What's the catch with Phoslock® & benthivorous fish?

How common carp interferes with attempted control measures to improve water quality.

Master thesis

Ing. F.M. (Chiel) Lauwerijssen

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Photo impression of enclosures at the time of placement and removal.

Summary

In a search for innovative methods to control cyanobacterial blooms, a manipulative field study with enclosures has been conducted in Stiffelio Pond in Eindhoven under the Water Framework Directive-innovation project: mitigating cyanobacterial nuisance (2009-2011). This field experiment aimed to examine the effects of three control measures to reduce internal eutrophication, dredging and/or the phosphate-fixative Phoslock®, on several water quality variables (like pH, oxygen and nutrients). Furthermore, a long term toxicity test (48 days) has been conducted with common carp and Phoslock® to examine possible accumulation of trace metals and its effects on the same water quality variables.

Of all treatments, Phoslock® had a significant negative and carp a positive effect on both total and cyanobacterial chlorophyll-*a* concentrations. Further, transparency had not significantly increased as a result of attempted control measures and transparency was lower in enclosures stocked with carp. Over the course of the experiment, turbidity was circa twice as high in enclosures stocked with carp (> 40 NTU) compared to control enclosures. Furthermore, dissolved oxygen concentrations were negatively affected by carp.

Surprisingly, despite an overdose of Phoslock®, no significant reduction in orthophosphate and total phosphorus concentrations were indicated among control measures. Furthermore, total and filterable lanthanum concentrations were significantly higher in enclosures stocked with carp. Moreover, the Dutch Standard for filterable lanthanum (10.1 µg La l^{-1}) was violated from day 43 in enclosures with carp and Phoslock®. Alarming is the violation of the Dutch Standard with circa 5 µg La l^{-1} (ca. 50%) in undredged enclosures treated with Phoslock® 70 days after application.
Moreover, lanthanum concentrations were significantly higher in the dry weights of both the gastrointestinal tract and mixtures of organs and tissues of carps exposed to Phoslock®. Lead concentrations, on the other hand, were significantly lower in mixtures of exposed carps.

Overall it is evident that none of the treatments improved the water quality to a good ecological potential/status. Moreover, this enclosure study also indicated a violation of the Dutch Standard for filterable lanthanum concentrations and accumulation of lanthanum in common carp. Therefore, it is recommended to look more into toxicological effects of Phoslock® and especially for inter species differences and possible bioaccumulation of lanthanum in the environment.

Finally, violation of the Dutch Standard and accumulation of lanthanum in carp might pose a major drawback in the applicability of Phoslock® as a mitigating measure in eutrophication control; for water managers it might be better to be reticent in applying Phoslock® until other studies have proven otherwise.

Samenvatting

In de Stiffelio vijver te Eindhoven is vanuit het Kader Richtlijn Water innovatieproject ‘’Bestrijding Blauwalgenoverlast’’ (2009-2010) een manipulatief veldexperiment uitgevoerd met enclosures. Het doel van dit experiment was om de effecten van drie beheermaatregelen om interne eutrofiëring te verlagen, baggeren en/of het fosfaatfixatief Phoslock®, op verschillende waterkwaliteitsvariabelen te onderzoeken (zoals pH, zuurstof en nutriënten). Daarnaast is een lange termijn toxiciteitstest (48 dagen) met karper en Phoslock® uitgevoerd om de mogelijke accumulatie van metalen en tegelijkertijd de effecten van karper op dezelfde waterkwaliteitsvariabelen te onderzoeken.

Van alle behandelingen had Phoslock® een significant negatief en karper een positief effect op zowel totaal- als blauwalgen chlorofyl-*a* concentraties. Verder was het doorzicht niet significant toegenomen als gevolg van de ondernomen beheermaatregelen en was het doorzicht lager in de enclosures met karper. De troebelheid was gedurende het experiment circa twee keer zo hoog in de enclosures met karper (> 40 NTU) als in de controle enclosures. Daarnaast hadden karpers een negatief effect op opgeloste zuurstofconcentraties.

Verrassend was dat ondanks een overdosis Phoslock® er geen significante reductie werd aangetoond voor totaal- en othofosfaatconcentraties als gevolg van de beheermaatregelen. Verder waren de totaal- en filtreerbaar lanthaanconcentraties significant hoger in enclosures met karper en werd de norm voor filtreerbaar lanthaan (10.1 µg La l^{-1}) overschreden vanaf dag 43 in enclosures met karper en Phoslock®. Alarmerend is het overschrijden van de norm in ongebaggerde enclosures met Phoslock® met circa 5 µg La l^{-1} (ca. 50%), 70 dagen na het toevoegen van Phoslock®.
Bovendien waren lanthaanconcentraties significant hoger in zowel de drooggewichten van het maagdarmkanaal als in het mengsel van organen en weefsels van karpers die aan Phoslock® blootgesteld waren. Loodconcentraties in het mengsel van de blootgestelde karpers, daarentegen, waren significant lager.

Over het algemeen heeft de waterkwaliteit in de enclosures als gevolg van de beheermaatregelen duidelijk niet de/het goede ecologische toestand/potentieel bereikt. Bovendien is uit dit enclosure experiment ook gebleken dat de norm voor filtreerbaar lanthaan werd overschreden en dat lanthaan accumuleert in karper. Daarom verdient het aanbeveling om de toxicologische effecten van Phoslock® nader te onderzoeken en dan met name voor verschillende soorten en voor mogelijke bioaccumulatie van lanthaan in het milieu.

Concluderend zouden het overschrijden van de norm van filtreerbaar lanthaan en accumulatie van lanthaan in karper bezwaarlijk kunnen zijn om Phoslock® toe te passen als beheermaatregel voor het verlagen van interne eutrofiëring; voor de waterbeheerder zou het beter zijn om terughoudend te zijn in het toepassen van Phoslock® totdat andere studies het tegendeel hebben bewezen.

Preface

This thesis report is written as part of my master study Hydrology & Water Quality at Wageningen University. I conducted my thesis research at the Aquatic Ecology and Water Quality Management Group of Wageningen University, where my work was supervised by Dr. Ir. Miquel Lürling. This research was conducted under the Water Framework Directive-innovation project: mitigating cyanobacterial nuisance (2009-2011), in which Wageningen University collaborates with three Dutch water boards (''Aa en Maas'', ''Brabantse Delta'' and ''De Dommel'') and ''Stichting Toegepast Onderzoek Waterbeheer'' (STOWA).

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1. Introduction

Surface waters including streams, rivers, lakes, estuaries and coastal waters are under increasing ecological stress due to anthropogenic activities worldwide (UNEP, 1999), leading to the enforcement of major environmental policies, including the European Water Framework Directive 2000/60 (EC, 2000). Nutrient over-enrichment (eutrophication) is one of the stressors, which has altered ecosystems completely. Increasing nutrient availability due to eutrophication has increased chlorophyll-*a* levels and has led to the proliferation of cyanobacteria and accumulation of biomass in nuisance scums (Fogg, 1969; Reynolds & Walsby,1975; Reynolds, 1987; Paerl, 1988, 2008). Because several cyanobacteria can produce a variety of very potent toxins, they pose potentially serious environmental and human health problems (Codd *et al*., 2005; Dittmann & Wiegand, 2006; Paerl, 2008; Paerl & Huisman, 2008).
Like eutrophication, benthivorous fish may have a positive effect on phytoplankton biomass as well (Meijer *et al*., 1990; Breukelaar *et al*., 1994; Nalewajko & Murphy, 1998; Zambrano & Hinojosa, 1999). As a result of benthic feeding, these fish release nutrients from sediments (Havens, 1991; Shormann & Cotner, 1997) and hamper sediment consolidation (Delgado *et al*., 1991; Breukelaar *et al*., 1994; Scheffer *et al*., 2003), which negatively affects water column transparency (Roozen *et al*., 2007).

Need for innovative methods

Sediment removal is often unsuccessful in controlling internal nutrient loads sufficiently and reducing algal biomass on the long term (Peterson, 1982; Welch & Cooke, 2005). Furthermore, dredging is an expensive and disruptive control measure. In a search for innovative methods to control cyanobacterial blooms, Wageningen University collaborates with three Dutch water boards (‘‘Aa en Maas’’, ‘’Brabantse Delta’’ and ‘’De Dommel’’) and ‘’Stichting Toegepast Onderzoek Waterbeheer’’ (STOWA) in the Water Framework Directive-innovation project: mitigating cyanobacterial nuisance (2009-2011).
So far, manipulative field studies have been conducted on different scales (lakes, ponds, compartments and enclosures) and have resulted in new insights and unexpected results. Innovative methods include combinations of fish stock management, flocculation of algae, the phosphate-fixative Phoslock®, sediment removal and planting different macrophyte species.

Phoslock®

Phoslock® is the registered trade mark of a phosphate-fixative that is developed by Phoslock® Water Solutions. It consists out of bentonite clay that has been modified with the rare earth element lanthanum and is manufactured by making use of the cation exchange capacity of the clay minerals (see figure 1.1). Lanthanum ions are exchanged with exchangeable cations that are present between clay sheets (Haghseresht, 2005).

Figure 1.1: Cation exchange (Adapted from Haghseresht, 2005).

With a molar ratio of 1:1 (see equation 1), lanthanum has a highly efficient phosphorous uptake (Douglas *et al.*, 2004). When oxyanions like orthophosphate are present in the environment, it is shown that a stable mineral known as rhabdophane ($LaPO_4.nH_2O$) is formed (Haghseresht, 2005).

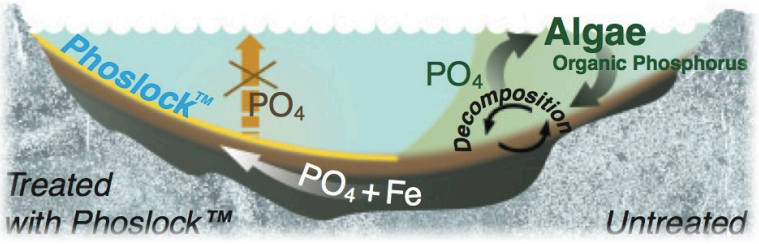


Figure 1.2: Effects of Phoslock® on phosphorus (Adapted from Greenop & Robb, 2001).

$La^{3+} + PO_4^{3-} \Rightarrow LaPO_4$ (1)

Phoslock® has a negative effect on phytoplankton biomass (see figure 1.2) by stripping dissolved phosphorus from the water column and intercepting phosphorus released from the sediments (Douglas *et al.*, 1999; Robb *et al.*, 2003; Akhurst *et al.*, 2004; Ross *et al.*, 2008). It has already been applied to more than 100 large water bodies in 20 countries, including the Netherlands (Groves, 2007).

Enclosure experiment

In this thesis study, a manipulative field study with enclosures has been conducted to examine the effects of three control measures to reduce internal eutrophication and improving water quality: dredging and/or Phoslock®. During the experiment a variety of water quality variables has been examined (e.g. pH, oxygen and nutrients). Furthermore, a long term toxicity test (48 days) has been conducted with common carp and Phoslock® to examine possible accumulation of trace metals and its effects on the same water quality variables.

Bioaccumulation of trace metals

According to Persey *et al.* (2006) lanthanum does not appear to be toxic when applied to humans. Though, it is unknown if bioaccumulation of lanthanum occurs. Donald & Sardella (2010) detected lanthanum in muscle tissue and ovaries of fish. Since benthivorous fish hamper sediment consolidation and lanthanum may be released from the bentonite clay-La^{3+} complex when added to water (Lürling & Tolman, 2010), they may store lanthanum in their body due to a Phoslock® application. As a consequence, this lanthanum might then be transferred up further in the food web.

Phytoplankton

It is shown that an increase in chlorophyll-*a* is accompanied by changes in phytoplankton community structure in terms of total abundance, species richness and evenness (Tsirtsis & Karydis, 1998; Tsirtsis *et al.*, 2008). Due to its fast population response to changes in water quality, hydrology or climate, phytoplankton is an efficient indicator of changes in nutrient loads and also effective in evaluating responses to many other environmental stressors (Domingues *et al.*, 2008). According to the European Water Framework Directive (EC, 2000), phytoplankton metrics that are fundamental in defining and classifying the ecological status of surface waters are biomass (as chlorophyll-*a*), community changes (composition and species abundance) and increase in the frequency and intensity of blooms (Spatharis & Tsirtsis, 2010).

2. Study area

The manipulative field study was conducted in Stiffelio Pond (N 51°48'96.57''/ E 5°47'65.31''), a man-made pond on the northern side of Eindhoven (Noord-Brabant) in the Blixembosch neighborhood. It has a rectangular shape and two ditches are connected perpendicularly to the pond (see figure 2.1). The shores are quite steep and totally timbered. Average water depth is about 1.4 meters and the surface area is about 7086m^2 (Kalkman, 2009).

Figure 2.1: Areal photographs of Stiffelio Pond and enclosures (right photograph).

Sediment

Stiffelio Pond is over-enriched with nutrients due to fish bait, bread and dog poop. As a consequence, low oxygen concentrations, fish kills and cyanobacterial blooms have evolved in the pond (see figure 2.2). Also, the sediment's pore water is nutrient rich and contains approximately 0.46 g P kg^{-1} dry sediment. .

Fish stock

Stiffelio Pond is intensively used by sport fishermen and the fish stock was estimated by Kalkman (2009) at 927 kg ha^{-1} or 5563 # ha^{-1} in 2009. Species richness is eight: bream, carp, gibel carp, perch, pike, roach, rudd and tench. In terms of weight, carp (48%) and gibel carp (24%) attributed most to the catch; roach (72%) and bream (16%) in terms of abundance .

WFD-innovation project

Under the Water Framework Directive-innovation project: mitigating cyanobacterial nuisance (2009-2011), six squared compartments (20x20m) were installed in Stiffelio Pond in the summer of 2009. In these compartments five different control measures to improve water quality are being examined. The enclosures used for this experiment were installed parallel to the compartments (see figure 2.1).

Figure 2.2: Stiffelio Pond; Sign 'Avoid contact with water because of cyanobacteria and/or botulism'.

3. Materials & methods

Experimental design

On 29 July 2010, 18 polycarbonate enclosures that were open to the sediment and the atmosphere, were placed in Stiffelio Pond in Eindhoven at a water depth about 1.4m. The enclosures were 2.25m in length and consisted out of two smaller enclosures that were 1.05 meter in diameter and respectively 1.35m and 0.9m in length. In order to make the connection impermeable for water, both parts were connected with self vulcanizing tape (50mm) on the outside and waterproof tape (40mm) on the inside. Also, three orange straps were bound around the enclosures to keep both parts in place and the enclosures manageable for placement and removal. To facilitate sampling, enclosures were placed alongside of a gangway and circa 1m apart from each other. Furthermore, to prevent litter from being thrown into the enclosures, they were covered with chicken wire (1cm meshes) during the experiment (see page 4 for photo impression).

After positioning the 18 enclosures circa 0.4m into the sediment, they were treated as follows:

I. Three enclosures were left unharmed (controls);
II. Three enclosures were stocked with one common carp;
III. Three enclosures were stocked with one common carp and then treated with Phoslock®;
IV. Three enclosures were treated with Phoslock®;
V. The top sediment layer was removed from three enclosures and then remaining sediment was treated with Phoslock®;
VI. The top sediment layer was removed from three enclosures.

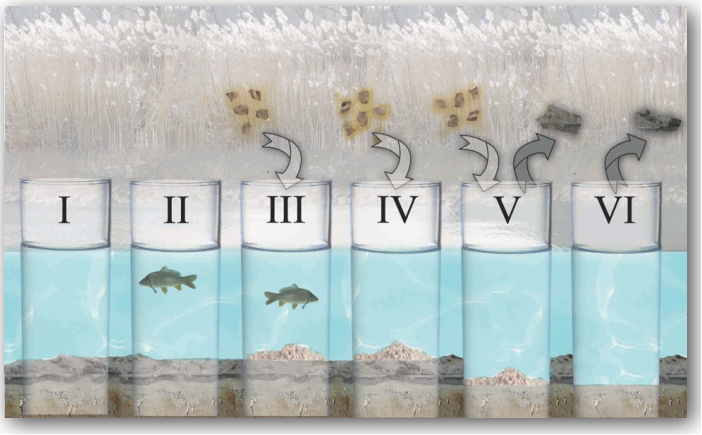


Figure 3.1: schematic representation of the six different treatments.

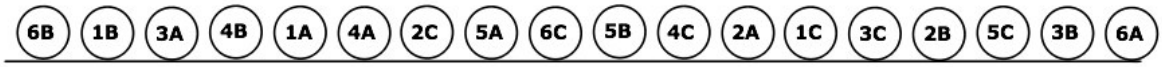


Figure 3.2: emplacement of enclosures: numbers 1-6 represent treatments I-VI; letters A-C represent replicates within each treatment.

To summarize, a balanced, fully randomized experimental design with three factors (sludge, fish and Phoslock®), 2 levels (presence/absence) and three repetitions per level was carried out (see figures 3.1 and 3.2).

Sediment removal

The top sediment layer of circa 5cm was scooped from six enclosures with a macrofauna scoop-net of 29 by 15 cm and a mesh size of 0.5mm. Sediment was removed in two days, August 1 and 2 (2010), in order to take out most of the sediment that had been stirred up the first day.

Carps

Nine common carps (*Cyprinus carpio* L.,1758) of circa 35cm in length and circa 750 grams were used for the experiment and bought from hatchery "Visweekcentrum Valkenswaard" (see figure 3.3). Carps were used, because they were easy to obtain in the required size, originate all over the Netherlands and can even tolerate dissolved oxygen concentrations of 2 mg l^{-1}(Van Emmerik & De Nie, 2006). Three carps were used as a negative control and six were separately put in an enclosure for 48 days. Three out of six enclosures with carps were then treated with Phoslock®. Carps were removed from the enclosures with a scoop-net, immediately euthanized and transported to Wageningen UR after the experiment for further analyses.

Figure 3.3: Carps used for the experiment.

Phoslock® application

The Phoslock® dose applied, was circa 1364 grams of Phoslock® per enclosure (0.87m^2 sediment, 1.21m^3 water). This dose was based on the application of circa 450kg Phoslock® per 400m^3 water in the compartments within Stiffelio Pond by Phoslock Water Solutions Ltd. (Van Goethem, 2009). Phoslock® was applied to the enclosures by mixing it in a bucket with circa five liters of pond water in advance.

Field & lab measurements

Field measurements

At least once a week, from 3 August 2010 until 20 September 2010, two liters of water were taken with a water sampler (1L reservoir) circa 0.7m below surface from both the enclosures and Stiffelio pond (see figure 3.4). Right after Phoslock® application sampling occurred more frequently, resulting in 10 measurements by the end of the research period. Sampling dates were: August 3, 4, 6, 12, 19 and 25; September 1, 8, 15 and 20, 2010.

After sampling, the following variables were measured in the enclosures:

- Conductivity[1] (µS cm^{-1}), ca. 35cm below surface;
- Oxygen[2] (mg l^{-1} and %saturation), ca. 35cm below surface;
- pH[3], ca. 20cm below surface;
- Temperature[2] (^oC), ca. 35cm below surface;
- Secchi depth[4] (m).

Equipment:

[1] WTW cond 115i
[2] OxyGuard Handy Polaris
[3] WTW pH 315i
[4] Secchi-disk, 20cm in diameter

Figure 3.4: Taking water samples from the enclosures.

Lab measurements

At Wageningen University water samples were subsequently analyzed for chlorophyll-*a*, nutrients, lanthanum and some for cyanobacterial toxins and phyto- and zooplankton. The carps were examined on the presence of trace metals.

Total and cyanobacterial chlorophyll-a

Total chlorophyll-*a* (µg l^{-1}) was used as a proxy for phytoplankton abundance and determined according WUR ESG Protocol ''Chlorophyll-*a* Analysis'' that is based on NEN-6520 (see appendix 1A). Furthermore, cyanobacterial chlorophyll-*a* (µg l^{-1}) was determined with a Phytoplankton Analyzer System (PHYTO-PAM System), developed by company Heinz Walz GmbH, Effeltrich, Germany. This PHYTO-PAM System contained the standard Power-and-Control-Unit (PHYTO-C) and the Portable Emitter-Detector Unit (PHYTO-ED). More information about this analysis is included in appendix 1B: WUR ESG Protocol ''Chlorophyll-*a* measurement with PHYTO-PAM''.

Cyanobacterial toxins

Seston was collected on a Watchman GF/C filter (0.6-1.2µm) after filtering 75 or 100 ml of water sample. Samples collected from the enclosures on 12-08-10 and from Stiffelio Pond on 30-07-10 and 12-08-10 were examined on the presence of microcystins. Microcystins were determined on a LC-MSMS according to WUR ESG protocol ''Sample preparation RPmix filters'' (see appendix 1E).

N.B. Since detection limits of microcystin concentrations were unknown, but are approximately negligible (circa 1-63 ng l^{-1}), concentrations were set to zero if detected below detection limits (best-case scenario).

Nutrients and lanthanum

Nutrients and lanthanum in water samples were determined on either ICP-MS or colourimetrically on a Continuous Flow Analyzer (CFA) and corresponding detection limits are shown in table 3.1.

Table 3.1: Detection limits.

Measurement	Detection limit
Ammonium (CFA)	0.02 mg N l^{-1}
Filterable lanthanum (ICP-MS)	0.02 µg La l^{-1}
Filterable phosphate (ICP-MS)	1 µg P l^{-1}
Nitrite and nitrate (CFA)	0.01 mg N l^{-1}
Total lanthanum (ICP-MS)	0.2 µg La l^{-1}
Total nitrogen (CFA)	0.2 mg N l^{-1}
Total phosphate (ICP-MS)	6 µg P l^{-1}

N.B.
1. If nutrient concentrations were detected below detection limits, they were replaced by those limits (worst-case scenario).
2. Filtrates for lanthanum and phosphate analyses were prepared with Whatman GF/C filters (0.6-1.2 µm).

Phyto- and zooplankton

For phytoplankton analysis, water samples were taken from Stiffelio Pond on 3 August 2010 and from both the enclosures and the pond on 20 September 2010 (20 samples in total). The unfiltered water was collected in 100ml PE bottles and fixed with Lugol's iodine solution. Then phytoplankton species composition and abundance was scored as it is written in the Water Framework Directive by Van der Molen & Pot (2007) for a 'shallow buffered lake' (code M14).

For zooplankton analysis, one liter of water sample was filtered through a plankton-net (55 micron meshes) and the filtrate was fixed with Lugol's iodine solution. Next, zooplankton species composition and abundance were determined of samples taken from the enclosures and the pond on August 3, 4, 6, and 12; September 20, 2010.

Trace metals in carp

The carps were washed in tap water and frozen at -20 ^{o}C after capture. First, carp's total length and weight were measured after defrosting. Then the gastrointestinal tract was removed and freeze-dried. The carps's remains (tissues & organs) had been cut into small pieces and put frozen into a blender to make a homogeneous mixture of each carp (see figure 3.5). Circa 50 grams of each mixture was subsequently freeze-dried like the GI-tracts according to WUR ESG freeze-drying protocol (see appendix 1C). Next, all freeze-dried samples were pulverized and circa 30 milligrams of each blend was destructed on a destruction block according to the WUR ESG protocol ''Preparation for identification of trace metals in macrofauna and zooplankton'' (Appendix 1D).
Finally, the following five trace metals with its detection limits were determined on ICP-MS: cadmium (0.005 µg l^{-1}), copper (0.1 µg l^{-1}), lanthanum (0.02 µg l^{-1}), lead (0.04 µg l^{-1}) and zinc (0.3 µg l^{-1}).

Figure 3.5: Preparing the carp's GI-tract (left) and mixing its remains (tissues & organs).

Data analysis

- Data was collected with Microsoft Excel 2011 for MAC, version 14.0.0.
- Graphs were created with SigmaPlot for Windows, version 11.0.
- Figures were created with Adobe Illustrator and Photoshop CSS Extended for MAC, version 12.0 x64.
- Statistics were carried out with PASW Statistics (formerly SPSS) for Mac, version 18.0.3.

Statistics

Univariate analyses were performed to indicate differences among treatments. Only for total and cyanobacterial chlorophyll-*a* concentrations repeated measures ANOVAs were carried out over different periods. Since for these two variables differences among treatments were larger over the first three weeks, the following periods were analyzed separately:

1. The first nine days;
2. From start until day 29;
3. The entire research period (48 days).

If preconditions for a repeated measures ANOVA could not be met, a Kruskal-Wallis test was carried out over the entire research period instead.

Equality of means were tested with either a one-way ANOVA or Student's t-test. If preconditions could not be met a Kruskal-Wallis or Mann-Whitney U test was used instead.
Subsequently, if either one-way ANOVA or Kruskal-Wallis test indicated a significance difference, this test was followed by a Games-Howell , Scheffe or Tukey post-hoc test (dependent on preconditions).

N.B.

- The statistical significance was defined as $P < 0.05$;
- Stiffelio Pond is plotted in some of the figures, but was not taken along in statistical analyses.

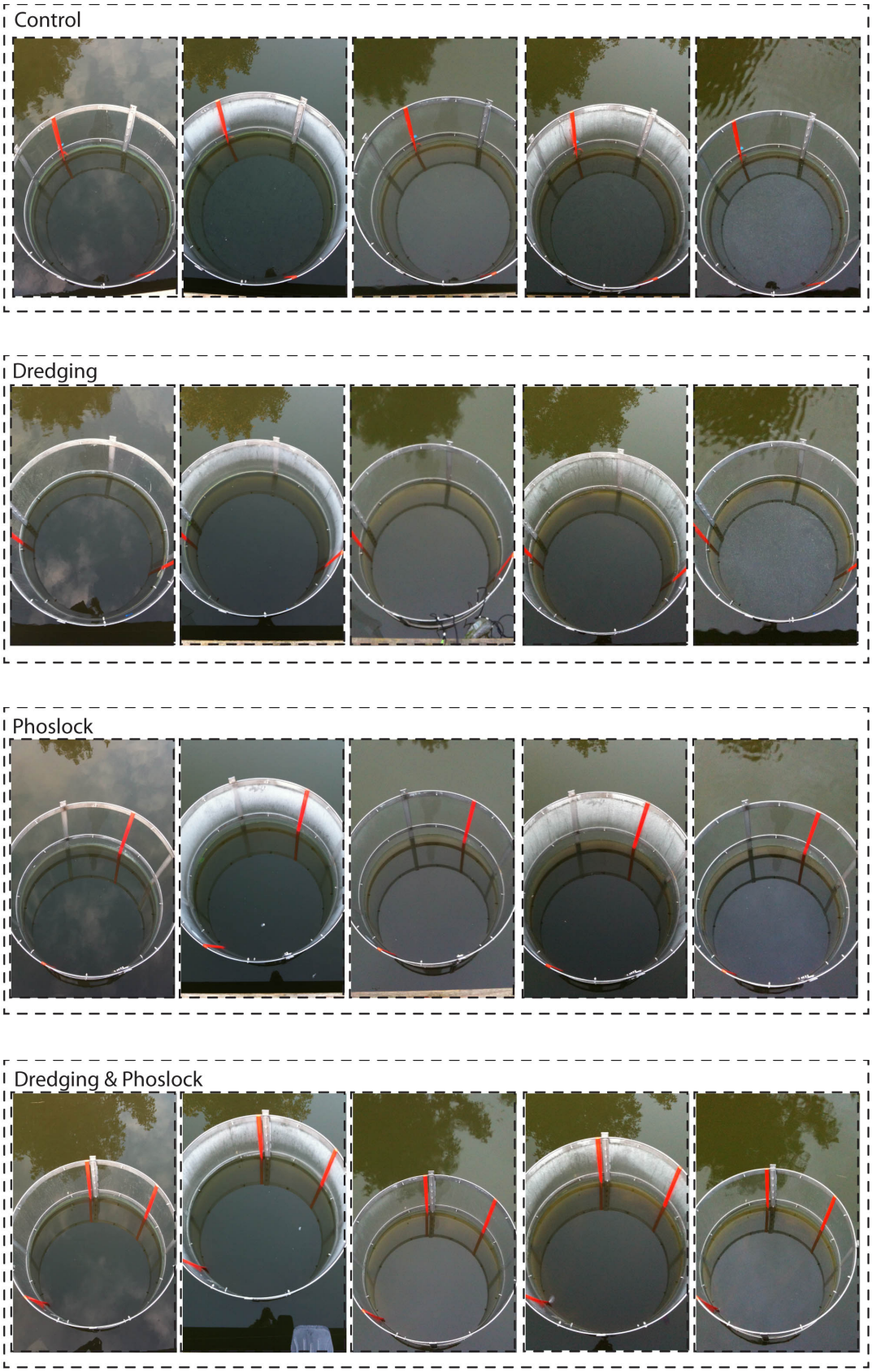


Figure 3.6: Top views of enclosures of the four treatments in chapter 4: Control, Dredging, Phoslock and Dredging&Phoslock on days 16, 29, 36, 43 and 48 (from left to right).

4. Three control measures to improve water quality

In this chapter the results of an enclosure study with three control measures to reduce internal eutrophication and to improve water quality are presented (see figure 3.6 for photo impression). In order to compare the effects of attempted control measures, the following four treatments were examined:

1. Three enclosures were left unharmed (Control);
2. Three enclosures were treated with Phoslock® (Phoslock);
3. The top sediment layer was removed from three enclosures and then remaining sediment was treated with Phoslock® (Dredging&Phoslock);
4. The top sediment layer was removed from three enclosures (Dredging).

Chlorophyll-*a*

Total chlorophyll-*a* was used as a proxy for algal biomass. In order to quantify the share of cyanobacteria in total chlorophyll-*a*, cyanobacterial chlorophyll-*a* concentrations have been examined as well. Both total chlorophyll-*a* data and its cyanobacterial share were analyzed separately and presented in tables 4.1 and 4.2. To analyze within and between treatment effects, repeated-measures ANOVAs were carried out for the entire research period (48 days) and for two parts of this dataset, namely the first nine days and from start until day 29.

Total chlorophyll-a

Mean total chlorophyll-*a* concentrations (see figure 4.1) were significantly lower in undredged enclosures treated with Phoslock® compared to dredged enclosures without Phoslock® (Tukey post-hoc test: P = 0.013, after RM ANOVA over 48 days). However, differences were not large and mean concentrations were between circa 65 and 90 µg l^{-1}.

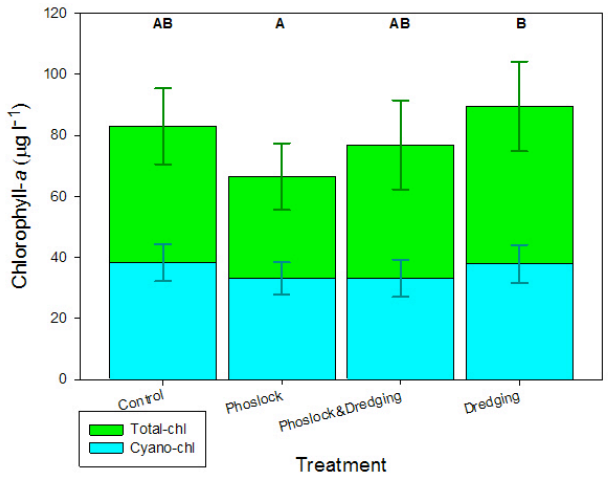


Figure 4.1: Bar graph of mean total and cyanobacterial chlorophyll-a concentrations (µg l^{-1}) over research period (48 days) for the four treatments: Control, Dredging, Phoslock and Phoslock&Dredging. Error bars represent 95% CI and identical letters (A, B) indicate homogeneous groups that are not different at the 95% level for Total-chl (Tukey post-hoc test). No significant differences were indicated for Cyano-chl (Tukey post-hoc test).

Repeated-measures ANOVAs indicated a significant time effect for all datasets and significant time*dredging and time*dredging* Phoslock® interaction effects for parts of the dataset (see table 4.1). So, total chlorophyll-*a* concentrations changed significantly over time and developed differently in dredged enclosures with and without Phoslock® compared to undredged enclosures with and without Phoslock®. Also, a significant effect of Phoslock® on total chlorophyll-*a* concentrations was indicated for all datasets and an effect of dredging for parts of the research period. This effect is also illustrated by figure 4.2 in which the green bars (Total-chl) of the enclosures treated with Phoslock® are lower than the enclosures without Phoslock®.

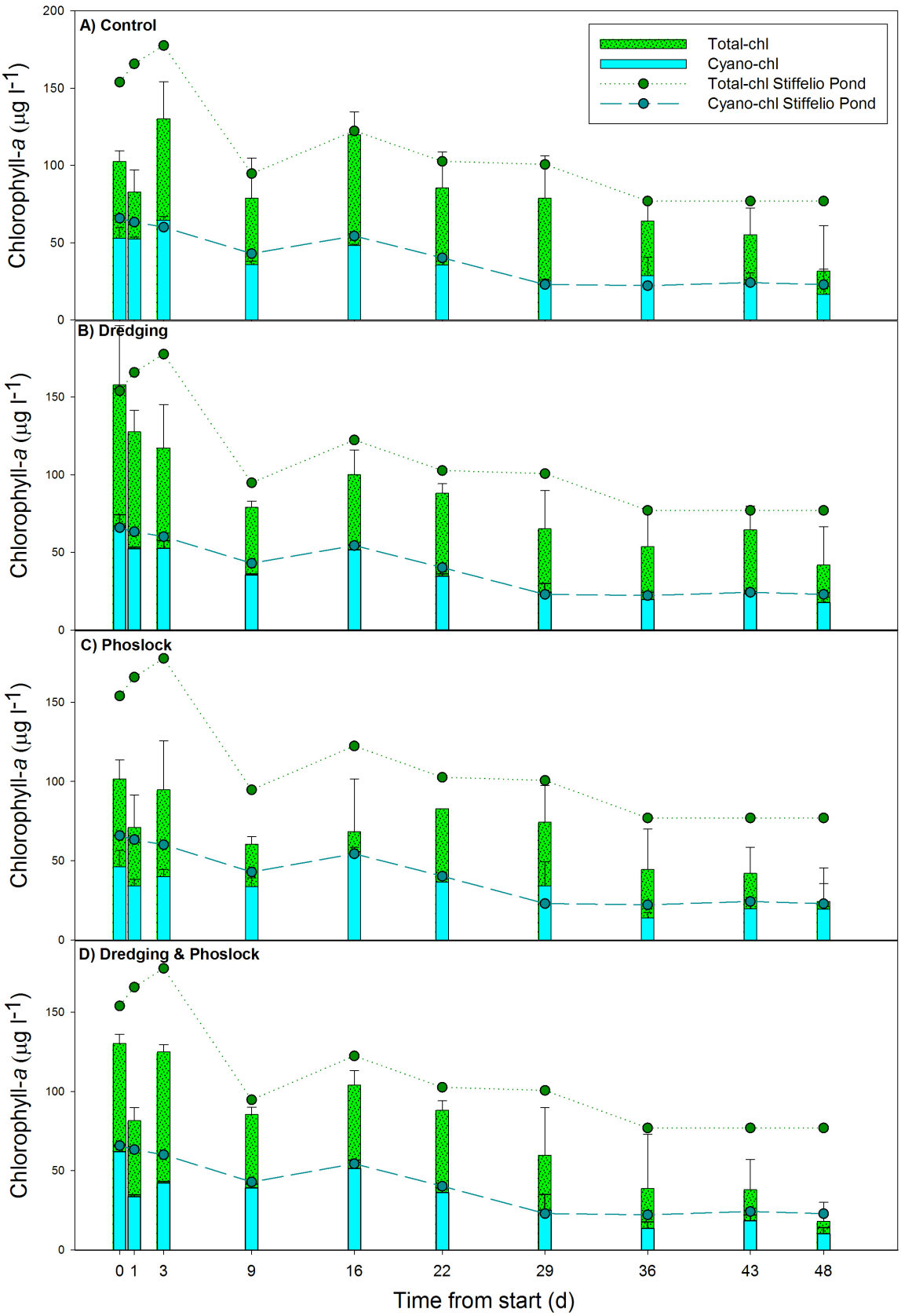


Figure 4.2: Total and cyanobacterial chlorophyll-a concentrations (µg l-1) in Stiffelio Pond, in controls (A), in dredged enclosures (B), in undredged enclosures treated with Phoslock® (C) and in dredged enclosures treated with Phoslock® (D). Error bars represent 1 SD (n = 3).

Table 4.1: Results from repeated-measures ANOVAs for total chlorophyll-a concentrations.

	9 days from start				29 days from start				48 days from start			
	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]
Mauchly's Test of Sphericity	7,66	5	0,180	-	46,54	20	0,002	1,000	-	44	-	0,285
Within subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Time	3,00	5866	16,62	**<0,001**	6,00	4749	12,53	**<0,001**	2,57	38222	26,80	**<0,001**
Time*Dredging	3,00	700	1,98	0,143	6,00	959	2,53	**0,033**	2,57	2791	1,96	0,158
Time*Phoslock	3,00	277	0,78	0,515	6,00	311	0,82	0,560	2,57	782	0,55	0,629
Time*Dredging*Phoslock	3,00	1092	3,09	**0,046**	6,00	855	2,26	0,053	2,57	2264	1,59	0,226
Error	24,00	353			48,00	379			20,55	1426		
Between subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Dredging	1	6165	17,91	**0,003**	1	3346	8,53	**0,019**	1	2158	4,74	0,061
Phoslock	1	2970	8,63	**0,019**	1	3695	9,42	**0,015**	1	6354	13,95	**0,006**
Dredging*Phoslock	1	11	0,03	0,862	1	451	1,15	0,315	1	118	0,26	0,624
Error	8	344			8	392			8	455		

a. May be used to adjust the degrees of freedom for the averaged tests of significance.
If epsilon <0,75, or nothing is known about sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity.
If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.

Cyanobacterial chlorophyll-a

Repeated-measures ANOVAs indicated significant time and time*Phoslock® interaction effects for all datasets; a significant time*dredging interaction effect for parts of the research period (see table 4.2). So, cyanobacterial chlorophyll-*a* concentrations changed significantly over time and developed differently in with Phoslock® treated enclosures compared to untreated enclosures. The same counts for the development of cyanobacterial chlorophyll-*a* concentrations in dredged enclosures compared to undredged enclosures.

Also, a significant effect of Phoslock® on cyanobacterial chlorophyll-*a* concentrations was indicated for all datasets. However, Tukey post-hoc test did not indicate significant differences among mean cyanobacterial chlorophyll-*a* concentrations (figure 4.1).

Table 4.2: Results from repeated-measures ANOVAs for cyanobacterial chlorophyll-a concentrations.

	9 days from start				29 days from start				48 days from start			
	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]
Mauchly's Test of Sphericity	17,70	5	0,004	0,459	43,24	20	0,004	0,331	-	44	-	0,267
Within subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Time	1,38	2125	71,70	**<0,001**	1,98	3991	48,17	**<0,001**	2,40	9512	62,97	**<0,001**
Time*Dredging	1,38	476	16,05	**0,001**	1,98	414	5,00	**0,021**	2,40	399	2,64	0,089
Time*Phoslock	1,38	571	19,26	**0,001**	1,98	850	10,25	**0,001**	2,40	752	4,97	**0,014**
Time*Dredging*Phoslock	1,38	71	2,41	0,145	1,98	125	1,51	0,251	2,40	165	1,09	0,366
Error	11,01	30			15,88	83			19,21	151		
Between subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Dredging	1	115	2,71	0,139	1	23	0,75	0,413	1	1	0,02	0,894
Phoslock	1	1257	29,47	**0,001**	1	449	14,24	**0,005**	1	736	14,14	**0,006**
Dredging*Phoslock	1	84	1,97	0,198	1	7	0,22	0,648	1	2	0,03	0,857
Error	8	43			8	32			8	52		

a. May be used to adjust the degrees of freedom for the averaged tests of significance.
If epsilon <0,75, or nothing is known about sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity.
If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.

Transparency and turbidity

Transparency

Figure 4.3 illustrates how transparency changed over time in Stiffelio Pond and enclosures. Over the course of the experiment, transparency in Stiffelio Pond remained below 0.3m; from day 22, transparency increased in all enclosures. Further, no significant differences were indicated for transparency measurements among treatments (Kruskal-Wallis test: χ^2 (3) = 0.20; P = 0.977).

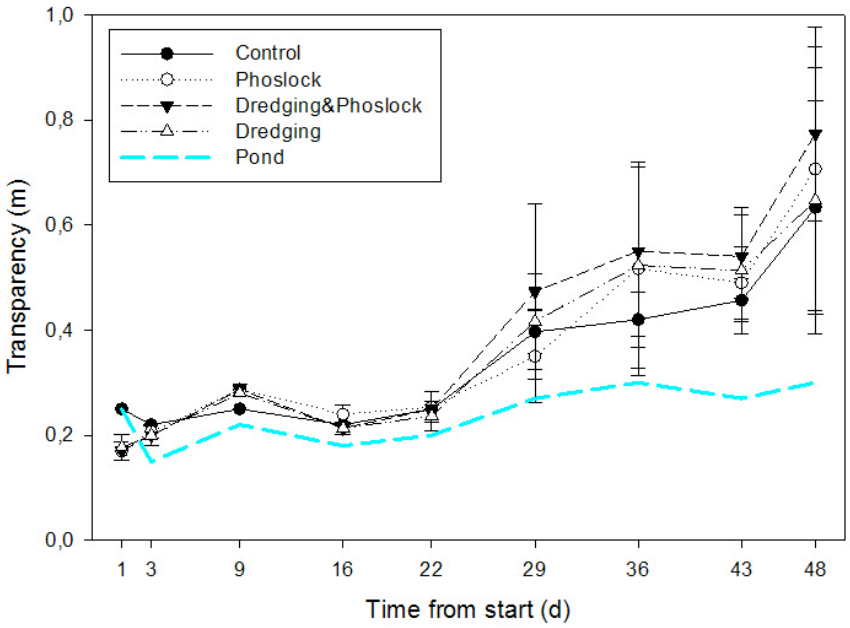
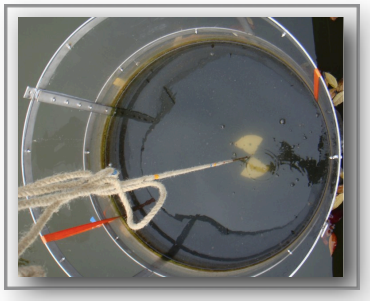


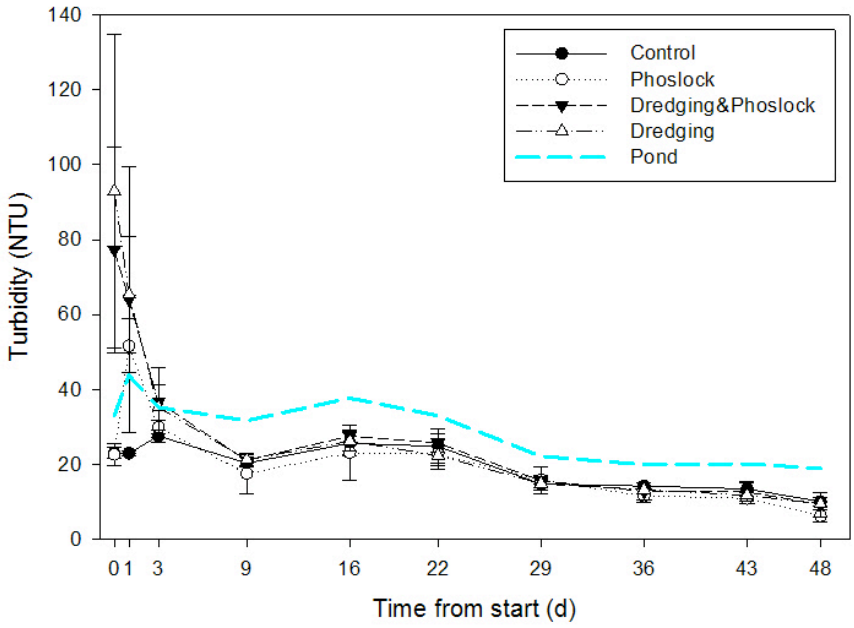
Figure 4.3: Transparency (m) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).



Using the Secchi-disk to determine transparency.

Turbidity

Initially, turbidity was high as a result of dredging and/or Phoslock® (see figure 4.4). Due to dredging small soil particles were stirred up and remained in suspension for a few days. It also took a few days for Phoslock® to settle down in the enclosures. Nine days from start, turbidity in enclosures did not deviate much and remained below Stiffelio Pond's turbidity over the course of the experiment. There were no significant differences indicated in NTU values among treatments (Kruskal-Wallis test: χ^2 (3) = 2.30; P = 0.513).



Turbid water in dredged enclosure without Phoslock® two days from dredging (one day from start).

Figure 4.4: Turbidity (NTU) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Conductivity, Oxygen, pH and Temperature

Conductivity

Conductivity followed a pattern similar to the pond and decreased from circa 280 to 210 µS cm^{-1} over the course of the experiment (see figure 4.5). Furthermore, conductivity seemed to be higher in undredged enclosures treated with Phoslock®. Though, standard deviations were large and no significant differences were indicated among treatments (Kruskal-Wallis test: χ^2 (3) = 5.31; P = 0.150).

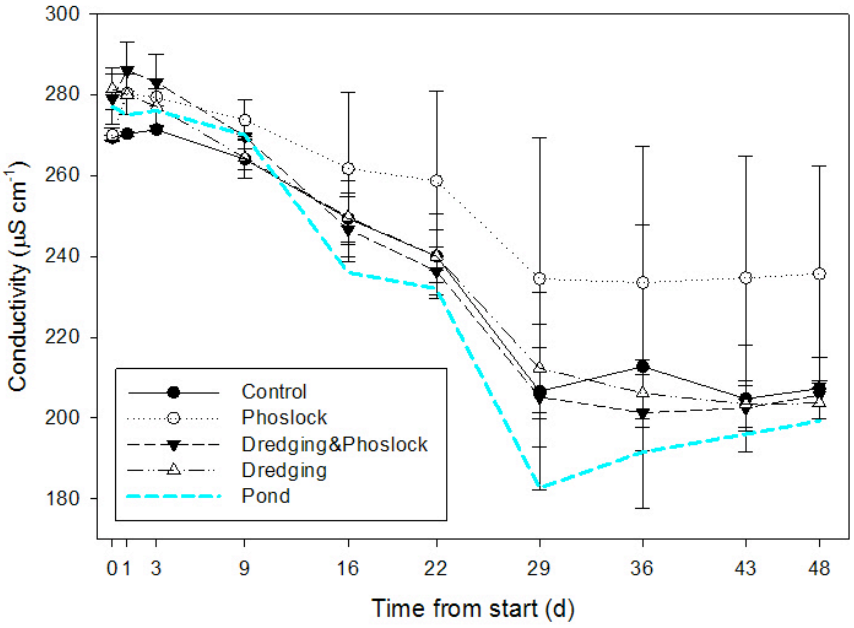


Figure 4.5: Conductivity (µS cm^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

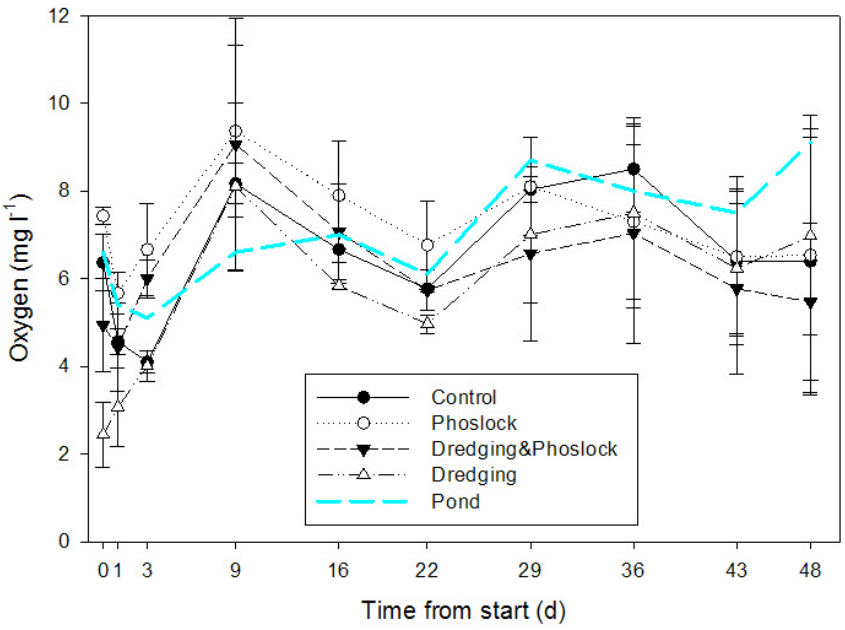


Figure 4.6: Oxygen concentrations (mg l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Oxygen

Initially, dissolved oxygen concentrations were low in dredged enclosures (ca. 4 mg l^{-1}), but from day 3 oxygen concentrations followed a pattern similar to the pond (see figure 4.6). Further, oxygen concentrations were significantly higher in undredged enclosures with Phoslock® compared to the dredged ones without Phoslock® (see figure 4.7, Scheffe post-hoc test: P = 0.015, after Kruskal-Wallis test: χ^2 (3) = 10.74; P = 0.013).

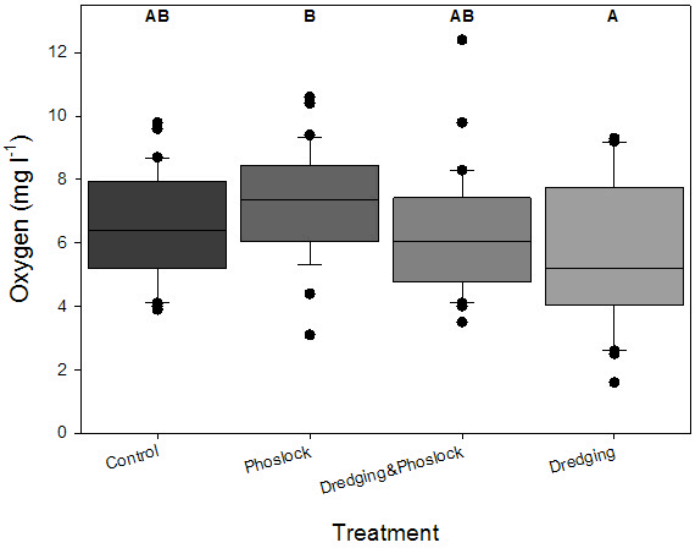


Figure 4.7: Box- and-Whisker plot of Oxygen concentrations (mg l^{-1}) for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Scheffe post-hoc test). The bottom and top of the box represent the 25th and 75th percentile (the lower and upper quartiles, respectively), and the band near the middle of the box the 50th percentile (the median). The ends of the whiskers represent the 10th and 90th percentiles, and the black dots are data points that lie outside these ends (outliers).

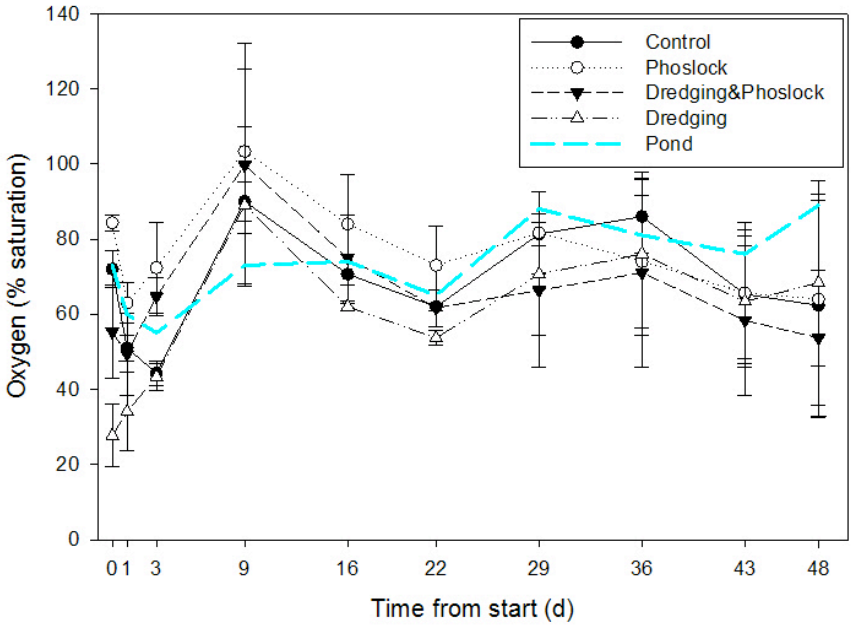


Figure 4.8: Oxygen (% saturation) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Oxygen saturation followed the same pattern as dissolved oxygen concentrations (see figure 4.8). Also, the same homogeneous groups were indicated (see figure 4.9).

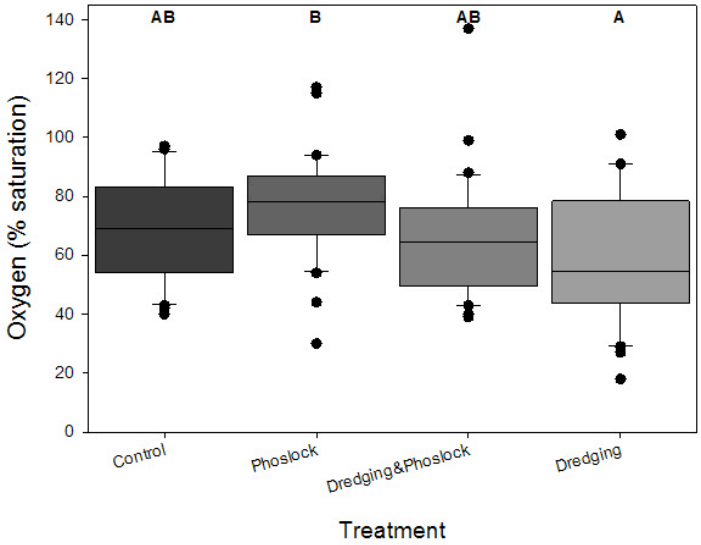


Figure 4.9: Box- and-Whisker plot of Oxygen (% saturation) for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Scheffe post-hoc test).

pH

For most of the time, pH values were higher in Stiffelio Pond (see figure 4.10). Despite an huge overlap in standard deviations, pH values were significantly higher in undredged enclosures treated with Phoslock® compared to dredged enclosures without Phoslock® (Scheffe post-hoc test: P = 0.033, after Kruskal-Wallis test: χ^2 (3) = 12.40; P = 0.006).

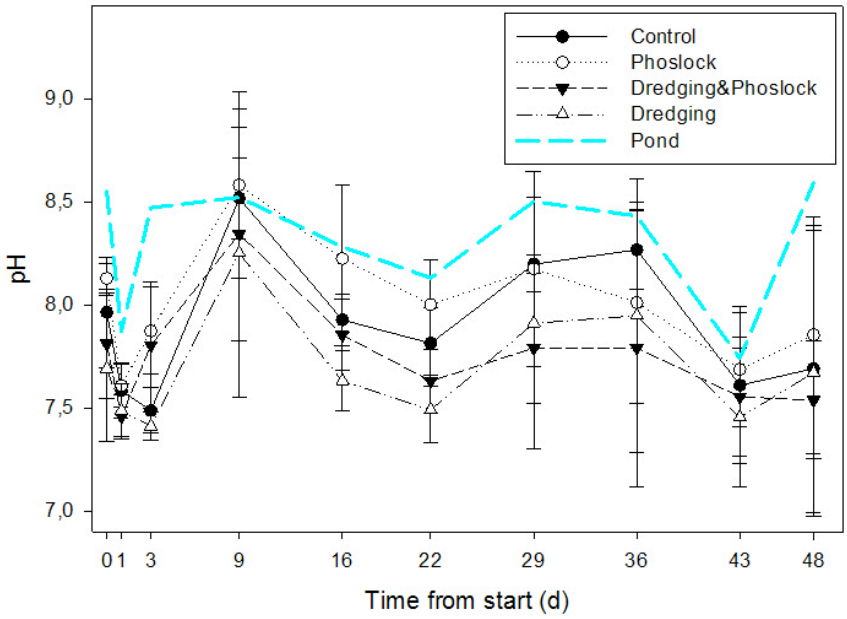


Figure 4.10: pH over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

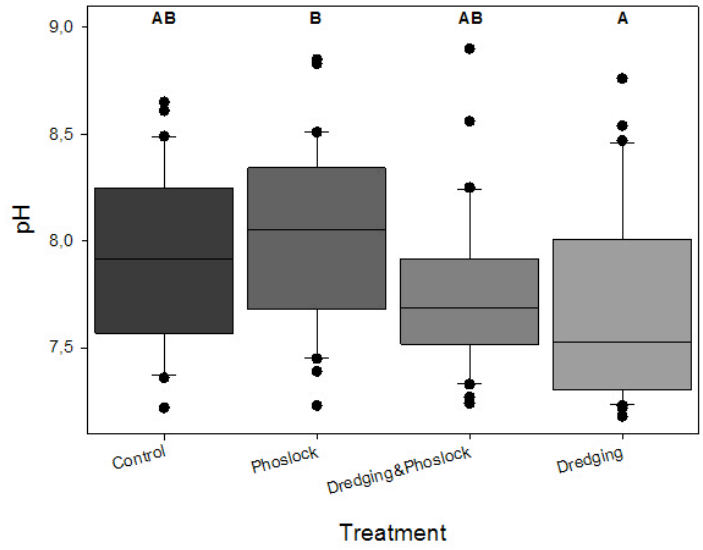


Figure 4.11: Box- and-Whisker plot of pH for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Scheffe post-hoc test).

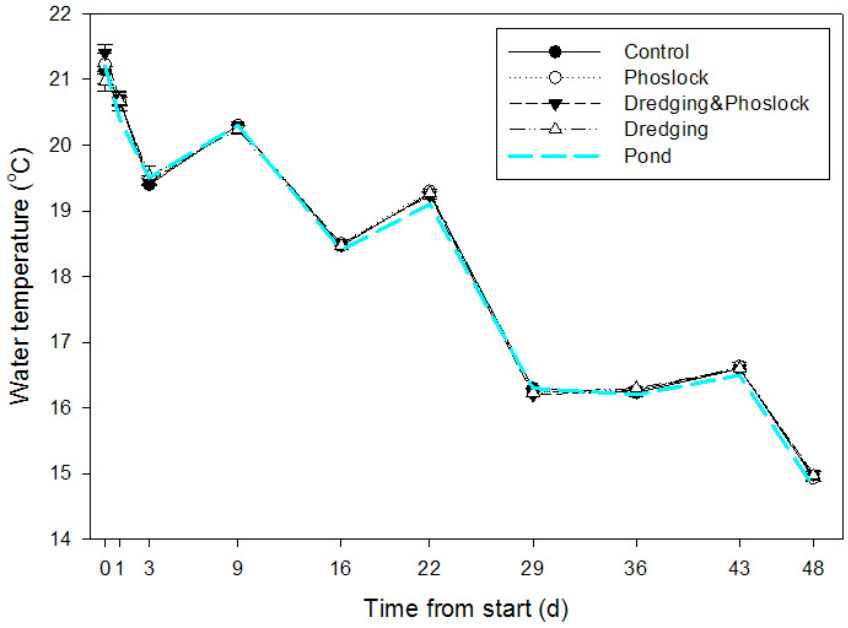


Figure 4.12: Water temperature (°C) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Temperature

Water temperature followed a pattern similar to Stiffelio Pond and decreased from circa 21 to 15 degrees Celsius over the course of the experiment (figure 4.12). Also, no significant differences were indicated among treatments (Kruskal-Wallis test: χ^2 (3) = 0.01; P = 1.000).

Total and filterable lanthanum

Total lanthanum

Initially, total lanthanum concentrations were as high as circa 2500 µg l^{-1}, but dropped to circa 100 µg l^{-1} within 9 days (see figure 4.13). Moreover, total lanthanum concentrations were equal between dredged and undredged enclosures treated with Phoslock® (Mann-Whitney U-test: U = 341.50; P = 0.109). In undredged enclosures concentrations stabilized around 50 µg La l^{-1} and in dredged enclosures around 13 µg La l^{-1}.

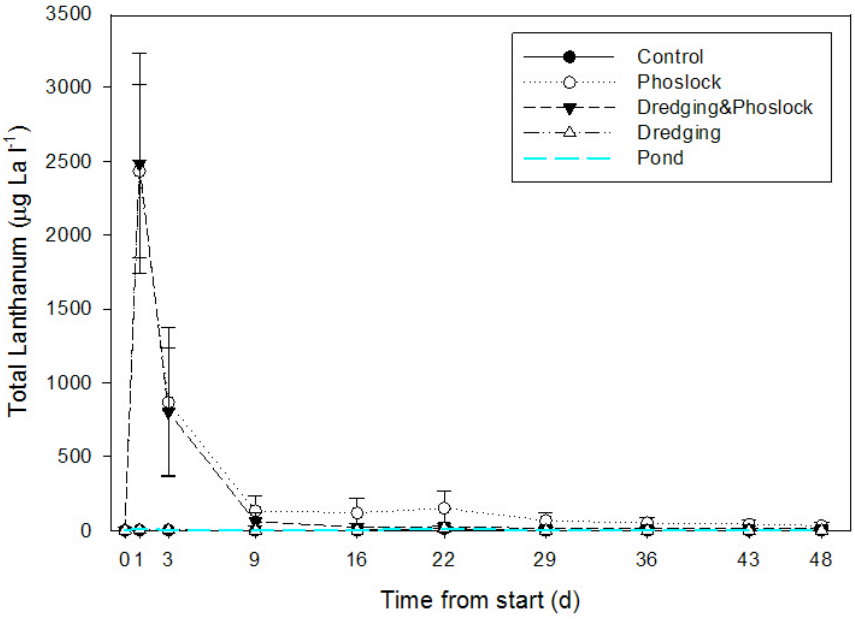


Figure 4.13: Total lanthanum concentrations (µg La l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

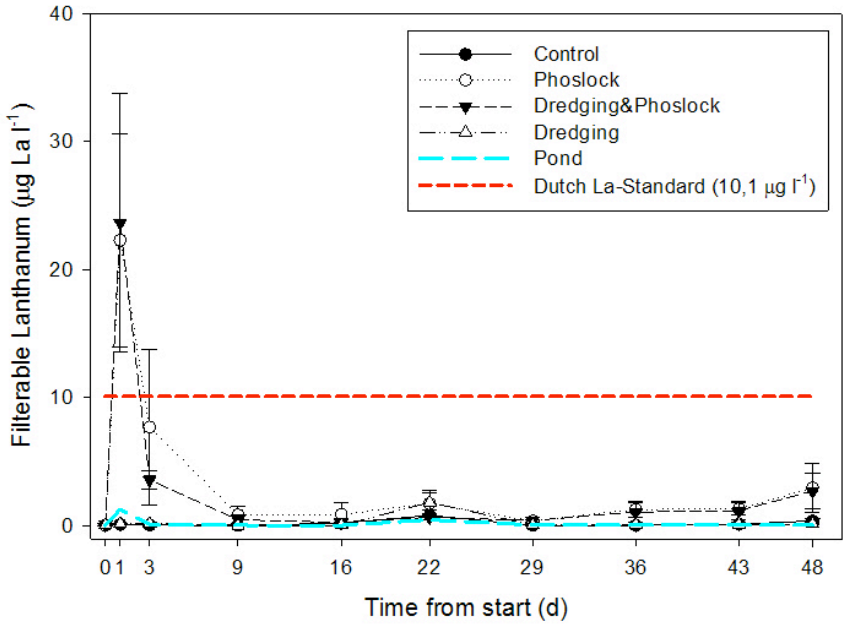


Figure 4.14: Filterable lanthanum concentrations (µg La l-1) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Filterable lanthanum

Initially, filterable lanthanum concentrations exceeded the Dutch Standard (figure 4.14). Filterable lanthanum concentrations were equal between dredged and undredged enclosures with Phoslock® (Mann-Whitney U-test: U = 390.00; P = 0.375).

Since filterable lanthanum concentrations showed an increase from day 29 (see figure 4.14), additional water samples were taken on 12 October 2010 (70 days after Phoslock® application). Figure 4.15 shows a further increase of filterable lanthanum concentrations to above (undredged enclosures with Phoslock®) and almost (dredged enclosures with Phoslock®) the Dutch standard.

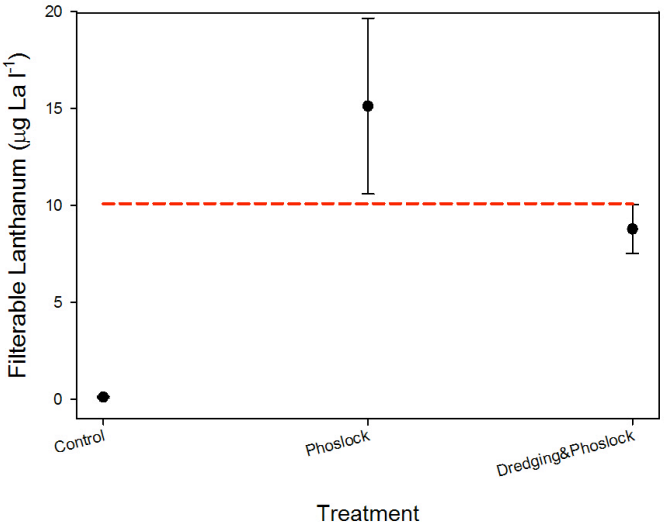


Figure 4.15: Filterable lanthanum concentrations (µg La l-1) on 12 October 2010 for the treatments: Control, Phoslock and Dredging&Phoslock. Dashed red line indicates Dutch La-Standard (10.1 µg La l-1). Error bars represent 1 SD (n = 3).

Nutrients

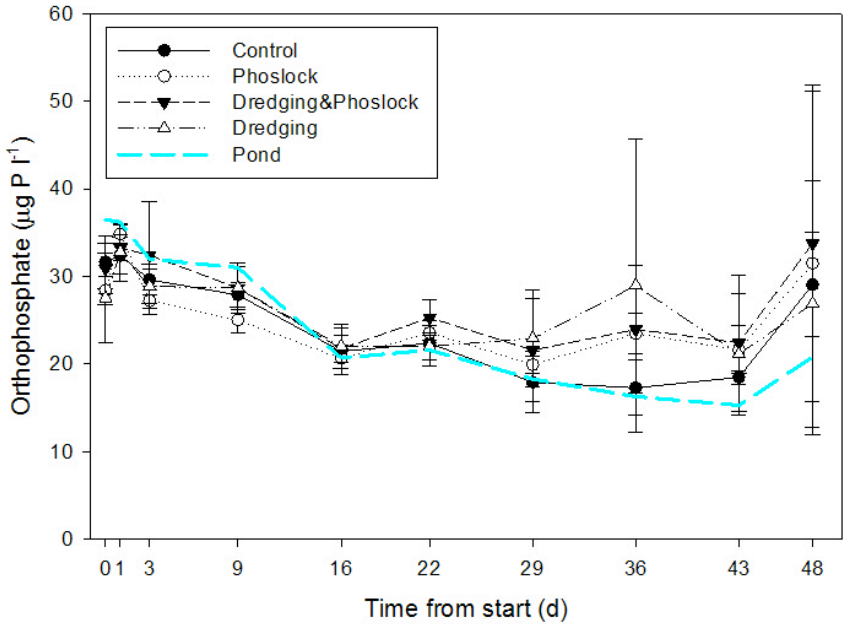


Figure 4.16: Orthophosphate concentrations (µg P l-1) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Orthophosphate

Orthophosphate concentrations followed a similar pattern and remained between circa 15 and 35 µg l^{-1} (see figure 4.16). Also, no significant differences were indicated among treatments (Kruskal-Wallis test: χ^2 (3) = 1.72; P = 0.632).

Total Phosphorus

Initially, total phosphorus concentrations were reduced by 20-30% in enclosures treated with Phoslock®. Dredging, on the other hand, caused a short increase in total phosphorus concentrations (figure 4.17). However, from day 16 total phosphorus concentrations followed a similar pattern and no significant differences were indicated among treatments (Kruskal-Wallis test: χ^2 (3) = 5.61; P = 0.132).

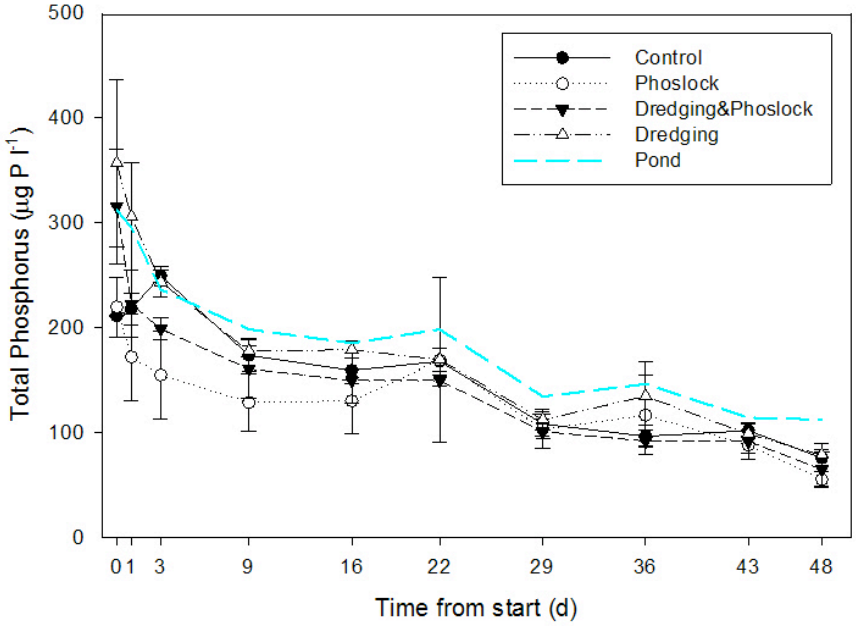


Figure 4.17: Total phosphorus concentrations (µg P l-1) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Nitrogen

Although, ammonium concentrations increased in enclosures treated with Phoslock® from day 22 (see figure 4.18), Kruskal-Wallis test did not indicate significant differences in ammonium concentrations among treatments at the 5% significance level (χ^2 (3) = 7.10; P = 0.070).

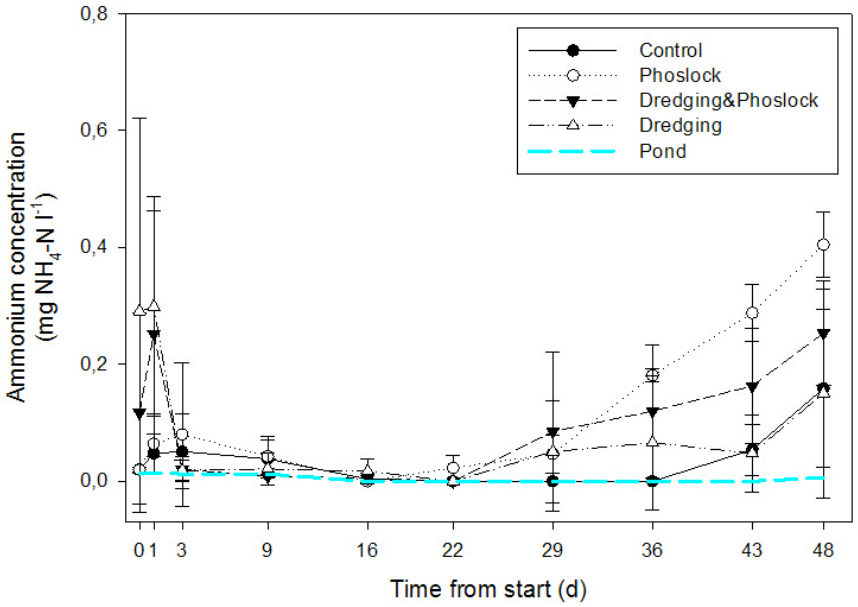


Figure 4.18: Ammonium concentrations (mg NH_4-N l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

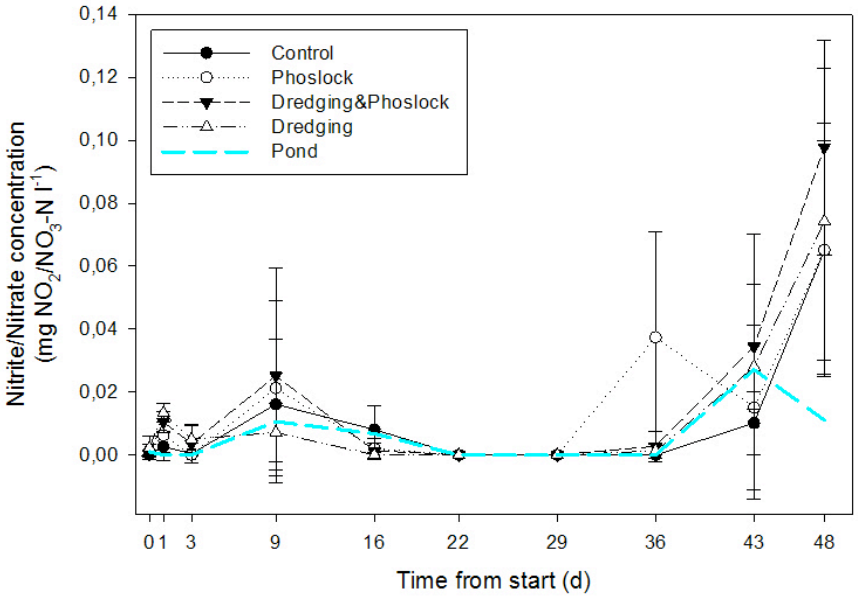


Figure 4.19: Nitrite and nitrate concentrations (mg NO_2/NO_3-N l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Dredging, Phoslock and Dredging&Phoslock. Error bars represent 1 SD (n = 3).

Nitrite and nitrate concentrations showed an irregular pattern and standard deviations were large (see figure 4.19). Also, concentrations increased from day 29, but no significant differences were indicated among treatments (Kruskal-Wallis test: (χ^2 (3) = 0.85; P = 0.838).

Total nitrogen concentrations showed a quite irregular pattern (see figure 4.20). Also, no significant differences were indicated among treatments (Kruskal-Wallis test: $\chi^2(3) = 2.65$; $P = 0.449$).

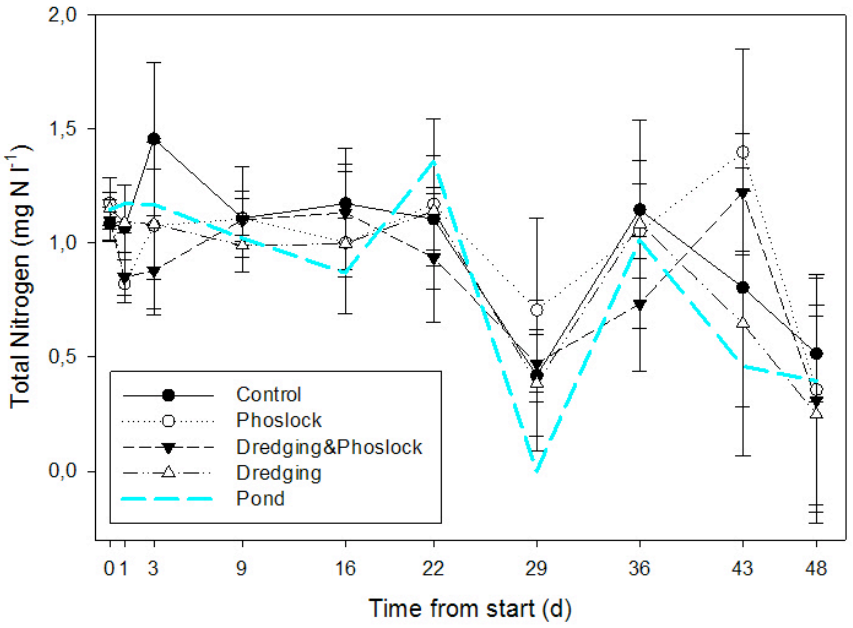


Figure 4.20: Total nitrogen concentrations (mg N l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

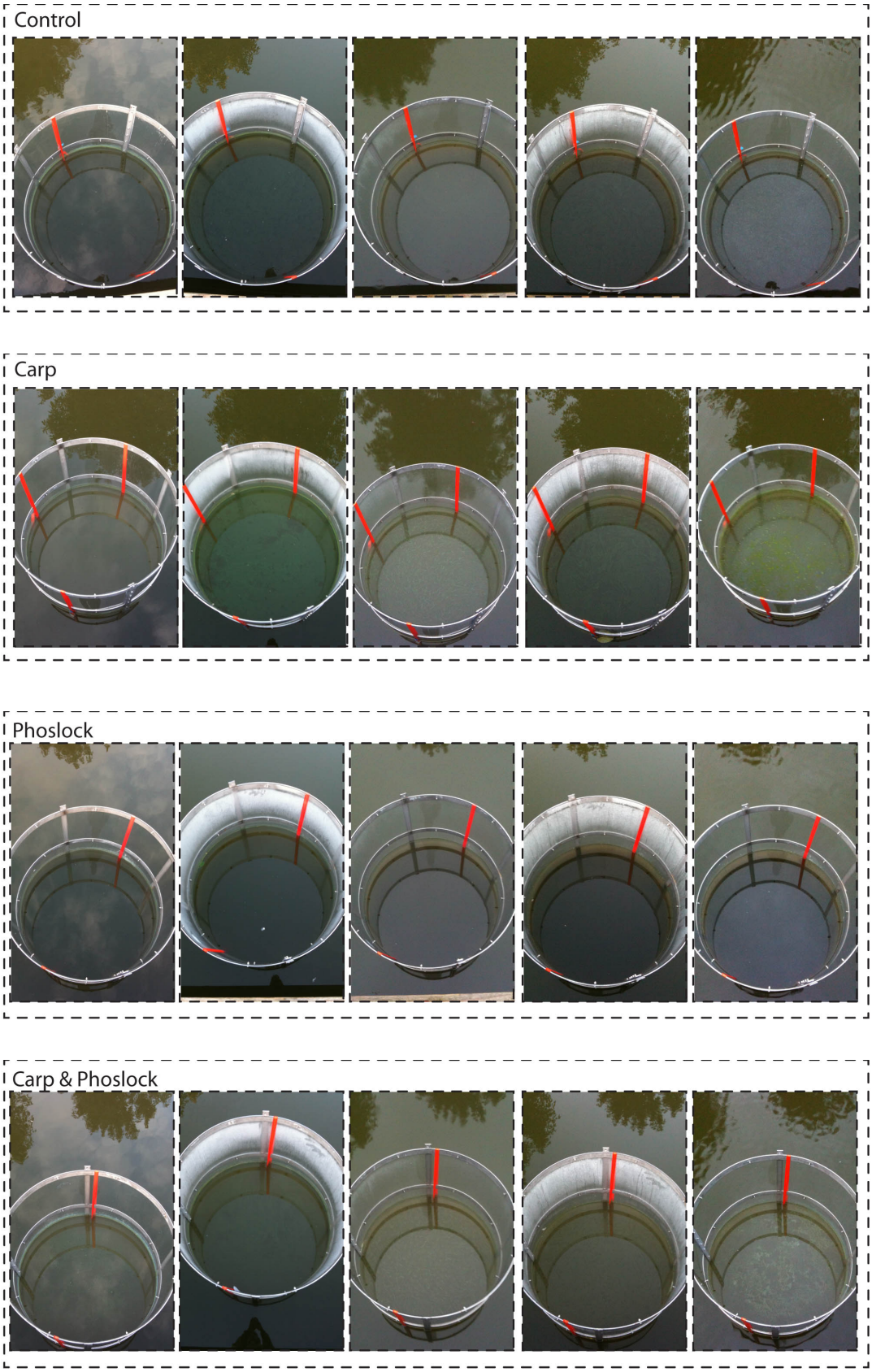


Figure 4.21: Top views of enclosures of the four treatments in chapter 5: Control, Carp, Phoslock and Carp&Phoslock on days 16, 29, 36, 43 and 48 (from left to right).

5. How carp deteriorate water quality

In this chapter the results of an enclosure study with common carp (*Cyprinus carpio* L.,1758) are presented. In order to compare the effects of carp on a variety of water quality variables, the following four treatments were examined:

1. Three enclosures were left unharmed (Control);
2. Three enclosures were stocked with one common carp (Carp);
3. Three enclosures were stocked with one common carp and then treated with Phoslock® (Carp&Phoslock);
4. Three enclosures were treated with Phoslock® (Phoslock).

Chlorophyll-*a*

Total chlorophyll-*a* was used as a proxy for algal biomass. In order to quantify the share of cyanobacteria in total chlorophyll-*a*, cyanobacterial chlorophyll-*a* concentrations have been examined as well. Both total chlorophyll-*a* data and its cyanobacterial share were analyzed separately and presented in tables 5.1 and 5.2. To analyze within and between treatment effects, repeated-measures ANOVAs were carried out for the entire research period (48 days) and for two parts of this dataset, namely the first nine days and from start until day 29.

Total chlorophyll-a

Mean total chlorophyll-*a* concentrations were significantly higher in enclosures with carp with and without Phoslock® compared to controls (see figure 5.1, Tukey post-hoc test: P = 0.001 and P = 0.004 for regarding pairwise comparisons, after RM ANOVA over 48 days).

Repeated-measures ANOVAs indicated significant time and time*carp interaction effects for all datasets (see table 5.1). So, total chlorophyll-*a* concentrations changed significantly over time and developed differently in enclosures stocked with carp compared to enclosures without carp.

Also, a significant effect of carp on total chlorophyll-*a* concentrations was indicated for all datasets. Further, significant effects of the combination carp&Phoslock® on total chlorophyll-*a* were indicated over the entire and part of the research period. These effects are also clearly illustrated by figure 4.21 (green color) and figure 5.2 in which the green bars from enclosures stocked with carp are above the dotted green line (Total-chl Stiffelio Pond) for most of the time.

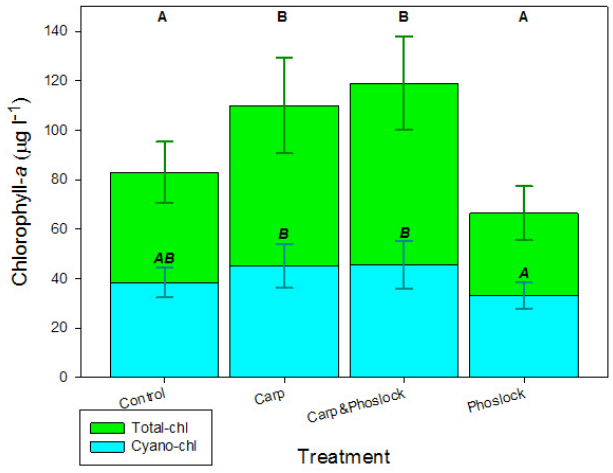


Figure 5.1: Bar graph of mean total and cyanobacterial chlorophyll-a concentrations (µg l-1) over research period (48 days) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 95% CI and identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Tukey post-hoc test). Letters posted in italics belong to cyanobacterial chlorophyll-a concentrations.

Table 5.1: Results from repeated-measures ANOVAs for total chlorophyll-a concentrations.

	9 days from start				29 days from start				48 days from start			
	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]
Mauchly's Test of Sphericity	7,96	5	0,162	-	17,14	20	0,705	-	-	44	-	0,875
Within subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Time	3,00	5711	9,44	**<0,001**	6,00	4782	8,87	**<0,001**	7,88	16135	23,63	**<0,001**
Time*Carp	3,00	5732	9,48	**<0,001**	6,00	3450	6,40	**<0,001**	7,88	3706	5,43	**<0,001**
Time*Phoslock	3,00	192	0,32	0,813	6,00	344	0,64	0,699	7,88	318	0,47	0,873
Time*Carp*Phoslock	3,00	530	0,88	0,467	6,00	624	1,16	0,344	7,88	488	0,71	0,676
Error	24,00	605			48,00	539			63,02	683		
Between subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Carp	1	40551	91,84	**<0,001**	1	53174	126,89	**<0,001**	1	47463	118,08	**<0,001**
Phoslock	1	828	1,87	0,208	1	392	0,94	0,362	1	428	1,07	0,332
Carp*Phoslock	1	844	1,91	0,204	1	3872	9,24	**0,016**	1	4883	12,15	**0,008**
Error	8	442			8	419			8	402		

a. Used to adjust the degrees of freedom for the averaged tests of significance.
If epsilon <0,75, or nothing is known about sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity.
If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.

Cyanobacterial chlorophyll-a

Mean cyanobacterial chlorophyll-*a* concentrations were significantly higher in enclosures with carp with and without Phoslock® compared to undredged enclosures treated with Phoslock® (see figure 5.1, Tukey post-hoc test: P = 0.017 and P = 0.019 for regarding pairwise comparisons, after RM ANOVA over 48 days).

Repeated-measures ANOVAs indicated a significant time*carp interaction effect for all datasets (see table 5.2). Also, a significant time effect was indicated over the entire and part of the research period for which a significant time* Phoslock® interaction effect was found as well. So, cyanobacterial chlorophyll-*a* concentrations changed significantly over time and developed differently in enclosures stocked with carp compared to enclosures without carp. For part of the research period cyanobacterial chlorophyll-*a* concentrations developed differently in enclosures treated with Phoslock® compared to untreated enclosures.
Also, a significant effect of carp on cyanobacterial chlorophyll-*a* concentrations was indicated for all datasets. Furthermore, a significant effect of Phoslock® on cyanobacterial chlorophyll-*a* concentrations was indicated over the first nine days.

Table 5.2: Results from repeated-measures ANOVAs for cyanobacterial chlorophyll-a concentrations.

	9 days from start				29 days from start				48 days from start			
	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]
Mauchly's Test of Sphericity	29,83	5	<0,001	0,857	52,75	20	<0,001	0,809	-	44	-	0,869
Within subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Time	2,57	425	3,16	0,053	4,85	1755	15,67	**<0,001**	7,83	4026	33,09	**<0,001**
Time*Carp	2,57	1186	8,79	**0,001**	4,85	892	7,96	**<0,001**	7,83	765	6,29	**<0,001**
Time*Phoslock	2,57	330	2,45	0,100	4,85	354	3,16	**0,018**	7,83	249	2,04	0,056
Time*Carp*Phoslock	2,57	84	0,63	0,584	4,85	81	0,72	0,609	7,83	58	0,47	0,867
Error	20,57	135			38,82	112			62,60	122		
Between subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Carp	1	5306	42,49	**<0,001**	1	4331	39,55	**<0,001**	1	2767	19,24	**0,002**
Phoslock	1	738	5,91	**0,041**	1	139	1,27	0,293	1	177	1,23	0,299
Carp*Phoslock	1	304	2,44	0,157	1	146	1,33	0,282	1	230	1,60	0,242
Error	8	125			8	110			8	144		

a. May be used to adjust the degrees of freedom for the averaged tests of significance;
If epsilon <0,75, or nothing is known about sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity;
If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.

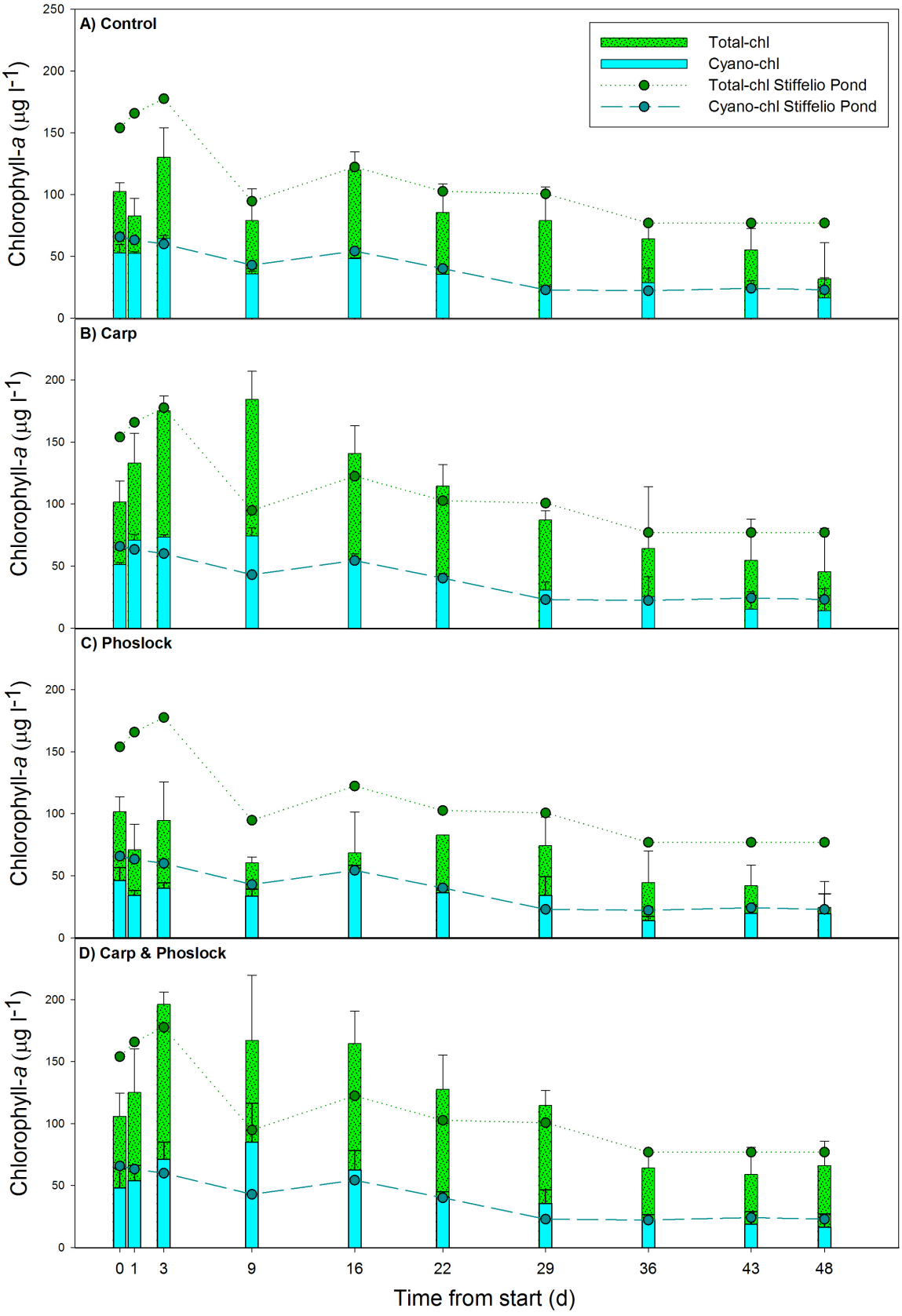


Figure 5.2: Total and cyanobacterial chlorophyll-a concentrations (µg l-1) in Stiffelio Pond, in controls (A), in enclosures with carp (B), in enclosures treated with Phoslock® (C) and in enclosures with carp and Phoslock® (D). Error bars represent 1 SD (n=3).

Transparency and turbidity

Transparency

Figure 5.3 illustrates changes in transparency in Stiffelio Pond and enclosures over time. Over the course of the experiment, transparency remained below 0.3m in Stiffelio Pond and below 0.2m in enclosures stocked with carp. This is in contrast with other enclosures in which transparency increased from day 22. Transparency measurements were significantly lower in enclosures stocked with carp with and without Phoslock® compared to the enclosures without carp (see figure 5.4, Games-Howell post-hoc: $P < 0.001$ for all pairwise comparisons, after Kruskal-Wallis test: $\chi^2 (3) = 69.74$; $P < 0.001$).

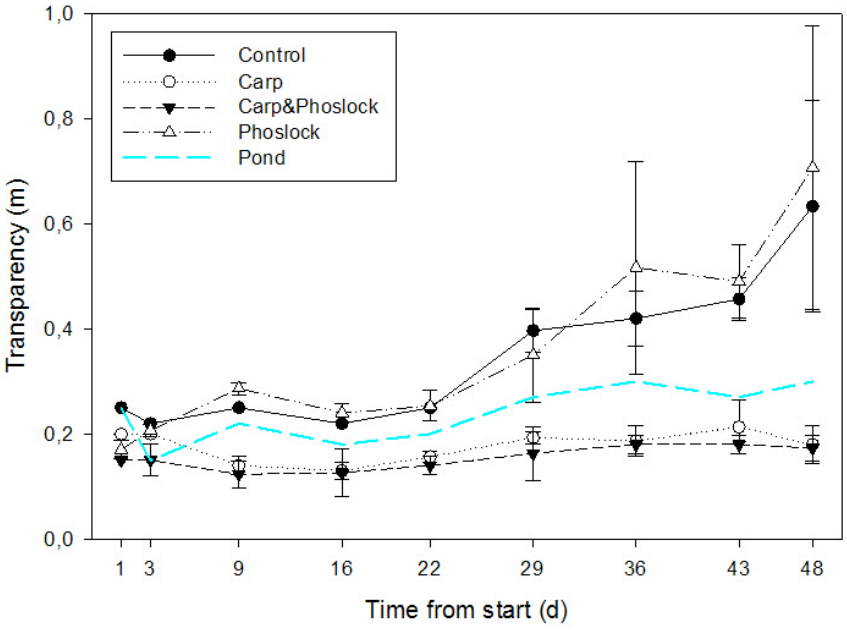


Figure 5.3: Transparency (m) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

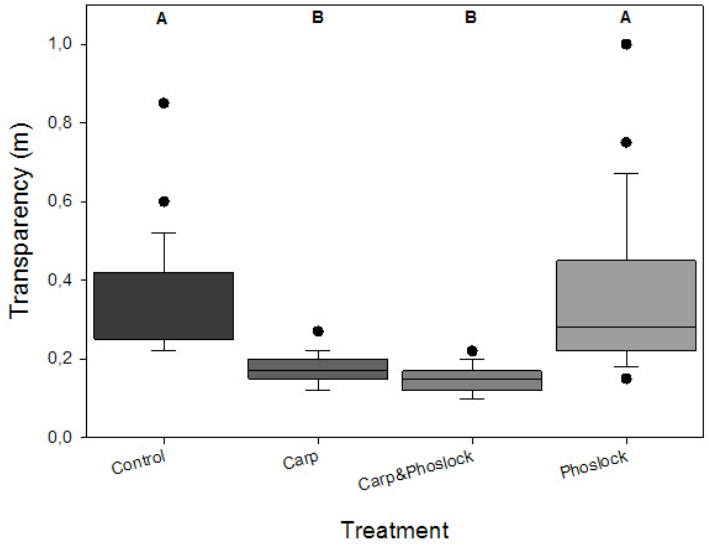


Figure 5.4: Box- and-Whisker plot of transparency (m) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Games-Howell post-hoc test).

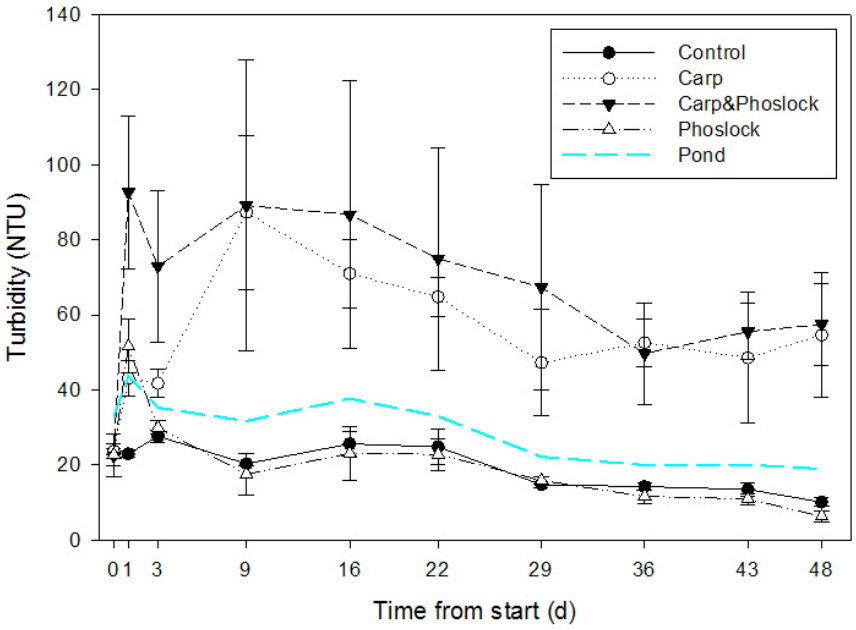


Figure 5.5: Turbidity (NTU) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

Turbidity

Turbidity was lower in undredged enclosures treated with Phoslock® and controls (< 30 NTU) than in Stiffelio Pond over the course of the experiment. In enclosures stocked with carp, on the other hand, turbidity remained circa twice as high as in the other enclosures (> 40 NTU) and stayed above Stiffelio Pond's turbidity (see figure 5.5).

NTU values were significantly higher in the enclosures stocked with carp with and without Phoslock® compared to enclosures without carp (see figure 5.6, Games-Howell post-hoc: $P < 0.001$ for all pairwise comparisons, after Kruskal-Wallis test: $\chi^2 (3) = 74.03$; $P < 0.001$).

Enclosure with carp and Phoslock® right after application.

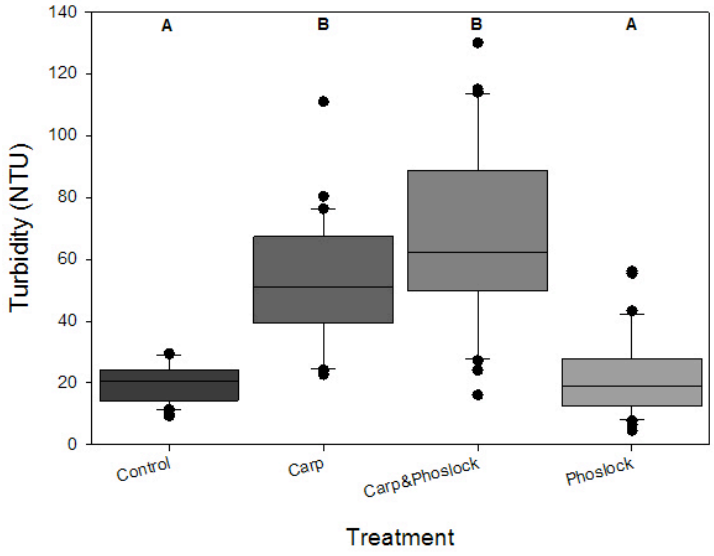


Figure 5.6: Box- and-Whisker plot of turbidity (NTU) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Games-Howell post-hoc test).

Conductivity, Oxygen, pH and Temperature

Conductivity

Conductivity followed a pattern similar to the pond and decreased from circa 280 to 200 µS cm^{-1} over the course of the experiment. Further, conductivity seemed to be higher in undredged enclosures treated with Phoslock® and in enclosures with carp and without Phoslock® (see figure 5.7). Though, no significant differences were indicated among treatments at the 5% significance level (Kruskal-Wallis test: χ^2 (3) = 7.38; P = 0.061).

Measuring conductivity, oxygen, pH and temperature.

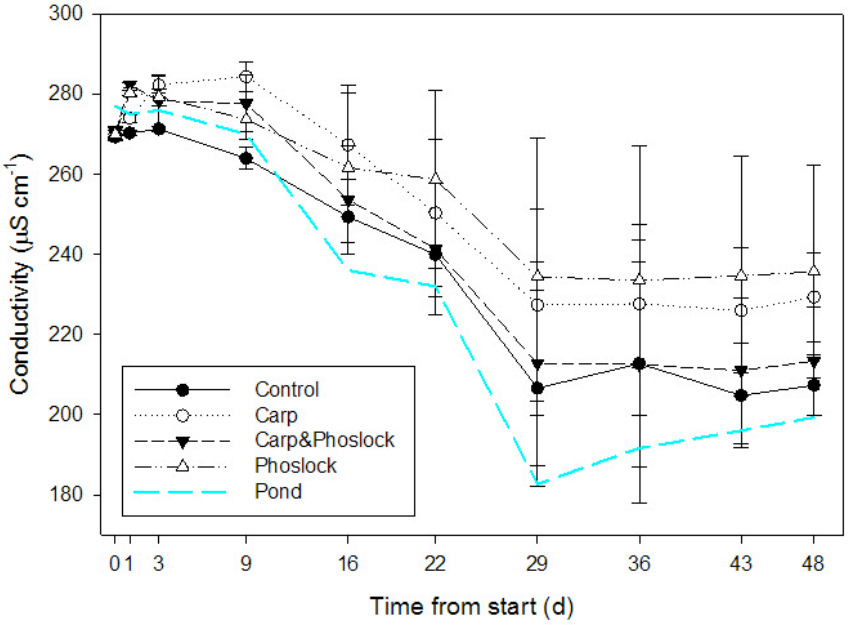


Figure 5.7: Conductivity (µS cm^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

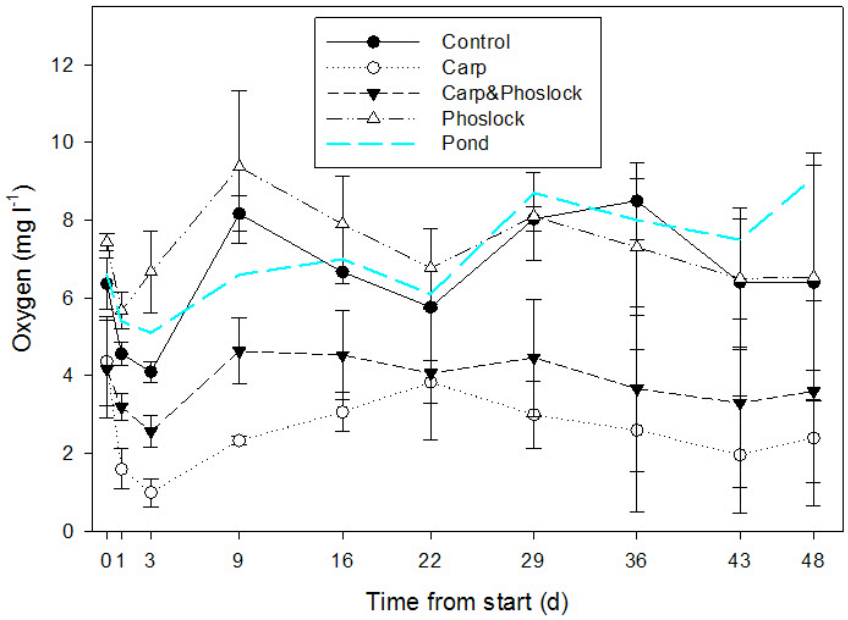
Oxygen

Repeated-measures ANOVAs indicated a significant time effect for dissolved oxygen concentrations over the research period (see table 5.3), which means that oxygen concentrations had changed significantly over time.
Also, significant negative effects of carps on oxygen concentrations were indicated over the research period.

Table 5.3: Results from repeated-measures ANOVAs for oxygen concentrations.

	Oxygen (mg/l)				**Oxygen (% saturation)**			
Mauchly's Test of Sphericity	χ^2	df	P	Epsilon[a]	χ^2	df	P	Epsilon[a]
	.	44	.	0,523	.	44	.	0,576
Within subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Time	2,37	34	8,28	**0,002**	2,53	3833	9,67	**<0,001**
Time*Carp	2,37	10	2,48	0,103	2,53	1018	2,57	0,091
Time*Phoslock	2,37	5	1,15	0,345	2,53	535	1,35	0,285
Time*Carp*Phoslock	2,37	4	0,93	0,427	2,53	395	1,00	0,403
Error	18,99	4			20,25	396		
Between subject effects	*df*	*Mean Square*	*F*	*P*	*df*	*Mean Square*	*F*	*P*
Carp	1	398	43,75	**<0,001**	1	44622	46,18	**<0,001**
Phoslock	1	28	3,07	0,118	1	3287	3,40	0,102
Carp*Phoslock	1	2	0,19	0,677	1	178	0,18	0,679
Error	8	9			8	966		

a. May be used to adjust the degrees of freedom for the averaged tests of significance.
If epsilon <0,75, or nothing is known about sphericity, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity.
If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.



Oxygen concentrations remained below 5 mg l^{-1} in enclosures stocked with carp over the research period (see figure 5.8). Further, oxygen concentrations in enclosures with carp with and without Phoslock® were significantly lower compared to control enclosures (see figure 5.9, Tukey post-hoc test: $P < 0.001$ for both pairwise comparisons, after one-way ANOVA: $F_{3,116} = 59.41$; $P < 0.001$).

Figure 5.8: Oxygen concentrations (mg l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

Furthermore, oxygen concentrations were significantly higher in enclosures with carp and Phoslock® compared to enclosures with carp and without Phoslock® (Tukey post-hoc test: $P = 0.017$, after one-way ANOVA: $F_{3,116} = 59.41$; $P < 0.001$). Moreover, oxygen concentrations near the sediment were about 0 mg l^{-1} in enclosures stocked with carp (not plotted in graph).

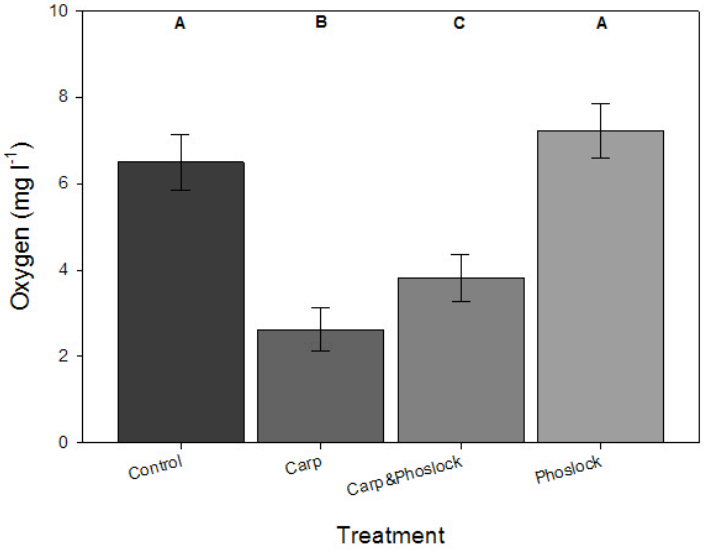


Figure 5.9: Bar graph of mean oxygen concentrations (mg l^{-1}) over research period (48 days) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 95% CI and identical letters (A, B, C) indicate homogeneous groups that are not different at the 95% level (Tukey post-hoc test).

Oxygen saturation followed the same pattern as dissolved oxygen concentrations (see figure 5.10). Also, the same homogeneous groups were indicated (see figure 5.11).

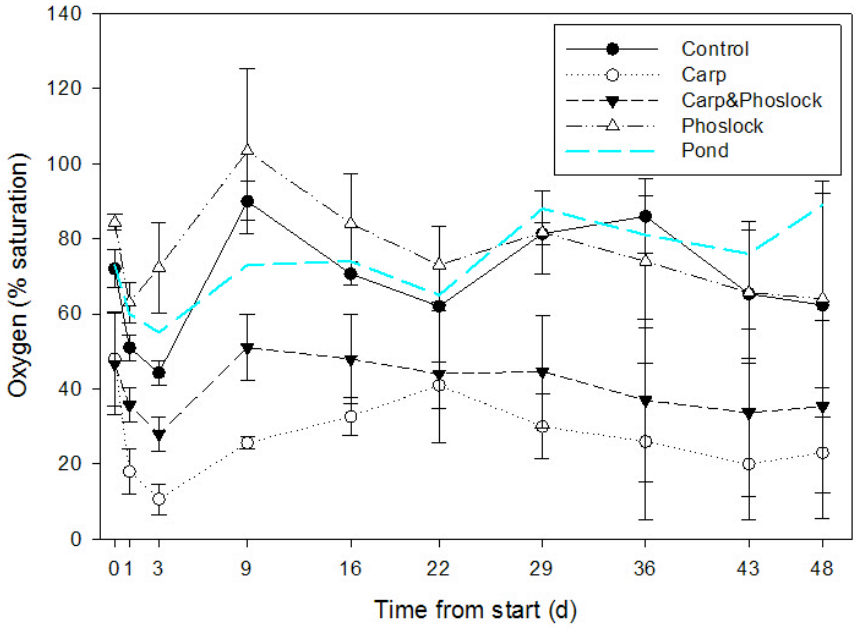


Figure 5.10: Oxygen concentrations (% saturation) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

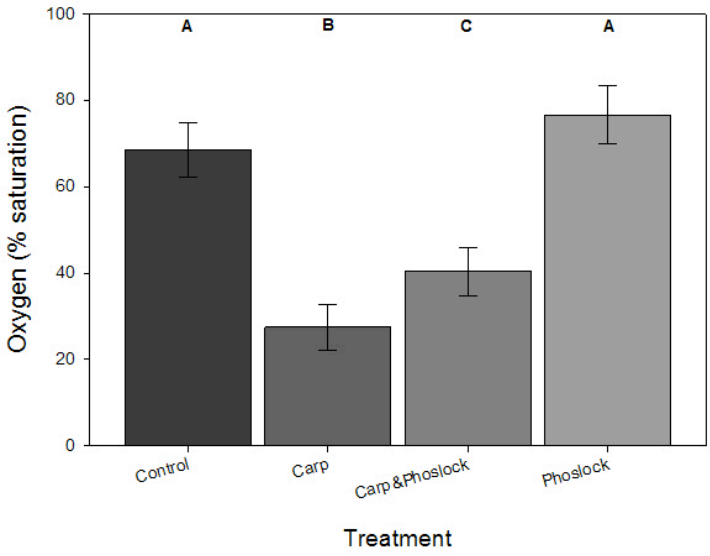


Figure 5.11: Bar graph of mean oxygen concentrations (% saturation) over research period (48 days) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 95% CI and identical letters (A, B, C) indicate homogeneous groups that are not different at the 95% level (Tukey post-hoc test).

pH

Repeated-measures ANOVAs indicated significant time and time*carp interaction effects for pH (see table 5.4), which means that pH changed significantly over time and that pH developed differently in enclosures with carp compared to enclosures without carp.
Also, a significant effect of carp on pH was indicated over the research period.

Table 5.4: Results from repeated-measures ANOVAs for pH.

	pH			
	χ^2	df	P	Epsilon[a]
Mauchly's Test of Sphericity	.	44	.	0,753
Within subject effects	*df*	*Mean Square*	*F*	*P*
Time	6,78	0,61	12,35	**<0,001**
Time*Carp	6,78	0,25	5,11	**<0,001**
Time*Phoslock	6,78	0,02	0,45	0,863
Time*Carp*Phoslock	6,78	0,05	1,06	0,401
Error	54,23	0,05		
Between subject effects	*df*	*Mean Square*	*F*	*P*
Carp	1	9,47	18,92	**0,002**
Phoslock	1	0,19	0,39	0,551
Carp*Phoslock	1	0,02	0,05	0,837
Error	8	0,50		

a. May be used to adjust the degrees of freedom for the averaged tests of significance. If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.

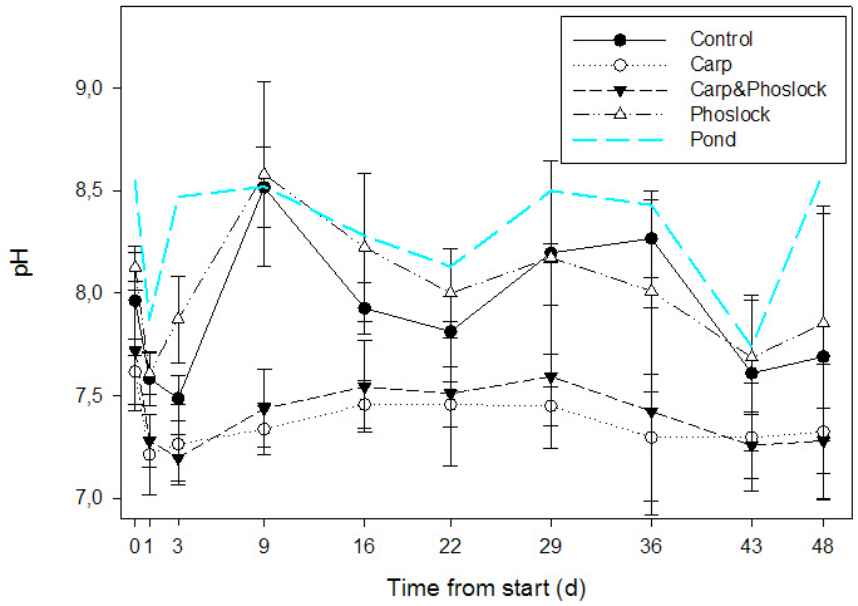


Figure 5.12: pH over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

For most of the time, pH values were higher in Stiffelio Pond and lower in enclosures stocked with carp (see figure 5.12). Also, significantly lower pH values were indicated for enclosures with carp with and without Phoslock® compared to enclosures without carps (see figure 5.13, Games-Howell post-hoc test: $P < 0.001$ for all pairwise comparisons, after one-way ANOVA: $F_{3,116} = 28.65$; $P < 0.001$).

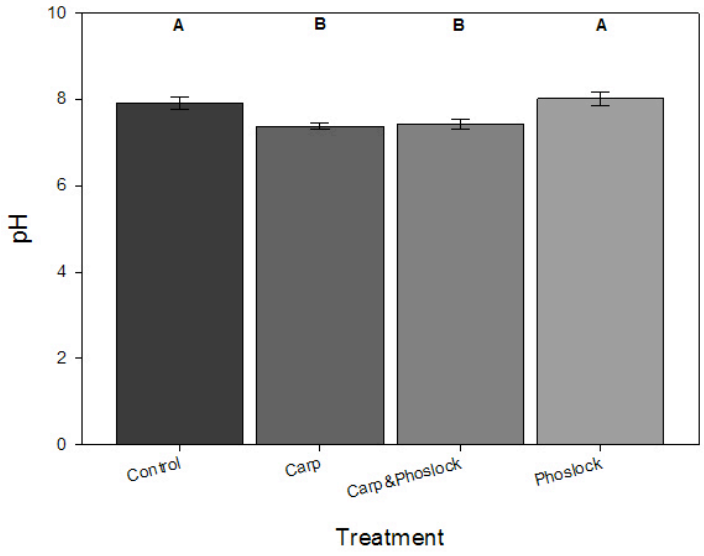


Figure 5.13: Bar graph of mean pH values over research period (48 days) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 95% CI and identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Games-Howell post-hoc test).

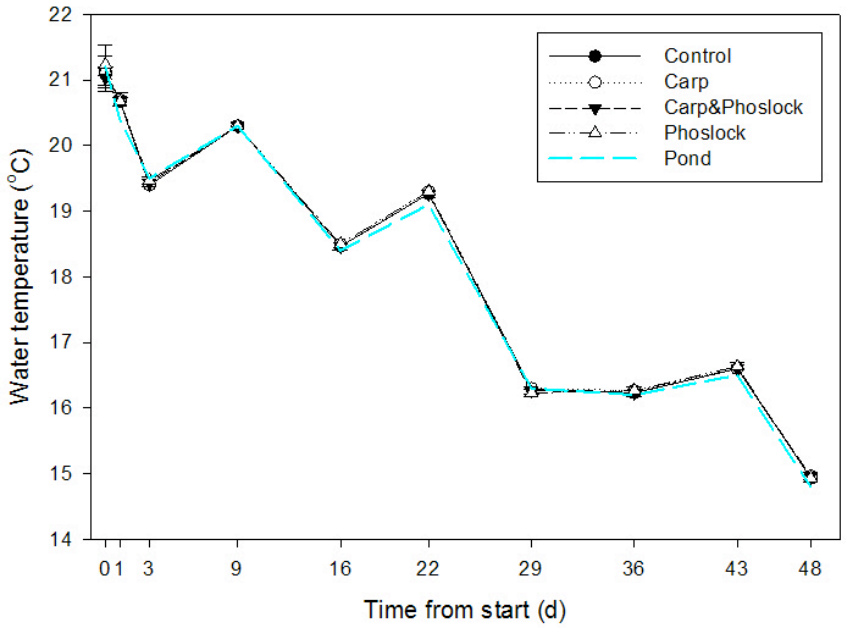


Figure 5.14: Water temperature (°C) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

Temperature

Water temperature followed a pattern similar to Stiffelio Pond and decreased from circa 21 to 15 degrees Celsius over the course of the experiment (see figure 5.14). Also, no significant differences were indicated among treatments (Kruskal-Wallis test: χ^2 (3) = 0.02; P = 0.999).

Total and filterable lanthanum

Total lanthanum

Initially, total lanthanum concentrations were as high as circa 2500 µg l^{-1} in enclosures without carp, but had dropped to circa 100 µg l^{-1} in nine days and stabilized around 50 µg l^{-1} (see figure 5.15). In enclosures stocked with carp, on the other hand, total lanthanum concentrations were initially as high as circa 4500 µg l^{-1} and stabilized around 500 µg l^{-1}. Furthermore, total lanthanum concentrations were significantly higher in enclosures with carp and Phoslock® compared to enclosures without carp and with Phoslock® (Mann-Whitney U-test: U = 199.00; P < 0.001)

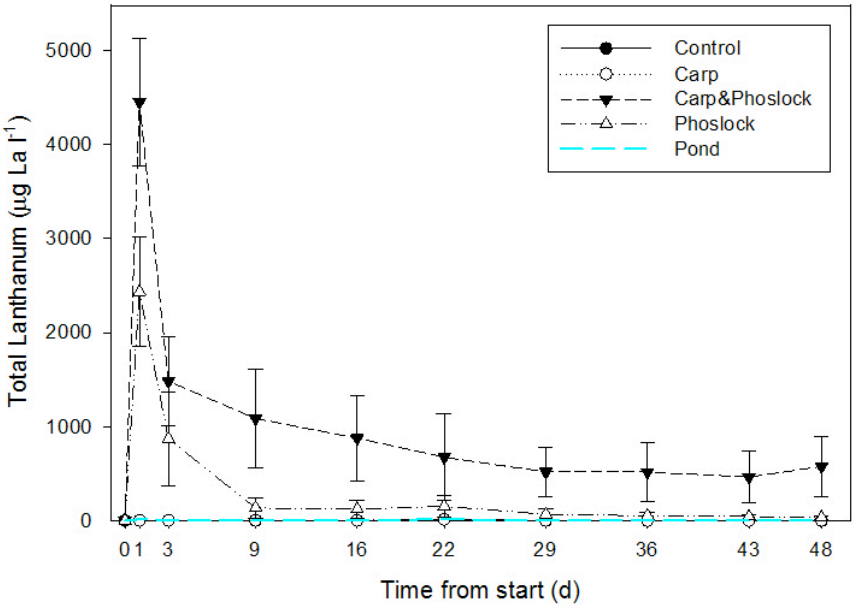


Figure 5.15: Total lanthanum concentrations (µg La l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

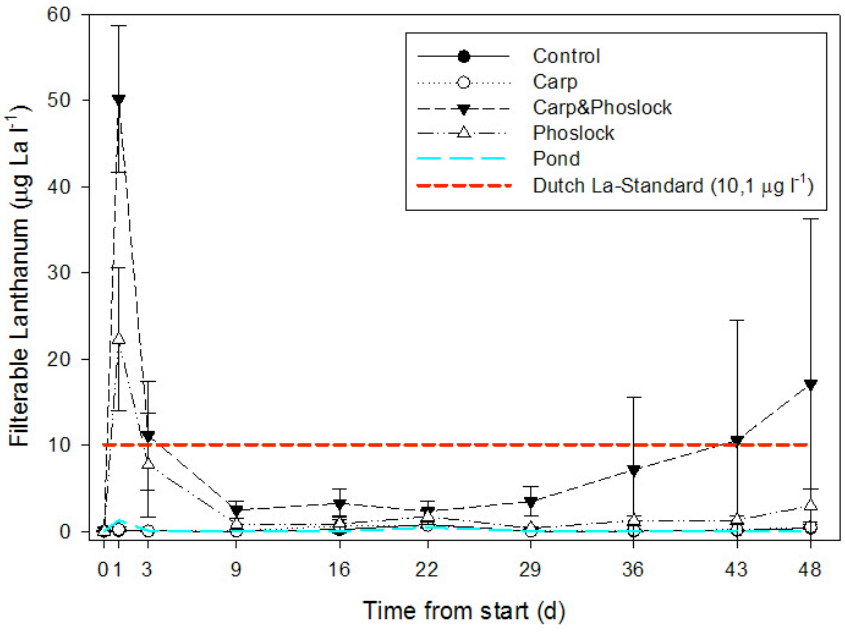


Figure 5.16: Filterable lanthanum concentrations (µg La l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

Filterable lanthanum

Initially, filterable lanthanum concentrations exceeded the Dutch Standard in undredged enclosures treated with Phoslock® (see figure 5.16). In enclosures stocked with carp, on the other hand, the Dutch Standard was exceeded both initially and from day 43. Also, filterable lanthanum concentrations were significantly higher in enclosures with carp and Phoslock® compared to enclosures without carp and with Phoslock® (Mann-Whitney U-test: U = 247.50; P = 0.003).

Nutrients

Orthophosphate

Orthophosphate concentrations followed a pattern similar to the pond and remained between circa 15 and 35 µg l^{-1} (see figure 5.17).

Although, orthophosphate concentrations in with Phoslock® treated enclosures did not differ significantly from control enclosures, orthophosphate concentrations were significantly lower in enclosures with carp and Phoslock® compared to enclosures with just carp (see figure 5.18, Scheffe post-hoc: P = 0.021, after Kruskal-Wallis test: χ^2 (3) = 10.08; P = 0.018).

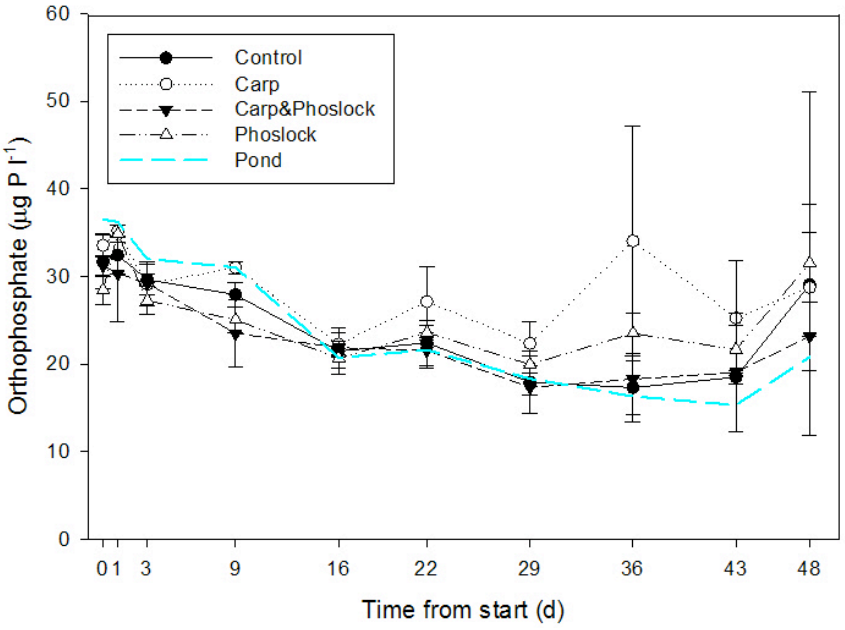


Figure 5.17: Orthophosphate concentrations (µg P l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

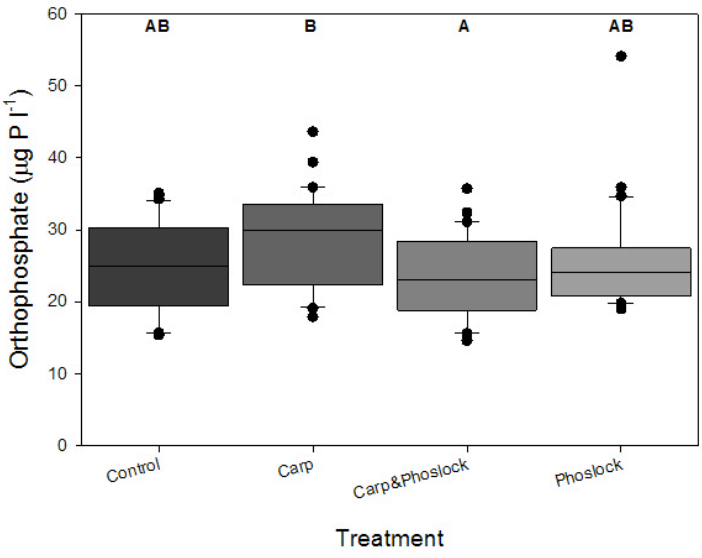


Figure 5.18: Box- and-Whisker plot of orthophosphate concentrations (µg P l^{-1}) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Scheffe post-hoc test).

Total Phosphorus

Initially, total phosphorus concentrations decreased in undredged enclosures treated Phoslock® (see figure 5.19). Total phosphorus concentrations were higher in enclosures stocked with carp and remained between circa 180 and 350 µg l^{-1} over the course of the experiment.

Furthermore, total phosphorus concentrations were significantly higher in enclosures stocked with carp with and without Phoslock® compared to enclosures without carp (see figure 5.20, Tukey post-hoc test: $P < 0.001$ for all pairwise comparisons, after one-way ANOVA: $F_{3,116} = 25.03$; $P < 0.001$).

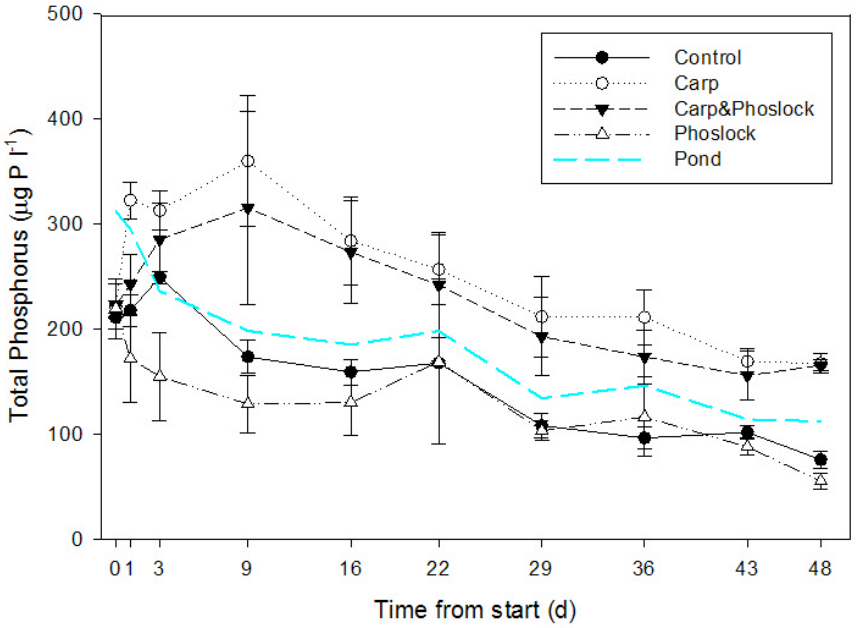


Figure 5.19: Total phosphorus concentrations (µg P l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

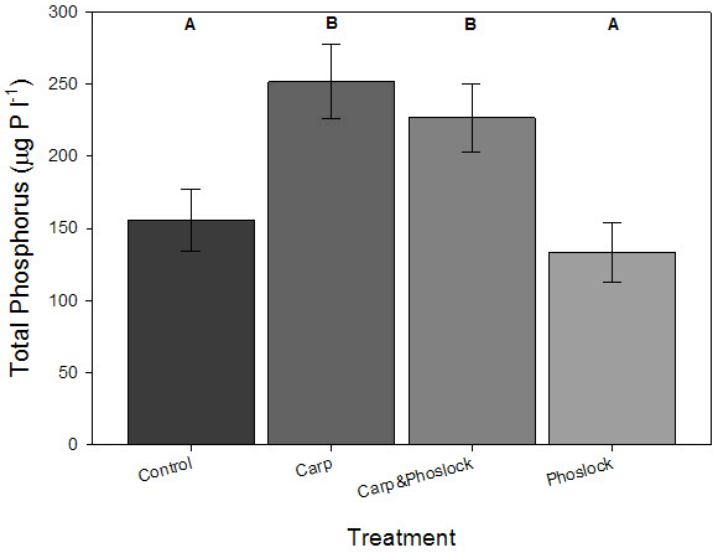


Figure 5.20: Bar graph of mean total phosphorus concentrations over research period (48 days) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 95% CI and identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Tukey post-hoc test).

Table 5.5: Results from repeated-measures ANOVAs for total phosphorus.

	Total Phosphorus			
Mauchly's Test of Sphericity	χ^2	df	P	Epsilon[a]
	.	44	.	0,833
Within subject effects	*df*	*Mean Square*	*F*	*P*
Time	7,50	36431	34,56	**<0,001**
Time*Carp	7,50	7548	7,16	**<0,001**
Time*Phoslock	7,50	2032	1,93	0,076
Time*Carp*Phoslock	7,50	1008	0,96	0,475
Error	59,97	1054		
Between subject effects	*df*	*Mean Square*	*F*	*P*
Carp	1	266963	93,54	**<0,001**
Phoslock	1	16851	5,90	**0,041**
Carp*Phoslock	1	46	0,02	0,902
Error	8	2854		

a. May be used to adjust the degrees of freedom for the averaged tests of significance.
If epsilon >0,75, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity.

Repeated-measures ANOVAs indicated significant time and time*carp interaction effects for total phosphorus (see table 5.5). So, total phosphorus concentrations changed significantly over time and developed significantly different in enclosures stocked with carp compared to enclosures without carp.
Also, significant effects were indicated for carp and Phoslock® on total phosphorus concentrations over the research period.

Nitrogen

Initially, nitrite and nitrate concentrations were similar, but increased in enclosures with carp and without Phoslock® from day 29 (see figure 5.21). Though, standard deviations were large and no significant differences in concentrations were indicated among treatments (Kruskal-Wallis test: χ^2 (3) = 3.34; P = 0.342).

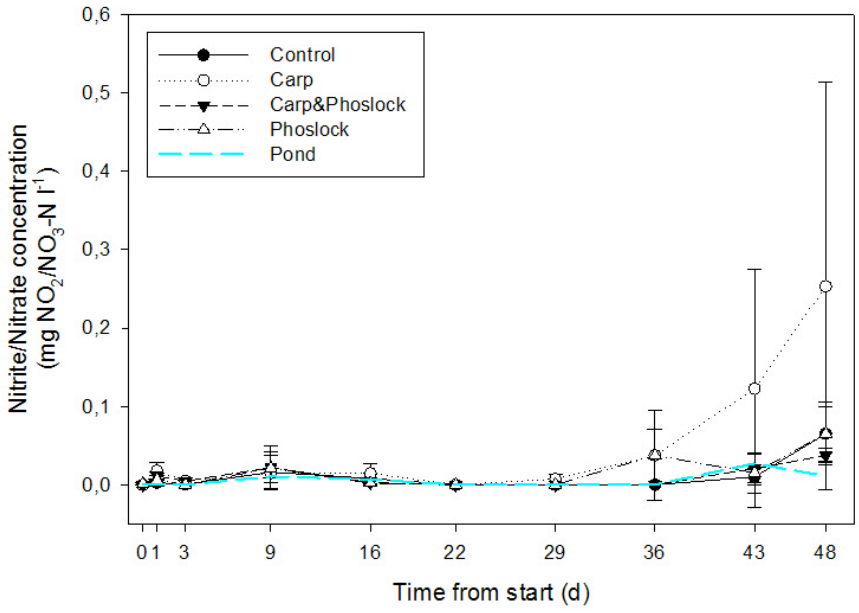


Figure 5.21: Nitrite and nitrate concentrations (mg NO_2/NO_3-N l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

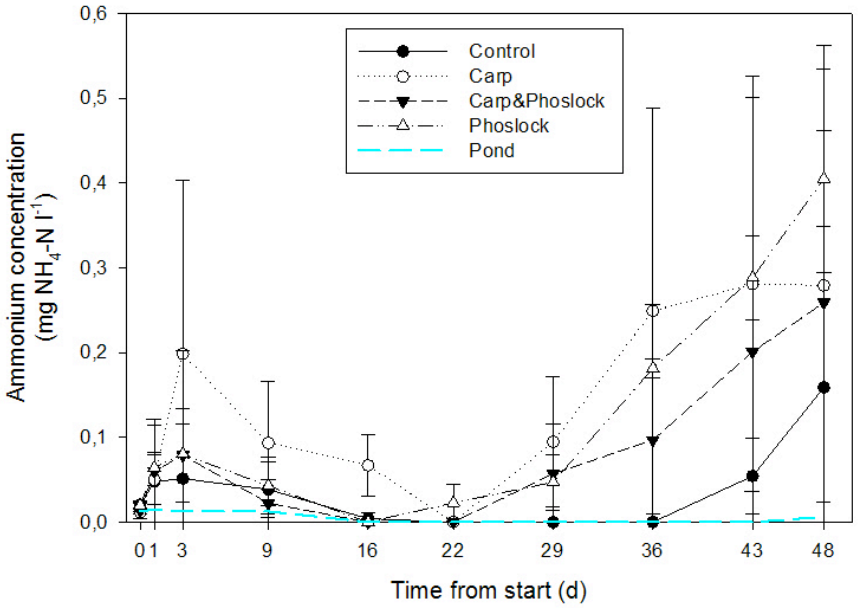


Figure 5.22: Ammonium concentrations (mg NH_4-N l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

Initially, ammonium concentrations showed a decreasing trend, but increased from day 22 until the end of the research period (see figure 5.22). Furthermore, ammonium concentrations in undredged enclosures treated with Phoslock® were significantly higher compared to control enclosures (see figure 5.23, Games-Howell post-hoc: P = 0.046, after Kruskal-Wallis test: χ^2 (3) = 9.80; P = 0.020).

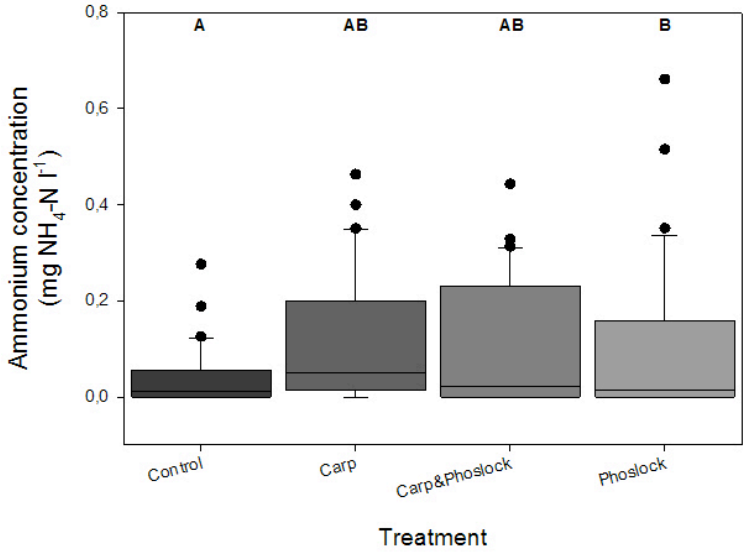


Figure 5.23: Box- and-Whisker plot of Ammonium concentrations (mg NH_4-N l^{-1}) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Games-Howell post-hoc test).

Total nitrogen concentrations showed a quite irregular pattern (see figure 5.24). Repeated-measures ANOVA indicated a significant time effect for total nitrogen, which means that total nitrogen concentrations changed significantly over time ($F_{7.6, 0.84} = 12.32$; $P < 0.001$). Furthermore, no significant differences in total nitrogen concentrations were indicated among treatments (one-way ANOVA: $F_{3,116} = 0.98$; $P = 0.406$).

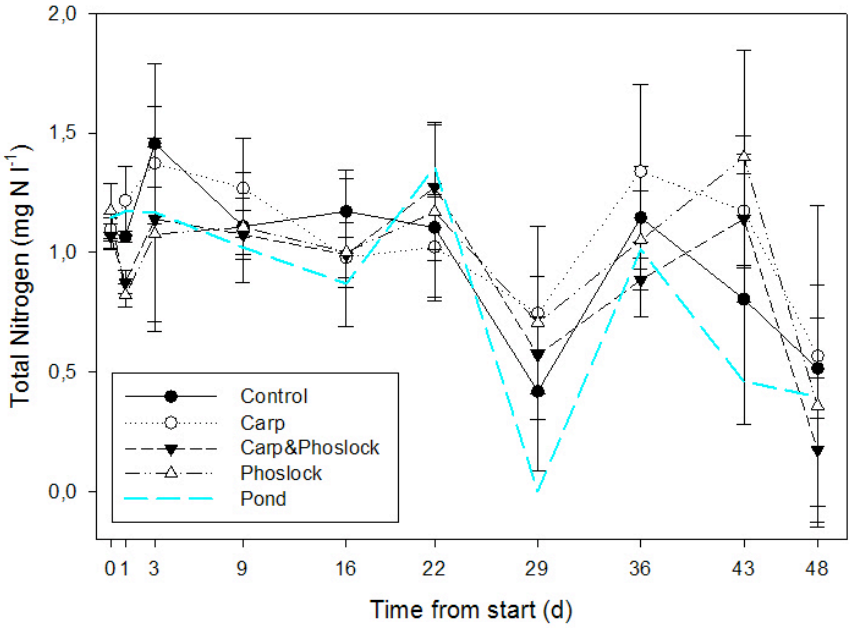


Figure 5.24: Total nitrogen concentrations (mg N l^{-1}) over research period (48 days) in Stiffelio Pond and for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Error bars represent 1 SD (n = 3).

6. Ecological Quality Ratios for phytoplankton

In table 6.1 phytoplankton scores are shown for water samples taken from the enclosures on 20-09-10 and from Stiffelio Pond on 03-08-10 and 20-09-10. For ecological quality assessment, phytoplankton is like other water quality elements ascribed an 'Ecological Quality Ratio' (EQR) in the Water Framework Directive. This EQR lies between 0 and 1, from which ''1'' is the best ratio (highest quality) to reach. A ratio of ''1'' corresponds to phytoplankton species composition and total chlorophyll-*a* concentrations in an undisturbed, natural, small lake (natural reference). For each treatment, average chlorophyll-*a* concentrations (Avg. Chl-*a*) were calculated over the research period. If Aphanizomenon filaments exceeded the threshold of 1000 filaments per ml, 0.55 was taken as EQR, since algal blooms #24 and #25 were indistinguishable (see appendix 2: Phytoplankton scoring according WFD).

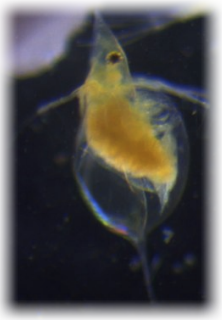
Highest scores for phytoplankton were reached in dredged enclosures with and without Phoslock® (resp. 0.37 and 0.39). Enclosures with carp with and without Phoslock® scored the lowest, respectively 0.22 and 0.23. Finally, Stiffelio Pond's score had decreased from 0.33 to 0.28 over the research period.

Table 6.1: WFD phytoplankton scores.

Sample	Treatment	Avg. Chl-*a*	Taxa	#cells/colonies/ filaments per ml	Type of bloom	Score species composition	Score abundance	EQR
Pond 20-09-10	Pond	115	Microcystis	543307	**8***	0,4	0,16	0,28
Pond 20-09-10	Pond		Dichotomococcus	7546	15	0,4		
Pond 20-09-10	Pond		Monoraphidium contortum	3953				
Pond 20-09-10	Pond		Tetrastrum staurogeniaeforme	1437				
Pond 20-09-10	Pond		Tetrastrum	1437				
Pond 20-09-10	Pond		Didymocystis	719				
Pond 20-09-10	Pond		Chlorophyta	12217				
Pond 20-09-10	Pond		Merismopedia minutissima	1437	23	0,5		
Pond 20-09-10	Pond		Aphanothece	719				
Pond 20-09-10	Pond		Aphanocapsa	7905				
Pond 20-09-10	Pond		Merismopedia	1437				
Pond 20-09-10	Pond		Aphanizomenon	1437	25	0,6		
***Determines EQR of algae bloom**								
Pond 03-08-10	Pond	115	Aphanothece	359	23	0,5	0,16	0,33
Pond 03-08-10	Pond		Merismopedia	719				
Pond 03-08-10	Pond		Chroococcus	2875				
Pond 03-08-10	Pond		Aphanocapsa	6827				
C2	Control	80	Aphanizomenon	2234	24/25	0,55	0,26	0,41
C5	Control	88	Pseudanabaenaceae	32648	4	0,2	0,23	0,21
C13	Control	81	Aphanizomenon	1233	25	0,6	0,26	0,43
								0,35
C7	Carp	123	Aphanizomenon	4928	24/25	0,55	0,14	0,34
C12	Carp	100					0,19	0,19
C15	Carp	107					0,17	0,17
								0,23
C3	Carp&Phoslock	119	Aphanizomenon	1902	25	0,6	0,15	0,37
C14	Carp&Phoslock	119					0,15	0,15
C17	Carp&Phoslock	119	Aphanizomenon	1530	25		0,15	0,15
								0,22
C4	Phoslock	63	Aphanizomenon	1006	25	0,6	0,33	0,47
C6	Phoslock	68	Pseudanabaenaceae	60727	4	0,2	0,31	0,26
C11	Phoslock	68					0,31	0,31
								0,34
C8	Dredging&Phoslock	86	Aphanizomenon	5473	24/25	0,55	0,24	0,39
C10	Dredging&Phoslock	66					0,32	0,32
C16	Dredging&Phoslock	79	Aphanizomenon	3765	24/25	0,55	0,27	0,41
								0,37
C1	Dredging	96	Aphanizomenon	2857	24/25	0,55	0,20	0,37
C9	Dredging	90	Aphanizomenon	3114	24/25	0,55	0,22	0,38
C18	Dredging	82	Aphanizomenon	2586	24/25	0,55	0,25	0,40
								0,39

7. Changes within three zooplankton groups

The following three zooplankton groups were examined: Cladocera, Copepoda and Rotifera. Changes in average numbers of concerning zooplankton as a result of attempted control measures or carps are plotted in separate graphs, but joined in the same figure. Furthermore, relative changes in average numbers of zooplankton among treatments are presented in pie charts.



Daphnia sp. in one of the water samples.

Cladocera

Compared to control enclosures the average numbers of Cladocera were higher in dredged enclosures treated with Phoslock® and in enclosures with carp with and without Phoslock® (see figure 7.1). However, standard deviations were large and no significant differences in numbers of Cladocera were indicated among attempted control measures (Kruskal-Wallis test: $\chi^2(3) = 1.14$; $P = 0.767$) or in the presence of carps (Kruskal-Wallis test: $\chi^2(3) = 5.02$; $P = 0.919$)

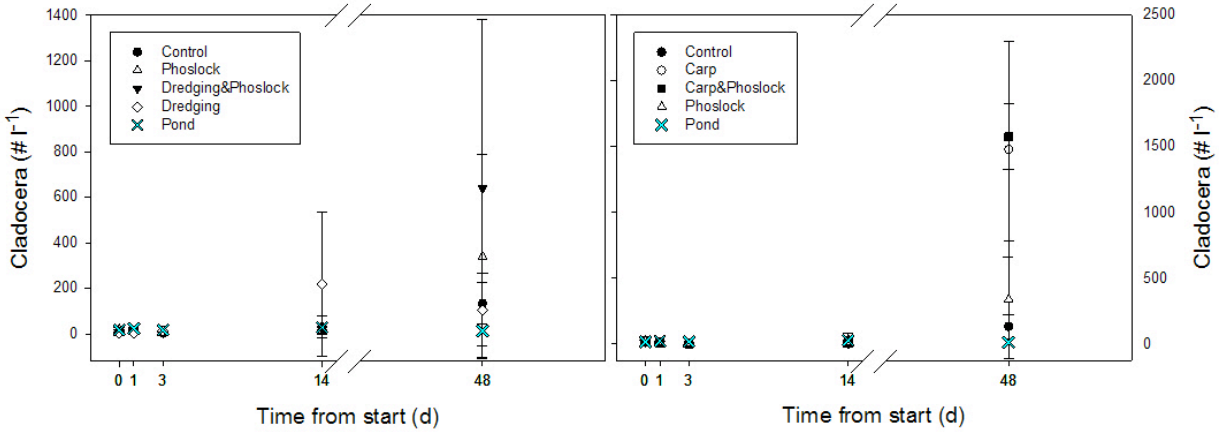


Figure 7.1: Changes in average numbers of Cladocera (# l-1) as a result of attempted control measures (left) or carps (right) on days 0, 1, 3, 14 and 48. Error bars represent 1 SD (n = 3).

Figure 7.2 shows a larger relative share of Cladocera in dredged enclosures without Phoslock® on day 14 (70%) and in enclosures with carp with and without Phoslock® on day 48 (resp. 37% and 34%) compared to other treatments.

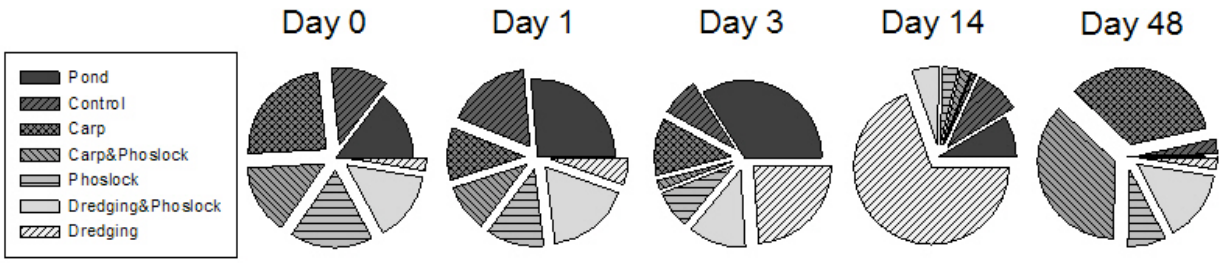


Figure 7.2: Proportional representation of differences in average numbers of Cladocera for the six treatments and Stiffelio Pond on days 0, 1, 3, 14 and 48..

Copepoda

On day 14, the average number of Copepoda was high in dredged enclosures without Phoslock® compared to controls (see figure 7.3). Though, standard deviations were large and no significant differences in numbers of Copepoda were indicated among attempted control measures (Kruskal-Wallis test: $\chi^2(3) = 5.14$; $P = 0.162$) The average numbers of Copepoda in enclosures stocked with carp and treated with Phoslock®, on the other hand, were significantly lower compared to controls and undredged enclosures treated with Phoslock® (see figure 7.4, Scheffe post-hoc: $P = 0.041$ and $P = 0.031$ for regarding pairwise comparisons, after Kruskal-Wallis test: $\chi^2(3) = 19.01$; $P < 0.001$).

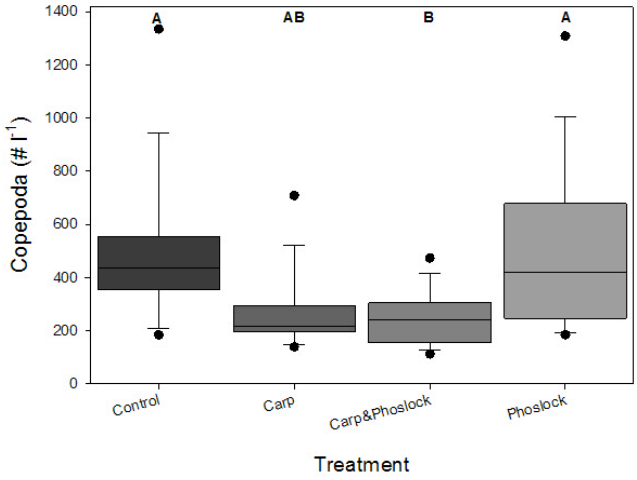


Figure 7.4: Box- and-Whisker plot of Copepoda (# l-1) for the four treatments: Control, Carp, Phoslock and Carp&Phoslock. Identical letters (A, B) indicate homogeneous groups that are not different at the 95% level (Scheffe post-hoc test).

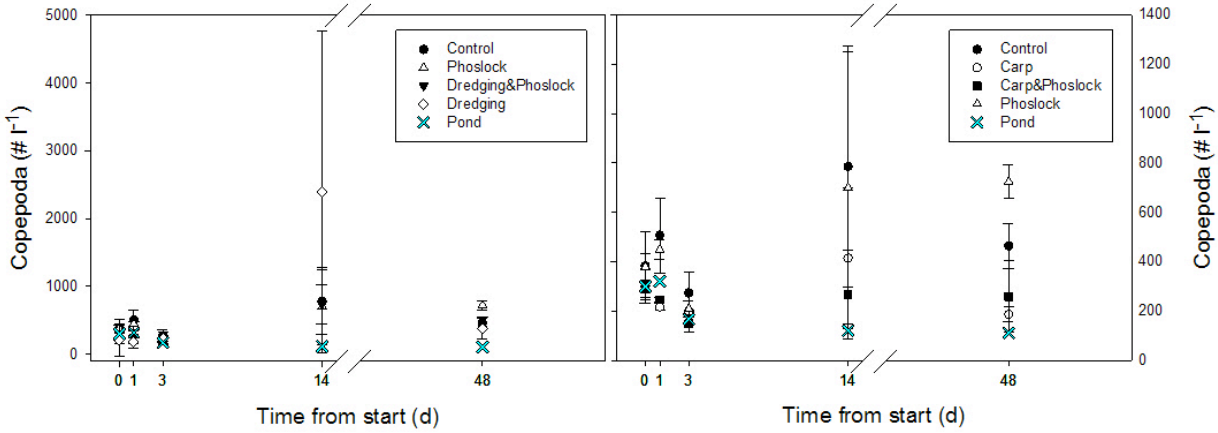


Figure 7.3: Changes in average numbers of Copepoda (# l-1) as a result of attempted control measures (left) or carps (right) on days 0, 1, 3, 14 and 48. Error bars represent 1 SD (n = 3).

Figure 7.5 shows a larger relative share of Copepoda in dredged enclosures without Phoslock® on day 14 (44%) and in undredged enclosures with Phoslock® on day 48 (29%) compared to other treatments.

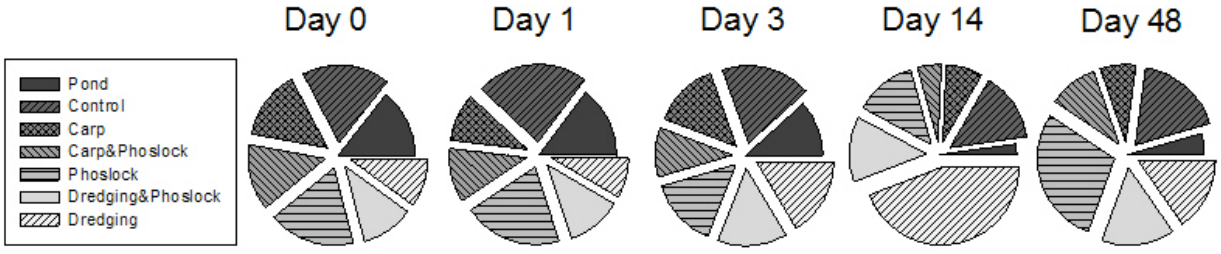


Figure 7.5: Proportional representation of differences in average numbers of Copepoda for the six treatments and Stiffelio Pond on days 0, 1, 3, 14 and 48.

Rotifera

Compared to control enclosures the average numbers of Rotifera were higher in Stiffelio Pond over the first three days and in dredged enclosures with and without Phoslock® (see figure 7.6). However, there were no significant differences in numbers of Rotifera indicated among attempted control measures (Kruskal-Wallis test: $\chi^2(3) = 6.48$; $P = 0.091$) or in the presence of carps (Kruskal-Wallis test: $\chi^2(3) = 7.31$; $P = 0.063$).

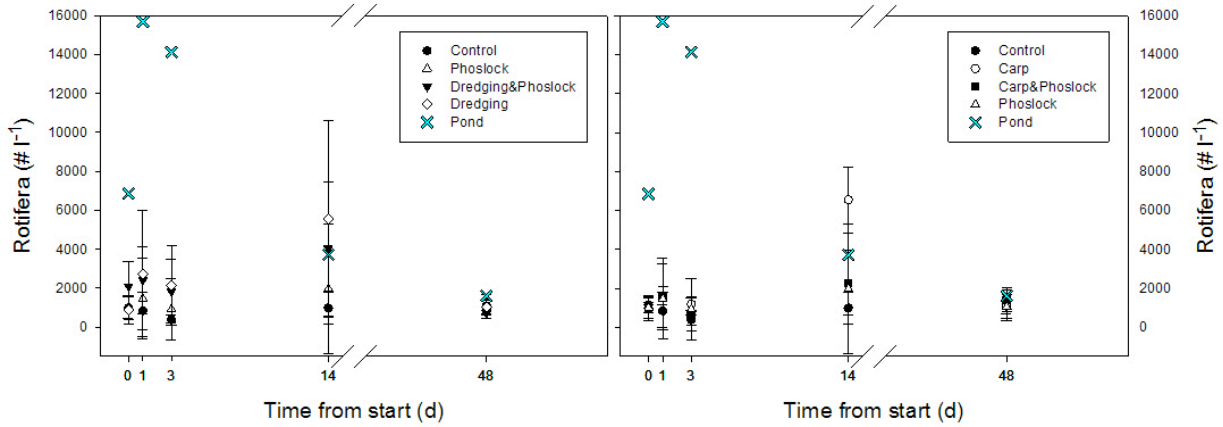


Figure 7.6: Changes in average numbers of Rotifera (# l-1) as a result of attempted control measures (left) or carps (right) on days 0, 1, 3, 14 and 48. Error bars represent 1 SD (n = 3).

Furthermore, the relative shares of Rotifera were larger in Stiffelio Pond compared to other treatments over the first three days (resp. 49, 60 and 66%). However, relative shares were more or less equal among treatments on day 48 (see figure 7.7).

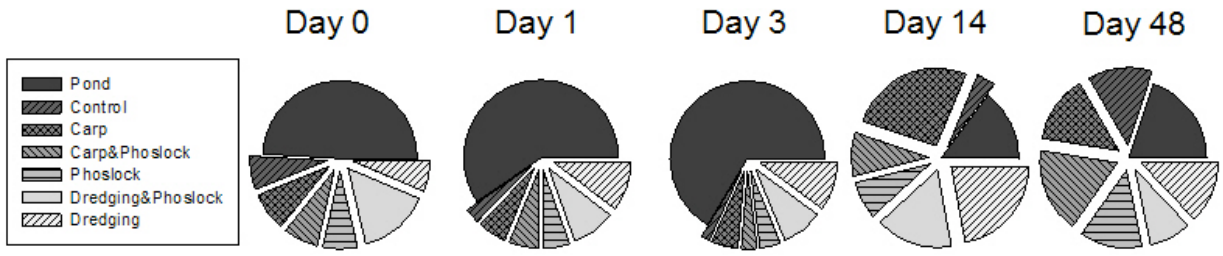


Figure 7.7: Proportional representation of differences in average numbers of Rotifera for the six treatments and Stiffelio Pond on days 0, 1, 3, 14 and 48..

8. Trace metals in carp

In this chapter the results of trace metal analyses are presented for the carp's gastrointestinal tract (GI-tract) as well as its remains (tissues & organs). The following five trace metals were determined: cadmium, copper, lanthanum, lead and zinc.

First, trace metals concentrations between carps euthanized at the start of the experiment (Hatchery) and carps caught from the control enclosures (Control) were compared. Since there were no significant differences indicated in trace metal concentrations among these two groups, these data were lumped to create one new control group with a larger sample size (n = 6). This group was then compared with carps exposed to Phoslock®.

Carp's GI-tract

Lanthanum concentrations in the carp's gastrointestinal tract were significantly higher in exposed (7.6 µg g^{-1} DW) compared to unexposed carps (0.1 µg g^{-1} DW) (see figure 8.1, Mann-Whitney U-test: $U < 0.001$; $P = 0.020$). Other metal concentrations did not differ among treatments (see figures 8.2 and 8.3).

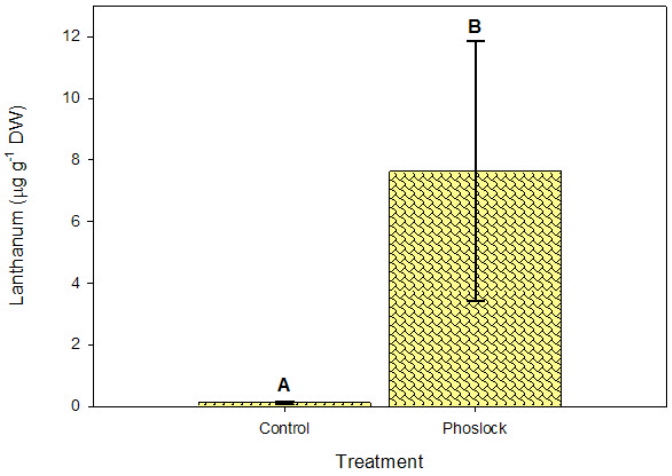


Figure 8.1: Bar graph of mean lanthanum concentrations in carps's GI-tract (µg Metal g^{-1} DW) for carps exposed (Phoslock) and not exposed to Phoslock® (Control). Error bars represent 1 SE.

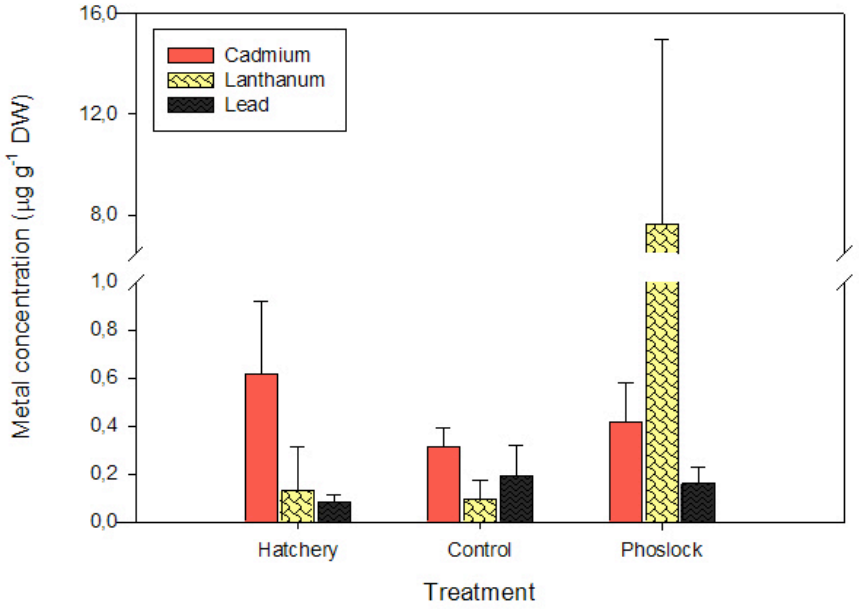


Figure 8.2: Cadmium, lanthanum and lead concentrations in carps's GI-tract (µg Metal g^{-1} DW) for the three treatments: Hatchery, Control and Phoslock. Error bars represent 1 SD (n = 3).

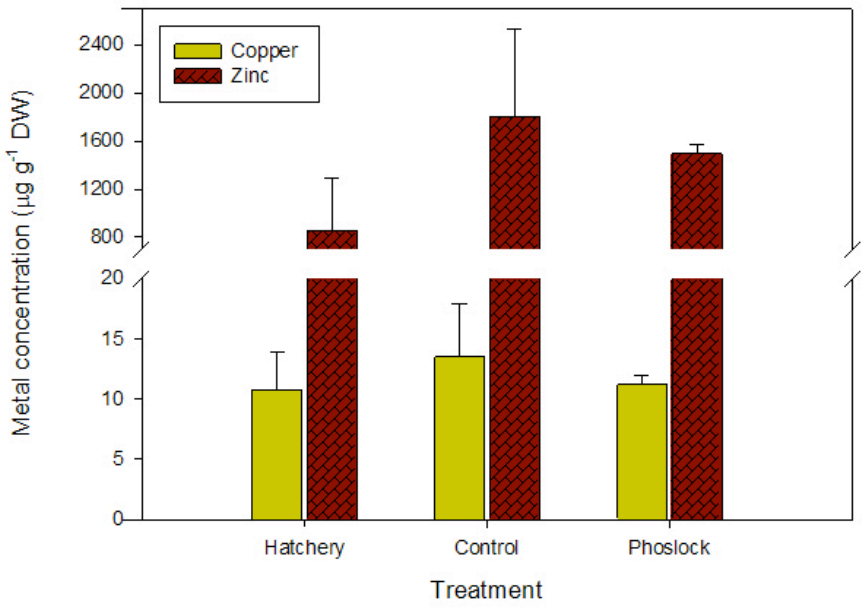


Figure 8.3: Copper and zinc concentrations in carps's GI-tract (µg Metal g^{-1} DW) for the three treatments: Hatchery, Control and Phoslock. Error bars represent 1 SD (n = 3).

Carp's tissues & organs

Lanthanum concentrations in the carps's remains (tissues & organs) were significantly higher in exposed (0.61 µg g^{-1} DW) compared to unexposed carps (0.06 µg g^{-1} DW) (see left bar graph in figure 8.4, Mann-Whitney U-test: U = 1.00; P = 0.039). Lead concentrations, on the other hand, were significantly lower in exposed (0.05 µg g^{-1} DW) compared to unexposed carps (0.11 µg g^{-1} DW) (see right bar graph in figure 8.4, Mann-Whitney U-test: U = 1.00; P = 0.039). Other metal concentrations did not differ among treatments (see figures 8.5 and 8.6).

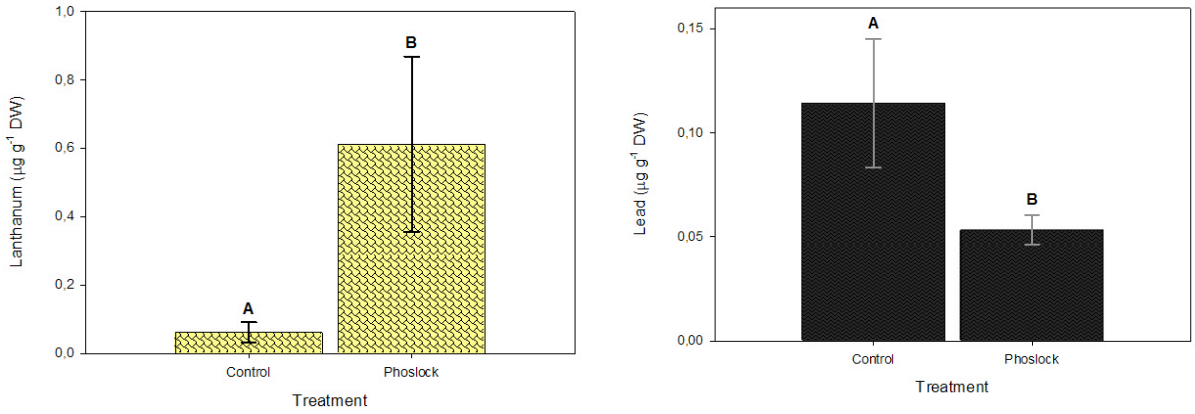


Figure 8.4: Bar graphs of mean lanthanum (left) and lead concentrations in carps's remains (µg Metal g^{-1} DW) for carps exposed (Phoslock) and not exposed to Phoslock® (Control). Error bars represent 1 SE.

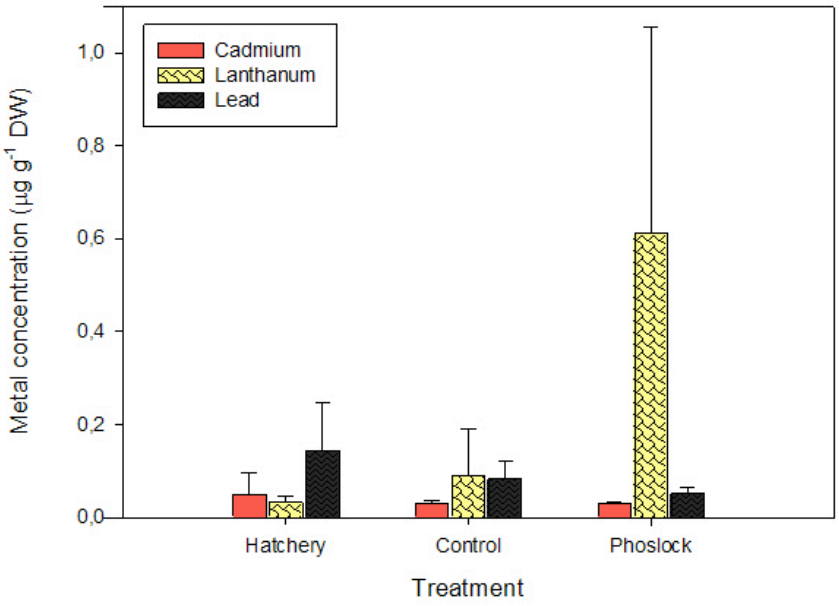


Figure 8.5: Cadmium, lanthanum and lead concentrations in carps's remains (µg Metal g^{-1} DW) for the three treatments: Hatchery, Control and Phoslock. Error bars represent 1 SD (n = 3).

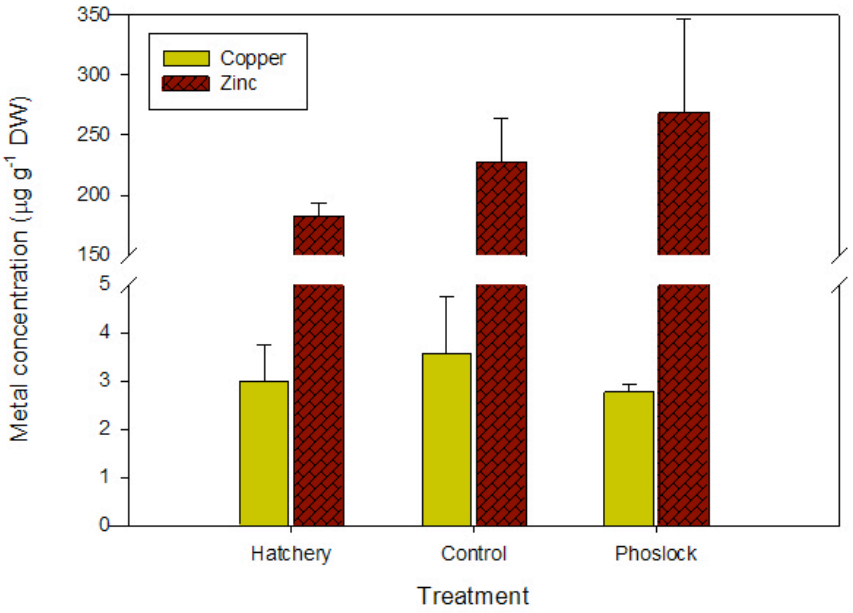


Figure 8.6: Copper and zinc concentrations in carps's remains (µg Metal g^{-1} DW) for the three treatments: Hatchery, Control and Phoslock. Error bars represent 1 SD (n = 3).

9. Cyanotoxins in enclosures and Stiffelio Pond

Water samples collected from the enclosures on 12-08-10 and from Stiffelio Pond on 30-07-10 and 12-08-10 were examined on the presence of microcystins. Four microcystin variants were detected in the samples: dmRR, RR, LR and YR. However, last two variants were undetectable in samples taken on 12-08-10. As can be seen in figure 9.1A, microcystin concentrations seemed to be lower in enclosures treated with Phoslock®. However no significant differences in microcystin dmRR concentrations were indicated among treatments (Kruskal-Wallis test: χ^2 (5) = 4.82; P = 0.439). Also, no significant differences in total microcystin concentrations were indicated among treatments (Kruskal-Wallis test: χ^2 (5) = 4.75; P = 0.447).
In Stiffelio Pond (figure 9.1B) total microcystin concentrations decreased from 3.04 to 0.67 µg l^{-1}. Microcystin variants LR (0.81 µg l^{-1}) and YR (0.21 µg l^{-1}) were present on July 30, but were undetectable on August 12.

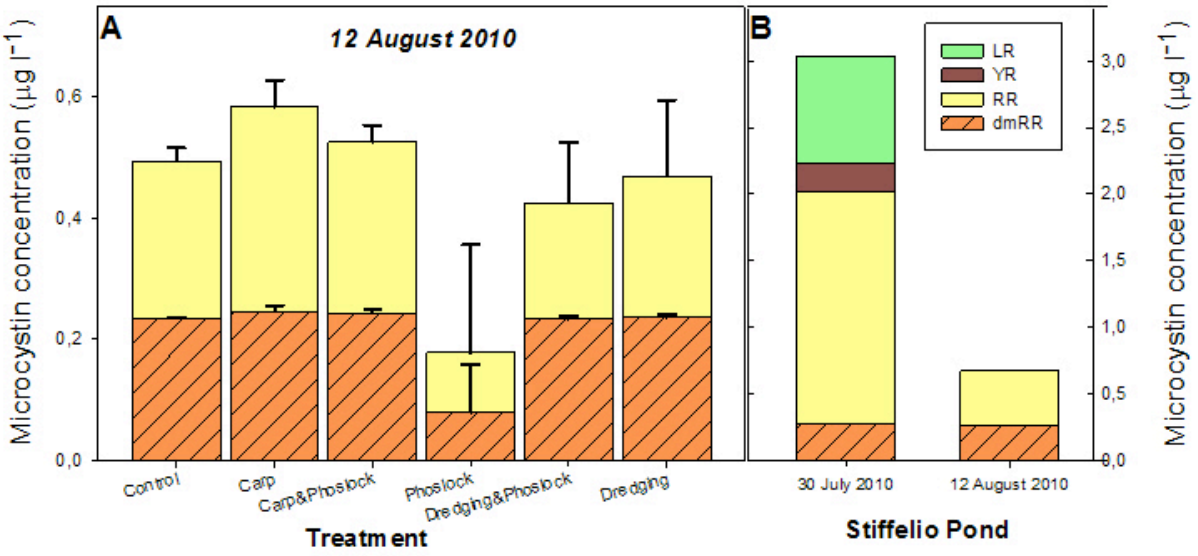


Figure 9.1: Microcystin concentrations in enclosures (A) on 12-08-10, and in Stiffelio Pond (B) on 30-07-10 and 12-08-10. Error bars in figure A indicate 1SE (n = 3).

10. Discussion

A manipulative field study with enclosures has been conducted in Stiffelio Pond in Eindhoven in order to examine the effects of three control measures to reduce internal eutrophication, dredging and/or the phosphate-fixative Phoslock®, on several water quality variables. Furthermore, a long term toxicity test (48 days) has been conducted with common carp and Phoslock® to examine possible accumulation of trace metals and its effects on the same water quality variables.

Total and cyanobacterial chlorophyll-*a*

Increasing nutrient availability due to eutrophication increases chlorophyll-*a* levels (Fogg, 1969; Reynolds & Walsby,1975; Reynolds, 1987; Paerl, 1988, 2008). In line with expectations, Phoslock® had a significant negative effect on both total and cyanobacterial chlorophyll-a concentrations. Though, a larger reduction of both total and cyanobacterial chlorophyll-a concentrations was expected to occur. It is possible that Phoslock® limited algal growth by immobilizing orthophosphate (Haghseresht, 2005).

Carps, on the other hand, had a positive effect on both total and cyanobacterial chlorophyll-*a* concentrations. This effect was also pointed out in other studies (e.g. Meijer *et al.*, 1990; Breukelaar *et al.*, 1994; Nalewajko & Murphy, 1998; Zambrano & Hinojosa, 1999). As a result of benthic feeding, carps might have transported nutrients from the sediment to the water column to the benefit of phytoplankton (Breukelaar *et al.*, 1994; Shormann & Cotner, 1997; Roozen *et al.*, 2007). Secondly, algal biomass might have increased because of nutrient recycling through fish excretion (Qin & Threlkeld, 1990; Attayde & Hansson, 1999). Thirdly, since sedimentation is one of the major loss processes operating in phytoplankton (Reynolds & Wiseman, 1982; Reynolds *et al.*, 1982), it is possible that resuspension of settled algal cells by fish could have occurred (Scheffer, 2004).

Surprisingly, total chlorophyll-*a* concentrations decreased to approximately the same levels for all treatments after four weeks. Possibly, an increased shading effect in the enclosures caused by Bryozoa growing on the enclosures in combination with decreasing water temperatures, limited algal growth from this time.

Transparency and turbidity

In contrast with expectations, there were no significant differences in transparency indicated between attempted control measures and controls. It is most likely that water and sediment closed in by the enclosures was hardly influenced by wind and water currents, and provided sediment enough time to consolidate.

Transparency of enclosures stocked with carp, on the other hand, decreased as soon as carps were put in and remained below 0.2m ever since. It is clear that resuspension of sediment and/or Phoslock® and maybe settled algal cells was probably the major factor causing decreased transparency (Scheffer, 2004; Roozen *et al.*, 2007).

Oxygen & pH

Carp negatively affected dissolved oxygen concentrations: oxygen concentrations were about 0 mg l^{-1} above the sediment and remained below 4 mg l^{-1} near the surface over the course of the experiment. Even under these conditions, all carps survived the experiment and kept feeding and stirring up the sediment. Oxygen was likely to be depleted by both carp's respiration together with increased oxygen demand of stirred up organic matter.

Oxygen concentrations were significantly higher in undredged enclosures treated with Phoslock® compared to dredged enclosures with Phoslock®, which is probably caused by initially

lower oxygen concentrations in dredged enclosures. Surprisingly, oxygen concentrations were also significantly higher (ca. 1 mg l^{-1}) in enclosures with carp and Phoslock® compared to enclosures with carp and without Phoslock®. Apparently, Phoslock® had a minor positive effect on oxygen concentrations in the presence of carps. It might be possible that less organic matter was decomposed in the presence of Phoslock®.

Although there were no huge contrasts for pH among treatments, significantly higher pH values were measured in enclosures treated with Phoslock®. Since measurements were taken in the morning before 10am, it might be possible that due to lower chlorophyll-*a* concentrations, respiration of algae was lower in those enclosures and as a consequence, pH higher.
In enclosures with carp, on the other hand, pH values were significantly lower. A possible mechanism could be increasing CO_2 concentrations as a result of respiration by carps and algae and/or decomposition of stirred up organic matter.

Ammonium

Compared to controls ammonium concentrations were higher for all treatments. However, only significant differences in ammonium concentrations were indicated for undredged enclosures treated with Phoslock®. Possibly, higher ammonium concentrations are measured as a result of ammonification: decomposition of particulate and dissolved organic nitrogen, like dead algae (Kalff, 2003). The initial molar N:P ratio in the control enclosures at the start of the experiment based on the total nitrogen and total phosphorus concentrations was about 11:1, potentially suggesting N-limitation. Enhanced availability of ammonium could therefore have caused stimulated phytoplankton growth in enclosures stocked with carp.

Phosphorus locked?

There were no significant differences in total phosphorus and orthophosphate concentrations among attempted control measures. Surprisingly, in the pairwise comparison between enclosures stocked with carp with and without Phoslock®, orthophosphate concentrations were significantly lower in enclosures where Phoslock® was added. So, Phoslock® did immobilize some of the orthophosphate in the water column. Also, total phosphorus concentrations were significantly higher in enclosures with carp. Peaks in total phosphorous concentrations can be explained by the same peaks for total chlorophyll-*a* concentrations. Again, in the pairwise comparison between enclosures stocked with carp with and without Phoslock® a significant difference was indicated: total phosphorus concentrations were significantly lower in enclosures where Phoslock® was added. So, under these disturbing and almost anaerobic conditions phosphorus was possibly not released from the Phoslock®.

Negative effects of Phoslock® on orthophosphate concentrations were shown after treating two Dutch lakes: ‘’De Rauwbraken’’ and ‘‘De Kuil’’ (resp. Lürling & Van Oosterhout, 2009; Van Goethem, 2010). These lakes were first treated with a flocculant and secondly with Phoslock®. Although, no flocculants were used in this enclosures experiment no significant differences in orthophosphate concentrations were indicated. This is remarkable, especially despite the huge overdose of circa 30 times more lanthanum than orthophosphate in the water column. Moreover, the Phoslock®:P ratio in the enclosures was about 3233:1, which is far higher than the proposed dosage of 200-230:1 (Martin & Hickey, 2004; Haghseresht, 2005; Ross *et al*., 2008) and might have been a sustainable solution.

Also, in a similar enclosure experiment conducted by Lürling (2010a), Phoslock® was also less efficient in stripping orthophosphate from the water column. Despite an overdose of Phoslock® only 25% of orthophosphate was immobilized.

It is known that adsorption capacity of Phoslock® decreases at pH values higher than 9 and is lower in algae-containing lake water than in prepared reverse osmosis water solution, possibly because of humic acids (Ross *et al*., 2008). At the time of application, pH was circa 7.5 so this should have hardly affected phosphorus uptake. Average chlorophyll-*a* concentrations were circa 120 µg l^{-1} at the time of application, so this might have negatively affected phosphorus uptake. Since humic acid concentrations were unknown, it might be possible that those had interfered with phosphorus uptake as well. In a controlled experiment with Phoslock® at pH 9, circa 70% of filterable reactive phosphorus (FRP) was locked in the absence of humic acids and circa 30% was locked in the presence of humic acids (Haghseresht, 2005).

Violating the Dutch Standard

Since total and filterable lanthanum concentrations were significantly higher in enclosures stocked with carp, it is likely that carps stir up Phoslock®. This fish-induced resuspension of Phoslock® could be an explanation for shown violations of the Dutch Standard for filterable lanthanum of 10.1 µg La l^{-1} (Sneller *et al*., 2000) from day 43 in enclosures with carp and Phoslock®. Alarming are the results from water samples taken at 12 October 2010, 70 days after Phoslock® application. The Dutch Standard had been violated in undredged enclosures with circa 5 µg La l^{-1} (ca. 50%) and had almost been violated in dredged enclosures treated with Phoslock®. Apparently, even in the absence of carp, lanthanum is released from Phoslock® in this sheltered environment. Lürling (2010a) encountered the same release of filterable lanthanum concentrations in an enclosures experiment. Moreover, leaching of lanthanum from Phoslock® has been reported in other studies as well (e.g. Pablo *et al*., 2009; Gibbs *et al*., 2010; Lürling & Tolman, 2010). Furthermore, violation of the Dutch Standard might pose a major drawback in the applicability of Phoslock® as a mitigating measure in eutrophication control.

(Bio)accumulation of lanthanum in common carp

A long term term toxicity test (48 days) with common carp revealed the uptake of Phoslock® and accumulation of lanthanum. Lanthanum concentrations in the carps's remains (tissues & organs) were significantly higher in exposed (0.61 µg g^{-1} DW) compared to unexposed carps (0.06 µg g^{-1} DW). Lead concentrations, on the other hand, were significantly lower in exposed (0.05 µg g^{-1} DW) compared to unexposed carps (0.11 µg g^{-1} DW). Carps were exposed to Phoslock via water as well as via the oral route. Since carps hampered Phoslock® consolidation and lanthanum may be released from the bentonite clay-La^{3+} complex when added to water (Lürling & Tolman, 2010), it might be possible that lanthanum ions in water led to uptake and absorption of lanthanum in the carp's eyes, skin and/or gills (Ahmed & Bibi, 2010). Also, lanthanum concentrations in the carp's gastrointestinal tract were significantly higher in exposed (7.6 µg g^{-1} DW) compared to unexposed carps (0.1 µg g^{-1} DW). The continuous process of water and Phoslock® ingestion, digesting and absorption might have led to accumulation of lanthanum in intestines with the passage of time. Persey *et al*. (2006), on the other hand, observed ultralow absorption of lanthanum by the gastrointestinal tract after oral intake in humans. Though, humans possibly metabolize lanthanum differently than carps.
A significant decrease of lead concentrations could be the result of detoxification of lead taken up from the environment in the hatchery. Metals could be cleared from the fish's body by binding

to metal binding proteins, such as metallothionein (Roesijadi & Robinson, 1994; Canli *et al*., 1997; Kotze, 1997; Ahmed & Bibi, 2010).

Since lanthanum accumulates in carp, it may accumulate in different (fish) species as well. Pauwels (2009) already observed lanthanum uptake by *Asellus aquaticus* and reduction in numbers/reproductive capacity of some other organisms. Maybe Phoslock® could be harmful to more aspects of the ecosystem than is claimed by commercial parties (Martin & Hickey, 2004; Afsar & Groves, 2009; Watson-Leung, 2009). Hopefully, more research will be conducted to look more into the toxicological effects of Phoslock® and bioaccumulation of lanthanum in the environment. Since Phoslock® is applied in aquaculture as well (Afsar, 2008), it is certainly recommended to pay attention to lanthanum concentrations regarding these products.

Phyto- and zooplankton

In this research, Ecological Quality Ratios (EQRs) for phytoplankton were used to compare differences among treatments. Highest EQRs for phytoplankton were reached in dredged enclosures with and without Phoslock® (resp. 0.37 and 0.39). Those higher scores were above all the result of lower average total-chlorophyll-*a* concentrations for these treatments. Furthermore, enclosures stocked with carp with and without Phoslock® scored the lowest, respectively 0.22 and 0.23. Lowest scores for carp were mostly due to higher average total-chlorophyll-*a* concentrations and the absence of algal blooms that could have increased the EQRs for carp.

However, it should be noticed that not all preconditions were met for scoring the samples according the Water Framework Directive as is described by Van der Molen & Pot (2007). Research period was too short compared to the required period described in the directive (April 1st until September 30th) to calculate proper average total chlorophyll-*a* concentrations. Also, sampling occurred only once within each treatment which is in contrast with the directive that describes at least two samplings (Faber *et al*., in prep.). In this case, it is maybe even justified to use the EQR of Stiffelio Pond on 3 August 2010 as a second measurement, because it would not have differed that much from the water in the enclosures prior to the treatments. The final EQR would than have been the average value of both Stiffelio Pond's EQR 3 August 2010 and the EQR from each treatment at 20 September 2010. If calculated, numbers would have been different of course, but between treatment contrasts would have been more or less the same.

The average numbers of Copepoda were significantly lower in enclosures stocked with carp and treated with Phoslock® compared to undredged enclosures with and without Phoslock®. It is known for many fish species to shift to less profitable foods when preferred food sources become depleted (Balcombe *et al*. 2005; Balcombe & Humphries, 2006). So, it is possible that in the absence of benthic macroinvertebrates, carp's feeding niche has shifted from near the bottom of the enclosures to the water column where they spend some of their time and fed principally on zooplankton (Rahman *et al*., 2010).

How further?

Overall it is evident that none of the treatments improved the water quality to a good ecological potential/status. Phoslock® had a minor negative effect on both total and cyanobacterial chlorophyll-*a* concentrations and dredging did not generate a positive result at all. For the combination of dredging and Phoslock® synergetic effects were expected to occur, since both control measures should have improved water quality. It is possible that the nutrient rich sediment layer had not been removed completely by making use of a macrofauna scoop-net. For the first

nine days dredging had a positive effect on phytoplankton by stirring op nutrients to the benefit of phytoplankton. Also, prior to the Phoslock® application total chlorophyll-*a* concentrations were higher in dredged enclosures, which has negatively affected the average chlorophyll-*a* concentrations in dredged enclosures.

On the long term, the combination of dredging and Phoslock® could have been the most effective control measure, but still contrasts between treatments were small. Since there are contrasts between enclosure studies conducted under the WFD innovation project, it is recommended to conduct further research. Controls in compartment studies seem to mimic the pond's water quality better (Lürling, 2010b). Probably, because there are less confounding factors involved in these studies, they might be better to evaluate the effects of attempted control measures.
This enclosure study indicated a violation of the Dutch Standard for filterable lanthanum concentrations and accumulation of lanthanum in common carp. Therefore, it is recommended to look more into toxicological effects of Phoslock® and especially for inter species differences and possible bioaccumulation in the environment.
So, violation of the Dutch Standard and accumulation of lanthanum in carp might pose a major drawback in the applicability of Phoslock® as a mitigating measure in eutrophication control; for water managers it might be better to be reticent in applying Phoslock® until other studies have proven otherwise.

11. Conclusions

In this chapter significant results and highlights are summarized and presented as bullet-points.

- Carps had a significant positive and Phoslock® a negative effect on both total and cyanobacterial chlorophyll-*a* concentrations.
- Dredging and/or Phoslock® did not significantly decrease turbidity or increase transparency.
- Carps with and without Phoslock® significantly decreased transparency and increased turbidity.
- Carps had a significant negative effect on dissolved oxygen concentrations. Phoslock®, on the other hand, significantly positively affected oxygen concentrations, even in the presence of carps.
- Significantly higher pH values were measured in undredged enclosures treated with Phoslock®. In enclosures stocked with carp with and without Phoslock®, on the other hand, significantly lower pH values were measured.
- Ammonium concentrations were significantly higher in undredged enclosures treated with Phoslock®.
- Despite the huge overdose of circa 30 times more lanthanum than orthophosphate in the water column, no significant differences in orthophosphate and total phosphorus concentrations were measured as a result of attempted control measures. Furthermore, total phosphorus concentrations were significantly higher in enclosures with carp with and without Phoslock®.
- Total and filterable lanthanum concentrations were significantly higher in enclosures stocked with carp.
- The Dutch Standard for filterable lanthanum was violated from day 43 in enclosures with carp and Phoslock®. Also, 70 days after Phoslock® application the Dutch Standard had been violated in undredged enclosures and almost in dredged enclosures treated with Phoslock® (enclosures with carp were not examined at this time).
- Lanthanum concentrations were significantly higher (76 times) in the gastrointestinal tract of carps exposed to Phoslock®.
- Lanthanum concentrations were significantly higher (10 times) in mixtures of organs and tissues of carps exposed to Phoslock®. Lead concentrations, on the other hand, were significantly lower (2 times).
- Highest Ecological Quality Ratios for phytoplankton were reached in dredged enclosures with and without Phoslock® and lowest in enclosures stocked with carp with and without Phoslock®.
- The average numbers of Copepoda in enclosures stocked with carp and treated with Phoslock® were significantly lower compared to controls and undredged enclosures treated with Phoslock®.

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Appendix 1: WUR ESG Protocols.

A: Chlorophyll-a Analysis

B: Chlorophyll-a measurement with PHYTO-PAM

C: ‘’Gebruikers handleiding vriesdroger’’

D: Preparation for identification of trace metals in macrofauna and zooplankton

E: Sample preparation RPmix filters

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Chlorophyll-a Analysis,

Date: 2008
Page(s): 2

Introduction
Protocol based on NEN 6520

Methods/Measurements

- Filtrate V litre of sample on a GF/C filter, until is almost dry.
- Usually the filter will be stored for some time.(max. 3 months at –20 ºC) Place the filter in a small plastic petri-dish, label the dish, pack in aluminium foil, freeze the sample (-20 ºC).
- Take samples from freezer and keep the petri-disks wrapped in foil until further processing in the dark room.
- Switch on temperature of water bath at 75 °C.
- Switch on cooled centrifuge at 5 °C. and set on pre cool.

From this moment on all steps should be performed using green light (Splendor flood persglas, 80 Watt) and at 20 °C.

- Switch on spectrophotometer, at least a half hour before starting measurements.
- Remove filter from foil and petri-dish.
- Roll up filter and put it inside a 10 ml centrifuge tube.
- Add 80% 10 ml ethanol and close tube with a cap.
- Shake tube on a vortex for 15 sec. and 80 rps.
- When finished the series, put them in a tube rack and subsequent put the rack in the water bath at 75°C for 5 minutes exactly.
- Cool down the rack with the tubes in ice water or under cold running water rapid to roomtemperture, to prevent the chlorophyll-a from degradation.
- Shake tube on vortex for 15 sec, at 80 rps.
- Centrifuge for 5 minutes exact at 3000 rpm, at 5°C. (Set centrifuge-time at 7 minutes)
- Carefully pipette sample into a glass cuvette. Whip dry whit tissue.
- Measure extinction at 750 (E_{750}) nm and maximum at 665 (E_{665}) nm. Lower pH with a drop 0.28 M HCl, put lit on cuvette and shake. (pH 2.6-2.8)
- Measure extinction again at 665 (E_{665HCl}) and 750 (E_{750HCl}) nm.
- Empty cuvette into waste container and rinse cuvette with a small amount alcohol.
- Introduce next sample.

DU 530 Beckman Spectrophotometer.
Calibration: when the machine is plugged in a self-testing program (wavelength and system test) starts automatically.
Operations:

step 1, main menu, select USER PROGRAM mode.
step 2, recall program 1, push ENTER; program ready to use
step 3, insert blank; add 2ml 80% ethanol to cuvette
step 4, set blank, blanking automatically
step 5, push READ to control
step 6, insert sample and read absorbance at 750 and 665 nm push READ
step 7, add a drop HCl, and read absorbance again

Calculation chlorophyll-a:

Chl-a ($\mu g.l^{-1}$) = $29.6\{(E_{665} - E_{750}) - (E_{665HCl} - E_{750HCl})\} * v/(V*L)$

and	*v* =	volume extract in ml (= 10 ml)
	V =	volume sample water in liter
	L =	way length cuvet in cm
	E=	extinction

Equipment
Spectrophotometer (Beckman DU 530)
Vortex (Eckli Electronic, type 6005)
Waterbasin (Memmert W350, serialnr. 800 528)
Cooled centrifuge (Harrier 18/80r)
Glass centrifuge tubs Klimax 10 ml
Cuvets: 1 cm quartz or glass, and/or 5 cm quartz or glass

General Remarks
Use green light during measurements.

Chemicals
Ethanol 80% (V/V): dilute 833 ml ethanol [96% (V/V)} to 1000ml demi-water
Hydrochloric acid, 0.28 mol/l: dilute 7.0 ml HCl 12 mol/l(=1.19 g/ml) to 300 ml demi-water

Remark
De pH van het aangezuurde extract moet 2,6 – 2,8 bedragen. Daarvoor is de zuurconcentratie ten opzichten van het NEN protocol aangepast.
Volgens NEN 6520: 0,2 ml 0,4 M HCl toevoegen aan 20 ml extract. Inhoud cuvet is ca 3.5 ml en volume van druppel is 0.05 ml dus moet de zuurconcentratie 0.28M zijn. Controleer af en toe de pH en pas zonodig het volume aan.

WAGENINGEN UNIVERSITEIT
OMGEVINGSWETENSCHAPPEN

Chlorophyll-a measurement with PHYTHO-PAM

Date: 2008

Principle

The chlorophyll-a fluorescence intensity below 500 µg l^{-1} is proportional to the chlorophyll-a concentration, which is applied in the PHYTO-PAM phytoplankton analyzer (Heinz Walz GmbH, Effeltrich, Germany) such that the signal amplitude gives direct information on the chlorophyll content. The PHYTO-PAM uses 4 different excitation wavelengths, which allows a separation between cyanobacteria, green algae and diatoms.
The relationship between fluorescence and chlorophyll depends on the species composition, growth conditions of the sample and the photosynthetic processes. The minimal fluorescence measured in the dark, F_0, is not dependent on photosynthetic processes and will give the best correlation with chlorophyll-a This means, however, that filling the cuvette with the sample in ambient light requires some adaptation to the measuring light in the cuvette until the fluorescence signal approaches F_0. The PHYTO-PAM is equipped with a 'status-LED'

Methodology

- Switch on the computer and PHYTO-PAM.
- Start Phyto-win programme, select COM1 – press Enter; select Phyto-ED – press Enter. Go to worksheet "ALGAE"
- Press red button on Phyto-ED Emitter-Detector-Unit, open black hood.
- Fill the cuvette with approximately 2 ml of sample. **Use pipette**. Whip cuvette dry whit soft tissue. Close black hood and press green button.
- Measuring in process, press the gain button on the computer if the signal is too low, or when the device indicates it is too high (overload"-warning will pop-up).
- Wait until the status-LED(computer screen) is green and values in the upper cells are more or less stable.
- Press CHL[MF32} button
- Write values that appear in CHL cells for 'blue', 'green' and 'brown'. Values are chlorophyll-a concentrations in **µg l^{-1}** for three major algal groups.
- Press red button on Phyto-ED Emitter-Detector-Unit, open black hood. Empty cuvette in waste container, rinse whit demi-water, introduce new sample.
- After measuring; rinse cuvette properly and fill whit demi-water. Whip cuvette dry whit soft tissue and close black hood.
- Switch off computer and PHYTO-PAM
-

Gebruikers handleiding vriesdroger

Protocol number:

Date: januari 2009
Authors: Frits Gillissen/ Wendy Beekman-Lukassen

1. **Tap het evt. achtergebleven dooiwater af, plaats daarvoor een lekbakje onder de slang en open het kraantje (linksvoor) lekbak leeg maken en kraantje sluiten.**

2. **Zet power vacuümpomp aan (controleer hierbij het oliepijl, te laag →bijvullen: vraag FG)**

3. **Zet vriesdroger aan. Display geeft temperatuur en druk aan. (A is Atmosferische druk)**

4. **Laat vriesdroger ca 15 minuten koelen, temp. moet rond -55 °C worden Hierbij staat de vacuümkraan open!**

5. **Verwijder voorzichtig pexiglazenkap.**

6. **Plaats de bevroren monsters, afdekken met tissue vastgezet met elastiekje, op het rek in de vriesdroger. (Afhankelijk van het aantal en grote van de monsters kan het rek worden aangepast)**

7. **Zet de kap op zijn plaats.**

8. **Sluit de vacuümkraan, controleer of de kap goed staat, de druk gaat langzaam naar beneden tot ca 0.05 mbar. Nu begint het vriesdroogproces, de duur is afhankelijk van monster grootte en hoeveelheid vocht.**

9. **Als de monsters droog zijn. Open langzaam vacuümkraan, pas na opheffen vacuüm de pomp uitschakelen(ivm terugslag) Laat de vriesdroger op druk komen. Als de display A aangeeft open en verwijder de kap. Schakel de power uit!**

10. **De monsters zijn klaar voor verdere behandeling**

11. **Rek en kam even schoonmaken en de kap op zijn plek zetten**

12. **Na ontdooien water aftappen en apparaat droog maken met doek.**

WAGENINGEN UNIVERSITEIT
OMGEVINGSWETENSCHAPPEN

AQWA waterkwaliteitsbeheer aquatische ecologie

Preparation for identification of trace metals in macrofauna and zooplankton

Protocol number: C8, E6 Project: SSEO
Date: version 1, August 22, 2000
Authors: C. van Griethuysen, C.T.A. Moermond, J. van Baren
Page(s): 1

Introduction

The trace metal content in benthic and pelagic macrofauna is determined after a digestion of the tissue with a combination of HNO_3 and H_2O_2. Bioaccumulation of trace metals can be assessed in this way. In the rest of this protocol, oligochaetes will be the target species, but the method can also be used for bulk macrofauna and zooplankton samples. The method is developed at the UvA (Aquatic Ecology and Ecotoxicology). Some of the materials and all reagents used are derived from the UvA.

Methods/Measurements

- About 20 mg of dry sample is weighed exactly on an analytical balance into the precision tips.
- 200 µl of Ultrex HNO_3 (65 %) is added to the sample and it is placed on the heated destruction block for 2 hours at 94 ° C. Before the addition to the first sample, the pipette point is pre-rinsed with HNO_3.
- When the HNO_3 of the previous step is almost evaporated, repeat the previous step (addition of 200 µl HNO_3). When the liquid is almost transparent, 100 µl of Ultrex HNO_3 is enough.
- The destruction block is cooled down till 65-70 °C, when the HNO_3 of the previous step is almost evaporated.
- Then, 100 µl H_2O_2 (30-35 %) is added to the samples. If the reaction is very strong, take the sample out of the destruction block and/ or add some nanopure water (as less as possible).
- The temperature is increased to 94° C for three hours.
- When the samples are completely evaporated, they can be taken out of the destruction block (the moment, at which complete evaporation is reached, can differ for different samples).
- 2 ml of nanopure water is added to the samples. Before dilution the samples **must** be thoroughly mixed with a vortex apparatus. When there is a long period of time between filling up and diluting, vortexing directly after filling up is desirable in addition to vortexing before dilution.
- Samples are diluted 1:10 (1 ml of sample, 9 ml of 0.1 M of Ultrex HNO_3) by means of a dilutor.
- Diluted solutions are measured on ICP-MS, (detection limit roughly 5 ppb at 10 times dilution) at the Soil Science and Plant Nutrition Group.

Equipment

- destruction block' for 60 samples
- reaction vials (Eppendorf precision tips, 2 ml)
- automatic pipette 50-200 µl, vortex apparatus, dilutor apparatus
- ICP-MS (Elan 6000, Perkin Elmer)

General Remarks

For sample collection and preparation see protocol numbers C1, C2 en C3.

Chemicals

- HNO_3 conc. (65 % ~ 14.3 M), Ultrex quality (UvA)
- H_2O_2 30- 35 %, Ultrex quality (UvA)

Special precautions

In this analysis, strong acids are used. Therefore, always work with a laboratory coat and work in a fume hood. When making reagents, also use gloves and safety glasses.

Sample preparation RPmix filters
20-9-2010

Objective
Extract samples (cyanobacteria on filters) for microcystins and nodularin analysis on LC-MSMS.

Materials
- 75% MeOH / 25% NP
- 100% MeOH
- 2 ml eppies without filter
- Eppies with cellulose acetate filter
- 12 ml glass tubes marked with diamond pencil
- 8 ml glass tubes
- Glass pipettes

Extraction
1. Fold the filters and put them in a 12 ml glass tube, marked with diamond pencil. Prepare maximum 26 samples and one blank at a time.
2. Turn water bath on at 60 degrees
3. Freeze dry samples for at least 2 hours
4. Add 4 ml 75% MeOH / 25% NP to samples
5. Vortex for 15 seconds
6. Put samples in waterbath for 30 minutes
7. Vortex for 15 seconds
8. Centrifuge tubes for xx minutes at xx rpm
9. Transfer supernatant to 8 ml ('small') glass tube, use glass pipette
10. Repeat step 4 to 10, in total you will need two glass tubes per sample. Turn on coldtrap speedvac during second extraction step.
11. Put tubes in speedvac (Pump and rotor on, Manual run, no preheat, T 50 °C, no Radiant cover)
12. When the supernatant fractions are dry, reconstitute eppies:
    - Add 400 ul of 100% MeOH to one of the two tubes, vortex and mix well
    - Transfer this 400 ul to the other tube, vortex and mix well
    - Transfer this 400 ul to a new eppie with filter
    - Repeat previous steps twice, but use 200 ul for last step. Final volume in eppie with filter is 1000 ul
13. Centrifuge eppies with filter in microcentrifuge for 5 minutes at 16*g
14. Transfer filtrate to amber vial for analysis.

Calibration standards

stocks		premix		
Compound	Conc mg/l	V naar premix ul	Massa in premix ng	conc in premix ug/l
MCLR	11.5	150	1725	958
MCRR	11.5	150	1725	958
NOD	8.6	150	1290	717
MCdmLR	7.07	150	1061	589
MCLW	5.486	300	1646	914
MCdmRR	8.11	150	1217	676
MCLF	8.156	300	2447	1359
MCLY	3.667	300	1100	611
MCYR	6.279	150	942	523
		1800		

Calibration standards: dilutions						
	Use st	V st (ul)	V MeOH (ul)	Dilution	% max	Vtot (ul)
st0	-		600		0	600
st1	st2	300	450	2.5	2	750
st2	st3	400	400	2	5	500
st3	st4	400	600	2.5	10	600
st4	st5	500	500	2	25	600
st5	st6	500	500	2	50	500
st6	premix	1100	0	1	100	600

Appendix 2: Phytoplankton scoring according WFD.

DEELMAATLAT CHLOROFYL-A

OVERZICHTEN VAN DE KLASSENGRENZEN VOOR CHLOROFYL-A; CONCENTRATIE IN µG/L.

De beoordeling vind plaats aan de hand van de chlorofyl-a concentraties in het zomerhalfjaar op een representatief meetpunt in het waterlichaam. Bij meren (behalve M32) loopt dat van 1 april tot en met 30 september; bij overgangs- en kustwateren en meer-type M32 van 1 maart tot en met 30 september (7 maanden). Bij meren (behalve M32) wordt de gemiddelde concentratie beoordeeld, bij overgangs- en kustwateren en type M32 wordt beoordeeld aan de hand van de 90-percentiel.

TABEL A **MAATLATGRENZEN VOOR CHLOROFYL-A VOOR ZOETE EN BRAKKE MEREN (GEMIDDELDE CONCENTRATIE)**

Type	0,0	0,2	0,4	0,6	0,8	1,0
M20	80	40	20	10	5,8	3,2
M14, M21, M23	184	95	46	23	10,8	6,8
M27	200	100	50	25	11,8	7,4
M30,M31	480	240	120	60	40	30

TABEL B **MAATLATGRENZEN VOOR CHLOROFYL-A VOOR OVERGANGS- EN KUSTWATEREN EN M32 (90-PERCENTIEL)**

Type	0,0	0,2	0,4	0,6	0,8	1,0
O2, M32	144	72	36	18	12	8
K1, K2	168	84	42	21	14	9,3
K3	120	60	30	15	10	6,7

DEELMAATLAT BLOEIEN IN MEREN

De deelmaatlat voor algenbloeien is een toets op ongewenste antropogene invloeden, zoals een excessieve belasting met nutriënten of de inlaat van gebiedsvreemd water. Deze deelmaatlat omvat een lijst met relevante fytoplanktontaxa en de bijbehorende indicatie van de waterkwaliteit.

Om bloeien van fytoplankton vast te stellen worden monsters op de taxa getoetst uit de lijst in tabel b, waarna de beoordeling van de bloei wordt getoetst in tabel a. Wanneer één of meer soorten van een bepaald bloeitype aanwezig zijn met een (gezamenlijke) hogere abundantie dan aangegeven in de kolom 'criterium' en in de kolom van het watertype staat bij het bloeitype een B vermeldt, dan is er sprake van een bloei en wordt een ecologische kwaliteitsratio uit de kolom EKR toegekend.

Van twee bloeitypen wordt niet de abundantie in het monster als criterium gebruikt, maar de aanwezigheid van een drijflaag. Dit gegeven wordt niet in het monster waargenomen maar bij de monstername vastgesteld. In tabel a staat hiervoor een D vermeld.

Bij sommige bloeitypen staan verschillende abundantiecriteria vermeld. Een bloei kan in zo'n geval meer of minder ernstig zijn met ook een verschillend kwaliteitsoordeel.

Wanneer alleen een genusnaam staat vermeld, dan geldt het criterium voor alle soorten van dat genus, behalve voor de soorten waarvan dat expliciet is aangegeven. Wanneer behalve genusnaam ook soortnamen staan vermeldt dan worden daarmee de soorten aangegeven die meestal een dergelijke bloei vormen.

TABEL A **OVERZICHT VAN BLOEITYPEN EN HUN BEOORDELING**

Nr	Bloeitype	EKR	criterium	eenheid	M14	M20	M21	M27	M30
1	Persistente bloei van *Planktothrix agardhii*	0.1	10000	fil/ml	B	B	B	B	B
2	Tijdelijke bloei van *Planktothrix agardhii*	0.3	4000	fil/ml	B	B	B	B	B
3	Bloei van *Planktothrix rubescens*	0.1	10000	fil/ml	B				
4	Bloei van dunne filamenteuze blauwalgen (LPP-groep)	0.2	20000	fil/ml	B	B	B	B	B
5	Bloei van *Thalassiosira pseudonana*	0.2	30000	cel/ml					B
6	Bloei van *Stephanodiscus hantzschii*	0.2	30000	cel/ml	B	B	B	B	B
7	Hevige bloei van *Microcystis* met omvangrijke drijflaag	0.2	100000	cel/ml	B	B	B	B	B
8	Matige bloei van *Microcystis* met weinig tot geen drijflaag	0.4	20000	cel/ml	B	B	B	B	B
9	Bloei van *Microcystis wesenbergii*	0.6	20000	cel/ml	B	B	B	B	
10	Soortenarme bloei van *Scenedesmus*	0.2	20000	cel/ml	B	B	B	B	B
11	Bloei van *Cyclotella meneghiniana*	0.3	5000	cel/ml					B
12	Bloei van *Stephanodiscus binderanus*	0.3	10000	cel/ml	B	B	B	B	
13	Bloei van *Gonyostomum semen*	0.3	1000	cel/ml				B	
14	Bloei van *Aphanizomenon gracile*	0.4	2000	fil/ml	B	B	B	B	B
15	Soortenrijke bloei van kleine Chlorococcales	0.4	20000	cel/ml	B	B	B	B	B
16	Bloei van *Anabaenopsis*	0.4	10000	fil/ml					B
17	Bloei van *Prymnesium* met kans op toxische effecten op vis	0.4	60000	cel/ml					B
18	Bloei van *Prymnesium*	0.6	10000	cel/ml					B
19	Bloei van kleine Cryptophyceae	0.4	10000	cel/ml	B	B	B	B	B
20	Bloei van *Cryptomonas*	0.4	2000	cel/ml	B	B	B	B	B

Nr	Bloeitype	EKR	criterium	eenheid	M14	M20	M21	M27	M30
21	Bloei van *Skeletonema*	0.4	10000	cel/ml	B	B	B	B	B
22	Bloei van *Diatoma tenuis*	0.4	6000	cel/ml	B	B	B	B	B
23	Soortenrijke bloei van kleine Chroococcales (ACM-group)	0.5	10000	kol/ml	B	B	B	B	B
24	Langduriger bloei van *Aphanizomenon flos-aquae* met kans op drijflaagvorming	0.5	2000	fil/ml	B	B	B	B	B
25	Kortdurende bloei van *Aphanizomenon flos-aquae* met kleine kans op drijflaag	0.6	1000	fil/ml	B	B	B	B	B
26	Bloei van *Anabaena*	0.5	800	fil/ml	B	B	B	B	B
27	Bloei van *Aulacoseira granulata* en/of *A. ambigua*	0.5	10000	cel/ml	B	B	B	B	B
28	Bloei van *de sieralg Staurodesmus extensus*	0.5	2000	cel/ml				B	
29	Bloei van de sieralg *Teilingia granulata*	0.5	10000	cel/ml				B	
30	Bloei van *Ankyra*	0.6	10000	cel/ml	B	B	B	B	B
31	Bloei van *Monomastix*	0.6	10000	cel/ml				B	
32	Bloei van *Pedinomonas*	0.6	10000	cel/ml				B	
33	Bloei van *Pyramimonas*	0.6	10000	cel/ml					B
34	Bloei van *Woronichinia naegeliana*	0.6	20000	cel/ml	B	B	B	B	
35	Bloei van *Chrysochromulina parva*	0.6	10000	cel/ml	B	B	B	B	B
36	Bloei van *Cyclotella radiosa*	0.6	1000	cel/ml	B	B	B	B	B
37	Bloei van *Asterionella formosa*	0.6	6000	cel/ml	B	B	B	B	
38	Drijflaag van *Gloeotrichia natans*	0.6			D	D	D	D	
39	Drijflaag van *Aphanothece stagnina* of *A. nidulans*	0.6			D	D	D	D	
40	Bloei van *Aulacoseira islandica* en/of *A. subarctica*	0.6	10000	cel/ml	B	B	B	B	
41	Bloei van *Cyclotella ocellata*	0.7	1000	cel/ml	B	B	B	B	B
42	Bloei van *Chaetoceros*	0.7	10000	cel/ml					B
43	Bloei van *Synura*	0.7	1000	cel/ml	B	B	B	B	
44	Bloei van *Mallomonas.*	0.7	1000	cel/ml				B	
45	Bloei van *Dinobryon*	0.7	1000	cel/ml	B	B	B		
46	Bloei van *Ochromonas*	0.7	10000	cel/ml				B	
47	Bloei van thecate dinoflagellaten *(Ceratium)*	0.7	200	cel/ml	B	B	B	B	
48	Bloei van thecate dinoflagellaten *(Peridinium)*	0.7	500	cel/ml				B	
49	Bloei van *Desmidium swartzii*	0.7	20000	cel/ml				B	

TABEL B **OVERZICHT VAN TAXA DIE VOOR DE VERSCHILLENDE BLOEITYPEN VERANTWOORDELIJK ZIJN**

nr	Bloeitype	Taxa	
1	Persistente bloei van *Planktothrix agardhii*	*Planktothrix agardhii*	
2	Tijdelijke bloei van *Planktothrix agardhii*	*Planktothrix agardhii*	
3	Bloei van *Planktothrix rubescens*	*Planktothrix rubescens*	
4	Bloei van dunne filamenteuze blauwalgen (LPP-groep)	*Limnothrix*	*Planktolyngbya limnetica*
		Limnothrix amphigranulata	*Planktolyngbya undulata*
		Limnothrix obliqueacuminata	*Prochlorothrix hollandica*
		Limnothrix planctonica	*Pseudanabaena*
		Limnothrix redekei	*Pseudanabaena acicularis*
		Planktolyngbya	*Pseudanabaena catenata*
		Planktolyngbya capillaris	*Pseudanabaena galeata*
		Planktolyngbya contorta	*Pseudanabaena limnetica*
5	Bloei van *Thalassiosira pseudonana*	*Thalassiosira pseudonana*	
6	Bloei van *Stephanodiscus hantzschii*	*Stephanodiscus hantzschii*	
7	Hevige bloei van *Microcystis* met omvangrijke drijflaag	*Microcystis*	*Microcystis flos-aquae*
		Microcystis aeruginosa	*Microcystis microcystiformis*
		Microcystis botrys	*Microcystis novacekii*

nr	Bloeitype	Taxa	
		Microcystis dimorpha	*Microcystis viridis*
8	Matige bloei van *Microcystis* met weinig tot geen drijflaag	*Microcystis*	*Microcystis flos-aquae*
		Microcystis aeruginosa	*Microcystis microcystiformis*
		Microcystis botrys	*Microcystis novacekii*
		Microcystis dimorpha	*Microcystis viridis*
9	Bloei van *Microcystis wesenbergii*	*Microcystis wesenbergii*	
10	Soortenarme bloei van *Scenedesmus*	*Scenedesmus*	*Scenedesmus gutwinskii*
		Scenedesmus aculeolatus	*Scenedesmus incrassatulus*
		Scenedesmus acuminatus	*Scenedesmus intermedius*
		Scenedesmus acutus	*Scenedesmus linearis*
		Scenedesmus armatus	*Scenedesmus longispina*
		Scenedesmus asymmetricus	*Scenedesmus magnus*
		Scenedesmus bicaudatus	*Scenedesmus maximus*
		Scenedesmus brasiliensis	*Scenedesmus naegelii*
		Scenedesmus brevispina	*Scenedesmus nanus*
		Scenedesmus caudato-aculeatus	*Scenedesmus obliquus*
		Scenedesmus columnatus	*Scenedesmus obtusus*
		Scenedesmus communis	*Scenedesmus opoliensis*
		Scenedesmus costato-granulatus	*Scenedesmus pannonicus*
		Scenedesmus denticulatus	*Scenedesmus protuberans*
		Scenedesmus dimorphus	*Scenedesmus quadricauda*
		Scenedesmus dispar	*Scenedesmus serratus*
		Scenedesmus ecornis	*Scenedesmus spinosus*
		Scenedesmus ellipticus	*Scenedesmus subspicatus*
		Scenedesmus falcatus	*Scenedesmus tenuispina*
		Scenedesmus flavescens	*Scenedesmus verrucosus*
		Scenedesmus granulatus	
11	Bloei van *Cyclotella meneghiniana*	*Cyclotella meneghiniana*	
12	Bloei van *Stephanodiscus binderanus*	*Stephanodiscus binderanus*	
13	Bloei van *Gonyostomum semen*	*Gonyostomum*	*Gonyostomum semen*
14	Bloei van *Aphanizomenon gracile*	*Aphanizomenon gracile*	
15	Soortenrijke bloei van kleine Chlorococcales	*Chlorophyta <5 µm*	*Pseudodictyosphaerium*
		Crucigenia tetrapedia	*Pseudodictyosphaerium jurisii*
		Dichotomococcus	*Pseudodictyosphaerium minusculum*
		Dichotomococcus curvatus	
		Didymocystis lineata	*Raphidocelis*
		Diplochloris	*Raphidocelis sigmoidea*
		Diplochloris lunata	*Siderocelis sphaerica*
		Marvania geminata	*Siderocelopsis kolkwitzii*
		Monoraphidium circinale	*Tetrastrum komarekii*
		Monoraphidium contortum	*Tetrastrum staurogeniaeforme*
		Monoraphidium tortile	
16	Bloei van *Anabaenopsis*	*Anabaenopsis*	
17	Bloei van *Prymnesium* met kans op toxische effecten op vis	*Prymnesium*	
18	Bloei van *Prymnesium*	*Prymnesium*	
19	Bloei van kleine Cryptophyceae	*Chroomonas*	*Planonephros*
		Chroomonas acuta	*Rhodomonas*
		Chroomonas coerulea	*Rhodomonas lacustris*
		Cryptophyceae	*Rhodomonas lens*
		Plagioselmis nannoplanctica	*Rhodomonas minuta*
20	Bloei van *Cryptomonas*	*Cryptomonas*	*Cryptomonas ovata*
		Cryptomonas acuta	*Cryptomonas platyuris*
		Cryptomonas curvata	*Cryptomonas rostrata*
		Cryptomonas erosa	*Cryptomonas rostratiformis*
		Cryptomonas erosa var. reflexa	*Cryptomonas tetrapyrenoidosa*

nr	Bloeitype	Taxa	
		Cryptomonas marssonii	
21	Bloei van *Skeletonema*	*Skeletonema*	*Skeletonema potamos*
		Skeletonema subsalsum	*Stephanodiscus subtilis*
22	Bloei van *Diatoma tenuis*	*Diatoma tenuis*	
23	Soortenrijke bloei van kleine Chroococcales (ACM-group)	*Aphanocapsa*	*Cyanogranis*
		Aphanocapsa conferta	*Cyanogranis ferruginea*
		Aphanocapsa delicatissima	*Cyanogranis irregularis*
		Aphanocapsa elachista	*Cyanonephron*
		Aphanocapsa elegans	*Cyanonephron elegans*
		Aphanocapsa holsatica	*Cyanonephron styloides*
		Aphanocapsa incerta	*Lemmermanniella*
		Aphanocapsa planctonica	*Lemmermanniella flexa*
		Aphanocapsa stagnalis	*Lemmermanniella pallida*
		Aphanothece	*Lemmermanniella parva*
		Aphanothece bachmannii	*Merismopedia*
		Aphanothece clathrata	*Merismopedia ferrophila*
		Aphanothece minutissima	*Merismopedia minutissima*
		Aphanothece pseudoglebulenta	*Merismopedia punctata*
		Aphanothece smithii	*Merismopedia tenuissima*
		Chroococcus aphanocapsoides	*Merismopedia vangoorii*
		Chroococcus batavus	*Pannus*
		Chroococcus microscopicus	*Pannus punctiferus*
		Coelomoron pusillus	*Pannus spumosus*
		Cyanocatena planctonica	*Radiocystis*
		Cyanocatenula	*Radiocystis aphanothecoidea*
		Cyanocatenula calyptrata	*Radiocystis elongata*
		Cyanodictyon	*Radiocystis geminata*
		Cyanocatena	*Snowella*
		Cyanocatena imperfecta	*Snowella lacustris*
		Cyanodictyon filiforme	*Snowella litoralis*
		Cyanodictyon intermedium	
		Cyanodictyon planctonicum	
24	Langduriger bloei van *Aphanizomenon flos-aquae* met kans op drijflaagvorming	*Aphanizomenon flos-aquae*	
25	Kortdurende bloei van *Aphanizomenon flos-aquae* met kleine kans op drijflaag	*Aphanizomenon flos-aquae*	
26	Bloei van *Anabaena*	*Anabaena*	*Anabaena macrospora*
		Anabaena aequalis	*Anabaena mendotae*
		Anabaena affinis	*Anabaena miniata*
		Anabaena catenula	*Anabaena minutissima var. attenuata*
		Anabaena circinalis	
		Anabaena compacta	*Anabaena mucosa*
		Anabaena crassa	*Anabaena nana*
		Anabaena curva	*Anabaena perturbata*
		Anabaena cylindrica	*Anabaena planctonica*
		Anabaena delicatula	*Anabaena scheremetievii*
		Anabaena echinospora	*Anabaena sigmoidea*
		Anabaena elliptica	*Anabaena smithii*
		Anabaena farciminiformis	*Anabaena solitaria*
		Anabaena flos-aquae	*Anabaena spiroides*
		Anabaena fusca	*Anabaena variabilis*
		Anabaena inaequalis	*Anabaena veneta*
		Anabaena lapponica	*Anabaena viguieri*
		Anabaena lemmermannii	
		Anabaena lemmermannii var. minor	

nr	Bloeitype	Taxa	
27	Bloei van *Aulacoseira granulata* en/of *A. ambigua*	*Aulacoseira*	*Aulacoseira ambigua*
		Aulacoseira granulata	
		Aulacoseira granulata var. angustissima	
28	Bloei van de sieralg *Staurodesmus extensus*	*Staurodesmus extensus*	
29	Bloei van de sieralg *Teilingia granulata*	*Teilingia granulata*	
30	Bloei van *Ankyra*	*Ankyra*	
31	Bloei van *Monomastix*	*Monomastix*	
32	Bloei van *Pedinomonas*	*Pedinomonas*	
33	Bloei van *Pyramimonas*	*Pyramimonas*	
34	Bloei van *Woronichinia naegeliana*	*Woronichinia*	*Woronichinia naegeliana*
35	Bloei van *Chrysochromulina parva*	*Chrysochromulina*	
		Chrysochromulina parva	
36	Bloei van *Cyclotella radiosa*	*Cyclotella radiosa*	
37	Bloei van *Asterionella formosa*	*Asterionella formosa*	
38	Drijflaag van *Gloeotrichia natans*	*Gloeotrichia natans*	
39	Drijflaag van *Aphanothece stagnina* of *A. nidulans*	*Aphanothece nidulans*	
		Aphanothece stagnina	
40	Bloei van *Aulacoseira islandica* en/of *A. subarctica*	*Aulacoseira islandica*	*Aulacoseira subarctica*
		Aulacoseira islandica ssp. helvetica	*Aulacoseira subarctica f. recta*
41	Bloei van *Cyclotella ocellata*	*Cyclotella ocellata*	
42	Bloei van *Chaetoceros*	*Chaetoceros*	
43	Bloei van *Synura*	*Synura*	*Synura petersenii*
			Synura uvella
44	Bloei van *Mallomonas*	*Mallomonas*	*Mallomonas akrokomos*
		Mallomonas acaroides	*Mallomonas caudata*
45	Bloei van *Dinobryon*	*Dinobryon*	*Dinobryon pediforme*
		Dinobryon bavaricum	*Dinobryon sertularia*
		Dinobryon cylindricum	*Dinobryon sociale*
		Dinobryon divergens	
46	Bloei van *Ochromonas*	*Ochromonas*	
47	Bloei van thecate dinoflagellaten *(Ceratium)*	*Ceratium*	*Ceratium hirundinella*
		Ceratium furcoides	*Ceratium cornutum*
48	Bloei van thecate dinoflagellaten *(Peridinium)*	*Peridiniopsis*	*Peridinium goslaviense*
		Peridiniopsis balticum	*Peridinium inconspicuum*
		Peridiniopsis borgei	*Peridinium lomnickii*
		Peridiniopsis edax	*Peridinium palatinum*
		Peridiniopsis elpatiewskyi	*Peridinium pusillum*
		Peridiniopsis penardiforme	*Peridinium raciborskii*
		Peridiniopsis penardii	*Peridinium raciborskii var. palustre*
		Peridiniopsis polonicum	*Peridinium tabulatum*
		Peridinium	*Peridinium umbonatum*
		Peridinium aciculiferum	*Peridinium umbonatum var. centenniale*
		Peridinium beroliensis	
		Peridinium bipes	*Peridinium umbonatum var. umbonatum*
		Peridinium cinctum	
		Peridinium cunningtonii	*Peridinium willei*
		Peridinium deflandri	
49	Bloei van *Desmidium swartzii*	*Desmidium swartzii*	