with grass carp in 1999 in comparison with both the control year 1998 and the same pond, but unstocked in 1999. Average macrophyte biomass decreased in the pond stocked with grass carp from 108.8 g/m^2 in 1998 to 32.8 g/m^2 in 1999, whereas it decreased only slightly in the control pond, from 87.7 g/m^2 in 1998 to 71.1 g/m^2 in 1999. Grass carp influenced the biomass of aquatic plant species selectively.

Stocking of grass carp significantly decreased the biomass of *Cladophora globulina* Kütz., *Eleocharis acicularis* L. and *Potamogeton pusillus* L.. The most preferred plant was the filamentous alga *Cladophora globulina*, the biomass of which decreased from 66 g/m to 0.4 g/m in the pond stocked with grass carp. On the contrary, the biomass of *Spirogyra* sp., *Myriophyllum spicatum* L. and *Ceratophyllum demersum* L. and Lemnaceae (*Spirodela polyrhiza* (L.) Schleid + *Lemna minor* L.) increased significantly after grass carp stocking. The biomass of the other macrophytes was not affected significantly, i.e. *Potamogeton pectinatus* L. (for which only the change of cover was significant), *Ranunculus trichophyllus* Chaix., *Sparganium emersum* Rehm. and *Elatine hydropiper* L. (Pípalová, 2002).

Indirect consequences of grass carp stocking

Water chemistry

A significant change occurred only in NO_3-N concentration (F(1,10) = 6.6, $P = 0.03$) after grass carp stocking. The average NO_3-N concentration decreased after grass carp stocking (from 0.99 mg/l in 1998 to 0.56 mg/l in 1999) but increased in the control pond in 1999 (from 0.67 mg/l in 1998 to 1.06 mg/l in 1999) (Table 1). Most of the hydrochemical parameters, i.e. alkalinity, apparent alkalinity (acid neutralizing capacity: $ANC_{8.3}$ and $ANC_{4.5}$), acidity (base neutralizing capacity: $BNC_{8.3}$), COD_{Mn} and the concentrations of NH_4-N, NO_2-N, TN, PO_4-P and TP were not affected by the stocking of grass carp. The mean PO_4-P and TP concentration significantly increased (impact of year: F(1,10) = 8.4, $P = 0.02$; F(1,10) = 9.9, $P = 0.01$, respectively) in both ponds in April 1999–March 2000 when compared to the time before grass carp stocking (April 1998–March 1999) (Figure 1). Water pH measured three times a day in each month during the growing seasons (1998 and 1999) decreased significantly (F(1,337) = 7.4, $P = 0.01$) in the pond stocked with grass carp in 1999 (Figure 1). Average pH was lower in both ponds in 1999 than in 1998 (Table 1). No differences in oxygen concentration and water temperature between the years and ponds were recorded.

Table 2. Chemical analyses of the upper (u) and lower (l) sediment layers (to the depth of 0.2 m) in 1998 and 1999 (mean ± SD expressed per 1 g of sediment dry weight; *n* = 3)

Parameter	Layer	Before grass carp May–July–Sep. 1998	Stocked with grass carp May–July–Sep. 1999	Control	
				May–July–Sep. 1998	May–July–Sep. 1999
Thickness (cm)	u	6	6	3	3
pH	u	6.90 ± 0.36	6.88 ± 0.27	7.02 ± 0.57	6.98 ± 0.24
Loss on ignition (%)	u	11.3 ± 1.9	12.1 ± 1.1	11.1 ± 2.3	15.3 ± 1.6
	l	6.9 ± 1.1	7.6 ± 1.0	7.5 ± 18.7	8.2 ± 1.0
TC (mg/g)	u	41.1 ± 6.9	37.8 ± 6.2	37.8 ± 3.9	51.6 ± 7.4
	l	18.1 ± 5.9	14.1 ± 3.6	13.5 ± 10.8	16.1 ± 5.4
TP (mg/g)	u	1.0 ± 0.3	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.1
	l	0.9 ± 0.1	0.9 ± 0.1	1.6 ± 1.3	0.9 ± 0.2
PO_4-P(H_2O) (μg/g)	u	2.1 ± 1.8	1.0 ± 0.6	2.0 ± 0.7	2.1 ± 1.5
	l	9.7 ± 3.5	9.1 ± 3.6	27.3 ± 33.3	7.2 ± 7.2
PO_4-P($NaHCO_3$) (μg/g)	u	32.7 ± 24.3	29.4 ± 12.7	37.0 ± 26.9	55.4 ± 22.7
	l	47.4 ± 14.6	32.5 ± 8.2	96.0 ± 112.1	34.3 ± 9.6
NH_4-N(KCl) (μg/g)	u	36.6 ± 43.2	17.6 ± 22.8	31.2 ± 44.8	51.8 ± 51.2
	l	7.6 ± 7.6	4.4 ± 5.6	5.1 ± 1.3	5.8 ± 5.2
NO_3-N(H_2O) (μg/g)	u	1.5 ± 1.2	5.4 ± 6.5	0.8 ± 0.9	25.1 ± 41.0
	l	0.3 ± 0.3	3.7 ± 4.8	0.1 ± 0.2	3.1 ± 4.1

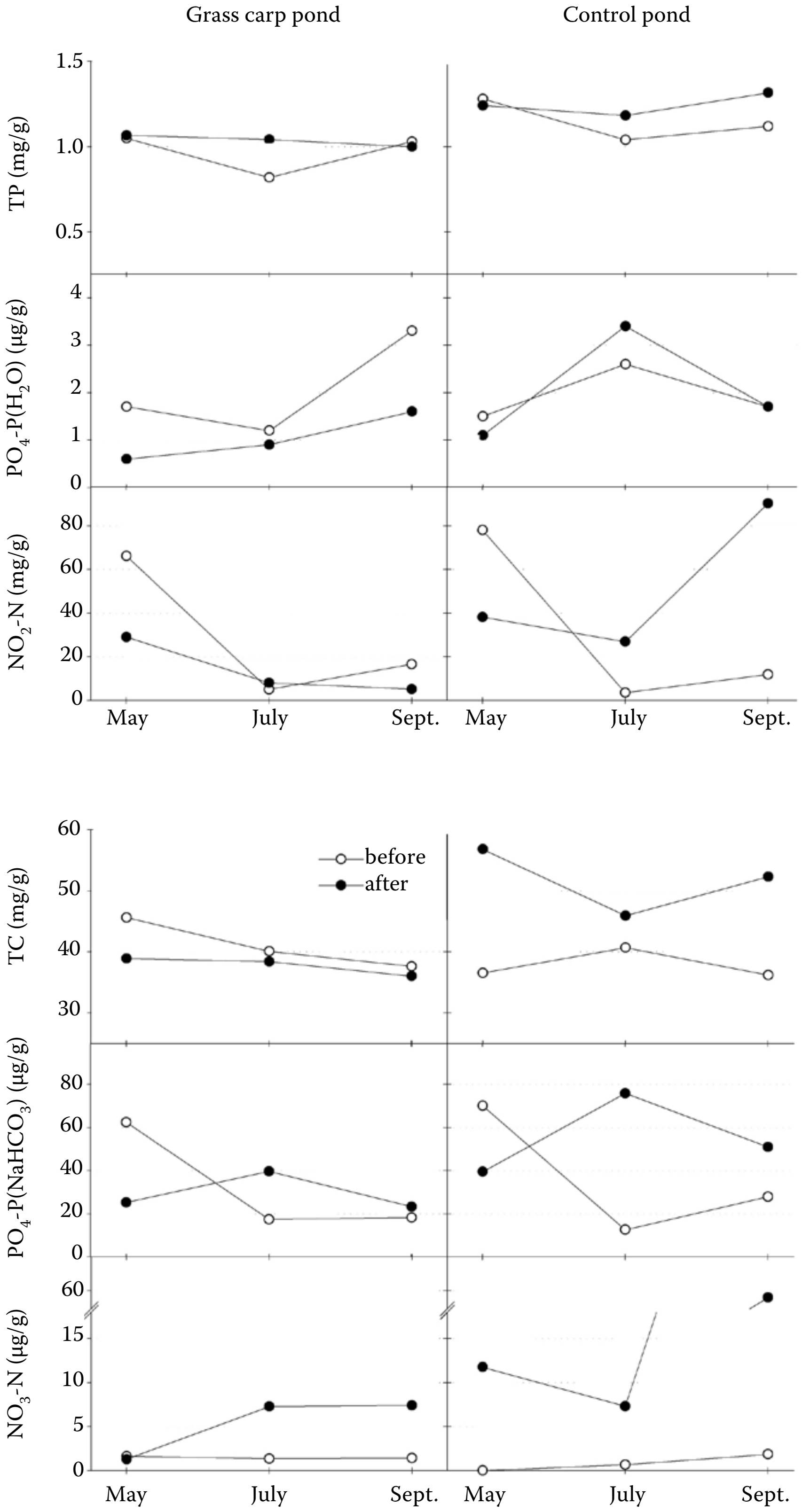

Figure 2. Concentrations of TC, TP (mg/g) and PO_4-P(H_2O), PO_4-P($NaHCO_3$), NH_4-N and NO_3-N (µg/g) in the upper sediment layer in the pond stocked with grass carp and in the control pond before (May–July–September 1998) and after grass carp stocking (May–July–September 1999)

Sediment chemistry

Grass carp stocking significantly influenced the content of organic matter loss on ignition (F(1,32) = 8.2, $P = 0.01$) and thus also the TC concentration (F(1,32) = 14.6, $P = 0.001$) and NO_3-N concentration (F(1,32) = 5.6, $P = 0.024$) in the upper sediment layer. Loss on ignition and TC concentration increased in the control pond in 1999 while they remained almost the same in the pond stocked with grass carp. The concentration of NO_3-N was higher in both ponds in 1999 than in 1998, but these differences were more pronounced in the unstocked control pond (Table 2 and Figure 2). The chemical composition of the lower sediment layer remained unchanged.

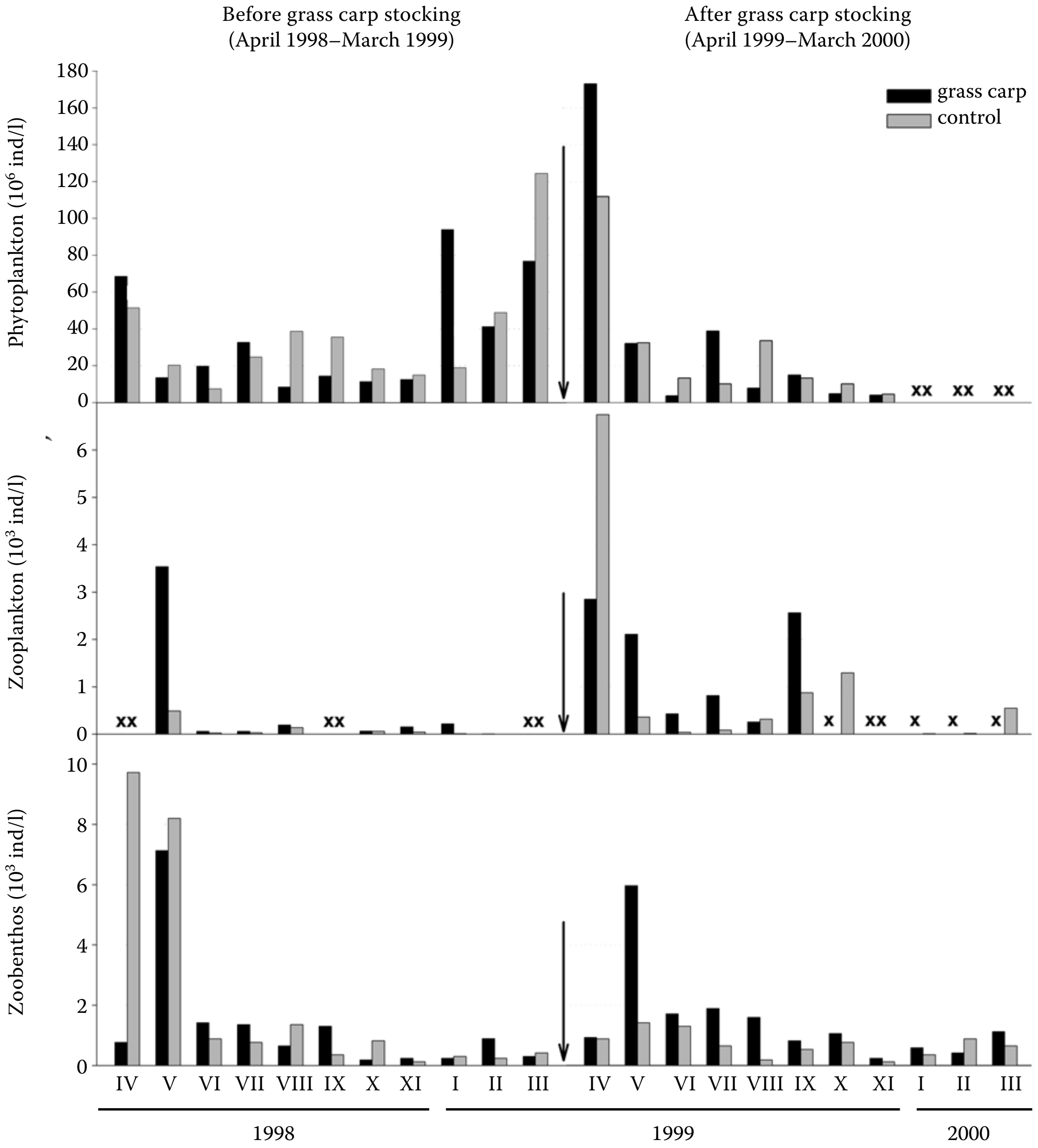

Figure 3. The total abundance of phytoplankton (10^6 ind/l), zooplankton (10^3 ind/l) and zoobenthos (10^3 ind/m^2) before and after grass carp stocking in the grass carp pond and in the control pond; **x** = no data (see methods); ↓ grass carp stocking

Abioseston

In the phytoplankton samples, a significant increase (F(1,7) = 19.8; $P = 0.003$) was recorded in the numbers of small (up to 3 μm) Fe particles (from a mean of 450 per ml before grass carp stocking to 1 900 per ml after it) while in the control pond their numbers decreased (from 1 450 per ml to 1 050 per ml).

Phytoplankton abundance and biomass

The impact of the grass carp on chlorophyll-a (chl-a) concentration was not significant (F(1,76) = 0.4; $P = 0.52$) although the mean chl-a concentration increased from 12.70 mg/m^3 to 20.86 mg/m^3. In the control pond the average chl-a concentration was similar in both years (20.56 mg/m^3 in 1998 and 22.96 mg/m^3 in 1999). Neither did the mean water transparency change significantly (from 72 cm to 66 cm) in the pond stocked with grass carp and remained the same in the control pond during both years (82 cm).

Grass carp stocking influenced neither the total abundance (Figure 3) nor the abundance or the number of species of any of the phytoplankton divisions (Cyanophyta, Dinophyta, Cryptophyta, Chromophyta, Euglenophyta, Chlorophyta and Mycophyta). The dominant divisions in the phytoplankton samples of both ponds were Mycophyta (especially *Saccharomyces* sp.), Chromophyta (Chrysophyceae and Bacillariophyceae – especially the species: *Chrysomonas* sp., *Stephanodiscus hantzschii* and *Chrysococcus rufescens*), Chlorophyta – Chlorophyceae, Ulvophyceae and Zygnematophyceae (Conjugatophyceae – especially *Monoraphidium contortum*, Chlorococcales – *Actinastrum hanzschii*) and Cyanophyta (especially the species: *Aphanizomenon flos-aquae*, *Limnothrix redekei* and *Pseudanabaena limnetica*).

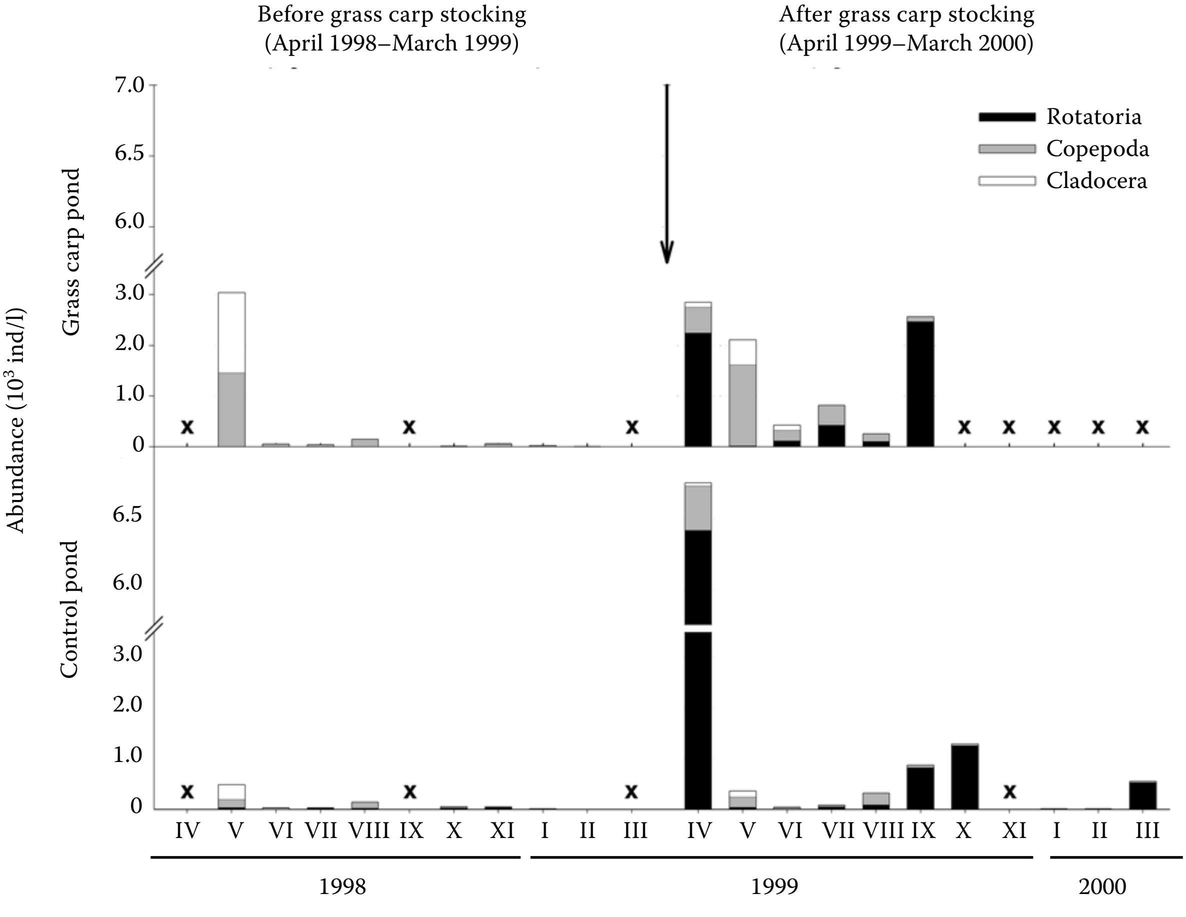

Figure 4. Abundance of Rotatoria, Cladocera and Copepoda (10^3 ind/l) before (April 1998–March 1999) and after grass carp stocking (April 1999–March 2000) in the grass carp pond and in the control pond; **x** = no data (see methods); ↓ grass carp stocking

Zooplankton abundance

Stocking of grass carp influenced neither the total abundance of zooplankton nor the abundance of each of Rotatoria, Cladocera and Copepoda (Figure 3). The increase in zooplankton abundance, especially that of rotifers (Rotatoria), was recorded in both ponds in 1999 compared to 1998 (Figure 4). Rotifers were a dominant group in both ponds (11 species, with prevalence of *Keratella cochlearis*, *K. quadrata*, *Polyathra* sp., *Brachionus angularis*, *Asplanchna* sp. and *Filinia* sp.), followed by copepods (3 species; especially nauplii and adults of *Eudiaptomus vulgaris* and *Cyclops vicinus*) and cladocerans (9 species; especially *Daphnia galeata* and *Bosmina longirostris*).

Zoobenthos abundance and biomass

The impact of grass carp stocking on total abundance and biomass of zoobenthos was insignificant (F = 2.9; *P* = 0.12 and F = 0.1; *P* = 0.77, respectively). The grass carp influenced neither the abundance of dominant groups of zoobenthos (Chironomidae, Oligochaeta) nor the species composition of zoobenthos (Figure 5). Oligochaeta (i.e. Tubificidae) dominated especially during the first half of the growing seasons in both ponds (Figure 5). Total biomass of zoobenthos increased from 6.34 to 15.02 g DW/m^2 in the pond stocked with grass carp and from 4.91 to 15.73 g DW/m^2 in the control pond.

DISCUSSION

Direct consequence of grass carp stocking Biomass of aquatic macrophytes

The grass carp stocking density (29 kg/ha) was intentionally chosen at about one third of the density usually recommended in temperate regions (Krupauer, 1989) in order to save aquatic macro-

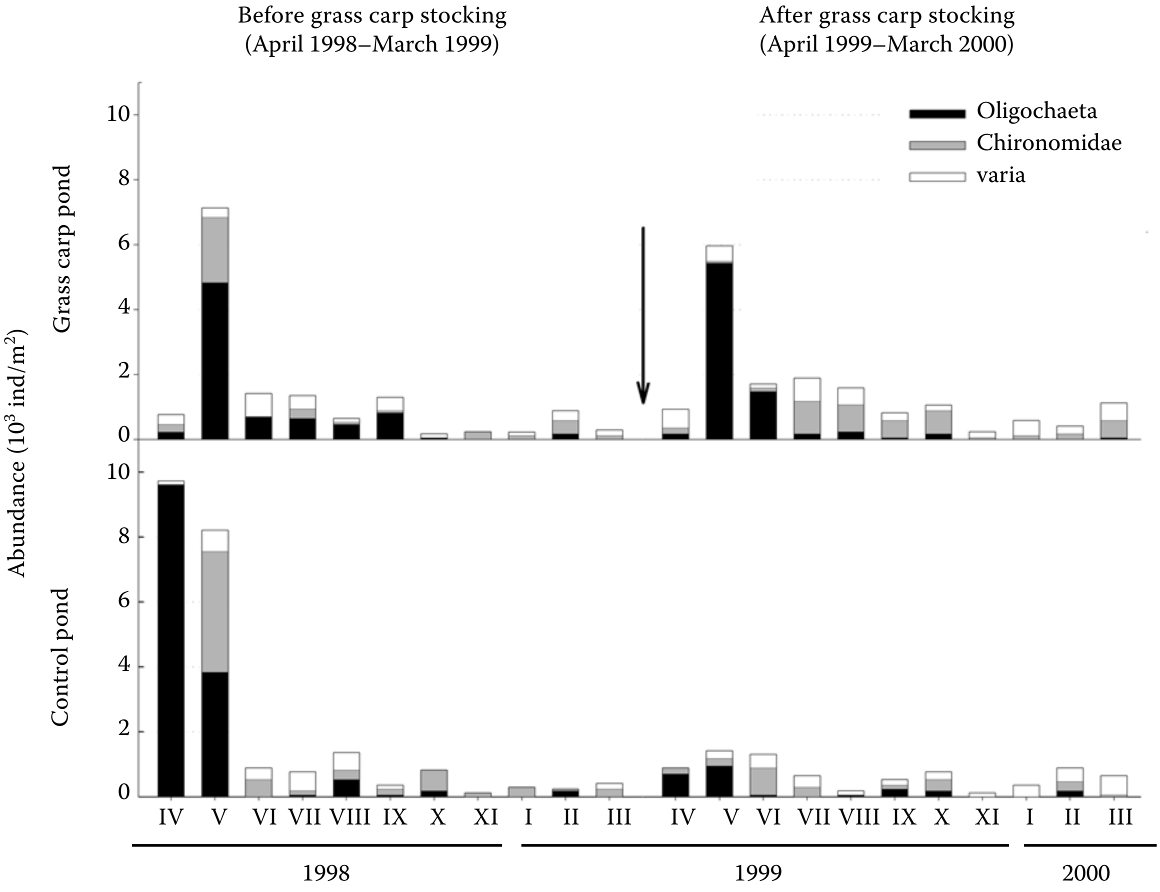

Figure 5. Abundance of Oligochaeta, Chironomidae and other zoobenthos groups (varia) (10^3 ind/m^2) before (April 1998 to March 1999) and after grass carp stocking (April 1999–March 2000) in the grass carp pond and in the control pond; ↓ grass carp stocking

phytes from a complete elimination and thus to study feeding preferences of the grass carp. In spite of this fact, the mean biomass of aquatic macrophytes decreased about 3 times (from 109 g/m^2 to 33 g/m^2, i.e. by about 70%) in the pond with grass carp after their stocking. These data are mostly in general agreement with those reported for small water bodies with similar stocking densities of grass carp (Terrell and Terrell, 1975; Lembi et al., 1978; Blackwell and Murphy, 1996) (Table 3). One year after the grass carp stocking (27 kg/ha), the vegetation standing crop (1 627 kg DW) measured in its annual peak was reduced by 89% in a small pond (3.6 ha) in Georgia (Terrell and Terrell, 1975). *Eleocharis* was a dominant macrophyte species and some *Utricularia* was also present in their pond. Maximum standing crop in our experimental pond (161 kg in June 1998) was reduced by 95% (to 9 kg in June 1999) or by 59% (to 66 kg in August 1999, when some regrowth of vegetation occurred) after the grass carp stocking (calculated according to Pípalová, 2002). The small discrepancies between our data and figures published by Terrell and Terrell (1975) could be caused especially by a difference in water temperature and by the water body depth, as their experiment was carried out under conditions very similar to our study conditions (Table 3). The low vegetation biomass, pre-treated with the grass carp stocking density of 11 kg/ha one year before the experiment, probably resulted in its faster (20 or 40 days) elimination (Lembi et al., 1978) than observed in our experiment. Large grass carp (mean total length 53 cm, mean weight 1.5 kg) with the stocking density of 11 kg/ha nearly eliminated all macrophytes within 5 months in the pond (Sandstone Lake) while the vegetation remained the same even after one-year impact of the grass carp in Jackson Lake (Blackwell and Murphy, 1996) (Table 3). The reasons could be in different depth of the two ponds or in compositions of macrophyte communities, which were dominated only by *Najas quadalupensis* in Sandstone Lake and which composed of *Ceratophyllum demersum* and *Najas guadalupensis* (1:1) in Jackson Lake. In our experiment *Ceratophyllum demersum* was also one of the plant species not preferred by the grass carp. Jackson Lake was stocked with additional grass carp (2 kg/ha, individual weight 0.4 kg), thus the overall stocking density could be similar to that used in our experiment (Table 3). Maximum dry biomass of aquatic macrophytes was then reduced from 236 g/m^2 to 75 g/m^2 (i.e. by 68%) within one year (Blackwell and Murphy, 1996). The grass carp stocking density is only one of the important factors which may influence the intensity of aquatic macrophyte control using grass carp. Water temperature (grass carp feeding activity), water body type (area, depth and hydrological regime), biomass and species composition of aquatic macrophytes available, grass carp size and duration of their impact are also crucial for the results of aquatic macrophyte control (Pípalová, 2006).

The aquatic biomass decreased by 72% the filamentous alga *Cladophora globulina* being reduced, which was the most preferred plant species in our experiment. Pine and Anderson (1991) and Kirkagac and Demir (2004) also reported *Cladophora* as the first plant disappearing after the grass carp introduction. Filamentous algae except for *Spirogyra* and *Mougeotia* (Prowse, 1971) are considered as the most preferred plants by the grass carp (Krupauer, 1968).

Indirect consequences

Water chemistry

The average concentrations of TP, PO_4-P, TN, NH_4-N and NO_2-N remained unchanged under the impact of grass carp in our study. The change of NO_3-N concentration in the water was statistically significant due to the increase in NO_3-N concentration in the control pond water and also due to a relatively small change (decrease) in its concentration in the pond stocked with grass carp (Table 1). The increase in NO_3-N concentration in the control pond water corresponded well with its increased accumulation in the sediment (Table 2). When comparing NO_3-N concentrations in the water only for the growing seasons (May–September in 1998 and 1999) when the grazing of grass carp should be most intensive, its changes were not significant. Seasonal variation (increase in both ponds in 1999) of TP concentration in the water did not allow us to prove the impact of grass carp stocking. In the control pond, nutrients were tied up mostly in the biomass of filamentous algae, from where they were released back to the water after they died off at the end of the growing season. In the experimental pond, grass carp consumed filamentous algae and the nutrients were continuously released into the water from their excrements. The photosynthesis increased pH values but a gradual consumption of filamentous algae could possibly

Table 3. Direct impact of grass carp low stocking density in small ponds

Author(s)	Our experiment	Terrell and Terrell (1975)	Lembi et al. (1978)		Blackwell and Murphy (1996)	
Grass carp stocking density (kg/ha)/ individual weight (g)	29/230	27/550	24/491	23/450	11 + 2/1476 + 405	11/1476
Vegetation before grass carp – max. dry biomass (g/m)/max. total dry biomass (kg)	201/161 (June 1998)	–/1 627 (August 1972)	52/– (June 1976)	17/– (June 1976)	228/– (July 1991)	68/– (July 1991)
After grass carp – max. dry biomass (g/m^2)/max. total dry biomass (kg)	82/66 (August 1999)	–/179 (August 1973)	0 (July 1976)	0 (August 1976)	236 → 75 (July 1992 → July 1993)	0 (September 1991)
Biomass reduction by (%)	95/59 (June/August 1999)	89	100	100	0 → 60	100
Species of vegetation	*Cla. g.*, *Pot. pu.*, *Pot. pe.* and *Ele. a.*	*Ele.* and *Utr.*	*Pot. fo.*, *Pot. cr.*, *Myr. s.*, *Char.* and *Spi.*		*Naj. g.* and *Cer. d.* (1:1)	*Naj. g.*
Length of experiment	1 year	1 year	20 days	40 days	1 → 2 year	5 months
Water body	grass carp pond	Whittimore	A-2 (1976)	A-3 (1976)	Jackson Lake	Sandstone Lake
Area (ha)	0.08	3.6	0.2 – 0.3		0.8 – 30	
Mean depth (m)	0.5	4.8 (max.)	0.7 – 1.2 (max.)		about 1.8[a] (max.)	about 0.6[a] (max.)
Mean water T (± SD) (°C)	19.0 ± 1.8[b]	Georgia	Indiana		Central Texas	

a = estimated from Secchi disc transparency; b = during the growing season 1999; *Cer. d.* = *Ceratophyllum demersum*, *Char.*: Chara, *Cla. g.* = *Cladophora globulina*, *Ele. a.* = *Eleo-charis acicularis*, *Myr. s.* = *Myriophyllum spicatum*, *Naj. g.* = *Najas guadalupensis*, *Pot. cr.* = *Potamogeton crispus*, *Pot. fo.* = *P. foliosus*, *Pot. pe.* = *P. pectinatus*, *Pot. pu.* = *P. pusillus*, Spi. = Spirogyra and Utr. = Utricularia cause the pH decrease in our experiment (Figure 1). Most of the significant changes in water chemistry were reported from long-lasting experiments in American lakes (Mitzner, 1978; Small et al., 1985; Maceina et al., 1992) or from the Danube River branch (Tomajka, 1995), especially following the total eradication of macrophytes.

Studies dealing with indirect changes in water bodies after grass carp stocking are usually complicated by problems with experimental design mentioned in the introduction. Furthermore, the water quality changes as a result of plant removal by the grass carp are mostly affected by the intensity of direct impact and also depend on whether there remained some aquatic macrophytes after the grass carp removal. Lembi et al. (1978) and Venter and Schoonbee (1991) reported changes in water chemistry following the grass carp stocking under conditions comparable to those in our study. Venter and Schoonbee (1991) did not find any significant changes in water chemistry (turbidity, pH, dissolved oxygen, alkalinity, total hardness, concentrations of NH_4^+, NO_2^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) in the Florida Lake (near Johannesburg, 26 ha) even though the dry biomass of aquatic plants decreased almost 6 times (from 20.6 g/m^2 to 3.5 g/m^2) during the one-year study. Unfortunately, this biomass decrease could be caused by the feeding activity of grass carp only partly. A dramatic reduction in *Potamogeton pectinatus* and *Lagarosiphon* might be due to the plant sampling campaign before grass carp stocking, when irregularly dense patches of both plants were sampled. Furthermore, the very low stocking density of grass carp (35 ind/ha, i.e. 2.1 kg/ha) of low individual weight (59 g) was used and thus the grass carp could be endangered by predation. Lembi et al. (1978) reported that only water turbidity increased when compared to ponds without fish in the small Indiana ponds (0.21–0.30 ha) with a low stocking density (11 kg/ha) during a 4-month study. This effect was not observed in ponds with higher grass carp stocking densities (20 and 69 kg/ha). The initial differences in turbidity (and also in other parameters: alkalinity, TP, PO_4-P, NO_3-N, NH_4-N, $N_{org.}$) among the control ponds and ponds with all 3 stocking densities of grass carp (11, 20 and 69 kg/ha) were not reported.

Mean total phosphorus and nitrogen amounts in water were 37 g (93 µg/l × 400 m^3 water volume) and 1 056 g (2.64 mg/l × 400 m^3) before grass carp stocking, respectively. The maximum standing crop of aquatic macrophytes before the introduction of grass carp (June 1998) calculated from the data of Pípalová (2002) was 161 kg DW in the experimental pond. Assuming the phosphorus content of 0.4% and nitrogen content of 2.5% (Dykyjová, 1979), the maximum of 600 g P and 6 400 g N could become available for a later release into the pond ecosystem. Grass carp consumed 95% of this amount (June 1999), thus the degree of nutrient enrichment by grass carp feeding activities was probably within the nutrient buffering capacity of the pond.

Sediment chemistry

In our experiments no changes in the chemical composition of the sediment (TC, TP, PO_4-P(H_2O), PO_4-P($NaHCO_3$), NH_4-N and NO_3-N) were recorded in the stocked pond (Table 2). The markedly higher increase in organic matter content and NO_3-N concentration in the upper sediment layer in the control pond could be caused by the accumulation of the dead filamentous alga (*Cladophora globulina*) since the middle of the growing season in 1999. Only Terrell (1975) evaluated sediment chemistry 1 year after grass carp stocking and found out significant increases in Fe, Mg and P-PO_4 concentrations in the sediment in Georgia ponds (USA). No significant changes in phytoplankton and zooplankton numbers and structure or in water chemistry were detected (Terrell, 1975). He assumed that the nutrients released by grass carp were incorporated into the sediment. Although we did not measure the Fe concentration in the sediment, we recorded an increase in the Fe particle amount in the phytoplankton samples. The concentration of PO_4-P in the sediment did not change in our experiment, while Terrell (1975) determined a more than twofold concentration of PO_4-P (8.9 µg/g) in ponds stocked for one year with grass carp when compared to those without fish (3.7 µg/g). Unfortunately, Terrell (1975) did not report the stocking density of grass carp. Furthermore, the concentrations of Fe, Mg, Mn, Ca and PO_4 in 4 ponds stocked with grass carp were compared to those without fish not reporting the differences between ponds before the experiment.

The mean total phosphorus content in the upper sediment layer (to the depth of 4 cm) was estimated to be 8.2 kg in the pond before grass carp stocking. This amount is 10 times higher than the amount of phosphorus bound in plants (0.6 kg) and thus no significant changes in nutrients in the sediment can be expected.

Phytoplankton abundance and biomass

No significant changes in either species composition or biomass (chl-a) of phytoplankton were found out. The increase in phytoplankton biomass has usually been explained by better availability of nutrients (especially phosphorus) released from grass carp excrements. Since no significant changes in water quality were found, the nutrients resulting from feeding on aquatic macrophytes by grass carp were not significantly demonstrated in phytoplankton. Furthermore, some species of aquatic plants remained ungrazed, thus being able to compete for nutrients with phytoplankton. Buck et al. (1975) showed in his tank experiment that *Ceratophyllum demersum* was able to compete for nutrients even with phytoplankton. Cassani et al. (1995) also found that the annual mean chlorophyll-a concentration remained stable in the Florida urban impoundments (0.8–45.3 ha) where macrophytes were only suppressed, but varied greatly at some sites where they were eliminated.

The water transparency did not differ significantly in our experiment either, which corresponds with insignificant change in the chl-a concentration and incomplete reduction of aquatic macrophytes. However, there was a tendency to higher chl-a concentration and lower water transparency in the pond stocked with grass carp. The decrease in water transparency (increase in turbidity) following from the grass carp stocking was related either to abiotic factors (sediment movements due to the wind and/or fish searching for food) (Lembi et al., 1978; Leslie et al., 1983; Mitchell et al., 1984; Bonar et al., 2002) or to biotic factors (phytoplankton biomass) (Maceina et al., 1992). The source of turbidity mostly depends on the intensity of macrophyte biomass reduction. An increase in abiotic turbidity is often connected with elimination or great reduction of aquatic macrophytes biomass. When only non-preferred plants or even no plants are left, grass carp can then search their food in the sediment and thus elicit increased water turbidity. The effect of wind on the sediment without plant canopy could also be intensive. The biomass of phytoplankton remains mostly the same probably due to the shading effect of turbid water. On the other hand, the phytoplankton has a shorter turnover rate than aquatic macrophytes and sometimes can successfully compete for nutrients and light during the process of macrophyte reduction by grass carp.

Zoobenthos and zooplankton abundances

In our experiment, the grass carp influenced neither the total abundance of zooplankton and zoobenthos nor the abundance of any zooplankton and zoobenthos species. This is in agreement with the research conducted e.g. by Terrell (1975) and Maceina et al. (1992). Direct consumption of zooplankton and zoobenthos by grass carp is insignificant, even when no plants are available (e.g. Opuszyński, 1972). The zooplankton biomass may increase after the grass carp stocking because of the increase in the phytoplankton amount (Richard et al., 1985) and the presence of grass carp faeces with bacteria attached to them (Takamura et al., 1993). Changes in zooplankton following the grass carp stocking can be selective. Crustaceans were replaced by rotifers when they had lost their shelters from size-selective fish predation (Vranovský, 1991).

Changes in benthos corresponded with a reduction of aquatic vegetation, which stabilises sediments, provides additional substrate (roots and decaying material) and water quality changes (Gasaway, 1979). Zoobenthos became more than twice as abundant as it had been before the grass carp introduction in the reservoirs of the Amudarja River (Turkmenistan). Because the annual vegetation die-off was prevented by the presence of grass carp, oxygen and water quality were improved (Aliev, 1976). Although the gradual grazing of aquatic vegetation by grass carp prevented the accumulation of dead plant biomass, the abundance and biomass of zoobenthos did not change in our experiment. This corresponds with insignificant changes in water and sediment chemistry in the pond stocked with grass carp.

More detailed experiments using the replicated BACI design (i.e. similar control ponds and similar ponds stocked with grass carp) with more frequent logging of indirect changes in the water and sediment chemistry and in biotic communities are necessary to understand and quantify the impact of lower grass carp stocking densities on the water body ecosystem.

CONCLUSION

Indirect changes and consequences (increased nutrients in the water and sediment, changes of phytoplankton, zooplankton and zoobenthos) caused by the low stocking density of grass carp (29 kg/ha) were rather negligible although the biomass of aquatic macrophytes (especially filamentous algae) was reduced three times. Nutrients released by grass carp were probably reused in the water and/or sediment by macrophytes left in the pond. Furthermore, the changes in water and sediment chemistry were greater in the pond without grass carp due to the presence and subsequent die-off of the filamentous alga *Cladophora globulina* and thus the changes caused by grass carp stock were not statistically significant.

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