

Strength of phytoplankton–nutrient relationship: evidence from 13 biomanipulated ponds

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Received: 28 January 2011 / Accepted: 1 May 2011
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Abstract Phytoplankton biomass–nutrient relationship is widely used by lake managers to assess the eutrophication impact and to set the nutrient targets. Submerged vegetation and large zooplankton grazing have long been identified as factors weakening the relationship by decoupling phytoplankton from nutrients. Proving this decoupling unambiguously is difficult because, in natural systems, many factors act together, blurring each other's effect. In this article, we present the results of continuous monitoring of 13 ponds where the effects of submerged vegetation and zooplankton grazing were enhanced by biomanipulation (fish removal). The monitoring allowed these effects to be assessed and compared with the pre-biomanipulation situations when phytoplankton biomass was mainly nutrient driven. The comparison showed a strong weakening effect of submerged vegetation and large zooplankton grazing on the chlorophyll *a*–total phosphorus relationship suggesting that a considerable degree of ecological quality of ponds affected by eutrophication can be

restored even when nutrient-loading reduction is not feasible.

Keywords Phytoplankton · Nutrient · Macrophyte · Zooplankton grazing · Pond

Introduction

Factors regulating biomass of phytoplankton, often represented by chlorophyll *a* (Chl *a*) concentrations, received considerable attention from aquatic ecologists. Nutrients are usually considered as the main determinants of the level of phytoplankton biomass in lakes and ponds. Higher nutrient levels generally imply higher phytoplankton biomass (Dillon & Rigler, 1974; Phillips et al., 2008). Because phosphorus is often the limiting nutrient for primary producers in fresh waters, it is used for the classification of lakes and ponds based on their nutrient richness, i.e. trophic status (UNEP, 2003; Bronmark & Hansson, 2005). Prediction of Chl *a* by total phosphorus (TP) is widely used for lake management. Some studies show, however, that the Chl *a*–TP relationship is weaker in shallow lakes and ponds than in deep lakes because in the former both turbid and clear-water states can exist over a wide range of nutrient levels (Moss et al., 1996; Scheffer et al., 2001). It becomes increasingly evident that the main factors weakening the relationship are large zooplankton grazing and submerged vegetation, which

Guest editors: D. Boix, B. Oertli, R. Céréghino, T. Kalettka, J. Biggs & A. P. Hull / Pond Research and Management in Europe – Proceedings of the 4th conference of the European Pond Conservation Network (Berlin 2010)

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are known to inhibit phytoplankton growth through a number of associated mechanisms, such as grazing, nutrient cycling, stabilisation of the water column, shading, and allelopathy (Hansson & Carpenter, 1993; Sondergaard & Moss, 1998; Cottingham & Schindler, 2000; van Donk & van de Bund, 2002).

Although the effects of zooplankton grazing and submerged vegetation on the Chl *a*-TP relationship are well known (Sarnelle, 1992; Steiner, 2002; Dokulil & Teubner, 2003), they have rarely been investigated and confirmed unequivocally because of their complex interaction with other factors blurring them in natural systems. This sometimes led to ambiguous results, e.g. difficulties to separate phytoplankton responses to planktivory-related changes in zooplankton from responses to changes in nutrient cycling caused by fish, macrophytes or bottom-up physical factors (Carpenter & Kitchell, 1992; DeMelo et al., 1992; Gragnani et al., 1999).

Ponds are less than a few hectares in size (Sondergaard et al., 2005b) and, therefore, are easier to study and manipulate than lakes. Except for size, depth and residence time, however, almost all other variables having bearing on phytoplankton biomass in the ponds studied could be greater than in lakes (nutrient levels, zooplankton size, vegetation coverage, fish density, etc.). Therefore, ponds “magnify” the processes that occur in lakes, which helps us have better understanding about them. Planktivorous fish removal or piscivorous fish addition, generally referred to as biomanipulation, further enhances the effects of zooplankton grazing and submerged vegetation on phytoplankton biomass (Shapiro, 1990; Perrow et al., 1997).

Forty-eight eutrophic to hypereutrophic ponds from the Brussels Capital Region (Belgium) were studied from 2004 to 2009. The results obtained confirmed the importance of submerged vegetation and large zooplankton grazing for phytoplankton biomass control (Peretyatko et al., 2007b, 2009). Thirteen ponds have been biomanipulated during the study period to prevent the occurrence of noxious algal blooms and improve their ecological quality. Biomanipulation generally enhances the effects of zooplankton grazing and submerged vegetation on phytoplankton biomass, thus underlining the importance of trophic interactions and competition for nutrients and light for phytoplankton biomass control. Continuous monitoring of these ponds have allowed

the effects of these factors on nutrient-phytoplankton relationship to be assessed. The objective of this study was to compare the strength of the Chl *a*-TP relationship corresponding to situations where phytoplankton biomass was mostly nutrient driven with situations where it was mostly determined by submerged vegetation and/or large zooplankton grazing.

Methods

Forty-eight urban and peri-urban ponds from the Brussels Capital Region (Belgium) have been studied between 2004 and 2009. All the ponds studied are artificial, created by damming of low order streams in the twentieth century or earlier. They are all shallow (maximum depth <3 m) and flat-bottomed and range in surface area from 0.1 to 6 ha. A number of ponds are used for different recreational activities of which fishing and boating are the most common. The ponds are populated by fish communities typical of northern Europe. Many of them have large stocks (often more than 500 kg ha⁻¹) of plankti-benthivorous fish (mainly common carp: *Cyprinus carpio*, and bream: *Abramis brama*).

In varying degrees, however, all of the ponds studied are affected by eutrophication caused by the historical and current urban pollution. Many of them are, therefore, prone to mass developments of phytoplankton generally dominated by bloom-forming cyanobacteria. This often leads to a formation of extensive surface scums and occasional fish and waterfowl kills (Peretyatko et al., 2010).

The 11 ponds most affected by eutrophication (persistent phytoplankton blooms often dominated by bloom forming cyanobacteria, recurrent anoxic/hypoxic conditions, fish and waterfowl kills) were biomanipulated (emptied with fish removal) between 2005 and 2008 to improve their ecological quality, to reduce phytoplankton biomass and to prevent the formation of noxious cyanobacterial blooms. Two other ponds, less affected by eutrophication, were temporarily emptied for infrastructure works. Continuous monitoring of these ponds allowed for the assessments on the effects of fish removal.

During the warm season (May–September) of the study period, the 13 biomanipulated ponds were sampled on 6–22 occasions for phytoplankton,

Table 1 Sampling and statistical comparisons designs

Site	Year of monitoring						Site	Year of monitoring					
	2004	2005	2006	2007	2008	2009		2004	2005	2006	2007	2008	2009
Beml			3	5*	3	3	Beml			bb1/2	ab2	ab1	
Dens			3	5*	3	3	Dens			bb1	ab1		
Leyb-a			3	5*	3	3	Leyb-a			bb1	ab1		
Leyb-b			3	5*	3	3	Leyb-b			bb1/2	ab1	ab2	
MIKI	4	5	5*	5	3		MIKI		bb1/2	ab1	ab2		
NrPd1			3			3*	NrPd1			bb1			ab1
PRB1			3		3	3*	PRB1					bb1	ab1
PRB2			3	5*	3	3	PRB2			bb2	ab2		
Sbsk			3	5*	3	3	Sbsk			bb1	ab1		
VKn1	4	5*		5	3		VKn1	bb1	ab1				
VKn2	4	5		5*	3	3	VKn2		bb1		ab1		
WPk1	4	5		5*	3	3	WPk1		bb1		ab1		
WtMI	4	5*			3	3	WtMI	bb1	ab1				

Left: number of sampling occasions per pond per year; right: pond-year cases used in the Wilcoxon comparisons; asterisk (*) indicates the first summer sampled after biomanipulation; black cells show years/cases before biomanipulation; grey cells show years/cases after biomanipulation with submerged vegetation cover $\geq 30\%$ and/or mean large Cladocera length ≥ 1 mm; white cells show years/cases after biomanipulation with submerged vegetation cover $< 30\%$ and mean large Cladocera length < 1 mm

zooplankton, main nutrients and submerged vegetation (Table 1). They were sampled monthly or at least on three occasions per warm season and at least 1 year before and 1 year after biomanipulation. The other Brussels ponds used in the analyses were sampled according to the same methodology (see Peretyatko et al., 2010 for details).

Integrated water column samples based on 10 random sub-samples were taken from each pond with a plastic tube sampler. After stirring the collected water, dissolved oxygen, pH, conductivity and temperature were measured with a portable meter (WTW 340i), from which 500 ml was taken for the identification and enumeration of phytoplankton and 1 l for the analysis of Chl *a*.

For the determinations of soluble reactive phosphorus (SRP), ammonium and dissolved inorganic nitrogen (DIN), a 100-ml water sample was filtered through a GF/C glass microfiber filter and stored in a cooler. In the laboratory, the samples were frozen until analysis on a Quattro-segmented flow analysis system (Seal Analytical limited) according to the manufacturer's procedures. For TP determination, a 100-ml water sample was stored in a cooler. TP was determined using the persulphate digestion method (APHA-AWWA-WEF, 1995). The samples for Chl *a* determination were kept in a cooler until delivery to the laboratory where they were immediately filtered onto GF/C glass microfibre filters. Filters were stored at -18°C for several days before analysis. Pigments were extracted in 90% acetone in the dark, at 4°C ,

overnight. Chl *a* concentrations were measured spectrophotometrically with due correction for pheophytin *a* (APHA-AWWA-WEF, 1995).

For zooplankton, a combined 10-l sample consisting of 10 random sub-samples of 1 l was taken from each pond at the same location and with the same sampler as used for nutrients and phytoplankton. Zooplankton samples were filtered in the field through a 64- μm mesh net and preserved in 4% formaldehyde (final concentration) before being identified and counted with an inverted microscope. Different levels of identification were used: cladocerans were identified with respect to genus level; copepods were divided into cyclopoids, calanoids and nauplii; rotifers were not discriminated. For the analyses, cladocerans were divided into two groups: 'large' (*Daphnia* spp., *Eurycerus* spp., *Sida* spp. and *Simocephalus* spp.) and 'small' (*Acroperus* spp., *Bosmina* spp., *Ceriodaphnia* spp., *Chydorus* spp., *Moina* spp. and *Pleuroxus* spp.) (Moss et al., 2003). Predatory cladocerans, *Leptodora* spp. and *Polyphe-mus* spp., that feed mainly on other zooplankters (Reynolds, 2006), were not included in the group of large cladocerans. Because of their rarity, few individuals were only occasionally observed in a small number of ponds, their impact on large cladocerans was considered as negligible. Copepods and rotifers had no significant negative relationship with phytoplankton biomass. For these reasons, only large cladocerans were treated here. The length of large Cladocera species was measured and taken as

an indicator of grazing intensity and size-selective predation (Pourriot, 1995; Carpenter et al., 2001). At least 20 or, if less, all present individuals of large cladocerans were measured in each sample. Zooplankton's size and composition was considered as a proxy of fish community structure (Pourriot, 1995).

The total surface cover of aquatic vegetation was mapped visually from a boat during each field visit. The presence/absence of the vegetation was verified with a rake when water was not sufficiently transparent. Because submerged macrophytes were often associated with filamentous green algae, which are also known to inhibit phytoplankton growth (Irfanullah & Moss, 2005; Peretyatko et al., 2007a), their combined surface cover was used in statistical analyses.

Some ponds were recolonised by fish after biomanipulation (mainly juvenile planktivorous fish). Owing to the size of the fish being generally very small, it was difficult to make reliable quantitative estimates of fish density. The presence of fish in the biomanipulated ponds was assessed visually and by fishing with a landing net (4-mm mesh size) during each field visit. Ponds where fish reappeared in large numbers were considered as recolonised by fish.

Hydraulic retention time was estimated on the basis of the outlet discharge (measured on each field visit during the period after biomanipulation) and the corresponding pond volume. Because retention time affected phytoplankton composition more than biomass, even in ponds with low retention time, it was not considered in the analysis of the Chl *a*-TP relationship.

For statistical analyses, the data from biomanipulated ponds were averaged per pond per year and divided into subsets corresponding to three situations: (1) before biomanipulation; (2) after biomanipulation with submerged vegetation cover $\geq 30\%$ and/or mean large Cladocera length ≥ 1 mm; and (3) after biomanipulation with submerged vegetation cover $< 30\%$ and mean large Cladocera length < 1 mm. The thresholds for submerged vegetation cover and large Cladocera length, seemingly the main controlling factors of phytoplankton biomass, beside nutrients, were derived from the analysis of the data collected from the 48 ponds studied and confirmed by other reports (see "Results" section for the threshold corroboration). Because large Cladocera length was not measured in 2004, there are less number of cases

used in the analysis where large Cladocera length was involved. The statistical comparisons of the two different outcomes of the biomanipulation (ab1, ab2) with the before biomanipulation situation (bb1, bb2) were done using Wilcoxon matched pairs test; only 1 year before biomanipulation and 1 year after biomanipulation were used in the comparison (see Table 1 for details).

The strength of the Chl *a*-TP relationships for the three situations was estimated by linear regression analysis of the log-transformed data. The slopes were compared using Tukey HSD test in the homogeneity of slopes model (StatSoft, Inc. STATISTICA version 8.0).

Results

All the ponds studied were characterised by high nutrient levels (Peretyatko et al., 2010). The biomanipulated ponds were particularly affected by eutrophication with TP concentrations well above $100 \mu\text{g l}^{-1}$ before fish removal. Dissolved inorganic nitrogen concentrations varied substantially from pond to pond, but often exceeded the level of $50\text{--}100 \mu\text{g l}^{-1}$ (Table 2) at which phytoplankton growth is likely to be slowed down due to nitrogen limitation (Reynolds, 1998).

The data from the 48 ponds studied showed threshold relationships between Chl *a* yield per unit of TP and submerged vegetation cover and large Cladocera length, 30% and 1 mm, respectively. The thresholds are outlined by the minimum yields. The minimum values and mean Chl *a* yields are markedly lower above the thresholds (Figs. 1, 2). These thresholds are consistent with the submerged macrophyte threshold reported by Meijer et al. (1999) where low Chl *a* yield coincides with macrophyte cover exceeding 25% and *Daphnia* length threshold reported by Mazumder (1994) and Mazumder and Havens (1998) where Chl *a* yield is significantly lower in the presence of *Daphnia* exceeding 1 mm in size. Therefore, for the analyses, the data from the biomanipulated ponds were split into the groups corresponding to these thresholds.

Before biomanipulation, the 13 biomanipulated ponds lacked submerged vegetation (mostly no or $< 30\%$ cover) and large-bodied cladocerans (mean large Cladocera length < 1 mm; bb1, bb2; Fig. 3).

Table 2 General characteristics of the 13 biomanipulated ponds averaged over the study period; range values are given when appropriate

Site	SA (m ²)	MD (m)	RT (day)	Chl <i>a</i> (µg l ⁻¹)	TP (mg P l ⁻¹)	SRP (mg P l ⁻¹)	DIN (mg l ⁻¹)	NH ₄ ⁺ -N (mg l ⁻¹)
Beml	4300	1.0	>300	23.4	0.342	0.141	0.564	0.511
				1.1–112.1	0.104–0.861	0.017–0.479	0.051–1.398	0.008–1.351
Dens	3300	0.7	>300	32.4	0.234	0.052	0.643	0.575
				1.3–170.0	0.084–0.493	0.005–0.093	0.010–2.744	0.008–2.552
Leyb-a	2800	0.5	127	144.9	0.730	0.452	0.209	0.145
				1.9–867.2	0.128–1.691	0.004–1.156	0.007–1.442	0.001–1.375
Leyb-b	2900	0.8	192	150.8	0.338	0.127	0.145	0.067
				1.1–604.5	0.039–0.922	0.002–0.353	0.013–0.629	0.001–0.378
MIK1	9600	0.9	11	84.1	0.359	0.121	0.137	0.091
				2.7–464.2	0.098–0.865	0.003–0.565	0.006–0.597	0.000–0.517
NrPd1	52200	0.9	33	151.8	0.710	0.194	1.592	0.855
				3.9–327.1	0.313–1.572	0.033–0.389	0.026–3.699	0.022–3.472
PRB1	2500	1.1	30	129.5	0.627	0.307	0.114	0.070
				5.1–491.3	0.178–1.760	0.020–0.677	0.012–0.549	0.009–0.378
PRB2	6200	0.8	>300	116.5	0.350	0.036	0.231	0.160
				51.3–222.1	0.178–0.861	0.001–0.243	0.014–0.632	0.011–0.567
Sbsk	2500	0.8	>300	28.5	0.257	0.110	0.424	0.355
				2.9–181.4	0.059–0.624	0.005–0.313	0.024–1.441	0.019–1.215
VKn1	4400	1.3	34	12.0	0.159	0.049	0.168	0.088
				1.1–51.1	0.044–0.326	0.000–0.278	0.014–0.678	0.001–0.524
VKn2	1100	1.0	4	31.7	0.196	0.063	0.269	0.075
				4.0–100.4	0.046–0.405	0.001–0.257	0.011–1.058	0.004–0.313
WPk1	23000	1.1	41	23.8	0.171	0.030	0.352	0.182
				1.6–73.6	0.050–0.360	0.000–0.160	0.002–2.076	0.000–1.515
WtMl	29,700	1.2	7	9.7	0.152	0.039	0.193	0.063
				2.4–28.7	0.032–0.308	0.002–0.124	0.014–0.982	0.000–0.429
Site	pH	<i>T</i> (°C)	Cond (µS cm ⁻¹)	SV (% cover)	LCD (ind l ⁻¹)	LCL ^a (mm)		
Beml	7.6	20.2	852	4	42	0.9		
	7.4–8.2	15.6–25.9	528–987	0–30	0–144	0.5–1.9		
Dens	7.9	20.8	415	0	78	1.1		
	7.5–8.7	12.7–28.0	285–517	–	0–292	0.5–1.7		
Leyb-a	8.6	20.5	572	48	29	0.7		
	7.7–9.4	13.5–26.8	453–729	0–95	0–182	0.3–1.3		
Leyb-b	8.4	20.2	602	32	17	0.8		
	7.8–9.3	13.7–25.9	480–711	0–85	0–74	0.8–2.0		
MIK1	8.3	19.4	484	0	72	0.8		
	7.7–9.0	15.0–24.7	336–594	–	0–250	0.5–1.6		
NrPd1	8.4	22.9	546	17	27	0.8		
	7.8–9.7	16.8–28.7	399–684	0–60	0–67	0.6–1.6		
PRB1	8.5	21.6	875	15	26	0.5		
	8.0–9.1	18.8–26.6	746–945	0–40	0–162	0.5–0.8		
PRB2	8.0	20.4	690	0	29	0.5		
	7.7–8.3	14.3–26.6	546–881	–	0–160	0.4–0.7		

Table 2 continued

Site	pH	<i>T</i> (°C)	Cond ($\mu\text{S cm}^{-1}$)	SV (% cover)	LCD (ind l^{-1})	LCL ^a (mm)
Sbsk	8.0	20.4	661	42	181	0.8
	7.3–8.7	14.7–26.3	480–872	0–100	0–1680	0.5–2.3
VKn1	7.7	18.1	468	47	36	1.0
	7.2–8.3	13.0–22.7	363–561	0–90	0–126	0.9–1.3
VKn2	7.6	17.3	519	47	4	0.6
	7.3–8.0	12.2–22.1	398–607	0–99	0–32	0.5–1.3
WPk1	7.8	20.1	911	8	99	0.8
	7.4–8.0	15.1–25.4	796–1023	0–60	0–326	0.4–1.7
WtMI	8.0	20.3	500	49	61	0.9
	7.4–8.8	15.7–24.4	333–596	2–85	0–266	0.7–1.0

^a Ranges of LCL represent min and max of averages per sample

SA surface area, MD mean depth, RT hydraulic retention time, *Chl a* chlorophyll *a*, TP total phosphorus, SRP soluble reactive phosphorus, DIN dissolved inorganic nitrogen, *T* temperature, Cond conductivity, SV submerged vegetation, LCD large Cladocera density, LCL large Cladocera length

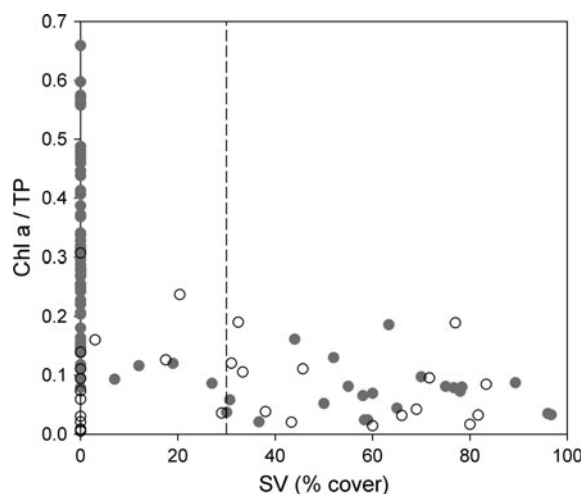


Fig. 1 Chlorophyll *a* yield per unit of TP versus submerged vegetation cover. Dark circles refer to the situation with mean large Cladocera length <1 mm. Open circles refer to the situation with mean large Cladocera length \geq 1 mm. Data from 48 Brussels ponds averaged per pond per year; a dashed line indicates the threshold used in statistical analyses

Fish removal resulted in marked decrease of phytoplankton biomass in 12 out of 13 biomanipulated ponds and general increase in density and size of large cladocerans as well as restoration of submerged vegetation (submerged macrophytes and/or filamentous green algae) in nine ponds (Peretyatko et al., 2009). Submerged macrophytes were mostly represented by *Potamogeton* spp., *Chara* spp. and *Ceratophyllum* sp., filamentous green algae by

Spirogyra spp. The magnitude of phytoplankton biomass decrease was highly dependent on the size of large cladocerans and abundance of submerged vegetation. Submerged vegetation cover above 30% and/or large Cladocera length exceeding 1 mm were associated with a significant decrease in *Chl a* concentrations (bb1–ab1; Fig. 3). Phytoplankton biomass was low in the presence of both extensive submerged vegetation and large-bodied cladocerans as well as either of them alone.

The size of large cladocerans appeared to be much more important than their density for phytoplankton control (Fig. 2). Large cladocerans were generally smaller (<1 mm) in the 10 ponds that were recolonised by planktivorous fish at different times after biomanipulation (Peretyatko et al., 2011). When large cladocerans were small, whether numerous or not, and submerged vegetation was lacking, *Chl a* level was not significantly different from the pre-biomanipulation situation (bb2–ab2; Fig. 3).

Biomanipulation led to a significant decrease in TP concentrations in the ponds where phytoplankton biomass was reduced (bb1–ab1; Fig. 3). It should be noted, however, that TP level remained high (mostly above $100 \mu\text{g l}^{-1}$) owing to high concentrations of SRP. When phytoplankton biomass was not reduced the TP level was not significantly different from that of the pre-biomanipulation situation (bb2–ab2; Fig. 3).

Soluble reactive phosphorus showed an opposite trend to that of TP as a result of biomanipulation (Fig. 4). The increase in SRP concentrations was

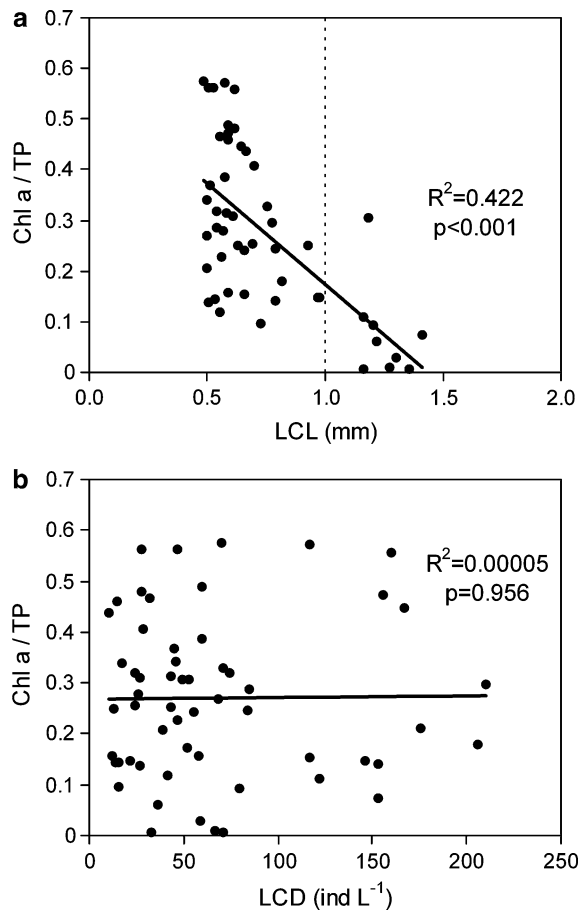


Fig. 2 Relationships between chlorophyll *a* yield per unit of TP and **a** mean large Cladocera length, **b** mean large Cladocera density, in ponds where submerged vegetation was absent. Data set from 48 Brussels ponds averaged per year per pond. Only cases with large Cladocera density ≥ 10 individuals per litre are shown; a *dashed line* indicates the threshold used in statistical analyses. Two outliers with LCD > 400 ind L⁻¹ were removed

significant in ponds with submerged vegetation cover above 30% and/or large Cladocera length exceeding 1 mm (bb1–ab1; Fig. 4). In ponds where submerged vegetation was lacking and large cladocerans small, the difference was not significant (bb2–ab2; Fig. 4). The differences in DIN concentrations between the pre-biomanipulation and after biomanipulation situations were not significant in either set of comparisons (bb1–ab1 and bb2–ab2; Fig. 4).

Regression analysis showed that Chl *a* had significant relationship with TP in all the three situations investigated: pre-biomanipulation, after biomanipulation without and with large-bodied cladocerans and extensive

submerged vegetation (Table 3; Fig. 5). The strength of the relationship, both in terms of slope and R^2 , appeared to be dependent on the presence of extensive submerged vegetation and/or large-bodied cladocerans. The test of homogeneity of slopes rendered significant differences (Tukey HSD test; $P < 0.05$) between the situations characterised by the lack of submerged vegetation and large-bodied cladocerans (situations 1 and 3) and that characterised by the presence of extensive submerged vegetation and/or large-bodied cladocerans (situation 2; Table 3; Fig. 5). There was no significant difference between the pre-biomanipulation situation and after biomanipulation situation lacking submerged vegetation and large-bodied cladocerans (situations 1 and 3). TP explained 51% of the variation in Chl *a* before biomanipulation, 77% after biomanipulation when submerged vegetation and large-bodied cladocerans were lacking and 28% in the presence of extensive submerged vegetation and/or large-bodied cladocerans.

Discussion

Human-induced increase in the productivity of ponds and lakes caused a considerable degradation of their water quality often resulting in noxious phytoplankton blooms (Paerl, 1988; Hudnell, 2008). Adequate management decisions require the effects of elevated nutrient levels to be quantified. The Chl *a*–TP relationship is often used by managers to assess eutrophication impact and to establish nutrient targets. A considerable degree of Chl *a* variability, however, is not accounted for by TP concentrations or loadings thus diminishing the reliability of this relationship (Carpenter et al., 1985; Phillips et al., 2008). Although there is a number of factors that can potentially affect the Chl *a*–TP relationship, submerged vegetation and large zooplankton grazing seem to be the most important ones in the ponds studied as well as in other shallow waterbodies (Lammens et al., 1990; Jeppesen et al., 1997). Both the submerged vegetation and large zooplankters are quite sensitive to the presence of plankti–benthivorous fish that play a central role in ecosystem structuring (Scheffer, 1998; Lammens, 1999). Biomanipulation by fish removal has a cascading effect on phytoplankton biomass owing to large zooplankton release from fish predation that results in more efficient phytoplankton control (Carpenter & Kitchell, 1992). Drastic decrease in benthivory and

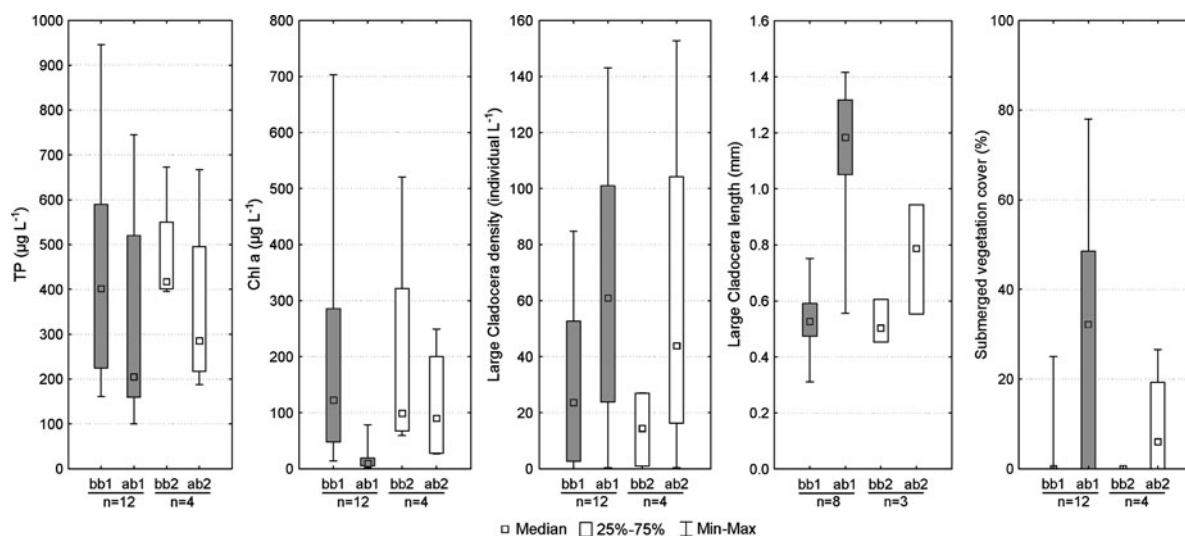


Fig. 3 Total phosphorus, Chl *a*, large Cladocera density and length and submerged vegetation cover corresponding to the two different outcomes of biomanipulation (ab1: with submerged vegetation cover $\geq 30\%$ and/or mean large Cladocera length ≥ 1 mm; ab2: with submerged vegetation cover

$< 30\%$ and mean large Cladocera length < 1 mm) as compared to the respective pre-biomanipulation situations (bb1 or bb2 lacking vegetation and large cladocerans (see Table 1 for details). Grey colour indicates significant difference; *n* shows the number of ponds used in each comparison

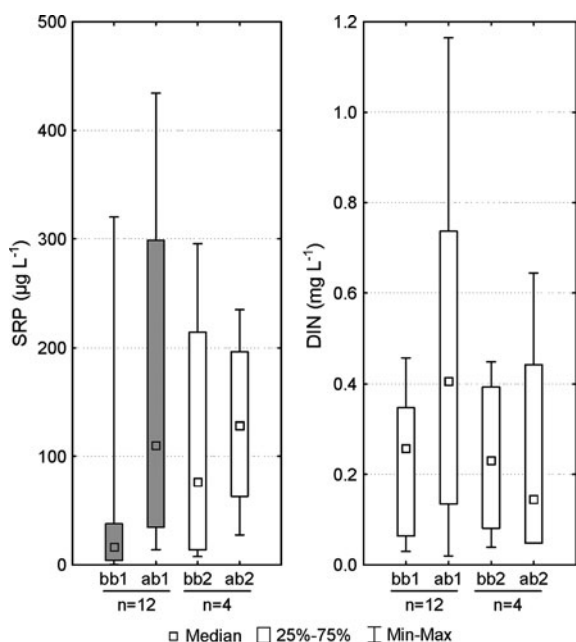


Fig. 4 Soluble reactive phosphorus and DIN concentrations corresponding to the two different outcomes of biomanipulation (ab1: with submerged vegetation cover $\geq 30\%$ and/or mean large Cladocera length ≥ 1 mm; ab2: with submerged vegetation cover $< 30\%$ and mean large Cladocera length < 1 mm) as compared to the respective pre-biomanipulation situations (bb1 or bb2 lacking vegetation and large cladocerans (see Table 1 for details). Grey colour indicates significant difference; *n* shows the number of ponds used in each comparison

improved light climate resulting from biomanipulation also promote restoration of submerged vegetation (Perrow et al., 1997; Scheffer, 1998; Peretyatko et al., 2009).

Biomanipulation and continuous monitoring of a large number of ponds characterised by a wide range of TP concentrations within the eutrophic–hypereutrophic part of the trophic spectrum allowed for assessing the effects of submerged vegetation and large cladocerans on Chl *a*–TP relationship in a nutrient rich context. All the biomanipulated ponds were very productive ecosystems. Before biomanipulation, they lacked submerged vegetation and large-bodied cladocerans and were prone to persistent phytoplankton blooms often dominated by bloom-forming cyanobacteria. The Chl *a*–TP relationship was relatively strong as phytoplankton biomass in these ponds was mostly nutrient driven. Despite the high productivity potential, fish removal led to a marked phytoplankton biomass decrease in the presence of extensive submerged vegetation and/or large-bodied cladocerans. Significant difference in slopes and marked difference in R^2 in the presence and absence of these factors indicate their strong weakening effect on Chl *a*–TP relationship and explain the low Chl *a* values at elevated TP concentrations.

Table 3 Regression equations for the Chl *a*–TP relationships corresponding to the three situations shown on Fig. 5

Situation	Equation	R^2	number of pond year	P	Number of ponds	Predicted Chl <i>a</i> ($\mu\text{g l}^{-1}$)
1.	$\text{Log}_{10} \text{ Chl } a = -2.043 + (1.568 \text{ Log}_{10} \text{ TP})$	0.51	17	0.001	13	154.5
2.	$\text{Log}_{10} \text{ Chl } a = -0.777 + (0.765 \text{ Log}_{10} \text{ TP})$	0.28	28	0.004	12	19.4
3.	$\text{Log}_{10} \text{ Chl } a = -2.304 + (1.673 \text{ Log}_{10} \text{ TP})$	0.77	7	0.009	4	162.7

For the comparison of logarithmic slopes, the predicted Chl *a* concentrations are given when $\text{TP} = 500 \mu\text{g l}^{-1}$

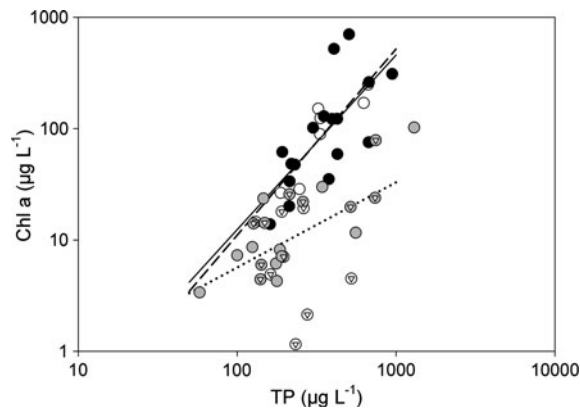


Fig. 5 Relationships between chlorophyll *a* and TP concentrations. *Black circles* before biomanipulation; *white circles* after biomanipulation submerged vegetation cover <30%; *grey circles* after biomanipulation submerged vegetation cover $\geq 30\%$; *diamonds* large Cladocera length ≥ 1 mm. *Solid regression line* samples before biomanipulation (situation 1, Table 3); *dotted regression line* samples after biomanipulation with submerged vegetation cover $\geq 30\%$ and/or large Cladocera length ≥ 1 mm (situation 2, Table 3); *dashed regression line* samples after biomanipulation with submerged vegetation cover <30% and large Cladocera length <1 mm (situation 3, Table 3). Data from 13 biomanipulated ponds averaged per pond per year and plotted on log–log scale

The negative effect of submerged vegetation on phytoplankton biomass through associated mechanisms, such as increased grazing by large zooplankters finding refuge in plant beds, competition for nutrients, stabilisation of the water column and allelopathy, is well established (Jeppesen et al., 1997; Sondergaard & Moss, 1998; van Donk & van de Bund, 2002; Irfanullah & Moss, 2005). This is supported by the results obtained in this study as indicated by generally lower Chl *a* yields per unit of TP in the presence of extensive submerged vegetation as compared to the situation when submerged vegetation was lacking (Fig. 1). It should be noted that the effect of extensive submerged vegetation cover

on the Chl *a* yield does not seem to depend on the size of large cladocerans suggesting that, when submerged vegetation is restored, phytoplankton can also be controlled by mechanisms other than large zooplankton grazing.

The effect of large cladocerans on phytoplankton biomass is less well elucidated. Some controversy exists as to the edibility/grazing resistance of phytoplankton in general and cyanobacteria in particular (e.g. Gliwicz, 1990b; Benndorf et al., 2002). The majority of the studies, however, use number or biomass of large Cladocera or *Daphnia* to assess their effect on phytoplankton. The results of this study indicate that density of large cladocerans has a less straightforward effect on phytoplankton biomass than their size. The non-vegetated ponds, where phytoplankton was mostly controlled by nutrients and zooplankton grazing, often showed markedly lower Chl *a* concentrations in association with smaller numbers of large-bodied daphniids than in association with high numbers of small-bodied daphniids. This is clearly reflected on the Chl *a* yield per unit of TP that has visibly stronger negative relationship with size than density (Fig. 2). The stronger effect of larger cladocerans on the Chl *a* yield is consistent with the idea that smaller individuals have narrower food size spectrum (Burns, 1968); therefore, they rather shift phytoplankton community towards grazing resistant species than effectively control phytoplankton biomass (Cottingham & Schindler, 2000; Benndorf et al., 2002; Steiner, 2002).

Apparent biomanipulation success, however, is not necessarily caused by large zooplankton grazing or submerged vegetation presence, but can be caused by a number of other factors related to fish presence (DeMelo et al., 1992). Fish presence contributes to the nutrient pool through excretion, bioturbation, and sediment resuspension (Brabrand et al., 1990; Breukelaar et al., 1994; Vanni, 2002). Changes in

zooplankton community structure caused by fish removal can also alter the N/P ratio in a way that would inhibit phytoplankton growth (Sterner et al., 1992). Therefore, fish removal may result in substantial decrease in nutrient availability and subsequently in phytoplankton biomass reduction. The marked increase in SRP and the insignificant change in DIN concentrations after biomanipulation in the ponds where it resulted in phytoplankton biomass decrease show, however, that fish removal did not result in a decrease in nutrient availability in the ponds studied.

There are also many reasons why biomanipulation might not work. Insufficient fish removal, phytoplankton composition shift to grazing resistant species, the presence of predator macroinvertebrates and high nutrient concentrations are generally considered as the main reasons of biomanipulation failures to reduce phytoplankton biomass (Meijer et al., 1999; Sierp et al., 2009). The drawdown of the biomanipulated ponds suggests that insufficient fish removal cannot be considered as the cause of the failure. Besides, ponds that were rapidly recolonised by fish could maintain low phytoplankton biomass in the presence of extensive submerged vegetation. When submerged vegetation was lacking, fish recolonisation was often associated with relatively smaller large cladocerans and phytoplankton assemblage bias towards bloom forming cyanobacteria. In the absence of fish, however, large cladocerans alone could control phytoplankton (Fig. 5; Peretyatko et al., 2009) suggesting that when released from predation pressure, large cladocerans can grow big enough (in the fishless ponds the length of large cladocerans exceeded at times 3 mm) to be capable of efficient control of phytoplankton, including bloom-forming cyanobacteria, and thus can prevent bloom formation. This is consistent with the report of Gliwicz (1990b) stating that *Daphnia* can control bloom-forming cyanobacteria below critical filament concentration.

Despite the removal of fish, no marked evidence of large Cladocera control by macroinvertebrates, as reported by Benndorf et al. (2000) and Hosper & Meijer (1993), was observed during the study period. The fluctuations of large Cladocera densities in the three completely fishless ponds seemed to be related to food availability rather than predation. It is possible that macroinvertebrates preyed preferentially on smaller cladocerans and thus biased their size

structure towards bigger individuals (Pinel-Alloul, 1995) as cladocerans in these two ponds were the biggest in size. This, however, can also be attributed to the absence of predation by fish as well as low food availability that gives larger filter-feeders a competitive advantage (Gliwicz, 1990a).

The significant positive relationship between Chl *a* and TP in the biomanipulated ponds with extensive submerged vegetation and large-bodied cladocerans confirmed that the success of biomanipulation depends to a certain degree on nutrient level. Indeed, the capacity of submerged vegetation and large cladocerans to buffer the effects of eutrophication diminished at higher nutrient levels as indicated by the markedly higher Chl *a* values corresponding to elevated TP concentrations. This is consistent with the idea that the clear-water state becomes less stable as nutrient levels increase (Scheffer et al., 2001). Therefore, to ensure biomanipulation success in ponds excessively polluted with nutrients, reduction of nutrient loading might be indispensable.

In the ponds that are not excessively polluted by nutrients, trophic (large zooplankton) and competitive (submerged vegetation) interactions seem to be more important predictors of phytoplankton biomass than nutrient availability. In the absence of fish, low phytoplankton biomass could be maintained by large Cladocera grazing alone. Because large zooplankters are very susceptible to fish recolonisation, such situation is very unstable and not sustainable in the longer term, except for ponds with recurrent anoxic conditions unsuitable for fish. Conversely, extensive submerged vegetation can withstand some degree of fish recolonisation and thus buffer, to certain extent, the effects of eutrophication. Therefore, submerged vegetation restoration seems to be crucial for the maintenance of low phytoplankton biomass on the longer term. This has important implications for the restoration of ponds affected by eutrophication, as it shows that considerable degree of their ecological quality can be restored by biomanipulation even when nutrient loading reduction is not feasible in the short term, as is often the case because of considerable phosphorus accumulation in the sediment (Jepesen et al., 2005; Sondergaard et al., 2005a) and surface and ground water contamination by urban pollution.

The slopes of the regression lines obtained by the analysis of the data from the Brussels ponds are

within the range of the slopes based on the data from temperate lakes (Phillips et al., 2008), suggesting that the ponds studied function in a similar way. Therefore, these results can also be used to get insights into the Chl *a*-TP relationship in lakes.

Conclusions

In ponds lacking submerged vegetation and large-bodied cladocerans, phytoplankton biomass is mainly nutrient driven and therefore the Chl *a*-TP relationship is strong. The presence of extensive submerged vegetation and/or intense grazing by large-bodied cladocerans can considerably weaken the Chl *a*-TP relationship by inhibiting phytoplankton growth despite high nutrient availability. As biomanipulation of ponds leads to the restoration of submerged vegetation and enhances large zooplankton grazing, it can be used to restore a considerable degree of their ecological quality even when nutrient loading reduction is not feasible. The capacity of submerged vegetation and large zooplankton grazing to buffer eutrophication effects diminishes at higher nutrient levels; therefore, to ensure biomanipulation success in ponds excessively polluted with nutrients, reduction of nutrient loading might be indispensable.

Acknowledgements This study was supported by the Prospective Research for Brussels (2003–2006), the Research in Brussels (2006), the Belgian Science Policy (project BBlooms 2: Cyanobacterial blooms: toxicity, diversity, modelling and management, contract SD/TE/01A) and Brussels Institute of Environment (BIM/IBGE). We thank the two anonymous reviewers for their valuable comments that helped us improve the manuscript substantially.

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