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EFFECTIVE CONTROL OF NUISANCE CYANOBACTERIAL BLOOMS BY BIOMANIPULATION

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Abstract

Eutrophication is a general problem of small and large lakes. Excessive growths of algae especially cyanobacteria in natural and man-made lakes regularly present problems for recreational, industrial and municipial users of freshwaters. In freshwaters cyanobacteria cause the most serious deterioration of waters due to their hepatotoxin and neurotoxin production. Methods of controlling algae by removal of nutrients with tertiary sewage treatment is quite expensive. Thus there is a need for algal control method that is environmentally acceptable and inexpensive. The food and feeding of silver carp (*Hypophthalmichthys molitrix* Val.) attracted significant interest in the 1970s because this fish species was considered a potential tool for controlling eutrophication. Studies in lakes, ponds and experimental enclosures led to contradictory results. In some cases silver carp decreased the phytoplankton biomass, however other studies showed that silver carp did not reduce the phytoplankton biomass. Analysis of the food ingestion and the feeding selectivity of silver carp demostrated that its food selectivity is a passive function of the filter morphology. The lower limit for available food particles is about 10 µm. A logical consequence of the above-mentioned result is that silver carp can not control the total algal biomass, but will modify the size structure of algal communities. *In vitro* experiments with digestive enzymes of this species resulted in a very fast disintegration of non-mucilaginous cyanobacteria, digestion of diatoms and cryptophytes was also effective, but mucilaginous cyanobacteria and green algae (*Chlorococcales*) proved practically indigestible. A large hypertrophic shallow reservoir (Marcali reservoir, Hungary) was stocked with 1+ fish (approximately 500 kg ha^{-1}) consisted of 80% of silver carp. Size-selective filtration of the dense silver carp population completely inhibited the growth of filamentous nitrogen fixing cyanobacteria.

Key words: biomanipulation, cyanobacteria, eutrophication, silver carp, water quality control

Introduction

Nuisance algal blooms seriously deteriorates the water quality all over the world. In temperate and tropical freshwaters mass development of colonial or filamentous cyanobacteria is a common phenomenon. The problems of algae produce include impedance of flow, blockage of filters and production of taint and toxins. In freswaters cyanobacteria cause the most serious deterioration of water quality due to their hepatotoxin and neurotoxin production. Algae are difficult to control and the most effective methods -primarily the removal of nutrients with tertiary sewage treatment- are very expensive. Copper sulphate has been used as algicide for many years but it is now banned in many countries because of its toxicity.

One of the alternative tool for water quality management is the biomanipulation (the control of natural aquatic organisms to improve water quality instead of chemical treatment or nutrient management). Most efforts to reduce the algal development in lakes and reservoirs have focused on manipulation of zooplanktivorous fish and zooplankton populations to increase grazing pressure on phytoplankton while filter feeding phytoplanktivorous fish had much less attention.

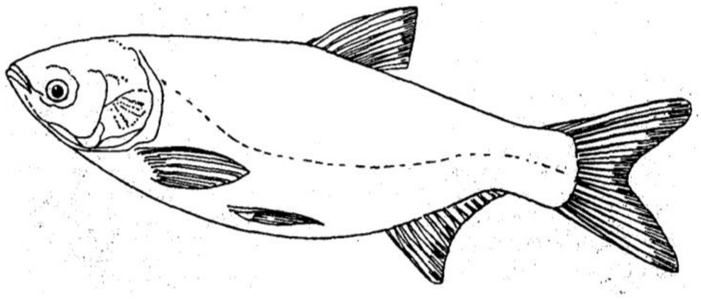

Fig. 1. Silver carp (*Hypophthalmichthys molitrix*)

In the early seventies silver carp (*Hypophthalmichthys molitrix*) (Fig. 1) attracted significant interest because this species was considered a potential tool for controlling eutrophication and therefore have been introduced worldwide to control algae and/or enhance fish production in lakes. At that time studies in lakes, ponds and experimental enclosures led to contradictory results. In some cases silver carp introduction caused insignificant decrease of phytoplankton biomass while in other cases the introduction of these fish enhanced the algal development (Januszko 1974, 1978; Kajak *et al.* 1975).

Analysis of the food ingestion and the feeding selectivity of silver carp supports the hypothesis that its food seletivity is a mechanical, passive function of its filter morphology. Distances between the silver carp's gill rakers range from 12 to 26 μm (Hampl *et al.* 1983) which is consistent with experimental results that the lower limit for available food particles is about 10 μm (Cremer and Smitherman 1980). A logical consequence of the above-mentioned results that silver carp grazing can not control the whole algal community, but will modify the size structure of phytoplankton by the selective filtration of large sized algae (Smith 1989). In a whole lake experiment (eutrophic shallow lake -Lake Donghu, China) stocking silver carp, and bighead carp at high densities caused dramatic changes in the lake ecosystem where the silver and bighead carp filtration significantly decreased the *Microcystis* biomass and led to the dominance of small sized green algae (Miura 1990). Enclosure experiments in a tropical reservoir demonstrated the successful reduction of *Cylindrospermopsis raciborskii* biomass in presence of silver carp (Starling and Rocha 1990). In Hungary in a shallow hypertrophic reservoir during a ten year long experiment we investigated the food and feeding of silver carp and their effect on the phytoplankton structure and development. In this paper we summarise the most important results of this long term study.

Materials and methods

General characteristics of the reservoir

Marcali reservoir with a surface area of 4 km^2 and average depth of 1,8 m was built in 1984. The reservoir has elongated shape (length: 5 km, average width: 0.8 km), with one inflow at the upper part and one regulated outflow at the dam. It was filled and emptied biennially. At the beginning of these cycles, the reservoir was heavily stocked with planktivorous fishes. Three species of fish (age 1$^+$) were introduced regularly. About 80% of the stocked and harvested fish population was silver carp (*Hypophthalmichthys molitrix* Val.), 15% bighead carp (*Aristichthys nobilis* Rich.) and 5% common carp (*Cyprinus carpio* L.).

In the different farming cycles the introduced fish biomass varied between 500 and 1800 kg ha^{-1} but the net fish production show little variation with an average of 1060 kg ha^{-1} 2 years^{-1}. This result was achieved without food addition while in the common fish farms in the Balaton region the yearly total production varied between 500-700 kg ha^{-1} year^{-1}.

The catchment area is 586 km^2 and the average water residence time is about 4 months and the total phosphorus load was found to be 73 kg ha^{-1} year^{-1}. Due to the high phosphorus load from point- and non-point sources the reservoir water has hypertrophic character where the annual average chlorophyll-a concentration usually exceeds the 100 µg l^{-1}. The water temperature is normally above 20 °C from the end of May to early September. During hot spells it may increase to 30 °C. Limestone and dolomitic rocks predominate in the catchment area, consequently the water discharged to the reservoir carries Ca^{2+}, Mg^{2+} and HCO_3^- in high concentrations. The conductivity of the water usually varies between 460 and 900 µS cm^{-1}, with an average of 600 µS cm^{-1}. The pH of the water is normally about 8.3. The concentration of dissolved humic substances, estimated by colour measurement, ranges between 19 and 67 mg Pt l^{-1} (V.-Balogh and Vörös 1997; Vörös *et al.* 1998).

Water quality monitoring

There has been a regular water quality monitoring of the reservoir since its construction. The water discharge and more than 30 chemical parameters (including N and P forms) were measured fortnightly in the inlet and the outlet. The quantity and composition of phytoplankton were determined monthly. Water samples were preserved with Lugol-solution for the nanophytoplankton- and microphytoplankton measurements. For the quantitative determination of phototrophic picoplankton the subsamples were fixed with formaldehyde (1.5 % final concentration). Algal taxa larger than 2 µm were counted with an inverted microscope (Utermöhl 1958). The quantity of picoplankton (< 2 µm) was determined by epifluorescence microscopy (Caron 1983). Biomass (fresh weight) of different algal taxa was calculated from cell volumes and abundances (Vörös *et al.* 1997).

The quantity of introduced fish populations was measured at the beginning and at the end of every 2-year working cycle. The net fish production was calculated by the differences of these values. Before the end of every 2-year farming cycle, the total biomass of macrophytes was estimated. The nitrogen and phosphorus content of the dominant plant and fish species were determined.

Gut content analysis

In 1988 parallel with the phytoplankton sampling, silver carp (age 1+) was collected with fishing nets from the three sampling sites. The fish samples were immediately dissected for the collection of gut contents. Five specimens of silver carp were examined at each collection from each sampling area. The whole intestine contents were homogenised. Aliquots were resuspended with filtered lakewater and preserved with formaldehyde solution (5 % final concentration). After this treatment the microscopic investigation of these diluted samples was identical with phytoplankton samples.

The index of electivity (*E*) for each of the different food components (algal taxa) was calculated by Ivlev's formula (Ivlev 1961):

$$E = \frac{g_i - w_i}{g_i + w_i}$$

Where g_i = relative frequency of phytoplankton species in the gut of fish, w_i = relative frequency of the same species in the surrounding water. The E values can range between –1 (total neglect) and +1 (total preference), whereas the $E = 0$ value shows an phytoplankton uptake corresponding to its frequency of occurrence in the surrounding water.

Experiments

For the *in vitro* digestion experiment of phytoplankton the reservoir's water was concentrated approximately by 100 times with centrifugation. Filamentous, nitrogen fixing blue-green algae were collected from Lake Balaton. For the same experiment starved silver carps (2+) were dissected and the gut juice was collected from the first 30 cm part of the intestine. Half a ml of the freshly collected particle free gut juice (GF/C filtered) and an equal volume of the concentrated phytoplankton suspension was mixed and incubated 0, 2, 4, 8, 15, 30, 60 and 120 minutes in test tubes at 25 °C. The incubations were stopped with formaldehyde (35%) addition. The biomass changes of different algal taxa during the incubation was followed by microscopic investigation. The digestion process was characterised by the quantitative changes of different algal taxa during the incubation time (Vörös *et al.* 1997).

Selenastrum capricornutum bioassay (US EPA 1971) was used for the estimation of biologically available dissolved phosphorus in the reservoir water in 1991 (Vörös 1991). Three experimental enclosures (3 m in diameter and 2 m in height) were installed in the open water close the outflow of the reservoir in July 1994. The enclosures were free of fish while in the open water the total fish stock was approximately 1000 kg ha^{-1} at the same time. In these enclosures the effect of NO_3-N enrichment on the phytoplankton biomass and composition was investigated (Présing *et al.* 1997).

Results and discussion

Size selective filtration of algae by silver carp

The morphology of the silver carp's filtering apparatus predicts a lower limit (about 12 µm) of the available particle size (Hampl *et al.* 1983). Cremer and Smitherman (1980) found the predominance of *S. caprinornutum* in the plankton samples but not in the intestinal content. The absence of *S. caprinornutum* in the intestinal content was probably reflective of its size, generally being less than 10 µm. Feeding experiments with different particle sizes (Smith 1989) support this hypothesis.

Our simultaneous size structure analysis of natural phytoplankton and algae of gut contents of silver carp in Marcali reservoir were found in accordance with these results. Planktonic algae larger than 100 µm did not occur in the reservoir during the observed period. The contribution of algae smaller than 10 µm to the total phytoplankton biomass in May was low (20 %) and later it was observed higher (30-50 %). The 10-30 µm size algal group had a similar importance as it was observed at the previous group, and the contribution of algae larger than 30 µm in most cases was unimportant (Fig. 2a). In spite of the permanent and significant contribution of small phytoplankters (<10 µm) to total phytoplankton biomass the carp's guts rarely contained algae smaller than 10 µm, and the frequency of larger form were much higher than in the surrounding water (Fig. 2b). The silver carp positively selected the >10 µm phytoplankton size fraction, but species composition of this algal size group showed large difference between the natural phytoplankton communities and in the gut content. These dissimilarities are partly explainable by the increasing filtration rate with the increasing particle diameter (Smith 1989). Probably another factor, mainly the taxon specific digestion was also responsible for these differences.

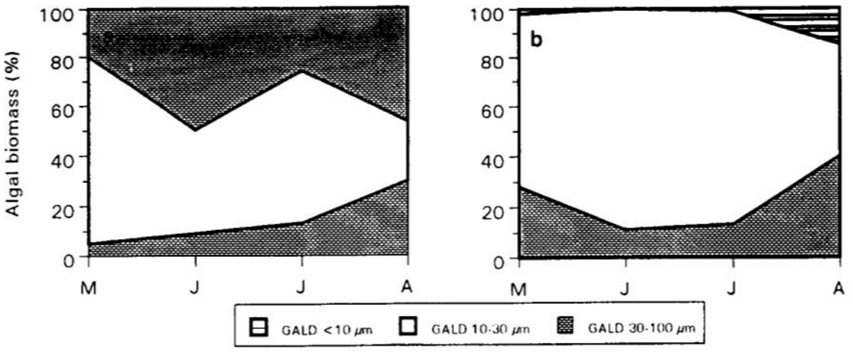

Fig. 2. Temporal changes of phytoplankton size (GALD) classes (% of biomass) in open water (a) and in gut content of silver carp (*Hypophthalmichthys molitrix*) (b) in Marcali reservoir (GALD = greatest axial linear dimension)

Taxon specific digestion of algae by silver carp

During the *in vitro* digestion experiment carried out with the reservoir algae (Fig. 3) the biomass of *Chlorococcales* green algae (*Scenedesmus* sp., *Tetraedron* sp., *Tetrastrum* sp., *Pediastrum* spp., *Oocystis* spp.) only slightly decreased. At the end of the

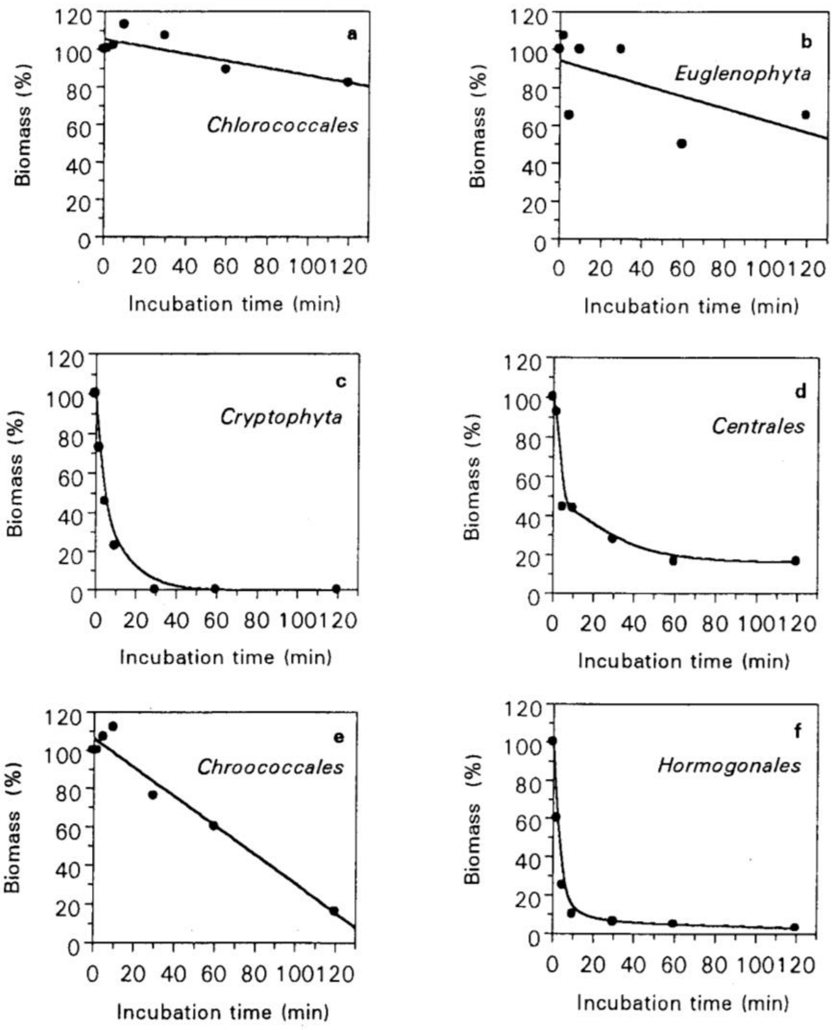

Fig. 3. In vitro digestion (remained biomass in % of initial algal biomass after incubation periods) of different phytopankton groups from Marcali reservoir (a-e) and cyanobacteria from Lake Balaton (f) by gut juice of silver carp (*Hypophthalmichthys molitrix*)

experiment more than 80 % of their original biomass were present. Their cells were found intact, and they did not show any damage (Fig. 3a). The biomass of *Euglenophyta* (*Euglena* sp., *Phacus* sp., *Trachelomonas* sp.) showed also a moderate, 40 % decrease during the incubation period (Fig. 3b). All the observable *Euglena* cells became spherical. The biomass of *Cryptomonas* and *Rhodomonas* cells decreased rapidly and after 30 minutes they completely disintegrated (Fig. 3c). The behaviour of centric diatoms (*Cyclotella* sp. and *Stephanodiscus* sp.) was similar, but about 20 % of them were resistant against the silver carp digestion enzymes (Fig. 3d).

The disappearance of *Chroococcales* blue-green algae showed a linear decrease and at the end of the experiment less than 20 % of the original biomass was detectable (Fig. 3e). In case of nitrogen fixing cyanobacteria (*Aphanizomenon flos-aquae* and *Cylindrospermopsis raciborskii)* the enzymatic action was extremely rapid, in ten minutes about 90 % of their biomass disappeared. At the end of the incubation time the presence of *A. flos-aquae* and *C. raciborskii* was not detectable (Fig. 3f). The digestion process of filamentous blue-green algae was observed under microscope too. It was well visible that the filaments disintegrated completely very fast (30-60 second enzyme treatment).

Our *in vitro* digestion experiments gave a very good explanation for the absence of some phytoplankton taxa in the fish guts. Studying zooplankton feeding, Porter (1973) observed three groups of phytoplankton that cut across the taxonomical lines: non available, available but non digestible and available and digestible. In the case of silver carp and probably for other filter feeding fishes this classification could be valid also, but the size limits and the digestibility of different taxa can be alter. According to our results in the case of silver carp these phytoplankton groups were the following:

1. Non available algae (<10 µm): autotrophic picoplankton (*Synechococcus* type), *Rhodomonas minuta*, small centric diatoms, several *Chlorococcales* green algae are the dominant members of this group of phytoplankton.

2. Available (>10 µm) but non digestible algae: the most common *Chlorococcales* green algae belong to this group. Besides the *Scenedesmus, Tetraedron, Tetrastrum, Pediastrum, Oocystis, Coelastrum* and *Chodatella* species some mucilagenous *Chroococcales* blue-green algae and *Euglenophyta* were not easily digested.

3. Available (>10 µm) and digestible algae: inside this size category, non mucilaginous filamentous cyanobacteria (*Aphanizomenon flos-aquae, Cylindrospermopsis raciborskii*), *Cryptomonas* species and diatoms proved to be well digestible.

Depending on the species composition of phytoplankton, the gut content analysis and the index of electivity (Table 1.) may lead to the importance of group 2 being considerably overestimated, and furthermore may not be able to reveal the importance of group 3 or may lead to its being considerably underestimated. This group (available (>10 µm) and digestible algae) has high feeding importance. This means, that the analysis of gut content and the index of electivity can result only information about the size selectivity of the fish filtration process if the sizes of the food particles are known.

Table 1. **The common algal taxa in the water of Marcali reservoir and in the gut content of silver carp (*Hypophthalmichthys molitrix*). The species are ranked by decreasing values of electivity index (E).**

Taxon	Electivity (E)
Cyclotella meneghiniana Kütz.	0,91
Stephanodiscus astraea (Ehr.) Grun	0,89
Planctonema lauterbornii Schmidle	0,66
Other species	0,60
Nitzschia sublinearis Hust.	0,53
Chlorella sp (10 μm)	0,35
Nitzschia acicularis W. Smith	0,20
Melosira granulata (Ehr.) Ralfs	0,05
Stephanodiscus hantzschii Grun.	0,01
Scenedesmus quadricauda (Turp.) Bréb.	-0,03
Crucigenia quadrata Morren	-0,24
Tetrastrum staurogeniaeforme (Schröd.) Lemm.	-0,27
Gomphosphaeria lacustris Chod.	-0,28
Trachelomonas volvocina Ehr.	-0,39
Scenedesmus sp.	-0,48
Ankistrodesmus angustus Bern.	-1,00
Chlamydomonas sp. (10 μm)	-1,00
Cryptomonas sp. (25X15 μm)	-1,00
Cryptomonas sp. (40X25 μm)	-1,00
Didymocystis planktonica Kors.	-1,00
Euglena pisciformis Klebs	-1,00
Lyngbya limnetica Lemm.	-1,00
Pediastrum boryanum (Turp.) Menegh.	-1,00
Peridinium inconspicuum Lemm.	-1,00
Phacotus lenticularis Ehr.	-1,00
Phacus longicauda (Ehr.) Duj.	-1,00
Phacus pyrum (Ehr.) Stein	-1,00
Picocyanobacteria	-1,00
Rodomonas minuta	-1,00
Scenedesmus acutus Meyen	-1,00

Effect of silver carp on water quality

The above mentioned results suggest that the silver carp grazing has a complex effect on the phytoplankton structure. The size selective filtration can control the large sized and digestible algae, including the bloom forming cyanobacteria. The size of the most common filamentous nitrogen fixing cyanobacteria ranges between 100 and 200 μm There is much experimental evidence for this treatment. Miura (1990) reported the effective control of *Microcystis* sp. by silver and bighead carp filtration leading the dominance of green algae smaller than 10 μm.

Enclosure experiments in a tropical reservoir demonstrated the successful reduction of *C. raciborskii* biomass in the presence of silver carp (Starling and Rocha 1990). Our results are in accordance with the view that the growth of small sized phytoplankton ($<10\mu m$) is significantly enhanced in the presence of silver carp because the size selective filtration of fish controls their large sized algal competitors and grazers (Kajak 1979; Spataru and Gophen 1985; Drenner *et al.* 1986). It is well known that silver carp is able to reduce the biomass of zooplankton significantly (Kajak *et al.* 1975; Opuszynski 1979; Spataru and Gophen 1985).

In deep lakes the silver carp filtration can decrease the biomass of algae belonging to the second group (available ($>10\mu m$) but non digestible algae) because faecal pellets sink into the non-photic layer. In shallow lakes the egested faecal pellets continuously resuspend in the euphotic zone. Consequently in these lakes ingestion can be advantageous to these non digestible species because they can take up mineral nutrients in the fish gut. Porter (1973, 1976) explained the resistance of several algal species to the digestive enzimes of zooplankters by the presence of gelatinous sheath. Its mucopolisaccharide sheats acted as molecular sieves, allowing small molecular and ionic nutrients to enter while keeping out large digestive enzymes. We found that not only can the mucilage protect the algal cells against the digestive enzymes, but also that in case of *Chlorococcales* green algae their cellulose cell walls are impenetrable to the digestive enzymes of the silver carp.

In the Marcali reservoir there was a large permanent phosphorus pool which was not utilized by algae or macrophytes. The average soluble reactive phosphorus concentration in the water was quite high ($\approx 100\ \mu g\ l^{-1}$) while the inorganic nitrogen was the limiting nutrient. During the summer months the inorganic nitrogen to soluble reactive phosphorus ratio was far below the Redfield-ratio (5.7 weight/weight) and varied about 1.0. By this ratio the reservoir should be suitable for nitrogen fixing filamentous cyanobacteria and their dominance could be expected.

The nitrogen deficiency was experimentally demonstrated. By the result of *Selenastrum capricornutum* bioassay the SRP pool in the reservoir was biologically available. Nitrogen enrichment of the filter sterilised reservoir water resulted in significant increase of *S. capricornutum* biomass. This increase exceeded threefold-eightfold times the total phytoplankton biomass existed in the reservoir. This result was supported by enclosure experiments in the lake (Présing *et al.* 1997).

In Lake Balaton and in several hypertrophic water bodies in Hungary the mass development of filamentous N_2-fixing cyanobacteria occurs regularly during the summer months, while in the Marcali reservoir it has never been observed between 1984 and 1995. The intensive filtration of silver carp population (1000-2000 kg ha^{-1}) completely eliminated the large sized algae (including the 100-200 µm sized filamentous nitrogen fixing cyanobacteria), therefore 80-90% of total phytoplankton biomass was consisted of algae smaller than 30 µm. It means that the dense population of silver carp in eutrophic water bodies can be an useful tool to control the water quality preventing the mass development of bloom forming and often toxic cyanobacteria.

Acknowledgments

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